A word from the editor

The book series “DISEASES OF MARINE ANIMALS” (organized and edited by Otto Kinne and contributed to by numerous outstanding experts over years) is now freely available with online Open Access.

Published in the years 1980 to 1990, the copyright for “DISEASES OF MARINE ANIMALS” was graciously transferred by the original publishers, to the editor Otto Kinne.

The technical problems involved in the re-publication of the treatise were mastered by Konstantin Kambach (Inter-Research). Unavoidably, the print quality of the final product is somewhat inferior to the original.

Otto Kinne
Oldendorf/Luhe
21.01.2009

DISEASES OF MARINE ANIMALS

Volume I GENERAL ASPECTS, PROTOZOA TO GASTROPODA

Volume II INTRODUCTION, BIVALVIA TO SCAPHOPODA

Volume III INTRODUCTION, CEPHALOPODA, CRUSTACEA, etc. TO UROCHORDATA

Volume IV INTRODUCTION, PISCES, REPTILIA, AVES, MAMMALIA

DISEASES OF MARINE ANIMALS

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VOLUME IV, PART 1
Introduction, Pisces

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FOREWORD

Volume IV* of 'Diseases of Marine Animals' summarizes and evaluates the present status of our knowledge on the diseases of those Pisces, Reptilia, Aves and Mammalia that live permanently or temporarily in marine or brackish habitats. The volume centers on biotic diseases, proliferative disorders, structural abnormalities, and impairments due to environmental stressors. While Volume IV concentrates on marine forms, it incluces data on species migrating between marine, brackish and limnic habitats. Wherever considered desirable for completeness and comparison, disease phenomena in fresh-water living relatives are also referred to.

Volume IV consists of 2 parts containing the following chapters:

Part 1

Introduction to Volume IV, Part 1: Pisces
Chapter 1: Diseases of Pisces

Part 2

Introduction to Volume IV, Part 2: Reptilia, Aves and Mammalia
Chapter 2: Diseases of Reptilia
Chapter 3: Diseases of Aves
Chapter 4: Diseases of Mammalia: Carnivora
Chapter 5: Diseases of Mammalia: Pinnipedia
Chapter 6: Diseases of Mammalia: Sirenia
Chapter 7: Diseases of Mammalia: Cetacea

I could not avoid having Volume IV appear before Volume III. With all contributions to Volume IV in my hands and knowing that the preparation of Volume III would take another year, I decided not to let the Volume IV contributions age on my desk but to make them available to the scientific community as fresh and early as possible — even at the risk of being critized for breaking the fundamental rule of chronological sequence.

There is more knowledge available on the diseases of fishes than of any other group of aquatic organisms. This fact is mirrored by the large size of Chapter I.

Although drawing on different sources, talents and disciplines, Volume IV maintains, as much as possible, the principles of organization and perspectives outlined in the introduction to Volume I.

I acknowledge with pleasure and gratitude the assistance and support received from all contributors to this tome; from Helga Witt, Seetha Murthy and Nancy Norris-Bauer in matters of technical editing; from Martin Söhl and Frau Schritt in the search for literature information.

Oldendorf/Luhe, September 16, 1984 O. KINNE

* For technical reasons Volume IV will be published before Volume III.

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OF
VOLUME IV, Part 1: Pisces

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INTRODUCTION
PISCES

INTRODUCTION TO VOLUME IV, PART 1: PISCES

O. KINNE

GENERAL SCOPE

Although a multi-author achievement, this book was conceived, planned and prepared with the aim to perpetuate – for the benefit of consistency, clarity and easy orientation – the organizational concept developed at the outset of organizing this treatise (for details consult Kinne, 1980a, b). It was considered equally desirable, however, to maintain the individuality and specificity of the contributions based on the authors' professional backgrounds and experiences. Hence, Volume IV is a compromise between consistency in style and format on the one hand, and the diversity of talents, expertises and perspectives of its contributors on the other.

SUMMARIES OF CHAPTER CONTENTS

Comments on Fish Diseases Caused by Microorganisms

Microorganisms constitute a most important source of disease in fish. In fact, over millions of years microorganisms have achieved mastership in gaining access to, and in the utilization of, organic materials synthesized and stored in the bodies of living organisms. Where the latter fail to prevent or to counteract excessive microbial attack, disease prevails.

In this tome, diseases of fishes due to parasitic microorganisms receive extensive and detailed attention. Diseases caused by viral forms have been documented and discussed by K. Wolf (p. 17); those due to bacteria by D. A. Conroy (p. 48); and those due to fungi by G. Lauckner (p. 89).

Diseases due to Viral Agents

There can hardly be any doubt: while our present knowledge on fish diseases caused by Virales is still quite limited, important breakthroughs can be expected in the foreseeable future. In fact, viruses may be shown not only to be the primary cause of numerous diseases not yet fully explored or even known, but also to function as pacemakers for numerous other secondarily effective disease agents. For these reasons, viruses require special attention in future research.

K. Wolf has summarized and evaluated the knowledge available on the diseases of marine, anadromous and catadromous fishes due to virus, visualized virus and virus-like particles. Some 50 such agents are now known to science. They exhibit the same basic characteristics as the Virales of homeotherms and they comprise the same virus groupings, e.g. herpesviruses, iridoviruses, rhabdoviruses and reoviruses. Additional fish-disease agents are considered to be adenovirus, coronavirus, calicivirus and leucovirus. Still others await investigation. Wolf predicts that also some of the major virus groups not yet isolated

from fish but known to exist in homeotherms will eventually be found to inhabit fish hosts. The overwhelming number of reports on virus-caused fish diseases stem from captive fish, i.e., hosts kept under environmental conditions which deviate from those to which the fish host has adapted during its evolution. Some 87 % of the viruses detected in fish tissues inhabit hosts (mostly fish raised in fresh-water aquaculture farms) utilized as human food. The remaining 13 % have been reported from tropical fishes, mostly pet fish (ornamental fish) kept in home aquaria.

Thus far, viruses inhabiting fish tissues have not been shown to be infectious to man or other homeotherms. However, the effects viruses exhibit in fishes and the general patterns of agent-host relations are similar in both cases. Two fish-inhabiting viruses, a leucovirus-like agent of esocids and a herpesvirus of *Oncorhynchus masou,* are oncogenic and other viruses, observed in neoplasms, might also turn out to be oncogenic (i.e., tend to induce tumors).

Wolf reviews the knowledge available on virus-caused fish diseases in a systematic exposition, introducing subtitles such as: Pathologic Changes, Biophysical Virus Properties, Diagnosis, Isolation and Identification, Transmission and Incubation, Geographic Range and Control Measures. Among the marine fishes, Wolf considers cod ulcus syndrome (p. 18), lymphocystis disease (p. 20), menhaden spinning disease (p. 23), turbot herpesvirus infection (p. 24) and viral erythrocytic necrosis (p. 25); among anadromous fishes, chum salmon reovirus (p. 27), herpesvirus salmonis disease (p. 28), infectious hematopoietic necrosis (p. 30), infectious pancreatic necrosis (p. 33) and *Oncorhynchus masou* virus (p. 36); among catadromous fishes, eel viral kidney disease (p. 38), eel rhabdovirus infection (p. 39) and eel virus-2 (p. 41). A special section is devoted to visualized viruses and virus-like particles.

Diseases due to Bacteria

Present in each drop of water, each cubic millimeter of sediment, attached to suspended particles and living in large numbers on and in all other organisms, bacteria are of basic importance for the functioning of natural aquatic and terrestrial ecosystems. While most bacteria known so far are benign, a large number have been shown to be the immediate cause of disease or to be secondarily involved in the development of disease phenomena.

D. A. Conroy reviews diseases due to bacteria in euryhaline and stenohaline marine fishes. He considers the disease phenomena reported in the scientific literature in the context of syndromes rather than as specific infections caused by a defined (i.e., taxonomically identified) etiological agent. Over the last few decades, research on bacteria-caused diseases of fishes has received unique impetus from man's rapidly increasing efforts to provide additional food through aquaculture. Intensive fish culture thrives under conditions which tend to change dramatically the natural agent-host balances, typically, in favor of the former. In addition to viral agents, this is particularly so for bacteria. The future of commercial fish culture depends decisively on the development of handy methods for diagnosis, prevention, control and treatment of fish diseases due to scores of bacterial agents.

Conroy pays special attention to bacteria-caused diseases of mugilids and salmonids, a reflection of his own professional background. He reviews in detail the following groups of disease phenomena: haemorrhagic septicaemia (p. 54), myxobacterial infections (p. 74),

streptococcal infections (p. 77), acid-fast bacterial infections (p. 79) and anaerobic bacterial infections (p. 85).

Diseases due to Fungi

Of the numerous marine fungi only a few associate closely with marine fishes. Thus far, *Ichthyophonus (hoferi)* is the only marine fungus that has been shown to cause diseases in fishes. On the basis of detailed and careful literature research, G. Lauckner traces and corrects numerous misquotations, misinterpretations and errors regarding the taxonomic identity of this organism. He concludes (p. 92) that *Ichthyophonus* is a plant taxon, with *I. hoferi* as type species (should, however, *Ichthyosporidium gasterophilum* Caullery et Mesnil be shown to be a fungus of the genus *Ichthyophonus,* and should it turn out to be different from *I. hoferi,* then *Ichthyophonus gasterophilum* would have to be designated as type species). *I. hoferi* invades its hosts via their alimentary tract by thick-walled, multinucleate 'resting spores' liberated from disintegrating, infected tissues. Transmission occurs from fish to fish. Ichthyophoniasis is a systemic infection (similar to tuberculosis or mycobacteriosis), characterized by granulomas in the affected organs. It can develop into a severe disease resulting in heavy mortalities in acutely and chronically infected fish. In *Clupea harengus,* external signs of the disease include a 'sandpaper appearance' of the skin due to minute reddish pustules (pathogen cysts in lateral muscles). In addition, white necrotic areas may form on the skin, as well as ulcerations from which spores are released into the ambient water. *Ichthyophonus* disease can also manifest itself in sex reversal, exophthalmia, hyperaemia and other eye infections, etc. From the marine environment, reports on fish invading fungi other than *Ichthyophonus* sp. are rare.

In contrast, numerous instances of fish diseases caused by fungi have been reported from fresh-water habitats. Members of Oomycetes, Deuteromycetes and Ascomycetes can cause severe diseases and heavy losses among fresh-water-living fishes, especially those reared and raised in aquaculture farms.

As with other diseases, prevention and treatment of fungus diseases is possible only under controlled conditions in captive water bodies. Prophylactic measures such as water-quality control, sanitary care, selection of uninfected food material and a rigid quarantine regime seem the primary methods of choice.

Comments on Fish Diseases Caused by Protistans

Of the ca 24,000 species of protozoans known to science, some 6,000 to 7,000 live during part or all of their life in close, intimate association with other organisms and obtain one-sided benefits (energy, matter, living space) from such symbioses at the expense of their partner (host). Hence these species are classified as parasites. The vast majority of protozoan parasites thus far studied live in fresh-water. Numerous marine and brackish-water protozoans await thorough study; only a few have been investigated in depth.

Most of the about 800 protozoan parasites known to live on or in marine fishes have received attention not primarily under etiological aspects or as disease-causing agents, but with an eye on their taxonomy, morphology or life-cycle dynamics. In fact, although seemingly of paramount significance as potential causes of fish diseases, parasitic protozoans have been studied less extensively than helminths (p. 193) or crustaceans (p. 321).

J. Lom summarizes and evaluates a large body of information concerned with the disease-causing potential of flagellates (p. 120), amoebic and opalinid forms (p. 127),

Apicomplexa (p. 127), Microspora (p. 138), Myxosporea (p. 148) and Ciliata (p. 157). In addition, he considers in detail nutrition, proliferation, life cycles, transmission, host relations, pathogenicity, host defense and ecological aspects of protozoan infections. Special attention is paid to diseases in cultivated fishes. Lom concludes that research on fish diseases due to protistan agents is still in its infancy.

As is the case with other biotic disease agents, protozoan species benign under undisturbed, natural conditions may succeed in obtaining considerable benefits (energy, matter, space) from fish hosts under stress (e.g., pollution, crowding in intensive culture), and consequently in inflicting severe damage. In nature, serious lesions have been reported to be due to tissue infecting forms. Ectoparasitic protozoans may be particularly harmful due to rapid flare, no need for intermediate hosts and little or no host specificity. The pathogenicity of protozoans (Flagellata, Amoebae) infesting the intestinal lumen of fishes tends to be quite limited. However, more experiments must be conducted in order to examine whether or not coccidians — which are common in fish intestines, but have not yet been shown to cause serious diseases — can in fact exert pathogenic effects in their respective hosts.

Comments on Fish Diseases Caused by Protophytans

To all we know, Protophyta (algae) do not qualify as important disease agents of marine fishes. According to G. Lauckner's literature research, only the dinoflagellates have evolved representatives — members of the genera *Amyloodinium, Crepidoodinium* and *Ichthyodinium* — which parasitize fishes. Thus, *A. ocellatum* is the agent of 'velvet disease' (p. 172) known from temperate and warm-water, marine fish species. It invades primarily the gills, in heavy infestations also the skin. Its rhizoids inflict cell damage and may cause hyperplasia, inflammation, haemorrhages and necrosis. *C. cyprinodontum,* although not penetrating the hosts' epithelial cells, may also cause gill hyperplasia and necrosis (oodiniasis, p. 172), but thus far has not been shown to produce direct mortalities in cultivated fish. *I. chabelardi,* observed to parasitize ova and freshly hatched larvae of sardines *Sardina pilchardus,* does not seem to cause large-scale mortalities among infested eggs and larvae.

Non-parasitic, free-living, red-tide-causing dinoflagellates — such as *Gymnodinium breve* — when present in large populations with high individual densities ('blooms'), may cause large-scale mortalities in fish and other taxa by releasing toxic substances into the ambient water.

In addition to dinoflagellates, members of the Cyanophyceae, Chlorophyta, Phaeophyta and Rhodophyta may associate with fishes. The role of these algae as fish symbionts remains to be investigated.

Comments on Fish Diseases Caused by Cnidarians

Intimate associations between cnidarians and fishes are rare. G. Lauckner summarizes the evidence available for a number of cnidarian–fish symbioses. These range from commensalism to parasitism. Several species of the athecate genus *Hydrichthys* have been shown to live parasitically on fishes (mainly juvenile or small-sized species), primarily affecting the hosts' body surface (epidermis, fins, opercula, gills), but some also invade the

fish body via wounds caused by fish-associated copepods and exploit deeper tissues and blood vessels.

Comments on Fish Diseases Caused by Helminths

The heterogeneous group of Helminthes – consisting of Turbellaria, Monogenea, Trematoda, Cestoda, Nematoda, Acanthocephala and Hirudinea – constitutes the largest, most numerous and most diversified assembly of parasites associating with marine fishes. However, only a few helminth species have been studied in depth and little is known about agent–host interactions, especially not in terms of the flow routes and rates of energy and matter between parasite and host. Parasite effects at the individual or population levels, agent virulence, host defense, host range, host specificity, etiology, as well as disease prevention and therapy await thorough attention from pathologists and ecologists alike.

His detailed review leads K. Rohde to conclude that most experimental studies conducted on helminth biology are concerned with non-marine helminths. The taxonomy and life cycles of many species remain to be analysed. Pathogenicity and detailed agent effects have been explored in only a few selected species, and practically nothing is known about helminth–fish relations in the unconstrained environment. That reliable reports on mass mortalities in natural fish populations due to helminth parasites are very rare, need not necessarily be indicative of the absence of such events, but may be due to the difficulties involved in the sampling of diseased, and hence weakened, as well as of dead hosts (see also Kinne 1984, p. 654 and pp. 10–11 of this tome).

His own studies and those reported in the scientific literature suggest to Rohde that most helminth species associated with marine fishes inhabit warm surface waters, particularly in the Indo-Pacific Ocean. While no clear evidence has been produced yet to indicate that helminth-caused fish diseases are also more varied in tropical waters than in non-tropical waters, in analogy to the established greater variety of helminth-caused diseases of man and domestic animals in the tropics, Rohde suggests that such a relation appears not unlikely.

Helminth-caused effects on fish hosts range from mild to severe to lethal. Several Trematoda may cause popeye (exophthalmia). Monogenea may exert severe effects on gills and skin; they can cause destruction of the eye, and sometimes death. Cestodes may damage the intestinal wall, and larvae of anisakid nematodes reduce the relative weight of the liver as well as the condition factor. Parasitic Acanthocephala can perforate the intestinal wall, and leeches may retard growth rates and transmit parasitic protozans. Following artificial introduction, the monogenean *Nitzschia sturionis* produced mass mortalities in sturgeon populations in the Aral Sea.

Helminth-caused diseases are more obvious, easier to study and often also more frequent in captive fishes (research cultivation and commercial cultivation), than in their free-living counterparts. Interestingly, cultured fish have been shown to lose many of their helminth parasites, while other parasites affect them in increased numbers. Such shifts in agent–host relations are indicative of differential environmental requirements of agent and host or of lack of intermediate hosts under culture conditions (see also Volume I: Kinne, 1980b).

Comments on Fish Diseases Caused by Crustaceans

Considering the role of marine Branchiura, Copepoda, Isopoda, Amphipoda, Cirripedia and Ostracoda as causes of fish diseases, Z. Kabata presents here the first detailed review on the subject. This contribution is based on his earlier review (Kabata, 1970) comprising both limnic and marine forms. As is the case with other biotic disease agents of fishes, more information is available on freshwater species — both agents and hosts — than on their marine relatives. In addition, few investigators have explored crustaceans with a view on their role as fish symbionts and as potential causes of fish diseases.

Among the crustacean groups considered, copepods display the widest range of adaptation to life on or in fishes. As in other crustacean fish parasites, small and mobile ectoparasites do not tend to be highly pathogenic; the intensity of their impact depends primarily on parasite density and host distribution. While larger, mobile ectoparasites may cause lesions, except for species of *Argulus* the effects inflicted are usually not severe. In general, crustacean gill parasites are capable of causing extensive damage to their hosts. However, from the marine environment no evidence is available to date for severe respiratory impairments. Serious host damage may be caused by mesoparasitic copepods. In fact, their association with fishes is believed to often result directly in fish mortality. Crustaceans living endoparasitically in fishes have been shown to cause a variety of effects, depending primarily on body size and taxonomic identity of agent and host, site and duration of the infestation and, of course, the balance between agent virulence and host defense.

Kabata considers in detail a variety of local parasite effects (e.g., on gills, skin, muscle, skeleton, sense organs, heart, liver, kidneys, gonads, alimentary canal) and general effects (e.g., on fish weight and chemical composition, growth, metabolism, blood, reproduction, behavior). General effects are easy to define and classify but difficult to assess in terms of their exact causes (p. 381). Secondary infections facilitated by crustacean parasites are insufficiently investigated. This holds for their importance relative to cases of intimate coexistence between fish hosts and additional groups of parasitic symbionts, as well as for defining cause–effect relations.

A brief consideration of impacts on fishes due to free-living crustaceans and of the economic consequences of detrimental parasite effects in fishery and aquaculture conclude Kabata's review. While attempts have been made to estimate the losses in quality and numbers of fishes suffered by commercial fishery and aquaculture ventures, and while the losses may be considerable, sound assessments require more solid data than are presently available.

Comments on Fish Diseases Caused by Neoplasia

Neoplasia ('new growth' or 'new formations') comprise local swellings (tumors) due to excessively growing tissues, benign or malignant. In recent years the study of neoplasms in aquatic organisms has been greatly intensified. An increasing number of organisms have been shown to carry tumors, especially those maintained in captive bodies of water (i.e., under modified environmental conditions) and those living in natural bodies of water subject to severe man-made pollution.

N. Peters' contribution on neoplasms of marine fishes is organized according to the

tissue type of tumor origin (epithelial, mesenchymal, pigment-cell, neural tumors) and the type of tumors formed (e.g., papilloma, fibroma, lymphoma, melanoma, neurilemmona, etc.). Peters uses wide-spread and well-investigated cases of fish neoplasia to document the present state-of-the-art. Wherever considered useful, he includes pertinent information on freshwater fishes.

Neoplasia of fishes are similar to those reported from higher vertebrates. Tumors are wide-spread among different fish taxa, ranging from Cyclostomata to Chondrichthyes to Teleostei. Almost all organs may be affected. In some cases, tumorous fish diseases have attained epidemic proportions and thus economic importance. Most of our present knowledge of fish neoplasia stems from investigations on freshwater forms. Of the different types of tumors reported, several have not yet been observed in marine fishes. Certain kinds of tumors can be produced experimentally via crossbreeding: they develop regularly in hybrids of different tumor-free parents.

As other disease phenomena, tumor formations are subject to natural selection. Since most fishes reproduce throughout their life, even tumors forming in old individuals participate in affecting the evolution of counteractive mechanisms (e.g., DNA repair, immunological defense). Peters concludes that tumor diseases may assist in programming the gene pool of a population towards a high level of resistance against tumor formation (p. 422).

While the occasional occurrence of neoplasia appears to be a natural phenomenon, rates of tumor diseases exceeding 1 % in a given population require explanation. According to Peters, they may be attributable to: (i) interbreeding resulting in genotypes with reduced tumor resistance; (ii) infections (virus) of non-adapted (non-immunized) fish; (iii) sudden environmental change (man-made water pollution; invasion of new habitats).

As with other diseases, it is usually the combination of different causes acting in concert that induces tumor growth. Like organisms themselves, tumors respond to the total accumulative impact of their envrionment.

Comments on Fish Diseases Caused by Environmental Stressors

The term 'stress', in its general connotation, refers to 'strain', 'pressure' or 'tension' exerted upon a body. In biology the term 'stress' to many investigators implies forces (e.g., sub– or supranormal intensities of temperature and/or salinity, oxygen availability, injury, disease-agent impact, social dominance) acting upon an organism or its parts, significant enough to interfere with normal functions. Such forces may produce non-specific or specific responses. Non-specific responses are — regardless of the causative agent — similar in quality (general adaptation syndrome: Selye, 1950, 1951); specific responses more or less differ from each other, depending on the types of stress concerned. In practice, such differentiation may be difficult to make because a given organism always responds to the summative, concomitant impact of all stress-causing forces encountered. Unfortunately the terminology employed for describing organismic responses to environmental stress — including that due to coexisting, foreign, free-living or parasitic organisms — is inconsistent.

G. A. Wedemeyer and C. P. Goodyear employ the term 'stress' to designate the response of an organism to an impact; the term 'stressor', to identify the causative agent. While extreme intensities of stressors, acute or chronic, tend to be lethal, sublethal stressor

intensities can induce a variety of negative deviations in the normal functions and structures of living organisms; they constitute the main subject of the review. Examples of sublethal stressor effects are: increased susceptibility to disease, reduced tolerance to other stressors, diminished rates of growth and reproduction, and a decreased capacity for intra- and interspecific competition. Effects of stressors at the community level are more difficult to establish. They manifest themselves through changes experienced at the individual level and may be masked by a variety of compensatory mechanisms effective at supra-individual levels of integration.

Fishes challenged by stressors respond by compensative measures at the molecular, subcellular, cellular, endocrine, blood, tissue and organ levels. While such measures tend to be similar in different species, species-specific differences have been reported. Typically, a stressor induces the central nervous system of the responding fish to release 'stress hormones' which, in turn, initiate the compensatory measures available and necessary.

Based on Wedemeyer and McLeay (1981), 3 levels of stress responses are distinguished: the primary level involving the endocrine system; the secondary level comprising blood and tissue chemistry; and the third level manifesting itself in individuals and populations. Reliable assessments of population-level responses are difficult to make, except in cases of severe impact and damage.

Considering the potentially pathogenic dimensions of stressor effects, Wedemeyer and Goodyear review and discuss reports which document or suggest that stressful environmental impacts (including those due to pollution) may predispose fishes to biotic and non-biotic diseases. Also behavioral factors can affect the fishes' disease predisposition, e.g., through excessive intensities of social dominance or of crowding. In aquaculture, vibriosis, viral erythrocytic necrosis, myxobacterial gill disease and bacterial hemorrhagic septicemia have become classic examples of stress-mediated infectious diseases.

Stress-mediated fish diseases may serve as indicators of environmental deformations, including those due to man's activities (i.e., in biological monitoring). Infectious diseases due to bacteria (aeromonads, pseudomonads, Myxobacteria) appear to be particularly well-suited because these pathogens are widely distributed in marine and inland waters. Non-infectious fish diseases that may hold promise as indicators of environmental deformation include chromosomal and structural abnormalities of eggs and larvae, skeletal anomalies and neoplasms (p. 400).

Wedemeyer and Goodyear document and discuss host-pathogen-environment relations, mechanisms of infectious and non-infectious diseases, as well as the effects of fish diseases at the population level. They conclude that lack of basic ecological data is hindering the definition of management alternatives for solving many acute fishery resource problems.

CONCLUSIONS

Basic Considerations

It seems safe to assume that the origin and evolution of life on earth was accompanied – right from the beginning – by the need of the newly-formed living substance to develop means for coping with and counteracting a variety of life-endangering circumstances and for defending the organic material synthesized and contained in a discrete body of life (individual) against other living entities – competitors for life-supporting substances. In other words: the phenomenon of disease appears to be as old as life itself. This statement applies to all the different basic categories of disease causes: (i) critical intensities of abiotic environmental factors; (ii) damage due to coexisting organisms; (iii) nutritional disorders; (iv) physical injuries; (v) detrimental circumstances internal to the individual involved (innate, idiopathic or genetic diseases).

While a given disease reveals itself to the observer at the individual level, its ecologically most significant effects appear to lie at the population or species level: adaptive changes in the gene pool, affected by selection of organismic variability and directed towards counteracting, or adjusting to, the cause of the disease. In response to environmental change and in concert with comparable adjustments in competing species such evolutionary changes lead to ever new levels of newly achieved balance and quasi-stationary harmony among different coexisting forms of life. Endangering the very existence of the individual, disease thus acts as a competitive, stabilizing mechanism at the population or species level. In fact, disease is a major motor and denominator of evolution.

This positive aspect of disease is no longer fully effective in man. Here, our attempts to safe-guard the individual against disease must be paid for, in the long run, by potentially incurable deficiencies at the population level, i.e., in our genetic material. It seems surprising: few, if any, of us who celebrate medical achievements appear to realize that success in controlling disease in individuals will ultimately produce shortcomings in our gene pools, i.e., will constitute the very cause of disease at the population or species level.

Individuals are the torch-bearers of life. They are time-space gestalten in which life temporarily manifests itself and in which it becomes formable in terms of diversification, specialization and selective directivity. All our own experiences are those of individuals, and our behavior, emotions or ethics are unthinkable unless rooted in individuals. Just as unthinkable would be the denial of medical help to an individual – a family member, a friend, a neighbor, or in fact, any member of the species *Homo sapiens* – on the grounds that such help may ultimately reduce healing forces developing through selection and evolution.

While this is not the place to deepen the argument, I wish to say here that I, personally, am even more concerned about the long-term effects of man-induced degradation of our genetic material than about the man-made pollution of our envionment. Most of our technological „achievements" and our seeming superiority over other forms of life may, in the end, turn out to work against us. In fact, how can any organism that has evolved over millions of years as a fully integrated member of earth's ecosystem succeed permanently in escaping the system's firmly established rules, laws and controls? Unless, that is – unless that organism commands unique abilities, not only in terms of technological intelligence, but also in terms of ethic and moral strength. But then, how could ethic and moral strength evolve in

an integrated ecosystem member? Nature does not select such properties. There is no mechanism of biological evolution that would support the development of ethical properties of the intensity and depth required for long-term survival of modern man (Kinne, 1984b, pp. 36–37). The ethical properties required are (i) *modesty,* i.e., self-control of man's unique dominance over all other forms of life and self-restriction in utilizing nature as a resource; (ii) *tolerance* of differences among men (racial, political, religious, philosophical), i.e., sympathy for, not suppression of, diverging thought and conduct; (iii) development of a *universal moral responsibility* for fellow creatures. All these properties are foreign – yes, even detrimental – to fully integrated members of earth's ecosystems, but they are a must for a species that has overcome essential integrating ecosystem forces and that now exerts a unique impact on, and control over, the system (Kinne, 1984b). In the absence of ecosystem-based evolutionary forces, the properties needed for man's new role in the overall system 'nature plus man' must be developed *de novo,* as an act of 'free will'. The substrate for such development comprises: recognition of the need for change; the firm intention to change; and a titanic ethical effort.

Modern man's basic dilemma is his apparent inability to develop from the status of a fully integrated ecosystem component to the status of an ecosystem 'controller' or 'conserver'. This inability is likely to restrict the life time of modern man – in geological terms – to an ephemeral existence. There appears to be no permanent niche between ecosystem member and ecosystem controller (Kinne, 1984b). In fact, the role of ecosystem controller is already occupied: Nature herself seems to control the system very effectively, so much so that her pull of gravity will, in time, bring back and reabsorb any would-be escapist.

Research on Fish Diseases – Importance, Problems and Shortcomings

Fishes are important members of aquatic ecosystems and are of paramount economic importance, both in fisheries and aquaculture. Especially in the last 2 decades increasing portions of public funds have been chanelled into supporting research on the diseases of fish species used in the production of protein for the world-wide increasing human population. While the body of information available is, in most cases, still grossly inadequate for in-depth assessments of disease causes and consequences, and for potential therapy measures, we know more about the diseases of fishes than of any other group of aquatic organisms.

Unfortunately, the overwhelming amount of the knowledge available on fish diseases is restricted to very few species – less than some 2 % of the total number of fish species presently known to science. As in other fields of biology, such heavy emphasis on a few commercially important or easily culturable forms is likely to distort the real picture, to falsify assessments of the ultimate ecological significance of diseases phenomena in free-living fish populations, and to mask the effect of diseases on the overall deformability of nature due to the impacts suffered from modern human societies.

Another problem complicates a sound analysis of the causes and consequences of disease phenomena in fishes under *in situ* conditions: As other diseased organisms, diseased fishes tend to disappear quickly and completely; hence they cannot be collected quantitatively (see also Kinne and Bulnheim, 1984a; Kinne, 1984a, p. 654; Lester, 1984). Disease of an organism tends to reduce (i) the energy available for sustaining essential functions and structures; (ii) resistance to other concomittantly effective stressors (natural

and man-made); (iii) capabilities for defense and escape. Consequently, disease renders the individual involved more available to its predators and to dislodgement from its original habitat due to water-current transport or sinking. Furthermore, disease diminishes the potential for competition and for counteracting additional disease-causing entities. Since long-term data on population dynamics are difficult to obtain and are in most cases not available, and since increasing pollution loads, especially in near-coastal areas and estuaries, induce superimposed influences and changes in ecosystem dynamics, the ecological net effects and the significances of disease phenomena in natural populations and in ecosystems are extremely difficult to assess and to quantify.

This situation contrasts sharply with that prevailing in captive fishes, both those used for physiological or ecological experimentation and those reared and raised in commercial aquaculture farms. Here, numerous carefully and convincingly documented accounts have become available on disease-causing entities, and a rapidly increasing body of knowledge is emerging on etiologies, as well as on disease diagnosis, prevention and therapy.

A captive body of water always undergoes a number of changes which ultimately alter the biological, chemical and physical properties of the water concerned and, hence its life supporting qualities. For many organisms, such changes lead to augmented stress, for others they increase the opportunities for establishment and development. In many cases, the first group comprises larger, multicellular organisms, usually referred to as hosts; the latter group, the more resistant microorganisms, among them a variety of disease-causing agents. The natural balance between symbionts tends to become distorted under conditions of captivity, and such distortions frequently result in disease phenomena. In order to compensate for detrimental changes in captive water bodies, water-quality management becomes necessary (Kinne, 1976a, b), i.e., planned and controlled improvements of water characteristics, often tailor-made for the support of selected target organisms.

Differential effects of environmental change on agent and host may also rid the host from its parasite. In the microsporidian parasite *Octosporea effeminans* and its host, the amphipod *Gammarus duebeni,* for example, the salinity ranges tolerated are not parallel. In increased salinities, agent effects diminish progressively until, in 30 ‰, the parasite tends to disappear,while the host survives without demonstrable harm (Bulnheim, 1969, 1975, 1978).

In commercial aquaculture farms environmental conditions are usually very different from those prevailing in natural ecosystems. The aquaculture entrepreneur strives to manage environmental conditions, flow patterns of energy and matter, and, wherever possible, the genetic constitution of his target organisms in such a way as to maximize and economize the mass production of marketable foods or raw materials – no matter how much the conditions in his culture enclosures deviate from those to which the cultivated organisms have adapted during their evolution (e.g., Kinne, 1976a, b, 1977; Kinne and Rosenthal, 1977). Of course, such deformation of natural requirements, conditions and equilibria is likely to affect the balance between host predisposition and agent virulence. Hence, host–agent relations and disease manifestations may be different in culture and in nature. This is an important point to keep in mind when studying and interpreting case histories and etiologies.

There is an additional point – already referred to in Volume I (p. 16; see also Kinne, 1984a, p. 653) – which needs to be emphasized again. Disease has been defined as 'a demonstrable, negative deviation from the normal state (health) of a given organism'

(Volume I, p. 14). In this definition, 'negative' implies an impairment, quantifiable in terms of a reduction in the ecological potential of the organism concerned. The negative deviation may be functional or structural or both, and it may result from a single cause or – more often – from several causes acting in concert. Of course, in a biotic disease, all partners involved may 'get sick'. Identifying themselves automatically with the larger partner (the 'host'), laymen and researchers alike have introduced here a uniquely subjective concept into the description and investigation of biotic disease phenomena, i.e., diseases based on the intimate coexistence of different forms of life (symbiosis; Volume I, p. 18). A fish and a nematode, for example, may live together in a balanced symbiosis without negatively affecting each other. Where such a balance shifts significantly in favor of the nematode, the fish will tend to suffer demonstrable deviations in functions and/or structures, i.e., it will exhibit a nematode-caused disease. The same, of course, may happen to the nematode if the balance shifts in favor of the fish. But I have never heard anybody talk about a fish-caused nematode disease in such a case.

Changes in the balance between agent and host are induced through genetic, environmental or nutritional factors. Unless disproven, we may postulate that the ecological dynamics which govern the relations between symbionts – including those identified as potential disease agents – are subject to principally the same rules that govern the interrelations among the free-living members of an ecosystem. In both situations, the key for comprehending the processes involved and their causes, as well as for the correction of damage (disease; environmental degradation) lies in the identification of (i) the forces responsible for destabilizing and changing established equilibria; (ii) the mechanisms of resilience (maintenance of existing balances).

A major shortcoming in fish disease research is the frequently confusing taxonomic status of the organisms involved, especially among the protozoan agents, but also in taxa of multicellular parasites. Numerous authors have described the parasitic species concerned with insufficient accuracy, others have quoted previous work incorrectly, and still others have failed to trace the taxonomic history of a given species with accurate care. This state of affairs has been documented in this Volume especially by G. Lauckner. The situation is similar in other agent-host groups (e.g., Lauckner, 1980, 1983).

There is much need for broadening the basis of disease research: we must apply modern ecological concepts; determine and quantify the flow patterns of energy and matter between the partners involved in a biotic disease; study the factors responsible for establishing and/or destabilizing equilibria between antagonistic forces; strengthen the cooperation between marine, brackish and limnic researchers; and include in our research programs non-commercial organisms of a wide taxonomic variety. Since life originated in an aquatic medium, the deepening of our understanding of disease phenomena in aquatic organisms can be expected to contribute significantly to the analysis, comprehension, prevention and treatment of diseases in general, i.e., including the diseases of organisms now living on land, and man. For this reason, a new International Journal has been established which is being directed by a board of Subject Editors with diverse professional backgrounds and of high international reputation. Hopefully, this new Journal* will assist in strengthening the foundations for modern disease research.

(*"Diseases of Aquatic Organisms". Inter-Research, POB 1120, D-2124 Amelinghausen, Federal Republic of Germany).

Special Significance of Viruses and Bacteria as Causes of Fish Diseases

Of particular importance as causes of disease phenomena in fishes are microorganisms, especially members of the universally-wide-distributed viruses and bacteria. For microorganisms, the organic material synthesized by, and temporarily stored in, the body of a living organism constitutes a unique source of life-supporting energy and matter. In the course of their evolutionary history, microorganisms have developed an impressive variety of 'ingenious' methods and tricks to gain access to these resources, and the organisms thus attacked have, in turn, 'invented' equally sophisticated mechanisms to counteract the intruders and to defend themselves. What we begin to discover today is a fascinating documentation of a millions-of-year-old game of 'outsmarting' each other, between parasitic microorganisms and their living target substrates (hosts).

Typically, the opposing or complementary roles and strategies of co-evolving agent–host pairs appear to follow 'restricted conflict rules', characterized by periods of sustained over-all equilibria. However, such equilibria are disrupted by distortions due to endo- or exogenously induced changes in the ecological potentials of the partners. Distortions are often especially pronounced in evolutionary young (insufficiently buffered) interrelations. Unbalanced agent–host relations tend to induce demonstrable negative deviations from the normal state in one (or more) of the partners involved, i.e., disease.

Ranging in size from some 18 to 410 nm, viruses depend entirely on living animal, plant or bacteria cells for maintenance, growth and development. Within the large organizational spectrum of living and non-living materials present on Planet Earth, viruses occupy a special position. They are composed of a protein coat, a nucleic-acid core and, in some cases, carbohydrate and lipid materials. Several of these intra-cellular inhabitants may inflict diseases on their hosts. Their detrimental effects range from rather harmless infections to severe cell damage to the induction of cancer. As is the case with other disease agents, the etiology depends on a variety of factors, including site and route of infection, agent virulence, host susceptibility, effectiveness of host defense, and the biological history of both agent and host.

The ecological potentials of agent and host, and hence agent–host dynamics, may be modified by such factors as age, physiological condition, environment and nutrition. Consequently, as in the other biotic diseases, the final course and outcome of a given virus infection is difficult to predict, and the development of agent–host dynamics may be complex.

Modern virology has received decisive stimulation from the discovery that viruses may infect bacteria (bacteriophages, phages). In fact, experimentation with bacterial viruses has opened up entirely new perspectives in biological research and laid the foundation for molecular biology. In the last 3 decades, numerous detailed analyses have revealed a surprising diversity of virus structures (shape, composition, size) and functions, and produced a rapidly growing body of knowledge on patterns of viral growth and development, as well as on the types of infection and dynamics of pathogenicity. Viral genetics (mutation, genetic mapping, deciphering of genetic codes), viral pathogenicity and the involvement of viruses in tumor development now occupy an increasing number of researchers.

Information on virus-controlled fish diseases has increased significantly in the last decade. Most of the knowledge now available was obtained from captive fishes. Especially

in aquaculture farms, many of the viruses that presumably persist in wild host populations in states of reduced activity were apparently favored by the diminished defense capacities of their fish hosts which suffered from suboptimal culture conditions (see also Hetrick, 1984; Sindermann, 1984a, b). The number of fish diseases shown to be due to viral agents is increasing (e.g., Sindermann, 1970, 1979) and the role of the viruses as pacemakers or secondary agents in disease phenomena primarily due to other causes requires careful attention.

Similar to viruses, an increasing spectrum of bacteria has been shown to cause, or to be involved in, fish diseases. Again, most pertinent information comes from fishes kept in captivity.

Viral and bacterial infections are major hazards confronting the commercial aquaculturist. The instrumentarium for microbial disease control in captive fishes comprises the development of effective vaccines and of general immunization techniques (disease prophylaxis via immunization), use of monoclonal antibodies, genetic engineering, employment of polyvalent bacterins whose properties can be adjusted to newly developing agent strains, chemical treatment, improvements in quarantine techniques and, of course, the reduction of captivity stress as well as the development of adequate diets. In addition, our present diagnostic techniques require improvement.

Uni- and Multicellular Disease Agents

About one quarter of all known protistan species may be assumed to be capable of inflicting disease phenomena on other forms of live (facultative plus obligate parasites), providing the conditions for their establishment are favorable. Like bacteria, protozoans command a pronounced affinity for coexisting intimately with foreign, usually larger, organisms. They constitute a tremendous potential for inflicting diseases on all groups of multicellular animals, including the fishes.

While a considerable number of fresh-water-living protozoans associated with fishes have received attention with respect to their parasitic potentials, their marine counterparts remain to be investigated much beyond the present level of our knowledge in order to assess their role as disease agents with more solidity. Life cycles, nutritional requirements, routes of host invasion, strategies for counteracting host defense, agent reproduction and agent distribution – many of these important facets of biotic diseases have remained in the dark. We know less about the role of marine protistans and protophytans as causes of fish diseases than about the roles of a number of multicellular agents, notably the helminths. Progress in fish pathology will depend to a large extent on a more intensive exploration of the disease-causing roles of viruses, bacteria and protistans.

A classical object of parasitology, the helminths (Turbellaria, Monogenea, Trematoda, Cestoda, Nematoda, Acanthocephala, Hirudinea) have attracted attention from numerous researchers for decades (see also Rohde, 1984a, b). The body of knowledge produced, expecially on life cycles, taxonomic identities and distribution patterns is impressive. Much less is known about metabolic aspects of agent–host relations (flow routes of energy and matter, and the materials involved; physical and chemical aspects of agent virulence and host defense) and – in captive fishes – of disease prevention, disease control and disease therapy. As in all other groups of organisms known to be able to cause diseases in fishes, more information has accumulated on fresh-water-living forms than on

their marine counterparts, and we know much more about parasites and hosts kept in captivity than about those living under natural conditions.

While numerous cases of intimate living together with other forms of life have become known from a variety of crustaceans, the major evolutionary stream of crustacean development points towards the free-living state and towards the establishment of multilateral, rather than bilateral, interrelations of organismic coexistence. Among the relatively few parasitic members of the group, ectoparasites dominate. Most of the facultative and obligate crustacean parasites of fishes are not highly pathogenic, except in fish with diminished defense capacities – e.g., senile individuals or those suffering from excessive stress (extreme intensities of environmental factors, captivity, pollution). In most cases crustacean effects are not severe and remain sublethal. However, extensive damage may be inflicted by species of *Argulus*, some gill parasites (fresh-water) and several mesoparasitic copepods. Crustacean endoparasites inhabiting fishes may be capable of causing severe damage. Their role in fish pathology requires more attention.

Further work is necessary to define the role of crustacean parasites of marine fishes more clearly and to assess the potential of crustacean parasites for facilitating infections of microorganisms and protozoans, as well as infestations of other multicellular parasites.

Abnormal Cell Growth

As other multicellular organisms, fishes suffer from a variety of disease phenomena due to abnormal cell growth, induced by genetic and/or non-genetic factors. Abnormal cell growth results from impairments of mechanisms which integrate and coordinate cellular division, differentiation and cooperation. Such mechanisms are the basic prerequisite for the evolutionary development of multicellular plants and animals. Diseases due to their impairment are, so to say, the price to be paid for multicellularity.

Excessive, abnormal, local cell division and growth produces a tumor (swelling), which may be benign or malignant. Both these forms of tumors are collectively referred to as neoplasms ('new growth' or 'new formations'). Neoplasms may be composed of quasi-normal body-own cells, abnormal body-own cells and/or foreign cells. Abnormal body-own cells exhibit one or more of the following negative deviations from the normal state: (i)anaplasia, i.e., degeneration of cellular characteristics and growth patterns; (ii)hyperplasia, i.e., autonomous and insufficiently restrained growth of normal cells leading to local increase in cell numbers; (iii)hypertrophy, i.e., increase in cell volume. The study of neoplasms is known as oncology.

The causes for negative deviations in cellular division, differentiation, gowth and coordination include circumstances internal to the individual involved (genetic predisposition, genetic disorder, senility), environmental factors (foreign organisms: viruses and other parasites; chemicals; injuries; irradiation; chronic irritation; severe stress) and nutritional circumstances (dietary deficiencies or excessivenesses). In most cases several of these causes appear to act in concert, thus increasing the total summative impact on the target individual. With the exception of external swellings (tumors of the body surface) many malignant forms of neoplasia (cancers) are beyond chances for effective, long-term repair by the time they are definitely diagnosed.

Cells of malignant tumors are characterized by their capacity to leave the site of their

origin and to migrate, or to allow themselves to be transported, to almost any other part of the body, where they may establish new centers of cancerous growth (metastases).

None of the neoplasia thus far reported from fishes differs essentially, in terms of characteristics and host-effects, from comparable tumors in other groups of organisms, invertebrate or vertebrate. There are reports of tumorous fish diseases of epidemic proportions, and a growing body of evidence suggests that the incidence of neoplasia in wild fish populations tends to increase in heavily polluted waters. Hence, critical studies are necessary to test the hypothesis that tumor development may be supported, directly or indirectly, by man-made pollutants.

Some fish tumors have been produced experimentally by cross-breeding. While the parents used were tumor-free, the resulting hybrids developed tumors with great regularity. Such experiments witness the significance of genetic factors for tumor development. For a review on genetic aspects of cancer development consult Anders and Anders (1984).

Extreme Intensities of Environmental Factors

Environmental stressors may not only be the immediate cause of a variety of diseases, but can also significantly modify the ecological potentials of both agent and host. Hence, they may play an important role in the destabilization of equilibria between disease-causing and disease-counteracting forces.

In addition to a number of environmental qualities identified as potential stressors (e.g., extreme intensities of temperature, salinity, oxygen availability, water movement), a host of chemical substances, including those made and released by man, must be expected to be capable of inducing stress-mediated diseases in fishes and other organisms (see also Dethlefsen, 1984; Möller, 1984; Wolthaus, 1984).

The ecological potentials of agents and hosts not only depend on acutely prevailing environmental circumstances, but also on their environmental histories. It may turn out that the status of acclimation (non-genetic adaptation) of a given organism can be of much more importance for the development of disease phenomena than is hitherto being accounted for. No disease can be explored adequately without taking environmental stressors, present and past, and nutritional disorders into account.

1. DISEASES OF PISCES

DISEASES CAUSED BY MICROORGANISMS

Agents: Virales

KEN WOLF

This section reviews and evaluates critically the diseases caused by virus, visualized virus, and virus-like particles in marine, anadromous and catadromous fishes. It is restricted to viral conditions. The term 'disease' refers to any deviation from the normal state (health); it includes such pathological conditions as result from genetic, physiological, nutritional, or environmental factors plus the more commonly considered infectious conditions caused by parasites; for a more detailed definition consult Volume I: Kinne (1980, p. 14). The term 'viral conditions' includes infections wherein a viral agent has been isolated and shown by fulfillment of Rivers' postulates to be the cause of a pathological condition. The subchapter further includes material on viruses that have been isolated but whose role is as yet unknown — unequivocal virus forms seen and known only by electron microscopy and still other abnormal intracellular forms best termed 'viruslike particles'. Further research is likely to show that some of the viruslike particles are indeed viruses but some others will almost certainly be found to be artifacts or unusual cellular particles of non-virus nature.

At present, about 20,000 different species of fish are known. It would be artificial to restrict this section to marine species, especially because some fish live in marine habitats at one time, and at other times migrate to or from freshwaters. Accordingly, I include viruses of catadromous fishes, notably the anguillid eels that migrate from estuarine and freshwater habitats to spawn in the sea as well as viruses of anadromous species, which spend the major portion of their life in marine waters but spawn in freshwater — typically, but not exclusively — the salmonids.

The term 'fish' as used here includes teleosts, bony fishes, plus the cartilaginous elasmobranchs (sharks, skates, and rays), and cyclostomes (lampreys and hagfishes). With one exception — virus forms seen in erythrocytes of a shark — viruses of elasmobranchs and cyclostomes have yet to be documented. That does not mean that these 2 classes are exempt from viral susceptibility, but rather that the more primitive fishes have not been adequately investigated virologically. After all, viruses are known from invertebrates as well as from teleost fishes and all the higher vertebrate classes.

The first fish virus was isolated from diseased trout in 1957 (Wolf, 1983). Twenty-five years later, an overview showed that viruses isolated from fishes, plus other bona fide viruses found only by electron microscopy and still others best termed 'virus-like,' collectively numbered about 50 (Wolf, 1983).

Viruses of fishes, where characterized, conform to major virus groupings known from

homeotherms. Fish have herpesviruses, iridoviruses, rhabdoviruses, and reoviruses. Other fish agents are provisionally considered to be adenovirus, coronavirus, calicivirus or leucovirus. Of course, still others remain to be isolated and characterized, and undoubtedly some of the remaining major virus groups will have representatives living in fish. A possible exception — at least for the present — is that no virus agent from fish seems to have the size and distinctive morphology of the poxviruses found in mammals and birds.

At least 25 viruses have been isolated from fishes and about 15 others have been visualized unequivocally in fish tissues. Conservatively, 87% of all viruses come from fish species that are important as human food; most of the viruses stem from fish raised in freshwater aquaculture facilities. Viruses from truly marine species amount to about one-fourth of the 87%, and the remaining 13% of all fish viruses come from tropical species, often those kept in aquaria.

The larger number of viruses, particularly among fish raised in aquaculture, reflects the influence of economics. Fish in husbandry are typically under regular observation. When diseases of problem proportion occur the condition is usually seen and remedial actions are taken to reduce mortality and hence the loss. In some cases when control measures were wholly ineffective, certain of the refractory infectious diseases were shown to have a viral cause. In other cases of patently infectious disease, microscopic pathogens were absent and appropriate examination showed a viral etiology.

Some generalizations are in order concerning the effects of fish viruses on their hosts. Just as occurs in virus-host relationships in homeotherms, the effects in fish range from acute or peracute disease with attendant mortality to infections that are mild or even inapparent. Viruses can be present in the absence of clinical signs of disease. Two viruses, a leucovirus-like agent of esocids and a herpesvirus of the Japanese salmon *Oncorhynchus masou* (OMV), are clearly oncogenic. Other fish viruses are present in certain neoplasms and might eventually be shown to be oncogenic also.

Whereas the general patterns of virus-host relationships in fish is like that in homeotherms, some exceptions should be noted. (i) No fish virus yet identified has been shown to require or to use regularly an invertebrate as a vector. (ii) Iridovirus, cause of lymphocystis disease, (there is evidence for distinct types) causes a unique and often spectacular cellular hypertrophy that has no counterpart among homeotherm diseases. (iii) Whereas vertical and horizontal transmission of fish virus occurs, venereal transmission is unknown, no doubt because intromission between fish is a rare exception.

The overall pattern of viruses in fishes is like that of viruses in warm-blooded vertebrates, with one notable exception. Viral zoonoses result from certain infected birds and mammals. Thus far, viruses of fishes are not infectious for man, neither do they result in disease when injected into homeotherms for the production of specific antisera.

Marine Fishes

Cod ulcus syndrome

Definition. The cod ulcus syndrome is a pathological condition consisting of focal lesions of the skin of yearling and older Atlantic cod *Gadus morhua* taken in Denmark's coastal waters. Viruses have been isolated from some specimens.

Signs and pathologic changes. Fish were collected with commercial gear, and no mention was made of possible behavior changes. Danish investigators have described 5

developmental stages in the course of the disease: (i) papulovesicular, (ii) erosive, (iii) early ulcerative, (iv) late ulcerative, and (v) healing (Jensen and Larsen, 1979).

Externally, Stage 1 lesions are multiple 2 to 8 mm diameter papules and occasional vesicles distributed along the lower part of the body but never on fins or head. Some papules have an apical hemorrhagic area. Papules progress to become vesicles that are larger and contain serous fluid.

Stage 2 lesions are perforate and crater-like with depressed centers and raised rims. The color is pink tinged grey to yellow.

Stage 3 ulcers are reddish and concave; they have hyperplastic tissue margins and necrotic centers.

Stage 4 ulcers (2 to 8 cm diameter) are the most severe form and progress from single lesions or from fusion of adjacent foci. The worst ulcers penetrate the abdominal wall.

Stage 5 is one of healing and recovery. The lesions are devoid of scales and pigment and thus are white. Understandably, the recovery stage is more apt to follow Stages 1 and 2 than after lesions have perforated.

Internal findings of affected cod have not been reported; accordingly, it is assumed that the overall appearance of the visceral mass was normal.

Histologic features of the lesion have been described and illustrated by Jensen and Larsen (1979).

Pathologic changes of Stage 1 lesions occur in the stratum compactum, but more so in the overlying tissues. The stratum spongiosum is markedly edematous and the surface epithelium intact but rough. Fluid fills the scale pockets and in turn those are surrounded by infiltrating granulocytes. Hyperemia and small hemorrhages are pronounced.

In Stage 2, the underlying musculature is involved; it is edematous and infiltrated. Epithelial margins of lesions are thickened and the loose connective tissue infiltrated.

Stage 3 lesions show a markedly edematous stratum compactum and beginning formation of granulation tissue in loose connective tissue. In some cases, the musculature is exposed.

Stage 4 lesions always have a breached stratum compactum. Granulation tissue has increased, and the exposed musculature has become necrotic, edematous, and infiltrated.

Stage 5 lesions show a covering of epithelium, but a lack of scales and pigment cells. Infiltration has lessened and fibroblasts have moved in. Obvious signs of recovery are present.

Biophysical properties of the viruses. Two morphologically distinct viruses have been isolated, but neither has been characterized. Typical rhabdovirus particles have dimensions of 55 × 175 nm (range 50 to 80 × 115 to 195 nm). At 15 °C in the PS line of pike sarcoma origin, the rhabdovirus attains a titer of about 10^5 $TCID_{50}$ ml^{-1}. In the EPC cell line the titer is only 10^1 $TCID_{50}$ ml^{-1}, and in BF-2, FHM, and RTG-2 lines no replication occurs (Jensen and co-authors, 1979).

The iridovirus occurs only in cell cytoplasm. It has a diameter of 145 to 150 nm and a nucleoid of 100 nm. The EPC cell line replicates the agent to a maximal titer of about 10^5 $TCID_{50}$ ml^{-1} and the PS line only 10^2 $TCID_{50}$ ml^{-1}.

Diagnosis. The etiology of lesions remains to be demonstrated; therefore, within the limitations of existing information, a pathologic diagnosis of the cod ulcus syndrome based on gross and histologic features of the lesions is the best that can be accomplished. Although 2 viruses have been isolated from pooled lesions, neither agent has been shown

to be present in all cases, and the effect of viruses on fry and fingerling cod — or related gadid fish — needs to be determined. It is possible that one or both agents are virulent for young cod and that fish which survive to maturity simply carry the virus.

Isolation and identification of the viruses. Larsen and Jensen (1979) carried out bacteriological examination of fish with ulcus syndrome. Among 350 fish examined, about half had sterile lesions, the others harbored *Vibrio anguillarum,* but that organism failed to evoke the subject skin ulcers under a variety of methods of experimental challenge.

The possibility of viral involvement came to light when a homogenate of 20 pooled papules from 7 cod was examined virologically and found to contain the 2 viruses. Neither agent was evident in original cultures but required 2 or 3 passages to show effects and then only in specific cell lines. The general effect of viruses *in vitro* was lytic.

On an individual basis papules from 33 affected cod were examined, and iridovirus was found in only one. Examination of 20 control cod showed no evidence of virus.

At present, identification can only be made on the basis of virus size, shape, and differential response in the EPC and PS cell lines. Antigenic relationship to other rhabdoviruses and iridoviruses remains to be determined.

Transmission and incubation. There seems to be little doubt that the ulcus syndrome is a contagious disease and, although Rivers' postulates have not been fulfilled, experimental evidence supports the proposition that the iridovirus has at least a contributing if not a causal role. Jensen and Larsen (1982) have carried out extensive trials, the results of which convincingly eliminate *Vibrio anguillarum* as anything but a secondary invader. Also, the disease could not be induced when the rhabdovirus was used as inoculum. On the other hand, a 100% transmission was achieved in 9 to 11 days when 6 healthy cod were held with 4 that had Stage 1 papules. A 36% transmission was obtained when papule homogenate was applied to scarified skin of 11 test fish. The incubation time was 9 to 12 days and new lesions appeared where skin had not been abraded. However, intraperitoneal injection of papule homogenate did not give a consistent response. In one trial, 40 fish were used but none developed lesions. In a second trial, 4 of 31 injected fish — only 13% — developed the ulcus syndrome. Four trials were conducted in which culture-grown iridovirus was injected. Twenty fish that received virus intracardially and 20 that were injected intraperitoneally all failed to show evidence of the disease. In 2 other trials wherein iridovirus was injected intracardially some of the recipients developed the ulcus syndrome. Collectively, 113 cod had been injected intraperitoneally and 21, or about 19%, developed the disease.

The health history of the wild caught test fish is completely unknown. The apparent resistance of many could be the result of prior exposure and immunity.

Host and geographic range. The only fish known to exhibit the disease is the Atlantic cod in waters off Denmark. It seem possible that the range will be extended. Also, related gadids might also be found susceptible.

Control measures. Not applicable.

Lymphocystis disease

Definition. Lymphocystis disease, or simply lymphocystis, is a chronic, dermatotropic, but trivial, viral infection found only in the more highly evolved orders of fishes — both freshwater and marine. The infection is typically superficial and results in a unique,

terminal, and spectacular cell giantism or hypertrophy with distinctive DNA inclusions in the cytoplasm.

Signs and pathologic changes. Behavior and activities of infected fish are normal. Typical lesions are external and overwhelmingly located on skin and fins; they consist in some cases of individual hypertrophied cells, but more usually of clusters that have a warty or pebbled appearance (Nigrelli and Ruggieri, 1965, Templeman, 1965). Lymphocystis lesions are usually covered with a layer of epithelium, which matches in pigmentation the coloration of underlying normal epithelium. Accordingly, lesions can be white, cream-colored, gray, or brownish; and additionally, if massive, the lesions can show a reddish hue due to vascularity.

In a few cases, lymphocystis cells can be found internally.

Mature lymphocystis cells are spherical to ovoid but those in clusters are moderately distorted by reciprocal pressure between adjacent cells during their growth. At all stages, except the earliest week or two, lymphocystis cells are significantly larger than normal cells, and at full development they commonly measure 100 to 1000 or more microns in greatest dimension.

Key features of the mature cell are a thick hyaline capsule; an impressively enlarged, distorted and generally centrally located nucleus; an enlarged, basophilic nucleolus; and prominent basophilic cytoplasmic inclusions that give a positive reaction for DNA. Morphologically, the cytoplasmic inclusions are of 2 types: (i) For convenience referred to here as 'mullet or flounder type', the first is well illustrated by Alexandrowicz (1951) and Murchelano and Bridges (1976). This type is comparatively massive and cord-like but with fenestrations and blebs. The location tends to be distant from the nucleus. (ii) The 'plaice type' is more lace-like and has scattered small condensations of chromatin. Its distribution is throughout the cytoplasm; its morphology is well illustrated in the report of Templeman (1965).

Lymphocystis cells apparently stimulate proliferation of adjoining noninfected tissue cells, for the infected cells are typically embedded in a matrix of connective tissue. Dunbar and Wolf (1966) described and illustrated the development of experimental lymphocystis in a freshwater fish at constant 25 °C.

Biophysical properties of the virus. Lymphocystis virus is a large cytoplasmic icosahedron with a genome of DNA and thus a member of the iridovirus group. The virus is heat- and ether-labile, but does not hemagglutinate. Infectivity persists for years in lesion homogenates that are either simply dessicated or lyophilized. Virion size differs with the fish of origin. In *Pleuronectes* (Pleuronectidae), Walker and Weissenberg (1965) found the particle size to be 130 to 150 nm and noted that the virions were significantly smaller than in two other genera. The largest virions, 280 to 330 nm, were found in *Bairdiella* (Sciaenidae) by Lawler and co-authors (1974). Other virions from other genera are intermediate in size.

Whether *in vivo* or *in vitro,* replication of lymphocystis virus takes place in fibroblastic cells; epithelioid cells seem incapable of supporting growth of the virus. The cytoplasmic inclusions are the site of viral synthesis and assembly. Wolf and Carlson (1965) carried out a one-step growth curve of lymphocystis in the bluegill *Lepomis macrochirus* at 25 °C and correlated the findings with morphological development of infected cells. A near maximum level of infectivity — about 10^7 ID_{50} ml^{-1} — was present by Day 15, but cell size continued to increase without appreciable increase in infectivity for 2 weeks.

The virus is readily replicated in susceptible cells *in vitro*. In that process, infected cells enlarge, show enlarged nuclei and nucleoli, and develop basophilic cytoplasmic inclusions. Virus from marine fish in cell cultures of marine fish origin was reported by Wharton and co-authors (1974, 1977), but infectivity was rapidly lost in passage. In contrast, a system using freshwater fish cell cultures and virus, infectivity was maintained and Rivers' postulates fulfilled (Wolf and co-authors, 1966). In that system, maximum infectivity — $10^{6.5}$ ID_{50} ml^{-1} — closely approached levels attained *in vivo*.

Diagnosis. The usual cases of lymphocystis can be diagnosed by macroscopic examination. In exceptional cases, the condition might be confused with chlamydial epitheliocystis disease or with small clusters of trematode cysts. With slight magnification, however, trematodes can be seen to move or their form and suckers recognized in fixed specimens. Epitheliocystis cells are much smaller than those of lymphocystis, and they are typically white and abundant. In sections, the nucleus of epitheliocystis cells is located peripherally.

Histologic examination clearly and unequivocally establishes the identity of lymphocystis disease. It is, in fact, a situation where an absolute virological identification can be made histologically. It should be noted that senescent lymphocystis cells can lack nuclei and inclusion bodies. Such cells are large and possess the hyaline capsule. The interior is filled with a homogeneous mass of eosinophilic cytoplasm.

Isolation and identification of the virus. The masses of hypertrophied cells contain abundant virus and homogenates thereof are infectious for susceptible fish and cell cultures. As inocula for isolations in cell cultures, homogenates should be decontaminated, and if membrane filtration is used, a 0.45 μm porosity is suggested so as to not lose excessive infectivity. Incubation temperature should approximate that of the environment from which the source specimen was taken. Incubation should be effected for 2 or more weeks, but it is doubtful if blind passage of apparently negative cultures has any value. For susceptible *in vitro* systems, cell cultures should be from species providing the virus, or at least from a member of the same family.

Presumptive identification of the virus can be made as was mentioned by finding lymphocystis cells in histologic section, or alternatively by demonstrating after suitable staining, their production *in vitro*. Demonstration of the virions by electron microscopy adds to and reinforces the presumptive identification.

Serological identification of lymphocystis virus from a freshwater fish was reported by Walker and Hill (1980) who used an indirect fluorescent antibody technique and noted that in cell cultures, the inclusion bodies were reactive.

Because differences in size are known, it seems likely that lymphocystis virus will be found to consist of strains or types of virus with at least some distinctive antigenic determinants.

Transmission and incubation. There is no doubt that waterborne transmission is the principal means of communicating virus; that can be demonstrated experimentally. Virus enters the host through abrasions or wounds such as those caused by nets, territorial defense, or spawning combat. It has been amply demonstrated that gills are also a portal of entry; virus being applied thereto will show up as lymphocystis lesions on skin and fins. In spite of considerable logic and speculation on the matter, limited experimentation has thus far failed to show transmission of lymphocystis with or by metazoan parasites.

As a generalization, incubation is prolonged and usually requires one to several weeks for lesions to become apparent. Factors involved include temperature, which when low

prolongs incubation; viral genome — some lymphocystis virus seem to have a slower growth cycle than others — and method of examination or determination. Greater length of time is required for lesions to become visible if macroscopic examination is used instead of microscopic.

Host and geographic range. Reinforcing part of what was given in the definition, lymphocystis is peculiar to the more highly evolved orders of fishes. Also, younger fish are more susceptible than older specimens of the same species. Currently, about 30 families of marine fishes are known to have one or more susceptible species. The infection is common among Perciform, Pleuronectiform, and Tetraodontiform fishes. Listings of susceptible species are given in the reports of Nigrelli and Ruggieri (1965) and Lawler and co-authors (1977).

Geographically, there are no known restrictions to the occurrence of lymphocystis.

Control measures. Not applicable for marine or anadromous species.

Menhaden spinning disease

Definition. Menhaden spinning disease is a long-recognized condition that occurs annually and causes mortality among *Brevoortia tyrannus* in coastal waters of eastern United States. The etiology has for years been an enigma, but recently a virus was isolated and claims made that the agent produced spinning disease in experimentally injected menhaden.

Signs and pathological changes. In epizootics, signs preceding death consist of erratic swimming and exophthalmia (Sindermann, 1970a). Menhaden injected intraperitoneally with low passage of the isolated virus developed signs of spinning disease. Histopathologic examination was not carried out, but hemorrhages occurred in eyes, fin bases, and along the body. Fish darkened, swam in circles, and died in a matter of 3 to 5 days (Stephens and co-authors, 1980).

Biophysical properties of the virus. The menhaden isolant has the major biophysical and chemical properties of infectious pancreatic necrosis virus; the menhaden agent is stable in the presence of chloroform, unaffected by antoganists of DNA synthesis, and in negatively stained preparations show icosahedra with a mean diameter of 58 nm. The buoyant density in CsCl is 1.33 g cm^{-3} (Stephens and co-authors, 1980).

Diagnosis. At present, menhaden spinning disease can be diagnosed in cases when large numbers of the species are found afflicted with erratic circular swimming behavior and the attendant mortality is great. Thus far, outbreaks have occurred during spring to autumn in coastal waters of eastern United States. The diagnostic value of hemorrhages is uncertain, for that sign occurs in bacteremias and in parasitemias. Likewise, the relevance of the IPN-like virus as a postulated etiologic agent needs to be critically assessed. Confirmation of original isolations have yet to be reported by other investigators.

Isolation and identification of the virus. The menhaden agent was isolated on a menhaden kidney cell line using as inocula homogenates from brain tissue and pancreas and adipose tissues, the latter two presumably in combination. Serologically, the menhaden virus is completely neutralized by polyvalent anti-IPNV serum and, in all aspects except one, it is a strain of IPNV.

The one unusual feature of the menhaden isolate is that it showed CPE, vacuolation, and cytoplasmic inclusions only after 1 or 2 weeks, and only on the menhaden kidney cell line. At 20 °C, that delay in appearance of CPE is most unlike the in vitro effect of IPNV.

However, transfer of fluids from the menhaden cell line to BF-2, CHSE-214, and RTG-2 cell lines resulted in a lytic CPE. The EPC line was refractory. Non-menhaden cell lines are refractory to original inocula.

The peculiar situation in which the menhaden kidney cell line was the only culture to show CPE on first inoculation has changed; additional isolations have been made and lytic effects were evident in original cultures.

Transmission and incubation. Nothing is known about transmission and incubation during epizootics. Environmental factors such as changes in salinity, pollution, and reduced oxygen due to high temperature could be involved or even be the prime cause of mortality. The virus could be a chance association.

Host and geographic range. Thus far, the spinning disease is known only in the Atlantic menhaden and only in Atlantic waters off North America. Related clupeid fishes in other waters might experience similar disease signs and mortality.

Control measures. Not applicable.

Turbot herpesvirus infection

Definition. Turbot herpesvirus infection is a mild to moderately serious giant cell condition of the skin and gill epithelium of young *Scophthalmus maximus* under husbandry in warm effluent from a nuclear power generating plant (Buchanan and co-authors, 1978).

Signs and pathologic changes. Affected specimens are anorexic and lethargic. Their posture is abnormal, for instead of lying flat on the bottom they are laterally flexed so that head and tail are raised. Other behavior changes and external signs have not been noted, neither has the internal appearance deviated from the normal.

Specific signs of the disease are evident only with microscopy. Giant cells ranging in size from about 9×15 μm to 70×130 μm are present in skin and gill epithelium (Richards and Buchanan, 1978). The giant cells are more numerous on the upper side than on the side in contact with the bottom. Cells occur singly, but in heavy cases several layers can be present. Most of the cells have a single nucleus, and in those cases that nucleus is large and occupies as much as 90 % of the cell. Other cells are multinucleated, apparently having been formed by fusion.

The significance of the infection is greatest when gills are involved and when many cells are present (Richards and Buchanan, 1978). The presence of giant cells in gills causes hyperplasia of surrounding tissue. When heavy infection is present, lamellae fuse and malpighian cells become hyperplastic. Vascular stasis and thrombosis occur and result in a considerable reduction in the functional respiratory area.

Fish with heavy infection must be considered in marginal condition, and stress conditions such as handling, transport, temperature fluctuation, and high chlorine levels (from fouling control measures in the plant's cooling system) can precipitate mortality.

Biophysical properties of the virus. The virus is known only from electron microscopy (Buchanan and Madeley, 1978, Buchanan and co-authors, 1978). The size and shape and apparent mode of replication indicate that the turbot agent is in all probability a herpesvirus; accordingly, the discoverers have proposed the name *Herpesvirus scophthalmi* (Buchanan and co-authors, 1978). The authors noted 18 nm petal-like projections on negatively stained preparations of enveloped particles and call attention to the similarity of such projections on coronaviruses. Coronaviruses however, are replicated only in cytoplasm and are not found in nuclei.

Herpesvirus scophthalmi particles measure about 100 nm in diameter — some have a 25 to 30 nm nucleoid, others are empty, but both occur in cell nuclei. Abundant, and at times membrane enclosed, enveloped particles measuring about 200 nm occur only in the cytoplasm and extracellularly. Micrographs showing actual envelopment were not found, and the agent has not been isolated.

Diagnosis. Considering the usual high degree of host specificity of herpesviruses, the turbot infection can be recognized by the pathognomonic giant cells in skin and gills of the host species. Light microscopy of gills alone might not always distinguish the condition from that of epitheliocystis. Electron microscopy, however, will clearly distinguish between herpesvirus and chlamydia of epitheliocystis.

Isolation and identification of the virus. Buchanan and Madeley (1978) attempted to isolate the virus, but they were unable to find a cell line that would replicate the agent. In their survey of fish cell lines and viruses, Wolf and Mann (1980) found no extant cell line of bothid fish origin. Primary cultures of turbot cells could easily be set up in several hours using procedures described by Wolf and Quimby (1976a, b). Application of fin epithelial cultures to isolation of the turbot virus would be most interesting.

Transmission and incubation. The giant cell condition has been found in the wild, and the infection is considered enzootic. In addition to the presence of giant cells, the skin of some infected individuals showed crypts that were considered to be sites where giant cells had been destroyed (or from which infected cells had sloughed). In either case, virus might have been released to initiate new infections.

Regarding incubation time, Richards and Buchanan (1978) noted that mortality occurred within 5 days after young turbot were transported. One must assume that the infection was well underway and that considerable gill hyperplasia was present in fish that would die so soon. The very size of the giant cells argues for a chronic course of infection.

Host and geographic range. Young turbot in waters off Scotland and Wales and in the fish production facility of the power station at Ayrshire, Scotland, are the only known susceptible host. It is possible, however, that closely related pleuronectiform fishes in the subject marine environment could also be infected. The turbot herpesvirus giant cell condition is reminiscent of giant cells of Pacific cod that are described by McArn and co-authors (1978).

Control measures. It has been suggested that the only means of minimizing mortality among infected turbot is to apply enlightened husbandry, i.e., to minimize handling, provide optimal nutrition, and reduce or eliminate stress factors.

Viral erythrocytic necrosis (Piscine erythrocytic necrosis)

Definition. Viral erythrocytic necrosis (VEN) is manifest, as the name indicates, only as pathological alterations of erythrocytes. The disease occurs in poikilotherm vertebrates from reptiles through elasmobranchs. Among teleost fishes, the host response varies from inapparent or mild to moderately severe. Also, in fishes, VEN is almost exclusively a viral infection of marine species.

Signs and pathologic changes. Behavior changes in infected fish have not been reported. The only consistent gross signs of the disease were seen by Evelyn and Traxler (1978) who found pale gills and generally pale visceral organs in Pacific salmon (genus *Oncorhynchus*) with VEN. Blood of affected specimens clotted slowly if at all, and

hematocrits were abnormally low. Histologically, hematopoietic tissue of Pacific salmon was hyperactive.

Specific pathologic alterations occur only in circulating erythrocytes. When fixed and stained with hematologic stains, blood films show 1 of 2 major changes: (i) Typical of Pacific salmon, a small (1–4 μm) eosinophilic inclusion is present in the cytoplasm or alternatively as a bleb on the nucleus. (ii) Typical of Atlantic cod *(Gadus morhua)*, cytoplasmic inclusions can also be present, but the more obvious change is in the nucleus that is vacuolate or shows various degrees of necrotic degeneration or karyolysis.

The degree of infection in individual fish can range from a low of less than 1 % to the most severe cases in which 100 % of the erythrocytes show pathological change. Leucocytes, however, are not affected.

Biophysical properties of the virus. The agent universally found in erytrhocytes of fish with VEN is a large heat-labile, cytoplasmic icosahedron with a genome of DNA. Accordingly, by those criteria, the agent is an iridovirus, but it has not been isolated in cell culture, and neither have Rivers' postulates been fulfilled.

Much like the size variability of another well-known fish iridovirus, the lymphocystis agent, the VEN virus in different hosts has shown a range of size from about 150 to 350 nm. The differences in size cannot be attributed to use of different procedures. Reno and co-authors (1978) found the size to be 145 nm in Atlantic herring *Clupea harengus,* while in 2 species of *Oncorhynchus* Evelyn and Traxler (1978) found the size to be 190 nm. The largest particles in a teleost were found in Atlantic cod, where the size was variously reported as 260 to 360 nm (Appy and co-authors, 1976; Walker and Sherburne, 1977; Reno and Nicholson, 1981).

The sole report of virus in an elasmobranch is that of a VEN virus-like agent seen in erythrocytes of a shark *Scyliorhinus canicula*; the size of that agent is given as 450 to 500 nm (Johnston, 1975).

Diagnosis. Presumptive diagnosis of VEN is made by staining films of peripheral blood and finding the distinct small eosinophilic cytoplasmic inclusions or erythrocytes with obvious lytic or degenerative changes in the nucleus or both. Confirmation of the diagnosis can be had by electron microscopy and findings of cytoplasmic icosahedral virions in cells showing nuclear damage and inclusions. Smail and Egglestone (1980) have reported a rapid method of demonstrating the virus electron microscopically in preparations of lysed erythrocytes.

Isolation and identification of the virus. Although various fish cell lines widely used in fish virology have been tried, none proved capable of supporting replication of the VEN virus, and the agent has not been isolated. Seemingly an obvious approach, cultures of hematopoietic tissue or cells have not been tried.

The virus — or viruses — can only be identified by electron microscopy, and then only to the extent of the assumed iridovirus grouping.

Transmission and incubation. Transmission in the marine environment is probably waterborne but other means are not excluded. That assumption is based on the fact that waterborne transmission was demonstrated between salmonids in freshwater experimentation (MacMillan and Mulcahy, 1979). In that work, the minimal incubation time was 7 days and the longest more than 3 months.

Transmission has also been achieved by injection of virus and, in that case, incubation time was a week or less.

Host and geographic range. Marine species constitute the primary host group for VEN infections, and anadromous species are secondary. Within a species, young fish apparently are more susceptible than older fish.

Walker and Sherburne (1977) list 12 species of fish known to have the infection and an additional five species in which the disease is considered possible. The listing shows that several families are represented by two or more susceptible species. Moreover, iridoviruses of poikilotherm vertebrates are not known for host specificity. Accordingly, it seems most appropriate to list families of fishes containing species known to be susceptible to VEN (Table 1).

Control measures. Not applicable.

Table 1-1
Families of fishes having one or more species known to be susceptible to VEN (adapted and expanded from Walker and Sherburne, 1977)

Scientific name	Common name	Scientific name	Common name
Scylliorhinidae	Cat sharks	Sciaenidae	Croakers
Clupeidae	Herrings	Cottidae	Sculpins
Salmonidae	Salmon, trout	Hemitripteridae	Sea ravens
Osmeridae	Smelt	Liparidae	Sea snails
Anguillidae	True eels	Labridae	Wrasses
Gadidae	Codfishes	Blennidae	Combtooth blennies
Paralichthyidae	Bastard halibuts		

Anadromous Fishes

Chum salmon reovirus (CSV)

Definition. Chum salmon reovirus (CSV) is a newly recognized apathogenic agent isolated once from 60 kidney and spleen samples of normal appearing *Oncorhynchus keta* on their return to Hokkaido, Japan to spawn (Winton and co-authors, 1981). The virological examination was carried out as a requirement for certification for importation of salmonid eggs into the United States. It is stressed that additional search for the agent has been conducted but, thus far, unsuccessfully.

Signs and pathologic changes. Source fish were sexually mature anadromous specimens; accordingly, they were assumed to be normal in appearance and behavior. Experimentally, Winton and co-authors (1981) intraperitoneally inoculated fry of chum salmon, chinook salmon (*Oncorhynchus tschawytscha*), and kokanee (*O. nerka*) with culture-grown virus. The fish were held at 12 °C and samples assayed for virus during a 42 day period of observation.

Neither behavior changes, nor signs of disease occurred. Replication of the virus — at most about 100 times greater than input — occurred in the young fish, more in the chum salmon fry than in the other 2 species. Histologically, focal necrosis of transient duration was found in livers of chum and chinook fry. The foci were first found in chum fry at about Day 8. At Day 14, the lesions were acute and numerous, but by Day 21 healing had begun. Chinook salmon fry were less severely afflicted and kokanee not at all. All other organs appeared normal.

Biophysical properties of the virus. The chum salmon reovirus has the major attributes of the Reoviridae. It is an acid- and chloroform-stable, doubly encapsidated icosahedron with a 75 nm diameter. Treatment of CSV with alpha-chymotrypsin results in subviral particles of 50 to 55 nm and preparations with more than a 10-fold increase in infectivity. The RNA genome is double-stranded and preliminary results indicate that the buoyant density is 1.33 to 1.369 cm^{-3}.

The CSV lacks 2 characteristics found in other reoviruses; it does not agglutinate human 0+ erythrocytes, and it is not stable at 56 °C.

Initial isolation was made on CHSE-214 cells, whereas RTG-2 cells showed no CPE. The range of *in vitro* replication is 10 to 20 °C and the optimum about 15 °C. With the exception of RTG-2 cells all of the widely used fish cell lines replicate virus. The highest titers — 10^6 $TCID_{50}$ ml^{-1} — are produced in cell lines of salmonid origin. The most evident cytopathic effect is the production of syncytia. Those syncytia could be confused with effects of OMV and *Herpesvirus salmonis* except that CSV will induce syncytia in cells at 18 °C, a nonpermissive temperature for *H. salmonis* and only marginally permissive for OMV.

Diagnosis. In the absence of a disease state, diagnosis is an inappropriate term; instead, virus isolation and identification will determine the presence (or absence) of the chum salmon reovirus.

Isolation and identification of the virus. The CCH-1 cell line of chum salmon origin and the CHSE-214 line of chinook salmon origin are suggested for isolation. Incubation should be at 15 °C for at least 7 to 10 days and in the absence of syncytia, or any other CPE, material should be blind passaged.

The CSV has not been serologically typed, accordingly its possible relationship to human and avian reoviruses remains unknown. The chum salmon isolant is somewhat similar to the golden shiner virus (GSV), but the 2 can be distinguished by the fact that GSV does not grow at 15 °C and CSV does not grow at 30 °C, which is near-optimal for GSV (Schwedler and Plumb, 1982).

Transmission and incubation. Nothing is known of transmission in nature, and the only experimental work employed intraperitoneal injection of fry size (1 to 2 g) fish with 10^4 $TCID_{50}$ each — a rather massive amount considering the small size of the animals.

Based on the first appearance of focal necrosis in livers of chum salmon fry and titers measured in chum salmon and kokanee fry, the minimal incubation time at 12 °C is 8 to 10 days.

Host and geographic range. The chum salmon returning to Hokkaido is the only known source of the virus, and that fact is further restricted by the finding of virus in only 1 of 60 5-fish pools of kidney and spleen tissues.

Control measures. Not applicable.

Herpesvirus salmonis disease

Definition. Herpesvirus salmonis disease is a subacute systemic disease leasing to high mortality among experimentally infected fry to fingerling rainbow trout *Salmo gairdneri* and chum salmon *Oncorhynchus keta* following injection of culture-grown virus. The virus was originally isolated from ovarian fluids of adult rainbow brood stock at a single location in the State of Washington, USA (Wolf and co-authors, 1978). The presence of the virus elsewhere has not been reported.

Signs and pathologic changes. In young rainbow trout, external signs of the disease include anorexia and lethargy, abdominal distension, exophthalmia, hemorrhages in orbits and fin bases, abnormal darkening and thick mucoid casts trailing from the vent. Internally, moribund victims contain abundant ascitic fluid, and the overall appearance of visceral organs is pale. The liver is pale or mottled, and its texture is friable. Food, if any, is present only in the posterior intestine. Peripheral blood contains immature erythrocytes and blast cells of uncertain nature.

Histologically, pathological changes are found in many organs, but particularly in renal, respiratory, cardiac, hepatic, intestinal, and pancreatic tissues. In some specimens, pancreatic acinar cells fuse to form syncytia, which are pathognomonic for the infection and known for no other fish disease. Kidneys show edema, hyperplasia of hematopoietic tissue, congestion, and necrosis, the latter also quite commonly in hematopoietic tissue as well as in tubules. Gill epithelium is edematous and hypertrophied. Pseudobranches are, to various degrees, edematous and some are grossly necrotic. Cardiac tissue is edematous and necrotic. Leucocytic infiltration is evident as are localized hemorrhages. Livers usually show edema, necrosis and hemorrhage or congestion. Posterior intestine or rectum shows necrotic mucosa that sloughs into the lumen and leucocytic infiltration of the submucosa. The sloughed mucosa undoubtedly constitutes the bulk of the mucoid cast that one sees trailing from the vent. Illustrated details of the histopathology are reported by Wolf and Smith (1981).

Biophysical properties of the virus. As the name indicates, *Herpesvirus salmonis,* or salmonid herpesvirus, possesses the attributes of the herpesvirus 1 group and is officially recognized as such (Roizman and co-authors, 1981).

Herpesvirus salmonis is replicated only by salmonid fish cell lines such as the CHSE-214 and RTG-2. Optimal temperature for replication is 10 °C. Maximal titer is about 5×10^4 PFU ml^{-1} for released virus and about 10-fold greater for cell-associated virus. A one-step growth cycle requires more than 96 h at 10 °C. Susceptible cell response to the virus results in cyncytia followed by limited lysis. Syncytia are produced at 0 °C, but at that temperature replication does not occur. Incubation at 15 °C results in inconsistent replication (Wolf and co-authors, 1978). Higher temperatures are not at all permissive.

Diagnosis. Herpesvirus salmonis disease is an experimentally induced condition, but the agent was originally isolated from a population of adults with a history of high postspawning losses and in which other causes could not be recognized. Accordingly, the virus has a potential for serious disease and mortality under hatchery conditions and possibly in the wild.

The disease can be suspected if young rainbow trout show the foregoing external and internal signs and mortality. When present, syncytia in pancreatic acinar cells are diagnostic, because other viral diseases of salmonids typically cause necrosis of the pancreas but not cell fusion. Absence of other viral agents of salmonids — IHNV, OMV, VHS, and IPNV — should be determined. Production of syncytia in salmonid cell cultures is helpful because at present OMV is the only other salmonid virus that has that ability.

Isolation and identification. Young, nearly confluent cultures of salmonid cells should be used for isolation. Their pH should be 7.3 to 7.5 and virus inocula should be adsorbed on drained cells sheets for 2 h at 10 °C. Care should be taken to avoid dehydration of cells during adsorption.

Kidneys, liver, stomach, and intestine of young fish harbor the greatest amount of

virus and are suggested for homogenization, decontamination, and use as inocula. Incubation at 10 °C should continue for at least 7 to 10 days after which material should be blind passaged if CPE is not evident.

Syncytia on salmonid cell lines is only presumptive evidence of virus, and only 2 salmonid herpesviruses produce that effect — *Herpesvirus salmonis* and OMV. Accordingly, serum neutralization is required for definitive identification. *H. salmonis* and OMV do not cross-react in cross-neutralization tests (T. Kimura, pers. comm.). The chum salmon reovirus also produces syncytia in certain fish cell lines, but that agent can be separated from *H. salmonis* and OMV by its RNA genome and ability to grow at 20 °C.

Transmission and incubation. Experimentally, fish-to-fish transmission could not be demonstrated among young rainbow trout at 8 to 10 °C. Infection, disease, and mortality were produced only by injection of virus, and first mortalities began 25 or more days after the fish were inoculated. Mortality continued for up to 50 days.

Nothing is known about transmission and incubation of the infection in the single source lot of brood stock; the fish were disposed of when it was learned that they harbored a new virus. It is postulated that the virus persisted to adulthood so that it would be present as a successful herpesvirus to infect the next generation of hosts.

Host and geographic range. Thus far, the sole location where the virus existed is the Winthrop (Washington) National Fish Hatchery. A 2-yr virological survey of hatcheries that received eyed eggs from the Winthrop brood stock failed to reveal progeny fish with the virus.

Young rainbow trout are demonstrably susceptible to injected virus. However, under the same conditions young brook trout *Salvelinus fontinalis,* brown trout *Salmo trutta,* Atlantic salmon *Salmo salar,* and kokanee *Oncorhynchus nerka* were not susceptible. On the other hand, T. Kimura (pers. comm.) found that chum salmon *(O. keta)* are susceptible to *Herpesvirus salmonis* by injection.

Control measures. Because only 1 source was known and that fish stock disposed of, discussion of control is somewhat academic. Avoidance can be recommended — if one knows the source. Were a new focus to be found, temperature sensitivity of the agent might be exploited and the disease controlled by elevating temperature to 15 °C or higher.

Chemotherapy with anti-herpesvirus compounds might be effective. T. Kimura (pers. comm.) has been able to effect control of OMV with continuous feeding of Acyclovir.

Infectious hematopoietic necrosis

Definition. Infectious hematopoietic necrosis (IHN) is an acute rhabdoviral disease and typically a cause of high mortality amoung young rainbow trout *Salmo gairdneri,* chinook salmon *Oncorhynchus tschawytscha,* sockeye salmon *O. nerka,* and certain species of Asiatic salmonids. In older literature, IHN was variously known as chinook salmon virus disease, Oregon sockeye disease, and sockeye salmon virus disease.

Signs and pathologic changes. The first sign is a sudden rise in mortality among fry or fingerlings. Affected fish avoid the stronger currents, move to sides of raceways or troughs, and are carried down current. Brief episodes of frenzied swimming can occur. Victims develop exophthalmia, abdominal swelling, abnormal darkening, anemia, and hemorrhages at fin bases. A thick mucoid cast or exudate trails from the vent of some victims. Internally, the visceral mass is pale and can show multiple petechial hemorrhages. The digestive tract is devoid of food but can contain yellowish or cloudy mucus-like fluid.

The dominant histopathologic change — by which the disease was named — is marked necrosis of renal hematopoietic tissue and tubules. Pancreatic necrosis can also be present, but a more distinctive feature that is pathognomonic is necrosis of the acidophilic granular cells of the intestinal stratum compactum and granulosum (submucosa). Stained imprints of kidney tissue show abundant cellular debris. Still other changes occur and have been described in a comparative review of the histopathology of fish viral diseases (Yasutake, 1975).

Amend and Smith (1975) carried out detailed physiological studies on the response of fish to IHNV and concluded that renal failure resulted in electrolyte imbalance, hemodilution, and death.

Biophysical properties of the virus. The agent of IHN is a typical rhabdovirus that measures about 160 × 90 nm. The genome is RNA, and the virus is labile to ether, acid, heat, and glycerol. Like others of the fish rhabdoviruses, structural proteins of IHN virus have been studied and uniformly found by several groups of investigators to contain L, G, N, M_1 and M_2 components. Slightly different values were reported for the respective molecular weights of each protein, but the profile of IHN virus resembled that of the rabies type rhabdovirus more than that of vesicular stomatitis virus. Other aspects of the molecular biology of IHNV and other fish viruses are to be found in reviews of McAllister (1979) and Pilcher and Fryer (1980).

The infectivity of IHNV persists longer in distilled water than in physiological salines or seawater. In similar fashion when dried from water, infectivity persisted longer than 1 week — the duration of infectivity when preparations were dried from physiological saline or even from homogenized tissue. This pattern of survival is consistent with the epizootiology of a virus that is transmitted in fresh water environments. Recommended storage of IHNV is at −20 °C or preferably at −70 °C or lower, and in the presence of serum or other protein.

Consistent with the biology of its hosts, IHNV is replicated at low temperatures. *In vitro*, the range is 4 to 20 °C, but the optimum is 13 to 18 °C. Maximal titer is about $10^{7.5}$ PFU ml^{-1}.

Diagnosis. A presumptive diagnosis of IHN can be made based on findings of the aforementioned clinical signs and supporting epizootiological data. These data include occurrence in fry or fingerlings of salmonid species known to be susceptible and in geographic areas where IHN is enzootic. The disease should also be considered if appropriate findings are present in the progeny of eggs that come from stock in geographic areas where IHN is enzootic.

Added confidence in the accuracy of a diagnosis can be had if histologic examination of victims shows marked renal and hematopoietic necrosis and especially necrosis of the eosinophilic granular cells of the digestive tract.

Susceptible lines of fish cells show a pattern of grape-like rounding before necrosis sets in. However, margination of chromatin is a more specific change. If sought, that feature can be seen both in stained and unstained preparations. Where specific antiserum is not available, a presumptive diagnosis can also be made based on cytopathic effects of IHNV. The reader is cautioned, however, that IHNV can occur in fish already carrying the virus of infectious pancreatic necrosis and that a definitive diagnosis requires that the virus be isolated and identified serologically.

Isolation and identification of the virus. Virus is abundant in victims and typically

reaches and usually exceeds 10^5 PFU g^{-1} of whole fish homogenate. The virus is readily isolated in cultures of commonly used fish cell lines such as CHSE-214, RTG-2, FHM, and others. The FHM line is preferred by some workers, but not all lineages retain susceptibility. Accordingly, the FHM cell line should be monitored to ensure that it will reveal IHNV if it is present.

Regardless of the cell line used, cultures should be young and vigorous. At most, cultures should be but several days old and only about 90 % confluent. Adsorption of suspect material on drained cell sheets is preferred for greatest sensitivity and, also to that end, the pH should be in the range of 7.6 to 8.0. With epizootic material, CPE will usually appear with 48 h at 12 to 15 °C and should reach a plateau in 4 to 5 days.

Identification of the virus is most commonly done with serum neutralization test. Fluorescent antibody techniques have been developed, but they have not found widespread use.

Egtved virus, the agent of viral hemorrhagic septicemia of rainbow trout, is presently the only other rhabdovirus known from a salmonid fish, and Egtved virus is not neutralized by IHNV antiserum.

Transmission and incubation. The virus is carried by survivors of the disease and is shed by adults at the time of spawning. The virus is associated with sex products of males and females, but is more abundant with eggs than with sperm. Although virus has not been demonstrated as actually within the egg, the association is intimate and, in effect, vertical or at least generation-to-generation transmission occurs. Supporting that concept is the fact that outbreaks of IHN have occurred among progeny of eggs that came from carriers but were disinfected with an iodophore, the most effective product known for that purpose. The virus is waterborne and also transmitted by contact and by ingestion.

Incubation time from exposure to first mortality is variable and depends on factors of temperature, route of infection, amount of virus, and age of fish. The usual range of incubation time is 5 days to about 2 weeks after fish have been exposed by contact.

Host and geographic range. The disease is native to and enzootic in the Pacific Northwest (USA) from California to and including most of coastal Alaska. The disease has been introduced to Japan with virus contaminated eggs from North America, and it has apparently become established both on Japan's Honshu and Hokkaido Islands.

In addition to rainbow trout, chinook and sockeye salmon, Japanese chum salmon, amago *Oncorhynchus rhodurus,* and masou salmon *O. masou* have sustained epizootics. Atlantic salmon *Salmo salar* have proven susceptible to IHNV when given by injection.

Control measures. Measures that contribute to avoidance or prevention should be implemented. Where possible, fish stocks known to harbor the virus should be avoided, but that measure is practical only with hatchery fish such as rainbow trout. In situations where anadromous stocks of fish are known to harbor IHNV and virus-free alternative populations are not available, several measures can be applied to reduce mortality even though the virus can persist.

The first such measure is to disinfect virus-contaminated eggs; iodophores are recommended for that purpose. Suggested disinfection is with 100 ppm available iodine for 10 to 15 min. The pH of the disinfecting solution should be 6.0 or higher.

In some, but not all, situations where water supplies are contaminated and IHN cannot be avoided, incubation of eggs and fry at 15 °C or higher has resulted in significantly reduced mortality. Although incubation at elevated temperature does not

eliminate the virus, the measure has been effective in minimizing losses of chinook salmon fry in California. Ironically, Idaho's Snake River Valley has constant 15 °C water and IHN outbreaks among rainbow trout have occurred there regularly during the last several years.

In theory, ultraviolet irradiation or ozonation of contaminated water supplies should be effective in controlling IHN. Still another measure results in reduced mortality. Returning stocks of salmon are examined virologically and eggs from individual pairings are incubated in separate lots. Where high levels of virus are found in one or both parents, the lots are discarded. The measure requires a disciplined approach in testing, and use of decontaminated or virus-free equipment for each pairing. Also, cell cultures must be incubated for a minimum of several days before results are available. Nevertheless, losses among progeny of parents that shed little or no virus are much less than losses of progeny of parents with high virus titers. Drastic reduction in virus levels seems to be the operative factor.

Methods of immunization are in development and hold promise for the future. Virus has been attenuated by repeated cell culture passage at elevated temperature and when administered by immersion the result has been to provide protection against virulent challenge. A 2 day immersion time was most effective (Pilcher and Fryer, 1980). Use of the vaccine has not yet found widespread application.

Infectious pancreatic necrosis

Definition. Infectious pancreatic necrosis (IPN) is an acute, widely disseminated and commonly virulent disease of young trout (termed fry or fingerlings) in freshwater facilities. The causal agent is a bisegmented double-stranded RNA virus with icosahedral morphology and a particle size of about 60 nm. Viruses indistinguishable from INPV have been isolated from anadromous, catadromous, and marine fishes. However, in only 1 kind of host, the eel, is the agent demonstrably virulent (see eel virus kidney disease, this section). In the case of menhaden spinning disease, the putative causal agent's virulence remains to be confirmed. A virus indistinguishable from IPNV has been isolated from another marine fish, the southern flounder *Paralichthyes lethostigma;* however, virulence for the source species or for brook trout *Salvelinus fontinalis* the species most susceptible to IPN, could not be demonstrated experimentally. Uncertainty over the nature of viruses like or distinguishable from IPNV is further clouded by the fact that additional IPNV-like agents have been isolated from marine molluscs and crustaceans (Hill and Torchy, 1981).

Signs and pathologic changes. The disease IPN occurs among young trout — fry and, in some cases, fingerlings. The first indication of a health problem is a sudden onset of mortality. Often, the largest and most vigorous fish are affected and victims exhibit an unusual swimming behavior in which they rotate about their long axis. Such agonal behavior alternates with prostration during which the respiration is shallow and rapid. Exophthalmia, abdominal distension, and darkened pigmentation are common signs, and some victims trail a thin strand of mucoid material from their vent.

Internally, food is absent from the digestive tract and the stomach is whitish. In some, but not all cases, petechial hemorrhages are present between pyloric caeca. The most significant finding — one that with the foregoing signs is pathognomonic — is the presence of a clear to whitish mucoid gel in the stomach and anterior intestine.

As the name of the disease indicates, pancreatic necrosis is the dominant histo-

pathological feature. The original report of IPN concerned the disease in brook trout and noted that necrosis occurred in both the acinar and islet tissue. Other organs were without significant change (Wood and co-authors, 1955). According to later authors, necrosis of islet tissue is an exception; instead small foci of mild necrosis is common in hematopoietic tissue of the kidneys. McKnight and Roberts (1976) investigated IPN in rainbow trout and found necrosis of renal hematopoietic tissue and intestinal mucosa. The latter sloughed into the lumen and constituted the catarrhal exudate or fecal cast.

Biophysical properties of the virus. No doubt, in part because it was the first virus isolated from a fish but also because of some of its unusual properties, IPNV has been one of the most intensively researched and characterized of the fish viruses. The viral genome is RNA, but double-stranded and composed of 2 segments. Those features are also found in Tellina virus, oyster virus, drosophila X virus, and in the virus of infectious bursal disease of chickens. Accordingly, it has been proposed that a new virus group name Birnaviridae be applied (Dobos and co-authors, 1979).

The IPN virus is an icosahedron about 60 to 65 nm in diameter and lacking an envelope. It is ether- and glycerol-stable and the latter feature is a decided advantage in shipping diagnostic specimens, infectivity survives even at ambient summer temperatures. Infectivity persists for 1 yr or more in 50 % glycerol at 4 °C and in serum containing cell culture medium at 4 °C or lower if the pH is 7.0 or less. Infectivity is readily preserved for years at −20 °C or lower or, alternatively, after lyophilization. Some isolants of IPNV are vulnerable to freezing and thawing if the pH is slightly alkaline. An acidic pH is decidedly beneficial in stabilizing infectivity.

The virus is remarkably stable, and infectivity can persist for weeks in environments that are suitable for rearing trout. Even when dried, infectivity can be demonstrated after several days. The virus is generally vulnerable to commonly used disinfectants such as chlorine, formalin, iodophores, and ozone (Pilcher and Fryer, 1980).

Additional information on the physical and chemical properties is to be found in the report of Dobos and co-authors (1979) and the reviews of McAllister (1979), Pilcher and Fryer (1980), and Dorson (in press).

Diagnosis. A clinical or presumptive diagnosis of IPN can be made if young trout evidence a sudden onset of significant mortality and show the aforementioned behavioral and clinical signs. Other causes of mortality, such as bacterial gill disease or parasitoses, should be ruled out and additional confidence in accuracy can be had if histopathologic changes are found that conform to those of IPN.

The best diagnosis is obtained if virologic examination is carried out and IPNV is isolated and serologically identified. However, mere presence of the virus is not sufficient to assign to it the cause of mortality. When IPNV causes mortality, the amount of virus per gram of whole victim fish is usually 10^5 PFU or greater. It should also be noted that a dual infection can occur with either infectious hematopoietic necrosis or viral hemorrhagic septicemia. Careful plaque assay on RTG-2 cells can reveal the presence of 2 salmonid viruses if they are present.

Isolation and identification of the virus. With the possible exception of the rhabdoviruses, IPNV is the most easily isolated of the fish viruses, for it is replicated with marked lytic CPE by most of the commonly used fish cell lines (Wolf and Mann, 1980). The virus is replicated at 4 to 26 °C, but 20 °C is commonly used and at that temperature CPE will generally be evident within 24 to 36 h — if the virus is causing mortality. Higher

temperature is used by some investigators in order to obtain an overnight appearance of CPE.

When investigating suspect cases of IPN mortality, homogenates of whole victim fry — fresh, frozen, or glycerinated — or viscera from fingerlings are prepared. Dilutions of 1:50 to 1:100 are used to minimize toxicity to cell cultures and decontamination carried out with centrifugation, treatment with antibiotics or membrane filtration. Inocula are adsorbed onto drained cell sheets or added directly to medium overlaying the cells. If CPE occurs and progresses in virus-like manner, serologic testing will provide identification.

It has been variously noted that there exists among fish at least 3 and possibly as many as 7 strains of IPNV (Hill, 1982). The strains differ in innate virulence, and a geographic pattern exists. Strains Sp and Ab are dominant in Europe, but the Ab strain has low virulence. Strain VR-299 of the American Type Culture Collection is the prototype virus. It is virulent and prevalent in North America. Regardless of their origin, all strains show some degree of cross-reactivity and polyvalent antisera are preferred for identification.

Serum neutralization test is the most widely and reliably used method of identification. Fluorescent antibody techniques are next most popular followed by complement fixation and immunoperoxidase. Recently, ELISA has been successfully applied and other methods are sure to be applied. For further reading see Dorson (in press).

Transmission and incubation. Infection is transmitted principally horizontally, but there is convincing evidence that vertical transmission also occurs. During epizootics, virus is shed in the mucoid cast material of victims and massive amounts of waterborne infectivity are readily demonstrable. Adult carriers are the prime source of virus, and infectivity can be shed in urine, feces, or sex products, or in all 3. The presence of virus associated with sex products long provided circumstantial evidence for vertical transmission. Although proof of virus within eggs is still lacking, careful iodophore disinfection of eggs from carriers does not prevent IPN outbreaks among resulting progeny.

Transfer or transport of the virus is possible through mammals or birds that might eat IPN victims, for infectivity has been demonstrated in feces of homeotherm carnivores following experimental feeding.

Incubation time to death depends on species and age of fish, but also on the strain of virus, temperature, portal of entry, and possibly on the amount of virus (Hill, 1982). At about 12 °C, fry of brook trout will show mortality 5 to 7 days after being fed the virus. As the fish grow older, incubation time is extended to 10 or more days, but when much beyond 6 months of age the fish can be infected but not show signs of disease. If virus is injected, incubation time is shortened by several days.

Compared with brook trout, rainbow trout are a bit more resistant to IPN, but they too show the pattern of development of resistance with age (Dorson and Torchy, 1981). As could be expected, temperatures of 4 to 6 °C prolong the incubation time, but in addition low temperature has a sparing effect in that mortality is reduced.

Host and geographic range. A marked difference exists in the pattern of resident or original strains of IPNV found in North America and those found in Europe. Accordingly, it seems likely that the disease is native to both continents in the northern hemisphere. The virus is also found in Japan and Chile, but epizootiological data fully support the contention that the pathogen was introduced.

The nominal victim of IPN disease is young trout, but virtually all salmonids are infectable, but with few exceptions the result is wholly sub-clinical.

Over the years, a growing array of nonsalmonid fish — freshwater, anadromous, catadromous, and marine — have yielded IPN virus. The groups involved are: anguillids, cichlids, clupeids, cyprinids, percids, and even a petromyzontid — a lamprey. In some instances, virus was found during investigation of mortality but in other cases the source fish appeared to be normal.

Control measures. The most effective measure for control of IPN is avoidance, but that approach requires that the water supply and the brood stock both be free of the virus. Although rarely practiced, virus-contaminated water supplies can be decontaminated by clorination followed by dechlorination, by ozonation, or by UV irradiation. Where captive brood stocks are known to harbor carriers, nondestructive virological testing of feces and sex products will reveal most of those that shed virus and should be culled. The fertilized eggs of each pair of presumptively clean parents should be incubated in separate containers and the resulting progeny observed and assayed for evidence of IPN. Where the available supply of virus-free water is limited, eggs can be incubated and fish reared to advanced fingerling age before transfer to contaminated water. In that way, age resistance is permitted to develop and mortality is reduced. The fish, however, will become infected and some could become carriers.

Where virus cannot be avoided, the practice of some operators is to incubate an additional number of eggs in order to offset anticipated mortality. The economic loss of fry is least and the year's production commitments or plans are ensured.

Antiviral drugs have been tested under laboratory conditions but hatchery-scale applications have been extremely limited. Incorporation of polyvinylpyrrolidone iodine in trout ration has reduced somewhat the mortality from IPN epizootics. Prospects for chemical therapy or prophylaxis of any viral disease of fish are presently very dim.

Immunization for control of IPN is an attainable goal, and although considerable effort is underway a practical product has not yet been developed. For economy and ease of application, the vaccine will probably be attenuated or low virulence virus that can be added to food or water. The product ought to be identifiable as a vaccine strain so as not to confound ongoing programs of inspection and certification.

Unpublished work on selective breeding of brook trout in Pennsylvania has resulted in a strain that has 95 % survival under challenge with homologous IPN virus. The most susceptible strain has a 5 % survival. Response to heterologous strain virus is not known.

Oncorhynchus masou virus

Definition. Oncorhynchus masou virus (OMV) is a herpesvirus that produces systemic disease and mortality in young Pacific salmon (Kimura and co-authors, 1981c). The virus is hepatotropic and, more significantly, oncogenic. A high percentage of OMV disease survivors develop epithelial papillomas on and about the head. The tumors show some sign of being invasive and metastasizing (Kimura and co-authors, 1981a). The virus and its disease were reviewed by Kimura and co-authors (1981b).

Signs and pathologic changes. The virus was isolated from normal appearing masou salmon *Oncorhynchus masou,* but the effects of OMV are best known from experimental infections of young chum salmon *O. keta.*

Infected young chum salmon develop anorexia, and some become exophthalmic or develop visible petechiation, but neither in behavior nor appearance are signs diagnostic. Internally, the liver is mottled with white areas and in extreme cases the organ is pearly

white. The digestive tract is devoid of food, the spleen of some is swollen, but kidney — usually a prime target for fish viruses — appear normal.

Histologically, the liver showed multiple foci of severe necrosis and areas of fused cells — syncytia. The spleen also showed some necrosis, and edema was present in the heart. Consistent with the gross appearance, kidneys were normal as were the pancreas. More extensive details of histopathology were not reported.

As many as 60 % of survivors of experimental infection develop epithelial tumors of papillomatous from about 4 to 6 months after initial infection (Kimura and co-authors, 1981b). Neoplasms occur preferentially on and about the mouth but also on eyes, opercles, other areas of the head, and occasionally on the caudal fin. In a single case, a tumor was found in kidneys.

Tumors consist of squamous cells with a fine connective tissue stroma. Mitoses are common. The single renal tumor, in contrast to external neoplasms, showed internal necrosis and displacement of normal kidney tissue. Nuclear size varied, but electron microscopy failed to reveal the presence of virus (Kimura and co-authors, 1981b).

Biophysical properties of the virus. The OMV, or salmonid herpesvirus 2, is unequivocally a member of the Herpesviridae and officially recognized as such (Roizman and co-authors, 1981). As could be expected, the salmon pathogen is replicated only by salmonid cell lines such as RTG-2, CHSE-214, KO-6, HIME, CHH-1, SE, and YNK (Kimura and co-authors, 1981a, c). *In vitro* effects begin as cell rounding followed by development of syncytia and a terminal lysis. Optimal temperature of incubation is 15 °C, but the virus is replicated at 5 to 18 °C but not higher. Peak titer *in vitro* — about 10^6 $TCID_{50}$ ml^{-1} — is reached at about 9 days.

The virus is somewhat labile; all infectivity is lost within 17 days if material is stored at 15 °C or higher, and even at – 20 °C most of the infectivity is lost by the 17th day. At −80 °C, infectivity persists for at least 6 months. T. Kimura (pers. comm.) carried out serological comparisons of OMV and *Herpesvirus salmonis* and found that the 2 were distinct agents.

Diagnosis. Disease caused by OMV can be considered when significant mortality occurs among young masou salmon, when an infectious process is obviously involved, and when other pathogens cannot be found. It is possible that the virus is virulent for other Asiatic salmonids. However, virulence for young masou salmon is predicated solely on the fact that the pattern of fish viral disease is one of causing mortality in the young and after which survivors develop a carrier state into maturity.

In vitro development of syncytia in salmonid cell lines coupled with findings of hepatic syncytia gives reasonable assurance of an accurate diagnosis. The most accurate assessment of cause is made by isolating the agent and identifying it serologically.

Isolation and identification of the virus. Ovarian fluids from masou salmon and homogenates of whole victims of experimental infection have yielded virus when inoculated on epithelial or fibroblastic cell lines of salmonid origin. Temperature of incubation of 15 °C is suggested. Young victims yield about 10^6 $TCID_{50}$ g^{-1} of body weight. In limited trials, virus was isolated from 1 of 11 tumors and in another case from primary culture of a tumor that underwent degeneration after several days.

Serological identification is achieved with rabbit anti-OMV serum. Earlier, in Japan, Sano (1976) isolated a herpesvirus from *Oncorhynchus nerka* and termed the agent NeVTA for nerka virus Towada lake Akita and Aomori Prefectures. For a while it was

thought that NeVTA was *Herpesvirus salmonis,* but now it appears (T. Sano, pers. comm.) that NeVTA might be the same as OMV.

Transmission and incubation. The original finding of the virus in ovarian fluids suggests that vertical transmission could be involved. More certainly, however, horizontal transmission takes place, because infection can be initiated experimentally by simple immersion. Kimura and co-authors (1981c) used a 1 h immersion of young chum salmon in a concentration of 100 $TCID_{50}$ ml^{-1} of virus at 10 °C. Three-month-old fry were highly susceptible; after incubation for about 11 days mortality began and by 65 days reached 60 %. Incubation time was nearly double — 20 days — in 5 month-old fry, and mortality reached 35 %. Age-dependent susceptibility was clearly apparent; 8 month-old chum salmon showed neither disease nor did they sustain mortality — even after a second attempt wherein they were injected intraperitoneally with 200 $TCID_{50}$ of virus.

Host and geographic range. Thus far, the only natural occurrence of OMV has been among adult masou salmon in Japan. However, the likelihood that NeVTA virus (herpesvirus of himemasu) is the same as OMV, extends the natural host range to nerka salmon *Oncorhynchus nerka,* but again in Japan. Experimentally, *O. nerka* was susceptible to OMV as were rainbow trout *Salmo gairdneri.*

Considering the geographic locations of Japan and nearby Soviet Union, one suspects that OMV and IHNV might well occur among anadromous salmon in the Far East of the USSR.

Control measures. Avoidance is the universal control measure for viral diseases of fishes. That measure is particularly appropriate for captive populations, but hardly for anadromous species. T. Kimura (pers. comm.) has recently achieved control of OMV by continuous feeding of Acyclovir. Also, and in time, it is anticipated that methods of vaccination will be developed.

Catadramous Fishes

Eel viral kidney disease

Definition. Eel viral kidney disease or branchionephritis is an acute and virulent infection of young eels of the genus *Anguilla* under conditions of husbandry in Japan. The disease characteristically occurs during cold months. The causal agent is an IPNV-like virus known as eel virus European (EVE or EEV) because the disease was first recognized after elvers were imported from Europe (Sano and co-authors, 1981).

Signs and pathologic changes. Significant mortality occurs and moribund fish show transient muscle spasms or rigidity. Gills are swollen and congested. Lamellae show hyperplasia that results in fusion and clubbing of filaments. Internally, food is absent from the gut. In some, kidneys are hypertrophied and ascitic fluid is present. Histologically, the condition is characterized as a proliferative glomerular nephritis. Renal tubular cells show hyaline droplet degeneration. Focal necrosis occurs in renal interstitial tissue, in some livers and in most spleens.

Biophysical properties of the virus. The causal agent, EVE, is unequivocally IPNV-like and thus it has characteristics of the Birnavirus group. Okamoto and co-authors (1983) compared the antigenic relationships of EVE and selected well-recognized strains of IPNV. The comparisons showed that EVE was most like the Danish strain Ab. Interestingly enough, the Ab strain is of low virulence for trout and although experimentally virulent for young eels, the EVE strain is also avirulent for trout.

Diagnosis. Eel viral kidney disease should be considered if mortality of significance occurs among young eels — particularly during the cold season — and if the aforementioned signs and pathologic changes are present. Bacteriologic and parasitologic examinations should be essentially negative, but virologic examination should readily reveal virus having characteristics of IPNV.

Isolation and identification. The RTG-2 cell line has been used for isolations; however, considering the IPNV-like properties of EVE, other commonly used fish cell lines would probably be susceptible and therefore appropriate. Temperature of incubation is not critical and can be anywhere in the range of 10 to 20 °C. Sano and co-authors (1981) prepared inocula from gills and kidneys. In the case of very small eels, homogenates could be prepared from whole elvers.

The eel isolant is readily neutralized by polyvalent anti-IPNV serum. It is only distantly related to Group I isolants typified by ATCC strain VR-299. It is more closely related to Group II isolants — those that are like Sp strain, but most closely related to the Ab strain (Okamoto and co-authors, 1983).

Transmission and incubation. Young eels are readily infected by intraperitoneal injection of 10^7 $TCID_{50}$ virus or more and after an incubation time of 6 to 8 days, sustain losses of 50 to 75 % during the following 10 to 12 days at 8 to 14 °C. Under similar conditions, rainbow trout were refractory (Sano and co-authors, 1981). The pattern of mortality is much like that of IPNV in young trout. In addition to injection, young eels are also susceptible to waterborne virus and can be infected by immersion for 1 h in virus at about 10^6 $TCID_{50}$ ml^{-1}. Incubation time and cumulative mortality are much like that in injected eels. Vertical transmission can be considered.

Host and geographic range. Thus far, the hosts known to be susceptible to EVE disease are *Anguilla anguilla* and *A. japonica.* Also, the disease has been recognized thus far only in Japan. Considering that the virus was presumed to have been introduced from Europe, and that European eels are shipped elsewhere in the world, eel viral kidney disease could well occur wherever eels are propagated.

It is conceivable that eel viral kidney disease is simply the expression of cross-infection of eels by strain Ab of IPNV from trout that are also widely propagated.

Control measures. If sources of EVE are known, avoidance is the most reliable control measure. Eels raised in proximity to trout that harbor IPNV — particularly strain Ab — might be at risk. For additional reading, the text section on IPN is suggested.

Eel rhabdovirus infection

Definition. Eel rhabdovirus infection is an expedient collective term for a group of 4 serologically related viruses isolated from *Anguilla* species. Two of the viruses were designated according to their geographic origin: EVA (eel virus America), and EVEX (eel virus Europe, unknown) but were actually isolated in Japan (Sano, 1976; Hill and co-authors, 1980). The other 2 viruses were designated B_{12} and C_{30} and were isolated in France (Castric and Chastel, 1980). The EVA isolant was isolated from elvers that had a hemorrhagic disease and sustained nearly a 60 % mortality. Experimentally, however, the virulence of EVA for young eels has yet to be demonstrated. The other isolants — EVEX, B_{12}, and C_{30} — were isolated from apparently healthy eels and produced no signs of disease in elvers that were challenged by several methods (Castric and Chastel, 1980). Sano (1976) found that EVEX was pathogenic for young rainbow trout.

Determination of the virulence or nonvirulence of eel rhabdoviruses might be resolved if young unexposed elvers can be obtained and appropriately tested. It is worth noting that most of the fish rhabdoviruses are virulent for young of the hosts in which they were originally found.

Signs and pathologic changes. Sano (1976) described afflicted elvers from which he isolated EVA as showing marked vascular congestion in pectoral and anal fins and a more diffuse congestion on the abdomen. Behavior changes were not reported, but the head was abnormally flexed downward. Histologically, skeletal muscle was hemorrhagic and degenerated, gills were hyperemic, and hemorrhage or leakage of proteinaceous fluid was present in Bowman's space and kidney tubules. Necrosis was evident in kidney tubules, liver, and spleen.

Biophysical properties of the virus. In their size and morphology, the 4 isolants are unequivocally members of the rhabdovirus group. In their polypeptide composition two of the isolants, and by inference a third, are distinctly different from agents of viral hemorrhagic septicemia of trout and spring viremia of carp (Hill and co-authors, 1980). Serologically, the C_{30}, EVA, and EVEX isolants are quite closely related, but at best only distantly related to B_{12} (Castric and Chastel, 1980).

All isolants are replicated by the EPC cell line at 14 °C, but at that temperature the B_{12} isolant requires 5 days to produce plaques, whereas the other 3 isolants require only 3 days and additionally will plaque in 2 days at 20 °C. Maximum titers are about 10^7 PFU ml^{-1} except for the B_{12} isolant that attains 10^8 PFU ml^{-1}.

The RTG-2 cell line replicates the 3 closely related isolants at temperatures as high as 20 to 25 °C, but B_{12} not at all. Hill and co-authors (1980) favored the BF-2 line that replicated EVA and EVEX at titers as high as 10^9 PFU ml^{-1}.

Diagnosis. The fact that 3 isolants were made from healthy eels and that only the fourth was from diseased fish and not demonstrably pathogenic, clouds the matter of eel rhabdoviruses as agents of disease. When eels show evidence of frank disease and other pathogens are not involved, virus should be considered. Separation of eel viral kidney disease and rhabdoviral infection can only be done reliably by isolation and identification.

Isolation and identification of the viruses. The EPC line is suggested for isolation of any of the presently known eel viruses but more specifically for isolation of the eel rhabdoviruses. Temperature of incubation should not exceed 14 °C. The RTG-2 and BF-2 lines will replicate 3 of the rhabdovirus isolants reliably and are probably suitable for isolations of all but the B_{12} agent. Serological identification of the eel rhabdoviruses is by neutralization test and to that end, Hill and co-authors (1981) described the preparation of antisera.

Transmission and incubation. Nothing is known, but it is assumed that waterborne infection is the rule.

Host and geographic range. Thus far the viruses have been isolated only in Japan and France, but one Japanese isolation was made from Cuban elvers at their Tokyo port of entry. The common eel *Anguilla anguilla* as known in America or *A. rostrata,* the European race, is the only known host. Susceptibility of the Japanese eel *A. japonica,* has not been determined.

Control measures. Because the etiologic role of viruses remains to be established, discussion of control measures is not appropriate.

Eel virus-2

Definition. Eel virus-2 (EV-2) is a virus of uncertain grouping, possibly an orthomyxovirus. The virus was isolated from 1 *Anguilla anguilla* taken in Germany and bearing oral neoplastic tissue known as stomatopapilloma or 'cauliflower disease' or 'Blumenkohlkrankheit' (Nagabayashi and Wolf, 1979). The role of virus in eels has not been determined. A similar agent was also isolated from young eels in North America's coastal waters (Nagabayashi and Wolf, 1979).

Signs and pathologic changes. The source eel was one of 12 that bore oral papillomas and were sent from Germany for virological investigation. The eels were otherwise normal in appearance and activity, but the source specimen became weak and so was selected for early examination.

Infectivity trials with the virus and elvers from North America were inconclusive; therefore, the role of virus in production of disease or of neoplasia remains to be determined.

Biophysical properties of the virus. The EV-2 virus is a pleomorphic particle, 80 to 140 nm in diameter, and has an RNA genome. In sucrose gradient, the buoyant density is 1.19 g cm^{-3}. The virus is replicated in FHM cells but not in BB, BF-2 or RTG-2. The range of temperature for replication is 10 to 25 °C and the optimum is 15 °C. Syncytia are produced at 15 °C, but at 20 to 25 °C the CPE is one of pyknosis and lysis. A one-step growth curve shows an initial CPE at about 10 h, and a plateau of infectivity of about $10^{5.3}$ PFU ml^{-1} is reached at about 20 h. The agent is ether-, pH-, and heat-labile, and its heat lability is not reversed by $MgCl_2$. The virus agglutinates chick and sheep red blood cells but only after infectivity is concentrated to 10^6 PFU ml^{-1} or greater.

Diagnosis. Because a disease has yet to be related to the presence of EV-2, diagnosis is not applicable.

Isolation and identification of the virus. Original isolations were designated EV-1 and were made in both FHM and RTG-2 cell lines (McAllister and co-authors, 1977). However, virus recovered from frozen stocks, again cloned and designated EV-2, failed to produce CPE in RTG-2 cells. Virus was isolated from homogenates of internal organs and from papillomatous tissue.

New efforts at isolation should consider using antisera against IPNV and against the eel rhabdoviruses to inhibit those viruses if they are present and thus to enhance finding EV-2. The EV-2 is presumptively identified from its eel origin and its ability to evoke syncytium formation in FHM cells. That characteristic is shared with the golden shiner virus, but the golden shiner virus is clearly an icosahedron.

Transmission and incubation. Very little is known about transmission and incubation except that transmission does occur, but whether in fresh water or marine environments has not been determined. When North American elvers were injected with virus about half died within 3 months and virus could be recovered from only about 25 % (Nagabayashi and Wolf, 1979).

Host and geographic range. The original source was *Anguilla anguilla* from Germany, but presumptively similar isolations were made from young *A. anguilla* from North American (Delaware) coastal waters. Serological comparisons, however, were not made. Considering that European and North American eels both spawn in adjacent areas of the Sargasso Sea and that elvers from North America and Europe are exported to Japan,

viruses similar to or identical with EV-2 might be found in Asia and in eels other than *A. anguilla*.

Control measures. Not applicable.

Visualized Viruses and Virus-like Particles

Atlantic cod adenovirus

Definition. Atlantic cod adenovirus is an intranuclear icosahedron seen in cells of localized areas of hyperplastic epidermis of *Gadus morhua* from the Baltic Sea (Jensen and Bloch, 1980).

Signs and pathologic changes. Raised flat and transparent skin lesions, 3 to 20 mm in diameter, are found on the body but preferentially in the caudal region. Thickness of epidermis within the lesions is about 4 times greater than that of normal skin and few, if any, mucus cells are present. Grossly, the hyperplastic cells appear to be normal, but cells near the germinal layer are shorter than normal. Vascularity within the lesion is greatly increased.

Intranuclear viral forms are not universally present. They are found in some of the outermost cells of the lesion. The inference is that virus is causal.

Biophysical properties of the virus. The agent was visualized but not isolated. Its provisional placement as an adenovirus is based solely on its 77 nm size, intranuclear location, and icosahedral morphology.

Diagnosis. Recognition of the condition requires finding skin lesions and distinguishing them from those of the cod ulcus syndrome, which occurs in the same waters. Gross and histological features are useful, but until such time as isolation is made, the most accurate determination additionally requires demonstration by electron microscopy of the intranuclear icosahedra of adenovirus-like size.

Isolation and identification of the virus. The literature has long been without a candidate adenovirus from fish. Jensen and Bloch (1980) qualified their findings by terming the first such agent 'adenovirus-like'. Nevertheless, the agent is the best candidate from a fish for a member of that virus group.

It is suggested that when isolations are attempted that cultures include normal skin epithelial cells and primary cultures of mixed cell morphology from the cod or a closely related gadid fish. In addition, an array of the commonly used established fish cell lines — epithelial and fibroblastic morphology — should be used.

Transmission and incubation. Nothing is known.

Host and geographic range. Thus far, the only report is that of finding the agent in Atlantic cod taken in the Baltic Sea off Denmark.

Control measures. Not applicable.

Atlantic salmon papillomatosis

Definition. Atlantic salmon papillomatosis is a condition of chronic plaque-like areas of proliferated epidermis. The plaques occur at multiple sites and seasonally, particularly on some captive populations of parr and young *Salmo salar,* but mostly among fish in freshwater. The etiology has not been demonstrated, but infection has been postulated, and virus-like particles have been reported by Carlisle (1977).

Signs and pathologic changes. Behavior of affected fish is normal. All signs of the

condition are external, and the subject lesions are transparent to whitish, wart-like, several millimeters thick and as large as 40 mm in diameter, but usually smaller. Histologically, the neoplastic cells are epithelial, but normal tissue architecture is deranged, mucus cells are scarce and the growth is supported by a fibrovascular dermis. Mitoses are common and cytoplasmic inclusions occur in some growths where cells are degenerate (Carlisle and Roberts, 1977). At the ultrastructural level, Carlisle (1977) found the neoplastic cells to have nuclei that were more spherical than normal epithelial cells. In addition, margination of chromatin was prominent.

Biophysical properties of the virus. Carlisle (1977) attempted isolation of virus on the AS cell line of Atlantic salmon origin, but was not successful. He described and illustrated the virus-like particles found in small numbers in degenerating cells. The particles measured 125 to 150 nm in diameter and had an electron-dense coat and internally, separated by an electron-lucent area, a 70 to 95 nm body that was nucleotid-like.

Diagnosis. Diagnosis can be made by findings of wart-like lesions on young Atlantic salmon. Histological features of the kind described and illustrated by Carlisle and Roberts (1977) support and confirm the diagnosis.

Isolation and identification of the virus. Isolation has not been achieved. The AS cell line is fibroblastic, but the dominant cell type in the lesion is epithelial. Accordingly, cultures of normal epithelial cells from Atlantic salmon should be tried and incubation should be prolonged to allow transformation to become evident if viral etiology is involved.

Transmission and incubation. Some attempts at experimental transmission have been made, but none were successful.

Host and geographic range. The disease is known from young salmon in Scandinavia and the United Kingdom where it is fairly common in husbandry facilities. On infrequent occasions it has been found in young salmon in streams in North America's Northeast.

Control measures. Not applicable.

Atlantic salmon swim bladder fibrosarcoma

Definition. Swim bladder fibrosarcoma of Atlantic salmon *Salmo salar* is a neoplasm of prominent proportions but of low incidence and with low associated mortality. The condition involves subadult fish in mariculture, and an associated C-type or leucovirus is present in the tumor (McKnight, 1978; Duncan, 1978).

Signs and pathologic changes. The condition became evident when mortality began among cage-reared fish in a loch. Affected individuals were sluggish and in poor physical condition. No external lesions were evident.

Internally, hard nodular tumors were distributed along the swim bladder, in some cases along its entire length. Individually, the nodular masses measured 15 to 30 mm in diameter; they protruded from the swim bladder and occupied much of the body cavity. No invasiveness, metastasis or adhesion formed (McKnight, 1978).

Histologically, the tumors consisted of well differentiated interlacing bundles of fibroblastic cells with elongate nuclei and cytoplasmic processes. The tumors arose at the junction of the smooth muscle layer and the loose areolar tissue. Centers of the nodules were not well vascularized and accordingly showed ischemic necrosis. In contrast, outer portions of the tumors were vascularized. Mitoses were fairly common, and McKnight (1978) classified the growths as leiomyosarcomas.

An electron micrographic study was carried out by Duncan (1978) who found 2 kinds of particles — one extracellularly, the other in the process of budding from cell membranes.

Biophysical properties of the viruses. Numerous electron-dense particles measured about 110 nm in diameter and were found both in aggregates enclosed in membranes and located extracellularly. The second type of particle measured about 120 nm in diameter and had C-type or leucovirus morphology and apparent mode of budding from cell membranes. No attempt was made at virus isolation.

Diagnosis. Recognition of the disease is based on host species and gross and histopathological findings.

Isolation and identification of the virus. The agents have not been isolated. Identification of the particles — particularly that of the leucovirus — is presently possible and at that only presumptively by electron microscopy.

Transmission and incubation. Nothing is known.

Host and geographic range. One yr-old smolts and 2 yr-old salmon in cage culture in a Scottish loch were involved in the sole case reported.

Control measures. Not known.

Gilthead sea bream virus-like particles

Definition. Gilthead sea bream virus-like particles are indeed convincing viral forms found in the cytoplasm of cells of benign oral papillomas that occur in *Sparus aurata* taken in Spain (Gutierrez and co-authors, 1977).

Signs and pathological changes. Papillomata were found in 7 of 39 fish taken and lesions were located about the mouth and on the maxillae. Tumors were slow growing, but in some cases interfered with feeding. Neither invasion nor metastases were evident. Internally, tumors showed no necrosis, and the basement membrane was intact.

Biophysical properties of the virus. The virions had morphological properties of leucovirus or C-type particles. The stated size of 35 to 65 nm is smaller than that of leucovirus. The particles occurred singly and in groups, and in some areas appeared to bud from cytoplasmic membranes. Centers of some particles showed evidence of an electron-dense nucleoid and particle surfaces had regular stud-like or peg projections reminiscent of cornavirus. Isolation was not attempted, and the author has not pursued the work.

Diagnosis. Findings of oral papillomata on the gilthead sea bream and accompanying evidence of leucovirus-like particles are suggested as being presumptively diagnostic. The provisional nature of that statement is based on the fact that the host specificity of the leucoviruses of fish — and there is a growing number of them — is not known. Also, the role of leucoviruses in fish is thought to be causal.

Isolation and identification of the virus. Thus far, the virus is identifiable only by its morphology and apparent mode of replication in tumor cells. Isolation was not reported.

Transmission and incubation. Presence of the virus suggests that the tumor is transmittable. The nature of the neoplasm and the apparent grouping of the virus further suggest a lengthy incubation time.

Host and geographic range. At present the agent is known only from the single report involving one species in Spain. The sea bream, however, is widely propagated; accordingly, similar cases might occur elsewhere. If so, neoplasms should be examined virologically.

Control measures. Not applicable.

Opaleye calicivirus

Definition. Opaleye calicivirus can be termed a 'minimal fish virus', because it occurs in the marine teleost *Girella nigricans,* and experimentally it has been shown to be replicated in the fish — in both cases without any evidence of harm to that host (Smith and co-authors, 1981). The virus has also been isolated from a liver fluke taken from a dead sea lion, and it is in that animal and other marine pinneped mammals that the calicivirus causes disease and mortality.

Veterinary virologists know the agent as San Miguel sea lion virus (SMSV), and SMSV shares common antigens with the virus of vesicular exanthema of swine (VES). The ocean, in fact, is the likely source of the agent that caused VES epizootics that swept much of the United States in decades past (Smith, 1981).

Much of the story of calicivirus in fish, parasite, and marine mammal has resulted from investigations of Smith and co-authors (1978, 1980a, b). Smith (1981) reviewed the history of investigations, and in the same volume, Bankowski (1981) reviewed diseases in swine and pinnepeds. Bankowski considers the pinneped and swine viral diseases to have the same etiology.

Signs and pathological changes. Effects of calicivirus on the opaleye in the ocean are not known; however, based on results of experimental infections, the virus has minimal impact. In a limited trial with 4 opaleyes, San Miguel sea lion virus Type 5 was inoculated into small specimens kept at 15 °C. The fish showed neither behavior change nor evidence of disease. Virological assay of several tissues and organs showed that the agent was replicated, that highest titers were produced in spleens, that virus persisted for more than 1 month, but that no mortality occurred (Smith and co-authors, 1981). Histopathologic examination was not done.

Readers interested in effects of the virus in swine and marine mammals are referred to the review of Bankowski (1981).

Biophysical properties of the virus. Caliciviruses have a genome of single-stranded RNA and are members of the Picornaviridae. Particle size is variously cited as being 20 to 40 nm and the morphology as icosahedral. The agents are stable to ether but labile to acid and heat. *In vitro* replication of the opaleye isolant occurs at 15 and 37 °C, and peak titers reach 10^7 $TCID_{50}$ ml^{-1}. Multiple related serotypes are known among isolants from marine mammals, the opaleye, a liver fluke, and swine.

Diagnosis. Inasmuch as the virus evokes no clinical signs in the fish, diagnosis does not fit the situation; instead, detection of the agent becomes the goal or purpose. That approach requires isolation and an appropriate serological method of identification.

Isolation and identification of the virus. Surprisingly, fish cell cultures, have not been tested for their ability to replicate the opaleye isolant. Instead, the Vero line of African green monkey kidney cells was used in the investigations of Smith and co-authors (1980b). Clarified tissue homogenates were used as inocula and adsorbed on cell sheets, which were incubated at 37 °C. Cytopathic effects were seen after 3 passages. The fish isolants were presumptively identified by determining their biophysical properties, then definitively identified and typed using cross-neutralization tests. The results showed that fish isolants were serotypes of SMSV, but they were not neutralized by the VESV typing serums that were used.

Primary cultures of fish cells are very easily set up (Wolf and Quimby, 1976a, b), and

more than 60 fish cell lines have been developed and are available to investigators (Wolf and Mann, 1980). Accordingly, it would seem essential to the study of calicivirus SMSV in fish to determine the effect of the agent in fish cell cultures. Perhaps the virus-fish relationship is one of long standing and the virulence of the agent for mammals one of recent encounter and hence of virulence.

Transmission and incubation. Nothing is known of the biological dissemination and incubation in fish. Smith and co-authors (1980a) have shown a possible lung worm linkage between fish and marine mammal. The lung nematode uses fish as an intermediate host and mammals as final host. Based on increase in titer in opaleyes that were experimentally inoculated, one can postulate an approximate incubation time of 2 weeks for significant replication (Smith and co-authors, 1981).

Aspects of incubation time of SMSV in fur seal pups were discussed by Smith and co-authors (1980a). Bankowski (1981) reviewed infections in swine.

Host and geographic range. Thus far, the sole marine fish host is the opaleye. That species inhabits coastal Pacific waters from southern California into Mexico. Virological examination of other fish species associated with the opaleye would be interesting.

Control measures. Not applicable to free-living species, nor warranted where the effect on the host is apparently benign.

Pacific cod herpesvirus

Definition. Pacific cod herpesvirus is of herpesvirus-like size, shape, and apparent sequence of replication from nucleus to cytoplasm. The agent is visualized in cyst-like bodies (also called giant cells) within raised skin lesions of *Gadus macrocephalus* taken in the Bering Sea (McArn and co-authors, 1978; McCain and co-authors, 1979).

Signs and pathologic changes. Nothing is known of possible behavior changes because the affected specimens were taken by trawl. In one case, a lesion incidence of about 4 % was found among 2000 cod.

The virus is found in cyst-like bodies as McCain and co-authors (1979) describe them, or as giant cells, the term used by McArn and co-authors (1978). The cellular forms harboring virus are within raised lesions in the skin of fish. Fish may bear 1 to 5 such lesions that measure up to 50 mm in diameter. The lesions consist of a 5 to 20 mm wide cream-colored strip surrounding a round patch of normal appearing epidermis. Only the epidermis and the stratum spongiosum are affected.

Histologically, the cyst-like bodies are about 4 times larger than normal mucus cells; they possess a vacuolated basophilic center surrounded by an eosinophilic margin. Within the margin and internal basophilic core, small eosinophilic bodies of unknown identity can be found. Infiltrating inflammatory cells occur in the stratum spongiosum near some of the cyst-like forms.

Biophysical properties of the virus. McArn and co-authors (1978) describe 80 to 110 nm particles in various stages of development within the nucleus of the 'hypertrophic cells'. Herpes-like virus particles measuring 120 × 170 nm were found within cytoplasmic vesicles. The authors note that the virus containing enlarged cells somewhat resembled lymphocystis and epitheliocystis cells.

Diagnosis. Mere presence of raised skin lesions is not diagnostic, only suggestive. Lesions should be sectioned and their histology shown to conform to that described by

McCain and co-authors (1979). Demonstration of virions in the cyst-like structures is the definitive approach at present.

Isolation and identification of the virus. The agent has not been isolated, but it would appear simple to attempt isolation with at least primary cultures of cod skin epithelium. Identification requires electron microscopy.

Transmission and incubation. Nothing is known. If live specimens could be held, opportunity to observe horizontal transmission and incubation would be available. Also, experimental transmission with lesion homogenate as inocula would be possible.

Host and geographic range. Pacific cod is the only known host; affected specimens have been taken from the Bering Sea off Alaska.

Control measures. Not applicable.

Agents: Bacteria

D. A. CONROY

The remarkable achievements which have come about as a result of the constant development of productive aquaculture in brackish, estuarine and coastal marine waters, a biotechnology variously known as 'mariculture', 'ocean ranching' or simply 'farming the sea', have brought in their train an increasing realization of the potential importance of bacterial and other diseases of euryhaline and stenohaline marine teleosts. Scientists the world over, as much in developed countries as in developing nations, are now actively working on research projects designed to investigate numerous aspects of the bacterial diseases which adversely affect the species of fish selected for cultivation. In the same way, efforts are being made to identify novel techniques for the diagnosis, prevention and control of infectious diseases in an attempt to make available a practical methodology compatible with the practices and priorities of salt water fish farming. Sufficient experience is now available to indicate that 'disease' as an entity is a factor of singular importance which limits at the least, or annuls at the most, the productive potential and commercial profitability of such aquaculture enterprises (see also Volume I: Kinne, 1980a, b).

This section identifies a number of disease problems associated with bacteria in euryhaline and stenohaline marine fish species, and discusses aspects of particular importance in relation to aetiological, epizootological, clinical and pathological considerations.

The disease conditions considered are dealt with in the context of syndromes rather than as individual infections broken down artificially in accordance with the present taxonomic status of the corresponding aetiological agent. For this reason, it has been deemed preferable to include important diseases such as furunculosis, vibriosis and pasteurellosis under the general heading of bacterial haemorrhagic septicaemia; in the same way, tuberculosis and nocardiosis have been grouped together under the heading of acid-fast bacterial infections. The special consideration given here to the bacterial diseases of mugilids and salmonids is a reflection of the personal experience and professional interests of the author, in addition to which it is emphasized that the catadromous mugilids and the anadromous salmonids, as representatives of euryhaline species of particular relevance to present-day salt-water aquaculture are, in addition, of exceptional value as 'biological models' with which to demonstrate the impact of bacterial infections on teleosts maintained in conditions of variable salinities during the process of their capture, confinement, and/or production as food for human consumption.

Grey mullets (Mugilidae) are most important examples of euryhaline and eurytherm fish, the biology and behaviour of which — already widely capitalized upon for aquaculture purposes — enable certain basic concepts underlying the aetiology of bacterial diseases of fish in fresh water and in sea water to be more clearly understood. As species of circumglobal distribution in suitable tropical, sub-tropical and temperate waters, the grey mullets in general possess feeding habits which may be broadly defined as herbivorous with marked detritivorous preferences. Payne (1978) has investigated the relationship between the gastro-intestinal pH and the digestive strategy of the species *Liza dumerili, L.*

falcipinnis, Mugil cephalus and *M. curema* in estuarine environments of Sierra Leone, West Africa. This worker reported a gastric pH of 2 to 5 in *Liza falcipinnis,* in which species 65 % of the readings lay between pH 3.5 and 4.5, whereas in *L. dumerili* the value was pH 7.6 to 8.5 and that of *M. cephalus* and *M. curema* was pH 8.5. No differences were detected in the readings from the cardiac and pyloric portions of the stomach, and in these 4 species of mugilids the caecal and intestinal pH values were consistent at 8.5. In the particular case of the striped mullet *M. cephalus,* Moriarty (1976) demonstrated that on a basis of muramic acid determinations of the biomass in individuals feeding on sea-grass flats, approximately 15 to 30 % of the organic carbon present in the stomach was made up of bacteria. It seems likely, therefore, that the grey mullets do not utilize the secretion of acid or the production of enzymes to facilitate the breakdown and digestion of the bacteria which they consume. More likely, they make use of mechanical grinding by the walls of the muscular stomach, combined with the abrasive effects of sand grains, with protection of the gastric epithelium being afforded by a process of permanent secretion of a neutral mucopolysaccharide, as has been demonstrated in Brasilian grey mullets by Castro and co-authors (1961).

Hamid and co-authors (1978) investigated experimentally the effects of different environmental salinities on the intestinal microflora of the striped mullet *Mugil cephalus.* The fish were transferred from fresh water to sea water through a 1:1 mixture of fresh water and sea water, and the process was subsequently repeated in reverse order. Samples from the intestinal tract of mullet in fresh water yielded species of *Bacillus, Enterobacter* and *Micrococcus.* After the mullets had been acclimatized to sea water, the predominant bacterial flora of the intestine was represented by the genera *Aeromonas, Pseudomonas* and *Vibrio.* When the fish were transferred back into fresh water, the only genus of bacterium to be recovered was *Enterobacter,* a finding which led Hamid and co-authors to conclude that the remaining genera had been incapable of withstanding the transitional process through the various salinities used in the experimental schedule. Lesel (1982) postulated that the genus *Enterobacter* constitutes the only genuinely autochthonous taxon in the digestive tract of the striped mullet *Mugil cephalus* in sea water. Other bacterial groups were considered to form part of an allochthonous flora of a temporarily sedentary nature.

The variations in the bacterial flora of the skin surface of apparently healthy and visibly diseased striped mullet in natural environments in Queensland, Australia, have been investigated and documented by Burke and Rodgers (1981). These workers captured adult mullet by means of gill nets in the mouth of the River Noosa (salinity: 15 to 35 ‰) and in Lake Cootharaba (0 to 10 ‰) during the period August 1976 to October 1977. The bacterial isolates were identified to generic level; the distribution of the genera encountered from healthy skin is summarized in Tables 1-2 and 1-3. In samples from healthy skin, the bacterial counts ranged from 1.5×10^2 to 1.6×10^3 cm^{-2}, whereas in diseased mullet the counts were in the range of 4×10^5 to 2.5×10^6 cm^{-2}. A predominance of pseudomonads was detected in the skin flora of healthy mullets captured in the mouth of the River Noosa in April 1977, as was also the case with healthy mullets from Lake Cootharaba in June 1977. In August 1976, however, Burke and Rodgers observed that micrococci were the predominant bacteria in the skin flora of healthy mullets from Lake Cootharaba. Diseased mullets from the mouth of the Noosa River had a skin flora composed of 100 % *Vibrio* sp., whilst in similarly diseased fish from Lake Cootharaba the

Table 1-2
Microbial flora of the skin of apparently healthy striped mullet *Mugil cephalus* from the mouth of River Noosa, Queensland, Australia, during April 1977 (Based on Burke and Rodgers, 1981)

Identity of isolate	Incidence of occurrence (%)
Coryneforms	10
Flavobacterium	18
Micrococcus	14
Moraxella	4
Pseudomonas	37
Vibrio	14
Yeasts	3

Table 1-3
Microbial flora of the skin of apparently healthy striped mullet *Mugil cephalus* from Lake Cootharaba, Queensland, Australia, during August 1976 and June 1977 (Based on Burke and Rodgers, 1981)

Identity of isolate	Incidence of occurrence (%)	
	August 1976	June 1977
Acinetobacter	6	–
Aeromonas	–	4
Alteromonas	–	4
Coryneforms	–	–
Enterobacteria	–	4
Flavobacterium	–	5
Micrococcus	59	5
Moraxella	6	–
Pseudomonas	17	69
Staphylococcus	–	5
Streptococcus	6	–
Vibrio	–	–
Yeasts	6	4

predominant bacterial flora of the skin was composed of *Micrococcus* sp. (39 %) and *Vibrio* sp. (37 %) in June 1977, as opposed to *Aeromonas* sp. (63 %) and Enterobacteriaceae (23 %) in August 1976.

A relation between the feeding habits of fish in brackish water and the incidence of *Vibrio parahaemolyticus* has been established by Natarajan and co-authors (1979). These workers isolated *Vibrio parahaemolyticus* from more than 1,000 specimens of fish from Porto Novo, southern India, and found that the incidence of this bacterium was higher in the gills and intestinal contents than on the skin. The occurrence of *Vibrio parahaemolyticus* was reported as being far higher in detritivorous fish than in the carnivorous, herbivorous and planktonivorous species which were examined.

Again with reference to euryhaline and catadromous species of fish, Kanai and co-authors (1977) investigated the composition of the intestinal bacterial flora of apparently healthy and visibly diseased Japanese eels *Anguilla japonica* cultured in fish ponds in

Shizuoka Prefecture. In the apparently healthy eels, the intestinal flora contained higher levels of *Aeromonas hydrophila* and other aeromonads in winter, whilst in spring and autumn the predominant flora was composed of Enterobacteriaceae. *Vibrio* sp. invariably occurred during October, and *Streptococcus* sp. was present at levels varying between 20 and 80 % in the intestinal contents of eels which were feeding. Diseased eels, in contrast, gave appreciably higher bacterial counts, and the relative incidence of *A. hydrophila* in these fish was particularly marked.

Yoshimizu and co-authors (1976a, b) have reported on the bacterial flora of the intestine of salmonids in Japan. In their studies on the flora of fresh-water salmonids, they used 55 specimens of masou salmon *Oncorhynchus masou*, 3 sockeye salmon *Oncorhynchus nerka* and 6 white-spotted char *Salvelinus leucomaenis* collected in 2 rivers and 2 lakes in the south of Hokkaido. The viable bacterial counts of the water samples were less than 1×10^3 ml^{-1}; the flora included representatives of the genera *Achromobacter, Aeromonas, Cytophaga/Flavobacterium, Pseudomonas* and members of the family Enterobacteriaceae. The viable counts of the salmonid intestinal contents ranged from 10^2 to 10^8 g^{-1}, with higher counts in spring and summer than in winter. This intestinal flora was less variable than that of the ambient water, with *Aeromonas* sp. and Enterobacteriaceae predominating. Further studies with mature anadromous salmon were carried out with 10 pink salmon *Oncorhynchus gorbuscha* and 20 chum salmon *Oncorhynchus keta* caught at the mouth of a river, and 10 cultured masu salmon. Viable counts in the intestinal slime of chum and pink salmon ranged from 10^2 to 10^8 g^{-1}, those of cultured masu salmon from 0 to 10^5 g^{-1}. The genera *Aeromonas, Pseudomonas* and *Vibrio* predominated in the intestinal slime of wild-caught chum and pink salmon; all of the vibrios were of a marine-halophilic type, the pseudomonads were of a halophilic-terrestrial type, and most of the aeromonads were of a terrestrial type. *Aeromonas* sp. proved to be the predominant type in the intestinal flora of masu salmon cultured in fresh water.

The relation between the bacterial flora of the environment and that of farmed marine fish has been investigated in the United Kingdom. Gilmour and co-authors (1976a, b) have studied the composition of the bacterial flora of the skin and intestinal tract of cultured plaice *Pleuronectes platessa* and of the power station effluent water used to cultivate these fish. Plaice were divided into 4 groups of 10 individuals each, and minced fish with a different binding agent was provided as food for each experimental group. The skin samples yielded pseudomonads of Groups 1, 2 and 4, *Vibrio* sp., Enterobacteriaceae and *Moraxella* sp. The intestinal flora, in addition to the aforementioned varieties, also contained *Acinetobacter* sp., *Aeromonas* sp., micrococci, coryneforms and unidentified oxidase-negative motile Gram-negative rods which failed to ferment glucose. The binding agents used had no effects on the composition of the skin or intestinal microflora. The power station effluent yielded a microflora composed primarily of arthrobacters, *Alcaligenes,* agrobacteria and pseudomonads, a finding which led Gilmour and co-authors (1976b) to conclude that eubacteria tend to predominate in coastal marine environments.

Austin (1982) published a significant report on the bacterial flora of a coastal marine fish-farming facility in England, a study which includes information on the taxonomic status and seasonal variations of the bacterial types in the water as well as on the surface and in the internal organs of apparently healthy and diseased turbot *Scophthalmus maximus*. In almost 600 isolates of heterotrophic aerobes, Austin detected 8 major and 43 minor phena. Five of the major phena were equated with *Acinetobacter calcoaceticus,*

Photobacterium phosphoreum and 3 groups of *Vibrio* spp., while of the remaining unidentified phena 1 was classified as Gram-variable rods and the other 2 were considered to occupy an intermediate position between the *Cytophaga/Flexibacter* and *Flavobacterium* groups. The surface bacterial flora of apparently healthy turbot included *Alcaligenes faecalis, Bacillus firmus, Photobacterium angustum, 'Photobacterium logei'* and *Pseudomonas fluorescens.* Pure cultures of *Alteromonas haloplanktis* and an unidentified budding bacterium were isolated from the internal organs and muscle tissue of turbot showing signs of a pathological condition. Austin found that budding bacteria and vibrios were present throughout the sampling period (January–November 1979), whereas coryneforms, Enterobacteriaceae and pseudomonads predominated in summer and *Alteromonas, Lucibacterium* and *Photobacterium* spp. predominated in winter. Organisms which could be classified as *Vibrio anguillarum* were conspicuous by their complete absence from the environmental samples studied by Austin, even from turbot showing pathological signs considered to be characteristic of clinical cases of vibriosis.

Shin and co-authors (1976) carried out a series of studies on the bacterial flora of Japanese coastal waters and its relation to the seasonal occurrence of *Vibrio parahaemolyticus* in 1974 and 1975. During summer, counts of 10 to 10^4 cells 100 ml^{-1} were obtained for *V. parahaemolyticus,* whereas this species was not detected during winter. In viable plate counts, performed at 25 °C for 7 days, species of *Pseudomonas* and *Vibrio* predominated irrespective of the season of the year, although most of the isolates made in summer could grow at 35 °C while those isolated in winter could not. Plate counts carried out at 42 °C showed a close correlation between total count and incidence of *V. parahaemolyticus.* Pseudomonads and vibrios formed a high percentage of the isolates during summer, whereas in winter the predominant flora was composed of Gram-positive rods. Further studies revealed that most of the vibrios isolated at 42 °C were *V. alginolyticus,* and that higher numbers of this species accompanied *V. parahaemolyticus* in the coastal water samples investigated during summer.

Wakabayashi and co-authors (1976) examined water samples from eel ponds in Yoshida, Shizuoka Prefecture, Japan, for the presence of bacterial fish pathogens, with special reference to *Aeromonas* spp. and *Edwardsiella tarda.* Throughout 1 yr, the bacterial counts ranged from 10^2 to 10^5 ml^{-1}, with a minimum in January and a maximum in September. Aeromonads and enterobacteria were the predominant bacterial types in all samples analyzed. Of 232 strains of aeromonads identified, 224 were *Aeromonas hydrophila* and 8 were *A. punctata.* The enterobacteria isolated included 78 strains of *Citrobacter,* 46 of *Enterobacter,* 9 of *Edwardsiella* and 4 of *Escherichia.* Although several eels *Anguilla japonica* were found to harbour *Edwardsiella tarda,* this bacterium was comparatively rare in the pond water samples tested. Wakabayashi and Egusa (1979) effected bacteriological studies on the internal organs of diseased Japanese eels from ponds in the Yoshida region. They observed that 96 eels harboured potential pathogens such as *A. hydrophila, E. tarda* and *Pseudomonas anguilliseptica,* the incidence of which was 77 % in the heart blood, 84 % in the spleen, 89 % in the liver and 94 % in the kidney. They concluded that the kidney is one of the most important organs which should be examined in order to detect and isolate such bacterial pathogens from diseased eels.

It is necessary to bear in mind, from the aetiopathological point of view, that the interaction of one or more predisposing factors is frequently of importance in the appearance of outbreaks of bacterial diseases in salt-water fish, as much in the natural

environment as in conditions of confinement and artificial rearing. Bacteria present in the water, on the surface, or in the organs of a fish are frequently capable of invading the host when it is subjected to injury or to biological, chemical and/or physiological stress factors which serve to reduce its resistance to infection. The effect of ectoparasites, for example, bears an important relation to the occurrence of bacterial infections in marine and estuarine fish. Muroga (1979) studied an outbreak of an ulcerative disease affecting grey mullet *Liza akame* in the estuary of the Ashida River near Fukuyama City, Japan, during 1977. Thirty-five of the 36 mullet specimens examined were parasitized by the copepod *Caligus orientalis,* the presence of which was believed to have inflicted superficial wounds on the skin which were subsequently invaded by bacteria. The microorganisms isolated from these skin lesions included *Citrobacter* sp., *Edwardsiella tarda, Klebsiella* sp., *Moraxella* sp., *Proteus vulgaris, Pseudomonas* sp. and *Vibrio* sp., genera which are often implicated in disease outbreaks among fish in fresh, brackish and coastal waters. For example, Kusuda and co-authors (1976) have described an epizootic due to *E. tarda* in *Mugil cephalus* from Okitsu Bay, in the Sea of Japan; another epizootic due to *E. tarda* has been reported by Kusuda and co-authors (1977) from crimson sea bream *Evynnis japonicus* cultured in Japan. Sato and co-authors (1982) have described a systemic bacterial infection associated with the presence of *Citrobacter freundii* which affected sunfish *Mola mola* maintained in a marine aquarium in Japan.

The aquatic pathobiologist who becomes involved in studies on the bacterial diseases of salt-water fish can expect to encounter a challenging situation which offers a number of intellectual attractions and a tremendous degree of job satisfaction to those possessed with the stamina to follow through and break new ground in a relatively new scientific discipline closely associated, as a key support activity, with the development of marine aquaculture as a source of food for mankind. The requirements of this industry include the application of novel or more precise techniques in the detection and diagnosis of disease problems, as well as in the utilization of a suitable methodology to ensure the effective prevention and control of such problems under operating conditions in the field.

One difficulty which frequently arises in practice is in relation to the correct taxonomic status of the bacteria which may be isolated from diseased marine or euryhaline fish. Many such isolates are motile Gram-negative rods whose precise characterization and classification at both generic and specific levels is a subject often surrounded by controversy or, at the very least, is a subject in relation to which differences of opinion and criteria exist. In general terms, these bacteria are identified as species of *Acinetobacter, Aeromonas, Beneckea, Pseudomonas* and *Vibrio.* The taxonomy of bacteria of marine origin is now becoming a subject of increasing interest to many specialists and, for this same reason, the 'traditional' nomenclature used to describe some of the better known bacterial pathogens of salt-water fish is susceptible to modification in the light of more sophisticated taxonomic schemes. For example, attention is drawn to the work of Baumann and co-authors (1971) who recommended that many Gram-negative, facultatively anaerobic and motile rods of marine origin capable of fermenting glucose anaerogenically should be assigned to a redefined genus *Beneckea.* The adoption of this recommendation would have led to the suppression of many workaday names now so 'familiar' to the fish pathologist as *Vibrio alginolyticus, V. anguillarum* and *V. parahaemolyticus,* and their replacement with terms such as *Beneckea alginolyticus* (Miyamoto and co-authors) comb. nov. and *Beneckea parahaemolytica* (Fujino and co-authors) comb.

nov. Baumann and co-authors (1980) have, however, proposed that the genus *Beneckea* be abolished altogether.

The Shakespearean adage 'a rose by any other name' must have an obvious appeal to all hard-working aquatic pathobiologists called upon to investigate and provide immediate practical solutions to problems associated with the causes and effects of bacterial diseases in species of teleosts identified as candidates or potential candidates for aquaculture operations in brackish and coastal marine waters.

Bacterial Haemorrhagic Septicaemia

One of the most widespread and possibly economically important diseased conditions in marine and euryhaline teleosts is bacterial haemorrhagic septicaemia, a syndrome in which various types of bacteria intervene as aetiological agents. The term 'bacterial haemorrhagic septicaemia' is used here to include diseases also known as vibriosis, furunculosis, pasteurellosis, and infections caused by aeromonads and pseudomonads, the clinical signs of which are strikingly similar but which are differentiated by the characterization of the aetiological agent involved in the corresponding outbreak.

Furunculosis *per se* is a specific form of bacterial haemorrhagic septicaemia caused by *Aeromonas salmonicida; sensu stricto* it is a disease which affects salmonids in fresh water. With the development of salmon and trout culture in sea water, however, increasing evidence is being accumulated to suggest that furunculosis is likely to become an important potential disease in salt water. Furunculosis is sufficiently well known as to require little further comment here; the reader is referred to Bullock and co-authors (1971) for a detailed description. Mention of furunculosis is confined here to reports on aspects of particular importance in relation to salt-water salmonid culture.

Nomura and Kimura (1981) have investigated the incidence of *Aeromonas salmonicida* in kidneys of apparently healthy anadromous salmonids captured in rivers in Hokkaido, Japan. The bacterium was isolated from 148 of 1,280 chum salmon *Oncorhynchus keta* taken from 12 rivers where the incidence in each river varied from 0 to 60.5 %, and from 66 of 592 pink salmon *O. gorbuscha* taken from 9 rivers where the highest incidence was 36.6 %. *A. salmonicida* was also isolated from kidneys of 116 specimens of masu salmon *O. masou* and 60 specimens of sockeye salmon *O. nerka*.

Novotny (1978) reported that epizootics of furunculosis caused by *Aeromonas salmonicida* constitute a very real hazard in salt-water salmonid culture in the State of Washington, USA. In this situation it appears that furunculosis can occur together with vibriosis as a dual or mixed infection, one outbreak of which caused mortalities of approximately 80 % in the course of a 5 month period among 300,000 chinook salmon *Oncorhynchus tshawytscha* maintained in 2 sea-water cages. The strain of *A. salmonicida* involved proved to be resistant to oxytetracycline and sulphonamides, although it was sensitive to furazolidone. Mixed infections were extremely difficult to treat in practice. Novotny (1978) was of the opinion that *A. salmonicida* is introduced together with fish from fresh water, but that the disease reached epizootic proportions in the conditions of confinement typical of cage culture. This worker placed particular emphasis on the need to effectively control cases of furunculosis during the phases of salmonid culture which are carried out in fresh water, thereby avoiding the unnecessary introduction of the pathogen into the sea water culture facilities.

Scott (1968) successfully demonstrated that *Aeromonas salmonicida* is pathogenic to

salmonids in marine and brackish waters. Sea trout and brown trout *Salmo trutta* maintained in salinities of 25.4 to 33.1 ‰ at 5.6 to 14.5 °C were infected. Scott made the interesting observation that the infected trout, when allowed to remain in fresh water, retained the typical clinical signs of furunculosis as evidenced by the presence of boil-like lesions of a ragged appearance and deep-red colour, whereas in fish maintained in sea water these lesions 'became clear-cut and their contents were washed away, leaving undamaged muscle tissue'. Scott concluded from this work that the transmission of furunculosis by direct infection from a diseased fish to a healthy one is a distinct possibility in brackish and coastal waters, in which the disease produces a generalized type of infection without the characteristic cutaneous lesions so typical of the disease. One very important conclusion of Scott is that anadromous salmonids might not only play an important role in the transmission of furunculosis, but also serve as a permanent reservoir of the pathogen as a result of the occurrence of latent or sub-clinical infections in feral stocks.

Smith and co-authors (1982) provided important information on the transmission of *Aeromonas salmonicida*. Routine examinations at 2 sea-water fish farms on the West Coast of Ireland, revealed the bacterium at a latent or sub-clinical stage in up to 100 % of *Salmo salar* smolts produced at one farm. Following the introduction of these smolts into sea-water cages, mortalities of 40 to 50 % occurred over a 2 to 12 day period, after which the infection subsided. By the end of the summer, however, the total cumulative mortality approached 70 %. When infected hatchery-reared smolts were introduced, mortalities were experienced some 20 days afterwards among smolts which had been produced at another hatchery. Within 43 days the mortality in the stock stood at 3 to 4 % *per diem,* to reach a total of 70 % by the end of the summer. These findings led Smith and co-authors to postulate that lateral transmission of furunculosis had occurred, a supposition which was further supported by data subsequently obtained from another fish farm. In this latter case, fish with a 100 % latent infection on introduction into sea water experienced mortalities of 40 % within 20 days after entering sea water. The strain of *A. salmonicida* isolated from these outbreaks had a 40 MD drug resistance plasmid coding with respect to spectinomycin, streptomycin, sulphonamide, tetracycline and trimethoprim, a characteristic which facilitated recognition of the strain as to its origin in one of the hatcheries from which the smolts had been received. Smith and co-authors postulated that this strain was capable of survival in the sea-water farm for at least 6 months in the absence of latent carrier smolts from the hatchery. They were unable to determine, however, whether introduction of fish from an uninfected hatchery had contributed to the persistance of the infection as carriers, or whether the bacterium had been introduced from wild fish in waters adjacent to the farm. An additional postulate was that the organism may have been derived from bottom sediments under the cages themselves. Smith and his colleagues draw attention to the importance of the propagation of furunculosis by one or more of these means in Atlantic salmon culture operations in sea water. Lubieniecki and Zawadzi (1981) have reported that furunculosis proved to be a contributory factor in mortalities of rainbow trout maintained in cages in brackish waters of Puck Bay, Baltic Sea.

One of the best known manifestations of the bacterial haemorrhagic septicaemia syndrome in euryhaline and stenohaline teleosts is a vibrio-associated disease variously known as 'Red Pest', 'Red Disease', *pestis rubra anguillarum, erysipelosis anguillarum,* 'Cod Pest', ulcer disease, eye disease, 'Boil Disease', bacterial dermatitis and salt water

furunculosis — *inter alia* popular terms which rightly correspond to the all-embracing designation of vibriosis (Feddersen, 1897a, b; Inghilleri, 1903; Bergman, 1909, 1912; Aaser, 1925; Schäperclaus, 1927; Bruun and Heiberg, 1932, 1935; Wells and ZoBell, 1934; ZoBell and Wells, 1934; Franco, 1938; Bagge and Bagge, 1956; Rucker, 1963; Kusuda, 1966; Akazawa, 1968; Wood, 1973).

Red pest ('*peste rossa*') of European eels was of considerable economic importance in Italy during the 18th and 19th centuries, where it produced catastrophic mortalities in sea-water ponds and coastal lagoons during 1825, 1850, 1864, 1884, 1885, 1889 and 1892 (Hofer, 1904; Drouin de Bouville, 1907). What appears to be the first recorded description of a bacterial fish disease is the report of an outbreak of red pest affecting eels in Italy in 1718 given by Bonaveri in 1761 (cited by Drouin de Bouville, 1907). Other early European records include reports of outbreaks of red pest in eels from Scandinavian waters from 1880 onwards, records which have been documented by Bruun and Heiberg (1932).

The first successful isolation of the aetiological agent of red pest of eels was effected by Canestrini (1893) in Italy; he described the organism as *Bacterium anguillarum.* Outbreaks of red pest in Swedish coastal waters in 1907 were investigated by Bergman (1909), to whom the species designation *Vibrio anguillarum* is due in regard to the organism which he isolated from the diseased eels. Much of the now classical work on vibriosis in fish has been carried out by European workers (Inghilleri, 1903; Bergman, 1912; Schäperclaus, 1927, 1934; Bruun and Heiberg, 1932, 1935; Nybelin, 1935; Franco, 1938).

The earliest records of what is currently recognized as vibriosis in the American continent are those published by Wells and ZoBell (1934) and ZoBell and Wells (1934). These workers described a disease characterized by signs of infectious dermatitis in natural populations of top smelts *Atherinops affinis,* killifish *Fundulus parvipinnis,* gobies *Gillichthys mirabilis* and blennies *Hypsoblennius gilberti* from coastal waters in the State of California, USA. When infected killifish were captured and transferred to aquaria during summer, mortalities of up to 90 % frequently occurred. The aetiological agent of the disease was isolated and described as *Achromobacter ichthyodermis* by Wells and ZoBell (1934). A similar organism was isolated from diseased plaice *Pleuronectes platessa* in Northeast Scotland by Hodgkiss and Shewan (1950), who classified it as *Pseudomonas ichthyodermis.* Shewan and co-authors (1960) have subsequently affirmed that this isolate should now be recognized as a species of *Vibrio.* David (1927) refers to the isolation of an organism described as *V. piscium* from a disease outbreak in fresh water fish, but it is doubtful whether this is a vibrio *sensu stricto* since it fails to attack carbohydrates.

Nybelin (1935) investigated the properties of strains of *Vibrio anguillarum* available at that time, and proposed 3 biotypes: (1) *Vibrio anguillarum forma typica* (Type A); (2) *V. anguillarum forma anguillicida* (Type B); (3) *V. anguillarum forma ophthalmica* (Type C).

Larsen and Jensen (1979) have described 2 additional biotypes of *Vibrio anguillarum,* designated Types D and E, on a basis of strains isolated from skin lesions and faeces of cod *Gadus morhua* and from invertebrates and particulate matter in Danish coastal waters. The criteria used to differentiate between the biotypes of *V. anguillarum* include the production of indole and the fermentation of mannitol and saccharose (Table 1-4).

Many workers have described isolates of *Vibrio anguillarum* from diseased fish as

Table 1-4
Criteria used for the characterization of *Vibrio anguillarum* biotypes (Based on Nybelin, 1935; Anderson and Conroy, 1970; Larsen and Jensen, 1979)

Biotype	Biochemical reactions		
	Acid from mannitol	Acid from saccharose	Production of indole
A	+	+	+
B	−	−	−
C	+	+	−
D	−	+	+
E	−	−	+

+ Positive reaction; − negative reaction

Types A, B or C. Strains from an epizootic detected in chinook salmon *Oncorhynchus tshawytscha* in USA have been reported as *V. anguillarum* Type A by Cisar and Fryer (1969), whereas strains from trout and other salmonids in Europe and USA have been classified as *V. anguillarum* Type C by Smith (1961) and Ross and co-authors (1968). According to Smith, the strain described as *V. piscium* var. *japonica*, and isolated from rainbow trout in Japan by Hoshina (1956, 1957), would also correspond to Type C. Strains of *V. anguillarum* isolated from cod and eels have been variously designated Types A and B (Bagge and Bagge, 1956; Lagarde and Chakroun, 1965; Muroga and Egusa, 1967; Larsen and Jensen, 1979). Larsen and Jensen (1979) reported the isolation of Types A and C from ulcers, mucus and faeces of diseased cod, in addition to Types B and E from faeces, and Type D from ulcers in these fish. The organism termed *V. ichthyodermis* would, on a basis of its reported biochemical reactions, be designated *V. anguillarum* Type A in the case of Hodgkiss and Shewan's (1950) strain, or *V. anguillarum* Type C in the case of Wells and ZoBell's (1934) strain. Anderson and Conroy (1970) isolated more than 40 strains of vibrios from different species of diseased marine fish and found that on a basis of the biochemical reactions of these strains, they would fall into the category of biotypes of *V. anguillarum* in some instances and *V. ichthyodermis* in others.

Much effort has been given, perhaps unnecessarily, to assigning biotypes to the strains of *Vibrio anguillarum* isolated from diseased fish purely on a basis of biochemical characteristics of the isolates. In this respect, one of the most significant contributions is the paper by Evelyn (1971), in which many confusing aspects of the taxonomy of *V. anguillarum* are carefully analyzed and a tentative archtype of the species is proposed. Evelyn's comprehensive studies establish a reference standard for *V. anguillarum,* a species which is not included in Bergey's Manual of Determinative Bacteriology (Breed and co-authors, 1957) but which is included as *V. anguillarum* Bergman, 1909 in the Index Bergeyana (Buchanan and co-authors, 1966).

With reference to the putative existence of biotypes, Evelyn (1971) recommended that this system of classification be discontinued and that henceforth such types should be considered as variants of *Vibrio anguillarum sensu stricto.* Recognizing that the system of biotype classification stressed the existence of variations among strains of one and the same species, and forestalled the artificial creation of other species on a basis of a limited

number of biochemical differences, Evelyn emphasized that the new archtype of *V. anguillarum* would include strains isolated from disease processes in fish and other marine organisms variously classified as *V. alginolyticus, V. anguillarum, V. ichthyodermis* and *V. parahaemolyticus*. This proposal would also enable *V. anguillarum* to be distinguished from other bacteria reported as vibrios without sufficient concrete criteria upon which to base this designation. The archtype of *V. anguillarum* proposed by Evelyn (1971) is defined as:

> "a facultatively anaerobic, non-sporing (asporogenous), actively motile, polar monotrichous flagellate, Gram-negative rod; capable of growth on conventional media with salt levels as low as about 0.07 % NaCl but growing optimally with 1–3 % NaCl; growing well, or best, at 20–30 °C, and poorly, or not at all, at 37 °C; non-luminescent; unable to digest agar; may sometimes cause fish disease."

The biochemical properties of the proposed archtype are given in Table 1-5.

Isolates of vibrios from diseased fish have frequently been classified as *Vibrio alginolyticus, V. anguillarum* and *V. parahaemolyticus,* and the salient characters used to differentiate between these 3 species and *Aeromonas* spp. of marine origin have been given by Larsen and Jensen (1979); they are summarized in Table 1-6. Evelyn (1971) recognized the need to differentiate between *V. anguillarum* (the proposed archtype of which would now include the organisms previously known as *V. alginolyticus* and *V. parahaemolyticus*) and *Vibrio*-like anaerogenic aeromonads and plesiomonads. The principal biochemical characters which could be used for such differentiation are summarized in Table 1-7. Evelyn's recommendations permit the adoption of an operational strategy whereby the diagnosis and identification of isolates of *V. anguillarum* comb. nov., anaerogenic aeromonads and plesiomonads can be effected in a routine manner.

It is generally recognized that *Vibrio anguillarum* does not ferment lactose, even though a small number of lactose-fermenting strains have been reported (see Evelyn, 1971). Tison and co-authors (1982) compared strains of vibrios isolated from diseased Japanese eels to clinical and non-clinical isolates of the lactose-positive *V. vulnificus*. The eel isolates were phenotypically different from *V. vulnificus* in their failure to produce indole, to ferment mannitol and sorbitol, to decarboxylate ornithine and to grow at 42 °C. They were, however, pathogenic to eels, a characteristic not shown by the *V. vulnificus* strains studied. In the light of Evelyn's proposal, these strains could conveniently be accomodated within the species *V. anguillarum*. Variability among strains of *V. anguillarum* will obviously remain a constant feature as new isolates are investigated. Håstein and Smith (1977) studied a total of 163 strains of *V. anguillarum* isolated from diseased fish in Norway. These strains could be divided into 2 main groups in relation to their ability to ferment arabinose. Strains originally isolated from salmonids cultured in sea water generally fell into Group I, while most strains isolated from saithe *Gadus virens* and other wild marine fish were classified in the arabinose-negative Group II.

The salinity tolerance of vibrios associated with epizootics among wild and cultured fish is a matter of practical concern to the fish pathologist, and various workers have commented on the sodium chloride requirements for growth shown by *Vibrio anguillarum* (Bergman, 1909; Nybelin, 1935; Wolter, 1960; Lagarde and Chakroun, 1965; Muroga and Egusa, 1967; Muroga and Motonobu, 1967; Anderson and Conroy, 1970; Yasamuga and Yamamoto, 1977). Nishibuchi and Muroga (1977) studied an organism which they

Table 1-5
Biochemical properties of the emerging (proposed) archtype of *Vibrio anguillarum* (Based on Evelyn, 1971)

Test	Reaction
Anaerogenic fermentation of glucose	+
Acid from arabinose	(+)
Acid from xylose	−
Acid from adonitol	−
Acid from glucose	+
Acid from fructose	+
Acid from galactose	+
Acid from mannose	+
Acid from rhamnose	−
Acid from dulcitol	−
Acid from mannitol	+
Acid from sorbitol	(+)
Acid from cellobiose	(+)
Acid from lactose	(−)
Acid from maltose	+
Acid from sucrose	+
Acid from trehalose	+
Acid from inositol	−
Acid from raffinose	−
Acid from dextrin	+
Acid from inulin	−
Acid from salicin	−
Acid from glycogen	(+)
Acid from glycerol	(+)
Kovács oxidase test	+
Sensitivity to 0/129	+
Sensitivity to penicillin	−
Catalase	+
Reduction of nitrate	+
Hydrolysis of gelatine	+
Arginine dehyroxylase	+
Lysine decarboxylase	−
Ornithine decarboxylase	−
Phenylalanine deaminase	−
Urease	−
Production of indole	(+)
Production of acetoin	(+)
MR	(−)
Production of hydrogen sulphide	−
Cellulose attacked	−

(+) 20 % or more strains give positive reaction
(−) 20 % or more strains give negative reaction

tentatively designated *V. anguillicida,* and which had been isolated from a disease outbreak in Japanese eels. The organism was found to have a tolerance for 0.1 to 6 % sodium chloride; the percentage of cells with multiple flagellation was directly proportional to the prevailing salt concentration (although the cells were consistently monotrichous when grown in nutrient broth). Nishibuchi and Muroga believed their isolate to be very similar to *V. fischeri.* Strains of *Vibrio* sp. isolated from diseased rainbow trout in Japan

Table 1-6
Salient differential characteristics of *Aeromonas* sp., *Vibrio alginolyticus, Vibrio anguillarum* and *Vibrio parahaemolyticus* (Based on Larsen and Jensen, 1979)

Test	*Aeromonas* sp.	*Vibrio alginolyticus*	*Vibrio anguillarum*	*Vibrio parahaemolyticus*
Gram	–	–	–	–
Motility	+	+	+	+
Morphology	rod	rod	rod	rod
Catalase	+	+	+	+
Oxidase	+	+	+	+
Anaerogenic fermentation of glucose	+	+	+	+
Pigmentation	–	–	(+)	–
Haemolysis	–	–	+	v
Swarming	–	+	–	–
Arginine attacked	+	–	+	–
Lysine decarboxylated	–	+	–	+
Ornithine decarboxylated	–	+	–	+
0/129 sensitivity	–	+	+	+
Growth in TCBS*	–	+	+	+
Growth in 6 % NaCl	–	+	–	+
Growth in 8 % NaCl	–	+	–	+
Growth in 10 % NaCl	–	+	–	–
V-P reaction	–	+	v	–
Inositol fermentation	–	–	–	–
Acid from arabinose	–	–	v	v
Starch hydrolysis	–	+	+	+
Gelatine liquefaction	+	+	+	+

* Thiosulphate citrate bile salts sucrose agar
+ Positive; – negative; (+) most strains positive; v variable

Table 1-7
Principal biochemical characteristics which could be used to differentiate between *Vibrio anguillarum, Aeromonas formicans* and other vibrio-like anaerogenic aeromonads and *Plesiomonas shigelloides* (Based on Evelyn, 1971)

Test	*Vibrio anguillarum*	*Aeromonas formicans* and other anaerogenic aeromonads	*Plesiomonas shigelloides*
Acetoin production	+	–	–
0/129 sensitivity	+	–	–
Inositol fermentation	+	–	+
Gelatine liquefaction	+	+	–
Lysine decarboxylase	–	–	+

+ Positive reaction; – negative reaction

survived more than 4 wk in sea water; they died within a matter of hours when maintained in fresh water (Ohnishi and Muroga, 1977).

Evelyn (1971) demonstrated conclusively that strains of *Vibrio anguillarum* grow at sodium chloride concentrations of 0.1 to 8 %, and that they die within 2 h when suspended in distilled water alone. This worker also made the interesting observation that when organic matter, in the form of 0.1 % peptone, was added to filtered and autoclaved sea water, the survival of the bacteria could be extended indefinitely, whereas in the presence of sea water alone survival was limited to 2 to 3 weeks. This might explain how a disease recognized as a marine phenomenon is capable of causing epizootics in fresh water environments, perhaps through the introduction of vibrios from a salt water habitat (e. g. the use of marine fish as food) in conditions which limit direct contact between bacteria and fresh water (Ross and co-authors, 1968; Evelyn, 1971; Muroga, 1975).

Several workers endeavoured to utilize the antigenic properties of vibrios pathogenic to fish in attempts to identify isolates from different sources (Bruun and Heiberg, 1935; Nybelin, 1935; Rucker, 1959). However, strong cross-reactions between strains isolated from different outbreaks and geographical areas are not always present. Pacha and Kiehn (1969) studied a number of fish pathogenic vibrios, and were able to differentiate them into 3 serotypes in accordance with the geographical area from which they had been isolated. Novotny (1978) detected 2 distinct serotypes of *Vibrio anguillarum* as aetiological agents of severe mortalities experienced in the culture of chinook salmon *Oncorhynchus tshawytscha* in floating cages in Puget Sound, Washington State, USA. Strout and co-authors (1978) identified 3 distinct antigenic groups of *V. anguillarum* in coastal waters of Maine and New Hampshire, USA, and found that 2 of these groups were antigenically similar to 2 strains isolated from the West Coast but that the third group bore no obvious antigenic relation to either East Coast of West Coast serotypes. Rucker (1959) reported a similar phenomenon in relation to a serotype of *V. anguillarum* isolated from Pacific herring *Clupea pallasi,* which showed virtually no antigenic relation to strains of *V. anguillarum* from Pacific salmon in the same geographical area. None of the isolates of *V. anguillarum* from gilt-head sea bream at Elat, Israel, gave a clear-cut agglutination reaction with a *Vibrio anguillarum* antiserum prepared in Great Britain (Colorni and co-authors, 1981).

Variations in the pathogenicity of isolates of *Vibrio anguillarum* from different species of fish have been commented upon by several workers. Egidius and Andersen (1978) found that strains of *V. anguillarum* isolated from diseased rainbow trout in Norway, whilst highly pathogenic for salmonids, failed to give rise to the disease in saithe *Gadus virens,* whereas strains from saithe were pathogenic to that species but not to the cultured salmonids. Paperna and co-authors (1977) administered intra-peritoneal injections of *V. anguillarum* cells suspended in saline to 40 healthy gilt-head sea bream *Sparus aurata* with a mean weight of 80 g. The fish were injected with strains of the organism, but failed to develop signs of the disease over a 4-week period of observation. The bacterium was never recovered from the peripheral blood of these experimentally infected fish, and it was concluded that the organisms constitute opportunistic invaders requiring the presence of some degree of physiological stress in the host which encourages them to act as pathogens. One possible explanation which Paperna and co-authors offered was that the bacteria need to maintain their virulence through continued fish-to-fish passage. Muroga (1975) found that different serotypes of *V. anguillarum* isolated from fish diseases in Japan, when

injected via the intra-muscular route into Japanese eels *Anguilla japonica,* were capable of producing a generalized septicaemia and 90 % mortalities within 1 week when a dose of 8×10^8 cells $(100\ g)^{-1}$ was used. In eels injected with 8×10^7 cells $(100\ g)^{-1}$, however, the bacteria had been eliminated from the tissues within the course of 72 h, and no clinical signs were present. Strout and co-authors (1978) observed that a single group of *V. anguillarum* isolated from wild winter flounders *Pseudopleuronectes americanus* was highly pathogenic to coho salmon *(Oncorhynchus kisutch)* smolts reared in fresh water. The uncertain nature of the pathogenicity of *V. anguillarum* has led Evelyn (1971) to recommend, very wisely, that this characteristic be considered of minor importance as a criterion upon which to base a concrete description of the organism.

Evidence has accumulated that, under certain circumstances, the use of contaminated food may serve as a potential source of infection in aquaculture operations. This hypothesis was first advanced by Ross and co-authors (1968) to explain how an outbreak of vibriosis came to occur in a fresh water rainbow trout farm, and infected marine trash fish were suspected of having been the vehicle of infection on being fed to the trout. Colorni and co-authors (1977) detected a strain of *Vibrio anguillarum* pathogenic to sea bream in the fish meal used to prepare the pelleted food on which the bream were fed, and a similar situation has been detected in Venezuela where strains of *V. anguillarum* identical to those causing epizootic disease in pompanos (*Trachinotus* spp.) in floating cages have also been isolated from frozen engraulids used to feed the pompanos (Cairoli and Conroy, unpubl.).

The histological manifestations of vibriosis in Japanese eels *Anguilla japonica* have been studied and documented by Miyazaki and co-authors (1977) with reference to an outbreak in Tokushima Prefecture. Clinically, the disease was characterized in the initial stages of infection by the presence of small haemorrhagic areas on the body surface or caudal fins, and gave rise to extensive haemorrhagic lesions and necrosis in advanced stages of the infection. The liver showed signs of congestion, the spleen was swollen and deep red in colour, the kidney was necrotized, the visceral blood vessels were dilated, and the intestine was reddish in colour with desquamation of the epithelium.

In the early lesion, the bacteria penetrated the dermis, sub-cutaneous adipose tissue and red muscle tissue, and in advanced cases the bacteria invaded the myoseptum and lateral musculature. The lesions became haemorrhaged and necrotized, and showed extensive dilation of the blood vessels and serous exudation. Systemic infections gave rise to metastatic necrotic lesions involving the gills, heart, kidney, liver and spleen. Desquamation of the intestinal epithelium was a particularly marked feature in advanced infections.

In yearling rainbow trout affected by vibriosis, Miyazaki and Kubota (1977) reported the presence of erosive lesions or small boils on the head or body surface during early stages of the infection. In advanced cases, however, the disease produced extensive ulcerative lesions on head and body surface, with exophthalmos, and splenomegaly. The early lesion was associated with considerable multiplication of the bacteria. Oedematous dissociation of the loose connective tissues of the dermis was detected, and inflammatory cell infiltration was observed. In the more advanced ulcerative lesions, the bacteria had spread extensively throughout the interstitial muscle tissues, and gave rise to desquamation or sloughing of the epithelium, infiltration by inflammatory cells, and degenerative necrosis of the muscle fibres. Masses of bacteria described as emboli were detected in the

hepatic sinusoids, renal haematopoietic tissue and capillaries of the gill lamellae. The histopathological manifestations of exophthalmos included pronounced dilation of the coroid capillaries, detachment of the retina with necrosis of the pigmented layer, degeneration of the cornea and necrosis of the iris.

Horne and co-authors (1977) reported an outbreak of acute oedematous disease in juvenile turbot *Scophthalmus maximus* characterized by swelling of abdomen and orbits. Clinical and pathological manifestations of the infection, which gave rise to high mortalities, were described as consistent with a circulating toxaemia affecting heart, orbit and kidney. *Vibrio anguillarum* was isolated in pure culture from all organs of the fish, and the characteristics of the isolate were considered to be almost identical to those of the type strain NCMB 6. Antibiotic therapy was ineffective, but when a small group of the fish was held at temperatures below 10 °C, the losses were reduced from 20 to 5 % in comparison to those observed in the untreated control group.

Vibriosis has been reported as one of the most significant diseases in the artificial production of juvenile red sea bream *Pagrus major* in Japan (Muroga and Tatani, 1982). Strains of *Vibrio anguillarum* were isolated from epizootics affecting this species of marine fish in 2 hatcheries, and identified as aetiological agents of the infection. Bacterial haemorrhagic septicaemia, frequently associated with *Aeromonas* and *Pseudomonas* spp., and more particularly with *Vibrio* spp., has been considered of sufficient importance as to constitute a limiting factor in the culture of gilt-head sea bream *Sparus aurata* in Israel (Paperna and co-authors, 1977). Further observations have again demonstrated the importance of acute bacterial haemorrhagic septicaemia as a contributing cause to mortalities in sea bream in that country (Colorni and co-authors, 1981). These workers isolated a total of 116 bacterial strains from the peripheral blood of 88 moribund sea bream over a 16-month period, using trypticase soy agar (TSA) and thiosulphate citrate bile salts sucrose agar (TCBS) prepared with 25 % filtered and autoclaved sea water as primary isolation media. A battery of more than 30 different tests was performed to facilitate the identification of the isolates, the great majority of which were classified as *V. alginolyticus, V. anguillarum* or closely related forms, and *V. parahaemolyticus*. Strains of *Aeromonas* and *Pseudomonas* spp. were also isolated from the blood and skin of moribund sea bream.

The gross pathology in the diseased fish included lethargy, dark coloration of the skin, lepidorthosis and haemorrhagic ulcerative lesions of the skin and muscle tissue. Congestion of the liver and of the capillary network of the intestine, swim bladder and peritoneum was detected, and the rectum was filled with clear mucoid material. Anaemia was another important clinical manifestation of the disease. All isolates were sensitive *in vitro* to chloramphenicol, furaltadone (tartrate salt) and nitrofurazone. Nitrofurazone 10 % powder, routinely used as a dip at a concentration of 50 ppm active ingredient for an exposure period of 1 h, was effective in controlling the condition in practice.

Colorni and co-authors (1981) endeavoured to reproduce experimentally the disease in healthy sea bream by intra-peritoneal injection of 10^9 cells suspended in saline into 40 fish (mean weight 80 g). The 3 strains of *Vibrio alginolyticus* used, designated Elat 1, 2 and 3, failed to give rise to signs of septicaemia over a 4-week observation period, and the organisms were never recovered from the peripheral blood of these injected fish.

A marine bacterium designated *Aeromonas proteolytica* was isolated by Merkel and co-authors (1964) from the intestine of the isopod *Limnoria tripunctata*. Schubert (1969)

considered this organism to be a halophilic variety of *A. hydrophila* which he named *A. hydrophila* subspecies *proteolytica* on the basis of its biochemical characteristics and polar flagella. This bacterium has been studied in detail by McCarthy (1975), who showed conclusively that it presents both peritrichous and polar flagella, fails to ferment galactose, is unique amongst the previously known aeromonads in its ability to grow in the presence of 9 % sodium chloride and, more important still, fails to produce precipitin bands when tested with 6 *A. hydrophila* antisera obtained from rabbits. Sandvik and Hagan (1968) have also been unable to detect serological cross-reactions between *A. proteolytica* on the one hand, and *A. hydrophila* and *A. salmonicida* on the other hand. McCarthy (1975) and Popoff and Véron (1976) concluded that *A. proteolytica* is not a true aeromonad and should be excluded from the genus until such time as its precise taxonomic status can be determined.

Hawkes (1976) carried out a survey of the diseases of pompanos *Trachinotus carolinus* and striped bass *Morone saxatilis* cultivated in earth ponds fed with brackish water (2 to 25 ‰ S) in Alabama, USA, from 1974 to 1975. These studies were complemented by others involving striped bass fingerlings in fresh-water ponds. Samples from kidney, liver, spleen, and lesions of the fish were streaked onto brain infusion agar plates incubated at 28 °C for 48 h. The isolates were subsequently purified and identified in accordance with the techniques outlined by Bullock (1971). Heavy infections of the kidney and liver of pompanos in brackish water were reported as being caused by *Aeromonas hydrophila,* and light to moderate infections caused by the same species of bacterium were also detected in fins, kidney and liver of striped bass in brackish water and in fresh water conditions. *A. hydrophila* and *Vibrio anguillarum* were isolated from healthy and diseased pompanos. Hawke also reported that cases of bacterial haemorrhagic septicaemia and fin rot caused by *A. hydrophila* and *V. anguillarum* were diagnosed in striped bass fingerlings maintained in indoor concrete containers supplied with warm (32 °C) brackish water, and concluded that under such intensive maintenance conditions serious epizootics due to both of these species of bacteria could occur.

An outbreak of a condition described as ulcer disease was investigated by Larsen and Jensen (1977) in cod *Gadus morhua* from net impoundments in marine and brackish water localities on the coast of Denmark. An organism identified as an aeromonad was consistently isolated in pure culture from the ulcerative skin lesions and kidney of the fish, as well as from faeces, gills and mucus in occasional cases. The characteristics of 22 of these isolates were compared with those of *Aeromonas hydrophila* ssp. *anaerogenes* (ATCC 15467), *A. hydrophila* ssp. *hydrophila* (ATCC 7966), *A. punctata* (NCMB 74), *A. salmonicida* ssp. *salmonicida* (NCMB 1102) and *A. salmonicida* ssp. *achromogenes* (NCMB 1110), in addition to those of 11 strains of *Vibrio anguillarum* isolated from the diseased cod. Larsen and Jensen's findings are summarized in Table 1-8.

Larsen and Jensen (1977) found that all strains of *Aeromonas* spp. investigated were capable of growth in 4 % sodium chloride. The cod aeromonads and strain ATCC 7966 grew in 5 % sodium chloride, but neither of them was capable of growth at a concentration of 6 % sodium chloride. Intra-peritoneal injection of the aeromonad into cod and rainbow trout produced acute septicaemia and mortalities in both teleost species. Whilst recognizing that the ulcer disease of cod seems to comprise a highly complex situation, Larsen and Jensen (1977) pointed to their finding that the aeromonad was more frequently isolated in pure culture than *Vibrio anguillarum* from the kidney of cod with signs of septicaemia.

This observation led them to conclude that the aeromonad was more capable of causing a generalized infection than *V. anguillarum.* The cod aeromonads were clearly distinguishable from *V. anguillarum* in their lack of sensitivity to the vibriostatic compound 0/129, and Larsen and Jensen were of the opinion that their strains belonged to the '*Aeromonas hydrophila — Aeromonas punctata* group'. These aeromonads from cod ferment glucose anaerogenically and produce gas from glycerol; they are 2,3-butanediol and lysine decarboxylase-positive. Strains of anaerogenic aeromonads have been isolated from bacterial haemorrhagic septicaemia in gilt-head sea bream *Sparus aurata* cultured in earth ponds and in floating cages in the Red Sea, Israel, by Colorni and co-authors (1981). These workers also found many of their anaerogenic aeromonads to be lysine decarboxylase and 2,3-butanediol positive, the former reaction being similar to that obtained with their isolates of *Vibrio* spp., all of which were 2,3-butanediol negative. The positive lysine decarboxylase reaction reported by Colorni and co-authors for their isolates identified as '*Vibrio anguillarum* and closely related forms' conflicts with the findings of Evelyn (1971) that *V. anguillarum* is lysine decarboxylase-negative.

Lee and co-authors (1981) mention a group of halotolerant bacteria frequently isolated from estuarine environments, which they had variously described as 'marine aeromonads' or 'Group F vibrios'. Phenotypically this group of bacteria appears to occupy an intermediate position between the genus *Aeromonas* and certain species of *Vibrio,* including *V. anguillarum.* The minimum inhibitory concentration (MIC) of the organisms to vibriostatic compound 0/129 (2,4-diamino-6,7-di-isopropyl pteridine phosphate) was 10 to 50 mcg ml^{-1}, whereas the MIC of *Aeromonas* was > 320 mcg ml^{-1} and that of *V. anguillarum* was 1 to 5 mcg ml^{-1}. Lee and co-authors commented that it was often difficult to distinguish between 'Group F vibrios' and members of the genus *Aeromonas* or vibrios such as *V. anguillarum* which possess arginine dehydrolase. A total of 154 type strains and original isolates of aeromonads, photobacters, plesiomonads and vibrios were investigated, many of which had been obtained from molluscs, crustaceans, fish, river and sea water, and from 'sea food'. Lee and co-authors confirmed that the 'Group F vibrios' are true vibrios, and designated them as members of the new species *V. fluvialis.* Salient characters useful for the identification of *V. fluvialis* are listed in Table 1-9.

The species description by Lee and co-authors (1981) for *Vibrio fluvialis* is as follows:

> "Gram negative short rods; axis straight or curved; sides usually parallel; ends rounded; occurring singly, in pairs or occasionally in short chains of 3 to 4 organisms; may be pleomorphic. Motile by means of a single polar sheathed flagellum in liquid media. On solid media lateral, unsheathed flagella of shorter wave length may be produced. Sodium chloride may be required for growth and the optimum concentration for growth is 1–3 % (w/v). Colonies on heart infusion agar are opaque, shiny, smooth, round, domed, entire, may be mucoid and are 2–3 mm in diameter after 18 h at 30 °C. No pigments are produced. Facultative anaerobe. Metabolism of glucose is fermentative and gas may be produced. Kovács oxidase positive. Reduce nitrate to nitrite. Grow on simple mineral media on a variety of organic carbon sources. The mol% G + C content of the DNA ranges from 49.3–50.6."

Two biovars, designated I and II, were recognized; their distinguishing characters are given in Table 1–10. Both these biovars are widely distributed in the aquatic environment, particularly in brackish and estuarine waters. The holotype of *Vibrio fluvialis* has been

Table 1-8
Differential biochemical characteristics shown by strains of *Aeromonas* spp. and *Vibrio anguillarum*
(Based on Larsen and Jensen, 1977)

Test	*Aer. hydrophila* ssp. *hydrophila* ATCC 7966	*Aer. hydrophila* ssp. *anaerogenes* ATCC 15467	*Aeromonas punctata* NCMB 74	*Aer. salmonicida* ssp. *salmonicida* NCMB 1102	*Aer. salmonicida* ssp. *achromogenes* NCMB 1110	*Aeromonas* sp. from cod	*Vibrio anguillarum* from cod
Acid from glucose	+	+	+	+	+	+	+
Gas from glucose	+	−	+	+	−	−	−
Acid from fructose	+	+	+	+	+	+	+
Acid from galactose	+	+	+	+	+	+	+
Acid from mannose	+	+	+	+	+	+	+
Acid from rhamnose	+	+	−	−	−	−	−
Acid from dulcitol	−	−	−	−	−	−	−
Acid from mannitol	+	+	+	+	−	+	+
Acid from sorbitol	−	−	−	−	−	−	+
Acid from arabinose	+	+	+	+	−	−	(+)
Acid from xylose	−	−	−	−	−	−	−
Acid from adonitol	−	−	−	−	−	−	−
Acid from cellobiose	−	+	+	−	−	−	(+)
Acid from lactose	+	+	+	−	−	−	−
Acid from maltose	+	+	+	+	+	+	+
Acid from melibiose	−	−	−	−	+	−	−
Acid from sucrose	+	+	+	−	+	+	+
Acid from trehalose	+	+	+	−	+	+	+
Acid from inositol	−	−	−	−	−	−	−
Acid from glycerol	+	+	+	+	+	+	+
Acid from raffinose	−	−	−	−	−	−	−
Acid from cellulose	+	+	+	−	−	+	+
Acid from dextrin	+	+	+	+	+	+	+
Acid from inulin	−	−	−	−	−	−	−

Acid from glycogen	+	+	+	+	+	+	+
Acid from starch	+	+	+	+	+	+	+
Acid from aesculin	+	+	+	+	−	−	−
Acid from salicin	+	+	+	+	−	−	−
Catalase	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+
Reduction of nitrate	+	+	+	+	+	+	+
Urease	−	−	−	−	−	−	−
MR	+	−	+	−	−	−	(+)
VP	+	−	−	−	−	+	+
2,3-butanediol	+	−	−	−	−	+	+
Haemolysis	+	−	+	−	−	+	+
Brown pigment	−	−	−	+	−	−	(+)
Arginine dehydroxylase	+	+	+	+	+	+	+
Lysine decarboxylase	−	−	−	−	−	+	−
Ornithine decarboxylase	−	−	−	−	−	−	−
Phenylalanine decarboxylase	−	−	−	−	−	−	−
Production of indole	+	+	+	−	+	−	(+)
Hydrolysis of gelatine	+	+	+	+	+	+	+
Sensitivity to 0/129	−	−	−	−	−	−	+
Sensitivity to penicillin	−	−	−	−	−	−	−
Sensitivity to novobiocin	−	−	−	(+)	(+)	(+)	+
Growth in 0 % NaCl	+	+	+	+	+	+	(+)
Growth in 4 % NaCl	+	+	+	+	+	+	+
Growth in 5 % NaCl	+	−	−	−	−	(+)	+
Growth in 6 % NaCl	−	−	−	−	−	−	+
Growth at 5 °C	+	+	+	+	+	+	+
Growth at 10 °C	+	+	+	+	+	+	+
Growth at 37 °C	+	+	+	−	−	+	+
Growth at 43 °C	+	+	+	−	−	+	(+)
Growth on TCBS agar	−	−	−	−	−	−	+
Growth on SS agar	+	+	+	+	+	+	−
Growth on MacConkey agar	+	+	+	+	+	+	+

+ Most strains gave positive reaction

Table 1-9
Useful characters for the identification of *Vibrio fluvialis* (Based on Lee and co-authors, 1981)

Test	Reaction
Gram	−
Motility	+
Glucose fermentation	+
Oxidase	+
0/129 sensitivity (150 mcg $disc^{-1}$)	+
Novobiocin sensitivity (5 mcg $disc^{-1}$)	−
Thornley's arginine	+
Møller's ornithine	−
Møller's lysine	−
V-P	−
Indole production	−
Growth in 0 % NaCl	v
Growth in 6 % NaCl	+
Growth in 8 % NaCl	v
Growth in 10 % NaCl	−
Growth in glycine	+
Growth on propionate	+
Growth on NH_4 butyrate	+
Growth on chi-ketogutarate	+
Growth on ethanol	+
Growth on arabinose	+
Growth on sucrose	+

+ Positive reaction; − negative reaction; v variable reaction

Table 1-10
Characters which distinguish between *Vibrio fluvialis* biovars I and II (Based on Lee and co-authors, 1981)

Test	Biovar I	Biovar II
Gas from glucose	−	+ (89 %)
Hydrolysis of aesculin	v (72 %)	−
Growth on cellobiose	v (63 %)	− (4 %)
Growth on glucuronate	+ (94 %)	− (7 %)
Growth on citrulline	+ (97 %)	− (4 %)
Growth on putrescine	v (31 %)	+
Growth on delta-NH_4 valerate	−	v (63 %)

Figures in parentheses: percentage of strains possessing the corresponding character

deposited in the National Collection of Marine Bacteria, Britain, as NCMB 11327. In addition, NCMB 11328 is recommended by Lee and co-authors (1981) as a working type for Biovar II. These workers pointed out the importance of correctly identifying *V. fluvialis* with reference to the ecology of the genus *Aeromonas*, since at present it is almost impossible to differentiate between *V. fluvialis* and anaerogenic, lysine decarboxylase-negative strains of aeromonads. Re-examination of old cultures previously identified as

Aeromonas sp. enabled Lee and co-authors to assign approximately one third of such anaerogenic, lysine decarboxylase-negative strains to the new species *V. fluvialis.*

West and Lee (1982) studied the distribution of vibrios from 2 sites in Kent, England, during 1978–1980. One of the sampling sites was a fresh-water stream which received domestic sewage treatment effluents; the other was a static brackish water ditch which drained agricultural land reclaimed from an estuary and which was uncontaminated by human faeces and sewage. *Vibrio anguillarum* and *V. fluvialis* were isolated infrequently from the fresh-water source, whereas *V. anguillarum* was repeatedly isolated from the brackish-water source throughout the sampling period.

An important bacterial infection of Japanese eels *Anguilla japonica* in brackish water ponds is known as 'Red Spot Disease', the aetiological agent of which is *Pseudomonas anguilliseptica.* Outbreaks tend to occur principally in spring and when the water temperature is below 20 °C; they give rise to heavy mortalities. In advanced cases of this disease, the condition produces petechial haemorrhages on body surface and fins; internally there is hepatomegaly, atrophy of haematopoietic tissues of kidney and spleen, and pericarditis/epicarditis. Miyazaki and Egusa (1977) investigated the histopathological changes which occur in 'Red Spot Disease'. The lesions are located in the dermis, sub-cutaneous adipose tissue and interstitial tissues of the musculature. Similar haemorrhagic lesions are present in the interstitial tissues of the *bulbus arteriosus,* heart muscle and blood vessels. *Pseudomonas anguilliseptica* grows rapidly in the lesions, in which the bacteria produce an inflammation characterized by the presence of a serous exudate and by the presence of cellular proliferation involving mesenchymal cells, with a leucocytic infiltration composed of macrophages and neutrophils. Numerous small haemorrhagic foci are present in the loose connective tissue of the dermis and in the intraepithelial papillary tissue.

Pseudomonas anguilliseptica has been characterized by Muroga and co-authors (1977a). The organism shows optimum growth in a salinity of 0.5 to 1 % sodium chloride (range: 0 to 4 %) at 15 to 20 °C (range: 5 to 30 °C) and pH 7 to 9 (range: 5.3 to 9.7). The survival of *P. anguilliseptica* is increased with a corresponding increase in salinity; it withstands more than 200 days exposure in full-strength sea water and in diluted sea water (Cl = 1.9 %). When grown in diluted sea water (Cl = 5.6 %), the bacterium survived for more than 40 days at 27 °C and below, but died within 7 days when held at 30 °C and above. An increase in incubation temperature led to a gradual decrease in motility of the pseudomonad. Muroga and co-authors concluded that the characteristics of *P. anguilliseptica* determined serve to explain why outbreaks of 'Red Spot Disease' occur in brackish water ponds when the prevailing water temperature is below 20 °C.

Jo and co-authors (1975) reported on an outbreak of 'Red Spot Disease' caused by *Pseudomonas anguilliseptica* in European eels *Anguilla anguilla* cultured in Tokushima Prefecture, Japan, during 1974. External clinical signs of the infection — characterized by petechial haemorrhages on the skin of the mouth, opercula and ventral body surface — were less obvious than in Japanese eels, and mortalities were less severe in *A. anguilla* than in *A. japonica.* According to Muroga and co-authors (1973) this condition occurs principally in brackish-water ponds below 20 °C, and ceases when the water temperature rises to 26 to 27 °C. Outbreaks occur in spring and autumn, but subside in summer. In addition to infection with *P. anguilliseptica,* some eels also harbour *Aeromonas liquefaciens* (= *A. hydrophila*) and *Vibrio anguillarum.*

Muroga and co-authors (1975) injected *Pseudomonas anguilliseptica* by the intra-

muscular route into Japanese and European eels. All Japanese eels died, but 29 % of the European eels survived, a fact which led Muroga and co-authors to conclude that the latter species is less susceptible to infection. Muroga (1978) recommended that European eels be cultured in preference to Japanese eels in ponds with brackish waters and temperatures below 27 °C as a means of preventing outbreaks of 'Red Spot Disease', since European eels are apparently more resistant to the infection.

Stewart and co-authors (1983) described an outbreak of 'Red Spot Disease' in approximately 70,000 elvers of the European eel from River Severn, south-West England. When the elvers were introduced into a closed-circuit fresh-water system in Scotland containing about 3,900 healthy adult eels carrying *Vibrio anguillarum* and *Aeromonas hydrophila,* within 3 weeks both eels and elvers began to die. In total, 96 % of the elvers and 39 % of the eels succumbed. No external signs were observed in the elvers, but adult eels developed small petechial haemorrhages over most of their ventral body surface. Pure cultures of *Pseudomonas anguilliseptica* were isolated from muscle tissue, liver, spleen, kidney and heart. Following the method of control employed in Japan, the water temperature was raised to 26 to 27 °C for 2 weeks, after which it was gradually lowered to the original temperature of 21 °C. No further cases of 'Red Spot Disease' occurred in the ensuing 5 months. Ellis and co-authors (1983) studied histopathological changes in European eels affected by 'Red Spot Disease'. The fish had petechial haemorrhages in the skin, particularly on the unpigmented ventrum. The epidermis, when present, was detached from the basement membrane below which the superficial collagen was haemorrhagic and oedematous. Where the epidermis had been lost, exposed dermal collagen was haemorrhagic and necrotic in appearance. The liver was pale, with congestion of the hepatic sinuses, petechial haemorrhages and, in 1 specimen, liquefactive necrosis of the tissues underlying the liver capsule. Slight vasodilation was observed in gills and kidney, but no lesions were detected in heart, spleen, pancreas or intestine. The lesions were described as less intense than those reported for Japanese eels, and no bacteria were seen in the histological sections.

Nakai and co-authors (1981) investigated the serological properties of 96 strains of *Pseudomonas anguilliseptica* isolated from European and Japanese eels. On the basis of agglutination tests carried out with rabbit antisera, it was shown that all of the 96 strains possessed a common heat-stable 0 antigen. When 0 antisera prepared against heatkilled cells were used, the strains could be divided into 2 antigenically separate types. This work also demonstrated that the use of a slide agglutination test is of value in the rapid diagnosis of isolates of *P. anguilliseptica*..

A mass mortality affecting white perch *Roccus americanus* occurred in Chesapeake Bay, Maryland, USA, during the summer of 1963 and was investigated by Snieszko and co-authors (1964b). The epizootic started in Potomac estuary, from whence it spread rapidly to other widely separated locations. In addition to white perch, of which approximately 50 % of the population was lost, the outbreak also affected striped bass *Morone saxatilis* in the same localities. Snieszko and his colleagues isolated 30 bacterial cultures of an organism identified as *Pasteurella* sp. from the blood and internal organs of 17 moribund white perch and 3 striped bass. The bacterium was a pleomorphic and non-motile Gram-negative rod, with polar staining characteristics. It was cytochrome oxidase positive, and fermented glucose, maltose, fructose and sucrose anaerogenically. Primary isolation was effected on blood agar, but it was subsequently found that agar prepared with

1 to 3 % sodium chloride, or half-strength sea water, supported growth *in vitro*. Optimum growth temperature ranged from 20 to 30 °C, with no growth at 37 °C. In nutrient broth, the organism grew at salinities of 0.5 to 5 % sodium chloride. Negative reactions were obtained with amylase, gelatine liquefaction, hydrogen sulphide production, indole production and urease tests, although the production of 'a trace' of acetyl-methyl-carbinol was reported.

Pasteurella sp. was further investigated by Janssen and Surgalla (1968) on the basis of 27 of the strains originally isolated by Snieszko and co-authors (1964b), all of which were found to be identical in every respect. The organism was designated *P. piscicida*. It was sensitive to vibriostatic agent 0/129, grew at 17 to 31 °C, and exhibited optimum growth at 1.5 % sodium chloride. the salinity tolerance of *P. piscicida* ranged up to 2.5 to 3 % sodium chloride, and at least 0.5 % sodium chloride was required for growth in culture media. When suspended in sterile brackish water (NaCl = 1.7 %) obtained from Chesapeake Bay, the organism died within 3 days. For these reasons, Janssen and Surgalla concluded that *P. piscicida* is poorly adapted to survival outside the body of the host fish *per se*.

Toranzo and co-authors (1982) carried out experiments on the survival of *Pasteurella piscicida* in waters of varying salinities. Using *P. piscicida* strain ATCC 17911 in filtered or autoclaved Chesapeake Bay water (12 ‰ S) and fresh water, they demonstrated that the organism was very labile both in fresh and estuarine waters. In fresh water, the viable bacterial cell numbers decreased by 4 logs within 24 h, and no organisms were detected after 48 h incubation at 20 °C. In contrast, survival of the bacterium in estuarine water was 4 to 5 days. These workers concluded that direct fish-to-fish transmission is likely to be of significance for the maintenance of the pathogen as an infective agent in fish from estuarine conditions.

Bacterial haemorrhagic septicaemia associated with *Pasteurella piscicida* has regularly been reported from cultured yellowtail *Seriola quinqueradiata* and black sea bream *Mylio macrocephalus* from Japanese fish farms. In Japan the disease is also known as bacterial pseudotuberculosis (Kusuda and Yamaoka, 1972; Simidu and Egusa 1972; Koike and co-authors, 1975; Muroga and co-authors, 1977b; Ohnishi and co-authors, 1982). Lewis and co-authors (1970) reported the occurrence of a *Pasteurella*-like bacterium in association with an epizootic affecting menhaden *Brevoortia tyrannus* and striped mullet *Mugil cephalus* in Galveston Bay, Texas, USA. The affected fish had a purulent material in the abdominal cavity, and a pleomorphic bipolar staining Gram-negative rod was isolated. This organism, which differed in several of its characteristics from *P. piscicida*, was experimentally transmitted to striped mullet by exposure of the fish to infected water.

The importance of pasteurellosis as a clinical entity within the concept of bacterial haemorrhagic septicaemia as a syndrome is illustrated by the report of Håstein and Bullock (1976) of an outbreak of the disease in Atlantic salmon *Salmo salar* and brown trout *Salmo trutta* from Norwegian fish farms. The first outbreak was detected on one single occasion during 1968 in fingerling brown trout raised at a fresh-water fish farm near Oslo, Norway, and gave rise to average mortalities of 58 %. Further observations in 1969 led to the detection of the infection in Atlantic salmon yearlings, smolts and brood stock at a fish farm on the West Coast of Norway. The fish farm concerned was supplied by a mixture of fresh water and sea water. Mortalities occurred throughout the year, but the death rate was highest from mid-March to mid-August, when the water temperature ranged between 10

and 20 °C. In this period, the mortalities were in the region of 3 to 8 % mo^{-1} for yearlings and 2-yr old salmon; Håstein and Bullock mentioned that the disease had been transferred to other Norwegian fish farms through the movement of infected smolts.

Few differences were detected in the clinical manifestations of the disease in 700 specimens of Atlantic salmon and brown trout. Main pathological changes in the trout included the presence of superficial lesions on one or both sides of the body; the lesions had the appearance of small blisters which, on rupture, produced superficial ulcers affecting the skin. Ulcerative areas were greyish-white in colour, with haemorrhagic areas and a slightly-raised whitish-coloured zone demarcating the ulcers from the normal skin. Gills were palid and showed evidence of petechial haemorrhages. Both kidney and spleen were swollen, and petechiae were frequently observed in intestine and liver. Ascitic fluid was occasionally present in the abdominal cavity, and exophthalmos was detected in the trout.

Håstein and Bullock (1976) reported that while no external clinical disease manifestations were observed in some of the Atlantic salmon, in other specimens lesions similar to those in the trout were present. Older salmon showed boil-like necrotic lesions in the muscle tissue containing a viscid haemorrhagic and purulent material strikingly similar to that found in cases of furunculosis and vibriosis. The microscopical examination of stained smears from the affected areas showed the presence of numerous small Gram-negative rods, erythrocytes and necrotized cell debris. *Pasteurella*-like bacteria were consistently isolated in pure culture from kidney, liver, spleen and necrotic lesions of diseased fish. A mixed flora was isolated from superficial lesions, in which *Pasteurella*-like organisms predominated. An interesting finding reported by these workers is that *Vibrio anguillarum* was occasionally isolated from diseased salmon in brackish water, a fact which led them to conclude that both pasteurellosis and vibriosis can occur simultaneously in the same fish under such environmental conditions.

Following a study of the morphology and other characteristics of 36 isolates of the *Pasteurella*-like organism from diseased salmonids, Håstein and Bullock (1976) were able to classify their isolates as being similar to *Pasteurella piscicida*. Reactions of Norwegian strains were compared to those reported by Janssen and Surgalla (1968), Kusuda and Yamaoka (1972) and Simidu and Egusa (1972) for strains isolated from white perch in USA and from yellowtail in Japan. The findings are summarized in Table 1-11.

Pasteurellosis is now recognized as a major problem in marine aquaculture operations in Japan, where it is of particular importance in cultured yellowtail. An outbreak of the disease has been reported by Muroga and co-authors (1977b), Ohnishi and co-authors (1982), and Sugiyama and co-authors (1977) from black sea bream *Mylio macrocephalus* reared in floating cages. In this instance, 8,000 of 9,000 black sea bream were lost. The pathogenicity of the organism was demonstrated by the intra-muscular injection of 9 sea bream, 8 of which succumbed to the infection. The aetiological agent was identified as *Pasteurella piscicida*.

Fukuda and Kusuda (1980) investigated certain aspects of the humeral immune response of yellowtail to *Pasteurella piscicida*. Following an outbreak of the disease in a population of cultured yellowtail, they found that the serum contained agglutinating antibodies against whole cells and cell extracts of the bacterium, with peaks occurring in mid-July and towards the end of October, when the water temperatures were in the range of 23 to 25 °C. A similar increase in the serum gamma-globulin was also detected. Fukuda

Table 1-11
Properties of *Pasteurella piscicida* as reported in the literature (Based on Håstein and Bullock, 1976)

Test	Janssen & Surgalla (1968)	Kusuda & Yamaoka (1972)	Simidu & Egusa (1972)	Hastein & Bullock (1976)
Motility	−	−	−	−
Acid from arabinose	−	−	−	−
Acid from xylose	−	−	−	−
Acid from adonitol	−	−	.	−
Acid from glucose	+	+	+	+
Acid from fructose	+	+	+	(+)
Acid from galactose	+	+	+	(+)
Acid from mannose	+	+	+	(+)
Acid from rhamnose	−	−	−	−
Acid from dulcitol	−	.	−	−
Acid from mannitol	−	−	−	(+)
Acid from sorbitol	−	−	.	(+)
Acid from cellobiose	−	−	−	(+)
Acid from lactose	−	−	−	−
Acid from maltose	+	−	−	(+)
Acid from sucrose	+	−	−	(+)
Acid from trehalose	−	−	−	(+)
Acid from inositol	−	−	.	−
Acid from raffinose	−	−	−	−
Acid from dextrin	−	−	−	(+)
Acid from inulin	−	−	−	(+)
Acid from aesculin	−	−	−	(+)
Acid from salicin	−	−	.	(+)
VP	−	(+)	(+)	−
MR	+	+	+	(+)
Hydrolysis of gelatine	−	−	−	(+)
Production of indole	−	−	−	−
Reduction of nitrate	−	−	−	(+)
Urease	−	−	−	−

(+) Variable reaction

and Kusuda concluded that infected yellowtail produce antibodies against *P. piscicida* during early summer and late autumn, a finding which suggests that some degree of protective immunity is conferred on those fish which do not succumb to the initial infection. According to Kusuda and co-authors (1978), the bacteria isolated from epizootics affecting yellowtail in various locations in Japan show a similar immuno-diffusion pattern, and should thus be considered as being of the same serotype. The immuno-diffusion pattern, as determined by the Ouchterlony technique, coincides with that of the organism defined as *P. piscicida* by Janssen and Surgalla (1968) in USA. Mori and co-authors (1976) carried out a field survey in Miyazaki Prefecture to detect the presence of pasteurellosis in cultured yellowtail. *P. piscicida* was detected by means of the direct fluorescent antibody technique used, and isolated *in vitro* from the fish in the absence of macroscopical signs of lesions in kidney and spleen. These workers concluded that the

fluorescent antibody test could prove of diagnostic value in the detection of pre-clinical or sub-clinical cases of the disease.

Myxobacterial infections

Myxobacteria are of particular interest to fish pathologists since, with the possible exception of an anaerobic myxobacterium capable of producing experimentally cytopathogenic effects on bruised human mouth tissue (Dworkin, 1966), these organisms are known only as pathogens of fish and certain aquatic invertebrates such as crustaceans. Higher vertebrates appear to be completely refractory to myxobacterial infections.

The myxobacteria are slender, long, procaryotic Gram-negative rods which exhibit a highly characteristic gliding motion associated with the production of abundant quantities of mucoid material which facilitates their movement. In nature, various types of myxobacteria are widely distributed in fresh and marine waters, and in the soil. Myxobacterial infections, or myxobacterioses, affect a wide variety of fresh-water fish in artificial and natural habitats. Among the diseases they cause are columnaris, cold-water or peduncle disease, and bacterial gill disease. Bullock (1968) suggested that myxobacteria may play an important role in relation to necrotic processes occurring in outbreaks of fin rot in brook trout *Salvelinus fontinalis* in fresh water. The importance of various myxobacterioses in relation to fish diseases has been reviewed comprehensively by Anderson and Conroy (1969).

The principal myxobacterial disease encountered in fish from salt-water environments is 'salt-water columnaris' or 'salt-water myxobacteriosis'. Its external clinical manifestations are strikingly similar to those found in columnaris disease *sensu stricto* of fresh-water fish. Among the first workers to have described salt-water myxobacteriosis are Rucker and co-authors (1953), who reported an outbreak affecting young chinook salmon *Oncorhynchus tshawytscha* reared in sea water on the North-West Pacific coast of USA. These workers commented on the close similarity between the clinical signs of this condition and those of fresh-water columnaris, and identified a halophilic and highly fastidious myxobacterium as the aetiological agent of the outbreak. Borg (1960) observed a similar disease in pink salmon *Oncorhynchus gorbuscha* raised in sea water; the disease is characterized by necrotized areas on the caudal peduncle. Numerous myxobacteria were detected in scrapings from affected areas and lesions. Wood (1973) mentioned that the salient clinical signs of salt-water columnaris in Pacific salmon and rainbow trout from sea water include rough and necrotic lesions, of variable size, affecting skin and superficial tissues. The disease has also been reported from coho salmon *Oncorhynchus kisutch* held in estuarine conditions in Maine, USA (Sawyer, 1976). In this instance, the outbreak caused the death of 50,000 coho salmon, approximately 30 % of the total stock. The diseased fish showed signs of progressive erosion of the cartilage of the snout, lower jaw and mouth; the severity of the infection increased with a corresponding increase in water temperature. Yellowish-coloured sheets of myxobacteria were detected in the buccal, pharyngeal and pneumatic duct tissues, and were believed to have been responsible for the infection in the absence of other known bacterial fish pathogens. A preliminary attempt to study the pathogenicity of the myxobacterium was inconclusive, since only 2 of 18 experimentally infected salmon developed typical clinical signs of the infection. It is of interest to mention in this context that Nigrelli and Hutner (1946) described fresh-water columnaris in mummichogs *Fundulus heteroclitus,* euryhaline cyprinodonts common to waters of the southern USA. The

possibility of columnaris disease *sensu stricto,* caused by *Flexibacter columnaris,* occurring in susceptible species of fish in low salinity estuarine conditions cannot, therefore, be completely disregarded.

Anderson and Conroy (1968, 1969) investigated the causes of a disease which they designated 'eroded mouth' in a population of 1-yr old rainbow trout reared in floating cages in a sea water loch on the West Coast of Scotland. The principal clinical signs observed included severe necrotic lesions which gave rise to erosion of the upper jaw of the trout. The entire population showed evidence of anorrexia and listlessness, and mortality reached 10 %. Histological changes included destruction of epidermis and parts of the dermis, with consequent exposure of the underlying cartilaginous tissues. Sections through the affected areas revealed masses of myxobacteria in the connective tissue, in which the bacteria were arranged in sheets. No evidence of any involvement of gills or other organs was apparent.

Japanese workers (Masumura and Wakabayashi, 1977) reported myxobacterial infections in hatchery-raised red sea bream *Pagrus major* and gilthead *Acanthopagrus schlegeli.* The disease affected 15 to 60 mm long fry reared in floating cages, producing mortalities of 20 to 30 %. Salient clinical signs included erosion of the mouth together with marked fin and tail rot. A halophilic myxobacterium was isolated, and when apparently healthy specimens were brought into contact with the organism following smearing of the surface of mouth and tail, similar symptoms were reproduced. Colorni and co-authors (1981) described cases of 'gill rot' in gilt head sea bream *Sparus aurata* cultured in floating cages in the Red Sea at Elat, Israel. Large numbers of myxobacteria were isolated from the diseased fish following the inoculation of Anacker and Ordal's medium and 2 % fish muscle-sea water broth. In the former medium the bacteria quickly lost their characteristic morphology and motility, but these features were retained to a greater degree in the fish muscle-sea water broth. Due to the difficulties inherent in the isolation and cultivation of these bacteria, Colorni and co-authors based their diagnosis on the detection of myxobacteria in fresh smears from the diseased areas of the fish.

Campbell and Buswell (1982) described a condition designated 'black patch necrosis' causing heavy mortalities in 0 and I group Dover sole *Solea solea* during 1974–1978 in a marine fish farming facility in Great Britain. When diseased and healthy tissues were investigated microbiologically, a myxobacterium was consistently isolated from diseased fish but not from healthy ones. The organism was a long and filamentous Gram-negative rod, closely resembling *Flexibacter columnaris,* and was identical to reference strain NCMB 1038 of *F. columnaris.* The bacterium produced 100 % mortalities in experimentally infected Dover sole maintained at 17.5 °C over a 96-h period, and was therefore identified as the aetiological agent of 'black patch necrosis' in this flatfish. Hikida and co-authors (1979) also recorded *Flexibacter*-like bacteria as pathogens of marine fish in Japanese waters.

From the foregoing, it is convenient to consider salt water myxobacterioses as non-systemic cutaneous infections which give rise to abrasive and shallow lesions on the skin of the flanks of the body, caudal peduncle and/or upper jaw of the fish. Salt-water myxobacteriosis is thus far known from anadromous salmonids being raised in sea water from the Atlantic and Pacific coasts of North America and from the West Coast of Scotland (Rucker and co-authors, 1953; Borg, 1960; Rucker, 1963; Anderson and Conroy, 1968, 1969; Pacha and Ordal, 1970; Wood, 1973; Sawyer, 1976), as well as from strictly marine

species cultured in sea water cages and artificial environments in Britain, Israel and Japan (Masumura and Wakabayashi, 1977; Hikida and co-authors, 1979; Colorni and co-authors, 1981; Campbell and Buswell, 1982).

The aetiological agent of salt-water myxobacteriosis in salmonids was successfully isolated by Borg (1960) on a 1.5 % (w/v) agar medium containing a small amount of sterile unheated fish extract and prepared with equal amounts of 3 % (w/v) sodium chloride solution and full-strength sea water. In their studies on salt-water myxobacteriosis of rainbow trout in Scotland, Anderson and Conroy (1968, 1969) were initially unsuccessful in isolating the organism on the medium of Anacker and Ordal (1959) containing 25 % (v/v) sea water. Successful isolation and subsequent maintenance of the bacterium in pure culture was achieved by the use of a medium containing 0.1 % (w/v) peptone, 0.1 % (w/v) yeast extract, 0.9 % (w/v) agar and 5 % (v/v) enzymic extract of fish muscle tissue. This medium was prepared with full-strength sea water and, following inoculation, was incubated at 10 °C. Subsequently, however, the bacterium was successfully maintained on a routine basis in sub-cultures at 20 °C. The isolates had an obligate requirement for sea water and failed to grow in the medium when this was prepared with sodium chloride alone. *In vitro,* the Scottish isolates were highly proteolytic, gave a positive reaction with Kovác's oxidase test, failed to reduce nitrates, produce indole or to attack glucose, maltose and saccharose. Varying degrees of sensitivity to chloramphenicol, nitrofurantoin, penicillin and streptomycin were observed.

The epizootics reported from USA and from Great Britain share the fact that 'a halophilic and highly fastidious myxobacterium' is involved as aetiological agent of the condition in salmonids. North American workers (Pacha and Ordal, 1970; Wood, 1973) refer to the causative agent as long and slender rods corresponding to a marine species of the genus *Sporocytophaga* which is capable of forming microcysts and also of producing resting cells directly from the vegetative cells themselves, without any need to develop fruiting bodies. The isolates studied by Anderson and Conroy (1969) from Scotland also produced round bodies, but were considered to be somewhat distinct from the strains of *Sporocytophaga* sp. isolated in North America. Since no information is currently available on the serological characteristics of the American and European strains, it might be prudent to reserve judgment on the precise generic status of the organisms until such time as more detailed research can be conducted. In general terms, however, the organism can be described as a halophilic and highly fastidious myxobacterium which produces yellowish-coloured colonies on solid or semi-solid (= 'sloppy') agar media prepared with full-strength sea water. It is recommended that initial isolation be attempted by inoculation and incubation of the medium at 10 °C, and that sub-cultures be maintained by incubation at 20 °C. In older cultures, round bodies or microcysts may be observed together with the vegetative cells.

As experimental pathogenicity tests have not been carried out on a large scale, it can only be postulated at this juncture that the infection occurs when myxobacteria invade areas affected by physical or mechanical abrasion when the fish rub against the sides of the floating cages. The euryhaline characteristics of the anadromous salmonids have been used to good effect in the elimination of the strongly halophilic myxobacteria from infected tissues. Anderson and Conroy (1968, 1969) transferred 200 infected rainbow trout from sea water to fresh water, and it was no longer possible to detect or to isolate any myxobacteria. However, these workers recognized that such treatment is impracticable in

large-scale aquaculture operations involving thousands of salmonids in sea water, particularly when the degree of stress imposed on the fish by the osmotic shock of transfer from sea water to fresh water and *vice versa* is taken into consideration. In practice, Anderson and Conroy (1968, 1969) achieved an acceptable level of control of the infection by exposure of the diseased trout to dips of 2 min duration in copper sulphate at a concentration of 1:2,000 in fresh water, on each of 3 consecutive days. It is also possible that outbreaks could be controlled by the use of baths in 1 to 2 ppm oxytetracycline or another suitable wide-spectrum antibiotic or nitrofuran derivative.

Streptococcal Infections

Plumb and co-authors (1974) documented an important mass mortality of fish in estuarine areas of Alabama and Florida, USA. It affected sea catfish *Arius felis,* menhaden *Brevoortia patronus,* silver trout *Cynoscion nothus,* pin fish *Lagodon rhomboides,* spot *Leiostomus xanthurus,* Atlantic croaker *Micropogon undulatus* and striped mullet *Mugil cephalus.* The stingray *Dasyatis* sp., an elasmobranch, was also affected. Mortalities were described as of an acute to chronic nature, occurring over 72 km of the north-western coast of the Gulf of Mexico, and tens of thousands of fish were stated to have died.

Moribund fish swam erratically at the water surface, and frequently exhibited whirling movements. Haemorrhagic lesions were detected on skin, opercula, in the buccal area, at the base of the fins and in the perianal regions. Signs of abdominal distension were observed, associated with the presence of a bloody fluid in the body cavity and with exophthalmos. Internally, the kidneys of most fish had a normal appearance, although the livers were pale and the spleens were of a deep red colour. Presence of haemorrhagic enteritis was confirmed in many of the fish, where the lumen of the intestine was filled with a bloody fluid which tended to be extruded via the anus.

Attempts to isolate the aetiological agent were made on brain/heart infusion (BHI) agar plates inoculated with material from eyes, kidney, liver, peritoneal fluid and skin lesions. Ninety to 100 % of the fish sampled yielded pure cultures of colonies subsequently identified as strains of a non-haemolytic, Lancefield Group B, type I_b, *Streptococcus* sp.

Plumb and co-authors (1974) made the interesting observation that, with the exception of Escambia Bay, Florida, all sampling sites from which fish were obtained possessed similar topographical characteristics in that a narrow passage exists at the mouths of the affected areas at their zone of confluence with larger bodies of water in the estuarine bays. The principal fish species affected was the menhaden, upon which sea catfish — a scavenger — were seen to feed. It was suggested that the kills might have been due to a lack of flushing by fresh or tidal waters, creating a stress situation, and that sea catfish and other ichthyophagous species could have become infected through the consumption of dead menhaden.

Cook and Lofton (1975) carried out pathogenicity tests with a strain of non-haemolytic, Lancefield Group B, type I_b *Streptococcus* sp., referred to as Strain 992, which they isolated from the kidney of a moribund menhaden captured in Alabama. This strain was shown to be serologically identical to the one isolated by Plumb and co-authors (1974). Pathogenicity studies were undertaken with this and other streptococci (*Streptococcus agalactiae, S. durans, S. faecalis, S. liquefaciens* and 1 strain each of a Lancefield Group A and a Lancefield Group D streptococcus), using sea catfish, menhaden, spot, Atlantic croaker and striped mullet as experimental fish. The fish were maintained in

aquaria containing 20 to 40 l of water (15 ‰ S; 22 to 25 °C) and were injected via the intra-peritoneal route with 0.1 ml of Todd-Hewitt broth to give a dose of 10 to 10^7 cells $fish^{-1}$. Strain 922 produced 100 % mortalities in menhaden and striped mullet injected with 10^4 to 10^6 bacteria. Mortalities in Atlantic croaker, sea catfish and spot ranged from 70 to 90 %, 0 to 40 % and 57 to 100 % respectively at a dose of 10 to 10^6 bacteria $fish^{-1}$. The results indicated that menhaden was the most susceptible and sea catfish the least susceptible of the fish species tested. Mortalities of Atlantic croaker and spot were observed when these species were injected with high (10^4 to 10^7 cells $fish^{-1}$) doses of *Streptococcus agalactiae, S. faecalis* and *S. liquefaciens,* a finding which prompted Cook and Lofton (1975) to conclude that such non-fish-related streptococci 'may simply have overpowered the protective mechanisms in some fish and caused death'. Their findings confirm, however, that *Streptococcus* sp. Strain 922 is pathogenic to euryhaline fish.

Streptococcal infections of marine fish such as yellowtail (*Seriola* spp.) are of such importance to aquaculture operations in Japanese coastal waters that the Japanese Society of Fish Pathology organized a Symposium on the subject at the University of Mie, during October 1971 (Japan, 1982). Kusuda and Komatsu (1978) carried out a series of comparative studies on 16 strains of streptococci isolated from disease outbreaks affecting yellowtails, eels, and other marine and fresh water fish in Japan and USA. The American strains were identified as *Streptococcus agalactiae,* whereas the Japanese strains — in spite of the fact that they could not be classified serologically as members of Lancefield's Group D — bore many features in common with both *S. faecalis* and *S. faecium,* being classified as a new species. It is of interest to mention that Hoshina and co-authors (1958) isolated a non-haemolytic Lancefield Group D streptococcus, classified as *S. faecalis,* from a disease outbreak affecting rainbow trout at a fresh water fish farm in Japan. In the absence of any obvious faecal contamination of the incoming water, it was concluded that the infection may have arisen from the use of contaminated food.

The pathogenicity of streptococcal infections of yellowtail has been investigated with reference to *Streptococcus* sp. Strain YT-3 by Kusuda and Kimura (1978). Following oral administration, the bacteria gave rise to high numbers of organisms in the blood and internal organs within a 10 min period, but had been eliminated from all organs except the intestine after 24 h. When administered by the per-cutaneous route, the streptococci were retained at a level of 10^7 cells g^{-1}, in the kidney tissue, but decreased from 10^5 to 10^6 cells g^{-1} in other organs between 10 min and 24 h of administration, following which the numbers rapidly increased and produced an infection. An interesting observation by Kusuda and Kimura is that the intestine yielded counts of 10^7 cells g^{-1} 72 h after inoculation, a finding which bears a close relation to that of Kanai and co-authors (1977) where feeding Japanese eels had an intestinal microflora composed of 20 to 80 % streptococci.

With reference to streptococcal infections of yellowtail, Shiomitsu and co-authors (1980) demonstrated that all strains of *Streptococcus* sp. isolated from disease outbreaks in 1 yr old specimens from marine fish farms in Kagoshima and Kochi Prefectures were sensitive *in vitro* to ampicillin, chloramphenicol, erythromycin and tetracycline. The minimum inhibitory concentration (M.I.C.) of erythromycin was 0.1 to 0.2 mcg ml^{-1}. A dose of erythromycin incorporated into the food at a concentration of 25 to 50 mg kg^{-1} live weight d^{-1} was capable of controlling clinical outbreaks of the infection, and of curing infections not controlled by ampicillin. No clinical abnormalities were observed in a

population of approximately 1,000,000 yellowtails treated with erythromycin, from which Shiomitsu and co-authors concluded that the compound appears to hold promise for the practical control of streptococcal infections in species of yellowtail reared in sea water.

A beta-haemolytic streptococcus, reported as being identical to that causing disease and mortalities in yellowtails, has been isolated from ayu *Plecoglossus altivelis* and amago salmon *Oncorhynchus rhodurus* cultured in fresh water ponds in Tokushima Prefecture, Japan, by Ohnishi and Jo (1981).

To conclude this section, mention must be made of the work of Kusuda and Sugiyama (1981), by whom a staphylococcus was isolated as aetiological agent of disease epizootics among cultured red sea bream *Chrysophrys major* and yellowtail *Seriola quinqueradiata* in Japan during 1976–1977. The diseased fish showed clinical signs of exophthalmos, congestion and swollen lesions on the caudal peduncle. On the basis of morphological, biological and biochemical studies undertaken with 6 strains of the organism, it was identified as *Staphlococcus epidermidis.* Three strains from red sea bream were classified as Baird-Parker's sub-Group II, whereas 3 strains from yellowtails were classified as Baird-Parker's Staphylococcus sub-Groups II, V and VI respectively.

Acid-fast Bacterial Infections

The term 'tuberculosis' is generally used to describe a chronic disease of man and other vertebrates caused by bacteria belonging to the genus *Mycobacterium.* These organisms are termed acid-fast because of their ability to resist the decolorizing action of alcohol and mineral acids. Tuberculosis *sensu stricto* is a disease characterized by the presence of cellular lesions, or tubercles, produced by the irritating action of bacteria in the sites where they are held and ingested by phagocytes. The tubercle frequently undergoes a process of caseation which commences in the centre and gradually extends to the outer margin of the nodule. Miliary tuberculosis occurs when the mycobacteria are released into the blood stream from the primary focus of infection, thereby reaching other organs such as kidney, liver and spleen, in which they produce secondary foci of infection. Because several differences have been reported in the clinical manifestations of mycobacterial infections in man and fish, as well as in the morphology of the corresponding aetiological agents, Parisot and Wood (1960) proposed that the disease in fish should be referred to as 'fish mycobacteriosis' rather than 'fish tuberculosis'. The present writer, however, has elected to retain the latter term since it is possibly more familiar to the general reader.

The first workers to investigate the occurrence of tuberculosis in marine fish were Bertarelli and Bocchia (1910), who failed to detect acid-fast bacterial infections in a number of wild-caught sea fish on sale in Italian fish markets. They concluded, therefore, that the disease is either absent or extremely rare in marine fish.

Alexander (1913) and Johnstone (1913) have the distinction of being the first to describe a naturally occurring case of tuberculosis in a marine fish. The species concerned was the cod *Gadus morhua* captured at sea and landed at the port of Fleetwood, England. Alexander described the case as resembling lupus in man, and isolated acid-fast bacteria from a dark-coloured patch on the skin of the fish, without being able to identify the isolate. Johnstone reported cutaneous lesions affecting caudal peduncle, fins and skin of the cod, and noted that these lesions measured up to 5 mm in diameter, were circular in shape, slightly rasied, and of a dark greyish to black colour. The case was investigated

histologically, and Johnstone reported evidence of morbid tissue formation in the cutaneous lesions of a type indicative of an infectious granuloma. The skin lesions, many of which appeared to be healing, were described as of a tubercular nature which principally affected the thick layer of coarse fibrous connective tissue of the integument. Several masses of acid-fast bacteria lying within small cavities surrounded by concentric layers of fibrous tissue were present, and in some instances encapsulated masses of bacteria were also detected. Cellular elements termed giant cells were similarly described, but could not be positively identified as giant cells *sensu stricto,* since they mainly consisted of ruptured erythrocytes, fragments of necrotized tissue elements and dense masses of acid-fast bacteria. The granulomatous tissue, which was not highly vascularized, contained melanin granules either in stellate form or, more frequently, as discrete rounded bodies.

Sutherland (1922) studied a naturally occurring case of tuberculosis in halibut *Hippoglossus hippoglossus,* and noted the presence of numerous greyish-white nodules in liver and spleen of the fish. Similar nodules were also detected in the ovary, where they had produced a moderate degree of caseation. The gastro-intestinal tract was normal in appearance, but muscular and sub-cutaneous tissues were of a yellowish colour and soft to the touch. In liver, ovary and spleen, a total replacement of the glandular tissue had occurred in many areas by the formation of a fibrous stroma composed of masses of fasciculi of flattened cells. Several tubercular lesions were present in these fasciculi, and were composed of a central group of round cells with well-stained nuclei, surrounded by concentric layers of flattened cells which gradually merged with the stroma. Different degrees of necrosis were apparent. The superficial lesions in the muscle tissue were located in the loose connective tissue between the muscle bundles, and in these locations the tubercles were more distinctly circumscribed than in the internal organs. Acid-fast bacteria were demonstrated in the tubercles and in the affected tissues. Johnstone (1927) encountered a further case of tuberculosis in a halibut, and in this specimen the ovary was small and undeveloped and the kidney was swollen and covered with yellowish-coloured tubercles and granulomatous masses. The histological structure of the lesions present in the kidney and the ovary was interpreted as being characteristic of a tuberculosis-like granuloma. Fibrosis had occurred to a marked extent, and the nodules were filled with a caseous substance. No giant cells were detected, but small numbers of acid-fast bacteria were demonstrated in the lesions.

Aronson (1926) investigated the causes of mortalities in various species of fish maintained in aquaria at the Philadelphia Zoo, in USA. An acid-fast bacterium designated *Mycobacterium marinum* was isolated from livers and spleens of croakers *Micropogon undulatus,* sea bass *Centropristes striatus* and sergeant-majors *Abudefduf mauritii* affected by the disease. Eyes, gills, kidneys, ovaries, spleens and pericardia had greyish-white tubercular nodules measuring up to 18 mm in diameter. Circular areas of focal necrosis, not sharply circumscribed, were detected in the liver but seemed to bear little relation to the vascular supply. Occasional giant cells were observed in the periphery of these areas of focal necrosis, and in certain cases the normal liver cells had been replaced by large mononuclear cells in which the nucleus had ruptured or had disappeared altogether. These mononuclear cells contained masses of acid-fast bacteria, which also occurred in clumps in the necrotized areas. The spleen showed evidence of necrosis, as did the endothelial cells of the blood vessels — the lumena of which were replete with acid-fast bacteria and yellow-coloured pigment granules. Winsor (1946) described tubercular

lesions from a single specimen of smelt *Osmerus mordax* and a single specimen of weakfish *Cynoscion regalis* from Philadelphia, USA; both specimens had been obtained as wild-caught fish from a local market.

A survey of halibut landed at British ports failed to reveal cases of tuberculosis (Anon., 1962, 1963, 1964, 1965), and over the decade 1940–1950 only 6 cases of the disease were observed among marine fish landed at Hull, England, one of which was in a plaice *Pleuronectes platessa* and the remaining 5 were in turbot *Rhombus maximus* (Rhodes, cited by Hodgkiss and Shewan, 1950).

Reichenbach-Klinke (1955c) detected cases of tuberculosis in fish from marine aquaria. Clinical signs of the disease included ulcerative lesions on jaw and skin, and a granular appearance of liver and spleen. In sea perch *Morone labrax,* numerous flattened and shrunken cells were detected in histological sections from the affected skin tissue. The epidermis was 'notched' and the mucus bed dark and thin. Giavenni (1982) found that tuberculosis was present in 130 of 653 specimens of tropical marine aquarium fish investigated in Italy, corresponding to 41 different species, and identified the aetiological agent as *Mycobacterium marinum.* Conroy (1965) reported a case of tuberculosis in a single specimen of horse mackerel *Trachurus picturatus* caught in the South-West Atlantic Ocean. No obvious disease signs were visible externally, but when the fish was opened the presence of several discrete nodules containing acid-fast bacteria was detected in the muscle tissue adjoining the vertebral column.

Bucke (1980) described an acid-fast bacterial infection from a single specimen of mackerel *Scomber scombrus* caught in British waters; further studies on the disease in this teleost species have recently been published by Hastings and co-authors (1982). These workers carried out routine examinations on specimens of mackerel from the northern part of the North Sea, the western English Channel and the Minche. Tubercular nodules were detected in heart, kidney, liver, spleen and walls of the gastro-intestinal tract, in addition to the mesenteries and connective tissue, in several of these fish. No marked variation was reported in the prevalence or distribution of the disease from the sampling sites, and the incidence of the disease was 80 to 100 % in mackerel of the 1 + and older age groups. On a histological basis, Hastings and co-authors were able to distinguish 3 different types of nodule, designated Types A, B and C respectively, in kidney and spleen. Type A nodules possessed an intact centre and were completely surrounded by a discrete capsule composed of epithelioid cells and fibrous tissue. These nodules also contained numerous melano-macrophage cells with acid-fast bacteria. Type B nodules possessed a solid caseous necrotic mass in the centre, frequently containing a few acid-fast bacteria and melanin granules; the mass was encompassed by a fibrous capsule surrounding a discrete layer of epithelioid cells. Type C nodules closely resembled Type B nodules, the main difference being that in the former the contents of the epithelioid cells and fibrous capsule were 'of a more amorphous appearance'. Hastings and his colleagues postulated that the acid-fast bacteria in kidney and spleen of the mackerel may be carried within macrophages towards the melano-macrophage centres from whence the nodules develop in the renal and splenic tissue. Nodules in heart, liver and mesenteric tissues were similar to Type B and C nodules of kidney and spleen, save that the former did not contain melanin, a finding which was interpreted as an indication that such nodules can occur in other tissues even in the absence of melano-macrophage centres. The contents of Type B and C nodules gave a positive periodic acid-Schiff (PAS) staining reaction. Hastings and co-authors found that 2, 3 and 5

yr old mackerel affected by tuberculosis showed evidence of a decreasing body length in relation to an increasing intensity of infection. They also observed that a higher proportion of females than males was affected. Neither Bucke (1980) nor Hastings and co-authors (1982) detected giant cells in the cases of tuberculosis which they investigated in mackerel.

The question as to whether giant cells are involved or not in fish tuberculosis has recently been investigated experimentally by Timur and co-authors (1977) with reference to plaice *Pleuronectes platessa*. Twelve 1 yr old plaice were obtained from a marine fish farm and were injected by the intra-muscular route with a saline suspension of *Mycobacterium* sp. (NCMB 1484) grown on Dorset's egg medium. This strain had originally been isolated from a case of tuberculosis in halibut. The experimentally-infected plaice were maintained at 10 °C and were sacrificed over a 12 to 60 days period post-infection. The first lesions produced included muscle-fibre necrosis extending over a wide area of the myotome. Destruction of myofibrils was observed, and macrophages were present. Occasional lymphocytes, polymorphonuclear neutrophils and plasma cells were detected, and acid-fast bacteria were present within the macrophages and free among the cells. By Day 18 post-infection, masses of macrophages containing acid-fast bacteria were present and some of them showed a typical epithelioid cell morphology. Occasional Langhans-type multinucleate giant cells were reported at this stage, the numbers of which increased by Days 24 and 28 post-infection. The typical epihtelioid granuloma of fish tuberculosis was present from Day 25 of the experimental infection, and thereafter the lesion was characterized by the presence of numerous typical epithelioid granulomata or tubercles, some of which had a central focus of caseation containing numerous acid-fast bacteria. The giant cells became less frequent after 28 days, and by Day 60 the lesion was formed primarily of fibrious tissue, epithelioid cells and central necrotic foci containing large numbers of acid-fast bacteria. Numerous lymphocytes were also present in the stroma of the tubercles, and this infiltration coincided closely with the development of caseation in the centres of the granulomata. The paper by Timur and co-authors is an important contribution to our understanding of the pathogenesis of fish tuberculosis, since it confirms that mycobacteria of piscine origin are capable of inducing the transient production of Langhans-type giant cells in marine teleosts during the course of an infection. Timur and co-authors suggested that the failure to detect giant cells in cases of tuberculosis of marine and migratory fish studied by other workers may be explained by the fact that these cases were advanced stages of the disease at which giant cell production had been suppressed.

Sato (1962) carried out histological studies of tuberculous nodules from several types of marine fish, and demonstrated that these were granulomata in which the acid-fast bacteria occurred both in and around the monocytes. This worker observed 2 types of nodule; the first was a slowly-developing lesion composed of epithelial cells with a surrounding fibroblast capsule, the second, a rapidly-produced lesion containing large numbers of histiocytes filled with acid-fast bacteria.

Interest in tuberculosis of migratory fish has centred principally on the finding of the disease in anadromous salmonids from the Pacific coast of North America. The presence of acid-fast bacteria in the kidney tissue of chinook salmon *Oncorhynchus tshawytscha* was first reported by Earp and co-authors (1953) for adult fish ascending the Columbia River from the sea during 1952. The infection was further observed in migrating chinook salmon in the Williamette River in 1953, 1954, 1955 and 1956 (Wood and Ordal, 1958) as well as in *O. keta, O. kisutch, O. nerka* and rainbow trout from coastal and lacustrine US waters of

Alaska, California, Idaho, Oregon and Washington; (Ross and co-authors, 1959; Ross, 1963).

Parisot (1958) gave a general description of the clinical signs and gross pathology of salmonid tuberculosis, and stated that the external signs usually include abnormally bright silver coloration, stunted appearance, and lack of normal development of the gonads. Numerous discrete greyish-white granulomata are present in the kidney, the posterior portion of which is swollen and shows evidence of necrosis. Similar granulomata are present in liver, spleen, pyloric caeca and wall of the intestine. These necrotic foci contain masses of acid-fast bacteria. The histopathology of tuberculosis in chinook salmon was described by Parisot and Wood (1960). In juvenile salmon, the disease was characterized by the presence of a massive infection involving the adipose connective tissue surrounding the intestine and in the serosa, in which the bacteria formed layers. No evidence of inflammation was detected either in early or in advanced infections. The haematopoietic tissues of the anterior and mid-kidney, liver and spleen contained small groups of acid-fast bacteria within the cytoplasm of fixed macrophages.

In older fish, the disease had extended well beyond the stage observed in juveniles. Massive involvement of kidney, liver and spleen was present, and the gills and cardiac tissue of adults were also affected by areas of focal necrosis associated with the presence of acid-fast bacteria. The total absence of an inflammatory response in salmonids infected by tuberculosis, as reported by Parisot and Wood (1960), has recently been questioned by Timur and co-authors (1977) on the grounds that the material examined may have been from an advanced stage of the infection beyond that when giant cells are prevalent. The presence of a giant cell response in fish, as demonstrated by Timur and co-authors, provides further grounds for utilization of the term fish tuberculosis in preference to fish mycobacteriosis when reference is made to this condition.

All workers who have thus far described cases of tuberculosis from marine and migratory fish reported the presence of acid-fast bacteria in the lesions, even in instances when these bacteria have not been actually isolated. The genus *Mycobacterium* comprises Gram-positive, acid-fast and non-motile rods which can usually be isolated *in vitro* from the lesions on Lowenstein-Jensen, Petragnani or other suitable medium. *Mycobacterium marinum* (Aronson, 1926) and *Mycobacterium salmoniphilum* (Ross, 1960) were described from marine fish and migratory Pacific salmon respectively. Several workers have detected the related genus *Nocardia* in fresh water fish and migratory salmonids (Valdéz and Conroy, 1963; Conroy, 1964; Snieszko and co-authors, 1964a; Campbell and MacKelvie, 1968; Ghittino and Penna, 1968). Japanese workers have reported that nocardiosis due to *Nocardia kampachi* is of major importance as a cause of mortalities in yellowtails (*Seriola quinqueradiata, S. purpurascens*) reared in marine fish farms (Kariya and co-authors, 1968; Kubota and co-authors 1968; Matsuzato, 1968). Wolke and Meade (1974) reported on cases of systemic nocardiosis detected in 2 chinook salmon smolts. The fish showed granulomatous oral masses which maintained the mouths open. These masses measured 1.0 × 0.75 cm in one specimen and 1.0 × 1.5 cm in the second specimen. Histopathological studies revealed that the masses were composed mainly of reticulo-endothelial cells closely resembling mammalian histiocytes, with a fine, sparse network of collagen fibres present within the cells. A fibrous capsule surrounded the mass. Small lymphocytes and erythrocytes were present in the tissue, but multinucleate giant cells were very infrequent. Randomly distributed necrotic foci were detected in the reticulo-endothe-

lial cell mass, and these foci were characterized by central areas of sparsely distributed necrotic debris and inflammatory cells surrounded by histiocytes. Granulomatous inflammatory tissue, necrotic areas and acid-fast bacteria were detected in gill tissue, myocardium, pericardium, spleen, kidney, pancreas, mesentery, pyloric caeca and anterior part of the gut. When the Fite-Faraco staining technique was used, the bacteria were filamentous, branched and beaded. Wolke and Meade commented that the proliferative tissue response, unique morphology and characteristic staining properties of the nocardiae provide a sufficient basis upon which to arrive at a diagnosis of nocardiosis in cases where it is impossible to isolate the aetiological agent. These workers suspected that, in this particular instance, the bacteria may have gained access to the fish through wounds in the buccal cavity, from which the infection subsequently spread systemically.

Yellowtails *Seriola quinqueradiata* were inoculated experimentally with *Nocardia kampachi* by Ikeda and co-authors (1976), and changes characteristic of a chronic suppurative inflammation were produced. The total leucocyte, neutrophil and monocyte counts increased, and other increases were detected in the A/G ratio, total protein and alkaline phosphatase levels of the serum. Slight increases were detected in the erythrocyte and reticulocyte counts and in the haemoglobin level of the blood. The effects of the organism on the kidney, liver and spleen were considered to be of limited significance, although it was concluded that increases in the neutrophil count, serum albumin level and alkaline phosphatase activity preceded the appearance of external clinical signs of the disease in the infected fish.

Kusuda and Nakagawa (1978) demonstrated that strains of *Nocardia kampachi* were capable of surviving for 1 to 2 days in sea water, but that survival increased to 6 to 8 days in water samples taken from the immediate vicinity of floating cages in which yellowtails were cultured. These workers injected yellowtails with heat- or formalin-killed bacteria and demonstrated the presence of serum antibody titres of up to 1:10,000 following 6 wk post-inoculation. Strict hygiene was recommended in view of the fact that survival of the nocardiae was enhanced by overcrowding of the fish, the presence of food in the water and factors which contributed to localized pollution.

In everyday practice, it is sufficient to make a tentative diagnosis of fish tuberculosis or nocardiosis on a basis of the demonstration of acid-fast bacteria in smears from the lesions and/or infected organs. A simple modification of the Petroff technique was successfully developed by Conroy (1966) for use in the detection of acid-fast bacteria in asymptomatic carriers in fresh water aquaria, and this technique has also enabled acid-fast bacteria to be detected in apparently healthy cod held in sea water tanks (Conroy, 1970).

Various hypotheses have been advanced in an attempt to elucidate the manner in which fish tuberculosis is transmitted. Winsor (1946) suggested that acid-fast bacteria may gain entry to fish maintained in aquaria through the use of infected marine fish provided as food. Ross (1959) and Wood and Ordal (1958) demonstrated conclusively that the incidence of tuberculosis in Pacific salmon was directly related to the practice of feeding untreated salmon offal to the young fish in the hatchery. Transovarian transmission of tuberculosis was demonstrated in the viviparous Mexican platyfish *Platypoecilus maculatus* by Conroy (1966), a finding of interest since Baker and Hagan (1942) had isolated *Mycobacterium platypoecilus* from this species of teleost, and also because the platyfish is a member of the family Cyprinodontidae which contains species possessing euryhaline characteristics that occur in brackish waters and in coastal environments. Transovarian

transmission of tuberculosis in chinook salmon, an oviparous species, could not be decisively demonstrated by Ross and Johnson (1962), although recent evidence from Australia (Ashburner, 1977) suggests that tuberculosis in this salmonid may be passed to the F_1 generation by the ovarian route, possibly by means of infected ovarian fluid. An interesting observation by Timur and co-authors (1977) is that *Mycobacterium* sp. (NCMB 1484) is capable of producing a generalized tuberculosis in hatchery-reared plaice when the bacterium is administered via the intra-venous route. Whatever the mode of transmission, fish tuberculosis is characterized by a chronic course which produces pronounced pathological changes in kidney, liver, spleen and reproductive organs, and thus it is likely that the acid-fast bacteria may possess a low degree of pathogenicity to the fish.

Anaerobic Bacterial Infections

The literature on fish pathology contains surprisingly few references to obligate anaerobes occurring as the aetiological agents of diseases or mortalities in fish from marine and other salt water environments. During 1976, however, mass mortalities linked with anaerobes affecting striped mullets *Mugil cephalus* and redfish or red drums *Sciaenops ocellata* from Biscayne Bay, Florida, and from the Texas coast of the Gulf of Mexico were investigated in USA. These observations are of particular interest to our knowledge and better understanding of the role of anaerobic bacteria in relation to epizootics affecting populations of fish in natural salt water environments.

Henley and Lewis (1976) isolated anaerobic bacteria which they tentatively classified as *Catenabacterium* sp. from blood, brain, kidney and liver of striped mullets and redfish affected by disorientation, debility and disordered swimming movements at the water surface. This condition was of epizootic proportions in the coastal waters of the State of Texas, and gave rise to massive mortality levels in these fish. The bacteria were isolated on thioglycollate agar and on salt bovine blood agar containing 40 mcg ml^{-1} of gentamycin. Both of these were useful as selective media for primary isolation of the bacteria. The anaerobe was experimentally pathogenic to striped mullets and sea catfish *Arius felis,* but not to channel catfish *Ictalurus punctatus* or to white mice.

Udey and co-authors (1976, 1977) reported that the salient clinical manifestations of the epizootic which they investigated in striped mullets from Biscayne Bay included the presence of an abnormal behaviour in the fish characterized by 'twirling', a feature assumed to indicate impairment of the normal neurological functions. Few other external pathological signs were in evidence in live or moribund mullets. The examination of stained sections of the brain revealed the presence of large Gram-positive rods with a tendency to form long unbranched filaments, distributed throughout the brain tissue. Samples from the brain and other organs were aseptically removed and cultured on brain heart infusion agar and in Brewer's thioglycollate medium incubated at room temperature for 7 days. Obligate asporogenous anaerobes were isolated in pure culture from the brain. Udey and co-authors also isolated 'occasional vibrios, biochemically resembling but serologically distinct from *Vibrio anguillarum*' in samples taken from liver and kidney tissue of the mullets on plates incubated under anaerobic conditions.

Five representative cultures from the brains of 5 striped mullets were selected for further study and characterization. One of these cultures was deposited with the American Type Culture Collection as ATCC 29255. The organism has been classified as *Eubacterium tarantellus* n. sp.; its characteristics are summarized in Table 1-12.

Table 1-12
Principal characteristics of *Eubacterium tarantellus* strain ATCC 29255 (Based on Udey and co-authors, 1977)

Test	Reaction
Gram	+
Production of spores	–
Motility	–
Catalase	–
Fermentation of glucose	(+)
Fermentation of lactose	(+)
Fermentation of maltose	(v)
Fermentation of mannitol	–
Fermentation of mannose	(v)
Fermentation of arabinose	–
Fermentation of amygdalin	–
Fermentation of cellobiose	–
Fermentation of fructose	(+)
Fermentation of melezitose	–
Fermentation of raffinose	–
Fermentation of rhamnose	–
Fermentation of salicin	–
Fermentation of sucrose	–
Fermentation of trehalose	–
Fermentation of xylose	–
Hydrolysis of starch	–
Hydrolysis of aesculin	–
Deoxyribonuclease production	+
Hydrolysis of gelatine	–
Lecithinase production	+
Lipase production	–
Reduction of nitrate	–
Production of indole	–
Production of hydrogen sulphide	–
Production of NH_4	–
Haemolysis of sheep blood	+ (beta)
Growth temperature (°C)	25 (15–40)
Salinity growth range (%)	0.5–2.0
pH growth range	5.6–8.0
Sensitivity to erythromycin	+
Sensitivity to chloramphenicol	+
Sensitivity to gentamycin	–
Sensitivity to kanamycin	–
Sensitivity to neomycin	–
Sensitivity to novobiocin	+
Sensitivity to penicillin	+
Sensitivity to polymyxin B	–
Sensitivity to streptomycin	(+)
Sensitivity to tetracycline	+
Sensitivity to vancomycin	+

(+) Weak positive reaction; (v) variable reaction

Henley and Lewis (1976) had tentatively classified 2 strains of the anaerobe which they isolated from moribund striped mullets and redfish captured in Texas coastal waters as *Catenabacterium* sp. Udey and co-authors (1977), after pointing out that the genus *Catenabacterium* Prévot, 1938 is no longer recognized as valid, considered that the similarities between the characteristics of the strains isolated in Texas and those isolated in Florida respectively were such that all of these isolates should rightly be placed in the genus *Eubacterium* Prévot, 1938. This proposal is strengthened by the finding that both the Texas and the Florida isolates possess a common antigen, as demonstrated by the indirect immuno-fluorescent technique, and on this basis Udey and co-authors suggested the possibility of these strains being biotypes or biovariants of the species *E. tarantellus*. Udey and his colleagues also isolated *E. tarantellus* from the brain tissue of no less than 10 additional, although unnamed, estuarine species of fish from Florida, without detecting the anaerobe in strictly marine teleosts which never enter bays or other environments subject to fluctuating salinities.

In discussing their findings, Udey and co-authors (1977) point out that the failure of *Eubacterium tarantellus* to grow in salinities above 2 % sodium chloride may indicate that the organism is limited to estuarine, rather than to marine environments. They suggested that the anaerobe grows slowly in the host fish, as evidenced by the fact that neurological manifestations are chiefly present in mature specimens. On the other hand, they made the alternative suggestion that the bacteria may cause a sub-clinical or chronic disease which only becomes manifest when the infected fish are subjected to stress.

With specific reference to possible stress factors in relation to these mass mortalities of striped mullets in Florida and Texas, mention must be made of the histozoic myxosporidian *Myxosoma cephalis,* first reported and described by Iversen and co-authors (1971) from dead and moribund striped mullets collected during 1964 from water with a salinity of 16 to 45 ‰ in the Everglades National Park, Florida, USA. Cysts of this parasite were detected in the cerebral meninges, gill arches, buccal cavity, jaw bone and crop tissues of the mullets, a finding which led Iversen and co-authors to speculate that the myxosporidian 'may have been responsible for the epizootic'. Skinner (1975) subsequently reported the presence of *M. cephalis* from gill arches, gill filaments, outer walls of the oesophagus, stomach and intestine, as well as on the surface of the liver, in mesenteries and in the brain of striped mullets from Biscayne Bay. According to Skinner 1 specimen of striped mullet had skin lesions above the mouth and soft skull bones, and cysts and individual spores of *M. cephalis* were detected in the brain cavity and in the brain tissue itself. Approximately 20 % of the striped mullets examined by Udey and co-authors (1977) harboured spores of *M. cephalis* in their brain cavity, in addition to low levels of the digenetic trematode *Bucephalus* sp. in a small (unquantified) sample of the fish. The precise role played by the myxosporidian in the aetiology of this 'twirling' syndrome cannot be ascertained, since pathogenicity experiments have not been carried out with the parasite. However, as Udey and co-authors (1977) reported, *Eubacterium tarantellus* is experimentally pathogenic to channel catfish, though not to guinea pigs, so that the available evidence points to the bacterium as being the prime aetiological agent of the condition.

The pathology of what was described as a case of 'whirling disease' was investigated by Brown (1970) in 1 specimen of 20,000 juvenile pompanos *Trachinotus carolinus* maintained in an outdoor pool provided with circulating sea water in the State of Florida, USA. The pompano showed signs of continuous anti-clockwise whirling movements. Abnor-

malities were confined almost exclusively to the cerebellum, the structure of which had been markedly disrupted and showed signs of encephalomalacia. Groups of several hundred bacteria each, and nervous system cellular debris, were encountered in the affected area of tissue. The single histological section examined by Brown had been stained with haematoxylin and eosin, so it became necessary to destain and restain it by the MacCallum-Goodpasture technique, with which groups of Gram-positive rods were demonstrated. In other affected areas, however, the rods appeared Gram-negative, a finding which led Brown to conclude that Gram-variable bacteria were involved although, as he pointed out, the irregular staining characteristics may have been due to the fact that the section had been destained and then restained. It was concluded that the condition was a bacterial encephalitis with cerebellar encephalomalacia associated with dysfunction of the central nervous system. Whilst bacteriological studies were not carried out with the pompano, it is tempting to associate the pathological manifestations of the 'whirling disease' reported by Brown (1970) with those described by Udey and co-authors (1976, 1977) and by Henley and Lewis (1976) in striped mullets and redfish infected by anaerobic bacteria.

The potential importance of these observations cannot be underestimated, since the fish pathologist now has at his disposal a methodology for the detection, isolation and characterization of *Eubacterium tarantellus* and other obligate anaerobic bacteria from estuarine and marine fish. This methodology will henceforth need to be utilized on a routine basis in all investigations relating to epizootics and 'fish kills' involving fish in coastal and estuarine waters.

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Agents: Fungi

G. LAUCKNER

Fungus diseases of fish are of considerable concern in freshwater. Most of the known fish-pathogenic fungi are members of the class Oomycetes. A few belong to the Deuteromycetes (Fungi Imperfecti) or the Ascomycetes, or are of uncertain taxonomic status and presently not classifiable. Recently, Neish and Hughes (1980) have critically reviewed the vast body of literature existing on the fungal diseases of fish. As expected, most of the information stems from freshwater species.

Although a vast number of fungi — pathogenic and non-pathogenic — exist in oceans and coastal waters (Johnson and Sparrow, 1961), relatively few species have been reported to occur in marine fish. Only a single species (or species complex), *Ichthyophonus (hoferi),* is known to be of ecological significance and to cause economic losses among commercially exploited stocks (Sindermann, 1966, 1970a).

The organism(s) commonly considered to represent *Ichthyophonus* display(s) a perplexing physiological adaptability to a wide range of environmental conditions and hosts. It has thus far been reported from more than 80 species of freshwater and marine fish from tropical to temperate waters (Lederer, 1936; Reichenbach-Klinke, 1954, 1956a, 1957b, 1980). There has been much confusion with respect to the identity of this organism, which has a long and fascinating history, full of errors and misinterpretations. The literature reflects the notorious habit of writers and reviewers of scientific publications to avoid the study of original publications (by some disqualified as 'fossil literature'!) and to copy misquotations and false informations from each other. As one of the consequences of this disgusting conduct, the organism(s) in question is (are) sometimes referred to as *Ichthyophonus* and, at other times, as *Ichthyosporidium*. The latter genus, however, is a valid taxon in the Microspora (see below).

Although *Ichthyophonus* is generally classified as an entomophthoracean phycomycete, there is no definite evidence favouring this viewpoint. It might not be a fungus at all. In a review of the taxonomy of the Entomophthorales, Waterhouse (1973) listed *Ichthyophonus* as a doubtful genus. Neish and Hughes (1980, p. 7) even concluded:

> "We have no clear conception of what *Ichthyophonus hoferi* really is, and although it is called a fungus by most researchers working with it, there is little firm evidence to support this viewpoint."

Ichthyophonus hoferi was named by Plehn and Mulsow (1911) in honour of B. Hofer (1893), who first reported the agent from diseased cultivated brown trout *Salmo trutta* and brook trout *Salvelinus fontinalis* in Germany, and who described the disease produced by this organism. Although being brief and devoid of illustrations, Hofer's description is very clear. Upon autopsy of diseased fish, tiny white 'cysts' became discernible at the surface of affected organs, mainly the kidney, liver and heart. When ruptured, these cysts liberated large numbers of very small 'grains' believed to be 'sporozoan' spores. Hofer tentatively assigned the organism to the Gregarinia — not to the Fungi, as corroborated even by modern textbook authors (e. g., Reichenbach-Klinke, 1980).

Hofer (1893) did not apply a specific name to the disease syndrome described by him but merely stated that affected trout typically perform 'torkelnde Bewegungen' ('staggering movements'). Subsequently, in his textbook on fish diseases, Hofer (1904) termed the condition 'Taumelkrankheit' ('stagger disease'), which now stands, in the literature, as a synonym of *Ichthyophonus* disease. As already pointed out by Plehn and Mulsow (1911), 'staggers' are not a characteristic sign of the disease. Therefore, 'Taumelkrankheit' or 'staggers' should be replaced by 'ichthyophoniasis', a term correctly applied by Schäperclaus (1979).

Apparently unaware of Hofer's (1893) brief note, Caullery and Mesnil (1905a, b) described 2 'haplosporidians' from marine teleosts at Wimereux and Saint-Martin, France — *Ichthyosporidium gasterophilum* from 5-bearded rocklings *Motella (= Ciliata) mustela* and sea snails *Liparis liparis,* as well as *I. phymogenes* from gilt-head *Crenilabrus melops.*

Doflein (1909) was the first who linked Hofer's (1893) 'gregarines' with Caullery and Mesnil's (1905a) 'haplosporidians' *Ichthyosporidium* spp., thereby clearing the way for an error which has persisted in the literature until today. Similarly, Robertson (1908, 1909) reported on a 'haplosporidian' of the genus *Ichthyosporidium,* which she first saw in marine teleosts — in a flounder *Platichthys flesus* and in a haddock *Melanogrammus aeglefinus* — and subsequently found to cause fatal disease in sea trout *Salmo trutta.* The British author is now generally believed to have been studying a fungus, in all probability *Ichthyophonus* (Sprague, 1965). There are several other early reports on the occurrence of what might have been *Ichthyophonus* or a closely related fungus in marine fish hosts. Thus, Johnstone's (1906) report on a presumed entomophthoracean fungus, believed to be related to the (plant-parasitic!) genus *Conidiobolus,* is highly suggestive of *Ichthyophonus.* Williamson (1913) described an organism from *Melanogrammus aeglefinus, Gadus morhua* and *Brosme brosme* in Scottish waters under the name *Dokus adus.* According to Reichenbach-Klinke (1957b) and Priebe (1973) this was clearly an *Ichthyophonus* infection. Alexeieff (1914) restudied what he believed to be Caullery and Mesnil's (1905a) *Ichthyosporidium gasterophilum* in *Ciliata mustela* from Roscoff, France. Unaware of the above records, Ellis (1928), describing an *Ichthyophonus* infection in *Pseudopleuronectes americanus,* claimed to report the fungus for the first time from the New World and from a marine host. As early as 1916, however, P. Cox had shown the presence of the fungus (which he regarded as a myxosporidian) to be associated with mass mortalities of herring in the Gulf of St. Lawrence.

In 1910, Laveran and Pettit reported on a epizootic disease of trout in France, which they believed to be identical with the condition reported by Hofer (1893) in Germany. Although exhibiting distinct affinities to the plant kingdom, the causative agent was tentatively assigned to the 'Haplosporidia'. Subsequently, Mulsow (1911) and Plehn and Mulsow (1911) reisolated what appeared to be the same organism from diseased trout, clearly demonstrated its fungal nature by means of cultivation in artificial media, and named it *Ichthyophonus hoferi.* The authors likewise believed it to be identical with Hofer's (1893) agent. Although making reference to Caullery and Mesnil's (1905b) publication, they did not, however, associate their agent with the latter authors' *Ichthyosporidium.* Pettit (1911), in spite of agreeing with Plehn and Mulsow (1911) in that the organism in question is a phycomycetous fungus, used both generic designations, *Ichthyosporidium* and *Ichthyophonus,* interchangeably, but in a later publication (Pettit, 1913) challenged the nomenclature in naming the fungus *Ichthyosporidium hoferi.*

Neresheimer and Clodi (1914), who reexamined *Ichthyophonus hoferi* in great detail, confirmed and extended Plehn and Mulsow's (1911) findings and emphasized the organism's distinctiveness from Caullery and Mesnil's (1905a, b) *Ichthyosporidium*. Concomitantly, Swarczewsky (1914), who restudied *Ichthyosporidium phymogenes* Caullery et Mesnil, 1905, in detail, believed it to be identical with a microsporan placed by Thélohan (1895) among the 'myxosporidiens glugéidées' and initially named *Glugea gigantea*, a view later confirmed by Sprague and Vernick (1968, see below). In a previous study, Swellengrebel (1911, 1912) had already restudied the same parasite, which he included in the microsporan genus *Pleistophora* as *P. gigantea*.

Léger and Hesse (1923) found a pathogenic fungus, apparently exclusively confined to the intestinal tract of trout, which they believed to be related to Caullery and Mesnil's (1905a, b) 'haplosporidian' *Ichthyosporidium gasterophilum* but to differ from Hofer's (1893) agent, and which they reclassified as *Ichthyophonus intestinalis*. In 1924, Léger described what he considered a different species from the digestive tract of *Lota lota*, another freshwater fish, and named it *I. lotae*. Léger (1927, 1929b) noted close similarities between *Ichthyophonus* and *Basidiobolus* spp., fungi parasitic in amphibians, reptiles and fish. He (1929a) even used the designations *'Ichthyophonus (Basidiobolus) hoferi'* and *'Basidiobolus (Ichthyophonus) hoferi'* interchangeably. At the end of a discussion of the *Basidiobolus — Ichthyophonus* problem, Neish and Hughes (1980, p. 64) concluded that

> "there is little reason to suspect that *Basidiobolus* and *Ichthyophonus* will ever be shown to be congeneric."

In spite of the evidence which became available through the detailed studies of Plehn and Mulsow (1911), Neresheimer and Clodi (1914), Swarczewsky (1914) and Léger (1924, 1927, 1929a, b), subsequent workers remained unaware of the fact that *Ichthyosporidium* and *Ichthyophonus* refer to 2 totally different organisms — a protozoan and a fungus — and continued to apply both generic names interchangeably to *Ichthyophonus hoferi*, or even listed *Ichthyophonus* as a synonym of *Ichthyosporidium*.

Eventually, Sprague (1965) made it clear that there are *two* original species in the genus *Ichthyosporidium* Caullery et Mesnil, 1905. In all probability, and as far as the French authors' incomplete description permits to decide, *I. gasterophilum* is not a protozoan at all, but a fungus. *I. phymogenes*, in its turn, was found to be clearly a protozoan, either a haplosporidian or a microsporan. Consequently, Sprague transferred *I. gasterophilum* to the (fungus!) genus *Ichthyophonus* Plehn et Mulsow, 1911, and reserved *Ichthyosporidium* for the protozoans, selecting *I. phymogenes* as type-species. Later, Sprague and Vernick (1968) confirmed the specific identity of *I. phymogenes* with *Glugea gigantea* Thélohan, 1895, and *Pleistophora gigantea* Swellengrebel, 1911, which had been identified as a species of *Ichthyosporidium* (Swarczewsky, 1914; see above). After initially having expressed some doubts about the validity of genus *Ichthyosporidium* (Sprague, 1966, 1969), Sprague and Vernick (1974) reestablished *Ichthyosporidium* with *I. giganteum* as type-species. Sprague and Hussey (1980) finally demonstrated the identity of Schwartz' (1963) *Ichthyosporidium* sp. — a haplosporidian from spot *Leiostomus xanthurus* in Chincoteague Bay, Maryland — with the type-species and introduced a new concept of the structure and host-parasite relations of the genus. Sprague (1977a) lists *Ichthyosporidium* in the family Nosematidae.

Hence, there can be no doubt that *Ichthyosporidium* is a valid genus in the Microspora, and that a fungus with the name *Ichthyosporidium hoferi* has never existed. The

genus *Ichthyophonus,* on the other hand, is clearly a taxon in the plant kingdom, with *I. hoferi* as type species (however, should it be proven that *'Ichthyosporidium gasterophilum'* Caullery et Mesnil is, in fact, a fungus of genus *Ichthyophonus,* and should it prove to be different from *I. hoferi,* then *Ichthyophonus gasterophilum* would have to be selected, by subsequent designation, as type species).

In spite of the information extractable from the above-cited original publications, and in particular from Sprague's (1965) clear delineation, the nomenclatorial confusion about this organism notoriously persists even in several of the present-day textbooks on fish diseases (Reichenbach-Klinke and Elkan, 1965; Amlacher, 1970; Mawdesley-Thomas, 1972; Roberts, 1978; Reichenbach-Klinke, 1980). Amlacher (1981) even went so far as to argue that, according to the rules of nomenclature, *Ichthyosporidium* has priority over *Ichthyophonus.* Richards (1977a, p. 149) reasoned:

> "There is considerable argument as to whether the organism responsible for this disease, *Ichthyophonus (Ichthyosporidium) hoferi,* is a haplosporidian parasite or a fungus."

As stated, *Ichthyophonus* occurs in freshwater and sea water as well. Apparently, its development proceeds along different lines, depending on host species and — probably — environmental factors, which has led to the distinction between 'salmonid' or 'cold-water forms', 'tropical freshwater-fish' or 'aquarium fish forms' and 'marine forms' (Schäperclaus, 1953a; Reichenbach-Klinke, 1956a, c, 1961; Herkner, 1961). At least some of these actually represent cases of mycobacterioses (see below). While it is generally believed that the freshwater and the marine forms of *Ichthyophonus* are conspecific, Johnson and Sparrow (1961) point out that *I. hoferi* has, apparently, never been formally circumscribed. The authors cautiously conclude (p. 557) that the extreme polymorphism of the various forms 'makes it highly possible that *"Ichthyosporidium hoferi"* is not a single species' — a view also shared by other workers. Thus, MacKenzie (1979, p. 5) states:

> "*Ichthyophonus* is the collective name for a fungus infection reported from many species of marine and freshwater fish. Most records are attributed to *Ichthyophonus hoferi* (Plehn & Mulsow), but it is not clear if this specific name embraces one or several species."

Neish and Hughes (1980) consider the name *Ichthyophonus hoferi* as a 'wastebasket' taxon with poorly defined species limits. In fact, there appear to be strong arguments in favour of the validity of the specific name *hoferi* for the freshwater or 'salmonid' form of ichthyophoniasis, while to the marine forms merely the generic name *Ichthyophonus* (in the collective sense) should be applied until it will be proven by application of several independent methods available today that both (or all) forms are specifically identical and referable to *I. hoferi.* Neish and Hughes (1980) feel that, on the basis of the information presently available, and the absence of extant cultures of organisms thought to be *I. hoferi,* a resolution of these problems is not possible.

Development and pathology of *Ichthyophonus hoferi* in freshwater fish have been studied by Plehn and Mulsow (1911), Neresheimer and Clodi (1914), Reichenbach-Klinke (1954), Dorier and Degrange (1961), and that of *Ichthyophonus* in marine fish by Daniel (1933a), Fish (1934), Sproston (1944), Sindermann and Scattergood (1954), Reichenbach-Klinke (1956c, 1957b) and Chien and co-authors (1979a, b, c). The general life-cycle pattern of the fungus in freshwater salmonids, as outlined by Dorier and Degrange (1961), may be summarized as follows (Fig. 1-1):

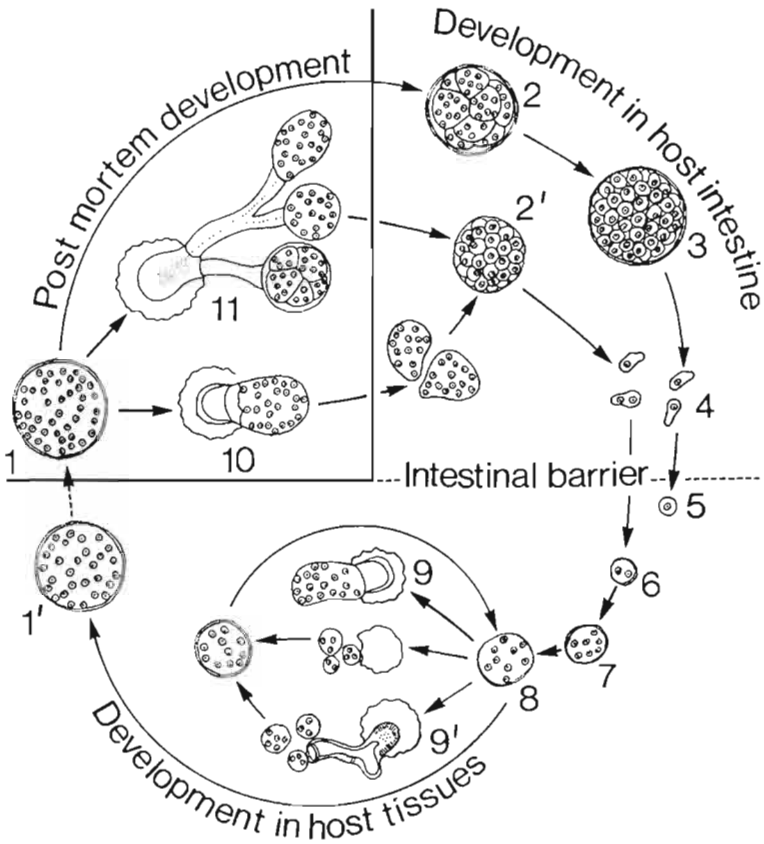

Fig. 1-1: *Ichthyophonus hoferi*. Life cycle. For explanation see text. (After Dorier and Degrange, 1961; modified.)

Infection of the suscept occurs *via* the alimentary tract by thick-walled multinucleate 'resting spores' ('kystes en latence'; Fig. 1–1:1, Fig. 1-2) liberated from disintegrating infected tissue. Division of their contents leads to the production of 'amoeboblasts' (Fig. 1–1: 2, 3) from which uni- or binucleate motile 'amoeboid forms' (4) develop. These plasmodia penetrate the intestinal barrier and are transported by the blood stream to their final location in host organs or musculature where they transform into initially uni- or binucleate spherical 'cysts' (5, 6), a process in which the host tissue actively participates. Rapid growth, accompanied by nuclear divisions and accumulation of reserve material (7, 8) eventually leads (in most cases) to the formation of new 'resting spores' (1'), which can reinitiate the cycle (1'–1). From some of these 'cysts' (7, 8), however, 'plasmodia' may hatch, which in their turn produce 'endospores'. These are either liberated by rupture of the spore wall (9) or by escape from the tips of irregular, stout, non-septate hyphae (9'). These 'endospores' reinitiate what may be termed an 'endogenous' or 'secondary' cycle. This mode of multiplication eventually produces 'resting spores' in enormous numbers, which rapidly overwhelm the entire host organism and may cause its death.

During post-mortem decay of infected host tissue, 'resting spores' may germinate into multinucleate plasmodia (10) or hyphae (11) which, in their turn, eventually lead to the

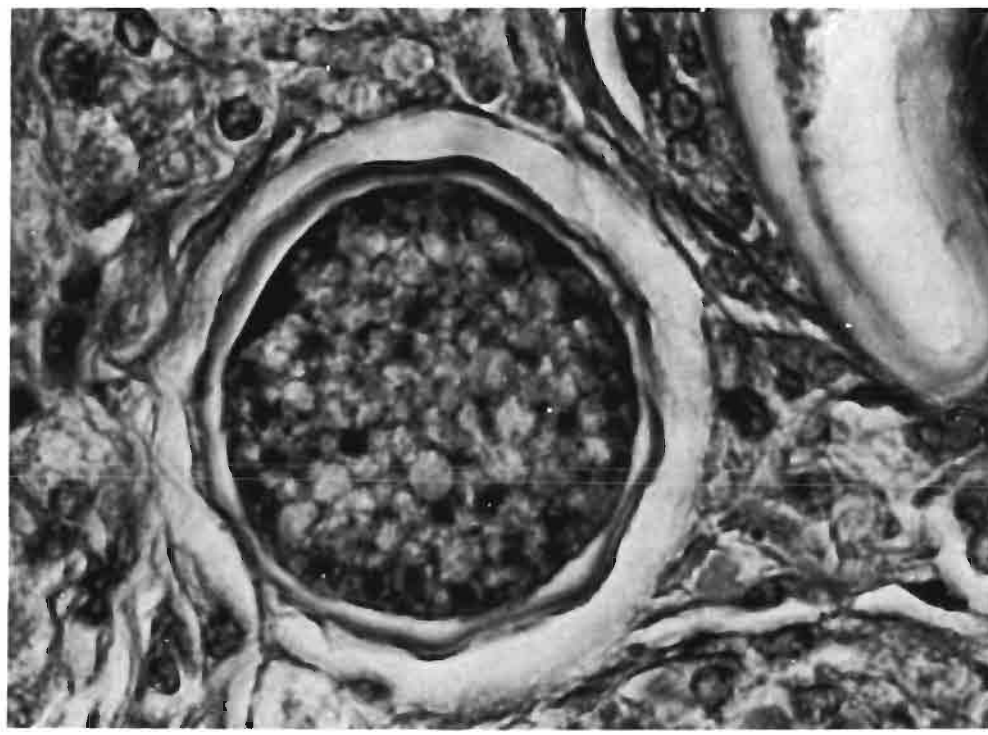

Fig. 1-2: *Ichthyophonus*. Typical 'resting cyst' ('resting spore') with multinucleated plasmodium from liver of yellowtail flounder *Limanda ferruginea*. (After Ruggieri and co-authors, 1970.)

formation of 'amoeboblasts' (2'), which are smaller and less thick-walled than those (2) developing directly from 'resting spores'. The hyphae growing from 'resting spores' may be highly polymorphic (Fig. 1-3).

In sea water, a somewhat simpler sequence of life-cycle stages of *Ichthyophonus* has been described from herring *Clupea harengus* by Sindermann and Scattergood (1954), and a more complex situation from mackerel *Scomber scombrus* by Sproston (1944). Among the developmental stages of the fungus, the occurrence of which apparently varies with the host species involved, are multinucleate stout hyphae which may or may not branch

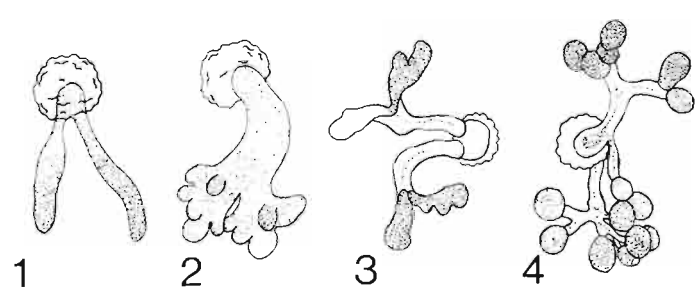

Fig. 1-3: *Ichthyophonus hoferi*. 1, 2: Filamentous *post mortem* germination; 3: condensation of hyphal contents; 4: formation of 'amoeboblasts'. (After Dorier and Degrange, 1961.)

(Fig. 1-3), nonseptate 'macrohyphae' measuring 7 to 15 μm in width, 'microhyphae' measuring 2 to 3 μm in width, brownish 'resting stages' with heavy cyst walls, chlamydospores, 'endoconidia' 1.5 to 4 μm in diameter, plasmodia measuring 0.2 to 2 μm in diameter, and similar or analogous structures, some of which are difficult or even impossible to interpret (for details consult Daniel, 1933a; Fish, 1934; Sproston, 1944; Sindermann and Scattergood, 1954; Dorier and Degrange, 1961; Chien and co-authors, 1979c). As pointed out by Neish and Hughes (1980), it is difficult to adopt a standardized terminology for some of the structures described by the various authors because they may not be homologous.

Some of the more 'exotic' stages reported and figured by Sproston (1944), including stages of sexual reproduction, have never been seen again, which led Johnson and Sparrow (1961) to the conclusion that the developmental pattern of *Ichthyophonus* in *Scomber scombrus* departs radically from that observed in *Clupea harengus* and other species. According to Neish and Hughes (1980), Sproston's (1944) *I. hoferi* remains an enigmatic organism.

The numerous inconsistencies in the above descriptions of what is considered *Ichthyophonus hoferi* have been discussed in detail by Johnson and Sparrow (1961) and Neish and Hughes (1980) and will not be considered here further. However, these discrepancies make it indeed hard to believe that all of the above authors dealt with one and the same organism. Neish and Hughes (1980, p. 86) conclude:

> "It appears that much of the supposed polymorphism attributed to *I. hoferi* may be related to problems in distinguishing among *Ichthyophonus* infections and infections caused by other organisms which also elicit a chronic, proliferative, granulomatous response."

Regardless of the above-mentioned problems and details in the life history of the presumed fungus (or fungi), the 'resting spores' are the most conspicuous stage common to all reported cases. They vary in size between 10 and 150 μm; the nuclei of the contained multinucleate plasmodium (the early 'amiboblastes' of Dorier and Degrange, 1961) have a diameter of roughly 2 to 4 μm. The cytoplasm gives a positive PAS and Bauer reaction, which indicates that it contains glycogen, a common reserve carbohydrate in fungi. The wall of the 'resting spore' gives a strong PAS reaction, which indicates that it is composed of polysaccharides as, indeed, it would have to be if we were going to entertain the notion that *Ichthyophonus* is a fungus (Neish and Hughes, 1980).

As stated, *Ichthyophonus* gains entrance into the host body *via* the digestive tract, the 'resting spores' germinating in the stomach, from which the plasmodia enter the blood stream by migrating into the vasculature of the gastric wall. As pointed out by Bendele and Klontz (1975), the most heavily affected organs are those receiving the richest blood supply, i. e., liver, kidney, heart and spleen. In advanced stages of the disease, any organ can be involved. The host's internal defense system appears to be effective against low numbers of invasive particles, as indicated by Sindermann's (1965) finding of the non-transmissibility of *Ichthyophonus* disease by low spore doses and single applications. The developing plasmodia are phagocytized but, when overwhelming the body in large numbers, are apparently not readily destroyed by the lysosomal enzymes of the macrophages.

As the fungal organisms overhwelm the macrophages in newly invaded tissue, a zone of leukocytes surrounds them, and renewed phagocytosis occurs. These parasite-loaden

areas then become centres of granulomas (Fig. 1-4), as reticulo-endothelial cells and fibroblasts surround the foci. The developing plasmodia cause local tissue necrosis and die within the developing granulomas. In affected organs, recurrent episodes of necrosis with accompanying proliferative inflammation occur until the normal tissue has been replaced by granulomatous and fibrous elements (Bendele and Klontz, 1975).

In culture, *Ichthyophonus* grew best on Sabouraud agar inoculated with material taken from the musculature and heart of diseased herring. The temperature required for fungal development was found to range from 3 to 20 °C, with an optimum at about 10 °C. Germination of the resting spores usually occurred 24 h after inoculation, but in rare cases took up to 7 days. By successive passage, the fungus could be maintained in culture for a period of up to 14 months (Sindermann and Scattergood, 1954). Fish (1934) successfully cultivated *Ichthyophonus* on 4 types of agar slants. More recently, artificial cultivation of *Ichthyophonus* has been achieved by Chien and co-authors (1979b).

Transmission of *Ichthyophonus* occurs from fish to fish. Experimentally, infections may be obtained by either feeding fish massive doses of resting spores (see below) or infected fish flesh or viscera (Plehn and Mulsow, 1911; Pettit, 1913; McVicar, 1977; Egusa, 1980). Sindermann and Scattergood (1954) succeeded in transmitting the fungus to *Fundulus heteroclitus* by force-feeding. Similarly, Herkner (1961) achieved a 20 % infection in tropical freshwater fish by feeding them infected flesh of marine fish. It appears, however, that the agent can also invade and survive in marine copepods,

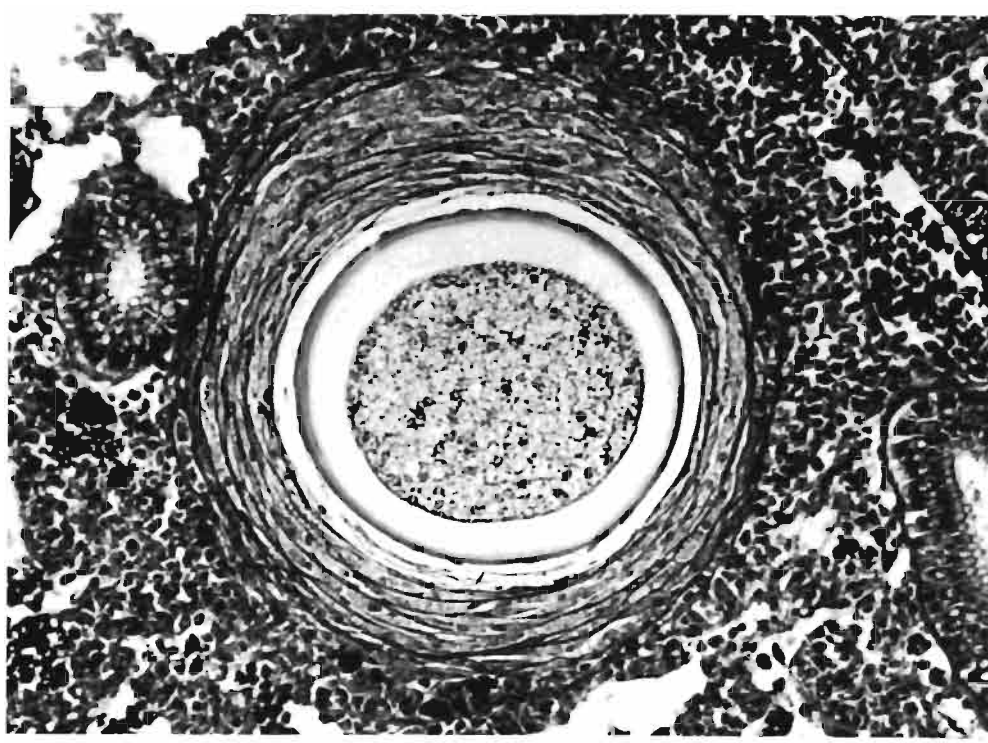

Fig. 1-4: *Ichthyophonus*. Granuloma-like reaction around 'resting spore' in kidney of *Limanda ferruginea*. Note accumulation of histiocytes and degenerative changes of kidney tubules due to pressure exerted by granuloma. (After Ruggieri and co-authors, 1970.)

apparently without producing overt signs of disease in these crustaceans. Chatton (1920) reported on an organism, believed to represent a species of *'Ichthyosporidium'*, in the general cavity of *Acartia clausi, Paracalanus parvus* and *Clausocalanus arcuicornis*. Jepps (1937a) saw a similar parasite in *Calanus finmarchicus*. Previously, Apstein (1911) had tentatively assigned his enigmatic 'Parasites No. 6, 7 and 8' from the same copepod host to *'Ichthyosporidium'*, but Jepps (1937b) doubted the correctness of this allocation. Chatton (1920), on the other hand, felt Apstein's (1911) 'Parasite No. 7' to be correctly classified and, in addition, identified the latter author's 'Parasite No. 10' from *C. finmarchicus* as a typical form of *'Ichthyosporidium'*. As stated by Apstein, infected copepods appeared quite vigorous. Whether the infection of marine copepods with what — from the above descriptions — appears to be *Ichthyophonus* is merely accidental, or whether these crustaceans act as normal (transport?) hosts for the fungus, is unknown. At least copepods should be considered as a possible infection source for plankton-feeding fish, such as herring and mackerel. Sindermann and Scattergood (1954) obtained experimental *Ichthyophonus* infections in *C. finmarchicus,* but herring fed copepods exposed to the fungus did not acquire the disease in two series of experiments. Reichenbach-Klinke (1957b) claimed to have achieved a "35 % *Ichthyophonus* infection" (= 4 of 11 test animals!) in *Crenilabrus melops* fed copepods which, upon microscopic inspection, had proved to be "partially infected by fungi". However, his experimental procedure is so dubious that the reported results should be evaluated with caution. One may cautiously conclude, — particularly in view of the role of spore dose in the transmission of *Ichthyophonus,* as established by Sindermann (1965) —, that concentrations of spores or invasive developmental stages of the fungus presumably present in the copepods used by Sindermann and Scattergood (1954) were not high enough to produce the disease in the experimental herring, but that copepods may nevertheless be capable of transmitting *Ichthyophonus* under suitable conditions in the field.

According to Reichenbach-Klinke and Elkan (1965), freshwater copepods have not yet been found to harbour *Ichthyophonus*-like fungi; but Pérez (1903, 1905) described a similar (or identical) agent from freshwater daphnids. Named *Blastulidium paedophthorum,* the organism was hesitatingly assigned to the Haplosporidia. Chatton (1908) showed it to be a fungus, probably a chytridiomycete, and Alexeieff (1914) emphasized its striking similarity with *'Ichthyosporidium gasterophilum'*. Therefore, the possibility of transmission of ichthyophoniasis *via* planktonic crustaceans may also exist in freshwater.

Normally, however, transmission of *Ichthyophonus* is effected from fish to fish by the heavy-walled resting spores, which have been found to survive in sea-water cultures for over 6 months with no loss of viability. Resting spores have been demonstrated in inshore bottom sediments during fungus epizootics in herring. Since, in the course of an outbreak, up to 27 % of the fish population may harbour the fungus, and since most diseased individuals die either from acute or chronic infection, the spore load in mortality areas can be very high (Sindermann, 1963, 1965).

Knowledge about the effects of increasing infection pressure on the manifestation of ichthyophoniasis in susceptible hosts has been gained by experimental infection of Atlantic herring *Clupea harengus*. In a pilot experiment, 2-year-old laboratory-held individuals were challenged repeatedly with varying doses of *Ichthyophonus* spores. Each experimental group consisted of 50 fish, maintained in 950-l sea-water tanks. Spores were obtained from naturally infected hosts and tested for viability by culturing in Sabouraud-serum agar.

Spore suspensions were added to the food just prior to feeding the herring. The experiment was terminated after 90 days. Low spore doses either failed to establish infections or resulted in chronic, and heavy doses in acute infections. Wide individual variation in susceptibility occurred in response to identical spore doses (Table 1-13; Sindermann, 1965).

Table 1-13
Clupea harengus. Response of 2-year-old herring to experimental *Ichthyophonus* infection (After Sindermann, 1965)

Ichthyophonus Exposure and dosage schedule	***Clupea harengus*** Response to experimental infection
2×10^5 spores in single exposure	No gross or histological evidence of disease after 90 days
2×10^5 spores on each of 3 consecutive days	After 90 days, 4 of 50 fish had subacute or chronic infections; spores few and encapsulated; other fish uninfected
2×10^5 spores on each of 5 consecutive days	After 20 days, 1 fish dead with massive acute infection; extensive tissue necrosis; little host response After 90 days, 10 of remaining 49 fish with subacute or chronic infections of varying severity; 39 fish uninfected
2×10^5 spores on each of 7 consecutive days	After 15 to 30 days, 5 fish dead with massive acute infections After 90 days, 12 with subacute infections of varying severity; 33 fish uninfected
Control (no spore exposure)	No gross or histological evidence of disease

With experimental demonstration of the effect of increasing spore dosage or infection pressure on prevalence and severity of *Ichthyophonus* disease, a large-scale laboratory experiment was set up. Challenge of 2,000 one-year-old herring with 2×10^5 spores on each of 4 consecutive days resulted in infection of 23 % of the population — 8 % acute and 15 % subacute cases. The disease was terminal in all acute cases within 30 days. Chronic and subacute infections resulted in death of all but very light cases within 6 months. At the termination of the experiment, 18 months after initial exposure, all surviving individuals were examined; only 3 lightly infected *Clupea harengus* were found (Sindermann, 1965).

Ichthyophoniasis is a systemic infection characterized by the formation of numerous granulomas in affected organs. The pathology caused by this disease can be severe, as mirrored by the heavy mortalities in acutely or chronically infected fish. In signs and internal appearance, ichthyophoniasis is essentially similar to tuberculosis (mycobacteriosis). Histologically the fungal granulomas resemble those produced in other granulomatous diseases when observed in tissue sections stained with hematoxylin and eosin. For differential diagnosis, Bendele and Klontz (1975) therefore recommend a PAS stain, because it allows detection of PAS-positive organisms in the granulomas and in free macrophages. As mycobacterial granulomas may also contain PAS-positive material, an acid-fast stain is essential for differential diagnosis in some cases. If no acid-fast organisms are seen on examination of sections or smears, it is very likely that *Ichthyophonus* is the organism involved (Richards, 1977a). Neish and Hughes (1980) suggest that material suspected to be an *Ichthyophonus* infection should be stained by the Ziehl-Neelsen

method for acid-fast bacteria, especially when involved with tropical freshwater fish. Occasionally, ichthyophoniasis can be diagnosed from the occurrence of the characteristic hyphae in wet tissue smears. Hyphae regularly appear in infected tissue after 12 h storage at room temperature and permit positive diagnosis even in difficult suspect cases (Richards, 1977a; McVicar, 1980; Möller and Anders, 1983). Schäperclaus (1953a) employed polarized-light microscopy in diagnosing ichthyophoniasis.

Developmental stages of the fungus may be found in any organ; the picture of the disease varies from species to species. In *Clupea harengus,* heart, lateral muscles and liver are most frequently affected. Acute infections are paralleled by massive tissue invasion, necrosis, and death of the victims within 30 days. Chronic cases exhibit cellular infiltration, progressive connective-tissue encapsulation of spores and accumulation of melanophores. Heavy acute infections sometimes result in partial decay of affected fish even before death. In *Scomber scombrus,* kidney and spleen, and in *Gadus morhua,* liver and kidney are main sites of the fungus (Figs 1-5 and 1-6). Also the gills, ovaries and the nervous system may be attacked. External signs, if present, comprise a 'sandpaper' appearance of the skin, produced by minute reddish pustules representing stages of the pathogen encysted in the superficial parts of the lateral muscles. In addition, white, necrotic areas may be seen on the skin, and ulcerations occur from which spores are released into the water (Daniel, 1933b; Sproston, 1944; Sindermann, 1956, 1963; Möller, 1974b).

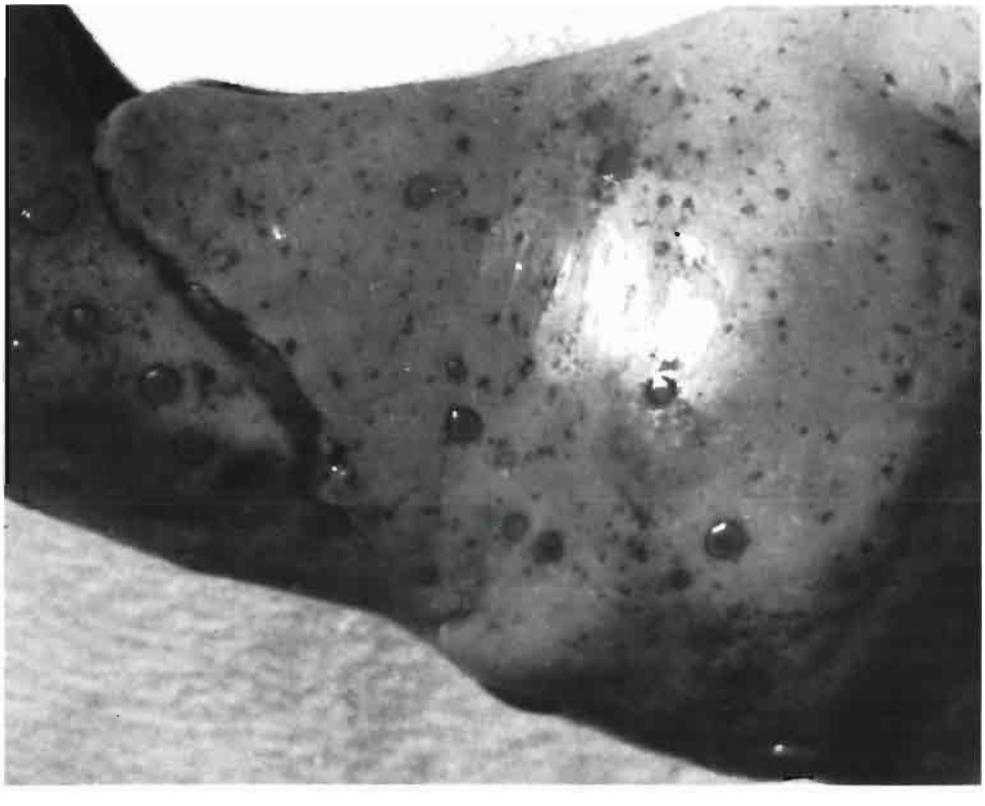

Fig. 1-5: *Ichthyophonus.* Granulomas on liver of *Gadus morhua.* (After Möller, 1974b.)

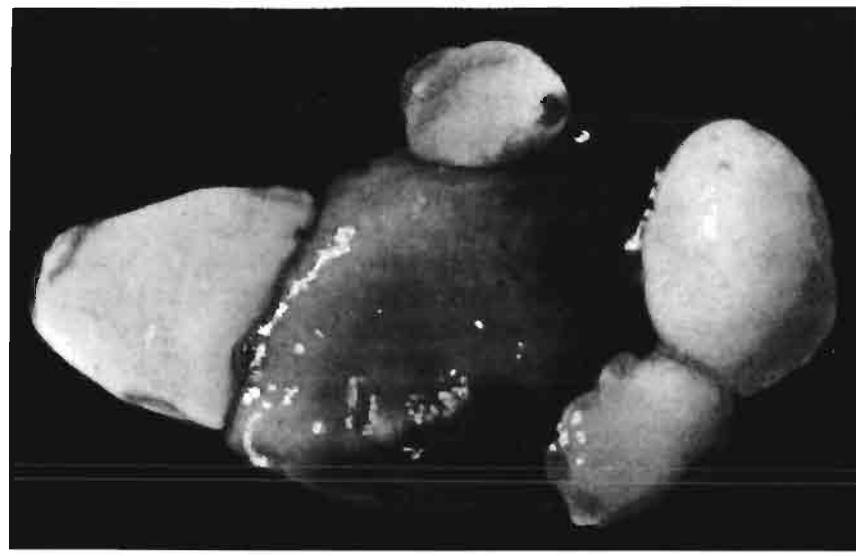

Fig. 1-6: *Ichthyophonus.* Three giant 'cysts' on heart of *Gadus morhua.* (After Möller, 1974b.)

Frequently, fish with severe internal lesions exhibit no external signs of the disease (Machado-Cruz, 1961). It furthermore appears that in fish species but lightly affected by *Ichthyophonus,* macroscopic inspection of organs for possible infection foci may not be sufficient to detect light cases of ichthyophoniasis. Thus, Machado-Cruz (1961) states that the liver of a haddock caught off Greenland appeared to be entirely normal on macroscopic inspection and revealed its fungal affection only upon microscopic study. In most cases, however, distinct lesions of internal organs are immediately apparent on gross macroscopic inspection. Typically, *Ichthyophonus* infection is accompanied by formation of 'granulomas' — white to yellow lesions, which may have a cheesy or hard consistency or even be calcified. They result from chronic inflammation, with development of a proliferative lesion progressing to fibrosis (Roberts, 1978). (Since similar defects occur in response to mycobacteria and other pathogens, differential diagnosis of ichthyophoniasis should rely upon cultivation of the agent or differential diagnosis employing appropriate staining methods; see above).

According to Johnstone (1913, p. 205), who diagnosed an *Ichthyophonus* infection in a mackerel,

> "the liver was hard to the touch and granular in appearance, the whole surface being marked by slight rounded elevations from 1/4 to 1 mm in diameter."

About three-fourths of the hepatic tissue had disappeared in this heavily affected fish. When the gills are affected, haemorrhages occur resulting in marked anaemia (Egusa, 1980). In blue whiting *Micromesistius poutassou,* the heart was the most common site of *Ichthyophonus* infection, but liver, kidney, spleen, swimbladder, gills and body muscles were also affected in different fish. Infected hearts were covered with a layer of tough nodular tissue, the nodules containing fungal spores (MacKenzie, 1979). *Ichthyophonus*-infected haddock *Melanogrammus aeglefinus* are sometimes rendered unsaleable by their unsightly appearance and unpleasant smell (McVicar, 1977).

Ichthyophonus disease has many faces, the site of the parasite within the host body and the severety of affection varies with the fish species involved and also varies in individual fish. Among the more exotic manifestations of the mycosis, sex reversal in female *Lebistes reticulatus (Poecilia reticulata)* may be mentioned. In body size, the affected individuals were similar to females, but in shape and colouration resembled males and had well-developed gonopodia. Developing ova and embryos were killed by *Ichthyophonus* (Wurmbach, 1951). Similar observations have been made by Reichenbach-Klinke (1955a). According to Neish and Hughes (1980), these observations and their association with ichthyophoniasis require confirmation.

Exophthalmia, hyperaemia and other eye affections in Mediterranean *Polyprion americanum, Serranus (Epinephelus) guaza, S. scriba* and *S. cabrilla* from the Neapolitan Marine Aquarium have been attributed to *Ichthyophonus* infection. Four of the diseased serranids were totally blind. Fungal hyphae and other developmental stages were detected in diseased eyes and in the adjacent connective tissue, as well as in the liver. The main damage occurring in the eyes was believed to be due to clogging of the blood vessels supplying the eyes by masses of fungal spores, which apparently led to a degeneration of these sense organs (Reichenbach-Klinke, 1957b).

Ichthyophonus infection of marine teleosts has, thus far, mostly been reported from the North Atlantic and adjacent waters. Apparently, the fungus has not yet been reported from the South Atlantic (Neish and Hughes, 1980). Reichenbach-Klinke (1954, 1957b, 1958) found it to be of frequent occurrence in Mediterranean fish. However, some of these records may be regarded as dubious. In Japanese waters, as well as in marine fish species cultivated in that country, *Ichthyophonus* was first reported by Kubota (1967). Thereafter, it has been encountered in various areas, but the incidence was usually low. In cultivated fish, it occurs mainly early in the first year of life of seed fish (Egusa, 1980). According to Reichenbach-Klinke (1955a) and Chlupaty (1962), *'Ichthyosporidium'* is widespread among coral fish. This curious statement would imply the existence of special warm-water strains of the pathogen. However, most 'mycoses' reported from captive tropical fish may actually represent cases of mycobacterioses, as suggested by Amlacher (1965).

Ichthyophonus is of considerable economic concern, since it is capable of producing mortalities of epizootic proportions in marine fish populations. *Clupea harengus* appears to be particularly susceptible to the fungus. At least 6 epizootics, caused by this agent, have occurred in the western North Atlantic during the past 80 years, resulting in widespread mortalities and affecting the abundance of the species in that area (Fig. 1-7; Cox, 1916; Fish, 1934; Scattergood, 1948; Leim, 1955; Sindermann, 1956, 1957b, 1958, 1963). It has been postulated that *Ichthyophonus* mycosis may be the most important single limiting factor to population growth of *C. harengus* in the western North Atlantic (Sindermann, 1958, 1966, 1970a).

During the 1930–31 outbreak in the Gulf of Maine, the average incidence in all age groups of herring was about 70 %, falling precipitously to approximately 18 % after July, 1931. Subsequently, Gulf-of-Maine fishermen reported an unusual scarcity of herring in that area. Alewives *Alosa pseudoharengus* and winter flounders *Pseudopleuronectes americanus* were also affected, but to a much lesser extent (Fish, 1934). During the 1954–55 epizootic, herring mortalities caused by *Ichthyophonus* (Fig. 1-7) extended along hundreds of miles of Gulf-of-Saint-Lawrence coastline. An estimated 50 % of the herring population in the Gulf was killed by the disease in these 2 yr, and subsequent landings

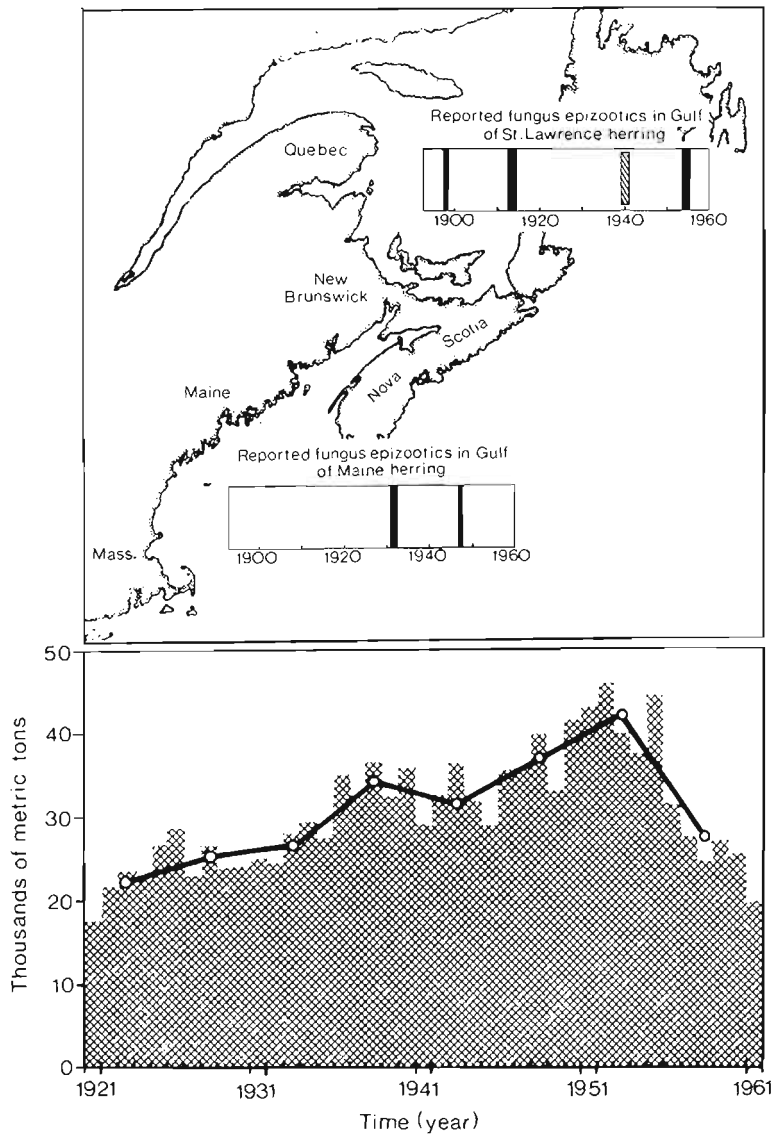

Fig. 1-7: *Ichthyophonus.* Time sequence of fungus epizootics and herring landings in the Northwest Atlantic. (After Sindermann, 1963.)

declined drastically. *A. pseudoharengus,* present in inshore waters, and *Scomber scombrus* also became infected in sufficient numbers so that mortalities were observed and reported. *Gadus morhua,* on the other hand, did not acquire infections, but fed on infected and dying herring to such an extent that their growth rate — as indicated by scale growth zones — exceeded anything experienced previously. Consequently, *G. morhua* landings almost doubled during the period immediately following the herring epizootic, due almost entirely to increased weight of individual fish landed, rather than to increased numbers of fish taken (Sindermann, 1963, 1965).

The observation that *Gadus morhua* did not become infected by *Ichthyophonus* during the 1954–55 Gulf-of-Saint-Lawrence *Clupea harengus* epizootic appears noteworthy, since it raises the question of the existence of either a variation in host susceptibility or of different strains (or even species) of *Ichthyophonus* commonly grouped under the name *I. hoferi*. For example, *Pseudopleuronectes americanus* generally exhibited low levels of fungus prevalence during the 1930–31 Gulf-of-Maine herring epizootic, and were hence regarded as accidental hosts for the fungus. In Lord's Cove (Deer Island, New Brunswick, Canada), however, where winter flounders were given occasion to feed on large quantities of fungus-diseased herring discarded by local fishermen, as many as 60 % of the flounders exhibited internal fungal lesions (Fish, 1934). *Ichthyophonus* infections in *P. americanus* have also been reported by Ellis (1928).

More recently, yellow-tail flounders *Limanda ferruginea* from western Sable Island Bank and Western Bank (off Nova Scotia, Canada) have been found to carry *Ichthyophonus* or a closely related fungus. Of 146 flounders, 20.6 % were found infected; 15.1 % exhibited low fungus prevalence, as indicated by the presence of small numbers of granulomas on the liver; 4.8 % had moderate infections characterized by confluent granulomas covering more than half of the liver surface; and the remaining 0.7 % were heavily affected, with the liver surface completely matted with cysts (Figs 1-8 and 1-9). Granulomas, up to 2 mm in diameter, were also found on most of the internal organs including heart, intestinal tract, spleen and kidneys, but the musculature was never attacked (Figs 1-10 and 1-11). In some cases, the heart was completely covered with cysts; some infected flounders had grossly distorted and enlarged kidneys. Even heavily infected individuals revealed no external evidence of the fungus. *Ichthyophonus* infection of *L. ferruginea* was first observed in July 1966, and three subsequent samplings on Sable Island Bank have confirmed that the disease was still present in May 1967. High mortalities among *L. ferruginea* have been attributed to the mycosis, and surveys indicated that yellow-tail flounder landings from that region have declined subsequently (Powles and co-authors, 1968; Ruggieri and co-authors, 1970; Hendricks, 1972).

While, according to Sindermann (1963, 1965), *Gadus morhua* from the Gulf of Saint Lawrence did not acquire *Ichthyophonus* infections, individuals of the same host species from waters off Greenland, the Northwest Atlantic, the northern North Sea and the Baltic Sea were found to be positive for the fungus (Machado-Cruz, 1961; Hendricks, 1972; McVicar and MacKenzie, 1972; Möller, 1974b).

Of 541 *Gadus morhua* from Kiel Fjord (western Baltic Sea), 15.2 % exhibited macroscopically visible signs of *Ichthyophonus* infection. Granulomas were most frequently encountered in the liver, but were also seen in the spleen and body musculature; the heart was rarely infected. Cyst diameters reached 6.5 mm in liver and 8.1 mm in heart tissue (Fig. 1-5). Heavily infected cod showed external signs of emaciation and had up to 30.7 % underweight. The average condition factor was 0.7787 in healthy and 0.7526 in infected *G. morhua,* the difference being statistically significant at the 5 % level. In one individual of 30.3 cm length and a condition factor of 0.5716, the liver weight was only 6.3 g, as opposed to 13.1 g (average of 8 determinations) in healthy cod of the same length. Infections appeared to be in a chronic state, since all observed cysts were found to be covered by a thick wall (Möller, 1974b). Ninety-two percent of *G. morhua* that had died during experiments with net cages suspended in the open water in Kiel Fjord, had *Ichthyophonus* infections (Kock, 1975).

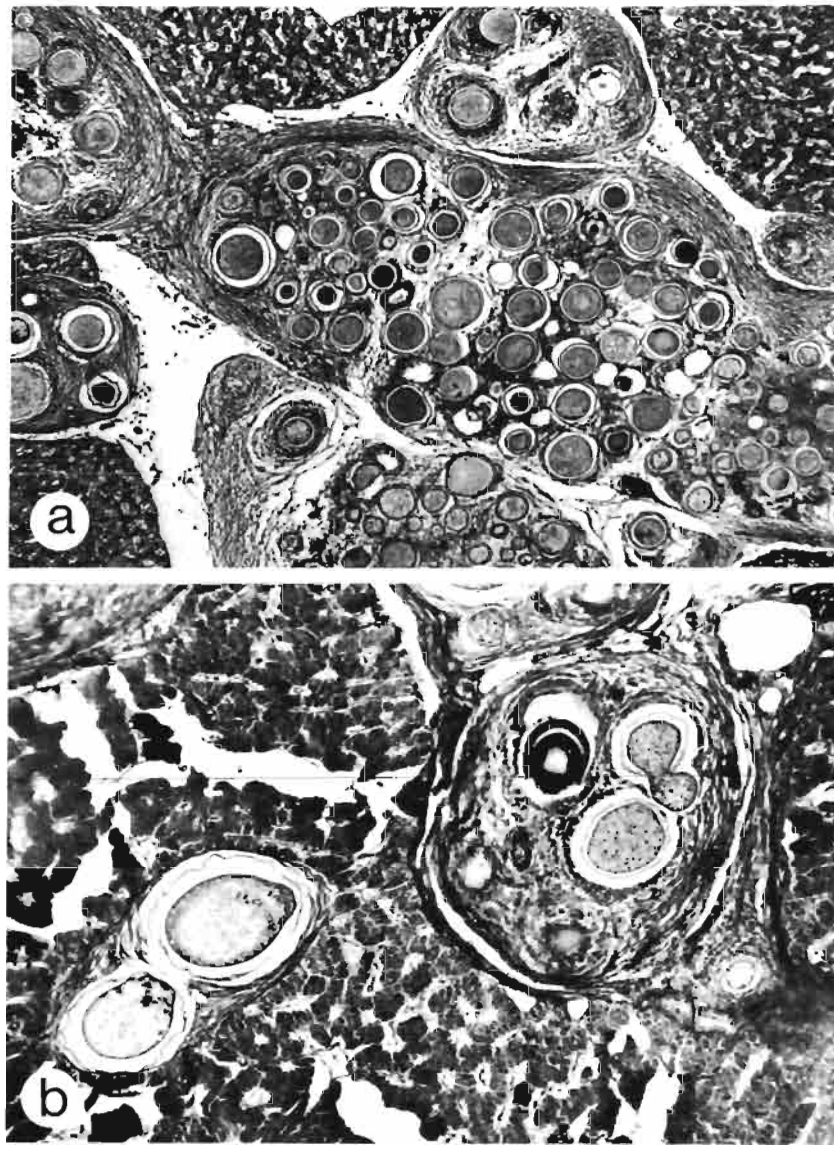

Fig. 1-8: *Limanda ferruginea*. Liver pathology caused by *Ichthyophonus*. (a) Densely packed 'resting cysts' causing extensive damage and development of connective tissue; (b) area of liver showing necrosis and distortion of parenchymal architecture. (After Ruggieri and co-authors, 1970.)

In pollock *Pollachius virens* from Iceland, *Ichthyophonus* infection produced extended necrotic areas in the body musculature, characterized by greenish discolouration. *Post-mortem* hyphal germination was observed in squash preparations of host tissue (Priebe, 1973).

Ichthyophonus is very common in food fish from Scottish waters. Species affected include herring, mackerel, cod, haddock, blue whiting, plaice and salmon. While in most of the northern North Sea 2 to 12 % of the *Melanogrammus aeglefinus* population are

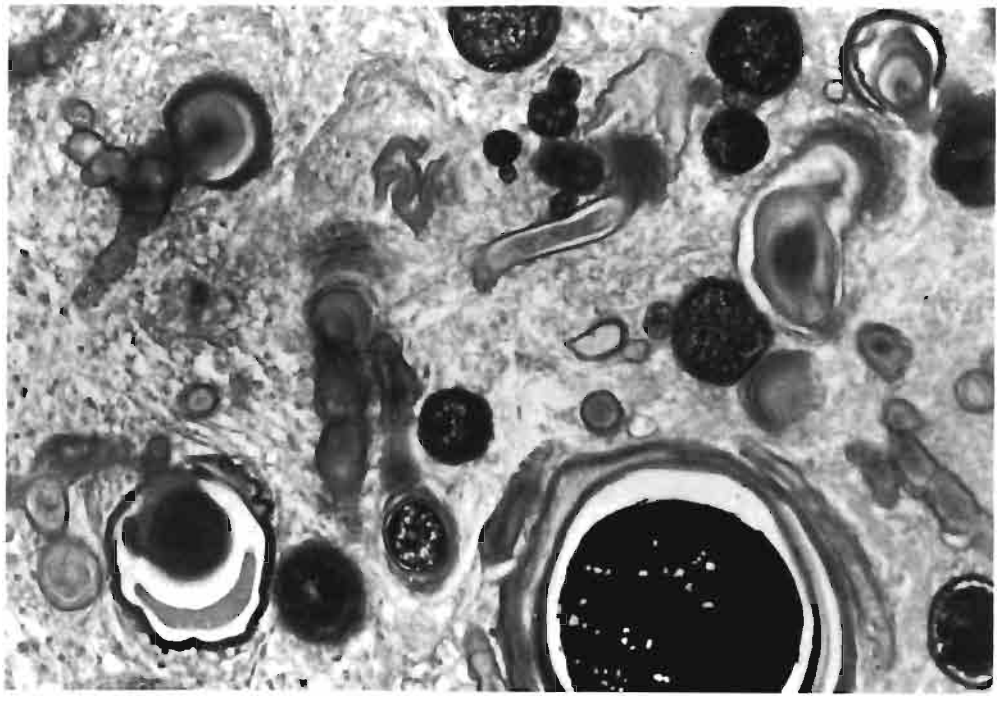

Fig. 1-9: *Ichthyophonus*. Germination and hyphal development in liver of *Limanda ferruginea,* accompanied by extensive parenchymal necrosis. Note absence of typical inflammatory reaction. Resting cysts strongly PAS-positive. (After Ruggieri and co-authors, 1970.)

infected, incidences reach 85 % in the Orkney-Shetland-Cape Wrath area. As a consequence, a significant percentage of haddock from some fishing grounds is rejected for human consumption. Infestation levels were lower in *Pleuronectes platessa* (max. 25 %) but showed the same geographic pattern. In infected haddock, a distinct cellular host-defense response (inflammatory reaction, encapsulation of fungal stages) was present and the pathogenicity of the disease was apparently low. Although a strong humoural antibody response was elicited by plaice, there was but a poor encapsulation response, and infected individuals were typically heavily parasitized, emaciated and thin. There was evidence that ichthyophoniasis was terminal to those flatfish within a few months. In areas of high disease prevalence a serious effect to the population could be estimated (McVicar, 1977, 1979, 1980, 1981).

In the western Baltic Sea, *Ichthyophonus* appears to prefer *Gadus morhua* as host. Macroscopic inspection of 228 *Anguilla anguilla,* 700 *Zoarces viviparus,* 208 *Pholis gunnellus,* 300 *Taulurus bubalis,* 101 *Myoxocephalus scorpius* and 2,183 *Platichthys flesus* revealed not a single case of fungus infection in these fish (Möller, 1974b). Similar 'apparent immunity' to *Ichthyophonus* infection (which, however, may rather be due to differences in susceptibility and response to the pathogen, as well as in the ecology of the respective hosts) has also been noticed by Fish (1934) and others. Spatial variation in infection of susceptible hosts renders *Ichthyophonus* suitable as a biological tag for the discrimination between fish populations of different geographic origin (Sindermann, 1961a, b, 1965).

Agius (1978) provided the first record of an '*Ichthyophonus*-like' fungus in deep-sea

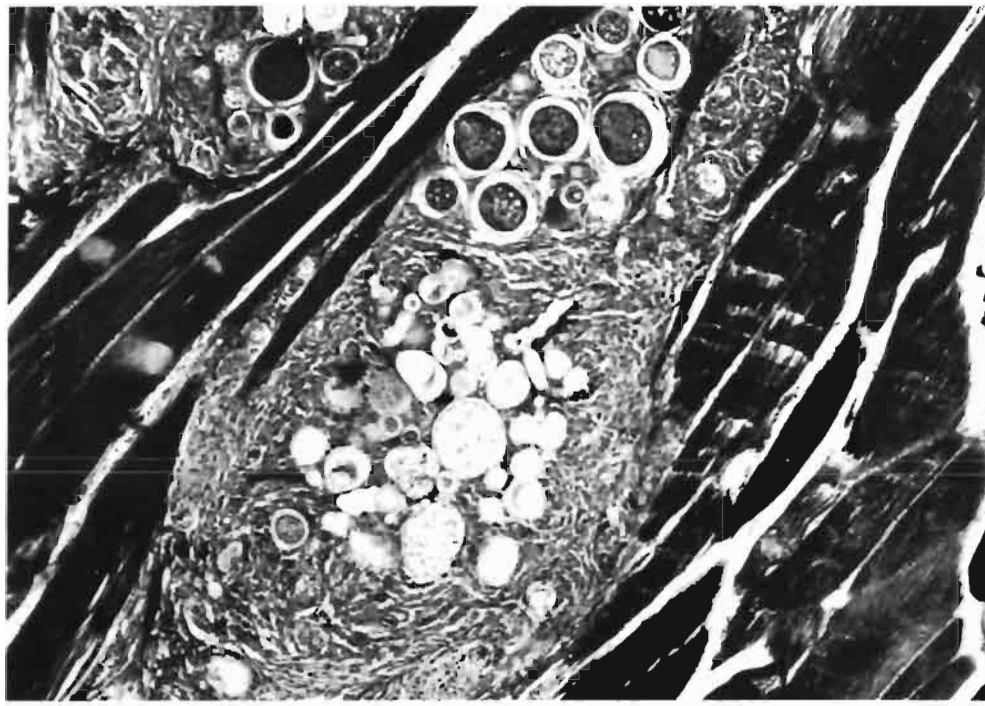

Fig. 1-10: *Limanda ferruginea*. Myocardial degeneration caused by *Ichthyophonus*. Note extensive connective-tissue development resulting in fibroid swelling. (After Ruggieri and co-authors, 1970.)

scabbard fish *Aphanopus carbo,* trawled from depths of 735 m and 980 m, respectively, in the Northeast Atlantic. No gross lesions were visible on any of 85 fish caught, but in 5 of 6 *A. carbo* selected at random from the catches and examined microscopically, all of the organs carried granulomas. Two of the fish were severely affected. In extreme cases, the inflammatory lesions had replaced much of the normal tissue. The granulomatous reaction type was indistinguishable from that found in ichthyophoniasis in gadoids and clupeids. How fish at such depths become infected with the fungus remained unknown.

Provided that identifications are correct, *Ichthyophonus* also affects elasmobranchs. Lederer (1936) reported the fungus from the shark *Scyliorhinus caniculus,* and Reichenbach-Klinke (1957b) from the eagle ray *Myliobatis aquila.* In the latter host, fungal development was observed in the uterus and in the liver.

The above records may be incomplete because fungal lesions, particularly chronic infections, may not have been recognized as such. Recent evidence suggests that chronic *Ichthyophonus* infections have a much higher prevalence in marine fish than previously expected. It is also possible that the reports contain erroneous records possibly encompassing microsporan, mycobacterial or other granulomatous diseases. In most of the numerous reports, positive identification of the causative agent(s) by cultivation in artificial media has not been made.

How problematic the distinction between developmental stages of fish-invading microsporans and fungi can be, particularly if statements have to be based on original descriptions rather than on the examination of fresh material, is vividly illustrated by

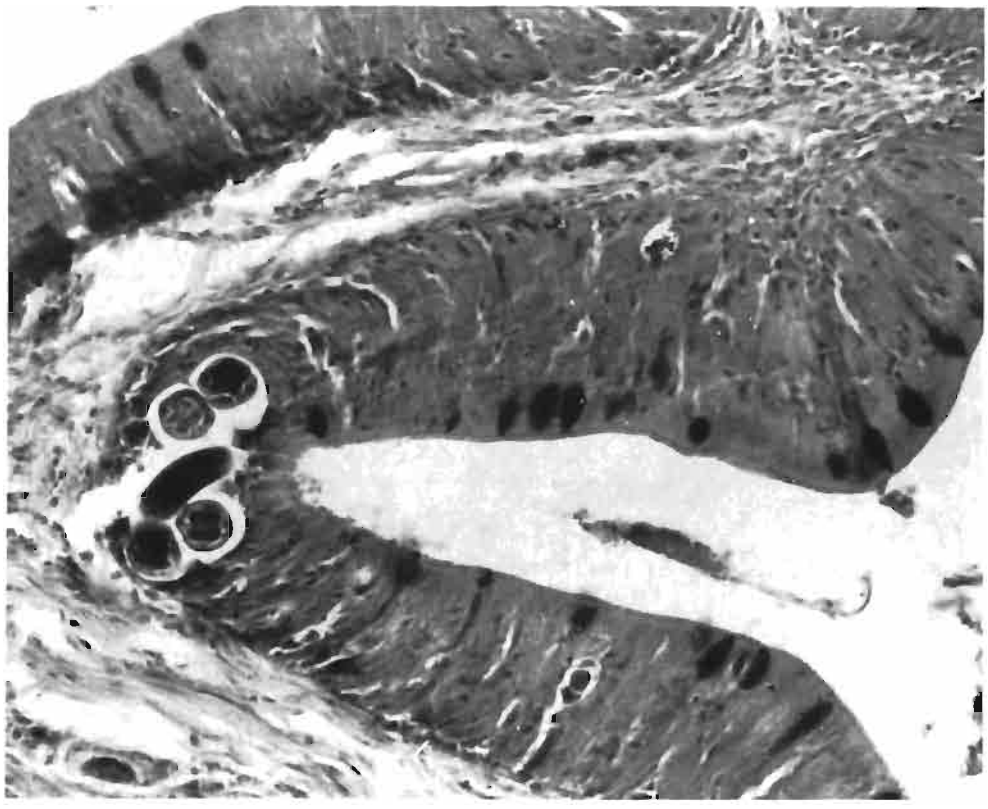

Fig. 1-11: *Limanda ferruginea.* Cysts of *Ichthyophonus* in mucosa of large intestine. Basement membrane has been penetrated. PAS stain. (After Ruggieri and co-authors, 1970.)

several examples documented in the scientific literature. Alexeieff (1914), for instance, studied what he believed to be *Ichthyosporidium gasterophilum* Caullery et Mesnil, 1905 (i. e., an organism now believed to be a fungus close to, or identical with, *Ichthyophonus;* see Sprague, 1965). Johnson and Sparrow (1961), comparing Alexeieff's organism with the latter fungus, stated (p. 562) that

> "it should be noted that Alexeieff's organism (1914–1915), reported as *Ichthyosporidium gasterophilum,* is incorrectly named."

Another *'Ichthyosporidium*-like' organism was found to cause considerable losses among commercially exploited stocks of ocean pout *Zoarces anguillaris (= Macrozoarces americanus)* on the U.S. Atlantic coast (Sandholzer and co-authors, 1945). Although clearly recognized as a protozoan (Fischthal, 1944), and later identified as a microsporan by Nigrelli (1946) and named *Pleistophora macrozoarcidis,* Johnson and Sparrow (1961) discussed it as a synonym of *Ichthyophonus hoferi.* Similarly, Reichenbach-Klinke (1956c), apparently unaware of the pertinent state of knowledge and the literature then available, regarded the above-mentioned protozoans and others as fungi, and confusingly compared them with *I. hoferi.*

Tumorous outgrowths on the body surface or on infected organs, produced by mycobacteria, *Pasteurella* species or actinomycetes, are macroscopically indistinguishable

from mycotic infections. Thus, a special form of 'aquarium fish ichthyophonosis' (Schäperclaus, 1953a), was later (Amlacher, 1965, 1968) identified as fish tuberculosis (mycobacteriosis) caused by *Mycobacterium* spp. As stated, a reliable proof of the involvement of *Ichthyophonus* can be obtained by a 12-h storage of diseased tissue at room temperature and subsequent inspection for the presence of fungal hyphae, or by special microscopical preparation, as outlined above.

In some cases, early stages of 'xenomas' produced by microsporans (Weissenberg, 1922a, 1949) may be mistaken for *Ichthyophonus* granulomas, or *vice versa*. Differential diagnosis is facilitated by search, in squash preparations of formalin-preserved material, for the characteristic spores, which are ever-present in microsporan infestations (Möller and Anders, 1983).

In fact, 64 or more species of microsporans, representing 7 genera, are presently known to invade fish (Lom and co-authors, 1980). Some of these can cause mass mortalities comparable to those produced by *Ichthyophonus*. Although Hofer (1893) is generally believed to have been studying a fungus, and although Plehn and Mulsow (1911) state that the 'cysts' described by the former author and later figured in his book on fish diseases (Hofer, 1904) are essentially like those produced by *Ichthyophonus hoferi,* the remote possibility exists that Hofer actually dealt with a microsporan infestation. It should be recalled that Hofer (1893) found what he believed to be 'gregarine spores' in trout lesions. Did he mistake the uninucleate plasmodia hatching from *Ichthyophonus* 'resting spores' for protozoan spores, or did he see true microsporan spores? The question cannot be answered with certainty. At least the very small size of the 'spores', reported by Hofer (1893) to be 'an der Grenze der Sichtbarkeit' is somewhat suggestive of a microsporan infestation. Microsporans of the genus *Pleistophora* have been reported from salmonids (Putz and co-authors, 1965; Putz and McLaughlin, 1970; Sprague, 1977b).

Reports on marine fish-invading fungi other than *Ichthyophonus* are scarce. Mycotic infection of plaice *Pleuronectes platessa,* associated with serious mortality, has been observed in culture tanks at Port Erin (England). The disease was characterized by the occurrence of whitish cysts on the liver, kidney and mesenteries. Studies were made from preserved material only (Johnstone, 1906). The agent was — with some reservation — placed with the Entomophthoraceae and believed to be related to genus *Conidiobolus* (a fungus parasitic on higher plants, however). Its identity with *Ichthyophonus* cannot be ruled out; at least it may be closely related to the latter. It should be recalled, in this context, that Léger (1927, 1929a, b) assigned *I. hoferi* to the Entomophthorales (see above), in which order it has remained until today (see Wolke, 1975). Johnstone (1913), describing what appeared to be an *Ichthyophonus* infection in a female mackerel, stated that the fungus in the latter was different from that observed by him (1906) in plaice; but he may have been misled by the differing appearance of the same parasite in different hosts.

Apstein (1910) found a fungus in the stomach wall of 52 of 96 lumpfish *Cyclopterus lumpus* from the North Sea. The organism formed thread-like filaments, 97 to 125 μm long, which developed from colonies of hyaline, unsegmented hyphae, 22 μm in diameter. When fully grown, the hyphae were capable of producing endogenous spores, which were globose or nearly so, hyaline, aplanetic, and up to 15 μm in diameter. The agent was named *Cyclopterícola marina* but was not further identified. Being short of examining the fungus on the basis of fresh material, Johnson and Sparrow (1961, p. 352) state that

> "... there is no way of placing the species into a family category, let alone into a specific order or class. In gross aspects, the fungus resembles an eccrinid, but apparently no hold-fast is produced. The endogenous spore formation suggests both certain of the Fungi Imperfecti and the conidial Phycomycetes; the nonseptate hyphae of *Cyclopterícola marina* seem phycomycetous."

There are several reports on the occurrence of Deuteromycetes (Fungi Imperfecti) or of agents believed to belong to this group, in fish. Although these organisms may not be regarded as typical fish pathogens, there is increasing evidence of their involvement in fish diseases, mainly in freshwater. Neish and Hughes (1980) have assembled a list of reported cases. The authors believe that records of infections by imperfect fungi will continue to increase in the future. Deuteromycete infections are usually fatal and, at present, there is no practical way to predict, prevent or treat them. Fortunately, reported incidences of infection are usually low (Neish and Hughes, 1980).

The Fungi Imperfecti are — artificially — grouped into Blastomycetes (including ascomycetous and basidiomycetous yeasts), Hyphomycetes (an assemblage of diverse mycelial forms) and Coelomycetes (not represented in marine fish).

The occurrence of yeasts, including forms potentially pathogenic for humans, in sea water and on the body surface of clinically healthy marine animals is not uncommon (see Vols I and II). Bruce and Morris (1973) isolated yeasts of the genera *Candida, Cryptococcus, Debaryomyces, Rhodotorula, Torulopsis* and *Trichosporon* from the skin of marine teleosts taken off the Scottish coast. However, true yeast infections of fish are virtually unknown (Neish and Hughes, 1980).

A hyphomycete, believed to be a member of the Dematiaceae and related to genus *Cladosporium,* has been found to be associated with epithelial hyperplasia in the gills of a single individual of *Gadus morhua* from the North Sea. Externally, septate hyphae, up to 8 μm in width and 140 μm in length, as well as brownish conidia measuring 8 to 24 × 8 to 10 μm, were seen (Fig. 1-12; Reichenbach-Klinke, 1955b, 1956b). The agent was not studied further. Johnson and Sparrow (1961, p. 84) comment that

> "Other than the unqualified statement by Reichenbach-Klinke [= 1956b] that the fungus is a parasite, there is no substantiating evidence that the organism is a primary invader of living tissue."

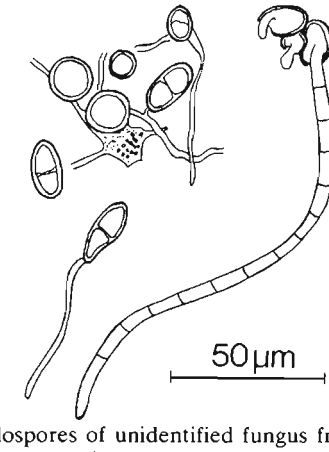

Fig. 1-12: Hyphae and chlamydospores of unidentified fungus from gill lesions in *Gadus morhua.* (After Reichenbach-Klinke, 1956b.)

There are several other reports on the occurrence of hyphomycetes in fish tumours and granulomas but, as emphasized by Neish and Hughes (1980), it is often uncertain whether these lesions are caused by the fungus or whether the fungus is taking advantage of the situation.

Otte (1964) isolated a hyphomycete, tentatively identified as a species of *Pullularia* (or as being very close to that genus) from a stingray *Dasyatis pastinaca* that had died of unknown causes in an aquarium. The infection appeared to be confined to the liver, which contained necrotic foci consisting of cellular debris, fat deposits and septate hyphae. Pure isolates of the agent, intraperitoneally injected into carp, provoked a lethal infection in these fish. Histologically, the lesions observed in the carp were similar to those in the stingray. The fungus could be reisolated in pure culture from the experimentally infected carp. Unfortunately, the agent was not further identified. Neish and Hughes (1980) assume that it could probably be included in the genus *Aureobasidium*. *Pullularia* is a synonym of *Aureobasidium*.

Dematiacean fungi of the genus *Exophiala* were identified as causative agents of systemic mycoses in channel catfish *Ictalurus punctatus* in freshwater (Fijan, 1969; McGinnis and Ajello, 1974) and similar conditions in hatchery-reared cut-throat trout *Salmo clarki* (Carmichael, 1966). More recently, Richards and co-authors (1978) described an outbreak of an *E. salmonis* infection in Atlantic salmon *Salmo salar* maintained in sea-cages at a marine fish farm in Scotland. During the entire course of the outbreak only 1 cage, originally stocked with 1,000 salmon about 55 g in average weight, was affected; similarly stocked cages, anchored only a few feet away, remained unaffected.

Infected *Salmo salar* exhibited marked abdominal swelling caused by kidney enlargement. Greyish-white nodules, up to 3.5 cm in diameter, were present on affected kidneys. In many fish, considerable quantities of straw-coloured fluid had accumulated underneath the kidney capsule, displacing the capsule and swimbladder ventrally. In some cases, necrotic lesions were seen extending deeply into the adjacent dorsal musculature. Lesions of variable extent also occurred in the heart, liver, spleen and pancreas. The essential reaction to the presence of fungal material was the formation of granulomas, although there was no apparent correlation between the extent of the lesions and the amount of fungus present. Fungal hyphae were usually seen at the centre of the granulomas.

The route of *Exophiala salmonis* entry into the fish remained obscure. Verdun (1903) suggested that ascending fungal infections in carp might occur *via* the ureters. Richards and co-authors (1978) suspected that, in the case studied by them, infection probably occurred through the food, since focal areas of fungal contamination have been found in bags of food from the same supplier. Approximately 10 % of the fish in the affected cage succumbed to the *E. salmonis* infection.

Blazer and Wolke (1979) isolated a fungus from a cod *Gadus morhua* that had died in a public aquarium, displaying gross lesions reminiscent of *Mycobacterium* infection. The dark green to black fungus growing in culture on Lowenstein-Jensen media at 25 °C was tentatively identified as an *Exophiala* species and subsequently found to be *E. pisciphilus*. The organism was also found to occur naturally in aquarium-held *Xanthichthys ringens, Hippocampus hudsonius, Amphiprion sebae* and *Stenotomus versicolor,* and was experimentally transmitted to *Tautogolabrus adspersus, Fundulus heteroclitus* and *Pseudopleuronectes americanus.*

Two fish, *Xanthichthys ringens* and *Hippocampus hudsonius,* had external dermal

masses attributable to the fungus. These were 3.0 and 0.5 mm in diameter and non-ulcerated. *Stenotomus versicolor, Gadus morhua* and *Tautogolabrus adspersus* had internal gross lesions. Raised (1–5 mm), round, yellow to white areas were present on the liver, kidneys, myocardium, swimbladder and spleen of the naturally infected *S. versicolor* and *G. morhua,* and hepatic lesions were seen in the experimentally infected *T. adspersus.* Histological examination revealed inflammatory reactions, which were either acute (non-proliferative) or chronic (proliferative). In *G. morhua,* the latter were characterized by focal granulomas resembling mycobacteriosis (Blazer and Wolke, 1979). Thus far, *Exophiala* infections have not been reported from feral fish.

Fungi may also affect the hard structures of marine animals including fish. One such infection, initially diagnosed as 'caries', appeared as tiny, chalky-white spots on the surface of teeth of a stingray *Raja clavata* from the North Sea. Microscopic inspection of a thin cut of a diseased tooth revealed the presence of a dense network of channels bored by a presumed fungus with the "Habitus eines *Actinomyces*" (Peyer, 1926). Roux (1887) had proposed the name *Mycelites ossifragus* for similar structures observed in fossilized remains of various vertebrates. Today considered a heterogenous assemblage of micro-organisms encompassing fungi and algae (Peyer, 1945), *'Mycelites ossifragus'* has also been believed to be responsible for abnormally heavy dental attrition in wolffish *Anarhichas lupus* from the North Sea. The lesions were studied *post mortem.* Although no living remains of the agent were found, it was believed to be a fungus (Schmidt, 1954, 1955). As shown by Kerebel and co-authors (1979), only the external dental material of *A. lupus* is affected, the agent apparently penetrating the substrate by means of localized enzymatic dissolution of the tooth substance in its immediate vicinity. Therefore, the condition is different from the more diffuse bacterial decalcification typical of dental caries.

Oomycetes of the order Saprolegniales, which pose considerable problems in freshwater (for review and literature consult Neish and Hughes, 1980), are not known from genuine marine fish. Reports on the survival of saprolegniaceous fungi in brackish water are still controversial (Dick, 1968; Stuart and Fuller, 1968a, b). There are, apparently, several members of this group, which definitely tolerate at least lower salinities. Thus, Stuart and Fuller (1968a) were able to cultivate *Saprolegnia parasitica,* a serious pathogen of freshwater fish, in sea-water base media (however, *'S. parasitica'* may, in fact, encompass a complex of species, as shown by Willoughby, 1978; for details consult Seymour, 1970, who published a monograph of the genus *Saprolegnia*). Te Strake (1959) demonstrated that members of the Saprolegniaceae can grow and sporulate in slightly brackish water, provided that temperature and nutritional levels are suitable. The author concluded that salinity, *per se,* does not inhibit planont production, as presupposed.

Aleem and co-authors (1953) described an infection of *Atherina riqueti* from Étang de Salses (French Mediterranean coast) by a saprolegniaceous fungus similar to or identical with *Isoachlya parasitica.* The mean salinity in that lagoon is 14.6 ‰ S. Since, however, these euryhaline fish may also enter estuaries and even freshwater, the infection may have been acquired in these habitats. In fact, it is not uncommon for parasites and diseases of freshwater origin, including fungi, to be carried downstream with the anadromous host to the estuary and the sea where survival is often limited (Sindermann, 1966). *Saprolegnia* infections, acquired by Pacific salmon in freshwater, disappeared when the fish were transferred to sea water (Earp and co-authors, 1953). Chlupaty (1962), on the

other hand, reported *Saprolegnia* as a stress parasite in aquarium-held coral fish, particularly pomacanthids. White patches of mycelial growth developed rapidly — sometimes within a few hours — on the body surface of freshly shipped fish, but gradually disappeared upon lowering of the water temperature.

Similar external symptoms of fungus invasion have been seen in individuals of *Alosa pseudoharengus* and *Osmerus mordax,* as they entered the sea after spawning (Sindermann, 1966). Stuart and Fuller (1968a) observed saprolegniasis in returning *Salmo salar* caught in estuarine waters, and Roberts and co-authors (1969, 1970, 1971a, 1973) reported secondary *Saprolegnia* sp. invasion of lesions associated with tagging and ulcerative dermal necrosis in anadromous *S. salar* from Scottish waters. Although saprolegniasis is usually considered to be secondary to bacterial or viral diseases (Wolke, 1975; Richards, 1978) or the consequence of physical damage to the body surface of the fish, such as tagging, there is a small body of evidence to suggest that, under certain conditions, *Saprolegnia* spp. may act as primary pathogens (Richards and Pickering, 1978; Willoughby, 1978; for discussion see Neish and Hughes, 1980).

As with other microbial fish maladies, prevention and treatment of fungal fish diseases and epizootics in the open ocean is virtually impossible. There is an urgent need for more detailed studies of the biology of *Ichthyophonus,* the most destructive marine fungus known. Epizootics caused by this pathogen have recurred in the western North Atlantic Ocean in a periodic fashion, with 14 to 25 years between peaks, since 1898 (Sindermann, 1963). If we were capable of predicting future outbreaks of the disease, measures could be taken to avoid excessive economic losses.

The fact that pelagic, plankton-feeding fish such as *Clupea harengus* and *Scomber scombrus* appear to be particularly susceptible to *Ichthyophonus* and have undergone recurrent mass mortalities, may point toward copepods as a possible primary infection source. From their mode of feeding, these curstaceans can function as ideal 'spore collectors', supplying their predators with the high spore doses required to establish patent *Ichthyophonus* infections. While laboratory trials using copepods in the transmission of the fungus have thus far remained equivocal, this does not necessarily mean that such an infection route could not exist in nature.

Routine inspection of pelagic copepods for fungal stages, including cultivation of copepod-squash preparations on artificial media, could possibly help to predict oncoming epizootics among plankton-feeding fish. With developing patency of infections and resulting fish mortalities, drastic periodic overfishing in localized areas of high disease prevalence could then assist in thinning out an affected herring population to a level at which transmission of the agent is markedly slowed down and dissemination to other areas inhibited or at least reduced.

In coastal areas, spread of *Ichthyophonus* infections to host species other than herring could, at least to some extent, be prevented by application of appropriate sanitary measures. As has been shown by Fish (1934), 60 % of a local population of *Pseudopleuronectes americanus* acquired *Ichthyophonus* infections by feeding on diseased herring carcasses dumped overboard by fishermen. In other areas, fungus prevalence in winter flounders was negligible.

Ichthyophonus has been introduced into fresh and sea-water fish cultures in which raw marine clupeoid fish, salmon viscera, infected trout viscera and infected marine forage fish were used as part of the diet (Sindermann, 1970b). McVicar and MacKenzie (1977)

emphasize the risk inflicted upon farmed fish to acquire *Ichthyophonus* infections from contaminated feed containing diseased fish flesh. They point out (p. 172) that

> "on some fishing grounds haddock are commonly infected, and since the flesh is one of the main sites of infection, a large proportion of infected fish are rejected and can appear in the reclaimed material available to fish farmers."

They suspect that incorporation of such untreated industrial or waste fish in the diet of cultivated stock was responsible for an outbreak of *Ichthyophonus* disease in plaice grown in an onshore aquaculture system at Hunterston, Scotland. Like previous workers, they (McVicar and MacKenzie, 1972) had shown that the fungus can be transmitted orally from one species of fish to another.

At present no therapy of ichthyophoniasis is available (McVicar, 1977; Neish and Hughes, 1980). Once established in a closed-water system, the fungus is difficult or impossible to eradicate. In freshwater-culture systems, it has been found that fish are sometimes enabled to wall off and kill the pathogen if the pH of the water is decreased and the temperature raised (Reichenbach-Klinke and Elkan, 1965). Fish exhibiting the slightest external signs of the fungus should be segregated or destroyed without delay and immediately on diagnosis (McVicar and MacKenzie, 1977; Richards, 1977a). When *Ichthyophonus* was identified in a residual plaice stock in an onshore aquaculture system at Hunterston, Scotland, McVicar and MacKenzie (1977) immediately advised the slaughter of all suspect stocks. The disease has not reappeared in the unit.

Various fungicidal drugs have been tested in the treatment of *Ichthyophonus* infections in freshwater-fish culture. Partial success has been obtained with phenoxetol and para-chloro-phenoxetol, but only in the early stages of the disease. Addition of 10 to 20 ml of a 1 %-stock solution (V/V) of phenoxetol per litre aquarium water has been recommended. Simultaneously, the fish are fed on pelleted food soaked in the stock solution. If para-chloro-phenoxetol is used, a 1 ‰-stock solution is prepared, of which 50 ml l^{-1} aquarium water is used, evenly distributed over 1 to 2 days. The water has to be changed after treatment (Reichenbach-Klinke and Elkan, 1965; van Duijn, 1967).

Saprolegniasis is more easily controlled. Fungi of this group respond to various conventional treatments, for example, potassium permanganate (1 g in 100 l of water for 90 min), salt baths (10 to 30 g NaCl in 1 l of water for 20 min), copper sulphate (1 to 5 g in 10 l of water for 10 to 30 min), and silver proteinate (collargol 0.1 mg l^{-1}, 20 min) (Reichenbach-Klinke and Elkan, 1965). Treatment with solutions of zinc-free malachite green has also been used successfully in controlling saprolegniasis (Martin, 1968; Carbery, 1969; Dunne, 1970; Roberts and co-authors, 1973; Atsushi, 1974; Richards, 1977b; and others).

Control of *Saprolegnia* on eggs of *Salmo gairdneri* has been achieved by the application of ozone (Benoit and Matlin, 1966). The inhibition of various disease agents including *Saprolegnia thuretii* by ultraviolet radiation has been tested *in vitro* by Vlasenko (1969); and Martin (1968) conducted *in vitro* studies on the susceptibility of 8 fish-pathogenic members of the Sparolegniaceae to varying concentrations of malachite green and acriflavine. Similar chemicals and methods may be, or have been shown to be, effective in maintenance and farming of estuarine and marine fish species. Neish and Hughes (1980) and Srivastava (1980) have recently reviewed the present status of knowledge of fish mycoses and their control. Most of the information at hand concerns freshwater forms.

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DISEASES CAUSED BY PROTISTANS

JIŘÍ LOM

Protistans are the simplest eucaryotic organisms, mostly unicellular, standing at the beginning of evolutionary lines which lead to multicellular organisms — animals, plants and fungi. This chapter focuses on protistans formerly classified as members of the now obsolete phylum Protozoa. Other protistan groups such as fungal or algal organisms are dealt with in the preceding sections. Fish are hosts to a bewildering variety of these simple, mostly single-celled 'protozoan' organisms which are also agents of the most common parasitic diseases causing troubles in fish cultures. Until the present, protozoans of freshwater fishes were far better known than those of marine fishes. Parasites of freshwater fishes are more conspicuous, especially in pond and river fisheries. They are more accessible to control measures, and their study was prompted a long time ago by the need to control frequent epizootics in pond fisheries.

Until recently, the supposedly formidable supplies of the marine environment fostered the belief that the study of marine fish parasites including protozoans was of little more than academic interest. Protozoan parasites of marine fish — of which there are about 820 species known to date — were studied for the attractivity they presented as zoological objects or as models for studies of host-parasite relationships. Most of the interest in these organisms was rather one-sided, concerning taxonomy and morphology. With the advent of marine pisciculture this attitude is gradually changing. That protozoans have attracted less attention than helminth and crustacean parasites of marine fish hosts is due to greater technical difficulties in the study of these microscopical and fragile organisms. The monograph of Shulman (1984), though limited by its regional scope, offers comprehensive characteristics for identification. For more specialized techniques we refer to Bulla and Cheng (1976; microsporidans), Lom (1969; myxosporeans) and Lom and Laird (1969; trichodinid ciliates).

A good general protozoological background has been provided by Grell (1973), in the series edited by Kreier (1977–1978), and by Sleigh (1973). Useful data on marine fish protozoans can be found in a book of reviews edited by Snieszko (1970) and in the monograph by Sindermann (1970a). Relevant sources of taxonomic information are the monographs of Sprague (1977b; Microsporida), of Kudo (1920) of Shulman (1966; Myxosporea), of Pellérdy (1974; coccidia) and of Corliss (1979; ciliates); the annotated list of fish coccidia by Dyková and Lom (1983) and the review of fish infecting trypanosomes and trypanoplasms by Lom (1979).

Protozoans as Parasites of Fishes and Agents of Fish Diseases

After about 80 yr of constituting the single phylum 'protozoans', this is no longer a formal taxon but a vernacular term. 'Protozoans' have been divided into 7 new phyla according to the new classification proposed by Levine and co-authors (1980). Members of 5 protozoan phyla parasitize in fishes: Sarcomastigophora, Apicomplexa, Microspora,

Myxozoa, and Ciliates. New, even more dramatic changes in high-level taxonomy may appear in the near future (Corliss, 1981); however, in this chapter the classification of Levine and co-authors (1980) will be adhered to.

One of the phyla, the Sarcomastigophora, includes autotrophic phytoflagellates. Thus, the diversity of protozoan structures and their physiological and biochemical regimes is enormous. It ranges from small amoebas, several microns in size and of simple structure, to flagellates or ciliates with cells up to several millimeters in size and full of intricate, specialized organelles. The types of nutrition range from holophytic to holozoic. The principal evolutionary line in the protozoan phyla has been specialization of cell organelles e.g. for feeding, locomotion and reproduction. Parasitic forms evolved through adaptation to their hosts, including biochemical specialization and formation of new organelles, thus coping with the parasitic way of life. Principal features of defined groups will be mentioned in the appropriate subsections.

Nutrition and Proliferation in Protozoans

Some protozoan species, ectocommensals rather than parasites (e.g., the dinoflagellate *Crepidoodinium;* see section 'Protophytans') are mixotrophs, combining their algal-type autotrophy with the need to derive some of their essential nutrients from the host. However, all 'true' parasites are typical heterotrophs deriving all their nutrition from their hosts. Feeding may be osmotrophic, through the cell membrane (e.g., microsporans), pinocytotic (many groups, especially myxosporea) or phagotrophic. Particulate material may be phagocytized (amoebae) or ingested through the cytostome (e.g., apicomplexa) which may sometimes be equipped with very complex skeletal and ciliary organelles (flagellates, ciliates).

Most protozoans divide by binary fission. After a period of cytoplasmic growth, mitotic division of the nuclei and duplication of some cell organelles is followed by cleavage of the cytoplasm. The cleavage line may run along the longitudinal axis of the cell (flagellates), or transversally (ciliates). The process results in 2 equal daughter individuals which grow up to the original size. Rapid sequence of divisions without growth produces a large number of small infective individuals (e.g., *Amyloodinium* [see section 'Protophytans', *Cryptocaryon*). Budding, a process by which primitive 'larval' individuals are cleaved off the surviving mother cell, is not found in protozoans infecting marine fishes. Merogony is a special case encountered in microsporans and apicomplexans: the nuclei of a grown cell (meront) divide (up to tens or hundreds) without cell cleavage, whereupon the cell divides into a corresponding number of small cells (merozoites), often leaving a cytoplasmic residuum behind. In endodyogony, found in some coccidia, 2 daughter cells arise within 1 mother cell, whose remnants are discarded as the new cells emerge.

Life Cycles and Transmission

The term 'life cycle' refers to a repeated sequence of physiological and morphological changes in protozoans, continually perpetuated under appropriate conditions. Parasitic forms must produce large numbers of individuals to insure the continuous existence of the species.

The simplest life cycle comprises an alteration of trophic or growth phase (cell stage called trophozoite) and cell division. Sexual process, if existing, may occur at irregular intervals. Unfavourable periods may be overcome in the encysted state, when a dormant

protozoan, retracted to a spherical shape to occupy the least volume is shielded by a secreted envelope. This is the cycle of free-living and of most ectoparasitic and digestive-tract infecting protozoans. Transmission is direct, from one host to another. A new fish host is either actively searched for — by free swimming trophozoites or special migratory stages (in sessiline paritrichs) — or is reached passively. The host ingests either trophozoites of intestinal parasites that are able to survive a period of time in water or cystic stages serving for species dispersal. There are minimal structural transformations of the trophozoite *vs.* a cystic stage (amoebae), or of an attached trophozoite *vs.* a migratory free-swimming stage (*Ichthyobodo,* sessiline peritrichs).

In some ectoparasites, the growth phase on the host lasts for a long period of time, resulting in a huge cell volume of the trophozoite called here 'trophont'. It is followed by a rapid series of divisions outside the host producing large numbers of small migratory stages; and there is a marked shape transformation in the 2 stages.

In their search for new hosts, migratory stages of ectoparasites depend on many chances such as fish-population density or water currents. They are guided by chemical stimuli, some by positive phototaxy, keeping them afloat in the water column.

In parasites of tissues and cells, the life cycle is more complex. Blood flagellates are diheteroxenous. They need an invertebrate (leech) to complete the life cycle, and transmit their final, metacyclic infective stages to another fish host. They divide by binary fission. There is a slight degree of structural change in the vertebrate host as the infection develops; and a marked transformation of stages in the leech vector. Sexuality is not known. Microsporans and myxosporeans undergo a period of vegetative growth — expressed as proliferation of trophic stages or growth of a large trophozoite — followed by spore formation. Spores contain an infective germ together with structures important for hatching, all encased by a solid shell. Primitive sexual processes during sporogenesis and soon after spore hatching are difficult to study and require further investigation. Transmission occurs through ingestion of spores discharged from the host's body. Structural differences between trophic and spore stages are enormous.

Apicomplexans have the most complex life cycle involving a regular sequence of asexual trophic phase, sexual phase (formation and copulation of gametes) and spore formation, all comprising essential structural changes. Transmission may be direct, by spore ingestion (most coccidians) or indirect, involving an intermediate host. Some coccidia (e.g., *Calyptospora funduli*) need an invertebrate host to 'ripen', in order to become infective for the fish host which ingests the invertebrate with the sporozoites sojourning in its body. Haemogregarines need a leech vector in which they complete their sexual process and produce numerous infective stages which the leech transmits to a new fish host. In fish piroplasms, also transmitted by a leech vector, the sexual phase is not definitely known.

As a rule, spores discharged into ambient water remain viable for a long period of time. Some cell or tissue parasites produce spores within organs — such as spleen, cartilage or muscles — from which they cannot reach the external environment. Spores can be released from such sites only after death and decomposition of the host's body; or when the host is devoured by a predator, they may pass through its digestive tract to the outside. In several coccidian and microsporan species, autoinfection — mature spores hatch while still in the host's body and initiate a new infection — has been assumed by certain authors to explain some extremely heavy cases of infection.

Relation to Host and Pathogenicity

According to their relation to the host, protozoans affecting marine fishes can be assigned to 4 categories. The first includes free-living ciliates which can attack only injured or stressed fish, mainly fry. These facultative ectoparasites can seriously damage or even kill their hosts.

The 3 other categories comprise obligatory commensals or parasites. The second group consists of ecto- or endocommensals which do not produce evidence of pathogenicity. Ectocommensal ciliates or flagellates such as *Scyphidia* or *Crepidoodinium* and intestinal amoebae and flagellates *(Cryptobia)* belong to this group.

The third category includes protozoans which are just on the borderline between commensalism and parasitism, and may be taken for potential pathogens. Trichodinids on the surface of fishes may develop heavy infections only under certain conditions (stressed or debilitated fish), and then start feeding on disintegrated host cells and injure the host's epidermis. Similarly, intestinal flagellates of the genus *Hexamita* can become pathogens if the natural resistance of the host was lowered.

The fourth and largest group includes a wide variety of 'true' parasites depending completely on nutrients derived from their hosts. Their pathogenicity may, however, differ significantly. Some of them (many myxosporean species, especially those from urinary and gall bladders; some trypanosomes) are well adapted ancient parasites; and, except for depriving them of some nutrients, practically cause no harm to their hosts. In others, the pathogenicity depends largely on the outcome of the dynamic interaction of the parasite's virulence and the host's defence mechanisms. Some parasites, notably tissue invaders (microsporans, coccidians) easily establish an infection. Others, like *Amyloodinium* or *Cryptocaryon*, may cause a detectable infection only under conditions debilitating the host, e.g., in a fish farm or an aquarium.

Pathogenicity of protozoan parasites can be assessed primarily by clinical signs and morphologically detectable changes or lesions, microscopical or histological. In fishes infected with parasites and showing distress or disease, one has to evaluate carefully which is the primary pathogen, especially in ectoparasitoses. Findings of even massively occurring surface protozoans (e.g., trichodinids) can sometimes be misleading and conceal the primary bacterial, viral, or environmental agent responsible for the debilitated condition of the host, allowing secondarily for proliferation of the protozoon. Extensive lesions of parasite origin or drastically changed blood-image values prove the pathogenicity of the protozoan involved.

Immune Response of Fishes to Parasites

The fish immune system includes primarily humoral and cellular reactions (Volume I: Kinne, 1980b, pp. 47–55). Data is extremely scarce on fish immunity to protozoans. Most knowledge of fish humoral immunity, understandably, has been obtained from studies with viral, bacterial and protein antigens. At variance with warm-blooded vertebrates, antibody formation in fishes is temperature dependent. Below a certain level, specific for a given fish species, the antigen provokes no antibody production. There are many more differences, including the globulin fractions involved in antibody production (both β and γ globulins) or classes of immunoglobulins (IgM only in marine fishes) (Ellis, 1982). The immune system functions basically in the same way as in homoiotherms, e.g., specific antibodies may circulate in the blood for up to 1 yr and antibodies can be found not only in the serum but

also in the gut mucus. Premunition or superinfection immunity has been demonstrated among other infections also in those caused by *Calyptospora funduli* (Solangi and Overstreet, 1980). Still, fishes are considered to have a level of antibody formation lower than mammals, and hence may have a low degree of protection which is not sufficient under less favourable conditions, or stress. Therefore, they must have efficient compensatory mechanisms which insure their survival, such as substances involved in resistance to infections and in surface and intestinal mucus. This is especially necessary in aquatic environments which are permanent sources of infective stages of ectoparasites and other potential disease agents.

In healthy fishes, the soft, mucus-covered body surface acts as an effective barrier with non-specific protective ability to repel infective agents or to keep their number below a detectable level. In fishes with slightly lowered resistance, the surface can be settled only by obligatory symphorionts, ectocommensals or ectoparasites but is still refractory to all other potential protistan or microbial invaders teeming in the surrounding water. Only in fishes heavily stressed or injured does the protection break down. They then become subject to heavy parasitization. In extreme cases, stressed hosts may even be susceptible to invasions by various free-living protistans which can become facultative parasites.

Principal steps of cellular response have all been demonstrated in protozoan infections of fishes. Infections with blood flagellates, myxosporeans, coccidians or microsporans induce phenomena such as phagocytosis, encapsulation with connective tissue, hypertrophy and hyperplasia, inflammation, granuloma formation and finally, in favourable cases, tissue repair (Volume I: Kinne, 1980b, pp. 38–49). It is noteworthy that while still growing, cyst-like myxosporean trophozoites or myxosporean xenomas do not elicit any host cell response in the tissue. It is only the grown 'cyst' containing mature spores that sets up the inflammation and triggers the sequence of events leading eventually to the elimination of parasites from the tissue (Dyková and Lom, 1978, 1979, 1980). Low levels of antigenicity in myxosporeans is also indicated by the evidence that some species (*Myxosoma cerebralis*; and *Myxobolus exiguus* infections in mullets; Siau, 1980) probably mimic the antigens of their host.

Since natural, non-specific resistance depends on the condition of the fish host (which in turn depends on appropriate environmental conditions), and given that the immune response is closely related to ambient temperature, marked deterioration of environmental factors may upset the host-parasite balance and result in a dramatic decline in the host's defense capacities. New infections may then be established or chronic ones may flare up.

Ecology of Protozoan Infections of Fishes

Temperature is an all-pervading factor in protozoan infections of fish. It not only controls the immune response but also inhibits or enhances parasite proliferation; and it is one of the principal factors in the ecology and epizootiology of fish protozooses. The infection of *Glugea stephani,* with different species of *Haemogregarina* and *Cryptocaryon irritans* can only be realized above a certain temperature threshold. Temperature is also the primary factor in seasonal prevalence and intensity of infections in many protozoan agents. Kabata (1963a), for example, observed minimal infection of herring with *Eimeria sardinae* and *Goussia clupearum* in summer, while the heaviest infections occurred in winter.

In addition to temperature, there are other important abiotic factors that determine

the degree of parasitization of marine fishes: water currents, dissolved oxygen concentration, illumination, water depth and salinity. According to Polyansky (1958), decreased salinity in some areas is followed by a decline in the occurrence of some parasites of marine fishes, e.g., myxosporeans.

Also biotic factors influence the relationship between fish hosts and protozoan agents. The more varied the diet, the more parasites can affect the fish. Fishes living in littoral zones with abundant plant life are generally infected with a greater variety of parasites than pelagic fishes. The formation of large schools favours the transmission of many parasites. Extensive fish migrations can also increase the rate of parasitization. Also crowding increases the opportunity for infections. Petrushevsky and Shulman (1958) reported that in three-spined sticklebacks in isolated tide-pools, a large concentration of spores of *Glugea anomala* resulted in heavy infections and mass mortalities. During massive infections by nematodes, endoparasitic protozoans may be greatly reduced in number or be even absent. The presence of large numbers of parasites of one species in a given fish usually excludes the presence of large numbers of other species of parasite in that fish. On the contrary, simultaneous invasion of fish gills by monogeneans and trichodinids may exert a synergistic effect upon the population of the latter (Noble, 1963). Because the transmission of their propagative stages is direct and rapid, and because most species require no vector, protozoans are the first to invade young fishes, unless the juveniles live in schools separated from adults.

Distribution

Protozoan species specific for one host may be restricted to a part of its range or spread over the whole area of distribution of their host which may be enormous considering that tunas, for example, migrate over thousand of miles. Within their particular range of distribution, the protozoan parasite may be quite unevenly distributed due to various abiotic and biotic factors including isolation of host stocks. Therefore, its presence may be used as a convenient parasite tag serving for identification of various fish stocks.

There are indications that some protozoan infections are more abundant in cold waters. Khan (1983) states that the prevalence of blood parasites in marine fish in the Northwest Atlantic Ocean increases northwards from tropical waters; and that benthic, coldwater fish are more heavily infected than pelagic or warmwater fish.

Many species seem to exhibit a low degree of host specificity, and some are claimed to be cosmopolitan. *Haemogregarina bigemina* has been reported from over 60 host species from the North and South Atlantic, South Pacific, Mediterranean and Red Seas. *H. mugili* was described from the Brazilian coast and the South Pacific Ocean. *Myxidium incurvatum* allegedly infects more than 20 hosts belonging to different orders from various geographic areas. Whether this is really true or whether the taxonomic species differentiation is not on the appropriate level so that several slightly different species are lumped into one (especially in some *Myxidium* and *Haemogregarina* species) is not certain. This casts some doubts on zoogeographic speculations concerning protozoan parasites of fish. Polyansky (1958) made a zoogeographical analysis of parasites including protozoans infecting marine fishes in the seas around the USSR. He distinguished certain myxosporean and trichodinid species as arctic, arctic-boreal, boreo-mediterranean and amphiboreal elements.

In trichodinids, where a definite identification is possible, many species have been found on a variety of hosts in various geographic regions; e.g., *Trichodina jadranica* in the

Adriatic, Carribean and Baltic Seas and in the Pacific Ocean; *T. raabei* in the North and South Pacific Ocean, White and Black Seas; and *T. ovonucleata* in the Black Sea and the Pacific Ocean.

Agents: Flagellata

Flagellates — members of the subphylum Mastigophora Diesing, 1866, phylum Sarcomastigophora Honigberg and Balamuth, 1963 — comprise a vast assemblage of most varying protozoan forms. It is difficult to offer any all-encompassing definition other than a brief diagnosis: trophozoites with 1 or more locomotory flagella divide by longitudinal (symmetrogenic) binary fission. Fishes are infected by representatives of 5 orders.

Marine fishes are hosts to ectozoic dinoflagellates of 2 genera, *Crepidoodinium* Lom and Lawler, 1981, and *Amyloodinium* Brown and Hovasse, 1946. The first is represented by *C. cyprinodontum* (Lawler, 1967) Lom and Lawler, 1981, probably an innocuous ectocommensal. The representative of the second genus, *A. ocellatum* (Brown, 1931) Brown and Hovasse, 1946 is known as a serious pathogen of marine fishes held in captivity (e.g., Paperna, 1980b). There are probably several *Amyloodinium* species. For further details, consult the Section 'Agents: Protophytans'; Fig. 1-4.

All other flagellate parasites of fishes belong to the heterotroph zooflagellates, class Zoomastigophorea Calkins, 1909. Members of the order Kinetoplastida Honigberg, 1963 have 1 or 2 unequal flagella and are characterized by a single large tube-like mitochondrium which widens at one point to accommodate a large extranuclear DNA-nucleoid. This part of the mitochondrium is called the kinetoplast. The order Retortamonadida Grassé, 1952 comprises parasitic species with 2 to 4 flagella, one of which is associated with a conspicuous ventral cytostomal area. The order Trichomonadida Kirby, 1947 includes exclusively parasitic species, typically with 4 to 6 flagella, one of which is recurrent and may adhere to the cell to form an undulating membrane. There is 1 nucleus and a supporting rod (axostyle) running the length of the body. The order Diplomonadida Wenyon, 1926 unites parasitic flagellates with 2 sets of all cell organelles — 2 nuclei, 2 sets of 4 flagella and skeletal structures — all arranged in a bilaterally symmetrical way, making the impression of 2 flagellates merged into one entity.

In marine fish, flagellates occupy various niches in the host's body. They live on the body surface as harmless ectocommensals or as pathogenic ectoparasites. Two genera infect the blood system; their pathogenic potential has only recently been disclosed. A few species live as harmless endocommensals in the lumen of the digestive tract. A single species infects the ovaries (See the Section 'Agents: Protophytans').

Diseases of the Body Surface

There are 2 kinetoplastid flagellates whose occurrence in salt water has not been known until quite recently. Species of the genus *Cryptobia* Leidy, 1846 have been known from the intestine of marine fish and as ectocommensals from freshwater fish. They have 1 anterior and 1 long, recurrent flagellum with which they can adhere to the substrate, and an ellipsoidal kinetoplast. Although often accused of being the primary cause of diseased conditions in freshwater fish (*C. branchialis* Nie in Chen. 1956) (Fig. 1-13a, b), they are ectocommensals which feed on surface débris, bacteria and suspended particles which they ingest by their anteriorly located cytostome. It appears now that *Cryptobia* can tolerate a wide range of salinities and can live on marine fish. There are 2 reports on the occurrence

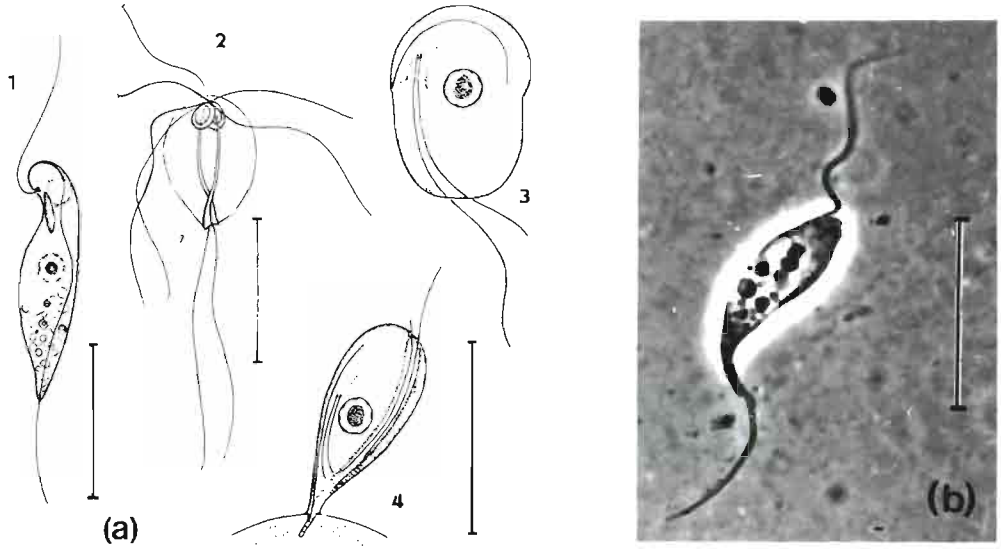


Fig. 1-13: (a) 1: *Cryptobia branchialis,* epibiont on gills of numerous species of freshwater fish and on some marine fish as well; contractile vacuole next to elongated kinetoplast; posterior end of cell filled with ingesta; scale 10 µm. 2: *Hexamita salmonis* from intestine of salmonids, probably identical with parasites recorded in many marine coastal fish; 2 nuclei, 2 sets of basal bodies, 6 anterior and 2 posterior flagella; scale 10 µm. 3 and 4: *Ichtyobodo necator;* 3 — free swimming stage; 4 — attached stage with cytostomeal tube plunged into the epithelial cell in the fish; scale 10 µm. (b) *Cryptobia dahlii*; scale 10 µm. (Original.)

of *Cryptobia* sp. (see Burreson and Sypek, 1981) on the gills of 6 species of fish from Chesapeake Bay, including the summer flounder *Paralichthys dentatus* which was heavily infested. The salinities ranged from 10.9 to 21.5 ‰.

Costiasis is a dreaded ectoparasitosis of freshwater fish, caused by *Ichtyobodo necator* (Henneguy, 1883) Pinto, 1928 (formerly *Costia necatrix*) (Fig. 1-13, 3 and 4). *Ichtyobodo* has two posteriorly directed, unequal flagella. When swimming, it is kidney-shaped; but when attached to the host, it assumes a pyriform shape with its cytostome being plunged into the host's epithelial cells. It is unique in having a multiple kinetoplast. It inflicts severe damage upon skin and gill epithelium resulting in mortalities especially in fish fry. Recently, it has been recorded infesting salmonids in saltwater (Wootten and Needham, 1978). Costiasis can be a significant disease in seawater salmonid culture (Ellis and Wootten, 1978). Heavily infested salmon smolts *(Salmo salar)* from sea-cages suffered from acute hyperplasia and fusion of secondary lamellae. There were mortalities at salinities ranging from 23 to 33 ‰. The mortality rate can be as high as 40 % (Poppe and Hästein, 1982). It was assumed that costiasis was contracted in freshwater prior to transfer into saltwater tanks. Disease control requires that smolts are uninfected on transfer. Formalin baths (1 : 4000, 20 min) have also proved useful.

The first case of *Ichtyobodo* infections in an exclusively marine host was recorded by Bullock and Robertson (1982) who found it in wild populations of juvenile plaice *Pleuronectes platessa* off Scotland. The prevalence was up to 30 % with some plaice being heavily infected. Curiously enough, *Ichtyobodo* thriving in seawater seems to contradict

control measures for freshwater costiasis using sodium chloride solutions, unless the flagellate in question is a physiologically or taxonomically different entity.

The dinoflagellate *Crepidoodinium cyprinodontum* (Lawler, 1967) Lom and Lawler, 1981 is a symphoriont rather than a parasite. The grass-green trophonts measure more than 600 µm in length. They attach by means of a sole-like holdfast organelle with short projections firmly adhering to — but not injuring — the cell membrane of the host's epithelial cells. It was found on the gills of several species of cyprinodontid fish (genera *Fundulus, Cyprinodon, Lucania*) in the estuarine environment on the coast of Virginia, USA. A fully developed chloroplast system within their cells and ample starch production suggest that this species is merely a specialized symphoriont, or an ectocommensal provided that it absorbs soluble nutrients diffusing through the gill surface (Lom and Lawler, 1973).

Amyloodinium ocellatum (Brown, 1931) Brown and Hovasse, 1946 is a well-known pest of marine fishes in captivity causing fatal epizootics under aquarium conditions. For further details consult the Section 'Agents: Protophytans'.

Flagellate Infections of Blood

Species of the kinetoplastid genus *Trypanosoma* Gruby, 1841 infect marine hagfishes (Agnatha), elasmobranchs and teleosts. Thus far, almost 50 species have been described. Many species were described under the earlier widely accepted assumption of strict host specificity of trypanosome species. This, however, is now in doubt. Trypanosomes have a slender, sinuous body. The single flagellum emerges from the flagellar pocket at the posterior end, and then — together with a pellicular fold — forms an undulating membrane extending to the anterior end. The anterior end of the flagellum is free. A single kinetoplast at the flagellum's basal body appears as a minute disc or lens. The largest trypanosomes yet recorded are those from skates: *T. gargantua* Laird, 1951 measures up to 130 and 131 µm (Fig. 1-14).

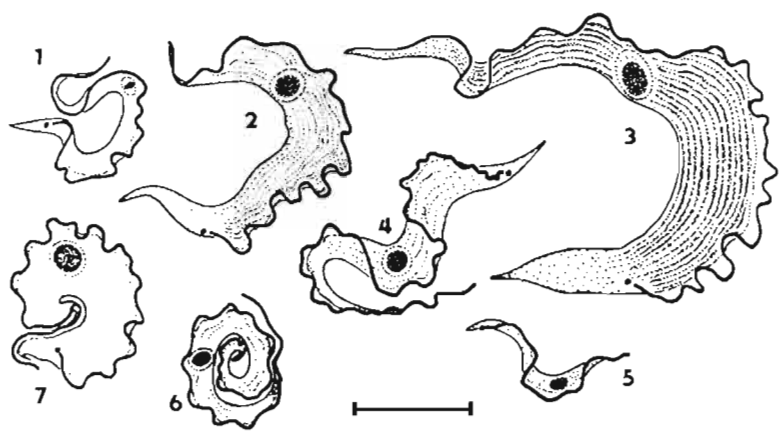


Fig. 1-14: Marine fish trypanosomes. 1, 2 and 3: young, intermediate and adult forms of *Trypanosoma gargantua* (after Laird, 1951); 4: *T. heptatreti* Laird, 1951 (after Laird, 1951); 5: *T. cephalocanthi* Ranque, 1973 (after Ranque, 1973); 6: *T. coelorhynchi* Laird, 1951 (after Laird, 1951); 7: *T. rajae* Laveran and Mesnil, 1902 (after Laird and Bullock, 1968); scale 20 µm.

The best known among species infecting marine fish, *Trypanosoma murmanensis* Nikitin, 1927 (Khan, 1976, 1978a) was originally described from cod *Gadus morhua*. It was later found to infect a total of 13 species of gadid, pleuronectiform, perciform and anguiliform fishes; and to have a geographically wide distribution ranging from the Barents Sea to the coast of Canada. Both in its wide host spectrum and geographic range, it is not unique among its kins. The same applies, for example, to *T. rajae* Laveran and Mesnil, 1902 from skates. *T. murmanensis* is transmitted by the leech *Johanssonia* sp. In its digestive tract it undergoes morphological transformation (Fig. 1-15). It involves a

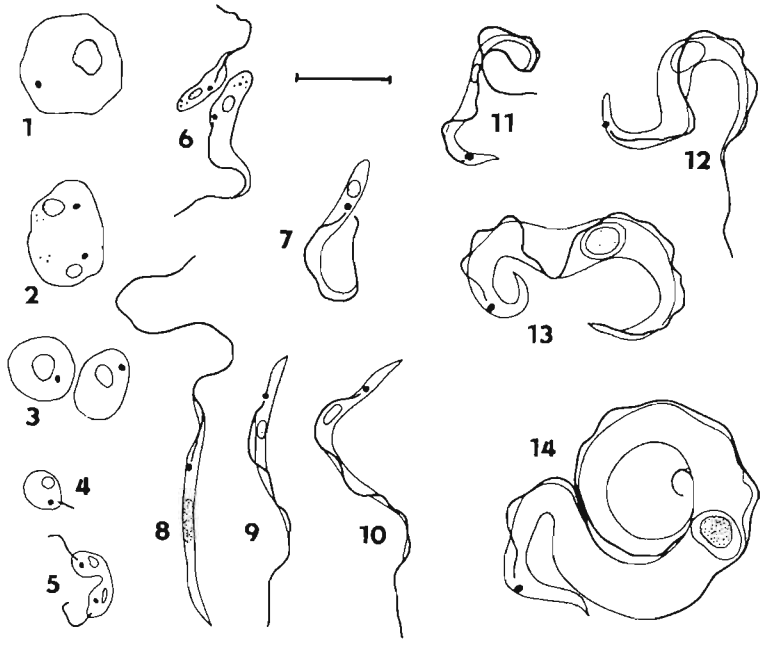


Fig. 1-15: *Trypanosoma murmanensis*. 1 to 14: developmental stages in the leech vector *Johanssonia* sp. 1: Amastigote (non-flagellated stage; nucleus and small dark kinetoplast visible) rounded up from a recently ingested trypomastigote; 2: amastigote about to divide; 3: daughter amastigotes; 4: spheromastigote with only a short free flagellum; 5: dividing spheromastigote; 6: epimastigote stages; 7: slender epimastigote; 8: long, slender epimastigote; 9 and 10: infective trypomastigotes (metatrypanosomes). 11 to 14: trypomastigote stages in blood of *Gadus morhua:* trypanosomes grow bigger and stouter. 11: 10 days post infection; 14: 55 days p.i.; scale 10 μm. (After Khan, 1976.)

sequence of round flagellumless amastigotes originating from trypomastigotes ingested with the blood meal, sphaeromastigotes with only a very short flagellum, slender epimastigote stages and, finally, long trypomastigotes. Amastigote and sphaeromastigote stages reproduce massively to provide an ample amount of metacyclic trypomastigotes — which are injected during the next feeding from the proboscis into a new fish host. The leech can harbour infective flagellates as long as 749 days after the initial blood meal. In the bloodstream of the new host, trypanosomes grow in size and change from a slender to a wide, stubby shape reaching a maximum of up to 84 μm by day 60 post infection. This polymorphism is common among trypanosomes from marine fish (see *T. gargantua* — Fig. 1-14). It involves as a rule, small, intermediate and large forms. The presence of the first

indicates a fresh infection, while large forms are those of chronic trypanosomiasis. The highest prevalence of *T. murmanensis,* 57 %, is in cod measuring 56 to 70 cm. However, in these chronic cases there may be as few as 3 trypanosomes 5 ml^{-1} of blood. The smallest fish infected measured 26 cm, with an infection rate of 25 % and a parasitaemia up to 5×10^5 trypanosomes ml^{-1}.

The prevalence of *Trypanosoma murmanensis* in different stocks in the Newfoundland area varies from 4 to 94 %. These differences are significant enough to be used as parasite tags, supporting conclusions that there are at least 6 different fish stocks in that area.

Until quite recently, both freshwater and marine fish trypanosomes were not assumed to exercise any effect upon their fish host. In freshwater trypanosomes, such harmlessness has already been challenged (Lom, 1979; Dyková and Lom, 1979). Now there is also some evidence of pathogenicitiy in marine species. The effect of *Trypanosoma murmanensis* infections has been examined in adult sculpins *Myoxocephalus octodecemspinosus* (Khan, Barrett and Campbell, 1980). The infection results in a long lasting decrease in hematocrit, hemoglobin and plasma protein levels and a temporary increase of lymphocytic cells. In plaice *Pleuronectes platessa* infected with *T. platessae* Lebailly, 1904 elevated levels of β-globulins were detected in the sera. This can be interpreted as part of the immune response. Most probably, the extremely low parasitaemia in chronic infections of marine fish with trypanosomes — when only a few, non-dividing flagellates can be detected in peripheral blood — are due to acquired host immunity. The pathogenicity of trypanosomes in fry and fingerlings of marine fish has not been investigated. These are the age classes in which the parasites — similar to the situation in freshwater fishes (Lom, 1979) — may have the greatest effect.

The prevalence of trypanosomes may fluctuate throughout the year. *Trypanosoma platessae* has a distinct maximum in March and a minimum in June-July (Cottrell, 1977b). This can be explained by differences in the activity of the temperature-dependent immune system. The March peak may also be linked with an increased contact of plaice with leeches on their spawning grounds in winter.

Trypanosoma cotti Brumpt and Lebailly, 1904 infects sea scorpions *Enophrys bubalis.* It is transmitted by the leech *Calliobdella punctata* in which only the amastigote stages divide. Most other trypanosomes of marine fish were described without any data on their biology.

Trypanoplasmosis, an infection in the marine environment much less common than trypanosomiasis, is caused by species of the kinetoplastid genus *Trypanoplasma* Laveran and Mesnil, 1902. Until quite recently, trypanoplasms have been considered synonymous with the genus *Cryptobia.* Trypanoplasms have a much better developed undulating membrane along their recurrent flagellum. The essential difference is, however, that they are diheteroxenous, i.e., they need a vector — a leech — to complete their life cycle and that in the gut of the vector they produce a series of morphologically and physiologically different stages (Fig. 1-16). Trypanoplasms are clearly pathogenic. Only 4 marine fish-infecting species have been recorded to date.

Trypanoplasma bullocki (Strout, 1965) Lom, 1979 (Fig. 1-16) is known to infect 10 species of heterostomatid, perciform and cyprinodontid fishes along the North American Atlantic coast. It is transmitted by the estuarine leech *Calliobdella vivida.* In this vector, flagellates divide in crop and postcaeca. They assume a rounded shape and divide by

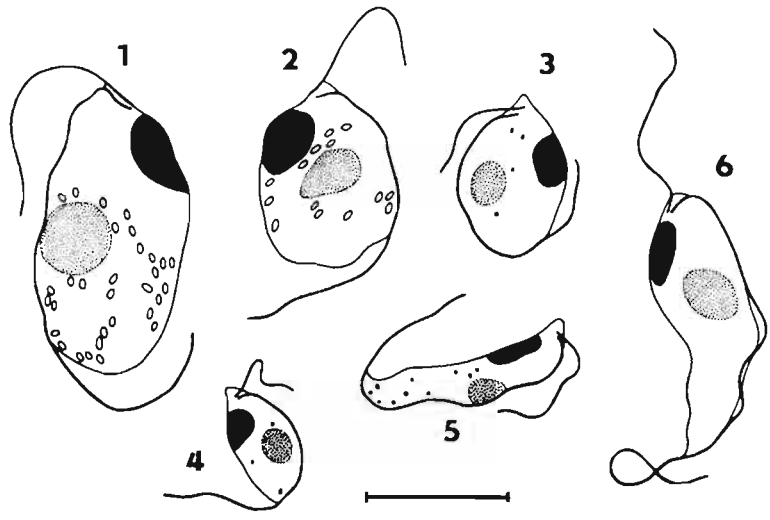


Fig. 1-16: *Trypanoplasma bullocki.* 1 to 5: stages in crop of the leech vector *(Caliobdella vivida);* 1 — 24 h after ingestion; 2 to 4 — proliferative stages; 5 — infective stage; 6 — flagellate in the blood of *Trinectes maculatus.* Dotted structure: nucleus; dark structure: kinetoplast; scale 10 μm. (After Burreson, 1982.)

binary fission. Their final, metacyclic, infective stages are found in the proboscis and are injected into a new host at the next feeding. Infected leeches retain flagellates through 3 subsequent feedings on uninfected fish. The development in the leech can be retarded or accelerated by low or higher temperatures. In the fish, flagellates divide in the peripheral blood and in internal organs. Parasiteamias range from < 300 mm^{-3} to > 2000 mm^{-3} although in lethal infections, they may be as high as 92,200 mm^{-3} (Burreson and Zwerner, 1982). Peak parasitaemia is determined by temperature.

Experimental infections proved high pathogenicity of *Trypanoplasma bullocki* for hogchoker *Trinectes maculatus*; and during winter, for summer flounder juveniles *(Paralichthys dentatus).* In the latter, hematological parameters decreased significantly. The only pathological manifestation was an ascites, grossly distending the abdominal cavity of all infected fish. All infected flounders died within 11 weeks of infection. In nature, over half of the yearling flounder that spend the winter offshore may be infected, many revealing severe pathological changes.

Trypanoplasmosis due to *Trypanoplasma salmositica* (Katz, 1951) Lom, 1979 is common in Pacific anadromous salmonids. It is a disease of the freshwater phase of salmonid life history, although the infection evidently persists in the marine phase.

Infections of the Digestive Tract

The digestive tract of fishes has an extremely poor flagellate fauna, in contrast to other lower vertebrates, e.g., amphibians. There is no ready explanation for this fact (Alexeieff, 1910; Lavier, 1936b). The reasons why some fish species harbour intestinal protozoans while others do not are not obvious. The differences do not seem to be due to diet, both herbivores and carnivores being among those who have intestinal flagellates, or to morphology of the intestine. The taxonomic position of the fish is also not important. Two

families whose members are relatively rich in intestinal protozoans (Gadidae, Sparidae) are not closely related.

One of the few exceptions is the Mediterranean sparid fish *Box boops* which harbours 3 flagellate and 1 opalinid species. The trichomonadid flagellate with 3 anterior and 1 recurrent flagellum, *Protrichomonas légeri* (Alexeieff, 1910) Alexeieff, 1911 (Fig. 1-17), is a harmless endocommensal in the stomach — feeding, among other things, on a fellow endo-commensal, the kinetoplastid flagellate *Cryptobia intestinalis* Léger, 1905.

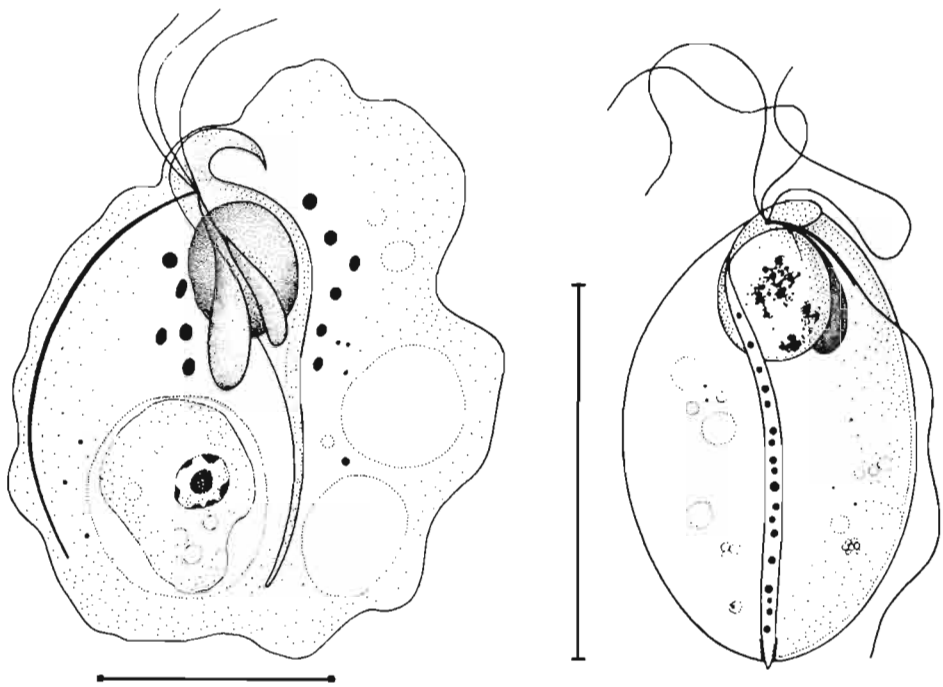


Fig. 1-17: *Protrichomonas legeri* from stomach of *Box boops;* digestive vacuole contains an ingested specimen of *Cryptobia intestinalis;* scale 5 µm. (Modified from Brugerolle, 1980.)

Fig. 1-18: *Monocercomonas molae* from intestine of *Mola mola;* 3 anteriorly directed flagella; 1 recurrent flagellum; conspicuous axostyle, supporting rod running the length of the cell; scale 10 µm. (Redrawn from Noble and Noble, 1966.)

Five more *Cryptobia* species have been found in the stomach of marine fish, e.g., *C. dahlii* (Fig. 13b) (Möbius, 1888) Alexeieff, 1912 has invariably been found in all specimens of lumpfish *Cyclopterus lumpus* in the Northern Atlantic Ocean. Deep-sea fishes in the Eastern Pacific Ocean also have stomach cryptobiids, e.g., *Coryphaenoides acrolepis* harbours *C. coryphaenoideana* Noble, 1968. Transmission of the parasites is probably accomplished by direct transfer from the stomach of the host through the mouth into the water swallowed by another fish (Noble, 1968).

The trichomonadid genus, *Monocercomonas* Grassi, 1879 inhabits the hindgut of several species of marine fish. They too are endocommensals in nature. *M. molae* Noble and Noble, 1966 lives in the sunfish *Mola mola* in California coastal waters (Fig. 1-18).

Chilomastix mediterranaeus Lavier, 1936 is an endocommensal retortamonadid flagellate living in the intestine of *Phycis mediterranaeus.*

Several species of fish in the Mediterranean Sea were found by Lavier (1936b) to harbour flagellates of the genus *Hexamita* Dujardin, 1838 in their intestine. The exact identity of these flagellates is not clear. Some of them — *H. bovis* Lavier (1936b) from *Box boops, H. motellae* Lavier (1936b) from *Gaidropsarus mediterraneus* and *H. copelani* Lavier (1936b) from *Gadus copelanus* – resemble the species *H. salmonis* (Moore, 1923) Kulda and Lom, 1964 (Fig. 1-13) to an extent that they might be conspecific (Kulda and Nohýnková, 1978). *H. salmonis* causes heavy epizootics in freshwater cultures of salmonids. If it is capable of infecting various fish species in the marine environment, it might cause problems in mariculture.

Intestinal flagellates are endocommensals without any pathogenic effect on their host.

Agents: Amoebic and Opalinid Forms

Thus far, marine fishes have been known to harbour only amoebae belonging to the genus *Entamoeba* Casagrandi and Barbagallo, 1895 (order Amoebida Ehrenberg, 1830, class Lobosea Carpenter, 1961, subphylum Sarcodina Schmarda, 1871, phylum Sarcomastigophora Honigberg and Balamuth, 1963). Amoebida are naked, very simple protozoans without any skeleton or fixed cell shape and uninucleate, without a flagellated stage. They move by pseudopodia or locomotive protoplasmic flow. Mostly free-living, they comprise several parasitic genera such as *Entamoeba.* While some *Entamoeba*-species from mammals or reptiles are pathogenic, the species known from marine fish are obviously harmless commensals in the digestive tract, ingesting particles of intestinal contents.

Entamoeba gadi Bullock, 1966 inhabits the rectum of pollock *Pollachius virens* from the Atlantic Ocean. It is up to 24 μm large and is a cannibalistic cystophage, devouring its own cysts (Fig. 1-19, 2 to 5). *E. molae* Noble and Noble, 1966 inhabits the hindgut of sunfish *Mola mola* in the Pacific Ocean.

Opalines (subphylum Opalinata Corliss and Balamuth, 1963, phylum Sarcomastigophora Honigberg and Balamuth, 1963) are neither ciliates nor flagellates. They resemble the former in having a uniformly ciliated, relatively large body. Like the latter, they have nuclei of one type, syngamy as sexual process, divide by longitudinal binary fission, lack any cytostome and have osmotrophic or pinocytotic food uptake. They are all endocommensals in the posterior portion of the digestive tracts of amphibians with the exception of very few species infecting other lower vertebrates. Marine fishes harbour 2 species. As endocommensals, they are not detrimental to the health of their hosts., e.g., *Gadus capelanus* from the Mediterranean Sea is a host to *Protopalina dubosqui* Lavier, 1936a (Fig. 1-19, 1).

Agents: Apicomplexa

The phylum Apicomplexa Levine, 1970 (formerly Sporozoa or Telosporea) comprises parasitic protozoans equipped in one or most of their life cycle stages with an apical complex – a complex structure consisting of a conoid, polar ring, and other organelles designed for better penetration into the host cell. As a rule, they live intracellularly and have a fixed sequence of proliferative, sexual and sporogonic stages. Infective stages are vermicular sporozoites, formed within spores and/or oocysts. The life cycle of many of

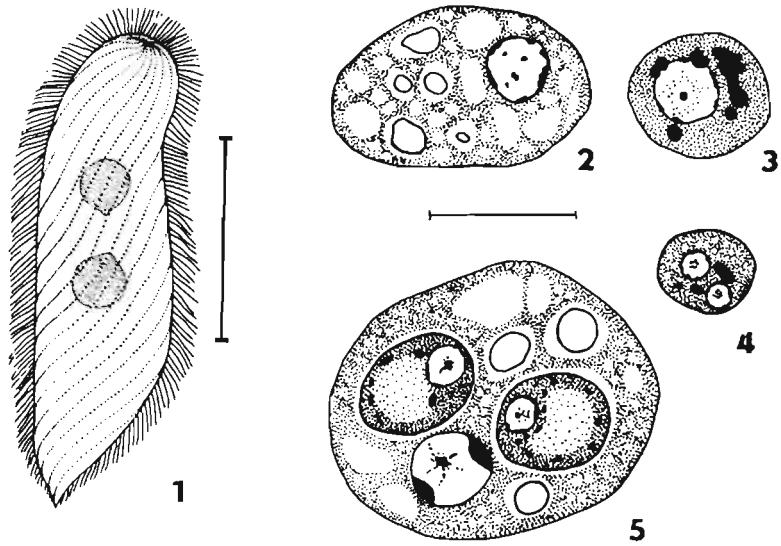


Fig. 1-19: 1: *Protopalina dubosqui* from *Gadus capelanus;* cell with 2 nuclei covered with spiral rows of cilia; scale 50 μm (after Lavier, 1936a); 2 to 5: *Entamoeba gadi* from rectum of *Pollachius virens;* 2 — trophozoite with food vacuoles; 3 — uninucleate and, 4 — binucleate cyst with chromatoidal bodies; 5 — large trophozoite with 2 ingested cysts both having a glycogen vacuole; scale 10 μm. (After Bullock, 1966.)

them requires 2 hosts. They are generally immobile although some stages exhibit gliding or flexing movement. Their feeding is by osmotrophy, pinocytosis and/or through a cytostome. The phylum includes an immense variety of forms.

In marine as well as freshwater fish live representatives of 2 subclasses: Coccidia Leuckart, 1879 and Piroplasmia Levine, 1961. Coccidia of marine fish comprise 2 groups: haemogregarines and coccidians.

Coccidioses

Fish infecting coccidians belong to the families Eimeriidae Poche, 1913 and Cryptosporidiidae Léger, 1911, suborder Eimeriina Léger, 1911. Their life cycle involves as a rule only the fish host, comprising 3 separate phases: (i) massive reproduction (merogony); (ii) formation of structurally different gametes: flagellated microgametes and oocyte-like macrogametes and their copulation (gamogony); (iii) development of the zygote into oocysts with sporocysts and sporozoites (sporogony). Cryptosporidia differ from eimeriids mainly in living epicellularly, within a hypertrophied microvillus of the intestinal epithelial cell (to which they are attached by a special attachment knob); and in forming non-flagellated microgametes. Thus far, only a single species is known from the marine fish, *Cryptosporidium nasoris* Hoover and co-authors, 1981.

Eimeriid coccidia are common parasites in marine fish — more than 50 species have been encountered. They belong to 5 genera: *Eimeria* Schneider, 1875 with sporocysts equipped with a Stieda body plugging a polar aperture for sporozoite hatching (Fig. 1-20, 1) and with a completely intracellular life cycle; *Epieimeria* Dyková and Lom, 1981 with sporocysts *Eimeria*-like but with epicellular schizogony and gamogony; *Goussia* Labbé, 1896 with the sporocyst wall consisting of 2 valves separating in the moment of

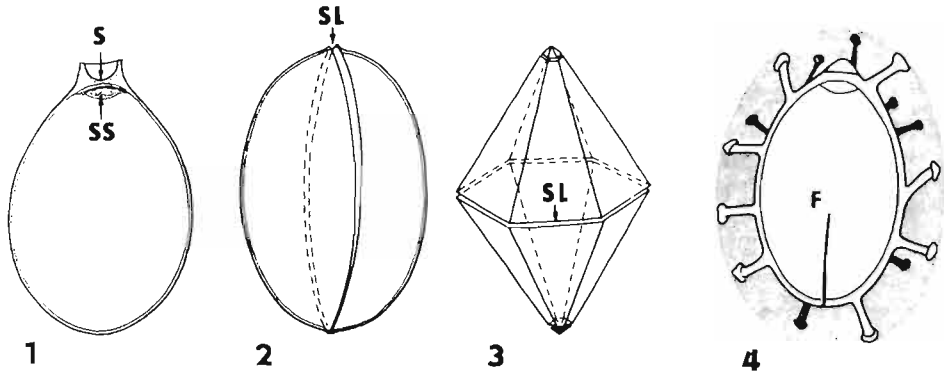


Fig. 1-20: Fish infecting coccidia. Four types of sporocysts. 1: Genus *Eimeria* (S Stieda body, SS sub-Stieda body); 2: genus *Goussia* (SL suture line of the 2 shell valves); 3: genus *Crystallospora;* 4: genus *Calyptospora* (F fissure to be opened for sporozoite release. After Duszynski and co-authors, 1979, modified.)

hatching along a meridian line (Fig. 1-20, 2); the monospecific, enigmatic *Crystallospora* Labbé, 1896 with a crystal-shaped sporocyst wall opening along an equatorial line (Fig. 1-20, 3); and *Calyptospora* Overstreet, Hawkins and Fournie, 1984 with sporocysts covered by a thin veil supported by surface projections or sporopodia and with an apical opening with an incomplete suture (Fig. 1-20, 4). All have oocysts with 4 sporocysts, each with 2 sporozoites. Unlike species from warm-blooded vertebrates, the oocyst wall of fish coccidia is an extremely thin, delicate membrane. As a rule, sporogony is completed already in tissues of the host. Oocysts are shed sporulated, often within 'yellow bodies' which are remnants of host cells destroyed by the parasite. Finally, although mostly localized in the digestive tract, extraintestinal sites of infection are quite common. The infection route is peroral. Transmission is direct by oocysts or sporocysts but there are claims that some species have an invertebrate as an intermediate, probably paratenic, host (Landau and co-authors, 1975 in *Eimeria* sp. with stages in a mysid crustacean; Solangi and Overstreet, 1980 in *Calyptospora* with stages in grass shrimp *Palaemonetes pargio;* MacKenzie, 1978 in a species similar to *Goussia clupearum* with stages supposedly in an euphausiid crustacean).

Coccidia of marine fish are much more common than could be expected from records. Detailed scrutiny would probably reveal them in most if not all of the coastal fish.

Intestinal coccidioses

The degree of pathogenicity in species infecting the digestive tract seems to be rather low; at least there have been no reports of severe lesions or disease symptoms in infected fish. *Eimeria variabilis* (Thélohan, 1893); Reichenow, 1921 (Fig. 1-21) infects pyloric caeca and rectum of *Cottus bubalis* in the North Sea. *E. ivanae* Lom and Dyková, 1981 (Fig. 1-22, 2) infects pyloric caeca of the comber *Serranus cabrilla* in the Mediterranean Sea. Sporulated oocysts are discharged within the yellow bodies. *Goussia lucida* (Labbé, 1893) Labbé, 1896 infects the posterior part of the intestine of dogfish *Scyliorhinus canicula* in the Mediterranean Sea. Macrogametocytes develop within the nuclei of epithelial cells (Fig. 1-23, b) which are thus destroyed. *Eimeria* (= *Goussia*?) *squali* Fitzgerald, 1975 inhabits the spiral valve of dogfish shark *Squalus acanthias* along the

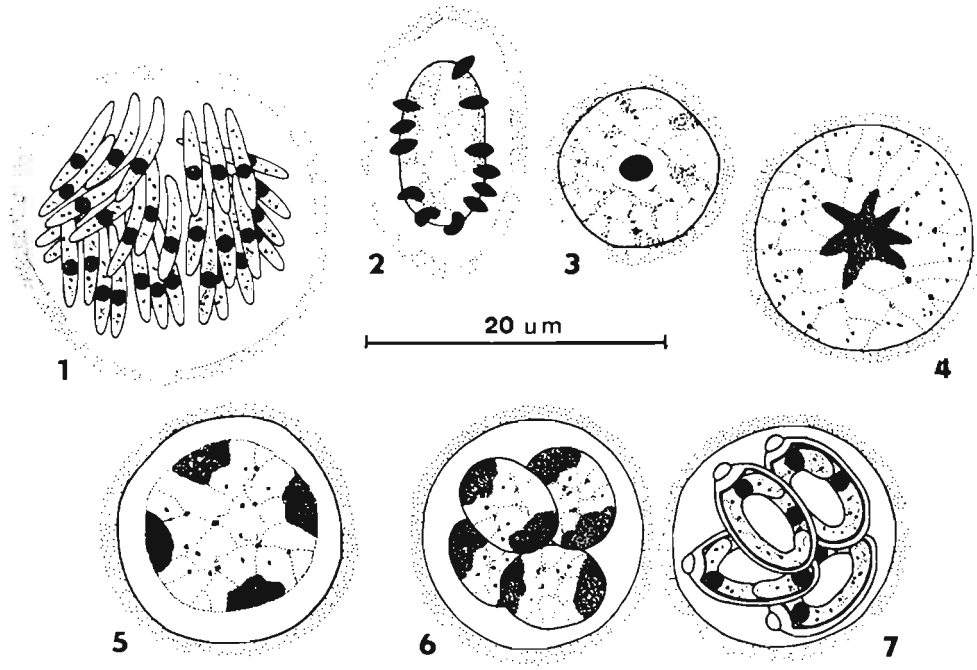


Fig. 1-21: *Eimeria variabilis.* Developmental stages in pyloric caeca of *Cottus bubalis.* 1: merozoites; 2: microgametocyte; 3: macrogamete; 4: zygote starting sporulation series; 5, 6 and 7: sporulation resulting in formation of the oocyst with four sporocysts; scale 20 µm. (After Davies, 1978.)

Pacific coast of the northern USA. It has an ornamentally pitted oocyst wall (Fig. 1-22, 3) and sporulates outside the host. *Epieimeria isabellae* Lom and Dyková, 1981 infects conger eel *Conger conger* in the Mediterranean Sea. It has, similar to *E. anguillae* (Léger and Hollande, 1922), an epicellular development (Fig. 1-24, e to g) except for sporogony which is intracellular. *Cryptosporidium nasoris* developing epicellularly, differs from other marine fish eimeriids infecting the intestine by being obviously pathogenic. An infected tropical marine fish, *Naso lituratus,* was affected by intermittent anorexia, regurgitation of food and digestion disturbances, resulting in severe emaciation (Hoover and co-authors, 1981). *Crystallospora crystalloides* (Thélohan, 1893) Labbé, 1896 is a harmless parasite in the intestine of the rockling *Gaidropsarus mediterraneus* in the Mediterranean Sea.

In *Eimeria brevoortiana* Hardcastle, 1944 infecting the menhaden *Brevoortia tyrannus* in the Atlantic Ocean, merogony and gamogony take place in the epithelium of pyloric caecae, but oocysts are found only in the testes, i.e., only in males. Hardcastle (1944) suggests that the zygote migrates actively into the testicle where the sporogony is completed. The degree of damage to the testicle was not assessed. Final proof that coccidia from gut and testes really constitute a single species has yet to be presented. Oocysts are discharged with the milt during spawning.

Extraintestinal coccidioses

In fish, coccidia living extraintestinally are much more common than in higher vertebrates and sites of infection may vary greatly. As a rule, these species are more

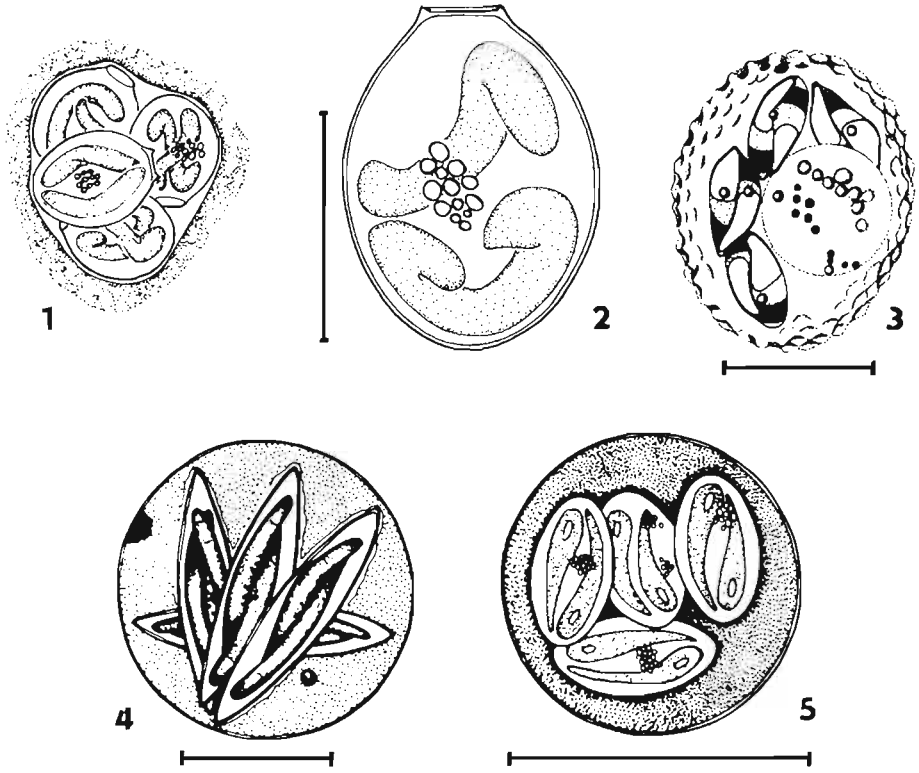


Fig. 1-22: 1, 2: *Eimeria ivanae* from intestine of *Serranus cabrilla*. 1: thin-walled, compressed oocyst with 4 sporocysts inside a 'yellow body'; (original.) 2: sporocyst; 3: oocyst of *Eimeria squali* from *Squalus acanthias* has pitted surface and a voluminous residual body; scale 10 µm (after Fitzgerald, 1975); 4: oocyst of *Eimeria sardinae* from *Clupea harengus;* scale 20 µm (after Möller and Anders, 1983); 5: oocyst of *Goussia clupearum* from clupeoid fishes; scale 20 µm. (After Möller and Anders, 1983.)

pathogenic than intestinal coccidia and infections sometimes may be fatal. Several species of clupeoid fishes, almost all European clupeoids (*Clupea sprattus, C. harengus* and *Sardina pilchardus* in particular), are infected by *Eimeria sardinae* (Thélohan, 1890) Reichenow, 1921 (Fig. 1-22, 4) localized in the epithelial lining of seminiferous tubules in the testis. Heavy infections cause serious lesions with large areas of the tissue suppressed by the parasite, indicating high pathogenicity and an adverse effect on reproduction. Extreme cases may result in sterility of the host due to parasitic castration (Pinto, 1956). In view of the common occurrence of this parasite, it could have an unfavourable effect on the reproduction of an affected population. *E. sardinae* is common along most of the European coast and in western and eastern parts of the Atlantic Ocean. According to Dogiel (1940) it is a stenohaline parasite absent in seas of low salinity such as the eastern part of the Baltic Sea or the Black Sea. Its rare occurrence in the White Sea may be due to low temperature. In the Far East, clupeoids are infected by very similar species: *E. nishin* Fujita, 1934 and *E. etrumei* Dogiel, 1948.

Oocysts of *Eimeria sardinae* were often found in human faeces and erroneously described by Dobell (1919) as a species specific to man: *E. oxyspora.*

Goussia clupearum (Thélohan, 1894) Labbé, 1896 (Fig. 1-22, 5) infects the liver of

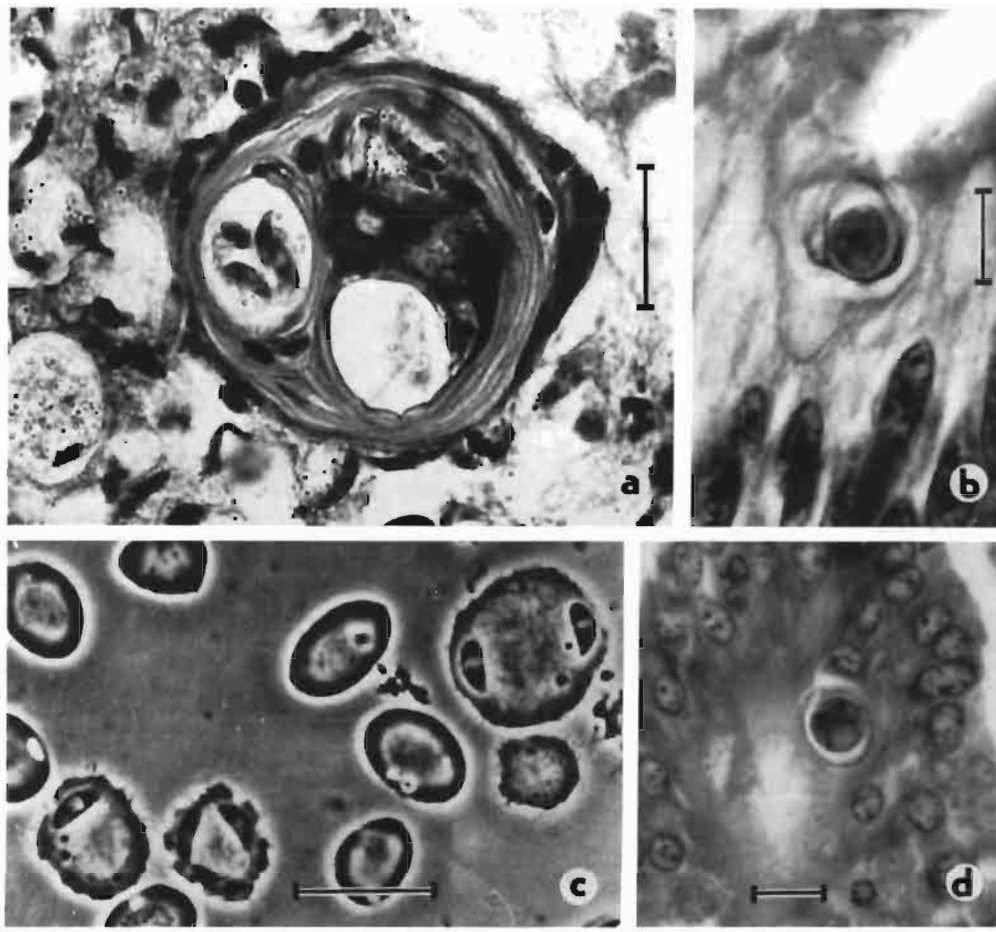


Fig. 1-23: (a) *Eimeria* sp. from the liver of *Micromesistius poutassou;* oocyst encapsulated as a result of host-tissue reaction; haematoxylin-eosin; scale 30 μm. (b) Zygote of *Goussia lucida* developing within the nucleus of an epithelial cell in the intestine of *Scyliorhinus canicula;* scale 10 μm. (a, b — original). (c) *Haemogregarina sachai* merozoites in white blood cells of *Rhombus maximus;* phase contrast, fresh mount; scale 20 μm (after McVicar, 1978). (d) Zygote of *Eimeria nucleocola;* developing within the nucleus of an epithelial cell in the intestine of *Myoxocephalus scorpius* (after Lom and Dyková, 1981); haematoxylin-eosin; scale 10 μm.

many clupeoid fish (e.g., *Clupea harengus, C. sprattus, Sardina pilchardus, Alosa sardina*). Although infections used to be heavy, it is not known as a serious pathogen. An *Eimeria* sp. was found by MacKenzie (1978) in the liver (Fig. 1-23, a) of all adult poutassou *Micromesistius poutassou* caught in the Atlantic Ocean, northeast of Scotland. It would be identical with *G. clupearum,* were it not for the larger oocysts. Curiously, females were infected more heavily than males and were in poorer health, possibly — but without definite evidence — due to the infection. Some of the infected fish had heavy liver lesions, possibly a manifestation of coccidiosis. The infection commences as the fish mature and is cummulative with host age. MacKenzie (1978) suggests that there may be a reservoir host

— a poutassou's prey — in the parasite's life cycle to concentrate the infective stages which would explain the high level of infection. Similarly heavy infections of *Goussia gadi* were explained by Fiebiger (1913) by autoinfection.

A perfect model for experimental work is the species *Calyptospora funduli,* common along the southern Atlantic US coast and in the Gulf of Mexico where it parasitizes in all adult specimens examined of the estuarine killifish *Fundulus grandis* and in some other species of the genus (Duszynski and co-authors, 1979; Solangi and Overstreet, 1980). It is a well described species, and its life cycle can be easily perpetuated in the laboratory. The fact that an infection can be achieved only by feeding shrimp from an infected area, never by direct ingestion of sporulated oocysts, led Solangi and Overstreet (1980) to postulate that shrimp are intermediate hosts. *C. funduli* infects liver and pancreas and rarely also other organs. Infection provokes an inflammatory cell reaction. Later the infected foci are encapsulated along with the appearance of lipofuscin. Ultimately, up to 85% of liver and pancreas are replaced by mature oocysts. In the late seventies, infections reached panzootic proportions in *F. grandis.* Because of its pathogenicity, the parasite can have a detrimental effect on the host population. *C. funduli* was susceptible to treatment with Monensin which, injected or administered perorally, reduced the infection dramatically to 50–70% of that in controls. Premunition probably exists, too. Fish with sporulated oocysts in their organs did not exhibit further development upon reinfection.

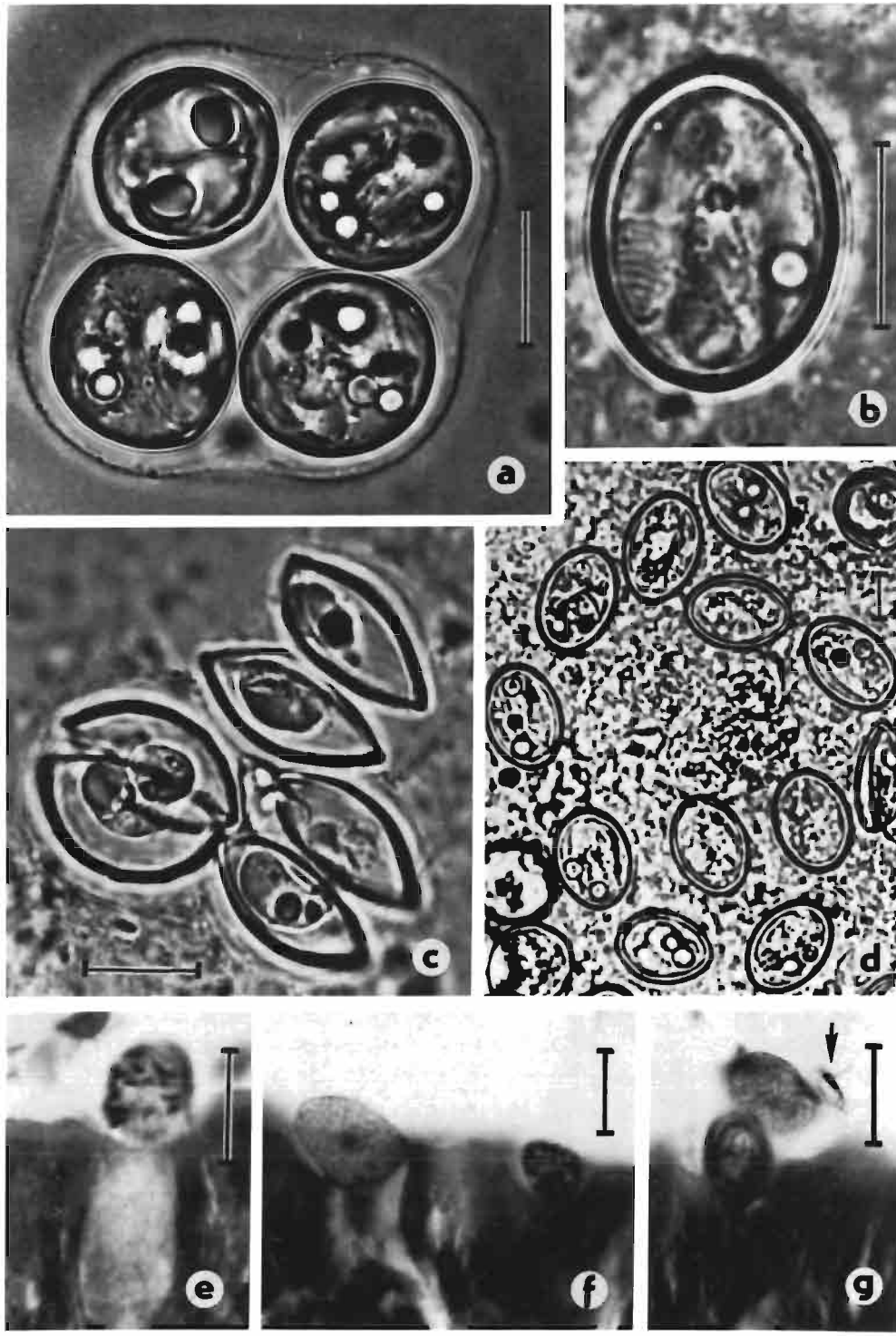
Another pathogenic coccidian, *Goussia cruciata* (Thélohan, 1892) Labbé, 1896, often causes macroscopically visible lesions in the liver of *Caranx trachurus* along the western coasts of France.

In *Goussia gadi* (Fiebiger, 1913) Grassé, 1953 — encountered in the gadids cod *Gadus morhua,* haddock *Melanogrammus aeglefinus,* and pollock *Pollachius virens* — the whole development takes place in the wall of the swimbladder. Mature oocysts fall into the swimbladder lumen where they accumulate in large masses to form — together with lytic debris, fibrous, cellular and lipoid material — a creamy or waxy substance until the bladder is completely filled. Findings of empty sporocyst shells and free sporozoites in such swimbladder contents are interpreted in favour of autoinfection. Fiebiger (1913) found *G. gadi* in up to 5% of cod in a Hamburg (FRG) fish market. Odense and Logan (1976) reported *G. gadi* from up to 58% of haddock on Nova Scotia fishing banks; but, curiously, not from the other 2 fish hosts listed above. There is also a record from Baltic cod *G. morhua callarius* (Shulman, 1962). Heavy infection renders the swimbladder functionless. This impairs buoyancy regulation, swimming and spawning behaviour. Fiebiger (1913) assumed that infected cod might die during the spawning migration when coping with increased physical stress. Odense and Logan (1976) conclude their study by claiming that the infection is fatal.

A similar species, *Goussia caseosa* Lom and Dyková, 1981 (Fig. 1-24, a–d), infects the roughhead grenadier *Macrourus berglax.* Its swimbladder becomes filled with a pasty yellow substance full of oocysts which may also be found in different stages of development in the gas gland. Oocysts may also occur in the blood stream, intestinal submucosa, muscular layer, and other organs. Such infections suggest a pathogenic effect.

Eimeria southwelli Halawani, 1930, infecting the intestinal spiral valve of the elasmobranch *Aetobatis narinari,* was found by Halawani (1930) intracellularly in fish embryos. Its possible way of entrance is not known.

Fish coccidia have an enormous potential for becoming pests in fish mariculture farms.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Further studies on their occurrence, host specificity, transmission and pathogenicity are very desirable.

Haemogregarinoses in Marine Fishes

Haemogregarines, the most common blood parasites of marine fishes, belong to the family Adelaidae Léger, 1911, subclass Coccidia. Their life cycle involves proliferative stages (merogony) in cells of the circulatory system of a vertebrate host, and gamogony and sporogony stages in a blood sucking invertebrate vector. Gamogony is characterized by the association of 2 gametocytes which mature together into 1 female macrogamete and 4 non-flagellated male microgametes, of which 1 copulates with the macrogamete. The zygote produces anywhere from several to many free sporozoites within a thin oocyst envelope. Since the first discovery in 1901 of a haemogregarine by Laveran and Mesnil in *Solea solea* — *Haemogregarina simondi* — more than 80 species of *Haemogregarina* Danilewski, 1885 have been described from red and white blood cells of marine teleosts and elasmobranchs. Contrary to the earlier reported absence of any indication of pathogenicity, it now appears that at least some species of haemogregarines may be serious pathogens.

Our still incomplete knowledge of the life cycle of fish haemogregarines is based on observations of spontaneous infections (Laird, 1953; Davies, 1982: *Haemogregarina bigemina* Laveran and Mesnil, 1901: many teleosts, Fig. 1-25; Khan, 1972b: *H. delagei*; Robertson, 1906: skates, Fig. 1-26; Kirmse, 1978, 1979: *H. sachai;* Kirmse, 1978: *H. simondi* from flatfish, Fig. 1-27). In the vertebrate host, oval crescentic or vermicular schizogony stages develop as a rule in white blood cells (lymphocytes, monocytes, neutrophils) producing through binary fission or true merogony 2 to 8 merozoites per host cell (in *H. sachai* occasionally up to 36). After schizogony cycles, merozoites — mostly vermicular — escape from white blood cells and enter erythroblasts and erythrocytes. There they divide by binary fission or merogony into 2 *(H. bigemina),* 4 (*H. quadrigemina* Brumpt and Lebailly, 1904 from *Callionymus lyra*), 8 *(H. simondi),* or 16 (*H. polypartita* Neumann, 1909 from *Gobius paganellus*) merozoites having the form of stubby or elongated vermicules. Later, these stages differentiate into micro- and macrogametocytes which may then be found freely in the blood plasma. These are species of the 'bigemina' group (Laird, 1952). In other species, intraerythrocytic stages do not divide but transform directly into gametocytes.

Transmission by invertebrates to new hosts was not directly observed in fish haemogregarines, but findings of developmental stages — i.e., gametes, zygotes, oocysts and sporozoites in leeches *(Hemibdella soleae)* or crustaceans (isopod *Gnathia maxillaris* or copepods of the genus *Lernaeocera*) — indicate that these animals act as vectors. The fish is most probably infected during the next feeding of the leech or by eating the isopods which harbour mature sporozoites in their intestine.

Fig. 1-24: a–d: *Goussia caseosa* from *Macrourus berglax*, fresh mounts. (a) maturing oocyst with delicate wall and fine inner membranes; scale 10 µm; (b) sporocyst with sporozoites; their anterior end has a wrinkled surface; scale 10 µm; (c) opened sporocysts with separated shell valves; scale 10 µm; (d) sporocysts in the viscous mass of cell debris in swimbladder contents; scale 10 µm; e–g: *Epieimeria isabellae* on epithelial surface in the intestine of *Conger conger;* (e) developing microgametocyte; scale 10 µm; (f) developing micro- and macrogametocyte; scale 10 µm; (g) microgametocyte with mature microgametes close to a macrogamete; arrow: a released microgamete; scale 10 µm. (Original.)

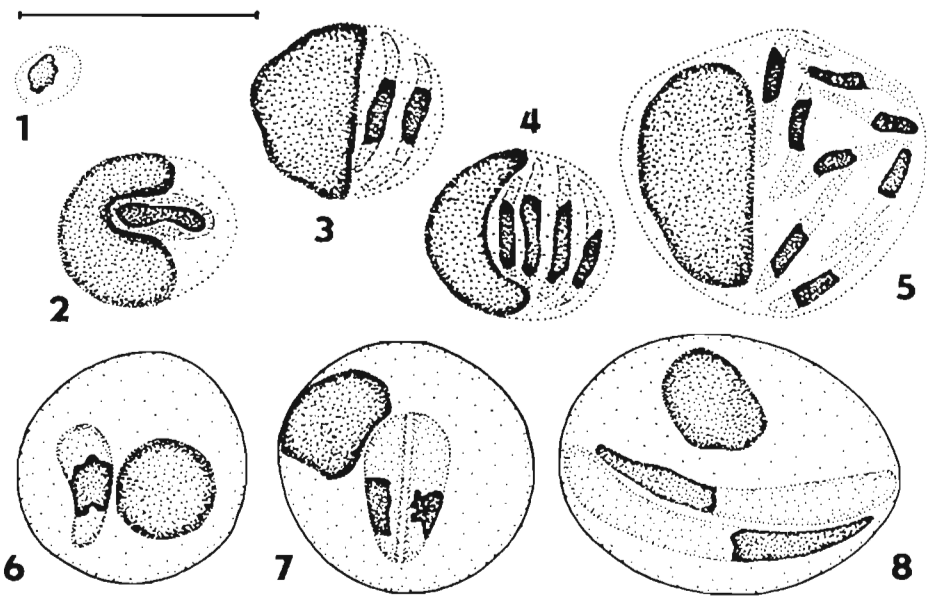

Fig. 1-25: *Haemogregarina bigemina*. 1: Small merozoite, free in the blood plasma; 2 to 5: intraleucocytic stages, starting with a dividing merozoite (2) and resulting in 8 merozoites; 6 and 7: intraerythroblastic trophozoite dividing into 2 gametocytes; 8: 2 grown gametocytes within an erythroblast; scale 10 µm. (After Laird, 1953.)

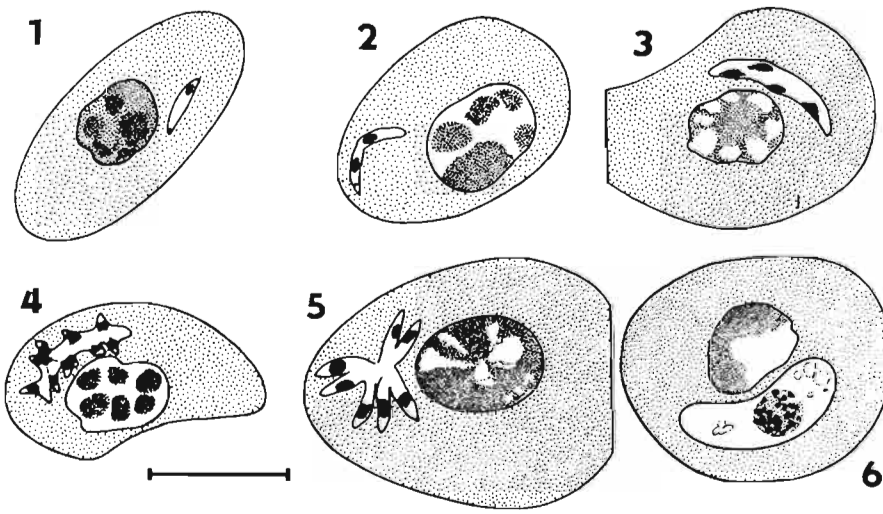

Fig. 1-26: *Haemogregarina delagei*. Intraerythrocytic development in *Raja radiata*. 1: Trophozoite; 2 to 4: merogony; 5: erythrocyte with 7 merozoites about to separate; 6: gametocyte; scale 4 µm. (After Khan, 1972b.)

Haemogregarines producing many, but not more than 100, sporozoites per oocyst were separated by Lainson (1981) in the genus *Cyrilia*. Khan (1978b) describing *C. uncinata* infection in eelpouts *Lycodes vahlii* found several cycles of intraerythrocytic

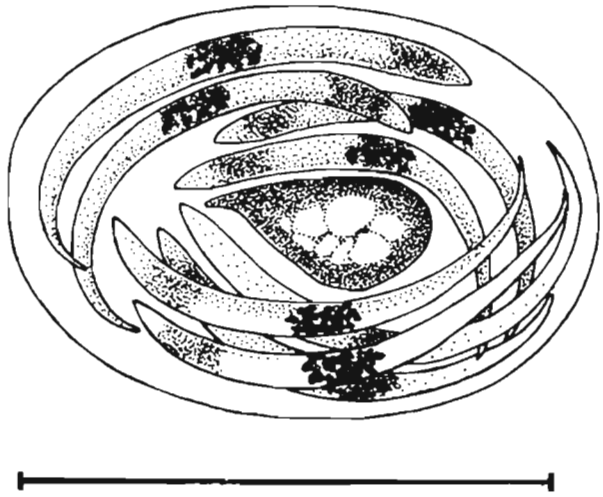

Fig. 1-27: *Haemogregarina simondi.* Erythrocyte of *Solea solea* with 8 merozoites; scale 10 μm. (After Kirmse, 1979.)

merogony (up to 30 small merozoites per erythrocyte). Gamogony takes place in the digestive tract of the leech *Johanssonia* sp., with the formation of 2 to 4 microgametes while oocysts develop intracellularly within intestinal epithelium.

Some haemogregarines are not host-specific and are of almost cosmopolitan distribution. *Haemogregarina bigemina,* for example, known from European and North American coasts, South Africa and the South Pacific Ocean, is claimed to invade more than 60 host species. The distinction between haemogregarine species, however, is not always easy or safe.

Invaded blood corpuscles are hypertrophied, have a distorted shape, a displaced and disfigured nucleus, and may ultimately disintegrate. Most of the infections are very slight and may be chronic (e.g., 16 parasites per 1000 erythrocytes in *C. uncinata*) likewise the damage may be negligible. Laird (1953) observed in young specimens of *Ericentrus rubrus* — not in older fish — a very high prevalence (80%) with 85% of lymphocytes and monocytes infected with *Haemogregarina bigemina.* The resulting damage to young fish may be quite considerable, as may be the case in other haemogregarine infections.

In mariculture, haemogregarine infections may result in severe disease. Ferguson and Roberts (1975) described 3 yr old cultured turbots *Scophthalmus maximus* in Scotland with lethal myeloid leucosis due to *Haemogregarina sachai* (Fig. 1-23, c). Ten percent of the stock examined were affected. Clinical signs were subcutaneous or ulcerated nodules. On the body surface, severely affected fish had space-occupying internal lesions up to 4 cm in size, ascites due to abdominal lesions and exophthalmos associated with retro-bulbar lesions. Lesions were yellow to creamy white with small ones being firm and large ones soft with liquefied centers. They consisted of parasites containing macrophages within a fibrous capsule, at varying stages of degeneration resulting in liquefactive necrosis in the centre of the lesion. Blood of affected fish showed an increase in immature leucocytes. Up to 75% of immature monocytes were parasitized.

Piroplasms in Marine Fishes

Members of the subclass Piroplasmia Levine, 1961 differ from all other apicomplexans in having a simplified apical complex without conoid, and in the lack of oocysts and spores in their life cycle. Typically, they are minute, often highly pathogenic, parasites of blood corpuscles or fixed cells of the circulatory system of higher vertebrates. They are transmitted by ticks in which they undergo sexual reproduction.

Marine fish harbour 3 genera with — as far as known — 5 species. They are very unsufficiently explored, and data on their pathogenicity is lacking. The best known genus is *Haemohormidium* Henry, 1910 thanks to the description of the life cycle of *H. beckeri* So, 1972 by Khan (1980) (Fig. 1-28). This species infects 4 species of perciform fish along the

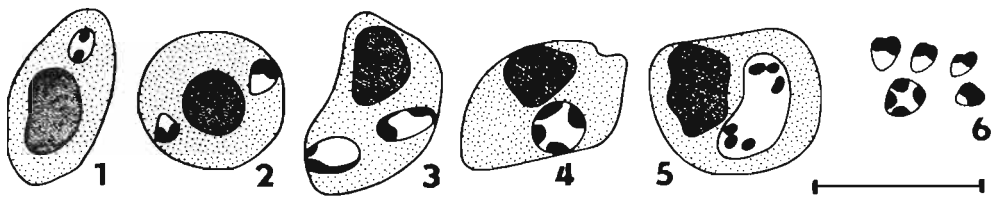

Fig. 1-28: *Haemohormidium beckeri.* 1 to 5: Proliferation of intra-erythrocytic stages from the blood of *Lycodes lavalei;* 1 — binucleate stage; 2 and 3 — nuclei in division; 4 — multi-nucleate schizont; 5 — schizont with 6 nuclei; 6 — intracellular stages from the gut of the leech *Johanssonia arctica,* suggestive of multiple fission; scale 10 μm. (After Khan, 1980.)

coast of Labrador and divides by binary fission and merogony in erythrocytes. Fish from other orders are resistant to infection. No gametocytes could be identified. The leeches *Platybdella obriti* and *Johanssonia arctica* are vectors. Developmental stages were discovered in their gut and proboscis. These infect new hosts at the next feeding of the leech. Infections reached the level of 60 parasites 1000^{-1} erythrocytes. Such a high parasitaemia elicits a leucocytic response consisting mainly of monocytes which phagocytize the merozoites.

Haematractidium scombri Henry, 1913 is a uninucleate parasite with a centrally located nucleus found in erythrocytes of the mackerel *Scomber scombrus* in the Irish Sea and on the US Atlantic coast (Fig. 1-29). *H. scombri* infects up to 45% of the mackerels examined. Up to 5% of erythrocytes may be infected (MacLean, 1980). The life cycle is unknown. The parasite may increase the fragility of the erythrocytes or perhaps lyse them.

The genus *Babesioma* Jakowska and Nigrelli, 1956, sometimes claimed to be synonymous with *Haemohormidium* (see discussion in Becker, 1970), is a parasite of erythrocytes characterized by forming up to 4 merozoites from cruciform or rosette-shaped schizonts. *B. rubrimarensis* Saunders, 1960 was found in the blood of 6 host species of the genera *Lethrinus, Cephalopolis, Scarus* and *Mugil* in the Red Sea (Fig. 1-30).

Agents: Microspora

Members of the phylum Microspora Balbiani, 1882 are strictly intracellular parasites of most animal groups including fish. Their spores, refractile in the fresh state and mostly ovoid, have a thick wall (without any opening) composed of a chitin-protein complex.

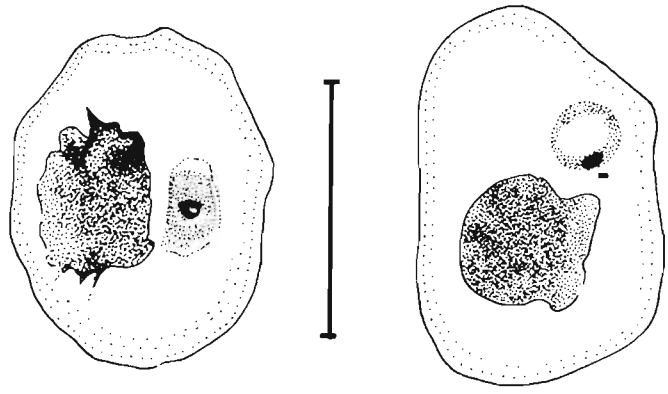

Fig. 1-29: *Haematractidium scombri* next to distorted nucleus in lysed erythrocytes of *Scomber scombrus;* scale 10 µm. (After MacLean, 1980.)

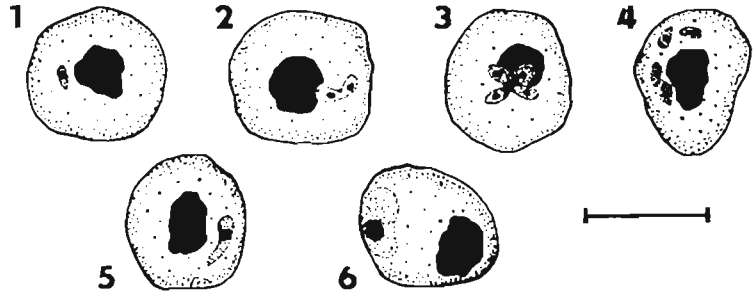

Fig. 1-30: *Babesiosoma rubrimarensis.* Stages in erythrocytes of *Scarus harid.* 1: Trophozoite; 2: binucleate schizont; 3 to 4: merozoites arranged as a cross or (4) free in the erythrocyte; 5 and 6: supposed micro- and macrogametocytes; scale 10 µm. (After Saunders, 1960.)

They contain an infective germ, the sporoplasm and a special extrusion organelle for spore hatching – a hollow, coiled polar tube capable of extremely rapid eversion through the apex of the spore. A large posterior vacuole in the spore and a laminar organelle (polaroplast) together supply the force for extrusion and for driving the sporoplasm through the entire length of the polar tube. This occurs after the spore has been ingested by a specific host. The sporoplasm is then literally injected into the host cell. There it migrates to the final site of infection and starts the proliferative phase by merogony (or binary fission) producing an enormous number of cells (meronts). The final product of merogony is a sporont which initiates sporogony.

In some genera, several to many spores develop within a common membrane or sachet and then stick together in this sporophorous vesicle or pansporoblast. Microspora live within the host-cell cytoplasm. The way in which the trophozoites spread into other organs or body areas is not quite understood. Probably young meronts are capable of migration (active or passive?) and some authors have suggested autoinfection. Some species stimulate the infected cell to an enormous, hypertrophic growth, finding inside it a favourable milieu for growth. The parasite forms, together with the infected cell, a functional and structural unit, a xenoparasitic complex or xenoma (Fig. 1-31). Xenomas appear as whitish 'cysts' up to several mm in size. Ultimately they are transformed into an

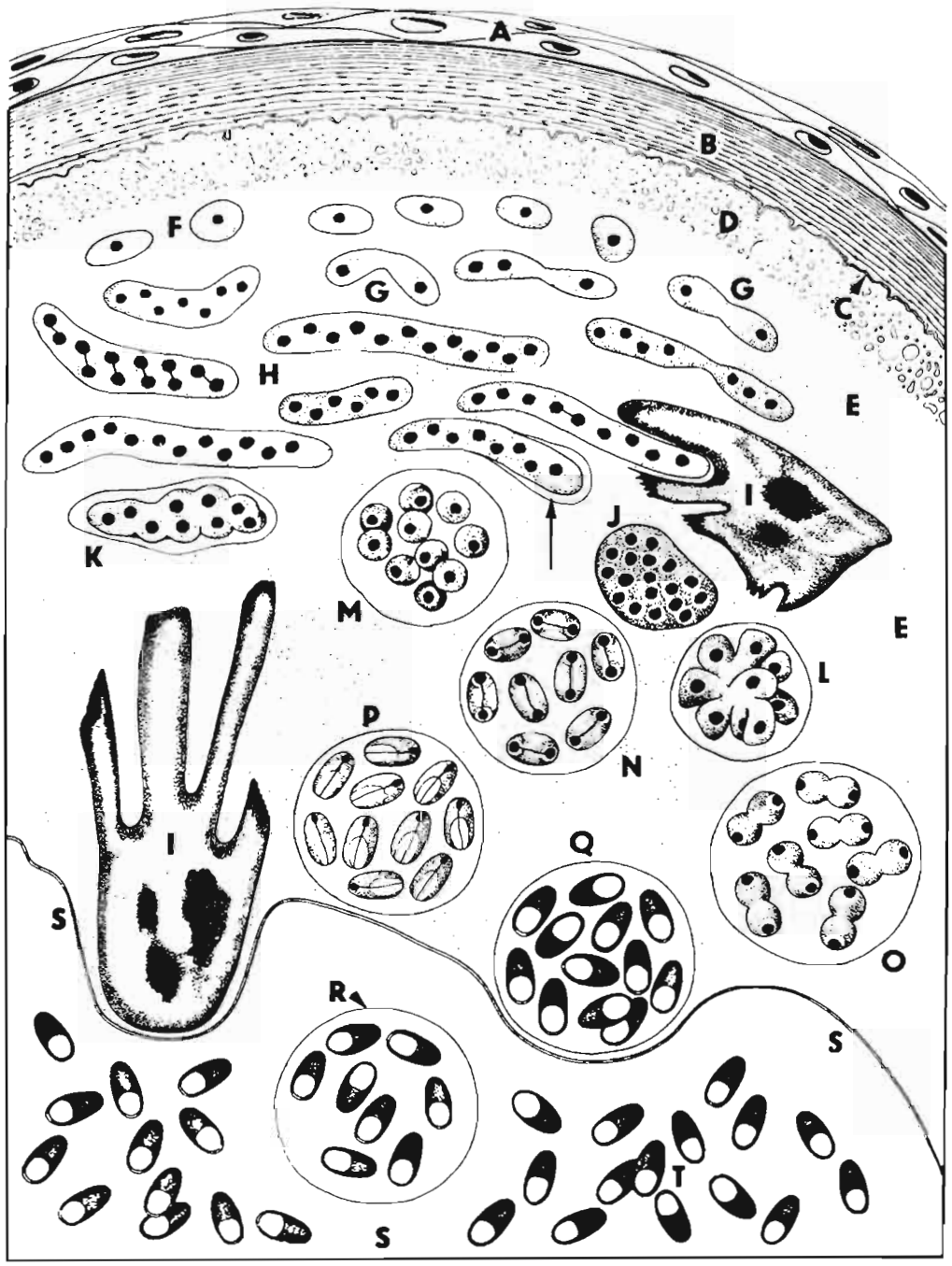

Fig. 1-31: *Glugea anomala.* Diagrammatic representation of a part of the xenoma. A: Layer of apposed connective tissue cells, product of host response; B: refractile xenoma wall; C: cell membrane of xenoma; D: peripheral layer of xenoma with increased pinocytotic activity; E: host-cell cytoplasm; F, G, and H: division of uninucleate stages and larger meronts of *Glugea;* I: host-cell nuclei; J: rounded meront; K to P: sporont starting sporogony within sporophorous vesicles and producing spores (Q); R: sporophorous vesicle wall breaks down later and spores (T) accumulate in the central space (S) of the xenoma. Arrow: initial stage of the formation of the sporophorous veside wall; (Original.)

envelope filled with a huge number of mature spores. By producing the xenomas, the host possibly checks any further spread of the parasite in its organs.

Classification is based on the structure of spores, developmental cycle and type of sporogony. Fishes are infected by representatives of the order Microsporida Balbiani, 1882. Sixty species allotted to 11 genera are known from marine fishes, including the collective group Microsporidium Balbiani, 1884 comprising unsufficiently described species or those with unclear affinities. Data on host specificity are missing in many species, so that differentiation of species with spores of similar shape and structure is rather tricky. These parasites can produce in their hosts most spectacular lesions and some are highly pathogenic.

Microsporidoses Due to Species Producing Xenoparasitic Complexes ('Cysts')

Species of the genus *Glugea* Thélohan, 1891 infect macrophages and other cells of mesenchyme origin. Infected cells settle mostly in connective tissues or in various body organs where they undergo considerable hypertrophy forming large xenomas. Merogony proceeds at the periphery of the xenoma. Masses of mature spores, at first in groups of 8 or more in delicate sporophorous vesicles, ultimately accumulate in the centre. Heavy invasions have disastrous consequences to the host.

A model for the study of host-parasite relation in Microsporidia is *Glugea anomala* (Moniez, 1887) Gurley, 1893 (Weissenberg, 1922b, 1968; Dyková and Lom, 1979; Canning et al., 1982). It infects subcutaneous tissue, body wall, intestine and other organs in sticklebacks *Gasterosteus aculeatus* and *Pungitius pungitius* both in fresh and estuarine waters (Fig. 1-32, a, d, e, f). *Glugea gasterostei* Voronin, 1974 from *Gasterosteus aculeatus* and *Glugea weisenbergi* Sprague and Vernick, 1968 from North American *Apeltes quadracus* are probably synonymous with *G. anomala*. Cyst-like xenomas cause serious dysfunctions of internal organs, deformation of the body and, finally, even mass mortality (Petrushevsky and Shulman, 1958).

Glugea hertwigi Weisenberg, 1911, similar to *G. anomala* both in structure and high salinity tolerance, is an agent of epizootic infections. It attacks the intestine and all other body organs in European and American smelts *Osmerus eperlanus* and *O. mordax* and some other euryhaline salmonids. The prevalence of infection in a smelt population may be 90% (Nepszy and co-authors 1978) or even almost 100% (Weissenberg, 1913: Island of Rügen, Baltic Sea). In severe infections, the body cavity is packed with cyst-like xenomas, up to 250 per fish and (exceptionally) up to 9 mm in diameter, seriously impairing the viability of the host. The intestinal lumen may be completely occluded by the xenomas sticking together in a large compact mass. Intestinal tissue may disintegrate resulting in a general septicaemia or intoxication. Infection of gonads may cause parasitic castration if blocking of the gonadal pore renders discharge of eggs or sperm impossible (Fig. 1-33, a). Resulting mass mortalities are thus due to the combined effect of mechanical and pathophysiological causes. In Canada, annual losses may be as high as several tens of millions of fishes.

Infections due to *Glugea stephani* (Hagenmüller, 1899) Woodcock, 1904 occur in 11 species of flatfish in the Mediterranean, Black, White, Baltic and Northern Seas, and in the Atlantic and Pacific Oceans; however, *G. stephani* depends on warmer waters. Temperatures of about 11 °C or lower inhibit its development (Olson, 1976). Site of infection is the intestine which, in heavy infections, becomes massively pervaded by up to 0.5 mm large

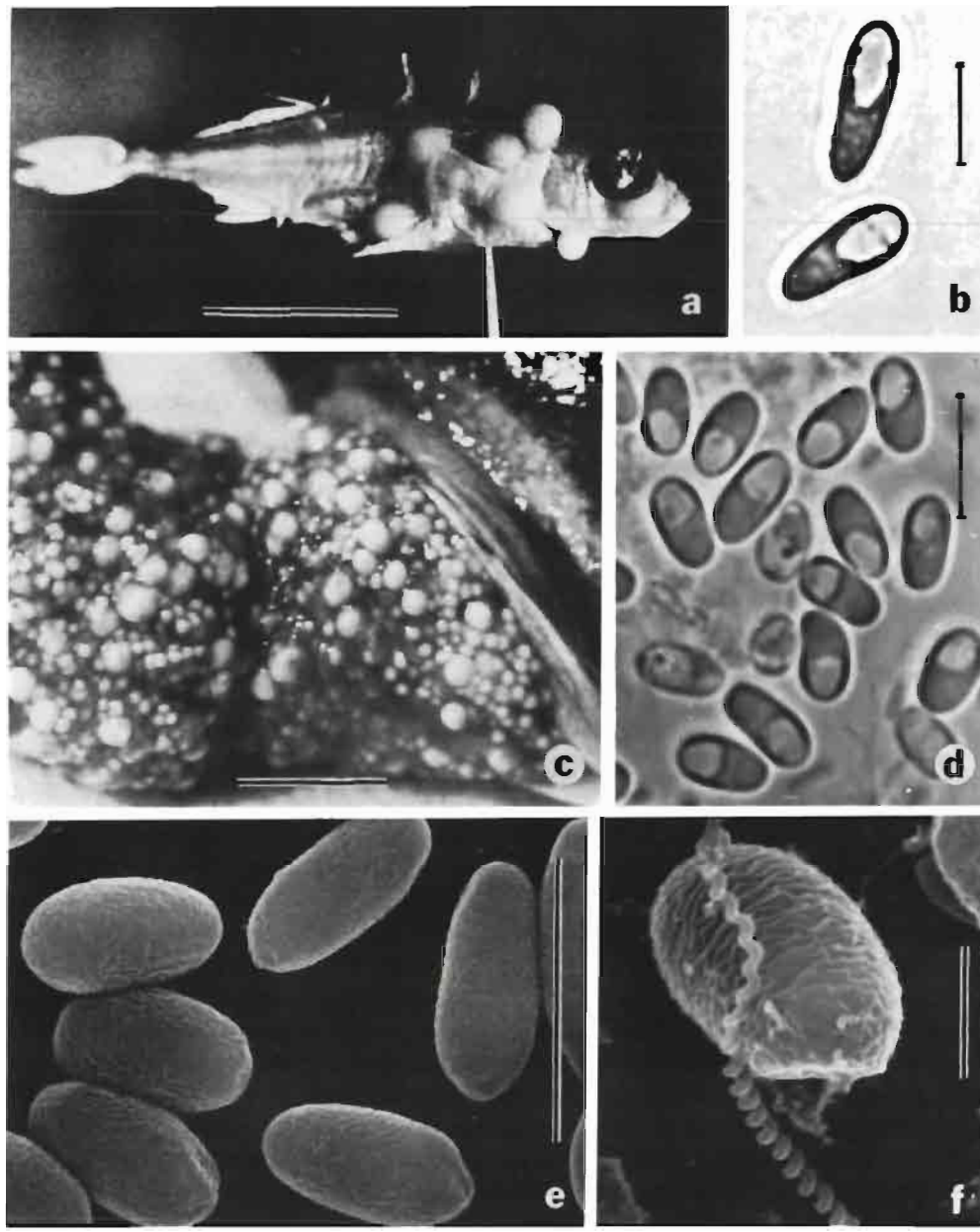

Fig. 1-32: (a) Stickleback *Gasterosteus aculeatus* infected with *Glugea anomala;* large subcutaneous xenomas protrude above the body surface; scale 2 cm; (after Möller and Anders, 1982.) (b) Fresh spores of *Glugea berglax* from intestinal wall of *Macrourus berglax;* scale 5 µm. (c) Intestine of flounder pushed out from anal opening; tissue heavily pervaded by xenomas due to *Glugea stephani;* scale 5 mm; (after Möller and Anders, 1982). (d) Fresh spores of *Glugea anomala* from stickleback; scale 5 µm. (e) *G. anomala* spores as seen in scanning electron microscope; scale 2 µm. (f) *G. anomala* spore with incompletely extruded, still coiled polar tube; scale 1 µm; (original.)

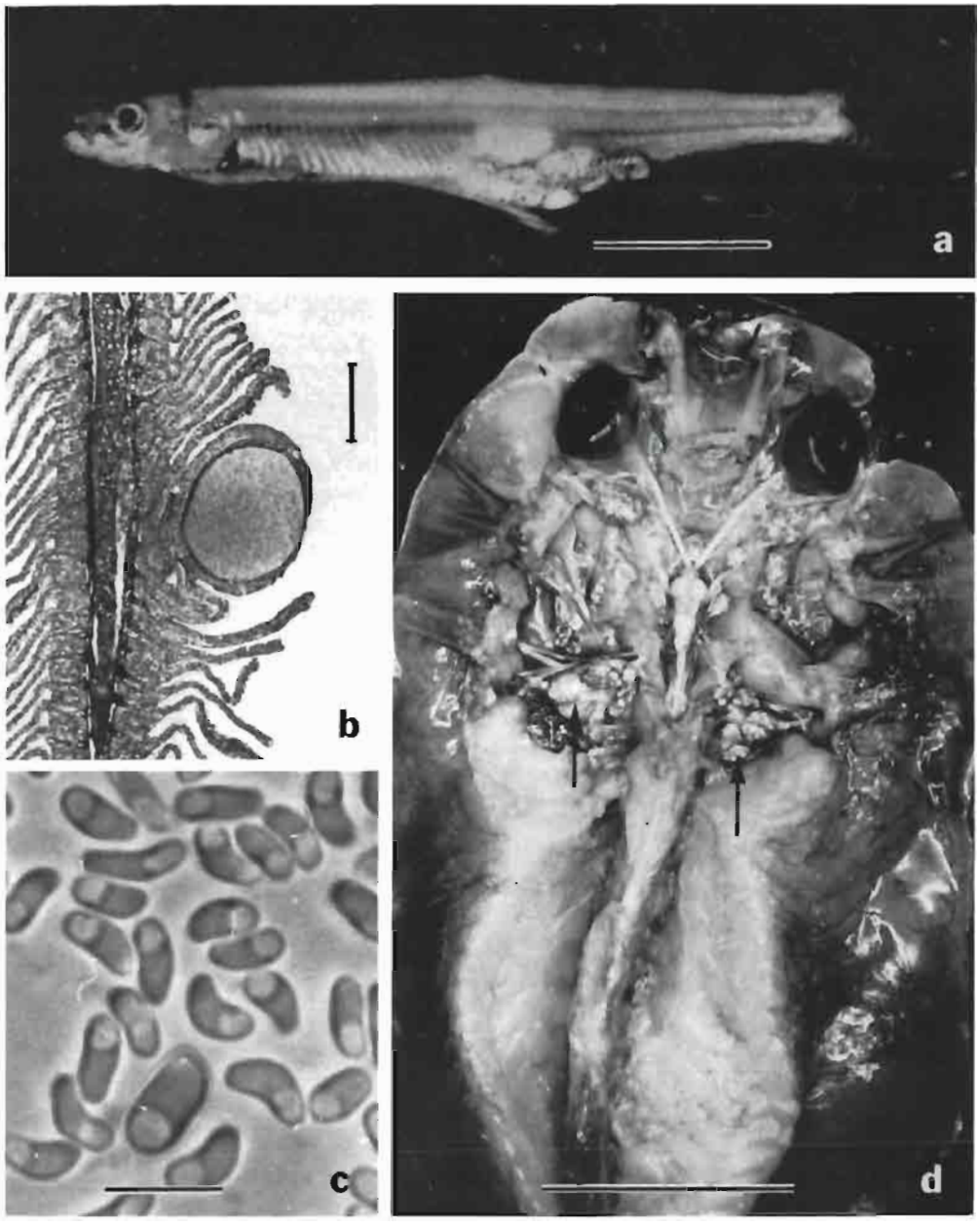

Fig. 1-33: (a) *Osmerus eperlanus mordax* seriously infected with *Glugea hertwigi*. Cyst-like masses of xenomas at rectum show through body wall; scale 1 cm; (photo courtesy of Dr. C. Delisle.). (b) *Loma branchialis* xenoma within secondary gill lamella of *Melanogrammus aeglefinus;* haematoxylin-eosin; scale 200 µm. (c) Spores of *Spraguea lophii* from *Lophius americanus* are dimorphic: small, curved ones and large, oval ones (bottom, centre); fresh mount; scale 5 µm; (b, c–Original.). (d) *L. americanus* infected with *L. lophii,* dissected to show neural ganglia (arrows) full of cyst-like agglomerations of large xenomas; scale 10 cm; (photo courtesy of Dr. S. Jakowska.)

xenomas and their aggregates (Fig. 1-32, c). The gut appears largely covered by layers of cysts, chalk-white in appearance and with rigid and thickened walls. Ovaries, liver and mesenteries may also be affected. Intestinal failure finally results in death. Prevalence in a given wild population may vary from 1.2% (in *Pseudopleuronectes americanus;* Stunkard and Lux, 1965) to 60% (in *Pleuronectes flesus;* Hagenmüller, 1899). Juveniles are particularly susceptible to infection and those heavily infected may not survive their first year. Survivors which appear healthy have a mean carrying rate of infection of about 3.5 to 8.8% (see also Takvorian and Cali, 1981). In grown plaice, the level of infection falls in time, indicating effective host reaction (McVicar, 1975a; Dyková and Lom, 1981). In experimentally infected juvenile plaice, mortalities set in within 2 months of spore administration (McVicar, 1975a).

Enormously large xenomas, up to 13 mm in diameter, are produced in sand smelts *Atherina boyeri* in brackish lagoons along the French Mediterranean coast, infected with *Glugea atherinae* Berrebi, 1978. The infection rate is up to 21%. The agent affects mainly the intestine which becomes partially obstructed. Large xenomas cause pressure atrophy of affected organs, thus impairing the viability of the host. There is an indication that the fish become infected in their first year. Infected specimens probably succumb to the disease and the surviving population contracts the infection once again the following year.

Pressure atrophy of intestinal and ovarial tissue is the result of infection with *Glugea capverdensis* Lom, Gaevskaya and Dyková, 1980 in lantern fish *Myctophum punctatum,* due to a great number of up to 2 mm large xenomas. In many other *Glugea* species producing small xenomas, the pathogenic effect on the host is not noticeable, e.g., in *G. berglax* Lom and Laird, 1976 infecting the intestine of roughhead grenadier *Macrourus berglax* (Fig. 1-32, b).

Conspicuous infections with xenomas similar to those of *Glugea*-type are caused by microsporidans assigned to separate genera, due to differences in the structure of the xenoma or sporophorous vesicle. *Loma branchialis* (Nemeczek, 1911) Morrison and Sprague, 1981 with only 1 or 2 spores per sporophorous vesicle is frequently found on the gills of various gadid hosts (e.g., *Gadus morhua, Melanogrammus aeglefinus*) of boreo-arctic distribution (Fig. 1-33, b). Xenomas about 1 mm in size may cause moderate pathological changes in the infected gill lamellae. However, Kabata (1959) did not observe any harmful effects in fishes with heavily infected gills. *Tetramicra brevifilum* Matthews and Matthews, 1980 produces 4 spores from 1 sporont. It forms small interlocking xenomas which give rise to several-mm-large cystic formations in skeletal muscles of turbots *Scophthalmus maximus* along British coasts. Heavy infections can lead to the inactivation of a substantial proportion of the body musculature and impairment of swimming. This parasite is a potential threat to turbot farming.

Infections with 3 other genera bring about the origin of xenomas of quite different nature. *Spraguea* (= syn. *Nosema*) *lophii* (Doflein, 1898) Sprague, 1977b infects ganglion cells chiefly in the cerebro-spinal region of angler fish, *Lophius piscatorius, L. americanus* and *L. gastrophysus* in the Mediterranean Sea, the North Sea and the Atlantic Ocean. It is a very common parasite with prevalences ranging from 32 to 100%. In large, market-sized fishes off the Icelandic coast, the prevalence was 46.5% (Priebe, 1971b). The parasite invades a part of the ganglion cell near the point of its exit or the axon itself, producing a xenoma without a special envelope where numerous xenomas coalesce to form macroscopic, grape-like tumours characteristic of this species (Fig. 1-33, d). The xenoma has an

inner core with slender, curved spores and a peripheral layer with larger oval spores (Figs. 25 c and 27 a). *S. lophii* has evidently (Loubès and co-authors, 1979) dimorphic spores. In spite of the serious effects on the central nervous system, the parasite seems to exert no harm to its host.

Most conspicuous masses of spores are found in fishes infected with *Ichthyosporidium giganteum* (Thélohan, 1895) Swarczewsky, 1914. Corkwings *Crenilabrus melops* and spots *Leiostomus xanthurus* show enormous abdominal swellings (Fig. 1-34, a) due to a parasite mass consisting of numerous, up to 4 mm large, xenomas. These feature a peculiar cytoplasmic envelope originating in a very complicated way in hyperplastic connective tissue and producing large quantities of spores. *Microsporidium cotti* (Chatton and Courier, 1923) comb. n. produces lesions in the testes of sculpins *Cottus bubalis,* including a xenoma with brush-border up to 0.7 mm in size.

Microsporidoses Due to Other Microsporidans

These are chiefly due to species of the genus *Pleistophora* in which a large, multinucleate sporont (sporogonial plasmodium) segments into irregular numbers (> 16) of spores within a thick-walled sporophorous vesicle. Spores of different size (micro- and macrospores) may be produced simultaneously. The parasite infiltrates the tissue diffusely. Infected myocytes are not delimited from healthy tissues except for connective tissue capsules in advanced and terminal stages of infection. In marine fishes chiefly skeletal muscles are infected.

In ocean pouts *Macrozoarces americanus, Pleistophora macrozoarcidis* Nigrelli, 1946b induces large tumour-like masses measuring up to 8 cm or more in diameter (Nigrelli, 1946b). Wherever the parasite penetrates, complete hyalinization and destruction of the muscle results, with eventually only granular débris and spores remaining (Fig. 1-34, b). Occasionally, the host lays down an extensive protective layer of fibrous connective tissue. Ulcerative condition was not observed. Autoinfection may be possible (Nigrelli, 1946b) and the infective germs may set up new centres of merogony in other body regions. This may be a new spreading parasite. The prevalence also seems to increase with fish age class.

A closely related species — *Pleistophora ehrenbaumi* Reichenow, 1929 — infects wolf-fish *Anarhichas lupus* in the North Sea. Similar to the preceding species, it forms large unsightly tumour-like swellings, the size of a man's fist. The muscle tissue is reduced to débris pervaded by the host's phagocytes which ingest the spores. Claussen (1936) found the same parasite in *A. minor,* where it formed similarly large tumours, and tried to explain such heavy proliferation by autoinfection. *P. duodecimae* Lom, Gaievskaya and Dyková, 1981 infects, considerably enlarges and inflicts heavy damage to muscle fibres in rat-tails *Coryphaenoides nasutus* in the Atlantic Ocean (Fig. 1-35, b).

In Japanese mariculture farms, a microsporidian provisionally named *Microsporidium seriolae* Egusa, 1982 (possibly a *Pleistophora*) has caused serious outbreaks of 'Beko' disease among juveniles of the yellowtail *Seriola quinqueradiata.* The microsporan formed fibrous-membrane bounded masses in the trunk muscles. Serious destructions of musculature with resultant ulceration may be caused by *Pleistophora gadi* Polyansky, 1955 in cod *Gadus morhua.* It may be considered highly pathogenic to cod fingerlings. Massive infections may be fatal (Polyansky, 1955a). Heavy muscular invasion of *Theragra chalcogramma* in the Sea of Japan have been recorded repeatedly since Akhmerov (1951). They

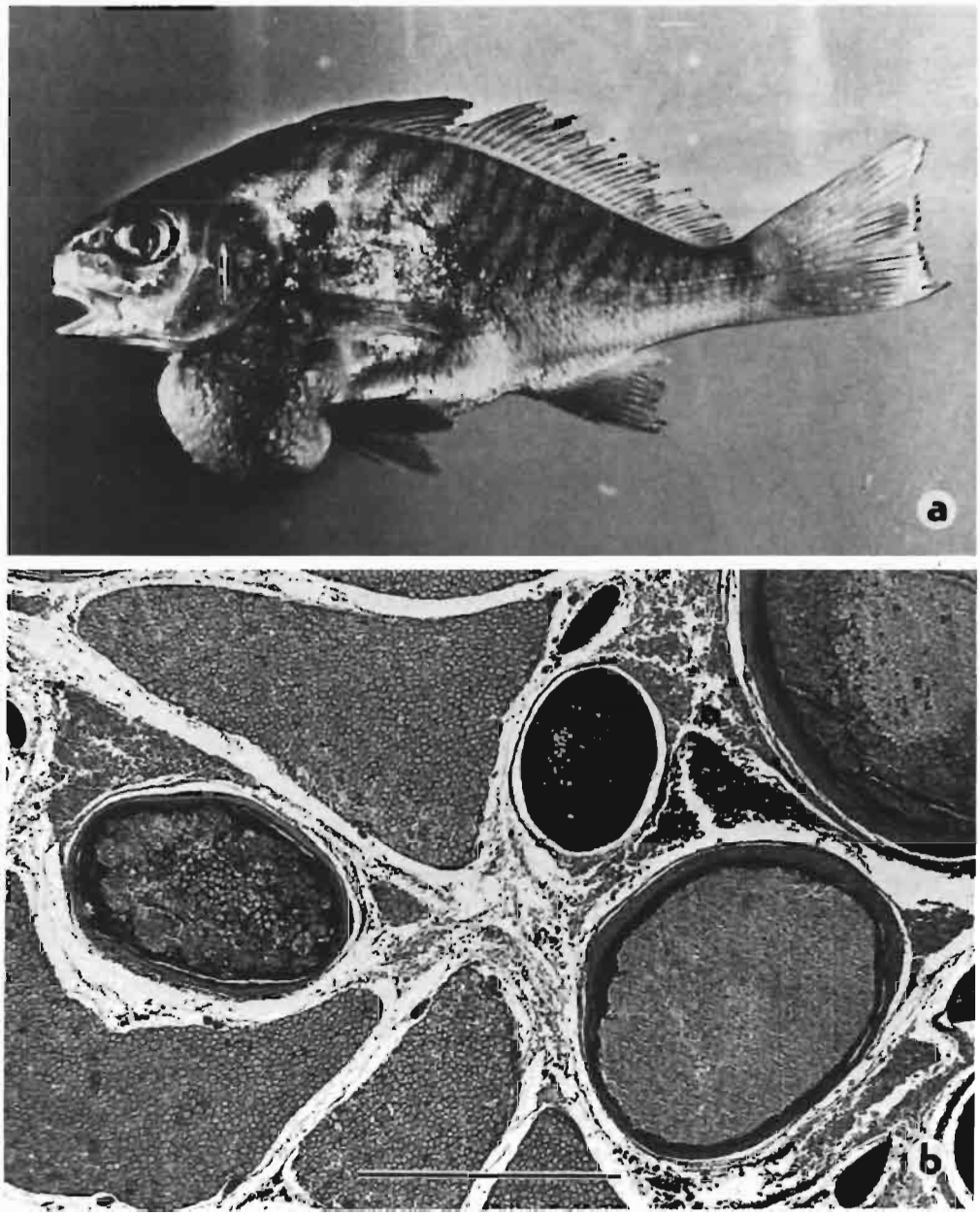

Fig. 1-34: (a) Spot *Leiostomus xanthurus* with ventral bulge due to tumour-like parasitic masses induced by infection with *Ichthyosporidium giganteum;* length of fish 11 cm; (photo: Dr. F. J. Schwartz.). (b) *Pleistophora macrozoarcidis* infection of muscle fibers of *Macrozoarces americanus;* sarcoplasm completely replaced by parasite; some fibres surrounded by connective tissue envelope; haematoxylin-eosin; scale 500 µm. (Original.)

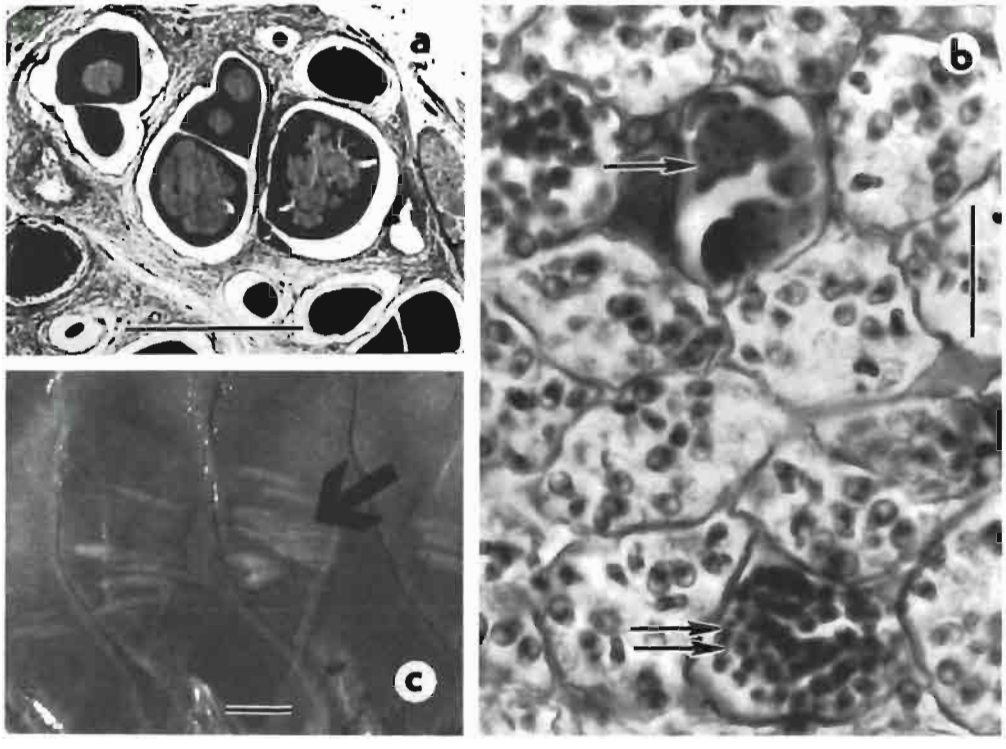

Fig. 1-35: (a) *Spraguea lophii* xenomas in spinal ganglion of *Lophius piscatorius* seen within clear halos (fixation artifacts); xenomas have dark periphery (= oval spores) and less dark core (curved slender spores); haematoxylin and eosin; scale 1 mm. (b) *Pleistophora duodecimae* from muscles of *Coryphaenoides nasutus;* walls of sporophorous vesicles encase typically a large number of spores; arrow: sporogonial plasmodia cleaved into sporoblasts (double arrow); scale 20 μm. (a, b — originals.) (c) 'Cysts' of *Pleistophora* sp. in muscles of *Micromesistius poutassou;* scale 3 mm; (after Möller and Anders, 1982.)

are due to a hitherto undescribed *Pleistophora* species. Fin muscles of *Drepanopsetta hippoglossoides* and of other flatfish from the North Sea often have nodules up to 2.5 by 10 mm in size, containing spores of *P. hippoglossoideos* Bosanquet, 1910.

An unidentified *Pleistophora* species was found to produce macroscopic intramuscular cysts in the body wall overlying the viscera in larval and postlarval herring *Clupea harengus harengus.* The parasite occurred in up to 3% of the samples taken in coastal waters of the Gulf of Maine (Sindermann, 1961c). A similar species is illustrated in Fig. 1-35, c.

Musculature of cottid fish and blennies may reveal whitish streaks 10 by 3 mm consisting of white threads, i.e., muscle fibres infected with *Pleistophora typicalis* Gurley, 1893 and with the closely related *P. littoralis* Canning and Nicholas, 1980. Passing mostly unnoticed, these infections seem to be quite common and may be deleterious to the host.

In addition to muscle infecting species, there are 'xenoma-less' microsporidans infecting other body organs. *Thelohania baueri* Voronin, 1974, a species of a genus in which each sporophorous vesicle contains 8 mature spores, affects ovaries of 3- and 9-spined sticklebacks. The cytoplasmic contents of the ovum may be completely replaced by

the microsporidan. *Microsporidium ovoideum* (Thélohan, 1895) Sprague, 1977 — a parasite in the liver of *Cepola rubescens* and *Gaidropsarus mediterranaeus* — was recognized by Raabe (1936) as a serious pathogen in *Mullus barbatus*. The liver was full of haemorrhages and of white cysts about 1 mm in size. *M. ovoideum* seems to be a frequent parasite with low host specificity. It was recorded from Scotland, and in *Merluccius gayi* and *M. hubbsi* on the coasts of Peru and Patagonia.

Agents: Myxosporea

Myxosporea Bütschli, 1881 — a subphylum of the phylum Myxozoa Grassé, 1970 — constitute a vast assemblage of about 1100 species belonging to 42 genera and except for a few species living in reptiles, amphibians and invertebrates, they all infect fish. By being multicellular for almost all of their life cycles, with structural and functional differentiation of their cells, they clearly exceed the unicellular level of most protozoan phyla. Two other distinctive features are the presence of polar capsules in their spores and endogenous cell cleavage in both trophozoite and sporogony stages. Myxosporea are usually immobile intercellular parasites of which only a few have intracellular stages in their life cycles in addition to 4 genera living intracellularly within myocytes of skeletal muscles.

Spores contain 1 to 6 polar capsules, 1 binucleate or 2 uninucleate amoeboid cell-infective germs or sporoplasms, within a shell composed of 2 to 6 valves which adhere together along suture lines. The valves may have a smooth or ridged surface and bear various projections or be coated with a mucous envelope keeping them afloat within the water column. Polar capsules contain a coiled polar filament capable of rapid extrusion driven by pre-built pressure. The polar filament everts inside out. When everted, it is sticky, thus probably fixing hatching spores to the host's intestinal surface.

In the digestive tract of a suitable fish host, polar filaments are extruded, shell valves separate, the released sporoplasm penetrates the intestinal epithelium and — probably via blood or lymph vessels — reaches the final site of infection in tissues or in organ cavities. The sexual process of autogamy takes place after hatching by fusion of the 2 nuclei of sporoplasm or of the 2 sporoplasms. Development at the final site of infection may be preceeded by little known proliferative processes in tissues or bloodstream.

The sporoplasm with nuclei fused into 1 synkaryon is the only uninucleate cell stage in the life cycle. This trophozoite may start proliferation in 2 ways. Some species produce large numbers of small stages with 2 or slightly more nuclei which divide to produce many parasites before sporogony begins. In other species the nucleus divides to produce a large plasmodium (up to several mm in size) inside which many generative cells arise by internal cleavage. Trophozoites are of variable shape, have osmotroph, pinocytotic or, rarely, phagotroph nutrition. Some exhibit a certain degree of amoeboid movement.

In small uninucleate or binucleate trophozoites, internal cleavage produces sporogonic cells which aggregate to produce one or more sporoblasts. In large plasmodia, generative cells associate pairwise, one enveloping the other to produce sporoblasts.

In the mature sporoblast, there are 1 to 2 capsulogenic cells transforming into polar capsules, 2 to 6 cells transforming into shell valves (according to various genera) and 1 or 2 cells giving rise to sporoplasms.

Trophozoites living in cavities of the gall bladder or urinary tract may float in the lumen or attach to the epithelium and may obstruct passages, e.g., of the bile. Trophozoites

in the tissue, if small may be dispersed among the tissue cells in a state of 'diffuse infiltration'. Large trophozoites, enveloped by connective tissue cells, may form cyst-like structures. Light infections are apparently harmless with most of the species. Some myxosporeans, however, are known as serious pathogens or agents deteriorating the quality of fish meat.

The present taxonomy of the subphylum is rather artificial. It is based solely on spore structure (Figs. 1-36, 1-37, 1-38, 1-39). There are 2 orders: the large order Bivalvulida Shulman, 1959 featuring spore walls with 2 valves, and the small order Multivalvulida Shulman, 1959 having spores with 3 to 6 valves (Kudo, 1920; Shulman, 1966; Sprague, 1982; Lom and Noble, 1984).

Marine fishes are hosts to myxosporeans belonging to 29 genera of which 17 occur solely in marine fish.

Myxosporeoses of Organ Cavities

In marine fishes, the gall-bladder and the urinary tract (i.e., urinary bladder, ureters and even renal tubuli) are common sites of myxosporean infections, and representatives of a total of 19 myxosporean genera are found in these organs. According to Shulman (1966), the gall-bladder of marine fishes is the site where the most primitive myxosporeans originally settled and from where they passed in the course of evolution, into other body organs and other hosts. Infections of the gall-bladder and urinary bladder seem more or less permanent, but exact long-term studies of infected fish have not been performed thus far. Trophozoites continue their vegetative reproduction while mature spores are released to the outside via the intestinal tract. During the reproductive cycle, trophozoites become temporarily attached to or, rarely, may even develop within the gall-bladder and urinary bladder epithelium although surprisingly little damage and host reaction develop. This may be interpreted as a result of a long history of mutual adaptation between parasite and host. For example, one of the common gall-bladder parasites, *Myxidium incurvatum* Thélohan, 1892, is assumed to invade almost 30 hosts belonging to different orders (Noble, 1941; Laird, 1953; Shulman and Shtein, 1962) from pelagic and littoral zones in various geographic areas but only sometimes causes obstruction of bile ducts by a mass of parasites (Doflein and Reichenow, 1953).

Only in some cases, or upon massive infection, may pathologic manifestations in the gall-bladder be apparent. There are no records of marked lesions in the urinary bladder. Noble (1957) noted changes in the bile. The contents of the gall-bladders of several fish species infected with myxosporeans of the genera *Ceratomyxa, Leptotheca* and *Myxidium* (Fig. 1-36, c, e, h) may be of a white, cheese-like consistency. In fishes from New Zealand, Meglitsch (1960) observed several degrees of gall-bladder damage caused by various species of *Ceratomyxa* ranging from altered appearance of the bile to swelling, hyperaemia and irritation of the bladder or inflammation of bile ducts. A heavy infection with *Myxidium sphaericum* Thélohan, 1892 in the gall-bladder of *Merlangus merlangus* from the North Sea (Kabata, 1967) can usually be recognized with the naked eye. The enlarged bladder is pale and has hyperplastic, hard and non-contractile walls. *Leptotheca infirmus* Auerbach, 1910, invading *Mola mola* in Atlantic Ocean and North Sea, may induce partial degeneration of the gall-bladder due to disintegration of epithelial cells. Gall-bladder infection with some myxosporean species (e.g., of the genus *Ceratomyxa*) may also result in the epithelium being coated with a thick layer of amorphous substance in which the

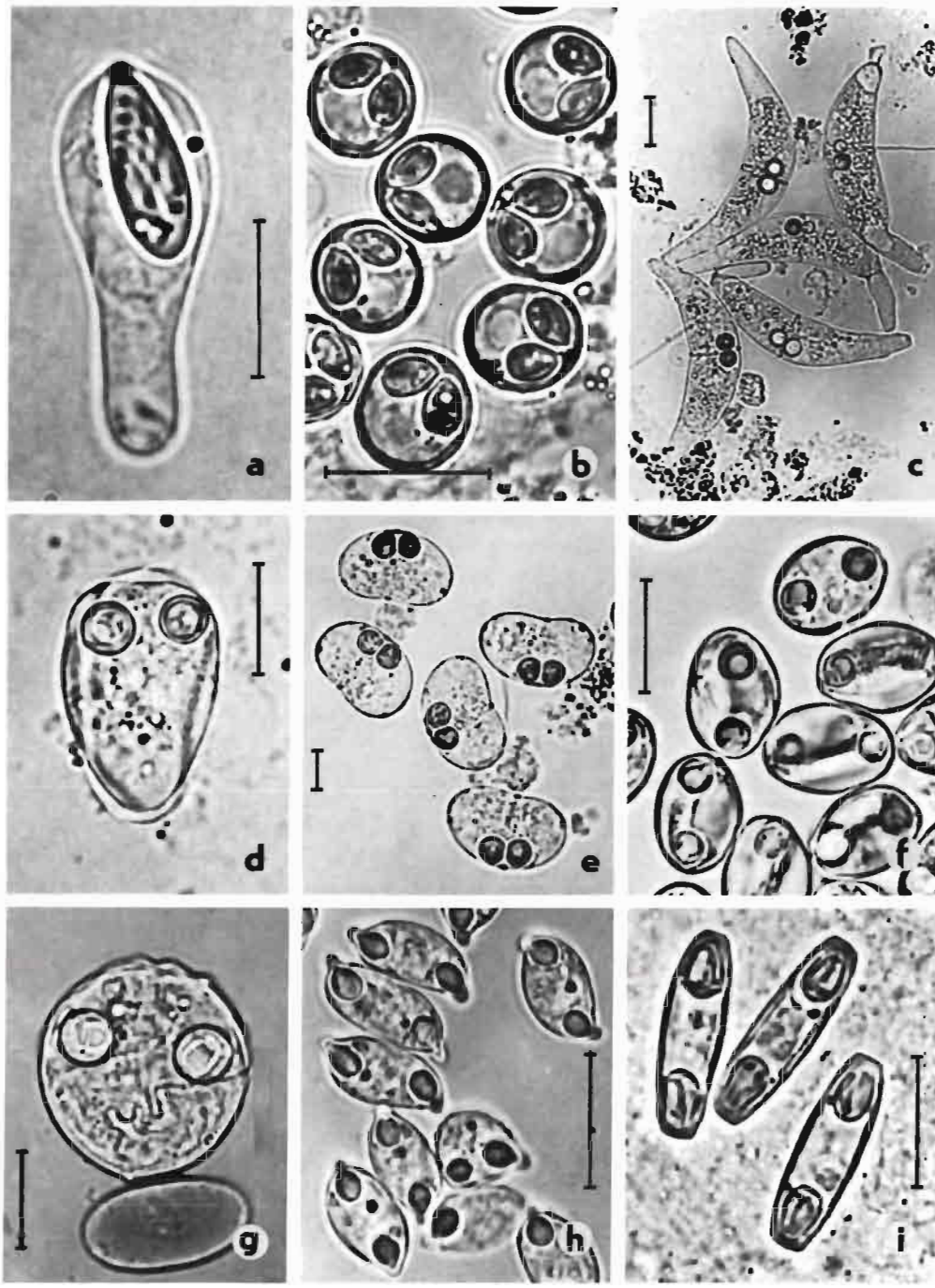

Fig. 1-36: Micrographs of fresh spores show morphological diversity in myxosporean genera infecting marine fishes. In all cases scale is 10 µm. (a) *Auerbachia pulchra* Lom, Noble & Laird, 1975 from gallbladder of *Macrourus* berglax. (b) *Myxobolus aeglefini* from gill cartilage of *Cyclopterus lumpus*. (c) *Ceratomyxa drepanopsettae* Awerinzew, 1908 from gallbladder of *Rheinhardtius hippoglossoides*. (d) *Myxoproteus* sp. from urinary bladder of *R. hippoglossoides*. (e) *Leptotheca macrospora* Auerbach, 1909 from urinary bladder of *Sebastes marinus*. (f) *Zschokkella* sp. from urinary bladder of *Gadus morhua*. (g) *Sinuolinea* sp. from urinary bladder of *Myoxocephalus scorpius*. (h) *Myxidium gadi* Georgevitch, 1916 from gallbladder of *Melanogrammus aeglefini*. (i) *Sphaeromyxa balbianii* from *Triglops murrai*. (Original.)

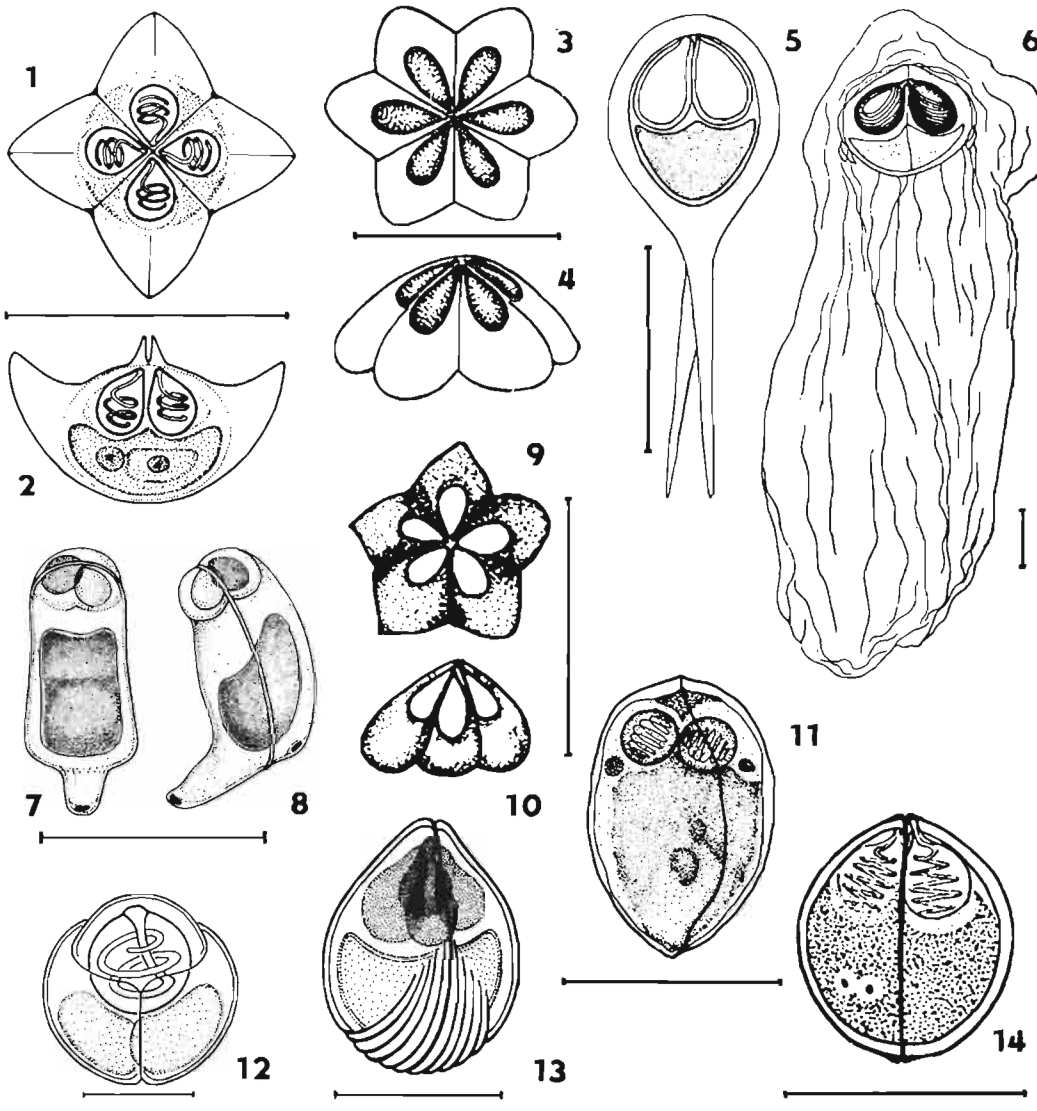

Fig. 1-37: Variety of spore shapes in myxosporean genera infecting marine fish. 1 and 2: *Kudoa lunata* Lom & Dyková, 1983 from skeletal muscles of *Arnoglossus* spp.; apical and side views. 3 and 4: *Hexacapsula neothunni* from muscles of *Neothunnus macropterus;* (after Arai and Matsumoto, 1953). 5: *Henneguya ocellata* Iversen & Yokel, 1963 from the intestine of *Sciaenops ocellatus;* (after Iversen and Yokel, 1963). 6: *Palliatus mirabilis* Shulman, Kovaleva & Dubina, 1979 from urinary bladder of *Xenodermichthys socialis;* (after Shulman and co-authors, 1979). 7 and 8: *Parvicapsula unicornis* Kabata, 1962 from urinary bladder of *Callionymus lyra;* side and frontal view. 9 and 10: *Pentacapsula shulmani* from muscles of *Nemipterus japonicus;* (after Naidenova and Zaika, 1970). 11: *Myxoproteus mexicanus* Yoshino & Noble, 1973 from kidneys of *Coelorhynchus scaphopsis;* (after Yoshino and Noble, 1973). 12: *Unicapsula seriolae* Lester, 1982 from muscles of *Seriola lalandi;* (after Lester, 1982, modified). 13: *Chloromyxum* cf. *leydigi* Mingazzini, 1897 from gallbladder of *Raja clavata.* 14: *Sphaerospora renalis* Bond, 1938 from kidneys of *Fundulus heteroclitus;* (after Bond, 1938). Scale in all cases 10 μm, except for 12 where it equals 3 μm.

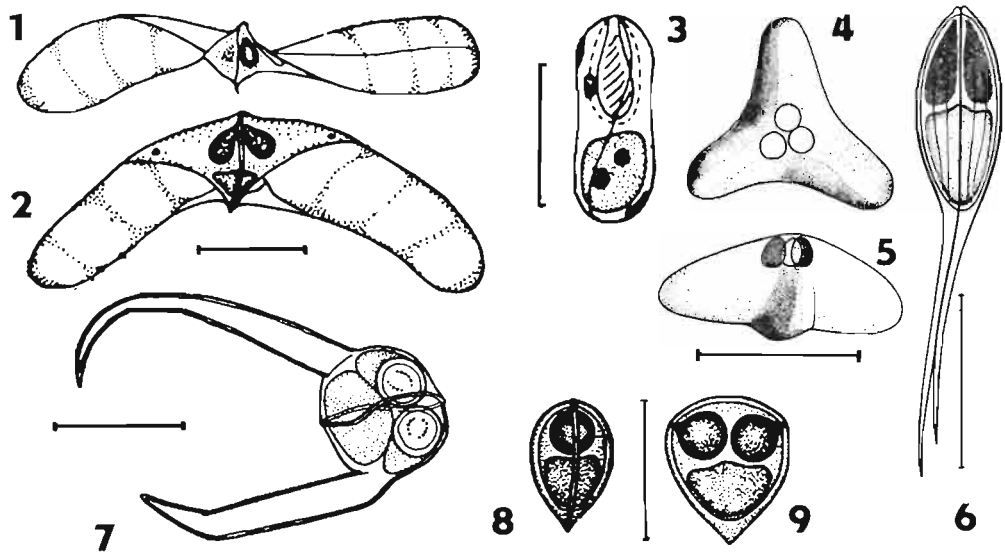

Fig. 1-38: Variety of myxosporean spore shapes (continued). 1 and 2: *Alatospora samaroidea* Shulman, Kovaleva & Dubina, 1979 from gallbladder of *Chlorophthalmus atlanticus;* (after Shulman and co-authors, 1979). 3: *Coccomyxa morovi* Léger & Hesse, 1917 from gallbladder of *Sardina pilchardus.* 4 and 5: *Trilospora californica* Noble, 1939 from gallbladder of *Typhlogobius californiensis;* apical and side view; (after Noble, 1939). 6: *Myxobilatus gasterostei* (Parisi, 1912) Davis, 1944 from renal tubules of *Gasterosteus aculeatus;* (Original.) 7: *Davisia ophiodoni* Zaika, 1966 from urinary bladder of *Ophiodon rochei;* (after Zaika, 1966). 8 and 9: *Ortholinea orientalis* Shulman & Shulman-Albova, 1953 from urinary bladder of clupeoid fish; (after Shulman and Shulman-Albova, 1953). Scale in all cases 10 μm.

parasites reside. The final outcome of such myxosporean affections of the gall-bladder is its partial, or even complete functional elimination.

Species of some genera, e.g., *Sphaerospora* and *Parvicapsula* (Fig. 1-37, 7, 8, 14), which live in the urinary tract may proceed to the renal tubuli. We have no data on their role in the kidneys of marine fish, but in freshwater hosts members of both genera were found to exert considerable pathogenicity in kidney tissues.

Sometimes, gall-bladder infections may also affect the surrounding tissue. As reported by Shulman and Shulman-Albova (1953), *Myxidium oviformis* Parisi, 1912 — seemingly a harmless gall-bladder parasite of many species of marine fishes — may cause serious liver damage in *Salmo salar* which is not its typical host. This latter fact may account for the seriousness of the infection. In addition to gall-bladder inflammation, the liver tissue is full of liquefied necrotic foci, the host being badly emaciated. Migration into freshwater did not free the salmon from this infection.

Myxosporeoses of Tissues

Histozoic myxosporeoses, as a rule, produce much more serious effects, sometimes inflicting upon their hosts lethal injuries or rendering their flesh unpalatable for humans. Even with these parasites there is at most only a very small host reaction during the initial stages of parasite growth in the host tissue. Only after the fully grown trophozoite ('cyst') has produced a mass of spores the tissue reaction sets in, and an inflammatory response develops.

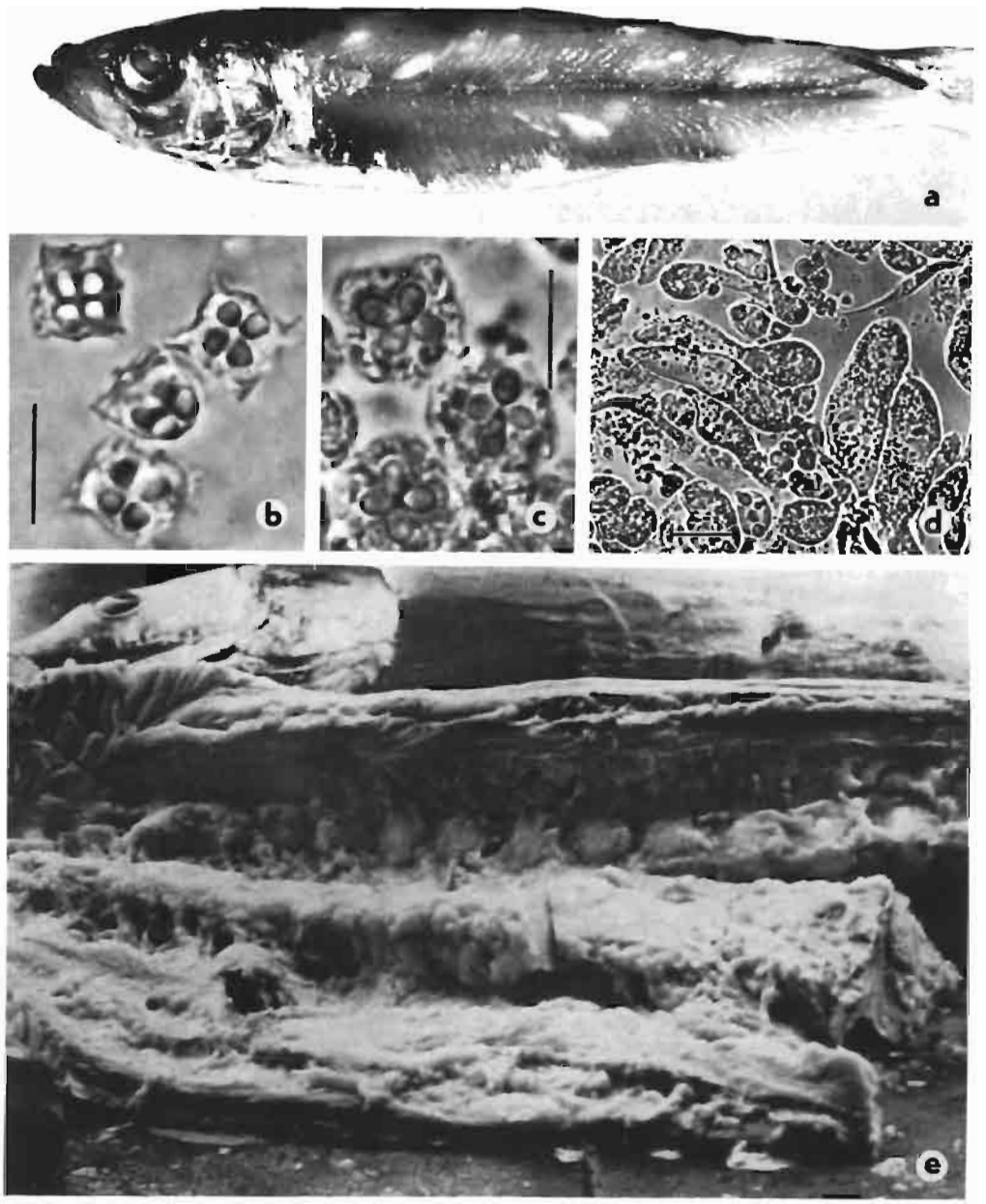

Fig. 1-39: (a) *Kudoa clupeidae*. 'Cysts' in flesh of herring; photo courtesy of Dr. C. Sindermann; length of fish 11 cm. (b) *Kudoa kabatai* Shulman & Kovaleva, 1979; formalin-fixed spores from muscles of *Zeugopterus punctatus;* scale 10 µm. (c) *K. clupeidae*; formalin-fixed spores from *Thunnus thunnus;* scale 10 µm. (d) *Myxidium gadi;* fresh trophozoites from gallbladder of *Melanogrammus aeglefinus;* scale 10 µm; (b to d – Original.) (e) *Xiphias gladius;* opened trunk; whole body musculature liquefied by action of *Kudoa musculoliquefaciens;* photo courtesy Dr. Matsumoto; size of fish about 1.5 m.

Most important pathogens are species of the order Multivalvulida which have radially symmetrical spores with more than 2 (3 to 6) shell valves and polar capsules. There are 3 valves but only 1 capsule (2 others are reduced during spore development; the spore is asymmetrical) in *Unicapsula* Davis, 1924 (Fig. 1-37, 12); 4 shell valves and capsules in *Kudoa* Meglitsch, 1947 (Fig. 1-37, 1 and 2); 5 valves and capsules in *Pentacapsula* Naidenova and Zaika, 1970 (Fig. 1-37, 9 and 10); and 6 in *Hexacapsula* Arai and Matsumoto, 1953 (Fig. 1-37, 3 and 4). As a rule, these parasites infect myocytes and have a fully intracellular propagative cycle. The infected muscle fibre becomes greatly enlarged. Its sarcoplasm is gradually replaced by the parasite until it is transformed into an oval, but mostly spindle-shaped pseudocyst – a cyst-like formation ultimately filled with mature spores which may be encapsulated by the host's connective tissue layer. Only rarely are interfibrillar connective tissue or other sites infected.

Muscle fibres of halibut *Hippoglossus stenolepsis* from the Pacific Ocean are invaded by *Unicapsula muscularis* Davis, 1924. Whitish-opaque enlarged muscle bundles filled with spores resemble small worms and hence the name of the disease, 'wormy halibut'. Most multivalvulids belong to the genus *Kudoa* comprising about 31 species which infect marine fishes, in addition to many still unidentified species encountered in various fish hosts. Commercially important clupeoid fishes *(Clupea harengus, Alosa aestivalis, A. pseudoharengus, Brevoortia tyrannus)* from the Atlantic coast of North America are commonly infected by *Kudoa clupeidae* (Hahn, 1917) Meglitsch, 1947. Sindermann (1970a) found that as many as 75% of the 1 yr old Atlantic herring were parasitized, while adults were uninfected. While Linton (1901) speculated that the diseased fishes were less viable and more subject to predation, Sindermann suggests that the infection may pass away after mature spores are released from opened cysts. The observations by Sindermann and Rosenfield (1954) on 'ulcer disease' in Atlantic herring confirm this suggestion. The musculature of first-year-group fishes revealed spindle-shaped white cysts up to 5 mm large (Fig. 1-39, a and e). Older fishes had pus pockets which finally opened to the outside by sloughing off the overlying epidermis and by the formation of ulcers. Ulcers reach up to 1 cm in diameter, have well-defined margins and often ooze white to yellow necrotic material. Up to 5% of a herring population may be ulcer-marked. In agreement with the assumption that this kuodasis is a disease of young fish only, Kovaleva and co-authors (1979) suppose that *K. clupeidae* is pathogenic for young *A. pseudoharengus* but not for adult fish, in which they never found any ulcerations.

Hakes *Merluccius hubbsi* in the Patagonian- and Falkland shelfs are commonly infected with *Kudoa rosenbuschi* (Gelormini, 1944) Meglitsch, 1947 and the prevalence may reach up to 52%. No ulceration was observed (Kovaleva and co-authors, 1979). North Pacific hakes *Merluccius productus* are hosts to *K. paniformis* Kabata and Whitaker, 1981. In the course of host-tissue reaction, which ultimately achieves complete destruction of the parasites within pseudocysts, melanin granules are deposited around the infected fibers. This is a feature unusual in other kudoases.

At variance with *Kudoa clupeidae,* the species *K. alliaria* Shulman et Kovaljova, 1979 infects older-age classes of its hosts while it is absent in young ones. In 1970–1975 it was commonly found in blue whiting *Micromesistius australis, Notothemia ramzay, N. conica* and *Macruronus magellanicus* in the region of the Falkland-Patagonian shelf. Blue whiting was infected in up to 100% with up to 56 infection foci per fish. Grabda (1978) reported probably the same species from Atlantic blue whiting from up to 90% of specimens

examined in 1977. Pseudocysts reached the size of 20 × 5 mm. The most widely distributed species of the genus is *K. nova* Naidenova, 1975 covering such a wide host range and geographic area, that one wonders if it is really a single *Kudoa* species. It infects 20 host species of various orders, including gobiid fish, as well as some commercially important species such as tunas, in various areas of the Atlantic Ocean, Mediterranean, Black and Azov Seas. According to Naidenova (1974), *K. nova* infection is supposed to be the cause of death of gobiid males exhausted when protecting their newly hatched offspring on the spawning grounds in the Black and Azov Seas. *Pagellus acarne* in the Central Atlantic Ocean is infected in up to 65%. *K. kabati* Shulman and Kovaleva, 1979 produces cysts up to 2 mm in size in muscles of *Zeugopterus punctatus.*

Not all *Kudoa* species infect muscles. Paperna (1982) reported on an unidentified species infecting up to 85% of renal glomeruli of hatchery bred *Sparus auratus* on the French Mediterranean coast. In the same host from the Gulf of Acaba, he found mesenteries and peritoneum infected with another *Kudoa* species. An exceptional site of infection characterizes *K. pericardialis* Nakajima and Egusa, 1978 infecting yellowtails *Seriola quinqueradiata* cultured in Japanese marine fish farms. As the name implies, very numerous trophozoites up to 27 × 1.2 mm in size float free in the pericardial cavity or are attached to proliferating connective tissue of epicardial or pericardial origin.

Pentacapsula schulmani Naidenova and Zaika, 1970 (Fig. 1-37, 9 and 10) form 2 mm large cysts in muscles of *Nemipterus japonicus* from the Indian Ocean. There is evidence that more species of this genus await to be discovered in marine fish.

There are several multi-valvulid myxosporeans which provoke dramatic changes in the flesh of heavily infected fish. They are said to have tiny trophozoites pervading the muscular tissue in a poorly documented way designed as 'diffuse infiltration'. Some others produce pseudocysts similar to those known in the above-mentioned species. In live fish, the effects of the parasites are restricted. The muscles appear either mottled by numerous parasite 'cysts' or, when touched, appear less elastic than usual. After capture (i.e., death of the fish) within up to 24 h, the flesh rapidly softens and may even turn into a thick, viscous mass or into a thin 'milky', jelly-like substance without any odour. The liquefaction is most striking when heavily infected fish are frozen while still appearing normal. When defrosted, the flesh is already jellified. According to existing evidence, myxosporeans are the cause of these changes. Bacterial lysis has not been proven. According to Willis (1949) the powerful proteolytic enzymes released by the parasites are continuously removed by the bloodstream in live hosts — or the enzymes may be localized strictly within the pseudocysts (Patashnik and co-authors, 1982) — and thus have only a localized effect within the affected muscle fibre. After death, however, they accumulate and/or diffuse outwards and cause the autolysis of host flesh, proceeding from infected to non-infected areas. There is only 1 record of *Kudoa*-induced liquefaction of the musculature in living fish, i.e., by *Kudoa* sp. in *Atherestes evermani* from the Bering Sea (Krasin, 1976).

The name *Kudoa histolytica* (Pérard, 1928) Meglitsch, 1947 is suggestive of the effect this parasite has on muscles of the Atlantic and Mediterranean *Scomber scombrus.* Also well known is the 'milky barracouta', a disease of *Thyrsites atun,* caused by *K. thyrsitis* (Gilchrist, 1924) Meglitsch, 1947 which produces a milky appearance of the flesh. Infected fish were caught on the South African and Australian coasts. According to Willis (1949) the agent was found in up to 7% of fish in the latter region. The same parasite was reported to bring about a similar milky condition in *Merluccius capensis* in South African waters

(Fletcher and co-authors, 1951). In *M. productus* along the Canadian Pacific coast, *K. thyrsitis* is probably responsible for the rapid softening of the flesh after capture (Kabata and Whitaker, 1981). *K. thyrsitis,* as described from *M. productus* and *Thyrsites atun* forms pseudocysts within the muscle fibres as any of the 'non-jellifying' *Kudoa* species. *K. thyrsites* was also reported from *Zeus faber* from waters off South Africa. Seventy-five % of the fish population were infected with 25% being infected heavily enough to render the fish unsuitable for filleting.

There are other remarkable accounts of muscular liquefaction. *Kudoa musculoliquefaciens* Matsumoto and Arai, 1954 induces it in specimens of *Xiphias gladius* of about 100 kg in weight (Fig. 1-39). There is a 'diffuse infiltration' throughout the muscular fibres and the outcome of heavy infections may be lethal. The same Japanese authors (1954) observed jellification in *Labeolabrax japonicus* caused by *K. cruciformum* Matsumoto and Arai, 1954. The flesh was perforated by numerous cavities, 5 × 10 mm in size, containing a jellified substance full of spores. The flying fish *Cypsilurus ago* and the dorado *Coryphaena hippurus* from the Japanese Sea are attacked by another jellifying species of *Kudoa* (Dr. Matsumoto, pers. comm.). There are 2 more unidentified 'histolytic' species of *Kudoa*. The first is responsible for the 'mushy' halibut (softening of the musculature of *Hippoglossus stenolepis*; Thompson, 1916) and the second for the 'milkiness' of *Parophrys vetulus* from the Canadian Pacific coast (Margolis, 1953; Patashnik and Groninger, 1964).

The action of *Hexacapsula neothunni* Arai and Matsumoto, 1953 on fish muscles is quite similar to that of 'jellifying' *Kudoa*. It infects *Neothunnus macropterus* and, pervading the muscles by diffuse infiltration, it turns large parts of muscles into cavities filled with a creamy substance suggestive of nasal mucus and full of parasites.

Marine fish are subject to infections with a number of myxosporean species belonging to genera known also from freshwater fish. *Sphaerospora platessae* Woodcock, 1904 — a member of a genus characterized by spherical spores with 2 polar capsules opposing the level of the shell-valve suture line — invades the head cartilage in *Pleuronectes platessa*. The genus *Myxobolus* Bütschli, 1882 which has spores with 2 polar capsules set in the level of the suture line, is represented by many species. A common species is *M. aeglefini* Auerbach, 1908 which erodes the head and gill cartilage of many hosts, e.g., *Melanogrammus aeglefinus* (Fig. 1-36, b), *Pleuronectes platessa, Merluccius merluccius, Gadus callarias* and *Merlangius merlangus. Myxobolus exiguus* Thélohan, 1895 — known as a rather harmless parasite of a number of freshwater fish — also infects marine and estuarine species of the genus *Mugil* along the Atlantic and Mediterranean coasts of France. It causes heavy lesions of the epidermis and gills in 5 species of the genus *Mugil* on the Tunisian coast (Siau, 1978). Petrushevsky and Shulman (1958) reported extremely heavy epizootics in *M. auratus* and *M. cephalus* in the Black and Azov Seas. The latter host was washed ashore dead in quantities up to 600 kg km^{-1}. Gill filaments packed with cysts were completely dysfunctional. Death occurred as a consequence of asphyxia and bleeding from injured gills.

Members of the genus *Henneguya* Thélohan, 1892 — differing from *Myxobolus* in having 2 caudal projections on the spore — also cause lesions in marine fish. *H. ocellata* Iversen and Yokel, 1963 (Fig. 1-37, 5) can inflict heavy damage upon *Sciaenops ocellata* in Florida waters. The pseudocysts may cover completely the pyloric caeca and beginning of the intestine. *H. lagodon* Hall and Iversen, 1967 forms pseudocysts in tissues surrounding the eyes, often forming conspicuous external bulges (Fig. 1-40, a). There are many more

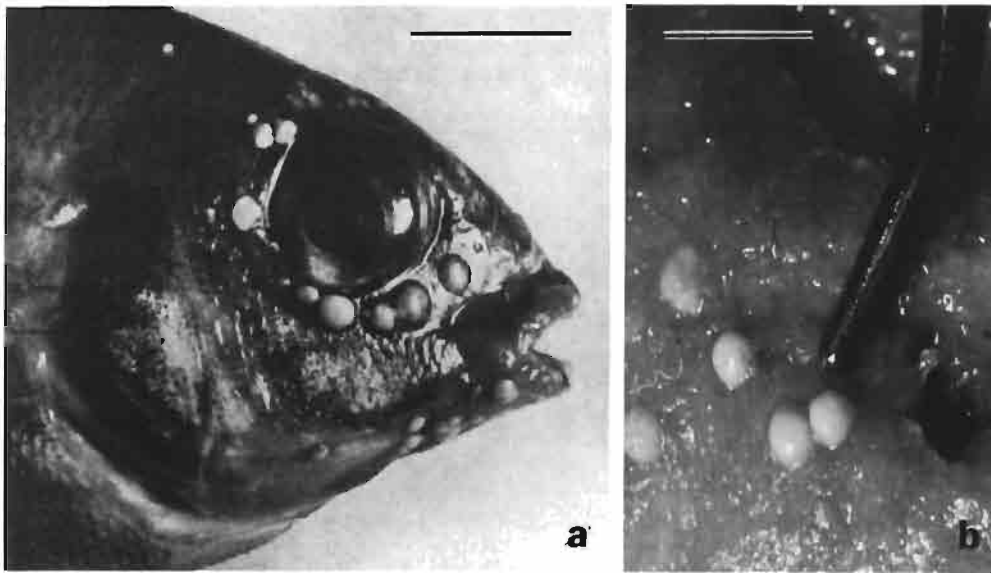

Fig. 1-40: (a) *Henneguya lagodon.* 'Cysts' on head of *Lagodon rhomboides;* scale 5 mm. (b) *Kudoa crumena* Iversen & van Meter, 1967. 'Cysts' in musculature of *Scomberomorus maculatus;* scale 4 mm. (Photo: J. W. Stephens, courtesy of Dr. Iversen.)

findings of this and other myxosporean genera as pathogens in marine fish — mostly isolated observations of rather sporadic infections. Future research will prove to what extent they can play a role in natural populations or, possibly, in aquaculture.

Agents: Ciliata

The phylum Ciliophora Doflein, 1901 comprises relatively large Protozoa, up to several mm in size, with simple cilia or compound ciliary organelles used for locomotion and food acquisition, present at least at some stage of their life cycle. They are usually mobile although in adult state some are sessile and/or lack ciliature. Infraciliature is always present as a complex meshwork of fibrillar systems associated with ciliary basal bodies, extending beneath the pellicle. The pellicle is composed of 3 membranes, 2 of which delimit a system of subpellicular alveoli. Ciliates have 2 types of nuclei, diploid generative micronuclei and enormously polyploid vegetative macronuclei. As a rule, ciliates divide by transversal binary fission, but exceptionally by budding or multiple fission. A typical sexual process is the conjugation of 2 individuals involving the exchange of haploid pronuclei. As a rule, particulate material is ingested by means of a very complex buccal apparatus. A contractile vacuole, active in osmoregulation, is usually present.

There are more than 7600 species known to date. Their classification is based primarily on the structure of the oral apparatus, its ciliary organelles and their morphogenesis. Because ciliates possess a large number of easily discernible characteristics, their present classification is the most complex among all protozoan phyla; and is still in a state of change. Most of the ciliates are free-living although some are commensals, symphorionts or true parasites. Most of their groups are subject to far-reaching mor-

phological adaptations to their particular mode of life: connected with a sedentary mode of life, changes in body polarity, secondary loss of ciliature or of oral apparatus, or complicated life cycles of some parasitic forms involving even diheteroxeny.

In marine fish, mostly ectozoic or ectoparasitic ciliate species are known. According to their relation to the host, they belong to several categories. Symphorionts (or epibionts) take advantage of their hosts chiefly as a mobile support keeping them within a constant water flow bringing organic particles, e.g., bacteria, on which they feed. Ectocommensals feed on water-born particles as well as on debris on the host's surface. Some may turn into dangerous ectoparasites on a stressed fish with impaired natural resistance. The rest are facultative and obligatory ectoparasites.

Ciliates as Symphorionts and Ectocommensals

Ciliates of this category all belong to the subclass Peritrichia Stein, 1859 comprising ciliates in which somatic ciliature is, in the adult, reduced completely or to 1 or 3 locomotory ciliary wreaths. Buccal ciliature consists of a double ciliary spiral, encircling the oral surface, which drives food particles into the buccal infudibulum. Peritrichs need a living or non-living substrate. They are either permanently attached to it by a special holdfast organelle at the aboral body end, the scopula (suborder Sessilina), or they glide over the surface being only temporarily attached by a sucker-like adhesive disc surrounded by 2 or 3 locomotory ciliary girdles (Mobilina).

Symphoriontic sessiline peritrichs of marine fish attach themselves to their hosts directly by means of scopula without any secreted stalks. The desmosome-like junction of scopular surface with cell membrane of the host epithelial cells (Lom, 1973) does not harm the surface tissue. They feed on dispersed particles and are never abundant on a non-stressed host. Even if present in great numbers they can never be regarded as primary pathogens.

The genus *Scyphidia* Dujardin, 1841 comprises ciliates with a stout conical body, and a coiled ribbon-like macronucleus. *S. arctica* Zhukov, 1964 epifaunates *Liparis gibbus* in the Bering Sea and is found also on cottid fish off the Pacific and Atlantic coasts of North America (Fig. 1-41, a). *S. adunconucleata* MacKenzie, 1969 lives on cultured plaice *Pleuronectes platessa* in Scottish waters. Several more species were recorded as symphorionts on the gills of various species of marine fish. *Ambiphrya miri* Raabe, 1952, of a genus displaying an equatorial trochal band of cilia, is found on *Nerophis ophidion* in the Baltic Sea (Fig. 1-42, 1).

Members of the genus *Calliperia* Laird, 1953 attach in different ways. They bear 2 long processes at the scopular end which join to form an attachment ring fastened around a piece of secondary gill filament. *C. brevipes* Laird, 1959 lives on the edge of gill filaments of *Raja erinacea* in Newfoundland waters (Fig. 1-42, 2). *Clausophrya oblida* Naidenova and Zaika, 1969 is a symphoriont with a similar way of attachment, living on *Proterorhinus marmoratus* in the Black Sea.

Ectocommensal ciliates are represented by mobiline peritrichs mostly referred to as trichodinids. Species of the genus *Trichodina* Ehrenberg, 1931 are the most frequently encountered ciliates on the surface of marine fish. A total of almost 70 such species have been described to date. In contrast to the situation in freshwater fishes, species of *Trichodinella* Šrámek-Hušek, 1953 (of which only 4 species were recorded on marine fish), *Paratrichodina* Lom, 1963 (2 species on marine fish — Fig. 1-41, f) and *Trichodina*

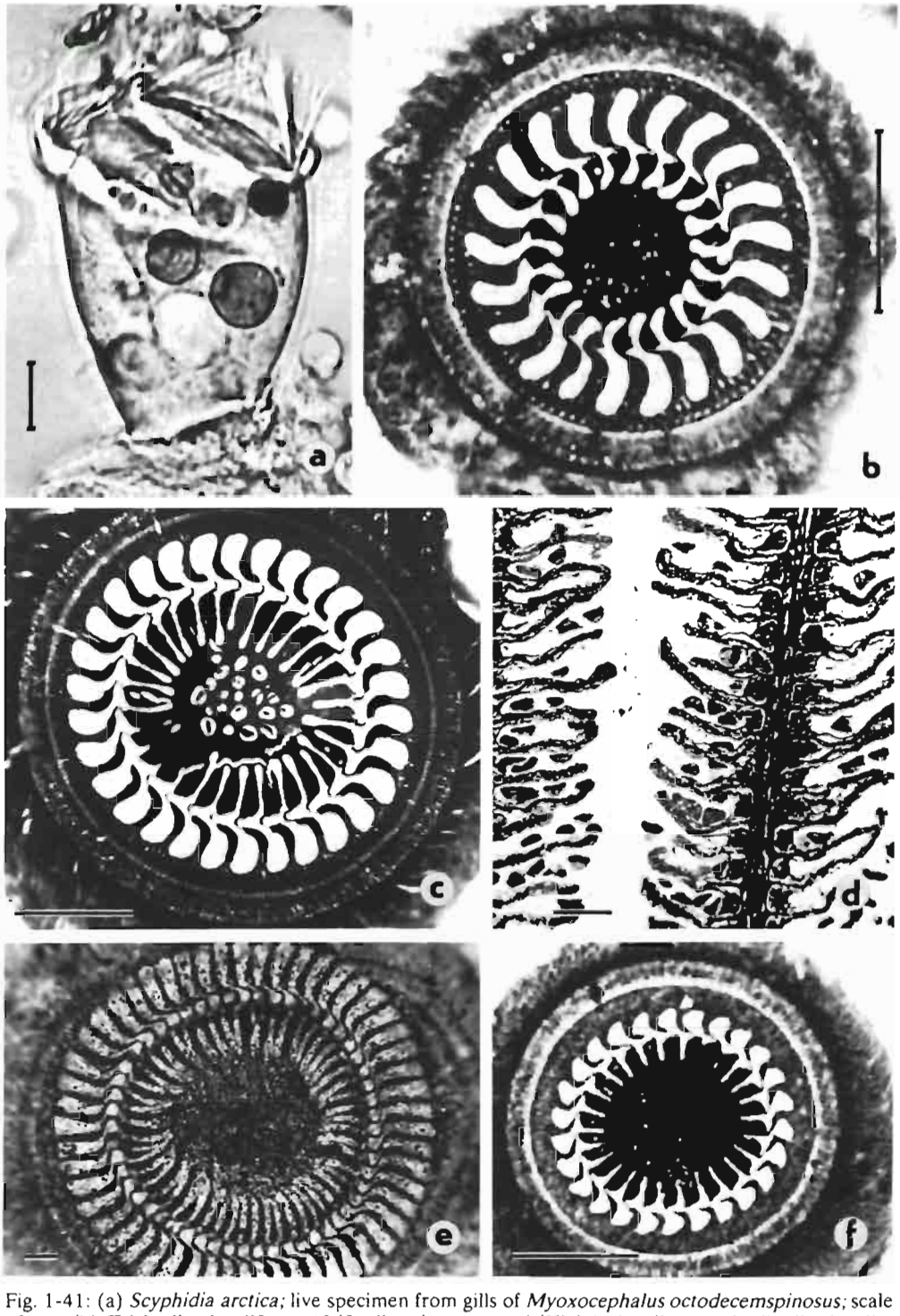

Fig. 1-41: (a) *Scyphidia arctica;* live specimen from gills of *Myoxocephalus octodecemspinosus;* scale 10 μm. (b) *Trichodina lepsii* Lom, 1962; silver impregnated (Klein's dry silver method) adhesive disc from gills of *Mugil auratus;* scale 10 μm. (c) *T. puytoraci* Lom, 1962; impregnated adhesive disc from *Mugil saliens;* scale 10 μm. (d) Longitudinal section through gill filament of a fish moderately invaded by *Trichodina;* scale 100 μm. (e) *T. oviducti;* impregnated adhesive disc from *Raja clavata;* scale 10 μm. (f) *Paratrichodina globonuclea* Lom, 1963; impregnated adhesive disc from gills of *Ophidion barbatus* (× 2000). (Original.)

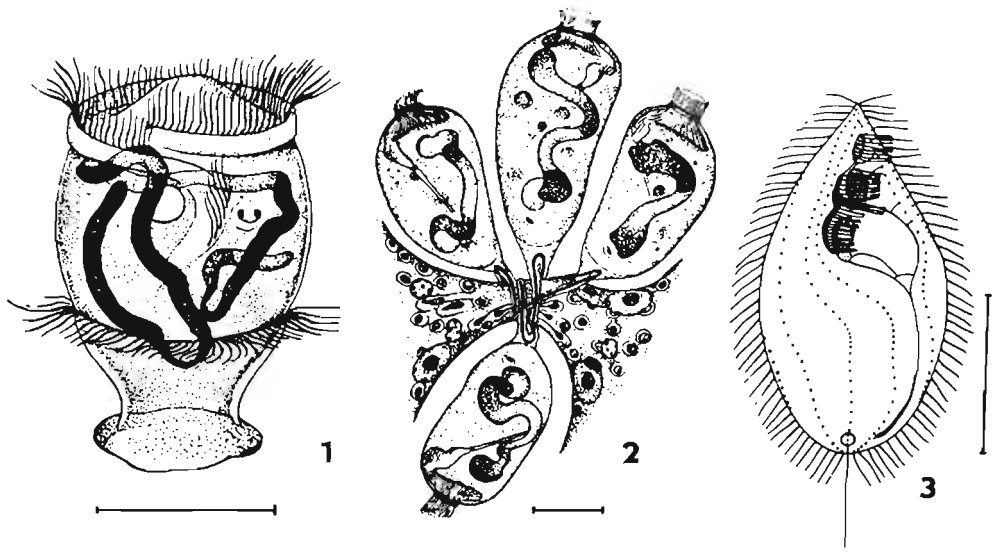

Fig. 1-42: 1: *Ambiphrya miri* from gills of *Nerophis ophidion;* scale 20 µm; (after Raabe, 1952). 2: *Calliperia brevipes* attached to a piece of gill tissue of *Raja erinacea* by means of their ring-like caudal processes; scale 20 µm; (after Laird, 1959). 3: *Miamiensis avidus* from sea horses; scale 15 µm; (after Thompson and Moewus-Kobb, 1964; modified.)

live all mainly on the gills (Fig. 1-41, b, c, d). In non-stressed hosts trichodinids occur in small numbers or seem to be completely absent. On a weakened host — and this can be easily proven by keeping a freshly caught fish in a small aquarium — their reproduction can attain massive proportions and they irritate the gills by attaching themselves to epithelial cells. Sharp borders of the adhesive disc may ultimately damage the epithelium and the trichodinids then feed on particles from disintegrated epithelial cells. At this stage the trichodinids become true and harmful ectoparasites, causing extensive damage to the gill epithelium and large skin lesions which may result in mortalities. The first known case of marine fish trichodiniasis was reported by Padnos and Nigrelli (1942). Gill epithelium of *Sphaeroides testudineus* was completely destroyed, thus causing death of the host. Trichodiniasis is presently one of the major problems in flatfish mariculture (e.g., Pearse, 1972; McVicar, 1978). In aquaria with marine ornamental fish, however, serious outbreaks of trichodiniasis are not common although fatal infections were recorded by Nigrelli (1940, 1943). Lawler (1977b) observed fatal infection of juvenile pinfish *Lagodon rhomboides* with *Trichodinella lawleri* Lom and Haldar, 1977.

Most marine fish will harbour at one time or another a trichodinid population on their gills with the intensity of infection varying in relation to ecological conditions. Lom and Laird (1969) found the percentage of fish species infected in cold seas to be far below the prevalence of infection in temperate zones. So far there is no evidence for a seasonal variability of incidence which is known in freshwater species. Trichodinids are transmitted directly, being able to survive for a certain time in the free water. As yet, it is not known whether some marine fish trichodinids can live alternatively on invertebrate hosts as is the case in freshwater species. Some euryhaline trichodinids (e.g., *Trichodina domerguei* from sticklebacks) tolerate great differences in salinity.

Widely distributed, *Trichodina rectuncinata* Raabe, 1958 is known from more than 15 host species in the Adriatic, Black and Caribbean Seas. Another widely distributed species is *T. jarmilae* Lom and Laird, 1969, recorded from about 20 host species in the Pacific and Atlantic Oceans. Mugils in the Black Sea are commonly parasitized by *T. puytoraci* Lom, 1962 (Fig. 1-41, c).

A morphologically and biologically different group of trichodinids invades the urogenital tract of elasmobranchs. They are large, with a great number of skeletal elements (denticles) of the adhesive disc. Endozoic trichodinids are pathogens rather than endocommensal. *Trichodina oviducti* Polyansky, 1955 infects 5 species of the skate genus *Raja* in the Atlantic Ocean (Fig. 1-41, e). The ciliates are present in urinary sinuses, rectal gland and rectum, in the seminal groove of males, and in the oviducts and copulatory sac of females. A yellowish-mucoid exsudate was invariably associated with heavy infections. The ciliates also sloughed and fed on the epithelium of the copulatory sac. They are most likely transmitted from one host to the other during copulation (Khan, 1972a).

Ciliates Causing Diseases of Body Surface

Facultative parasites

Heavily stressed or injured fish may become susceptible to invasion by various free-living ciliates which can colonize their body surface as facultative parasites. This is well-known in freshwater fish debilitated and weakened after an unfavourable winter period (e.g., various hymenostome ciliates, several species of the genus *Tetrahymena* or sessiline peritrichs, e.g., *Vorticella*). In marine fish, such cases mostly pass unnoticed except in mariculture or aquaria. Purdom and Howard (1971) recorded 3 species of ciliates — *Helicostoma buddenbrooki* Kahl, 1931, *Uronema* sp. and *Euplotes* sp. — attacking young *Zoarces viviparus,* and 0-group sole and plaice. The ciliates are attracted to skin abrasions — natural or due to fin-biting — expanding them into large lesions and thus causing lethal injuries. Other ciliates can penetrate deep into the fish body. *Miamiensis avidus* Thompson and Moewus, 1964 is a scuticociliatid ciliate (Fig. 1-42, 3) isolated from sea horses *Hippocampus atlanticus* from Florida waters (Moewus-Kobb, 1965). It thrives well in fish-tissue cultures and prefers the lower osmolalities inside the fish to the higher ambient salinity levels. It may thus tend to become an obligatory parasite. Another closely related facultative parasite, *Uronema marinum* Dujardin, 1941, caused enzootics and mortalities in the New York Aquarium (Cheung and co-authors, 1980) affecting 9 species of marine fish from 4 families. The ciliates were found in musculature and on the skin. In sea horses they also pervaded kidney, urinary bladder, neural canal, blood vessels and gills. They are highly destructive to host tissues, ingesting blood cells and tissue debris. No inflammatory response was observed.

Obligatory parasites

Two genera of serious ciliate pathogens belong to the class Kinetophragminophorea de Puytorac et al. 1974, order Cyrtophorida Fauré-Fremiet, 1956, i.e., *Chilodonella* Strand, 1928 and *Brooklynella* Lom and Nigrelli, 1970. Both have a dorsoventrally flattened body, with ciliary rows only on the ventral side. The cytostome is reinforced by supporting skeletal rods (nematodesmata) and equipped with 3 short ciliary rows (kinetofragments). *Chilodonella cyprini* (Moroff, 1902) Strand, 1928 is a notorious pathogen of

freshwater fish, infecting most species almost indiscriminately. It can also live in estuarine and brackish environments, e.g., in the eastern Baltic Sea (Calenius, 1980). *Brooklynella hostilis* Lom and Nigrelli, 1970 is the marine counterpart of *Chilodonella* and a parasite typically found in aquaria, but not in wild fish (Fig. 1-43, a, b). It differs in having a greater number (up to 36) of ventral ciliary rows and a ventral organelle secreting viscous material. In marine aquaria, *B. hostilis* attacks various hosts from different orders. The infection seems to be limited to the gills, the symptoms being indications of respiratory difficulties. The lesions range from mild inflammatory reaction to epithelial desquamation of secondary gill lamellae, accompanied by infiltration of macrophages and resulting in fusion of lamellae. Severe lesions are primarily responsible for the death of the host.

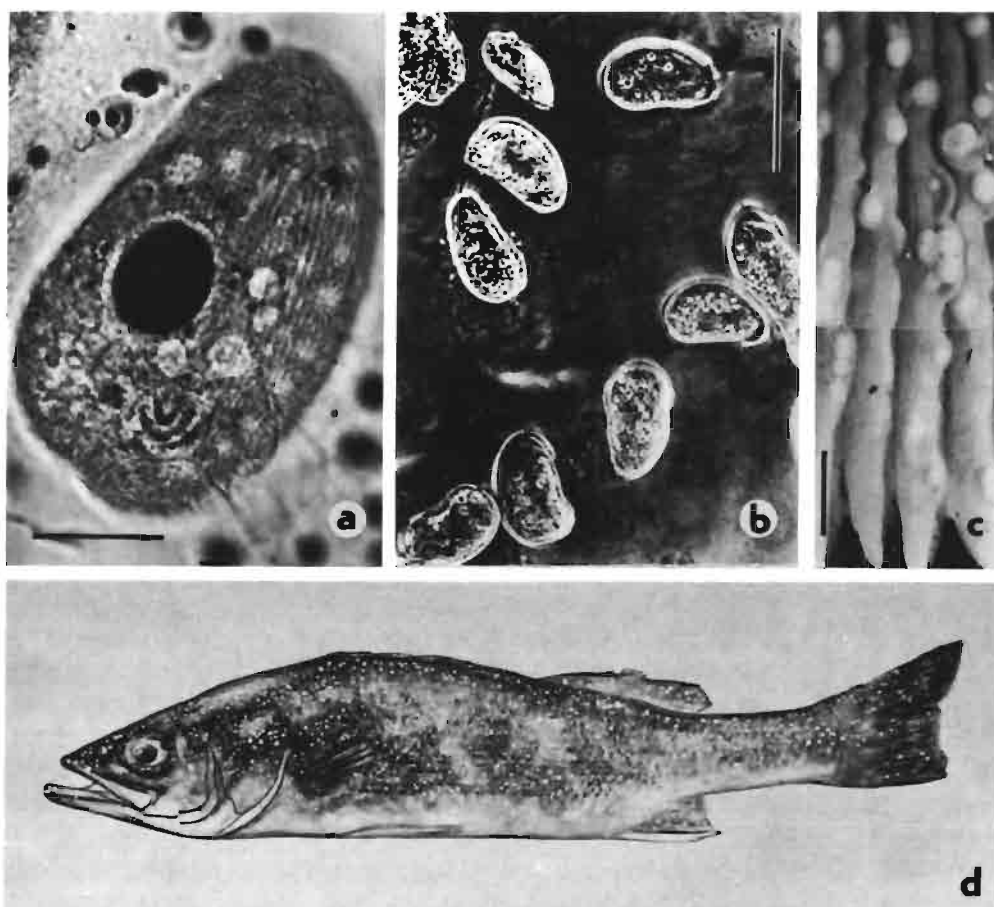

Fig. 1-43: (a) *Brooklynella hostilis.* Stained with haematoxylin; next to oval macronucleus are several minute micronuclei; arched structure: secretory attachment organelle; scale 20 μm. (b) *B. hostilis;* a group of ciliates in a fresh mount; phase contrast; scale 100 μm. (a, b — original). (c) *Cryptocaryon irritans;* large trophozoites, burrowing under epithelium of gill filaments of *Siganus fuscenscens;* scale 1 mm. (After Sikama, 1938). (d) Cryptocaryoniasis (white spot disease) on skin of *Labeolabrax japonicus;* (after Sikama, 1938.)

Cryptocaryoniasis, white spot disease of marine fishes, is caused by *Cryptocaryon irritans* Brown, 1951. This is the marine counterpart of *Ichthyophthirius,* the causal organism of the 'ich' disease of freshwater fishes, known in old China since the Sung Dynasty (964–1126 A. D.). A member of the class Oligohymenophora de Puytorac et al., 1974, order Hymenostomatida Delage and Hérouard, 1896, it has a densely ciliated body, an alternately quadri-partite or ribbon-shaped macronucleus, and a complex buccal apparatus including 3 membranelles and 1 paroral membrane. Its life cycle (Fig. 1-44)

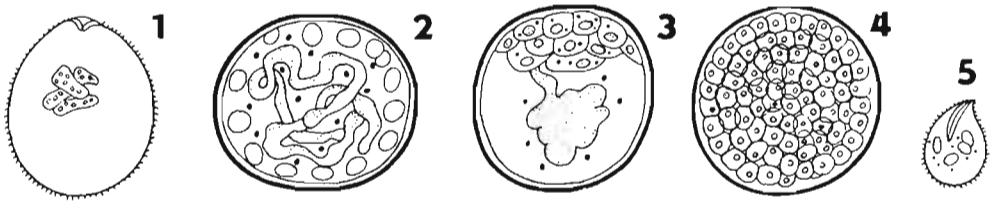

Fig. 1-44: *Cryptocaryon irritans.* Diagrammatic representation of life cycle stages. 1: Fully grown trophozoite; 2: encysted stage (tomont) with ribbon-like macronucleus and numerous small dark micronuclei; 3: unequal division; 4: cyst full of small tomites; 5: released infective stage, theront, with 4 small macronuclei. (After Brown, 1963; redrawn and modified; not to scale.)

includes the trophont stage, living in the skin of the fish. After reaching considerable dimensions (up to about 500 μm), it detaches from the tissue and encysts on a substrate (tomont stage) producing, by a series of binary fissions, up to 200 or more tomites. The latter emerge from the cyst and swim about (theront stage) in search of another susceptible host. If successful, the cycle starts all over again.

Cryptocaryon is probably at home in warmer seas, as indicated by the optimal temperature range (25 to 30 °C) for encystment and tomite production (Cheung and co-authors, 1979). Once introduced into marine aquaria, it affects practically all marine teleosts kept at 20 to 26 °C. Elasmobranchs, however, are resistant. Under natural conditions, *Cryptocaryon* is very rarely encountered. Laird (1956) examined 36 wild fish species in Fiji and found this ciliate on only 1 species, *Epimephelus messa.*

Cryptocaryoniasis has become a problem in public marine aquaria, where it often reaches enzootic proportions. In Japan, 44 of 53 species of fishes in marine aquaria were attacked (Sikama, 1938). Nigrelli and Ruggieri (1966) reported mortalities in 27 species of marine fish, and similar reports exist on aquarium epizootics from different parts of the world. Outbreaks of cryptocaryoniasis are evidently due to crowding and other factors reducing host resistance in aquaria. Infected fish are restless, exhibit respiratory distress and may occasionally scratch on the hard substrates. The surface of infected fish reveals numerous macroscopic whitish pustules or minute greyish vesicles which are the nests of trophonts burrowing under the epidermis. They feed on the host's cells, undermine the epithelium and cause heavy irritation resulting at first in excessive mucus production and epithelial hyperplasia followed by inflammation and haemorrhages. In the gills (Fig. 1-43, c), the ciliates eventually completely destroy the secondary lamellae and cause their dysfunction. On the skin (Fig. 1-43, d), considerable lesions result in the destruction of large epidermis areas which can be seen falling off the host in string-like masses. The trophonts may also invade the eyes, eventually causing blindness. Heavy infections

invariably result in death of the host while secondary infections tend to complicate the situation.

Effective disease treatment (Nigrelli and Ruggieri, 1966) is a combination of copper sulfate and citric acid solutions (0.15 to 0.2 ppm) together with sufficient amounts of methylene blue to produce a clean blue colour. Since *Cryptocaryon* is sensitive to changes in salinity, osmotic schocks can be used to kill tomites and to get rid of trophonts. Either lowered salinities (below 16‰) or hypersalinity (45‰) can be used for treatment, depending on salinity tolerances of the host (Cheung and co-authors, 1979; Huff and Burns, 1981). The latter authors recommend hypersalinity combined with the use of quinine-HCl or chloroquine and minimal handling of the fish.

Protozoan Diseases in Cultured Marine Fish

Diseases in Mariculture

Parasite-caused diseases of cultured marine fish are still poorly documented and less well known than those of cultivated freshwater fish. While parasite diseases of marine pet fish have been studied for a longer time than diseases in mariculture, our present knowledge is mainly limited to ectoparasites. In both mariculture and pet-fish culture, the infection-enhancing and disease-provoking factors are essentially identical: crowding and environmental stress which seem to be much more pronounced than in freshwater cultures.

Crowding due to high population densities is common to all culture systems (e.g., Kinne, 1976b, 1977). Often, in combination with weeds, pollution and contamination from uneaten food, it may result in lowering host resistance, easier spread of ectoparasites and increased chance of contracting a parasitosis by high densities of infectious stages (e.g., spores). Parasitic disease agents are easily introduced — e.g., via stocking of already infected fish collected at sea, contact with wild fish and/or with contaminated water. In net cages the ample food supplied for cultivated fish may attract wild fish from far away and thus increase the risk of infection. An analysis of environmental factors involved in the parasite build-up in mariculture was presented by McVicar and MacKenzie (1977). Temperature is a very important environmental factor (Kinne, 1970). Warmer waters generally tend to support parasitic diseases. Thus, *Glugea stephani* requires temperatures for development above those in Scottish coastal waters; therefore, it can thrive in plaice cultured in Scotland in heated water (but not outside the tanks) after being introduced from southern, warmer waters. Salinity controls especially the ectoparasites. Stenohaline parasites can be eliminated by exposing fish to salinities acceptable to the host but not to the parasite.

In intensive cultures, such as cage cultures, the variety of parasite species as compared with wild populations may be reduced, but the number of certain species may increase greatly producing considerable harm and even mortalities (e.g., Williams and Phelps, 1976). In cultivated striped mullet the increase in numbers of certain parasites is more pronounced in mono-species cultures than in polycultures. Culture conditions tend to increase parasite densities and thus create favourable environments for disease agents.

The high prevalence of protozoan parasites in mariculture does not necessarily induce a pathologic condition in the host. Infected fish may appear healthy, but protozoans constitute a potential threat should the host-parasite balance continue to prevail against the host. Parasites may also contribute to the death of the fish by worsening the already

debilitated condition of the host. Anderson and co-authors (1976) noticed such a role of *Myxidium* sp. in plaice with diet-induced hepatorenal syndrome (For details on agent-host interactions consult Volume I: Kinne, 1980b.)

Ectoparasites of the genera *Amyloodinium, Cryptocaryon* and *Brooklynella* are notorious pests not only in aquaria, but also in mariculture, especially in warmer regions such as the Red Sea (Paperna and Laurencin, 1979). Species of the genus *Trichodina,* ectocommensals turning ectoparasites on fish with lowered resistance, may provoke mortalities in fingerlings of hosts of the genera *Sparus, Mugil* and *Liza* in warm-water cultures such as in the Gulf of Akaba (Paperna, 1977), in estuarine fishes in brackish waters (Ghittino, 1974) or even in plaice cultivated in more northern regions of the Irish Sea (Pearse, 1972; McVicar and MacKenzie, 1977). Sessiline peritrichs of the genera *Scyphidia* and *Apiosoma,* although possibly present in large numbers (Ghittino, 1974; MacKenzie and co-authors, 1976) are harmless ectocommensals. Heavy infections with some other ectoparasites were also recorded. In mullets from the Red Sea, the flagellate *Bodomonas* sp. was reported (Paperna and Overstreet, 1981) and *Dicentrarchus labrax* was infected with flagellates tentatively identified as *Colponema* sp. (Paperna and Laurencin, 1979). The histophagous ciliate *Helicostoma buddenbrooki* caused mortalities in small, post-metamorphosis soles in Scottish maricultures (Purdom and Howard, 1971). In Chinese maricultures of garrupa *Epinephelus akara,* a chlamydodontid ciliate described as *Petalosoma epinephelus* and possibly identical with *Brooklynella,* caused mortalities with symptoms of skin speckle disease (Huang Qiyau and co-authors, 1981).

Intestinal infections have rarely been noted in mariculture. *Hexamita* sp. was recorded intra-epithelially in mullets (Paperna and Overstreet, 1981) while *Entamoeba* sp. and *Balantidium* sp. in the intestinal lumen of several species of the genus *Sigarus* (Paperna, 1977).

Frequent infections are those by myxosporeans. Kudoasis of skeletal muscles due to *Kudoa amaniensis* is a common disease of yellowtails cultivated in Japanese coastal waters (Nakajima and Egusa, 1978). Sources of infection are allegedly coral fishes sparsely infected with the same species. Myxosporeans, reminiscent of *Hexacapsula neothunni,* caused mortalities in cultured sea bass in Japan by infecting their brain (Yasunaga and co-authors, 1981). High infection rates with *Sphaerospora irregularis* (up to 90 %) were noticed in plaice in Scottish waters (MacKenzie and co-authors, 1976). These and other myxosporeans — e.g., *Henneguya* sp. in pompano or *Kudoa cerebralis* in striped bass (Sindermann, 1974) or species of *Myxobolus, Henneguya* in mullets and *Sigarus* (Paperna, 1977) — have a pathogenic potential although they have not been reported to be directly involved in mortalities. Microsporidans known to produce damage in flatfish maricultures are *Glugea stephani* and *Microsporidium* (syn. *Nosema*) *ovoideum* in plaice intestine (McVicar and MacKenzie, 1977). 'Beko' disease of cultured yellowtail juveniles *(Seriola quinqueradiata)* in Japan is caused by *Microsporidium seriolae* invading trunk muscles of the fish.

A haemogregarina, ill-noted for its pathogenicity, is *Haemogregarina sachai* from Scottish plaice (Ferguson and Roberts, 1976). Paperna (1979) observed a similar species as agent of nodular disease of *Sparus auratus* and *Sigarus luridus* cultured in the Red Sea.

No remedies other than prevention have been proposed for diseases due to endoparasitic protozoans in mariculture. It pays to stock cultures with parasite-free, hatchery-reared fry, to use non-contaminated water, to feed parasite-free food and, of

course, to prevent contact with wild fish. Various baths and dips which have been developed to control ectoparasites in aquaria may also be applied in mariculture tanks. In plaice cultures, a formalin bath (1 : 20000 parts of seawater for 24 h) was successful against *Helicostoma* (Purdom and Howard, 1971). Fresh-water dips or sprays were found to be more effective for control of trichodinids and other ectoparasites than formalin solutions, since the tolerance limits of host and parasite to the latter may not differ greatly.

Diseases in Marine Aquaria

Agents of protozoan diseases of fishes in marine aquaria have all been scrutinized in preceding paragraphs. Ectoparasites are the most widespread and hazardous parasites. Nigrelli (1940, 1943) published reports on causes of diseases and death of fishes in the New York Aquarium, one of the largest establishments of its kind. He found that in 1939, *Amyloodinium* alone was responsible for 32.6 % of all mortalities and, curiously enough, *Trichodina* sp. for 10.3 % while in 1940 and 1941, these 2 agents caused only 4 % and 16 % mortalities, respectively. Interestingly, at that time Nigrelli did not record *Brooklynella* or *Cryptocaryon*. The latter established itself later as one of the principal pathogens in marine aquaria (see also section 'Protophytans').

Studies of marine fish in captivity, first in aquaria and more recently in mariculture, have frequently lead to detections of protozoan parasites previously unknown in nature (e.g., species of *Amyloodinium, Cryptocaryon* and *Brooklynella,* and many *Trichodina* species). In the future, such studies will continue to be indispensable for advancements in the understanding of protozoan and parasitic diseases of fish, life histories of their agents, their epizootiology, ecological relations and, most important, their possible cure.

Economic Impact of Protozoan Fish Diseases

An estimate of financial losses due to protozoan infections of marine fish — in wild populations or in mariculture — is thus far not available. Existing evidence justifies the assumption, however, that the losses may sometimes reach quite considerable levels. Direct effects of diseases may be reflected in the reduction of numbers of commercial fish available to the fishery and in rejection of unsightly or unpalatable fish by consumers. Indirect effects are weight loss in diseased individuals, growth retardation and limitation of reproductive capacity. These background effects may concern the majority of the fish population affected and thus, in the final analysis, may be more important than mortalities. For example, infections with *Eimeria* sp., a major agent contributing to loss of condition in blue whiting in the Atlantic Ocean north and west off Scotland, belong to this category.

Easiest to detect are losses caused by protozoans producing macroscopically visible changes on the fish body such as 'cysts' in flesh or bowels, tumour-like swellings and the like. Other diseases — e.g., infections of the body surface and blood infections — are less conspicuous and as yet we have few reports on their intereference with fishery.

The group of pathogens responsible for direct losses is perhaps lead by *Glugea hertwigi* infecting smelts. In 1972, Nepszy and Dechtiar reported that dead smelts were washed ashore along a 120 km stretch representing 70 tons of dead fish. Such kills may result in the complete loss of a stock of smelts, an important prey supporting the growth of commercial fish. The microsporidan *G. stephani* has caused estimated losses among first-year flounders *(Pseudopleuronectes americanus)* of up to 40 to 50%. It may be a limiting

factor in the growth of natural populations of plaice and flounder, and a potential hazard for farms as well. Damage to *Atherina boyeri,* an important commercial fish in southern France, caused by *G. atherinae* may also be a matter of concern. The flagellate *Trypanoplasma bullocki* can seriously affect year-class strength of summer flounder *Paralichthys dentatus,* especially when they spend the winter in such areas as the Chesapeake Bay (USA). Infections with the coccidian *Calyptospora funduli* can reach panzootic proportions in the Gulf killifish *Fundulus grandis,* serving as an important forage for mammals, birds and fishes and widely used at present as commercially propagated bait. Such infection may have a detrimental effect on the killifish population and may reduce its economic potential.

There are diseases due to myxosporeans and microsporidans for which data on actual mortalities are not available, but whose economic importance can still be felt. Heavy lesions of affected fish cause their rejection by dealers or consumers. A great part of filleted *Merluccius hubbsi* prepared by the Argentinian fishery for sale in USA had to be discarded due to infection with *Kudoa rosenbuschi.* Blue whiting *Micromesistius poutassou* netted by the Soviet fishery fleet in the region of the Patagonian and Falkland shelves may be largely unsuitable for marketing due to up to 100% prevalence of heavy infections with *K. alliaria* (Kovaleva and co-authors, 1979). Development of the Pacific hake fishery has been hampered by rapid softening of the flesh occurring soon after capture of the fish (Kabata and Whitaker, 1981) due to *Kudoa*-infection. Considerable damage is due to infections with other species, e.g., *K. nova* in *Thynnus obesus* and *Trachurus trachurus.* Damage caused by other multivalvulids has already been mentioned in the preceding paragraphs.

Pleistophora ehrenbaumi, a microsporidan, causes destruction of body muscles of catfish *Anarhichas lupus,* producing large, unsightly tumour-like swellings. Mann (1954) mentioned that 10% of a large catch of catfish landed at Hamburg in 1952 had to be discarded as unpalatable. A high rate of infection (30%) of ocean pouts *Macrozoarces americanus* with *P. macrozoarcidis* is an obstacle to successful marketing of ocean pout as food fish in the USA (Sheehy and co-authors, 1974).

All these examples are drawn from ocean or sea fisheries. Some of the economically important diseases in mariculture have already been listed in the preceding paragraphs. The losses in mariculture are not less serious, but are easier to assess and, what is important, also to prevent in the future.

Diseases Caused by Protistans: Conclusions

The agents of protistan diseases of marine fishes have been studied for a long time as objects of zoological research. Keeping marine fish in captivity proved an important pathogenic potential of parasitic protozoans — first in marine aquaria and later in commercial mariculture. A good example is supplied by haemogregarines. Once a group of rather obscure parasites of blood cells, they are now known to include species *(Haemogregarina sachai)* of an enormous pathogenic potential. Thus, under the impact of recent studies, protozoans emerged as one of the foremost groups of pathogens of marine fish. They can cause diseases in mariculture and may play an important role in marine biocenoses as one of the limiting factors for population growth of their hosts.

In natural environments, serious lesions are caused by tissue infecting protozoans.

Fish under stress in captivity develop heavy infections with ectoparasites of which marine aquaria supply a good example. Fish in commercial mariculture may suffer from both external and internal affections. Infections caused by ectoparasitic protozoans are especially dangerous due to rapid flare, no need for intermediate hosts, and almost no host specificity of their agents. Infections of the intestinal lumen tend to be unimportant (flagellates, amoebae). Future studies must show whether or not groups such as intestine-infecting coccidia, very common but not known yet to cause serious diseases, have a pathogenic potential.

Research on protozoan diseases of marine fish is still in its infancy and case reports prevail. In the future, investigations should concentrate on the study of agent transmission, host specificity, pathogenicity and ecological dependencies of the agents, and on host resistance. This is a prerequisite for assessing the exact ecological role of these diseases in the marine environment and — supplemented by improvements in control measures — for coping with them successfully in mariculture.

DISEASES CAUSED BY PROTOPHYTANS (ALGAE)

G. LAUCKNER

With the exception of parasitic dinoflagellates, Protophyta (algae) are not known as significant fish-invading disease agents. There are a few scattered reports on associations of plants — mainly blue-green (Cyanophyceae) and green (Chlorophyta) algae — with fish. The majority of these cases, which range from epizoism (phoresis) to parasitism in nature, are from freshwater hosts, with only a few records from marine teleosts and elasmobranchs. Edwards (1978) has reviewed the literature on algal-fish associations.

The fish-pathogenic micro-algae — parasitic dinoflagellates — have been accommodated in phylum Sarcomastigophora, subphylum Mastigophora, class Phytomastigophorea, order Dinoflagellida by Levine and co-authors (1980) in their 'Newly Revised Classification of the Protozoa'. Lom (1981) presented a synopsis of fish-invading dinoflagellates. Representatives of 3 genera — *Amyloodinium, Crepidoodinium* and *Ichthyodinium* — are known to affect marine fish.

Amyloodinium ocellatum, a blastudinid peridinean first described as *Oodinium ocellatum* by Brown (1931), is a parasite of temperate- and warm-water marine fish causing 'velvet disease'. Its parasitic trophont stage is a spherical to oval, non-pigmented, unicellular sac-like organism containing scattered starch granules, conspicuous digestive vacuoles with particulate food, and a large nucleus. The trophont possesses a characteristic red stigma located near the base; hence the specific name *'ocellatum'*. A flattened attachment disc, radiating into numerous filiform projections (rhizoids) and provided with a stomopode (Fig. 1-45, 1), attaches the parasite to the host-epithelial cell. Upon growing

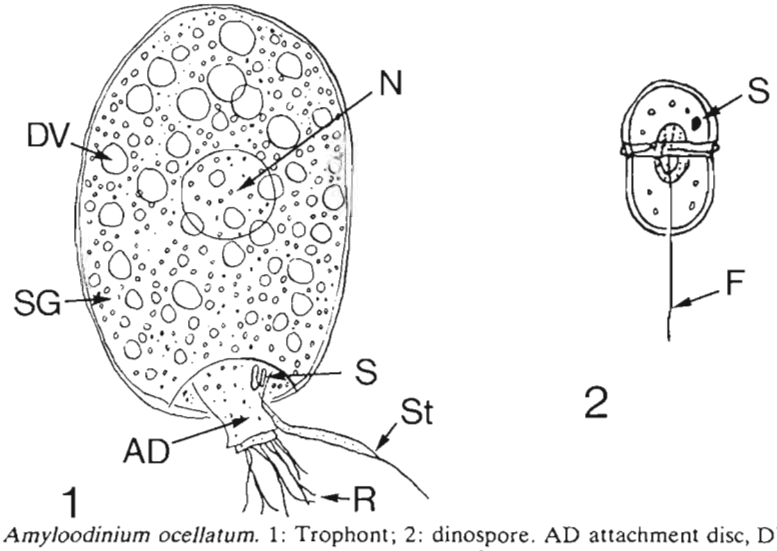

Fig. 1-45: *Amyloodinium ocellatum.* 1: Trophont; 2: dinospore. AD attachment disc, DV digestive vacuole, F flagellum, N nucleus, R rhizoids, S stigma, SG starch granules, St stomopode. (After Nigrelli, 1936.)

to a size of some 150 µm (maximum 350 µm), the organism withdraws its penetrating processes, develops a cellulose cyst wall, and drops off the host to transform into an encapsulated, non-parasitic dividing stage (palmella stage, tomont). Cellular division (without tomont growth) continues until the 128-cell stage. The cells then divide once more and develop into 256 yellowish-green pigmented dinospores, about 11.6 to 15.4 µm long and 10.4 to 14.5 µm wide. Each of these 'swarmers' can attach to a fish, develop into a trophont, and repeat the cycle. The free-swimming dinospores (Fig. 1-45, 2) display the characteristic red stigma in the hypocone; they constitute the stage that allows proper classification of the parasite, the sac-like trophont exhibiting little if any morphological resemblance to the Dinoflagellida. Details of the life cycle of *A. ocellatum* have been worked out by Brown (1934), Nigrelli (1936) and Brown and Hovasse (1946). The ultrastructure, particularly of the holdfast organ, has been studied by Lom and Lawler (1971, 1973).

Amyloodinium ocellatum invades primarily the gills, in heavy infestations also the skin of the fish, its rhizoids causing damage to the affected epithelial cells. Overt disease signs include gill hyperplasia, inflammation, haemorrhages and, eventually, gill necrosis. Infested gills are pale, not deep red as in healthy fish. Bacteria and fungi may appear as secondary invaders of prominent epithelial lesions. Heavy *A. ocellatum* infestations are usually terminal. Death is believed to result from acute anoxia and suffocation (Brown, 1934; van Duijn, 1967; Lawler, 1977b). Penetration of *A. ocellatum* beneath the epithelial layer, as claimed for fish-invading freshwater *'Oodinium'* species, has not been observed (Lom, 1970).

Lom and Lawler (1971, 1973) studied the ultrastructure of the mode of attachment in 2 species of parasitic dinoflagellates isolated from the gills of 3 species of cyprinodontid fish collected in estuarine waters around Gloucester Point, Virginia. One of the flagellates had grass-green trophonts and was identified as *Crepidoodinium cyprinodontum* (Fig. 1-46, 1), previously described by Lawler (1967) as *Oodinium cyprinodontum;* the other

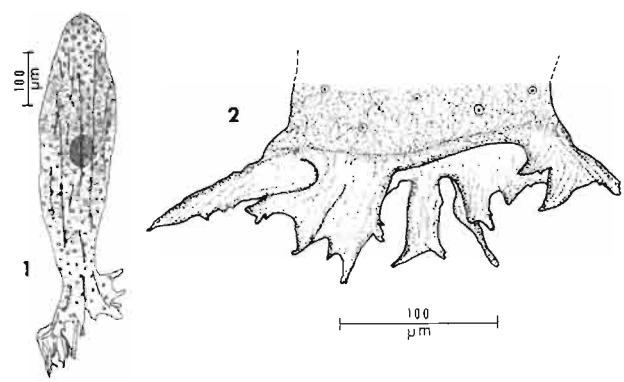

Fig. 1-46: *Crepidoodinium cyprinodontum.* 1: Trophont; 2: holdfast. (After Lom and Lawler, 1973.)

one was non-pigmented and found to be a species of *Amyloodinium,* later (Lawler, 1980) considered to be *A. ocellatum.* The cytoplasm of *C. cyprinodontum* contains numerous typical dinoflagellate chloroplasts and abundant starch granules but shows no traces of ingested host material. The trophont merely adheres to the gill filament by a flattened part

of the cell, which ramifies into major and minor branches that bear finger-like rhizoids, the tips of which are attached to the surface of the host cell, but without pentrating it, in a way resembling a simple type of cell-to-cell junction of epithelial cells (Fig. 1-46, 2). There is no stomopode.

Amyloodinium ocellatum, in contrast, completely lacks chloroplasts. The filiform rhizoids of its flattened attachment disc (Fig. 1-47) are firmly anchored in the gill-epithelial cells, but there is no evidence that they are directly involved in the feeding

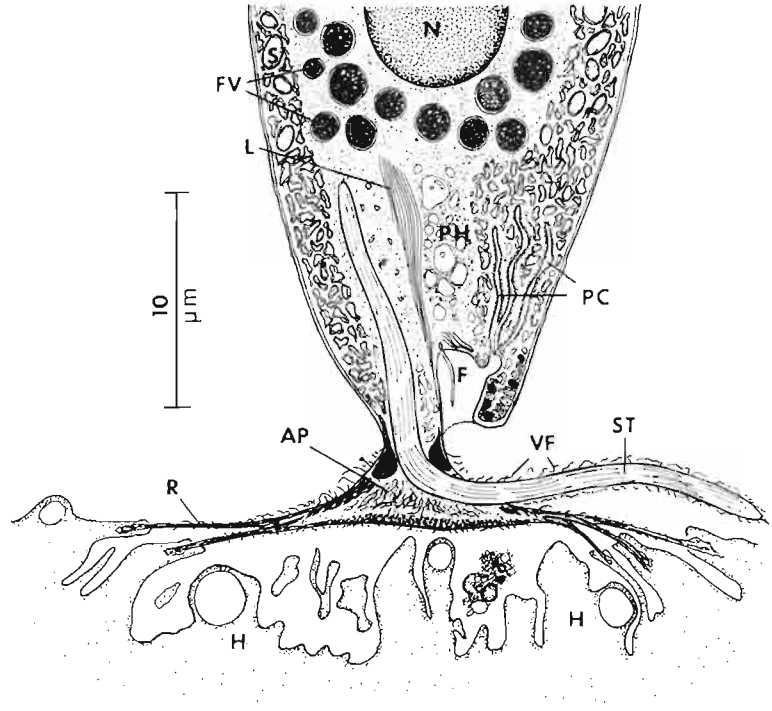

Fig. 1-47: *Amyloodinium* sp. Schematic reconstruction of basal portion of attached trophont. AP attachment plate, F flagellum, FV food vacuoles, H host cell, L fibrillar ledge, N nucleus, PC pusular canal, PH phagoplasm, R rhizoid, ST stomopode tube, VF velum-like pellicular folds. (After Lom and Lawler, 1973.)

process. However, due to their tension inflicted upon the host-cell walls, the gill-epithelial cells are heavily damaged, elongated, separated from each other, and fragmented. The parasite feeds on the contents of the host's cell. Ingestion of host material appears to be aided by the mobile, tentacle-like stomopode, which protrudes from the attachment disc. This organelle might produce exoenzymes or lytic bodies and/or bring host-cell material to a presumed phagocytic region, a slit-like opening at the attached end of the trophont (Fig. 1-47). Digestive vacuoles, up to 10 µm in diameter and containing lumps of host cytoplasm, are present in the trophont's cytoplasm (Lom and Lawler, 1971, 1973).

There can be no doubt that both dinoflagellates differ fundamentally with respect to their mode of attachment and energy uptake. The photosynthesizing *Crepidoodinium cyprinodontum* exhibits ample starch production and appears to be an autotrophic

symphoriont rather than a parasite, although it might utilize some unknown soluble substance derived from the fish or excreted through the gills. The heterotrophic *Amyloodinium ocellatum,* in its turn, is clearly a true ectoparasite. Its pathogenicity may be due to its destructive effect on the host-epithelial cell in which the rhizoids are embedded (Lom and Lawler, 1971, 1973). However, Paperna (1980b), who made a detailed histopathological study of *A. ocellatum* infestation in *Sparus aurata,* observed that — apart from the immediate damage caused to the parasitized cell — there are widespread pathological changes throughout the gill epithelium. Hyperplasia, followed by oedematous changes ('spongiosis'), as well as extensive cellular degeneration and necrosis, were believed to be more detrimental than the focal damage induced by the parasite. Although definitely not penetrating host-epithelial cells, *C. cyprinodontum* may cause gill hyperplasia and necrosis. However, in contrast to *A. ocellatum,* this dinoflagellate has not yet been found to cause deaths in aquarium fish (Lawler, 1980).

Oodiniasis, as the disease is termed, is transmitted directly from fish to fish *via* the motile dinospores, which closely resemble free-living dinoflagellates (Fig. 1-45, 2). Spore development and liberation of the invasive stages into the environment takes 1 to 3 days, depending on water temperature, fouling, and other factors. *Amyloodinium ocellatum* dinospores retain their infestivity for at least 15 days after release. Growth of the developing trophonts is rapid. Due to the large number of invasive particles produced — 256 dinospores per mature tomont — there can be a very rapid build-up of dinoflagellates in closed systems. It is not uncommon to find more than 200 trophonts per gill filament (Fig. 1-48). In heavy infestations trophonts may also occur on the skin, fins, eyes, pseudobranchs, membranes of the branchial cavity and around the teeth, as well as in the lateral line pits, nasal passages, oesophagus and intestine (Lawler, 1977b, 1980). The skin of such badly affected fish may assume an abnormal tinge, thus justifying the designation of the condition as 'velvet disease'. Death of fish may occur in as little as 12 h after introduction of previously healthy individuals into tanks containing numerous dinospores (Lawler, 1977b; Overstreet, 1978).

Onset of an *Amyloodinium ocellatum* infestation can be recognized by a change in the behaviour of affected fish. Croakers, for instance, gasp and congregate near the water surface with heads up; hogchokers and clingfish, when heavily infested, can no longer stay attached to the tank walls; sea robins and blennies, which are usually demersal, swim almost constantly at the surface and attempt to jump out of the water. A summary of most of the disease signs, as reported by various authors, has been presented by Lawler (1977b, 1980).

The symptoms of *Amyloodinium ocellatum* infestation, which may vary from species to species, include (i) rapid gasping for air, irregular opercular beat, mouth opened; (ii) congregation near water surface; or at bottom of container, upside down and gasping; (iii) rapid swimming to surface, then sinking back to bottom; (iv) squirting water or 'coughing' in order to backflush gills (anoxia); (v) spastic movements or sluggishness; (vi) little or no interest in food; (vii) scratching against objects in tank; (viii) appearance of tiny white spots on fins and skin, visible in heavy infestations with the unaided eye.

Brown (1934) believed *Amyloodinium ocellatum* to be a dinoflagellate indigenous to fish from warm latitudes, particularly from Bermuda and the West Indies. Nigrelli (1936), on the other hand, showed it to occur abundantly on fish from Sandy Hook Bay, New York. The parasite was isolated from members of the Carangidae, Pomatomidae,

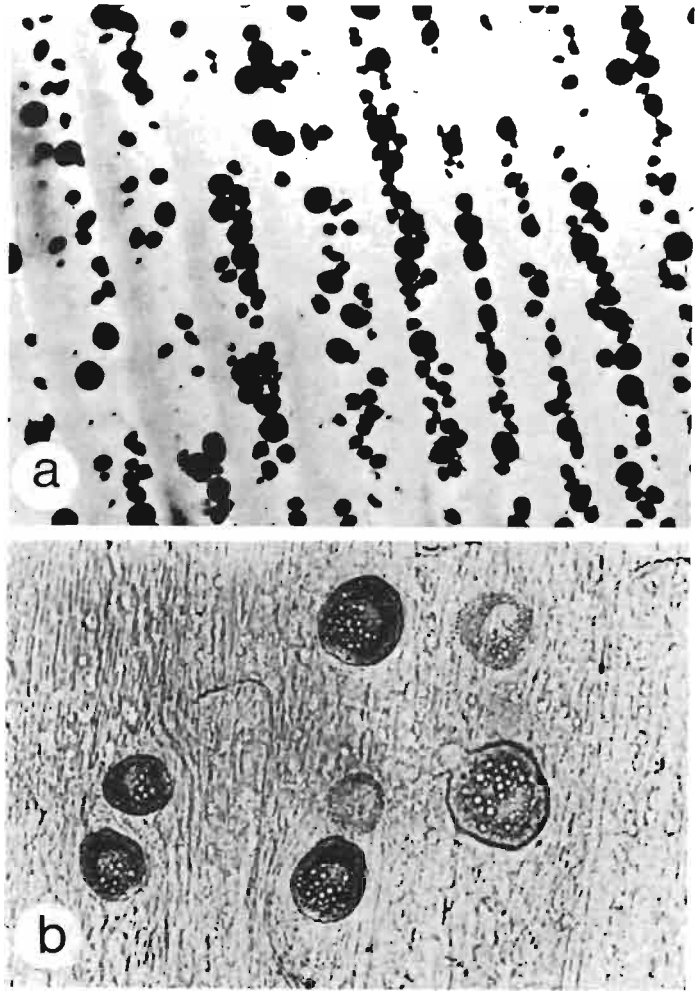

Fig. 1-48: *Amyloodinium ocellatum*. (a) Trophonts attached to gill filaments of *Sparus aurata*. Fresh preparation, immersed in Lugol, × 100. (b) Trophonts attached to caudal fin of postlarval *S. aurata*. Unstained, × 450. (After Paperna, 1980b.)

Sparidae, Sciaenidae, Tetraodontidae, Diodontidae and Triglidae. Infestations were mild in all fish with the exception of the northern puffer, *Sphaeroides maculatus,* and the spiny boxfish, *Chilomycterus schoepfi.*

Lawler (1979, 1980) found 16 of 43 fish species — representing 11 families — from Mississippi Sound to carry natural *Amyloodinium ocellatum* infestations. With a few exceptions, parasite numbers per host (on the gills) were moderate to low. Under experimental conditions, however, 71 of 79 fish species (representing 39 families and including 1 elasmobranch, the stingray *Dasyatis sabina*), exposed to *A. ocellatum* dinospores, were susceptible, and eventually succumbed, to the dinoflagellate. Further fish hosts of *A. ocellatum* have been listed by Brown (1934), Nigrelli (1936, 1940), Brown and Hovasse (1946), Kingsford (1975); and others (for references consult Lawler, 1980). Curiously, *Crepidoodinium cyprinodontum* appears to be restricted to cyprinodont fish,

from which it has been reported by Lawler (1967, 1968a, b) and Williams (1972a). No *C. cyprinodontum* were found on 160 specimens of 37 other families of fish examined by Lawler (1968b).

From the above host records it appears, that *Amyloodinium ocellatum* is virtually non-specific in host selection (Brown, 1934; Sindermann, 1970a, b). Lawler (1980), however, demonstrated experimentally that some Mississippi Sound fish species may not be susceptible to this dinoflagellate, and others may become less susceptible with increasing size (age resistance?). Individuals of 9 species (including *Anguilla rostrata, Opsanus beta, Menidia beryllina* and several Cyprinodontidae) survived massive exposure to *A. ocellatum* dinospores for extended periods of time, although numerous other fish introduced into the same tanks succumbed to the parasite. The survivors were believed to have some resistance to *A. ocellatum* — a view supported by observations of Paperna (1980a, b; see below). Brown (1934) also noted that some fish species appear to be more susceptible to the agent than others. Lawler (1977b) observed a correlation between apparent resistance to *A. ocellatum* and the hosts' tolerance to low oxygen levels, which suggests that in most cases the primary cause of death may be anoxia and asphyxiation.

Tomonts of *Amyloodinium ocellatum,* sometimes found in the fish intestine, had apparently been swallowed after dislodging from the gills (Lawler, 1977b). It appears reasonable to assume that these stages are normally passed to the exterior without affecting the fish; whether they remain viable after intestinal passage and can cause reinfestation, is not known. Cheung and co-authors (1981) reported on the unusual occurrence of *A. ocellatum* in internal tissues of several heavily parasitized pork fish *Anisotremus virginicus* from the New York Aquarium. Internal trophonts were not detected in fresh preparations but were readily seen in H&E-stained sections. The organism exhibited the characteristic staining reaction of a pre-cystic trophont. No division stages were seen in the tissues examined. The parasites were present in the pharyngeal submucosa, muscle and connective tissue, and also in the haematopoietic tissue of the head kidney, Stannius corpuscle and mesentery adjacent to the liver but not in the liver proper, the spleen, posterior kidney, urinary bladder, intestine, brain or heart. No observable host-cellular changes were associated with the presence of the internal trophonts; this was believed to indicate host-parasite compatibility. The varied sizes of the trophonts (17 × 17 μm to 78 × 86 μm) may suggest that the parasites entered the host tissue at different times. The mechanism by which the presumed dinoflagellates reached these unusual sites has not been determined.

Unfortunately, Cheung and co-authors (1981) did not specifically report the presence of the red stigma typical of *Amyloodinium ocellatum* in these internal trophonts, but this may be due to the fact that only sectioned material has been examined, in which the section level may have missed the level of that organelle. The authors' observation, which may require confirmation, is somewhat reminiscent of a similarly unparalleled case of internal infestation of a white shrimp *Penaeus setiferus* by (heterotrophic!) diatoms *Amphora coffaeformis,* as reported by Overstreet and Safford (1980).

While apparently displaying low to moderate virulence in the sea, *Amyloodinium ocellatum* has been found to cause mortalities of epizootic proportions in aquarium-held fish. 'Coral fish' were particularly susceptible to infestation with this dinoflagellate (Chlupaty, 1962; Paccaud, 1962). In the New York Aquarium, *A. ocellatum* was shown to be responsible (together with ciliates *Trichodina* and monogeneans *Epibdella*) for more

than 60% of the deaths of marine fish that had occurred in 1939 and for 26% of the losses in 1940 (Nigrelli, 1940, 1943). *A. ocellatum*-caused mortalities of similar magnitude have been observed in the Aquarium of the Zoological Society of London (Brown, 1934) and in a Singapore aquarium (Laird, 1956). In 'Danmarks Akvarium', Charlottenlund (Denmark), *A. ocellatum* has been the only organism which "really caused trouble", until a suitable treatment of the disease was developed (Højgaard, 1962). This treatment involved the addition of a dilute copper sulphate solution to the aquarium water, giving a concentration of approximately 0.5 mg ionic Cu l^{-1}. Higher doses, as recommended by previous authors, are not tolerated by sensitive fish, such as *Pomacanthus* spp. (Dempster, 1955; Chlupaty, 1962). Other chemicals tested at 'Danmarks Akvarium' gave negative results, but even with copper sulphate, *A. ocellatum* could not be eradicated completely, although losses were minimized (Højgaard, 1962).

In addition to favourable temperature, crowding of susceptibles in aquaria with recirculating water is evidently a factor contributing to the onset of *Amyloodinium ocellatum* epizootics, which usually spread rapidly. The observation that fish in the field are generally but lightly infested and presumably do not succumb to the parasite has led to the belief that *A. ocellatum* attacks primarily stressed or weakened hosts. However, in view of the fact that, in closed systems, death of fish that were healthy and vigorous prior to introduction into dinospore-rich tanks may occur within 12 h, rather suggests that the sequence of events is reversed: Overwhelming of the host with dinospores is the *cause* of the rapid debilitation, not the *consequence* — at least, as far as previously healthy fish are concerned, and with excessively high dinospore concentrations. In infestations developing more slowly, Paperna (1980b) observed that even among fish in heavily contaminated tanks *A. ocellatum* was distributed in an overdispersed pattern; this suggests host differences in susceptibility. In some tanks, 'hyperinfestation' (in the sense of excessively high parasite numbers) was restricted to a few, sometimes retarded or deformed, fish.

Although dinoflagellates are known to produce exotoxins (see below), the possible involvement of such substances in *Amyloodinium ocellatum* kills has, with the exception of a brief mention by Paperna (1980b), never been taken into consideration. Such toxins could easily and readily be absorbed by the host, since the parasite inhabits the type of epithelium of the fish body with the highest permeability for organic molecules — the gill.

Initially recognized as a disease of aquarium fish, oodiniasis is increasingly becoming a problem in modern mariculture (Ghittino, 1977; Lawler, 1977b, 1980). Paperna (1980a, b) and Paperna and co-authors (1980) consider *Amyloodinium ocellatum* to be the most serious pathogen of cultivated marine warm-water fish. In an Israelian mariculture facility at Eilat, Red Sea, the agent first appeared in 1977, and since has caused severe outbreaks of mortalities among fry, fingerlings, brood stock and larvae in the hatchery. All fish species cultivated proved to be highly susceptible to infestation. Greatest losses caused by *A. ocellatum* in Eilat occurred among *Sparus aurata* larvae. In 1979, about 50% of the entire stock of 50 to 80-day-old individuals were killed. Eventually, however, reinfestations could be prevented and the agent be eliminated from the tanks. This eventual suppression of *A. ocellatum* was believed to be suggestive of the development of a sort of resistance to infestation in the fish (Paperna, 1980a, b).

Thus far, oodiniasis has been diagnosed in cultivated stock of gilt-head sea bream *Sparus aurata,* sea bass *Dicentrarchus labrax* and mullets *Mugil* spp. in Israel, Italy, Yugoslavia and France (Paperna and Baudin Laurencin, 1979; Paperna, 1980a,b; Paperna

and co-authors, 1980), as well as in pompano *Trachinotus carolinus*, striped bass *Morone saxatilis* and other Gulf of Mexico fish (Lawler, 1977b; Overstreet, 1978a).

In 1976, the Gulf Coast Research Laboratory (USA) lost about 300,000 juvenile (37 to 38-day-old) *Morone saxatilis*, held in a recirculating system, to *Amyloodinium ocellatum* infestation. This was 75 to 80% of the stock on hand. Apparently, the initial infestation had been established by dinospores contained in the water pumped from the outside (a small craft harbour) prior to stocking. Remarkably, the salinity of the water in which the disease developed was as low as 2‰ S (McIlwain, 1976). Further outbreaks of oodiniasis in several Gulf Coast mariculture facilities have, thus far, involved *Trachinotus carolinus* (Carangidae), *Morone saxatilis* (Percichthyidae), *Sciaenops ocellata* (Sciaenidae), *Lutjanus campechanus* (Lutjanidae), *Mugil cephalus* (Mugilidae) and *Aluterus schoepfi* (Balistidae). The latter 2 were being held for toxicity tests (Lawler, 1980).

Treatment of oodiniasis in closed-circuit mariculture systems is essentially the same as that employed in public aquaria. Therefore, experience in handling the disease on a large scale can be gained by aquarium experimentation. However, due to the larger bodies of water and the greater complexity of such systems, technical problems and costs may increase by an order of magnitude. Preventive measures minimizing the risk of introducing the pathogen into healthy stocks should include adequate water pretreatment by ozone or ultraviolet radiation (for review see Kinne, 1976b). Lawler (1977b) reports that UV light kills *Amyloodinium ocellatum* dinospores in 1.5 mm of water in small dishes within 4 min. In view of the limited survival time of the free-swimming dinospores, simple 'aging' or purification by continuous-flow centrifugation of the incoming water should suffice. New fish arrivals should be quarantined for at least 20 days, as should fish after successful treatment prior to retransfer to uncontaminated or disinfected(!) tanks. Since dinospores still enclosed in tomont cysts can seemingly survive passage through the fish intestine without apparent loss of viability — and in view of the fact that a single surviving dinospore can reestablish new epizootics, disinfective measures must be conducted with meticulous care.

It has been an unpleasant experience that, once introduced into a mariculture system, *Amyloodinium ocellatum* is difficult to eradicate, particularly because its life cycle consists of intermittent phases of an actively feeding stage (trophont), and an encapsulated, non-parasitic dividing stage (tomont), which eventually yields the free-swimming dinospores. The encapsulated tomont is resistant to most conventional parasiticides. Dinospore differentiation within the tomont was not inhibited by incubation at 100 ppm formalin and 4 ppm copper sulphate, and was only temporarily suppressed by exposure to 200 ppm formalin for 24 h. Division was resumed upon retransfer to pure sea water. Similarly, trophonts of *A. ocellatum* were found to tolerate formalin concentrations of up to 100 ppm and short-term exposure (up to 9 h) to 200 ppm formalin and 4 ppm $CuSO_4$ (Paperna, 1980a,b; Paperna and González, 1980; Paperna and co-authors, 1980).

Therefore, oodiniasis is very difficult to treat. *Amyloodinium ocellatum* can live and reproduce in salinities ranging from about 2.8 to 70‰ S and tolerates temperatures between 15 and 30 °C (Lawler, 1977b; Overstreet, 1978a; Paperna, 1980a,b; Paperna and co-authors, 1980). Below 10 °C dinospore production is suppressed, and the temperature for optimal growth of the organism appears to be slightly above 25 °C (Brown, 1934). Copper sulphate, the cure recommended by most workers, may be more effective in its complex (citrated) form. Fish to be treated should be exposed to 0.15 ppm Cu for 1 day

and to 0.3 ppm Cu for a further 5 days. Since copper is highly toxic to fish, invertebrates and algae as well, infested fish should be removed from their original containers and treated separately. Due to the hardiness of *A. ocellatum* and because of the generally weakened condition of fish before the disease is observed, treatments that remove the parasites often kill the fish. Therefore, as well as in view of the general toxicity of copper ions, freshwater baths of 2 to 5 min may be more suitable (Dempster, 1972; Kingsford, 1975; Lawler, 1977b; Overstreet, 1978a).

Whether all records of *'Oodinium'* (= *Amyloodinium*) *ocellatum* from marine fish are actually referable to that species, remains to be established; they may well include other, specifically undescribed or unrecognized members of the genus. Thus, a dinoflagellate found attached to the gills of inshore lizardfish *Synodus foetens* from Buttonwood Canal, South Florida, differed morphologically from *A. ocellatum* in being larger and lacking the red stigma. It also differed from *Crepidoodinium cyprinodontum* (Overstreet, 1968). Paperna and Zwerner (1976) detected an *'Oodinium* sp.' in low abundance in striped bass *Morone saxatilis* and *Fundulus* sp. from mesohaline waters of lower Chesapeake Bay. Lom and Lawler (1971, 1973) reported *Amyloodinium* sp. from cyprinodont hosts collected off Gloucester Point, Virginia, subsequently, although with some reservation, ascribed to *ocellatum* by Lawler (1980). An unidentified, green-pigmented dinoflagellate, devoid of a holdfast and stomopode, and found adhering to the gill and skin mucus of a single *Bagre marinus* individual from Mississippi Sound, is probably a free-living form (Lawler, 1980). The latter author lists a number of fish species on which dinoflagellates other than *A. ocellatum* have been found. Ultrastructural examination might be necessary to distinguish and characterize the above dinoflagellates specifically.

Another blastudinid dinoflagellate, *Ichthyodinium chabelardi,* has been identified as a parasite of ova and freshly hatched larvae of sardines *Sardina pilchardus* and other teleosts in the Mediterranean Sea. Young trophonts, spherical in shape, uninucleate and less than 8 μm in diameter, are the earliest stage of the parasite observed in low numbers (1 to 3) in the yolk mass of the host egg or in the yolk sac of the larva. The trophonts grow rapidly. Karyokinesis without plasmotomy produces giant multinucleate plasmodia or 'primary schizonts', which may attain a size of 100 to 120 μm. Eventually, the 'primary schizonts' cleave into elongated 'secondary schizonts'. These, in their turn, differentiate into subspherical units, 15 to 20 μm in diameter, which resemble the young trophonts observable at the early stage of infestation. At this stage (Fig. 1-49) the young fish die, and the parasite, released from the disintegrating host, undergoes 1 or 2 further divisions in the open water. The resultant free-swimming dinospores survive several days in culture, but their further fate remains uncertain. It was found impossible to obtain experimental infestations, either by exposing sardine eggs to spores or by direct inoculation of dinospores into the yolk mass (Hollande and Cachon, 1952, 1953). As far as the reviewer has been able to determine, there are no further records of *I. chabelardi* infestation in the literature. It appears that this dinoflagellate does not cause large-scale mortalities among fish eggs or larvae.

Although not invading fish directly, several free-living dinoflagellates are known to be detrimental. 'Red tides', produced by blooms of non-parasitic dinoflagellates, may cause large-scale mortalities of fish and other sea life, particularly in temperate and subtropical waters. The impact of *Gymnodinium breve,* the causative agent of 'Florida red tides', on

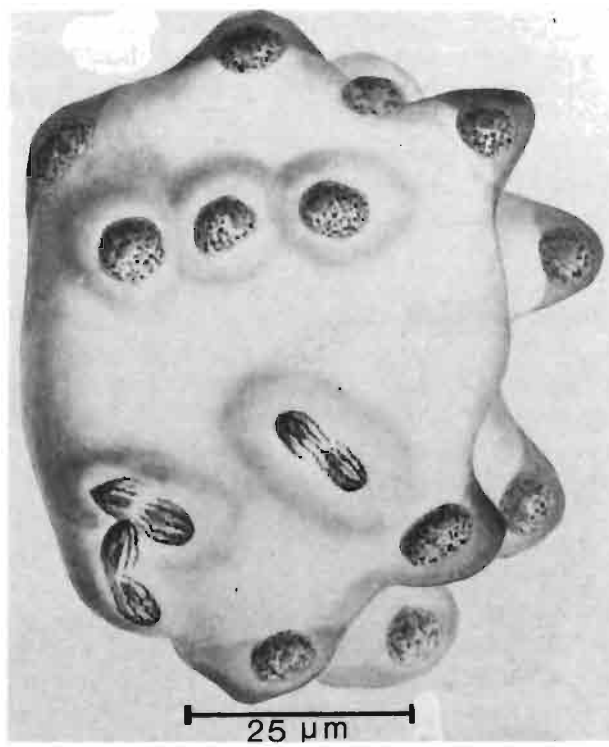

Fig. 1-49: *Ichthyodinium chabelardi.* 'Primary schizont' in process of differentiation into 'secondary' schizonts'. (After Hollande and Cachon, 1953.)

marine communities is particularly well documented. Ichthyotoxins have been shown to be present in these and other dinoflagellates (Brongersma-Sanders, 1957; Reish, 1963; Swingle, 1968; Ray, 1971; Loosanoff, 1973; Quick and Henderson, 1974, 1975; Blogoslawski and co-authors, 1975; Narahashi and co-authors, 1975; Schantz, 1975; Smith, 1975; Steidinger, 1975a,b; White, 1977, 1981; Munro, 1978; Tiffany and Heyl, 1978; Brown and co-authors, 1979; Hall and co-authors, 1981; Nishitani and Wakeman, 1981; and others; see also Vol. I, p. 312 and Vol. II, pp. 615 to 616).

Non-parasitic red-tide dinoflagellates may, in their turn, be killed by parasitic dinoflagellates of the genus *Amoebophrya* (see Vol. I, Chapter 3). Wakeman and Nishitani (1981) suggested that the rapid response of *Gonyaulax (Protogonyaulax) catenella,* a red-tide organism from the Northeast Pacific, to *A. ceratii* may indicate that the latter could serve as a biological control agent for paralytic shellfish poisoning.

In addition to dinoflagellates, algal members of the Cyanophyceae, Chlorophyta, Phaeophyta and Rhodophyta are known to associate, in some way or another, with fish. Most of these associations, the larger proportion of which occurs in freshwater, are fortuitous or dubious in nature (for literature review see Edwards, 1978).

A peculiar algal infestation has been reported from sevengill sharks *Notorhynchus maculatus.* Eight individuals, caught in San Francisco Bay, were maintained in the Steinhart Aquarium, San Francisco (USA), for several weeks, when first disease signs became apparent in 2 of the sharks. Lesions, initially observed on the flanks of the fish,

consisted of numerous pinpoint white, slightly raised foci on the skin, and spread quickly to involve the entire body surface. Many of the lesions enlarged to several mm in diameter, and within a few weeks all sevengill sharks became similarly affected. During this time they became anorexic, lethargic and eventually died. Curiously, other species of elasmobranchs — leopard shark *Triakis semifasciata*, horned shark *Heterodontus francisci* and brown smoothhound *Mustelus henlei* — maintained in the same 21,000 l holding pool, did not contract any infestation.

At autopsy, gross lesions were found to be limited to the skin. Smears taken from these lesions revealed numerous green spheres, 4 to 6 μm in diameter. Since the organisms contained chloroplasts, they were tentatively identified as algae. Tissue destruction was limited to the epithelium and did not involve the underlying dermis or the denticles. The epithelium was eroded; heterophils, erythrocytes, macrophages and epithelial cells could be identified in the resulting cellular debris. Algal cells were thick-walled and unicellular, and often occurred in pairs and occasionally in tetrads. It remained uncertain, however, whether these organisms belong to the Cyanophyceae or to the Chlorophyta. If they were members of the former, then they were most likely *Anacystis aeruginosa* (Blasiola and Turnier, 1979).

Cyanophyceae, usually classified as 'blue-green algae', should be grouped with the Bacteria in light of their typically prokaryotic structure (Davis and co-authors, 1973).

Burrowing organisms, variously ascribed to the Fungi or to the Algae, are known to invade animal-hard structures, such as molluscan shells (see Vols I and II) or the teeth of marine teleosts and elasmobranchs (see Section 'Agents: Fungi'). Peyer (1945) concluded that at least some of the numerous reports on the occurrence of the dubious 'fungus' *Mycelites ossifragus* in the skeletal parts of fish may include algae. Arsuffi (1939) reported on the presence of 'protozoan parasites' in minute tunnels excavated in the teeth of *Tetraodon maculatus*, which Schmidt (1954, 1955) believed to be algae.

Epizoic growths, involving 5 members of the Cyanophyceae, 2 of the Chlorophyta, 2 of the Phaeophyta and 3 of the Rhodophyta, have been reported from the beaks of 15 species of live parrotfish (Scaridae) from the Indopacific Ocean. A porcupine fish, *Diodon hystrix*, was also found to carry algae. No host-specific relation existed between the plants and the fish. It was concluded that the exposed beaks of the scarids merely seem to provide another type of substratum, i.e., a 'moving reef' on which the algae could grow. There was a positive correlation between fish length and the size of the algal thalli. Fouling of this special type is not uncommon for parrotfish. Thus, Micronesian fishermen often express the size of the fish caught in terms of the length of the algae growing on the beak (Tsuda and co-authors, 1972).

Although not invading fish directly, algae of various groups producing blooms or fouling of aquarium or pond water may cause fish kills resulting from gill clogging or exotoxin production. 'Blue greens', including species of *Microcystis*, are known for their capability of exotoxin production. The halophilic chrysomonadine phytoflagellate *Prymnesium parvum* has been blamed for large-scale fish losses in aquaculture facilities in Israel and elsewhere. Blooms may develop over 3 to 5 days. Affected fish die without gross pathological signs, presumably because of osmotic imbalance resulting from increased gill permeability induced by *P. parvum* exotoxins (Sarig, 1971; Munro, 1978; Richards, 1978).

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DISEASES CAUSED BY METAZOANS: CNIDARIANS

G. LAUCKNER

Among the Cnidaria, athecate hydroids are known to associate with a wide variety of marine invertebrates including Porifera, Cnidaria, Annelida, Mollusca, Crustacea and Urochordata (for reviews consult Hand, 1957; Rees, 1967; Lauckner, 1980a, b, c, 1983). The overwhelming majority of these symbioses represent cases of facultative or obligate commensalism or epiphoresis.

At first sight, associations between Cnidaria and fish might be expected to be fortuitous. However, although a rare phenomenon, such associations represent natural relations. Hydroids living on the body surface of fish have been reported from various parts of the World Ocean. The nature of these symbioses ranges from obligate commensalism to true parasitism.

The first and oldest-known account is a note by Cornish (1868), who found a colony of *Sertularia operculata* to be firmly attached to the second dorsal of a 'picked dogfish' *(Squalus acanthias).* Newman (1873) observed an abundant growth of 'parasitic' hydroids, believed to represent *Serialia lendigera*, on the head, neck and anterior body part of an aquarium-held sea horse *Hippocampus ramulosus.*

Alcock (1892) described the association of an Indian deep-water scorpaenid rock perch, named *Minous inermis*, with a hydractiniid gymnoblastic hydroid initially believed to be a species of *Podocoryne*, but subsequently assigned to the genus *Stylactis* as *S. minoi.* As pointed out by Heath (1910), *M. inermis* Alcock is a junior synonym of *M. monodactylus* Block and Schneider. Stechow (1908, 1909, 1913), who studied the hydroid in more detail, placed it in the genus *Podocoryne*, but later (Stechow, 1921) changed its name into *Podocorella minoi.*

As already noted by Alcock (1892), the hydroid resembles *Podocoryne* in general appearance (Fig. 1-50). The hydrorhiza (Hr) is composed of delicate filamentous stolons, some 30 μm in thickness and coated with a very thin and soft membrane. It branches out profusely, but does not form an extensive network. There are 2 kinds of polyps — gastrozooids (Ga) and blastostyles (Bl). The gastrozooids are about 3.5 mm in height when contracted and carry 30 to 40 tentacles when fully developed. Young polyps may be only 1 mm in height and support less than 10 tentacles. The characteristic blastostyles are only 0.5 mm in height, have no mouth opening and the tentacles are degenerated or may be lacking. The gonophores (Go) are attached to the tip of the thicker portion of the blastostyle columns and vary in number (up to 4) and in size. Small filamentous, slightly twisted structures arising directly from the stolons probably represent nematozooids (N) (Komai, 1932).

Podocorella minoi appears to be a constant and obligate associate of *Minous monodactylus* throughout its distributional range. Alcock (1892) found the fleshy polyps on 9 of 12 *M. monodactylus* trawled from 80 to 130 m of water at several stations along the Indian coast; but on none of the invertebrates or on any of the numerous other fish species present in the same hauls and sharing the habitat with *M. monodactylus*, including the

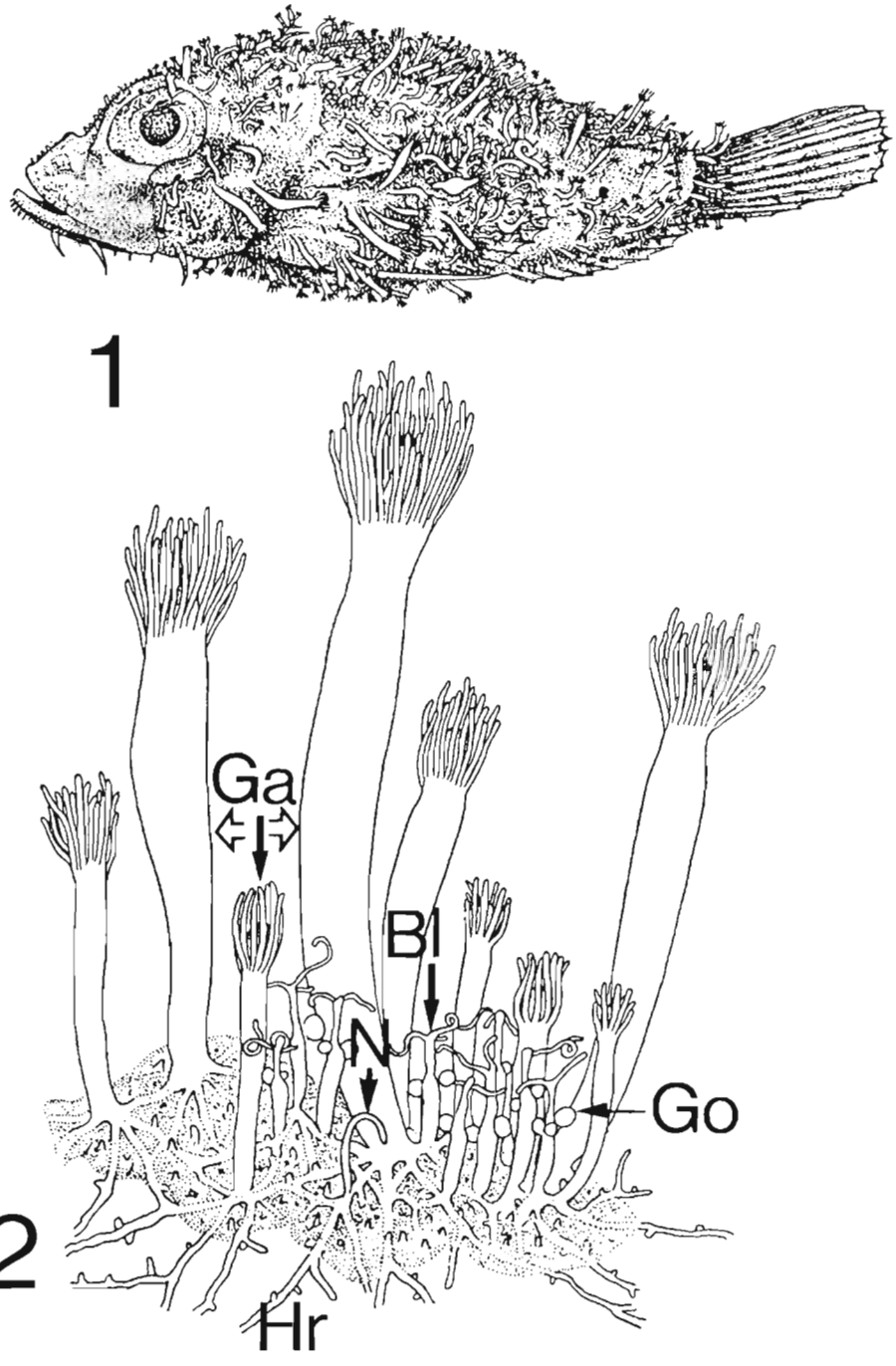

Fig. 1-50: *Podocorella minoi*. 1: Dense overgrowth on body surface of *Minous monodactylus*; 2: enlarged portion of young colony. *Bl* blastostyle, *Ga* gastrozooid, *Go* gonophore, *Hr* hydrorhiza, *N* nematozooid. (After Komai, 1932.)

closely related *M. coccineus*. Alcock concluded that *P. minoi* is specific to *M. monodactylus*. Similarly, Franz and Stechow (1908), Stechow (1909) and Komai (1932), who made their collections in Japanese waters, found *P. minoi* exclusively on this scorpaenid.

According to Alcock (1892), *Minous inermis (= M. monodactylus)* differs from other species of the genus in having a thinner skin and a more feebly developed spinal armature of the head. With respect to this unusual external morphology and to the nature of the hydroid-fish association (which he clearly recognized as commensalistic), he concluded (p. 214):

> "It appears more than probable that this lack of defensive armature stands in some sort of direct relation with the presence of the polyps, for the latter would disguise the fish from its enemies no less than from its prey."

Similarly, Hickson (1906, p. 268) concluded "that there is a mutual advantage in the association."

Another gymnoblastic hydroid, *Perigonimus pugetensis* (Fig. 1-51), was found to associate with Pacific *Hypsagonus (Myoxocephalus?) quadricornis*. Of 37 individuals dredged at 70 m of water in Puget Sound, Washington (USA), 10 were coated with hydroids of this species. In every specimen, the cnidarians were more abundant on the ventral body surface, especially in the axilla, and a luxuriant growth was usually found on the pectoral and ventral fins (Fig. 1-51, 1). There was no indication of a parasitic nature of the association, abundant food (entomostracans and undefinable organic material) in the gastric cavity of the hydranths showed the feeding processes to be those of free-living hydroids (Heath, 1910). Pointing out that several other species of *Perigonimus* are known to live attached to the tests of ascidians, crustaceans and molluscs, Gudger (1928) regarded the *P. pugetensis* — *H. quadricornis* association as probably more or less accidental. Host-epiphoront contact may be controlled by ecological factors, as none of the *H. quadricornis* dredged in the open ocean off the Washington coast and in the Bering Sea carried any hydroid (Heath, 1910).

Komai (1932) described *Stylactis piscicola*, a similarly structurally unmodified hydractiniid hydroid, from *Erosa erosa* living in shallow waters off Seto, Japan. The hydroid is an athecate of a rather primitive type (Fig. 1-52). The hydrorhiza (Hr) is made up of a stolon, which is some 0.1 mm in thickness, rather regularly reticulate, and coated with a thin and soft membrane. The gastrozooid (Ga) is very slender, and large ones may attain a length of 10 mm when fully extended. The tentacles, 15 to 25 in number, are very delicate. The blastostyle (Bl) does not differ much from the gastrozooid, except for being smaller and provided with fewer (usually 5 to 8) tentacles. There are 1 to 3 gonophores, about 0.4 mm in diameter, on each blastostyle, which are sporosacs of the eumedusoid type with the bell cavity filled with large eggs. No nematozooids were seen (Komai, 1932).

The polyps of *Stylactis piscicola* were particularly numerous on the dorsal body surface of the host. Some were found on the throat and belly, as well as on the basal parts of the dorsal and pectoral fins. Only the head and the pelvic and caudal fins were devoid of hydroids (Fig. 1-52, 1). The relation was diagnosed as being clearly commensalistic (Komai, 1932).

Podocoryne bella, a further unmodified hydractiniid hydroid, has its habitat on the body surface of pigfish *Congiopodus leucopaecilus* from Otago Harbour, New Zealand, together with a large array of other fouling organisms. According to Hand (1961, p. 91), "the animal life present [on the fish] reminds one of the surface of almost any alga-covered

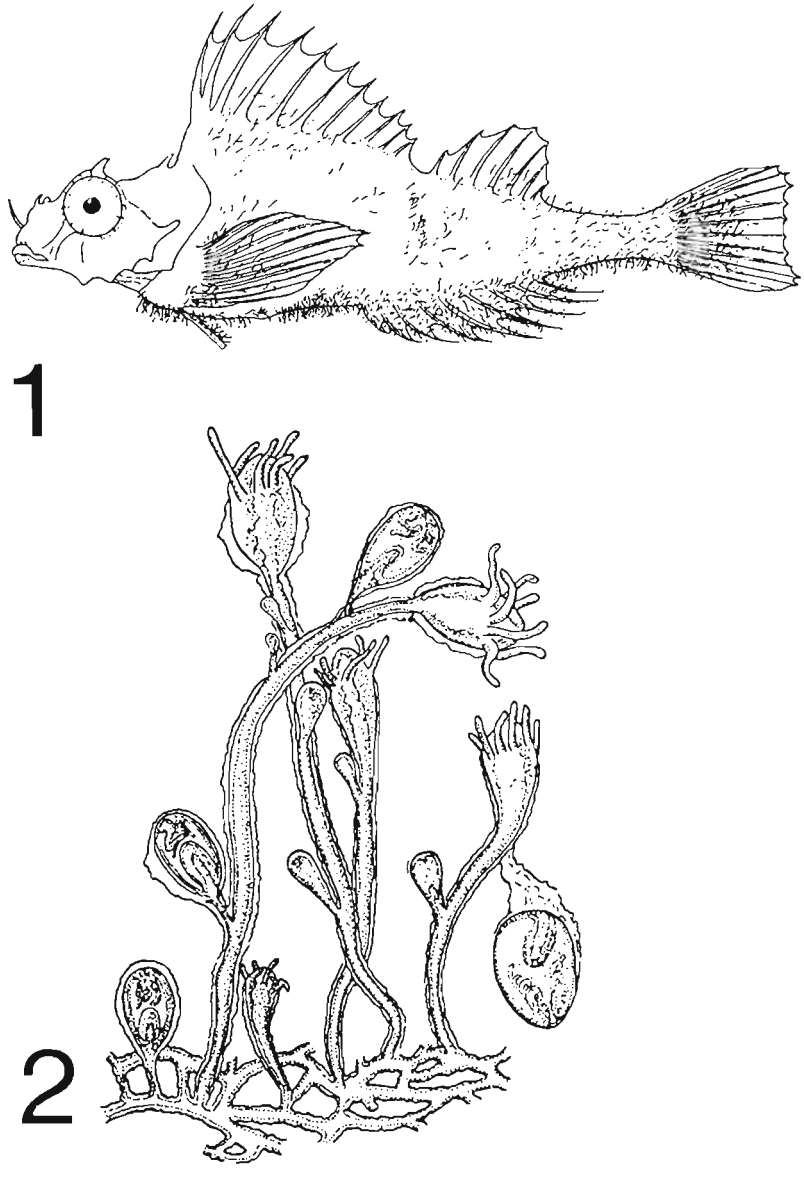

Fig. 1-51: *Perigonimus pugetensis*. 1: Hydroids growing on *Hypsagonus quadricornis*; 2: enlarged portion of colony. (After Heath, 1910.)

rock or piling along the shore". *P. bella*, however, was found on no other substrate than the pigfish.

It should be noted that the fish partners of the associations described — *Minous, Hypsagonus* and *Erosa* — are all scorpaenid rockfish. *Congiopodus* was at one time also considered to be a scorpaenid, but more recently has been classified as a member of the closely related family Congiopidae (Hand, 1961). As representatives of the Hydractiniidae, the above athecate hydroids are predisposed for a commensalistic or even

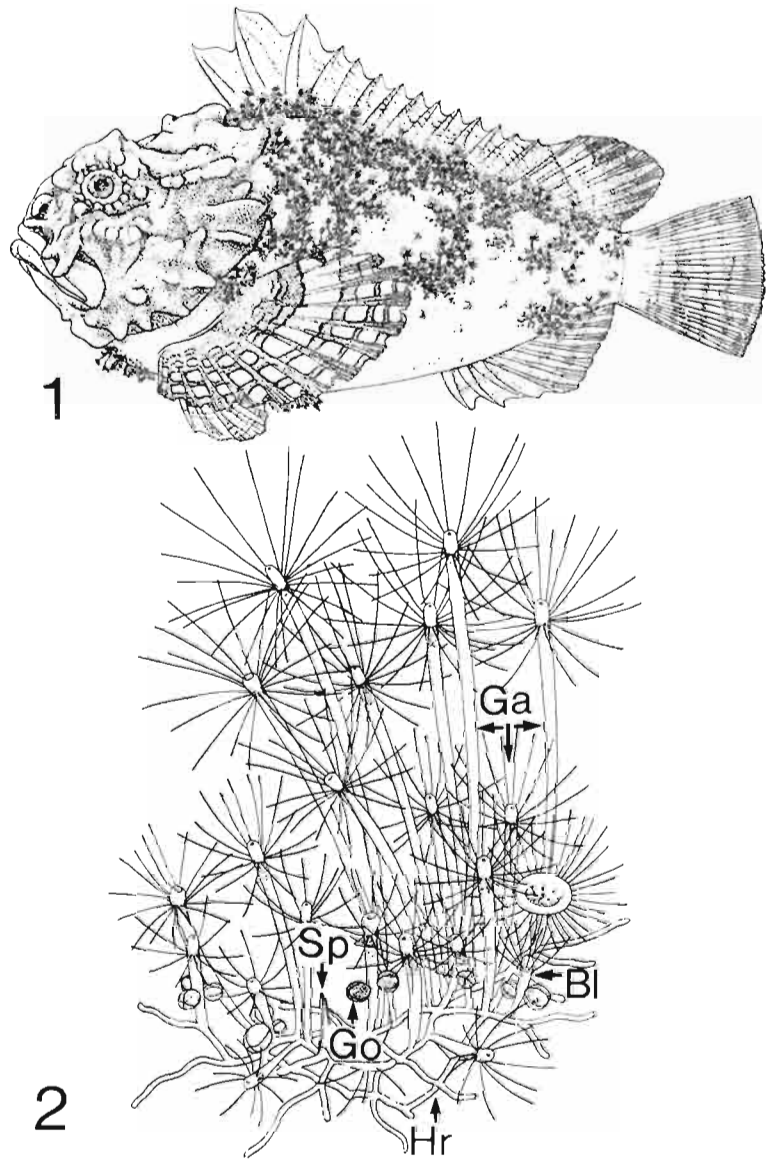

Fig. 1-52: *Stylactis piscicola*. 1: Colony growing on skin of *Erosa erosa*; 2: structure of colony. *Bl* blastostyle, *Ga* gastrozooid, *Go* gonophore, *Hr* hydrorhiza, *Sp* spine. (After Komai, 1932.)

mutualistic way of life. Various species of *Hydractinia, Hydrichthella, Stylactis, Stylactella* and *Podocoryne* enter into symbiotic associations with many different hosts in varying degrees of intimacy (Hand, 1957, 1961). Slow-moving, bottom-feeding fish, such as scorpaenids, provide an ideal substrate for these hydroids. On the other hand, dense upgrowths of colonial cnidarians afford excellent camouflage to their carriers. In addition, the toxins produced by the hydroids' cnidocysts may, at least to some extent, repel predators.

In contrast to the above-described athecates, members of the genus *Hydrichthys* are truly parasitic on fish. Fewkes (1887) observed a reddish patch, at first mistaken for a fungoid growth (Fig. 1-53, 1), on the body surface of a small carangid, *Seriola zonata*, captured off Newport, Narragansett Bay (Rhode Island, USA). Closer examination revealed the growth to be a colony of strongly modified hydroids. The fish was kept alive in an aquarium, and medusae were raised from the attached hydrozoans. When young, the medusae had 2 tentacles, but when fully developed, they had 4, and closely resembled those of the genus *Sarsia* (Fig. 1-53, 3).

Subsequently, the hydroid on the fish was studied in more detail, found to be a strongly modified athecate, and named *Hydrichthys mirus* (Fewkes, 1888a, b). In this species, 2 kinds of polyps arise from the hydrorhiza — 'gonosomes' (= blastostyles) and 'filiform bodies'. Both are devoid of tentacles, but the latter possess a terminal mouth. The author did not study the hydroid – fish interface microscopically and hence (Fewkes, 1888a, p. 225), was unable "to determine how much nourishment the hydroid *Hydrichthys* draws from the fish upon which it lives through the network of tubes from which the gonosomes and filiform bodies arise". Although the infested *Seriola* appeared to be well and healthy and lived for considerable time without exhibiting any inconvenience from the attached parasite, the muscles under the 'basal plate' of the colony appeared somewhat wasted.

Warren (1916) made a histological study of *Hydrichthys boycei*, a hydroid found to invade at least 3 different littoral fish, namely *Ambassis natalensis*, an unidentified species of *Mugil*, and an unidentified glyphidodontid, all obtained from Durban Bay, South Africa. The colonies of *H. boycei*, which may attach to various parts of the fish body, appear as reddish clusters of individuals, some 12 mm^2 in area and 2.5 mm in height. They consist of a plate-like hydrorhiza bearing elongated hydranths and branching gonostyles. The basal plate is capable of budding medusae directly withouth the intervention of any obvious gonostyle. It is composed of a network of branching or intercommunicating tubes, irregularly lobed and firmly attached to the surface of a fin or the body of the fish (Fig. 1-54).

The basal plate grows from the free edge and gradually extends over the surface of the fish. An outer layer of ectodermal cells, moderate in depth, continues around the free edge of the plate into the inner layer of ectoderm, which is in contact with the fish skin and consists of columnar cells of great depth with very large nuclei and granular cytoplasm. These cells are capable of 'eating away' the fish epidermis at the growing edge of the plate (Fig. 1-54). Also, this inner layer extends into haustorium-like projections, which can penetrate deeply into the dermis and may reach the muscular tissue. The inner ends of these elongated columnar cells send branching protoplasmic processes between the host cells, which in this way are absorbed.

The hydranths of *Hydrichthys boycei* are characterized by the entire absence of tentacles. Usually, the mouth opening is completely closed. Curiously, the hydranths are capable of bending down to the surface of the fish and forcing their mouth, which is then widely opened and reflexed, into the injured host tissues, thereby tapping the blood vessels (Fig. 1-54, 2). Host blood is sucked into the gastrovascular cavity in considerable quantity. In one case, a large mass of connective tissue was seen to be engulfed similarly. Fish-blood cells may be found in the coelenteron of any part of the hydroid. Their sometimes shrivelled and disintegrated appearance indicates that they are readily digested. Food in

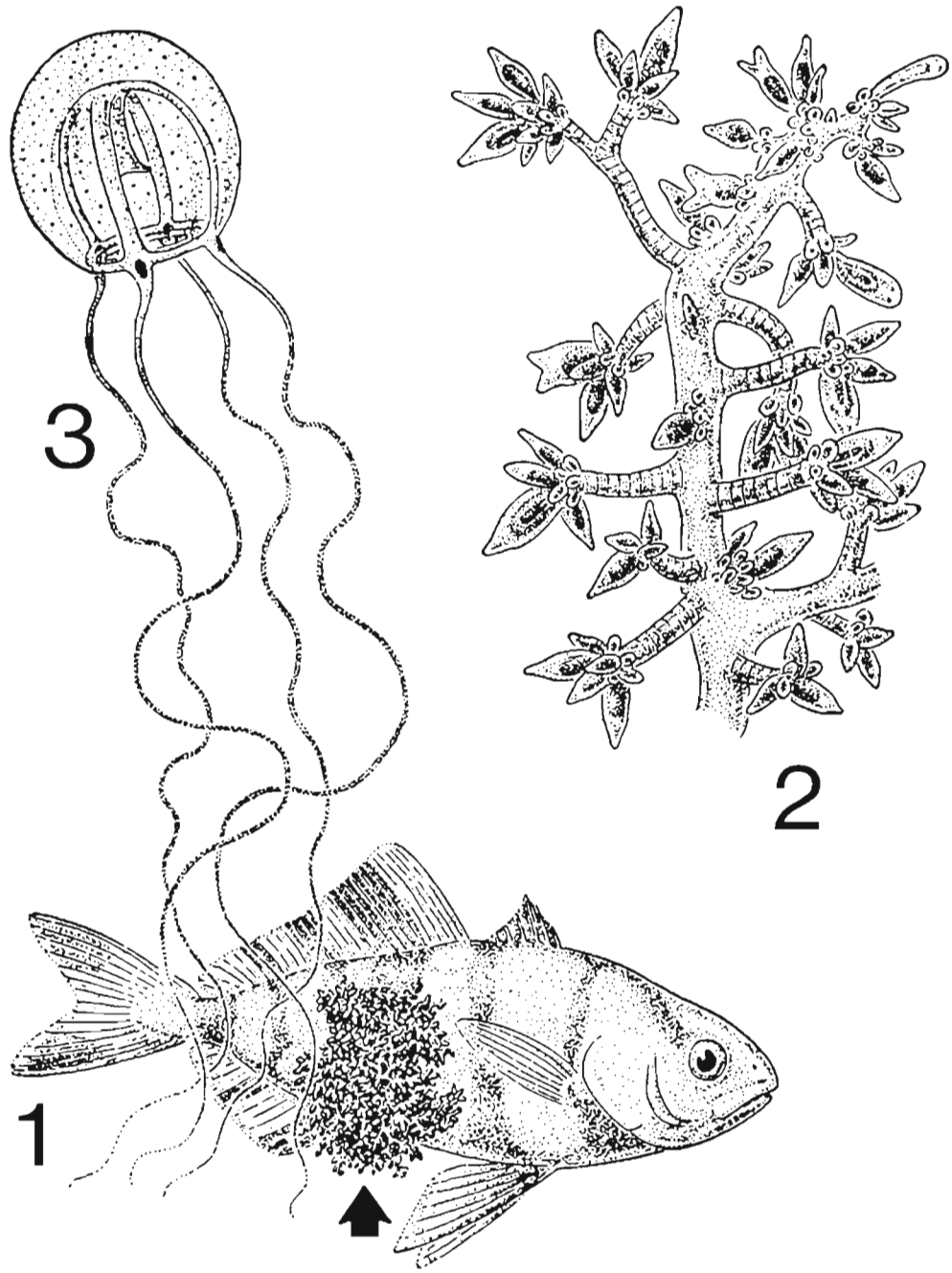

Fig. 1-53: *Hydrichthys mirus*. 1: Colony attached to *Seriola zonata* (arrow); 2: enlarged view of hydroid colony; 3: young medusa. (After Miner, 1950.)

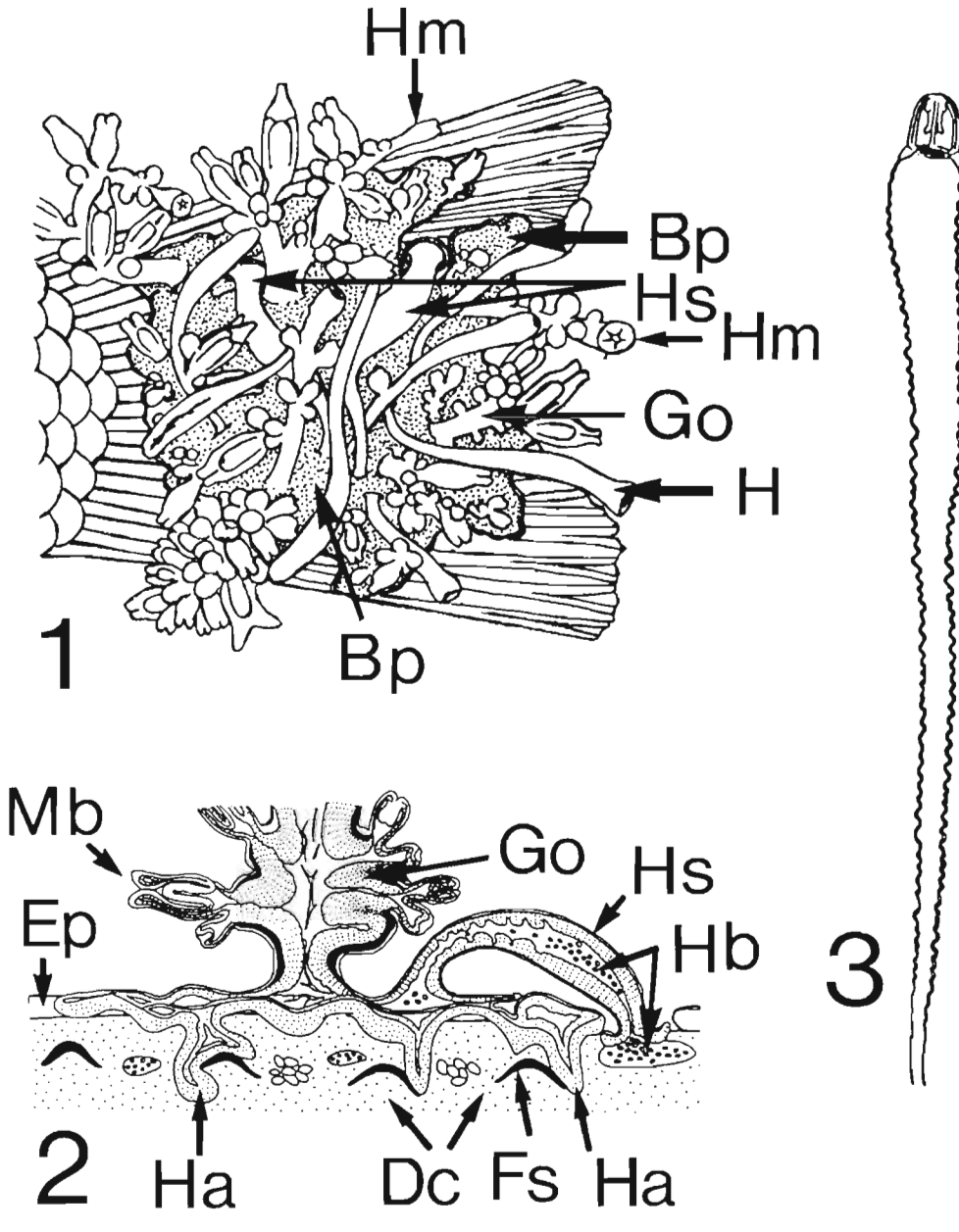

Fig. 1-54: *Hydrichthys boycei.* 1: Colony growing on caudal fin of *Mugil* sp.; 2: vertical section of hydroid-fish interface; note haustorium-like outgrowths (Ha) of basal plate penetrating host epidermis and hydranth (Hs) sucking blood; 3: young 2-tentacled medusa. *Bp* basal plate (hydrorhiza), *Dc* dead host-connective tissue, *Ep* host epidermis, *Fs* scale of fish, *Go* gonostyle, *H* hydranth, *Ha* haustorium-like outgrowth of basal plate, *Hb* host-blood cells, *Hm* hydranth bearing medusae buds, *Hs* hydranth sucking blood, *Mb* medusae buds. (After Warren, 1916.)

the form of captured prey was never seen in the gastrovascular cavity. It was concluded that the main, if not the entire, food supply was derived from the fish host (Warren, 1916).

A hydroid colony, resembling "a small white body something like a cotton piece (about 5 mm in extent)", was recovered from a larval *Chaetodon* sp. collected from a tide pool near the Seto Marine Biological Laboratory, Japan. It was found to be similar to *Hydrichthys mirus* and named *H. pacificus*; but was neither described adequately, nor was its relation to its fish host studied in any detail (Miyashita, 1941).

Several further species of *Hydrichthys* are known from mesopelagic myctophids and a gonostomatid. Colonies of *Hydrichthys cyclothonis* have been found on mesopelagic gonostomatids *Cyclothone signata*. The hydroids (Fig. 1-55) were invariably attached to 1 or 2 of the fins, which when infested had a frayed appearance or were even entirely degenerate. As in the other *Hydrichthys* species, the hydranths of *H. cyclothonis* lack tentacles, and their basal ectoderm is transformed into "un véritable épithelium digestif" (Damas, 1934). There remains no doubt that *H. cyclothonis* is a true parasite. Damas (1934) found it on about 1% of some 2,000 *C. signata*, captured in 300 to 2,100 m of

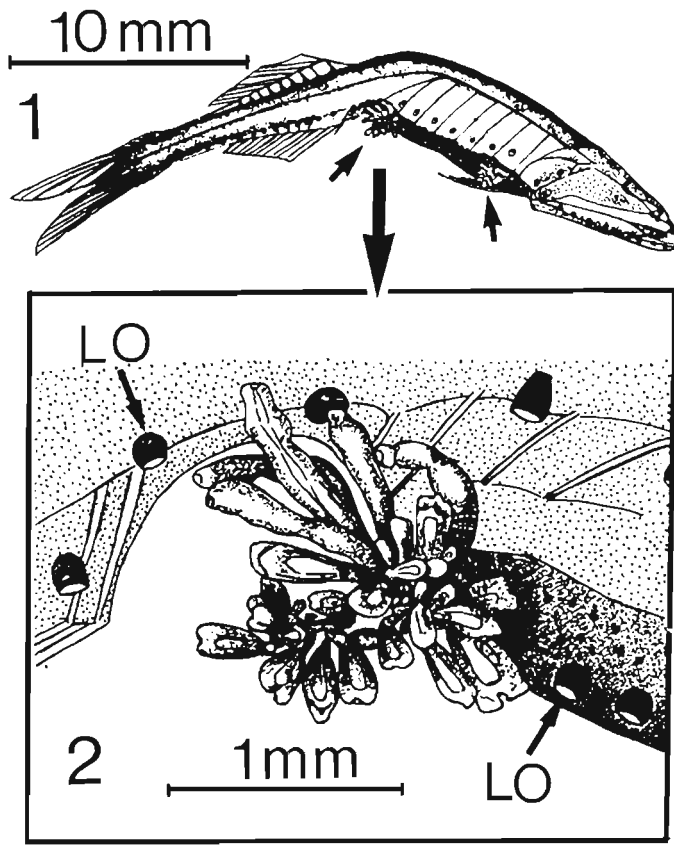

Fig. 1-55: *Cyclothone signata*. 1: Individual carrying 2 *Hydrichthys cyclothonis* colonies on right ventral and pectoral fins (arrows); 2: enlarged view of *H. cyclothonis* colony on ventral fin. Note almost complete destruction of fin at site of attachment. *LO* luminescent organ. (After Damas, 1934.)

water at several stations in the eastern Central Atlantic Ocean. Over 2,000 individuals of *Cyclothone*, including *C. signata*, taken by McCormick and co-authors (1967) off the Oregon coast in the Pacific Ocean, yielded not a single epizoic hydroid.

Hydrichthys pietschi (Fig. 1-56) is an ectoparasite of myctophids *Ceratias holboelli*, collected at 95 m of water off Oahu, Hawaii. Its basal plate was found to penetrate the

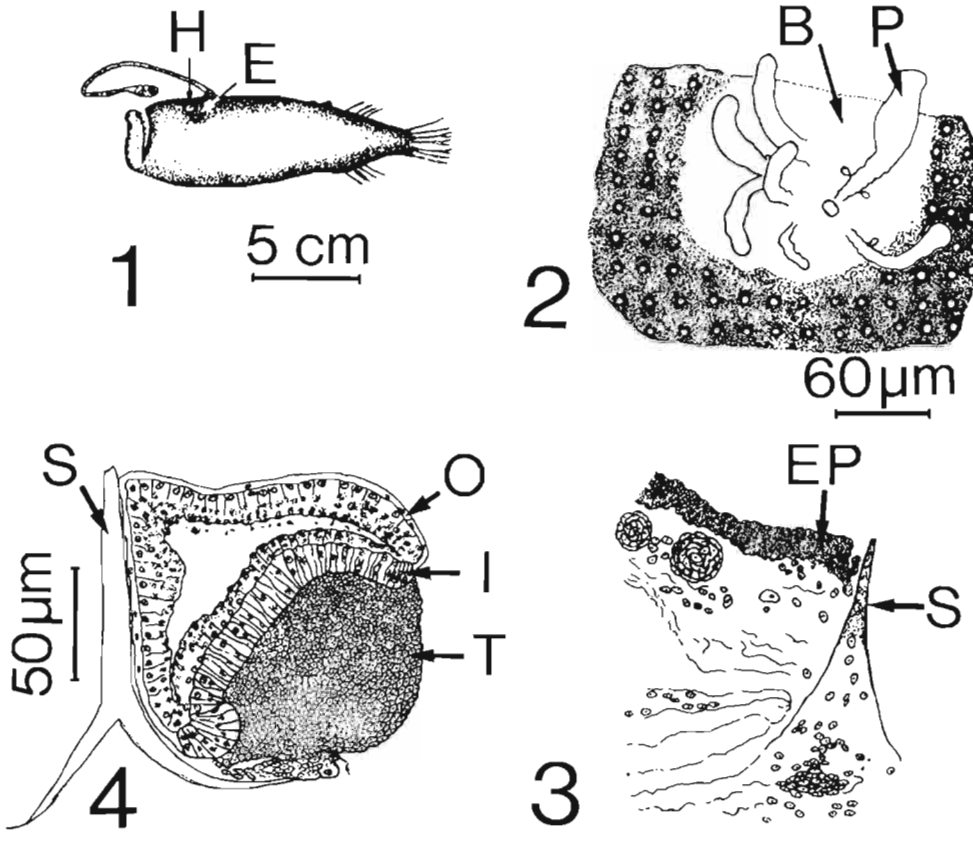

Fig. 1-56: *Hydrichthys pietschi.* 1: Hydroid colony (H) attached to skin of host, *Ceratias holboelli*, near eye (E); 2: enlarged view of colony; 3: section of uninfested host skin; 4: section through part of basal plate of *H. pietschi* and host skin. Note intensive host-tissue response. *B* basal plate, *EP* pigmented epidermis, *I* inner ectoderm, *O* outer ectoderm, *P* polyp, *S* spine-like scale, *T* host-tissue response. (After Martin, 1975.)

host's pigmented epidermis and some of the underlying tissues, to cause skin erosions; and to provoke a definite host-tissue response manifesting itself by a massing of cells beneath the inner ectoderm (Martin, 1975).

During a survey of more than 30,000 specimens of mesopelagic fish, collected off Oregon and representing 40 different species, 1 species of hydroid was found to invade 3 species of lanternfish (Myctophidae) and parasitic copepods on 2 of these (Fig. 1-57; McCormick and co-authors, 1967). The hydroids were closest to *Hydrichthys boycei,* but had no haustoria-like projections and did not appear to feed directly on host tissue, as

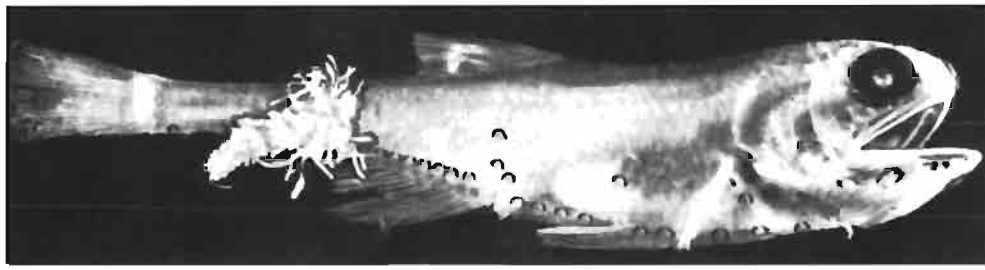

Fig. 1-57: *Tarletonbeania crenularis*. Individual carrying colony of *Hydrichthys* sp. × 3.5. (After McCormick and co-authors, 1967.)

reported for the latter species. *Hydrichthys* sp. was found on 12 of 2,772 *Tarletonbeania crenularis*, on 13 of 4,105 *Diaphus theta* and on 1 of 12,446 *Stenobrachius leucopsarus*. Additional 6 hydroids occurred on *Cardiodectes medusaeus*, a copepod living parasitically on the isthmus of the fish, with its anterior end embedded in the bulbus arteriosus.

On the myctophids, most of the *Hydrichthys* sp. colonies were located in the head region (gills, opercula, isthmus). Damage to the fish skin at the site of attachment was found to be slight. Infested copepods were sometimes deformed and showed erosion of the carapace, but the body wall was never penetrated (McCormick and co-authors, 1967).

In view of the strong modification of its hydroid, the occurrence of a species of *Hydrichthys* on fish-parasitic copepods and its manner of feeding appear enigmatic. Jungersen (1911a, b) described *Ichthyocodium sarcotretis* from a copepod, *Sarcotretes scopeli*, which in turn parasitized mesopelagic myctophids *Scopelus glacialis* (Fig. 1-58, 1). The 'hyperparasitic' hydroids were invariably found on the copepod parasites, but never on the fish host proper. The author was unable to offer any suggestion as to the mode of feeding of *I. sarcotretis*.

Jones (1966), who found hydroids (probably *Ichthyocodium sarcotretis*) attached to copepods *Sphyrion lumpi* parasitic on North Atlantic redfish *Sebastes mentella* (Fig. 1-58, 2), observed masses of cells, indistinguishable from *S. mentella* blood cells, in the coelenteron of some of the gastrozooids, which suggested food uptake in the manner described by Warren (1916) for *Hydrichthys boycei*. Referring to the *Scopelus glacialis* — *Sarcotretes scopeli* – *Ichthyocodium sarcotretis* association and to the close proximity of the polyps to the fish tissues, which had been damaged by the parasitic copepods, as described by Jungersen (1913a, b), Kramp (1921, p. 15) concluded:

> "I can see no other possibility, therefore, but that the hydroid must feed on the epidermis of the fish."

It appears that the hydroids use the parasitic copepods merely as substrate for attachment, but depend on the fish for nourishment, gaining easy access to the deeper tissues and blood vessels of their true host *via* the wounds inflicted by the crustaceans.

Damas (1934) and McCormick and co-authors (1967), who noted close similarities between *Hydrichthys* and *Ichthyocodium*, doubted the justification of the maintenance of *Ichthyocodium* as a distinct genus. Their view is supported by the observation that *Hydrichthys* sp. *(boycei?)* occurs both on myctophids and on their copepod parasites (see above). Probably all fish-parasitic and copepod-associated hydroids thus far described have to be included in the genus *Hydrichthys*. To these may have to be added *Nudiclava*

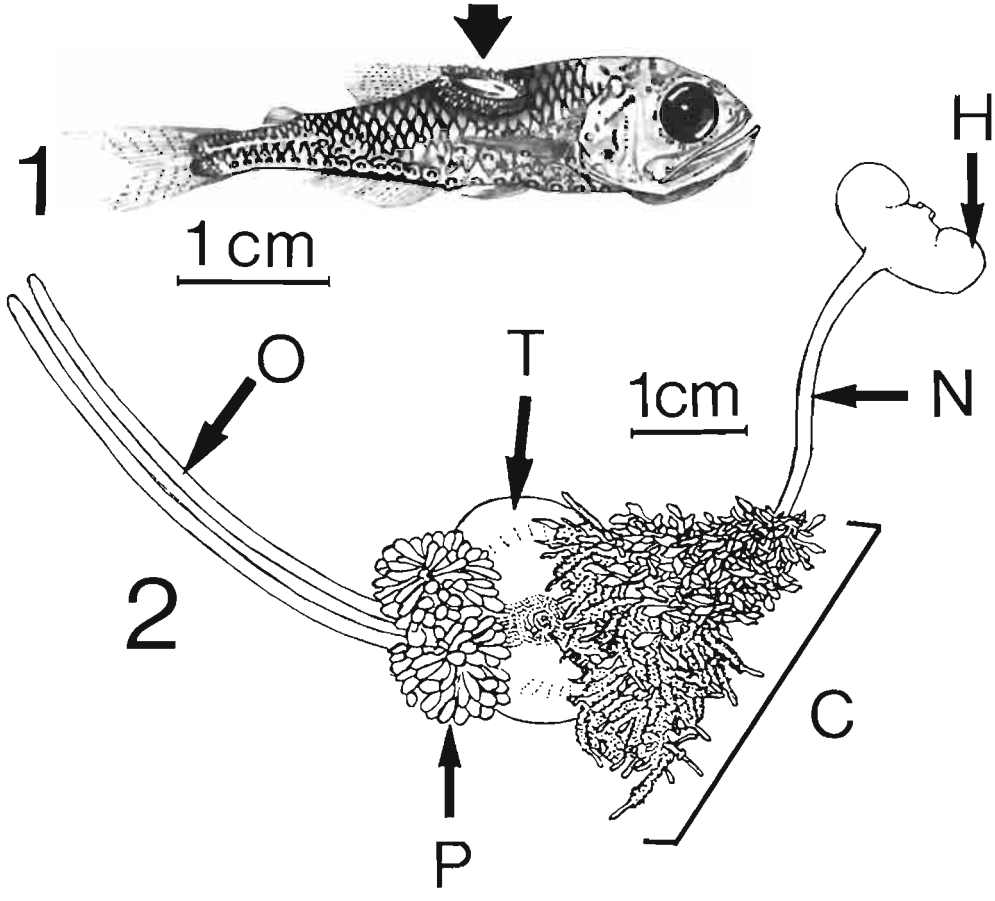

Fig. 1-58: *Hydrichthys (Ichthyocodium) sarcotretis* 'hyperparasitic' on fish-invading copepods. 1: Colony growing on *Sarcotretes scopeli* (arrow) infesting *Scopelus glacialis*; 2: colony growing on *Sphyrion lumpi* removed from host, *Sebastes mentella*. *H* head, *N* neck, *O* ovisacs, *P* posterior processes, *T* trunk of copepod; *C* colony of *H. sarcotretis*. (1 after Jungersen, 1911a; 2 after Jones, 1966.)

monocanthi, a hydroid parasitic on *Monocanthus tomentosus* in the Andaman Sea (Fig. 1-59; Lloyd, 1907).

In conclusion, fish-parasitic cnidarians are of rare occurrence and have little adverse effect on populations. *Hydrichthys* spp. appear to attack mainly young host individuals or small-sized fish species. Thus, Fewkes (1888a) reported *H. mirus* from "a small fish of the genus *Seriola*"; *Nudiclava (Hydrichthys?) monocanthi* was obtained from an 18-mm long *Monocanthus tomentosus* (Lloyd, 1907); Warren (1916) described *H. boycei* from "a small fish . . . about ¾ inch in length"; *H. cyclothonis* was recovered from a 3-cm long *Cyclothone signata* (Damas, 1934); and Miyashita (1941) reported *H. pacificus* from a larval *Chaetodon* sp. 3 cm in length. Myctophids and gonostomatids — favourite hosts for hydrichthyids — are all small fish. It appears that the epidermis of these young or small hosts is more easily penetrated by the cnidarians than the more rigid skin of larger fish.

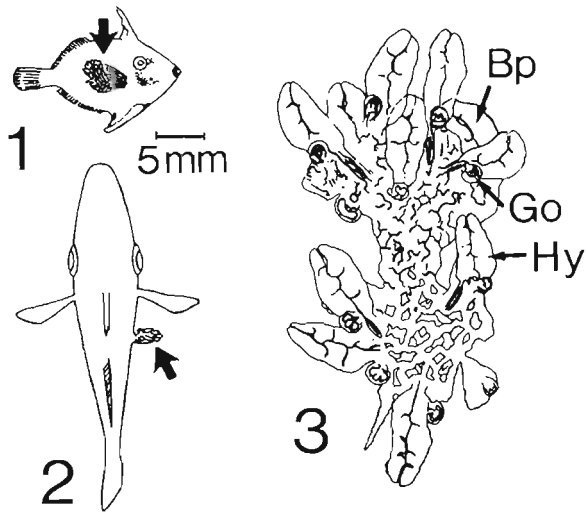

Fig. 1-59: *Nudiclava (Hydrichthys?) monocanthi.* 1: Colony (arrow) attached to skin of host, *Monocanthus tomentosus*; 2: dorsal aspect, with colony fully expanded; 3: enlarged view of colony. *Bp* basal plate, *Go* gonophore, *Hy* hydranth. (After Lloyd, 1907.)

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DISEASES CAUSED BY METAZOANS: HELMINTHS

K. ROHDE

Helminths in General

Present State of Research on Marine Helminths

The historical development of the study of fish parasites including the helminths shows that there always has been a strong emphasis on freshwater forms. The first books on fish diseases, i.e., those by Hofer (1904) and Plehn (1924), make only passing reference to a few marine species. In the third edition of the classical textbook by Schäperclaus (1954) only a few marine parasites are discussed. Emphasis on freshwater forms and our meagre knowledge of marine helminths are also shown by the following reviews and textbooks: Fujita (1937, 1943); Liaiman (1949, 1957); Altara (1953); Reichenbach-Klinke and Elkan (1965); Reichenbach-Klinke (1966, 1969); Amlacher (1970); Reichenbach-Klinke and Landolt (1973); Carvalho-Varela (1975); Bauer and co-authors (1977); Needham and Wootten (1978); Schäperclaus and co-authors (1979). The brief historical outline of fish parasitology in the USSR by Bauer (1973) reveals the same situation. Most of the helminth species of importance to fisheries in British Columbia (Canada) listed by Bell and Margolis (1976), are acquired in freshwater. At a symposium held in 1962 (European Symposium on Diseases of Fish and Inspection of Marine and Fresh Water Products, Torino) Christensen and co-authors (1963) and various other papers on the state of research in Western Germany, Denmark, Finland, France, Italy, Poland, Sweden, Switzerland and USSR, barely mention diseases of marine fishes. Oppenheimer (1953) and Oppenheimer and Kesteven (1953) emphasized the poor knowledge of marine parasites, Khalil and Young (1969) drew attention to the neglect of marine parasites compared with other branches of aquatic biology, and Paling (1968) characterized our knowledge of fish diseases as being 'equivalent to the state of human medicine in about the 17th century'. How little is known about the ways in which helminths affect marine fishes, is shown by reviews of fish biology. For example, Gibson (1969) reviewed the biology and behaviour of littoral fish. Less than 1 of the 44 pages deals with predators and parasites, and no information on any specific effect is given. Thomson (1966) wrote a 35-page review of the grey mullet. Little more than 1 page deals with parasites and mortalities. Apart from references to numbers and kinds of parasite species, only 1 brief paragraph deals with damage and mortalities due to infection, but none of the examples refers to a helminth species.

Grabda and Grabda (1959), in a review of parasitological problems in Polish fisheries, pointed out how little is known on the pathogenicity of fish parasites; less than 3 pages in Robert's and Bullock's (1976) review of pathological effects on the skin of fishes deal with parasites, and only a few marine helminths are mentioned (see also Kinkelin and co-authors, 1970).

Most experimental studies on helminths deal with non-marine forms. To mention only 2 examples, almost all studies on host and site-finding behaviour of larval and adult helminths until 1970 were done with non-marine forms (review by Ulmer, 1971); the

review on feeding of helminths by Markov (1946) refers only briefly to some marine forms. Read's (1968) review of the same subject does not mention marine species at all, that of Arme (1976), only a few.

The following recent reviews contain information on helminths of marine fishes: Sindermann (1966); Euzeby (1975); Gaevskaya and Kovaleva (1975b); Overstreet (1978a); Grabda (1981); Möller and Anders (1983). A very brief account of helminths of marine fish was given by Oppenheimer (1962). Ginetzinskaya (1958) reviewed the life cycles of fish helminths, including a list of parasites from USSR marine and freshwater habitats with their intermediate and final hosts. Möller's (1979) review of fish diseases in the Northeast Atlantic Ocean includes some references to parasites, but non-parasitic diseases are mainly discussed. Historical reviews on marine fish parasitology in the USSR, eastern Baltic Sea, the seas of the Far East and the Aral Sea were given by Polyansky and Bychowsky (1959), Shulman (1959), Zhukov and Strelkov (1959), and Osmanov (1959) respectively. McGregor (1963) listed the publications on fish parasites and diseases from 300 B. C. to A. D. 1923. In some cases, only 1 or 2 works of a more prolific researcher are cited. The progress reports by Snieszko (1972, 1973) contain some information on marine helminths. The following bibliographies contain some references on marine helminths: Williams (1967, review and bibliography on helminth diseases of fish, but largely of freshwater fish), Sindermann (1970c, more than 5,000 references on diseases and parasites of marine fish and shellfish); Margolis (1970a, parasites of fishes in Canada from 1879 to 1969); Möller (1975a, 496 references on diseases and parasites of marine fishes from the North and Baltic Seas, 1927 to 1975); Natarajan and James (1977, 531 references on parasites and diseases of marine and freshwater fish of India); Möller (1980, fungi and zooparasites in Northeast Atlantic since 1900). Millemann (1970) provided keys to helminths of fishes, including explanations of terms. Important check-lists of marine parasites including helminths have been provided by Loftin (1960, Northwest Florida); Margolis (1970a, Canada); Hewitt and Hine (1972, New Zealand); Love and Moser (1976, California); Lawler (1978, South Carolina); Margolis and Arthur (1979, synopsis of fish parasites of Canada); Moles (1982, Alaska). Some recent surveys of fish parasites were reviewed by Chubb (1981).

Relative Importance of Marine Helminths and Helminth Groups

The following surveys give some indication of the relative species numbers in the various groups of parasites of marine fishes.

According to data from Polyansky (1957), fishes in the Barents Sea harboured the following numbers of parasite species: Zoomastigophora, 2; Myxozoa, 21; Microspora, 3; Apicomplexa (sporozoans), 1; Ciliophora, 5; Monogenea, 21; Trematoda, 43; Cestoda, 24; Nematoda, 16; Acanthocephala, 4; Hirudinea, 3; Copepoda, 20; Isopoda, 1 (total 164). Paperna (1975) compiled species numbers of the various groups infecting mullet in 4 geographical regions (Table 1-14).

The checklist of Hewitt and Hine (1972) of parasites of mostly marine fishes of New Zealand shows that the following species numbers had been recorded until about 1970: Protozoa, 70; Monogenea, 37; Trematoda, 83; Cestoda, 38; Nematoda, 59; Acanthocephala, 4; Hirudinea, 6; Copepoda, 53; Isopoda, 2; Branchiura, 1 (total 353). Arthur and Arai (1980b) surveyed Pacific herring *Clupea harengus pallasi* in North American waters from 1975 to 1977 and performed complete necropsies on 175 juvenile and 594

Table 1-14
Mullet parasitofauna in 4 geographical regions. Figures indicate numbers of species (After Paperna, 1975)

Parasites	Eastern Mediterranean Sea	Northern Red Sea	Black Sea*	Gulf of Mexico**
Gill Protozoa	1	1	1	2
Myxozoa	1	3	2	1
Monogenea	4	4	2	5
Digenea, adults	5	7	4	5
Metacercariae	12	1	2	2
Nematoda	4	–	1	1
Cestoda, larvae	2	–	1	–
Acanthocephala	1	1	1	1
Copepoda	4	1	1	1
Isopoda	–	2	–	–
Hirudinea	–	–	–	1
Total	34	20	15	19

* Reshetnikova (1955); ** various sources

adult fish from various localities. They recorded the following species numbers: Protozoa, 6; Monogenea, 2; Trematoda, 9; Cestoda, 3; Nematoda, 6; Acanthocephala, 4; Copepoda, 2. Dollfus's (1953) monograph on parasites of cod *Gadus morhua* lists the following species numbers: Protozoa, 10; Monogenea, 2; Trematoda, 16; Cestoda, 9; Nematoda, 10 or 11; Acanthocephala, 7; Hirudinea, 3; Copepoda, 6; Isopoda, 7; Amphipoda, 1 (total 71 or 72).

The relative importance of the various parasite groups is also indicated by the data on prevalence of infection of marine and estuarine fish in India given by Tripathi (1965). Of 1453 fish examined, 1115 (76.7 %) had parasitic infections. Prevalence of infection was as follows: Monogenea, 39.4 %; Trematoda, 37.7 %; Cestoda, 18.2 %; Nematoda, 29.9 %; Acanthocephala, 13.4 %; Hirudinea, 0.9 %; Copepoda, 30.4 %; Isopoda, 7.7 %. Protozoa were not included in the survey.

All the data show that helminths represent the largest group of parasites of marine fishes.

Taxonomy of Helminths

Much confusion has arisen in some groups of helminths because of insufficient taxonomic data. Three major points to be considered in taxonomic studies of helminths are: (i) morphological changes with increases in size, particularly those due to allometric (relative) growth of various organs and body parts; (ii) host-induced morphological changes; (iii) site-induced morphological changes.

Early studies on allometric growth of parasitic nematodes have been presented by Inglis (1954, a very brief account of *Toxascaris leonina*) and Rohde (1961), on trematodes by Thomas (1965) and Rohde (1966). Subsequently, several authors have studied allometric changes in marine helminths. Scott (1969b) discussed such changes in the trematode *Lecithophyllum botryophorum,* and Fischthal (1978a, b) and Fischthal and co-authors (1980) in a large number of marine trematodes. It has become clear that most if not all

species of trematodes and nematodes undergo allometric growth. In species that grow chiefly 3-dimensionally, the allometric formula $y = bx^{\alpha}$, first developed by Huxley (see for instance Rohde, 1961), can be applied, where y = size of organ or body part; x = body length; b = fraction of the body size that the body part or organ represents; α = allometric exponent. In species that grow chiefly in 1 or 2 dimensions, like most nematodes, the formula becomes a linear one, $y = a + bx$, where a = constant. Many species descriptions which are based mainly on differences in relative organ sizes and body dimensions, are invalid. As suggested by Rohde (1966), in species descriptions measurements should be given individually (or at least separately for different size groups), in order to avoid false establishment of new species. Means and ranges of measurements are insufficient in all those species which are not of a very narrow size range.

With regard to the second point, host-induced variability, several studies have shown that trematodes at least may be strongly affected by their hosts, leading to morphological variability and incorrect description of several species. For example, Szuks (1975), in order to assess the variability in the trematode *Podocotyle atomon,* examined 989 specimens of 24 fish species. He found 301 trematodes in 8 of the species and analysed them statistically. Furthermore, 5025 specimens of 18 crustacean species and 1429 specimens of the snail *Littorina saxatilis* were examined for larval stages. On the basis of the material, Szuks considered the species *P. reflexa* and *P. olssoni* as invalid, and *P. syngnathi* as doubtful (see also Shulman-Albova, 1952; but see Gibson and Bray, 1982, who believe that more than 1 species is involved). Fischthal and co-authors (1980) compared allometric growth of the trematode *Metadena globosa* from 3 fish species and found some significant differences between worms from different hosts.

However, it must be kept in mind that the demonstration of differences between populations in different naturally infected host species does not necessarily imply that the differences are non-genetic and host-induced, even if a wide overlap between the populations exists. Only cross-infection experiments, and ideally cross-breeding experiments between parasite populations from different hosts, can show definitely whether such differences are inherited or not. Even if 2 helminth populations in 2 host species are identical, it is possible that we are dealing with 2 sibling species, whose existence can be ruled out only by cross-infection and cross-breeding experiments. Unfortunately, such experiments with helminths are technically difficult. Ulmer and Rohde (1981) have summarized recent developments in taxonomic studies of helminths. Chaetotaxy, biochemical and serological methods, computer and statistical methods are useful tools in taxonomy, and

> "it becomes increasingly apparent that ultimately the biological-species concept as used in most animal groups must also be applied to parasites reproducing by cross-fertilization. Although this may involve considerable technical difficulty, results eventually will prove to be far more meaningful to helminthologists (p. 159)".

In particular, host-induced and other morphological variability must be considered in taxonomic studies of helminths.

> In the meantime, it seems wise to follow Yamaguti's (1970) advice (p. 243) "when we are at a loss to decide whether 2 species are identical or not, we should be able to avoid the risk of misidentification . . ., if we regard them provisionally as distinct until we are better informed concerning the bionomi-

cal aspects of the host-parasite relationship in the 2 species. Synonymizing will often do more harm than splitting".

With regard to the third point, site-induced variability, few studies have been made. Fischthal and co-authors (1982) found significant differences in the allometric growth of testes and ovary of *Bucephalus gorgon* from 2 different sites, the pyloric caeca and the small intestine, in *Seriola dumerili* from near Jaffa, Israel. The same considerations made above for host-induced variability, are valid.

Zoogeography of Marine Helminths

There is a number of thorough surveys of the helminth faunas of marine fishes in various seas (e.g., Dogiel and Bychowsky, 1938: Caspian Sea; Shulman and Shulman-Albova, 1953: White Sea; Polyansky, 1955: Barents Sea; Zhukov, 1963: Bering Sea; Osmanov; 1976: Aral Sea; Parukhin, 1976: commercial fish of Southern Ocean; Campbell and co-authors, 1980: Northwestern Atlantic deep water). Extensive surveys are needed for other seas, particularly for tropical waters in the Atlantic and Indo-Pacific Oceans.

Rohde (1982) reviewed the zoogeography of marine helminths. Data for most groups are insufficient, but the following trends are clear:

(i) Species numbers of helminths are greater in the Indo-Pacific than in the Atlantic Ocean (shown for Monogenea, Trematoda, and Nematoda); (ii) endemicity is greater in the Indo-Pacific than in the Atlantic Ocean (shown for Monogenea and Trematoda); (iii) species numbers of helminths increase towards the equator (shown for trematode genera and monogenean species; in Monogenea there appears to be a *relatively* greater increase of species numbers towards the equator than of their host species; species numbers of Hirudinea decrease in warm waters, Epshtein, 1970); (iv) frequencies (prevalence) of infection increase toward the equator (shown for Monogenea); (v) intensities of infection with Monogenea vary greatly at all latitudes and no latitudinal differences are apparent; (vi) host ranges of Monogenea are more or less the same at all latitudes (with a possible small increase at low latitudes), host ranges of Trematoda are narrower at low latitudes; host specificity both of Monogenea and Trematoda does not significantly change with latitude (p. 200); (vii) niche width of helminths, as indicated by host specificity, numbers of microhabitats occupied and food, apparently does not change with latitude (shown for ectoparasites of marine fishes).

Rohde's (1982) review also shows that there are distinct seasonal fluctuations in some helminth infections of marine fishes (Monogenea) in cold-temperate waters, but studies are not sufficient for warm waters. With regard to differences between shallow and deep water, the data of various authors (e.g., Collard, 1970; Noble and Collard, 1970; Campbell and co-authors, 1980; Campbell, 1983) indicate that midwater fishes have far fewer species and numbers of parasites than inshore and open ocean surface fishes. There are very few adult helminths. The parasite fauna becomes poorer with depth until the benthopelagic zone is reached. Bottom-dwelling fishes have more species and greater numbers of parasites than midwater species. However, more studies are needed and it is possible that distinct vertical gradients only exist where surface waters are warm, as indicated by the findings of Campbell and co-authors (1980) whose large survey off the New York Bight demonstrated a relatively great diversity except at the greatest depth (see also Campbell, 1983). According to Noble and Collard (1970), macroscopic observations indicate that animal parasites of deep water marine fishes are rarely pathogenic, and with

regard to nematodes, "the intensity of the host-parasite relationship is probably not great since there was no observable evidence of parasite-host competition for energy (for growth of the worms, etc.)" . . . (p. 63). They did not observe damage by cestodes to their hosts, nor were the parasites ever found in various stages of destruction by the hosts. However, more studies are needed to verify these statements.

Parukhin (1975), on the basis of the examination of 6500 fish specimens belonging to 200 species in 100 families concluded that marine fishes in the northern hemisphere generally have a richer fauna of parasitic nematodes than in the southern hemisphere. In view of the very rich fauna of Monogenea found on the Great Barrier Reef (Rohde, 1976b, 1977a, 1978a, 1980b) and of helminths elsewhere in the southern Pacific Ocean (e.g., Rohde and co-authors, 1980; Byrnes, 1980; Hooper, 1980; Roubal, 1981; Roubal and co-authors, 1983), this conclusion cannot be accepted as a generalization for all helminths, although it may well apply to open-ocean fishes.

In summary, surface waters of tropical seas, and particularly of the warm Indo-Pacific Ocean, have the greatest number of helminth species and hence of possible disease agents. However, species numbers alone do not necessarily determine the variety of diseases. Other factors — like intensity of and resistance to infection — may be important, and so far differences in infection intensities have not been demonstrated between cold and warm seas and between the Indo-Pacific and Atlantic Oceans. Nevertheless, there are more types of the better known helminth diseases of man and domestic animals in the tropics, and by analogy one should not be surprised if warm seas harbour not only more kinds of helminths but also of helminth *diseases.*

With regard to individual species of fish, it seems that a fish which has been in a geographical area for a long time, carries a greater number of possible disease agents than one which has recently arrived. Although the data are scanty, some indication of this is the observation of Holloway and Spence (1980) that *Rhigophila dearborni* has a smaller number of helminth species and of species specific to it than *Trematomus centronotus.* Both species live in the same geographical area, McMurdo Sound in Antarctica, but whereas the first species is an immigrant to the Southern Hemisphere the second is not. The first species has 7 species of adult and 3 of larval helminths, the second has 10 species of adult and 4 of larval helminths.

Fish which migrate, retain many of their parasites for long periods. Such parasites can be used as indicators of the geographical origin of fish populations. For example, Margolis (1963, 1965) discussed in detail parasites as indicators of the geographical origin of sockeye salmon *Oncorhynchus nerka* and other salmon (further examples in Rohde, 1982).

Ecology of Marine Helminths

General ecological aspects of parasitism were discussed by Kennedy (1975), and by various authors in Taylor and Muller (1970) and Kennedy (1976). Rohde (1982, 1984) reviewed the ecology of marine parasites, and Overstreet (1982) briefly discussed abiotic factors affecting marine parasites.

Helminths of marine fishes exhibit a number of ecological characteristics, some of which are also shown by other parasites. Important characteristics are aggregated distribution, and specificity for hosts, micro-habitats (sites), age and sex of hosts, and for macrohabitats. Many species also were shown to undergo seasonal fluctuations (see above).

Marine helminths often have an aggregated (= contagious = overdispersed) distribution within the host population, i.e., many host individuals are uninfected whereas a few are more heavily infected than expected if the distribution was random. For example, most helminth species in the whiting *Melangius merlangus* showed an aggregated distribution (Shotter, 1976). The distributions conformed 'more or less' to a negative binomial distribution. A well documented example of an aggregated distribution of a helminth species in its host corresponding to a negative binomial distribution is that of the metacercaria of *Stephanostomum baccatum* in juvenile plaice (MacKenzie and Liversidge, 1975) (Table 1-15).

Table 1-15

Stephanostomum baccatum. Observed frequency distribution of metacercariae in juvenile plaice from Firemore Bay, with negative binomial fitted to data (After MacKenzie and Liversidge, 1975)

Number of parasites	Observed number of plaice	Fitted negative binomial distribution
0	136	125.9
1– 5	164	154.9
6– 10	50	60.1
11– 15	35	33.2
16– 20	12	20.2
21– 25	16	12.8
26– 30	3	8.4
31– 35	6	4.6
36– 40	1	
41– 45	2	
46– 50	1	
51– 55	1	
56– 60	2	
61– 65	1	
66– 70	1	
71– 75	0	13.0
76– 80	0	
81– 85	0	
86– 90	0	
91– 95	0	
96–100	1	
101–145	0	
146–150	1	

$\chi^2 = 11.386$; $P > 0.05$

Rohde (1982) gave a recent discussion of host specificity in marine parasites (see also Shulman, 1958). He distinguished host range and host specificity.

> "Host range is the number of host species found to be infected by a certain parasite species irrespective of how heavily and frequently the various host species are infected. Host specificity takes intensity and/or frequency of infection into account (p. 108)."

This is done by using host specificity indices proposed by Rohde (1980c). The host specificity index based on parasite density (= number of parasite individuals found in each host species/number of host individuals examined) is defined as

$$S_i \text{ (density)} = \frac{\Sigma \frac{{}^{x}ij}{{}^{n}j\ {}^{h}ij}}{\Sigma \frac{{}^{x}ij}{{}^{n}j}} \quad (1)$$

where S_i = host specificity of the ith parasite species; ${}^{x}ij$ = number of parasite individuals of ith species in jth host species; ${}^{n}j$ = number of host individuals of jth species examined; ${}^{h}ij$ = rank of host species j based on density of infection $\frac{{}^{x}ij}{{}^{n}j}$ (species with greatest density has Rank 1). The index for a whole parasite community is $S_c(\text{density}) = \frac{\Sigma S_i}{{}^{n}p}$ where ${}^{n}p$ = number of parasite species in the community. Using Equation 1, an index based on frequencies (prevalence) of infection can be defined (${}^{x}ij$ = number of host individuals of jth species infected with parasite species i; ${}^{n}j$ = number of host individuals of jth species examined; ${}^{h}ij$ = rank of host species based on frequency of infection). For epidemiological studies, it may be important to consider not only densities and frequencies of infection, but the population densities of the various host species as well. This can be done by introducing the factor α_j (= relative density of host species j in percent) into the formula, which now reads

$$S_i = \frac{\Sigma \frac{\alpha_j\ x_{ij}}{n_j\ h_{ij}}}{\Sigma \frac{\alpha_j\ x_{ij}}{nj}}$$

The formula can further be modified by introducing a factor which describes the egg or larval output of the parasite species in different host species.

Numerical values of the indices vary between 0 and 1. The closer to 1, the higher the degree of host specificity.

Using these indices, Rohde (1980c) has shown that host ranges of trematodes increase from low to high latitudes, but host specificity is similar at all latitudes and very great, because even though some trematodes infect a wide range of hosts, they usually infect 1 or a few host species more heavily. Host ranges and host specificities of Monogenea are similarly great at all latitudes (see also p. 197). The discussion in Rohde (1982) shows that restriction of marine helminths to certain host species is universal, but it is greatest among the Monogenea.

Restriction of helminths to certain sites is also universal, although the degree of site specificity varies among the various helminth groups and species (Rohde, 1982). Examples are shown in Fig. 1-60. Frequently, microhabitat preferences of larvae are less marked than those of adults (Table 1-16). Microhabitats of helminths often are not static, they sometimes vary with the age of the host. They also may be different for young and old parasites (e.g., Rohde, 1980a), or change seasonally (e.g., Scott, 1981: *Echinorhynchus gadi* in *Melanogrammus aeglefinus*), and they may also be affected by other parasite species. According to Shotter (1976), the acanthocephalan *Echinorhynchus gadi* is more restricted to the posterior intestine in the presence of the nematode *Hysterothylacium clavatum*, and conversely, *H. clavatum* is more restricted to the anterior intestine when *E. gadi* is present (Table 1-17). However, more often than not there appears to be no interaction between parasite species (examples in Rohde, 1982).

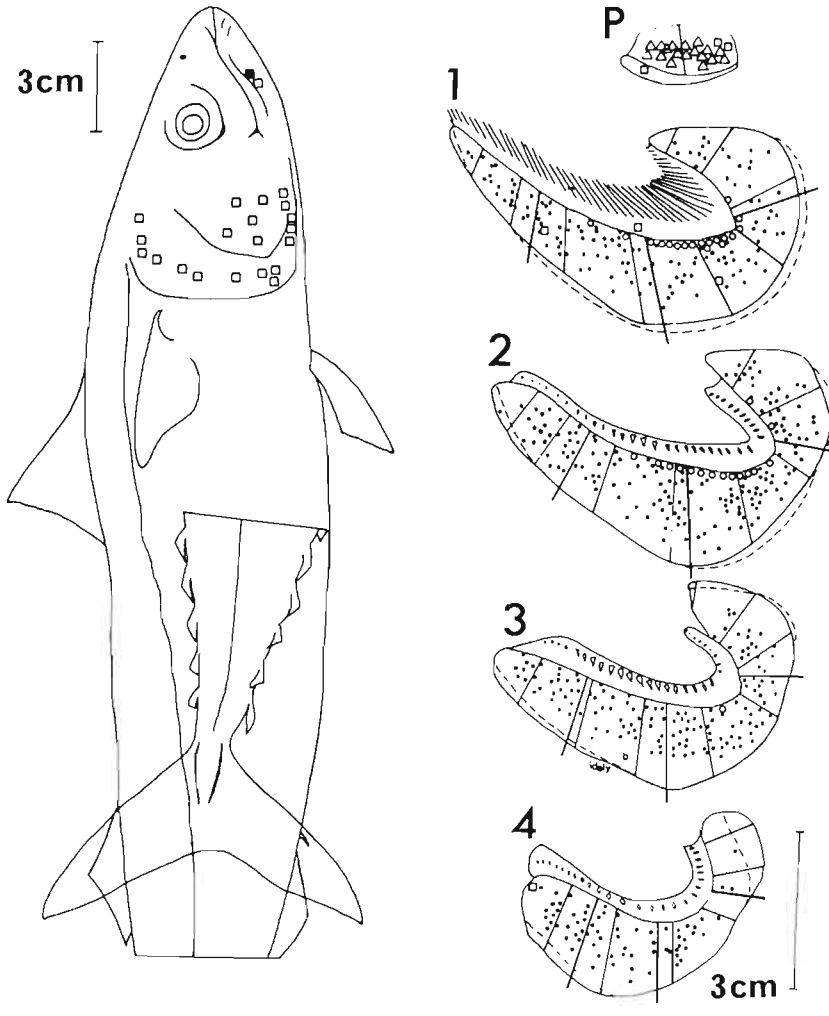


Fig. 1-60: Distribution of ectoparasites on the surface and in the mouth cavity of 122 *Scomber scombrus* at Helgoland, North Sea. □ *Caligus pelamydis* in mouth cavity and on gills; ■ *C. pelamydis* in external fold of mouth; ● cysts; ○ *Kuhnia scombri* (1 circle = approx. 5 individuals); △ *Kuhnia* sp. P = pleurobranch; 1-4 = gill numbers 1-4. (After Rohde, 1982.)

In several cases, positive associations between helminth species were observed, i. e., 1 species occurred more commonly when a host specimen was also infected with another species (references in Rohde, 1982). However, usually there is neither a significant association nor antagonism between parasites. An example can be found in MacKenzie's (1970) study of the monogenean *Gyrodactylus unicopula* on young plaice *Pleuronectes platessa* on the northwest corner of Scotland. The 3 common parasite species on the host are the monogenean and 2 species of ciliates. Statistical analysis showed that there was neither a positive nor a negative association.

A well documented example of age dependence of a helminth infection is that of larval *Anisakis (simplex?)* in *Clupea harengus pallasi* (Bishop and Margolis, 1955). In

Table 1-16
Stephanostomum baccatum, metacercariae. Distribution in tissues of 1965 year-class of plaice from Firemore Bay after experimental infection (After MacKenzie and Liversidge, 1975)

Site of infection	Number of metacercariae	Percentage of total number
Subcutaneous tissues of lower side		
Operculum	43	2.2
Myotomes	542	27.6
Pterygiophoral muscles	841	42.8
Subcutaneous tissues of upper side		
Operculum	1	< 0.1
Myotomes	91	4.6
Pterygiophoral muscles	303	15.4
Fin membranes		
Dorsal	49	2.5
Ventral	41	2.1
Pelvic	1	< 0.1
Tail	10	0.5
Gill muscles	9	0.5
Pericardium	1	< 0.1
Liver and mesentery	33	1.7

Table 1-17
Distribution and relationship of the nematode *Hysterothylacium clavatum* and acanthocephalan *Echinorhynchus gadi* in 219 whiting. Number of fish with both parasites = 60; number with *H. clavatum* = 147; number with *E. gadi* = 12. (After Shotter, 1976)

	Number in anterior intestine	%	Number in posterior intestine	%	Total
H. clavatum in the absence of *E. gadi*	667	74.4	229	25.6	896
H. clavatum in the presence of *E. gadi*	246	87.2	36	12.8	282
Total	913		265		1178
E. gadi in the absence of *H. clavatum*	10	27.8	26	72.2	36
E. gadi in the presence of *H. clavatum*	31	11.0	251	89.0	282
Total	41		277		318

1950/1951 and 1951/1952, there was a significant increase of the mean level of infection with age, 1-yr fish (fish in their first year of life) being uninfected. Grabda (1976) found a similar increase in frequency of infection with larval *Anisakis* and *Contracaecum* in Baltic cod (for other examples see Rae, 1958, 1963; Arthur and co-authors, 1982) (Figs 1-61 and 1-62). But not all species show an increase in infection with age. For example, Scott

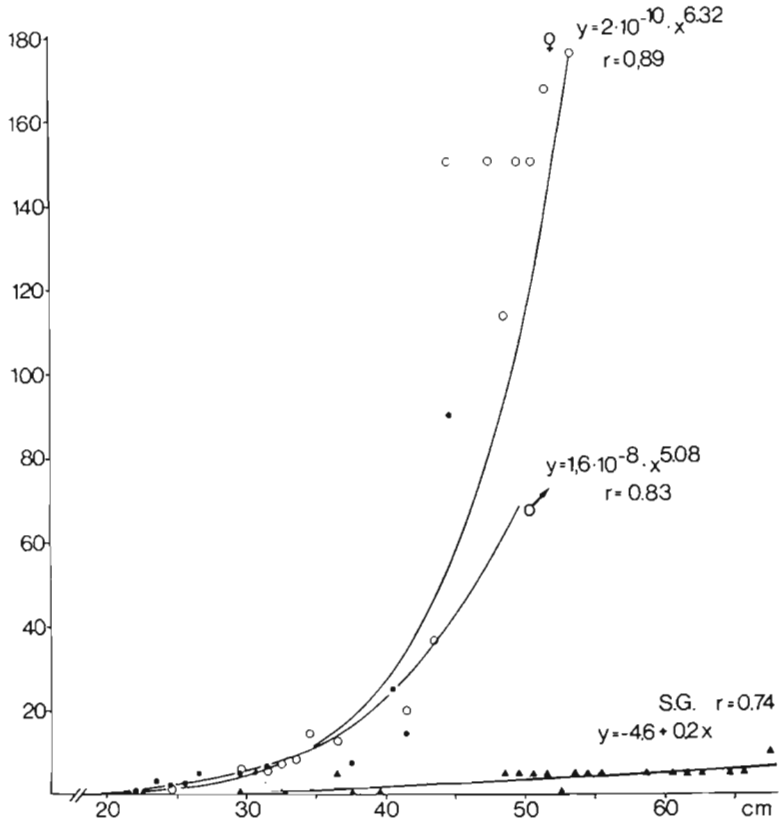


Fig. 1-61: Relation between infection intensity with *Contracaecum* sp. larvae and length of *Chaenocephalus aceratus* (males and females off the South Shetland Islands, S. G. = South Georgia). Abscissa: length in cm; ordinate: numbers of parasites per fish. (After Siegel, 1980.)

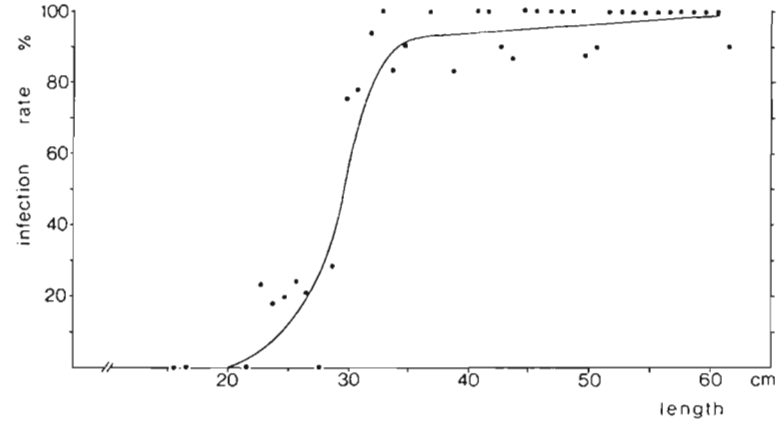
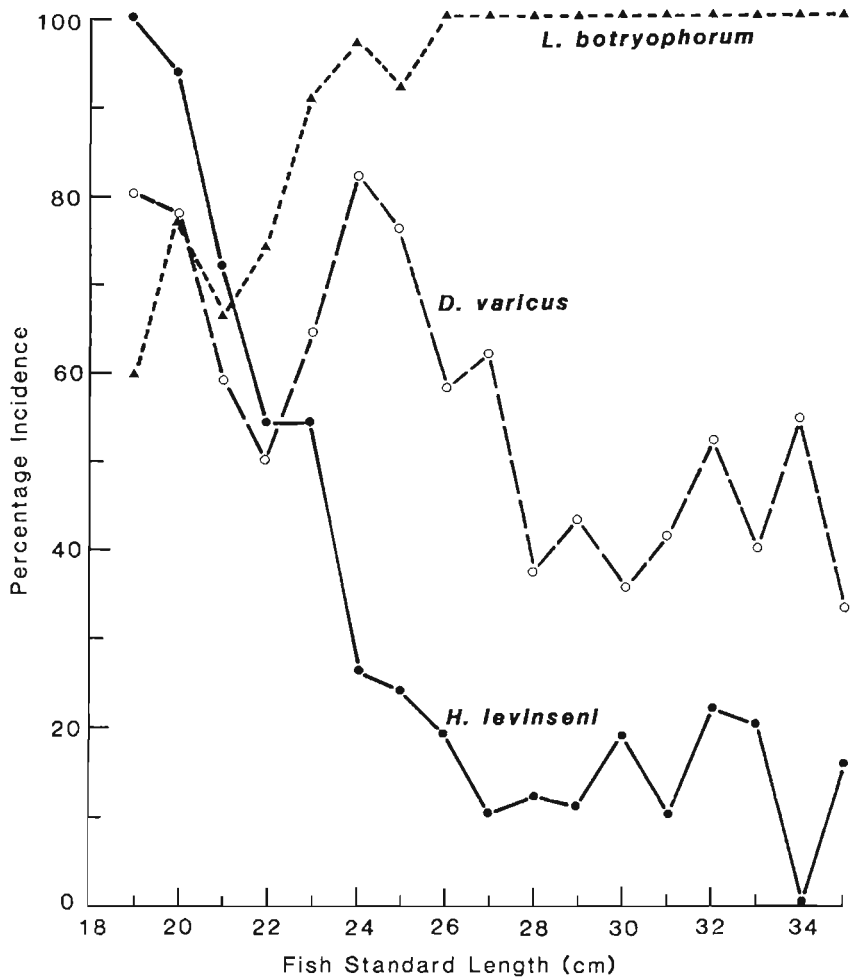


Fig. 1-62: Relation between infection rate with *Contracaecum* sp. and length of *Chaenocephalus aceratus* off South Shetland and South Orkney Islands (eyefitted). (After Siegel, 1980.)

(1969a) found that the digenean *Hemiurus levinseni* in 581 *Argentina silus* from the Northwestern Atlantic Ocean had a high intensity and prevalence in young fish, both parameters decreasing with age (Figs 1-63 and 1-64). *Lecithophyllum botryophorum*, on the other hand, infected more host individuals and occurred in greater intensities in larger fish. A third species of trematode, *Derogenes varicus*, was intermediate. Scott attributed the transition from heavy infection with *Hemiurus* to heavy infection with *Lecithophyllum* to migration of the fish with a corresponding change in diet. Young argentines apparently feed heavily on planktonic copepods in shallower water at the edge of the continental shelf, whereas fish approaching maturity move into deeper water and eat a higher proportion of other near-bottom organisms which are second hosts to *Lecithophyllum*. Infection with the trematodes *Derogenes varicus* and *Hemiurus communis*, the plerocercoid of *Grillotia erinaceus*, and the nematode *Hysterothylacium clavatum* in *Odontogadus merlangus*



Fi. 1-63: Percentage incidence of *Lecithophyllum botryophorum, Derogenes varicus,* and *Hemiurus levinseni* in different length groups of *Argentina silus*. Combined data from several samples. (After Scott, 1969a.)

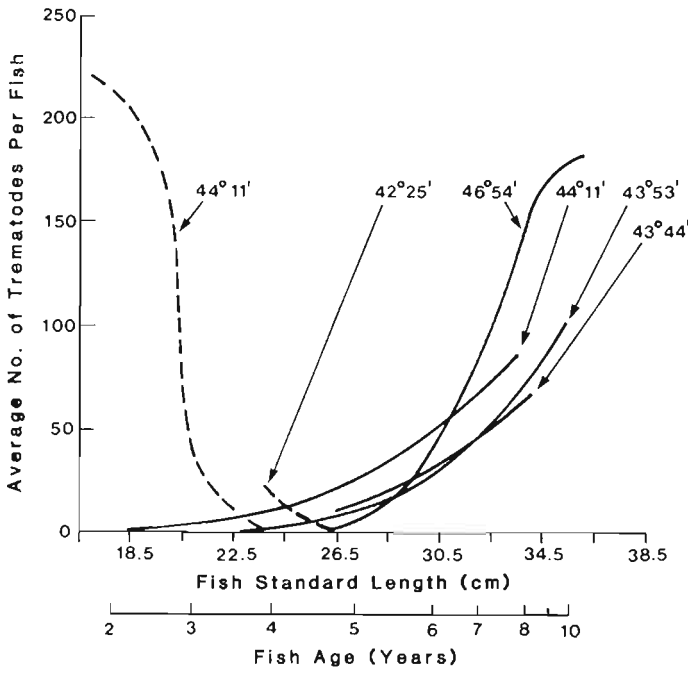


Fig. 1-64: Average numbers of *Lecithophyllum botryophorum* (solid line) and *Hemiurus levinseni* (broken line) in different length groups of *Argentina silus*. Numbers: northerly latitude in degrees and minutes at which the different samples of fish were taken. (After Scott, 1969a.)

increased with age, whereas infection with the trematodes *Lecithaster gibbosus, Stephanostomum pristis* and *Podocotyle atomon* decreased with age. Infection with the monogenean *Diclidophora merlangi* remained more or less the same (Shotter, 1973b).

Commonly there also is an increase in species numbers of parasites with the age of the host. For example, Rawson (1976) demonstrated an increase in the number of monogenean species on Class 0 and I *Mugil cephalus* at Sapelo Island, Georgia, USA (maximum 5 species).

Differences of infection between the 2 sexes of a host species are not common. According to Arthur and co-authors (1982), male walleye pollock *Theragra chalcogramma*, in British Columbia, Canada, were more heavily infected with the third stage larvae of *Anisakis simplex* and plerocercoids of *Nybelinia surmenicola* than females of similar lengths, although prevalence (frequency) of infection did not differ significantly. The other 2 helminth species examined, larval anisakids, infected both sexes equally. Of 11 species of parasites in Pacific herring *Clupea harengus pallasi* in coastal waters of British Columbia, only 1 protozoan parasite, but none of the 7 helminth species infected one sex more than the other (Arthur and Arai, 1980a).

Seasonal fluctuations have been shown for many fish helminths in cold temperate seas. For example, Möller (1974) found that most endoparasitic species of flounder *Platichthys flesus*, in the Bay of Kiel, Federal Republic of Germany, showed distinct seasonal fluctuations, and according to Möller (1975c), all intestinal parasites including 9 helminth species of *Gadus morhua* in the same area also exhibited seasonal fluctuations (see also p. 216).

Many if not all species of helminths have, to a greater or lesser degree, preferences for certain macrohabitats (for recent review see Rohde, 1982). For example, the acanthocephalan *Echinorhynchus gadi* and the digeneans *Podocotyle atomon* and *Cryptocotyle lingua* (metacercariae) were significantly less common in young whiting *Merlangius merlangus* from an offshore than an inshore location off the Isle of Man (Shotter, 1973a). Frequency of infection with larvae of *Anisakis* (probably *A. simplex*) in British Columbia herring *(Clupea harengus pallasi)* in 1950–51 and 1951–52 was 80 to 90 % in the Strait of Georgia and 90 to 100 % in Hecate Strait, on the west coast of Vancouver Island and in the mainland coastal area of Queen Charlotte Sound, respectively. Intensity of infection varied more distinctly in different localities (Bishop and Margolis, 1955). Careful investigations by Reshetnikova (1955) in the Black Sea and by Paperna (1975) in the eastern Mediterranean Sea showed that the parasite fauna of mullet changes throughout the life of the fish, corresponding to changes in habitat and feeding preferences. Fish smaller than 30 mm in coastal and estuarine waters have many didymozoid trematodes, cestodes and larval nematodes, acquired during planktonic feeding in the open water. They are replaced by trematodes and acanthocephalans acquired by benthic feeding and ectoparasites acquired from older fish with whom young fish now share the same habitats. In addition, there are certain infections restricted to certain localities. Thus, fish returning for spawning to the open sea acquire deep-water trematodes. Other examples were described by Polyansky (1957), Polyansky and Bychowsky (1959), Zhukov and Strelkov (1959), Gibson (1972), Noble (1972), Reimer and co-authors (1972), Scott (1975b), Olson (1978), Gaevskaya (1979), etc. Macrohabitat preferences facilitate use of helminths as biological tags indicating habitat and habits of the hosts.

Long-term studies have shown that parasite faunas change due to human activities which affect the macrohabitats. Thus, changes in the composition of the parasite fauna of fishes in the Aral Sea were observed by Osmanov (1975) from 1967 to 1971. Of the 215 species of parasites, 23 were introduced during acclimatization of fishes. Increase in salinity reduced the area of distribution and the population size of many species. A considerable time span may be necessary until effects due to pollution can be eliminated. Thus, Overstreet and Howe (1977) studied the effect of pollution on fish disease in the estuaries of the Mississippi; between June 1970 and January 1972, and again beginning in July 1975, about 100 Atlantic croaker *Micropogon undulatus* were examined at monthly or bimonthly intervals. Two localities, one highly polluted (less than 1 ppm of dissolved oxygen for half the year) until 1975 when pollution was reduced, the other relatively unpolluted were compared. One species of Monogenea, 1 acanthocephalan, 1 larval cestode and 1 trematode were considered. Except for the greater intensity of infection with the trematode in 1975 to 1976, none of the infection rates suggested that the river's community structure had become altered since improvement of water quality. The paucity of invertebrates obtained in trawls and dredges also suggests that much time may be necessary to establish a benthic community (Overstreet and Howe, 1977).

Polyansky and Bychowsky (1959) summarized the results of extensive studies by Russian workers. According to them, the following factors lead to an increase in the numbers of parasite species in a fish: (i) a great variety of food and consequently of possible intermediate hosts; (ii) a long life span; (iii) a large area over which a fish migrates and consequently an increase in contacts with other final hosts (exchange of parasites) and intermediate hosts; (iv) school formation; (v) a large body size. Of special interest is their

generalization that fry of marine fish first become infected with parasites using intermediate hosts. The reason is that there usually is spatial isolation between schools of differently aged marine fish and hence no exchange of parasites with direct development at an early age. On the other hand, freshwater fish of different age mix freely and hence acquire parasites by contact first.

Host-parasite Interactions

Most if not all infectious diseases are not the result of infection with a pathogen alone. A premise of modern epidemiology is that epidemic outbreaks are caused by an imbalance between the hosts, disease agents and the environment (Snieszko, 1972). According to Snieszko (1957), in many cases the pathogens and hosts can exist side by side without disease symptoms resulting. Such symptoms, leading to illness or death, appear only when the balance of mutual tolerance between the host and the pathogen is shifted in favour of the pathogen by such factors as poor nutrition, unfavourable physical and chemical conditions, and the genetic make-up of the fish.

Applied to parasite infections, we must consider 3 aspects to understand disease: (i) parasite, (ii) host and (iii) environment (see also Volume I: Kinne, 1980a).

Ad (i) A parasite may affect a host in 4 ways (Dogiel, 1964), that is by mechanical action, by withdrawal of substances necessary for the normal metabolism of the host, by toxic effects, and by facilitating entrance of pathogenic microorganisms. Körting (1975) gave examples for the 4 mechanisms in fish parasites. Mechanical action is exerted by ectoparasites that injure skin or gills by boring, pressure, penetration or sucking activities. Endoparasitic helminths attach to and penetrate into the intestinal wall, and heavy infections can block the intestinal lumen. Parasites may withdraw enzymes, carbohydrates, amino acids and lipids of the host. Excretion of toxic substances can lead to inflammation, oedema, necrosis, and to changes in the blood picture, resorptive activity of the digestive tract, growth and sexual maturation (however, some authors assume that generalized pathogenic phenomena are not so much due to toxic effects but to the loss of vitamins, proteins and hormones) (p. 256). Bacteria, viruses and fungi may enter the wounds caused by acanthocephalans that have perforated the intestine.

The mechanisms of pathogenesis are poorly understood for most helminths of fish, especially of marine fish. Even in helminth infections of man and domestic animals, the pathology of gastrointestinal helminthiases is little understood (Symons, 1969). With respect to toxins, Symons asks whether any parasites actually produce 'toxins'? There is perhaps circumstantial evidence that they do but if so, nothing is known about their chemical characteristics and how they affect the host.

Little is known about transmission of microorganisms by marine helminths, but non-marine nematodes, trematodes and cestodes have been repeatedly shown to be vectors of viruses, rickettsia, bacteria, and protozoans (for review see Lee, 1971). Marine leeches transmit blood protozoans (see sections on various helminth groups).

Ad (ii) Hosts respond to infection by protective reactions, i.e., immunity and tissue reactions. An important host factor frequently suppressing the outbreak of symptoms is immunity. Fish possess an immune system, although it is not as highly developed as in the 'higher' vertebrates (see recent reviews by Snieszko, 1969; Anderson, 1974; Corbel, 1975; some chapters in Roberts, 1978). Corbel's (1975) review shows that fish have non-specific immune mechanisms similar to those of other vertebrates, i.e., non-specific cellular

immunity and non-specific serum factors, and they also possess specific cell-mediated immunity and specific serum factors. All the reviews listed also show that immunity to helminths can be induced in fishes. Examples will be given in the various sections devoted to specific helminth groups.

Tissue reactions usually interact with immune reactions in the host's attempt to eliminate or neutralize a parasite. Cosgrove (1975) gave a very brief discussion of tissue reactions in fish infected with species of the various helminth groups. Typically, necrosis of cells due to infection leads to an acute inflammatory reaction (oedema, vasodilation, phagocytosis) and, if the parasite survives, to chronic inflammation. The latter often results in capsule formation around the parasite. Reichenbach-Klinke (1954, 1955a) discussed capsule formation in teleosts, including capsule formation due to helminths. His account shows that trematodes, cestodes, nematodes and acanthocephalans can induce capsule formation. Capsules of epithelial origin due to helminths had not been observed up to the time of his report. Reasons are that helminths are either ectoparasites which are never totally encapsulated, or they are endoparasites which penetrate into the mesenchyme. Mesenchymal capsules of helminths may occur in the most diverse parts of the body, including the skin. Occasionally, such capsules are even pushed outward through the epidermis. However, contact with the mesenchymal tissue is maintained. Beside such capsule formation, some helminths can induce capsule-like growths which do not enclose the parasite.

Ad (iii) The host's reactions depend on environmental conditions, particularly stress (for details see Subchapter 1.6). That stress may affect the immune system of fish and therefore their parasite fauna is indicated by the observation that both cell-mediated and antibody-mediated immune responses in fish depend upon environmental temperature, and that nutrition and behavioural patterns may affect the immune response (review by Corbel, 1975). That environmental 'stress' may lead to changes of the parasite fauna has been shown repeatedly for non-marine parasites (see review by Esch and co-authors, 1975). Both the infrapopulation (= all individuals of a single parasite species within an individual host) and the suprapopulation (all individuals of a single parasite species, in all stages of development, within all hosts of an ecosystem) may be affected. Stressors may be temperature, diet, inter- and intraspecific interaction, host density and mechanical irritation. The example of stress effects on infrapopulations, given by Esch and co-authors (1975) is seasonal temperature changes leading to density changes of the parasites. Decrease in parasite population density is often brought about by temperature, diet and inter- as well as intraspecific interactions. An increase in parasite density may be due to reduction of resistance, elimination of behavioural or ecological barriers which normally limit recruitment of parasites, and seasonal change which results in regular recruitment of parasites by their natural host, and host stress can directly influence the first 2 of these factors.

According to Esch and co-authors (1975), much less information is available on stress effects on suprapopulations. Nevertheless, it is clear that suprapopulations may be affected in several ways, leading to the development of new physiological tolerances or to the elimination of some species, or to survival because of a preexisting tolerance. For further discussions on the relation of environmental stress with fish disease see Wedemeyer (1970) and Wedemeyer and co-authors (1976) and Subchapter 1.6. Temperature does not only act via the immune system; there is also a direct effect on the parasites. The review by

Roberts (1975) shows that there is a strong correlation between optimum temperature for multiplication of parasites and outbreak of disease.

It also has to be kept in mind that some environmental factors affect disease agents simultaneously over wide geographical areas, leading to concurrent outbreaks of several apparently unrelated diseases. Sindermann (1963) drew attention to curious synchronizations of outbreaks of several diseases in different marine species, and Möller (1981), on the basis of a thorough examination of many individuals of 10 fish species from Danish and German coastal waters, concluded that there should be a general mechanism, independent of single pathogens, which determines the health status in the population by reducing its general resistance to many infective diseases. Möller considered mainly skeletal deformities, lymphocystis, cauliflower disease, dab papilloma, skin ulcers, fin rot, tumors and gill haemorrhages, and only the loss of one eye in smelt was assumed to be due to a parasite infection, i. e., an infection with larval trematodes *(Diplostomum spathaceum)* acquired in freshwater. Nevertheless, the possibility cannot be excluded that a general factor may lead to concurrent outbreaks of parasitic, including helminth, diseases.

Interesting in this context is Szidat's (1968) suggestion, based mainly on the study of terrestrial and freshwater parasites over a 40 yr period, that mass mortalities due to parasites occur predominantly near the minima of the sunspot curve. Minima occur in 11 yr intervals and are correlated with drought periods. The hypothesis needs verification by other authors, but it represents a 'general mechanism' as postulated above.

It is generally assumed that interactions of parasite, host and environment should lead to benign associations with little disease, if the system is well balanced. Casual observations of many authors seem to support the view that disease due to helminths of marine fishes is rare. For example, Grozdilova (1968) stated that, in general, intestinal helminths are not pathogenic for pink salmon in the White Sea and do not harm them. Möller (1974a) examined 2183 flounder *Platichthys flesus* in the Bay of Kiel and found little evidence for harmful effects by any of their helminths. The same author noticed no harmful effects due to 8 helminth species in 1082 *Zoarces viviparus* (Möller,1975b), and due to 9 helminth species except 1 larval nematode in *Gadus morhua* in the same area (Möller, 1975c). Berland (1980) even suggested that ascaridoid nematodes in fishes, marine birds and seals may be beneficial to their hosts by breaking up large chunks of food thus making them more easily digestible. Osmanov (1959), on the other hand, reported mortalities due to helminths in the Aral Sea (for further examples consult the various sections on helminth groups).

Interpretation of data both for absence and presence of harmful effects is difficult. Failure to observe disease may often be due to the fact that infected fish die rapidly or rapidly become so weakened that they are eaten by predators. Observation of disease outbreaks under *natural* conditions becames more and more difficult because few areas are left which are not affected by human activities. Some epizootics in the sea can be traced directly to human activities (p. 219).

Agents: Turbellaria

The reviews by Jennings (1971, 1974) show that many turbellarians of different groups are commensals or parasites of invertebrates, but very few are associated with fish. *Ichthyophaga subcutanea* lives subcutaneously in the anal and brachial regions of the

teleosts *Bero* and *Hexagramma* (Menitzkii, 1963). It probably feeds on blood. *Micropharynx parasitica* and *M. murmanica* live on the surface of skates, *Raja* spp.

An as yet unidentified turbellarian, probably of the order Eulecithophora and resembling *Ichythyophaga* in some respects, has recently become a problem in marine aquaria (Kent, 1981) (p. 315).

Agents: Monogenea

Biology

A number of authors have provided excellent general accounts of the Monogenea, e.g., Dawes (1956, Monogenea and trematodes of Europe, with emphasis on non-marine forms), Bychowsky (1957, general account of the morphology, biology, development, evolution and taxonomy of Monogenea), and Baer and Euzet (1961, general account). Llewellyn (1963, 1968) reviewed monogenean larvae, and Erasmus (1972) gave a well illustrated review of the biology of trematodes and monogeneans, but most data refer to non-marine forms. A bibliography of Monogenea was published by Hargis and Thoney (1983). Taxonomic synopses of Monogenea are by Sproston (1946, includes detailed host and geographical records) and Yamaguti (1963a). Yamaguti (1968) gave detailed descriptions of Hawaiian Monogenea.

So far, more than 1,100 species of Monogenea have been described, and many new species are being described every year. With few exceptions, Monogenea are ectoparasites of fishes, and frequently of marine fishes. They commonly live on gills, skin or fins, but some invade the rectal cavity and its vicinity in sharks and chimaeras, others the ureters, body cavity and even the blood system. There are 2 groups of Monogenea, the Monopisthocotylea and the Polyopisthocotylea. Species of the first group have a posterior attachment organ (opisthaptor) consisting of a sucker and/or hooks; species of the second group have an opisthaptor bearing complex clamps and sometimes also hooks. There are some detailed studies on how monogeneans attach themselves to their hosts.

The gill-dwelling monoposthicotylean *Diplectanum aequans,* according to Paling (1966) 'gaffes' the 2 pairs of hamuli (hooks) into the gill tissue of the host *Morone labrax* (Fig. 1-65). The squamodiscs, i.e., accessory sucker-like and sclerotinized attachment organs, tend to push the parasite out from between the secondary gill lamellae, and action of the hamuli counteracts this. Kearn (1964) described in detail the functional morphology of the opisthaptor of *Entobdella soleae*, another monopistocotylean. He concluded that attachment is brought about by hydraulic suction pressure generated within the sea-water filled cavity between the cup-shaped haptor and the skin of the fish. The posterior hooked region of each anterior hamulus pierces the epidermis and penetrates the dermis of the fish (Fig. 1-66).

Attachment of a polyopisthocotylean was described by Llewellyn (1957a). *Kuhnia scombri*, on the gills of *Scomber scombrus*, perforates 3 or 4 secondary gill lamellae with its hooks, and each clamp grasps 1 or 2 secondary lamellae (Fig. 1-67). (For further accounts of mechanisms of attachment see Llewellyn, 1956a, 1958.) Adults of many species do not move about (e.g., *Diclidophora denticulata*; Frankland, 1955), whereas others are not permanently attached, but may move around on gills or skin. Locomotion of the skin-dwelling *Leptocotyle minor* on the dogfish *Scyliorhinus canicula* was studied by Kearn (1965). The worm moves in a leech-like fashion, alternately attaching its head and posterior ends.

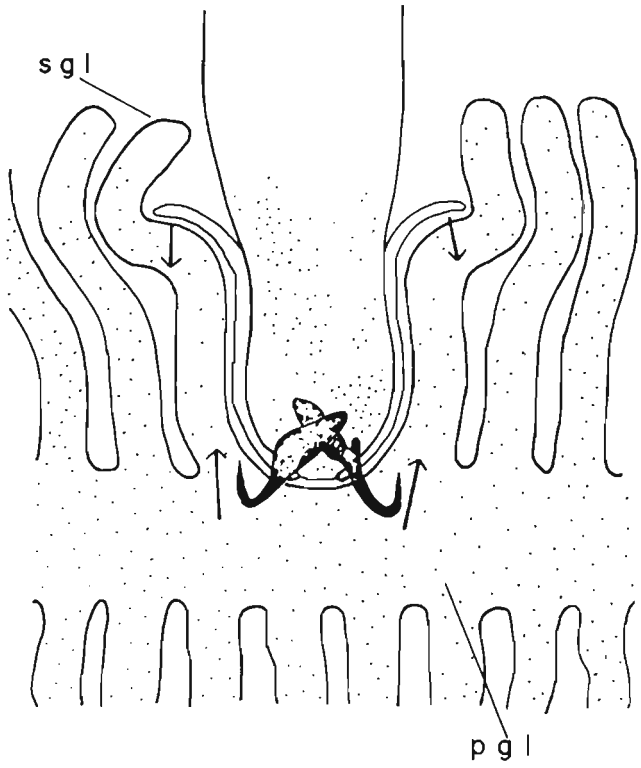


Fig. 1-65: *Diplectanum aequans.* Oblique, longitudinal section of haptor *in situ*, illustrating the complementary functioning of squamodiscs and hamuli. sgl: secondary gill lamellae; pgl: primary gill lamella. (Redrawn after Paling, 1966.)

Generally, Polyopisthocotylea feed on blood and Monopisthocotylea on mucus, epithelial cells, and sometimes also on blood. Thus, Llewellyn (1954), using spectroscopic and histochemical methods, showed that 8 species of polyopisthocotylean Monogenea feed on blood, among them the marine species *Hexabothrium appendiculata, Kuhnia scombri, Anthocotyle merluccii, Axine belones, Diclidophora merlangi* and *D. luscae.* For comparison, the 2 species of Monopisthocotylea, *Leptocotyle minor* and *Acanthocotyle* sp., were also examined. No evidence for blood-feeding was found.

Some Monopisthocotylea have pigments in the gut which resemble blood pigment. This is, for instance, the case in the monocotylid *Dendromonocotyle kuhlii* on the stingray *Amphotistius kuhlii* in eastern Australia. Kearn (1979) showed that it apparently does not feed on blood, its pigments being derived from the host's skin. The same probably applies to 2 other species examined by him.

Although Polyopisthocotylea are blood feeders, they may use additional food sources, as shown by Halton (1978) according to whom *Diclidophora merlangi,* in addition to ingesting blood, may also be able to absorb low-molecular weight organic nutrient directly from the sea water via the tegument.

The feeding process of 2 monopisthocotylean Monogenea, *Entobdella soleae* on the skin of the common sole *Solea solea* and *Acanthocotyle* sp. on the skin of *Raja clavata* was studied by Kearn (1963b). The retracted pharynx (feeding organ) is diagrammatically

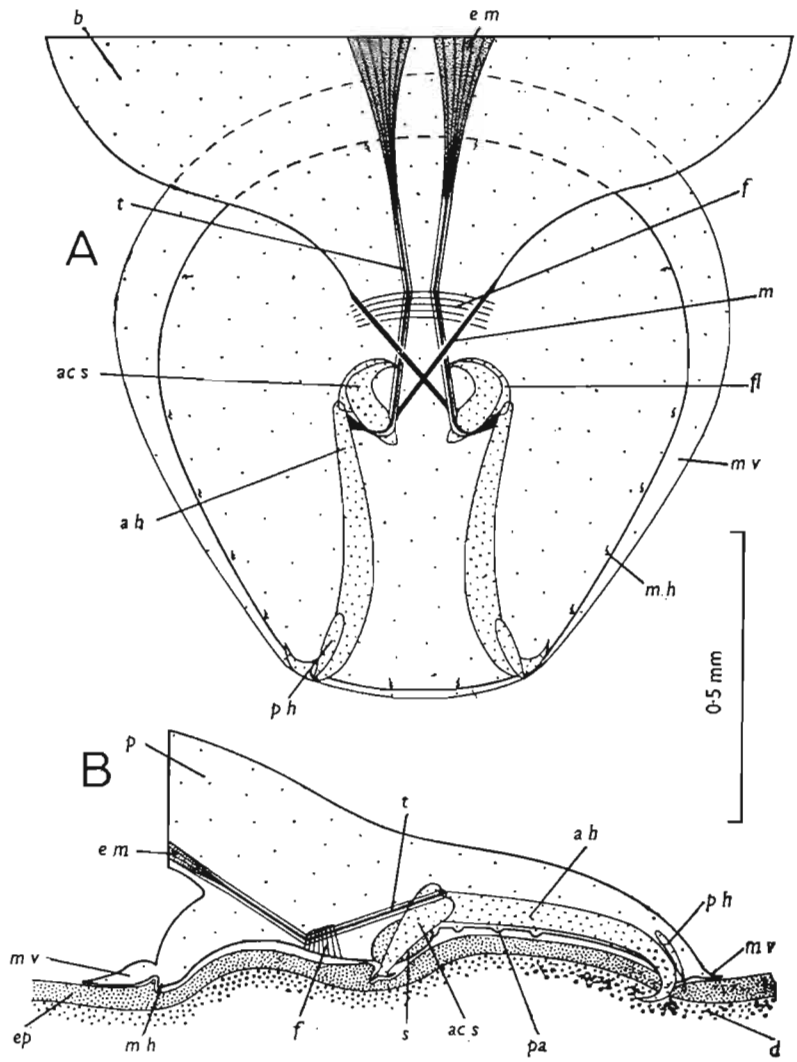


Fig. 1-66: *Entobdella soleae.* Adhesive apparatus. (A) Haptor in dorsal view. (B) Diagrammatic parasagittal section through haptor (sclerites drawn in the positions they occupy when attached to the skin of the host.) acs: accessory sclerite; ah: anterior hamulus; b: posterior body region; d: dermis of fish; em: extrinsic muscle; ep: epidermis of fish; f: fibres attaching tendons to ventral surface on the haptor; fl: flange; m: muscle responsible for orientation of accessory sclerite; mh: marginal hooklet; mv: marginal valve; p: peduncle; pa: papilla; ph: posterior hamulus; s: sheath enclosing accessory sclerite; t: tendon in tendon canal. (After Kearn, 1964.)

represented in Fig. 1-68. The anterior part of the feeding organ can be protruded during feeding. It is applied to the surface of the skin enclosing a circular area (Fig. 1-69). The sites of feeding are usually on the exposed outer surface of the ctenoid scales. Peristaltic contractions of the posterior, non-protrusible part of the feeding organ pump a colourless fluid from the feeding site into the gut. The parasite feeds on epidermis and mucus on the outer surface of the epidermis. The structure of the feeding organs of some other species of the Capsalidae indicates that they feed in a similar way on the hosts' epidermis.

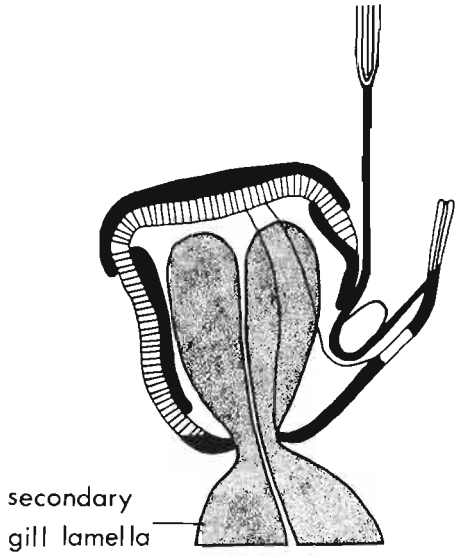


Fig. 1-67: *Kuhnia scombri.* Median vertical section of a clamp to show clamping mechanism. (Redrawn after Llewellyn, 1957a.)

Most Monogenea have a high degree of host and site specificity. Data on host specificity of Monogenea can be found in Bychowsky (1957), Hargis (1957), Llewellyn (1957b), Rohde (1978b, 1980c, 1982), Lawler (1981), etc. Tripathi's (1959) study of Monogenea from Indian fishes shows that of 105 species recorded by 1957, 1 species was found on 4 host species, 3 on 3 host species, 6 on 2 host species and 95 on a single host species. Of 435 species of marine Monogenea from various seas, 340 (78 %) were found on a single host species, 388 (89 %) on species of 1 genus, 420 (96 %) on species of 1 family, and 429 (98 %) on species of 1 order (Rohde, 1978b). Kearn (1967b) examined several hundred pleuronectids and elasmobranchs, but many hundred *Entobdella soleae* were found only on sole *Solea solea,* except for 2 specimens which apparently were transferred accidentally during trawling. Host specificity may be due to ecological barriers which may break down under artificial conditions. Thus, in the New York Aquarium, many fish species of 17 families were found to be susceptible to *Neobenedenia melleni* (reported as *Epibdella melleni*) which in nature is much more host specific. Nevertheless, many fish species of 18 families kept in the same aquarium did not become infected, and none of the elasmobranchs acquired the parasite (Jahn and Kuhn, 1932), indicating that other factors beside ecological barriers determine host specificity. According to Kearn (1967b), 1 such factor is the behaviour of the oncomiracidium larva. Larvae of *Entobdella solea* attach themselves to the skin of the normal host, *Solea solea*, in preference to that of other fishes (Kearn, 1967b). Larvae of some species were shown to hatch only when stimulated by a specific host factor (pp. 217–218).

Strict site specificity was documented by many authors, e.g., Llewellyn (1956b), Kearn (1971a); Rohde (1976a, 1977a, b, c, 1980a, 1982), Roubal (1981), Roubal and co-authors (1983). For example, certain species occur only on the pleurobranchs, others only at the base of the gill filaments, still others only on the fins. Young stages may have site

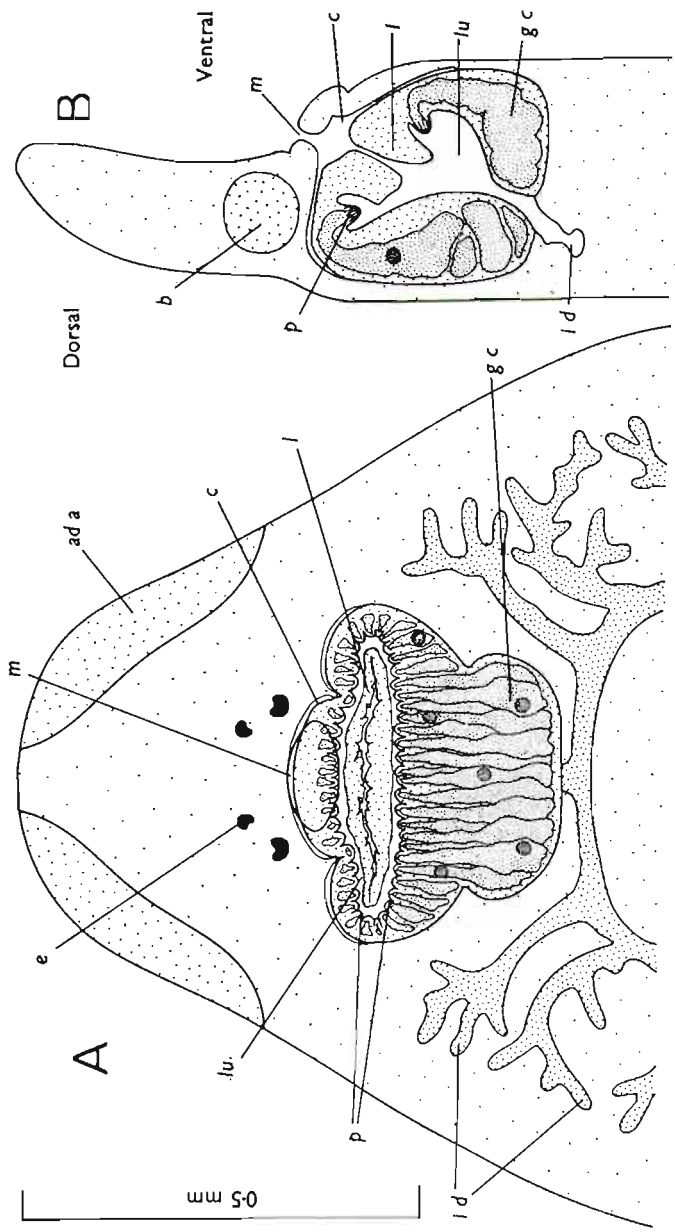


Fig. 1-68: *Entobdella soleae*. Morphology of feeding apparatus. (A) Retracted feeding organ, dorsal or ventral view. (B) Median sagittal section through retracted feeding organ. ad a: anterior adhesive area; b: 'brain'; c: cavity accommodating feeding organ; e: eye; gc: gland cell; id: intestinal diverticulum; l: lip, tucked inside lumen of feeding organ; lu: lumen of feeding organ; m: mouth; p: papilla. (After Kearn, 1963b.)

preferences different from older stages, and there may be constraints on the number of worms that can be accommodated in a microhabitat. Thus, according to Kearn (1978), young *Horricauda rhinobatidis* are found between the secondary gill lamellae of *Rhinobatos batillum* in Queensland, Australia. Before reaching sexual maturity, the worms establish themselves in the septal canals. With rare exceptions, only a single worm is found in each septal canal. In 1 host specimen 572 out of 675 canals contained a single adult parasite each, and only twice were 2 adults found in the same canal.

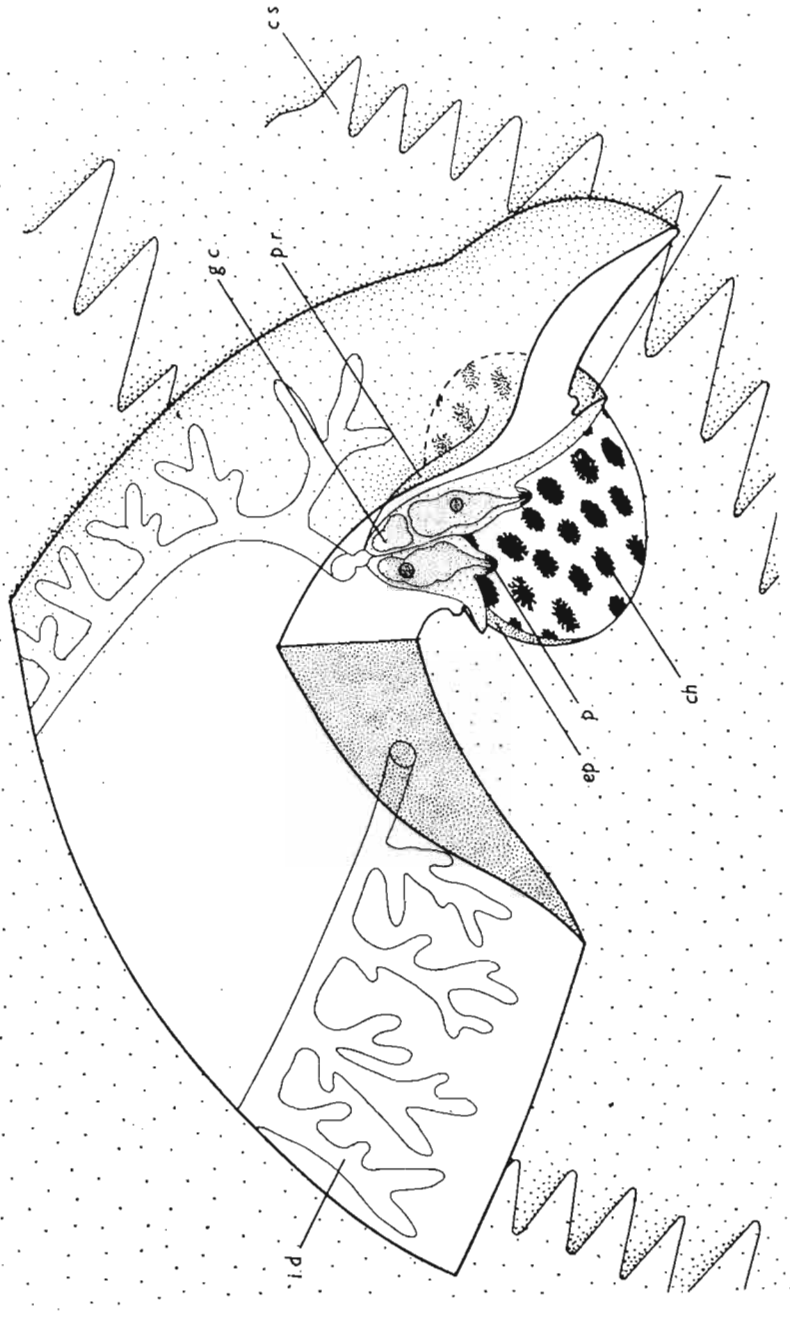


Fig. 1-69: *Entobdella soleae*. Diagrammatic reconstruction of anterior region of a parasite in the act of feeding. A sector of the head region of the parasite has been cut away to display the anatomy of the feeding organ. ch: chromatophore in dermis exposed by erosion of overlying epidermis; cs: ctenoid scale; ep: epidermis; gc: gland cell; id: intestinal diverticulum; l: lip of feeding organ; p: papilla; pr: posterior region of feeding organ. (After Kearn, 1963b.)

There may even be preference for one sex of the host. Williams (1965) did not find any *Calicotyle kroyeri* in female *Raja radiata* which contained eggs in the uteri.

Some species of Monogenea are restricted to certain macrohabitats. An example was given by Rohde (1976c): Whereas 4 species of Monogenea in *Scomberomorus commerson*

have a wide geographical distribution, 1 was found only in a small area of the Great Barrier Reef. Monogenea are generally lost when migratory fish move from a marine to a freshwater environment. Thus, in Tripathi's (1959) study of Indian Monogenea, only the species *Paramazocraes phasae* on *Setipinna phasa* was found both in brackish and freshwater areas of Chilka Lake and the river Ganges respectively.

Seasonality in egg production was demonstrated by Shotter (1972) for the monogenean *Diclidophora merlangi* on whiting *Merlangius merlangus* in the Irish Sea. The proportion of worms with eggs in the uterus was directly related to temperature. However, under experimental conditions egg production was greatest at 6 °C, and the conclusion that at higher temperatures egg production is increased could not be drawn.

The Monogenea have a direct life cycle. A larva (oncomiracidium) hatches from an egg and gradually transforms to the adult; normally no intermediate host is used. Only for some species has it been suggested that an 'intermediate' host may be involved in the life cycle. Bychowsky and Nagibina (1967; see also Popova and Gitchenok, 1978) provided circumstantial evidence for this. Young *Pricea* and *Gotocotyla* were never found on the final hosts, large pelagic predatory fishes, however 29 species of small fish contained young stages but no adults of the parasites throughout the year. The intermediate hosts are apparently eaten by the larger final ones whose gills they infect.

One of the best known life cycles is that of *Entobdella soleae* (Kearn, 1963a, 1967b, 1971b, 1973, 1974b, 1980; Kearn and MacDonald, 1976). The host is the sole *Solea solea* which is buried in sediment for some time each day and rarely moves more than a few centimeters from the sea bottom. The monogeneans lay eggs while on the lower surface of the fish. Eggs are attached to sand grains by an egg stalk with sticky droplets (Kearn, 1963a). Free-swimming oncomiracidia hatch and infect the anterior part of the upper surface of fish, the only part exposed to the larvae when the fish is buried. After a short period the worms migrate to the lower surface. For other life cycles of marine Monogenea see Jahn and Kuhn (1932), Frankland (1955) and Llewellyn (1962) (Fig. 1-70).

Oncomiracidia of *Benedenia seriolae* survive about 1 day without a host. Maturity is reached in 18 days at 21.8 to 26 °C, and in about 1½ months at 17 to 18 °C. Eggs do not hatch below 9 °C and above 30 °C (Hoshina, 1968). Free larvae of *Diclidophora denticulata* also survive for about 24 h, and the adult stage is reached within 6 months (Frankland, 1955), and according to Kearn (1967b), oncomiracidia of *Entobdella soleae* survive for about 24 h as well. In *Entobdella hippoglossi*, hatching takes 25 days at 12 to 13 °C and 46 days when kept at 4 to 5 °C for 3 to 5 weeks and subsequently at 7 °C. Free oncomiracidia live for more than 24 h (Kearn, 1974a). Survival time of hatched larvae of *Neobenedenia melleni* appears to be shorter. Jahn and Kuhn (1932) observed that they become exhausted and lose their cilia within 6 h, if they do not find a suitable host. According to MacDonald (1975), oncomiracidia of *Diclidophora merlangi* swam actively for 12 to 15 h at 13 °C. At 18 °C they swam actively for a minimum of 9 h and died within 30 h after hatching. The life span of free larvae of *Gastrocotyle trachuri* is less than 24 h (Llewellyn, 1964).

Some oncomiracidia in the egg may stay alive for long periods. Thus, MacDonald (1974) found that oncomiracidia of *Acanthocotyle lobianchi* at 13 °C remained alive for up to 83 days in the egg, although they apparently had fully developed at 15 days.

Seasonality of many infections with Monogenea indicates that the life span of the adults of many species is less than 1 yr. For example, according to Llewellyn (1962, 1964),

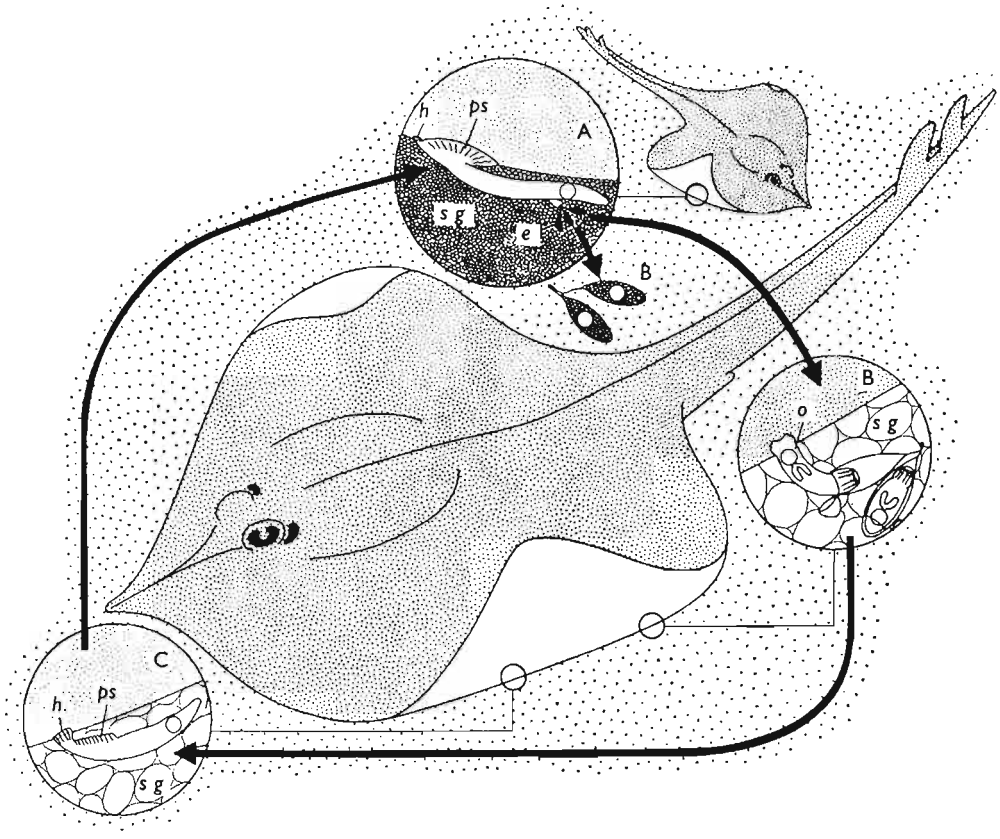


Fig. 1-70: *Acanthocotyle lobianchi.* Life-cycle. (A) Adult parasite on lower surface of host. (B) Eggs attached to sand grains; larvae have no cilia and are picked up when a ray settles on top of them. (C) Pseudohaptor (ps) developing. e: egg; h: haptor; o: oncomiracidium; sg: sand grains. (After Kearn, 1967a.)

Gastrocotyle trachuri and *Pseudaxine trachuri* on *Trachurus trachurus* normally do not live longer than 1 yr.

Oncomiracidia swim slowly compared with their fish hosts. Thus, Kearn (1967b) measured the swimming speed of larval *Entobdella soleae* as 5 mm s^{-1}, and Magnan (1930) found that the cruising speed averaged over hours of several marine fish species was 3.2 body lengths s^{-1}. Over short periods, fish may reach much greater speeds (e.g., Bainbridge, 1960). To ensure infection of the hosts in spite of this discrepancy in speed, monogeneans have a number of 'ingenious' infection mechanisms. Llewellyn (1968, 1972) reviewed the behaviour of larval monogeneans with special reference to host infection, but at the time of the reviews little was known and most of his examples are freshwater species. Kearn (1981) gave a brief but up-to-date review (further references therein).

For *Entobdella soleae* Kearn (1967b) demonstrated that a substance secreted by the skin of sole released a response in the larva, and experiments by Kearn and MacDonald (1976) showed that dilute solutions of urea or ammonium chloride in seawater stimulated hatching of the larva. The factor responsible under natural conditions is probably urea,

since the skin mucus of the host contains sufficient urea but insufficient ammonia to stimulate hatching. The host factor is non-specific, as urea from other fish species also induces hatching. The hatching induced by urea is superimposed on an endogenous hatching rhythm, which leads to hatching during the first hours of illumination (Kearn, 1973). This enhances finding of the host which is active during the night and rests on the bottom during the day. Kearn (1980) made thorough studies of light and gravity responses of the larva of *Entobdella soleae*. Oncomiracidia freshly hatched without chemical stimulations are positively phototactic, swimming to the top of a container and swimming there actively. Occasionally, larvae swim to the bottom of the container, a behaviour that becomes more frequent in older larvae. Alternating positive and negative phototaxis, together with horizontal passive transport by currents, leads to a pattern of searching for the host. When urea is used to stimulate the eggs and to bring about hatching, large numbers of larvae hatch, many being negatively phototactic during 30 min after stimulation. This enhances contact with bottom-living fish. In the dark, urea-stimulated larvae are negatively geotactic which also enhances host contact.

In the related species *Entobdella hippoglossi,* a skin parasite of the halibut *Hippoglossus hippoglossus,* hatching occurs mainly at the end of the first 2 h of the period of darkness (Kearn, 1974a). This again may be an adaptation to host-finding. The host may rest at night, although little is known about its behaviour. In another related species, *E. diadema* on the stingray *Dasyatis pastinaca,* rapid hatching can be induced by a reduction in light intensity (Kearn, 1982). In darkness, freshly hatched larvae are negatively geotactic. The host is a shallow-water fish and its environment is well illuminated. A resting or slowly swimming fish would darken the eggs, induce hatching and thus infection. Negative geotaxis would guide the larvae to the fish.

There may even be differences in the behaviour of different populations belonging to one species of Monogenea, apparently an adaptation to differences in behaviour patterns of different host populations. One population of *Diclidophora merlangi,* according to MacDonald (1975), hatched mainly during the first 4 to 6 h of illumination, the second population hatched mainly during the 2 h period before light. The difference may be related to differences in the behaviour of the host, the whiting *Merlangius merlangus,* which undergoes vertical migrations under experimental conditions in relation to light. It is possible that different stocks of fish show different behaviour and thus expose themselves to infection at different times of the light-dark cycle. *D. luscae* hatched over 'dusk' and *D. denticulata* after the light was switched off. Hosts of the 2 species are the pouting *Trisopterus luscus* and the coalfish *Pollachius virens,* respectively. Pouting is dispersed over the sea bottom at night (although it remains on the bottom during daytime forming tight shoals). Coalfish of the size used for obtaining parasites, descend to the sea bottom only at night. Hence, the hatching behaviour of both monogenean species may lead to an enhancement of infecting the fish. In neither of the first 2 species did mechanical disturbance or proximity of host tissues or muscles bring about hatching (MacDonald, 1975).

Dictyocotyle coeliaca has neither an endogenous hatching rhythm nor does skin mucus or body fluid of the host induce hatching (Kearn, 1975). This may indicate that the host, *Raja naevus,* has no well-defined, rhythmical, daily activity pattern.

The oncomiracidium of the acanthocotylid *Acanthocotyle lobianchi* is not ciliated and infection of the ray *Raja montagui* occurs when the host settles on them (Kearn, 1967a)

(Fig. 1-70). Hatching could be triggered by mucus from *Raja* (MacDonald, 1974), but not by mucus from *Solea*, that is the hatching factor is host specific (Kearn and MacDonald, 1976).

For *Gastrocotyle trachuri* and *Pseudaxine trachuri* on *Trachurus trachurus*, Llewellyn (1962, 1964) demonstrated a long-term seasonal pattern which enhances infecting the fish. Fish become infected when they descend to the sea bottom in October. Apparently as an adaptation to such seasonal migration pattern the parasites cease to produce larvae 'in anticipation' of the host's summer disappearance (Llewellyn, 1962).

Effects on Hosts

Gill-monogenea

Fernando and Hanek (1976) gave a brief review of the gills as a habitat for parasites. My own observations have shown that many species of gill monogeneans cause little or no obvious harm. Frankland (1955) did not observe any apparent harm caused by *Diclidophora denticulata* to its host, *Gadus virens*. Several authors, however, have reported damage due to gill-dwelling monogeneans.

Best known, because of its economic importance, are effects of *Nitzschia sturionis* on the spiny sturgeon *Acipenser nudiventris* in the Aral Sea (e.g., Lutta, 1941a; Petrushevsky and Shulman, 1961). Dogiel and Lutta (1937) reported that fish were dying in large numbers, due to the large (up to 2 cm long) gill-dwelling monogenean. In saltwater areas infection was 100 %, in areas with slightly reduced salinity almost 100 %. Maximum intensity of infection was 600. Worms did not only cover the gills, but had spread to the lips and into the mouth. Some worms even crept into the gut, where they remained active for some time. Fish with many worms had a reduced fat content, and there was a general emaciation of the body, as well as a jaundice with yellowish-green colouration of the organs adjacent to the gall bladder, and wrinkling of the liver. No other parasites were present, nor was there any sign of bacterial disease. *Nitzschia sturionis* was not found during a previous examination in 1930, and local fishermen had not seen the parasite previously. It was apparently introduced into the Aral Sea from the Caspian Sea. In 1933, 350 000 larvae and in 1934, 7 million larvae of Caspian stellate sturgeon were introduced into the Aral Sea. In addition, 90 spawners were introduced in 1934 without previous inspection for fish disease, and *Nitzschia* was known to occur in the Caspian Sea (Dogiel and Lutta, 1937). According to Osmanov (1959), mortalities due to the parasite in the Aral Sea occurred in 1936, and sturgeon fisheries attained no commercial significance for the next 20 years.

Lutta (1941b) made a detailed study of the effects of *Nitzschia sturionis* on *Acipenser nudiventris*. Its opisthaptor causes a mechanical irritation of the gill tissue. This and supposed action by toxic substances secreted by the worm, leads to an inflammation of the gills. Histological examination revealed mechanical destruction of the gill tissue, hyperplasia of the epithelium of the primary gill lamellae and to a lesser degree hypertrophy of the connective tissue, atrophy of the gill capillaries, and atrophy of the secondary gill lamellae. Morphological changes made gas exchange apparently partially or completely impossible, leading to acute disease and mass mortality.

Bauer and co-authors (1977), without giving details, mention *Gyrodactylus* spp. and *Discocotyle sagittata* as also infecting the gills of sturgeon. Infections with the latter species

are said to be rare, but in heavy infection the gills are pale and covered by mucus and haemorrhagic ulcers.

Eto and co-authors (1976) examined the effect on yellowtail *Seriola quinqueradiata* of the gill monogenean *Axine heterocerca*. Fish of 89 g body weight were fed for about 2 months. Eight abnormal fish with an average body weight of 177 g and with natural infections of *Axine*, and 10 normal fish with an average body weight of 511 g (and presumably without parasites) were selected for analysis. Parasitized fish showed sluggish movements, loss of appetite, poor growth, darkish skin and 'colourlessness' of muscles, gills, kidney and liver. Parasitized fish furthermore had a lower erythrocyte count (3.0 ± 1.1 vs. $4.7 \pm 0.4 \times 10^6$ mm^{-3}), lower haematocrit values (24.2 ± 12.0 vs. 50.7 ± 4.4 %), a reduced haemoglobin content (5.7 ± 3.0 vs. 13.8 ± 1.0 g dl^{-1}), and lower mean corpuscular constants. All these differences were statistically significant, but the significance level was not given. The mean corpuscular diameter was 9.12 μm in infected and 10.23 μm in uninfected fish. On the basis of the findings, the anemia was classified as a micro-cytotic-hypochromenia-type. The Price-Jones curve (frequency of erythrocyte diameters) of parasitized and normal fish showed that the parasites caused an anisocytosis. Contents of serum protein etc. were also lower in infected fish (Table 1-18),

Table 1-18
Chemical components of blood serum from parasitized and normal fish (After Eto and co-authors, 1976)

Component	Parasitized	Normal
Total protein (g dl^{-1})	2.96	4.83
Total cholesterol (mg dl^{-1})	183	636
Total bilirubin (mg dl^{-1})	0.29	0.41
BUN (mg dl^{-1})	16.4	12.1
Creatinine (mg dl^{-1})	1.31	0.89
ZTT (KU)[1]	0.50	0.80
Ca (mg dl^{-1})	12.4	15.4
Inorganic P (mg dl^{-1})	9.9	11.6
Ca/P ratio	1.1	1.6
LDH (WU)[2]	115	325
GOT (KU)[3]	96.5	88.8
GPT (KU)[3]	13.3	41.0
GOT/GPT ratio	7.3	2.2
LAP (G-RU)[4]	130	121
AIP (K-AU)[5]	2.75	3.20

[1] Kunkel Unit; [2] Wroblewski Unit; [3] Karmen Unit; [4] Goldbarg-Rutenburg Unit; [5] King-Armstrong Unit

and there were lower activities of serum enzymes and ZTT values. Contents of urea-N and creatinine as well as activities of GOT and LAP in the serum of infected fish were higher. The condition factor — body weight (g) $\times$ 100/(fork length [cm])^3 — was 1.23 ± 0.12 vs. 1.80 ± 0.10 g, and the percentage weights of liver and spleen were reduced significantly in infected fish. Percentage weights of heart and intestine were not reduced. With regard to the chemical composition of muscle, liver and vertebrae, the analysis showed that the lipid

and glycogen contents in the muscles and liver were reduced, but moisture and protein contents were increased. Unfortunately, the authors gave neither infection intensities nor information about possible other parasites present. It was not even stated whether 'normal' fish were entirely without the monogeneans.

Oliver (1977) described severe effects due to *Diplectanum aequans* on the serranid fish *Dicentrarchus labrax,* using histological methods and the scanning microscope. He observed hyperplasia of gills and haemorrhages where hooks of the parasites are inserted (Figs 1-71 and 1-72).

Roubal (pers. comm.) examined pathological effects of 2 species of Monogenea on the gills of *Acanthopagrus australis* in eastern Australia. *Lamellodiscus acanthopagri* does little damage. Reactions are restricted to surface effects caused by hooks and squamodiscs (Fig. 1-73). In some cases, there was slight swelling between secondary gill lamellae, but usually there were no marked tissue reactions. Upon infection with *Haliotrema spariensis,* the gill filament carrying the worm is fused with the adjacent filament (Fig. 1-74). Furthermore, there is a heavy hyperplasia between the secondary gill lamellae with hyperplastic and hypertrophic cells filling the spaces between the lamellae (Fig. 1-74). The opisthaptor of the worm is deeply embedded in hyperplastic tissue which has numerous haemorrhages and infiltrated cells. There is a slight oedoma with vasodilation (Fig. 1-75).

It is possible that pathogenic effects of 1 parasite species may be aggravated if others are present. Thus, Noble (1963) found that the ciliate *Trichodina* and the monogenean *Gyrodactylus* on the gills of *Gillichthys mirabilis* were positively associated, i.e., intensity of infection with one species was greater if the other was present. Noble suggested that the ciliate may feed on host tissue damaged by the monogenean. However, no direct evidence for this assumption was given. McVicar and MacKenzie (1977) also mentioned briefly, without any supporting evidence, that concurrent infection of *Trichodina* and *Gyrodactylus* had more serious pathological effects on plaice than single infections with either of the species.

Typical symptoms of double infections were in grey mucous-covered patches on the skin, leading to open lesions and secondary infections with bacteria and free-living or saprophytic protozoans.

Pathological effects due to gill Monogenea may also depend on water quality. Thus, Skinner (1982) studied infections with the monogeneans *Neodiplectanum wenningeri, Ancyrocephalus* sp. and *Ancyrocephalus parvus* on the fish *Gerres cinereus, Lutjanus griseus* and *Strongylura timucu* respectively and the associated gill pathology. Fish were from 2 habitats in South Biscayne Bay, Florida (USA), one heavily polluted with high amounts of ammonia, trace metals, and pesticides, and the other not polluted. Only fish from the polluted habitat were heavily infected and showed marked pathological effects (Table 1-19). *Neodiplectanum wenningeri,* in light infections, caused mechanical damage by deflecting gill lamellae. In heavy infections, the lamellae were covered with the monogeneans, and there was increase in mucus production and clubbing of filaments where parasites were attached. Similarly, heavy infection with the 2 species of *Ancyrocephalus* caused pathological changes at the site of attachment including epithelial hyperplasia, heavy mucus production, and loss of respiratory epithelium in some instances. Often the side of the filament opposite the worm attachment was also affected. Other effects were deflection and adherence of lamellae, and in severe cases clubbing of filaments and obliteration of normal filament structure. The affected filaments appeared white and

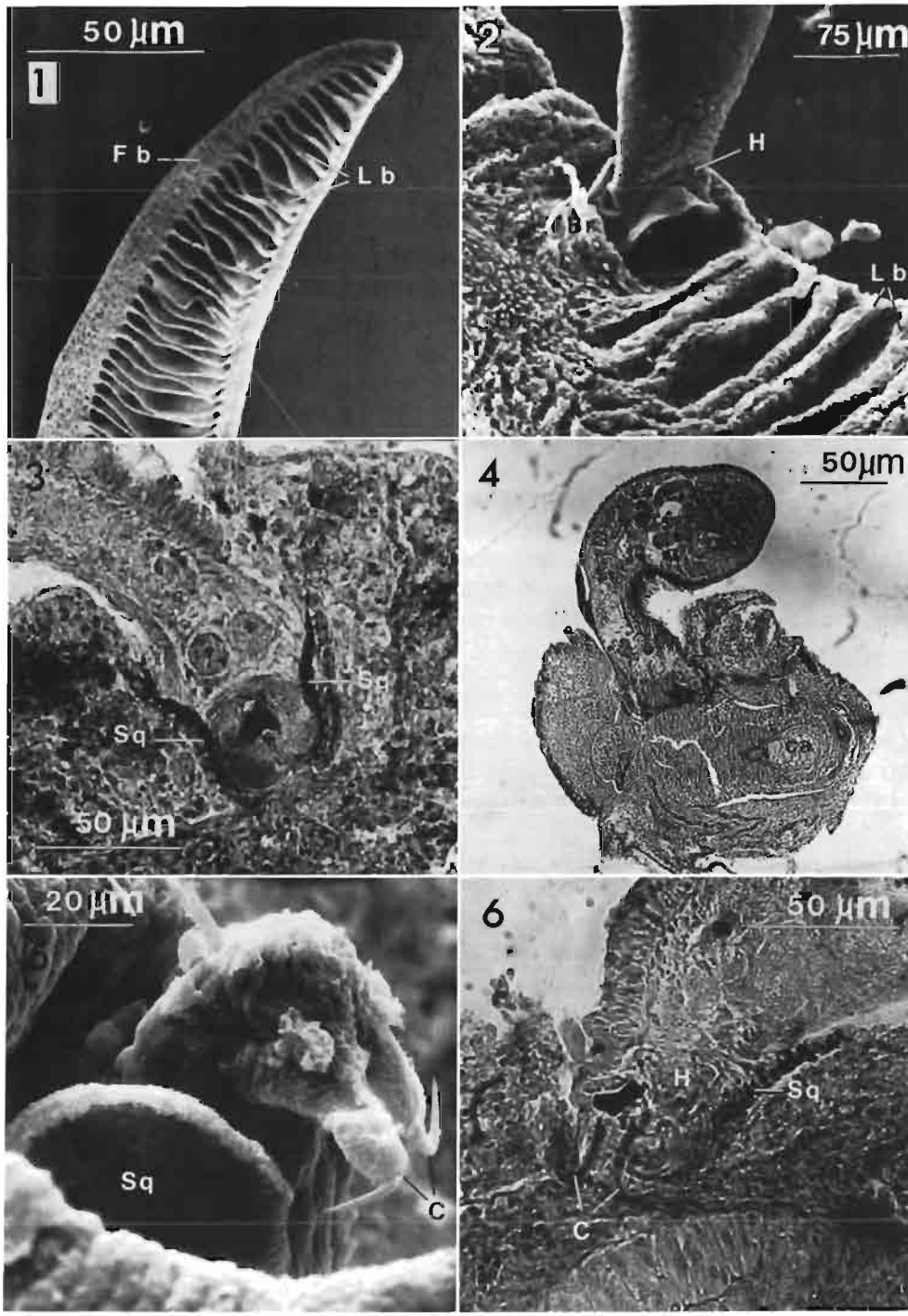


Fig. 1-71: 1. *Dicentrarchus labrax* (L., 1758); gill filament showing gill lamellae. 2: Attachment of *Diplectanum aequans* (Wagener, 1857) Diesing, 1858, with its haptor between 2 adjacent gill lamellae; note beginning of the formation of a tissue fold. 3: Tissue section showing the attachment of squamodiscs to the opposed surfaces of 2 adjacent gill lamellae. 4: Attachment of *Diplectanum aequans* to the gill filament of *Dicentrarchus labrax*. 5: Hamuli of *D. aequans;* note their 'opposed' position. 6: Insertion of the hooks of *D. aequans* into tissue of the gill filament. Br, tissue fold; C, hamulus; ca, cartilage; H, haptor; h, red blood cells; Hy, hyperplasia; Fb, gill filament; Lb, secondary gill lamella; Sq, squamodisc. (After Oliver, 1977.)

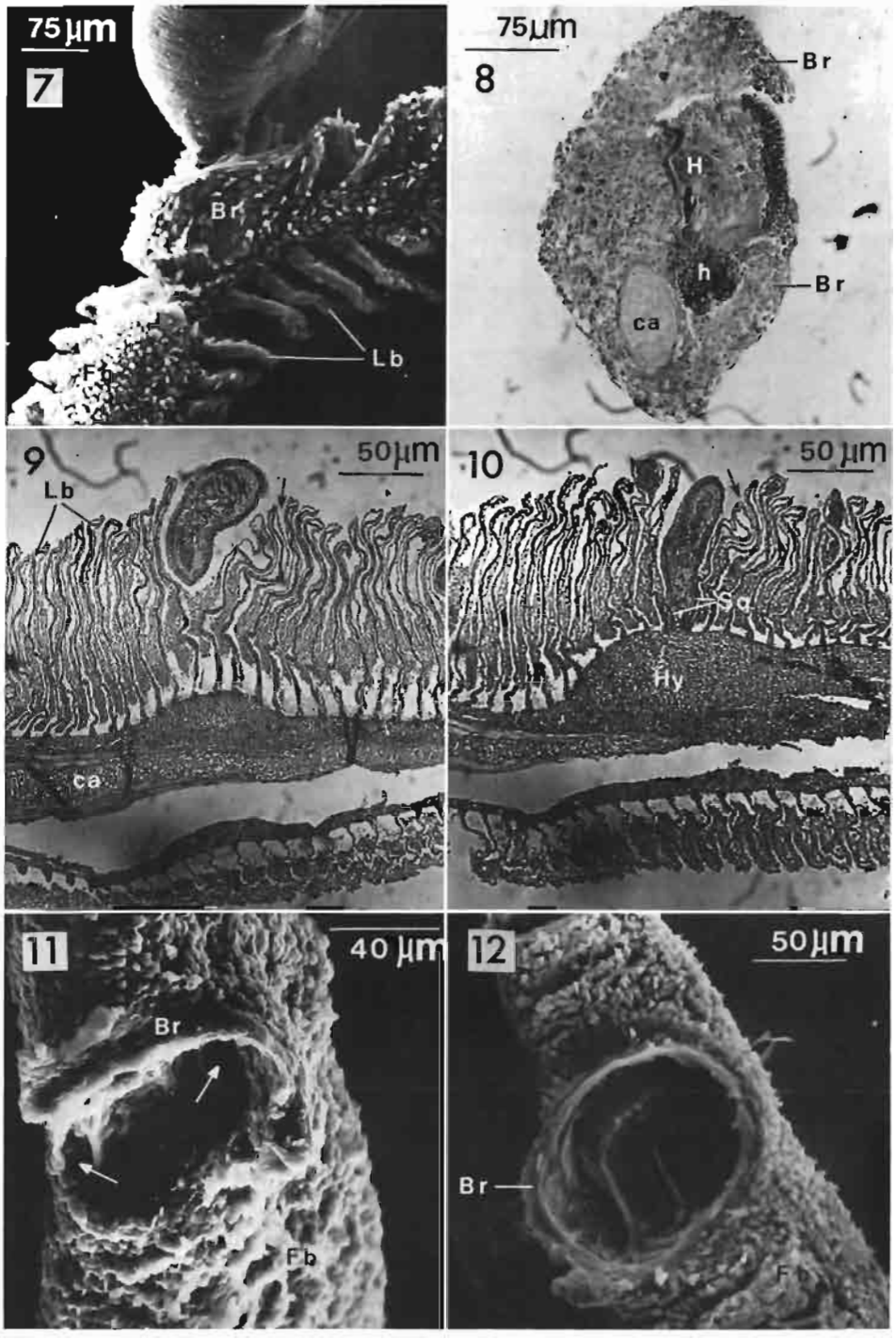


Fig. 1-72: 7: Tissue pad around the haptor of *Diplectanum aequans*. 8: Transverse section of a gill filament of *Dicentrarchus labrax* showing enclosure of the parasite by a tissue fold and a mass of red blood cells turning into a haemorrhage at the level of the large hamuli. 9 and 10: Hyperplasia of basal tissue of a gill filament at the level of attachment of *D. aequans*. 11 and 12: Scar on a gill filament left by a parasite. Arrows: penetration of the large hamuli. For symbols, see Fig. 1–71. (After Oliver, 1977.)

Table 1-19
Averages of some nutrients, trace metals, and pesticides in water samples, and Monogenea and gill pathology of the 3 host species in southeast Biscayne Bay and southwest Biscayne Bay at Black Creek Canal from May 1975 to August 1976 (After Skinner, 1982)

Component	Southeast Biscayne Bay				Southwest Biscayne Bay at Black Creek Canal			
	Min.	Avg.	Max.	No. of Samples	Min.	Avg.	Max.	No. of Samples
Total ammonia nitrogen (mg l^{-1})	0.00	0.00	0.012	6	0.03	0.45	1.0	11
Arsenic ($\mu g\ l^{-1}$)	0.00	0.00	0.00	6	2.0	2.0	2.0	3
Lead ($\mu g\ l^{-1}$)	0.00	0.00	0.00	6	4.0	12.5	20.0	4
Manganese ($\mu g\ l^{-1}$)	0.00	0.00	0.00	6	4.0	6.0	20.0	4
Mercury ($\mu g\ l^{-1}$)	0.00	0.00	0.00	6	0.2	0.28	0.5	4
Diazionon ($\mu g\ l^{-1}$)	0.00	0.00	0.00	6	0.02	0.026	0.06	3
2,4-D ($\mu g\ l^{-1}$)	0.00	0.00	0.00	6	0.00	0.09	0.27	3
Silvex ($\mu g\ l^{-1}$)	0.00	0.00	0.00	6	0.00	0.04	0.10	3
Parathion ($\mu g\ l^{-1}$)	0.00	0.00	0.00	6	0.00	0.01	0.02	2
Neodiplectanum wenningeri no per gill arch	0.00	0.625	5	69	25	72.5	> 100	52
Ancyrocephalus sp. no. per gill arch	0.1	1.4	8	57	69	124.75	> 500	80
Ancyrocephalus parvus no. per gill arch	0.3	2.25	4.5	44	61	89.25	> 200	54
Pathological changes*	None	None	Slight	170	Moderate	Severe	Severe	186

* Slight: mucus production above normal; moderate: heavy mucus production and epithelial hyperplasia; severe: fusion of lamellae, loss of structure

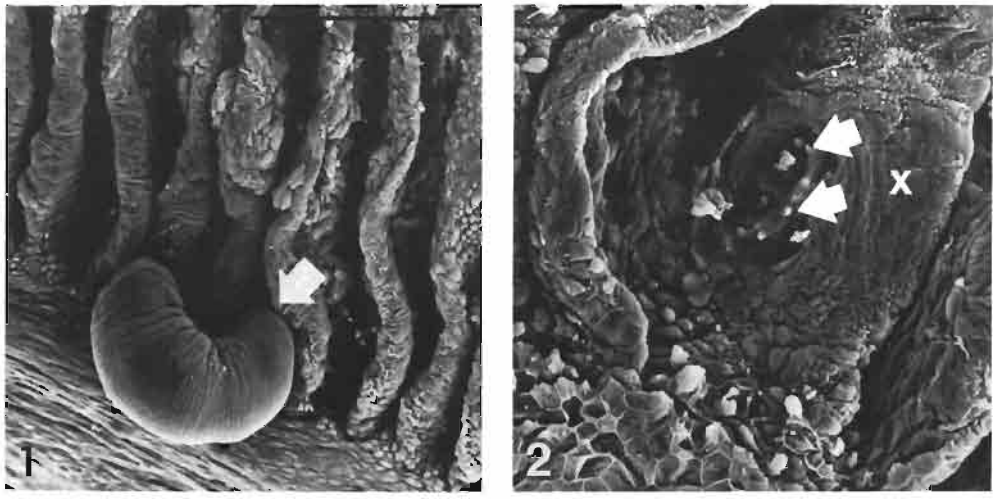


Fig. 1-73: *Lamellodiscus acanthopagri* on *Acanthopagrus australis*. 1: Worm attached between secondary gill lamellae; arrow: posterior, attached end; note: no marked changes around site of attachment; scale 100 µm. 2: Scar left at attachment site after removal of worm; note: papilla-like protrusions left by larval hooklets of worm (arrows), and smoother appearance of area to which squamodiscs had been attached (cross); scale 10 µm. (Original provided by F. R. Roubal.)

the gills were congested with mucus. Few fish from the non-polluted habitat had increased mucus and only fish from the polluted habitat had increased mucus production and moderate to severe pathological effects. Mucus-producing cells were concentrated, sometimes in several layers, at the tips of the gill filaments. Fusion of gill lamellae along entire filaments, epithelial hyperplasia, clubbing of lamellae or obliteration of lamellar structure, aneurisms, and clubbing of filaments occurred frequently, along with proliferation of cells at the bases of lamellae.

Skin-monogenea

The body surface of fishes as a habitat for parasites was discussed by Kearn (1976). Skin-dwelling monogeneans appear to move around frequently and the wounds produced by them heal rapidly, due to the great regenerative power of the fish skin. Kearn (1963a) observed that *Entobdella soleae* frequently changes its site on the sole *Solea solea* and thus may inflict many feeding wounds. However, the epidermis is apparently replaced so rapidly that feeding wounds were rarely seen on unpopulated parts of the fish. Kearn (1971b), reviewing his work on *Entobdella soleae*, stated that the depth of the feeding wounds varies. In some, epidermis is left at the bottom of the wound. Wounds may be shallower because the parasite did not digest its way through the surface more deeply, or because regeneration has begun. According to Kearn (1965), *Leptocotyle minor,* a skin parasite of the dogfish *Scyliorhinus canicula,* erodes the epidermis but leaves the dermis undamaged.

In spite of the relatively minor effects by many skin Monogenea, some authors reported harmful effects by some species. Jahn and Kuhn (1932) found that *Neobenedenia melleni* (reported as *Epibdella melleni*) does considerable damage to host fish, and death often results. In mild infections the cornea of the eye is attacked and if the infection is not

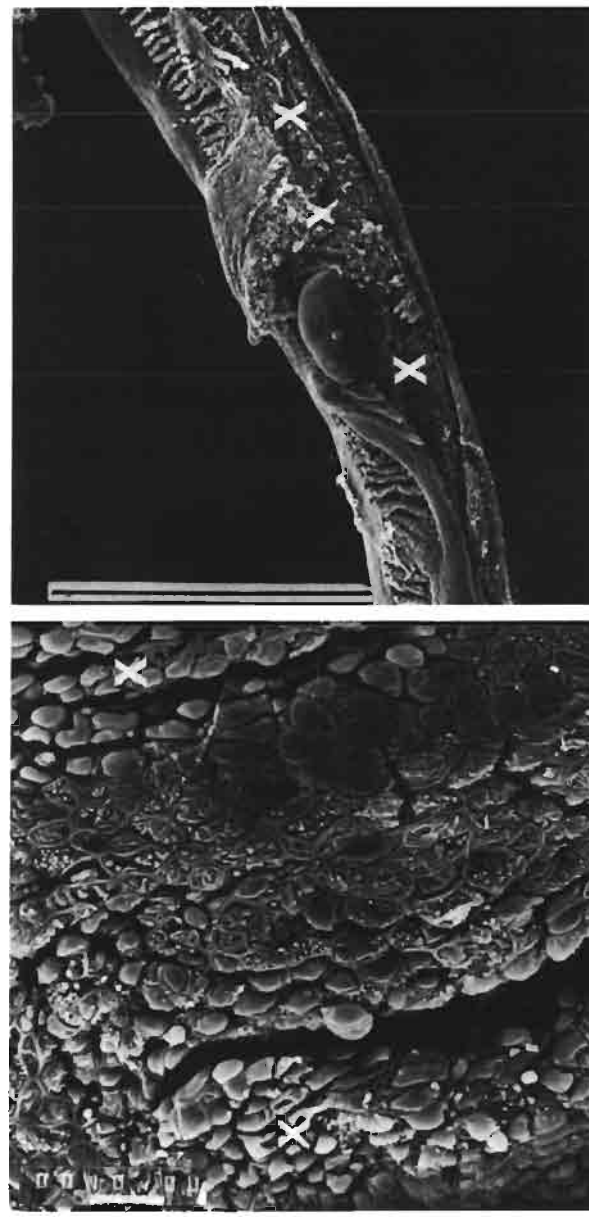


Fig. 1-74: *Haliotrema spariensis* on *Acanthopagrus australis*. Top: Worm attached to gill filament; note: destruction of filament surface along original area of fusion to adjacent filament (crosses); scale 1 mm. Bottom: Enlarged area of top; note: hypertrophic epithelial cells between normal secondary lamellae (X) filling interlamellar space; scale 10 µm. (Original provided by F. R. Roubal.)

treated, the eye is destroyed, probably due to the combined effect of the monogenean and secondary bacterial invaders. In very heavy infections the epidermis becomes so severely injured that the scales fall off, leading to exposure of large areas of connective and muscular tissues and subsequent death (see also Kearn, 1963b).

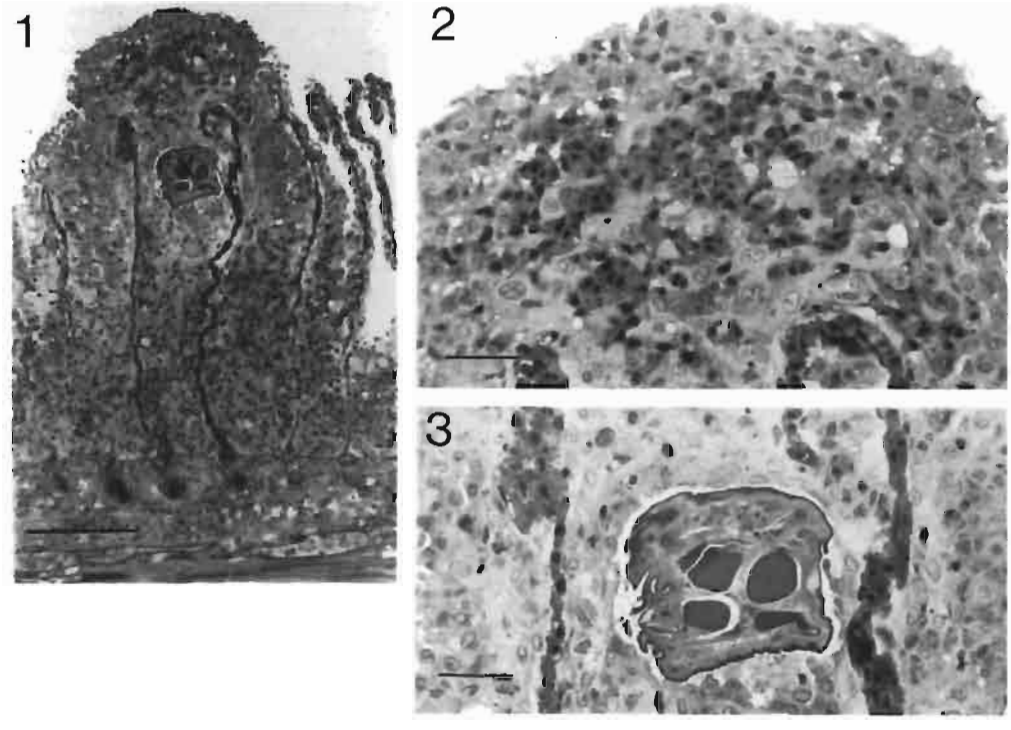


Fig. 1-75: *Haliotrema spariensis*. 1: Section through opisthaptor (X) of worm deeply embedded in hyperplastic epithelium of several adjacent secondary gill lamellae; note: normal lamellae only at right of section; scale 100 µm. 2: Surface of (1) more strongly enlarged; note: many darkly stained infiltrated cells and oedema indicated by numerous empty-looking spaces, pycnotic nuclei at surface; scale 20 µm. 3: Tissue around opisthaptor, more strongly enlarged. Note: enlarged blood vessels (X); scale 20 µm. (Original provided by F. R. Roubal.)

Nigrelli and Breder (1934) and Nigrelli (1935c) reported that several species of marine fishes acquire either a partial or a complete immunity to the infection with *Neobenedenia melleni,* and according to Nigrelli (1937), the 2 fish species *Trachinotus carolinus* and *T. falcatus*, both susceptible to the parasite, may acquire a permanent or partial resistance after several exposures. Worms placed in petri dishes containing mucus from elasmobranchs (not normally susceptible) and immune fishes died in a shorter time than worms placed in mucus from non-immune fishes or in seawater. This indicates that a factor in the mucus is responsible for the immunity (see also Nigrelli, 1935b). Attempts at immunizing fish by injecting worm material, or sera from immune fishes, failed.

Logan and Odense (1974) described the light-microscopic histology of the integument of the ocean sunfish *Mola mola* and lesions associated with a copepod and the monogenean *Capsala martinierei*. The monogenean is very large (2 to 3 cm in diameter), and lies on the surface of the skin. On gross examination, it did not appear to be embedded in it, and the area around it appeared for the most part normal. Only in front of the anterior end were small white areas lacking tubercles and mucus. Beneath the worms were similar white patches or most of the area was white and the stratum compactum was exposed. Microscopic examination revealed the major lesions to be beneath the anterior end.

Beneath the opisthaptor the epithelium was compressed, with some necrosis of the skin. In some areas, the stratum spongiosum was compressed and often inflamed. Effects of feeding were visible under the anterior part of the body, i.e., the epithelium was strongly compressed or absent, the stratum compactum was absent or haemorrhagic. In one case, even the inflamed stratum compactum was exposed. Cellular responses to the parasite occurred in the stratum spongiosum and the outer stratum compactum.

The lesions resulted primarily from feeding and to some extent from attachment. As histolytic debris was not found, the authors concluded that feeding effects are mechanical. The superficial lesions might result in secondary infections.

Paperna (1975) reported that the monogenean *Benedenia* sp. (*B. monticelli* acc. to Paperna and Overstreet, 1981) is pathogenic to the mullets *Mugil* and *Crenimugil* in the northern gulfs of the Red Sea. The worms are up to 5 × 1.5 mm in size and normally attached to the oral mucosa. Usually fewer than 6 worms per fish were found, and occasionally up to 12. Fish smaller than 60 mm in length were not infected. The lips and oral mucosa of infected fish were irritated with extensive submucosal haemorrhages. Mortalities occurred, but it was not clear whether environmental factors were involved besides the parasite. Captured infected fish began to die 2 wk after putting them into tanks. Fish infected experimentally began to die 1½ to 2 months after infection. In heavily infected fish, the worms had spread all over the body, with associated symptoms. All fish that had died of the infection in tanks, had perforations 'along the intermandibular groove and between the mandibulae and the basihyal'. The interskeletal tissue had been eaten by the worms.

Monogenea as vectors of microorganisms

Morris and Halton (1975) demonstrated, with the aid of the electron microscope, the presence in *Diclidophora merlangi* of bacteria and mycoplasma-like organisms. Whereas the bacteria appeared to be contaminants of the culture medium, the mycoplasma-like organisms occurred in the cytoplasm of gland cells associated with the anterior part of the digestive tract in an apparently hyperparasitic relationship. There is no evidence that the microorganisms can be passed on to the host, the whiting *Merlangius merlangus*.

Agents: Trematoda

Biology

Trematoda comprise one of the largest groups of parasites infecting marine fishes. Manter (1957) estimated the number of species described at 5,000; at least 1,500 of these were from fishes and approximately 1,000 from marine fishes. Manter estimated the total number of fish trematode species to be 10 times as high, that is, he assumed that only a small proportion of trematodes had been described at that time. Yamaguti (1958) listed 367 genera and 1390 species of trematodes from fishes. Yamaguti (1970) collected 314 species of adult digenetic trematodes from 144 species of marine fishes at Hawaii; 4 large pelagic and predatory fish – i.e., *Euthynnus yaito, Katsuwonus pelamys, Thunnus albacares* (syn. *Neothunnus macropterus*), and *Parathunnus sibi* – had the largest number of trematode species (16, 13, 22, and 18 species respectively).

General information on Trematoda can be found in Dawes (1956), Baer and Joyeux (1961), Ginetzinskaya (1968) and Erasmus (1972). Taxonomic reviews are by Dawes

(1947; trematodes of British fishes), Yamaguti (1958), Skrjabin and co-authors (1964; illustrated key to the trematodes), Schell (1970), and Yamaguti (1971; synopsis of trematodes of vertebrates; 318 of 802 pages on keys and descriptions of fish trematodes). A comprehensive review of the occurrence of metacercariae of fish in the USSR has been presented by Bychovskaya-Pavlovskaya and Petrushevsky (1963; mostly freshwater but also marine species), and data on larval trematodes in Japan and the northern seas can be found in Komiya (1965) and the volume edited by Polyansky (1966) respectively. Smyth (1966) gave a general account of the physiology of trematodes, but included few data on marine forms. According to Manter (1957), Digenea are primarily parasites of the digestive tract with the greatest number of species, genera, and families occurring in this location. Some of them occur, however, in practically any organ. Members of the large family Hemiuridae are parasites of the stomach, as are the Hirudinellidae and Azygiidae. Some hemiurids are found on the gills, sometimes perhaps the result of migration from the stomach after the death of the host. One hemiurid, *Gonocerca macroformis,* infects the ovary of the cod. The Syncoeliidae occur in the gill cavity or mouth except for 1 genus, *Paronatrema,* species of which have been found in the oviduct of *Squalus* and on the skin of the manta ray. There is no clearly valid record of a trematode from the spiral valve of selachians.

Any particular species of trematode is usually well restricted to a particular location although data are scanty. For example, some genera such as *Stephanostomum* (Acanthocoplidae) and *Proctotrema* (Monorchidae) usually inhabit the posterior part of the intestine. Trematodes living in the anterior portions of the intestine may be found occasionally in the caeca, and *vice versa*; those of the urinary bladder may occur in the cloaca. Fellodistomatidae typically are intestinal parasites, but a few infect the gall bladder. The Didymozoidae are tissue parasites, living, usually in pairs, encysted in the gills, skin, mouth, muscles, body cavity, or connective tissue. The Sanguinicolidae and Aporocotylidae are blood flukes, although one genus, *Deontacylix,* lives in the coelom (Manter, 1957).

Different trematode species in the same host often inhabit different sites, but sometimes there is much or complete overlap. Thus, Yamaguti (1970) observed that 2 species of didymozoid trematodes, *Didymocystis palati* and *D. superpalati,* throughout their later larval and entire adult stages, parasitize the palate of *Neothunnus macropterus* in Hawaii. Yamaguti (1970) suggested that occupation of the habitat is controlled by the principle "first come, first served". No displacement of 1 species by the other was observed throughout their parasitic life.

Most trematodes are restricted to 1 or a few host species. Generally, they have a wider host range in colder waters than in the tropics (Manter, 1957; Rohde, 1978b). However, if the intensity of infection is taken into consideration, their host specificity is equally great at all latitudes (Rohde, 1980c, 1982; p. 197). Manter (1957) gave the following data on host ranges of trematodes from 4 localities (Table 1-20).

Some species of Hemiuridae show a marked lack of host specificity, and among trematodes of Hawaiian fishes, Yamaguti (1970) recorded the greatest host specificity for some didymozoids.

Many trematode species exhibit a distinct preference for hosts of a certain age or length. Thus, frequency of infection of American plaice *Hippoglossoides platessoides* with the trematodes *Derogenes varicus* and *Stenakron vetustum* decreased with host length,

Table 1-20
Host range of Digenea of marine fishes as indicated by collection records in 4 localities (After Manter, 1957)

Locality	1 host	2 hosts	3 hosts	4 hosts	more hosts
Japan 296 species	226 (76.1 %)	36 (12.1 %)	20 (6.76 %)	5 (1.68 %)	9 (3. %)
Tortugas, Fla. 190 species	106 (55.6 %)	43 (22.6 %)	14 (7.4 %)	7 (3.7 %)	19 (10. %)
Mediterranean 107 species	68 (63.5 %)	20 (18.7 %)	8 (7.5 %)	3 (2.8 %)	8 (7.5 %)
British Isles 75 species	36 (48. %)	13 (17.3 %)	7 (9.3 %)	5 (6.6 %)	14 (18.6 %)

whereas infection with the trematodes *Steringotrema ovacutum* and *Zoogonoides viviparus* increased. Two other trematodes showed little change (Scott, 1975a).

Trematodes often are characteristic of certain ecological conditions, i.e. they are 'indicator' species for certain environments of their hosts. For example, according to Zhukov and Strelkov (1959), *Podocotyle reflexa, Neophasis oculatus, Opecoelus sphaericus, Stephanostomum baccatum, Tubulovesicula lindbergi* and *Lecithaster gibbosus* are open-sea indicators, whereas *Podocotyle atomon* and *Liliatrema skrjabini* indicate a coastal environment in the Far East. Similarly, the trematode fauna is greatly influenced by salinity (e.g., Reimer, 1964).

Only a few studies concerning feeding of trematode parasites of marine fishes have been made, but observations of flukes in other hosts permit some conclusions concerning what might be expected in marine fish. Observations on living worms, as well as histological and histochemical studies of flukes from lungs, intestine, rectum and urinary bladder of frogs, bile ducts of cattle and sheep, and veins of mice, led Halton (1967) to generalize that trematodes have developed a variety of nutritional adaptations enabling them to exploit a diversity of hosts and habitats. The gut-dwelling species examined fed on superficial epithelial tissues and associated mucus secretions of the host and sometimes also on blood and material in the intestinal lumen, whereas worms in lungs and blood systems fed exclusively on blood. Species in the urinary bladder fed predominantly on tissue from the bladder wall and small amounts of blood, those in bile ducts mainly on blood, but also on liver tissue, bile-duct epithelium and mucus. Encysted didymozoids, which are common in marine fishes, derive nutriment through the blood capillaries of the host which extend all over the cyst and also into the connective tissue septa projecting between the parts of the worm (Yamaguti, 1969). Some species of didymozoids ingest the host's blood cells, but most apparently feed on blood serum. Observations of gut contents suggested to Yamaguti (1970) that most didymozoid trematodes feed on blood plasma when encysted and on tissue fluid when unencysted. *Melanocystis kawakawa* and the unencysted form of *Angionematobothrium* feed on blood (see also Lester, 1980). The blood fluke *Aporocotyle simplex* ingests blood cells of its hosts, i.e., various species of pleuronectids (Thulin, 1980).

Trematodes in various vertebrate hosts have life spans from 1 wk to over 25 yr

(Dogiel, 1964), but few data are available on life span, growth and maturation in marine trematodes. Meskal (1967) examined monthly samples of cod *Gadus morhua* from western Norwegian coastal waters over 12 months and concluded that *Hemiurus communis* has an average life span of 8 months and a possible maximum life span of approximately 15 months. According to Balozet and Sicart (1960) a hemiurid species, thought to be *Hemiurus communis* in eels *Anguilla anguilla,* lives for several years. Margolis and Boyce (1969) suggested that a misidentification of the worm was possible, although the different microenvironment in the eel as compared with that in cod may contribute to the longer life of the parasite. Scott (1969a) concluded that the hemiurid *Lecithophyllum botryophorum* in *Argentina silus* in the northwestern Atlantic has a life span of 8 to 10 months. The hemiurid species *Tubulovesicula lindbergi* and *Lecithaster gibbosus* in Pacific salmon were examined by Margolis and Boyce (1969) using naturally infected young salmon captured within a few weeks of their entry into the sea and kept in tanks precluding further infection. The former species matures in 2 to 4 months and lives at least 31 months, growing from 0.6 to more than 4.5 mm; the latter species matures in 1 to 2 wk and lives approximately 2 to 9 months, growing from 0.5 to almost 1.9 mm (see also Boyce, 1967). The data, all for species of Hemiuridae, indicate that even within 1 trematode family there may be a considerable variability in life span.

With regard to the life span of metacercariae, Dönges (1969) on the basis of his own studies and those of others arrived at the following generalizations. There are 3 groups: Group A comprises species whose metacercariae encyst in the open; Group B, species whose metacercariae do not grow inside the second intermediate host; and Group C, species whose metacercariae undergo growth and metamorphosis before they enter the resting stage in the second intermediate host. Species belonging to Group A live for 3 to 7 months (exceptionally up to 1 yr). Species of Group B remain infective for 10 to 14 months, exceptionally up to 3 yr. Species of Group C live up to 7 yr. Although all species included in the discussion use freshwater or terrestrial hosts, it is likely that marine metacercariae have similar lifespans. Metacercariae of *Bucephalus haimeanus* survive for at least 10 months in the goby *Pomatoschistus microps* (Matthews, 1973b).

Timofeeva (1978) examined the lifespan of 3 species of cystophorous cercariae from the Barents Sea in sea water. The period of locomotory activity ranged from 2 to 5 days, the medium life span from 2 in 1 species to 4 to 5 and 20 to 25 in the others; maximum life span was 6 to 7, 10 to 14 and 35 to 40 days respectively. Cercariae of *Paucivitellosus fragilis* on the Great Barrier Reef, Australia, remain attached to a substratum and live for 3 to 4 days (Pearson, 1968).

The trematodes comprise 2 groups, Aspidogastrea and Digenea. Aspidogastrea were reviewed by Rohde (1972a). Only 1 life cycle of a marine aspidogastrean is known – that of *Lobatostoma manteri* (Rohde, 1972b, 1973). Final host is the snub-nosed dart *Trachinotus blochii* on the Great Barrier Reef, Australia. Eggs are shed in the faeces of the fish and ingested by the littoral snails *Cerithium moniliferum, Peristernia australiensis* and *Planaxis sulcatus.* Worms grow up to full size or almost full size but do not reach maturity in the snails. Maturation occurs after snails harbouring preadult parasites are eaten by fish. Narasimhulu and Madhavi (1980) found many shell fragments in the digestive tract of host fish infected with *Lobatostoma hanumanthai* and considered it therefore likely that this aspidogastrean has the same type of life cycle.

Digenetic trematodes have much more complex life cycles, with at least 1 intermedi-

ate host, usually a mollusc, and several larval stages. Data on life cycles of marine digenetic trematodes can be found in Polyansky (1966), Ginetzinskaya (1968), Odening (1969) and Yamaguti (1975). According to Holliman (1961), only 60 life cycles of the 334 species of marine cercariae known in January, 1960, had been partly or completely worked out, but much information has been gathered since that date. However, even today not a single complete life cycle of a didymozoid trematode is known, although larvae are probably ingested by the final host when eating prey (Cable and Nahhas, 1962; Nikolaeva, 1965) and a tentative scheme of development was presented by Yamaguti (1970). According to Gibson and co-authors (1981), death of a host may be necessary for the completion of the life cycle of the didymozoid *Halvorsenius exilis* which infects the connective tissue outside the pericardium and of the anteriorly adjacent 'throat' region, along the kidney side of the dorsal peritoneum, in and around the orbits of the eyes, and in the sub-cutaneous tissue of the opercula of the mackerel *Scomber scombrus* in the North Sea and the English Channel. Live worms occur only in young mackerel, but aggregations of eggs are found in older fish. Eggs appear to remain in the host's connective tissue, and are released only when the fish is eaten and digested or dies and decomposes. Death of the fish is apparently necessary to make larvae available for the first intermediate host.

Fish serve as second intermediate or final hosts. To increase the chances of getting into a fish host, production of cercariae in molluscs is sometimes very large. According to Meyerhof and Rothschild (1940), 3330 cercariae of *Cryptocotyle lingua* per day were produced in 1 *Littorina littorea* at the beginning of a 5 yr period, and 830 at the end. To increase larval output even further, metacercariae of a number of species are progenetic, i.e., they mature in the intermediate hosts and produce eggs (e.g., *Podocotyle atomon* in amphipods; Hunninen and Cable, 1943).

Fish acquire most species of trematodes by ingesting intermediate hosts harbouring metacercariae. There is a wide range of hosts for metacercariae (e.g. Uspenskaya, 1955; Ginetzinskaya, 1958; Komiya, 1965; Reimer and co-authors, 1971); such as amphipods (*Podocotyle atomon*; Hunninen and Cable, 1943; *P. enophrysi;* Ching, 1979), polychaetes (*Deropristis inflata;* Cable and Hunninen, 1943; several records from the Barents Sea; Amosova, 1955), copepods (a syncoeliid; Overstreet, 1970; *Lecithaster* sp.; Boyce, 1967), marine fishes (*Stephanostomum baccatum;* Wolfgang, 1954, 1955; *Cardiocephalus longicollis*; Prevot and Bartoli, 1980, final hosts are gulls; *Rhipidocotyle* spp.; Stunkard, 1976; *Bucephalus haimeanus* and *Bucephaloides gracilescens;* Mathews, 1968, 1973b, 1974a), sea urchins (*Zoogonus;* Stunkard, 1941), ctenophores, hydrozoans and scyphozoans (*Neopechona cablei;* Stunkard, 1980); molluscs (several Japanese records; Komiya, 1965), etc.

In a series of beautifully illustrated studies, Køie (1976, 1978a, b, 1979a, b, 1980, 1981) desribed the morphology and life cycles of some marine trematodes.

Zoogonoides viviparus uses the marine prosobranch *Buccinum undatum* as the first intermediate host. Metacercariae occur in brittle stars, polychaetes, lamellibranchs and gastropods. Several fish species, among them plaice and flounder, become infected by eating infected second intermediate hosts (Køie, 1967). *Stephanostomum caducum* undergoes early larval development in the prosobranch *Natica alderi,* and cercariae encyst below the epidermis of the mouth of gobiid fishes. Cod *Gadus morhua* eat the gobies and the adult worm develops in the pyloric caeca (Køie, 1978a). *Derogenes varicus* is one of the most widely distributed and common marine trematodes; more than 100 fish species have

been found to be infected (Køie, 1978b); it occurs in temperate, subarctic and subantarctic waters as well as in the deep water of warm seas. It has *Natica cateria* and *N. pallida* as first intermediate hosts. The free-swimming cercaria is ingested by calanoid or harpacticoid copepods, but other crustaceans, e.g., hermit crabs, may become infected. *Sagitta* was found to have natural infections with metacercariae of this trematode. It probably becomes infected by eating infected copepods. *D. varicus* may mature in crustaceans and chaetognaths, but the eggs are released into the water only after death of the host or ingestion by a fish. Fish and cuttlefish (Cephalopoda) may function as final host (Køie, 1978b, 1979a, Fig. 1-76). Adult worms may be transferred from one fish to another. *Monascus* (= *Haplocladus*) *filiformis* and *Steringophorus furciger* (family Fellodistomidae), use the bivalves *Nucula nitidosa* and *Nuculana* (= *Leda*) *minuta* respectively as first intermediate hosts. The forked cercaria of the first species is eaten by dab *Limanda limanda* and matures in the fish. When eaten by gobies, maturation of the cercariae did not occur, but horse mackerel *Trachurus trachurus* became infected when ingesting infected gobies. In nature,

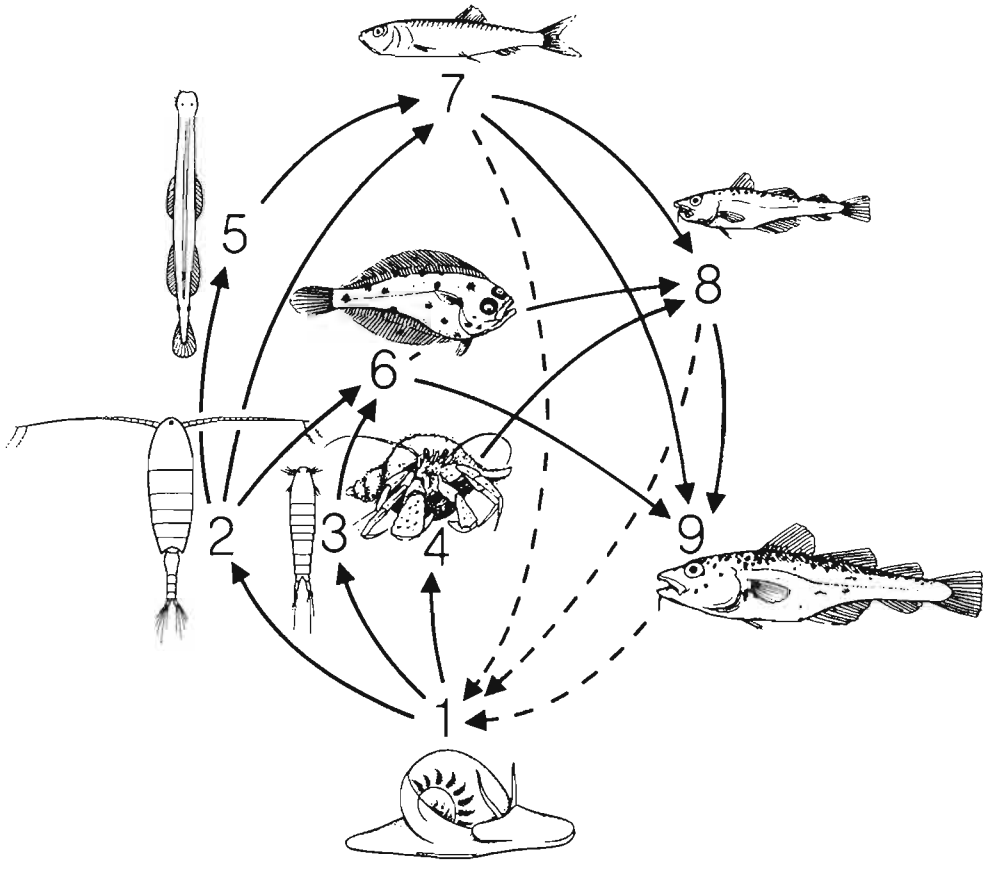


Fig. 1-76: *Derogenes varicus*. Life-cycle based on literature and experiments. 1: *Narica* spp.; 2: calanoid copepod; 3: harpacticoid copepod; 4: hermit crab and probably other decapods and barnacles; 5: *Sagitta* spp.; 6: small fishes, e.g., gobies and fry of other fishes; 7: planktophagous fishes, e.g., salmon, herring and juvenile gadid fishes; 8: benthophagus and piscivorous fishes, e.g., gadid fish and flatfishes and *Sepia officinalis;* 9: piscivorous fishes, e.g., large cod. (After Køie, 1979.)

many fish species are infected. The cercaria of *S. furciger* is also eaten by dab, in which it matures (Køie, 1979b, Fig. 1-77). *Steringotrema pagelli* develops in the bivalve *Nucula nitidosa*. The cercaria has 2 long contractile tail furcae. It is swallowed by the final hosts, species of flatfish. *Fellodistomum fellis* (syn. *Steringotrema pagelli*) uses *Nucula tenuis* as first intermediate host. Forked cercariae are eaten by brittle stars, and several fish species

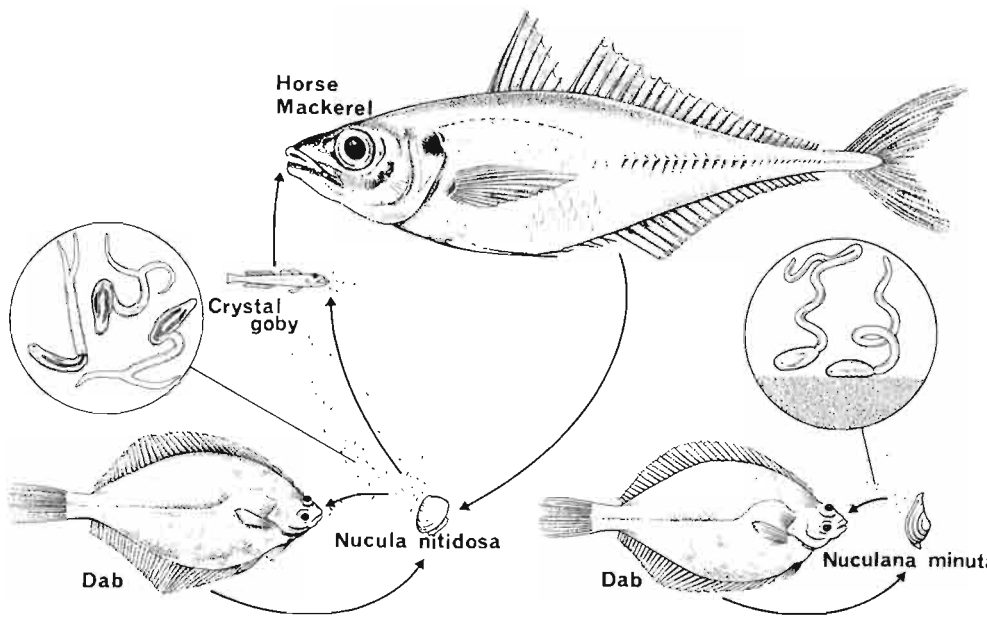


Fig. 1-77: Life-histories of *Monascus filiformis* (left) and *Steringophorus furciger* (right). Swimming behaviour of cercariae of *M. filiformis* drawn after electronic flash photographs. (After Køie, 1979b.)

function as final hosts. They become infected by eating the brittle stars (Køie, 1980, Figs 1-78 and 1-79). *Podocotyle reflexa* develops in marine prosobranchs (Køie, 1981). Metacercariae occur in various crustaceans, and many fish species become infected by eating the second intermediate hosts. Stunkard (1930) worked out the life cycle of *Cryptocotyle lingua* in the eastern United States. The littoral snail *Littorina littorea* is the first intermediate host (as it is in England; Rothschild, 1939). Fish serve as second intermediate, and gulls as final hosts. On the New England coast (USA), infection of snails and cercarial emergence show a marked seasonality, differing somewhat between snails from the low- and high-tide zone (Sindermann and Farrin, 1962). In the littoral zone, availability of cercariae coincides with the presence of herring which are an important second intermediate host.

Some trematodes have 2 so-called alternative life cycles. Such a life cycle was for the first time described for *Gymnophallus choledochus* by Loos-Frank (1969). However, fish are not involved in it.

Smith and Williams (1967) speculated that cercariae of the blood fluke *Aporocotyle spinosicanalis* probably penetrate the final host *Merluccius merluccius* directly, but no experimental evidence was given. Blood flukes of salmonids (*Cardicola klamathensis* and

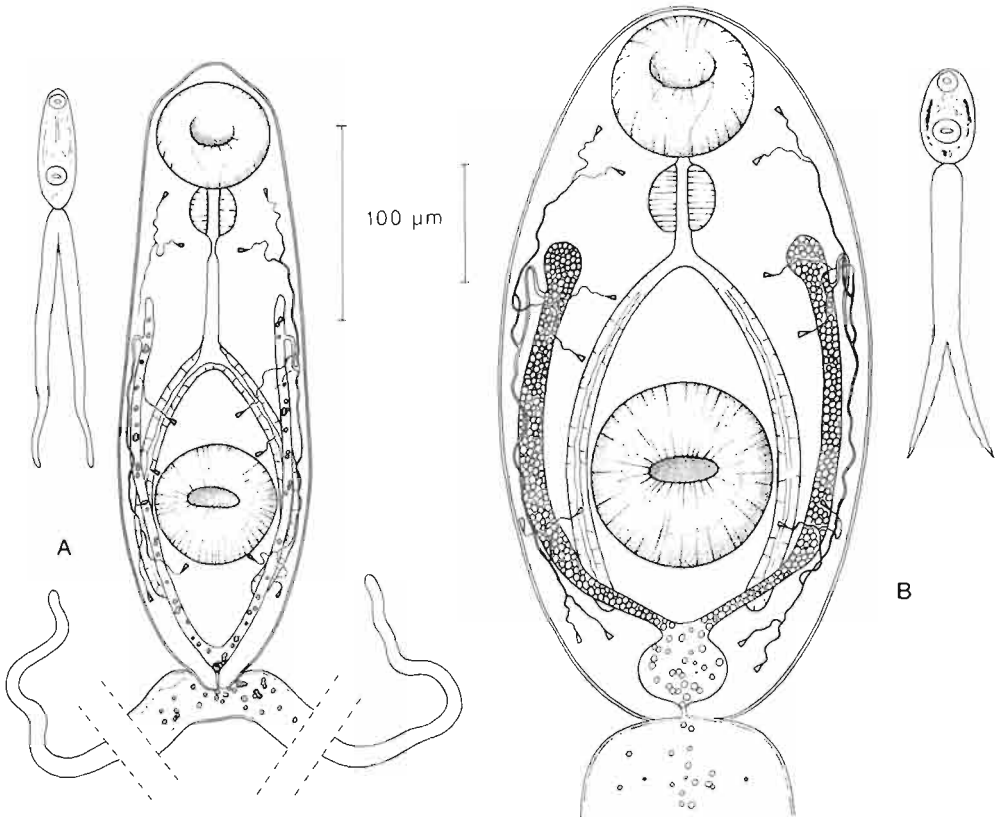


Fig. 1-78: Cercariae of *Steringotrema pagelli* (A) and *Fellodistomum fellis* (B). Ventral views of living flattened specimens. (After Køie, 1980.)

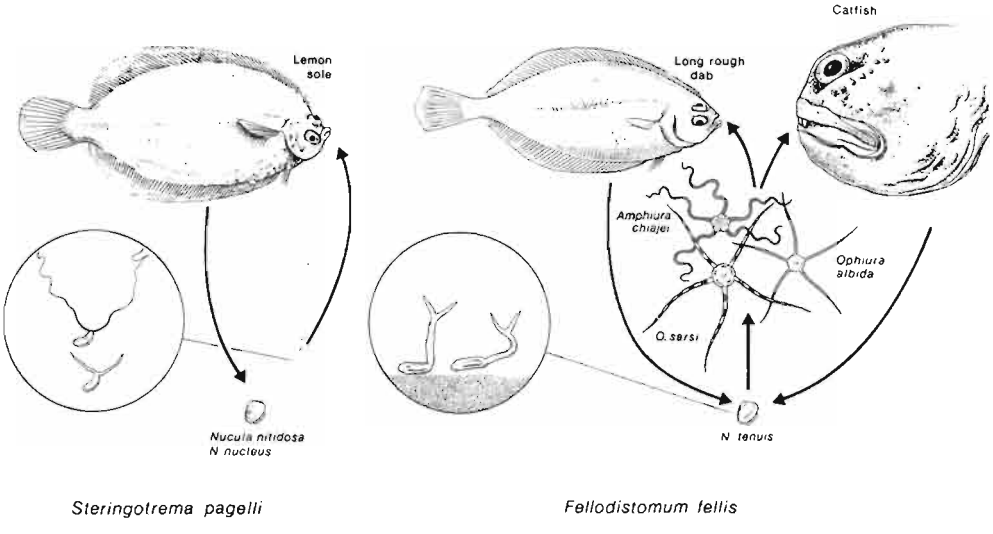


Fig. 1-79: Life-histories of *Steringotrema pagelli* and *Fellodistomum* fellis. (After Køie, 1980.)

Cardicola alseae) are acquired by the fish in freshwater by penetration (Meade, 1967 and Meade and Pratt, 1965 respectively; see also review by Smith, 1972). Some trematodes are acquired by the fish final host when free-living cercariae are eaten (*Paucivitellosus fragilis*; Pearson, 1968).

Penetration of cercariae into a fish intermediate host was described by Stunkard (1976). Cercariae of the bucephalid *Rhipidocotyle* spp. have tail furcae which are about as long as the body. They hang from the furcae which extend upward. Retraction of the furcae in a spiral manner raises the body and the cercariae are wafted about by currents. When the furcae of the cercaria make contact with a fish, they adhere firmly and contract until the base of the tail stem touches the surface of the fish. The body of the cercaria then attempts to enter the host. Penetration and encystment to form a metacercaria occurs. Many fish species are attacked, but development occurs apparently only in the silverside *Memidia memidia*.

Many marine cercariae have elaborate morphological adaptations to dispersal, such as mechanisms ensuring prolonged floating in the water (Fig. 1-80). One mechanism for dispersal is the retractible furcae of bucephalids (see above). In *Bucephaloides gracilis-*

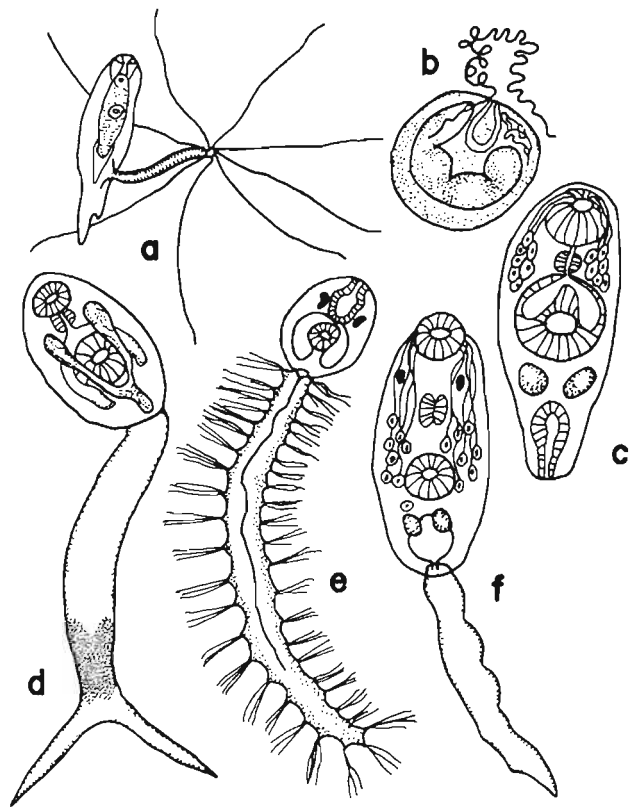


Fig. 1-80: Types of cercariae of marine trematodes. (a) Hemiuridae gen. sp. (from Chubrik); (b) *Lecithaster* (from Hunninen and Cable); (c) *Zoogonoides* (from Lebour); (d) *Fellodistomum* (from Chubrik); (e) *Opechona* (from Lebour); (f) *Neophasis* (from Chubrik). (Redrawn after Ginetzinskaya, 1958.)

cens, the extended furcae are 12 times the length of the body. In this position they tend to drift upwards and are caught in water currents, lifting the body off the bottom. Partial contraction of furcae results in simple swimming movements by which a cercaria can maintain its position just above the sea bottom (Matthews, 1974a) (Fig. 1-81).

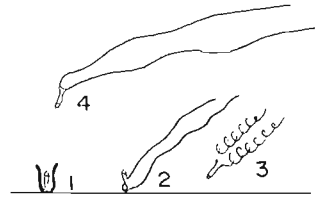


Fig. 1-81: *Bucephaloides gracilescens.* Cercaria showing furcae in different states of contraction. 1: Fully contracted, cercaria on bottom; 2: extending furcae being drawn upwards in water currents; 3: furcae undergoing rapid contraction in spiral fashion, cercaria sinking; 4: fully extended furcae, cercaria floating. (Redrawn after Matthews, 1974a.)

Effects on Hosts

Effects due to adult trematodes

There are few studies on the effects of adult trematodes on marine fishes. Martin (1960) reported that eggs of the blood fluke *Paracardicola hawaiensis* in *Tetraodon hispidus* damage the liver, but Smith and Williams (1967) found no apparent effect of the blood fluke *Aporocotyle spinosicanalis* in hake *Merluccius merluccius,* using histological sections and weight of infected fish. However, body weight may be too rough an indicator to detect minor effects, and presence of other parasites may have prevented the detection of minor effects. Smith (1972) reviewed the blood flukes of cold-blooded vertebrates and drew attention to the fact that very little is known about the biology and pathology of marine Sanguinicolidae, although 51 of the 91 species of fishes found to harbour them, are marine. With regard to freshwater forms, various authors have shown that carp and salmonids may suffer considerable damage as a result of the infection. According to Grabda (1977), Alaska pollack *Theragra chalcogramma* heavily infected with several parasite species, are anaemic, emaciated and of lower nutritional value. Parasites infecting the blood system, among them the blood fluke *Aporocotyle simplex,* are considered to be particularly hazardous, but no supporting evidence was given. Thulin (1980) described some effects of *Aporocotyle simplex* on flatfish, mainly *Hippoglossoides platessoides,* but also *Limanda limanda* and *Pleuronectes platessa.* Intensities of up to 174 worms per host were found, usually in the arterial blood system. The host's blood cells are ingested and on several occasions pathological effects were observed. Fragments of disintegrating flukes were observed in and at the openings into afferent gill filament arteries, and some such gill filaments appeared stunted and greyish. Once, 2 dead worms were found encapsulated on the surface of the liver. Eggs apparently also cause pathological effects, but no details were given. According to Thulin (1982), eggs of the sanguinicolid *Chimaerohemecus trondheimensis* induce heavy tissue reactions in gill filaments but more often in heart muscles of *Chimaera monstrosa* near Bergen, Norway.

Prosorhynchus crucibulum, a gasterostome trematode in the caeca of the 'seasnail' *Liparis atlanticus* on the North American Atlantic coast, causes light abrasion of the

mucosa (Munson, 1974). Similarly, damage due to the trematodes *Podocotyle reflexa* and *P. atomon* in the intestine of the same host was light, due to grasping of small areas of the mucosa with the suckers (Munson, 1974).

Transversotrema and some related forms are the only digeneans found on the surface of fish, both freshwater and marine. No information is available on pathological effects on marine fish, but Mills (1979) studied attachment and feeding of *T. patialense* on the freshwater zebra fish *Brachydanio rerio*. Two hosts were experimentally infected with 30 parasites each, and 7 days after infection the hosts were sacrificed. The epidermis from which a parasite was removed bore imprints of the parasite's acetabulum, and the spines of the parasites left indentations in the epidermis as well. Apparent feeding lesions could also be seen, but they were not serious and, as fish epidermis heals and regenerates rapidly, were probably repaired soon.

Among the most conspicuous marine trematodes are the didymozoids. Williams (1959) described the cysts of *Koellikeria filicollis*. They are 9.1 × 6.5 × 2.5 mm in size, each containing 1 to 5 'males' and 1 'female', although the sexes are not entirely separate. They are attached to the epithelium inside the operculum near the posterior gill of *Brama raii*. The cyst wall appears to be mainly a reaction of the host to the 'female' worm. The host's epithelial tissue has apparently degenerated to form the outer layer of the cyst. Oval cells of undetermined origin are embedded in fibres of loose vacuolated connective tissue. According to Lester (1980), *Neometadidymozoon helicis* in the dermis of the buccal cavity and gill arches of flathead *Platycephalus fuscus* in Australia also seem to be encapsulated by a discrete cyst, but sections stained for connective tissue showed little more than a parting of the dermal fibres to accommodate the parasites (Fig. 1-82, 1 and 2). *Nematobothrium spinneri,* in the musculature of *Acanthocybium solandri,* on the other hand, is enclosed by a thick wall of fibrous tissue (Fig. 1-82, 4 and 5). Connective tissue and blood capillaries are interdigitated between the coils so that the whole of the surface of the worm is in intimate contact with its host (Fig. 1-82, 6). Blood feeding by the parasite is indicated by partly digested host erythrocytes in the caeca.

Neometadidymozoon helicis shows seasonal occurrence in Moreton Bay, Queensland (Australia). Most of the parasites disappear between September and December, and 123 of 2,041 capsules examined at this time were red due to capillaries haemorrhaging around the worms. In the same period, 75 depressions in the mucosa, probably sites of former

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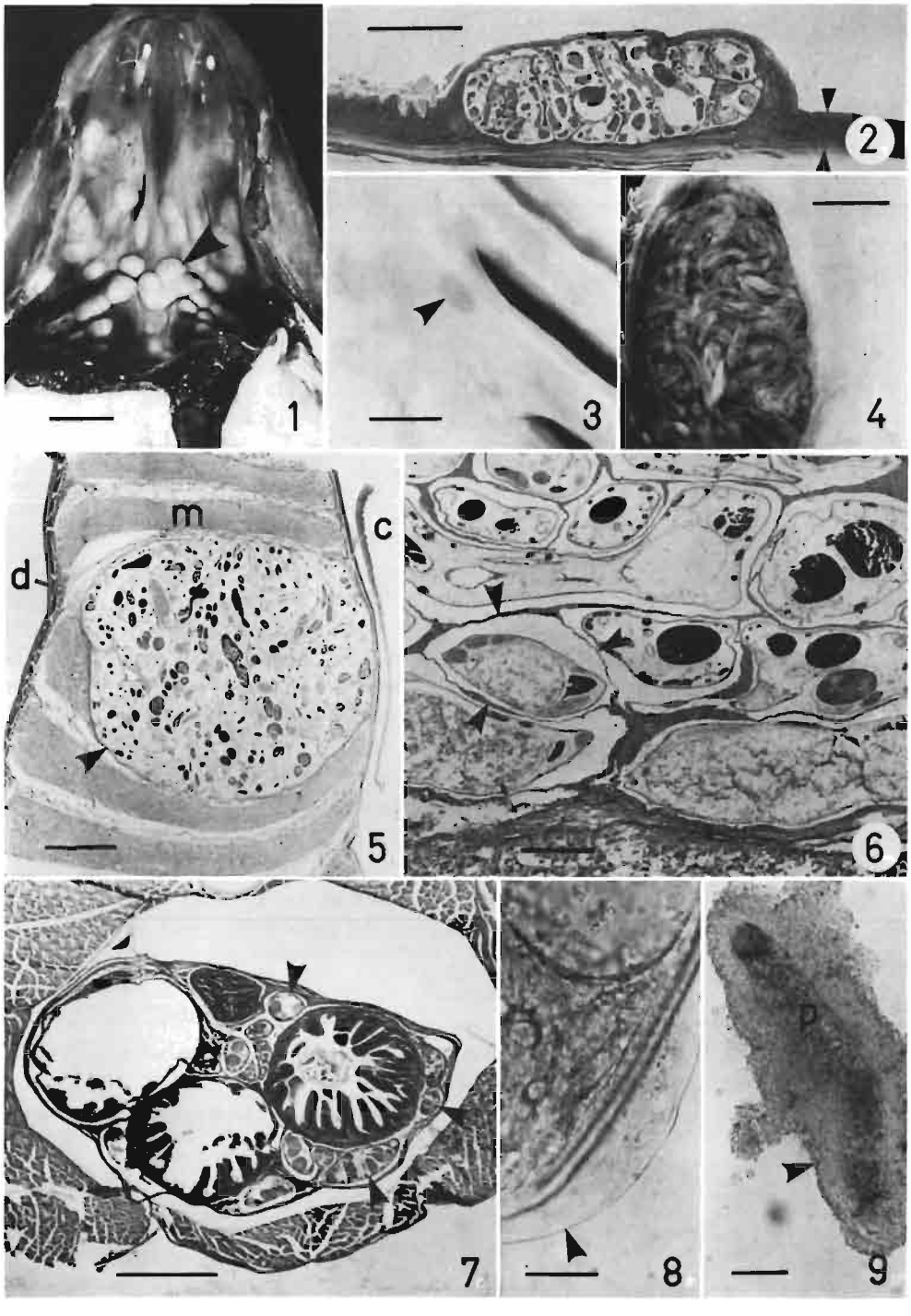
Fig. 1-82: (1) Palate of *Platycephalus fuscus* infected with *Neometadidymozoon helicis*. Capsules (arrow), unusually large in this specimen, in life are bright yellow; each contains 2 intertwined worms; bar = 10 mm. (2) Section across *N. helicis* capsule showing parasites coiled up within the dermis; dermis thickness indicated by arrows (Van Gieson stain; epidermis lost during processing); bar = 1 mm. (3) Scar (arrow) in dorsal surface of lower jaw marking site of former *N. helicis* capsule; bar = 5 mm. (4) Capsule of *Nematobothrium spinneri* in musculature of body wall of *Acanthocybium solandri;* bar = 10 mm. (5) Section of capsule of *N. spinneri* (Van Gieson stain); note fine band of connective tissue surrounding the worm (arrow); c: body cavity; d: dermis; m: myotome; bar = 3 mm. (6) Higher power showing the thin connective tissue wall and the connective tissue sheaths (arrows) enclosing the coils of the worm; bar = 50 μm. (7) Section of visceral mass of *Favonigobius exquisitus* containing larval didymozoids (arrows); note that none are in the gut lumen; bar = 400 μm. (8) Posterior end of larval didymozoid from viscera of *F. exquisitus* maintained for 32 days in the laboratory, showing thin connective tissue capsule (arrow); bar = 20 μm. (9) Dying larval didymozoid (p) from *P. fuscus* experimentally infected 2 days before; note thick wall of host leucocytes (arrow); bar = 100 μm. (After Lester, 1980.)


1
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infection, were found (Fig. 1-82, 3). Apparently, the capsule wall breaks down and the worms are released. Lester (1980) also observed larval didymozoids of several types in a number of host fish species. They occurred in the visceral mass (Fig. 1-82, 7) and were enclosed in a sheath of connective tissue 1 cell thick (Fig. 1-82, 8).

Overstreet and Edwards (1976) described benign subcutaneous mesenchymal fibromas possibly due to a didymozoid or nematode (page 292).

According to Paperna and Overstreet (1981), adult trematodes probably do little harm to mullets in natural habitats. However, when mullets are trapped with little water and many snails, effects may be more severe. By experimentally infecting juvenile *Mugil cephalus* with large numbers of *Dicrogaster fastigata,* hosts could be killed (Heard and Overstreet, cit. Paperna and Overstreet, 1981).

Effects due to larval trematodes

Metacercariae are sometimes extremely common with resulting severe effects. Dogiel and Bychowsky (1934) and Dubinina (1949) reported mass mortalities due to larval trematodes in brackish estuarine environments (Aral Sea, Volga delta). According to Arru and co-authors (1968) and Ceretto and Arru (1969), 30 % of red mullets, *Mullus barbus* and *M. surmulatus,* marketed in Sassani, Italy, had infections with metacercariae of *Stephanostomum* spp. Larvae occurred in the subcutaneous tissue, sometimes in heavy intensities, leading to reduced commercial value of the fish. Paperna (1975) found a maximum intensity of 6000 metacercariae g^{-1} of muscle tissue in mullets in Israel.

Early examples of observations on trematodes in marine fishes are those discussed by Gamble and Drew (1911) and Linton (1911). Linton found metacercariae of one and possibly several species in the skin of the cunner *Tautogolabrus adspersus,* the tautog *Tautoga onitis* and other fish at Woods Hole, Mass., USA. In certain localities the larvae were extremely common in the first 2 fish species. In heavily infected fish, the entire surface of the body including the cornea of the eye may be dotted with cysts. The 2 corneas of 1 tautog had 74 and 81 metacercariae.

One of the most studied trematode infections is that by *Cryptocotyle lingua.* Mawdesley-Thomas (1975) found no cellular response to the worm, when it was located in the skeletal muscles of fish. He suggested that some immune reaction dictated by the parasite may have persuaded the host that it is 'self' and therefore elicited no cellular response. However, nothing is known of such a reaction and that the 'persuasion' is at least not complete was shown by several authors who demonstrated immunity and tissue reactions to the parasite.

Cottrell (1975, 1976, 1977a) demonstrated precipitating antibodies in plaice *Pleuronectes platessa* to the metacercariae of *Cryptocotyle lingua* and *Rhipidocotyle johnstonei.* Serum from uninfected plaice formed precipitates around cercariae of *C. lingua* after 30 min, but all parasites remained active over 60 min. The immune serum totally immobilized all cercariae within 5 min. In fish kept at 5 °C, antibody response did not occur. Antibody formation to *R. johnstonei* was similarly temperature dependent (positive at 20 °C, negative at 5 °C), and developed with some delay to infection with metacercariae (Fig. 1-83). According to Cottrell (1975), antibody from immune sera of plaice combines both with the tegument and certain secretory glands of both trematode species. Antibody was not detected in the cutaneous mucus of plaice.

McQueen and co-authors (1973) examined the tissue response of plaice *Pleuronectes*

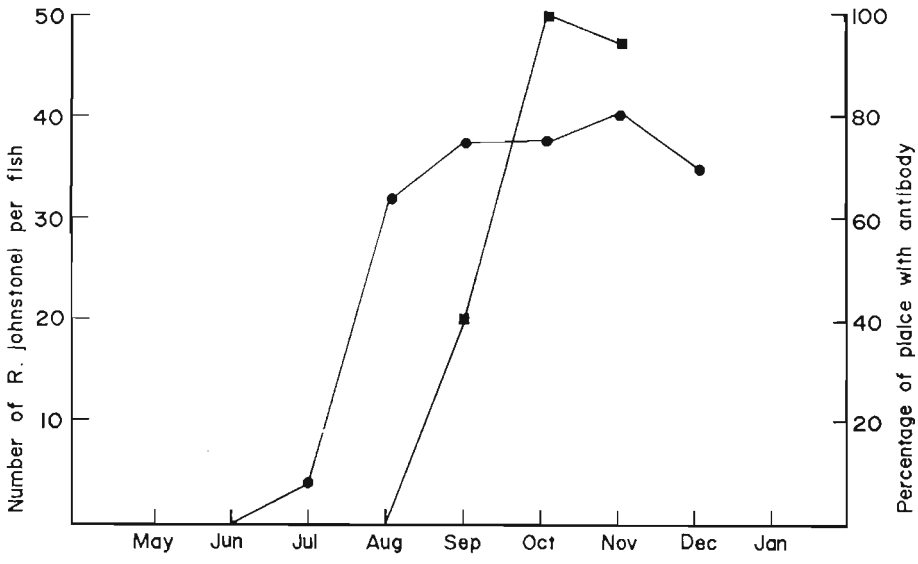


Fig. 1-83: *Rhipidocotyle johnstonei*. Average monthly number infesting plaice, May to December 1973 (●), and the antibody response, recorded as percentage of '0'-group fish with detectable precipitins to *Rhipidocotyle johnstonei* antigen (■). (After Cottrell, 1977a.)

platessa to experimental infection with metacercariae of *Cryptocotyle lingua* at 15 and 5 °C, using histological and histochemical methods. At 15 °C, independent of whether the parasites were in the dermis, musculature or paraspinal connective tissue, the response was essentially similar, consisting of the formation of a cellular capsule. In the epidermis, small hyperplastic foci were seen after more than 258 h infection; frequently, macrophages containing melanin were also observed. The authors assume that these nodules are a reaction to the passage of parasites. At 18 h, muscle degenerative change was seen. There was myofibrillar necrosis with flocculation and small vacuoles. At 138 h, many fibres had swollen sarcolemmal nuclei surrounded by basophilic material containing many colonies of bacteria. At 618 h, bacteria had disappeared and there was further degeneration of myofibrils and some replacement fibrosis (Fig. 1-84). In the intermuscular tissue, as early as 18 h a response could easily be detected in the form of swelling of the inter-muscular, fascial and epimysial connective tissues. Gradually the intermuscular fascial planes became much thicker than in normal fish. They became basophilic and apparently oedematous, but an inflammatory cell component was seen only at 258 h, when tissue histiocytes apparently used them as routes into the bacterial lesions. At this stage cells from the dermis containing melanin, or small melanin deposits around blood vessels also appeared to be migrating along these routes towards metacercariae. No further changes in the response were observed subsequently. The cyst wall was already secreted 6 h after infection. At 5 °C, development of the response and formation of the cyst was markedly slower, but otherwise similar. However, no massive degeneration and replacement fibrosis, fibre regeneration, or melanin were observed. Most remarkable, according to the authors, is the 'almost negligible inflammatory response to the infection', which is explained by the 'excellence of the adaptation' of the parasite to the host.

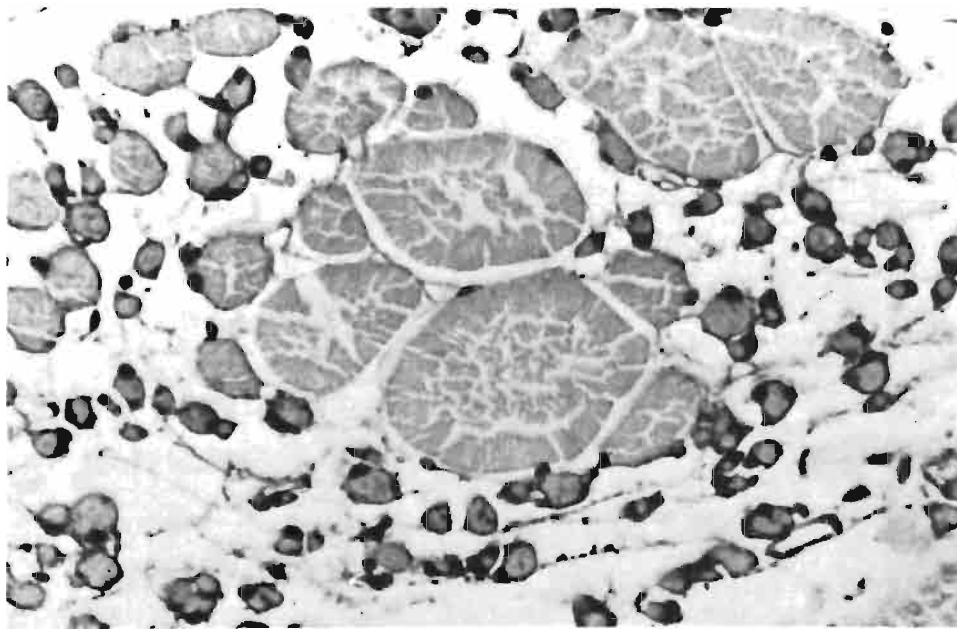


Fig. 1-84: *Pleuronectes platessa* infected with metacercariae of *Cryptocotyle lingua*. Final stage of myodegeneration, 678 h after infection (15 °C). The myofibrils are very small with some replacement fibrosis, but there is little cellular response and the area consists mainly of lipid. H. & E (X 200). (After McQueen and co-authors, 1973.)

A careful quantitative study under controlled conditions was made by Sommerville (1981), who examined tissue response to invasion and encystment of cercariae of *Stephanochasmus baccatus* in 4 experimentally infected species of flatfish *(Pleuronectes platessa, Scophthalmus maximus, Solea solea, Limanda limanda).* All fish were 1+ (in their second growing season) at the time of the experiments, and observations were made for up to 7 wk after infection. In plaice at 6 h p.i. larvae were on the scales and in the epidermis. There was no obvious damage to the epidermis and no signs of haemorrhage in the tissues and no parasite cysts were seen. At 19 to 24 h p.i. most and 48 h p.i. all larvae were encysted, with some disruption of connective tissue fibres and the superficial muscle caused by penetrating and migrating larvae, but no inflammation. At 48 h p.i., a distinct inflammatory cellular infiltrate consisting mainly of macrophages, was seen. A few tissue macrophages were aligned along the parasite membrane, forming the beginning of the capsule (Fig. 1-85). The larva increased in size as the result of an increase in the size of the posterior body cells. At 125 h p.i. a distinct host capsule was formed, whose innermost cellular layer consisted of infiltrated macrophages. At 8 days p.i., a marked inflammatory reaction surrounded the cyst and an outer cellular layer appeared to be formed by proliferating fibroblasts. From 11 days p.i. the capsule became more compact and the inner cell layer underwent degenerative change (Fig. 1-86). At 16 days p.i. the innermost layer of degenerating cells was clearly demarcated and an epithelioid layer which had begun to form from macrophages earlier became more distinct, containing small numbers of melanocytes (Fig. 1-87, a). At 23 days p.i. there was extensive degeneration of epithelioid

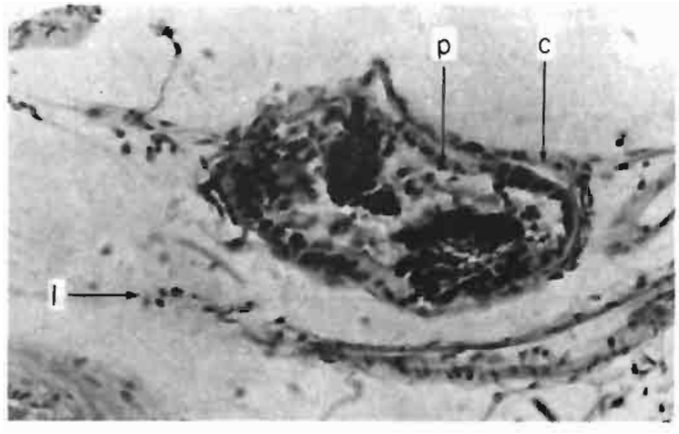


Fig. 1-85: *Pleuronectes platessa* infected with metacercariae of *Stephanochasmus baccatus*. Cyst at 48 h showing early capsule development. c: capsule; l: leucocytes; p: parasites (X 320). (After Sommerville, 1981.)

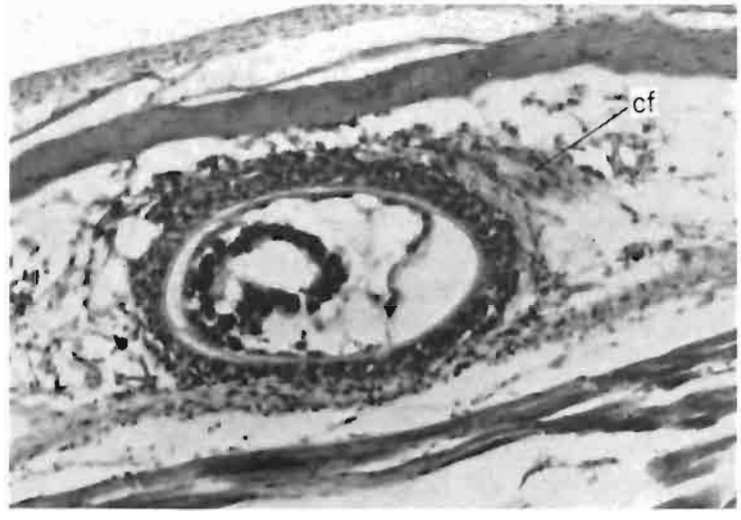


Fig. 1-86: *Pleuronectes platessa* infected with *Stephanochasmus baccatus*. Cyst at 11 days showing a well-defined, compact capsule and the appearance of collagen fibres in the outer capsule layers; cf: collagen fibres (X 200). (After Sommerville, 1981.)

tissue in the middle and inner parts of the capsule (Fig. 1-87, b), and at 28 days p.i. the capsule had the form of an extensive chronic granuloma with the appearance of giant cells (Fig. 1-88). The outer capsule showed vascular infiltration. At 40 to 45 days p.i. the capsule was much more fibrous. Muscle cysts had a less extensive layer of fibroblasts than fin cysts, and the innermost layer was less distinct, beside some other slight differences. In experimentally infected 0-group plaice (in their first growing season), the cercariae penetrated more deeply into the body as well as into the somatic muscles and fins. There was extensive muscle degeneration and invasion and proliferation of inflammatory cells. In the other host species, the tissue response was similar and also consistent with 'a chronic

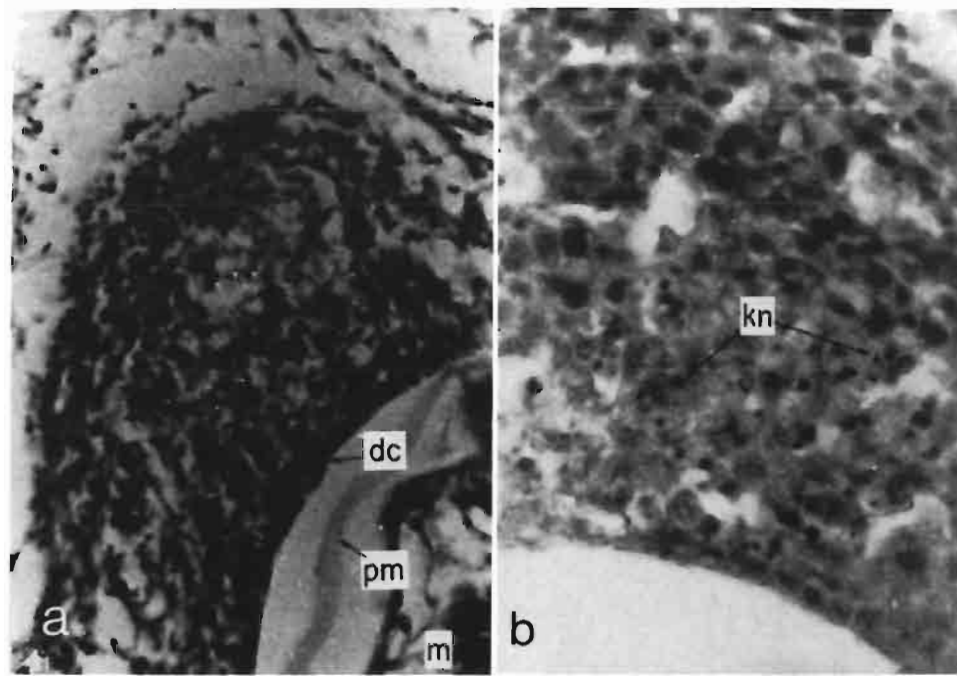


Fig. 1-87: *Pleuronectes platessa* infected with *Stephanochasmus baccatus*. (a) Section through capsule of cyst at 16 days post-infection showing an innermost layer consisting of degenerating cells. The epithelioid tissue of the middle layer shows some vacuolation at its centre, dc: degenerating cells; m: metacercaria; pm: parasite cyst membrane (H & E, X 280). (b) Extensive degeneration of capsule cells in a section of a capsule at 23 days; kn: karyorrhectic nuclei (X 70.)

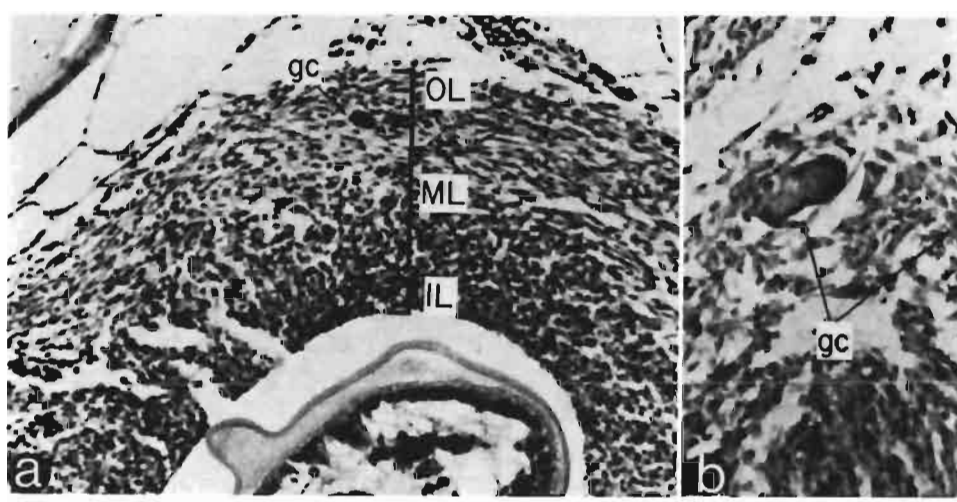


Fig. 1-88: *Pleuronectes platessa*. (a) infected with *Stephanochasmus baccatus*. Section through a capsule at 28 days post-infection showing 3 layers: inner layer (IL) consists of a necrotic ground substance in which are embedded karyorrhectic nuclei; middle layer (ML) is composed of degenerating epithelioid tissue on the periphery of which occur giant cells; outermost layer (OL) is comprised largely of fibroblasts and collagen; gc: giant cells (H & E X 175). (b) Section showing giant cells at periphery of middle layer of capsule at 28 days post-infection (X 286). (After Sommerville, 1981.)

granulomatous inflammatory reaction induced by resistant particulate foreign bodies'. Differences were seen in the strength and rate of the reactions.

Strength of the host's tissue reaction appears to depend on presence or absence of a cyst wall secreted by the parasite. Thus, according to Matthews (1973a), cercariae of *Prosorhynchus crucibulum* in experimentally infected turbot *Scophthalmus maximus* and dab *Limanda limanda* penetrate mainly at the margin of the fins and migrate to the base of the dorsal and ventral fins, although metacercariae may be found in the connective tissue and musculature in any region of the body. The metacercariae secrete a hyalin cyst within 3 to 4 h after penetration. However, the cysts break down and in 1-month old infections the larvae lie free in the tissue, evoking a strong tissue reaction which leads to formation of a fibrous capsule. According to Matthews (1974b), an intense host reaction occurs in *Pleuronectes platessa* and *Scophthalmus maximus* infected with metacercariae of *Rhiphidocotyle johnstonei* and *Prosorhynchus crucibulum* respectively. This appears to be related to the absence of a cyst wall secreted by the worms which feed directly on host tissue. No details were given.

Metacercariae of *Bucephaloides gracilescens* are found mainly in the cranial nerves and spinal cord of various Gadidae. They occur either in a hyaline cyst or free and there is no encapsulation by host tissue (Matthews, 1974a). This is probably related to the scarceness of fibroblasts in the nervous tissue.

Effects of metacercariae on the eye of marine fish have been reported repeatedly (e.g., Linton, 1911, p. 319). Such effects are popeye (exophthalmus), opacity of the lens (cataract), lesions and in extreme cases complete destruction of the eye. Popeye due to *Cryptocotyle lingua* infections in herring *Clupea harengus* was reported by Sindermann and Rosenfield (1954b). It was characterized by a distinct bulging outward of the cornea. There also was progressive opacity of the lens (Fig. 1-89) and in some cases the eyeball even burst. According to Mawdesley-Thomas and Young (1967), flounders from the south coast of England had heavy infections with metacercariae of *Cryptocotyle*. The eyes appeared cloudy and the fish were blind. Metacercariae of *Nanophyetus salmincola* also lead to exophthalmia and lesions in the retina and cornea of salmonids (references in Millemann and Knapp, 1970b). However, the infection is acquired in freshwater, although it is carried into the sea by migrating salmon. Millemann and Knapp, in contrast to previous findings by Ward and Mueller (1926), found only 4 to 21 worms in the eyes and optic nerves, but large numbers (1200 to 1400) in other tissues including the kidneys. They therefore concluded that the exophthalmia may be due to oedema resulting from kidney damage by the parasites (see also review on exophthalmia by Dukes, 1975a, b). Hsiao (1941, some early references therein) described a cod, *Gadus morhua*, heavily infected with metacercariae which remained unidentified but were possibly *C. lingua*. Metacercariae and melanophores were so abundant in the cornea that the fish was blind and the eye hardly distinguishable from the rest of the head.

Also repeatedly encountered has been 'pigment spot disease' (melanosis) of marine fishes due to metacercariae. An early record is by Ryder (1884), and it has been reported in herring *Clupea harengus* and other coastal fish on the Atlantic coast of the USA due to infection with *Cryptocotyle lingua* (Sindermann and Rosenfield, 1954a, b). The larvae are located just under the epidermis or occasionally deeper in the tissue, and pigment is deposited around them. In one strongly melanotic *Gadus morhua* with large numbers of metacercariae examined by Hsiao (1941, see above), even in the less densely pigmented

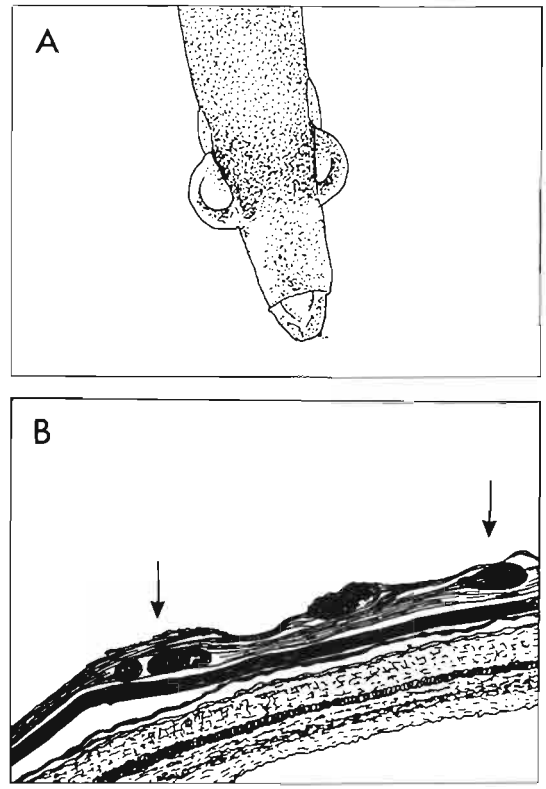


Fig. 1-89: 'Popeye' of herring due to larval trematode invasion. (A) Gross symptoms after 12 days continuous exposure to 100 infected snails. (B) Histological section of eye from fish pictured above; note larval worms in tissues. (Drawn after Sindermann and Rosenfield, 1954b.)

regions, the infected fish had about 6 times as many melanophores mm^{-2} on the head and 6 to 9 times as many on the paired and anal fins as a normal fish. The connective tissue in infected regions was 3 times as thick as normal. Heavy infection with larval *C. lingua* in flounders made the skin appear uneven and dirty (Mawdesley-Thomas and Young, 1967).

Patches of extensive hyperplasia of the surface epithelium were described by Smith (1935) in 2 winter flounders *Pseudopleuronectes americanus* on the North American Atlantic coast. Both fish harboured metacercariae, probably *Cryptocotyle lingua*. No direct evidence was found that the larvae were the cause of the disease, although some cysts were found directly in the lesions.

Reports on general effects by metacercariae on marine fish range from none over light to heavy and leading to death. For example, Abott (1968) reported that unidentified metacercariae in the brain of 25 *Fundulus heteroclitus*, mostly in the optic lobes, caused considerable compression and possible destruction of nervous tissue, but the behaviour of the fish, observed in aquaria, did not appear to be affected. Acording to Paperna (1975), all mullets of commercial size from marine and lagoon catches on Israel markets, in northern Sinai and apparently in Egypt have muscular infections with heterophyid metacercariae (maximum 6000 g^{-1} of muscle tissue). The most heavily infected fish were

also emaciated. In the bull-head *Gobus melanostomus* lipid content of muscles and liver, heavily infected with metacercariae of *Cryptocotyle concavum,* decreased (Shchepkina, 1981).

Massive continuous infection of herring reared with *Cryptocotyle lingua* under experimental conditions may cause death (Sindermann and Rosenfield, 1954a, b). Similarly, 0+ plaice, *Pleuronectes platessa*, experimentally infected with *Stephanostomum baccatum,* showed mortalities (MacKenzie and Liversidge, 1975). Older fish were less susceptible, possibly because of increased epidermal thickness with age, as shown by Roberts and co-authors (1971b). Steele (1966) and MacKenzie (1968) considered natural infections of 0-group plaice with *C. lingua* as contributing to mortalities, and experiments by MacKenzie (1971) showed that 7 of 10, and 4 of 10, plaice died within 10 and 17 days respectively when infected with 100 to 1000 *C. lingua* $fish^{-1}$. None of the control fish died. According to Matthews (1973b), metacercariae of *Bucephalus haimeanus* severely damage the liver of young gobies and plaice. Experimental infection with 50 or more cercariae killed larval plaice within 2 days. According to the review of Millemann and Knapp (1970b), metacercariae of *Nanophyetus salmincola* may cause damage and death to fish in freshwater. Symptoms apparently associated with heavy infections are exophthalmia (see above), lesions in the fins, gills, tail, retina, cornea, etc., as well as damage to muscles and other tissues. Studies of naturally and experimentally infected salmonids showed that different species have different 'sensitivity'. There may be a decrease in swimming activity, loss of equilibrium, drifting, erratic swimming, an increased respiratory rate and a vertical or horizontal tail curvature. Injection of homogenized cercariae showed that toxic substances are probably responsible for the effects.

Trematodes as vectors of microorganisms

The trematode *Nanophyetus salmincola* transmits microorganisms causing salmon poisoning disease of canids. Weiseth and co-authors (1974) recorded metacercariae of the fluke in 31 % of 542 king salmon *Oncorhynchus tshawytscha*, 53 % of 2,049 coho salmon *O. kisutch* and none of 35 pink salmon *O. gorbuscha* caught off the northwestern (Oregon) coast of USA. Various other authors have reported that the parasite is carried into the sea and retained there for several yr (review by Millemann and Knapp, 1970b). However, the infection is acquired in freshwater. Bennington and Pratt (1960) completed the life cycle of *N. salmincola.* First intermediate host in Oregon is the streamsnail *Oxytrema silicula.* Cercariae emerge and penetrate into fish where they encyst to form metacercariae. Millemann and Knapp (1970a, b) reviewed the pathogenicity of the parasite to fish and the biology of the species respectively. Salmonid and some non-salmonid fishes, as well as the Pacific giant salamander, carry the metacercariae, and birds and mammals are final hosts. Snail hosts for the Siberian subspecies *N.s. schikhobalowi* are the freshwater snails *Semisulcospira laevigata* and *S. cancellata.* All stages of the trematode (egg to adult) in North America carry Rickettsia-like organisms, i.e., *Neorickettsia helminthoeca* and EFF (elokomin fluke fever) agent. The 2 species, alone or together, are responsible for salmon poisoning disease of canids (Cordy and Gorham, 1950; Philip and co-authors, 1954; Farrell and co-authors, 1973). However, experimental proof for the contribution of the second species to the syndrome is still lacking.

Agents: Cestoda

Biology

There are about 1,500 described species of cestodes. A taxonomic synopsis of cestodes is authored by Yamaguti (1959) (see also Schmidt, 1970). General accounts on cestodes were given by Braun (1894–1900), Hyman (1951a), Wardle and McLeod (1952), Joyeux and Baer (1961a, b). Tapeworm research in the period 1950–1970 was reviewed by Wardle and co-authors (1974). Smyth (1969) discussed the physiology of cestodes, including the aspects of hatching, infection of hosts, tissue and immunity reactions. Few data on marine forms are given, and in 1964 almost nothing was known of the development or life cycles of marine tapeworms, and few plerocercoids had been described (Vik, 1964). Since then, more knowledge has accumulated, but aspects of marine life cycles are still often based on indirect evidence. Dollfus (1923) reviewed the role of plankton and other marine invertebrates as hosts for cestodes.

Table 1-21 contains data on the hosts of marine cestodes of the USSR. Crustacea are first intermediate hosts, and a second intermediate host is absent or is represented by invertebrates or fishes. Fishes are also important final hosts.

Both orders of Cestodaria, the Amphilinidea and Gyrocotyloidea, have representatives in marine fishes. Not a single life cycle of Gyrocotyloidea is known, and of the Amphilinidea only the life cycle of 1 species from fish, *Amphilina foliacea,* has been described. Freshwater amphipods serve as first intermediate hosts, and final hosts, sturgeon, become infected by ingesting infected amphipods. They retain the infection when migrating into the sea.

Of the Eucestoda, the following orders have been reported from marine fishes: Caryophyllidea, Spathebothriidea, Pseudophyllidea, Diphyllidea, Tetraphyllidea, Lecanicephalidea, Trypanorhyncha and Proteocephalidea.

Most records of Caryophyllidea are from freshwater fish. The only records from marine fish are from coastal brackish areas and include immature forms. Mackiewicz (1972) considers them accidental hosts. Infected freshwater fish may show severe pathological effects.

Of the other orders, only some species of the Tetraphyllidea, Spathebothriidea, Trypanorhyncha and Pseudophyllidea have been studied in some detail with regard to effects on hosts.

Life cycles of Tetraphyllidea are incompletely known. The plerocercoid stage occurs in the intestine of numerous marine fishes, and also in marine molluscs. It has been given the collective name *'Scolex pleuronectis'* or *'Scolex polymorphus'* (Fig. 1-90). Maximum intensity of infection with this larva in pink salmon in the White Sea was 7420 (Grozdilova, 1968). Sometimes, the larvae are red-pigmented (Koratha and Martin, 1962). Friedl and Simon (1970) reported the larva from a marine snail. Marine crustaceans, i.e., copepods, are probably first intermediate hosts (e.g. Reichenbach-Klinke, 1957a). Elasmobranchs, and for a few species teleosts, are final hosts.

Amphipods have been shown to harbour the plerocercoid stage of Spathebothriidea, which mature in fish.

Among the most common marine cestodes are the Trypanorhyncha. Plerocercoid larvae infect fishes and invertebrates, adults the digestive tract of elasmobranchs (Figs 1-91, 1-92). Aspects of the life cycle of the common species *Grillotia erinaceus* were

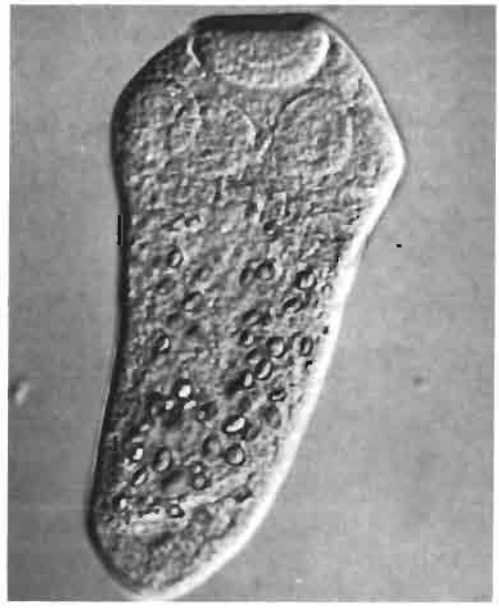


Fig. 1-90: *Scolex polymorphus.* (Original, courtesg R. M. Overstreet.)

Fig. 1-91: Individuals of trypanorhynch larvae (plerocercoids of *Dasyrhynchus* sp.) being cut out from tissue of the crevalle jack. The blastocyst has a long thin tail (caudal extension) and contains the plerocercoid. (After Overstreet, 1978a.)

worked out by Ruszkowski (1934). First intermediate hosts are various species of copepods, in which a procercoid larva develops from the ciliated first larva, the coracidium. Many species of marine fishes in the Atlantic and Pacific Oceans are second intermediate hosts, and elasmobranchs are final hosts. Aldrich (1965) reported observations on the ecology and life cycle of *Prochristianella penaei.* Larvae occur in shrimp, *Penaeus* spp., in the Gulf of Mexico. The life cycle of *Poecilancistrium caryophyllum* is represented in Fig. 1-93.

Infections with trypanorhynchs are sometimes heavy. For example, Linton (1907) reported that 1 butterfish *Prionotus triacanthus* on the North American Atlantic coast had

Table 1-21
Life cycles of cestodes infesting fishes of the USSR (After Ginetzinskaya, 1958; modified)

Species of parasite	First intermediate host	Second intermediate host	Final host
		(A) Fish as final host	
Amphilina foliacea (Rud.)	*Dikergammarus haemobaphes, Gammarus platycheir, G. pulex, Corophium curvispinum* and other spp. (Crustacea, Amphipoda) freshwater	Absent	Acipenseridae (*Acipenser ruthenus* and other spp.) migrate into sea
Eubothrium crassum (Bloch)	*Cyclops strenuus, Eucyclops serrulatus* (Crustacea, Copepoda) freshwater, also marine copepods	*Perca fluviatilis* freshwater	Salmonidae (*Salmo salar, Salmo trutta, Coregonus lavaretus,* etc.) some migrate into sea
Acanthobothrium coronatum (Rud.) (larva – *Scolex pleuronectis* Müll.)	*Calanus finmarchicus* (Copepoda, Crustacea)	*Sardina pilchardus, Engraulis encrasicholus*	*Scylliorhinus canicula*
Diplocotyle nylandica Schneider	*Gammarus locusta, Anonyx pugnax* (Crustacea, Amphipoda)	Absent	*Pleuronects flesus, Gymnacanthus tricuspis*

Bothriocephalus scorpii (Müll.)	*Eurytemora hirundo* (Crustacea, Copepoda)	*Gobius minutus, Gasterosteus aculeatus*	*Pleuronectes flesus, Rhombus maximus, Myoxocephalus scorpius*
Grillotia erinaceus (Van Beneden)	*Acartia longiremis, Pseudocalanus elongatus, Paracalanus parvus, Temora longicornis* (Crustacea, Copepoda)	Various spp. of fish	*Raja clavata, Raja punctata*
Nybelinia lingualis (Cuvier)	Unknown	*Sepia officinalis, Loligo loligo, Octopus vulgaris* (Mollusca, Cephalopoda)	*Raja clavata, Raja punctata, Acanthias vulgaris*
Eutetrarhynchus ruficollis (Eysenh.)	Unknown	*Hyas araneus, Cancer pagurus, Carcinus maenas* (Crustacea, Decapoda)	*Acanthias vulgaris, Mustelus hinnulus*
	(B) Fish as intermediate host		
Diphyllobothrium lanceolatum (Krabbe)	Unknown	*Gadus morhua*	*Erignatus barbatus, Phoca hispida* (Pinnipedia)
Diphyllobothrium schistochilus (Germanos)	Unknown	*Gadus callarias*	*Erignatus barbatus, Phoca vitulina* (Pinnipedia)
Pyramicocephalus phocarum (Fabricius)	Unknown	*Gadus morhua, Melanogrammus aeglefinus*, etc.	*Erignatus barbatus* (Pinnipedia)

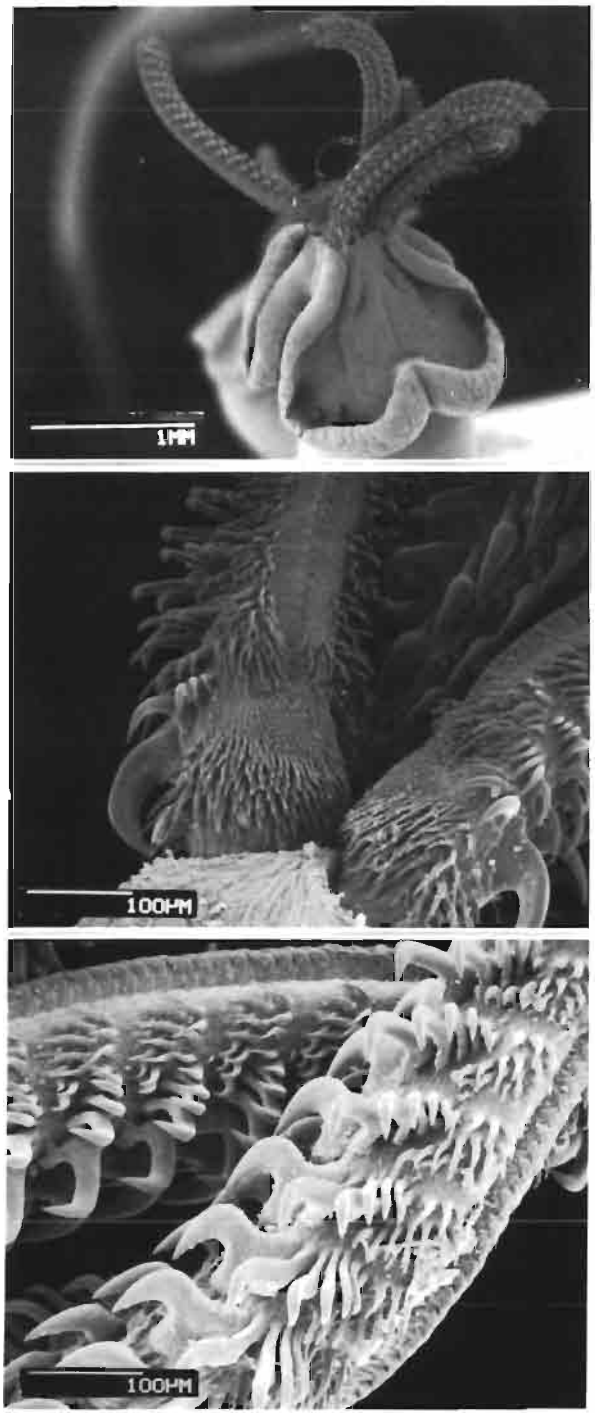


Fig. 1-92: Plerocercoid of *Dasyrhynchus* sp. (Top) scolex; (Middle) tentacles at base showing elaborate hook patterns and morphology; (Bottom) mid-area of a tentacle showing spiralling of hooks and lateral view of chainette (Original, courtesg T. L. Deardorff.)

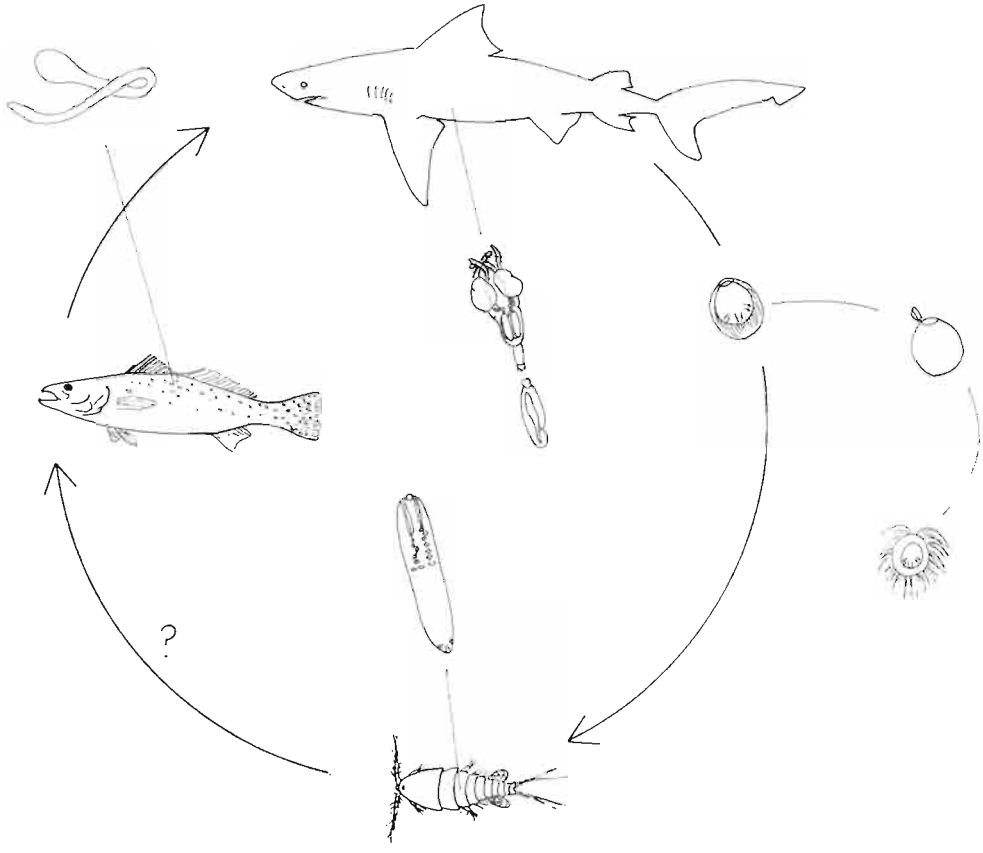


Fig. 1-93: *Poecilancistrium caryophyllum*. Life cycle of the trypanorhynch tapeworm that causes 'wormy trout'. The adult develops in the bull shark (or other related sharks). Released segments from the adult disperse eggs, each with a larva that infects a crustacean which in turn is eaten by a fish. When eaten by seatrout and related fishes, the larva develops further in flesh and becomes infective for sharks. (After Overstreet, 1978a.)

several thousand larvae of *Otobothrium crenacolle*, most densely concentrated in the flesh around the vertebrae (see also Linton, 1897 for records of larval cestodes). According to Reimer (1981), *Leionura (= Thyrsites) atun* off the south western African coast harboured an average of 12.4 *Gymnorhynchus gigas*. In 1 fish, the total length of the plerocercoids was 171 cm, and the average length of the plerocercoids 13.8 cm. Chandler (1954) reported that drum *Pogonius cromis* in the Gulf of Mexico were so heavily infected with trypanorhynch larvae, also called 'spaghetti worm', that authorities considered banning them from marketing. In Victoria and Tasmania, Australia, snoek or barracouta *Leionura atun* often are heavily infected with larval tapeworms *Gymnorhynchus thyrsitae* in the muscles. More than 10 % of the catch frequently has to be discarded. The condition is known as 'wormy couta'. Because of the same infection, there is little demand for the fish in New Zealand (Lester, 1978). Walleye pollock *Theragra chalcogramma* in the Northwestern Pacific Ocean is so heavily infected with larvae of *Nybelinia surmenicola* that it

was deemed unfit for human consumption (Oshmarin and co-authors, 1961). (Consult Sindermann, 1966 and Overstreet, 1977, 1978a, b for further data on trypanorhynchs).

MacKenzie (1975) examined the eyes of 24,000 northern North Sea whiting *Merlangius merlangus* for plerocercoid larvae of *Gilquinia squali.* He found 1 to 18 larvae fish^{-1} and there was apparently no seasonality. The left eye was consistently more frequently infected than the right eye, although the difference was significant only in some groups (Table 1-22). Infection begins when the fish are aged 1+ (fish in second growing season);

Table 1-22
Merlangius merlangus. Observed numbers with single left or right eye infections of *Gilquinia squali,* and results of significance tests (After MacKenzie, 1975)

Year-class of whiting	Age of whiting (yr)	Left eye only	Right eye only	*P*
1967	3+	133	99	<0.05
	4+	150	82	<0.001
	5+	87	75	>0.05
1961	3+, 4+	132	104	>0.05
1962	2+, 3+	332	288	>0.05

it increases to a peak at 2+ to 4+ (fish in fifth growing season) and thereafter decreases. Furthermore, there is a progressively increasing proportion of degenerate immature worms. Both these phenomena may be due to a developing immunity in older fish (see also Overstreet, 1977).

Infection with trypanorhynch larvae sometimes shows marked local differences. Thus, Hislop and MacKenzie (1976) distinguished stocks of whiting *Merlangius merlangus* in the northern North Sea by tagging experiments as well as by the presence of larval *Gilquinia squali.*

Some important studies on the effect of cestodes on host fish have been made on *Eubothrium salvelini* (Pseudophyllidea). Five of the 8 species of *Eubothrium* are exclusively marine, 1 *(E. crassum)* has freshwater as well as marine strains, and 2 (*E. rugosum* and *E. salvelini*) are acquired in freshwater but survive under marine conditions (Kennedy, 1978). Andersen and Kennedy (1983) provided a detailed discussion of the systematics of the genus.

Boyce (1974) demonstrated experimentally that various species of freshwater *Cyclops* (Copepoda) are first intermediate hosts of *Eubothrium salvelini.* Thus, infection of fish is acquired in freshwater, but the parasite survives when salmonid hosts migrate into the sea. The species is largely specific to *Salvelinus alpinus* in Eurasia, but many salmonid species in North America are infected (e.g., Kennedy, 1978). The related species *E. crassum* has freshwater copepods as first intermediate hosts, freshwater perch, *Perca fluviatilis,* as second intermediate hosts, and salmonids as final hosts in Europe (various authors, see Akhmerov, 1962). Akhmerov (1962) presented some circumstantial evidence that marine invertebrates may also act as first intermediate hosts of this species, and Fahy (1980) reported that trout *Salmo trutta* within weeks of first going to sea, accumulate large numbers of *E. crassum.* This led him to the conclusion that there is probably a marine

source of infection, as previously postulated by Kennedy (1978). Infection with certain species shows marked local differences. Thus, prevalence and intensity of infection of *Mallotus villosus* with *Eubothrium parvum* were higher in certain areas of the Barents Sea than in others (Kennedy, 1979).

For some cestodes, consistently low infection intensities have been demonstrated. Simmons and Laurie (1972) reported that infections with 2 individuals of *Gyrocotyle fimbriata* (Gyrocotyloidea) comprised 68 % and with 2 individuals of *G. parvispinosa* 56 % of all infections in *Hydrolagus colliei*. Single-worm infections constituted 21 and 44 % respectively. According to Williams and Halvorsen (1971), 44 of 62 cod and 12 of 24 large cod *Gadus morhua* harboured a single *Abothrium gadi* (Pseudophyllidea). Worms showed a preference for the pyloric openings adjacent to the bile duct opening. The authors suggested that the parasite's ability to limit its population size is not by premunition (prevention of reinfection by the worm already present), because sometimes several worms do co-occur. Instead they suggested that an 'intrinsic' factor prevents crowding and thus allows the worms to control their number in order to derive maximum benefit from the host. A similar mechanism was proposed for *Gyrocotyle* in *Chimaera monstrosa* (Halvorsen and Williams, 1968), but no experimental evidence for the existence of such an 'intrinsic' factor is available. It seems possible that the microhabitat of the host cannot support more than one or a few of the relatively large worms and that additional worms simply die. A small number of parasites may reduce adverse effects on the host, and thus the possibility must be considered that low infection intensities are an adaptation to reducing such effects.

Many cestodes of marine fishes show a distinct host specificity (e.g., Dollfus, 1957; Euzet, 1957; Williams, 1966). Host specificity is sometimes due to toxic substances in the serum. Thus, McVicar and Fletcher (1970) demonstrated that the serum of *Raja radiata* contains a factor which kills *Acanthobothrium quadripartitum,* a cestode specific to another ray, *Raja naevus* (Fig. 1-94). The intestinal and skin mucus of the right host species did not contain a factor harmful to the cestode.

Cestodes often also show marked preference for hosts of a certain age or size. For example, infection of *Raja naevus* with *Echinobothrium harfordi* (Diphyllidea) decreased with host length, 70 to 74.5 % of rays up to 30 cm were infected, but only 0 to 25 % of rays 45 cm and longer. The corresponding mean intensities of infection were 2.2 to 2.9 and 0 to 0.25 (McVicar, 1976). Larval cestodes occur in molluscs and a variety of crustaceans, and the decrease of infection in larger fish is probably due to a change in diet from predominantly crustaceans to fish.

Effects on Hosts

Rees (1967) reviewed pathogenesis of adult cestodes in vertebrates. Few marine examples are discussed, but the account is valuable to the marine biologist as it draws attention to aspects which need to be studied in marine tapeworms. According to the author, infection may have the following effects: (i) Large worms and large numbers of worms may lead to obstruction of the intestine (e.g., *Eubothrium crassum* in salmon); (ii) there may be traumatic and irritative action (e.g., *Parabothrium* and *Abothrium* in *Gadus* spp. cause fibrosis: Williams, 1960; large myzorhynchus of *Discobothrium fallax* in *Raja clavata* causes considerable damage, apparently author's observations; see also Euzet, 1959); (iii) migration to unusual sites may cause damage (no marine examples); (iv) there

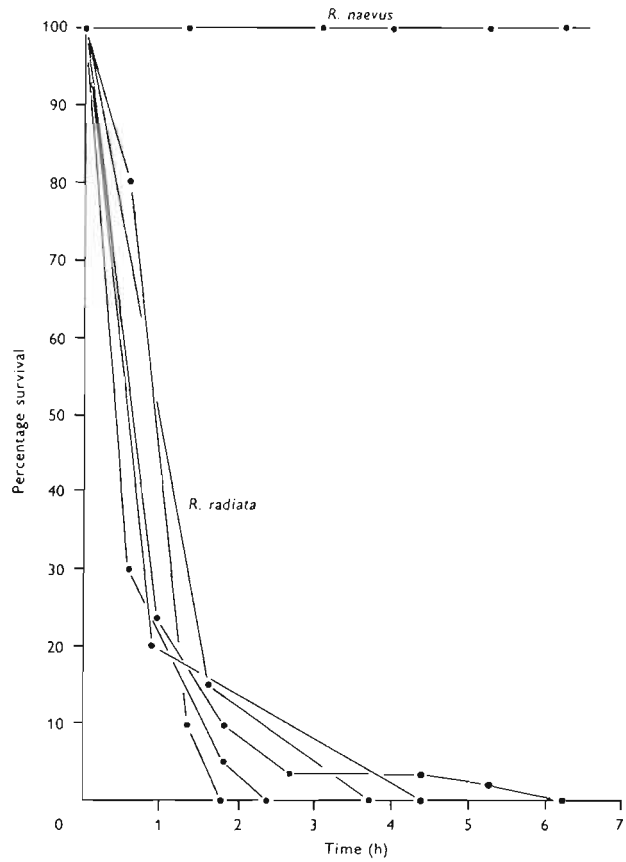


Fig. 1-94: *Acanthobothrium quadripartitum.* Survival in *Raja naevus* and *Raja radiata* serum. (After McVicar and Fletcher, 1970.)

may be pyogenic action (no marine examples); (v) there may be spoliative action (no marine examples); (vi) there may be toxic action (insufficient evidence, no marine examples).

Further according to Rees (1967), with regard to the symptoms caused, infection may lead to retarded growth, loss of weight and emaciation (e.g., *Eubothrium crassum* in salmon), but no marine examples have been studied for gastro-intestinal disturbances, changes in bone marrow and blood, and neurological disturbances. There are also no immunological studies using adult marine cestodes. Rees's conclusions with regard to adult cestodes in general are, that they have little effect on the host and are very rarely pathogenic.

> "As a general rule, in the absence of pathogenicity among adult cestodes a distinction must be made between infestation and disease. The latter is evidence of occasional maladjustment between parasite and host. This may be caused by excessive numbers resulting in the mechanical blocking of the intestine or of ducts, by traumatic and inflammatory action in rare instances . . .". "Cestodes may also deprive the host of some essential constitutents in the diet (p. 15)."

There are some early reports on the effects or the absence of effects of tapeworm infections on hosts which either do not give names of the parasites, or deal with multiple infections, or give insufficient quantitative data. For example, Matsui (1949) claimed a correlation between degree of infection with cestodes and *Ichthyobdella*, and the reduction in fat content of *Gadus macrocephalus* from one locality. However, only 47 fish of a length ranging from 44 to 97 cm were examined and the nematode burden was only estimated. Copepod infections were given only as + or –. The conclusion is therefore not acceptable.

Some careful studies have shown various degrees of damage due to adult and larval tapeworms. Rees and Williams (1965) described attachment of the tetraphyllidean *Acanthobothrium coronatum* in the spiral valve of *Scyliorhinus stellaris*. The hooked scolex is embedded vertically in narrow clefts of the mucosa, leading to a cylindrical cavity around the scolex. At the base of the cleft, the epithelium is torn. The epithelium lining the cavity is destroyed and small haemorrhages occur. The cavity is eventually lined by the stratum compactum but no inflammation results and the damage remains localized.

According to Williams (1968), the scolex of *Acanthobothrium quadripartitum* appears to have little destructive effect on the intestine of the ray *Raja naevus* although it adheres very closely to the mucosa. Each bothridium attaches to small areas of a villus causing a distinct imprint indicating the size and form of each loculus. Some epithelial cells are damaged by the prongs of the bifid hooks.

There are few studies on pathological effects due to helminths using the electron microscope. McVicar (1972) examined the ultrastucture of the parasite-host interface of 3 tetraphyllidean tapeworms in the intestine of *Raja naevus*. Attachment of a tetraphyllidean to the mucosa of a ray is diagramatically shown in Fig. 1-95. As observed earlier by

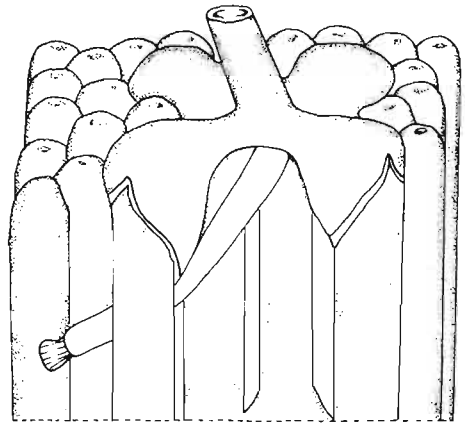


Fig. 1-95: *Pseudanthobothrium hanseni* attached to gut of *Raja radiata*. Scolex *in situ* on mucosa. (Redrawn after Williams, 1966.)

Williams (1968), *Acanthobothrium quadripartitum* does not appear to cause marked harmful effects. On the other hand, *Echeneibothrium* sp. and *Phyllobothrium piriei* severely damage the host. The only effect due to the first species was a compression of the microvillous border of the mucosal cells, and numerous gaps, mainly intercellular, just outside the area where the rim of the bothridium pinched deeply into the villus.

Echeneibothrium sp. completely destroyed the microvillous border of the gut and the outer limiting membrane was ruptured, in many places by the spines of the bothridia. Accumulation of membrane-bound electron-dense material was observed in such cells. Near the myzorhynchus the intestinal wall was most strongly damaged, the mucosa in contact with the apical pad being reduced to a homogeneous mass. *P. piriei* caused stunted and generally abnormal appearance of the microvilli to complete absence of microvilli (Fig. 1-96). Furthermore, its small bothridial spines ruptured the external membrane of the cells.

In the experiments of Rosenthal (1967), 10 to 15 % of herring larvae *(Clupea harengus)* harboured *Scolex pleuronectis* (larval Tetraphyllidea), but mortality due to the parasite was not observed. Only 1 larva with 8 parasites showed 'staggering' swimming movements. A similar species, classified as *S. polymorphis*, appears to be highly pathogenic to young mullet in Mississippi, when present in large numbers. As many as 4500 specimens were observed in the intestine of small mullet. Heavily infected fish did not have obvious food in their intestines (in aquaria, heavily infected mullet did not feed well and died; Paperna and Overstreet, 1981).

Munson (1974) made some observations on the effect of infection with *Spathebothrium simplex* (Spathebothriidea) in the intestine of the 'seasnail' *Liparis atlanticus*. Damage was light when the infection intensities were light, but in heavy infections the worms flattened the mucosa and eroded its brush border. No change in the number of goblet cells was noted.

Nakajima and Egusa (1972) introduced 20 larval trypanorhynchs *Callotetrarhynchus* sp. into the stomach of each of 35 sharks *(Triakis scyllia)*. Twelve sharks died during the experiment. Dying sharks always showed abnormal movements a few days before death and a severe intestinal inflammation was observed at autopsy.

With respect to larval trypanorhynchs, Linton (1912) found tapeworm larvae in the majority of 2000 butterfish *Prionotus triacanthus* examined. He never saw any signs of inflammation or secondary bacterial infections. However, a detailed pathological examination was not made.

Suzuki and Oishi (1974) examined the effect of infection with larvae of *Anisakis* sp. and *Nybelinia surmenicola* on the condition* of *Theragra chalcogramma*. There did not seem to be any effect.

Seyda (1976) described massive infections of *Brama raii* with larval *Gymnorhynchus gigas* off the Northwestern African coast. Thirty-one out of 33 fish were infected with a total of 170 larvae. Maximum intensity of infection was 17 worms; 72 % of the parasites were recovered from the ventral part of the body. Large numbers of worms caused 'a clear deterioration' of the consumptive value of the fish by loosening the tissue, but no histological examination was made. Shaharom and Lester (1982) described the larval trypanorhynch *Grillotia branchi* from the gill arches of *Scomberomorus commerson* in eastern Australia. Developmental stages were observed within and outside the blood vessels. Thousands of fully developed blastocysts were in the arches. Around the cysts was black pigment, and histological sections showed that the cysts filled most of the cavity in the arch. Fibrous tissue was found around the cysts, and there was extensive cellular

* $\frac{\text{Weight (g)}}{\text{Standard length (cm)}^3} \times 10^3$

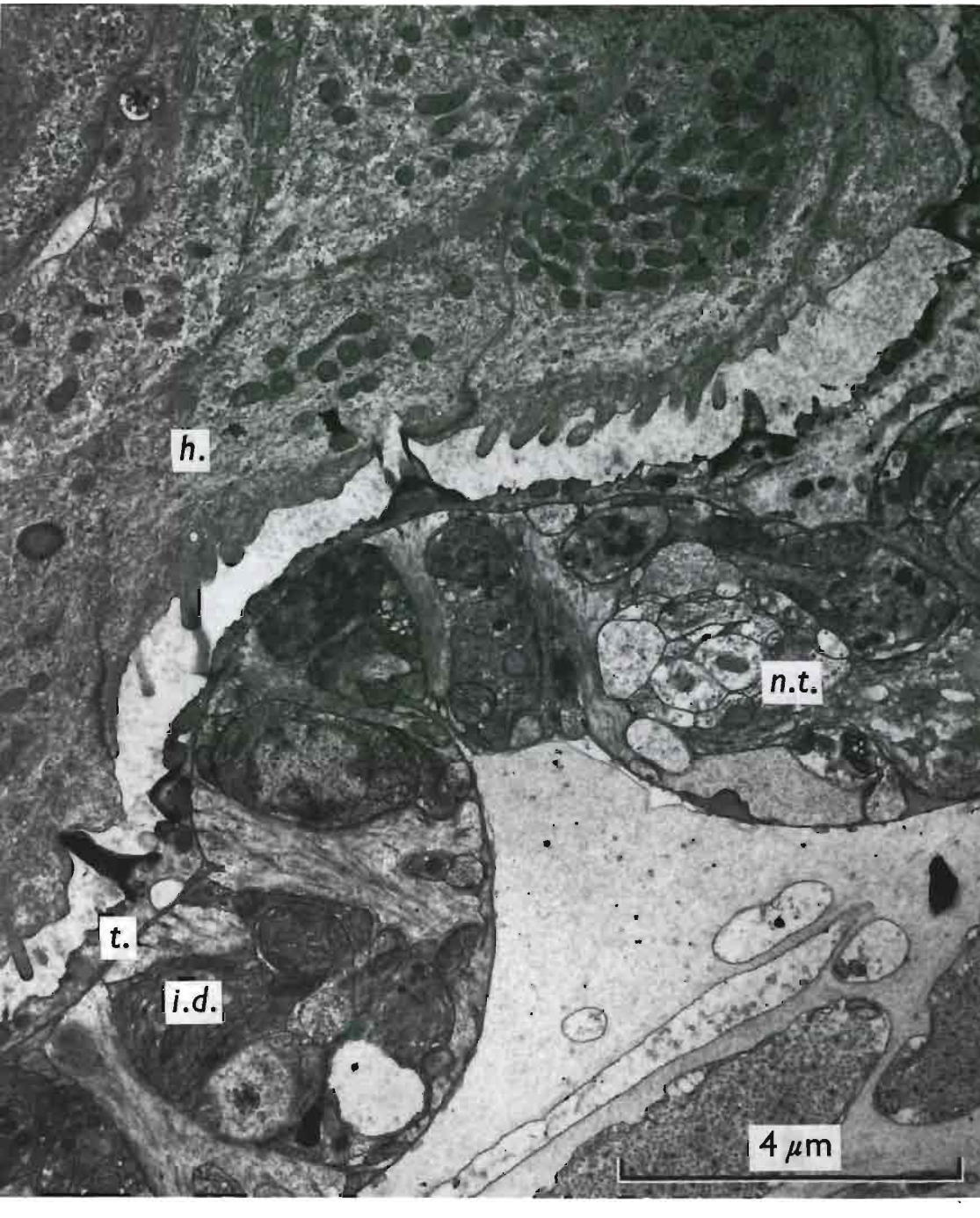


Fig. 1-96: *Phyllobothrium piriei.* Effect on mucosa of *Raja naevus.* t. = tegument of cestode; h. = host tissue; i.d. = intramyofibrillar dense material; n.t. = nerve tract. (After McVivar, 1972.)

infiltration suggesting inflammation. The bone of the gill arch adjacent to cysts was eroded, and 'masses of brown material . . . were evidently haemaglobin breakdown products from earlier sites of haemorrhage' (Fig. 1-97). Overstreet (1977) suggested that larval *Poecilancistrium caryophyllum* may cause death of juvenile fish, as indicated by the probably harmful sites occupied by the worms, but adult fish apparently are not harmed.

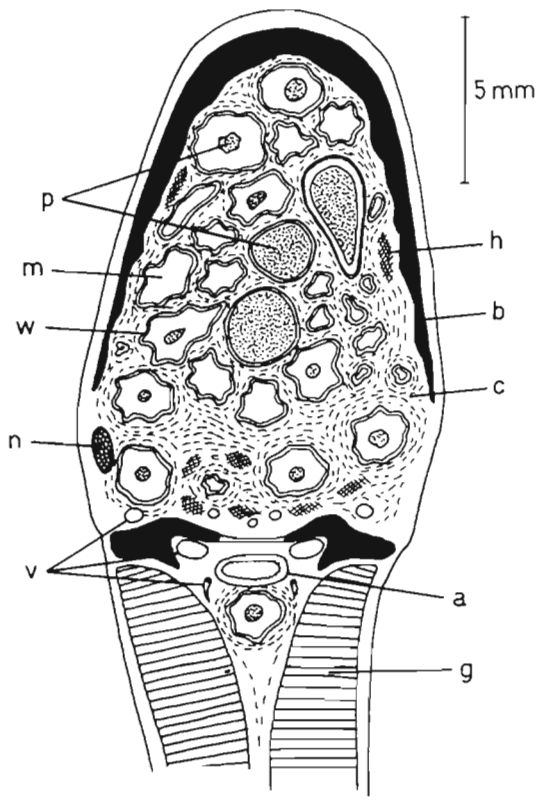


Fig. 1-97: *Scomberomorus commerson* infected with larval *Grillotia*. Diagram of section of gill arch showing metacestodes (p), gelatinous matrix (m), blastocyst walls (w), and areas of haemorrhage (h), Other symbols: a = afferent artery; b = bone; c = connective tissue; g = gills; n = nerve; v = blood vessels. (After Shaharom and Lester, 1982.)

Some authors have reported that certain species of Pseudophyllidea have little effect on their hosts. According to Rees (1958), *Bothriocephalus scorpii* and *Clestobothrium crassiceps* exert little effect on the mucosa of their hosts. *Bothrimonus* which lives less than 1 yr and may reach high infection intensities (maximum 1482 specimens in *Coregonus autumnalis*), did not lead to noticeable irritation in the intestine of *Pseudopleuronectes americanus*, although this host species also was sometimes heavily infected (Sandeman and Burt, 1972).

In certain pseudophyllidean species, the anterior end degenerates to form a so-called *scolex deformatus*. In *Parabothrium gadipollachii*, the *scolex deformatus* is approximately 13 to 15 mm long, 3 to 3.5 mm wide, with a bulbous swelling 3.5 to 4.0 mm wide near its base. The swelling probably has the function of wedging the *scolex deformatus* inside the

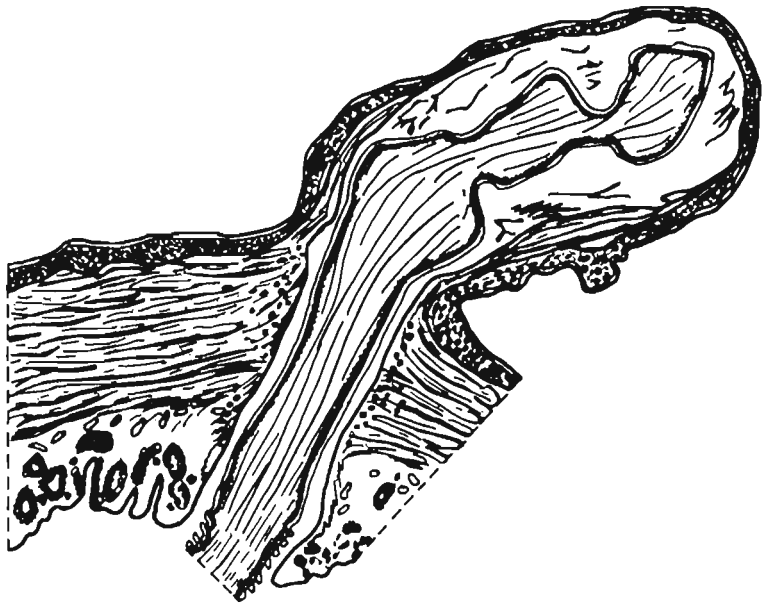


Fig. 1-98: *Parabothrium gadipollachii.* Section through the *scolex deformatus* embedded in the wall of the intestine. (After Williams, 1968.)

caecum (Fig. 1-98). In an advanced state of parasitism, the wall of the caecum disappears completely and the anterior end of the cestode protrudes into the body cavity (Williams, 1968).

In another pseudophyllidean, *Abothrium gadi,* also infecting gadid hosts, a well-developed *scolex deformatus* is not present, but some degeneration of the anterior end occurs (Fig. 1-99) (Williams, 1968). Both in infections with *Parabothrium gadipollachii* and *A. gadi,* the intestinal wall becomes damaged and discoloured. Whereas the serosa and longitudinal muscles, the 2 outermost layers of the wall, are apparently not affected, the next layer, i.e., a layer of circular muscles, undergoes extreme fibrosis. The following layer could not be identified because of extreme fibrosis, and of the mucosa and submucosa, the latter undergoes much fibrosis (Fig. 1-99).

Detailed studies have been made on the effect of *Eubothrium salvelini* on salmon. Susceptibility of salmon fry to *E. salvelini* (Pseudophyllidea) decreases with size of the fish (Boyce, 1974) (Fig. 1-100). Smith (1973) found no direct evidence that the parasite causes death of juvenile sockeye salmon, though several observations suggested that it may occur. Thus, a decline in overall infection intensity and changes in mean-size relations of infected and uninfected fish in consecutive sample periods could have resulted from mortality among heavily infected small fish. No obvious tissue damage, but complete blockage of the gut due to many worms was seen.

Mature worms infected about 30 % of smolts of the sockeye samon *Oncorhynchus nerka* in Babine Lake, British Columbia, and according to Dombroski (1955), infected smolts were smaller than uninfected ones (see also Smith, 1973; Smith and Margolis, 1970). However, observations of naturally infected fish could not rule out the possibility that the infection is not the cause of retarded growth but merely associated with it, for

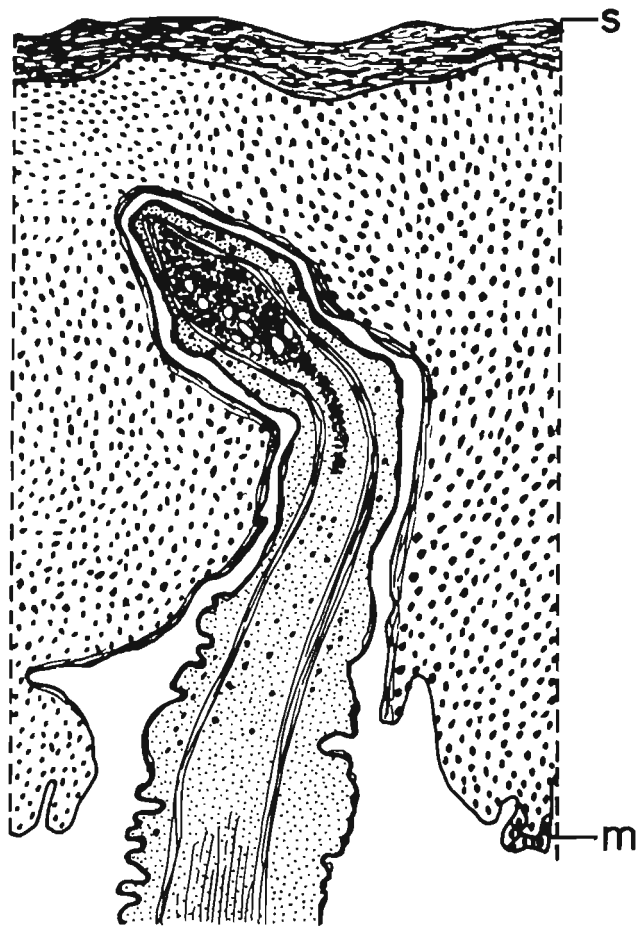


Fig. 1-99: *Abothrium gadi* from haddock, saggital section through anterior end embedded in the wall of the intestine. m = mucosa; s = serosa. After Williams, 1968.)

instance by making smaller and weaker fish more receptive to the infection. Boyce (1979), therefore, studied the effects of the tapeworm on the growth and vitality of sockeye salmon experimentally. He could show that infection affected survival in 1 of the 3 experimental groups (Fig. 1-101), growth in all 3 groups (example in Figs 1-102 and 1-103), and stamina as measured by speed of current against which fish could swim, in all 3 groups (Fig. 1-104). Differences between infected and uninfected fish were significant. Furthermore, sockeye salmon without the parasite were shown to be more resistant to zinc than infected smolts (Boyce and Yamada, 1977). There is little evidence that adult fish are affected by *Ebothrium salvelini* or the related *E. crassum* (Smith, 1973).

Mikhailova and co-authors (1964) examined tissue reactions to larval pseudophyllideans. Plerocercoids of *Pyramicocephalus phocarum* in the liver of haddock *Melanogrammus scorpius* and cod *Gadus morhua* led to proliferative reactions in the liver. Larvae were surrounded by connective tissue or they were in direct contact with the liver parenchyma. Three layers around the larvae could be distinguished: (i) an interior layer consisting of

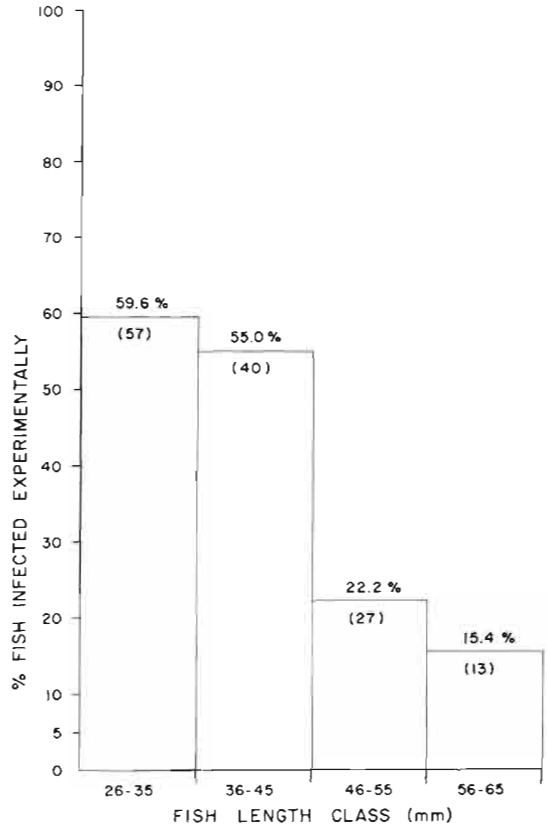


Fig. 1-100: *Oncorhynchus nerka* fry. Susceptibility to experimental infection with *Eubothrium salvelini* vs. size. Numbers in parentheses: sample size. (After Boyce, 1974.)

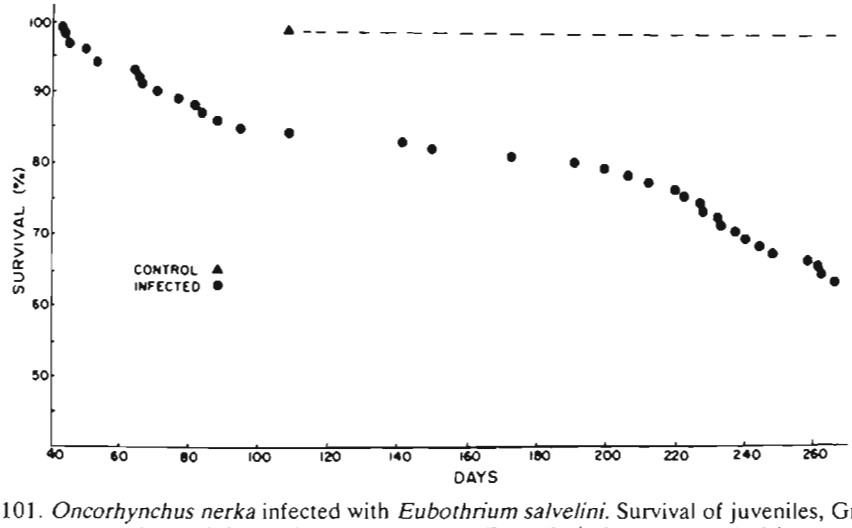
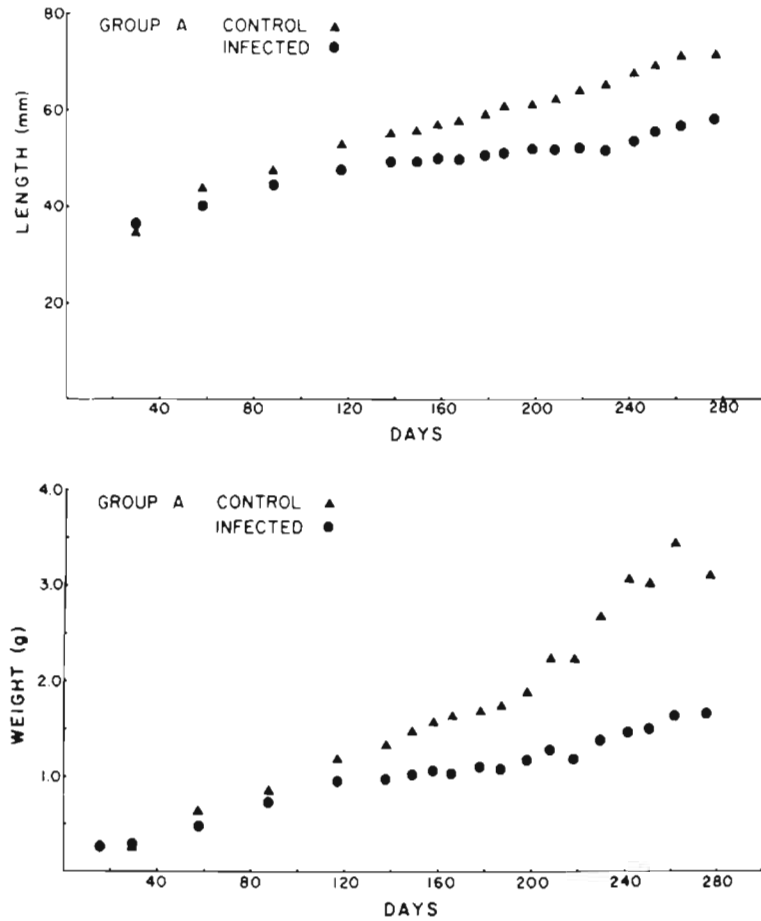


Fig. 1-101. *Oncorhynchus nerka* infected with *Eubothrium salvelini*. Survival of juveniles, Group A, controls and infected from postexposure Day 40. (After Boyce, 1979.)



Figs 1-102 and 1-103: *Oncorhynchus nerka* infected with *Eubothrium salvelini*. Growth of juveniles. (After Boyce, 1979.)

damaged connective tissue and liver cells; (ii) a middle layer consisting of porous connective tissue with many blood vessels; (iii) an outer layer consisting of relatively little changed liver tissue.

Reactions to plerocercoids of *Bothriocephalus* sp. in the mesentery of arctic char *Salvelinus alpinus* led to capsules around the parasites infiltrated by large numbers of leucocytes. According to Bauer and co-authors (1977), disease due to *B. scorpii* is of the greatest significance in Black Sea and Azov Sea plaice and turbot. First hosts of the tapeworm are crustaceans, and second hosts are fish. Forty-five species of marine fish, including plaice, bullhead and catfish, serve as final hosts. In the Black and Azov Seas, infection begins in the littoral zone in yearlings which have taken up the benthic mode of life (62 % of yearlings infected with 1 to 17 worms). Almost all old fish are infected (10 to 150, usually 20 to 50 worms fish^{-1}). In some fish the gut is blocked and it is possible that the most heavily infected fish die, although this is difficult to determine in the sea. In the Barents Sea, bullheads become infected when they begin predation. In the Atlantic Ocean,

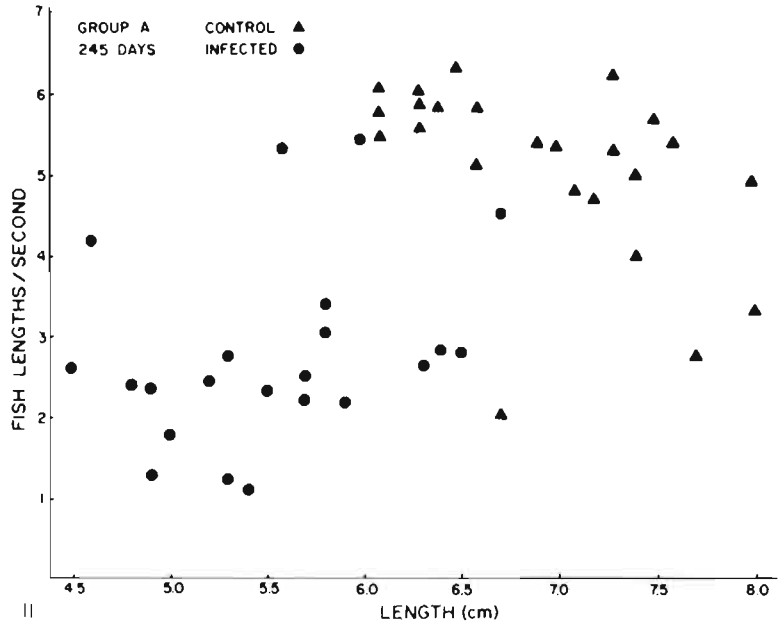


Fig. 1-104: *Oncorhynchus nerka* infected with *Eubothrium salvelini*. Critical swimming speeds of infected and control fish tested on postexposure day indicated. (After Boyce, 1979.)

virtually 100 % of Black Carp are infected. The parasite has also been found in the Mediterranean, Baltic, North, Laptev and Far Eastern Seas. Symptoms and pathogenesis have been little studied. Without giving details or supporting evidence, Bauer and co-authors state that heavy infections lead to reduced growth rate, and haemorrhages in the gut with acute hyperaemia of adjacent areas.

Rees (1969) reported that goblet cells in the intestine of *Synodus intermedius* heavily infected with *Acompsocephalum tortum* (Pseudophyllidea), tended to disappear adjacent to the worms. Where only a few worms were present, a visible effect on the mucosa could not be seen, but large numbers flattened the longitudinal folds of the mucosa apically or pushed them to one side. Columnar cells sometimes showed a slight decrease in height, but mechanical damage was not evident.

Agents: Nematoda

Biology

Nematoda comprise one of the largest phyla in the animal kingdom, with more than 10,000 described species, about half of them parasitic, numerous species remaining to be described. However, detailed studies of the biology have been made mainly of parasites of terrestrial vertebrates. General accounts of Nematoda were given by Yorke and Maplestone (1926), Schuurmans Stekhoven (1937), Chitwood and Chitwood (1950), Hyman (1951b), Chabaud (1965), DeConinck (1965), DeConinck and co-authors (1965), Nigon (1965) and Bird (1971). A general account of the physiology of nematodes has been presented by Lee (1965). It includes data on feeding etc., but there is little reference to

marine forms. Rogers and Sommerville (1963) reviewed the infective stages and the process of infection in nematodes, but not a single marine form is discussed. Nevertheless, many aspects of infection are probably similar in marine species and the review is therefore valuable to the marine biologist. Tissue reactions to nematodes were reviewed, again with no reference to marine species, by Poynter (1966). Useful also is the early review on feeding of parasites by Markov (1946), who discussed many nematodes but refers only briefly to marine helminths. Yamaguti (1961) provided an account of nematode taxonomy, and keys of various groups of marine nematodes were presented by Willmott (1974), Hartwich (1974), Chabaud (1975a, b), Petter (1976), Chabaud (1978) and Anderson and Bain (1982).

Adult nematodes may live in various organs and tissues of marine fishes, but they occur primarily in the digestive tract. Sexes are separate and the fertilized egg cell develops to a first larva. Altogether, 4 moults lead to a 2nd, 3rd, and 4th stage larva and finally to the adult. Life cycles are indirect including either 1 or 2 intermediate hosts. Crustaceans play an eminent role as first intermediate host. For example, among the Habronematoidea, species of *Ascarophis* are common parasites of marine fishes, which serve as final hosts (e. g. Appy, 1981: description of 3 North Atlantic species). Uspenskaya (1953) determined the life cycles of 2 species, *A. filiformis* and *A. morrhuae*, from the digestive tract of fishes in the Barents Sea. Both use decapod crustaceans as intermediate hosts, the former species mainly *Hetairas polaris*, the latter mainly *Pagurus pubescens* (Figs 1-105 and 1-106). Other crustaceans are infected to a lesser degree. Fish become infected by eating crustaceans containing infective larvae. Several species of decapod crustaceans were also

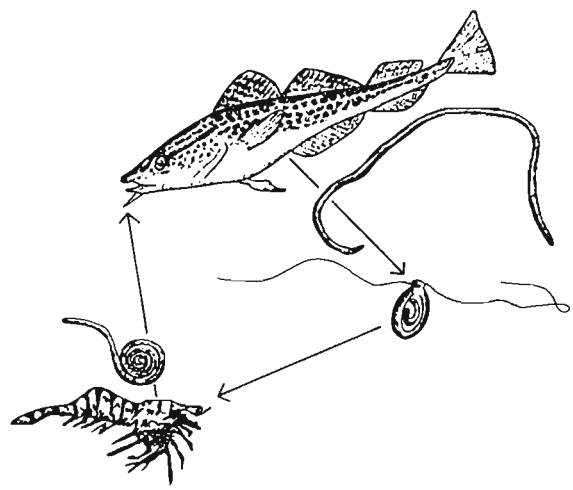


Fig. 1-105: *Ascarophis filiformis*. Life cycle. (Redrawn after Uspenskaya, 1953.)

found to be infected with larval *Ascarophis* on the coasts of California and Washington State (Poinar and Thomas, 1976). Data on nematodes of marine fishes in the USSR show that copepods, amphipods, decapods and other crustaceans act as first intermediate hosts (Table 1-23). Fish are second intermediate hosts which become infected by eating infected first intermediate hosts and are in turn eaten by and pass on the infection to the final hosts, larger predatory fish, birds or mammels. Alternatively they are final hosts.

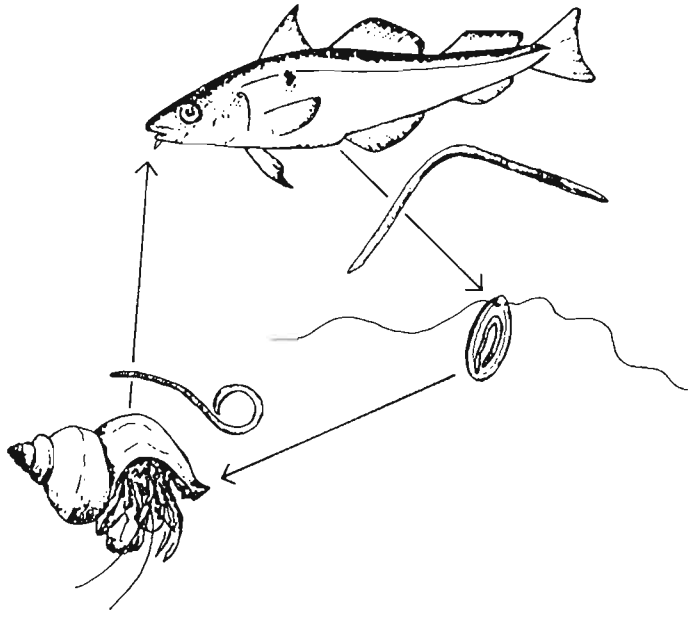


Fig. 1-106: *Ascarophis morrhuae.* Life cycle. (Redrawn after Uspenskaya, 1953.)

Nematodes feed either on food in the intestinal lumen of fish, on host tissue or blood. For example, *Salvelinema walkeri* in the swimbladder of *Oncorhynchus kisutch* may feed on blood (Margolis, 1967), and *Hysterothylacium bidentatum* feeds on the fluid contents of the stomach of fish (Geller, 1975).

Host specificity of marine nematodes varies greatly between different species, but most species are not strictly specific to 1 host species. Thus, data given by Polyansky (1955) for the Barents Sea show that of 12 nematode species, only 1 was restricted to a single host species, whereas 10 occurred in several fish species belonging to 1 or several families.

Taxonomy and Life Cycles of Anisakidae (Ascaridoidea) of Marine Fishes

Anisakids, and particularly larval anisakids, are among the most common nematodes of marine fishes. They affect fish by causing pathological symptoms and possibly mortalities, they reduce the commercial value of fish, and they may infect man producing harmful effects. For all these reasons, anisakids have been studied extensively, but much confusion still exists about their taxonomy and some aspects of their life cycles (for history of research on Anisakidae see Myers, 1976). Because of their importance, the taxonomy and life cycles of anisakids will be discussed in detail.

Important taxonomic reviews of the Ascaridoidea, to which the Anisakidae belong, were given by Hartwich (1954, 1974). The latter paper contains an illustrated key to the genera of Ascaridoidea. Templeman and co-authors (1957) discussed difficulties in identifying larval anisakids, and Myers (1975) gave an illustrated key to the larvae. Important characteristics used for generic distinction are the structure of the anterior part of the digestive tract and the position of the excretory pore (Fig. 1-107). The main features of important genera are illustrated in Fig. 1–108.

Table 1-23
Life cycles of nematodes infesting marine fishes of the USSR (After Ginetzinskaya, 1958; modified)

Species of parasite	First intermediate host	Second intermediate host (= accessory host)	Final host
		(A) Fish as final host	
Contracaecum aduncum (Rud.) Baylis (sp.?)	*Acartia bifilosa, Eurytemora affinis* (Crustacea, Copepoda)	*Gadus morhua, Pleuronectes limanda, Myoxocephalus scorpii,* etc. (about 40 spp. of fishes)	*Gadus morhua, Pollachius virens, Melanogrammus aeglefinus*
Proleptus acutus Dujardin	*Carcinus maenas, Pagurus* sp. (Crustacea, Decapoda)	Absent	*Raja clavata*
Proleptus obtusus Dujardin	*Eupagurus bernhardus, Carcinus maenas* (Crustacea, Decapoda)	Absent	*Scyllium canicula, Acanthias vulgaris*
Eustoma rotundata (Rud.)	*Lithodes* sp. (Crustacea, Decapoda)	Pleuronectidae, Gadidae	*Raja radiata* and other elasmobranchs
Ascarophis morhuae Van Beneden	*Pagurus* sp. and others (Crustacea, Decapoda)	Absent	*Gadus morhua, Melanogrammus aeglefinus*
Ascarophis filiformis Poljansky	*Hetairas polaris, Sclerocrangon* sp. and others. (Crustacea, Decapoda)	Absent	*Gadus morhua, Melanogrammus aeglefinus*
Cuculanellus minutus Rud.	Planktonic invertebrates	Fishes of the family Pleuronectidae (in gut wall)	Pleuronectidae (in lumen of gut)
		(B) Fish as intermediate host	
Contracaecum osculatum baicalensis Mosgovoj and Ryjikow	*Macrohectopus branickii* (Crustacea, Amphipoda)	*Cottocomephorus grewingki*	*Phoca sibirica* (Pinnipedia)
Pseudoterranova decipiens (Krabbe)	Crustacea, Copepoda, Isopoda, Mysidacea, Decapoda	Many spp. of marine and freshwater fishes	Piscivorous mammals (Pinnipedia)

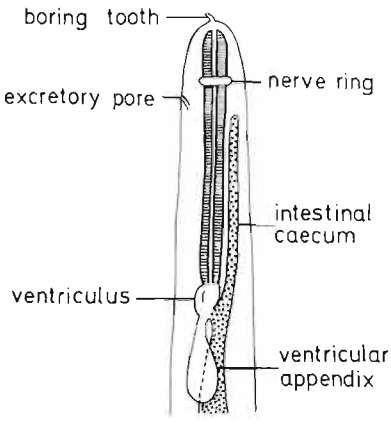


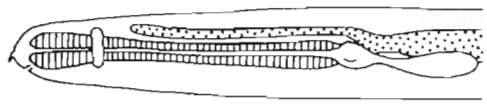
Fig. 1-107: Anterior digestive tract of larval anisakines. (After Myers, 1975, modified.)

Oishi and Hiraoki (1971) reviewed *Anisakis* and anisakiasis, Cheng (1976) reviewed the biology and life cycles of anisakids, and various aspects of anisakiasis, including the taxonomy of *Anisakis*, were discussed by van Thiel (1976) and Smith and Wootten (1978). Oshima (1972) reviewed anisakiasis and *Anisakis* in Japan, with a list of 247 references, and Margolis's (1970b) discussion of nematode diseases of marine fishes includes a detailed section on larval and adult anisakids in marine fishes.

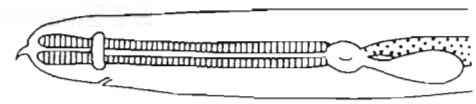
Larval anisakids in fish belong mainly to the genera *Hysterothylacium, Contracaecum, Anisakis, Porrocaecum, Phocanema, Raphidascaris* and *Terranova*. There may be several hundred larvae in 1 fish, and many fish species act as hosts. For example, Berland (1961a) found only 9 of 64 fish species (260 specimens) in Norwegian marine waters free of nematodes, and most widespread were larval Anisakidae of several genera. Cannon (1977) examined 123 species of marine fishes in southeastern Queensland, Australia, and found anisakid nematodes in 47 of them.

Deardorff and co-authors (1982) surveyed 134 species of fishes and 8 species of invertebrates caught near the Hawaiian Islands over a 26-month period and recovered 21,746 ascaridoid larvae of the genera *Anisakis* (2 types), *Hysterothylacium* (3 types), *Raphidascaris* (1 type) and *Terranova* (2 types), from fish. Only some further examples of papers that give data on anisakid larvae in fish can be cited here, e.g., Kahl (1940), Dollfus (1953), Skrjabin (1953), Bishop and Margolis (1955), Templeman and co-authors (1957), Grainger (1959), Shulman (1959), Yamaguti (1961), Tolgay and Tolgay (1965), Okumura (1967), Otsuru and co-authors (1968), Oishi and co-authors (1969), Otsuru and co-authors (1969), Petter (1969), Dollfus (1970), Kagei and co-authors (1970, 1971), Muravev (1970), Valter (1970), Parsons and Hodder (1971), Priebe (1971a, b), Sakaguchi and Katamine (1971), Oshima (1972), Reimer and Jessen (1972), Velasquez (1972), Berland (1973), Rokicki (1973), Sasaki (1973), Shiraki (1974), Sluiters (1974), Suzuki and Oishi (1974), Ono (1975a, b), Platt (1975), Soleim (1976), Cannon (1977), Grabda (1978), Torres and co-authors (1978), Wootten (1978), Carvajal and co-authors (1979), MacKenzie (1979), Myers (1979), Valter (1979), Holloway and Spence (1980), Sakaguchi and co-authors (1980), Siegel (1980), Dailey and co-authors (1981), Deardorff and Overstreet (1981b). Some further references will be given for individual genera. Unfortunately, the taxonomic status of some of the worms recorded is unclear, for instance

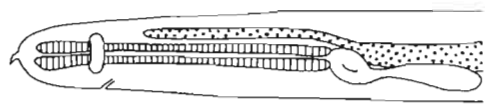
Contracaecum
(Marine mammals and birds)
Intestinal caecum and ventricular appendix present, excretory pore opening at the base of the subventral lips.



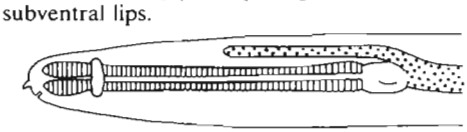
Iheringascaris (Fish)
Hysterothylacium (Fish)
Intestinal caecum and ventricular appendix present, excretory pore opening at the level of the nerve ring.



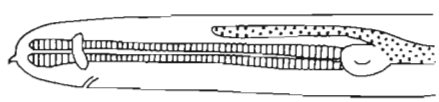
Raphidascaris (Fish)
Intestinal caecum absent, ventricular appendix present, excretory pore at the level of the nerve ring.



Pseudoterranova (= Phocanema)
(Marine mammals).
Terranova (Elasmobranchs, teleosts and reptiles).
Intestinal caecum present, ventricular appendix absent, excretory pore opening at the base of the subventral lips.



Porrocaecum (Birds, fish)
Intestinal caecum present, ventricular appendix absent, excretory pore opening at the level of the nerve ring.



Pseudanisakis (= Anacanthocheilus)
(Marine fish)
Anisakis (Marine mammals). Intestinal caecum and ventricular appendix absent, excretory pore opening at level of nerve ring.

Fig. 1-108: Generic characteristics of important larval anisakines from marine fishes. (After Myers, 1975, modified). The genus *Phocanema* has been suppressed in favour of *Pseudoterranova* for species in marine mammals by Gibson and Colin (1982); detailed reasons have not yet been published. The genus *Terranova* is retained for species in elasmobranchs, teleosts, and reptiles.

Contracaecum osculatum in the Baltic Sea has frequently been misidentified as *Hysterothylacium aduncum* (p. 284).

Anisakis. Davey (1971) and Smith and Wootten (1978) discussed the taxonomy of the genus *Anisakis*. The former author recognized only 3 valid species, i.e., *A. simplex, A. typica* and *A. physeteris* and 4 species inquirendae, i.e., *A. dussumierii, A. schupakovi, A. alexandri,* and *A. insignis*. According to the latter authors, some problems still remain, especially those concerning difficulties in distinguishing *A. simplex* and *A. typica*.

Various authors have distinguished a number of types of third-stage larvae of *Anisakis* (see review by Smith and Wootten, 1978, further references therein). For example, Japanese workers distinguished Types I-IV. The types differ in length of the body and some organs and body parts, as well as in their structure. Type I appears to be the larva of *A. simplex* (Pippy and van Banning, 1975; further references in Smith and Wootten, 1978).

According to Oshima (1972), the life cycle of *Anisakis simplex* in the North Pacific Ocean is as follows (Fig. 1-109). Final hosts are cetaceans, i.e., *Balaenoptera borealis,*

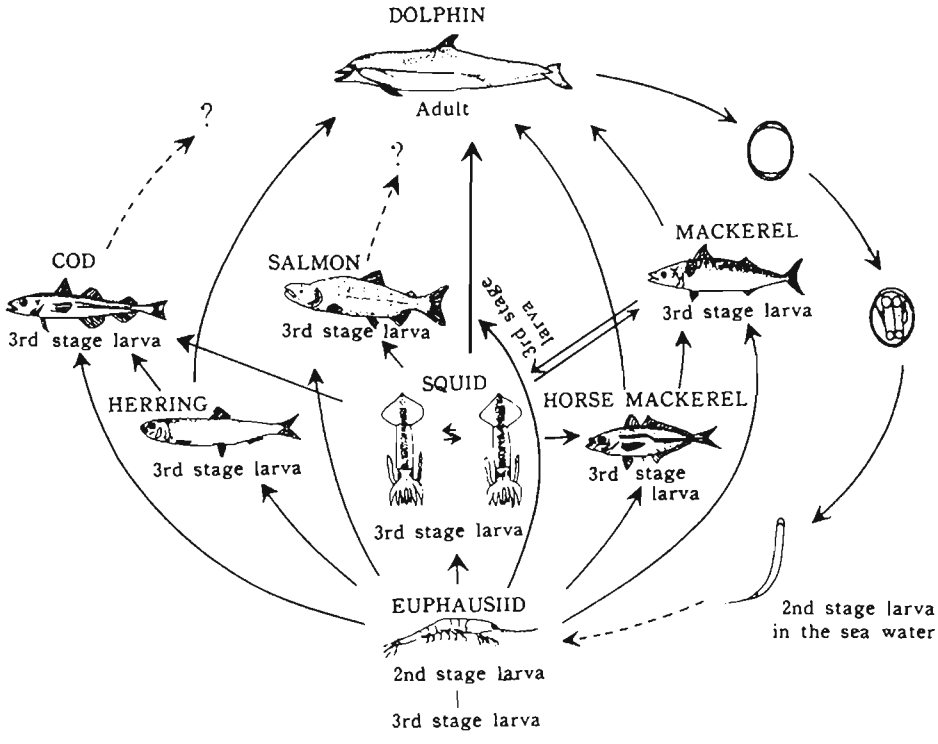


Fig. 1-109: *Anisakis simplex*. Life cycle. (After Oshima, 1972.)

Stenella caeruleoalba, Phocaena phocaena, Phocoenoides dalli, Physeter catodon, Steno bredaenensis, Globicephalus scammoni, Callorhinus ursinus, and probably *Tursiops gilli.* Pinnipeds are mainly hosts to *Terranova decipiens* and *Contracaecum osculatum* and do not play a significant role as hosts of *Anisakis*. Eggs leave the host in the faeces and develop at temperatures ranging from 2 to 27 °C. At 2 °C they hatch within 40 days. Hatched second-stage larvae are ensheathed in the cuticle of the first larva. Eggs and larvae are slightly heavier than sea water and sink gradually to the bottom. While sinking, they are dispersed by waves and currents. Larvae are eaten by various euphausiids, in which exsheathment occurs within 8 days. Moulting to the third larval stage occurs before they reach 6 mm in length. Larvae longer than 18 mm can survive in fish and squid when ingested and penetrate through the wall of their digestive tract. Larvae can be repeatedly passed on to other squid or fish without further moults, i.e. such squid or fish act as paratenic hosts. Gradually, larvae are concentrated in several large predatory fish species.

In British and adjacent waters, Cetacea also are the most common hosts of adult *Anisakis* (Young, 1972), but no *Anisakis* larvae were found by Kagei and Kureha (1970) in 3 species of whale (*Balaenoptera* spp.) from Antartica. Further references and a discussion of the life cycle of *Anisakis* can be found in van Thiel (1976), Cheng (1976) Smith and Wootten (1978). Although Euphausiacea appear to be the most important first intermediate hosts, (e.g., Smith, 1983) other planktonic crustaceans may also become infected (see review by van Thiel, 1976; Smith and Wootten, 1978). Smith and Wootten (1978) have pointed out that experimental proof concerning the hosts in which some

moults occur, is still lacking. Kulachkova (1978) showed that herring *Clupea harengus pallasi* n. *maris-albi* harbours stage II and III larvae as well as larvae moulting from III and IV. Larvae in herring not only grow, as indicated by measurements of stage III larvae, but they also undergo organogenesis. Herring is thus not a paratenic but a genuine intermediate host. According to Kulachkova (1980), circumstantial evidence suggests that larvae can live in the fish for about a year. However, evidence by others indicates that they can live for several years (Margolis, pers. comm.).

Hysterothylacium and Iheringascaris. Species now included in the genus *Hysterothylacium* were previously referred to as *Contracaecum* (occasionally *Contracoecum*) and *Thynnascaris*. Deardorff and Overstreet (1981a) established that most species that mature in fish are species of *Hysterothylacium*. One species, *inquies* (junior synonym *iheringascaris*) was placed in the genus *Iheringascaris,* and 8 species of adult and 12 species of larval *Contracaecum* were considered to be of uncertain status but possibly or probably referable to *Hysterothylacium*. In the following the species of *Hysterothylacium* parasitizing fishes are listed under their original names:

Ascaris adunca Rudolphi, 1802; *Ascaris aucta* Rudolphi, 1802; *Ascaris bidentata* Linstow, 1899; *Ascaris clavata* Rudolphi, 1809; *Ascaris cornuta* Stossich, 1904; *Ascaris fabri* Rudolphi, 1819; *Ascaris gadi* Müller, 1776; *Ascaris habena* Linton, 1900; *Ascaris increscens* Molin, 1858; *Ascaris incurva* Rudolphi, 1819; *Ascaris marina* Linnaeus, 1767; *Ascaris rigida* Rudolphi, 1809; *Contracaecum amoyensis,* Hsü, 1933; *Contracaecum arii* Yamaguti, 1954; *Contracaecum assi* Parukhin, 1973; *Contracaecum baylisi* Yamaguti, 1941; *Contracaecum chaunaxi* Olsen, 1952; *Contracaecum coiliae* Yamaguti, 1941; *Contracaecum (Simplexonema) cyclopteri* Kreis, 1952; *Contracaecum epinepheli* Yamaguti, 1941; *Contracaecum fortalezae* Klein, 1973; *Contracaecum gracile* Yamaguti, 1935; *Contracaecum hapalogenyos* Yamaguti, 1961; *Contracaecum histiophori* Yamaguti, 1935; *Contracaecum ilishae* Yamaguti, 1941; *Contracaecum longispiculum* Fujita, 1940; *Contracaecum magnum* Smedley, 1934; *Contracaecum melanogrammi* Smedley, 1934; *Contracaecum melichthysi* Olsen, 1952; *Contracaecum murrayense* Johnston and Mawson, 1940; *Contracaecum ogcocephali,* 1952; *Contracaecum okadai* Fujita, 1940; *Contracaecum pagrosomi* Yamaguti, 1935; *Contracaecum paralichthydis* Yamaguti, 1941; *Contracaecum rectum* Yamaguti, 1961; *Contracaecum saba* Yamaguti, 1941; *Contracaecum salvelini* Fujita, 1940; *Contracaecum scomberomori* Yamaguti, 1941; *Contracaecum seriolae* Yamaguti, 1941; *Contracaecum trichiuri* Thwaite, 1927; *Contracaecum zenis* Baylis, 1929; *Contracaecum zenopsis* Yamaguti, 1941; *Thynnascaris carangis* Kalyankar, 1971; *Thynnascaris dollfusi* Schmidt, Leiby, and Kritsky, 1974; *Thynnascaris haze* Machida, Takahashi, and Masuuchi, 1978; *Thynnascaris reliquens* Norris and Overstreet, 1975; *Thynnascaris rhacodes* Deardorff and Overstreet, 1979.

Contracaecum remains a valid genus comprising species that mature in mammals and birds. Soleim and Berland (1981) made a detailed light- and scanning-microscopic study of *Hysterothylacium aduncum* (see also Soleim, 1974). In spite of its common occurrence, even the life cycle of *H. aduncum* is incompletely known. Based on their own observations and those of other authors, Norris and Overstreet (1976) outlined the likely life cycle of the species as follows (Fig. 1-110) (see also Fagerholm, 1982): Eggs passed from the definitive host embryonate on a substratum. Second stage larvae hatch and are free living, until they are eaten by either an invertebrate or a fish. Within a suitable intermediate host, development proceeds to the third, and occasionally fourth stage, either of which is

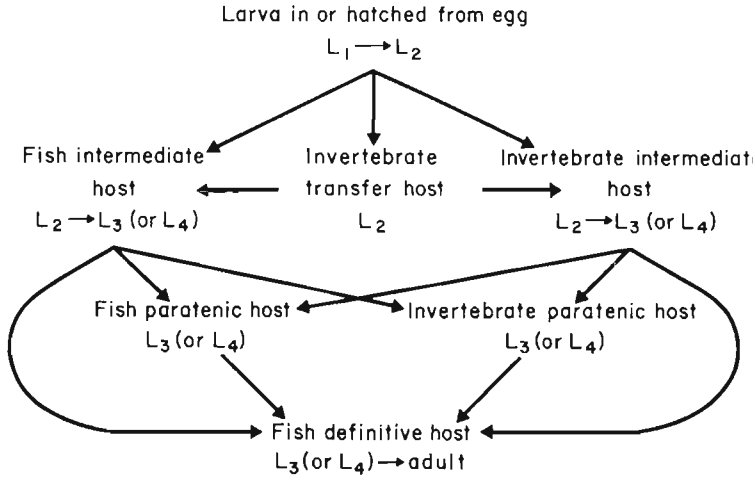


Fig. 1-110: *Hysterothylacium.* Diagrammatic life-cycle of species belonging to this genus. (After Overstreet, orig. reproduced by permission of Overstreet.)

infective to the definitive host. Certain other invertebrates, apparently unsatisfactory as intermediate hosts, act as transport hosts in which no development occurs. The larvae remain infective for a true intermediate host. These 'transfer' hosts, often copepods, provide the necessary link for infection of plankton-feeders such as herrings and anchovies. Fishes and invertebrates, after feeding on intermediate hosts, may serve as paratenic hosts, which maintain infective larvae without further development, and in which larvae typically occupy specific sites such as the mesentery, hepatopancreas, liver, or muscle, depending on the species of larva and host. Third or, occasionally, fourth stage larvae mature in the digestive tract of suitable definitive hosts.

Numerous marine invertebrates serve as intermediate hosts of *Hysterothylacium* spp., among them polychaetes (Popova and co-authors 1964; Popova and Valter, 1965, 1967), isopods (Valter, 1968a), amphipods (Valter, 1968b) and even hydromedusae (Lichtenfels, 1974). Valter (1968c) infected a range of invertebrates with larvae of *H. aduncum.* The highest incidence of infection was obtained in isopods (83.7 %) and polychaetes (65.1 %), and the maximum intensity in gastropods (5 to 2398). Oligochaetes were found not to be suitable hosts. Norris and Overstreet (1976) compiled a list of invertebrates known to be hosts of *Hysterothylacium* (Table 1-24). Some of the records mentioned may well refer to larval *Contracaecum* (see taxonomic discussion of *Contracaecum*). The genus has a world-wide distribution.

A unique recent observation on the life cycle of *Hysterothylacium ilishae* in the red sea bream *Chrysophrys major* in Japan was made by Sakaguchi and co-authors (1980). They stated that worms entered the stomach and then the midgut from where most penetrated into the body cavity. After growing, the worms re-entered the intestinal caecum and finally escaped through the anus into the water. This observation appears unusual and unlikely and needs verification. According to Geller (1957), eggs of *Hysterothylacium* in the sterlet, *Accipenser ruthenus,* eaten by other fish retain their ability for further development.

Table 1-24
Invertebrate hosts of Hysterothylacium spp. (After Norris and Overstreet, 1976). Note: at least some of the records (Baltic Sea) are probably of *Contracaecum osculatum;* see footnotes to table

Host	Parasite	Locality
COELENTERATA		
Hydrozoa		
Phialidium sp.	*Contracaecum* sp.	Not stated
Polyorchis penicillatus	*Contracaecum* sp.	California
Scyphozoa		
Ceriantharia	*Contracaecum* sp.[a]	North of Congo River
CTENOPHORA		
Pleurobrachia pileus	*Contracaecum* spp.[a]	New Zealand
	Contracaecum sp.[b]	North Sea
MOLLUSCA		
Gastropoda		
Cantharus cancellarius	*Thynnascaris* sp.	Mississippi
Cyclonassa neritea	*C. aduncum*	Black Sea
Margarites groenlandicus	*C. aduncum*	Experimental
Nassa reticulata	*C. aduncum*	Black Sea
Thais haemastoma floridana	*Thynnascaris* sp.	Mississippi
Cephalopoda		
Lolliguncula brevis	*Thynnascaris* sp.	Mississippi
Todarodes pacificus	*Contracaecum* sp.[a, d]	Japan
ANNELIDA		
Polychaeta		
Enoe nodosa	*Contracaecum* sp.[b]	White Sea
Gattiana cirrosa	*C. aduncum*	White Sea
Gattiana sp.	*C. aduncum*	White Sea
Harmothoe imbricata	*Ascaris* sp.[b]	Great Britain, Baltic Sea
	C. aduncum	White Sea, Experimental
Lepidonotus sp.	*C. aduncum*	White Sea, Experimental
L. squamatus	*C. aduncum*	White Sea, Experimental
Nereis helgolandica	*C. aduncum*	White Sea
N. pelagica	*Contracaecum* sp.[b]	White Sea
Nereis sp.	*C. aduncum*	White Sea
Tomopterus helgolandica	*Contracaecum* sp.	North Sea
ARTHROPODA		
Copepoda		
Ascartia biflosa	*C. aduncum*	Experimental
A. longiremis	*C. aduncum*	Experimental
Calanus finmarchicus	*C. aduncum*	Barents Sea
	Contracaecum sp.[b]	North Sea
Calanus sp.	Nematode[b]	North Sea
	Contracaecum sp.	North Sea
Euchoeta sp.	Nematode[b]	North Sea
	Contracaecum sp.	North Sea
Eurytemora affinis	*C. aduncum*	Experimental
Microsetella norvegica	*C. aduncum*	Experimental
Pseudocalanus elongatus	*C. aduncum*	Experimental
Pseudocalanus sp.	Nematode[b]	North Sea
Pseudocalanus sp.	*Contracaecum* sp.	North Sea
Temora longicornis	*C. aduncum*	Experimental

Table 1-24 (continued)

Host	Parasite	Locality
ARTHROPODA (cont'd)		
Malacostraca		
Mysidacea		
Erythrops erythrophthalma	*Contracaecum* sp.	Nova Scotia
Meganyctiphanes norvegica	*Contracaecum* sp.	North Sea
Mysis mixta	*Contracaecum* sp.	Nova Scotia
Neomysis americana	*Contracaecum* sp.	Nova Scotia
N. vulgaris	*Contracaecum* sp.[b]	North Sea
Isopoda		
Iaera albifrons ischiosetosa	*C. aduncum*	White Sea, Experimental
Amphipoda		
Caprella septendrionalis	*C. aduncum*	White Sea
Unidentified	*T. bidentatum*	Experimental
Euphausiacea		
Euphausiid	*Contracaecum* sp.[a]	Northern North Pacific Ocean
Nyctiphanes couchii	*Contracaecum* sp.	North Sea
Thysanoessa raschii	*Contracaecum* sp.	North Sea
Decapoda		
Clibanarius vittatus	*Thynnascaris* sp.	Mississippi
Emerita talpoida	*Thynnascaris* sp.	Mississippi
Pandalus borealis	*Contracaecum* sp.[a, c]	British Columbia
	C. aduncum	Barents Sea
Penaeus aztecus	*Contracaecum* sp.[a]	Florida
	C. habena	Florida
	C. habena	Texas
	Thynnascaris sp.	Northern Gulf of Mexico
	Thynnascaris spp.	Mississippi
P. brasiliensis	*Contracaecum* sp.[a]	Florida
	Thynnascaris sp.	Florida
P. californiensis	*Contracaecum* sp.	Mexico, Pacific Coast
	Nematode[b]	Dry Tortugas
P. duorarum	*Contracaecum* sp.[a]	Florida, North Carolina to Campeche Banks
	C. habena	Florida
P. setiferus	Nematode	Florida
	Contracaecum sp.[a]	Florida
	C. habena	Florida
	Thynnascaris sp.	Northern Gulf of Mexico
	Thynnascaris sp.	Mississippi
P. stylirostris	*Contracaecum* sp.[a]	El Salvador, Pacific Coast
P. vannamei	*Contracaecum* sp.	Mexico, Pacific Coast
	Thynnascaris sp.	Mexico, Pacific Coast
Sea crabs	*T. inquies*	India
Sicyonia dorsalis	*Contracaecum* sp.[a]	Dry Tortugas
S. typica	*Contracaecum* sp.[a]	Dry Tortugas
Solenocera atlantidis	*Contracaecum* sp.[a]	Dry Tortugas
Trachypenaeus constrictus	*Contracaecum* sp.[a]	Florida
T. similis	*Contracaecum* sp.[a]	Dry Tortugas
Xiphopeneus kroyeri	*Contracaecum* sp.[a]	Florida or adjacent water
ECHINODERMATA		
Asteroidea		
Lucidia clathrata	*Thynnascaris* sp.	Mississippi

Table 1-24 (continued)

Host	Parasite	Locality
CHAETOGNATHA		
Sagitta hipunctata	*Ascaris* sp.[a]	English Channel
	Contracaecum sp.[a]	New Zealand
S. elegans	*Contracaecum* sp.	White Sea, Scotland, North Sea
S. euxina	*Contracaecum*	Black Sea
S. friderici	Ascarid larva[d]	Moroccan Atlantic Coast
S. hispida	*Thynnascaris* sp.	Mississippi
S. inflata	*Ascaris* sp.[d]	Mediterranean area
S. setosa	*Thynnascaris* sp.	France
	Nematode[b]	English Channel
	Contracaecum sp.[b]	North Sea
Sagitta sp.	*Ascaris* sp.[b]	Mediterranean area
	Contracaecum sp.	Florida
	Contracaecum sp.[a, b]	North Sea
S. tennis	*Thynnascaris* sp.	Mississippi

Contracaecum aduncum belongs in the genus *Thynnascaris*
[a] Illustration or description indicates this species belongs to the genus *Thynnascaris*
[b] Considered by other authors as a species we consider belonging to *Thynnascaris*
[c] Adult egg-bearing worms were present, but infection considered by authors as atypical
[d] Considered by that author to be the same as a species we consider belonging to *Thynnascaris*

Contracaecum. A species often reported from various fish is *Hysterothylacium aduncum.* However, some larval stages in marine fishes were erroneously named *'Contracaecum' aduncum,* among them those from cod *Gadus morhua* in the Baltic Sea. Fagerholm (1978) identified nematode larvae in the liver of *G. morhua,* but also in *Salmo salar, Myoxocephalus quadricornis* and *Lota lota* in the northern Baltic Sea as *Contracaecum osculatum. H. aduncum* larvae were not found and previous identifications of this species were considered to be wrong. Identical larvae were also reported from seals. Fagerholm (1979) infected rats and a golden hamster successfully with 3rd-stage larvae from the liver of Baltic cod and obtained 4th-stage larvae morphologically similar to those in seals and identified as *C. osculatum.* Adult *C. osculatum,* but no other nematode species, were recovered from the seal *Halicoerus grypus.*

Of the *Contracaecum* species considered valid and described from seals, only *C. osculatum* has been recorded in areas adjacent to the Baltic Sea (Fagerholm, 1982). The same species is also known from seals in the northern Pacific Ocean (e.g., Oshima, 1972). Some studies on the pathology of *'Hysterothylacium aduncum'* infections were, in fact, made on *C. osculatum.*

Deardorff and Overstreet (1980a) clarified the taxonomic status of *Contracaecum multipapillatum* (junior synonym *C. robustum*) which infects the proventriculus of many amphi- American fish-eating birds. Larvae occur encapsulated in the liver, kidneys and mesentery of various species of mullet. Other species also found in the northern Gulf of Mexico are *C. micropapillatum, C. microcephalum* and *C. rudolphii.* The life cycle of *C. osculatum* is not completely known, but the main stages are as follows: egg — 2nd-stage

ensheathed larva into copepods (experimentally) — fish as 2nd intermediate hosts — seals as final hosts (Fagerholm, 1978, 1982; also Markowski, 1937).

Aspects of the life cycle of a related species, *Contracaecum spiculigerum*, the adult of which is parasitic in marine piscivorous birds, were worked out by Huizinga (1966). At 21 °C, eggs develop in seawater in about 5 days to 1st-stage larvae. In the egg, they moult to 2nd-stage larvae in 5–7 days. The cuticle of the 1st stage is retained, but the larva escapes from the egg and attaches to the substrate. After ingestion by copepods, the larva exsheathes and penetrates into the haemocoel where some growth but no moulting occurs. Fish were experimentally infected by feeding them 2nd-stage larvae or infected copepods. The larvae penetrate into the body cavity of the fish where development continues to an ensheathed 3rd-stage larva. The probable complete life cycle is illustrated in Fig. 1-111. However, no observations on the part of the life cycle in birds were made and this part of the diagram is therefore speculative.

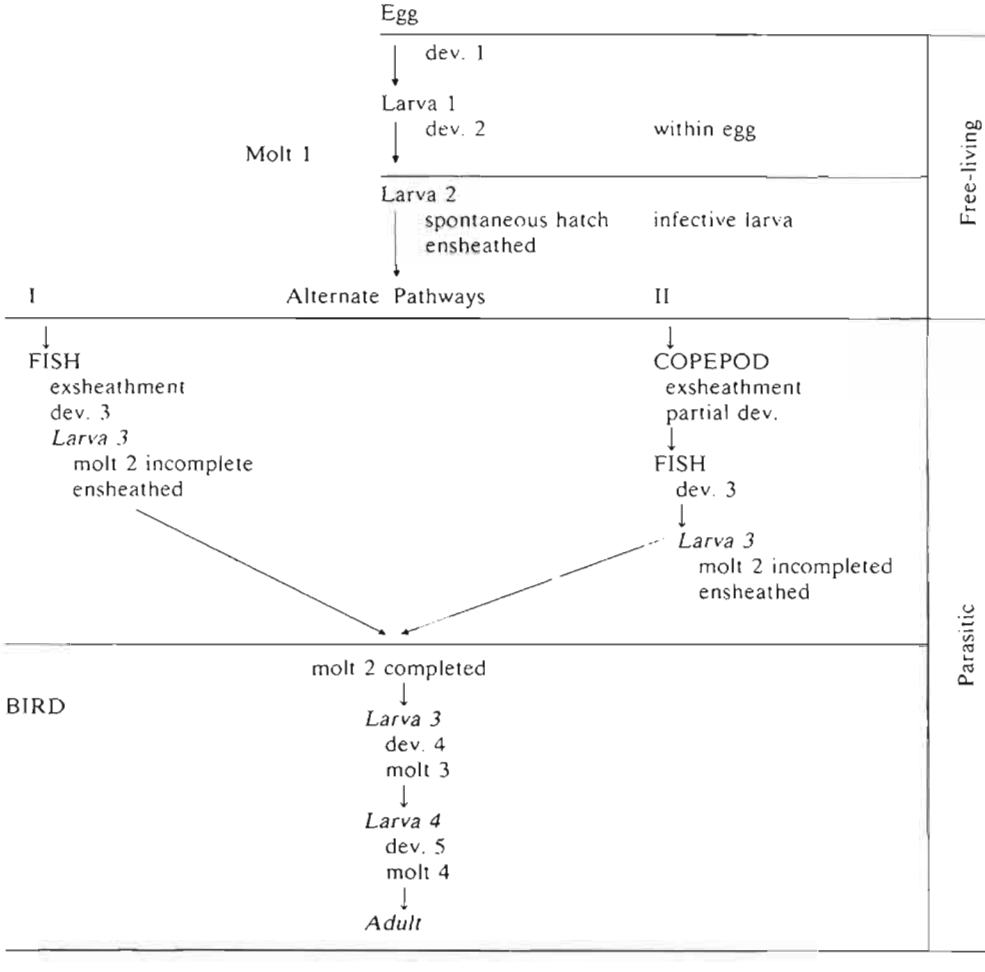


Fig. 1-111: *Contracaecum spiculigerum*. Life-cycle of parasite from piscivorous birds. (After Huizinga, 1966.)

***Pseudoterranova* (junior synonym *Phocanema*).** Gibson and Colin (1982) suppressed the genus *Phocanema* in favour of *Pseudoterranova* for species maturing in mammals. Detailed reasons will be published shortly. The species most often encountered in the literature is *P. decipiens (= Porrocaecum decipiens = Terranova decipiens).*

Eggs of *Pseudoterranova decipiens* sink to the bottom. Emerging ensheathed larvae attach themselves by their caudal extremities to the substrate (Scott, 1955; Myers, 1960; McClelland and Ronald, 1974; McClelland, 1982). Survival of ensheathed larvae in seawater ranges from 6 days at 17 °C to 140 days at 5 °C. At 20 °C, larvae died within 24 to 48 h; at 0 °C they survived for 90 to 120 days (McClelland, 1982). Larvae are ingested by benthic copepods, as indicated by the experiments of McClelland (1982), who successfully infected 12 species of harpacticoid copepods and 1 species of cyclopoid copepod. Two species of calanoid copepods did not become infected. Larvae were also found to infect naturally marine isopods, mysids, and decapods (various authors, see McClelland, 1982). Fish could not be directly infected with the larvae (Myers, 1960), and the smallest worms in naturally infected fish are 4 to 5 mm long (Palsson, 1979), which is further evidence that invertebrates act as obligatory first intermediate hosts. Bjørge (1979) examined 10 *Gadus morhua* from the Norwegian coast, and found an average of 53 *P. decipiens* fish^{-1}. Food items in their stomachs included 87 isopods, mainly *Idothea neglecta,* from which 1 *P. decipiens* was recovered. The author concluded that isopods represent a significant source of food for the fish and that they are a 2nd intermediate host of the parasites. Final hosts are seals (e.g., Scott, 1953: *Phoca vitulina*). In British and adjacent waters, only seals are significantly parasitized by *P. decipiens* (Young, 1972), and pinnipeds have been shown to be hosts also in the northern Pacific (e.g., Oshima, 1972).

***Pseudanisakis* (junior synonym *Anacanthocheilus*).** Myers (1975) considers *Anacanthocheilus* a junior synonym of *Pseudanisakis.* Kahl (1938b) reported *Anacanthocheilus rotundatum,* under the name *Eustoma rotundatum,* as a common parasite of many fish species in the North Atlantic Ocean. However, the species is probably *Anisakis* (e.g., Berland, 1961a). Williams and Richards (1968) made a detailed study of a species named as *Pseudanisakis rotundata,* but according to Margolis (1970b), the structure of the anterior end does not agree with that of *P. rotundata* and the identity of the worms requires clarification.

Effects on Hosts

Effects due to larval anisakids

Anisakid larvae of uncertain taxonomic status. An early report on effects of unidentified anisakid larvae on fish is by Agersborg (1918). He noticed the problems associated with such worms during a visit to northern Norway. Without giving any first hand evidence, he reported that fish (cod) with many worms encysted in the muscles become sluggish, lose the ability to catch food and become easy prey to predators. One must agree with Margolis (1970b) that, in the absence of any supporting evidence, one is left to wonder whether these are simply speculations. Agersborg (1918) further reported that due to nematode infections it was difficult to sell certain fish and that feeding of young fishes with the viscera of older infected fish may have been responsible for an increase in the infection which was not known a number of years earlier. The nematode larvae are either those of *Anisakis, Contracaecum* or *Hysterothylacium.*

According to Rosenthal (1967), about 10 % of thousands of herring larvae *(Clupea harengus)* reared in tanks and fed on plankton, were lost due to parasite infections. Parasites were anisakid larvae identified as *Contracaecum* sp. which were possibly *Hysterothylacium* sp., the cestode *Scolex pleuronectis* and the copepods *Lernaeocera* sp. and *Caligus rapax*. Herring larvae stopped feeding approximately 10 days after infection with *'Contracaecum'*. Intensity of intestinal peristalsis decreased and the nematodes tended to depress the lumen of the intestine, and to interrupt defaecation. The fish showed 'staggering' swimming motions. Death occurred about 11 days after infection, after intestinal peristalsis had ceased completely. The author attributed death primarily to the increasingly violent movements of the growing nematode which damages the intestinal wall. He also considered it possible that the fish were poisoned by 'defaecation products of the parasites'. Herring larger than 10 mm seemed to be more resistant, and some infected larvae survived for several weeks.

Anisakis. Many species of marine fish and squid may harbour larval *Anisakis* (e.g., Kagei, 1970 with list of records from marine fishes and squid in Japan; Davey, 1972; Rokicki, 1972; Tantaleán, 1972; Dornheim, 1973; Beverley-Burton and Pippy, 1977, 1978; Beverley-Burton, 1978; Kulachkova, 1978; Bussmann and Ehrich, 1979).

Prevalence of infection may be high (e.g., Kulachkova, 1980: 48.8 % of 1145 White Sea herring *Clupea harengus pallasi* n. *maris-alba,* in 13.1 % larvae in both the body cavity and muscles). Infection may increase with length of fish. For example, Hennig (1974) found such an increase in anchovy *Engraulis capensis* in Southwest Africa. Davey (1972), on the other hand, often found younger herring *C. harengus* more heavily infected than older fish and suggested that annual fluctuations either in the population of the first intermediate host or in the rate of feeding on it were responsible. There also may be marked short-term and long-term fluctuations. Thus, Reimer and Jessen (1972), on the basis of a comparison of their data with those of others, concluded that there was a marked increase in *Anisakis* infections in the North Sea during the 60's.

Banning and Becker (1978) have shown that such long-term fluctuations, for instance in *Clupea harengus* in the North Sea are conspicuous and well documented (Fig. 1-112). They were attributed to changes in the migration behaviour of the fish (see also Rae, 1972, for long-term fluctuations of larval anisakines in cod; according to Schultz 1911, in the 1880's infections of cod in Schleswig-Holstein, northern Germany, with nematode larvae were apparently particularly heavy). There are also distinct local differences in infection. For example, Grabda (1974), using infection levels with larval *Anisakis simplex* as well as otoliths and gonad maturity, distinguished 4 herring stocks appearing successively in the Pomeranian Gulf and adjacent areas of the Baltic Sea.

Larvae of *Anisakis* occur throughout the muscles and viscera of fish, but the distribution within the tissues varies between fish species (see review by Smith and Wootten, 1978; further references therein).

Several authors have reported on histological changes of naturally infected fish. Kahl (1938b) described reactions of *Gadus morhua* and *Sebastes norvegicus* to infections with larvae of *'Anacanthocheilus rotundatus'*. According to Berland (1961a) among others, the worms were *Anisakis* larvae. Larvae were surrounded by thin sheaths. They were suspended in the mesentery or had penetrated into the stomach wall. Occasionally, several larvae were found in one hole. Details of tissue reactions to larvae in the stomach wall, body cavity, musculature, liver and fat tissue were given. Such reactions are discussed

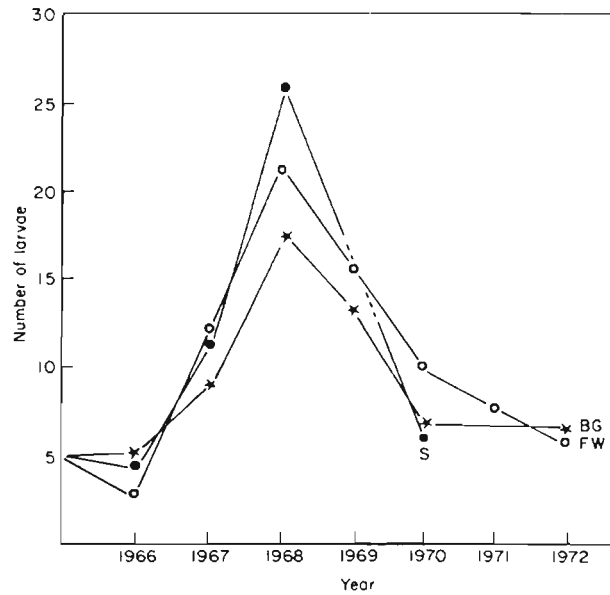


Fig. 1-112: *Anisakis larvae.* Mean numbers in 3 herring stocks: Sandettie (S), Botney-Gut (BG) and Flamborough-Whitby (FW). (After Banning and Becker, 1978.)

below based on more recent experimental studies. All larvae, except those in the stomach, were surrounded by changes in the adjacent tissue. Kahl (1938b) assumed that larvae migrated from the stomach through the stomach wall and body cavity into the musculature. But the migration is apparently so slow that the host can form a connective tissue capsule around the migrating larvae, as indicated by the observation that larvae in the stomach wall, body cavity and in the muscles all had connective tissue sheaths. Gradually, the larvae coil up. The form of the capsule depends on the amount of connective tissue present, and not so much on the host species. However, capsule size apparently depends on host size. Kahl speculated that the damage due to single larvae is probably not great, but that in heavy infections the health of the host may be affected. Especially conspicuous is the damage to the liver. Small portions of the liver show degeneration and disintegration. In some heavy infections, whole liver sections were functionless and shrunken. The muscles apparently are least affected. Occasionally there may be secondary bacterial infections.

Liver damage due to *Anisakis* infections has also been reported by Remotti (1933a, b; for taxonomy see Margolis (1970b); Akhmerov (1951); Petrushevsky and Kogteva (1954); Brian (1958); Torres and González (1978); Ramadan and co-authors (1981) (see also discussion in Margolis, 1970b) (Fig. 1-113).

Data on the effect of *Anisakis* infections on the condition of fish are contradictory. Neither Brian (1958) in his study of the effects of *Anisakis* on hake *Merluccius merluccius,* nor Hennig (1974) in his study of effects on the anchovy *Engraulis capensis,* nor Suzuki and Oishi (1974) in their study of the effects on the Pacific pollock *Theragra chalcogramma,* by larvae of *Anisakis* sp. and the cestode *Nybelinia surmenicola,* noticed any marked effect on the condition factor. Hennig (1974) also did not find an effect of the infection on the average length distribution and the average gonad weight. He examined

Fig. 1-113: Portion of liver from a gadoid fish heavily infected with encapsulated *Anisakis* larvae; scale in mm. (After Smith and Wootten, 1978.)

1850 fish over a 10 month period, of which 65 were parasitized. Petrushevsky and Kogteva (1954) on the other hand, reported that Fulton's coefficient of condition (p. 286) in shorthorn sculpin *Myoxocephalus scorpius* from the White Sea decreased in fish heavily infected with *Anisakis*. However, no data on the comparative length and age of the fish with heavy and light infections were given, and the conclusions are therefore not beyond doubt (Margolis, 1970b).

Arai (1969) observed large ulcers in the stomach wall of one lingcod *Ophiodon elongatus* from British Columbia, which contained larval *Anisakis*. The weight of the fish

was greatly reduced and the author considered it likely that the parasites were the cause of the emaciated condition.

Some detailed studies on the pathology and pathogenesis of *Anisakis* infections are discussed in the following.

To study the pathogenesis of *Anisakis* infection, Prusevich (1964) transplanted carmine-stained *Anisakis* larvae from cod liver into 19 to 23 cm long *Myoxocephalus scorpius.* Ten fish were infected with dead larvae (killed by 5 min boiling), and 10 fish with live larvae. Each fish was infected with 2 larvae. Pieces of liver were fixed at intervals for histological examination. Within 24 h after infection with live larvae, there was destruction of liver parenchyma and ruptures of the walls of some blood vessels, small haemorrhages and thrombus formation. The larvae were covered with fibrin infiltrated with leucocytes. Nine days after infection, a distinct net of fibrin fibers of varying thickness was present around the larvae, and a leucocyte ridge was formed. Thirty days after infection, 4 layers were visible: (i) the first layer consisted of dying cells and apparently corresponds to the leucocyte layer observed earlier; (ii) the second layer consisted of fibroblasts; (iii) the third layer consisted of diverse cell elements with fibroblasts predominating, and blood capillaries grew into this layer; (iv) the fourth layer consisted of collagen bundles, fibers and various cell elements, and was located at the border with the liver parenchyma.

The liver parenchyma near the capsule also changed, although only insignificantly. Liver cells redifferentiated, the amount of fat droplets decreased, and wandering cells were visible between the liver cells. In 2 cases, larvae penetrated more deeply into the liver tissue than normally. Reactions in these cases were more severe.

Fourteen to 24 h after transplantation of dead larvae, only insignificant haemorrhages and other tissue reactions occurred, and significant damage occurred later, probably resulting from decomposition of the parasites. Proliferation of fibroblasts and formation of collagen fibrils after infection with dead larvae was less than after infection with live larvae.

Mikhailova and co-authors (1964) made a histological study of the capsule around larval helminths, including *Anisakis* and *Contracaecum,* in haddock *Melanogrammus aeglifinus,* cod *Gadus morhua morhua,* shorthorn sculpin *Myoxocephalus scorpius,* and arctic char *Salvelinus alpinus.* Anisakid larvae are usually coiled up and encapsulated, causing impressions in the liver parenchyma. The formation of the capsule is 'obviously' as follows: larvae attach themselves to the liver surface, compressing and mechanically damaging the serous membrane and adjacent liver parenchyma. Larval products also 'obviously' have some effect. The serous membrane near the larvae is tens of times thicker than normal, and a connective tissue capsule is formed. Reactions in all 3 fish species are similar, and the capsule around *Anisakis* and *Contracaecum* is also similar. The layer directly in contact with *Anisakis* is narrow, consisting of damaged and sometimes pycnotic cells and disintegrating nuclei. The next layer contains more fibroblasts, partly undergoing degenerative changes, and often unusually elongated nuclei which are often fragmented and in amitotic division. The third layer usually consists of porous connective tissue with numerous blood vessels and various cell types. Bordering the liver parenchyma is relatively unchanged connective tissue, and the liver tissue adjacent to the capsule is little changed, except for some development of connective tissue and a greater number of blood vessels. In older infections, the connective tissue grows in the serous membrane of the liver close to the parasite, and more blood vessels penetrate into the capsule. The parasite becomes more deeply embedded in the liver tissue, due to intra-abdominal pressure and probably

due to reduced growth of the liver tissue. There are also changes in the capsule wall itself. Cellular elements disappear from the most interior layer, and a narrow tissue strip appears between the first and second layer, without 'cells' but sometimes with fibrillar structures. In the second layer, some of the cells are destroyed, and 2 zones are visible in the third and widest layer. The zone adjacent to the second layer contains blood vessels and degenerative cell elements in the connective tissue, and the second zone contains thick bundles of collagen fibres.

The authors also transplanted *Anisakis* larvae from cod to sculpin. Within 4 h, half of the larvae were attached, usually to the serous membrane of the liver. After 6 h, almost all larvae were attached, some already rolled up. After 12 h, a thin membrane had been formed around the larvae, and after 24 h, 'fixation' on the serous membrane was completed. Encapsulation of larvae killed by boiling, was much slower. Only 12 h after infection did attachment to the serous membrane occur.

Tissue reactions to live larvae differ considerably from those to dead bodies, the latter being encapsulated in a dense fibrous capsule. Live larvae, however, continue to provoke destructive changes of the cellular and fibrous elements of the capsules and proliferative processes which result in continuous formation of new fibroblastic layers, growth of blood vessels, and accumulation of numerous and diverse phagocytes (Mikhailova and co-authors, 1964). Parasite larvae use these responses for nutrition, excretion and defence, and the absence of a dense fibrous capsule also facilitates escape from the capsules.

Capsules around *Anisakis* and *Contracaecum* were also studied in the serous membrane of the stomach and intestine of haddock and cod. Basically, the same capsule layers could be distinguished, but the second layer is less well developed and sometimes absent, beside some other quantitative differences.

J. W. Smith (1974) radioactively labelled *Anisakis* larvae from *Salmo salar* and fed them to haddock *Melanogrammus aeglefinus*. Radioactively labelled *Anisakis* larvae from *Clupea harengus* were fed to whiting *Merlangius merlangus*. Larvae penetrated the wall of the stomach or the pyloric caeca. Twenty-four h after infection they were first seen in the body cavity, and a delicate capsule around them had been formed 34 h after infection.

Hauck and May (1977) examined histopathological alterations due to *Anisakis* larvae in Pacific herring *Clupea harengus pallasi*. Larvae were coiled up inside a connective tissue capsule at the surface of the pyloric caeca, pancreatic tissue, liver and large intestine. In some cases the capsule adhered to the tissue serosa, but generally it was separated by a layer of host exudate containing free macrophages and lymphocytes. In all cases there was a mechanical compression and displacement of the pancreas as well as host exudate at sites of close or direct larval contact. In moderate to light infections, larval concentrations near the liver did not exist. Fish with many larvae had parenchymal granulomas of the liver of unknown origin. There was mechanical compression of the liver in all cases where the liver was involved, but there was no necrosis associated with the compression. One fish with heavy infection had a severe and diffuse liver necrosis. Since compression of the parenchyma was not observed in this case, the author did not consider *Anisakis* larvae to be the cause. In one case there was mechanical injury to the muscularis externa of a pyloric caecum. The author concluded that *Anisakis* infection in Pacific herring was unlikely to cause critical organ dysfunction, and that the observed cellular components of the lesion indicated a chronic pathology which would only become serious in older, more heavily infected fish.

Berland (1981) observed clusters of larval *Anisakis simplex* in craters of the stomach wall of *Gadus morhua* (Fig. 1-114). He suggested that immune reactions of large hosts which have been infected previously by large numbers of larvae, prevent larvae from penetrating through the stomach wall, whereas in young fish with a low level of immunity penetration proceeds easily.

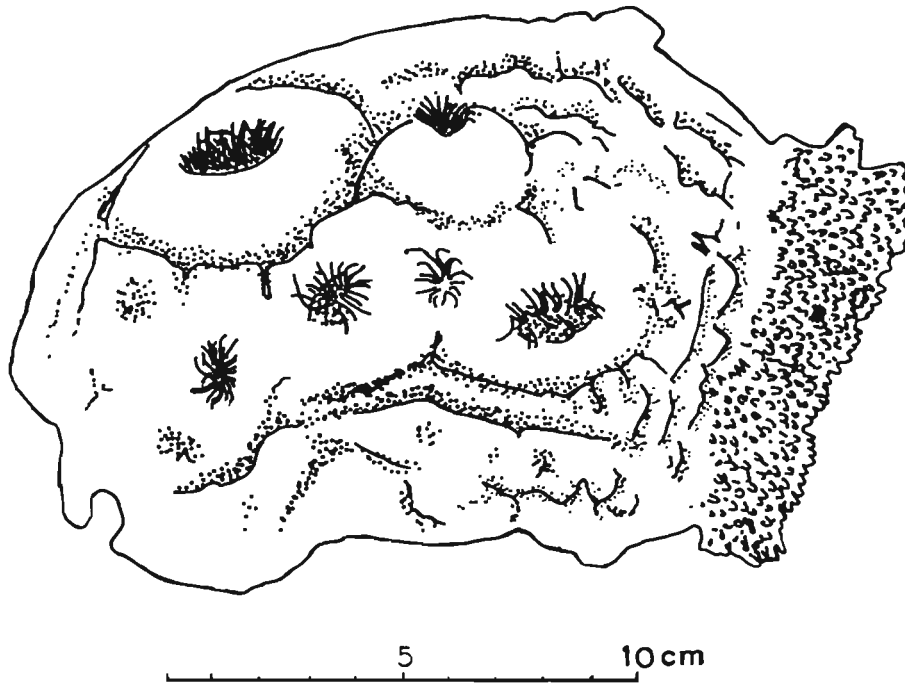


Fig. 1-114: *Gadus morhua*. Stomach of a very large cod with clusters of larval *Anisakis simplex* in craters. (After Berland, 1981.)

In summary, several detailed studies have shown that infections of fish with larval *Anisakis* lead to marked tissue reactions. However, data on the effects of the infection on the condition of fish are contradictory. We must agree with Smith and Wootten (1978) that there is little conclusive evidence that larval *Anisakis* are serious pathogens of fish, and that there is a need for carefully controlled experimental infections to determine with certainty the effect of larvae on fish.

Hysterothylacium. As pointed out in the taxonomic discussion of *Contracaecum*, a species identified as *aduncum* is in fact *Contracaecum osculatum*, at least in the Baltic Sea. Further studies may well show that some of the *Hysterothylacium aduncum* recorded from other seas have also been incorrectly identified. Species of *Hysterothylacium* are widespread and infect many species of marine fishes. For example, Deardorff and Overstreet (1981b) recorded 4 species (*H. reliquens, H. fortalezae, H.* type MB, *H.* type MD) in numerous fishes and invertebrates in the northern Gulf of Mexico (see also Norris and Overstreet, 1975). Shchepkina (1978) examined the lipid composition of the muscles and liver of Black Sea anchovy *Engraulis encrasicholus ponticus*, infected with *H. aduncum*.

The triglyceride fraction was found to be most affected. It was 34 %, 28 % and 29 % lower in the white muscles, red muscles and liver respectively of heavily (80 to 350 larvae) than of weakly (not more than 20 larvae) infected fish. This is one of the few studies in which statistical tests were used to evaluate the findings.

Hysterothylacium haze is mainly found in the body cavity of the goby *Acanthogobius flavimanus* in Japan, but it also penetrates into the liver, subcutaneous tissue and the orbit of the eye. In heavy infections, the worms cause visceral adhesions, liver enlargement and necrosis (Machida and co-authors, 1978).

Sometimes it is not clear whether a causal relation exists between presence of parasites and pathological effects. An example is the paper by Iversen and Kelley (1974). Ten of 114 blue marlin *Makaira nigricans* and 2 of 3 black marlin *M. indica* contained gastric ulcers. Seven stomachs with ulcers of the first species, and 2 stomachs with ulcers of the second species were histologically examined and nematodes were found in 1 and 2 of these stomachs respectively. T. Deardorff (pers. comm.) identified worms from the same host and locality as *Hysterothylacium incurvum*. Ulcers were either separate or in clusters and the bases were covered with a dark brown shaggy material. Nematodes were light gray, 5 to 7 mm long and 0.5 mm in diameter. The base of the ulcer was covered by granulation

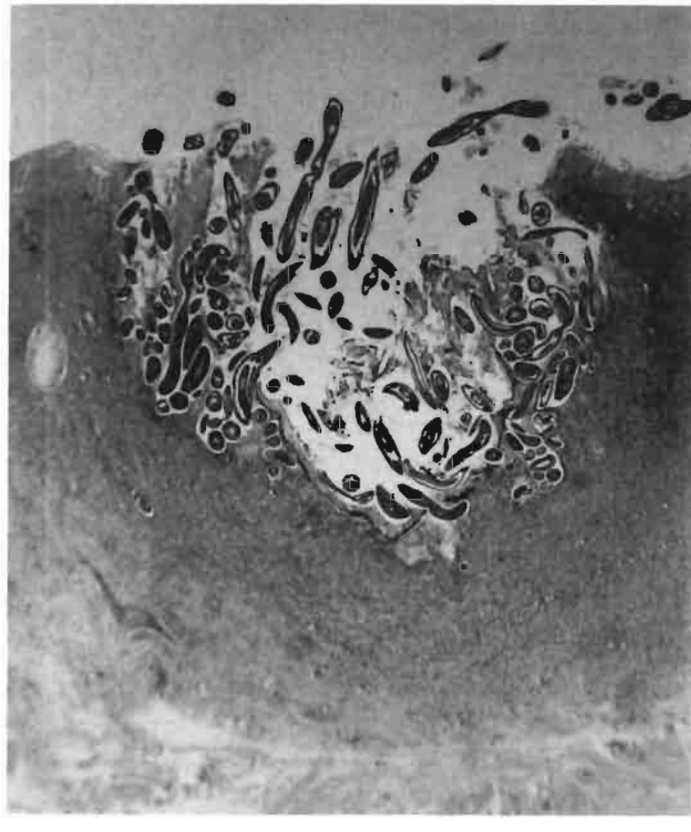


Fig. 1-115: *Rachycentron canadum*. Typical noninflamed, regenerating stomach tissue, showing nest of L4's of *Iheringascaris inquies*. (After Deardorff and Overstreet, 1981b.)

tissue, with many fibroblasts and an infiltration of acute and chronic inflammatory cells. The fibrous proliferation extended through the entire wall and obliterated the usual muscular layer. Generally, there was an intense granulomatous inflammatory reaction surrounding the parasites, which were found throughout the ulcer base. It is uncertain that the ulcers were caused by the nematodes. The possibility exists that ulcers were caused in other ways, for instance by sharply pointed food items, and that the nematodes took advantage of these. According to the authors, this appears not unlikely since nematodes have repeatedly been demonstrated in marlin without gastric ulcers.

Iheringascaris. According to Deardorff and Overstreet (1981b), adults of the only described species, *Iheringascaris inquies*, infect only the cobia, *Rachycentron canadum*. Fourth stage larvae live free in the lumen of the stomach or in the stomach tissue. Occcasionally, larvae invade regenerating host tissue (Fig. 1-115). Adults live in the lumen of the stomach and pyloric caeca.

Contracaecum. Petrushevsky and Kogteva (1954) gave data on the effects of helminths on various species of marine fish. To determine the coefficient of condition (condition factor), they used the formula

$$K = \frac{g \cdot 100}{L^3}$$

where K = condition factor; g = weight of fish; L = length of fish. Both Fulton's and Clark's methods were used. In the former, the total weight of the fish is used for determining K, in the latter, the weight of fish after removal of the internal organs.

Baltic cod *Gadus morhua callarias* infected with *Contracaecum osculatum* (identified by the author as *C. aduncum*, see taxonomic discussion of *Contracaecum*), showed a distinct decrease in Clark's condition factor:

No. of parasites	*K*
0	.941
1– 10	.909
10– 25	.861
25– 50	.846
50– 75	.829
75–100	.821
100–200	.793
200–300	.627

Altogether 594 fish were examined, of which 97.5 % were infected, but no data on the number of fish in each group were given.

Getzevitchute (1955) examined the livers of 1000 Baltic cod *Gadus morhua* of similar size (45 to 60 cm, 4+ yr old) from 2 localities in the Baltic Sea for infection with *Contracaecum osculatum* (identified by the author as *C. aduncum*, see taxonomic discussion of *Contracaecum*, p. 276). There was a distinct seasonal pattern of infection, prevalence and intensity of infection decreasing in winter (Grabda, 1976, found no seasonal pattern of infection with larvae of *Anisakis* and *Contracaecum* in Baltic cod). Correlated with these fluctuations were seasonal fluctuations in the percentage weight of the liver related to the body weight. It was higher in winter than in summer. Similarly, the condition factor decreased from 1.13 to 1.14 in winter to 0.80 to 0.97 in the summer and

autumn, indicating decreasing health with increasing worm infection. Petrushevsky and Shulman (1955) also showed that Baltic cod heavily infected with *C. osculatum* had a percent ratio of liver weight to body weight significantly lower than lightly infected fish. The condition factor was reduced from 0.941 to 0.627 in heavily infected fish. However, no statistical evaluation was made.

Many other authors have reported on the effect of *Contracaecum* infection on cod (e.g., Bazikalova, 1932; Shulman, 1948; Akhmerov, 1951; Shulman and Shulman-Albova, 1953; Bauer, 1958; Petrushevsky and Shulman, 1958; Shulman, 1959, Dogiel, 1964; Sindermann, 1966; review by Margolis, 1970b). Many of the infections studied probably were *C. osculatum,* others possibly *Hysterothylacium.* Although many of the studies are inadequate in some respects, for instance in the lack of statistical evaluation or the comparison of fish that were of different size, sex and spawning condition, or harboured other parasites, altogether the conclusion is justified that heavy infection of cod with *Contracaecum* leads to a decreased percent weight of the liver and a decrease in the condition factor. However, Margolis (1970b) pointed out that information on this liver disease of gadids has come solely from observations on naturally infected wild fishes. He felt that a series of well designed infection experiments using appropriate controls would greatly improve the reliability of quantitative data on the effects of the parasite.

Pseudoterranova. The codworm *Pseudoterranova decipiens* is frequently a serious industrial problem in many countries (e.g., Odense, 1978; some references in Sindermann, 1966). Margolis (1977) reviewed the potential of *P. decipiens* as a health problem. Several authors have shown that the infection in fish increases when seal herds increase (e.g., Berland, 1961a; Rae 1963; 1972; see also Chapter 4), and there may be marked local differences in infection. For example, Platt (1975) found that larvae of *P. decipiens* were common in *Gadus morhua* from Iceland and the Faroe Plateau, but virtually absent in the same species from Greenland, Faroe Bank and Arcto-Norwegian fisheries. Grainger (1959), by rearing larvae *in vitro* to pre-adult condition, identified nematodes in the body musculature of Icelandic cod as *P.* probably *decipiens.* Davey (1969) used ingestion of dyes by cultured larvae of *P. decipiens* obtained from cod to determine feeding pattern. He observed 2 feeding periods, one between ca. 24 and less than 36 h in the culture, after the new cuticle had begun to form, and one beginning about 4 days in the culture, when ecdysis normally took place. No feeding occurred between the 2 periods.

Some histopathological findings on the infection with *Pseudoterranova* were reported by Widera (1976) and Torres and González (1978). Kahl (1938a) reported that the distribution of *P. decipiens* larvae (named by him *Porrocaecum*) in the flesh differs in different fish species. In larger fish, such as *Gadus morhua* and *Molva vulgaris,* the worms are found most commonly in the musculature adjacent to the body cavity. In smaller fish, such as smelt *Osmerus eperlanus,* the worms are also found in head and tail regions. And whereas in *G. morhua, M. molva (= M. vulgaris), M. byrkelange,* and *Sebastes marinus (= S. norvegicus)* the larvae are found between the strong connective tissue septa, they are scattered randomly through the tissues of smelt. Some larvae are found free in the tissue, as already reported by Martin (1920), who made some early observations on capsule formation, but most larvae in *G. morhua* and *M. molva* are surrounded by a capsule of host origin. The capsule consists in its simplest form of loose connective tissue in which the worms are embedded. The connective tissue close to the worm is often denser, forming a sheath-like structure. Sheaths may show degenerative processes, as may the worms,

leading to a shrinking of the capsules and worms which appear brown in colour. In *O. eperlanus,* larvae were not surrounded by capsules. Instead, they were located in large cavities and caused extensive damage to the tissues. Kahl (1940) reported that infection in *G. morhua* increases with age and differs in different geographical regions. The paper also contains data on infection of various fish species with this parasite.

According to Mikhailova and co-authors (1964), larvae of *Pseudoterranova decipiens* are usually located deep in the liver parenchyma of *Myoxocephalus scorpius.* In young infections, blood vessels are destroyed and liver cells are compressed and some are destroyed. Capsule formation is common. The capsule wall has only 2 layers. The first layer probably corresponds to the first 2 layers of the capsule around *Anisakis,* (see discussion on *Anisakis*), the second layer consists of 2 zones and may be absent from parts of the capsule. The inner zone is without collagen fibres, the outer zone contains bundles of collagen fibres. Shulman and Shulman-Albova (1953) made some observations on the effect of *P. decipiens* on the liver of *M. quadricornis.* Of 3 fish 26 cm long, the liver weight in percent of body weight was 6.0 in 1 uninfected fish, 3.6 in 1 fish with 12 larvae, and 1.9 in 1 fish with 100 larvae.

Raphidascaris. A significant decrease in the fat content of *Atherina mochon pontica* from the Aral Sea infected with larvae of *Raphidascaris acus* was demonstrated by Dergaleva and Markevich (1977).

Effects due to adult anisakids

An adult nematode, *Pseudanisakis rotundata,* which infects *Raja radiata* in the North Sea, was studied by Williams and Richards (1968). Its taxonomy is not clear (see taxonomic section). They observed a prominent host tissue reaction around the anterior end of the worm, and conspicuous wedge-shaped strands of host tissue interlocking with the constriction. This interlocking device may be an adaptation to secure attachment (Fig. 1-116).

Deardorff (in press) described a new species of *Terranova* from the hammerhead shark *Sphyrna lewini* in Hawaii. In addition to 14 specimens that were free in the lumen of the stomach, adult specimens were also found firmly encysted in 2 fibrous nodules in the stomach wall. Worm aggregates were surrounded by necrotic tissue, and the periphery of the necrotic zones was heavily infiltrated with lymphocytes and monocytes.

The genus *Goezia* has representatives in freshwater and marine fishes. Deardorff and Overstreet (1980b) described observations on life history, pathology and attachment of some species. Worms are firmly attached to the stomach of hosts, but, according to the authors, evidence of worms associated with food in some hosts also containing lesions indicates that at least some species are able to detach, leave the site of attachment and return to the same site. It seems that these species feed on both host and its partially digested food. The species *Goezia pelagia,* parasitic in the marine fishes *Rachycentron canadum, Chaetodipterus faber* and *Ophicthus* sp. in the northern Gulf of Mexico, causes little damage and does not penetrate into the body cavity as reported for a freshwater species. In one case, the extended worm (Fig. 1-117, 1) penetrated the mucosa, nearly reaching the muscularis. There was some haemorrhaging, but no appreciable leucocytic inflammatory response. In the second case studied, an extensive vascularized, dense, collagenous capsule was present (Fig. 1-117, 2). The worm penetrated into the muscularis, evoking a chronic, inflammatory response. The mucosa was inflamed, degenerating, laden with bacteria and sloughing (Fig. 1-117, 3). In a third case, the collagenous capsule was

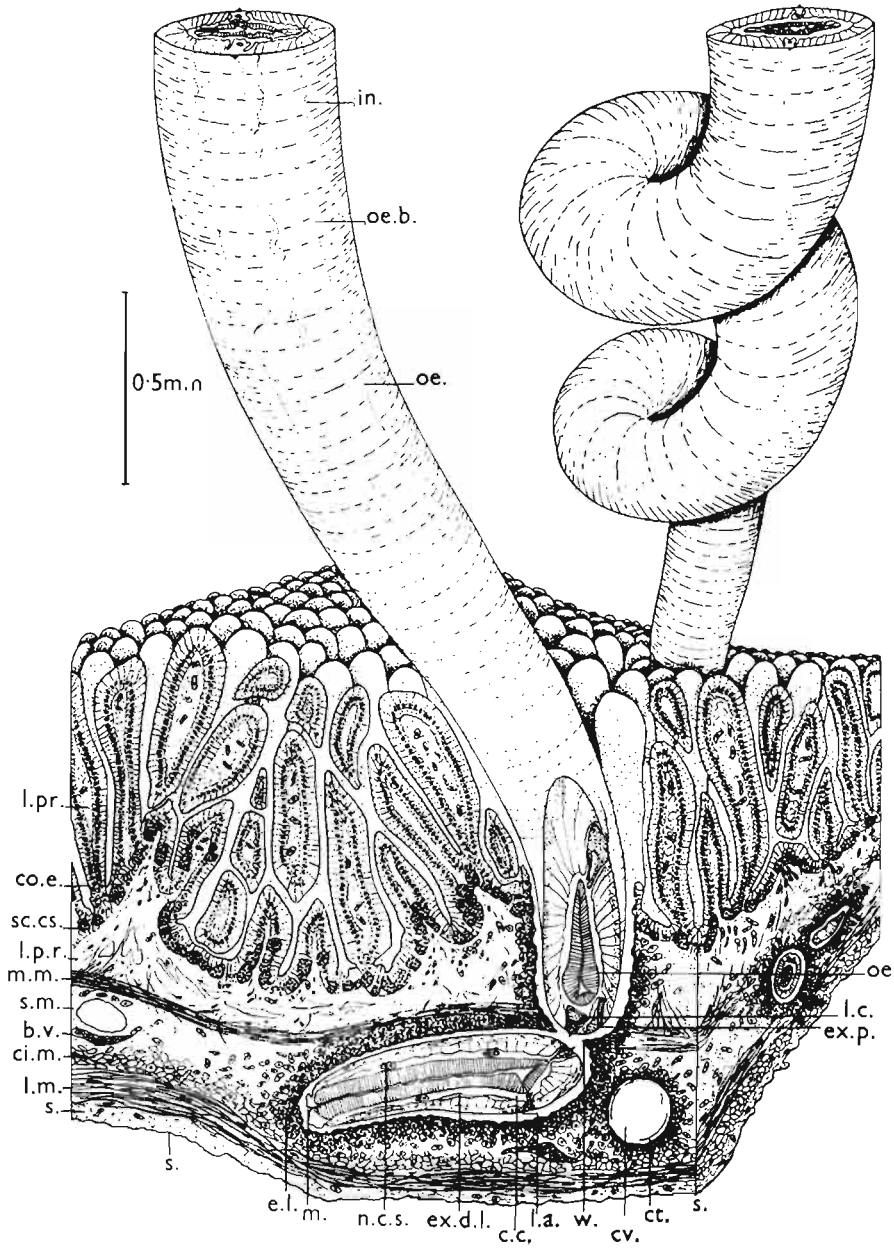
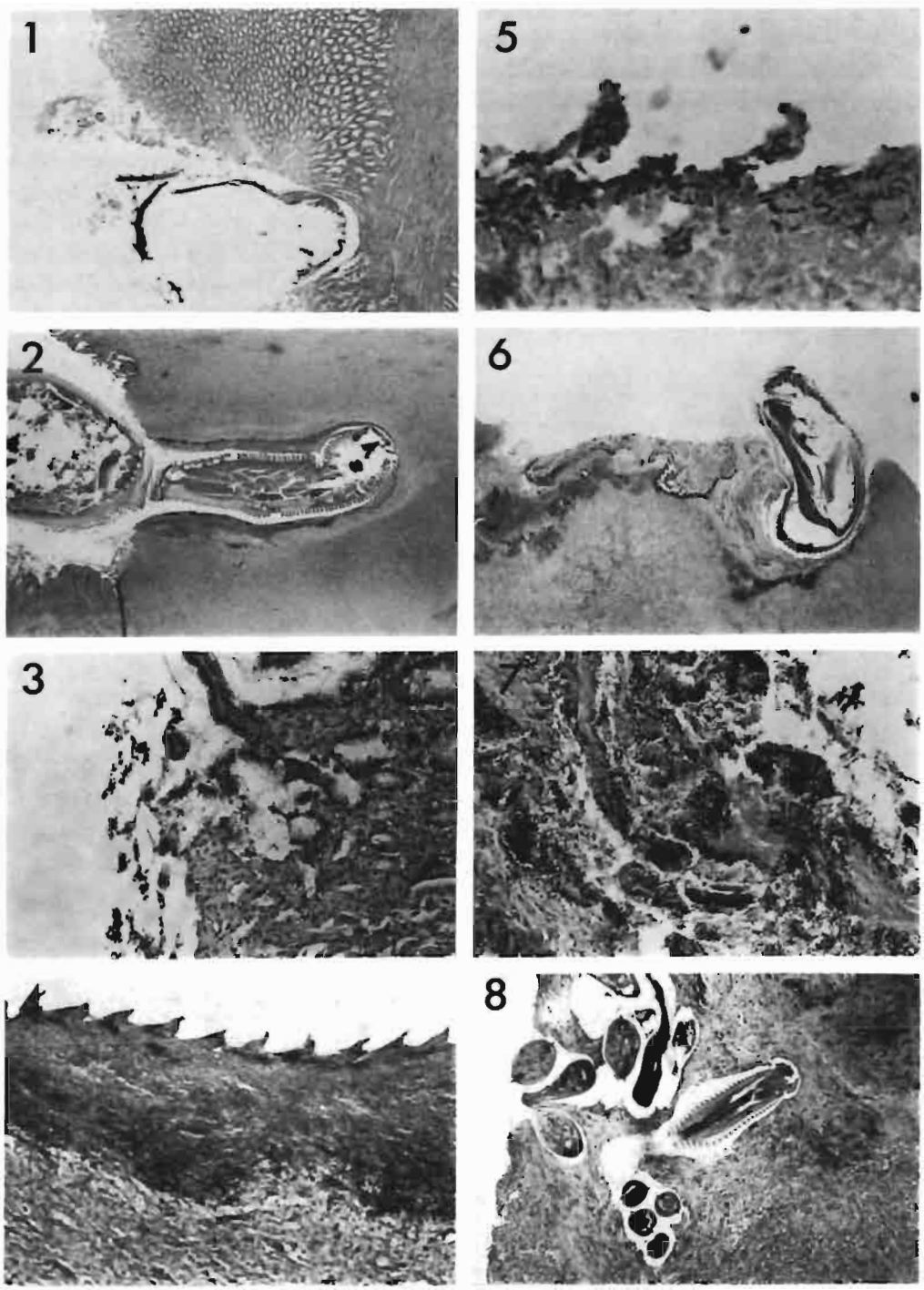


Fig. 1-116: *Raja radiata. In situ* drawing of anisakid nematode embedded in the intestinal wall. b.v.: blood vessel; c.c.: coelomic cavity; cl.m.: circular muscle; co.e.: columnar epithelium; ct.: cuticle; cv.: cavity left by nematode; e.l.: eosinophilic leucocytes; ex.d.l.: excretory duct-lateral; ex.p.: excretory pore; in.: intestine; l.a.: longitudinal ala; l.c. lateral cord; l.m.: longitudinal muscle; l.pr.: lamina propria; m.: mouth; m.m.: muscularis mucosa; n.cs.: nerve cells; oe.: oesophagus; oe.b.: oesophageal bulb; s.: serosa; sc.cs.: secretory cells; sm.: sub-mucosa; w.: wedge of tissue. (After Williams and Richards, 1968.)


1
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more extensive. In the last case, the collagen-rich irregular region was thicker than in the other cases (Fig. 1-117, 6). Much of it was necrotic with many bacteria (Fig. 1-117, 7). This tissue was continually sloughing into the cavity. Eggs of the parasite occurred deep in the capsule, and a degenerated worm was located near the base of the nodule. The nodule also contained several *Thynnascaris inquies* (Fig. 1-117, 8). The 4 cases seem to indicate progress from a recent invasion to a well-established nodule. Lizardfish *Synodus foetens,* infected with *Goezia minuta,* in Florida, have fibrotic nodules in the stomach wall similar to those described above. Of the fishes sampled monthly over 2 yr, 49.8 % had an average of 3 worms each.

Effects due to other nematodes

The genus *Philometra* (Dracunculoidea) comprises several species which occur in the gonads and other tissues of marine fishes, sometimes causing marked pathological effects. Kuitunen-Ekbaum (1933) described *Philometra americana* from 5 species of coastal fish at Nanaimo, British Columbia — Canada i.e., *Platichthys stellatus, Lepidopsetta bilineata, Epigeichthys atropurpureus, Pholis ornatus,* and the cling fish *Caularchus meandricus.* In the 2 flounder species, the parasites occurred between the rays of the dorsal and ventral fins and subcutaneously in the opercular region, gill cavity and occasionally in the caudal fin. In the 2 blenny species, the parasites were always found subcutaneously in the head or in the anterior part of the body. In 2 cling fish, the parasites infected the lateral wall of the body, and in a third the body cavity. In all cases the parasites were coiled and immobile, and in 1 blenny with 7 parasites in the head, the head of the fish was greatly swollen and turned upward. To extrude the embryos, the female worms must bore through the host tegument. A large worm in the dorsal fin of a flounder kept for 2 weeks in an aquarium, was seen to protrude through the skin of the fish. The following day the parasite had escaped, leaving a hollow space in the host tissue. Small empty spaces presumably formerly occupied by parasites that had escaped, were found 'a few times' in the fins of flounders during August. Other fish species were subsequently found to harbor the parasite, and infections were sometimes heavy. Olson (1972) reported that English sole *Parophrys vetulus,* 29 cm long, harboured 273 female *P. americana,* 15 to 49 mm long.

Paperna and Zwerner (1976) found pathological conditions associated with *Philometra rubra* in the striped bass *Morone saxatilis,* a commercially important and highly valued game fish. During the final phase of the life cycle of *P. rubra,* gravid females, previously living free in the visceral cavity, penetrated or became embedded in the mesenteries and the peritoneum causing visceral oedema and granuloma and extensive

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Fig. 1-117: Sectioned *Goezia pelagia* and associated nodules in the stomach of *Rachycentron canadum.* (1) Anterior of worm penetrating through mucosa and nearing muscularis; note absence of fibrotic nodule and inflammatory response; H & E, X 29. (2) Another worm with associated nodule; note narrow dense collagenous capsular layer adjacent to anterior end of worm; H. & E, X 13. (3) Degenerating mucosa at margin of nodule near trunk of worm; H. & E, X 137. (4) Bacteria-laden, plicated lining of capsule where cuticular rings of worm inserted; H. & E, X 132. (5) Similar region as in (4) but in different nodule; bacteria in photo restricted to plications; however, large nests of bacteria occurred elsewhere in capsule; Taylor's bacteria method, X 558. (6) Nodule with thicker and more irregular layer separating worm from portion with loose connective tissue; H. & E, X 16. (7) Close-up of irregular layer showing less restricted localization of bacteria than in previous nodules; Taylor's bacteria method, X 136. (8) A few individuals of the related *Thynnascaris inquies* burrowing into nodule of *G. pelagia;* note one worm entering the capsular layer; H. & E, X 51. (After Deardorff and Overstreet, 1980b.)

visceral adhesions. Males eventually died and disintegrated, while larvae invaded the adjacent tissues. In heavy infections, particularly in juvenile fish, extensive visceral adhesions appeared following the death of immature female worms which were subsequently absorbed into the mesenteries. Other effects were advanced fibrosis of the liver and spleen resulting from heavy infections with this and other helminth species, as well as 'black spot', that is aggregates of dark pigment, in the visceral mesenteries resulting from encapsulation and decomposition of dead worms (*P. rubra* and other species) and their eggs.

Overstreet and Edwards (1976) observed benign mesenchymal fibromas in 1 southern flounder *Paralichthys lethostigma,* and 1 sea catfish *Arius felis.* The tumours in the 2 fish were histologically similar and the authors considered it possible that in the flounder philometrid nematodes or didymozoids were responsible.

Philometra is common in the gonads. Nakajima and Egusa (1979) found an unidentified species of the genus in *Chrysophrys major* in Japan. In male fish immature female worms were located under the serous membrane, and mature worms in the connective tissue of the testes. All large masses of parasites were covered by a thin clear membrane derived from the serosa of the testes. In female fish, mature worms were free in the parenchyma of the ovary. Repeatedly, *Philometra* was shown to damage the ovaries, although infection does not always appear to have harmful effects. Thus, *Philometra* sp. and unidentified Spiruroidea infected the ovaries of approximately 90 % of mature skipjack tuna *Katsuwonus pelamis* in the eastern and western tropical Atlantic Ocean. No nematodes were found in immature fish. Maximum number of nematodes in a pair of ovaries was about 75, but no noticeable damage to the eggs was observed (Simmons, 1969). Annigeri (1962) recorded a case of infection of one *Otolithus argenteus* with *Philometra* sp. in the Mangalore area, India. Except in the anterior one-third of the left ovary and the apical region of the right one with a few immature eggs, all the space of the ovaries was occupied by a female parasite 537 mm long, extending from 1 ovary through the oviduct into the other. Ramachandran (1975) described the pathology of *Mugil cephalus* infected with a species of *Philometra.* The worms feed on blood. In 1 ovary, 14 female and several male worms were found. Heavily infected fish appear less glossy and more silvery and lack the flavour of healthy fish. Heavily infected ovaries are strongly swollen, and most developing ova atrophy. Ovarian tissue shows fibrosis, increased numbers of granulocytes, haemorrhages and a progressive deposition of black pigment. Infected testes are less seriously infected. Great damage to the ovary of skipjack tuna in the Indian Ocean by nematode larvae was also reported by Raju (1960). The nematodes were, however, not identified. One ovary contained 68,200 larvae, mainly in the distal part, with resulting destruction of most eggs. The right ovary was not infected and contained normal eggs.

Hooper (1980) never found more than 1 gravid female *Philometra pellucida* in each ovary of flathead *Platycephalus bassensis* from the New South Wales coast, Australia. He considered it possible that heavier infections were lethal. Infections were often accompanied by the deposition of black pigment on the outer surface of testes and ovaries. Ova were sometimes necrotic and others were discoloured by pigment, but damage to the ovaries was not extensive (Fig. 1-118). Testes did not show any pathological effects, other than discolouration. Gonad size and maturity was similar in infected and uninfected hosts. Hine and Anderson (1981), on the other hand, reported granulomata and atrophy of the

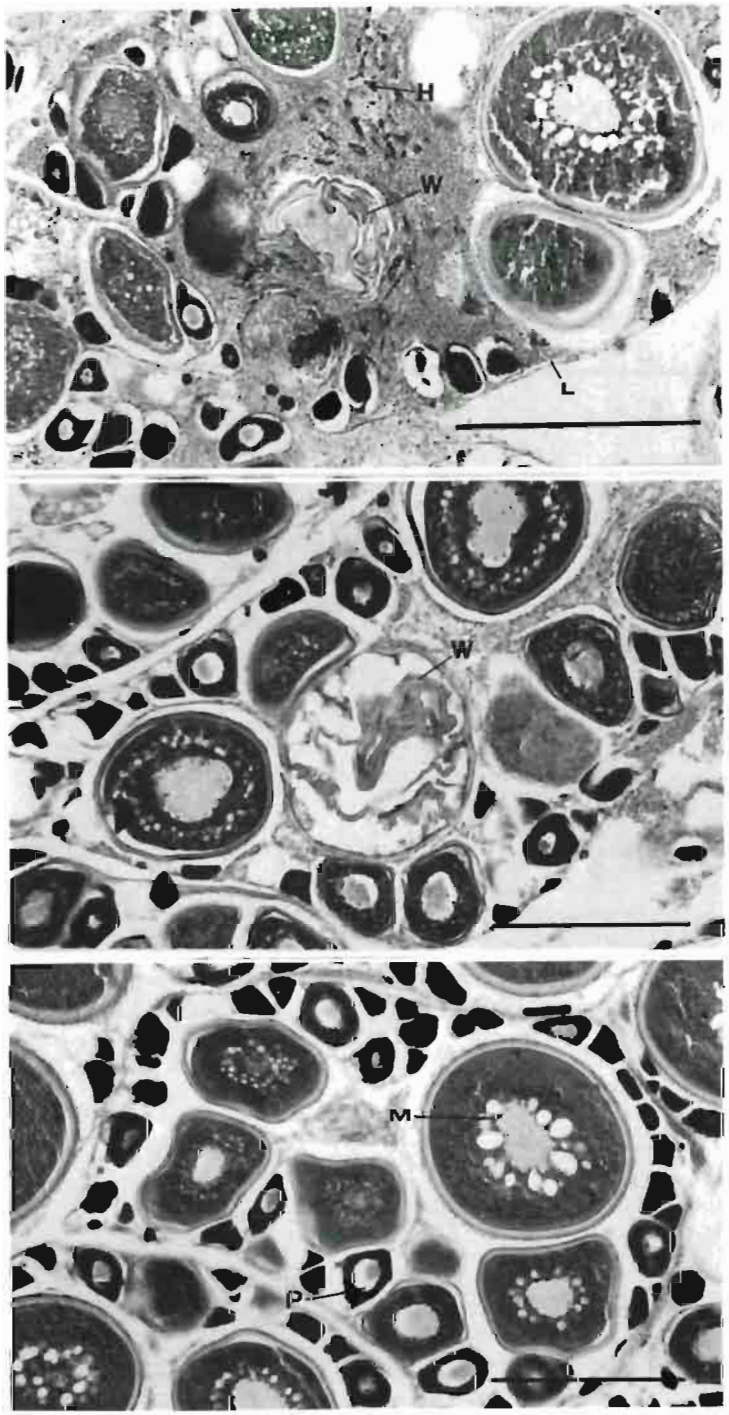

Fig. 1-118: *Platycephalus bassensis*. Photomicrographs of cross-sections through ovaries, comparing ovaries infected with female *Philometra pellucida* and an uninfected ovary. Sections were made 9 to 10 μm thick and stained with haematoxylin eosin. Top: Infected ovary, showing cross-section of anterior end of a gravid female worm, lying within interstitial tissue of an ovarian lobe; mature oocytes, primary oocytes and signs of inflammation and haematin pigments are shown surrounding the worm (scale 500 μm). Centre: Infected ovary, showing cross-section of gravid female worm, sectioned through its intestinal region (scale 500 μm). Below: Uninfected ovary, showing primary and developing oocytes (scale 450 μm). H: haematin pigment; L: ovarian lobe; M: mature oocyte; P: primary oocyte; W: worm. (After Hooper, 1980.)

distal testes in New Zealand snapper, *Chrysophrys auratus*. Males were more often infected with *Philometra* sp. than females (< 58 % vs. < 11 %). Degree of host reaction was not related to the volume of the parasites and in severe infections, all except the peripheral distal gonads were replaced by inflammation tissue.

Deardorff and Stanton (1983) observed heavy infections of 2 sharp-nosed puffer fish *Canthigaster jactator*, with *Philometra* sp. in Hawaiian waters. The large worms in the body cavity caused a conspicuous permanent protrusion of the abdomen in both fish (Fig. 1-119). The authors speculated that, because of the permanent distension, there may be

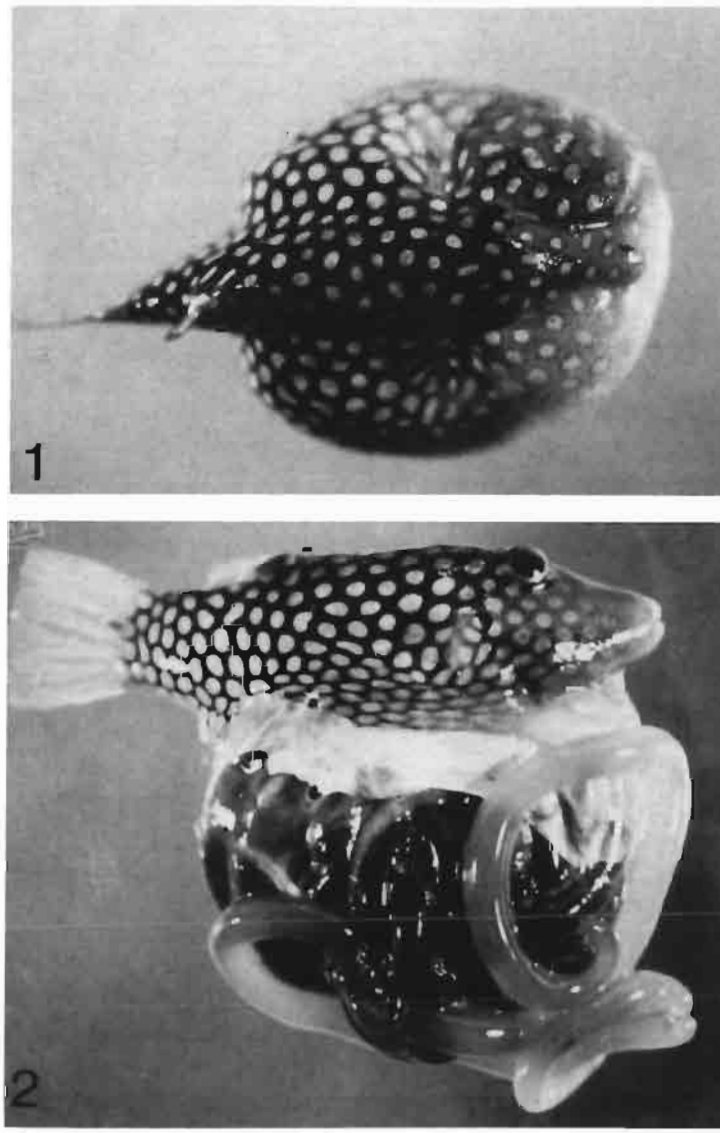

Fig. 1-119: *Canthigaster jactator*. Infection with *Philometra* sp. (1) Dorsal view, showing asymmetrical protrusion due to infection; (2) fish opened to show nematodes. (Original T. L. Deardorff)

significant effects on morbidity and mortality of the fish. One infected fish had a bouyancy problem, it rapidly ascended to the surface if not wedged into a crevice. Five additional infected fish kept in aquaria died, but no controls were kept.

Members of another dracunculoid genus, *Philonema*, have been reported from marine fishes. Platzer and Adams (1967) infected freshwater copepods *Cyclops bicuspidatus* with eggs of *Philonema oncorhynchi*, and salmon fingerlings with copepods containing infective 3rd stage larvae. Thus parasites are acquired in freshwater, but worms develop while the fish are in the sea. In naturally infected *Oncorhynchus nerka*, 4th-stage larvae were found in fish 26 months old. At 32 months, they moved into the coelom and moulted to the subadult stage. At this time, visceral adhesions developed. Worms apparently reach maturity when the fish return from the sea to spawn. Gravid worms are probably passed out with the eggs of the fish, burst in freshwater, and release their larvae which infect the freshwater copepods. Maturation of the worms may be controlled by host hormones (Platzer and Adams, 1967). Adams (1969) examined sections of young fingerling salmon and found that juvenile worms penetrate the gut wall mainly near the pyloric caeca. Most juveniles move through the coelomic cavity to the swim bladder, but some apparently migrate through the mesentery and associated tissues. The adhesions due to the parasites are apparently transitory and mortality of affected salmon does not seem likely in view of the continuing successful existence of sockeye salmon stocks with close to 100 % incidence and high intensity of infection (Margolis, 1970b). Several authors have reported adhesions in salmon caught on the high seas. For example, according to French (1965), a high percentage of sockeye and to a lesser degree chum salmon (*Oncorhynchus nerka* and *O. keta*) caught from 1957 to 1964 had multiple mesenteric and peritoneal adhesions. Other salmon species were not affected. *P. oncorhynchi* was recovered from some of the fish with adhesions, but it is not clear whether the adhesions were caused by the parasites. *P. okeni*, according to Bauer and co-authors (1977), causes exophthalmia in sea perch. However, no supporting evidence was given.

According to the findings of Ko and co-authors (1975), 11 of 15 rays *Aetabatus flagellum* were infected with altogether 107 adult nematodes *Echinocephalus sinensis* (Gnathostomatoidea, Gnathostomatidae) at Hongkong. Larvae infect a great proportion of oysters *Crassostrea gigas*, and rays apparently acquire the infection by eating oysters. Worms were found in the intestinal lumen, attached to the spiral valve or intestine, or in nodules. Nodules had a diameter of 2 to 10 mm and were of 2 types. The first type had a swollen periphery with a central opening of 0.5 to 2.0 mm in diameter (Fig. 1-120, 1). Worms were attached to tissue inside a cavity and protruded through the opening. In the second type the periphery of the nodule was not swollen and a cluster of worms protruded from a poorly defined cavity (Fig. 1-120, 2). The cavity of the nodules was sometimes oedematous, containing a fibrinous exudate with tissue debris (Fig. 1-120, 3). Mild infiltration with leucocytes was observed in the tissue surrounding the cavity containing worms.

According to Schuurmans Stekhoven and Botman (1932), the physalopteroid *Proleptus obtusus* in the dogfish *Scyllium canicola* is firmly attached to the intestinal wall. Worms ingest 'wound secretion' and tissue, but no blood, although there is some bleeding in the villi adjacent to the worms. The epithelium to which the worms had been attached is completely destroyed. However, the ulcers do not show even a trace of cellular infiltration, except for certain areas with strong infiltration, apparently where worms had been

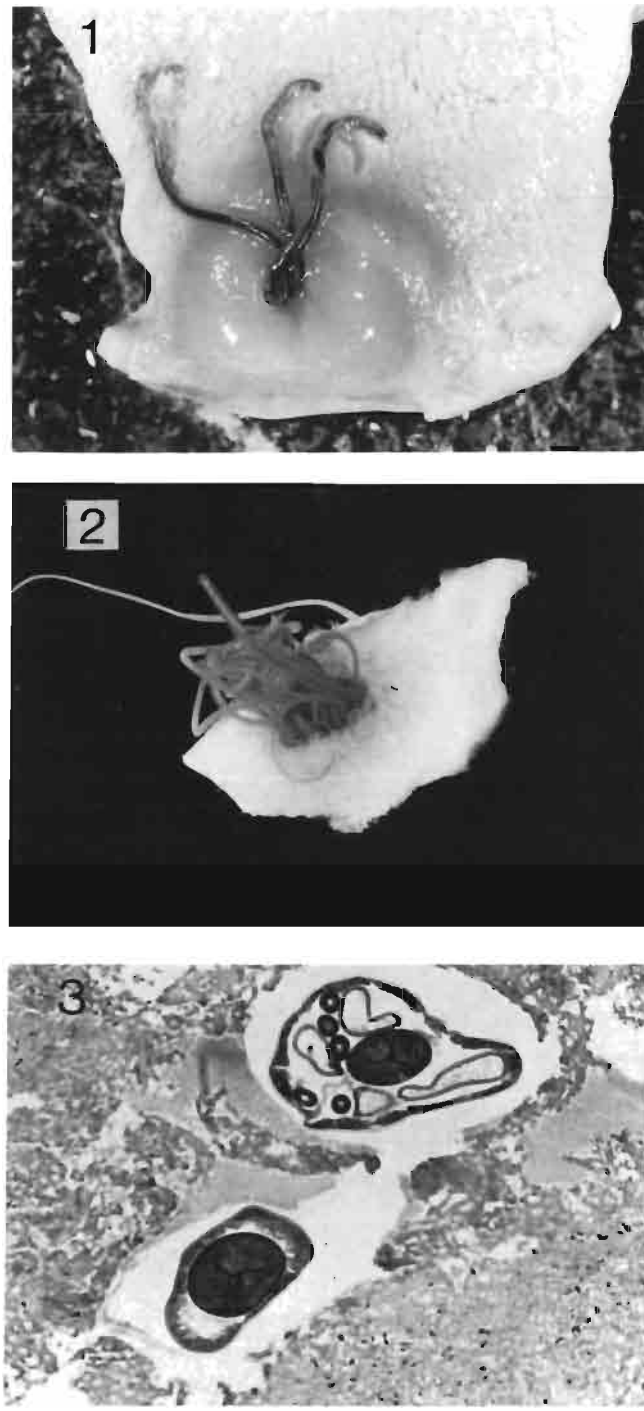

Fig. 1-120: Adult *Echinocephalus sinensis* in *Aetabulus flagellum*. (1) *E. sinensis* in Type I nodule; X 1.5. (2). *E. sinensis* in Type II nodule; X 1.5. (3). Oedematous fluid, fibrinous exudate, and leucocytes in cavity of nodule; X 60. (After Ko and co-authors 1975.)

attached some time earlier. Two characteristics of tumours can be recognized in these areas. There is a proliferation of connective tissue, and the epithelium contains aberrant cell types. The cytoplasm of goblet cells has conspicuously large secretory granules, and infiltration of leucocytes reaches the muscularis mucosae and slightly beyond it (see also Hoeppli, 1927). Janiszewska (1938) reported that adults live in the intestinal lumen and the first 3 larval stages of *Cucullanellus (= Cucullanus) minutus* (Seuratoidea) in the intestinal wall of *Pleuronectes (= Platichthys) flesus*. All 3 stages cause pathological changes, similar to an inflammation mainly of the submucosa. The earliest pathological symptoms are bleeding at the surface of the intestine, destruction of the mucous epithelium, vasodilation of the capillaries at the periphery of the mucosa, and of blood vessels of the submucosa. The part of the submucosa adjacent to the muscularis is strongly infiltrated with haemolysed erythrocytes, and in the middle of the submucosa are infiltrations of small lymphocytes and small necrotic foci. The larva is outside the inflamed areas, in the lumen of blood vessels. In some cases, the larvae migrate into larger blood vessels. After some time they encyst in the wall of the blood vessels of the mesentery, or outside the vessels.

At a somewhat later stage, the larva is partly in the serosa and partly in the connective tissue. It is surrounded by connective tissue strongly infiltrated with lymphocytes. The submucosa near the larva contains many lymphocytes, and the serosa is degenerated at the point of contact with the larva. In many flounders an inflammation of a large area of the submucosa was found. In the centre of the inflamed submucosa was a cavity which was partly filled with necrotic granulation tissue and contained the larva. The larva can apparently move within the cavity. After the larva has entered the lumen of the intestine, the inflammation subsides and regeneration occurs. A distinct capsule in the wall of the intestine is usually associated with degenerating larvae. Outside the intestine, i.e. in the mesentery and liver, larvae are always surrounded by a capsule consisting of loose connective tissue infiltrated with lymphocytes and histioblasts.

Eggs are usually laid into the lumen of the intestine and leave the host through the anus but several times they were found in the liver of the host together with females, both encapsulated. Females probably had penetrated through the wall of the intestine.

Species of the genus *Spirocamallanus* (Camallanoidea) are common parasites in the digestive tract of marine fishes (e.g. Fusco and Overstreet, 1978; Overstreet, 1978a; Rychlinski and Deardorff, 1982), but little is known about their pathology. Observations on the related genus *Camallamus*, which has been implicated in disease and death of aquarium fish, indicate that the worms may be harmful (Rychlinksi and Deardorff, 1982).

McVicar and Gibson (1975) recovered up to 27 nematodes of the species *Pancreatonema torriensis* (Thelazioidea) from the pancreatic ducts of *Raja naevus* in the North Sea. The worms were usually in the distant portions of the ducts, and only occasionally were some specimens located close to or just within the pancreas. In sections, worms were free in the lumen of the ducts and did not appear to attack the tissues of the duct wall. Distal portions of the ducts were, however, greatly distended.

Among the Trichinelloidea, *Trichinella* may occur in marine fishes, which probably act as transport hosts. This is indicated by some circumstantial evidence and experimental infections (Kozlov, 1971). In view of the serious effects which heavy infections have on warm-blooded vertebrates, further studies may show pathological effects on fish.

Capillaria cyprinodonticola occurred sometimes in massive infections in various

cyprinodontiform fishes in the Florida Keys, resulting in an extensive destruction of liver tissue and the deposition of large numbers of eggs (Huffman and Bullock, 1973). Atrophy or necrosis of the liver parenchyma due to eggs deposited by a species of *Capillaria* in *Scomber japonicus* was also reported by Yamaguti (1935).

At the base of the ventral and pectoral fins of *Mustelus mustelus* in the Mediterranean, Ruyck and Chabaud (1960) found tumours filled with larvae of the nematode *Phlyctainophora lamnae* (Dracunculoidea). They penetrated deep into the abdominal wall. Two large tumours with larvae were also found projecting into the mouth cavity. They penetrated into the intermuscular connective tissue and even into the cartilage. The tumours were thought to have contained gravid females which died, with only the larvae remaining.

Agents: Acanthocephala

Biology

So far, approximately 500 species of acanthocephalans have been described. Taxonomic synopses of Acanthocephala have been presented by Yamaguti (1963b) and Golvan (1969). General information on Acanthocephala was given by Hamann (1891), Meyer (1933), Hyman (1951b), Baer (1961) and Golvan (1964). The biology including the life cycles and pathology of acanthocephalans has been reviewed by Nicholas (1967, 1973). These publications show that very little is known about marine forms. Crompton (1970) reviewed acanthocephalan physiology from an ecological point of view.

Adult worms live in the intestine of vertebrates, particularly of fishes. They absorb intestinal contents of the host through their surface. Female worms lay eggs which are eaten by the first intermediate host, benthic crustaceans, in which the first larva, the acanthor, hatches, develops to the acanthella and further to the juvenile. The juvenile may encyst thus becoming a cystacanth. The vertebrate final host becomes infected by ingesting infected first intermediate hosts. Sometimes other invertebrates or fish may serve as transport hosts, in which the infective larvae survive and retain infectivity without undergoing further development. According to Crompton (1970), the 'transport host' of certain species may in fact be a necessary second intermediate host in which growth occurs.

Corynosoma semerme uses amphipods as intermediate hosts, fishes as transport hosts and seals as final hosts. However, this sequence is based only on circumstantial evidence. It may also be that undigested amphipods in fish rather than immature worms in the tissues of fish transmit the infection to the final host.

Corynosoma strumosum undergoes early development in marine amphipods and uses many species of fish as transport hosts. Seals and birds become infected by eating infected fish.

The life cycle of *Echinorhynchus lageniformis* was experimentally worked out by Olson and Pratt (1971). Shelled acanthor larvae are ingested by the amphipod *Corophium spinicorne*. Thirty days after infection at 23 °C, acanthella-larvae in the crustacean become infective to the final host, the starry flounder *Platichthys stellatus* (which is the host on the coast of Oregon, USA). The life span of the adult worm is about 1 yr, since older fish lose the parasites. Only young fish (4 to 18 cm long) feed primarily on amphipods and hence become infected.

Paratenuisentis ambiguus infects eels *Anguilla rostrata* in the tidal zone of the Oyster River, New Hampshire. *Gammarus mucronatus* and *G. tigrinus* are intermediate hosts. A

second intermediate host is apparently not required, although various other fish are possibly transport hosts and contribute to massive infections of eel (Samuel and Bullock, 1981). Freshwater and estuarine gammarids could be infected experimentally with larvae of *Pomphorhynchus rocci*. Cystacanths occurred in striped bass *Morone saxatilis* and the euryhaline white perch *Morone americana* (Paperna and Zwerner, 1976). According to Buckner and co-authors (1978), *Tegorhynchus furcatus* and *Dollfusentis chandleri* use amphipods as intermediate hosts (Fig. 1-121).

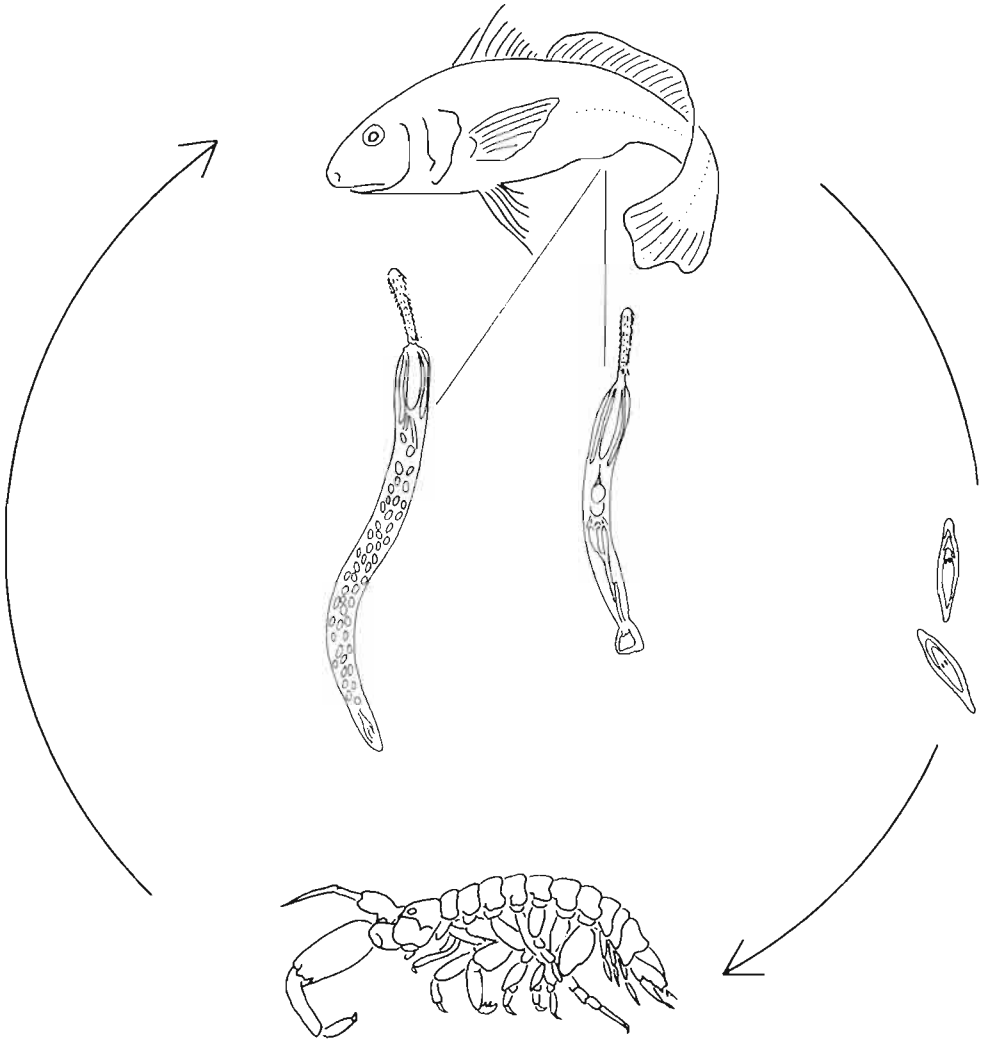

Fig. 1-121: *Dollfusentis chandleri*. Life cycle. Male and female worms live and mate in the rectum of the Atlantic croaker and other fishes. Specific amphipod crustaceans feeding on deposited eggs acquire a stage that ultimately produces a juvenile worm. Croaker become infected from eating these amphipods; however, some other acanthocephalans utilize different arthropods as hosts, and some use additional transfer hosts to bridge the apparent gap between an arthropod and a predator that feeds on relatively large prey. (After Overstreet, 1978a.)

Effects on Hosts

There are several records of harmful effects of acanthocephalans on fish hosts. Hofer (1904) and Schultz (1911) pointed out that echinorhynchs had been responsible for mass mortalities of fish in fish cultures and under natural conditions. *Pomphorhynchus laevis* (reported as *Echinorhynchus proteus*), for example, in the near-anal hindgut of flounder *Pleuronectes flesus,* perforates all layers of the intestine and the worms are covered only by the peritoneum of the intestine. In infections with several worms, the intestinal lumen is almost blocked making defaecation difficult. On the other hand, *Echinorhynchus gadi* (reported as *E. acus*) in *Gadus morhua,* according to the observations of Schultz (1911), causes much lesser damage. Usually the worms were found loose in the faeces of the posterior portion of the gut. In living fish they are probably less deeply inserted than *P. laevis,* since their proboscis is shorter. Occasionally, larvae of *E. gadi* occur in the body cavity, apparently after perforation of the intestinal wall.

Other authors have reported serious damage due to *Echinorhynchus gadi* which occurs in many fish species and has a wide geographic distribution (e.g., Linton, 1933; Yamaguti, 1963b; further references in Sindermann, 1966). For instance, Suzuki and Oishi (1974) observed intestinal lesions due to the parasite in *Theragra chalcogramma,* and Munson (1974) reported that it caused total destruction of the epithelium at the site of penetration in the 'seasnail' *Liparis atlanticus.* Nevertheless, penetration was never deeper than the lamina propria, which was slightly thickened near the penetration site, due to an increase in the number of fibroblasts and lymphocytes.

Janiszewska (1938) gave more details on the effect of *Pomphorhynchus laevis* on the flounder. There is strong development of connective tissue around the parasites, vasodilation, and tissue degeneration. *Echinorhynchus lageniformis,* which infects various species of pleuronectids on the North American Pacific coast (Ekbaum, 1983; Prakash and Adams, 1960; Olson and Pratt, 1973) extensively damages the gut wall of the starry flounder *Platichthys stellatus* (Prakash and Adams, 1960). More than a quarter of the fish caught near Vancouver, western Canada, were infected, and the parasites were most frequently recovered from the proximal loop of the intestine. The proboscis of the worms is usually deeply embedded in the submucosa, and sometimes reaches into the muscularis externa. Female worms are usually more deeply embedded than males. At the site of penetration, the columnar epithelium is completely denuded. The tissue around the insertion degenerates. Many fibroblasts tend to form a collagenic capsule. Depending on the degree of penetration, granulation tissue appears either as a dark-staining mass which extends from the mucosal layer to the submucosa or continues through the muscularis externa and serosa to form a polypoid protrusion outside the intestinal wall. This protrusion is not neoplastic but a chronic granuloma. Similarly, localized or extensive granuloma and epithelioidal changes around the intestine resulting from perforation of the lower intestine by adult *Pomphorhynchus rocci,* were observed by Paperna and Zwerner (1976) in striped bass *Morone saxatilis.*

Infections with *Serrasentis nadakali* cause hyperplastic, metaplastic and hypertrophic changes in the intestine of *Rachycentron canadus.* The worms destroy the villi of the intestine at attachment sites, and cause degeneration and necrosis of the mucosal epithelium. Inflammatory responses are characterized by aggregation of epithelioids, lymphocytes, macrophages and unidentified cells. There is also excessive mucus secretion (George and Nadakal, 1981).

According to George and Nadakal (1982), the acanthocephalan *Echinorhynchus veli* occurred in large numbers (maximum 112) throughout the year in the brackish water fish *Synaptura orientalis*. The worms are concentrated in the region 10 cm anterior to the cloaca, gravid worms occurring more anteriorly than the immature worms. Proboscis and neck of the worms are embedded in the wall of the intestine, rupturing the villi and submucosa and deeply penetrating into the muscularis. There is a proliferation of fibroblasts and fibrous tissue around the attachment sites, giving rise to spongy granulomatous

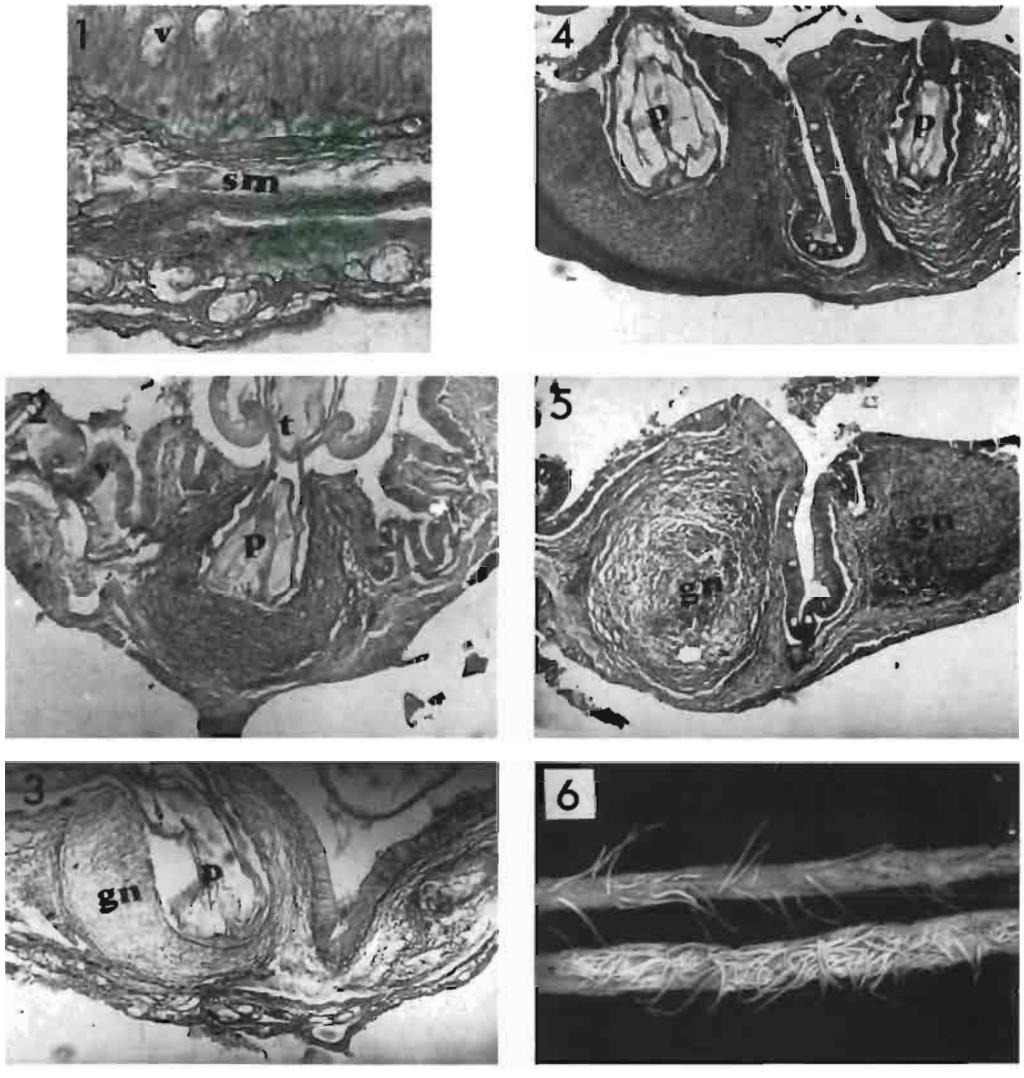

Fig. 122: (1) Uninfected intestine of *Synaptura orientalis* 400 X. (2) Infected area of intestine with *Echinorhynchus veli* 100 X. (3) An advanced stage of infection with *Echinorhynchus veli* 100 X. (4) Multiple nodulated area of infection 100 X. (5) Granuloma of the intestine of *Synaptura orientalis* developed by the infection of *E. veli* 100 X. (6) *Echinorthynchus veli* in the small intestine of *Synaptura orientalis* (Photo by T. I. Jacob) 1.5 X. v = villi; sm = submucosa; p = proboscis; gn = granulomatous nodule; m = mucosa; t = trunk. (After George and Nadakal, 1982.)

nodules (Fig. 1–122, 5), which press on the muscularis. In advanced stages of infection the enlarged nodules measured 480 to 500 × 670 to 700 µm (Fig. 1–122, 3), with total destruction of the mucosa and submucosa at the point of attachment. In addition, hypertrophic and hyperplastic changes occurred in the epithelium. Cell necrosis was slight. In heavy infections there was occlusion of the intestine, but there were no secondary bacterial infections and the mucous secretions appeared to be normal. Average weight of 10 uninfected fish was 35.6 (21 to 48), that of infected fish 20.7 (7 to 26) (apparently g, but not stated) in fishes 13 to 15 × 6 to 8 cm large. However, it is not stated whether other parasites were present. In *Saurus tumbil* infected with a species of *Serrasentis,* Tadros and co-authors (1979) observed damage to the intestinal wall. An inflammatory reaction in the vicinity of the hooks of the trunk was thought to be due to a secondary bacterial infection.

Andrejev and Markov (1971) reported on effects of the nematode *'Contracaecum',* the cestodarian *Amphilina foliacea* and the acanthocephalan *Leptorhynchoides plagicephalus* on the blood of sturgeon. Affected by the last 2 species were haemoglobin level, erythrocyte number, Cu, Co, Mn and Zn contents, index of colloidal stability, and fat contents, but the data are difficult to interpret because of multiple infections, possibly also with further parasite species not mentioned.

With regard to the effect of acanthocephalans on the condition of fish, Huizinga and Haley (1962) did not notice a measurable effect on the host condition as indicated by weight in infections of *Leiostomus xanthurus* with *Telosentis tenuicornis.* But they emphasized that a better measure of condition is required than host weight.

Petrushevsky and Kogteva (1954) used data supplied by Polyansky for 90 Murman cod *Gadus morhua morhua,* infected with the acanthocephalan *Echinorhynchus gadi* (62 % infected) and 3 species of larval nematodes, to calculate Fulton's condition factor (see discussion for *Contracaecum,* p. 286). For *E. gadi* considered separately, the following data were obtained:

No of E. gadi	*Fulton's K*
1–10	.912
10–25	.832
25–50	.798
greater than 50	.712

One fish with 60 *E. gadi* and 1431 nematodes had a K of .698. In 30 White Sea cod *Gadus morhua morhua* n. *hiemalis,* infected with *E. gadi,* Fulton's K was .993 in fish with 1 to 30, and .908 in fish with 20 to 50 parasites. Infections of cod with *E. gadi* reduce the condition of fish markedly, although some caution in interpreting the data is necessary, particularly because of the small number of fish, the simultaneous occurrence of other parasite species, and the lack of adequate statistical methods to evaluate the data.

Bauer and co-authors (1977), without giving any details, stated that *Echinorhynchus truttae* (syn. *Metechinorhynchus truttae*), leads to emaciation and death of fish in the North Sea.

Agents: Hirudinea

Biology

There are approximately 300 species of leeches, of which ca. three quarters feed on blood. The others are predators, and some of the haematophagous species feed also as animal predators. Most blood feeders attack vertebrates, and for some species presence of a substance has been demonstrated that prevents blood clotting. Mann (1962) wrote a monograph of the leeches, Lukin (1976) reviewed the leeches of fresh and brackish water and Epshtein (1970) discussed the zoogeography of marine leeches. Accounts of some aspects of the biology of marine leeches were given by Selensky (1923), Knight-Jones (1940), Llewellyn (1965), Gibson and Tong (1969), Sawyer (1970), Sawyer and Hammond (1973), Sawyer and co-authors (1975), Burreson (1976a, b), Khan and Meyer (1976), Penner and Raj (1977), and Khan (1982). Some species infect a wide range of hosts, others show host specificity of varying degrees. For instance, *Trachelobdella lubrica,* a piscicolid leech, has a wide geographical distribution, occurring in the Atlantic Ocean, Mediterranean Sea, Indian Ocean, Arabian Sea, Australian waters, Philippine and Hawaiian waters. So far, 30 species of fishes, most belonging to the Perciformes, have been reported as hosts (Epshtein, 1973). *Calliobdella carolinensis* infects various clupeids, occasionally non-clupeids and even elasmobranchs (Sawyer and Hammond, 1973).

Host specificity of leeches is indicated by the following observations of Khan and Meyer (1976). The piscicolid leech *Malmaiana scorpii* infects shorthorn sculpin *Myoxocephalus scorpius* and long-horn sculpin *M. octodecemspinosus* in Newfoundland. Three hundred leeches were removed from the fish and kept in an aquarium for 28 days or longer. Various fish (23 species) were added for 1 to 3 days. The only fish to which the leeches attached were the 2 sculpins. Even when leeches were directly put on other fish, attachment did not occur. However, several times leeches attached to dead objects covered with mucus from sculpins. Experiments by Khan (1982) showed that *Johanssonia arctica* feeds readily on a number of host species, less readily on some others, and not at all on still others. *Hemibdella soleae* in British waters apparently infects only *Solea solea* and mainly mature fish, but in the Mediterranean several other host species have been reported (Llewellyn, 1965). Some species show marked preferences for certain macrohabitats. Thus, among 14 piscicolid species from Newfoundland to Texas coastal waters, Sawyer and co-authors (1975) distinguished northern and southern brackish water and high salinity species.

For several species, a distinct site specificity has been demonstrated. Data on site preferences of some leeches of fishes in the Indo-Pacific Ocean were given by Sanjeeva Raj (1974). Throughout 3 yr of study, *Calliobdella carolinensis* were restricted to the mouth cavity of *Brevoortia tyrannus;* none was found on body or fins (Sawyer and Hammond, 1973). *Aestabdella abditovesiculata* occurs in the mouth of the staghorn sculpin *Leptocottus armatus* and of several other fish species. However, in heavy infections with more than 75 leeches in the mouth, parasites were also found on pelvic fins (Burreson, 1976a). Young *Oceanobdella blennii* attach themselves to *Blennius pholis* and move into the opercular cavity. Larger worms move to the body where they are sheltered by the pectoral fins (Gibson and Tong, 1969; Sawyer, 1970). Some species invade the internal organs of hosts. Thus, *Branchellion lobata* was recovered from the ventral surface of embryos of the Pacific angel shark *Squatina californica* and from the cloacal region and from the spiral valve of female sharks (Moser and Anderson, 1977).

In cold-temperate seas, where most studies have been made, many leeches show distinct seasonality. Thus, the piscicolid *Aestabdella abditovesiculata* in upper Yaquina Bay, Oregon (USA), appears in July, when water temperatures reach about 18 °C, is abundant during August and September at 20 °C, and disappears by the end of October near 12 °C. Other examples of seasonality in marine leeches were discussed by Gibson and Tong (1969: *Oceanobdella blennii* off north Wales most frequent in February to March), Sawyer and Hammond (1973: *Calliobdella carolinensis* off South Carolina, disappears from host fish in late winter and early spring), by Sawyer and co-authors (1975: 14 piscicolid species from Newfoundland to Texas often with marked seasonality), and by Daniels and Sawyer (1975: *Myzobdella lugubris* increase on catfish *Ictalurus catus* in South Carolina from October to January, but decrease from February to April).

Hemibdella soleae remains on its host, *Solea solea*, while attaching cocoons to shell fragments with which the fish camouflages itself (Llewellyn, 1965). However, most blood sucking leeches apparently leave their hosts during the breeding season and seek a solid substratum for cocoon deposition. Thus, *Calliobdella carolinensis* leave menhaden *Brevoortia tyrannus* in late winter and early spring and attach themselves to oyster clumps and associated seaweed *(Ulva)* (Sawyer and Hammond, 1973). According to Sawyer and co-authors (1975), leeches on the North American coast, toward the end of their reproductive period leave the host following a blood meal, deposit cocoons on a solid substratum, such as shells, rocks, or a crab, then die. Some species deposit their cocoons upon the hosts, and occasionally leeches occur on crustaceans for dispersal and for cocoon deposition (e.g., *Myzobdella lugubris* on the crab *Callinectes sapidus:* Daniels and Sawyer, 1975; *Johanssonia arctica* on the crab *Chionocetes opilio:* Khan 1982; Fig. 1-123). Most marine piscicolid leeches produce 1 embryo in a cocoon with the exception of some species of *Malmiona,* in whose cocoons 3 to 5 eggs may be present (Khan and Meyer, 1976). Some species live for several years. According to Khan (1982), for example, *Johanssonia arctica* lives 2½ yr at −1 to 2 °C.

Egg development in the cocoons depends on the species and temperature. Cocoons of the piscicolid *Aestabdella abditovesiculata,* which are loosely attached to the substratum and contain 1 egg, hatched in the laboratory in 30 days at 20 °C (Burreson, 1976a). *Trachelobdella oregonensis* also has cocoons with 1 egg which hatched in 104 days at 10 °C (Burreson, 1976b). In the latter species, all newly hatched individuals are mature males with immature female apparatus. Cocoons of *Oceanobdella blennii* off North Wales are laid on stones in April and May, and hatch in the following December and January (Gibson and Tong, 1969). *Calliobdella carolinensis* deposits its cocoons usually in clumps of 7 to 29. Each leech deposits about 21 cocoons, after which it dies (by May). No leeches are found until the following winter, when juveniles attach to fish (Sawyer and Hammond, 1973). *Hemibdella soleae* hatches in about 41 days at 17 °C. Juveniles can infect fish immediately after hatching, but they can also remain unfed for up to 8 weeks. Maturity is reached in about 23 days after infection, and full size in about 37 days (Llewellyn, 1965). Cocoons of *Johanssonia arctica* hatch in 176 to 253 days (Khan, 1982). Most leeches produce 1 generation each year but occasionally 2 as in *Oceanobdella sexoculata* (Khan and Meyer, 1978).

Leeches, corresponding to their complex structure, particularly of the nervous system, exhibit complex behavior patterns which help in host finding. Knight-Jones (1940) described the habits of the marine leech *Abranchus blennii.* It does not appear to swim but

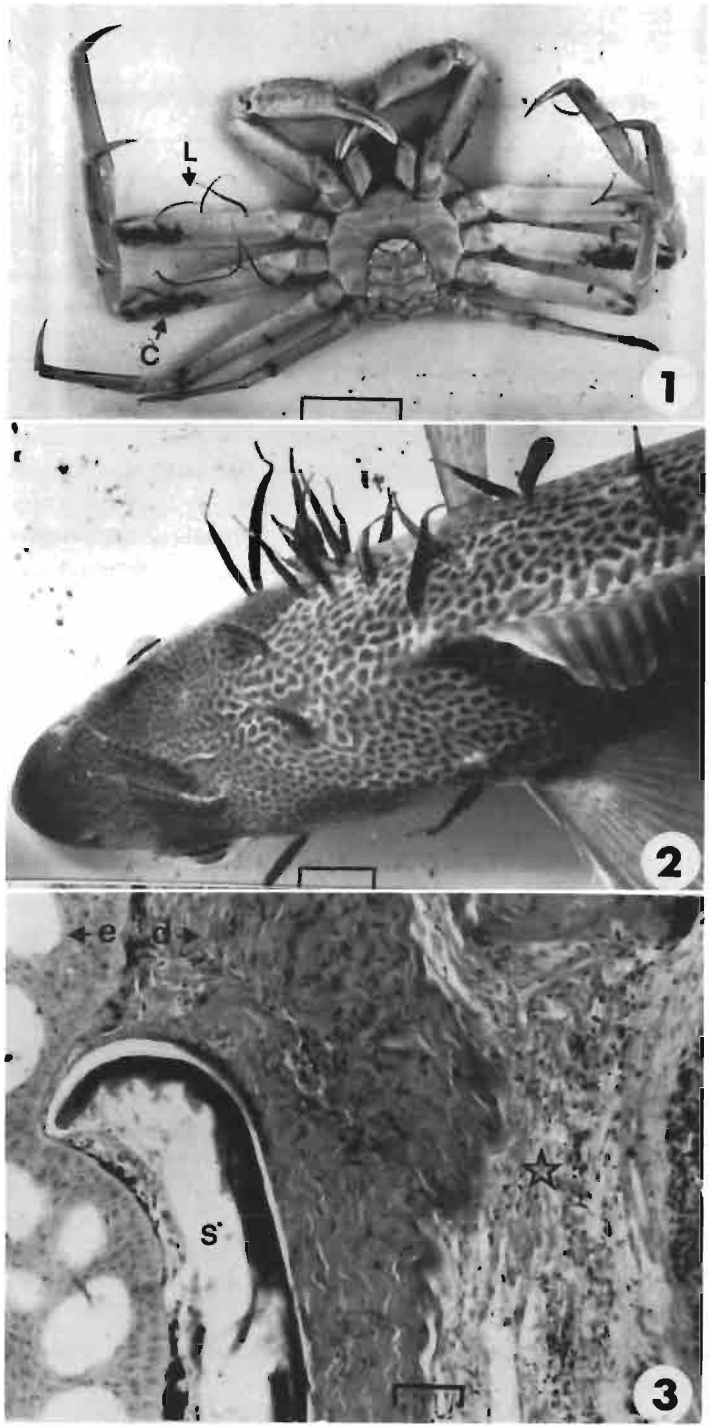

Fig. 1-123: (1) Ventral aspect of spider crab with leeches (L) and cocoons (C); note latter on distal femoral surfaces; scale bar = 5 cm. (2) Atlantic cod with engorged leeches; scale bar = 2 cm. (3) Subcutaneous haemorrhage (star) in the skin of Atlantic cod. d: dermis; e: epidermis; s: scale; scale bar = 10 μm. (After Khan, 1982.)

can move energetically by looping. Slight water movements or a shadow falling across it induce stretching to more than twice its resting length as well as random searching movements, characterized by swinging the attenuated rigid body in all directions, while attached with the posterior sucker. It attaches itself by means of its anterior sucker, if a fish is presented to it. According to Gibson and Tong (1969), host-finding in *Oceanobdella blennii,* a parasite on *Blennius pholis* on the coast of North Wales, is also facilitated by response to water movement and shadow. Newly emerged *Calliobdella carolinensis* are good swimmers. Laboratory observations showed that some probably find their hosts, the surface-feeding Atlantic menhaden *Brevoortia tyrannus,* by suspending themselves upside down on the surface film of the water. Others probably find their hosts by swimming toward a school of fish as it casts a shadow on the bottom (Sawyer and Hammond, 1973).

Feeding was observed in *Calliobdella carolinensis* by Sawyer and Hammond (1973). It lasted for at least 6 to 8 h and in some cases the fish reacted with a frenzied darting motion which occasionally dislodged the leeches. Rate of digestion was slow. At 9 °C, leeches lived for at least 10 weeks without a further feeding. *Johanssonia arctica* ingests from 2 to 4 times its body weight of blood. Time for digestion depends on the size of the leech and the quantity of blood ingested and varies from 47 to 105 days. Some leeches can fast for at least 1 yr (Kahn, 1982).

Infection intensities with leeches may be high. Thus, probably as a result of unusually cold water temperatures and a high level of turbidity, a population explosion of *Calliobdella carolinensis* occurred on the Atlantic menhaden *Brevoortia tyrannus* on the South Carolina coast in January to May 1971 (Sawyer and Hammond, 1973). Hundreds, if not thousands of leeches were found with almost every collection of menhaden. Infection intensities reached 348 leeches in the mouth cavity of 1 fish. Local fishermen verified that during this period the leeches accumulated on the decks of their boats in great numbers and were often found on the hands and feet of the fishermen. Prior to this population explosion only few records were known.

Effects on Hosts

Effects due to leeches often are light or not obvious. Lammert (1974), during the examination of 2405 *Solea solea* for ectoparasites, excluding those on the gills and under the fins, did not notice harmful effects by the leech *Calliobdella* (probably *nodulifera*).

Mace and Davis (1972) used carefully controlled experiments to study the energetics of the host-parasite relations of the leech *Malmiana brunnea* (= *nuda*) and the shorthorn sculpin *Myoxocephalus scorpius.* Fish of approximately the same age and size, fed exclusively with mantle tissue of a squid species, were used. After the experiment, dissections of the fish did not yield any other helminth species. The energy requirements of the parasites were calculated indirectly and compared with direct estimates from host energy anomalies. Energy values were assigned to all components of the fish energy equation, and the total of these values compared with energy input. Following a controlled growth period of five weeks, the difference between observed and expected growth rates of parasitized fish was assumed to be due to the presence of the parasite.

No wound was visible after removal of a leech, and feeding generally lasted from 2 to 3 h. The growth rate of infected fish was distinctly slower than that of non-infected fish, and it was concluded that the additional energy consumption of the host due to the parasite burden was approximately 750 cal g^{-1} leech $week^{-1}$. The energy loss of the fish was not

entirely due to the energy requirements of the leech, but partly to the increase in the metabolic rate of the fish probably as the result of physiological strain.

Paperna and Zwerner (1974) described the histopathology of the skin of a white catfish *Ictalurus catus* from the brackish part of York River, Virginia, USA, infected with large numbers of the leech *Cystobranchus virginicus.* They found 500 leeches attached, mainly to the sides of the mouth, along the gill aperture under the operculum, in the skin fold behind the lower jaw, and at the bases of the fins. In the attachment area, the white skin was densely dotted with red spots, becoming confluent in areas with the heaviest infection. Changes in the skin at attachment sites included extensive inflammation, displacement and erosion of the dermal layers, hyperplasia of the epithelium, and cellular infiltration in the epidermis, in the collagen layer of the dermis, and in some areas of the subcutis and underlying muscles. Cellular infiltration was most obvious in the opercular region and at the bases of the fins (Fig. 1-124, 1 and 2). The number of lymphatic spaces in the stratum germinativum increased, and haemorrhages were present beneath the epithelium in the upper dermal layer.

Although the epithelial surface was frequently intact, some localized erosion of the epidermis was extensive and disruptive, affecting the squamous and middle layers. The dermal collagen layer was often disoriented. Two sites from the ventral skin folds of the lower jaws exhibited massive papilloma-like hyperplasia of both the giant skin gland cells ('club-shaped glands' of Mittal and Munshi) and the epithelium. This hyperplastic epidermis contained an expanded network of connective tissue stromata and the surface of the epidermis was very eroded in this area (Fig. 1-124, 3 and 4).

In some areas the epithelium, as well as strands of dermal collagen and bundles of fibroblasts, was pushed into subdermal layers and sometimes even into the muscular layer. This epithelium proliferated extensively and penetrated adjacent tissues (Fig. 1-124, 2, 5 and 6.).

The normal habitat for fish and parasite is apparently freshwater, but brackish water can be tolerated. It appears that the leeches contributed significantly to the distressed condition of the fish. The infestation is potentially destructive to the fish, as reported from catfish in fishponds in the southeastern USA (for *Cystobranchus* sp.). Subcutaneous haemorrhages caused by *Johanssonia arctica* on Atlantic cod *Gadus morhua* (Fig. 1-123, 2), are shown in Fig. 123, 3.

Paperna and Overstreet (1981) noted lesions on mullet associated with attached leeches of the species *Myzobdella lugubris.* According to the authors, extensive blood loss probably occurs only with intense and prolonged infestations. Roubal (pers. comm.) observed thickening of the mucosa, lamina propria and submucosa (terminology by Groman, 1982) with heavy cellular infiltration in the mouth cavity of *Acanthopagrus australis* at the attachment site of an unidentified leech (Fig. 1-125).

Sanjeeva Raj (1974) provided brief information on pathological effects caused by leeches in fishes in the Indo-Pacific Ocean, based on various sources and his own observations, without including details or illustrations. According to that author, *Austrobdella bilobata* causes ulcerated patches and extensive scars on the body of its host, the flounder *Rhombosolea tapirina*, in Tasmania. *Branchellion parkesi* frequently leaves an eroded scar on its host.

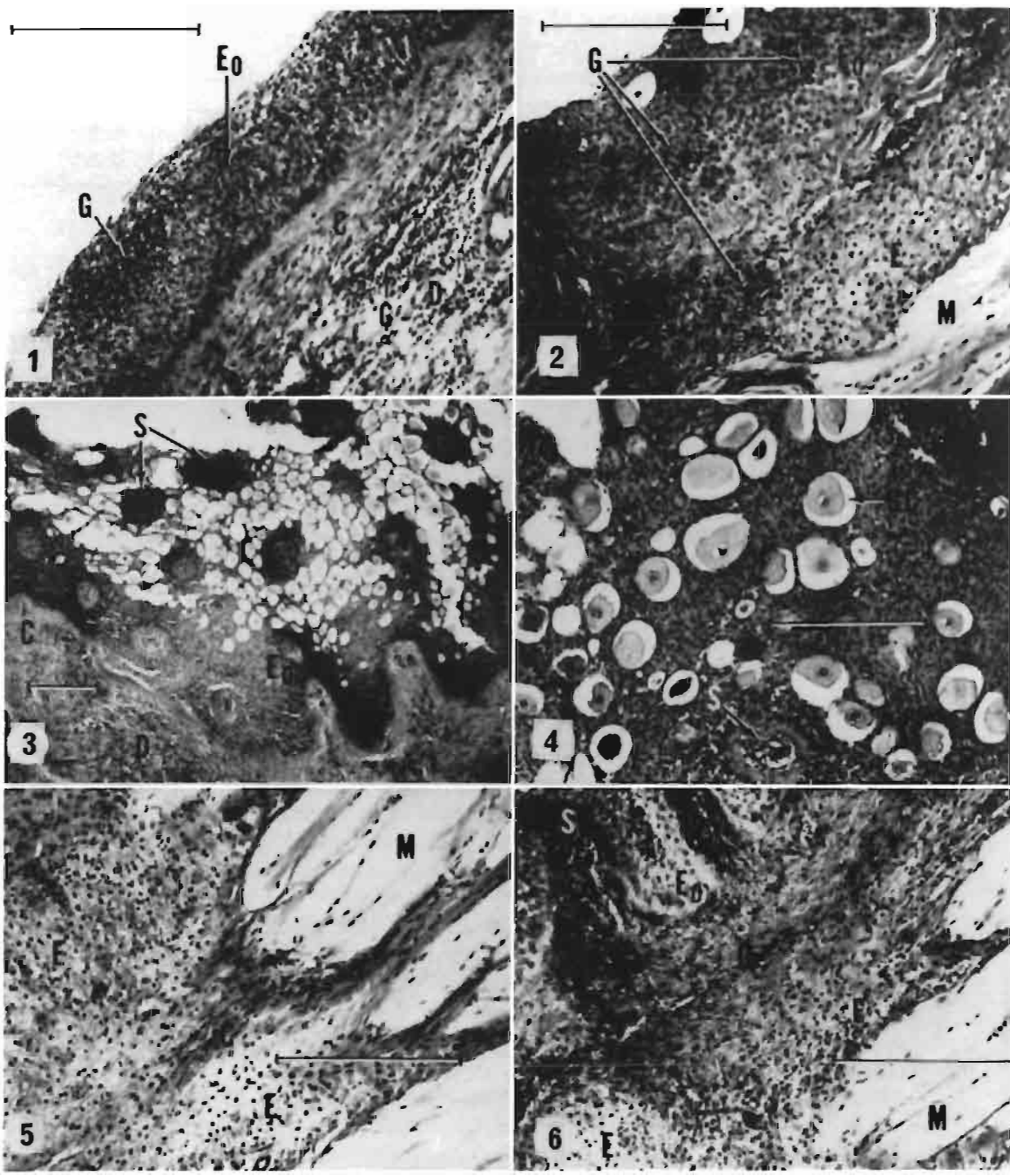

Fig. 1-124: *Ictalurus catus* infected with *Cystobranchus virginicus*. Histological sections of skin. Scale bars for 3 to 6 = 0.2 mm. C: dermal collagen; D: subdermis; E: displaced epithelium; E_0: surface epithelial layer; G: granulation sites; GC: club-shaped gland cells; M: muscle layer; S: connective tissue stromata in the epithelium. (1) Cross-section showing areas of granulation. (2) Cross-section showing additional areas of granulation and displacement of epithelium into the subdermis. (3) Oblique section showing proliferation of epithelium and club-shaped gland cells; note eroded surface layer. (4) Enlargement of area similar to 3 showing detail of club-shaped gland cells. (5) Cross-section showing displacement of epithelium into muscle layer. (6) Same section as above showing involvement in the deeper tissues. (After Paperna and Zwerner, 1974).

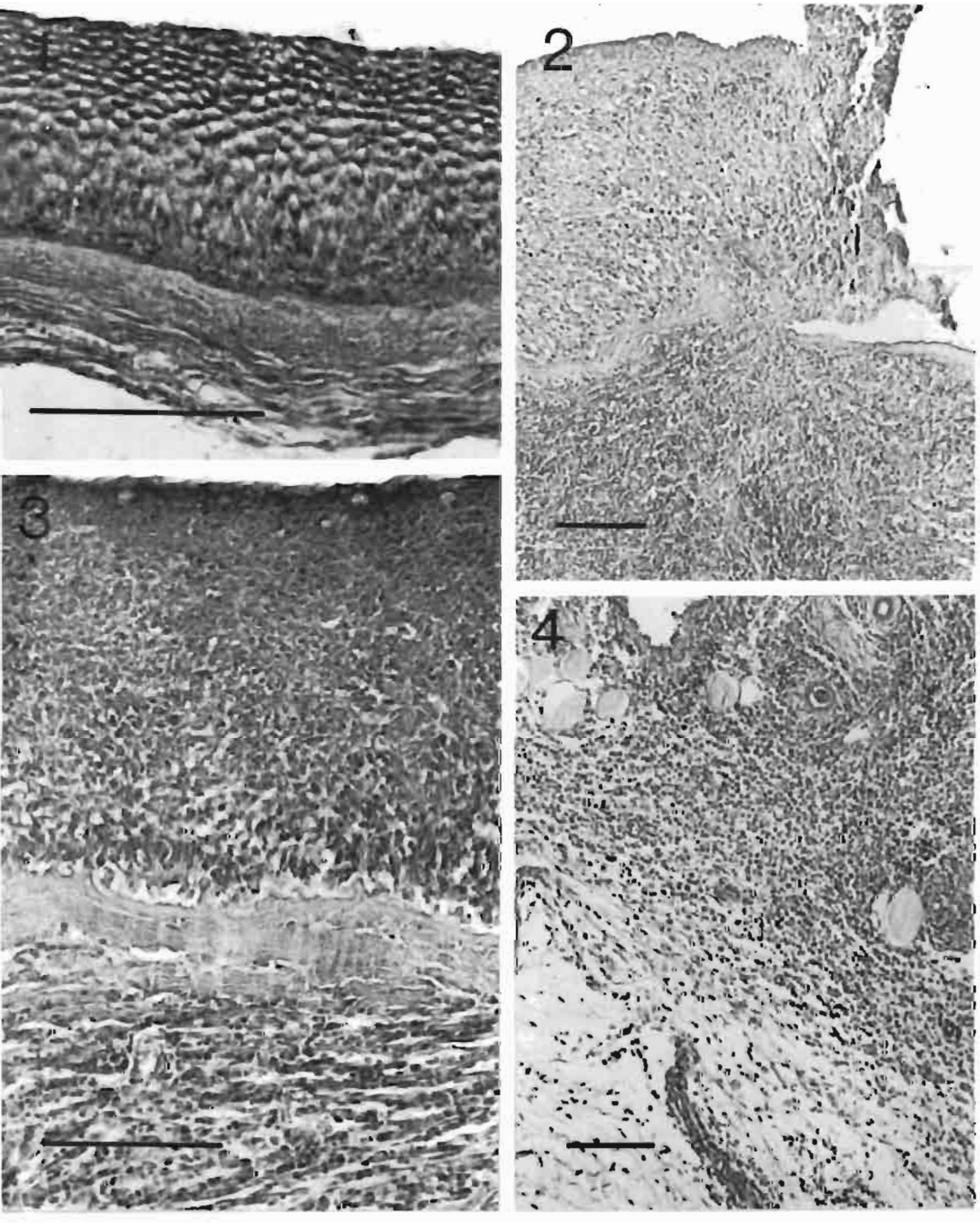

Fig. 1-125: Tissue reactions due to Hirudinea (unidentified) in mouth cavity of *Acanthopagrus australis*. (1). Upper palate, normal, scale 100 µm. (2) Attachment site with eroded mucosa, lamina propria exposed, scale 100 µm. (3) Feeding site of leech, note thickened mucosa, lamina propria and submucosa, scale 100 µm. (4) Submucosa below c; note heavy infiltration by cells; scale 100 µm. (Original F. R. Roubal.)

Leeches as Vectors of Blood Parasites

Marine fishes harbour a variety of haematozoa, e.g., species of the flagellate genera *Trypanosoma, Cryptobia,* and *Trypanoplasma,* and of the sporozoan *Haemogregarina* (see review by Becker, 1970; Khan and Newman, 1982; for early studies see Neumann, 1909). According to Bower (1982, in press), *Trypanoplasma* is a junior synonym of *Cryptobia.* Marine fishes also are hosts to *Babesia*-like organisms of unclear taxonomic status (e.g., Davies, 1980), and of piroplasms (e.g., Khan, 1980). Early studies on marine leeches as vectors of trypanosomes are by Brumpt (1906), Robertson (1907, 1910), and Neumann (1909) (see also brief review by Molyneux, 1977).

Khan (1976, 1977a, b, 1978a) showed that the marine leech *Johanssonia* sp. is the vector of a trypanosome, *Trypanosoma murmanensis,* of marine fishes. Infection in some leeches can persist through 5 to 6 feedings, that is 542 to 749 days after the blood meal containing the infective stages, provided the fasting periods do not exceed approximately 5 months. Depending on the size of the leech, the amount of blood ingested, and temperature, the developmental cycle in the leech lasts 40 to 80 days (at 0 to 1 °C, 62 days, at 4 to 6 °C, 42 days). Epimastigote stages which apparently do not divide, migrate into the proboscis of the leech and transform to metatrypomastigote stages which are transmitted to fish.

Burreson (1979) demonstrated experimentally that the leech *Malmiana diminuta* transmits the flagellate *Trypanoplasma* (= *Cryptobia?*) *beckeri* to the cabezon *Scorpaenichthys marmoratus.* Multiplication occurred in the leech over approximately 48 h; after 72 h large numbers were found in the proboscis sheath and also in the anterior crop. Eight days after inoculation, the parasites were first observed in the peripheral blood of the fish.

Trypanoplasma (= *Cryptobia?*) *bullocki,* widely distributed in marine fishes along the Atlantic coast of North America and in the Gulf of Mexico, is transmitted by *Calliobdella vivida* (see Burreson, 1982). Infective stages of the flagellates appeared in the proboscis sheath of the leech 10 days after feeding at 5 °C, and 24 h after feeding at 20 °C. Flagellates were retained by the leeches during 3 subsequent feedings on uninfected fish. The life cycle was demonstrated experimentally, but flagellates have been found in this species in nature, and a similar wide geographic and host range of parasite and leech support the view that the leech is the natural vector of the parasite (references in Burreson, 1982).

With regard to haemogregarines, Sawyer and Hammond (1973) suggested that the leech *Calliobdella carolinensis* is possibly a vector for haemogregarines and haemoflagellates, 'like most bloodsucking piscicolids examined', but no experimental evidence was given. According to Davies and Johnston (1976), *Haemogregarina bigemina* in *Blennius pholis* does not appear to be transmitted by leeches, since leeches did not occur intertidally in their main study area. Furthermore, of 7 specimens of the species *Oceanobdella blennii* which had fed on infected fish and were dissected immediately after feeding, only 1 contained parasites similar to gametocytes in the lumen of the posterior crop caeca, but no other stages were observed. Praniza larvae (Isopoda), on the other hand, fed on infected fish, yielded parasites tentatively identified as developmental stages of the haemogregarine. Khan (1978b), demonstrated development of *Haemogregarina uncinata* in the leech *Johanssonia* sp. Sporozoites migrate into the proboscis of the leech. Failure to

transmit the parasite to the sculpin *Myoxocephalus octodecemspinosus* and to 3 Atlantic cod *Gadus morhua* was explained as indicating host specificity of the parasite which was described from eelpouts, *Lycodes lavalaei* and *L. vahlii.* Leeches, *Platybdella olriki* and *Johanssonia arctica,* were found to harbour piroplasms of the species *Haemohormidium beckeri* after feeding on infected marine fish. Inoculation of their gut contents led to infection of uninfected fish. Transmission also occurred under natural conditions (Khan, 1980).

In summary, it seems that leeches play an important role as vectors of the haematozoa of marine fishes, similar to the role insects play as vectors of blood parasites of terrestrial vertebrates.

Effects of Helminths on Cultured Fish

General Considerations

Due to the crowded condition in fish cultures, disease is often more important than under natural conditions (see also Volume I: Kinne, 1980a, b). Because of the great and rising economic importance both of cultured pet fish and food fish, a knowledge of parasites that cause disease is essential. Ricker (1975), in a review of the activities of the Fisheries Research Board of Canada, expressed the view that the greatest problems of the growing aquaculture industry is the control of contagious diseases among fish or invertebrates held in crowded quarters (see also Snieszko, 1975). According to Conroy (1975) the extent of trade in ornamental fish and aquarium accessories in the USA for 1972 was broadly comparable to that involving cats and dogs combined:

	Ornamental fish	Cats	Dogs
Live animals	$250 million	30	220
Accessories	$350 million	55	300

The retail value of the live ornamental fish trade, including accessories, on a worldwide basis was US$ 4,000 million. Although wild-caught saltwater fish formed only ca. 1 % of the total ornamental fish imports into the USA during 1972 and 1973, the saltwater side of the industry appears to have a promising future. Many dealers held more than 100 marine species at any one time.

Ghittino (1972) gave some data on cultured food fish production. Four thousand tons of mullet a year were produced in Italy, and 20,000 tons of yellowtail in Japan. This compares with 700,000 (or > 1 million) tons of carp worldwide, 25,000 to 30,000 tons of channel catfish in the USA, 25,000 tons of eel a year in Japan, and 50,000 to 60,000 tons of trout in the world, indicating the greater importance of freshwater fish. According to Pillay (1979), production in aquaculture has risen from 2.6 million tons of fish in 1970 (partial estimate) to 3.7 million tons of fish in 1973 and to more than 4 million tons of fish in 1975. By 1985, an increase to 12 million tons was expected. The total production of aquaculture in 1975 represented about 8 to 9 % of the total catches of fish and shellfish (Pillay, FAO technical report 1976, but not in final paper, 1979). Although marine fishes represent a relatively small proportion of the total, their importance is rapidly increasing.

Brief discussions of the control of diseases in fish cultures have been presented by Snieszko (1957), Herman (1970, prevention and control of disease in freshwater hatcheries, but useful for mariculture), Herman (1972, principles of therapy), Marcato and Andreucci (1973, technological and pathological aspects of culturing marine fish, little on

helminths), Hoffman (1962, brief discussion of control of various parasite taxa), Sindermann (1977). Sarig (1971) discussed prevention and treatment of diseases of warm water fishes under subtropical conditions, with special emphasis on intensive fish farming. Of the helminths only trematodes are treated briefly and monogeneans are treated in more detail, but most species are freshwater ones. Ghittino (1973) briefly reviewed diseases of cultured marine fish. McVicar and MacKenzie (1977), in their discussion of the effects of different systems of monoculture on marine fish parasites, largely based on their own experience with *Pleuronectes platessa* and *Scophthalmus maximus,* stressed that little is known about parasite problems because of the short history of culturing marine fish.

The losses due to parasites in reared freshwater and marine fish are difficult to evaluate. According to Bauer and co-authors (1981), who gave a recent review of parasitic infections of cultured freshwater and marine fishes, such losses in some countries are estimated as 10 to 20 %, and in several cases they may be greater. In some areas the losses are chiefly caused by viral and bacterial diseases, but in some areas the ectoparasites cause greater loss. Nigrelli (1943) evaluated data from the New York Aquarium for 1940 and 1941 for causes of death of marine fishes. He attributed a significant proportion of mortality to helminth infections (Table 1-25).

The relatively common occurrence of helminths in cultured fish is shown by the data given for cultured plaice *Pleuronectes platessa* in Scotland (MacKenzie and co-authors, 1976; Tables 1-26 to 1-28). Whereas wild plaice had 18 species of helminths, 3 of Protozoa and 1 of Crustacea, the species numbers in 1 of 3 fish farms with the greatest number of helminths species was 5 helminths, 4 protozoans and 1 crustacean. It appears that at least for plaice under the conditions of the study, many helminth species did not establish themselves. Nevertheless, some species were maintained with a high prevalence and intensity of infection (Table 1-26), and one, the digenean *Cryptocotyle lingua,* was more common than under natural conditions. Probable reason was that the cages were close to rocks with many periwinkles which serve as first intermediate host of the parasite. Furthermore, plaice under natural conditions but not in the cages spend a considerable time buried in the sand which protects them against infection.

Keeping *Gadus morhua* for 46 days, and *Zoarces viviparus, Myoxocephalus scorpius* and *Platichthys flesus* for 76 days in aquaria, Möller (1976) found a reduction of infection intensities of 5 common intestinal parasites during these periods. Those species that feed on the host's predigested food and are not, or only slighly, attached to the intestinal wall, were most strongly affected, i.e., *Hysterothylacium aduncum* (93 %) and *Echinorhynchus gadi* (85 %) (see also Nigrelli, 1943; Williams and Phelps, 1976).

Williams (1972b) examined 20 specimens each of pompano *Trachinotus carolinus,* Atlantic croaker *Micropogon undulatus,* gafftopsail catfish *Bagre marinus,* and sea catfish *Arius felis* for parasites before and after keeping them in feeding cages. In pompano, infection with leeches was 10 % in preculture and 0 % in postculture fishes (intensities 'very light' and '0' respectively). The corresponding frequencies (intensities) of cestodes (primarily immature) were 5 and 75 % (very light and high), of digeneans 75 and 0 % (heavy and 0) and of nematodes 24 and 15 % (very light and very light). Similarly, in Atlantic croaker infections with leeches and digeneans disappeared in postculture fish, infection with cestodes increased from 10 to 30 % (light to very light), and infection with nematodes decreased from 75 to 25 % (very light to very light). Infection with larval nematodes in the mesentery remained consistent at 20 % (very light). In gafftopsail

Table 1-25
Causes of death of marine fishes. Parasitic and infectious diseases (After Nigrelli, 1943)

Diseases	1940	1941
Diseases of skin and gills		
1 Bacterial	8	23
2 *Oodinium* (Flagellate)	11	–
3 *Trichodina* (Ciliate)	20	24
4 Myxosporidia (Cnidosporida)	–	3
5 *Epibdella* (Monogenea)	99	2
6 *Microcotyle* (Monogenea)	44	14
7 *Diplectanum* (Monogenea)	2	–
8 *Argulus* (Branchiura)	2	–
9 *Livonica* (Isopoda)	1	–
Diseases of skin and internal organs		
10 Lymphocystis	9	–
Diseases of digestive system		
11 Enteritis and stenosis due to Acanthocephala	5	5
Diseases of circulatory system		
12 Pericarditis due to echinostome infection	–	1
Total	201	72
Non-infectious and Non-parasitic diseases		
Neoplastic diseases		
13 Nephroma	–	2
14 Thyroid tumor	1	1
Diseases of digestive system		
15 Prolapsed intestine with stenosis	1	–
16 Hepatic degeneration	7	10
17 Fatty degeneration of liver	–	11
Diseases of urinary system		
18 Kidney degeneration	–	5
Diseases of Reproductive System		
19 Ovarian degeneration	–	1
Diseases of circulatory system		
20 Cardiac degeneration	–	3
21 Fatty degeneration of heart	–	1
22 Ruptured myocardium	1	–
23 Gas embolism (cerebral hemorrhage)	61	4
24 Interal hemorrhage	–	2
Diseases of bone and organs of locomotion		
25 Tail atrophy	–	1
Diseases due to ill-defined causes		
26 General degeneration of internal organs	–	2
27 Edema	–	1
Violent and accidental deaths		
28 Killed in fighting	26	22
29 Jumped tank	2	3
30 Fractured skull	1	2
31 Multiple abrasions	10	8
Deaths due to external causes		
32 Temperature changes	–	2
33 Changes in water chemistry	85	2
Diseases of organ of vision		
34 Blindness	9	1

Table 1-25 (continued)

Diseases	1940	1941
Diseases due to nutrition		
35 Malnutrition	17	–
36 Fatty degeneration	4	–
Senility		
37 Deaths due to old age	–	4
38 Causes unknown	67	35
Total non-infectious diseases	292	123
Total infectious diseases	201	72
Grand total	493	195

Table 1-26
Comparison of parasite faunas of wild and farmed plaice *Pleuronectes platessa*. Number of species in each parasite group (After MacKenzie and co-authors, 1976)

Parasite group	Wild plaice*	Hunterston I & II	Ardtoe Pond	Moidart I & II
Protozoa	3	4	3	4
Monogenea	1	0	1	1
Digenea	10	3	1	2
Cestoda	4	0	0	0
Nematoda	2	0	0	2
Acanthocephala	1	0	0	0
Crustacea	1	0	0	1

* Data from MacKenzie (1968, 1971)

Table 1-27
Incidence of parasite infestation (%) in farmed plaice (After MacKenzie and co-authors, 1976)

	Hunterston		Ardtoe pond	Moidart	
	I	II		I	II
Number of fish examined	90	50	68	70	48
Protozoa					
Glugea stephani	8	6	0	0	0
Unidentified microsporidians	0	4	0	0	0
Sphaerospora irregularis	8	0	44	43	6
Scyphidia adunconucleata	4	2	2	0	0
Trichodina borealis	3	26	9	20	27
Monogenea					
Gyrodactylus unicopula	0	0	7	30	17
Digenea					
Cryptocotyle lingua	78	82	91	94	65
Hemiurus communis	4	8	0	1	0
Lecithaster gibbosus	1	0	0	0	0
Nematoda					
Contracaecum sp.	0	0	0	1	2
Anisakis sp. larva	0	0	0	1	0
'Chalimus' larva	0	0	0	1	0

Table 1-28
Intensity of infestation (mean level per infested fish) in farmed plaice. L = light, M = medium infestation (After MacKenzie and co-authors, 1976)

	Hunterston		Ardtoe pond	Moidart	
	I	II		I	II
Number of fish examined	90	50	68	70	48
Protozoa					
Glugea stephani	M	M	0	0	L
Sphaerospora irregularis	L	0	L	M	M
Scyphidia adunconucleata	M	L	L	0	0
Trichodina borealis	M	L	L	L	M
Monogenea					
Gyrodactulus unicopula	0	0	23.0	93.3	24.4
Digenea					
Cryptocotyle lingua	3.5	5.0	6.7	6.2	5.9
Hemiurus communis	1.0	1.3	0	1.0	0
Lecithaster gibbosus	1.0	0	0	0	0
Nematoda					
Contracaecum sp.	0	0	0	1.0	2.0
Anisakis sp. larva	0	0	0	1.0	0
Crustacea					
'Chalimus' larva	0	0	0	1.0	0

catfish, infections with cestodes were 10 and 15 %, and those with nematodes were 5 and 0 %. In sea catfish, the data were for digeneans 5 and 0 %, for cestodes 0 and 85 %, for nematodes 0 and 5 %. No detailed data were given for Monogenea, but in the 'Discussion' the author mentions that preculture croaker, gafftopsail catfish and sea catfish were heavily infected with monogeneans, and that pompano and the 2 species of catfish also harboured monogeneans after culture.

The results clearly show that, depending on the helminth species, infection may decrease or increase under farming conditions.

Turbellaria

An unidentified turbellarian, similar to *Ichthyophaga* except for the anteriorly located pharynx, has been observed on skin, fins and rarely on gills of aquarium fishes (Blasiola, 1976). It also occurs on wild fishes at Hawaii (Kent, 1981). According to Kent, in heavily infected aquarium fishes the skin is reddened, fish rub against objects, lie on the bottom breathing rapidly and soon die. The worms are most commonly observed on the yellow tang *Zebrasoma flavescens*, but also occur in other species of Acanthuridae, and similar worms were reported by Conde (1976) from Scaridae and Labridae; they also occur on fishes of other families. Gravid turbellarians leave the host. Actively swimming ciliated juveniles are discharged from a rupture in the body wall of the adult, which contains up to 160 young, and reinfect hosts. At 24.5 °C, development of young in the off-host stage requires approximately 5 days. On the host, the young grow from 70 to 350 μm before leaving the host ca. 6 days after infection. The pharynx of the worm penetrates the epithelium of fish and there is an associated dermatitis. Cultures of lesions from heavily

infected fish revealed a secondary infection with *Vibrio* sp. It is not known whether mortalities are due to the parasite itself or to the bacteria (Kent, 1981).

Monogenea

According to Paperna (1960) Monogenea are a frequent cause of high mortality in fish cultures especially among fry and fingerling (for mullet see Paperna and Overstreet, 1981). Effects depend on ecological factors like season and habitat, behaviour pattern and age of the host, and characteristics of the parasites. Overcrowding of fish in the cultures, lack of water movement and high temperature contribute to mortality. Although most references cited deal with freshwater fish, marine fish are similarly affected.

Infection intensities are sometimes heavy. For example, Kearn (1971a) reported a 'superabundance' of *Trochopus* on *Trigla hirundo* in a public display tank in Lisbon, and according to Wiskin (1970) in excess of 300 young *Rajonchocotyle emarginata* were found in the gill mucus of one *Raja clavata* in the Public Aquarium of the Marine Biological Association Laboratory at Plymouth (see also Kearn, 1967a).

According to McVicar and MacKenzie (1977), monogeneans are amongst the most serious pathogens of cultivated marine fish, mainly because of their direct life cycle. Various authors have reported problems in marine fish cultures due to monogeneans, i.e., Nigrelli (1943): *Neobenedenia* and *Microcotyle* on various fishes; Kubota and Takakuwa (1963), Hoshina (1968), and Tsutsumi and Hayashi (1969): *Heteraxine heterocerca* and *Benedenia seriolae* on yellowtail *Seriola quinqueradiata;* Anderson and Conroy (1968): *Entobdella soleae* on sole *Solea solea;* Ghittino (1973): *Microcotyle tai* on red sea bream *Chrysophrys major;* Sindermann (1974) and Lawler (1977a): *Bicotylophora trachinoti* and *Benedenia* sp. on pompano *Trachinotus carolinus;* Bardach and co-authors (1972): *Diclidophora tetrodontis* on puffers; Reiss and Paperna (1975): *Benedenia* on fingerling *Siganus;* MacKenzie and co-authors (1976): *Gyrodactylus unicopulua* on plaice, *Pleuronectes platessa;* Bauer and co-authors (1977): *Calceostomella inermis;* Paperna (1978): *Furnestia echeneis* on *Siganus auratus, Bivagina* sp. on *Siganus luridus, Benedenia monticelli* on mullet and *Tilapia nilotica;* Lawler and Cave (1978): *Aspinatrium pogoniae* on *Pogonias cromis.*

Nigrelli (1940, 1943) observed mortalities among various fish species held in the New York Aquarium due to *Neobenedenia melleni.* The number of deaths was reduced partly by limiting the number and segregating highly susceptible fishes, mainly Chaetodontidae, and by a consistent application of prophylactic and therapeutic measures (control of temperature and salinity) (see also Nigrelli, 1935a). Another monogenean, *Microcotyle,* although responsible for mortalities, never reached epidemic proportions. Fish could be cured by dipping in freshwater.

Gyrodactyliasis is common in freshwater fish hatcheries and ponds. In the marine environment, Dogiel and co-authors (1958) mentioned mass mortalities in sticklebacks stranded in White Sea shore pools. Infection intensities of up to 1000 fish^{-1} (of 2 species of *Gyrodactylus*) were apparently responsible for the death of most fish. According to MacKenzie (1970), the level of infection of young plaice with *G. unicopula* is kept down under natural conditions to a low level. However, under artificial conditions, e.g., in marine fish farms, the mechanism restricting parasite numbers may break down. Thus, at the Fish Cultivation Unit at Ardtoe (west coast of Scotland), 0-group plaice which had been kept in tanks, had to be treated for gyrodactyliasis. The species responsible was not

identified but it was probably *G. unicopula*, since this is the only member of the genus known from plaice (Fig. 1-126).

Sindermann (1974; see also Lawler, 1977a) listed infections of cultured pompano *Trachinotus carolinus* with the gill monogenean *Bicotylophora trachinoti* and with the skin monogenean *Benedenia* sp. as mariculture disease in USA. The infections rarely cause

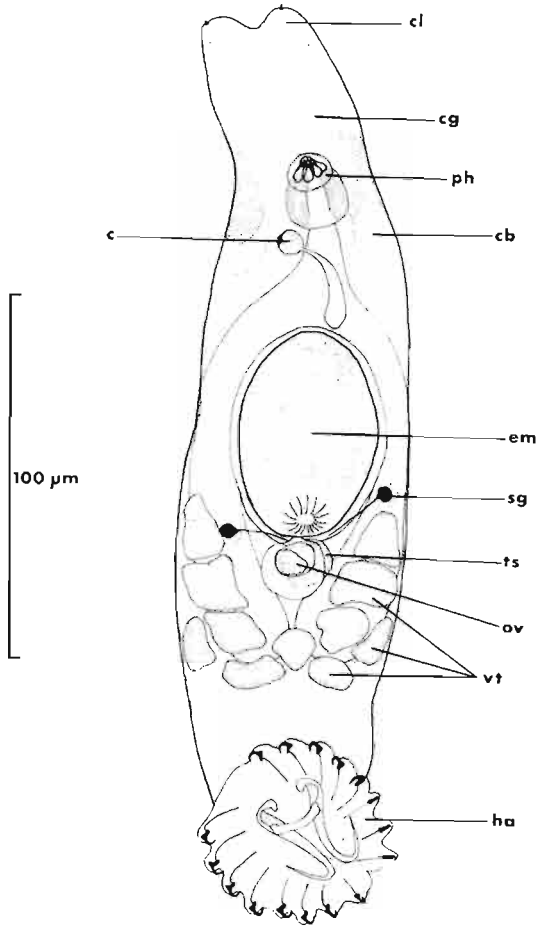

Fig. 1-126: *Gyrodactylus unicopula* from plaice at Loch Ewe, Scotland. c: cirrus, cb: contractile bladder, cg: cerebral ganglion, cl: cephalic lobe, em: embryo, ha: haptor, ov: ovary, ph: pharynx, sg: shell gland, ts: testis, vt: vitellaria. (After MacKenzie, 1970.)

direct mortality, but *B. trachinoti* damages the gill epithelium in heavy infections and parasites may contribute to the death of hosts, if other environmental stresses are present. Infection can be prevented or reduced by avoiding overcrowding or using formalin dips when numbers of worms become apparent. Suggested treatments are 35 min in 150 ppm formalin, or placing in water with reduced salinity.

According to Tsutsumi and Hayashi (1969), the life span of *Seriola quinqueradiata* in fish tanks was considerably reduced by infections with the dinoflagellate *Oodinium* and the

monogeneans *Axine heterocerca* and *Benedenia seriolae* (see also Kubota and Takakuwa, 1963; Hoshina, 1968). According to Hoshina (1968), fishes with many *Benedenia* receive injuries when they rub against enclosure nets to get rid of the parasites, in the form of haemorrhagic ulcerations which in severe cases may become secondarily infected. Infected fish become irritated, with anorexia, lowering of growth etc., but no details were given. Dipping into freshwater for several minutes was recommended as a control measure.

Paperna (1978), without giving details, listed the following helminths as being responsible for fatal epizootics in maricultured tropical fish at Eilat, Gulf of Aqaba, Israel: the monogeneans *Furnestia echeneis* on *Siganus auratus, Bivagina* sp. on *Siganus luridus,* and *Benedenia monticelli* on *Tilapia nilotica* adapted to seawater and on Mugilidae. In spite of overcrowded maintenance conditions, parasites were sustained at non-pathogenic levels interrupted by the episodic occurrence of morbid hyperinfections. Outbreaks appeared to be related to interruption of the normal growth pattern, and fish surviving an epizootic appeared to be refractory to reinfection for several weeks or months (see also Reiss and Paperna, 1975, for effects of *Benedenia* sp. on fingerling *Siganus*). According to Paperna and Overstreet (1981), microcotylids caused severe anaemia of *S. luridus* with a lowering of the haematocrit value to 15 % of its normal level. The occurrence of *Diplectanum aequans* on maricultured *Dicentrarchus labrax* was reported by Paperna and Baudin Laurencin (1979). No details on pathological effects were given.

In Israel, the mullets *Mugil capito* and *M. cephalus* are extensively cultured. Fry, 15 to 20 mm long, are caught on the coast and raised together with carp, silver carp and tilapia in freshwater ponds. The monogeneans *Ergenstrema ancyrocephalus* and *Ancyrocephalus vanbenedenii* are known to infect the cultured mullet, but no losses due to these parasites are known (Lahav, 1974).

Cheung and co-authors (1982) described a disease caused by the monogenean *Dermophthirius* sp. on lemon sharks *Negaprion brevirostris* kept in recirculating sea water aquaria. At 22 °C, the disease usually takes about 1 month to develop. Early signs of the disease include erratic swimming, flashing and rubbing against the bottom of the tank. Subsequently greyish patches and open wounds develop, and the scales become detached at the sites where the parasites are attached. The ulcerated skin lesions become secondarily infected with bacteria, presumptively identified as belonging to the *Vibro complex*. Sharks kept at 19 to 22 °C showed less flashing and rubbing, probably due to decreased activity and a smaller number of infective larvae produced at a lower temperature.

Trematoda

Among the trematodes, *Cryptocotyle lingua* was found to cause heavy infections in cultured plaice *Pleuronectes platessa* (Steele, 1966; MacKenzie, 1968). *Hemiurus communis* infected plaice at another locality (MacKenzie and co-authors, 1976). Ivanchenko and Grozdilova (1971) reported that *Brachyphallus crenatus* had pathogenic effects on cultured young herring and led to mortalities among larvae and fry. According to Lahav (1974), metacercariae of *Heterophyes* sp. infect mullet in the sea, in river estuaries and in fish ponds in Israel. The littoral snail *Pirenella conica* is the first intermediate host. *Mugil capito* of 250 g weight harboured 2300 to 6000 metacercariae per gram of flesh. *M. cephalus* of the same size had only 100 to 150 metacercariae g^{-1}. The larvae were said to cause damage, but no details on the pathology were given, and mortalities in the fish cultures were apparently exclusively due to copepods.

Larvae of red sea bream 8 to 11 mm long and cultured in Japan, were reported by Yamashita (1979) to be killed in large numbers by larval trematodes. The trematodes were not identified but were thought to use the top shell *Batillus cornutus* as their first intermediate host. The parasites were mainly found in the digestive tract, but some also in the urinary bladder, ureter and renal tube. Metacercariae of the salmon poisoning fluke *Nanophyetus salmincola* are known to have caused heavy losses of trout in hatcheries, in some cases with symptoms of popeye (Simms, 1933). However, the life cycle of the trematode is a freshwater one, although various salmonids retain the infection when migrating into the sea.

Cestoda

There are various reports on cestode infections, sometimes heavy, of maricultured fish (e.g., Nakajima and Syuzo, 1969: larval *Callotetrarhynchus* sp. in yellowtail; Ivanchenko and Grozdilova, 1971: *Scolex pleuronectis* in young herring; Nakajima and Egusa, 1978: larval *Callitetrarhynchus gracilis* in yellowtail, infection acquired by eating anchovies, *Engraulis japonica*). However, there are no reports of losses due to tapeworm infections.

Nematoda

Among the nematodes, larval *'Contracaecum' aduncum (Hysterothylacium aduncum)* (see taxonomic discussion of *Contracaecum,* p. 276), sometimes infects cultured plaice, *Pleuronectes platessa,* heavily (Steele, 1966; MacKenzie, 1968), and probably leads to mortalities (MacKenzie, 1968). Rosenthal (1967) reported a 10 % mortality of cultured herring larvae due to infection with *'Contracaecum'* sp. (for details see p. 279). According to Guiart (1938), *'Contracaecum'* contributed to mortalities among *Labrus festivus* and *Pagellus erythrinus* in the Aquarium of the Oceanographic Museum in Monaco.

Nakajima and Egusa (1979) found *Philometra* sp. in cultured red sea bream in Japan. Damage due to the parasite was not reported.

Diseases Caused by Helminths: Conclusions

Data available at present indicate that the greatest number of helminth species of marine fishes is found in warm surface waters, particularly in those of the Indo-Pacific Ocean. There is no evidence yet that helminth diseases are also more varied in tropical waters, since not only species numbers, but other factors like infection intensities of the parasites and resistance of the hosts determine whether a parasite leads to disease. However, in analogy with the greater variety of helminth disease of man and domestic animals in the tropics, it seems not unlikely that there is a greater variety of helminth diseases in tropical seas.

There are data on effects by helminths on individual hosts for all groups of helminths. Some effects may be severe and sometimes lead to mortality. For example, Monogenea may cause haemorrhages, hyperplasia of the gills and death; larval trematodes may cause popeye (exophthalmia), destruction of the eye and, under experimental conditions, death. Some cestodes were shown to damage the intestinal wall, others led to death under experimental conditions; among the nematodes, larvae of *Contracaecum* are responsible for decreased relative weight of the liver and a reduced condition factor. Acanthocephala may perforate the intestinal wall, and leeches were shown to retard the growth rate of fish.

The only well-documented case of a marine helminth species responsible for mass

mortalities in nature is that of the monogenean *Nitzschia sturionis,* which caused mass mortalities of sturgeon in the Aral Sea, into which it had been introduced by man. For some other species, only circumstantial evidence indicates that they may lead to large-scale mortalities. An example is the cestode *Eubothrium salvelini* which possibly causes death of salmonids (in freshwater).

In stress situations (pollution) helminth burdens and pathological effects were shown to have increased. Thus, damage due to three species of monogeneans was much more severe in a polluted habitat than in a similar non-polluted one.

In fish cultures and aquaria, fish lose many helminth species, whereas others occur in increased numbers. Most important among the helminths appear to be the Monogenea, many species of which have been found to be serious pathogens for captive fish and responsible for many mortalities.

Altogether, our knowledge of helminths as disease agents of marine fish is poor. Many species remain to be described, and the life cycles of most species have not been worked out. Pathological effects are known only for a few selected species, and practically nothing is known of how helminths affect fish populations in their natural environment. The fact that mass mortalities of fish in nature due to parasites have been observed so rarely, could have two explanations. Firstly, it could mean that mass mortalities are rare. Secondly, it could mean that fish weakened by disease are soon eaten by predators. Experiments under carefully controlled conditions simulating the natural environment, and extended observations in nature are necessary to improve our knowledge on this point.

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DISEASES CAUSED BY METAZOANS: CRUSTACEANS

Z. KABATA

It appears improbable, at first sight, that Crustacea should be agents of diseases, especially fish diseases. The popular concept of both fishes and Crustacea accords both groups of animals more or less equal biological status. Their interactions are, after all, very dissimilar to those between microorganisms and their hosts. We have become accustomed to seeing only the outcome of the latter interactions as a syndrome that merits the term 'disease' (Volume I: Kinne, 1980). It takes, however, only a second glance to perceive the superficiality of this view. To begin with, it does not take into account the astounding range of biological variability among Crustacea. It ignores the fact that, in their successful expansion and exploitation of numerous ecological niches, the crustaceans have experimented with, and succeeded in, interrelations with other living organisms, including fishes. In some instances these associations have had a deleterious impact on the fishes that can be defined as disease. Many would qualify this term by the adjective 'parasitic'. If one, therefore, avoids limiting one's view of Crustacea to the common large decapods, and if one allows a wide latitude in defining disease, one can accept the title of this chapter without qualms.

In his introduction to this treatise, Kinne (1980) has treated disease as an ecological phenomenon. His discourse applies to this chapter also, since the harmful effects of the crustaceans on fishes are an outcome of an interaction between 2 organisms, played out against the background of a changing, dynamic environment to which both are connected by complex ties, and which has the power of modifying both the circumstances and the results of the relations. This complexity was discussed, for marine fishes, by Polyansky (1961), who outlined some characteristic features of those relations and discussed their possible mechanisms. More recently, Rohde (1982) has dealt in some depth with the same topic (see also Section 'Helminths'). A special part of this section (p. 349) is devoted to the interrelations between fishes and Crustacea in their ecological and evolutionary context.

Perhaps the fact that it has been difficult for many to view crustaceans as pathogenic agents is responsible for the lack of attention to the role they play in diseases of fishes. With notable exceptions for devastating epizootics caused by some freshwater copepods (e.g., species of *Lernaea* and *Ergasilus*), the literature of the field is devoid of comprehensive reviews of this subject. The lack of easily accessible information is particularly acute for marine fishes.

Sindermann (1970a), Snieszko (1970), Gaevskaya and Kovaleva (1975a), Bauer and co-authors (1977), Schäperclaus (1979), Grabda (1981), and Möller and Anders (1983), all include sections on pathogenic crustaceans. None of them, however, treats them in a comprehensive manner. Most of these compendia limit themselves to brief taxonomic reviews of Crustacea parasitic on fishes; some add comments on their effects on the host. Kabata (1970) published the only full-scale review devoted solely to Crustacea as agents of fish diseases, but he included in it both marine and freshwater fishes. This section can be considered as an update on Kabata (1970), stripped of its freshwater component and an

account of treatment. It attempts a review of the salient features of the crustacean-fish systems, placed against their ecological background. It also devotes considerable space to the discussion of the impact of these associations on fishes. It does not intend to be a complete literature review.

It is hoped that the reader will find here a full illustration of the role played by Crustacea in their interactions with fishes. I begin this section with a brief review of the agents involved.

Agents: Branchiura

Previously considered as belonging to Copepoda, this small group of Crustacea differs from them sufficiently to constitute an independent major taxon. Its taxonomic relations with the copepods are difficult to determine, when confounding influences of the superficial morphological similarities are accounted for and many biological differences are properly weighed. Unlike Copepoda, the members of this group are predominantly (75 %) parasitic on freshwater fishes. Only the genus *Argulus* has marine representatives, but even of this genus about 70 % of the species live in fresh water. With our present state of knowledge, it is impossible to provide definitive data, but current inventories list between 30 and 40 species of marine *Argulus.*

Although *Argulus* is an obligate parasite, it has retained a large degree of freedom. It is capable of moving over the surface of the host, as well as of abandoning the host for various periods. It can move from fish to fish, and deposits its eggs in characteristic clusters on the bottom of the habitat where they are attached to suitable submerged objects.

While attached to the fish, *Argulus* remains associated with its exposed surfaces (though some records suggest that it might also venture into buccal and branchial cavities). The more or less permanent residence in an environment characterized by sweeping currents rushing past the flanks of the fish apparently prompted the evolution of the low-profiled silhouette and of a large, protective dorsal shield, covering all but the most posterior appendages. All of the most important functions of *Argulus* as a parasite are carried out under the protection of the shield. Seen in dorsal view, *Argulus* is immediately recognizable by the suborbicular or oval shape of the shield, often with more or less prominent posterolateral lobes, and by the bifid abdomen, extending posteriorly in pointed or rounded lappets. The general morphology of *Argulus* is shown in Fig. 1-127. Two features deserve special attention: (i) The main organs of attachment are in the form of 2 powerful suckers (modified first maxillae); attachment by suckers is not common among Crustacea, which usually have hook- or pincer-type attachment organs. (ii) The tube-like mouth is equipped with strongly armed mandibles. Also visible inside the mouth (Fig. 1-128) are 2 syphons, through which a copious flow of digestive secretions pours out over the surface of the host to prepare the tissues for maceration and ingestion. It is clear that extrabuccal digestion is prevalent in *Argulus.* Deeper penetration is achieved by secretions produced by glands situated at the base of the preoral sting, or stylet. As can be seen in Fig. 1-129, this long, needle-like structure is long enough to reach relatively deep layers. *Argulus* is unique among Branchiura in possessing a preoral stylet. The parasite feeds on tissue fluids, blood and predigested tissues of the fish. The presence of whole blood in the intestinal tract of *Argulus* was amply demonstrated by Bower-Shore (1940), who applied 3 blood tests to its contents, with positive results (Kastle-Meyer reagent, haemochromogen test and benzedene test).

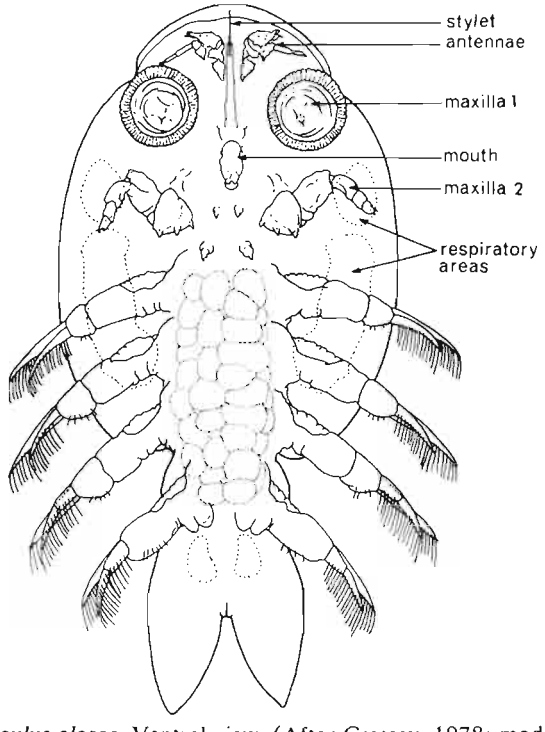

Fig. 1-127: *Argulus alosae.* Ventral view. (After Cressey, 1978; modified.)

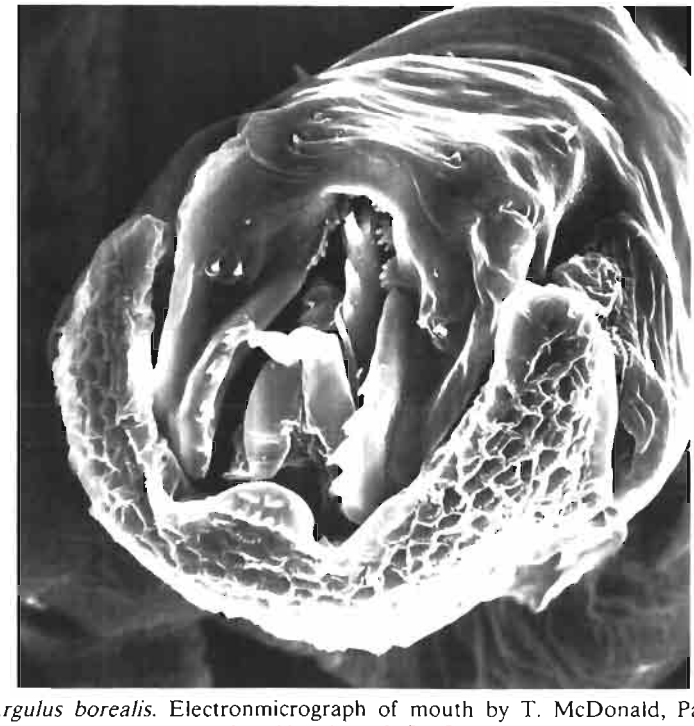

Fig. 1-128: *Argulus borealis.* Electronmicrograph of mouth by T. McDonald, Pacific Biological Station, Nanaimo, B.C., Canada

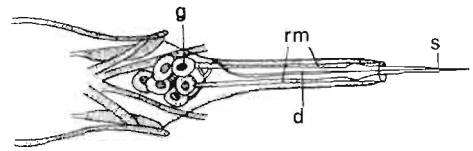

Fig. 1-129: *Argulus*. Preoral stylet. (After Wilson, 1902.)

The ventral surface of the shield carries 2 pairs of respiratory areas, 1 small and 1 large on each side of the shield. The relative sizes and positions of these areas constitute a good diagnostic feature and give one of the main clues to the identity of the species of *Argulus*. Continuous movements of the thoracopods cause the areas to be bathed in the oxygen-rich current of water, ensuring adequate ionic exchanges.

The remaining appendages comprise 2 pairs of antennae, 2 pairs of maxillae and 4 pairs of biramous thoracopods. The fourth pair might be partly covered by the dorsal shield, or be completely exposed in the dorsal aspect.

Argulus shows some sexual dimorphism, manifest mainly in the shape of the posterior part of the body and some modification of the appendages.

Although freshwater Branchiura have been demonstrated to be very harmful, even lethal, the existing evidence does not indicate that the effect of the marine *Argulus* is equally devastating. The impact is, however, far less known and cannot be definitely assessed.

Agents: Copepoda

The total number of copepod species parasitic on fishes is difficult to determine with precision. The known number keeps changing as new species are being continuously added to the list. On the other hand, the improving knowledge of copepod systematics causes redefinition of interspecific boundaries. Some nominal species are described in insufficient detail for assessing their validity. As a rough estimate, probably erring on the generous side, the number is somewhere between 1,600 and 1,800 species. Of these, about 75 % belong to the suborder Siphonostomatoida, 20 % to Poecilostomatoida and 5 % to Cyclopoida. Quoting these figures, Kabata (1979) speculated on the clear predominance of the siphonostomes among fish parasites, suggesting that the type of the mouth characteristic of this group may have been a preadaptation that permitted its to exploit fish as their substrate.

A census of about 1,500 species has shown that as many as 90 % of them are marine. These proportions are not the same in the 3 suborders mentioned. Siphonostomatoida are virtually all marine (only 3 % live on freshwater fishes), Poecilostomatoida are also predominantly marine, only about 1 % of their species dwelling in freshwater habitats. In contrast, Cyclopoida are exclusively freshwater animals (if one disregards 1 isolated record of *Lamproglena* from a Red Sea fish). Hence they will be left out of consideration in this review; so will the Harpacticoida — copepods very rarely parasitising fishes.

It is impracticable to attempt here a broad morphological and biological review of all copepods parasitic on fishes. A restriction to selected examples is necessary. This review comprises generalized descriptions of 23 genera (each examplified by 1 species), belonging to 16 families, 12 of the suborder Siphonostomatoida and 4 of Poecilostomatoida. They are listed in Table 1-29. The systematic arrangement follows that proposed by Kabata

Table 1-29
Examples of site selection and type of attachment of Copepoda to fish. 1: Elasmobranch gills; 2: Elasmobranch skin, superficial grasping and/or mobile; 3: Elasmobranch skin, superficial anchored; 4: Elasmobranch skin, burrowing; 5: Teleost gills, superficial anchored; 6: Teleost gills, superficial grasping; 7: Teleost skin, superficial anchored; 8: Teleost skin, superficial grasping; 9: Teleost surfaces, burrowing (diggers); 10: Teleost surfaces, burrowing (divers); 11: Teleost surfaces, mobile; 12: Teleost – endoparasitic (Original)

Copepod	1	2	3	4	5	6	7	8	9	10	11	12
SIPHONOSTOMATOIDA												
Dissonidae												
Dissonus		×									×	
Trebidae												
Trebius		×										
Pandaridae												
Pandarus		×										
Cecropidae												
Cecrops								×				
Caligidae												
Caligus											×	
Eudactylinidae												
Eudactylina	×											
Dichelesthiidae												
Anthosoma				×								
Hatschekiidae												
Hatschekia						×						
Lernanthropidae												
Lernanthropus						×						
Pennellidae												
Lernaeocera (juvenile)						×						
Lernaeocera (adult)									×			
Phrixocephalus										×		
Sphyriidae												
Sphyrion									×			
Lernaeopodidae												
Lernaeopoda	×		×									
Dendrapta			×									
Clavella					×		×					
POECILOSTOMATOIDA												
Bomolochidae												
Bomolochus											×	
Ergasilidae												
Ergasilus						×		×				
Chondracanthidae												
Acanthochondria			×					×				
Strabax									×			
Lernentoma									×			
Philichthyidae												
Colobomatus												×
Lerneascus												×
Sarcotaces												×

(1979). The selection was made to illustrate various types of parasitic adaptations, resulting from the diversity of host-parasite relations. The evolution of those relations and their impact on the fish will be discussed on p. 349.

As mentioned above, Siphonostomatoida comprise 75 % of the copepod species parasitic on fishes. A large component of this group is represented by 6 families living on the surfaces of their host, both in exposed areas and in protected ones, such as the buccal and branchial cavities. The members of these families are characterized by their low profiles, facilitating attachment in an environment dominated by an almost continuous flow of water. All of them are distinguished by their possession of the dorsal shield, a broad, flat structure formed from the coalesced terga of the anterior segments and resembling the anterior end of Branchiura. Being in essence an inverted saucer, the dorsal shield serves as a sucker and the main organ of attachment for these often mobile parasites. Their posterior halves, consisting of the genital complex and abdomen, float above the surface of the fish, when it is in motion, supported by the current generated by this motion. The evolution leading towards more secure attachment by suction is affected by the process of 'cephalization' (Kabata, 1979), in which successive segments are added to the cephalothoracic tagma, their terga contributing to the expansion of the shield. Kabata (1982) suggested that 2 parallel lines of cephalization can be identified, one exemplified by the sequence Pandaridae, Cecropidae and Euryphoridae, the other culminating in Caligidae. The former line originated possibly from siphonostomes, either free-living or associated with invertebrates, characterized by the extraordinary length of their mouth tubes. An example of such a possibly ancestral form of this line is *Scottocheres* (Fig. 1-130 A). The most primitive members of this group belong to the family Pandaridae and can be exemplified by *Pandarus* (Fig. 1-130 B). In this genus and its relatives only the first pedigerous segment has been incorporated into the cephalothorax, while the segments bearing legs 2 to 4 still retain their separate identity. In *Cecrops* the situation has been modified by the fusion of the second and third pedigerous segments into a small tagma, still separate from the cephalothorax, and in *Euryphorus* these segments have been incorporated into the cephalothorax. All members of this evolutionary series show a tendency to develop dorsal and/or lateral aliform expansions of the tergal sclerites. The other evolutionary line begins with Dissonidae, a monotypic family whose members live on the skin of both elasmobranchs and teleost fishes. *Dissonus,* like *Pandarus,* has a dorsal shield which extends from the anterior end of the body to the posterior margin of the first pedigerous segment. The segments bearing legs 2 to 4 are free (Fig. 1-130 D). In *Trebius* (Fig. 1-130 E) the second pedigerous segment has been incorporated into the cephalothorax and the shield has been correspondingly enlarged. The shield reaches its greatest development in Caligidae (Fig. 1-130 F), in which only the fourth pair of thoracopods is left outside the cephalothorax. The consequences of this progressive cephalization for the segmental appendages involved are described by Kabata (1979).

The reason for deriving the pandarid-cecropid-euryphorid series from the long-mouthed siphonostomes is provided by the structure of the mouth tube. When in repose, the tube is folded under the ventral side of the body, its orifice pointing backwards. To engage the substrate, the tube must be swung forwards. In all members of this series, however, the mouth tube is too long to assume a position perpendicular to the substrate. Consequently, it remains at an oblique tilt, the orifice 'dragging behind' the base (Fig. 1-131). For such a tube to be applied to the substrate, the plane of the buccal orifice must be

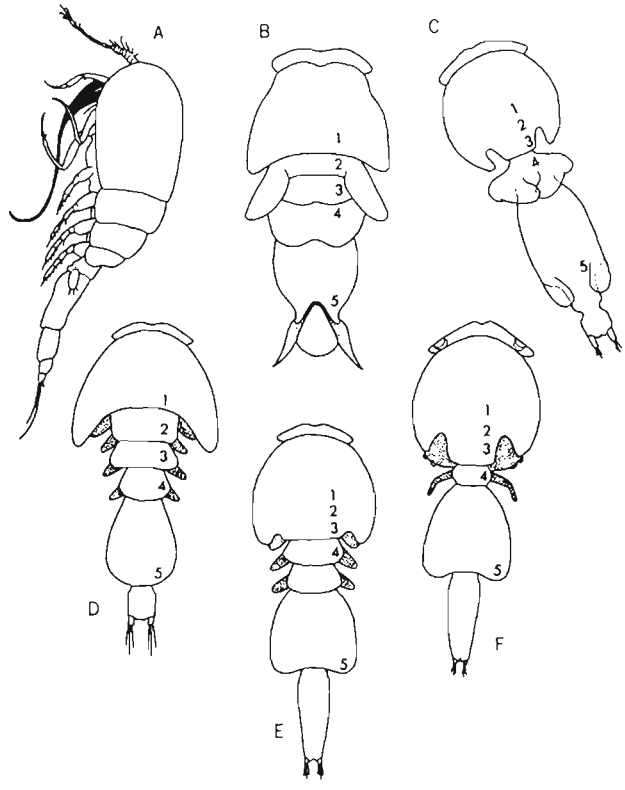

Fig. 1-130: Surface-dwelling caligiform copepods and their possible evolution. (A) *Scottocheres;* (B) *Pandarus:* (C) *Euryphorus;* (D) *Dissonus;* (E) *Trebius;* (F) *Caligus.* Numerals denote pedigerous segments. (After Kabata, 1979; modified.)

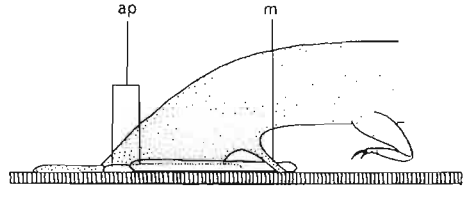

Fig. 1-131: Sagittal section through anterior end of pandarid copepod (diagrammatic). ap: adhesion pad; m: mouth tube. (After Kabata, 1979; modified.)

oblique to the long axis of the tube. This is achieved by the labrum being shorter than labium (Fig. 1-132). There is the tendency for the tube to become shorter in the course of evolution, but even in the comparatively short tube of *Euryphorus* the oblique position of the buccal orifice has been retained. The mechanism of ingestion in these copepods is still unknown.

In the dissonid-caligid line, the mouth tube does not appear to have been derived from the long-mouthed siphonostomatoids. The tube is short enough to swing into the perpendicular in relation to the substrate. Hence, the plane of the orifice can be at right angles in

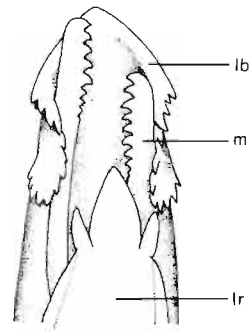

Fig. 1-132: *Pandarus bicolor.* Tip of mouth tube, anterior. lb: labium; lr: labrum; m: mandible. (After Kabata, 1979.)

the long axis of the tube. The mechanism of ingestion has been described for Caligidae by Kabata (1974). It involves abrasion of the surface of the host by a structure known as the strigil (Fig. 1-133) (a bar divided into 2 lateral halves and armed with a saw-like edge), dislodgement of the tissue debris by the flexible tips of the mandibles and its conveyance up the mouth tube by suction. The contractions of the intrinsic musculature of the labrum, by reducing the thickness of that part of the mouth tube, suddenly expand the lumen of the tube and cause a drop in intrabuccal pressure. A series of pumping waves is created, assisted by alternating push-and-relax motions of the mouth tube. The motions aid the process of food transfer up the tube and, at the same time, activate the action of the scraping strigil.

Although the members of these families are predominantly surface browsers, mobile animals capable not only of shifting their position on the host, but also of changing hosts, there are some among them that have become much more stationary. This is true

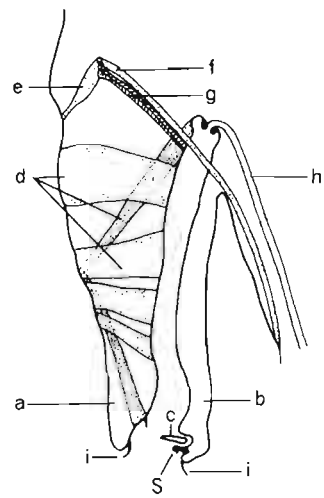

Fig. 1-133: *Caligus curtus.* Sagittal section through mouth cone. a: labrum; b: labium; c: mandible; d: intrinsic musculature of labrum; e: depressor labri; f: levator labri; g: apodeme; h: oesophagus; i: marginal membrane of mouth tube; S: strigil. (After Kabata, 1974; modified.)

particularly of the pandarid-euryphorid series, both parasitic on elasmobranchs and on teleosts (Cecropidae). Even the more mobile dissonid-caligid series comprises some stationary species, though comparatively fewer than among their relatives from the other series. As a rule, the gill-dwellers tend to be more sessile, for obvious reasons.

Stationary habits are prevalent among the primitive copepods belonging to Eudactylinidae and Dichelesthiidae. This small group of species (ca. 40), with the exception of *Dichelesthium,* a parasite of sturgeons, lives on elasmobranch hosts, mainly on the gills. A typical member of the group is exemplified by *Eudactylina* (Fig. 1-134). The primitive nature of this copepod is evident from its poorly developed tagmosis. The anterior tagma, the cephalothorax, incorporates only the first pedigerous segment. The segments bearing legs 2 to 5 are quite distinct and do not show a great disparity in size. All have very strong tergal sclerites. The sexual dimorphism shows itself in the structure of the abdomen, 2-segmented in the female and 3- or 4-segmented in the male. The first 4 pairs of legs are biramous, the 5th leg persists as a 1-segmented vestige. The legs of the male are less modified and retain more primitive characteristics, reminiscent of the free-living ancestry.

The main organ of attachment of Eudactylinidae is the maxilliped, which is quite uniquely chelate (Fig. 1-135). The chela of the appendage closes into a spoonlike expansion of the preceding segment. (This structure closely resembles that of the second antenna of the only slightly related *Kroyeria,* an interesting case of convergence). The mouth tube is conical and short, not departing significantly from the generalized siphonostome plan.

The family Dichelesthiidae, as constituted in the most recent review (Kabata, 1979), comprises the genera *Dichelesthium* and *Anthosoma.* The former is the more primitive of the 2, but shows a similarity to the latter in its general structural plan. *Anthosoma* (Fig. 1-136) has a prominent dorsal shield that, in contrast to the shields of the caligiform siphonostomes, is not adapted for adhesion to the substrate. The first pedigerous segment is incorporated into the cephalothorax. Three ill-defined segments are present between it and the genital segment. The 3 pairs of thoracopods present are modified into prominent, subcircular, foliaceous plates, interlocking around the circumference of the body with aliform outgrowths of the second pedigerous segment (in female). Together, these structures form an elastic 'skirt' protecting a large part of the body from external pressure. In the males the dorsal surface is left unprotected due to the absence of those elytra on the second pedigerous segment. The mouth of *Anthosoma* is a typical siphonostome tube, armed with the usual type of long and flexible mandible, amply denticulated at the distal end. The main organ of attachment is the second antenna, with a characteristic, flexible and contractile stem and short, sharply bent subchela (Fig. 1-137). The function of the protective skirt formed by the legs and dorsal elytra becomes apparent, when one sees *Anthosoma* in situ on its shark host. It buries itself, head down, in the musculature, with only the posterior end protruding externally. The cavity, within which the parasite lodges, is tight-fitting and its walls are capable of exerting pressure on the uninvited guest. Interestingly, *Dichelesthium,* which does not share the burrowing habits of its relative, has not developed expanded thoracopods. The tendency for such expansion is, however, evident from the structure of these appendages (Kabata, 1979). It is also foreshadowed in the appendages of the fossil dichelesthiid from the Lower Cretaceous, described by Cressey and Paterson (1973). Nothing is known of the biology of either eudactylinids or dichelesthiids, beyond descriptions of some early ontogenetic stages.

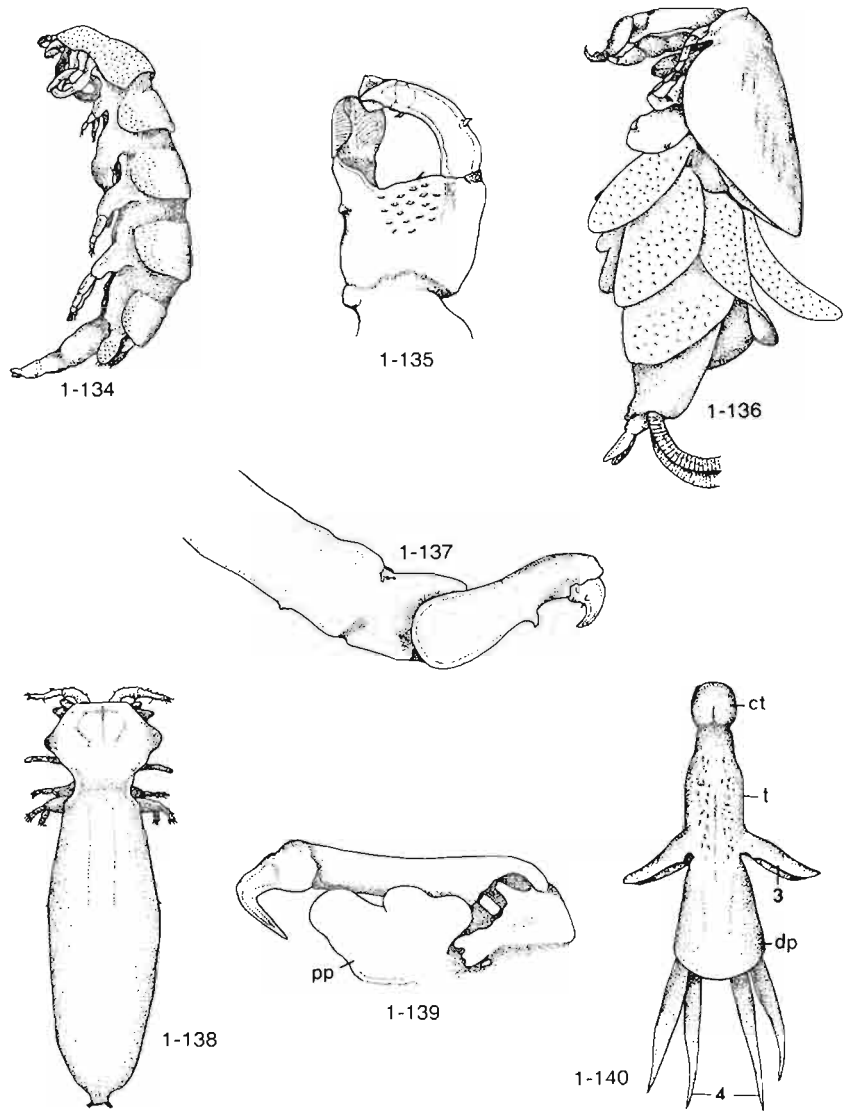

Fig. 1-134 to 1-140: Parasitic Copepoda. 1-134: *Eudactylina similis,* female, lateral. 1-135: *E. acuta,* maxilliped, ventral. 1-136: *Anthosoma crassum,* lateral. 1-137: *A. crassum,* second antenna, extended. 1-138: *Hatschekia labracis,* dorsal. 1-139: *H. labracis,* second antenna, ventral. 1-140: *Lernanthropus gisleri,* female, dorsal. ct: cephalothorax; dp: dorsal plate; pp: parabasal papilla; t: trunk; 3: third leg; 4: fourth leg. (After Kabata, 1979.)

Parallelling this group in the stationary mode of living and selection of the gills as their main habitat are Hatschekiidae and Lernanthropidae, 2 families of marine siphonostome copepods. They differ from them, however, in living only on teleost fishes. Between them, these 2 families presently number about 220 species, flourishing in lower latitudes and dropping off sharply in the temperate climatic zones. The most eye-catching difference

between them is the discrepancy in their sizes, Hatschekiidae being as a rule very small (less than 2 mm total length), while Lernanthropidae are distinctly larger.

The most common genus of Hatschekiidae is *Hatschekia,* represented by about 80 species. A typical member of the genus is *H. labracis* (Fig. 1-138). The body of *Hatschekia* has 2 distinct tagmata, cephalothorax and trunk, joined by an indistinct neck, sometimes showing traces of 1 or 2 segments, but in some species marked only by a narrow constriction. The only organ of attachment is the second antenna (Fig. 1-139), a well-developed, subchelate appendage, always accompanied by a parabasal papilla (pp), a unique structure unknown in other copepods and of unknown function. The female *Hatschekia* has no maxillipeds. This is also true of the other 4 genera currently included in Hatschekiidae. Its natatory appendages have become much reduced, only the first 2 pairs having retained some of their original biramous structure. The mandibles, typical siphonostome blades, have greatly reduced dentition. Sexual dimorphism is poorly marked, males resembling the females of the species, but tending to be smaller. They are also conspicuously less common, sometimes only isolated specimens being present in host populations of hundreds. *Hatschekia* produces characteristically few eggs, matured in uniserial egg sacs, sometimes containing fewer than 10 eggs each. The reproductive strategy of these copepods is not understood. It is obviously successful, in spite of the small number of eggs produced. It must be noted, though, that measured in terms of the biomass of eggs produced in relation to the biomass of the body of the egg-laying female the reproductive effort of *Hatschekia* is certainly not less than that of larger copepods which produce many more eggs.

The family Lernanthropidae comprises copepods of much larger sizes than *Hatschekia* and its allies. The type and the most abundant genus of the family is *Lernanthropus* (presently 115 species). A good example of the genus is provided by *L. gisleri* (Fig. 1-140). The body of *Lernanthropus* consists of cephalothorax, provided with a strong shield, trunk and a small, sometimes indistinct abdomen. The most characteristic features of the lernanthropid morphology are the third legs, usually shoe-horn-shaped and protruding from the body, the foliaceous fourth legs and a prominent dorsal plate of the fourth pedigerous segment. The relative size and shape of this plate is of great importance in taxonomic diagnosis at the species level, although it differs also with the age of the female and can be used diagnostically only for mature individuals. Some species of *Lernanthropus* are devoid of this plate, as are the males of all species. The ventral position of the plate is used as a generic discriminant within this family. The males are much smaller than the females. The buccal apparatus of *Lernanthropus* is typically siphonostome, its mode of feeding unknown. The usual attachment organs, the second antennae, are subchelate and strong. Their prehensile action is assisted, however, by the third legs, wedged between the gill filaments on each side of the filament gripped by the second antennae. The maxillipeds are also well developed, subchelate, and possibly assist in prehension. At any rate, the members of the family are able to maintain a very firm grip on the host. We know nothing about the biology of either Hatschekiidae or Lernanthropidae.

The next 2 families to be included here are unique among Siphonostomatoida. The uniqueness of these families, Pennellidae and Sphyriidae (together numbering about 160 species), lies in their host-parasite relations and morphological modifications required to suit them. Their members, in the course of their ontogeny, undergo extensive metamorphosis that leaves them so profoundly altered as to render them unrecognizable as

copepods. The best-known example of Pennellidae is *Lernaeocera*. Before the onset of metamorphosis, the female *L. branchialis* can be recognized as a typical free-swimming siphonostomatoid (Fig. 1-142). Its body consists of the cephalothorax that includes the first pedigerous segment and of the genital complex, long and cylindrical, fused with the abdomen. Between the cephalothorax and genital complex lie 3 distinct pedigerous segments. The copious transverse wrinkling of the genital complex indicates the looseness of the cuticle in this region, destined to undergo growth on a comparatively gigantic scale, requiring substantial expansion of the integument. The organs of attachment of *L. branchialis* at this stage are its subchelate second antennae. There are no maxillipeds. The buccal apparatus is a siphonostome tube, formed by the fusion, or partial fusion of labrum and labium, the latter consisting of 3 heavily sclerotised rings capable of telescopic contraction and extension. The tube houses blade-like, denticulated, flexible mandibles. There are 4 pairs of thoracopods, the first 2 pairs biramous, the second 2 uniramous. All the rami are 2-segmented.

The metamorphosis of the pennellid copepods is accomplished by the activity of 2 main growth centres (Kabata, 1979), one located in the cephalothorax, the other in the genital complex. The latter is by far the more vigorous and responsible for the expansion of that region to the point at which it constitutes as much as 90 % of the total body mass. The former produces a holdfast organ consisting of 1 dorsal and 2 lateral antlers, usually repeatedly branching at their tips. The cephalothorax of the metamorphosed female (Fig. 1-141) has its longitudinal axis at right angles or oblique to that of the following part of the body (in contrast with the premetamorphosis female; Fig. 1-142). The shape of the tagma is distorted by the development of the holdfast, but all the appendages retain their structure, though they have been dwarfed by the enormous growth of the female. The 4 pairs of thoracopods remain close together behind the cephalothorax, indicating that the segments they belong to take no part in the gigantism of the genital complex. The rest of the body, derived by differential growth from that complex, becomes a huge trunk, with narrow anterior neck (embedded in the host) and a sigmoid posterior part, much more bulky than the neck (protruding from the host). The egg sacs, containing flattened, coin-like eggs, are uniserial and irregularly looped around a central hyaline stylus by a mesentery-like membrane. The stylus itself is secreted by the shell glands that also produce the sacs themselves.

Another example of Pennellidae is *Phrixocephalus longicollum* (Fig. 1-143). The adult female of this copepod, although morphologically dissimilar from *Lernaeocera,* differs from it in only 3 significant features: a more elaborate holdfast, the presence of a secondary holdfast (sh) some distance from the main holdfast, and the absence of the sigmoid curvature of the trunk.

The genera of Pennellidae comprise 2 categories: those living in the branchial cavity of their hosts, and those inhabiting their exposed surfaces. *Lernaeocera* and *Haemobaphes* are examples of the first category. Living in the confined space, they evolved more or less elaborate folding of the exposed trunks and looped or coiled egg sacs, so as to get the greatest possible biomass into that space. Those of the second category (e.g., *Lernaeenicus, Cardiodectes, Sarcotretes*) have evolved long, slender trunks and egg sacs, an obvious adaptation to life in a continuous stream of water. As often happens, there are exceptions to this neat division. For example, the genus *Trifur* morphologically resembles *Lernaeocera,* yet lives on the outer surfaces of its host. The only explanation of this anomaly is in

the speculation that it is a recent migrant that has undergone a habitat shift as a consequence of its host's migration, accompanied possibly by the arrival of new competitors into the branchial cavity. The attachment organs, invariably of the holdfast type, vary from a simple pair of lobes *(Sarcotretes)* to the most profusely branching, root-like structures *(Phrixocephalus, Peroderma)*. In one genus, *Ophiolernaea,* the mouth tube itself becomes an organ of attachment (although 2 lateral lobes are produced by the cephalothorax), exceeding several times the length of the remainder of the body (Fig. 1-

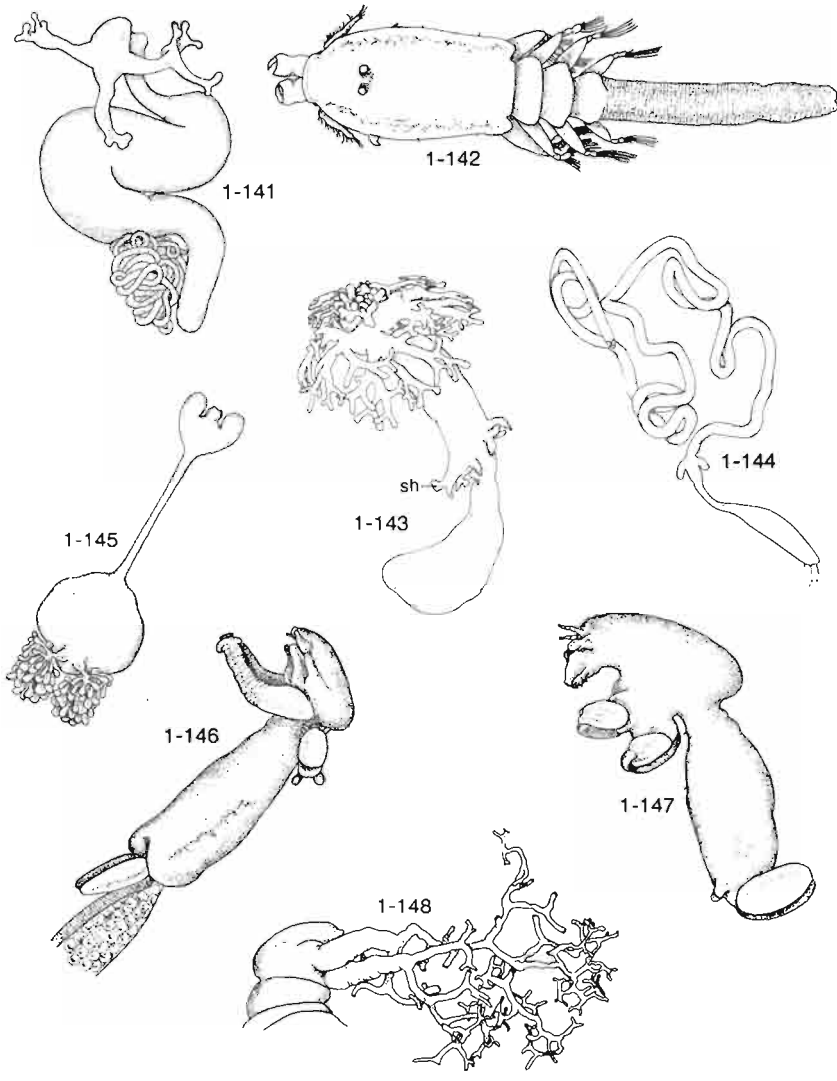

Fig. 1-140 to 1-148: Parasitic Copepoda. sh: secondary holdfast. 1-141: *Lernaeocera branchialis,* metamorphosed female. 1-142: *L. branchialis,* free-swimming female. 1-143: *Phrixocephalus longicollum,* metamorphosed female. 1-144: *Ophiolernaea longiceps,* metamorphosed female. 1-145: *Sphyrion lumpi,* female. 1-146: *Lernaeopoda galei,* female with male attached. 1-147: *L. galei,* male, lateral. 1-148: *Dendrapta cameroni,* female, cephalothorax, lateral. (1-141, 1-142, 1-146, 1-147 after Kabata, 1979; 1-143 to 1-145 and 1-148 after Kabata, 1970.)

144). Meandering apparently at random through the visceral cavity of the host, it comes to rest at the target site that provides a satisfactory food supply for the copepod.

The tendency to produce outgrowths is not restricted to the cephalothorax of the pennellid copepods. In *Creopelates*, dendritiform secondary holdfasts occur in the neck region of the trunk. In some genera the abdominal region is also characterized by a more or less well-developed 'brush', usually 2 multiple rows of dichotomously branching cuticular processes. The best known example of such a brush is found in the genus *Pennella* (a name derived from the feather-like appearance imposed by the brush). Among pennellids with sigmoid trunks, *Lernaeolophus* possesses a well-developed abdominal brush.

Although our knowledge of the biology of the pennellids is scanty, it is, nonetheless, more detailed than that of most other families. This is mainly due to the studies of *Lernaeocera branchialis* conducted since the second half of the 19th century (e.g., Metzger, 1868; Pedashenko, 1899; Sproston, 1941; Schuurmans Stekhoven, 1936; Capart, 1948; Kabata, 1958; Sundnes, 1970). Pennellidae are unique among Siphonostomatoida in having 2-host cycles. After passing through 4 chalimus stages (only 3 in *Cardiodectes*) on the intermediate host, on which the copepods mate and attain sexual maturity, the pennellids abandon it and become free-swimming again. During a brief period of their free existence, they must find a suitable definitive host, almost invariably a teleost fish (at least 1 species of *Pennella* lives on large cetaceans). The final metamorphosis of the female takes place on that host. In some instances at least, the males also become attached to the definitive host (Kabata, 1958), but die soon afterwards without undergoing any further development. The intermediate hosts of pennellids are known for only a few species. *L. branchialis* uses other teleosts in this capacity (Kabata, 1979, listed 13 species of these hosts, 8 of which were flatfishes), *Cardiodectes* develops to maturity on pteropod molluscs (Ho, 1966), *Pennella* on cephalopods (Wierzejski, 1877). Some pennellids, however, appear to be able to complete their life cycles on a single host, e.g. *Sarcotretes scopeli* (cf. Jungersen, 1911a).

The complicated relations between pennellids and their hosts are best reflected in the elaborate sequence of attachment processes. The initial prehension, effected by the second antennae of the copepodid on its first contact with the host, is soon replaced by a much more permanent anchoring with the frontal filament. The latter is abandoned when the copepod, having attained sexual maturity, leaves the intermediate host. The first contact with the definitive host brings the second antennae into play again, but their grip is soon rendered unnecessary by the burrowing of the copepod in the tissues of the host, followed, in most pennellids, by the development of the holdfast. The need to provide hatching larvae with immediate access to the external environment necessitates protrusion of the genital complex from the host and, as stated earlier, vigorous reproductive activity calls for massive enlargement of that part of the body. We must not, however, lose from sight the fact that, morphologically speaking, the pennellids are almost entirely embedded within the tissues of the host. Some become totally buried within the host during part of their ontogeny. Although they are not, strictly speaking, endoparasitic, it would be misleading to classify them as ectoparasites. To acknowledge this unique characteristic mode of life, Kabata (1976, 1979) coined for them the term 'mesoparasites'.

As regards feeding, it is certain that the blood and body fluids of the host are the main diet of the pennellids. Some genera are characterized by their predilection for the blood

vessels as target sites *(Lernaeocera, Haemobaphes, Cardiodectes)* and have been observed ingesting blood (Schuurmans Stekhoven, 1936; Kabata, 1981).

The second family in this group, Sphyriidae, is much smaller, containing fewer than 30 currently known species. Sphyriids are considerably less common than pennellids, perhaps partly due to the fact that they show a tendency to associate with deep-water teleosts, themselves not overly abundant. As an example of the family, however, a genus will be presented that lives on abundant mid-water fishes and occurs often enough to be familiar to North Atlantic fishermen. This genus is *Sphyrion*. Fig. 1-145 shows a mature female of *S. lumpi,* a copepod occurring most commonly on species of the genus *Sebastes* (Scorpaeniformes), although its range of hosts includes teleosts belonging to 4 orders (Kabata, 1979). Because of the absence of thoracopods in adult females, it is not possible to determine the morphological contents of the divisions of its body. Topologically, however, it is divided into a cephalothorax, with a transversely expanded holdfast consisting of a pair of massive lobes, a subcylindrical neck and a broad, posterior, dorsoventrally flattened trunk. The only other notable structure is a pair of processes, each producing, by multiple divisions, an aciniform aggregate of pyriform swellings. These processes are modified uropods; their function is unknown. The first and second antennae are reduced to small tubercles, whereas the buccal apparatus is a siphonostome tube. We do not know the mandible of *Sphyrion.* Its maxillae are digitiform, or conical, very small, while the maxilliped is subchelate. Egg sacs are straight, multiserial.

The presently known genera of *Sphyriidae* are distinguishable from one another by the elaboration of their holdfasts, ranging from a single subspherical swelling *(Opimia)* to a multilobate structure *(Lophoura)* and powerful lateral expansions *(Sphyrion).* Another distinguishing feature is the presence of secondary holdfasts in the neck area (*Periplexis* and *Paeonocanthus*). Most important also are the shape and the degree of development of the posterior processes. They are simple cylinders in *Opimia, Paeon, Tripaphylus* and *Paeonocanthus.* In *Periplexis,* these cylinders are divided by transverse constrictions into 3 parts. In *Lophoura* the processes consist of numerous branches arising from central axial stalks, while those in *Sphyrion* are even less regularly structured, as mentioned above. No known sphyriid is devoid of the posterior processes.

The males of the family are known only for very few species. They are dwarfs, morphologically closely resembling the males of Lernaeopodidae (see below).

Next to nothing is known about the biology of Sphyriidae. Jones and Matthew (1968) described the nauplius of *Sphyrion lumpi,* but otherwise their ontogeny and the manner of their metamorphosis are also unknown.

The last family of Siphonostomatoida to be mentioned in this series is Lernaeopodidae, a widespread and successful group of parasites, cosmopolitan in distribution and associated with both elasmobranch and teleost fishes. With the latter it has colonized and inhabits freshwater habitats, although it is predominantly marine. Of about 260 species of Lernaeopodidae now known, not more than 11 % live in fresh water.

The success of Lernaeopodidae was made possible by the fact that they have evolved a unique method of attachment to the fish, a method that combines the security of the sessile parasites with a degree of freedom second only to that possessed by freely mobile copepods. The lernaeopodid organ of attachment, the bulla, is an anchor-like structure, heavily sclerotised and variable in shape. It is produced by the maturing female in the frontal region of its head, possibly by a gland derived from the frontal gland originally

responsible for the development of the frontal filament, the almost universal larval attachment device of all parasitic siphonostomatoids. The bulla is manipulated by the copepod into a position of permanent contact with the tissues of the host (usually in a special implantation cavity, but sometimes on the surface of the integument), where it becomes solidly cemented. The second maxillae of the copepod are then inserted into special openings in the bulla and fuse with it permanently (Kabata and Cousens, 1972).

This new method of attachment brought in its train a completely novel evolutionary path that singled out Lernaeopodidae from all their relatives. The evolution centred around the second maxillae and their permanent fusion with the bulla. These appendages became a tether that bound the parasite to the host. The degree of freedom the parasite had was determined by the length of that tether and by the length of the cephalothorax, both contributing to the radius of the circle within which it could swing around the bulla, the centre of the circle. (For the evolution of Lernaeopodidae see Kabata, 1979).

To understand the structure of Lernaeopodidae, it is best to examine a primitive member of the family, a representative of the genus *Lernaeopoda*. *L. galei* (Fig. 1-146) is a common parasite of small sharks in the Atlantic Ocean, living usually in cavities on the outer surfaces of the host (cloaca, grooves in the claspers). Like most members of the family, *L. galei* consists of 2 tagmata: cephalothorax and trunk. The former is subtriangular in shape, with a broad base and tapering apex carrying all cephalic appendages (the maxillipeds are situated close to the mouth cone, near the base of the triangle, on its ventral surface). The cephalothorax is arranged obliquely, almost at right angles, to the long axis of the sacciform trunk, the part of the body that houses the reproductive organs and serves as the storage space for the eggs during the initial stages of their development. A deep constriction separates the 2 tagmata. In or near this constriction are located the bases of the second maxillae, the only cephalic appendages that have become displaced from the segmental sequence in the course of metamorphosis; they have grown into long, contractile, powerful, subcylindrical arms, linking at their apices with the bulla. The last conspicuous feature of this copepod is a pair of prominent posterior processes, modified uropods, located ventrally to the large, straight, multilateral egg sacs.

The buccal apparatus of *Lernaeopoda* (like that of all Lernaeopodidae) is typically siphonostome, with fairly short mouth cone and mandibles, the latter apparently rigid. Situated close to the buccal region, the second antennae are prehensile and might be used to hold the mouth in position while feeding. The exact mode of feeding is unknown, but it is generally assumed that *Lernaeopoda,* like all lernaeopodids, are surface browsers, living on the mucus and epithelial cells of the host.

The extensive metamorphosis of the female *Lernaeopoda,* which takes it from a copepodid resembling its free-living ancestors to the form illustrated in Fig. 1-146, is not parallelled by the development of the male. In Lernaeopodidae, the males are ephemeral dwarfs (the size discrepancy between the sexes can be seen in Fig. 1-146, in which the male is attached to the dorsal surface of the female, close to the constriction separating the cephalothorax from trunk). The 2 tagmata of the female are present also in the male (Fig. 1-147), but the male cephalothorax is only slightly shorter than the trunk. The cephalic appendages are all present, but the second maxillae are not modified into arms. Together with the maxillipeds, they form a 4-point attachment organ that also enables them to move over the surface of the host in search of the female (for the locomotion of a lernaeopodid male see Kabata and Cousens, 1973). The males usually die soon after copulation.

In 3 genera of Lernaeopodidae living on elasmobranchs, the bulla has been superseded by another, novel mode of attachment. These genera *(Brianella, Dendrapta, Schistobrachia)* still possess a vestigial bulla during the early adult life of the female, but this is soon replaced by outgrowths of the second maxillae. In *Dendrapta* (Fig. 1-148), these outgrowths become profusely branched and dendritic; they permeate a large portion of the host's tissues, sometimes larger than the body size of the copepod. It is not known whether they have functions (absorption?) other than the obvious prehension.

Among the best known lernaeopodids of the marine teleosts is a group of some 20 species belonging to the genus *Clavella.* About a half of them live in the North Atlantic Ocean; only 5 have been recorded from the southern hemisphere. (For a synopsis of the genus, see Kabata, 1979.) The most widespread and best known among them is *C. adunca,* recorded from the North and South Atlantic Ocean. Its most common hosts are the members of the family Gadidae, at least 10 species of which have been recorded in its host range. Isolated records of other, unrelated hosts have also been made. The usual sites of attachment are gills, fins, skin and buccal cavity of the fish. The morphology of female *C. adunca* (Fig. 1-149) differs from that of *Lernaeopoda* mainly in the proportions of the 2 tagmata and the relative size of the second maxillae. The cephalothorax is as long as, or sometimes longer than, the trunk. It is cylindrical with a slightly enlarged head that can be moved within an exploitable circle of substrate with the radius determined by the length of cephalothorax. The trunk is dorsoventrally flattened and its length-to-width ratio is very variable. There are no posterior processes, but a club-shaped genital process is present in the centre of the posterior margin. The male, when present, is usually attached to that process, which houses the vaginal orifices. The second maxillae are short and covered by a common cuticular sheath. The bulla, to which they are permanently attached, varies in structure depending on the conditions at the site of attachment. (For the difficulties which these differences have caused in the systematics of *Clavella,* see Kabata, 1963b.) In general, it can be argued that the structural plan of *Lernaeopoda* and *Clavella* differ from each other only in the relative emphasis on the individual components of the body.

The same cannot be said about the morphology of the males of these 2 genera. The male of *Clavella* (Fig. 1-150) has become considerably modified from the original plan represented by the males of the more primitive lernaeopodids. The modification has been achieved by the doubling-up of the male, so that its ventral surface has been eliminated. The entire surface of the male *Clavella* illustrated in Fig. 1-150, including its rounded posterior extremity, is dorsal. The genital orifice, from which the spermatophores are extracted, now lies close to the appendages that manipulate and insert them into the vaginae. The arching intestine ends blindly. The ephemeral life of the male allows it to dispense with feeding and removes the need for evacuation of solid wastes.

The buccal apparatus of *Clavella* resembles that of *Lernaeopoda.* It appears that *Clavella* feeds on the mucus and epithelial surfaces of the host, its mouth being held in place during that process by the maxillipeds. The second antennae, unlike those of the more primitive lernaeopodids, are no longer prehensile, having become only sensory, tactile appendages.

As has been mentioned earlier, the ontogeny of Lernaeopodidae includes an extensive metamorphosis. This is true also of *Clavella.* The life history of Lernaeopodidae is known from studies on only a few species, mainly freshwater ones. Kabata and Cousens (1973) produced a detailed account of the ontogeny of *Salmincola californiensis.* The more or less

fragmentary life cycle studies of other, marine genera allow us to postulate that the patterns of lernaeopodid ontogeny are more or less similar to one another. A glaring exception to this rule appeared to have been the genus *Clavella* (and, presumably, its close relatives of the *Clavella*-branch of the family). Heegard (1947) described the ontogeny of *C. adunca* as comprising none of the usual 4 chalimus stages, separated by moulting from one another. Instead, he saw the copepodid passing directly into a 'pupal' stage that continued to develop into an adult female without intervening moults. Later investigators confirmed Heegard's findings (Shotter, 1971). This fundamental difference between *Clavella* and other Lernaeopodidae has generated some doubts as to the appropriateness of placing it in the family. Tentative suggestions of a separate family Clavellidae were already made several decades ago (Gurney, 1934). However, recent work of Kawaton and co-authors (1980) has shown that these conclusions may have been premature. These Japanese authors discovered that *Alella macrotrachelus,* a close relative of *Clavella* with which it shares the abbreviated male, has a life cycle with the full complement of 4 chalimus stages. It must now be considered possible that the earlier work on *Clavella* produced misleading results. There are indications that the normal course of copepod life cycles (including that of free-living copepods) can be distorted by experimental conditions. Lernaeopodidae, *Clavella* included, might be more homogeneous biologically than has sometimes been supposed.

The suborder Poecilostomatoida, as stated earlier, has been less successful in developing systems of relationships with fishes. None the less, it, too, has evolved many species parasitic on marine fishes, with a wide range of differences in host : parasite systems. Several types of such relationships will be presented below, illustrated by members of 3 families.

The family Bomolochidae, comprising about 110 species, is exclusively marine, its members parasitic predominantly on teleosts. They are characteristically small, the females rarely exceeding length of 2 mm. They are all fairly mobile ectoparasites, usually capable of moving over the surface of the host, though they are normally restricted to well-defined habitats (e.g., inner surface of the operculum, surface of eyeballs, etc.). An example of the family is *Bomolochus bellones* (Fig. 1-151). Its first noticeable morphological feature is the low degree of tagmosis. The body of *Bomolochus* remains divided into segments, with the cephalothorax including only the first pedigerous segment. This tagma is the only one distinguished by general enlargement. It is also remarkable in parallelling the dorsal shield of the unrelated siphonostomes, the Caligidae, by its adaptation to prehension by suction. The cephalothorax is concave on its ventral side and rimmed on lateral margins with strips of membrane. The posterior side of the cephalothoracic suction cup is sealed off by the modified and expanded first legs (third in Caligidae). The cephalothorax is followed by 4 well-defined, pedigerous segments, usually diminishing in size from anterior to posterior, but with different size sequences from species to species. Posterior to these segments is the genital complex, small and subspherical. A 3-segmented abdomen ends in well-developed and armed uropods. The first 4 pairs of thoracopods are biramous, their rami mainly 3-segmented (2-segmented endopod in Leg 4). The fifth leg is reduced to a single, 1-segmented ramus, the sixth to only 3 setae. The mouth is of a typically poecilostome structure, with prominent, ornamented labrum but with a very small labium. The mandibles carry 2 apical processes. The mode of feeding is unknown but can be presumed to be of browsing type, utilising mucus and superficial epithelial debris.

Attachment is effected mainly by suction, though the second antennae (folded in the caligid fashion, a striking display of parallellism) and the maxillipeds might be used as auxiliary attachment organs. It is not known whether a relatively small hook, borne on the first antenna, has any prehensile function.

The genus *Bomolochus* is only slightly sexually dimorphous. The males can be distinguished from the females mainly by their narrower cephalothoraces and by having

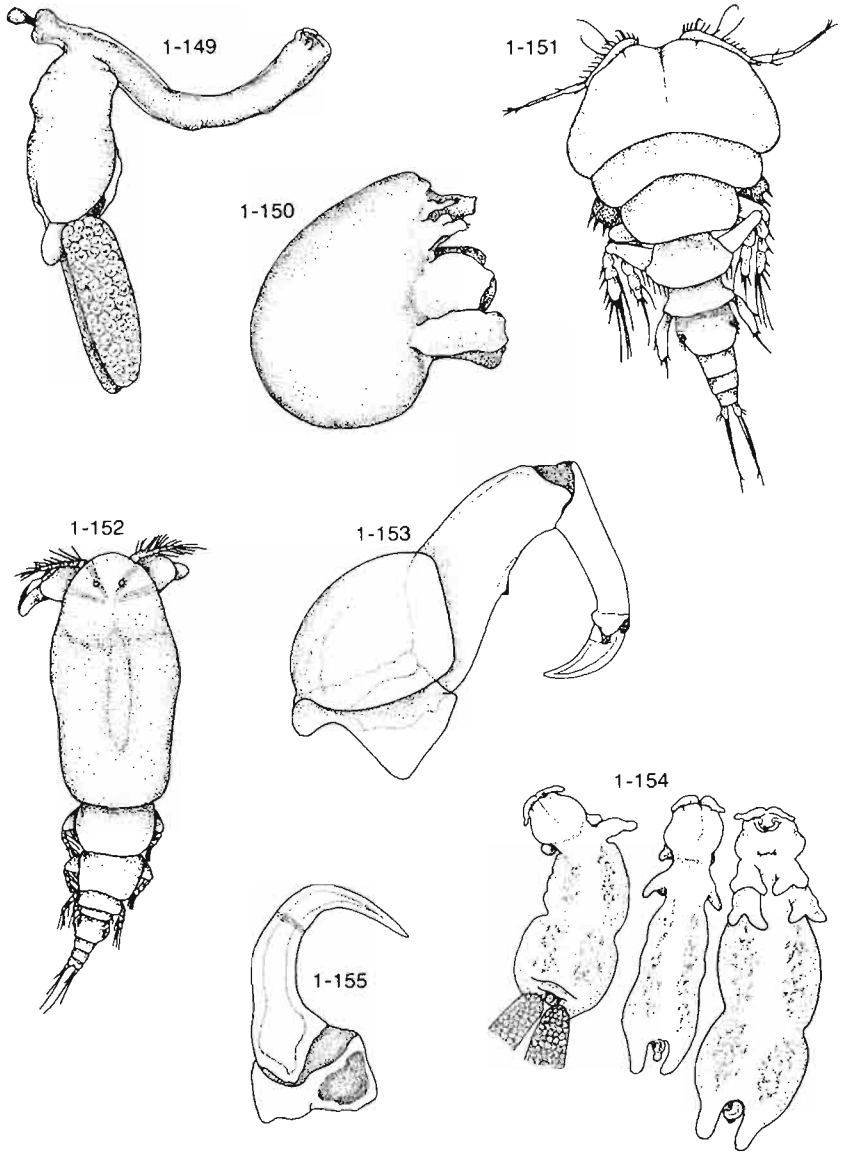

Fig. 1-149 to 1-155: Parasitic Copepoda. 1-149: *Clavella adunca,* female, lateral. 1-150: *C. adunca,* male, lateral. 1-151: *Bomolochus bellones,* female, dorsal. 1-152: *Ergasilus gibbus,* female, dorsal. 1-153: *E. gibbus,* second antenna, ventral. 1-154: *Acanthochondria cornuta,* females, two dorsal one ventral. 1-155: *A. cornuta,* second antenna. (After Kabata, 1979.)

1 segment less in the abdomina. Of the 19 species of *Bomolochus* recorded by Kabata (1979), 9 were found in the North Pacific Ocean, but the genus is present in all ocean areas and has a very wide range of hosts.

The family Ergasilidae is unique among the poecilostome parasites of fishes in having a large freshwater-living component. Almost all freshwater-living members of the family belong to the genus *Ergasilus,* now including 84 species. Of the 107 species of Ergasilidae, 63 % live in fresh water. Several brackish water species also exist, providing a tell-tale sign of the migration from one type of aquatic habitat to another.

Since *Ergasilus* is the most abundant genus of the family, it can be used as a good example. *E. gibbus* (Fig. 1-152) provides an illustration of the genus. The anterior half of its body is fused into the cephalothorax, a tagma consisting of segments up to and including the first pedigerous one. In some species of the genus there is still an incipient boundary between that last segment and the rest of the tagma; in others, this entire tagma expands into a relatively huge trunk (see Kabata, 1979). Next follow 4 distinct pedigerous segments, gradually diminishing in size. The genital complex, compact and subspherical, is followed by a 2-segmented abdomen (3-segmented in some species), the last segment carrying well-armed uropods. Like *Bomolochus, Ergasilus* has a typically poecilostome buccal apparatus, the entire oral region sometimes being elevated on a tubercular swelling above the level of the ventral surface. The mouth parts of *Ergasilus* are not adapted for vigorous maceration of firm tissues. The members of the genus, like many other poecilostomes, resort to extrabuccal digestion. Loosened, semi-digested cells are swept into the open buccal orifice by the concerted action of all the buccal appendages, as well as the first pair of thoracopods. The attachment organ of *Ergasilus* is its second antennae (Fig. 1-153), powerfully subchelate and providing good clues for identification at the specific level. Although *Ergasilus* is most commonly a gill parasite, some species are found also in other sites on the host, particularly those free of scale cover (fins, fin bases, etc.). Even a gill-dwelling *Ergasilus* will spill over to other sites when the gills become crowded. The antennae might serve as a clasping organ, embracing and constricting a gill filament, or they might together form a clamp, pinching between them a portion of the host tissue. The first 4 pairs of natatory legs are biramous, with 1- to 3-segmented rami; the fifth thoracopod is uniramous, consisting of 2 segments. The degree of sexual dimorphism is insignificant, the sexes being quite similar morphologically, although distinguishable from each other as adults. Biologically, however, there is much to distinguish between them. The male is non-parasitic. The female mates prior to settling on the fish and continues the reproductive part of its life cycle in the absence of the male.

The third family of Poecilostomatoida that must be mentioned is Chondracanthidae. Exclusively marine and parasitic predominantly on teleost fish hosts, the family numbers over 160 species. These species are grouped in over 30 genera, more than half of them monotypic. This fact alone testifies to the great morphological variety existing within the familial boundaries. Two tendencies in the evolution of the chondracanthids have contributed to this variety: a tendency to form cephalothoracic and trunk outgrowths, differing in numbers from species to species and, a less pronounced tendency, to penetrate deeply into the tissues of the host. More will be said about this later. Here, 3 species will be introduced, as illustrations of the range of the family's biological possibilities.

The most abundant genus in the family, comprising more than 50 species, is *Acanthochondria.* The genus is distinguished from all other genera of the family by its

morphological simplicity. The body of *Acanthochondria cornuta* (Fig. 1-154) consists of 3 tagmata: a small cephalothorax, a relatively large trunk and a diminutive genito-abdomen. The cephalothorax is dorsoventrally flattened, usually slightly narrower anteriorly and covered by a heavily sclerotised dorsal shield. It is separated from the trunk by a transverse constriction. The trunk expands posteriorly, its lateral margins uneven. The posterior extremity carries 2 posterolateral processes that extend backwards on both sides of the genito-abdomen. The latter is small, its genital and abdominal components are distinguishable from one another. The attachment organ of *Acanthochondria* is its second antennae, heavily sclerotised and rigid hooks (Fig. 1-155), with only limited freedom of articulation in hinge-like joints near the bases. These antennae act together as a staple, fastening the copepod securely and permanently to the tissues of the host. The first antennae are purely sensory, perhaps functionless. The buccal apparatus is typically poecilostome, with a soft, flexible, falciform mandible, unable to deal with firm tissues. Like *Ergasilus,* this chondracanthid and other members of the family use extrabuccal digestion as the means of obtaining their food (Chabanaud, 1951). Of the natatory appendages only 2 anterior pairs of thoracopods remain, grossly modified into prominent, bilobed limbs, well covered with fine spinulation.

The male *Acanthochondria* is a dwarf, usually measuring one-tenth the length of the corresponding female. Its inflated cephalothorax passes imperceptibly into the narrower posterior half, comprising thoracic segments and genito-abdomen. Segmentation is indistinct. Appendages are similar to those of the female, except for 2 pairs of flat, lappet-like legs.

The genus *Strabax* (Fig. 1-156) provides an example of invasive relations. The female of its only known species, *S. monstrosus,* in the course of its metamorphosis, becomes embedded in the tissues of the host. The anterior, lobate part, with all the cephalothoracic appendages is followed by a cylindrical, narrow neck, the first part of the body to protrude outside. The trunk expands posteriorly into 4 large, bifid processes. The mouth is of the usual poecilostome structure, but the legs are reduced to 2 pairs of uniramous vestiges. *Strabax* is obviously confined to a monophagous diet and can never be extricated from its place of final attachment. Nothing is known about its mode of feeding and its biology in general. The males of the genus resemble those of *Acanthochondria* and other chondracanthids. (The unique nature of *Strabax* underwent a change of status recently, with the discovery of 2 new genera, *Markevitchielinus* and *Auchenochondria,* both of which resemble it in their mode of attachment.)

A superficial resemblance to *Strabax* is shown by the genus *Lernentoma.* Like *Strabax,* it penetrates deeply into the tissues of the host. However, as can be seen in Fig. 1-157, this penetration is achieved by the expansion of the premandibular segments of the cephalothorax alone. As a result, the mouth of the copepod remains on the surface. The embedded part is a cylindrical stem, anteriorly expanded into 2 transverse lobes. Both pairs of antennae, now functionless, are carried in their usual locations. The mouth and its adnexa are situated ventrally at the base of the cylindrical stem and show no significant differences from the general chondracanthid type. The 2 pairs of legs are large and bilobed. The trunk is divided into 2 halves by a transverse constriction, each with one or more processes on both lateral margins. The male is a typical chondracanthid.

The penetration of the host by *Lernentoma* undoubtedly confers upon it security of attachment. It is difficult to see what other biological aims are served by it. In view of the

fact that most chondracanthids are completely secure using only their second antennae, the holdfast of *Lernentoma* appears to be a superfluous structure, an inexplicable sport of evolution. Considerably more must be learned about the biology of parasitic copepods, chondracanthids in particular, if the meaning of this development is to be clearly understood.

This account of the biological variety of parasitic Copepoda would not be complete without mentioning one more family, the endoparasitic Philichthyidae. This small group, numbering currently 46 nominal species, is still very poorly known. One can state without exaggerating that the true number of philichthyids is many times higher. Although some philichthyids reach relatively large sizes, most of them are very small and well secluded in their habitats. Only a specifically directed search of the lateral line canals and subcutaneous mucus ducts of teleost fishes is likely to disclose their presence. Indeed, concentrated search for them has usually been quite productive in the past, as exemplified by the work of Richiardi in the 19th century.

The most abundant genus of Philichthyidae is *Colobomatus,* constituting 70 % of species in the family. An example of the morphology of this genus is shown in Fig. 1-158, illustrating the female of *Colobomatus kyphosus.* The bizarre shapes assumed by these females and the advanced condition of tagmosis they display make it difficult, except in the broadest sense, to establish homologies of the individual parts of their bodies. Traces of segmentation are retained only in the abdominal region of some species. The common characteristic of all species of the genus is the presence of prominent cephalothoracic processes, the size, number and configuration of which vary greatly from species to species. A discussion of philichthyid morphology was published recently by Kabata (1979). The cephalothoracic appendages are reduced but their structure has retained its poecilostome characteristics. Two or 3 pairs of vestigial thoracopods are present in the adult females of some species.

Colobomatus is distinctly dimorphous, as are all other genera of Philichthyidae. The males are distinguished by their small size, better retention of external segmentation and less reduced appendages. Their cephalothoraces do not include the first pedigerous segments. The second pedigerous (third thoracic) segments bear 2 small lateral processes, which impart to them a 'winged' appearance. Otherwise, however, the males are slender and vermiform.

Little is known about the biology of *Colobomatus.* Izawa (1975) discovered that *C. pupa* goes in its life cycle through 5 nauplius stages and 1 copepodid. However, the parasitic segment of the cycle is still unknown. It can be surmised that the processes of the adult female, so luxuriously developed on the cephalothorax and trunk, aid in wedging the copepod in the lumen of the mucus ducts. The mode of feeding and type of food are unknown.

A philichthyid genus radically differing in its mode of life from *Colobomatus,* though

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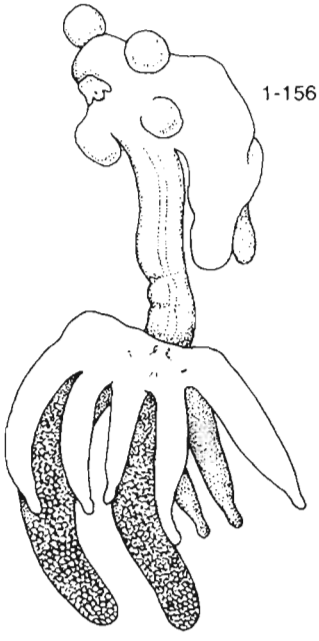
Fig. 1-156 to 1-162: Parasitic Copepoda and Isopoda. 1-156: *Strabax monstrosus,* female, ventrolateral. 1-157: *Lernentoma asellina,* female, lateral. 1-158: *Colobomatus kyphosus,* female, dorsal. 1-159: *Lerneascus nematoxys,* female, ventral. 1-160: *Sarcotaces* sp., female, ventral. 1-161: *Sarcotaces* sp., male, dorsal. 1-162: *Praniza* stage of *Gnathia* sp., ventral. (1-156 after Barnard, 1948, redrawn; 1-157 after Kabata, 1979; 1-158 after Sekerak, 1970, redrawn; 1-159 to 1-162 after Kabata, 1970.)



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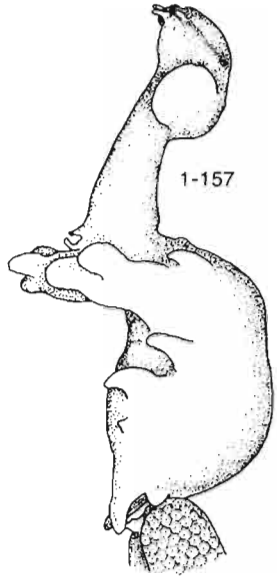

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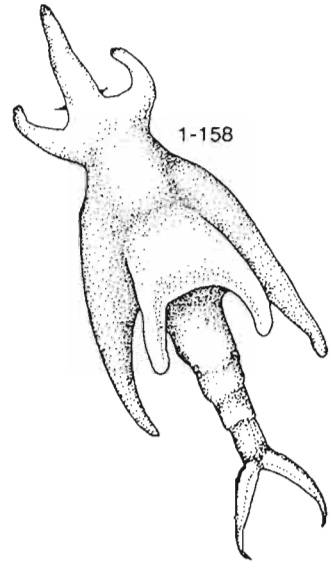

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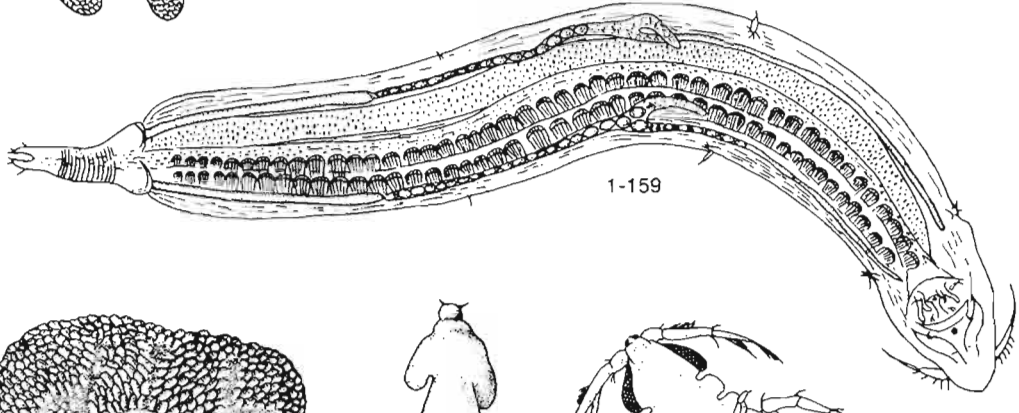

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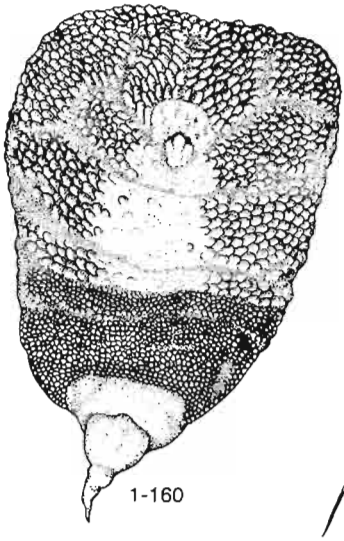

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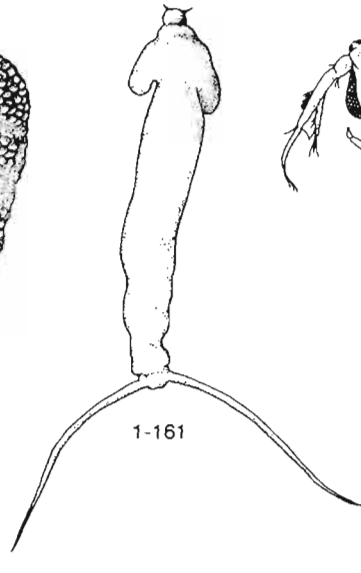

1-161

1-162

also endoparasitic, is the monotypic *Lerneascus* (Fig. 1-159). The adult female of this copepod is slender and vermiform, without any traces of the processes that dominate the appearance of *Colobomatus*. It might be speculated that this difference is due to the type of host-parasite relationship. Unlike *Colobomatus, Lerneascus* burrows under the scales of its teleost hosts (Claus, 1887) and is firmly held there by the surrounding tissues of the host. It has no need, therefore, for outgrowths or processes to hold it in position.

The female *Lerneascus* has lost its external segmentation. Its cephalothoracic appendages are philichthyid, its 3 pairs of thoracopods reduced to vestigial stumps. They are much better preserved in the diminutive male which, as in *Colobomatus* and other philichthyids, is far less modified and dwarfed.

Finally, *Sarcotaces,* a genus now containing 6 species, pursues its endoparasitic habits by producing pouches, budded in from the wall of the posterior part of the alimentary canal of the fish, or even from the external wall of the body. There the members of the genus dwell, permanently encased in host tissues, in pairs of 1 male and 1 female each. The female of the genus is a shapeless sac (Fig. 1-160), largely covered by warty outgrowths. Its segmentation is barely recognizable in some younger specimens, usually completely lost in adult females. The cephalothoracic appendages are much reduced and there are no thoracopods. As is usual for the family, the male (Fig. 1-161) has retained much more of the original structure.

The biology of *Sarcotaces* is also unknown. Izawa's (1973) pioneering work elucidated the early stages in its life history. The pre-parasitic phase of the cycle of *Sarcotaces* comprises 5 nauplius stages and one copepodid. Izawa provided evidence that *Sarcotaces* is a philichthyid. (Yamaguti, 1963c, placed it in a separate order Sarcotacidea.) This copepod still confronts us with unsolved puzzles, one of which is the origin of the thick, black liquid often present in the *Sarcotaces* zoocoecidia. The composition of this liquid suggests that it is derived from blood, though the source of the blood has not been identified. It has been postulated that *Sarcotaces* is a blood feeder. A lot more needs to be known before this assertion can be definitively accepted.

Agents: Isopoda

Unlike Copepoda and Branchiura, Isopoda belong to higher Crustacea, the Malacostraca. It may be argued that the higher level of organization, characteristic of Malacostraca, determines the extent to which they respond morphologically to the demands of parasitism, when this mode of life is adopted. Many parasitologists postulated that the magnitude of such a response is inversely proportional to the level of organization. Indeed, Isopoda, on becoming parasitic, undergo relatively few changes that would distinguish them from their free-living relatives. Their peraeopods become better adapted for prehension, some modifications occur in the buccal apparatus and the alimentary canal alters its structure to accomodate the parasitic mode of feeding. Development in confined spaces might bring about some distortion of the body symmetry, but there is no loss of segmentation and no changes that would deserve to be called metamorphosis.

Kabata (1970) quoted an estimate, according to which the number of isopod species parasitic on freshwater and marine teleosts, as well as on the elasmobranchs, is about 430 species. In the intervening decade more species have been added to the list, but the number has not risen sharply. There can be no doubt that this number is only a small

fraction of the real abundance and variety of isopods parasitic on fishes. Inadequate as our knowledge of copepods is, we know considerably less about isopods. The main reason for this unsatisfactory state of affairs is the fact that Isopoda appear to have a distinct distribution gradient, their numbers dropping from the equator towards the poles. Rohde (1982) summarized our knowledge about the latitudinal gradients of parasite distribution and of their host fishes and confirmed their general validity. In relation to Isopoda, it means that the great majority of their species occur in tropical waters. It is quite possible that in these latitudes they are the dominant group of crustacean ectoparasites of fishes. However, since these regions have been only very inadequately explored, we can be confident that a teeming multitude of isopods, far in excess of the number already known, awaits discovery.

Reviewing isopod parasites of fishes, Kabata (1970) distinguished 2 types, differing from each other by their modes of life, host-parasite relations and morphology. The first and decidedly smaller group comprises members of the family Gnathidae, numbering in excess of 50 species. The complicated life history of these crustaceans and their involvement with the fish has been described by Kabata (1970, p. 51) as follows:

> "The family Gnathiidae' . . . 'are parasites of a very different type. In fact, one is tempted to label them as *parasitoid,* since their parasitism is only a passing phase in their life cycles. The gnathiid cycle involves such drastic morphological changes that as many as three generic names were formerly used for the different stages of the genus *Gnathia.* The newly hatched larvae pass through a brief free-swimming period. Soon they metamorphose into the stage known as *praniza* (one of the old generic names) and become attached to teleost fishes, on the blood of which they feed. The gills are the favourite site of attachment, but the gnathiid *pranizae* are also found on the skin and in the mouth of their hosts. A characteristic feature of the *praniza* stage' (Fig. 1-162) 'is the enormous expansion of the mid-gut, adapted for the reception of huge blood meals and seriously disrupting the segmentation of the body. The parasitic stage over, *praniza* leaves its host fish and moults into the adult male or female. The sexes differ from each other so much that the male was originally described as a different genus *Anceus,* the name now being used to denote the adult male stage. Both male and female adults lead secluded lives, inhabiting sheltered places in the bottom of the sea, abandoned molluscan shells, etc. They do not feed. Nothing is known about the effects of the gnathids on the fish. Probably these effects are comparable to those caused by other blood-feeding parasites." (See, however, p. 397).
>
> "The scanty information available on the isopods of the family Corallanidae (about 40 species known), suggests that, although they are more closely related to the cymothoids, their life cycles are more like those of the gnathiids. They feed on the blood of fishes. It is presumed that the ovigerous females leave the host after they completed their feeding. Like adult Gnathiidae, they take up a secluded, demersal mode of life. At any rate, they have not been observed attached to the host."

However, isopods of the second type constitute the majority of fish parasites of that group. All of them are members of the suborder Flabellifera and fall into 3 families, constituting a heterogeneous group linked by a common structural plan, but lacking uniformity in finer

morphological details. They also differ in their host-parasite relations, particularly in the degree of constancy and intimacy of their associations with the host fishes.

The general structure of a flabelliferan isopod is shown in Fig. 1-163. Three regions can be identified. The most anterior one is the cephalon (c), unsegmented and bearing 2 pairs of antennae and a mouth. The buccal appendages are hidden from view by the

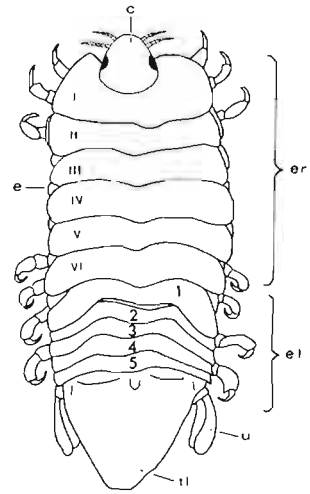

Fig. 1-163: General structure of flabelliferan isopods. c: cephalon; e: epimeres; el: pleon; er: paraeon; tl: pleotelson; u: uropod. (After Schultz, 1969; modified.)

largest and most external, the maxilliped, which usually offers a good clue to the identity of the species. The lateral margins of the cephalon are partly occupied by sessile eyes, which can be large or small; in some species they are absent. The cephalon is sometimes partly embedded in the anterior segment of the next region, as shown in Fig. 1-163. That region of the body is known as the peraeon (er) and consists of 7 distinct segments, covered by terga extending laterally into wing-like expansions, the epimeres (e). Each peraeonal segment carries a pair of appendages, the peraeopods, which can be ambulatory or, in parasitic species, prehensile. The third, the posterior region, is the pleon (el), consisting of 6 segments. Each of the first 5 segments carries a pair of biramous natatory limbs, the pleopods. The sixth segment, the pleotelson (tl), has the shape of a horizontal plate, flanked by the biramous uropods (u).

The interesting recent work of Avdeev (1981a, b) sheds some light on the gradually increasing intimacy of the relations between flabelliferan isopods and their teleost hosts. According to Avdeev, the 3 families associating with fish form a series, progressing from Aegidae to Cymothoidae. Aegidae are only facultative, temporary parasites that have no preference for a specific site on the host (skin, buccal or branchial cavity). Anilocridae, on the other hand, have become more specific in site selection, becoming limited to the skin of their hosts (usually above the lateral line in the mid-section of the body). Their association with the host fishes is obligatory and their attachment more permanent. The third family, the Cymothoidae, shuns exposed surfaces. Some of its genera live in buccal and/or branchial cavities, others form large pouches, the zoocoecidia, in the flanks of the host. These pouches communicate with the outside world by narrow, funnel-like orifices,

ensuring free irrigation and exit for the larvae. The parasites, a male and a female, inhabiting the zoocoecidia, are imprisoned in them for the duration of their lives (Avdeev, 1981a, b, considers them as true mesoparasites). The entrance to the zoocoecidium is directed forwards and is almost invariably located behind the pectoral fins, the movements of which maintain currents of water through the zoocoecidium, when the fish is not actively swimming. *Ourozeuktes bopyroides,* living on balistid fishes, cannot form a pouch behind the pectoral fin because of the bony armour covering that part of the fish. Its zoocoecidia are located near the level of the anus and its respiration can be ensured only by the protrusion of the posterior end of the parasite from the pouch.

Although there are no striking differences in the morphology of this group of isopods, the habitat of each species can be discerned from the strength of its exoskeleton and the degree of development of its prehensile peraeopods. Thus, Anilocridae, which live on the skin, have hard exoskeletons and long, powerful dactylopodids, the hooked terminal segments of the peraeopods. Dactylopodids of the cymothoid genera inhabiting the buccal cavities are much shorter, though thicker. Dwellers in zoocoecidia have their prehensile appendages markedly reduced. Thickness and hardness of the integument are also reduced with increasing security of the habitat.

Cymothoidae and their relatives are protandrous hermaphrodites; they pass through a male stage to become eventually females. The presence of a mature female appears to arrest the further sexual development of a male-stage specimen associated with it. It is not known whether the arrest continues after the death or detachment of the female. Many individuals probably pass rapidly through the male stage of development without functioning as males.

Agents: Amphipoda

Very few crustaceans belonging to this group are true parasites of fishes. One of the best known and well documented examples is *Laphysthius sturionis* (Fig. 1-164). First discovered on the skin of sturgeon (hence its specific name), it appears to associate with diverse fishes, both elasmobranch and teleost. Among its hosts are *Raja batis, Gadus morhua* and *Lophius piscatorius* in the North Atlantic Ocean. The prehensile appendages of *L. sturionis* allow it to maintain its hold on the host, but it shows a tendency towards selection of the protected sites on the skin (e.g., behind the pectoral fin). Like Isopoda, Amphipoda do not undergo extensive structural changes on becoming parasites. Apart from the slight modification of the prehensile and feeding appendages, they show no differences from their free-living relatives.

No information is available on effects of parasitic Amphipoda on their hosts. Feeding on external tissues of fishes, some of them are at least capable of inflicting large, open sores.

Agents: Cirripedia

Kabata (1970) reviewed this group of crustaceans in their role as parasites of fishes. It appears that little new information has been added since that time. The only cirriped truly parasitic on fish is *Anelasma squalicola* (Fig. 1-165), which lives on small sharks and dogfishes. This cirripedian attaches itself usually, though not exclusively, near the dorsal fin of its host, sometimes in clusters. A single *Etmopterus spinax,* one of the smallest sharks known, was found carrying 4 *A. squalicola.* The parasite passes through the normal

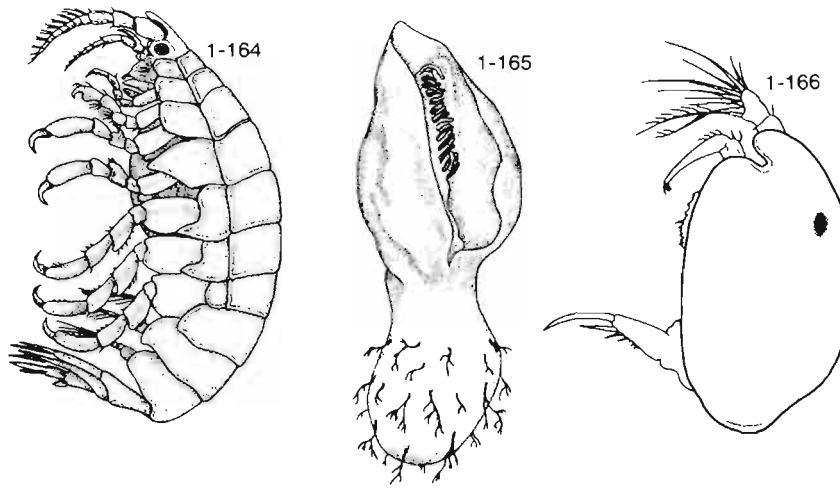

Fig. 1-164 to 1-166: Parasitic Amphipoda, Cirripedia and Ostracoda. 1-164: *Laphystius sturionis,* lateral. 1-165: *Anelasma squalicola.* 1-166: *Sheina orri,* male, lateral. (1-164 and 1-165 after Kabata, 1970; 1-166 after Harding, 1966; redrawn.)

cirriped life cycle stages and develops into a barnacle differing from its free-living relatives in absence of the calcareous plates in the capitulum and in deep purple coloration. Few changes can be seen also in its internal anatomy. The basal part of *A. squalicola* is a bulb inserted into the host tissue, where a large 'cavity of implantation' forms to accommodate it. This bulbous stalk is equipped with root-like, branching processes, apparently absorbent and enabling the parasite to feed on its host. Hickling (1963) proved conclusively that *A. squalicola* has debilitating effect on its hosts.

The literature, old and recent, contains scattered reports of free-living cirripeds occasionally becoming epizoic on fishes. As often as not, the cirripeds involved are barnacles of the genus *Conchoderma.* The associations of this type are haphazard, entirely non-specific and cannot be considered parasitic. The attachment of these barnacles can, however, result in superficial local tissue damage.

Agents: Ostracoda

Instances of parasitism in this group of Crustacea are rare. They have been summarized in a brief paper by Harding (1966). Wilson (1913) described *Vargula parasitica* from the gills and nostrils of *Sphyrna zygaena,* as well as from the gills of *Epinephelus adscensionis* and *Caranx crysos.* Some ostracods found in the nostrils of fishes later proved to be free-living species accidentally lodged in these easily accessible cavities. Harding himself described material from Queensland, Australia, collected from the gills of elasmobranchs, both sharks *(Hemiscyllium oscellatus)* and rays *(Taeniura lymna).* He established for his specimens a new genus and named them *Sheina orri* (Fig. 1-166). The genus *Sheina* is related to *Vargula.*

Harding did not bring up the subject of host-parasite relations and pathogenic effects of the ostracods on their hosts. He stated that *Sheina orri* was collected 'by scraping them

from the gills into sea water in which they at first swam actively' (p. 371–372). This statement leads to the speculation that the position of the parasite on the gills must have been superficial and its attachment not very secure. It is also likely that the reaction of the gill tissue was not strong enough to produce proliferative swellings that would bury the parasite, as they do, for example, the anadontan glochidia.

Information currently available does not allow any definite evaluation of Ostracoda as parasites of fishes, or of their impact on the possibly accidental fish hosts.

Pathogenic Effects as the Outcome of Host-Parasite Interaction

Nature and severity of pathogenic effects of Crustacea on fishes are determined by: (i) degree of intimacy of the interaction between host and the parasite; (ii) duration of contact between them; (iii) relative sizes of crustacean and fish, as well as absolute size of the crustacean; (iv) survival value to the fish of tissues or organs affected by the parasite; (v) evolutionary history of the relations between the 2 components of the system. It is not difficult to perceive these interactions as ecological.

The ecological approach to parasitology fosters the view that the host constitutes the immediate environment of the parasite. Interaction between parasite and fish affects both. The parasite adapts to the fish, with consequent morphological, physiological and behavioural consequences, whereas the fish is influenced by the presence and/or activities of the parasite. The effects are often detrimental to the point of being pathogenic.

Among crustacean parasites, Copepoda have the widest range of interactions with host fishes. For this reason they will be used here as examples, other groups of Crustacea being inserted at the appropriate places. The review is largely based on a recent discussion of the topic by Kabata (1982).

Since contact between the crustacean and the fish is a prerequisite for the establishment of a host-parasite system, it is inevitable that many parasitic Crustacea will enter into association with the skin of the fish, the broadest expanse of its exposed surfaces. (For Branchiura, this surface is an almost exclusive habitat on the fish, while many Isopoda also live clinging to the flanks of their fish hosts.) Virtually all copepods parasitising the outer surfaces of fishes belong to Siphonostomatoida. The possession of the frontal filament, a larval attachment organ that ensures the security of the vulnerable chalimus stages of the life cycle, is probably largely responsible for this state of affairs. The process of affixing that filament results in the first tissue damage inflicted by the settling copepod on the skin.

Speculating on the forces moulding the evolution of the interaction between skin of fish and copepods attempting to colonize it, Kabata (1982) made the following statements:

> "The living surface of the teleost skin is not a particularly nourishing substrate, offering only a moderate quantity of mucus and some epithelial cells as the sole source of food. A copepod settling to a life on such a surface is, therefore, faced with a clear choice: either it must increase its harvesting area by moving over the skin of its host, or it must seek more food by modifying its substrate. The implications of these 2 strategies are obvious and have very definite morphological and biological impacts.
>
> The conditions offered to a would-be parasite by the elasmobranch skin are rather different. As a substrate, it discourages movement. It is, therefore, predictable that some types of host-parasite associations possible for

copepod-teleost systems cannot exist between copepods and elasmobranchs" (p. 203).

Copepods now living on the outer surfaces of the teleosts have adopted both strategies mentioned above. *Caligus* and its relatives are able to slide over the skin of the fish and collect sufficient food over a wide area without inflicting much damage. Only those species that have become less mobile in their behaviour damage the substrate. For example, *Caligus macarovi* burrows under the scales of *Cololabis saira*, the Pacific saury, inflicting quite deep and extensive injuries. Some species can be either mobile or fairly stationary, e.g., *Caligus elongatus.* On the outer surfaces it causes no appreciable damage, but living in the buccal cavity it can cause noticeable irritation or erosion of the epithelium. This is obvious in sheltered places such as a patch of skin behind the vomer, where a group of *Caligus* can often be found, exploiting the protection the vomerine teeth provide against being ingested with the food. Even on exposed surfaces, however, the final effect depends on the intensity of the epithelial browsing. *Lepeophtheirus salmonis* has become a serious pest on salmon maintained in sea pens in Norway. The same species has been blamed for mass kills of salmon on the Atlantic coast of Canada (Kabata, 1970).

Crustaceans physically capable of changing their position on the skin of the host are not always markedly perambulatory. *Argulus,* a branchiuran, tends to remain in one spot sufficiently long to inflict serious damage to the skin in its vicinity. The damage it causes results not only from the devastating feeding process, unique to *Argulus,* but also from the pressure exerted by the very presence of the parasite. The latter effect is shared by *Argulus* with other crustaceans, particularly large ones, like Isopoda, or the Copepoda, mainly those belonging to the family Pandaridae, living on the skin of elasmobranchs.

It can be postulated as a general proposition that the extent and severity of damage inflicted on any particular part of the skin is directly proportional to the duration of contact with the exploiting crustacean. There are, of course, other modifying factors, e.g., the size of the crustacean, the type of its activity and the characteristics of the attacked skin. Thus, a *Caligus* is less damaging than an *Argulus. Clavella* on the skin is less harmful than on the fins, where it sometimes causes extensive erosion. When the stationary habits of the parasite go hand-in-hand with its large size, pressure builds up to exploit the substrate more efficiently. This situation sometimes engenders a tendency to deeper penetration of the host, with its concommitant increase in the severity of the lesions. It can be further aggravated by the colonial aggregations resulting at times from the stationary habits of some copepods (e.g., some Caligidae and Pandaridae).

A good example of a crustacean that evolved in the direction of stationary habits and deeper penetration of the host skin is *Anthosoma crassum,* a member of an ancient siphonostome family Dichelesthiidae. As mentioned earlier (p. 329), adult females of this species burrow through the skin of their shark hosts until they reach the underlying musculature, leaving only the posterior halves of their bodies projecting externally. Extensive lesions are formed. In this instance the injuries inflicted are due both to the feeding process of the parasite and the way in which it interacts with the host tissue to effect secure attachment. It is the latter need that brings about most of the damage caused to the surface of the fish by the copepods of the family Lernaeopodidae. The anchoring device unique to this family, the bulla, injures the fish tissues to an extent dependent on its size and shape. Some deleterious effects can also accrue as the result of the metabolic exchanges between parasite and host taking place through the bulla. Lernaeopodidae such

as *Dendrapta cameroni,* which have replaced the original bulla by dendritic ramifications of their second maxillae, penetrate with them a part of host tissue larger than their own body size and cause correspondingly extensive damage.

It is interesting to note that Poecilostomatoida, devoid of the frontal filament, the lifeline of the developing siphonostomatoids, have never successfully colonized the exposed surfaces of their hosts. Only quite exceptionally do the members of this suborder inhabit the skin. One finds them usually in sheltered places, where they are protected from dislodgement during the vulnerable early stages of their life cycles.

Among the members of this suborder parasitic on the surfaces of marine fishes, one finds both mobile and stationary parasites. The former are grouped mainly in the family Bomolochidae and its relatives. They are small and tend to choose surfaces with an ample supply of mucus. In addition to serving as food, mucus provides an important aid to attachment, acting as a kind of embedding medium, sealing the margins of the cephalothoracic suction cups. As a rule, these copepods cause no appreciable injury to the fish. This cannot be said about the stationary Poecilostomatoida, mainly belonging to the family Chondracanthidae. Although some of them have migrated secondarily onto the gill filaments, the majority are stationary in secluded areas, like the opercular folds and nasal cavities of the teleosts, the nasal fossae and cloacae of the elasmobranchs. Stapled permanently to the host with their second antennae, they often inflict serious injuries and cause tissue reactions. Their extrabuccal digestion erodes substantial portions of fish tissues in the immediate vicinity of the parasite, while the irritation caused by the parasite's presence and activity often causes proliferation of the adjacent cells, eventually producing swellings that cover the anterior end of the parasite. Some genera (e.g., *Lernentoma, Brachiochondrites, Strabax, Markevitchielinus, Auchenochondria*) have penetrated their hosts to a considerable depth, either by burrowing with their entire anterior ends in the tissues, or by producing a unique anchoring device in which only their premandibular segments are involved but which constitute about a half of the total body length of the parasite. The damage to the tissues of the fish is equally extensive in both instances.

The branchial chamber is also a frequent site for the isopods of the family Cymothoidae. These crustaceans, also inhabiting the buccal cavity of their teleost hosts, inflict injuries of quite a different kind. Their relatively large size makes it impossible for many of them to be present in the gill chamber, so that the infections are often single. However, even a single cymothoid is capable of exerting pressure on a large area of the gills and of causing 'crypting', erosion of gill filaments. The presence of a bulky crustacean also interferes with the normal irrigation of the gills. When in the buccal cavity, another of their sites of predilection, these parasites are an impediment to feeding, as well as causing the usual tissue damage.

The tendency for stationary crustaceans, particularly when large, to develop an ever-increasing intimacy of contact with the host tissues is engendered by the need to ensure the security of their attachment, as well as by their demand for food supplies sufficient to maintain the metabolism of their large bodies. (Pennellidae contain the largest copepods known; some species of *Pennella* exceed a length of 50 cm.) The neighbouring tissues of the host become inadequate for this purpose. Since the parasite is stationary, the food must, as it were, come to it. This can be accomplished only by tapping a source of liquid nutrient, such as the blood or tissue fluids that flow past the parasite's site in the course of the normal circulatory processes. Penetration in search of such a source is very characteris-

tic of the pennellid copepods. Schuurmans Stekhoven (1936) coined for this process the term 'arteriotropism'. The destruction of the host tissues along the path of penetration, and the defensive reactions it provokes, are among its pathogenic impacts, but the withdrawal of blood and tissue fluids often leads to more serious and generalized consequences.

Kabata (1982) recognized 2 types of pennellid penetration. The commonly known one is exemplified by *Lernaeocera*. The female, upon attachment to the definitive host, begins to burrow inwards. As it does so, it feeds intensively and its growth keeps pace with the rate of penetration. Consequently, the posterior end of the copepod, i.e. its genital complex, remains outside the body of the fish. When the target site has been reached and permanent contact with the source of liquid food established, the protruding part undergoes gigantic growth and the embedded part develops the complicated holdfast, the permanent anchoring device. In the second type of penetration, known to occur in *Phrixocephalus* (cf. Kabata, 1969) and in *Cardiodectes* (cf. Kabata, 1982), the parasite's penetration rate is more rapid than its growth. In consequence, during the early stages of attachment, the copepod is completely buried within the host. During the subsequent stage of accelerated growth, the posterior end of the copepod must traverse the host, sometimes along the path of the initial penetration, aggravating the damage already inflicted. *Phrixocephalus,* enclosed completely within the eye of the fish and anchored to the vascularised choroid layer of its fundus, usually must pierce with its posterior end a new exit through the iris and the corneal layers, the lens deflecting its path from the central part of the eye. Frequent failures to do so result in the death of the parasite and its decomposition within the eye, with catastrophic consequences for that organ. (Similar to Pennellidae in respect to the pathogenic effects are Sphyriidae, mentioned earlier (p. 335), to which many of the above comments apply.)

As mentioned earlier (p. 334), Kabata (1976) used the term 'mesoparasitism' for this type of host-parasite relation. He was unaware at the time that the same term had already been introduced by Feyzullaev (1971) for helminths parasitic in various cavities of the host's body, a completely different sort of host-parasite system. Avdeev (1981a, b) applied it to the peculiar association between teleosts and cymothoid isopods, which form zoocoecidia (see p. 346). The pathogenic effect on the fish is 2-fold. The immediate result is the pathological transformation of the part of the body wall that becomes the wall of the cavity. Probably more important, however, is the pressure exerted by the large zoocoecidium on the viscera. Tissue changes and general displacement of the organs involved can result in functional disturbances.

All examples of the host-parasite interactions related above, and their consequences to the fish, are the outcome of the initial encounter between the crustacean and the exposed surfaces of the fish. There exists, however, another way in which this first contact can be made. The free-swimming dispersal stages of many parasitic crustaceans, copepods in particular, can be drawn into the buccal and branchial cavities of the fish with the respiratory currents. In some instances (e.g., *Lernaeocera branchialis*), they can also show a negative reaction to the current and move against it to establish contact with the tips of the gill filaments. In both cases, the encounter provides the prospective parasite with a biologically rewarding substrate, the gills. Rich in a very readily available and abundant nutrient, this environment imposes only one strict requirement on the parasite: the absolute need for secure attachment. Even more than on the outer surfaces of the fish, the would-be colonizers of the gills are ever in danger of being swept away. This danger varies

with the type of the gills and of the branchial chamber, but it must be mastered by the crustacean. The response of the parasites was the development of a range of attachment organs that can be reduced to 2 types: grasping and superficial anchoring. Exceptionally, deep anchoring can occur. For example, *Lernaeocera lusci* introduces its holdfast deep into the tissues of the gill arches.

All initial contacts between parasite and gills fall, of course, within the first type, i.e., grasping. The crustaceans use for this purpose appendages that they have inherited from their free-living ancestors. Thus, the early stages of the female Pennellidae – such as *Lernaeocera* or *Haemobaphes* — grip the filaments with their chelate second antennae. For these copepods, however, the gills are only a pathway to their target sites, and they move along the filaments to their bases and on to the points ventral to the arches, where they can begin to burrow in the direction of the blood vessels. For many parasites, the grasping attachment to the gills remains the permanent method of maintaining contact with the fish. It appears that the strength of the grasping attachment is directly related to the size of the crustacean. In *Hatschekia,* one of the smallest siphonostomes (mainly less than 2 mm long), the second antennae are so sufficient for attachment to teleost gills that its females have lost their maxillipeds. The much larger *Lernanthropus* not only uses its second antennae and maxillipeds, but has pressed into service its third legs, modified into accessory attachment organs. A similar situation occurs on the gills of elasmobranchs. *Eudactylina* grasps the gills with its large, chelate maxillipeds. The larger *Nemesis,* whose maxillipeds are subchelate and appear less efficiently prehensile, has evolved peculiar first legs that are almost chelate and can be used for grasping the gills (see Kabata, 1979, p. 39). Also grasping are Ergasilidae (Poecilostomatoida), which use their disproportionately large second antennae as attachment organs. Chondracanthidae, on occasions, also attach themselves to the gill filaments of teleosts, but their second antennae, which staple them to the gill filaments, cannot strictly be described as grasping.

The gnathiid isopod *pranizae* often live on the gills, grasping as well as piercing them with their mouthparts. A unique mode of grasping is employed by ostracods; they use their valves as grasping mechanism. Finally, some copepods belonging to groups that are more typically mobile on the surface of fishes have adapted to life on the gills. *Lepeophtheirus thompsoni,* for example, grasps the filaments with its second antennae, but depends on its protected position between filaments for the retention of contact with the host.

The superficial anchoring type of attachment is less common on the gills, since the filaments are often too delicate to provide a firm substrate for anchoring structures. The most common group to exploit this method are Lernaeopodidae (Copepoda, Siphonostomatoida). Even they, however, will be found only on the gills of older and larger fish, whose gills are substantial enough to receive the anchoring bulla.

The pathogenic consequences of these actions by parasitic crustaceans are 4-fold. Firstly, they frequently result in interruption of normal branchial circulation. Secondly, they may cause 'crypting', i.e., erosion of gill tissues adjacent to the parasite; some erosion is caused also by the feeding activities of many crustaceans. Thirdly, they provoke a vigorous proliferative reaction of the gill epithelium, ultimately leading to its thickening and to respiratory disfunction. Finally, some of the gill-dwellers feed on blood, readily available in this location. The drain on blood, without provoking a marked local tissue response, can have serious general results, the severity of which depends on the size and number of the parasites present on the gills.

Endoparasitism is rare among crustacean parasites of fishes. It occurs only among Copepoda (and must be distinguished from micropredatory activities encountered in some groups, to be mentioned later). The family Philichthyidae (Poecilostomatoida) consists exclusively of endoparasitic species, interacting with their hosts in 1 of 3 ways. The most prevalent type of host-parasite relation is exemplified by the genus *Colobomatus.* The members of this genus and its relatives inhabit superficial ducts under the skin of fishes, in particular the lateral line canal and mucous ducts in the cephalic area. In the majority of cases the presence of the copepod is not discernible externally and its pathogenic impact is unknown. Occasionally, swelling is produced. *Leposphilus labrei* provokes pathological changes even in adjacent bone tissue. The second type of association, represented by *Sarcotaces,* is not unlike that involving cymothoid isopods, i.e., formation of coecidium-like pouches within tissues of the fish. Most commonly, these pouches are formed in the intestinal tract in its rectal region, though on occasion the entrance to the pouch is located on the outer surface of the body. Finally, phylichthyids can burrow in the tissues and cause lesions and/or swellings. The former effect is produced by *Lerneascus,* the latter by *Ichthyotaces.*

In the light of the above discussion the pathogenic impact of parasitic crustaceans can be predicted from the type of their interactions with their fish hosts. Small and mobile ectoparasites, inhabiting external surfaces, are not likely to be highly pathogenic, though the severity of their impact will depend on their abundance on an individual fish and on the condition and size of the fish itself. (This does not necessarily hold true in a freshwater environment.) Larger mobile ectoparasites are likely to cause local lesions, but (except for *Argulus*) are not otherwise a serious threat. Gill parasites can produce extensive gill damage (the same is true of larger parasites resident in the branchial chamber), but under marine conditions have not been reported to cause respiratory dysfunction. Mesoparasitic copepods, on the other hand, can be, and often are, a serious health hazard to their hosts and may be an indirect cause of mortality. Finally, endoparasitic Crustacea vary in their effects, depending on their size and the site of infection.

Local Effects

As the name implies, local effects of parasitic infections are pathogenic changes limited to the immediate vicinity of the parasite. As such, they might not present a serious threat to the health of fish. This is not to say that effects of a local nature cannot have a generalized impact on the state of health. Such impact, however, is a secondary result of the summation of local injuries.

Local effects are usually limited to tissue damage, the extent depending on the many factors discussed above. The effects will be presented here, grouped according to the site involved.

Damage to Gills

Reviewing potential pathogenic changes in the branchial apparatus of fishes infected with Crustacea, Kabata (1970) took into account the following factors: (i) Damage caused by feeding and attachment of the parasite; (ii) damage resulting from the mere presence of the parasite; (iii) response of the gills by a characteristic and uniform reaction of their epithelial and sometimes connective supporting tissues; (iv) atrophy of the tissues due to the pressure exerted by the parasite; (v) the fact that the same parasite might provoke a

strong reaction in one host and only a mild one in another, depending on the species of fish and the type of its association with the crustacean parasite in question.

The defensive reactions sometimes result in lowering the respiratory efficiency of the gills. The epithelium becomes thicker and less able to effect ionic exchanges.

Damage to gills can be classified, according to its immediate cause, under 4 headings: occlusion of branchial circulation, parasite feeding, pressure, and proliferation of gill tissues.

Occlusion of branchial circulation

This effect can be the outcome of the pinching of blood vessels by the attachment appendages of the parasite. It can be short or more prolonged, the extent of the damage being proportional to the size of the area deprived of blood supply and the duration of the occlusion. For example, young females of *Lernaeocera branchialis,* on their first attachment to the gills, grip the filament with their second antennae and often interrupt the flow of blood in a vessel. The occlusion becomes manifest in visible blanching of the filament distal to the point of attachment. Should the blockage be prolonged, necrotic changes are sure to take place in the deprived tissue. Although *L. branchialis* does not remain at the original contact point long enough to cause serious local damage, some ergasilid copepods certainly do. The second antennae being proportionately larger, their grip can pinch off a portion of the entire filament (e.g., *Ergasilus amplectens* on the gills of *Mugil cephalus*). Paperna and Zwerner (1982) observed that gill filaments of *Morone saxatilis,* heavily infected with *Ergasilus labracis,* were 'pale and swollen', suggesting extensive interruption of branchial circulation.

In addition to the eventual necrosis caused by deprivation of blood supply, the affected part of the filament often reacts by a copious production of mucus. Formation of a thick coating of this substance impedes gaseous exchange and diminishes the respiratory efficiency of the coated gill area.

Copepods of the family Naobranchiidae attach themselves to gill filaments of their teleost hosts by a firm embrace of the second maxillae, permanently fused into a flat-walled ring. In their case, however, the effect is rather a partial constriction than complete occlusion, judging by the absence of filament blanching *in vivo.* The same is probably true of *Lernanthropus,* a copepod that thrusts its third legs between the filaments to assist attachment.

Permanent blockage of branchial circulation can be caused by any breach in the integrity of the vessels that brings into play the blood clotting mechanism. The resulting thrombus is capable of lodging in the relatively small branchial vessels. The seriousness of such occlusion depends on the importance of the occluded vessel, as well as on the size of the area served by it.

In the process of occluding the circulation of the branchial vessels, attachment appendages commonly pierce the gill tissues. Punctures in filament tissues are naturally damaging, but they do not appear to cause serious injuries. According to Paperna and Zwerner (1982, p. 396), the second antennae of *Ergasilus labracis* caused the tissue to be 'pushed away or torn'. The collagen 'surrounding the connective tissue core of the gill filaments was also torn' at the point where the antennae were inserted. However, 'no epithelial or granular tissue reaction' was evident in the gill filament around the point of attachment.

Parasite feeding

There can be no doubt that feeding of crustacean parasites causes loss of gill tissues. There is no evidence, however, that this loss is serious. Most tissue-feeding gill parasites are small and their individual depredations relatively insignificant. Only very heavy infections are likely to result in damage serious enough to endanger the respiratory function of the gills. Such danger is particularly likely in infections with ergasilid copepods or isopod gnathiid *pranizae*. The former resort to extrabuccal digestion that causes partial lysis of the tissues. Even in their case, however, the actual loss of tissue is less significant than the reaction of that tissue. Hyperplasia of branchial epithelium was found to be particularly 'prominent in the tissue in the nearest proximity to the parasite's mouth', when *Ergasilus labracis* parasitised the gills of the sea bass (Paperna and Zwerner, 1982, p. 396). It must be concluded that, at present, very little is known of the direct effect of feeding by crustacean parasites on the gills of marine fishes.

Pressure

The most deleterious effect of pressure exerted by crustaceans on the gills of their fish hosts is the so-called 'crypting' (Friend, 1941). The greatest danger of crypting is not posed by parasites attached directly to the gills but by those present in the branchial cavity. This apparently strange phenomenon can be easily explained by the relative sizes of the crustaceans involved. As stated above, the crustaceans dwelling on the filaments tend to be small and their pressure is alleviated by the fact that the filaments are in constant motion. On the other hand, crustaceans of quite large sizes often inhabit the branchial chamber. Their larger size goes hand-in-hand with hardness of their exoskeleton. Consequently, they can exert greater and more constant pressure. The identity of these crustaceans is irrelevant, their effects quite non-specific. It is very likely that any large crustacean present in the gill chamber for a sufficiently long time will cause this type of pathological change. Menzies and co-authors (1955) reported crypting caused by the isopod *Lironeca convexa,* whose males often crowd the branchial chamber. Turner and Roe (1967) noted some gill erosion in *Brevoortia* harbouring another isopod, *Olencira praegustator,* in its branchial cavity. Lindsey and Moran (1976) studied *Lironeca ovalis* parasitic on several hosts in Delaware Bay, Atlantic coast of the USA (*Pomatomus saltatrix, Bairdiella chrysura, Cynoscion regalis, Morone americana* and *M. saxatilis*). They stated that out of 18 *M. saxatilis,* infected with this isopod the majority (15) of fish 'had only small sections of the gill filament destroyed, while 2 others sustained more extensive damage to the filaments' (p. 330). Rokicki (1982) reported partial or complete disappearance of gill filaments of *Selar crumenophthalmus,* a pelagic fish on the Mosambique shelf, caused by the pressure exerted by yet another isopod, *Lironeca indica.* The same can be true of the copepod *Lernaeocera branchialis* (cf. Kabata, 1970), although its pressure does not invariably cause crypting (e.g., Sundnes, 1970, failed to observe it on the gills of cod *Gadus morhua* infected with this parasite). Grabda (1975) found loss of branchial tissue in *Theragra chalcogramma,* a Pacific gadid fish, carrying another pennellid copepod, *Haemobaphes diceraus.* According to her 'the lamellae undergo a shortening or complete atrophy over the space filled up by the parasite's thick S-shaped genital segment' (p. 14–15).

No quantitative assessment of the proportion of the respiratory surface lost to the fish has been attempted for marine species. It is known from the freshwater examples that the

crypts can reach a depth of 5 to 6 mm from the margin. As stated above, they can be much deeper in some cases, the entire filament being in danger of disappearance. The area no longer functional as respiratory surface is further enlarged by the tissue reaction along the margins of crypts. This reaction is described below.

There is very little information on the effects of crypting on the respiration of fishes. It is generally known that, under normal circumstances, even substantial reduction in the respiratory surface does not provoke a crisis in this vital function. The situation might be different under stress. Lewis and Hettler (1968) experimented with temperature/salinity effects on the survival of young menhaden *Brevoortia tyrannus.* They found that the L_{50} of fish with eroded gills, at 35 °C, was only half of that in fish with normal gills (1 h, as compared with 2 h). It is reasonable to presume that such results might have more general application.

Pit-like depressions in the planar surfaces of gill filaments can also cause loss of respiratory epithelium. Kabata (1970) mentioned *Lepeophtheirus thompsoni,* a caligid copepod, as forming such pits in the gill filaments of *Scophthalmus maximus,* a commercially valuable flatfish in the North Sea. The respiratory folds disappear completely from the area on which this copepod settles. The presence of these often bulky crustaceans may prevent complete closure of the opercula and interfere with normal gill irrigation, thus contributing to respiratory dysfunction.

Proliferation of respiratory epithelium

As mentioned earlier, gill tissues and, in particular, the respiratory epithelium of filaments respond to irritation by proliferation of their cells in a process of hyperplasia. This reaction renders the responding area non-functional as respiratory tissue, because it increases the thickness of the irritated layers and affects ionic exchanges. The reaction, uniform in nature, moves the source of irritation farther away from the vital centres of fish; its deleterious effects on respiration is an unfortunate byproduct.

The histology of gill infection with *Ergasilus labracis* was studied by Paperna and Zwerner (1982). Examining the response of the gill tissue of *Morone saxatilis* (lower Chesapeake Bay, USA), they found that the 'tissue response to the parasite's presence took the form of epithelial hyperplasia along the filament adjacent to the copepod's body' (p. 396). 'The initially localized hyperplasia at the attachment site progressively spread to the entire gill filament, causing the major part of the entire gill filament to lose its lamellar structure.' The process of the response consisted of 3 elements: (i) increase in the number of epithelial cells, with consequent increase in the thickness of the originally one-celled layer; (ii) abundant proliferation of the epithelial mucus cells along the margins of the affected area and farther away, sometimes extending to neighbouring, uninfected filaments; (iii) 'intensive infiltration by macrophages, lymphocytes and eosinophils over the entire gill filament' . . . 'in some heavily infected gills' (p. 396). These authors found that in some filaments the intercellular spaces of the hyperplastic epithelium were filled with eosinophils. The defensive character of these reactions was quite evident.

Without histological investigation, Kabata (1970) described the effects of this process on gill filaments of *Microstomus kitt,* a flatfish from the European Atlantic seaboard, infected with larval stages of the pennellid copepod *Lernaeocera branchialis.* The tip of a normal filament is illustrated in Fig. 1-167B. The respiratory folds can be seen running along both planar surfaces. Fig. 1-167A shows the tip of a filament from which the

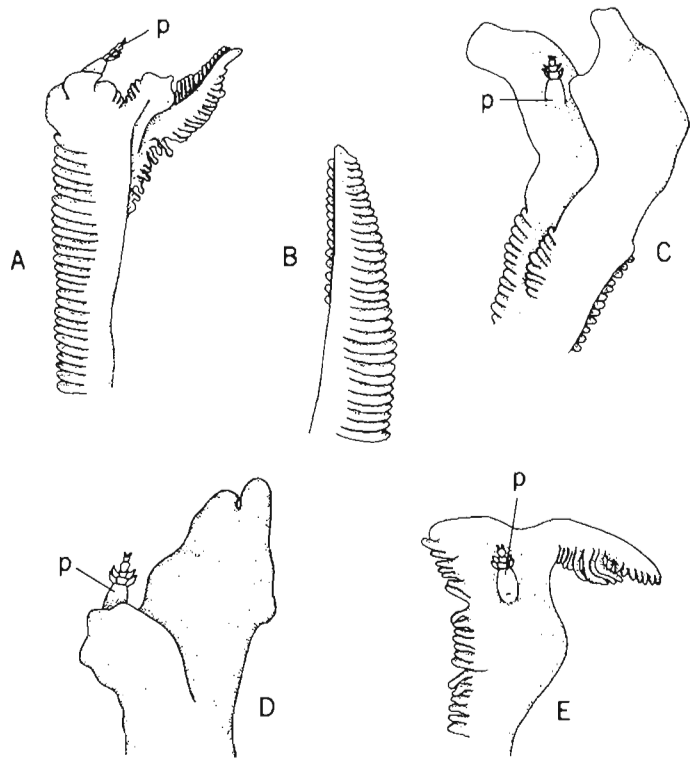

Fig. 1-167: Tips of gill filaments damaged by larval *Lernaeocera* (Copepoda). p: parasite. For further explanation consult text. (After Kabata, 1970.)

proliferation of the gill tissues has obliterated several respiratory folds. Under the impact of the, probably frequently recurring, stimulus of the copepod's attachment, a spurious sideways-projecting tip developed. Fig. 1-167C shows severe damage of the filament, about a quarter of which has lost its respiratory folds and has split into 2. Another spurious tip is shown in Fig. 1-167E, whereas Fig. 1-167D shows the most advanced stage of damage. No trace of respiratory folds remains, the filament is completely non-functional. It should be remembered that as many as 700 of these larvae might occur simultaneously on the gills of one fish.

The reaction of the gill tissues of elasmobranchs is much the same as that of teleost fishes. Benz (1980) examined histologically effects of the attachment of the siphonostome copepod *Nemesis lamna* to the gill filament of the shark *Isurus oxyrinchus*. Having noted that those relatively large copepods (females ca. 9 mm long) appear to show preference to the efferent arterioles of gill filaments as attachment sites, Benz reported pronounced hyperplasia occurring at these sites. 'Proliferation specifically occurred in the epithelial and connective tissue layers surrounding the efferent arterioles.' 'An increase in mucus cell density in the epithelial layer and fibrosis of the underlying connective layer were also exhibited' (p. 443). The affected areas appeared swollen, turgid and pale. A similar appearance was seen by Cressey (1967) at the sites of attachment of *N. aggregatus* on the

gills of *Alopias vulpinus.* Benz saw these reactions as 'natural protective responses'. The defensive nature of tissue proliferations has already been suggested above. Kabata (1970), having considered this defensive process as largely ineffective against crustacean parasites, cited one instance of its successful outcome. 'I have seen on one occasion' . . . 'an example of a successful removal of a copepod from the gills of an elasmobranch host, *Raja batis.* I found a juvenile female of *Charopinus dubius,* suspended from the gills on a digitiform outgrowth of soft tissue. The outgrowth was more than 5 mm long (about twice the length of the parasite) and was distinctly pedunculate. It appeared obvious that it would eventually drop off and that the parasite would be removed. I know of no other record of similar occurrence but I assume that the incident observed was not unique' (p. 61).

Although the mechanisms of the defensive reactions show a large measure of uniformity, the intensity of those reactions varies from species to species. It appears that the extent and vigour of the tissue response is specific to each host-parasite system. A good example of such differences is presented by the attachment of *Clavella adunca* (Copepoda: Lernaeopodidae) to the gill filaments of its gadid hosts (Fig. 1-168). On the gills of *Gadus*

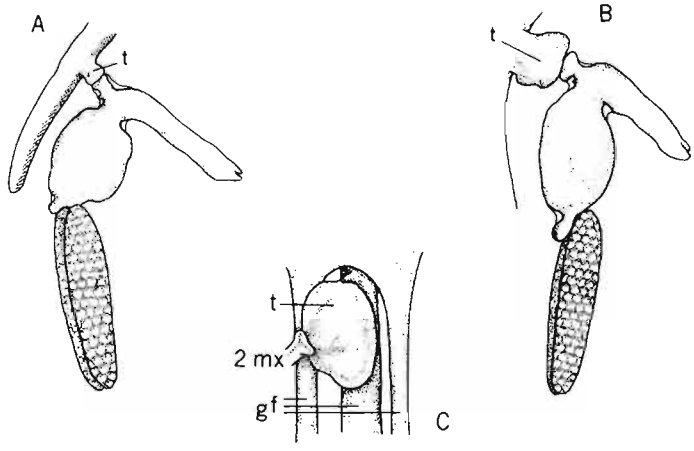

Fig. 1-168: *Clavella adunca* (Copepoda) attached to gills of gadid fishes. (A) *C. adunca* on gills of *Gadus morhua.* (B) Same, on gills of *Melanogrammus aeglefinus.* (C) Same, enlarged. gf: gill filaments; t: tumour; 2 mx: second maxilla of *Clavella.* (After Kabata, 1970.)

morhua this parasite causes the formation of a so-called 'tumour of attachment' of a relatively small size, sufficient to cover the bulla with hyperplastic tissue. The same parasite prompts the development of a much larger tumour on the gills of *Melanogrammus aeglefinus.* This difference has been at least once attributed to the existence of two different species of *Clavella* on these hosts.

In many instances, parasitisation of the gills by Crustacea causes more than one type of effect. A good example of such a case was given by Khan and Pitt (1974), who described virtual devastation caused by a heavy infection of the gills of *Lycodes lavalaei* by *Tanypleurus alcicornis,* a siphonostome copepod.

> "The parasites were attached to the dorsal surfaces of the branchial arches, and either destroyed and lay in place of or displaced the gill filaments. In one

> fish, most of the gill filaments in the right chamber as well as those of three gill arches on the left side were absent' (p. 470). 'Most small blood vessels were destroyed and there was evidence of inflammation, with necrotic tissue in some parts, leaving only traces of the normal structure. Some of the gill filaments were replaced by connective tissue. Few normal branchial filaments remained and these were pushed aside by the parasites. The former, almost white in color, suggested severe anaemia. The fish appeared extremely emaciated" (p. 471).

The widespread occurrence of gill damage caused by crustacean parasites has been referred to in many brief publications scattered in the recent literature. Examples include reports on the impact of *Pseudocycnus armatus* on gills of *Indocybium guttatum* (cf. Natarajan and Balakrishnan Nair, 1972b), of *Pseudergasilus zacconis* (Copepoda: Ergasilidae) on *Plecoglossus altivelis* (cf. Nakajima et al., 1974), of *Lernaeolophus sultanus* (Copepoda: Pennellidae) on *Priacanthus hamrur* (cf. Natarajan and Balakrishnan Nair, 1977), of *Clavella adunca* on Atlantic cod (Janusz, 1980), of *Ergasilus lizae* on various mugilid fishes (Paperna and Overstreet, 1981), and of *Lernantoma asellina* (Copepoda: Chondracanthidae) on *Trigla lucerna* (cf. Rousset and Raibaut, 1983).

Damage to Skin and Muscle

Impact of presence and activities of crustacean parasites on the integument and underlying tissues of the fish hosts produces effects, the variety of which appears to defy classification. Not only do the parasites differ from one another in many ways, but the skin itself displays numerous structural differences from one group of fishes to another. The computation of these differences, however bewildering in detail, can, nonetheless, be reduced to broadly defined categories by the application of equally broad definitions to the main structural components of fish integument and to the types of host-parasite relationships in which the integument is involved (p. 349).

In essence, the skin of fish comprises 3 components: outermost epidermis and 2 layers of dermis (stratum spongiosum and the innermost stratum compactum). This 3-layered arrangement is often complicated by the presence of scales, originating in dermal pockets and jutting out into the epidermis. Underlying the integument is the musculature. With this schema in mind, we can categorise the effects of crustaceans on the skin as falling into the following groups.

(i) Effects involving the epidermis only. They consist of loss of epithelial cells, counteracted by hyperplasia and resulting in thickening of the epithelial layer. The final outcome depends on the balance between the extent of the tissue loss and the vigour of the hyperplastic response. The effects are further compounded by the production of abnormally large numbers of epidermal mucus cells by the skin around the injury. Copious production of mucus can affect some important skin functions.

(ii) Effects reaching into the subepidermal layer, the *stratum spongiosum*. This layer is characterised by large intercellular spaces in the reticular matrix, spaces filled with tissue fluid. Tearing of the reticulum not infrequently leads to loss of that fluid, due to feeding of the parasite or simply to leaking. This loss, in turn, causes collapse and compacting of the affected area, followed by fibrotic response of the connective tissue. The connective tissue reaction is a standard response of the integument to the presence of, and/or irritation by, any foreign body and is apparently aimed at isolation and neutralisation of that body. In

addition, all effects described under (i) are in force, since penetration of the spongiosum is possible only through the epidermis.

(iii) Effects extending into the *stratum compactum* are similar to those listed under (ii) but more extensive and more vigorous. They often bring about local thickening of the skin, swelling at the site of damage and an inflammatory response.

(iv) When the integumental protection has been breached, the parasite affects the musculature. In addition to the variety of damage resulting from any of the 3 preceding categories, effects on the musculature might contribute to functional disturbances. The seriousness of such effects depends, of course, on the extent of the damage and on the importance of the damaged site to the survival of the fish.

(v) A special category of skin injury is the pathological change associated with the formation of zoocoecidia. The penetration of the parasite into the skin is rather shallow, but the structure of the integument is often seriously affected.

A phenomenon causing some confusion and discrepancies in literature reports is the diversity of effects produced on the skin by the same parasite species. These sometimes contradictory accounts, usually involving Caligidae and related copepods, attribute to them severe injuries, sometimes leading to large-scale mortalities, or, on the other hand, report no perceptible skin damage. Such confusion can be resolved, when one recalls the ecological basis for the host-parasite relations within systems involving fish skin. The caligiform copepods have been described above (p. 326) as being adapted to locomotion, the mobility being forced upon them by their need to browse upon the surface of the host fish. They do not remain feeding in one place long enough to cause serious damage. When the browsing area available to a single copepod is restricted (e.g., when the copepod population on the host becomes dense or when the fish is small), the intensity of browsing increases and the exploitation of the substrate becomes much more vigorous. Under those circumstances even a normally innocuous skin parasite is likely to inflict extensive injuries, reaching deep into the tissues. A good example of this is provided by *Lepeophtheirus salmonis,* a caligid copepod normally not seriously injurious. Nonetheless, there are reports of occasional grave damage caused by this species. The oft-quoted salmon kill in the western Atlantic Ocean (White, 1940) was directly due to the heavy infection with *L. salmonis.* Its depredations in Norwegian sea-pen salmon culture are well known and have called for the development of special countermeasures (Brandal and Egidius, 1977, 1979).

As a general illustration of the effects of the crustacean parasites on the skin of marine fishes, 2 recent descriptions are discussed below, dealing with 2 different types of skin.

Logan and Odense (1974) described skin lesions inflicted on *Mola mola* by the cecropid copepod *Philorthagoriscus serratus.* These stationary parasites penetrate deep into the skin of the fish, the lesions caused by several copepods sometimes coalescing to produce one large sore. Threlfall (1967) reported a lesion measuring 105 × 45 mm and containing 'a number of specimens of *P. serratus* lying deeply embedded in the subcutaneous tissues' (p. 169). Logan and Odense (1974) found these copepods in pits varying in depth from shallow to several mm below the surface. The bottoms of the pits were slanted, with the head of the copepod deeper than its posterior end. In fact, the posterior halves protruded above the surface (though the egg sacs were held to the surface of the fish by mucus). The histological structure of the skin was greatly modified in the area occupied by the copepod, particularly underneath its cephalothorax. The authors stated that the affected skin was almost devoid of mucus cells, melanophores and tubercles, except along

the outer rims of the lesion. The epithelium was compressed, or even absent. The stratum spongiosum lost its distinctness and appeared to be missing in some places. The outer stratum compactum was infiltrated and showed some degree of oedema. Fibrillar plates and the network of the connective tissue fibres were distorted, but no responsive fibrosis was observed. The posterior end of the lesion was slightly compressed, but the skin there retained all its component layers.

Logan and Odense also found pathological changes that could be attributed directly to the action of feeding and attachment appendages. Under the cephalothorax, a hole reaching into the stratum compactum was present in the area of the mouth tube. The margins of the lesions were covered by the epithelium which was deflected downwards into the lesion for a short distance. In some places the stratum compactum was exposed. Inflammatory reaction was evident but necrosis appeared minimal. Some signs of epithelial degradation were present. Haemorrhage was only slight and was present mainly at the points of insertion of the appendages.

The interesting part of this report is the absence of host response, other than localised inflammation. In particular, no epithelial hyperplasia or dermal fibrosis appeared to be present. The authors tentatively attributed this fact to the 'low level of cellularity in the stratum compactum' of *M. mola,* which contains only few fibroblasts.

Boxshall (1977) described the histopathology of the infection of the pectoral fin of *Platichthys flesus* with *Lepeophtheirus pectoralis.* This copepod is frequently found in stationary colonies covering considerable parts of the fin. The attachment is effected by the second antennae and the maxillipeds, the claws of which usually penetrate only the epidermis, though they sometimes pierce also the dermal layers of the skin. Severe erosion of the epidermis was observed in the area of the parasites' action, involving also the tips of the first maxillae. There was hyperplasia in the epidermis in areas bordering on the lesion. The most pronounced changes, however, occurred in the dermis. Fibroblasts proliferated and fibres were produced on massive scale in areas directly affected. Macrophages and lymphocytes infiltrated the lesions, together with fibroplasia causing formation of a dense granular tissue. This produced externally visible swellings under each parasite. Heavy infections were accompanied by dermal haemorrhages.

These 2 examples were chosen to illustrate the differences in the effects caused by the crustacean parasites on the integument of the host fishes. The differences are determined by the characteristic features of the host-parasite relationships within individual systems. They are mainly due to the nature and vigour of the host's reaction to the impact of the parasite. The integument of the fin of *Platichthys flesus* reacts very strongly, while the skin of *Mola mola* remains passive, for reasons tentatively suggested by Logan and Odense. Neither copepod penetrated deeply enough to break through to the subdermal tissues. Instances of deeper penetration are, however, not uncommon, in spite of statements made by Smith (1975), as will be made clear by the brief review below.

Kabata (1970) recorded irritation of the integument of the roof of the buccal cavity, particularly behind the vomerine teeth, caused by *Caligus elongatus* in Atlantic cod *Gadus morhua.* More serious injuries, referred to as 'skin ulcers', were caused by *C. orientalis* in *Liza akame* in Japanese waters (Muroga, 1979; Urawa and co-authors, 1979). The lesions were secondarily infected with bacteria (p. 395). Similar effects, caused by *C. amplifurcus* in *Caranx delicatissimus* and by an unidentified *Caligus* in *C. delicatissimus, Seriola quinqueradiata* and *S. purpurescens,* were documented in Japan a decade earlier (Kubota

and Takakuwa, 1963). These authors observed that portions of skin were lost altogether. White (1940) also reported that large patches of skin were lost by Atlantic salmon *Salmo salar* in heavy infection with *Lepeophtheirus salmonis*. Deeper wounds were also observed. Kabata (1970) observed a large *Lepeophtheirus salmonis* attached to the top of the cranium of *Oncorhynchus gorbuscha*. The copepod had chewed through the still rather soft cranium and was about to damage the fish's brain. Hastein and Bergsjo (1976) described and photographed an extensive occipital lesion, which may result in the loss of epidermis from the affected areas. In some instances the dermis also may be lost.

Some species, like the cecropid copepod *Philorthagoriscus serratus*, regularly cause deep lesions. *Caligus macarovi* which burrows its anterior half between the scales of its host, *Cololabis saira*, penetrates to the musculature and creates lesions up to 5 mm in diameter. Numerous elliptical scars are left on the flanks of the fish.

Lüling (1953) has described tissue damage due to *Elytrophora brachyptera* (= *Euryphorus brachypterus*) infecting the gill cavity of tuna. In the richly vascular region of the pseudobranch the copepods carpeted the epithelium with dense stationary colonies. The normally thick skin was seriously abraded, reduced to half its normal thickness, with extensive haemorrhages and large blood lacunae. Subdermal tissues were not involved. According to Lüling, such lacunae are characteristic of integumental injuries caused by most copepods, regardless of host-parasite relations. He pointed out their existence in infections with mesoparasites, as well as with ectoparasitic species. In light of information gathered during the last 30 yr, Lüling's statement seems rather sweeping. As mentioned above, some crustaceans can build up large populations in confined areas without causing serious haemorrhages or, indeed, provoking strong defensive reactions of the integument.

Many crustaceans, particularly those of small or moderate sizes, find the scales of the fish an effective deterrent to deeper penetration. Even relatively shallow implantation of the attachment organs might be impeded or prevented. Special adaptations are required to overcome this obstacle. Kabata (1970) described an example of such adaptation in the marine lernaeopodid *Clavella stellata*. This parasite, living on the skin of the European hake *Merluccius merluccius*, has evolved a flat bulla, the anchor of which has perforations arranged in a ring around the margin. The subanchoral surface of the bulla is applied to a scale and an adhesive substance, produced by the maxillary glands, cements it permanently in place. Surplus cement substance spills over through the perforations of the anchor to the supranchoral surface and forms a ring of plugs, reinforcing the bond between bulla and scale. Scales can be perforated by mesoparasites burrowing their way into deeper layers. Natarajan and Balakrishnan Nair (1972a, 1973) illustrated neat oval perforations pierced through the cycloid scales of *Hemirhamphus xanthopterus* by the pennellid copepod *Lernaeenicus hemirhamphi* (Fig. 1-169). The mechanism involved in these perforations is not known, but their smooth margins suggest that it is something other than simple mechanical abrasion. Entire scales might be lost and extensive lesions result from infections by other mesoparasites, e.g., *Lernaeenicus radiatus* (cf. Voorhes and Schwartz, 1979).

The largest areas of external surfaces devoid of scales are the fins, frequent sites of attacks by parasitic copepods. Kabata (1970) illustrated a case of complete bisection of a dorsal fin by the chalimus stage of *Caligus clemensi* in a young Pacific pink salmon *Oncorhynchus gorbuscha*. In addition to severing the fin, the larval copepod excavated a rounded cavity in the integument and muscle close to the base of its embedded frontal filament. Lernaeopodids such as *Clavella adunca*, attached to the fins of *Gadus morhua*

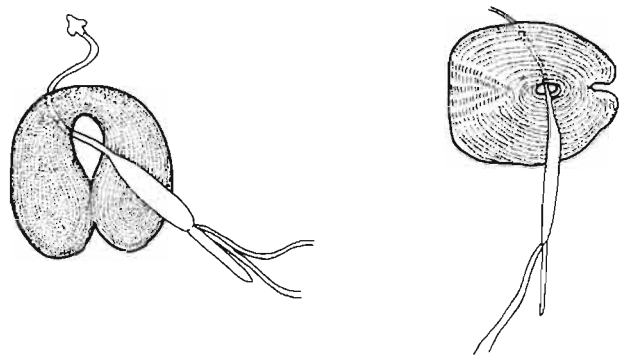

Fig. 1-169: Scales of *Hemirhamphus xanthopterus* perforated by *Lernaeenicus hemirhamphi.* (After Natarajan and Balakrishnan Nair, 1972a, 1973; modified.)

cause serious erosion of their margins. Deep notches with swollen, pale rims, are created within the reach of the copepods' cephalothoraces. This effect was documented half a century ago by Poulsen (1939); it has been observed repeatedly by the author.

Considering damage caused by crustaceans to fish integument, it is necessary to keep in mind injuries by some specialised crustacean appendages. One such appendage is the preoral stylet of the branchiuran *Argulus.* No detailed study of the effects of *Argulus* on a marine fish has yet been made, but ample literature on *Argulus*/freshwater fish interaction is available. There is no reason to suppose that the interaction differs significantly for marine fishes (for details see Kabata, 1970). In brief, injury due to the preoral stylet consists of mechanical damage caused by penetration of the stylet and the histopathogenic action of secretions of preoral glands. The latter is the main instrument of injury; its influence extends for some distance from the point of the stylet's entry into the tissues and in many cases transforms the local injury into a general, systemic effect.

The piercing mouth parts of the gnathiid *pranizae* (Isopoda) also cause local and rather small wounds. The wounds, however, cause haemorrhages. Multiplied many times during a severe attack by numerous *pranizae,* they not only lead to destruction of the skin, but raise the haemorrhage to life-threatening proportions. Paperna and Por (1977) described such attacks by *Gnathia piscivora* on many species of fishes. *G. vorax* carries out similar attacks. The authors observed these isopods in the Gulf of Aqaba, attached to skin, gills, and walls of pharyngeal and branchial cavities. Their attacks led to extensive injuries to skin, as well as to near-lethal or lethal loss of blood.

The defensive mechanisms of the integument, and in particular the proliferative reactions to the crustacean parasites, sometimes result in the formation of tumour-like swellings, already mentioned above (p. 359). Many copepods (e.g., Chondracanthidae) cause localised swellings — produced by proliferating epithelial cells — and partly, or completely, covering the cephalothorax of the parasite. Chondracanthids provoke this reaction partly because of their extrabuccal digestion that necessitates the pouring out of digestive secretions into the tissues of the host. Their lytic action is the main stimulus to the proliferative reaction. Other mechanisms are also responsible for reactions of this type. The copepod *Leposphilus labrei,* living in the mucus ducts of fish, causes a marked swelling, extending over the space of 2 to 3 scales. The most conspicuous swellings of this

type were described by Shiino (1932). The swelling produced on the surface of *Pterois lunulata* by *Ichthyotaces pteroisicola* (Fig. 1-170) are covered by tautly stretched skin. Shiino did not investigate the pathology of the infection, but its severely disfiguring effects are obvious. Each swelling acts as a protective sac for the parasite, an interesting example of the exploitation by the crustacean of the defensive reaction of its fish host. That this

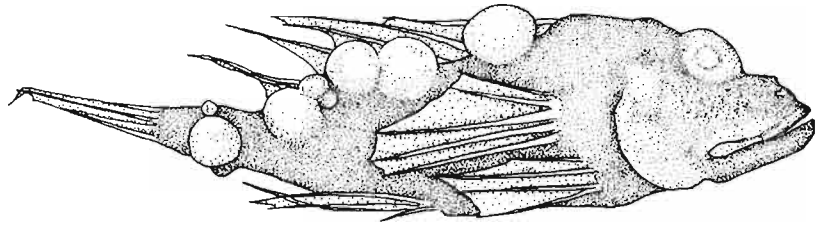

Fig. 1-170: *Pterois lunulata* disfigured by *Ichthyotaces pteroisicola*. (After Kabata, 1970.)

example is not unique can be seen from Lewis's (1964) description of a copepod, tentatively identified as *Nessipus costatus*, parasitic on the gills of many fishes belonging to the family Acanthuridae (also Labridae, Zanclidae and Pomacanthidae) in Hawaii. The male of this species, during the preadult part of its life cycle, lives on the fins, to which it is attached without the aid of a frontal filament. The copepod (Fig. 1-171A) evokes a very violent proliferative reaction of the integument. Lewis did not examine the histopathology of this reaction. Its extent, however, is sufficient to result in the formation of a large swelling that covers the entire copepod, a cyst reaching a length of 5.0 mm (Fig. 1-171B). Only the tail protrudes from the small orifice of this peculiar cyst. The copepod moults within it and leaves it on attaining adulthood. Empty cysts, some still containing exuvia, were found. Lewis reports that the cyst is eventually resorbed. This case offers an example of an adaptation of the parasite's life cycle to the pathological changes in the host.

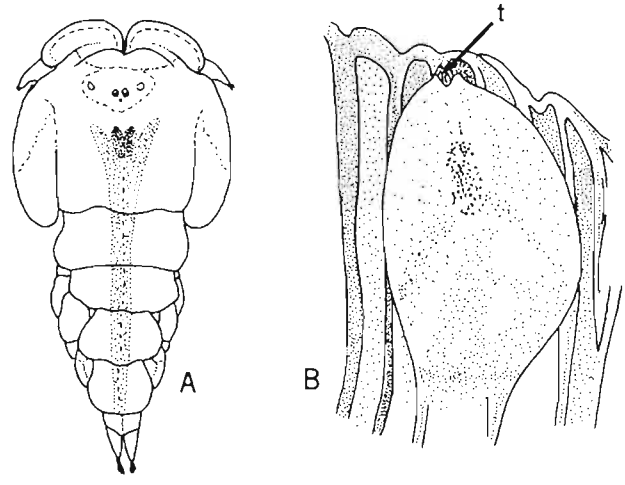

Fig. 1-171: (A) Juvenile male of *Nessipus costatus* (?), removed from its cyst on a fin of a teleost fish. (B) Cyst of *N. costatus*. t: protruding tail of *N. costatus*. (After Lewis, 1964; redrawn.)

Skin and muscle can be injured also by crustaceans that do not use them as target sites, but move through them on the way to deeper tissues. The effects of these, often mesoparasitic, crustaceans have been described in the work of Kabata (1970).

The parasites that penetrate the skin more deeply in the process of burrowing into deeper tissues do not cause significantly different damage. Both the skin and muscle are subject to: (i) mechanical damage caused by the parasite; (ii) the responsive reaction of the tissues, and (iii) the secondary infections.

Nigrelli and Firth (1939) gave a good description of pathological changes caused by *Sphyrion lumpi* (Sphyriidae), a copepod living on *Sebastes marinus* (Scorpaenidae). The reaction of the host tissues produces a connective tissue capsule, isolating the parasite. This reaction is similar to that occurring in most fishes under similar circumstances. The capsule is the outcome of a general inflammatory process. The incoming blood vessels dilate and the affected area is infiltrated by blood cells. Leucocytes, monocytes, erythrocytes move into the fibrin network, small lymphocytes and neutrophils, as well as granulocytes have been observed. *S. lumpi* can become attached to different sites on the fish (Fig. 1-172).

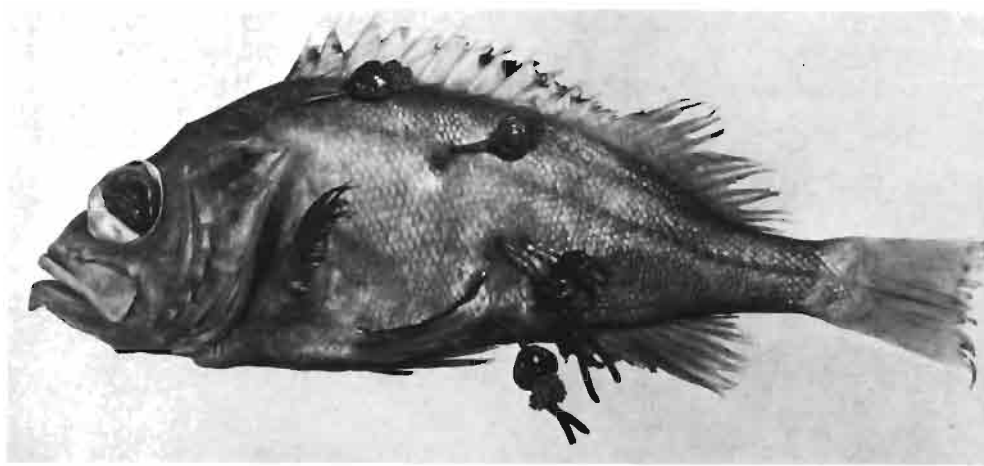

Fig. 1-172: *Sebastes marinus* parasitised by several *Sphyrion lumpi.* (After Templeman and Squires, 1960.)

Secondary changes are not common, but sometimes a black, tumour-like formation can be found at the point of attachment (Fig. 1-173). The black surface of this structure is due to melanophores. The skin surrounding it is pale. Deeper layers are composed of dense connective tissue and cellular infiltrate. Similar pigmented swelling was described by Baudouin (1917) around the point of attachment of *Lernaeenicus sprattae* (Pennellidae) to the Atlantic sardine.

The point of attachment can also become a sore, containing viscous exudate. Nigrelli and Firth (1939) found in such a sore many microorganisms resembling algae, as well as some flagellates. Bacteria were scarce. The bulk of the sore comprised blood cells and debris of muscle tissue. Similar sores were found on sardines infected by *L. sprattae.*

A very common and well-known mesoparasitic copepod is a pennellid *Lernaeocera branchialis.* On its way to the target site, the cardiac region of the fish, *L. branchialis*

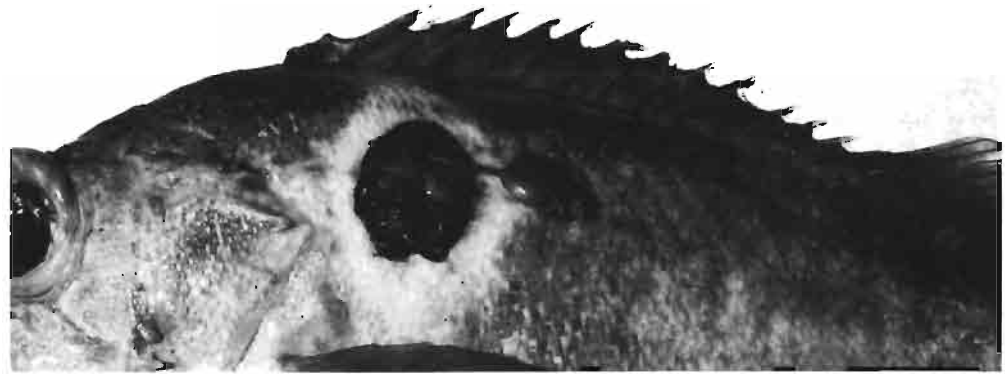

Fig. 1-173: Tumour-like formation around the point of attachment of *Sphyrion lumpi* to *Sebastes marinus*. (After Templeman and Squires, 1960.)

breaks through from the branchial cavity, damaging epithelium and subcutaneous tissues. Sundnes (1970) identified 2 main histopathological changes. The epithelium of the host in the vicinity of the parasite becomes superficially necrotic, and the subcutaneous tissues develop a granular connective tissue layer around the parasite, with some vascularisation. These changes are characteristic of chronic inflammation. A 'tumour' covered the part of the parasite embedded in the host. According to Sundnes, the tumour consists of necrotic epithelium surrounding the parasite's cuticula with intact epithelium between necrotic and granulated subcutaneous host tissue. Granulated tissue surrounds the whole tumour. The epithelium remains invaginated and the histology of the infested host area unchanged throughout the parasite's life. The invagination of the epithelium appears to follow the movement of the parasite through the tissues and interposes an epithelial barrier between it and the subepithelial layers. This observation led Sundnes to the conclusion that *L. branchialis* is an ectoparasite. Moser and Taylor (1978), examining the histopathology of the infection of the lanternfish *Stenobrachius leucopsaurus* with another pennellid copepod, *Cardiodectes medusaeus,* observed only a 'nonspecific fibrotic response'. They suggested that 'a type of host granuloma' might be formed under the influence of this parasite. Yet another pennellid, *Pennella hawaiiensis,* has been found by Kazachenko and Kurochkin (1974) to infect the commercially valuable fish *Pentaceros richardsoni.*

> "As the reaction of the organism to the presence of a foreign body, there forms around *Pennella* in the musculature of the fish a large area of dense, dark tissue, eventually becoming a connective tissue capsule, enclosing chitinous remains of the cephalothorax and a part of the neck of *Pennella.* Such focal infections of muscle tissue around each *Pennella* have the volume of 2 to 5 cm^3" (p. 50).

The copepods penetrate the musculature to the depth of 2 to 4 cm, but only occasionally break through to the visceral cavity.

At least in some cases, the capsule surrounding the parasite does not fit tightly, leaving a space between cuticle and capsule wall. This space is filled with liquid. Commenting on

the existence of such spaces around *Rebelula* (= *Lophoura*) *edwardsii,* a sphyriid copepod, Candeias (1952) speculated that the liquid might act as a cushion, damping down the forces generated by water current and tugging at the exposed part of the parasite's trunk.

All Pennellidae and Sphyriidae can be presumed to be responsible for injuries similar to those described above.

Injuries caused by digestive fluids of chondracanthid copepods have been mentioned earlier. It should be added here that those fluids also lyse muscle tissue, used by the copepods as food. Sometimes chunks of musculature, equalling or exceeding in volume the cephalothorax of the parasite, are dissolved.

The cirriped crustacean *Anelasma squalicola* forms large 'cavities of implantation' (Hickling, 1963) in the musculature of small sharks and dogfishes, most commonly in the vicinity of the dorsal fins. The embedded part of the parasite is provided with numerous dendritic outgrowths, to which the absorbent function has sometimes between attributed. No detailed study has been made of the histopathology of this infection. Sometimes a normally non-parasitic copepod settles on the skin of a fish, causing focal inflammation at the point of attachment.

The last to be mentioned in this account is the pathogenic change which Kabata (1970) described as one of the most unusual effects produced by a crustacean parasite in skin and muscle of fish. This statement was made in reference to the zoocoecidium, or pouch, made in the body wall of the fish by isopod crustaceans. These parasites infect both freshwater and marine fishes, but most information about pathological changes comes from studies of freshwater-living parasites. Assuming that effects of marine pouch-forming cymothoids do not differ significantly from those of their freshwater relatives, the latter are used below as a generalised illustration.

Kabata (1970) summarised the course of this strange process as follows: When a small isopod settles on the skin, it begins to exert pressure that causes an indentation in its surface. As the parasite grows, the pressure increases. Eventually, the parasite sinks well below the surface, becoming gradually enclosed in a pouch of skin that keeps stretching to accommodate its growth. Only a small opening remains to allow contact between the isopod and the external environment. Sections through stretched pouch wall reveal that in some areas the skin retains its stratified epithelium (Malpighian layer), basal membrane, pigment cells, scales, and the connective tissue layer. In other places the skin stretches so much that its thickness becomes reduced to less than one tenth of the normal. Most layers disappear, except for a trace of epithelium. Dermal scale pockets are still present as are scales, though reduced in size. The scales do not overlap; they are arranged side by side with their margins barely touching. Only the connective tissue layer is retained. The pouch wall contains some blood vessels. Apparently, the parasite feeds on the blood supplied by these vessels. The presence of granular contents in the gut of the parasite can be taken as evidence of blood feeding.

Huizinga (1972) studied the histopathology of the zoocoecidium produced by *Artystone trysibia,* a freshwater cymothoid. He found that the initial settling of a young isopod is accompanied by mechanical damage to the body wall of the fish and by resulting haemorrhages. The host response brings about massive inflammation, swelling around the perimeter of the injured area and copious, white, muco-cellular exudate. This reaction subsides about 30 days after attachment. The capsule around the crustacean consists of a

fibrous connective tissue stroma with scattered muscle fibres and stretched mucoid-epithelial cells, apparently derived from the original body wall. The stroma is infiltrated by only a few eosinophilic and basophilic granulocytes.

The 2 accounts contain some obvious differences and raise some questions. Not the least of these questions is the problem of host recovery, if any, following the death of the parasite. The pathology of the zoocoecidium is still in need of careful and detailed study.

Pouches are also formed by some philichthyid copepods. *Sarcotaces* produces them occasionally in the body wall of its host, as well as in sites such as the operculum, under the pectoral fin, in subcutaneous layers on the flanks of the fish and even on the top of the cranium (Dollfus, 1928; Komai, 1923; Kuitunen-Ekbaum, 1949; Moser, 1977). Their pathology has not been investigated.

Damage to Skeleton

The hardness of skeletal tissues, particularly in teleosts, makes them an unsuitable substrate for crustacean parasites. As a site for attachment, the skeleton is too firm; it is also poor and relatively inaccessible as a source of food. While host-parasite relations between fishes and crustaceans, in which the skeletal structures are directly involved, are rare, the skeleton is not immune from pathogenic influences of crustacean infections. The literature contains a number of reports of skeletal damage resulting from such infections. The damage, however, is usually indirect.

Generally speaking, 2 types of skeleton damage caused by Crustacea can be recognised: (i) perforation or erosion of skeletal tissues; (ii) swelling or distortion of bones. The former is more common. The only known specific association between a crustacean parasite and a skeletal structure of its marine fish host has been mentioned by Kabata (1970). The lernaeopodid copepod *Vanbenedenia kroyeri* invariably becomes attached to the dorsal spine of *Chimaera monstrosa*. Although the surface offered by this spine for attachment is very small, Kabata found 7 large females of this copepod (23 to 28 mm long) crowded together on the spine, ignoring other sites. By unknown means, cavities of implantation were excavated in the spine for the large bullae of the parasites.

Nearly a century old record (Joubin, 1888) describes destruction of the hypophyses and extensive damage to the vertebrae, caused by *Lernaeenicus encrasicoli*, a pennellid copepod. The damage resulted from the passage of the neck-like part of the parasite's cephalothorax between hypophyses of adjacent vertebrae. *L. hemirhamphi* was reported in a curious case of skeletal damage. It penetrated the operculum of its host, *Hemirhamphus xanthopterus* (Fig. 1-174), by passing its 'neck' between the subopercular and preopercular bones (Natarajan and Balakrishnan Nair, 1973). Having emerged in the gill cavity, it grew across the cavity space and came to rest with its 'head' anchored in the floor of the pharynx. How this could be accomplished in the presence of the continuous movements of the operculum, defies imagination. Four of the 240 parasites collected were associated with the hosts' opercula.

Grabda (1972) described and illustrated damage caused to the skeleton of *Pneumatophorus colias* by another pennellid, *Lernaeolophus sultanus*. Parasites were usually attached to the roof of the buccal cavity and pierced through it, destroying large parts of the palatine bone. They grew towards the eyes and nasal cavity of the fish. At the latter site they seriously damaged the proethmoid and lateral ethmoid bones, parts of which were entirely missing. The same parasite produced similar effects on the cranial

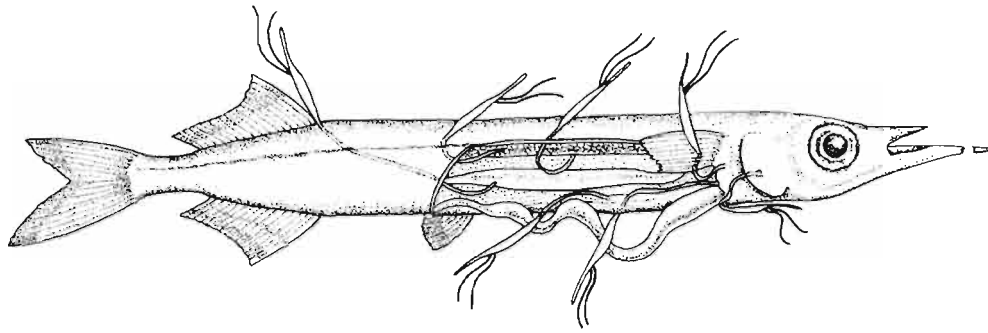

Fig. 1-174: *Lernaeenicus hemirhamphi* attached at various sites to its host *Hemirhamphus xanthopterus.* (After Natarajan and Balakrishnan Nair, 1972a; modified.)

skeleton of *Pagellus erythrinus.* Raibaut and Hedi Ktari (1971) described and illustrated damage caused to the palatine (Fig. 1-175A), vomerine, nasal (Fig. 1-175B) and lachrymal bones (Fig. 1-175C).

Swelling of osseous tissues was reported by Quignard (1968). The philichthyid copepod *Leposphilus labrei,* dwelling in the subcutaneous ducts in the frontal region of the head of *Centrolabrus exoletus,* was discovered to be the cause of large, dome-like swellings of the frontal bone situated on both sides of the duct. These swellings have been considered in the past by ichthyologists as a taxonomic characteristic (or as being associated with age, maturity or physiological condition of the fish) and referred to as the 'frontal gibbosities'.

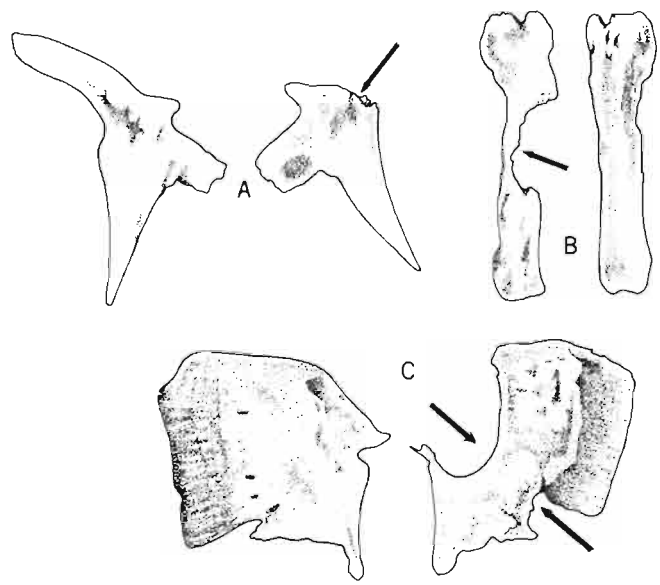

Fig. 1-175: *Pagellus erythrinus.* Skeletal damage inflicted on the cranium by *Lernaeolophus sultanus.* (A) Palatine bones. (B) Nasal bones. (C) Lachrymal bones. (After photographs of Raibaut and Hedi Ktari, 1971; redrawn.)

Bones can become misshapen when a parasite lodges itself near them in a young and actively growing fish.

The mechanism involved in the skeletal injuries is not known. As mentioned above for *Lernaeenicus encrasicoli,* mere proximity of the parasite to a skeletal structure suffices to provoke a pathological change. The only attempt to study the histology of skeletal injuries caused by crustaceans was made by Cressey and Colette (1970). They studied the infection of the preoperculomandibular canal of *Strongylura notata* with *Colobomatus goodingi* and found that reactions to *C. goodingi* ranged from almost none to nearly complete obliteration of the lateral line canal by granulation tissue, inflammatory infiltrate, and sometimes, new bone formation. Bone formation remained orderly and provided no evidence of neoplastic transformation. In one case the formation could be related to fracture of an adjacent bone trabeculum. The formation of new bone was always associated with marked chronic osteitis or osteitis combined with microfracture. The affected area was inflamed, the inflammatory infiltrates comprising mainly lymphocytes and macrophages. Mononuclear eosinophils were also present in fair numbers.

Damage to Sense Organs

Sense organs of host fishes suffer from parasitic crustaceans in 2 ways: they can be target sites, or might be 'accidentally' injured by activities primarily directed towards other sites. In almost all cases, the Crustacea involved are Copepoda. The effects differ from negligible to severe, depending on the intimacy of the association of the copepod with its substrate. The 3 sense organs involved are: nose, eye and lateral line.

The nose

Several copepod species specifically occupy the nasal cavities or fossae of teleost and elasmobranch fishes. Only some of them will be listed here as examples. The oldest record known is that of *Bomolochus soleae* (cf. Claus, 1864), living in the nose of the flatfish *Solea solea.* Often confused with this species is *Holobomolochus confusus,* a common inhabitant of the nasal capsules of several gadid fishes, mainly *Gadus morhua,* in the European Atlantic Ocean and the North Sea. The pathology of this nose infection has not been investigated, but I often found the infected nasal capsules full of opaque, pus-like mucus. Since the nose depends for its functioning on the uninterrupted flow of water through the capsules, the olfactory function of a nose infected with bomolochids is probably adversely affected. In the Pacific Ocean, 2 species of nose-dwelling *Chondracanthus* were described recently: *C. narium* in *Ophiodon elongatus* and *C. triventricosus* in *Sebastes alutus,* both off the Canadian coast. Among Siphonostomatoida, an example of a nose-specific parasite is *Albionella globosa,* a lernaeopodid commonly found in the nasal fossae of the nurse dogfish *Scyllium canicula.* This species, like the chondracanthids, inflicts damage on the olfactory epithelium with its attachment organ, the bulla. The size of these copepods alone is capable of disrupting the orderly flow of water through the nose and so of disturbing the process of olfaction.

Some species occur in the nose only occasionally. Among them is *Caligus fortis,* originally found in the nose of *Carangoides emburyi* in Queensland, Australia (Kabata, 1965), but subsequently seen also at other sites on the fish.

A good example of 'accidental' damage to the nose has already been mentioned in connection with the skeleton (p. 369). Grabda (1972), studying the effects of a pennellid

mesoparasite *Lernaeolophus sultanus* on its host *Pneumatophorus colias,* found the copepod penetrating the nasal cavity by growing into it from its original point of entry in the buccal cavity. She saw 1 or even 2 copepods in a nasal capsule. The olfactory epithelium was stretched by the pressure of the ingrowing copepod. Petechial haemorrhages were located near the buccal region of the parasite. In this case also the capsule was filled with mucus and very likely non-functional.

The eye

The many species of copepods known to be associated with the eye of their fish hosts can be divided into 3 groups, according to the intimacy of the association which can be superficial, shallow or deep. In addition, some copepods can cause damage to the ocular adnexa, without injuring the eye directly. Grabda (1972) described penetration of *Lernaeolophus sultanus* into the orbit of *Pneumatophorus colias.* The eye itself did not seem to be affected and the possible impact on vision was not clear.

The group of superficial parasites comprises those that are general surface browsers and are not limited to the surface of the eye, as well as those that are so limited. Heavy infections with caligid copepods, or with argulid branchiurans, can damage the surface of the cornea to the point of inducing opacity and obstructing the vision of the fish. Such damage is, however, rare. Few mobile ectoparasites are specific to the surface of the eye. The best examples come from scombrid and clupeid fishes. These 2 teleost groups are not only ecologically similar, but are both equipped with an adipose lid. The pocket formed between lid and cornea offers a safe refuge for some copepods, otherwise sliding freely over the eye. Kabata (1970) quoted as examples of such copepods *Pumiliopsis sardinellae* on *Sardinella albella, P. varunae* on *Anadontostomata chacunda, Pseudoeucanthus alosae* on a clupeid and *Bomolochus jonesi* on a scombrid, *Rastrelliger kanagurta.* Nothing is known of the possible pathogenic influence of these copepods.

The copepods that effect shallow penetration of the eye are invariably immobile and permanently anchored to the eye. The depth of the penetration and its consequences differ from species to species. The lernaeopodid copepod *Ommatokoita elongata* attaches its bulla to the cornea of the eye of the Greenland shark *Somniosus microcephalus.* Other species of sharks are less frequently attacked. Unlike most members of its family, *O. elongata* does not produce a deep implantation cavity for its small bulla, but causes this organ to be fixed to the cornea by means of an adhesive substance produced by the maxillary glands. The effects on vision appear to be negligible. Berland (1961b), who examined over 1,000 specimens of *S. microcephalus,* found that they were nearly all infected. As many as 84.4 % carried the copepod on both eyes. If *O. elongata* were seriously harmful to its host's vision, the effects would be disastrous on the population scale and might lead to virtual annihilation of the species.

Another copepod that penetrates below the surface of the eye is *Lernaeenicus longiventris,* not an obligate eye parasite. *L. longiventris* does sometimes enter the eye from the periphery of the cornea and grows more or less in parallel with the corneal surface, producing long loops of the 'neck' part of its thoracic region, clearly visible from outside. This infection, described in some detail by Carvalho (1953) does not appear to be seriously injurious to the eye.

Not normally an eye parasite, the siphonostomatoid *Sphyrion lumpi* also attacks the eye on rather rare occasions. Nigrelli and Firth (1939) found some specimens that pierced

the cornea of *Sebastes* and entered the anterior chamber of its eye. They suggested that a rather large opening in the cornea and the distortion of the corneal surface might cause some disruption of vision. Kabata (1970) thought that the effect of this injury is, at best, slight, because the corneal surface plays a very limited part in the visual process of fishes. There were signs of some host response to the presence of *Sphyrion* in the development of some fibrous connective tissue around the parasite. Neighbouring ocular vessels were dilated and the area around the point of implantation was infiltrated by 'numerous leucocytes'.

Most of the copepods that penetrate the eye are obligate eye parasites. Two well-known examples are *Lernaeenicus sprattae* and *Phrixocephalus cincinnatus,* both pennellid copepods. *L. sprattae* pierces the cornea of sprat, sardine and occasionally herring, grows across the entire eyeball and becomes embedded in the fundus of the eye under the retina. It derives its nourishment from the serum of the blood richly supplied by the choroid plexus of the eye. The damage consists of corneal injury and retinal distortion. The lens is not affected in most cases, but sometimes a cataract effect is produced, in the absence of a mechanical injury to the lens. Secondary bacterial infections were suspected of being instrumental in producing this effect. Earlier records of this infection were summarised by Kabata (1970).

The best-documented case of serious eye injury, leading inevitably to blindness of the infected eye, was that of *Phrixocephalus cincinnatus,* parasitic on the Pacific arrowtooth flounder *Atheresthes stomias.* The attachment to the eye and subsequent growth of this parasite are similar to those of *Lernaeenicus sprattae,* with one important difference: *P. cincinnatus* becomes completely immersed in the eye and must emerge from it again in the process of growth by pushing its way out with its posterior end. The damage to the eye is extensive in both corneal and retinal layers. Fig. 1-176 shows the region of penetration at the junction of the cornea and sclera. Around the puncture the cornea loses its normal structure and becomes swollen due to the inflammation. *P. cincinnatus* develops 2 pairs of lateral processes, contained within the eye and occasionally damaging the lens. The anterior extremity of the copepod punctures the retina (Fig. 1-177). The developing holdfast spreads through the choroid layer of the eye, provoking extensive tissue changes. Many capillaries are ruptured by the dendrites of this organ, as well as by the second antennae and the mouth parts. Small blood lacunae form in the affected area, sometimes coalescing into large haematomas around the copepod's cephalothorax. A section through this area (Fig. 1-177) shows the cephalothorax (H), its dendrites (A), and the haematoma (B) (Kabata, 1970). The holdfast of *P. cincinnatus,* pushing up against the bottom of the retinal layer, distorts it to the point at which normal focussing becomes impossible and the vision is impaired. The most severe impact of the infection is felt, however, when the parasite dies and its remains decompose within the eye. The lens is completely destroyed and eventually the eye is completely filled with pus, blood and tissue debris.

Complete destruction of the eyes of sharks is sometimes caused by *Anthosoma crassum,* a dichelesthiid parasite normally parasitic in the flesh of its hosts. I saw on one occasion a cluster of several females of this species embedded side by side in the eye, the condition of which resembled that described above for the terminal stage of infection with *Phrixocephalus cincinnatus.*

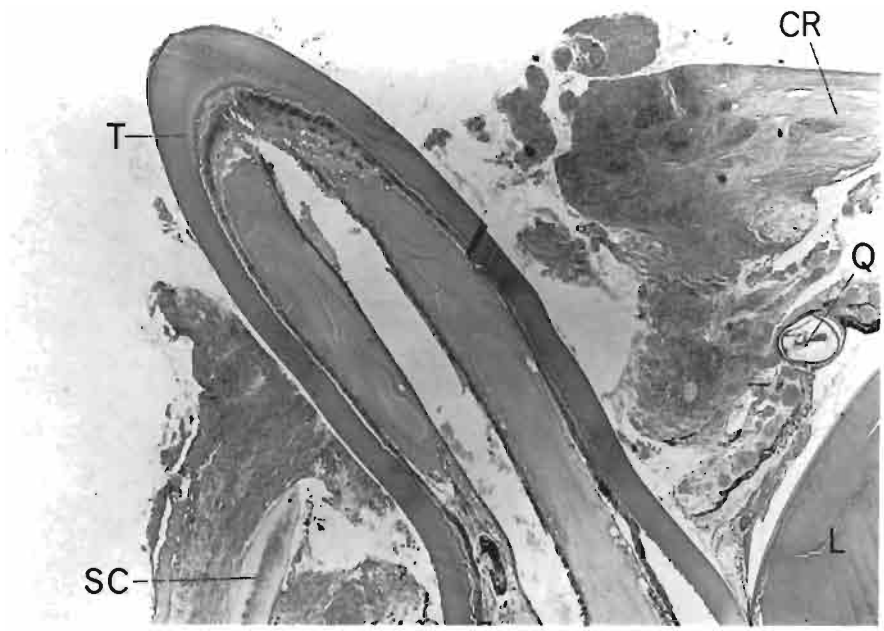

Fig. 1-176: Section through the point of entry of *Phrixocephalus cincinnatus* into the eye of *Atheresthes stomias*. CR: cornea; L: lens; Q: section through one of the parasite's dendrites; SC: sclera; T: parasite's trunk. (After Kabata, 1970.)

The lateral line

Infection of the lateral line canal with *Colobomatus goodingi* has already been mentioned in connection with skeletal damage (p. 371). Cressey and Colette (1970) found some canals nearly obliterated. While the normal epithelial lining of the canal was only a few cells thick and the cells were flat, the inflammatory reaction to the presence of the copepod caused the epithelium to thicken and to become diffusely infiltrated. Granulation tissue was found, in some cases, containing fairly discrete granulomas. The epithelial cells within these structures contained 'deposits of eosinophilic material, possibly derived from phagocytosis of the necrotic ova commonly present within the cavity' (p. 393). The effects of these pathological changes on the function of the lateral canal are as yet unknown.

Damage to Viscera

The prefatory statements to the preceding part of this section are largely applicable to this one also. The main difference between them is in the fact that the crustacean parasites of marine fishes seldom choose the viscera as their target sites. With the sole and inexplicable exception of the heart, the internal organs of fishes suffer pathological changes mainly as the result of indiscriminate activities of mesoparasitic and some ectoparasitic crustaceans. The heart is the only specific site for some copepod parasites. In general, damage might be due to mechanical injury to the tissues of an organ, caused by

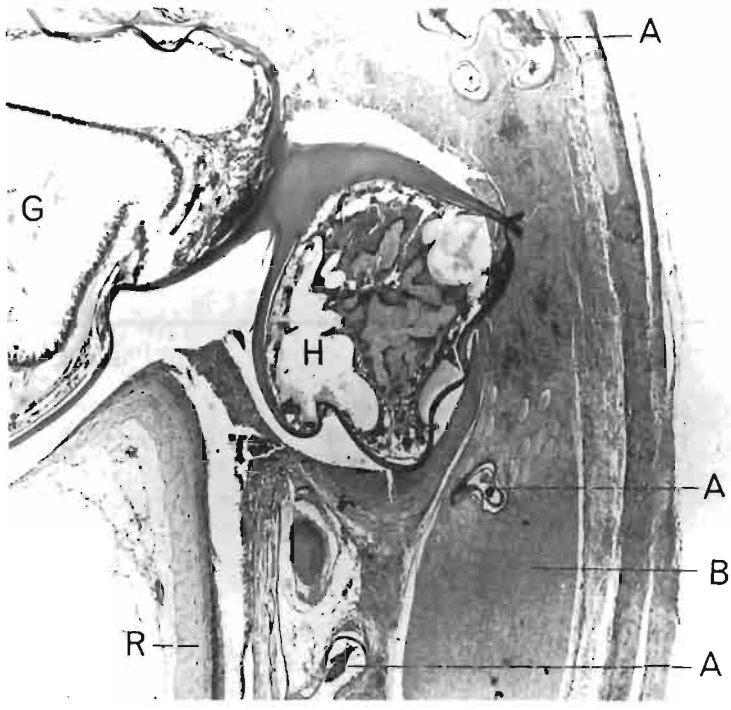

Fig. 1-177: Section through the point of attachment of *Phrixocephalus cincinnatus* to the eye of *Atheresthes stomias.* A: section through dendrites; B: haematoma; G: intestine of parasite; H: cephalothorax of parasite; R: retina. (After Kabata, 1970.)

penetration of the parasite's anchoring structures, or by its feeding activities; alternatively, it might result from the pressure exerted on them by a parasite located in close proximity. This section will discuss the effect of crustacean parasites on heart, liver, kidneys, gonads and intestinal tract.

The heart

Very few crustacean parasites are capable of causing injury to this most important organ. Surveying pertinent literature, one seldom finds references to associations between fish heart and crustacean parasite. Such as do occur are usually aberrant in nature. There is, however, an important exception to this picture. Some species, even entire genera, of mesoparasitic Pennellidae (Copepoda) are frequently, or invariably, found embedded in the cardiac region, though not necessarily in the heart itself. The *bulbus arteriosus* of the heart is the most common place of attachment. Kabata (1970) summed up our knowledge of this host-parasite interaction, pointing out that the bulbus is believed to be best suited to accommodate the parasite's holdfast; it comprises undifferentiated mesenchyma cells with great plasticity in their responses. Normally, the volume of the bulbus increases, so as to cover the cephalothorax of *Lernaeocera* and its holdfast. The lumen is sometimes drastically reduced (Fig. 1-178). For details of histopathological changes consult Kabata (1970). Sundnes (1970), who studied *Gadus morhua,* did not find *Lernaeocera branchialis* embedded in the bulbus or the pericardium of mature fish. Young fish in the Borgenfjord,

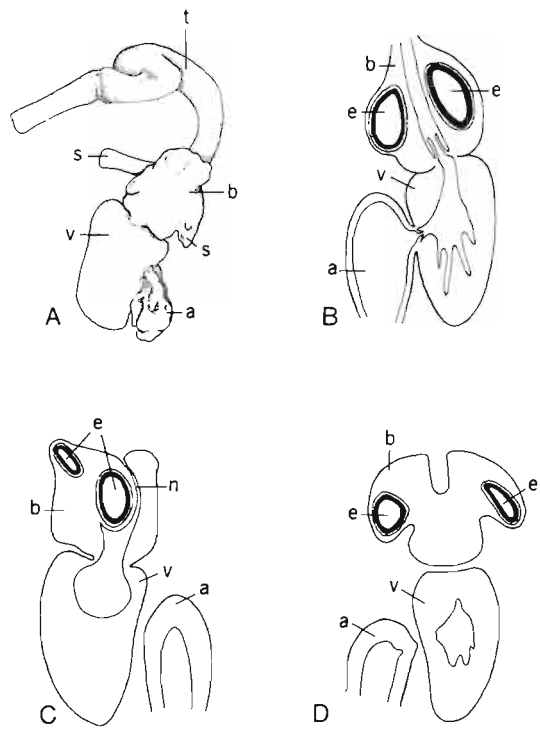

Fig. 1-178: Damage to heart of host caused by *Lernaeocera branchialis.* (A) External view, with parasite embedded. (B–D) Sections through infected hearts. a: atrium; b: bulbus arteriosus; e: section through parasite; n: reduced lumen of bulbus; s: bulbus walls pushed out by parasite's holdfast; t: trunk of parasite; v: ventricle. (After Schuurmans Stekhoven, 1936.)

on the other hand, had their hearts infiltrated by that copepod. The size of the host at the time of infection is clearly of paramount importance. Sundnes' observations on the pathological changes caused in the heart were similar to those described by Kabata (1970): *Lernaeocera* probably very seldom penetrates the lumen of the vessel. Such penetration could have disastrous consequences for fish and parasite alike, resulting in immediate thrombus formation followed by death of both.

There can be little doubt that the reduction in the lumen of the *bulbus arteriosus,* or of the ventricle, affects the efficiency of the heart, though it has not yet been possible to quantify the extent of such impact. Kabata's comments on the lethal effects of any breach in the walls of the cardiac chambers have been found, in the intervening years, not to be universally applicable. Arguments against them were provided by increase in our knowledge on the biology of *Cardiodectes.*

As its name indicates, the genus *Cardiodectes* is site-specific with regard to the heart of its host. Moser and Taylor (1978) found that *C. medusaeus* breaks into and becomes permanently embedded in the *bulbus arteriosus* of its host *Stenobrachius leucopsaurus.* The outcome is not fatal and the fish appears to function fairly normally, even showing at times a greater than normal rate of somatic growth. Kabata (1981) confirmed this finding. His diagrammatic illustration of the parasite's position within the host (Fig. 1-179) shows

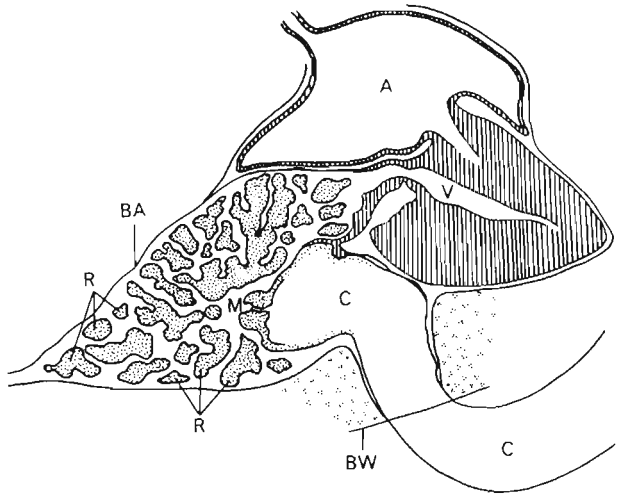

Fig. 1-179: Section through heart of myctophid fish with *Cardiodectes medusaeus* embedded in *bulbus arteriosus*. A: atrium; BA: bulbus arteriosus; BW: body wall; C: copepod; M: mouth; R: rhizoid holdfast processes; V: ventricle. (After Kabata, 1981.)

the enlargement and stretching of the walls of the bulbus, within which head and holdfast of the parasite are anchored. Blood pumped into the bulbus by the ventricle must percolate between the dendrites of the holdfast. It is necessarily slowed down and some of it is diverted into the mouth of the parasite. Fresh erythrocytes were found in the anterior part of the parasite's alimentary canal. It is not clear how the fish avoids thrombus formation and its consequences. Perhaps some anticoagulant is produced by the copepod and injected into the bloodstream.

Grabda (1975) investigated the pathology of infection with *Haemobaphes diceraus,* another pennellid copepod, in the heart of its host *Theragra chalcogramma,* a gadid fish in the northern Pacific Ocean. She illustrated the entry of *Haemobaphes* into the cardiac region diagrammatically (Fig. 1-180). The copepod enters the vascular system at some point on the gill arch (usually near the ventral end) and grows along the afferent vessel to its junction with the ventral aorta. It then follows the course of that vessel 'upstream' until it reaches the *bulbus arteriosus* or even the ventricle of the heart. (Hence the characteristic hairpin bend in the neck of the dissected copepod.) Like *Cardiodectes, Haemobaphes* fills

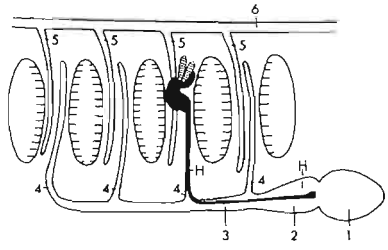

Fig. 1-180: Location of adult female *Haemobaphes* in cardiac region of its host. 1: ventricle; 2: bulbus arteriosus; 3: ventral aorta; 4: afferent branchial vessels; 5: efferent branchial vessels; 6: dorsal aorta; H: *Haemobaphes.* (Modified from Grabda, 1975.)

the lumen of the vessels along which it grows. Grabda pointed out the discrepancy between this fact and Kabata's (1970) comments alluded to above. Not only is there no thrombus formed, but the circulation maintains a level of efficiency compatible with the survival and normal functioning of the infected fish. How this can be accomplished, in view of the fact that the lumen of the infected vessel is completely filled by the parasite's cephalothorax (or even 2, in not uncommon double infections), can only be surmised. Grabda suggested that the answer lies in the elasticity of the arterial walls, permitting them to distend enough to allow the passage of blood along the blocked segments of the vessels around the blocking parasite. That the vessels could do this (and it appears that they must), is a testimony to their extraordinary resilience. Grabda's observations showed not only blockage of the vessels, but also damage to their tunica intima. Penetration of *Haemobaphes* into the ventricle also causes some damage to the cardiac valves, the muscular layer of which becomes much more 'loose'.

Finally, many of the cymothoid zoocoecidia, which have their points of entry behind the pectoral fin of the fish, in the process of their expansion come to abut on the pericardial cavity. Some degree of pressure and possibly displacement can be expected. However, the literature provides no information on the pathogenic impact of this host-parasite interaction on the heart of the host.

The liver

The only crustacean parasite that appears to use the liver as its target site is *Ophiolernaea formosana,* a pennellid with an oral region of unusual proportions. Carried at the apex of a tube much exceeding the rest of the body in length, the mouth of this parasite grows along a meandering course through the viscera of the host, until it comes to rest in its liver. The histopathology of this infection has not been investigated, but the fact that it involves the excavation of long passages through the liver suggests that there must be extensive damage to the hepatic tissue.

Kazachenko and Kurochkin (1974) found that yet another pennellid, *Pennella hawaiiensis,* usually attached to the musculature of *Pentaceros richardsoni,* occasionally breaks into the visceral cavity of the fish and penetrates its liver. They did not study the pathology associated with such cases.

The cymothoid zoocoecidia, filling a portion of the visceral cavity, usually impinge on the liver, the largest of the viscera. Huizinga (1972) observed that the pouches formed by one such isopod, *Artystone trysibia,* pressed on the liver, which was compressed; while the visceral organs were displaced, they revealed no cellular responses. More detailed investigations are required, but it seems that the liver is likely to remain unresponsive to pressure and displacement, a degree of which it often experiences under normal conditions.

The kidneys

There are no known crustacean parasites that use the kidneys of marine fishes as target sites. However, the occasional attachment of some mesoparasitic pennellid copepods to the kidneys has been documented and appears to be not uncommon. Natarajan and Balakrishnan Nair (1973) found the kidneys of *Hemirhamphus xanthopterus* to be second only to the muscles in preference as the site of attachment of *Lernaeenicus hemirhamphi* (Fig. 1-174). Almost 44 % of 155 copepods examined were embedded in the kidneys (46.4 % in the musculature). They found the renal tubules around the site of attachment to be degenerate and the blood vessels dilated. The 'cellular

nature' of the neighbouring tissue was lost. They did not mention any noticeable haemorrhages. Infected renal tubules were also found to contain deposits of pigment, the nature and origin of which were not identified. Local extravasations were, on the other hand, seen by Monterosso (1923, 1925, 1926) in kidneys attacked by *Peroderma cylindricum,* yet another pennellid copepod, parasitic in clupeid fishes. Fig. 1-181 shows 2 dendrites of the holdfast of this parasite embedded in the kidney and surrounded by masses of erythrocytes. The renal tubules are compressed and compacted in isolated islets. The function of the holdfast is not fully understood. It is, however, suspected that it serves other purposes in addition to the obvious one of anchoring the parasite. Absorption and excretion have been considered, but there are many conflicting arguments to be put forward both for and against these functions. Neither is the impact of the damage inflicted by the copepod

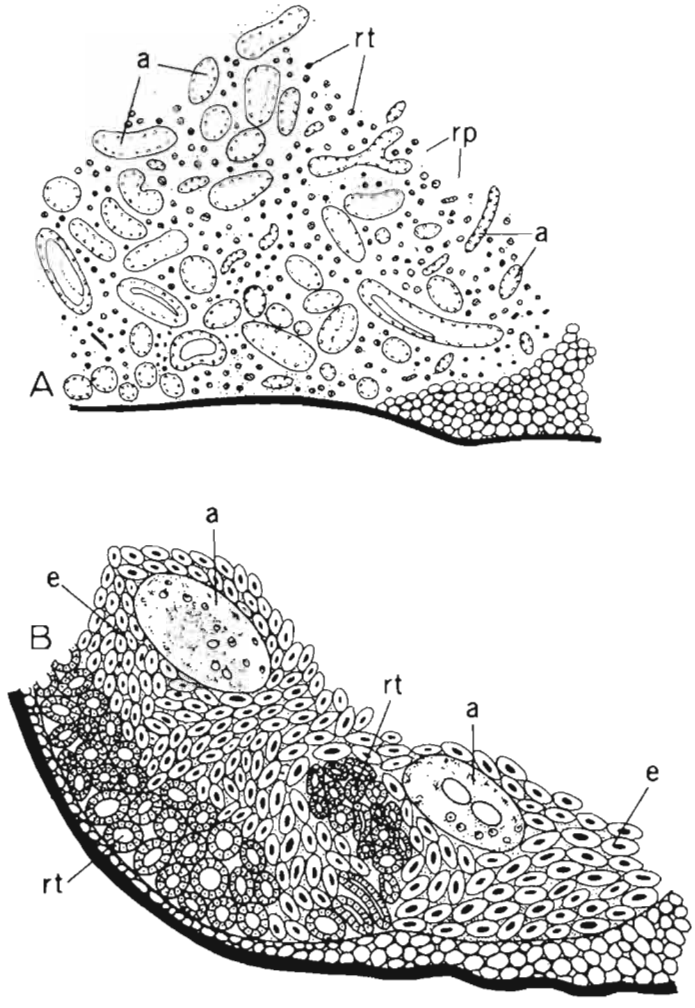

Fig. 1-181: (A) Section through part of kidney showing damage caused by *Peroderma cylindricum.* (B) Same, much enlarged. a: branches of copepod's holdfast; e: erythrocytes; rp: renal parenchyma; rt: renal tubules. (After Kabata, 1970.)

understood, even in approximate terms. The presence of some deleterious effects on the function of the kidneys is, however, assumed.

The gonads

Like the kidneys, the gonads are not known to be a specific site for any crustacean parasite. Incidental damage by mesoparasitic pennellids can be expected, but documentation is very scanty. Recent literature contains only a passing statement by Natarajan and Balakrishnan Nair (1975) of damage to the ovary of *Hemirhamphus xanthopterus* (Fig. 1-174) by *Lernaeenicus hemirhamphi*. Among the 155 copepods examined, one only was embedded in the ovary. As might have been expected, damage was inflicted on the *tunica albuginea*. The embedded cephalothorax was surrounded by a connective tissue capsule, its fibres extending outwards and surrounding also the developing oocytes in the vicinity of the copepod. No detailed histological study was made and we cannot even speculate on the effect of such injury on the functionality of the gonad.

The alimentary canal

Crustacean parasites very seldom choose the gut of the fish as specific target site. A copepod that comes most closely to being gut-specific is *Sarcotaces* (Philichthyidae). Although it has been found at other sites, it usually occurs in zoocoecidium-like pouches formed from the intestinal wall in the rectal region (Fig. 1-182). To accomodate the large,

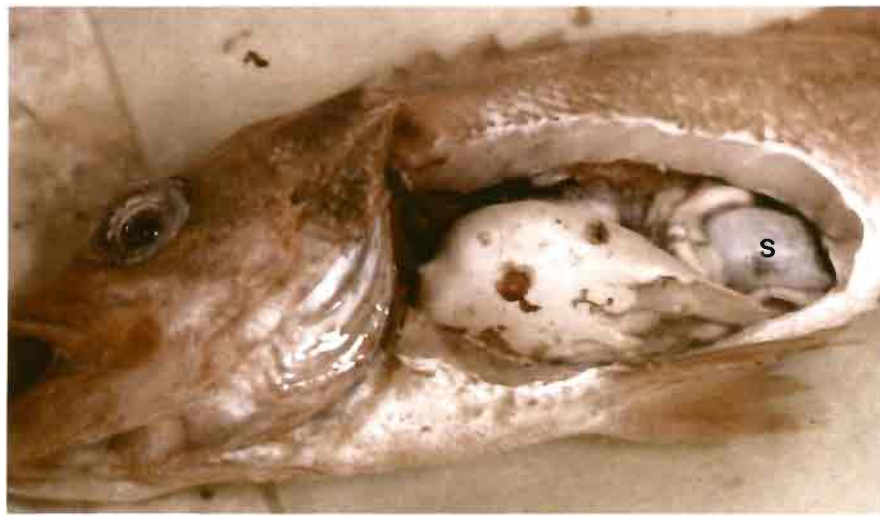

Fig. 1-182: *Sarcotaces* sp. in a capsule (S) formed from the intestinal wall of *Sebastes alutus*. (After Kabata, 1970.)

sac-like female and a diminutive male, the wall of the intestine must undergo drastic histological changes, never described in detail. The effect is multiplied when more then 1 couple of copepods become ensconced in the rectum of the same fish. The functional consequences of this type of pathogenic impact are unknown. The fact that living and apparently normally functioning fish can be found so infected, suggests that the impact is at least not immediately lethal.

Nigrelli and Firth (1939) recorded *Sphyrion lumpi* embedded in the rectum of *Sebastes* from the American Atlantic Ocean. Intestine and surrounding tissue responded by 'intensive inflammation', a non-specific reaction that might have been expected.

No details of damage to the alimentary canal of *Hemirhamphus xanthopterus* by *Lernaeenicus hemirhamphi* have been described by Natarajan and Balakrishnan Nair (1973), but they did record the attachment of that mesoparasite to various sections of the canal (pharynx, stomach) (Fig. 1-174). Five of the 155 specimens examined (3.2 %) were attached at these sites. It can be presumed that attachment tumours, described by these authors for other sites, were present also in the alimentary canal. The tumours were subspherical or reniform, somewhat less than 1 cm in diameter, white in colour but with black pigment deposited near the surface. They consisted of a dense connective tissue matrix interspersed with 'cells'.

Naturally, like other viscera, the alimentary canal is subject to pressure or displacement. The presence of large zoocoecidia or similar structures (Fig. 1-182) clearly disturbs the normal position of this vital organ. The effects, however, have never been assessed.

Other organs

Natarajan and Balakrishnan Nair (1973) reported that *Lernaeenicus hemirhamphi* becomes embedded in the swim bladder of its host. According to their observations, 8 of the 155 copepods (5.2 %) were attached to it. Not stating it explicitly, the report allows the conclusion that the parasite did not penetrate the lumen of the bladder, but formed attachment tumours, like those described above, in its wall. It can be presumed that the local histological changes resulting from this infection have no effect on the function of the bladder. The infection of the bladder, like that of other viscera, not mentioned in this account, can be considered as accidental outcome of the penetration by a mesoparasite that displays only limited specificity in its selection of the target site.

General Effects

Given the definition adopted for local effects on p. 354, one would have to define general effects of crustacean pathogens on fish as pathological changes not limited to the site of activity of the parasite. The changes are systemic in nature, and involve the entire body of the fish. They take place either because the parasite introduces into the body of the fish some diffuse-acting agent (e.g., a noxious chemical substance), or because its presence imposes an overload stress (see also Chapter 1.6), on a vital organ or system of organs. The general effects are unquestionably more threatening to the well-being, or even life, of the fish than the local effects that can be isolated and dealt with by a modest mobilisation of the defensive mechanisms.

While the general effects are easy to define, they are difficult to assess. Understood as deflections from the normal condition of the fish, they require, to be measured, our ability to fix the zero point on our scale of measurement, i.e., our ability to define with precision the normal condition. Such an ability exists only in a few cases, where natural populations of marine fishes are concerned. Intraspecific variations and scatter of data are usually of the dimension that makes it difficult to define the normal values for most parameters of conditions.

To evaluate the magnitude of the impact of a crustacean parasite, one must make sure

not only that it can be measured with reference to normal, but also that one does not measure any other factor at the same time. A noticeable effect, such as emaciation, is often attributed to the most obvious observable cause, e.g., the presence of a crustacean parasite. It is at least possible, however, that other parasites or pathogens might be simultaneously present and that their impacts add to the visible effect, confounding the observer. This fact, well known to the medical diagnostician, is only too often ignored by those interested in the health of fishes. It must not be forgotten, if we are to be able to weigh accurately the effect of any pathogenic factor (see also Volume I: Kinne, 1980a).

It must not be forgotten, either, that some parasites are secondarily superimposed on an otherwise debilitated fish. These so-called debility parasites exploit the condition of the fish, weakened for some other reason, to use it as a defenseless host. In other words, the question must always be asked: which came first, the parasite or the poor condition of the host?

Misinterpretation of the circumstances surrounding suspected changes in the health of the fish can arise, if one does not understand the biology of that fish in its broadest sense. This additional difficulty in assessing the general effect of many crustacean (and other) parasites can be illustrated by an example of what the reviewer has termed 'Janusz's dilemma'. Examining the possible effect of the ectoparasitic lernaeopodid copepod *Clavella adunca* on *Gadus morhua,* Janusz (1980) found that the weight of the infected fish differed from that of the uninfected. The differences, however, varied depending on the age of the fish. Infected fish age 4 were 32.9 % heavier than non-infected. Those age 5 were only 9.9 % heavier. By age 6, the situation was quite different; the infected fish were 5.9 % below the weight of uninfected. As the fish grew older, the apparent loss of weight fluctuated, but by age 9 it was 8.1 % in relation to the uninfected. Janusz was puzzled. He put forward 3 possible explanations of this phenomenon: (i) parasitisation has an initially stimulating effect, which wears off and is followed by gradually increasing debility; (ii) younger fish of poor conditions suffer higher mortality, only those in the best condition remaining in the infected part of the population and giving higher average weight values; (iii) younger fish of better condition join the adult stock earlier than their weaker cohorts and are exposed to parasitic infection for a longer period than less robust and smaller fish. Hence they have a higher prevalence of infection. The outcome, as in (ii), is to give relatively higher weight values to infected fish. Janusz resolved his dilemma by opting for the third scenario, having considered the ecology of the cod stocks in the area where he studied his problem. He gave us a clear example of the difficulties involved in understanding the effects of crustacean parasites on their fish hosts.

The most drastic effect of parasitisation by Crustacea is the death of the fish. Mortalities inflicted by crustacean, or any other, parasites of marine fishes are much more difficult to detect than those occurring in freshwater fish populations, especially those living in controlled habitats. Marine fish that succumb to the parasites are effectively removed from their populations and find themselves outside the field of observation. The records of lethal effects will be reviewed in a separate section, concluding this chapter. This section deals only with sublethal effects.

Summing up the requirements that must be satisfied if the general effects of crustacean parasites are to be fully assessed, Kabata (1970) listed the following salient points:

(i) The normal condition of the examined host population must be known. To establish this important baseline, one must take into account size:age relations of both

infected and uninfected fish; size:weight relations; condition of the internal organs, particularly the liver; blood picture and other haematological indices, etc.

(ii) The total deleterious effect on the fish must be examined, with the view of determining its increment directly attributable to the parasite in question.

(iii) The potential effects must be extrapolated from those observed. Stress induced by, for example, unfavourable environmental conditions might be unacceptable to infected fish, while being tolerated by uninfected ones.

(iv) The duration, or potential duration, of the parasite's impact must be known. Other things being equal, a long-lived parasite will be more harmful than a short-lived one.

(v) The impact of the parasite on an individual fish should be extrapolated to the population level.

To be fully evaluated, the effects should be, whenever possible, expressed in quantitative terms. Most frequently used for this purpose is the condition factor, in one of its varieties, or a similar expression of condition. There are no special formulae expressly in use for parasitised fish. Standard all-purpose practices are acceptable.

Effects on Weight and Chemical Composition

Loss of weight caused by parasitisation with a crustacean is probably the most common general effect recorded in literature. Many scattered references to this phenomenon can be found in papers otherwise not concerned with the condition of the host and thus not attracting the attention of fish health specialists. Most are only passing comments, without quantitative assessment. They are usually made when the loss of weight is striking enough to catch the eye. For example, Hewitt (1971) mentioned that *Caligus epidemicus,* infecting *Mugil cephalus, Aldrichetta forsteri, Liza argentea* and *Myxus elongatus* in the estuary of the Mitchell River (Victoria, Australia), had caused emaciation of some of its hosts. Kubota and Takakuwa (1963), who studied maricultured *Seriola quinqueradiata* in Japan, believed that an unidentified *Caligus* had caused emaciation of this fish, by irritating it to the point at which it lost its appetite. However, the authors also noted that this *Caligus* showed a tendency to choose fish with various abnormalities that predisposed them to a bad general condition. It is possible that 'Janusz's dilemma' could be invoked to offer an alternative explanation for the emaciation of the fish. Hotta (1962) noted that *C. macarovi,* when present in large numbers, depressed the condition factor of *Cololabis saira.* Observations of this type are not, nor are they intended to be, helpful in determining the impact of the parasite on the general state of the host. They are not interesting enough to be enumerated.

Caligus and its relatives (e.g., *Lepeophtheirus*) can be expected to cause generally debilitating effects only when in very large numbers, particularly when the fish is stressed. On the other hand, mesoparasitic pennellid copepods, attacking vital organs of their hosts, or otherwise causing extensive tissue damage, are more likely to generate such effects. Particularly well studied in this respect is the genus *Lernaeocera.* Mann (1953) investigated the effects of *L. branchialis* on several commercial species of Gadidae. His comparison of weights of the infected and uninfected fishes is shown below:

Fish species	% underweight	
	1 parasite	2 parasites
Merlangius merlangus (gutted)	0–20	19–42
Gadus morhua (gutted)	0–28	30
(ungutted)	0–31	0–35
Melanogrammus aeglefinus (gutted)	0–47	
(ungutted)	0–40	28–36

The data show pronounced weight loss. Kabata (1958) re-examined these results in respect to *Melanogrammus aeglefinus*. He found that the loss of weight, though present, seemed less severe than that found by Mann, the average being about 10 %. (Hislop and Shanks (1981) estimate only about 5 %.) More interesting was the fact that the onset of the infection appeared to promote a transitory gain in weight, accompanied by a rise in other observed indices (Fig. 1-183). Kabata interpreted this phenomenon as the result of

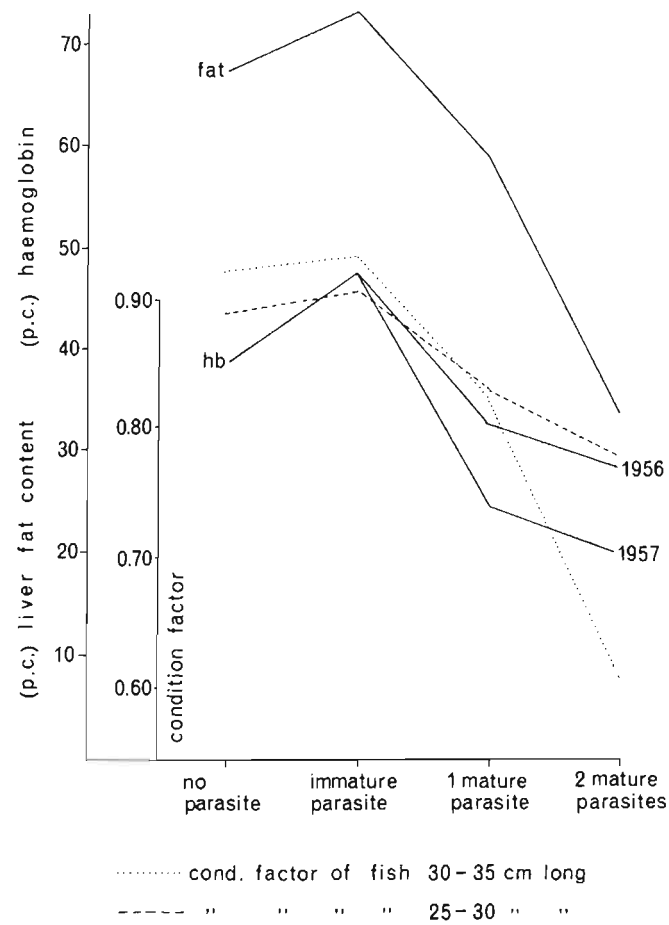

Fig. 1-183: Summary of effects of *Lernaeocera branchialis* on *Melanogrammus aeglefinus*. (After Kabata, 1970.)

overcompensation in the reaction to the secondary haemorrhage caused by the feeding of the parasite (p. 390). Sundnes (1970) evaluated very thoroughly the effects of this parasite on the Arcto-Atlantic population of *Gadus morhua* and confirmed the lowering of the condition factor of infected fish, the loss of weight becoming increasingly severe with the rise in the number of parasites on the fish. Sundnes also detected a transitory increase in weight with the onset of infection, but only in cod less than 40 cm long. Since these fish also tended to have fewer parasites per individual and since those they had were more often immature, Sundnes' findings open the way to a different interpretation of the effects than that offered by Kabata. Van den Broek (1978), who looked at the effects of the same copepod on *Merlangius merlangus,* found that its mature stages 'caused a significant weight reduction'. The term 'significant' was used in its statistical sense; the loss of weight appears to have been small. As Broek stated: 'the evidence that copepod parasites affect fish condition remains inconclusive. The crucial factor appears to be the period of association between the parasite and host; older infected whiting were emaciated and clearly lighter than uninfected contemporaries. This condition was not often seen in the first-year fish' (p. 714).

To this variety of views, all of which agree that *Lernaeocera branchialis* does cause some loss of weight, should be added also those that express the opposite opinion. Sproston and Hartley (1941) found that *L. branchialis* had no effect on the weight of *Merlangius merlangus* off the coast of southern England. Sherman and Wise (1961) found no effect on the weight of *Gadus morhua* in New England waters. It is clear that the reports mentioned above are not of equal value and cannot be compared. They spotlight the need for the adoption of standard examination practices, incorporating all the conditions mentioned (p. 382–383).

According to Mann (1964), another species of this genus, *Lernaeocera minuta,* caused loss of weight when attached to *Pomatoschistus minutus.* The loss, particularly in fish more than 7 cm long, varied from 0 to 21 %. It seemed to have been proportional to the duration of the period of parasitisation, as measured by the maturity of the copepod. Evans and co-authors (1983) in their study of *L. lusci,* came to the conclusion that it exerts no significant effect on the condition of 0-group *Trisopterus luscus.* The length:weight relations of infected and uninfected fish were 'remarkably similar'.

A related pennellid genus, *Lernaeenicus,* comprises species with widely differing sites of attachment. It is also likely, for that reason, that its members vary in the general effects they produce. Harding and Wheeler (1958) examined the effects of *L. sprattae,* attached mainly to the eye of its host, on the condition of *Clupea sprattus.* The results produced were rather paradoxical in suggesting that the infection had a slightly beneficial effect on the weight of the fish. Of the 42 infected fish, the shape factor used to calculate size:weight relations was below normal in 17 and above normal in 25. The duration of the exposure to the parasite by individual fish could not be estimated, neither were the effects of different attachment sites taken into consideration. Another species, *L. radiatus,* was studied by Voorhes and Schwartz (1979). This copepod, by becoming attached at different sites of the body of *Brevoortia tyrannus,* causes extensive local injuries, including severe tissue erosion and loss of scales. Some parasites burrowed from one flank to the other and protruded on the opposite side. In spite of these injuries, the general effects, expressed by the lowering of the condition factor (i.e., loss of weight) were slight. Uninfected fish had a condition factor 1.95, while those carrying 30 parasites had 1.65. Interestingly, fish infected with

smaller numbers of parasites had condition factors above the average for the uninfected. Those with 1 to 5 copepods had 2.01, those with 6 to 10 had 2.00 and those with 11 to 15 had 1.96. The apparent stimulus provided by the copepods, when present in numbers below critical, seems to have prompted some gain of weight.

Sardine infected with *Peroderma cylindricum* also appeared to lose weight. Dieuzeide and Roland (1956) weighed infected and uninfected fish, without taking into account the details of infection. The average weight loss was 3 %, but it was not uniform throughout the infected population. Only 52 % of infected fish showed it, whereas no effect was detected in 38 %. As many as 10 % of infected fish were heavier than the uninfected. It might be presumed that the last-named category represented fish stimulated by the early stages of infection. Crehuet and Val Cordon (1973) also observed some loss of weight in sardines infected with *P. cylindricum.*

Crustacea other than Copepoda have also been recorded as causing loss of weight in their hosts. Unidentified Isopoda infecting *Seriola quinqueradiata* in Japan were observed to lead to emaciation of that fish (Kubota and Takakuwa, 1963) and were judged to be, together with *Caligus,* the most injurious of the crustacean parasites of this commercially valuable fish. It is probable that most crustacean parasites are capable of producing similar results under comparable conditions.

Infected fish can have lower weight than non-infected cohorts, because it failed to grow normally. Effects on growth are discussed on p. 387. Alternatively, weight might be lost by a fish that originally was normal in this respect. This type of loss, akin to the effects of starvation, is accompanied by some general changes in the tissues of the affected fish. Several authors investigated these changes in relation to infection with the pennellid copepods. Mann (1960, 1964) examined differencial loss of weight by various parts of the body of *Merlangius merlangus* infected with *Lernaeocera branchialis.* As might have been expected, he found that the greatest loss took place in muscle and skin, but viscera were also affected, dropping from 17.1 % of body weight in uninfected fish to 15.0 % in those infected with more than 1 copepod. There were also obvious changes in fat and water content of muscle and other tissues. A serious loss of fat took place, the total content dropping from 1.08 % in healthy fish to 0.49 % in parasitised fish, a loss of about half of the body fat. At the same time, the water content rose slightly, from 79.4 to 81.8 %. Similar effects were produced by *Lernaeocera minuta* in *Pomatoschistus minutus.* Kabata (1958) examined the fat content of the liver of *Melanogrammus aeglefinus.* His findings were as follows:

Uninfected fish	67.4 %
Immature parasite	71.4 %
One mature parasite	57.8 %
More than one mature parasite	32.6 %

The serious drop in fat content of liver parasitised with mature copepods was preceded by a slight rise, parallelling that of the weight, during the early stages of infection. Effects of the same parasite on *M. merlangus* in British waters were investigated by Van den Broek (1978), with special reference to abnormalities induced in the chemistry of the host's liver. The relative weight of the liver was not significantly reduced, but the mean weight of lipids was 51.4 % less in fish infected with adult copepods. There were also differences in the individual components of the lipid content. For example, while there were no differences

in the phospholipids of the uninfected fish and those carrying immature parasites, in those infected with mature *Lernaeocera* they were reduced by about 30 %. No reductions were observed in the triglyceride content. On the other hand, the cholesterol level in fish with adult parasites rose to twice its normal level (though this fact was not definitively confirmed). No clear changes could be detected in the content of free fatty acids.

Other pennellids affect the tissue composition of their hosts. Natarajan and Balakrishnan Nair (1976) studied the effects of *Lernaeenicus hemirhamphi* on *Hemirhamphus xanthopterus* in India. In an extensive investigation, they examined effects on several components of various fish tissues. They concluded that no statistically significant changes occurred in water content and discovered that the fat content of the liver dropped drastically, by more than 50 % (more than 75 % in the ovary). From the muscles the fat practically disappeared, decreasing from 4.41 to 0.05 %. To show these effects, the fish had to be infected with 5 or more copepods. Fewer copepods produced correspondingly milder losses. The authors corroborated Kabata's (1958) findings that early stages of infection (i.e., presence of immature parasites) were characterised by an increase in fat content of all tissues. Some of the constituent protein was also lost by organs and tissues, as measured by nitrogen content; the loss was, however, small. A transitory increase during early infection was registered for all tissues. The carbohydrate content was disturbed. The liver lost up to $^1/_3$ of its carbohydrate. Losses in ovary and muscle tissue were also severe (44 and 35 % respectively). Natarajan and Balakrishnan speculated that, in addition to the depletion of the tissue reserves, drawn upon to withstand the stress of infection, the host's ability to absorb food could be reduced or completely suppressed. They could not rule out that the conversion efficiency of the host is affected as a result of infection.

Copepods are not the only Crustacea capable of producing effects of this kind. Infection with *Anelasma squalicola,* a parasitic barnacle, also caused reduction in fat content of its host's liver. Investigating this possibility, Hickling (1963) took into account other possible factors that might play a role in this change (host size, sex, sexual maturity). The host, *Etmopterus spinax,* shows clear differences in relation to its size. Smaller fish do not appear to be affected. Larger females lose about 30 % of their liver weight. Lack of impact on smaller (younger?) fish might be due to the shorter duration of their exposure to the parasite. (For elasmobranchs like *Etmopterus* the loss of liver fat might have an adverse affect on locomotory efficiency, since liver fat is also a factor in buoyancy.)

Romestand and Trilles (1979) did not find a significant impact from infection with the cymothoid isopods *Meinertia oestroides, M. parallela* and *Anilocra physodes,* on the weight of *Boops boops* and *Pagellus erythrinus.*

In summary, general effects expressed as loss of weight can be attributed to more than one cause. The most obvious one is the loss of food reserves drawn from various depots and other tissues to help in coping with the ravages of infection. The blockage of absorption and the breakdown of the conversion mechanisms is also possible, though not yet demonstrated. It can be assumed that wherever loss of weight occurs, tissue changes will also take place. It has been mentioned that the abnormally low weight of the infected fish might result from its failure to grow normally.

Effects on Growth

Kabata (1970) regretted the paucity of quantitative data on this subject. Most references turned out to be mere surmises. Few could offer quantitative evidence for or

against this effect. This situation has not altered significantly, especially for marine fish. One of the most serious obstacles to obtaining correct data on possible growth retardation induced by a crustacean parasite (or by any other cause) is the difficulty of determining the normal size-at-age parameters. The techniques of age determination are still cumbersome, time-consuming and requiring considerable expertise. They are seldom applied in parasitological investigations. Without knowing the age of the examined fish, it is impossible to know how well, or badly, it has grown.

An early attempt to determine the effect of *Lernaeocera branchialis* on the growth of *Merlangius merlangus* was made by Desbrosses (1948) in France. Using height:length and total length:head length ratios to compare infected and uninfected fish, he found general retardation of growth, manifest in the differences between proportions of the body of uninfected and infected fish. The head of the parasitised fish was relatively larger and the body shallower. In these features, the latter resembled the juveniles of its species. The signs were compatible with growth retardation. Desbrosses' findings were not corroborated by Mann (1953) or Kabata (1958), who discovered no differences in the proportions of *Melanogrammus aeglefinus* that could have been attributed to the impact of *Lernaeocera.* Mann concluded that (i) effects of the parasite on fish weight are greater than those on growth; (ii) fish infected later in life are less strongly affected. Kabata (1958) explained his failure to detect impact on growth by an analogy with the effects of human anaemia. The somatic growth is rarely affected.

More recent investigations also failed to provide strong evidence of the retardation of growth due to pennellid copepods. Rauck (1976) found no effect on growth of sprat *Clupea sprattus* infected with *Lernaeenicus sprattae* and/or *L. encrasicoli.* Moser and Taylor (1978), who studied the infection of *Stenobrachius leucopterus* with *Cardiodectes medusaeus,* discovered that the parasite promotes somatic growth of its host. Infected male and female fish were longer than their uninfected counterparts. On the other hand, Gjøsater's (1971) study of *Benthosoma glaciale,* a scopelid fish infected with *Sarcotretes scopeli,* suggested a growth-retarding impact. Infected fish were 2.8 % shorter than comparable uninfected ones. According to Crehuet and Val Cordon (1973), *Peroderma cylindricum* causes some growth retardation in *Sardina pilchardus.*

Students of parasitic Isopoda seem to be rather more definite in agreeing that these parasites retard the growth of fish. Sadzikowski and Wallace (1974) and Romestand and Trilles (1979) found that infected fish are shorter than uninfected ones of the same age. Thus, 1 yr old uninfected *Morone americana* were 126 to 135 mm long, while their infected cohorts measured only 85 mm, a difference of about 35 %. In 2 yr old fish this difference was about 22 %. The growth of *Boops boops* and *Pagellus erythrinus* was retarded by the influence of *Meinertia oestroides, M. parallela* and *Anilocra physodes.*

The above examples lead to the conclusion that our understanding of the impact of the crustacean parasites on the growth of their hosts is far from complete. What has become clear in the last decade is that the effects on growth depend on the type of host-parasite relation and the age of the fish at the time of infection. Mesoparasitic pennellid copepods, predominantly haematophagous, do not seem to have a pronounced retarding influence and might even act as a stimulus to somatic growth. Isopods, mainly cymothoids, appear to act as a retardant. This difference might stem from the fact that the former mobilise and bring into play defensive mechanisms based on the response of the haemopoietic organs of the fish to continuous haemorrhage, while the latter cause a general stress of crushing

proportions. Much more has to be known before the effects, and the mechanisms responsible for them, can be understood. When the apparent effects are assessed, the possible existence of 'Janusz's dilemma' (p. 382) must also be considered.

Metabolic Disturbances

We now turn to pathological changes in functions of the host that are not a direct result of the activities of the parasite. These indirect changes, often remote from the site of the direct impact, stem from 3 possible causes: The parasite might (i) introduce into the body of the fish a substance capable of rapid systemic diffusion and able to affect adversely the normal function of various organs that are not under the parasite's mechanical influence; (ii) interfere with the absorption by the fish of substances required for the normal performance of a function or functions; (iii) actively withdraw from the body of the fish substances sustaining various functions, or maintaining the energy balance.

The best example of the first category is provided by the branchiuran *Argulus*. Although no observations have been made on marine species of this genus, there is no reason to suppose that these species differ to any great extent from their better known freshwater congeners. Kabata (1970) summed up the effects of *Argulus* on the metabolism of their hosts. In essence, they are due to the injection of digestive fluids, produced by the glands of the preoral stylet (Fig. 1-129), into the tissues of the fish. The fluids are toxic and, depending on the size of the fish and the quantity of the toxin injected, capable of producing a wide range of effects, from changes in the function of the chromatophores to death. The poecilostome copepods, also practicing extrabuccal digestion, resemble *Argulus* in that they release their digestive fluids into the tissues. It appears, however, that their general effects are minimal, perhaps because of their inability to diffuse. Nothing is known about them. Neither is anything known about the nature of an anticoagulant believed to be released into the bloodstream of the fish by the mesoparasitic pennellids such as *Peroderma*. Its existence is being postulated, because there is no coagulation of the blood of the vessels, the walls of which have been breached by the copepod. The field is wide open for research.

The second type of mechanism — blockage of, or interference with, absorption — has not been demonstrated either experimentally or by observation. It should not be concluded, however, that it does not exist. Some indications of its existence in host-parasite systems involving helminths have been reported (e.g., Hiscox and Brocksen, 1973), though in still-tentative terms. Its existence in the system crustacean-fish should be investigated.

As regards the impact on the metabolism due to the withdrawul of substances, the attention of the researchers has been concentrated on the effects of the haematophagous mesoparasites. Mann (1953) found that *Lernaeocera branchialis* depressed the oxygen consumption of *Merlangius merlangus*. While uninfected fish consumed 0.17 mg O_2 (unit weight)$^{-1}$ h^{-1}, the corresponding value for infected whiting was 0.12 mg. Carrying out similar studies on the system *L. branchialis* — *Gadus morhua*, Sundnes (1970) subjected uninfected control fish to experimental, controlled haemorrhage. The artificially induced anaemia depressed consumption of oxygen and the level of energy metabolism dropped below standard. However, metabolic depression occurred only when haematocrit readings were less than 10. Sundnes failed to find readings below 20 in cod infected with *L. branchialis*. One might conclude that this parasite's impact on the metabolism was, at the most, slight. Moser and Taylor (1978) looked at the energetics of the infection of

Stenobrachius leucopsaurus with *Cardiodectes medusaeus*, by measuring the total caloric contents of infected and uninfected fish, as well as of the parasite itself. They concluded that the copepod's 'energy demands on the host appear negligible, at least in terms of its own somatic growth. There was no significant difference in caloric content between uninfected fish and infected fish minus copepods' (p. 2357). An interesting addendum to these data, though not directly relevant to marine fishes, is the report of Srinivasachar and Shakuntala (1975) on the increase in the oxygen consumption of *Lebistes reticulatus* infected with *Lernaea hesarangattensis*.

The overall picture is anything but clear and apparently contradictory. Parasites associated with their hosts in a similar relation seem to provoke diametrically opposite reactions or cause no reaction at all. Resolving this contradiction requires well-planned and carefully conducted studies (Kabata, 1981).

Effects on Blood

Thus far, the impact of crustacean parasites on the blood picture of marine fishes has been studied only for the parasitic copepod *Lernaeocera* (Pennellidae). The difficulties of this kind of study are obvious, but the lack of progress is regrettable. Kabata (1970) reviewed investigations by Mann (1953) and Kabata (1958). Mann found that, while the haemoglobin content of uninfected whiting *Merlangius merlangus* was 30 to 40 %, infected fish had only 20 to 22 % haemoglobin. The erythrocyte count was also reduced, from 902,500 ml^{-1} to 847,500 ml^{-1}. No account was taken of the size of the fish or the severity of infection. Kabata studied the haemoglobin content of haddock *Melanogrammus aeglefinus* in 2 consecutive years (Fig. 1-183). The level of haemoglobin of uninfected fish (38.3 and 38.4 %) suffered a substantial drop under the influence of multiple infection by mature *L. branchialis* (27.5 and 19.2 %). The onset of the infection was attended, however, by a marked increase in this value (46.7 and 47.0 %). Kabata concluded that this rise, due to overcompensation by the haemopoietic organs, is the trigger, prompting corresponding increases in other parameters (Fig. 1-183). Mann (1964) found reductions in the haemoglobin content of *Pomatoschistus minutus* under the influence of *L. minuta* (from 24 to 27 to 17 to 21 %).

Both Mann (1953) and Kabata (1958) implicitly accepted the suggestions of earlier authors, who saw *Lernaeocera* as a haematophagous parasite, taking in whole blood *per os*. Kabata (1970) attempted to make an estimate of the quantity of blood taken at a single meal by a population of 10 parasites on a single fish. The work of Sundnes (1970) has shown, however, that his predecessors had looked at the feeding process of *Lernaeocera* in an oversimplified manner. There appears to be no direct connection between the bloodstream of the fish and the alimentary canal of the copepod. Some erythrocytes occur occasionally in its gut, but they are apparently withdrawn from the granulation tissue surrounding the cephalothorax. Spectroscopic analyses have demonstrated that the copepod takes in only blood serum, i.e., some elaborate mechanism of extravasation-filtration-ingestion regulates its food intake. The lowering of the haemoglobin content and the drop in the erythrocyte count cannot, therefore, be attributed to simple loss of whole blood by the fish. A less direct mechanism must be responsible.

Although the effect on the blood of the host is less direct than has been supposed, it does, nonetheless, exist. Sundnes (1970) took haematocrit readings of infected and

uninfected fish of 2 size groups. In the 20 to 29 cm group the uninfected fish had a reading of 32.1. It went up to 34.0 in those carrying 1 immature parasite. A single adult copepod caused it to drop to 28.9, more than 1 depressed it to 26.8. In the 30 to 39 cm group the value dropped from 33.7 to 27.9, but there was no increase in the fish with immature parasites. In the over 40 cm group, Sundnes made no distinction between intensities of infection. Three populations of uninfected cod had haematocrit readings 32.8, 31.3 and 33.9. The corresponding values for infected fish were 36.3, 32.5 and 31.7. Thus, 2 out of the 3 populations showed higher haematocrit readings for infected than for uninfected fish. Similar inconsistencies occurred in the erythrocyte counts (in millions: uninfected 1.63, 1.57 and 1.60; infected 1.68, 1.50 and 1.59). Only 2 populations were checked for white blood cell count (in thousands: in 1 of them it dropped from 160 to 127, while in the other it rose from 136 to 139).

Khan and Pitt (1974), who described severe gill damage caused by *Tanypleurus alcicornis* (Copepoda: Siphonostomatoida) to *Lycodes lavalaei* (p. 359), also took note of the effect of this infection on the blood of the fish.

> "An abnormal blood composition was observed in the fish. The haematocrit readings varied from 5 to 11 (normal readings 18 to 22) and the haemoglobin content was reduced to 0.6–0.8 g (normal values 3.0–8.0 g/100 ml). Examination of cardiac blood smears revealed erythrocytic anisocytosis. There was an increase in erythrocytic precursors, the nuclei of which were often segmented' . . . 'Normal erythrocytes and erythrocytic precursors occurred in the ratio 16:1000 in one of the animals. Hyperplasia and hypertrophy of erythroid elements were evident in stained impression smears of the kidney" (p. 471).

The above information, particularly that provided by Sundnes — while making us aware of the complexities of host-parasite relations regarding the flow of nutrients — revealed that we really know very little of the impact of crustacean parasites on the blood of marine fishes.

Effects on Reproduction

The retardant influence of parasites on the reproductive capacity of their hosts is well known. 'Parasitic castration' has been documented in many and varied host-parasite systems reviewed by Kuris (1974). The reproductive function of the host can be depressed or even suppressed, depending on the severity of the castrating effect. Kabata (1970) lists 3 causes for this effect: The parasite may (i) attack gonads directly and cause mechanical destruction; (ii) produce toxins acting selectively on the gonads; (iii) cause general debility of the host, thus indirectly also depressing gonadal development and functions. Cause (i) is the least important in fish — crustacean systems, only occasionally occurring in infections with pennellid mesoparasitic copepods. Some free-living crustaceans are also implicated in the mechanical destruction of the gonads (p. 396). There is no evidence of chemical retardants of reproduction, though they should not be discounted out of hand. Cause (iii) is probably the most common one. Its effects are well known in many and varied situations, some of them far removed from fish — crustacean systems.

Studying gonad development in *Melanogrammus aeglefinus* infected with *Lernaeocera branchialis* and comparing it with uninfected fish, Kabata (1958) recorded lower weights of both male and female gonads of infected haddock. These preliminary observations were re-examined by Hislop and Shanks (1981), who took a much closer view of the

suspected depressant effect of the copepod. Having examined 119 females, a sample which proved to be 33.6 % infected, they compared the fecundity of its infected and uninfected components. Infected fish produced, on average, 21 % fewer eggs than their uninfected cohorts. Hislop and Shanks suggested that the effect is at least partly due to the lower weight-at-size of infected fish. When, however, fecundity per unit weight of the 2 groups was compared, that of infected fish was 17 % lower. The conclusion was that the parasite not only reduces the growth of the fish but also its relative fecundity. The implications for stock assessments of *M. aeglefinus,* based on calculations involving fecundity, are obvious. It should be noted, however, that Hislop and Shanks made no distinction between different levels of infection. Neither did they examine the effects on male gonads, reported by Kabata (1958).

Studying *L. branchialis* parasitic on Canadian Atlantic cod *Gadus morhua,* Templeman et al. (1976) also concluded that the copepod 'interferes with the onset of sexual maturity, and causes the fish to be immature at a larger size and presumably at a greater age' (p. 729).

The impact of another pennellid copepod, *Peroderma cylindricum,* on the ovaries of the European sardine has been studied by several authors. A quarter of a century ago, studies along the Spanish and French coasts (Bardan and Navarro, 1952; Dieuzeide and Roland, 1956) showed that 3 to 8 % of this commercially valuable fish were infected with the copepod. Of those infected, 22 % showed no effect on the gonads; 55 % were sexually retarded. Complete castration occurred in 23 % of infected fish. Fish were classed as castrated when their sex was no longer recognisable. Effects of multiple infections were not different from single-copepod ones, as might be expected from Kuris' (1973) review which clearly outlines the absence of additive effects of multiple infection. However, Dieuzeide and Roland (1956) found a relation between the severity of the castrating effect and the position of the copepod on the fish. Complete castration was mainly associated with the attachment of *P. cylindricum* to the anterior part of the fish. Kabata (1970) pointed out that the majority of *Peroderma* (85 %) are so attached and, therefore, might be expected to produce most of the cases of castration. Crehuet and Val Cordon (1973) took another look at the impact of *P. cylindricum* on the sardine. Their investigations were set against the background of new information. It appears that the sexual development of the sardine is closely reflected in its fat content in an inverse fashion. The development of the gonads is accompanied by a drop in fat, while high fat levels occur during the reproductively inactive periods. According to Crehuet and Val Cordon, in at least some infected sardines, fat content was high and the gonads quiescent. Generally speaking, however, they found no signs of castration. The only demonstrable effect of *P. cylindricum* was a lower rate of sexual maturation and a shorter spawning season. Interestingly, Crehuet and Val Cordon found that the sex ratio of infected and uninfected sardine was different. While in the uninfected fish 44 out of a 100 were males, among the parasitised fish this value was 58.5 in 100. They offered no explanations for this difference.

Examining infection of the scopelid fish *Benthosoma glaciale* with *Sarcotretes scopeli* (Pennellidae), Gjøsater (1971) found that, although the external secondary sexual characters of infected fish were normally developed, their gonads failed to mature. Similar effects were reported by Moser and Taylor (1978), who studied the effects of *Cardiodectes medusaeus* on *Stenobrachius leucopsaurus.* Regardless of their size, infected females produced oocytes only, while 2 % of the uninfected ones began to develop mature ova on

reaching the length of 3.5 cm. The authors also noted the absence of the additive effects of multiple infections, or of simultaneous infections with larval nematodes.

This effect is not restricted to Pennellidae, or indeed to Copepoda: according to Hickling (1963) the cirriped *Anelasma squalicola,* embedded in the dorsal musculature of its elasmobranch host *Etmopterus spinax,* causes statistically significant suppression of gonad maturation in both sexes. Of the 42 mature infected females, only 1 had active ovaries (2.4 %, compared with 58 % of uninfected). For mature males the corresponding values were 14.3 and 99 %. It should be pointed out that in this case the parasite's site and locus of activity are definitely divorced spatially from the gonads. Mechanical effects must be ruled out.

Abnormal Behavior

It is often difficult to determine the cause of behavioural abnormalities. To impute them to the influence of a specific parasite, one must resort usually to indirect evidence. However, observations have provided strong indications that in at least some instances the behaviour of the infected fish differs from that of its uninfected cohorts, and that the difference is to the disadvantage of the infected individual.

In broad terms, there are 3 ways in which a crustacean parasite can affect the behaviour of its host: it can (i) cause local irritation strong enough to elicit behavioural response aimed at the removal of the irritating agent; (ii) debilitate the host, inducing general malaise, lassitude and lack of responsiveness; (iii) cause damage to organs responsible for motor coordination, thus disrupting normal behaviour patterns. Any two can operate simultaneously, so that distinctions between them are not always clear.

The most common way in which a fish carrying an ectoparasite acts to rid itself of irritation is to perform abnormally fast, darting movements. Freshwater fishes rub themselves against the bottom or submerged objects, as if attempting to scrape the parasite off their surfaces. This recourse is not available to most marine fishes. However, some literature records mention short bursts of high speed and erratic movements, attributable to the irritating presence of a crustacean parasite. For example, Hotta (1962) observed *Cololabis saira* parasitised by *Caligus macarovi* breaking the surface and jumping clear out of the water, an action indicating both unusual speed and manner of swimming. Rosenthal (1967) watched larval herring, over 20 mm long, with *Caligus rapax* (= *C. elongatus*) attached to its lower jaw. Although no fatal effects were noted, the number of unsuccessful prey capturing attempts increased, compared to that in normal larvae and some larvae tried to get rid of the ectoparasite by bursts of swimming. Reactions of this kind are probably much more prevalent than records suggest.

Cursory observations on the possible effects of crustacean parasites on the behaviour of fishes are exemplified by Kroger and Guthrie (1972), who thought that the isopod *Olencira praegustator* might affect the behaviour of *Brevoortia tyrannus.* Such records are, however, of little help in the evaluation of behavioural effects. Other reports, though more specific, do not allow one to determine the cause of the behavioural abnormalities. For example, Lindsey and Moran (1976), writing about *Morone americana* infected with the isopod *Lironeca ovalis,* mentioned only 1 instance of visible effects. They were able to capture by hand a 10 cm long specimen 'swimming in circles on the surface of a tidal creek'. Locomotory disorientation of that fish might have been due to damage of the appropriate organs, but the fish was moribund and its erratic behaviour might have been only an expression of terminal disorientation.

Guthrie and Kroger (1974) described an interesting case of *Brevoortia tyrannus* infected with *Olencira praegustator,* involving behavioural abnormalities. They observed that 4 daylight catches by surface trawl in clear water netted only 11 fish, all harbouring the isopod. In contrast, 4 night catches in the same area took in 4,306 fish, only 0.5 % of them infected. The authors assumed that the uninfected fish were able to avoid the net during daylight, while the infected fish were incapable of doing so. During the night the fish could not see the net and none avoided it. Here again, one might wonder what was the reason for this lack of reaction of the parasitised fish. They might have been in a condition of lassitude, or might have suffered structural or functional damage preventing the defensive reaction. There is, however, another possibility. The infected fish might have been available to the net during the day, because they did not migrate with the healthy fish away from the area of fishing in the normal diel cycle of behaviour.

That some crustacean parasites can prevent the fish from carrying out normal migrations is evident from observations of Sproston and Hartley (1941) on *Merlangius merlangus* and *Pollachius pollachius* infected with the copepod *Lernaeocera branchialis* off the south coast of England. These fish become infected with the copepod during early, estuarine life and, when that stage is completed, fail to move offshore with the uninfected fish. It has been speculated that metabolic disturbances caused by the parasite necessitate a higher than normal intake of fluid by the fish, impossible under the conditions of higher salinity prevailing outside the estuary.

It can be concluded that, while the records at hand give ample grounds for believing that the crustacean parasites sometimes have an impact on the behaviour of their fish hosts, most of the evidence available has not risen above the anecdotal level. Systematic, well-designed studies are needed to provide an insight into this impact and its mechanism.

Secondary Infections

The possibility of secondary, potentially dangerous infections, facilitated by the crustacean parasites, has long loomed in the background of many general considerations of the effects these parasites have on their fish hosts. The authors concerned with fish health saw parasitic Crustacea as being capable of introducing or making possible such infections. In the former eventuality, they could act as vectors of microbial pathogens, transmitting them from host to host; in the latter, they could simply prepare the ground for such infections by depressing the efficiency of the defensive mechanisms of the fish, or by opening to the microorganisms paths of entry normally inaccessible.

These theoretical possibilities have not been borne out in practice, at least with regard to marine fishes. Nigrelli (1950) considered the role of *Ergasilus* as a putative vector for lymphocystis, a viral disease, using, as a supporting argument, the correlation between the distribution of both *Ergasilus* and lymphocystis. The correlation, however, does not necessarily imply a cause-and-effect relation. Nothing has been added to the records since Kabata's (1970) review.

Lowering of immunity of the fish by crustacean parasites has not been demonstrated. As to the infection by microorganisms of superficial lesions inflicted by Crustacea, very few observations have been recorded. The prevailing conditions under which examinations can be conducted usually render it impossible to make a distinction between pathogens and mere contaminants. Not enough is known about marine fishes in this respect to make a firm judgement.

Kubota and Takakuwa (1963) reported secondary infections of lesions caused by crustaceans *(Caligus)* with *Vibrio* sp. and *Pseudomonas* sp. in caged marine fish. A possibility of secondary bacterial infections, following the primary infection of the Atlantic salmon with *Lepeophtheirus salmonis* in sea pens, has been mentioned by Hastein and Bergsjo (1976). The evidence is not very strong. There is also no evidence of fungal diseases being facilitated by crustacean infections in marine habitats, although in fresh water they often follow superficial lesions of various origins. This is not to say, however, that the real state of affairs is accurately reflected by the literature on this topic. It is more likely that the paucity of records is due more to the difficulties of obtaining this type of information on marine fishes than to the innocence of Crustacea as causes of secondary infections.

Free-swimming Crustacea Harmful to Fishes

Marine fishes very often share their habitats with Crustacea. The pervading presence of crustaceans in most marine habitats means that fish encounter crustaceans throughout their life cycles. The encounters have developed into varied and complicated patterns in which the fish can become predator, prey or microenvironment, depending on the relative sizes and degree of development of the antagonists. Here we discuss 2 aspects of these varying relations. The impact of Crustacea on fish larvae will be touched upon briefly, followed by a discussion of the damage caused by Crustacea to adult fishes, in the type of interaction which Kabata (1970) referred to as 'micropredation'.

There can be no doubt that the survival of fish larvae, affected by many biotic and abiotic factors, is also influenced to some extent by predators. Among the latter, a significant role is played by planktonic copepods. While predation is outside the scope of this review, we include interactions in which the relative sizes of the species involved preclude the possibility of ingestion of the larvae. Attacks by crustaceans smaller than the attacked larvae lead to injuries not immediately fatal, although they might cause the injured fish to succumb later, or might impair their chances of survival. For the sake of convenience one could include here also the attacks by the free-swimming stages of parasitic copepods, in their initial search for, and settlement on, a suitable host.

Rosenthal (1967) observed herring larvae, 20 to 25 mm long, being attacked by copepods *(Lernaeocera)* in an aquarium. Since *Lernaeocera* never occur on adult herring and since their copepodids are difficult to distinguish from those of *Lernaeenicus,* a common parasite of herring, it is likely that the identification was inaccurate. All copepodids were able to locate a larva within 10 min of being released into the aquarium. Immediately on attachment they began feeding, with the effects on the herring being at once noticeable. The larvae became sluggish, their movements uncoordinated. Soon they stopped feeding and died a few hours later. Thereupon the copepodid detached itself from the corpse and swam off, searching for another host. Nishimura's (1960) observations in Japan established that poecilostomatous copepods caused injuries to larvae and pelagic fishes in the sea. Fishes such as sardine, anchovy and pearlside were among the victims of the attacks. It was impossible to determine whether the copepodids fed actively on the larvae, but there were strong indications that they did. It is very likely that the chances of survival of the larvae attacked were diminished. Scott (1904) recorded the only known instance of fish larvae being apparently attacked by an ostracod crustacean. Two *Podon leuckartii* were found attached to an early postlarval stage of a pleuronectid fish, probably

Pleuronectes platessa, in Scottish waters. One ostracod gripped the larva on the dorsal side, the other on the ventral directly opposite the first. There were no signs of tissue damage, but the movements of the larva must have been seriously hampered by this burden.

The fish does not become immune to attacks by Crustacea by surviving its larval stage. Juvenile *Oncorhynchus gorbuscha* and *O. kisutch* were attacked in an aquarium by the isopod *Rocinela belliceps pugetensis.* The fish swam erratically about, drifted listlessly to the bottom and died a few minutes later. The isopod attacked each young salmon laterally, just above or slightly posterior to its pelvic fin producing small but deep wounds (Novotny and Mahnken, 1971). Contrary to expectations, similar attacks were observed on at least 3 occasions in open waters. The fish attacked was *Oncorhynchus keta* and the isopod fastened on it in a vertical position, just behind the dorsal fin, its mouth close to the lateral line. The attacked fish was unable to maintain its position in the school, but swam in short bursts in various directions, sometimes abandoning the school altogether. Its chances of survival were judged to be poor. One *O. kisutch,* weighing about 200 g, with the isopod attached, showed signs of distress. Because of the size of the fish, however, the observers thought that the wound produced was not fatal.

Nevertheless, its size, however large, cannot save the fish from a concentrated attack of micropredators. These crustaceans, usually Isopoda or Amphipoda, sometimes quite big, attack fish even of the largest sizes. They seem to be attracted to fish whose poor condition becomes manifest in their behaviour. Fish struggling on the hooks of long lines are among their objects of attention. Kabata (1970) referred to complete stripping of the fish of all its tissues save the skin. Fishermen have given the name of 'Lousy Bank' to a locality off the west coast of Great Britain, where their lines bring up masses of crustacean micropredators, filling the skin of fish. Similar instances in the Mediterranean were recorded by Heldt (1952). Flatfishes and small sharks were completely devoured in the course of a night, their skins untouched, by the micropredators, amphipods belonging to 4 families (Lysianassidae, Calliopidae, Atylidae, Amphithoidae). Templeman (1967), working in Baffin Bay, off the Atlantic coast of Canada, found a large female *Raja hyperborea,* 76 cm long, with 'almost all the pelvic fins eaten away ventrally'. Two eelpouts *(Lycodes),* about 30 cm long, were eaten completely, only their jaw bones being left on the hooks. The micropredators, 4 of which were still attached to the large *Raja,* were identified as *Eurythenes gryllus* (Amphipoda).

Some micropredators show more discrimination in selecting the site of the attack. Entering the ovary via the oviduct, they feed on its contents, often without breaking the ovarian wall. Some ovaries become almost completely filled with the crustaceans. Raitt (1929) reported amphipods, *Tmetonyx cicada* and *Lepidepecreum carinatum,* and the isopod *Cirolana borealis* involved in such activities. Kabata (1970) observed similar incidents. They are probably rather rare, but are common enough to be reported in the literature from time to time. Halvorsen (1966) saw several of them, all within 2 months, involving *Cirolana borealis* in the roe of cod in Norway. Berland (1983) reported, for the first time, an attack of crustaceans on the reproductive organs of an elasmobranch. Two *C. borealis* were found in the body cavity of a female dogfish *Squalus acanthias* in Bergen, Norway. The isopods made their way into the uterus, where they damaged the yolk sacs of the 3 embryos present. The incident was atypical in that the micropredators then ruptured the uterine wall and fell into the body cavity.

Very similar to micropredation are the attacks on fishes by the *pranizae* of gnathiid isopods. Although these isopods are regarded as parasitic (p. 345), their association with the host comes very close to being predatory. Paperna and Por (1977) described the attack by *Gnathia piscivora* on the elasmobranchs *Dasyatis uarnak* and *Isurus oxyrhynchus*. The *pranizae* of *G. piscivora* attack the fish in large numbers in marine cages and impoundments, at night and in water usually less than 2 m deep. They become attached to skin, gills and walls of the pharyngeal cavity. Those attached to the skin become engorged within 2 to 4 h and abandon the fish; those on the gills tend to stay longer (1 or 2 days). The *pranizae* feed on blood (and presumably tissue fluids). They can kill the fish or, if the attack is less severe, leave them 'anaemic and stressed' to succumb within the next day. Rarely are there any survivors among the victims of the attack.

It is very likely that micropredation by Crustacea on fishes is much more common than existing records suggest.

Economic Impact

A substantial part of investigations into the effects of crustacean parasites on fish has been motivated by economic considerations. It has been deemed important to determine the extent of the impact of these parasites on fisheries. Even if one proved unable to prevent this impact, rational management of fisheries would obviously benefit from the knowledge of its importance.

Economic losses are incurred by: (i) loss of weight by the fish, which reduces the volume of the catch without reducing the number of fish taken; (ii) loss of condition and quality of the catch, reducing customer acceptance; (iii) reduction in the volume of the catch caused by mortality among the infected fish.

It is very difficult to assess quantitatively how much fish flesh is being denied to the fisherman who catches a certain number of fish, because those fish are of subnormal weight. An oft-quoted attempt to gain a vague idea of this loss was Kabata's (1955) calculation of losses inflicted by *Lernaeocera branchialis* on Scottish fishery for the haddock *Melanogrammus aeglefinus* in 1955. He estimated that if each infected fish had lost no more than 1 ounce (28.3 g), then the weight of the haddock landed would have been almost 5,000 tons less than might have been expected from the number of fish caught. In reality, the loss of weight was probably greater (p. 384). Fishery management economists can apply this example to other cases in which the parasite has been demonstrated to cause loss of weight. Wrzesiński (1982) made a similar calculation for the population of the Peruvian mackerel *Scomber japonicus peruanus* affected by the cymothoid isopod *Meinertia gaudichaudii*. The prevalence of this parasite, living in the buccal cavity of its host, varied from 1.0 to 32 % (mean 9.4 %) along the coast of Peru. The loss of weight attributed to the influence of this parasite averaged 15.38 % $fish^{-1}$. Accepting hydroacoustic estimates of the total biomass of mackerel as 1,185,000 tons, Wrzesiński calculated the loss of biomass to be 154 kg ton^{-1}, i.e., a total of 285,000 tons. Although both the above examples present rather simplistic pictures, they provide a good indication of real and serious economic losses inflicted by crustacean parasites, even when these parasites do not cause mortality.

Good examples of the effect the crustacean parasites can have on the quality of fish products were given by Kurochkin (1969). In 1968, the Soviet Pacific fleet made its first

landings of *Pseudopentaceros richardsoni,* substantial stocks of which were first discovered by a fishery scouting vessel on several banks in the north-western part of the Hawaiian Ridge. Good commercial quantities of the fish and its high comestible qualities made *P. richardsoni* an eminently suitable object of exploitation. Substantial catches were taken. It was later discovered, however, that 40 to 80 % of the fish were infected with a copepod, then identified as *Pennella* sp. Kazachenko and Kurochkin (1974), described it subsequently as *Pennella hawaiiensis.* The heavily sclerotised remnants of dead copepods were embedded in the flesh of the fish and were not readily detectable externally. The difficulties of processing such a heavily infected catch were such that some 36,000 tons of it stood in danger of outright condemnation. In addition to the loss of catch, Kurochkin listed other costs, such as extended cold storage, very expensive ships' time, a large research program, both biological and technological, required to clarify the feasibility of utilising *P. richardsoni.* The economic impact was real and significant. A similar, though less severe, impact was exerted by *Pennella* on the sailfish fishery conducted by the Soviet fleet in the eastern Pacific Ocean (Kurochkin, 1969), and by its relative, *Allotrifur,* on the Pacific hake *(Merluccius)* fishery (Nechaeva, 1970). The commercial value of the saury *Cololabis saira* for the Japanese fishing industry was depressed by the injuries inflicted on the fish by *Caligus macarovi* (p. 363) (Hotta, 1962).

Reduction in the abundance of stocks has an obviously adverse economic impact. It is difficult to determine to what extent fish mortality can be attributed to crustacean parasites. In some rare instances they have been blamed for mass kills. White (1940) reported such kill of Atlantic salmon, apparently caused by the caligid copepod *Lepeophtheirus salmonis.* Less spectacular effects are difficult to detect, let alone to evaluate quantitatively. These difficulties do not apply to cultured fishes where the impact and its extent are measurable. It is sufficient to quote mortality inflicted on brackish water milkfish *Chanos chanos* in the Philippines by *Caligus patulus* (cf. Lavina, 1977), and a fatal epizootic among *Mugil cephalus* in Israel, provoked by *Pseudocaligus apodus* (cf. Paperna and Lahav, 1974). In the latter case, a stock of 30 fish, averaging 1 kg in weight, was wiped out within a few weeks.

Some tentative assessments of mortality in wild fish populations, attributable to parasitic Crustacea, have been attempted. Thus, Sadzikowski and Wallace (1974) reported an estimate of survival of white perch *Morone americana* parasitised by the isopod *Lironeca ovalis,* and free of this parasite. Between ages 3 and 7, unparasitised males showed 46 % survival, females 42 %. Only 21 % of parasitised fish were older than 3 yr. Sadzikowski and Wallace speculated that these values might indicate parasite-induced mortality, although alternative explanations can also be entertained. Somewhat better based were the calculations of Sundnes (1970), who estimated that in one of the populations of cod he studied (Borgenfiord, Norway) the mortality rate of fish infected with *Lernaeocera branchialis* was 6 % higher than that of uninfected fish. Similarly, Raibaut and Hedi Ktari (1971) calculated that about 10 % of the total mortality of *Pagellus erythrinus* in the Gulf of Tunis is caused by the pennellid copepod *Lernaeolophus sultanus.* Kabata and Forrester (1974) found 10.7 % of young *Atheresthes stomias,* sampled off the coast of British Columbia, to have both eyes infected with *Phrixocephalus cincinnatus,* another pennellid copepod. This parasite causes blindness, and complete loss of sight leads to death of the fish. If the value for the infection of the young is accepted as applicable to the population of *A. stomias* along the entire coast of British Columbia, then

the parasite would be responsible for 10.7 % mortality. In 1982, when 526 tons of this fish were caught, this value would have meant the loss of about 56 tons of fish. Of course, a crustacean parasite can achieve reduction in the host population without inflicting mortality. Depression of fecundity of the fish, discussed earlier can lead to the same results.

This section, like several preceding ones, can best end with the statement that much more has still to be discovered before the full extent of the ecologic and economic impact on fisheries by Crustacea can be appreciated.

DISEASES CAUSED BY NEOPLASIA

N. PETERS

The term 'Neoplasia' is derived from the Greek and conveys the general meaning 'new formation'. In pathology, Neoplasia refers to new formation of tissue beyond normal requirements, involving at least a partial evasion of the organism's growth-regulatory mechanisms. Neoplasms or tumors (from the Latin, meaning 'swellings') are tissue enlargements resulting from excessive cell proliferation. Other kinds of tissue swelling due to accumulation of fluids, cell enlargement, or temporary aggregation of cells during infectious or regenerative processes are distinguished as edema, hypertrophy, hyperregeneration, or pseudotumors. As a rule, tumor formation is associated with increased mitotic activity and, because mitotic activity and cell differentiation are negatively correlated, with a more or less distinct dedifferentiation of the tissue involved.

Benign tumors grow relatively slowly. Their cells are only slightly dedifferentiated and, therefore, quite similar to those of the tissue from which they arise. Benign tumors are usually richly infiltrated by blood vessels and nerves, which form an essential part of their connective tissue stroma. Even in cases of excessive enlargement, such tumors remain confined to the locations (organs) of their origin. They are frequently encapsuled by connective tissue.

Malignant tumors, commonly referred to as cancer, usually grow rapidly. Their cells appear hardly, if at all, differentiated and are reminiscent of embryonic cells. Thus, their histological origin can no longer be recognized. They also grow invasively, infiltrating adjacent organs with a destructive effect. The resulting tissue growth appears chaotic. The spreading tumor masses are insufficiently supported and supplied by the stroma which results in the development of large necrotic regions. Thus, the cohesiveness of the tissue is reduced, allowing pieces of the tumor or individual cells to break away and form secondary tumors (metastases) in distant organs that they reach through body cavities, blood, or lymph vessels.

Frequently, tumors begin as benign growths and then become more and more malignant (malignancy increase). During such transition a tumor may not be clearly classifiable as belonging to one or the other category. In general, the differences between normal cells and those from benign and malignant tumors are more of a quantitative than a qualitative nature.

The external causes of tumor formation are frequently complex and can, therefore, be determined with certainty only in a minority of cases. Tumors can develop as a result of damage to the organism from radiant energy (ultraviolet, X-ray, or radioactive irradiation), thermal energy, mechanical disturbance, or chemical effects of numerous different substances. In addition, they can develop in response to hormonal disturbances, parasite infestation, or virus infection. Like every characteristic of an organism, tumors are products of both heredity and environment, being dependent upon genetic reaction norms plus environmental influences. Certain kinds of tumors can be produced in breeding experiments. They develop regularly in hybrids of different tumor-free parents.

With regard to the characteristics listed above, the tumors of fishes apparently do not differ from those of higher vertebrates. Comparable tumors in members of the various vertebrate groups show great similarity in their histological structure. Tumorous diseases are wide-spread among fishes, some reaching epidemic proportions and thereby gaining economic importance. Tumors have been observed among all fish taxa, from the Cyclostomata to the Chondrichthyes and Teleostei. Almost all organ systems are affected. It must be mentioned, however, that our present knowledge on this subject has been obtained chiefly from freshwater fishes. All the known forms of tumor formation have not yet been observed among marine fishes.

This section is organized mainly according to the type of tumor discussed and its organic origin. Wide-spread, well–investigated tumor diseases serve as examples and are treated more thoroughly. Rare or isolated reports of other tumor types are referred to only when described in detail. Pertinent information on freshwater fishes will be included when essential for the interpretation of corresponding findings in marine fishes. Systematic lists of fish tumor diseases can be found in reviews authored by Schlumberger and Lucké (1948), Wellings (1969), and Mawdesley-Thomas (1975), as well as in the Activities Reports Registry of Tumors in Lower Animals, maintained and continually amended by Harshbarger since 1965.

Epithelial Tumors

Papilloma, Carcinoma, Polyp

Whether facing the outside of the animal concerned or its inner body cavities, a true epithelium produces only 3 fundamental types of tumor.

Type 1: papilloma. The tumorous growth begins with a thickening of the epithelium. When the height of such hyperplasias is double or triple that of the normal epithelium, the underlying connective tissue begins to infiltrate the epithelial thickening with finger-like or folded processes, producing a wart-like stage. When the connective tissue component spreads to form a branched stroma, the tumor surface grows lobular or papillar. This papilloma is simply a benign tumor that enlarges itself toward the outside. Its epithelial portion and the tumor stroma remain separated by a continuous basal lamina.

Type 2: carcinoma. In fishes, as well as in other vertebrates, a local dissolution of the basal lamina can be observed with ageing or increasing size of the papilloma. The epithelial cells break loose and in peg-like groups, or completely diffused, penetrate the stroma. Eventually this leads to ulcerative disintegration of the prominent part of the papilloma, while the basal part develops into a highly malignant carcinoma which invades the body (Peters and co-authors, 1972).

Superficial carcinomas can also develop without going through a preliminary papillomatous stage, especially in areas exposed to vigorous mechanical or other local irritation that produces a deep, persistent wound in the epithelium. Carcinomas in fishes occur predominantly on the edges of the jaws (Peters and Peters, 1985).

Type 3: polyp. This is, to a certain extent, a special variant of the papilloma. While the epithelium of the typical papilloma is obviously thickened and the papillae or folds in its basal part coalesce, the polyp is in essence a section of skin surface that has folded itself into a form resembling a filigree tree. While papillomas are frequently found on fishes, polyps are rare. Both remain benign in most cases, and carcinomas do not develop.

Skin and Buccal Cavity

Neoplasms most often observed in fishes are skin tumors. They are more obvious than tumors of the inner organs because of their location on the body surface. This is one reason why they are so frequently recorded. On the other hand, the body surface is directly exposed to stimulants that are known to induce tumor formation. For example, harmful chemicals in the water may exert detrimental effects on the skin and may be concentrated locally by absorption processes. Skin areas subjected to increased mechanical impact, such as snout and edge of the mouth, are especially endangered. They are often exposed to the combined effects of chemical, mechanical, and other apparently detrimental factors responsible for inducing tumor growth (Peters and Watermann, 1979).

Skin papillomas are folded tumors of the epidermis, formed by an inordinate reproduction of Malpighian cells, the fundamental elements of vertebrate epidermis. Highly differentiated elements — such as goblet, club, or granular cells — become less abundant and reduced in size, compared to the normal epidermis. They may even be completely eliminated. A more or less obvious manifestation of dedifferentiation in the epithelial tumor tissue is the reduction in the number of tonofilaments in the Malpighian cells. Membrane interdigitations between neighbouring cells begin to even themselves out, or the membranes fully separate. Often large intercellular spaces form, and the cells become star-shaped, contacting each other only at the ends of their processes. The tissue thus assumes a spongy appearance.

In normal epidermis, leucocytes (predominantly small lymphocytes) are found in the intercellular spaces, especially in the basal layers. Apparently they immigrate from the supporting connective tissue. In epidermal papillomas, leucocytes are frequently present in large numbers. Epidermal tumors heavily infiltrated by these cells may show degenerative or even necrotic characteristics.

Among the most thoroughly investigated tumor diseases of fishes is the papillomatosis of the European eel *Anguilla anguilla.* Tumors that protrude high above the body surface consist superficially of lobes, folds, and papillae. *In toto,* they are often semispherical in shape. Their superficial appearance is responsible for the term 'cauliflower disease'. The tumors may reach a diameter of several cm. Their colour, determined by the number of melanophores in the stroma, varies from dirty red or grey to blue-black. The disease was named 'stomatopapillomatosis' because it is generally localized around the mouth or the snout; however, the tumors may also form in any other region of the body surface. Sites most often affected are those particularly exposed to mechanical irritation; besides mouth and nasal regions, especially throat and fin edges are subject to such exposure.

Although with few exceptions these tumors are benign (Peters and co-authors, 1972), they grow very rapidly. At temperatures above 15 °C, the tumors at least double in volume over a period of 4 wk (Peters and Peters, 1977). Small tumors can attain a volume 5 times the original during the same period (Lühmann and Mann, 1956). Several groups of small tumors can combine and cover large parts of the eel's head. Two or more large tumors are also frequently observed on the same fish.

Young eels with total lengths between 10 and 35 cm are most commonly afflicted. They have remained in coastal waters since arriving in Europe from the Sargasso Sea for 1 to 3 yr. The rate of affliction among large eels is much lower.

The disease occurs mainly in brackish water regions in which the salinity ranges

between 3 and 20‰. It has been observed most often in estuaries and coastal waters of the Baltic Sea (Peters and Peters, 1979; Schäperclaus, 1979). Afflicted individuals are only occasionally captured far upstream in rivers or in the open sea. Occurrence of the disease in lakes can apparently be ascribed to stocking.

Eels with large tumors in their mouth region appear to be hindered in respiration and, especially, in feeding. Such individuals tend to become obviously emaciated. In extreme cases their weight amounts only to one half of the norm, and their fat content may decrease to one fifth (Koops and Mann, 1969).

In European waters, eel papillomatosis occurs mainly during the warm period of the year. During the cold seasons, the tumors remain so small that they may not even be apparent and their rate of occurrence is low. However, in mid-summer the size and abundance of tumors tends to be many times greater. In the lower Elbe, for example, the rate of affliction varies from 12 to ca. 30% in summer while in winter it is only 3 to 7% (Peters, 1975). In autumn, summer values decrease to the winter level. During the year, the tumors undergo changes in structure and consistency. In summer, the epidermal tumor tissue is firm and clear, while in late autumn and winter, obvious signs of tissue degeneration or even cellular necrosis can be seen. Pieces of the tumor begin to break away. There is an increase in the percentage of eels with bloated bloody or slimy scars on the tumors. It can be assumed that some of the tumorous individuals succumb to general weakness or possibly to wound infections during these seasons (Peters, 1976). Such seasonal fluctuations in the growth or degeneration of the tumors can be induced experimentally by temperature variation (Peters and Peters, 1977).

Eel papillomatosis was first described around the turn of the century from the Baltic Sea region (Nagel, 1907; Wolff, 1912). According to reports from fishermen, isolated specimens afflicted with the disease were also captured in the lower Elbe at the beginning of this century. It is assumed that the rate of affliction did not exceed 0.5 to 2% at this time (Schäperclaus, 1953b). Significantly higher rates were first reported after the Second World War (Christiansen and Jensen, 1950; Lühmann and Mann, 1956). According to Koops and Mann (1969), the following rates of affliction were recorded among stocking eels (15 to 35 cm) from the lower Elbe region: 1957 to 59, 5.6%; 1960 to 63, 8.0%; 1964 to 65, 11.1%; 1967, 11.9%. These authors (pers. comm.) reported that the percentages are based on catches made chiefly during the summer. Investigations conducted from 1971 through 1981 revealed annual affliction rate maxima between 12 and 30% (Peters, 1975). Even though the data obtained by the different authors mentioned are not strictly comparable, it must be assumed that the disease has become more prevalent during the past decades, at least in the lower Elbe. According to Anders and co-authors (1977, quoted in Schäperclaus 1979), the affliction rate has also increased in the coastal regions of the German Democratic Republic, especially near the island of Rügen (Baltic Sea).

Papillomatosis of the European eel is wide-spread in Northern and Central European coastal waters (Sweden, Denmark, Poland, Germany, Holland). Recently, very high rates of affliction have been reported from Scotland (Hussein and Mills, 1982). In contrast, the disease does not seem to occur along the coasts of France, the Iberian Peninsula, and in the entire Mediterranean Region. Only the European eel is affected. The disease has not appeared among other eel species in America, Japan, or Africa.

Because the disease is limited in its distribution but spreading, it has long been assumed that a halophilic virus is responsible for the disease (Schäperclaus, 1953b; Koops

and Mann, 1969). However, attempts to induce the disease by injection or external application of tumor homogenate, or by transplantation of small pieces of tumor have remained unsuccessful. No inclusions have yet been found in the epidermal tumor cells that might be interpreted as virus particles. The round or polyhedral particles associated with bundles of needle-like structures described by Schubert (1969) were not found in tumor cells but rather in lymphocytic cells that had apparently immigrated. Experiments to isolate a virus using various fish cell cultures and a tumor homogenate (Pfitzner, 1969; Schubert, 1969) produced negative results. However, it was possible to engender cytopathological conditions in fish cell cultures (RGT-2 and FHM cells) and to detect polyhedral virus particles (55 nm diameter) inside the cells after addition of eel blood. Nevertheless, intramuscular injection of this virus material failed to produce the disease in healthy eels (Schwanz-Pfitzner, 1976).

Two other virus forms (EV-1 and EV-2) have recently been isolated from homogenates of tumors and various organs of the European eel (Wolf and Quimby, 1973; McAllister and co-authors, 1977), and another 2 (EVE and EVEX) have been found in specimens not afflicted with tumors (Sano, 1976). In all these cases, it is not known whether the virus has anything to do with eel papillomatosis.

While the cause of the disease remains unknown, the most likely explanation for its occurrence is multifactorial impact, possibly involving a virus plus harmful environmental factors that trigger viral activity. This would explain the slow spread of the disease and the increasing rate of affliction in certain coastal waters during the last decades. Additional factors that might contribute to causing the disease are the low salinity in the coastal regions and frequent irritation of skin areas.

Skin tumors occur regularly in about 20 species of Pacific flatfish from coastal waters of North America, Japan, and Korea (Stich and co-authors, 1977). They appear a few weeks after metamorphosis as so-called angioepithelial nodules, which are usually reddish and about 4 mm in diameter. These excrescences of the dermal connective tissue are richly supplied with blood vessels and covered by a slightly thickened epidermis (McArn and co-authors, 1968). During the following months, they develop into papillomas, pigmented to various degrees and exhibiting the typical structure of folded epidermal tissue resting on a branching connective tissue stroma. Such papillomas can attain a diameter of several cm and a height of 20 mm. Frequently, several tumors are distributed over both sides of the body and on the fins of a single fish. The average number of tumors on the pigmented upper side is greater than that on the underside, indicating that the agent responsible for initiating tumor growth acts on the fish after metamorphosis. Sometimes the angioepithelial nodules develop into polyps, i.e., folded growths on the epidermis without local thickening. Papillomas may also form without going through a nodule-like stage.

The flatfish afflicted are usually in the year classes 0 to 2 and measure 5 to 15 cm in total length. That individuals in the year classes 3 to 5 are only occasionally observed with tumors indicates high mortalities among tumorous fishes (Wellings and co-authors, 1964). Because fishes transitioning from year class 0 to 1 are most frequently afflicted, there is great seasonal fluctuation in the rate of tumor formation, depending on the spawning time of the species.

The structure of Pacific flatfish papillomas is extraordinarily complex (Peters and co-authors, 1978). As the tumors grow in size, an increasing number of rounded, voluminous cells, called X-cells, displace cells' Malpighian character in the epidermal portion (Brooks

and co-authors, 1969) and eventually become the dominant elements. The X-cells, especially the relatively large ones, give the impression of being degenerative. Their mitochondria are swollen and their ER is vesicular and degenerated. Sometimes both cell organelles can scarcely be recognized in the cytoplasm because they take on a foamy consistency (N. Peters and co-authors, 1981). X-cells are also characterized by an obvious nucleolus, by strongly contrasting cytoplasmatic and smaller karyoplasmic particles of various shapes in the EM pictures, and by a thickened plasmalemma. It has not yet been determined whether X-cells reproduce by mitosis or by another process. Their nuclei cannot be tagged with ^{3}H-thymidine (Kranz and co-authors, 1980).

X-cells of epidermal tumor tissue lack the desmosomes, tonofilaments, and membrane interdigitations that characterize Malpighian cells. They are separated from each other by the wing-like processes of the interstitial Malpighian cells in a typical fashion. They are also excluded from the basal lamina by the basal cells and separated from the tumor surface by deck cells both being of Malpighian character (Peters and co-authors, 1978, 1981).

X-cells with the same characteristics, but on the average somewhat smaller, occur individually or in small groups in the stroma of papillomas and in the connective tissue of angioepithelial nodules.

The origin of X-cells is still unknown. The fundamental question is whether X-cells are parasitic Protozoa, such as Amoebae, that form papilloma-like aggregates together with the tissues of the host or whether they are actually fish cells transformed by agents, such as viruses (Wellings and co-authors, 1977; Peters and co-authors, 1978, 1981; Yamazaki and co-authors, 1978). Evidence for the first mentioned assumption would be that the pseudobranchial tumors of cod, which are formed of X-cells and interstitial cells in the same manner as flatfish papillomas, contain X-cells that evidently engage in mitotic activity and also pass through encysted stages (p. 413). On the other hand, the second assumption is supported by observation of virus-like particles in the X-cells (Wellings and Chuinard, 1964; Wellings and co-authors, 1967; Stich and Acton, 1976; N. Peters and co-authors, 1981). Some of these virus-like particles were irregularly formed, while others were observed to consist of a capsid and nucleoid, similar to icosahedral virions. Furthermore, fibrillary, spherical nuclear inclusions, apparently composed of nucleic acid, were observed in papilloma X-cells from starry flounder *Platichthys stellatus* and lemon sole *Parophrys vetulus* (Peters and co-authors, 1983). Such nuclear inclusion bodies have been described from cells demonstrated to have been infected with viruses (Kim and co-authors, 1978).

No one has succeeded yet in infecting a fish with Pacific flatfish X-cell papillomatosis, either by inoculation with normal or tumor cells, or by injection of tissue homogenates with and without whole cells (Wellings and co-authors, 1976b). Since X-cell papillomatosis is confined to Pacific waters, it can nevertheless be expected that an infectious agent at least contributes to the outbreak of the disease.

Stich and co-authors (1977) ascertained that in juvenile lemon sole populations near large cities and industrial areas along the North American West Coast — such as San Francisco, Eureka, Seattle, Everett, Bellingham, and Vancouver — maximum annual infection rates ranged from 7 to nearly 60%, while elsewhere this rate was frequently less than 1%. Similar findings were reported for various species on the East Coast of Hokkaido, Japan. In other cases, however, the infection rates could not be correlated

directly with the degree of anthropogenic pollution. For example, while in the Bay of Bellingham (Washington, USA), 50 km south of Vancouver, both starry flounder and lemon sole populations are heavily infected (55 and 33%, respectively); in the mouth area of the Frazer River (Canada) infection rate of starry flounder is only 0.2%, but that of lemon sole nearly 60%. Furthermore, in waters off the northern coast of Queen Charlotte Island, which are nearly free of pollutants, the disease rate among sand sole *Psettichthys melanostictus* is as high as 30%, while lemon sole are completely free of the disease. In the Bering Sea, rock sole *Lepidopsetta bilineatus* are afflicted at a rate of up to over 10%. In this case, as well, no conclusions can be drawn.

In addition to Pacific flatfishes, this type of X-cell papilloma has been observed only in the goby *Acanthogobius flavimanus* from Japanese coastal waters (Oota, 1952; Imai and Fujiwara, 1959; Kimura and co-authors, 1967; Ito and co-authors, 1976). In these populations the papillomas develop mainly in the head region. The X-cells also contain strongly contrasting cytoplasmic particles, referred to as possible virus material (Imai and Fujiwara, 1959; Ito and co-authors, 1976).

Relatively flat, whitish thickenings of the skin reaching a diameter of over 10 mm and a height of several mm have been observed repeatedly on dab *Limanda limanda* from the North Sea and adjacent waters (Dethlefsen, 1978; Möller, 1979; Peters and Watermann, 1979; Dethlefsen and Watermann, 1980; Möller, 1981). These skin thickenings occur exclusively on older fishes, at least 2 to 3 yr old and measuring over 10 cm in total length. Frequently, a fish displays multiple growths of this sort, generally on the sides of its body and fins, but seldom on its head.

Some of these skin thickenings on dab are epidermal hyperplasias, which are relatively low and have a very even surface. The subdermal connective tissue remains unaffected. When folds or finger-like processes from the upper part of the subepithelial connective tissue *(stratum laxum)* develop into a slightly ramified tumor stroma, the hyperplasia becomes essentially a papilloma with a rather lobular surface. In this case, a dislocation of the *stratum laxum* can result, thereby causing deformation and displacement of the scales.

The rate of occurrence of this disease is generally low, usually under 1% and sometimes even below 0.1%. In some locations, however, the rate may reach several percent. For example, in the German Bight, about 12 nautical miles northwest of the island of Helgoland, Dethlefsen (1978) and Dethlefsen and Watermann (1980) determined rates as high as 6.6%. In this region, several hundred thousand tons of Ti O_2 waste water containing heavy metals and sulfuric acid have been disposed of by the titanium industry each year since 1969 from special dumping ships. The above-average rate of disease occurrence and the decrease of this rate with distance from the impact region can apparently be correlated with the presence of the waste water and its dilution as it spreads from the center of the dumping area. Prior to the investigations by Dethlefsen and Watermann, this kind of papilloma had not been reported for dab from the German Bight. However, as early as 1925, Johnstone, who investigated tumorous fishes caught near the British Isles for many years, observed several dab with wart-like structures that were apparently examples of the same condition. While the waste waters from the titanium industry therefore seem to promote the development of papillomatosis in dab, they are apparently not its only cause. Since average rates of disease occurrence in winter and early spring are significantly higher than in summer (Watermann, pers. comm.), seasonal causative factors must also be considered.

A special type of lip papilloma afflicts the white croaker *Genyonemus lineatus* in the coastal waters of Southern California. The tumors consist mainly of an immensely developed tumor stroma covered by a thickened epidermal epithelium that is usually flat but occasionally has rather deep indentations (Russell and Kotin, 1957; Phillips and co-authors, 1976). The stroma, that contains much collagen in places, is interspersed with cyst-like, fluid-filled cavities covered by epidermal epithelium. Epithelial pearls are also found in the stroma.

The rate of affliction averages 1%, but at some locations reaches 4.1% (Mearns and Sherwood, 1977). Contrary to the initial assumption of Russell and Kotin (1957) and of Young (1964), no relation could be found between disease rate and local degree of pollution. Only fishes with a standard length greater than 15 cm are afflicted, and the probability of the condition increases with body length. While young white croakers feed chiefly on plankton, older individuals (> 15 cm SL) consume mainly benthos. Apparently, white croakers over sandy sediments are more frequently afflicted with the papillomas than those living above muddy bottoms. This appears to indicate that increased mechanical irritation of the lip epithelium represents the primary stimulus for initiating tumor growth.

Young salmon *Salmo salar* are regularly afflicted with whitish flattened growths ranging from epidermal hyperplasias to papillomas with short stroma processes reaching a height of several mm and a diameter of 4 cm (Ljungberg, 1963; Wolf, 1966; Wiren, 1971). Young salmon develop tumors mainly during their stay in freshwater, when affliction rates as high as 50% have been observed. The rate decreases to below 10% during sea-ward migration and is less than ca. 1 or 2% at the time of return for first spawning (Needham, 1975, cited by Carlisle, 1975). The disease also occurs in salmon hatcheries.

Papillomatosis of young salmon develops mainly during summer; during late fall and winter, a healing process is observed. The disease persists over the whole year only in young salmon inhabiting ocean waters. Newly formed tumors contain only a few lymphocytes, mainly in the basal intercellular spaces of the epidermal portion. Later, there is a massive infiltration of lymphocytes and macrophages through the stroma followed by the appearance of some neutrophils (Carlisle, 1975). From a focus point in the central epidermal region near the stroma, the tumors become increasingly necrotic until they fully disintegrate, leaving an ulcerous lesion that rapidly heals. The rejection of the tumor tissue is reminiscent of a cell-mediated immune reaction. Cases of fatalities among young salmon with papillomatosis are probably due to occasional secondary infections, especially those caused by the fungus *Saprolegnia,* after tumor rejection.

Scarcely anything is known about the cause of this disease. The cytoplasm of degenerating tumor cells and the intercellular spaces of the necrotic region contain virus-like particles about 125 to 150 nm in diameter and with an electron-dense core measuring 70 to 95 nm. There is no other evidence for the involvement of viruses in the disease. In any case, the etiology is typical for an infectious disease, which heals after the host has developed immunity to the pathogen.

Kimura and co-authors (1981b) demonstrated experimentally that viruses can actually cause tumors, particularly skin papillomas, in fishes. They isolated a herpes virus, 115 nm in diameter, from the ovary of *Oncorhynchus masou.* Three to 5-month-old juvenile *O. keta* were infected with this virus by immersion. Some of the infected fish died of viral liver necrosis during the following weeks, and most of the survivors developed epithelial tumors within 4 to 8 months. These appeared in the region of the head and on

the tail fins, but the greatest number of them were skin papillomas around the mouth. Although the virus could not be detected in the tumor cells with an electron microscope, it was isolated from primary cultures of tumor cells. Similar results were obtained from experiments employing *O. kisutch.* This is the only case of a positive identification of a tumor virus from fishes yet reported.

Epidermal hyperplasias and papillomas are frequently observed among fishes kept for long periods in net cages and aquaria. Whitish thickenings on upper and lower fin margins are the growths most commonly observed on salmon kept in net cages. These are hyperplasias from irritation that apparently result from constant contact between fish kept at high stocking densities. Flatfishes (*Pleuronectes platessa, Scophthalmus maximum,* etc.) kept in containers with solid sides of cement or glass develop semispherical tumors on the tip of their lower jaw, the lower margin of their operculum, and on the lower edge of their body in the anal region (Peters and Watermann, 1979). This seems to be due to mechanical irritation of body areas exposed to maximum contact with the container walls during swimming. Initially, relatively small nodules (a few mm in diameter) with smooth surfaces develop. Histologically, these form as excrescences of the dermal connective tissue covered by highly hyperplastic epidermis. The epidermal basal cells are frequently of high prismatic shape, and the layers above are spongy. Typical papillomas reaching a diameter of 2 cm develop from such nodules. They contain a particularly large portion of stroma.

Frequently, the jaws of fishes kept in containers with solid sides are permanently injured. Ulcerative papillomas develop along the margins of the wounds. After a while, these can become massive ulcerous carcinomas that first deform and then produce an ulcerative degeneration of the jaw and adjacent structures. Even among wild fishes, ulcerous carcinomas have occasionally been observed on the jaws (Roberts, 1978). The epidermal tumor masses are characterized by their chaotic growth with disorderly entanglement of their connective tissue components, and by polymorphism of their cells. Cells with pycnotic nuclei are particularly obvious.

Stomach and Intestine

Fishes kept in Japanese aquaculture facilities — yellowtail *Seriola quinqueradiata,* sea bream *Pagrus major,* and eel *Anguilla japonica* — developed the so-called adenomatous polyps on their gastric mucous epithelium (Kubota and co-authors, 1974; Kimura and co-authors, 1976). These bushy excrescences usually develop as groups of tumors on raised parts of the stomach folds. Each is about the size of a pin head. The authors also conducted extensive investigations on Japanese species of the salmonid genus *Oncorhynchus* and determined that in freshwater culture facilities from a few percent to 89% were afflicted with such polyps. Two factors were considered possible triggers for producing the tumors: Particularly high rates of disease were found among fishes that (i) ingested gravel, causing repeated mechanical irritation or injury to their gastric mucous epithelium, and (ii) were simultaneously given feed containing aflatoxins. Although, possibly even dry feed (pellets) can produce mechanical irritation; in this case, chemical carcinogens (aflatoxins) and mechanical irritation seem to reinforce each other.

There are few other reports on epithelial tumors of fish stomachs and intestines. A fibrocarcinoma in the stomach of an angler fish *Borophryne apogon* was reported by Nigrelli (1947a), and an adenocarcinoma on the rectal gland of the gadid *Theragra*

chalcogramma described by Takahashi (1929). No tumors are known from the intestinal epithelium itself.

Adenomas, Adenocarcinomas

Benign tumors of secretory tissue are known as adenomas, while malignant tumors of this tissue are called adenocarcinomas. These are nodular, protruding excrescences, usually distinguishable from the secretory tissue by colour. They begin as swellings no more than a few mm in diameter and grow until many times the size of the glands and almost fully displacing the normal tissue. The histological structure of the adenoma is highly variable, depending on the tissue from which it develops. At least during the benign phase, its structure is lobular, follicular, or alveolar. Cystic adenomas sometimes form as a result of a duct blockage and subsequent enlargement of the gland lumen. At the end stages of malignant tumor growth, a chambered tumor vesicle can form as the center of the tumor disintegrates necrotically. The connective tissue contributes little to the formation of the tumor stroma.

Liver

Early stages of liver cell tumors stand out against the brownish to brownish-red fish liver as whitish or yellowish spots about 1 mm in diameter, just below the surface or deeper in the liver parenchyma. The nodules, often occurring multiply, consist of strings of liver cells that frequently exhibit an obviously trabecular growth through the enlargement of the sinusoids. These strings may be formed by a single or a double cell layer (Falkmer and co-authors, 1976; Pierce and co-authors, 1978). Histological differences between these tumors and the surrounding liver tissue include (i) different arrangement of liver cell strings (sometimes in form of a rosette); (ii) hypertrophy of cells; (iii) increase in the ratio of nucleus to cytoplasm volume; (iv) tendency of cells to stain extremely basophilically or, less frequently, eosinophilically. The liver tissue immediately surrounding the growth becomes compressed and atrophies, apparently due to enlargement of the nodule. Nodule cells give the impression of being well differentiated; they are largely uniform. These well delimited tumors are classified as hepatomas and can reach a considerable size (several mm in diameter) without losing their differentiated character.

Large nodules that form large tumorous masses reaching several cm in diameter, progressively lose the integrity of their liver cell strings and eventually become a disorganized mass of hepatocytes, that vary increasingly in shape. Occasionally, multinucleate cells are observed. Hemorrhages, edema, necrosis, and excrescences of the connective tissue, generally beginning in perivascular regions, also occur. Thus, the structure of the tumor becomes more and more variable. Sooner or later, the periphery of the tumor is no longer clearly delimited, and growth becomes extremely invasive. The larger the growth, the more it takes on the form of a chambered vesicle due to central necrosis. As soon as invasive growth can be observed, the tumor is designated a primary hepatocarcinoma.

Cholangiomas and cholangiocarcinomas, tumors of the gall ducts, are often associated with true liver cell tumors. Externally, they can scarcely be distinguished from the hepatoma. Histologically, they have a distinctly adenomatous structure, appearing follicular or cystic with little connective tissue stroma between the follicles, at least when in the benign state. The differences between the 2 types of tumor disappear more and more when they become malignant. There are also growths that include both forms in early stages of

development (Falkmer and co-authors, 1976). In the malignant state, both tumor types metastasize, producing secondary growths outside the liver, mainly in gills, kidneys, and spleen.

The occurrence of liver cancer in cultured salmonids (*Salmo gairdneri* and *Oncorhynchus masou*), that reached epidemic proportions in the 1950s and 1960s around the world and afflicted as much as 80 to 100% of a culture stock, was the original problem that prompted the initiation of detailed fish hepatoma studies (Ashley and co-authors, 1965; Halver, 1965; Sinnhuber and co-authors, 1965; Halver and Mitchell, 1967; Ghittino, 1976). The epidemic spread of the disease began with the transition from wet feed to dry pellets. Such feeds, especially those containing much vegetable protein and lipid originating as peanut and cottonseed meal, are especially prone to attack by the molds that produce the carcinogenic aflatoxins. After receiving feeds containing aflatoxins for 2 to 3 months, the fishes show the first histological changes in their liver tissue (aflatoxicosis). Within 6 to 12 months, hepatomas develop at the sites of the initial alterations. When administration of the toxins is discontinued, the period of latency increases. Females of *S. gairdneri* and *O. masou* are afflicted much more severely than males (Takashima, 1976). Other salmonids, such as *Salmo clarki* and *Salvelinus fontinalis,* are much less susceptible, apparently due to a lesser degree of genetic predisposition. Since feed pellets have been stored as dry and cool as possible, and since their aflatoxin content is controlled, liver cancer in culture facilities has been almost completely eliminated with occurrence rates dropping below 1%.

Recent investigations of marine fishes have indicated that liver tumors and other degenerative conditions of the liver are distinct bioindicators of excessive contamination of a water body, especially with chlorinated hydrocarbons. Falkmer and co-authors (1976, 1977) investigated for 5 yr 28,000 hagfish *Myxine glutinosa* from Gullmar Fjord in southwestern Sweden; they observed a decline in the rate of hepatoma occurrence from 5.8% in 1972, to 2.9% in 1973, and to 0.6% from 1974 through 1976. In the open sea, 12 km away, rates in parallel catches were 2.8% in 1972, and 0.9% in 1974. In 1971 and 1972, the use of PCBs had been prohibited by law in Sweden, and the use of DDT and its derivatives, DDD and DDE, had been drastically limited. The PCB concentration in the hagfish liver was ca. 5 mg kg^{-1} wet weight in Gullmar Fjord, and ca. 0.2 mg kg^{-1} wet weight in the open sea from 1974 through 1976. Analyses for aflatoxins gave negative results. A peculiarity in the findings was that the sediments at the sampling sites contained no detectable chlorinated hydrocarbons. The presence of these substances in cod liver made it appear very likely that the hagfish, as scavengers, had been contaminated through the food chain.

Fishes in river estuaries near industrial regions seem to be especially prone to liver tumors. Pierce and co-authors (1978) found that 92% of lemon sole *Parophrys vetulus* in the Duwamish River near Seattle, Washington (USA) showed signs of severe liver degeneration, such as dissolution of liver cell strings, blood congestion, excessive deposition of fat in the hepatocytes, cellular hypertrophy, and eventually development of necrotic foci. Among these fish, 32% were also afflicted with liver tumors ranging from benign nodules to invasive excrescences. Significantly less liver degeneration was found among lemon sole at the edge of Elliott Bay, into which the Duwamish River empties. No liver tumors occurred in these fish. These findings indicate that a causal relation seems to exist between severe liver degeneration and occurrence of liver tumors.

High PCB concentrations were also found in lemon sole from the Duwamish River (1.5 mg kg^{-1} dry weight of the whole body). The liver degeneration was similar to degenerative alterations observed in fish experimentally exposed to sublethal doses of PCBs and other chlorinated hydrocarbons (Johansson and co-authors, 1972; Hansen and co-authors, 1974; Couch, 1975). PCBs may cause liver tumors in several mammals, such as rats and mice (Fishbein, 1974; Kimbrough, 1976).

The Hudson River Estuary in New York, USA, is the spawning ground of a tomcod *(Microgadus tomcod)* population. Juvenile fish remain in the estuary until reaching sexual maturity at the age of about 1 yr, and most of the spawners are doing so for the first time (Smith and co-authors, 1979). Among these fish, 25% are afflicted with liver tumors in a range of malignancy from benign nodules to edematous carcinomas. According to preliminary investigations, only severely degenerative livers seem to be affected. PCB contents of tomcod livers range between 11 and 98 ppm.

Thyroid

The primary follicle of the thyroid gland is formed during embryological development by invagination and separation of epithelial tissue from the branchial chamber. Secondary follicles develop by budding, and in the fish thyroid, they are loosely distributed in the connective tissue around the *aorta ventralis*, mainly between *aorta ventralis* and the floor of the gill chamber. Benign tumors can be formed when budding begins again and produces numerous additional follicles. The soft tumors begin to protrude into the throat, but they can also produce external swellings (Baker and co-authors, 1955; MacIntyre, 1960). The initial reddish swellings, about the size of a pin head, grow into lobed or spherical tumors or tumor aggregations that can sometimes reach a diameter of several cm. Large tumors greatly impede respiration, especially when they protrude from the throat region into the gill chambers.

In the early stages, the histological structure of the excrescences resembles that of normally formed thyroid follicles with a central secretory (colloid) space. In some cases, the follicle walls can become thickened through change in the shape of follicle cells to high prismatic. In later stages, the follicles become increasingly irregular in shape; some of them lose their central lumen, others assume a growth typical of papillae or cysts. The glandular tissue, that eventually develops hose-like branches, begins to invade neighbouring tissues of the gill arches, heart region, and cartilage, bones, and muscle tissue in the pharyngeal region. Soon, highly irregular glandular follicles can be found in spleen, kidney, intestinal wall, and even in the body wall. This mostly indicates that the tumor has metastasized, but such follicles might also have developed as a result of wide-spread invasive growth. The continuity of the transition from benign to malignant is particularly evident in the case of thyroid tumors.

This disease has been observed mainly in aquarium-held fishes; and it is much more common among freshwater than marine species (Nigrelli, 1954; Harshbarger, 1965–81). This is explained by the fact that the cause of the adenomas and adenocarcinomas is usually a chronic lack of iodine, and that this element is relatively abundant in seawater. Tumor formation can be arrested at a relative early stage by administering inorganic iodine (e.g., with potassium iodide-iodine, $KJ \cdot J_2$) with the food (concentration ca. 1 : 2,500) or by simply adding it to the aquarium water (1 : 5,000,000). These treatments can even result in regression of the tumor. Conversely, by administering thiouracil, a substance that

blocks the formation of thyroxin by preventing chemical bonding of the iodine, the formation of benign tumors can be induced (Stolk, 1955).

Thyroid hyperplasias and tumors can also occur abundantly among cultured trout, especially juveniles (Marine and Lenhart, 1910a, b, 1911; Marine, 1914). In hatcheries, entire generations of such fishes are sometimes killed by this disease. According to Duerst (1941), this may be due to the high oxygen requirements of salmonids and the low concentrations frequently encountered in the water of the culture facilities (3 to 4 mg l^{-1} or less). An oxygen supply that is too low can, like lack of iodine, result in an underactivity of the thyroid gland. An additional complication is the especially high iodine demand of salmonids, which is at least 5 μg l^{-1} (Robertson and Chaney, 1953). Underactivity of the thyroid caused by lack of oxygen or iodine brings about an increase in secretion of TSH; this, in turn, stimulates production of thyroid secretory tissue and eventually leads to tumor formation. A detailed discussion of the causes of thyroid tumors was provided by MacIntyre (1960).

Among wild fishes, this disease seems to be extremely rare, especially in marine environments. Isolated occurrences among salmon (*Salmo salar* and *Oncorhynchus kisutch*) were reported, but in these cases, usually designated hyperplasias, development had scarcely proceeded beyond the early benign stages (Harshbarger, 1965–81). Early stages of the disease were also diagnosed among rays and sharks kept in zoological parks and public aquarium collections.

Pseudobranch

Pseudobranchial tumors of Atlantic *(Gadus morhua)* and Pacific cod *(G. macrocephalus)*, as well as of other gadids, have been reported by Lange (1973), Alpers and co-authors (1977), Lange and Johannessen (1977), and Morrison and co-authors (1979, 1982). The tumors generally occur as paired swellings that protrude into the throat cavity from the dorsolateral pharynx wall. These compact tumors measure from 1.5 to 4.5 cm in length and 1.0 to 3.5 cm in width; they can protrude up to 4.0 cm above the surrounding surface. Small growths are reddish; larger ones are yellowish or light pink and develop individual blood vessels that stand out prominently. The tumor surface is even, becoming slightly lobed in the later stages. The tumors are generally closely adjacent to the pseudobranch or fully enclose it, thereby partially or extensively displacing it. Although tumors on each side are normally similar in size, form, and colour, occasional asymmetrical development and even growth of tumors on only one side have been observed. The tumors sometimes protrude as far as the region of the anterior kidneys and the inner side of the opercula. Especially large, paired tumors can fuse along the dorsomedial line of the pharynx.

Histologically, the tumors mainly consist of lobes of large spherical cells. Like the Pacific flatfish papillomas (p. 405), the large spherical cells are separated by a network of flattened, branching intermediate cells. The ultrastructure of the spherical cells is remarkably similar to that of the X-cells in flatfish papillomas in that they possess a single faint nucleus with a prominent nucleolus, numerous strongly contrasting cytoplasmic particles, and a thickened cell membrane. Another similarity is that the small, obviously younger cells feature a relatively dense cytoplasm; while the cytoplasm of the larger cells is of a foamy consistency, where cell organelles, such as the mitochondria and ER can scarcely be recognized under the electron microscope. As in the case of X-cells of Pacific flatfish,

degeneration seems to occur. Another similarity with flatfish papillomas is the apparent epithelial origin of intermediate cells, indicated by the presence of tonofilaments and desmosomes, as in Malpighian cells. While their nuclei stain distinctly with Feulgen reagent, the nuclei of X-cells are Feulgen negative.

The division of pseudobranchial tumors into lobes occurs as clumps of X-cells and intermediate cells which are separated from each other by branching or anastomosing septa of connective tissue stroma. The main septa are continuous with a stiff, collagen-rich tumor capsule around the outside of the growth. This connective tissue tumor capsule is separated from the pharyngeal cavity by a covering of histologically unchanged epithelium. Invasive growth of the tumorous tissue has never been observed. The lobular structure of large tumors is locally disintegrated, and large masses of X-cells, round or amoeboid in form, float in fluid-filled cavities. A mechanical injury to such growths results in the streaming out of large tumorous regions.

In Atlantic cod, Watermann and Dethlefsen (1982) discovered inflamed, nodular swellings beneath the epithelium of the pharynx wall near the pseudobranches that might be preliminary stages of the tumors. At the edge of this region of inflammation, numerous cysts were observed consisting of several multinucleate cells with prominent nucleoli surrounded by dense capsules containing mucopolysaccharides. Around these were empty cysts interspersed with free multinucleate cells. Toward the center of the inflamed region, the number of nuclei in the cells decreased; finally, typical X-cells became numerically predominant. In contrast to flatfish papillomas, Dawe (1981) found that relatively small X-cells with rather dense cytoplasm sometimes show mitotic figures. Unlike in normal X-cell nuclei, the mitotic figures were Feulgen positive, and their DNA could be stained with fluorochromes as well. Large X-cells with foamy cytoplasm, which are predominant in large tumors, obviously do not reproduce. Nuclear inclusion bodies such as in flatfish papilloma X-cells have not yet been observed in X-cells of pseudobranchial tumors.

Once again the question arises whether X-cells of pseudobranchial tumors are transformed fish cells or parasitic Protozoa specialized for inhabiting host tissue, being enclosed by intermediate host cells and connective tissue stroma. While the presence of multinucleate cells and reproductive activity of X-cells provides evidence that they may be Protozoa, the regular incorporation of X-cells in the host tissue speaks against such an assumption. If the cells were Protozoa, it would remain unclear why the cysts, that should serve as resting or reproductive stages, develop at the beginning of the infection cycle, and why the great mass of X-cells in fully developed tumors are incapable of reproduction, degenerating fully instead of forming resistant or reproductive stages. Actual defensive host reactions (immune reaction or encapsulation) against the alleged parasites has not been observed, although occasional phagocytosis of degenerating X-cells by intermediate cells, or perhaps macrophages, does occur. In comparing X-cell papillomatosis with pseudobranchial tumors, it should be noted that the former occurs exclusively in the Pacific, while the latter is found in both Pacific and Atlantic Oceans.

The rate of occurrence among Atlantic cod amounts to a few percent. Morrison and co-authors (1982) reported an average rate of 2% in Canadian waters around Nova Scotia, with the values for individual sampling stations varying from 0.9 to 6.1%. In the Barent Sea, the rate of disease occurrence in various catches ranged from 0.4 to 1.7% (Egidius and co-authors, 1981). According to Watermann and co-authors (1982), the disease is confined almost exclusively to cod in the year classes I through III. Among specimens in

this age group from the North Sea (German Bight), the average rate of occurrence was reported to be ca. 1.5%. According to locality, this rate varied between 0.7 and 4.9%, apparently dependent on population density and the seasonal migration of cod in the German Bight.

Pseudobranchial tumors are apparently more abundant among Pacific cod than among those in Atlantic waters. Stich and co-authors (1976) reported rates of tumor occurrence between 4.5 and 11.4% in cod catches along the West Coast of Vancouver Island, Canada. Wellings and co-authors (1977) found an average rate of 7.4% in the Bering Sea, and local rates in excess of 15%. In the Pacific Ocean, it is also the lower year classes that are the chief victims of the disease.

Stich and co-authors (1976) suggested that the respiration of afflicted fishes is hindered; in this way, the disease retards growth and delays the onset of sexual maturity. According to McCain and co-authors (1979), walleye pollock *Theragra chalcogramma* (Gadidae) afflicted with pseudobranchial tumors are about 15% smaller than healthy fish of the same species of ages between 3 and 5 yr. As little is known about the later course of the disease and the fate of afflicted fishes, as is about the nature of the tumors and their causes.

Odontoma, Ameloblastoma

Odontomas are in general a class of tumors affecting the tooth-forming tissue, while ameloblastomas, also called adamantinomas, are tumors arising specifically in the epithelial portion, i.e., the ameloblasts of the enamel organ. Ameloblastomas form bulky tumorous masses on the edge of the jaws and on the tooth-bearing bones on the roof of the mouth. Initially, they prevent the mouth from fully closing, and eventually they result in the complete deformation of the jaw and snout region (Schlumberger and Katz, 1956; Dawe and Harshbarger, 1975). In contrast to epidermal carcinomas around the edges of the mouth, these tumors remain benign and ulceration does not occur.

The tumors consist of branching, lobed epithelial processes embedded in abundant loose connective tissue. The outermost layer of the processes is formed of high prismatic cells, arranged in a pallisade formation. The inside is filled with reticular cells. Occasionally, papilloma-like excrescences also occur in the pharyngeal epithelium. Their stroma is arranged as in the pulp of a tooth with peripheral odontoblasts and an amorphous intercellular substance between pulp (stroma) and epithelial parts. In other cases, the intercellular layer between odontoblasts and ameloblasts proved to be dentine (Harshbarger and co-authors, 1976). Finally, deformed tooth buds and even quite normally formed, but irregularly arranged, teeth can develop at this site. As soon as tooth-like structures or true teeth are formed, the complex tumor is designated an odontoma.

Ameloblastomas have been observed in only a few species, a good many of them salmonids of the genera *Oncorhynchus* and *Salmo* (Wellings, 1969). This suggests that salmonids may be genetically predisposed for this type of tumor (Schlumberger and Katz, 1956; Dawe and Harshbarger, 1975). All transitional forms from papillomas of the pharyngeal epithelium to ameloblastomas and complex odontomas have been found in Atlantic cunner *Tautogolabrus adspersus* from the estuary of the Sakonnet River near Portsmouth, Rhode Island, USA (Harshbarger, 1976). Cunner are benthos-feeders that also graze on barnacles and clams; hence the cause for tumors developing on the tooth-forming tissues could be a combination of the special mechanical charge and a high pollutant content in the benthos of the estuary.

Mesenchymal Tumors

Fibroma, Fibrosarcoma

Fibromas are tumors of mainly loose, but sometimes also dense connective tissue of all body regions. At first, the histological structure of the tumor seems generally normal. As malignancy increases, it becomes evident that the cellular portion of the tissue, consisting mainly of fibroblasts, is increasing, while the intercellular substance, especially the collagen fibers, is decreasing in quantity. In extreme cases, the tumor consists of dense masses of undifferentiated cells that are frequently spindle-shaped with little intercellular material. The tumorous mass no longer transitions to normal connective tissue at its edge or remains within a capsule, but rather penetrates the adjacent tissues, such as muscles, invasively. Such distinctly malignant fibrosarcomas are designated as spindle-cell sarcomas when the longitudinally extended cells are arranged parallel in bundles. If the cells lack organization, possess numerous processes, and are interspersed with many intercellular spaces, then the growth is referred to as a reticulosarcoma. Naturally, between the 2 there are intermediate forms. Especially among the various-shaped cells of the reticulosarcoma — which contain polymorphic nuclei, exhibit atypical mitosis, and tend to form giant cells — there are large numbers of other kinds of cells, such as lymphocytes, macrophages, and erythrocytes (Ashley and co-authors, 1969).

Fibromas and fibrosarcomas can be said to constitute their own stromas. They frequently develop from the stroma of other tumors by excessive development. In such cases we speak of a fibroepithelioma if the other tissue component is an epithelium; and of fibroadenomas, fibrohemangiomas, and fibrolipomas if the growth includes a simultaneous development of glandular, blood vessel, or adipose tissue, respectively. When the connective tissue forms the main proportion of the tumor, the names used are adenofibroma, adenofibrosarcoma, hemangiofibroma, etc.

Thus, fibromas and fibrosarcomas display many histological variations. The great majority of connective tissue tumors described from fishes are located in the subepidermal layers or body musculature. They are also frequently found in the mesentery and in the peritoneum of the body cavity (Wellings, 1969). These are probably simple fibromas and fibrosarcomas. They are macroscopically visible as swellings or lumps. Frequently, those on the body surface, digestive tract, or mesentery possess stalks of various lengths. Tumors in the musculature are covered by normal skin, while those in the dermis have a coating of normal epidermis.

Scarcely anything is known about the factors that induce the formation of connective-tissue tumors. Fibromas and fibrosarcomas occur in a wide variety of fish species. Tumors with diameters of several centimeters have been observed on coho salmon *Oncorhynchus kisutch,* Atlantic cod *Gadus morhua,* and Pacific halibut *Hippoglossus stenolepis.* In 1 yr, 1967, about 40 specimens of coho salmon with this type of tumor were caught in the Pacific Ocean near Newport, Oregon, USA (Wellings, 1969). This suggests that a virus might be the chief causative agent. Among a spawning population of the North American pike perch *Stizostedion vitreum* about 5% of the sexually mature fish were afflicted with multiple dermal fibrosarcomas. In the intercellular spaces of these growths, virus particles about 100 nm in diameter (Type C) were sparsely distributed, but found with great regularity (Walker, 1969). These virions form by budding from the cell membrane. Virus-induced fibromas and fibrosarcomas are also known from birds and small mammals.

Finally, it must be noted that the skin swellings (nodules) and skin papillomas of fishes kept in hard-walled tanks of culture facilities possess an excessively proliferated loose, or sometimes stiff, connective tissue beneath the proliferated or even papillomatous epithelium. These growths can therefore be referred to as fibroepitheliomas (Peters and Watermann, 1979). As mentioned on p. 408, the tumors form on the body surfaces that often come into contact with the tank walls as the fish swims about. It can therefore be concluded that, in this case, a mechanical stimulus is the apparent cause of the excessive development of the connective tissue.

Lipoma

Lipomas are usually solitary tumors of adipose tissue, but multiple lipomas have been observed on rare occasions. They are well delimited, soft, elastic tumors that always remain well differentiated, i.e., they display the normal structure of adipose tissue. The adipose cells contain large fat vacuoles and peripheral cytoplasm and nuclei; a soft stroma containing blood vessels is typical for the tissue. The tumors can develop wherever adipose tissue is located. In fishes, they are primarily observed as tumors of the dermal adipose tissue (Wellings, 1969). They are mostly spherical in shape and often extend above the body surface on stalks of various lengths.

Lipomas occur on fishes only in isolated cases and much less frequently than fibromas. Sometimes they form part of tumors involving other kinds of tissue. In one such growth, a fibrolipoma on a Pacific halibut *Hippoglossus stenolepis,* Wellings (1969) found islands of adipose tissue scattered in a firm dermal connective tissue mainly consisting of collagen fibers. In the case of an osteolipoma on the operculum of a specimen belonging to the same species, irregular trabeculae of bony tissue were embedded in an adipose tissue interspersed with fibers.

Chondroma, Osteoma, Osteosarcoma

Tumors in cartilage, called chondromas, are neoplasms ranging in form from nodular to highly convoluted. Apparently, they usually develop on the surface (appositional growth) when the spindle-shaped cells of the perichondrium form a cartilaginous matrix and transform in excessive numbers into chondrocytes (Nigrelli and Gordon, 1946). The tumorous cartilage tissue therefore contains an abnormally large number of chondrocytes, that are grouped together in nests or may even be thickly packed throughout the tissue leaving little room for the intercellular matrix. The size and shape of the chondrocytes is highly variable, and their arrangement is irregular. Sometimes 2 or more chondrocytes are present in a single cell capsule, possibly indicating a simultaneous interstitial growth of the tumor. Cartilage tumors can grow quite fast, but in fishes they probably remain benign. Fish chondromas have been observed only in exceptional cases (Wellings, 1969).

Osteomas are much more common in fishes. Included among them are numerous bone swellings, apparently with a very limited growth (Wellings, 1969). Such thickenings, which may be better designated as exostoses or hyperostoses, are found in fishes primarily on vertebral processes (hemapophyses and neurapophyses), on fin bones, and on fin rays (Thomas, 1932). They are reminiscent of hyperregeneration (callous formation) resulting from fractures. Numerous such hyperostoses have been found on the hemapophyses of *Pagrosomas major* (Takahashi, 1929). Schlumberger and Lucké (1948) conducted X-ray and histological investigations on 3 specimens of this species and concluded that there was no evidence of any previous injury to the hemapophyses displaying this condition.

In contrast to hyperostoses, that generally have a smooth surface, true osteomas in fishes are convoluted. The connective tissue periosteum contains irregularly arranged trabeculae or spicules of bone that proliferate and spread rapidly. During this process, the connective-tissue periosteum or stroma can be infiltrated by a stiff, fibrous connective tissue; by adipose tissue; or by a cartilaginous substance that displays a tumorous growth pattern (Nigrelli and Gordon, 1946; Wellings, 1969). The resulting compound tumors are designated fibro-osteomas, lipo-osteomas, and chondro-osteomas, respectively. When osteomas penetrate adjacent muscle tissue, as observed in the hake *Pollachius virens,* the resulting tumor is called an osteosarcoma (Williams, 1929). These tumors have not been observed to metastasize, at least not in fishes. If hyperostoses are excluded from the category of true osteomas and osteosarcomas, then this category of fish tumors is only occasionally found.

Myoma, Myosarcoma

Tumors of muscle tissue are also seldom encountered in fishes (Harshbarger, 1965–81; Wellings, 1969; Mawdesley-Thomas, 1975). Leiomyomas are well delimited tumors of the smooth musculature. They are, therefore, most frequently encountered on organs in the body cavity and can grow to considerable size. Histologically, they are formed of interwoven bundles of long, spindle-shaped smooth muscle cells that are still capable of contraction.

Rhabdomyomas are formed by not fully differentiated striated muscle fibers lying in alternate planes. The cells can be so dedifferentiated that the striations become unrecognizable and the tumor is increasingly dominated by arachnocytes, cells with many processes. Formation of metastases and invasive growth are possible (rhabdomyosarcomas). Rhabdomyomas of fishes occur mainly in the trunk musculature, in which they form massive tumor nodules that appear as lumps on the surface of the body or on the internal wall of the body cavity.

Hemangioma

Hemangiomas are formed by excessive proliferation of capillary-like blood vessels, which are so thickly packed that little room is left for the connective tissue stroma. In some cases, however, the blood vessels are greatly dilated and separated by connective tissue septa containing collagen fibers. Hemangiomas are blood-red benign tumors with a spongy structure. They are rare but very obvious growths that can occur singly or in groups. In fishes, they have been observed mainly in the musculature of the trunk (Wellings, 1969).

For many years, in the North Sea large proportions of the fish caught (50% or more) — especially gadids and clupeids — had blood traces in their eyes, sometimes associated with eye cloudiness. These were generally assumed to have resulted from mechanical damage associated with trawler operations. Recently, however, investigations have indicated that this condition is actually a hemangioma-like excrescence originating in blood vessels of the chorioidea or chorioideal body (Watermann and Dethlefsen, pers. comm.). This condition leads to extensive displacement and destruction of retina and vitreous body. Furthermore, lens and iris can also be destroyed. Fishes with scar-tissue-filled or covered eye sockets could be taken as evidence that this condition can result in the total destruction of the eye, finally also involving its ejection. More intensive investigations of this phenomenon are about to be undertaken.

Lymphoma, Lymphosarcoma

Fishes lack the specialized lymphatic organs of higher vertebrates, such as lymph nodes and bone marrow. Hemopoiesis occurs chiefly in the kidneys of teleosts (cephalic kidney), to a lesser extent in the spleen and intestinal submucosa. Kidneys and spleen are the most frequent sites of the so-called lymphomas or lymphosarcomas in fishes. Other primary sites include thymus, liver, dermal connective tissue, and other body regions, which the tumors may also penetrate invasively (Nigrelli, 1947b; Dunbar, 1969; Mulcahy, 1970; Sonstegard, 1975). These tumors consist of massive aggregations of uniform, round cells that appear to be lymphocytes differentiated to various degrees. Thus, some of the round cells may be relatively large with inflated nuclei containing several prominent nucleoli, while others resemble the small lymphocytes of fishes, with their very dense nuclei. The round cells probably originate as lymphocytes, but because this origin cannot (always) be demonstrated with certainty, the tumors have also been given the more neutral name round cell sarcomas (Wellings, 1969). Organs afflicted by these tumors generally display total swelling. In the skin, however, tumor nodules can also be found that later expand to form flattened swellings, and eventually ulcerate. From the thymus, located in the dorsolateral pharynx wall above the gill arches, sack-like growths can develop and cause a narrowing of the gill chamber.

In the central Baltic Sea, particularly in the coastal regions near Stockholm and along the northeast coast of Gotland Island, about 10% of the pike *Esox lucius* are afflicted with nodular or flattened lymphomas or lymphosarcomas in the dermal connective tissue (Ljungberg, 1976). The tumors consist of massive aggregations of lymphoid cells, 1.5 to 2 times as large as the normal lymphocytes. The tumors can be transmitted to healthy fishes by inoculation of cells containing tumor material. This can be accomplished by subcutaneous as well as intraperitoneal injection. In the latter case, the tumors form on the organs within the body cavity. Occasionally, lymphosarcomas are associated with epidermal hyperplasias that have intercellular spaces filled with large numbers of Type C virus particles, which apparently are formed by budding from the cell membrane (Winqvist and co-authors, 1968).

The lymphosarcomas of the pikes from the Baltic Sea correspond in all respects, including ultrastructure, to those of pikes *(Esox lucius)* and muskellunges *(E. masquinongy)* in Ireland and North America, which are wide-spread and have been extensively investigated to determine the course of the disease and its cause (Winqvist and co-authors, 1973; Mulcahy, 1976; Sonstegard, 1976). The disease appears initially as nodular aggregations of lymphoid cells in the skin of the head, flanks, or fins. The cells are similar in appearance to immature lymph or plasma cells. The nodules develop into flattened swellings of the skin that begin to ulcerate on the surface. Histologically, a deep infiltration reaching to the underlying muscle tissue can be detected. In late stages of the disease, kidneys and spleen and, finally, liver are affected. While the disease is fatal to more than 99% of the muskellunges, the lesions on *E. lucius* seem to heal in some cases, and recovery might even be the rule.

During the main part of the summer, muskellunges suffering from this disease can scarcely be found (< 0.5% afflicted). In autumn, increasing numbers with early disease stages are observed. The highest rates of affliction (as much as 21%) occur in the middle of spring, when the disease is in its late stages. Before early summer, the great majority of the

afflicted fishes have died. In open waters, the disease occurs only among sexually mature fish.

The disease has been successfully induced in both Irish and North American pikes, as well as in muskellunges, not only through transplant but also through injection of cell-free tumor homogenate (Mulcahy and O'Leary, 1970; Sonstegard, 1976). The incubation period is 5 to 7 months for transplants, and 7 to 18 months when the disease is transmitted with cell-free extract. The success in transmitting the disease with cell-free extracts is evidence for a viral etiology; but in spite of intensive investigations employing the electron microscope, no virus particles could be found in tumor cells *in vivo* or *in vitro*. Papas and co-authors (1976), however, were able to concentrate 100 nm-diameter Type C virus particles containing RNA from tumor homogenate by fractionation. These virus particles correspond in size and morphology to the virions regularly encountered in epidermal proliferations of pike and muskellunge. The occurrence rate of these plaque-like epidermal hyperplasias during spring was several percent. As already described for pike from the Baltic Sea, these epidermal hyperplasias are sometimes associated with lymphosarcomas; that is, they sometimes affect epidermis that covers lymphosarcomas.

It is thought that the disease spreads from one fish to another by skin contact. Because the pike has a solitary mode of life, such contact occurs almost exclusively during the breeding season. Naturally, only sexually mature individuals would be affected. After spawning in spring, there is an incubation period of several months, and the early stages of the disease appear in autumn (Sonstegard, 1976).

Ljungberg (1976) mentioned that the Baltic Sea region, in which the affliction rate of pike with lymphosarcomas is remarkably high, is among the most polluted regions, particularly with chlorinated hydrocarbons. The disease was practically unknown in the Baltic Sea until about 1950, when the great increase in chlorinated hydrocarbon pollution began.

It is known from investigations of mammals that the activity of tumor viruses can be triggered by environmental factors, including the presence of harmful substances. Apparently, tumor diseases of fishes can also be induced or their rate of incidence be increased by a combination of virus infection and presence of harmful chemicals. The rate of tumor occurrence among the fishes of a highly polluted inland water body (Fox River, Illinois, USA) was compared with that in an almost uncontaminated lake in the same geographic region (Lake-of-the-Woods, Ontario, Canada) by Brown and co-authors (1973) from 1967 through 1972. A total of 17 species were examined for a wide variety of tumors on their outer and inner organs. In all species, the rate of tumor occurrence was greater in highly polluted waters. The average rate was 4.4% (1.2 to 12.2%), compared to 1% (0 to 2.6%) in relatively unpolluted waters. Among the tumors identified were lymphosarcoma of pike and subdermal connective-tissue sarcoma of walleye, both of which show clear signs of a viral etiology. These tumors were also encountered 3 to 4 times more frequently in highly polluted waters.

Myxoma, Myxosarcoma

Myxomas can be considered a special variant of fibroma. The star-shaped cells are arranged like a wide-mesh net in an intercellular substance that appears slimy or gelatinous. Fibers, mostly collagen, are thinly scattered in the mucoid ground substance (Stolk, 1958). Compound tumors, especially fibromyxomas, also occur. The few reports of these

tumors are mostly relatively old and concern flatfishes (Wellings, 1969). The myxomas of fishes seem to occur mainly in subdermal connective tissues.

Pigment Cell Tumors

Although erythrophores, xanthophores, and guanophores can form tumors, these are relatively unimportant in comparison to the melanomas formed by melanophores. Primary melanomas can occur wherever cells containing melanin are normally found. Such cells not only occur in skin and eyes but also in the meninges of brain and spinal cord, peritoneum and mesenteries, on blood vessels and nerve sheaths, in capsules and ligaments of kidneys and gonads, in intestinal walls, in adipose tissue, in the periosteum, within the musculature, etc. In fishes, however, the main primary sites of the melanomas are in the dermis and the chorioid of the eye.

Structurally, melanomas are massive aggregations of cells containing melanin, which form a tight net-work supported by fine strands of connective tissue and blood vessels forming the stroma. In slow-growing melanomas, large dendritic or star-shaped melanocytes are predominant. These differentiate into melanophores, incapable of reproduction, only in a few parts of the tumor (Vielkind, 1972; Sobel and co-authors, 1975). In contrast, fast-growing tumors are dominated by small spindle-shaped or even rounded melanocytes, that display little differentiation and contain only lesser quantities of melanin. The melanocytes are frequently binucleate.

In the skin the highly malignant melanomas, also called melanosarcomas, especially grow invasively. At first the overlying epidermis remains unchanged; it then displays a slight hyperplasia and becomes somewhat spongiotic. As tumor growth continues, the epidermis becomes thinner, until only 1 or 2 layers of cells remain. When the layer breaks and the basal lamina is lost, the ulcerous stage is reached. Thus, the body of the fish can actually disintegrate due to the action of the rapidly spreading tumors. Frequently, fishes afflicted with melanomas have already lost large parts of their fins and trunk before the destruction reaches a vital organ with fatal results.

Counterparts of the highly malignant melanoma are the deep-black pigment flecks that scarcely project above the body surface. These flecks, designated as premelanomas, occur relatively often on wild fishes, including species of flatfish. Premelanotic flecks reaching several cm in diameter are known to occur on rosefish *Sebastes marinus* from the Irminger Sea. Most of them develop just above the pectoral fins (Kosswig, pers. comm.). They are formed mainly of fully differentiated melanophores.

In marine waters, the fish most frequently afflicted with skin melanomas is *Argyrosomus argentatus*. In the estuaries of various Japanese rivers, occurrence rates between 8 and 52% were recorded (Kimura and co-authors, 1974).

The best investigated pigment-cell tumors are those of the viviparous poeciliids. Tumors displaying all degrees of malignancy can be obtained by crossing various species (Kosswig, 1929; Gordon, 1959; Anders and co-authors, 1972; Sobel and co-authors, 1975; Schwab and co-authors, 1979). The tumors form from black spots and bars of the colour pattern that contain macromelanophores. The malignancy of the tumors depends on the degree of imbalance between colour genes and regulator genes combined in the hybrids. The highest degree of malignancy is reached by unpigmented tumors (amelanotic

melanomas) in crosses involving the albino factor; these tumors contain only the precursors of melanin (Vielkind and co-authors, 1969; Vielkind and Vielkind, 1982).

The investigation of pigment-cell tumors in poeciliids has revealed how much the degree of differentiation of pigment cells and related events of tumor formation and the attainment of a special degree of malignancy are influenced by environmental factors, including such fundamental ones as nutrition, temperature, surface tension, salinity, and conductance of the water (Proewig, 1954; Anders, 1967; Anders and co-authors, 1972; Vielkind and co-authors, 1977). Furthermore, tumor growth is greatly influenced by substances such as cyclic AMP, ACTH, MSH, and testosterone (Vielkind, 1972; Vielkind and Vielkind, 1973). It is conceivable that the formation of melanomas in wild fishes is also triggered by a combination of stimulating environmental factors, by hormonal imbalances or by genetic mutations that affect the differentiation of melanocytes and melanophores.

Erythrophoromas and xanthophoromas correspond to melanomas in structure as well as in growth. With increasing malignancy, the dendritic chromatophores are increasingly replaced by spindle-shaped or rounded cells. The tumors can also be produced in crossing experiments involving viviparous poeciliids, and they react to changes in the external and internal milieu similarly to melanomas. However, they do not appear to reach the melanomas' extreme degree of malignancy. Cases are known of erythrophoromas and xanthophoromas, that are already growing invasively, being infiltrated by melanophores or melanocytes to the extent that the primary tumor degenerates and is replaced by a highly malignant melanoma (Nigrelli and co-authors, 1951).

Pigment-cell tumors display an extremely invasive form of growth, but metastases are seldom formed (Smith, 1934; Stolk, 1959a, b). Because testosterone stimulates tumor growth, the average rate of occurrence among males is higher than among females; and number and degree of tumor malignancy in males are also greater. Guanophoromas have been observed only on extremely rare occasions; hence little is known about them (Takahashi, 1929; Stolk, 1959b).

Neural Tumors

Except neuroblastomas of the eye, no tumors of the fish central nervous system have been observed (Dawe and Harshbarger, 1975; Anders and Anders, 1978). In contrast, there are numerous reports of subcutaneous neurilemmonas and neurofibromas (Wellings, 1969). Neurilemmonas develop from Schwann cells surrounding the nerve fibers, while neurofibromas are formed from the endo- and epineurium, connective tissue that accompanies the nerves. Both of these tumors are very difficult to distinguish from ordinary fibromas (Scarpelli, 1969). In fishes, diagnosis is based on the following characteristics: the whitish nodules that frequently occur in groups are located chiefly in the dorsolateral subcutis of head, body, and caudal fin at points along the lateral line system or the nerves running to the free neuromasts. Histologically, the tumors consist mainly of elongated spindle-shaped cells penetrated by numerous neurites that mostly run parallel to the longitudinal axis of the neurofibroma cells. These spindle-shaped cells may be arranged in palisade-like layers in places. Even the nuclei are strictly arranged in transverse rows, giving the tissue a cross-hatched appearance in histological preparations which is characteristic of Antoni Type A neurilemmonas of mammals. Other tumors or parts of a tumor consist of loose nearly edematous tissue represented by reticular cells, corresponding to

the Antoni Type B neurilemmonas of mammals. The tumors can be so large that they protrude above the surface of the body like a hernial sack, always remaining covered by a layer of normal skin.

Multiple tumors of this sort were found by Lucké (1942) along the nerve branches from the head to the dorsal part of the trunk in 0.5 to 1.0% of the snappers *(Lutianus griseus, L. jocu, L. apodus)* collected from Florida coastal waters. Comparable tumors were not found in other species from the same region, nor were the 3 species of snapper afflicted to any significant degree with other types of tumor.

In a pond, Schlumberger (1952, 1957) found 10% of the goldfish *Carassius auratus* afflicted with multiple tumors of nerve sheaths in skin and visceral cavity. It is still disputed whether similar tumors in other goldfish populations and in brook trout *Salvelinus fontinalis* should be designated as fibromas or as neurofibromas and neurilemmonas (Young and Olafson, 1944; Duncan and Harkin, 1968; Wellings, 1969). In all other cases, neurofibromas and neurilemmonas have been reported as isolated occurrences. The same can be said of other tumors of the nervous system, such as ganglioneuromas of the spinal ganglia and neuroepitheliomas of the olfactory epithelium, which are still being diagnosed with uncertainty (Schlumberger and Lucké, 1948). Invasive growth of these tumors occurs occasionally (Finkelstein and Danchenko-Ryzchkova, 1965).

Diseases Caused by Neoplasia: Conclusions

Local dedifferentiation and escape from growth control, which occur during tumor formation, are deviations from the norm submitted to natural selection. This is especially true in animals that generally remain reproductively active for their entire lives, such as fishes. Thus, even tumors that first appear when the fish has reached an advanced age affect the process of natural selection. This fact has certainly contributed to the evolution of such mechanisms as DNA repair and immunological defense, that are able to correct disorders capable of promoting tumor formation, or even eliminate tumors that have already begun to form.

Evolutionary adaptation required to colonize and optimally exploit a habitat is a very slow process effected mainly by natural selection, which, in turn, depends on variability within biological populations. Variants have different degrees of advantage or disadvantage in their chances to reproduce. In spite of modifying environmental influences, variants are in general based on the genetic background. Tumorous individuals are among those variants that nearly always face a disadvantage in natural selection. Occasional tumor diseases among a population can be considered rare occurrences that serve, or are even necessary, to keep the gene pool of a population continually programmed for a high degree of resistance against tumor formation.

Whereas the occasional occurrence of tumor diseases may be a normal phenomenon, rates of tumor affliction at or above 1% or even epidemic outbreaks, can only be attributed to sudden changes in the organism-environment system. In such cases, not enough time has been available for a natural selection process to readjust and establish a new level of tumor resistance. Such changes can be classified in 3 categories:

(i) Two or more populations or subspecies of a single species, or of closely related species, increase their distributional ranges. Some of the individuals interbreed and produce unbalanced genotypes with reduced tumor resistance under prevailing

environmental conditions. Good examples of this are pigment-cell tumors in hybrid poeciliids (p. 420). The occurrence of neurilemmonas in 3 species of snapper of the genus *Lutianus* from the same coastal region off Florida could also have been the result of a partial mixing of the populations (p. 422).

(ii) An infectious agent, usually a virus in the case of tumor disease, reaches a host population and spreads rapidly. An example of such a case was unequivocally demonstrated by the experimental production of epidermal tumors on young *Oncorhynchus keta* by infection with a virus isolated from the ovary of *O. masou* (p. 407). But the spread of a virus-induced tumor disease can also be attributed to the triggering of a latent viral infection by a sudden change of environmental conditions (see below).

(iii) The environment of a species changes, or the species invades a new environment. The first case has probably become a world-wide problem because man-made pollution of environment and food with increasing quantities and numbers of different pollutants has reached enormous proportions in the past few decades. While it is difficult to establish such correlations in individual case histories, there is much evidence to demonstrate that such correlations exist. Examples include increases in numbers of skin tumors on dab near a TiO_2 wastes dumping area in the German Bight, North Sea (p. 406); of liver tumors in various species from highly polluted coastal waters and estuaries (p. 410–411); and the significantly increased rate of lymphosarcoma occurrence among pikes from coastal and inland waters heavily polluted with wastewater (p. 419).

The example of pike lymphosarcomas shows that a combination of carcinogenic factors is often required to produce a tumor disease. This is the only acceptable explanation for the observation that some tumor diseases, such as papillomatosis of the eel, are strictly limited to certain regions, while the host species inhabits a much greater range, most parts of which seem to be just as polluted as the area in which the disease occurs. Like other characteristic features of an organism, tumors react to the total effect of a variety of environmental factors, including temperature and salinity.

When a host population is not fully eliminated by the sudden spread of a tumor disease reaching epizootic proportions, it can be expected that conditions in a once more stabilized environment will normalize over the long term, so that the disease will again become of infrequent occurrence. This concerns mainly tumor diseases resulting in severe disadvantage or even mortality among the afflicted animals. On the contrary, it is remarkable how often such conditions as thyroid hyperplasias, hyperplastic liver nodules, and epidermal hyperplasias develop in many fish species without becoming tumors, displaying considerable structural modification and partially autonomic growth. Instead, the hyperplasias are resorbed, rejected, or possibly dismantled by phagocytic cells.

DISEASES CAUSED BY ENVIRONMENTAL STRESSORS

GARY A. WEDEMEYER and C. PHILLIP GOODYEAR

The use of the terms 'stress' and 'stressor' is sometimes inconsistent (e.g., Pickering, 1981). The term 'stressor' should be used to describe environmental or other factor intensities severe enough to require a compensatory response at any level of biological organization. A stressor is normally extrinsic. The term 'stress' indicates the organismic response initiated by the stressor, also at any level of biological organization. Thus, the original concept of Selye (1950) that stress is 'the sum of all the physiological responses by which an animal tries to maintain or re-establish a normal metabolism in the face of a physical or chemical force' has evolved into the concept that stress is the biological effect of any force that challenges homeostatic or stabilizing processes and extends them beyond their normal limits, at any level of biological organization — individual, population, or ecosystem (Esch and Hazen, 1978; Bayne, 1980).

At the individual level, biotic or abiotic factor intensities severe enough to be considered stressors are those that require physiological compensation (a stress response) by the affected fish. If tolerance limits are not exceeded, the stress response will succeed in maintaining health by establishing a new equilibrium between the changed environment and the fish. Thus, if fishes can achieve compensation (non-genetic adaptation), their probability of survival will be enhanced. In contrast, failure to achieve compensation, and death of individuals, may increase the probability of survival for a population as a whole. For example, mortality due to stress caused by crowding may benefit the group by reducing population density.

At present, the tolerance to a stressor and the limits of non-genetic adaptation are not well-defined for most species, even with regard to biotic or abiotic stressors that occur singly. The problem is exacerbated by the fact that fish and fish populations are normally subjected to multiple stressors: unfavorable or fluctuating physical and chemical conditions such as temperature, salinity, dissolved oxygen, light intensity, and varying degrees of water pollution. In addition, actual physical trauma may occur; for example impingement or entrainment of larval fishes in power-plant cooling systems (see Barnett and Hardy, 1984 for review). Stressors such as these can impose a considerable load on the physiological systems of fishes. Acute or chronic stress that substantially exceeds tolerance limits tends to be readily apparent because it soon causes death. Sublethal stressor effects are more common and because the resulting stress tends to be both indirect and delayed, cause-and-effect relations are more difficult to recognize. Increased susceptibility to diseases, reduced tolerance to subsequent stressors, and reduced growth, longevity, and reproductive success, are characteristic consequences of sublethal stress. If stressful conditions persist over long periods of time, adverse effects at succeedingly higher levels of biological organization will occur which may or may not be reversible (Kinne, 1962; Rosenthal and Alderdice, 1976; Lugo, 1978). For example, chronic stress that exceeds tolerance limits becomes manifested at the population level as reduced recruitment to succeeding life stages, and eventually, exhaustion of the compensatory reserve (Ryan and

Harvey, 1977; McFarlane and Franzin, 1978). The effects of stress at the community/ecosystem level may be expressed as species shifts due to changes in energy flow between trophic levels. Thus, the original conditions may be permanently lost even if the stressful alterations are eventually corrected.

Responses to Environmental Stressors

Effects of environmental stressors at any of the higher levels of biological organization begin with effects at lower levels. For example, there can be no effects on fish populations in the absence of effects on individuals. An understanding of the physiology of the stress response is important for an understanding of how the stressors affect the living system and for a definition of tolerable limits for environmental alterations.

When fish are challenged by stressors, substantial physiological changes may occur in the course of compensating for changed conditions. Compensation can be achieved by means of endocrine, blood, and tissue changes. These are similar in most teleosts, although species-specific differences do occur (Schreck, 1981; Thomas and co-authors, 1981a, 1981b; Peters, 1982). A stressor causes the central nervous system to trigger the release of catecholamine and corticosteroid 'stress hormones'. These, in turn, initiate metabolic changes required for compensation. Where the stressor intensity remains within the physiological tolerance limit, compensation may be successful, though at some bioenergetic cost. If the stressor exceeds the physiological tolerance limit, compensation will eventually fail.

Since stressor effects manifest themselves at succeedingly higher levels of biological organization, the stress response may be considered at 3 levels: primary, secondary, and tertiary (Wedemeyer and McLeay, 1981).

(i) Primary Level: Endocrine System. The central nervous system records a physical, chemical, or biological event that is severe enough to require compensation. Adrenocorticotrophic hormone (ACTH), catecholamines, and corticosteroids are released from the pituitary and interrenal tissues (Mazeaud and co-authors, 1977; Donaldson, 1981; Mazeaud and co-authors, 1981; Schreck, 1981).

(ii) Secondary Level: Blood and Tissue Chemistry. Hormone-induced physiological alterations occur as the stress response continues. Hyperglycemia, hyperlacticemia, hypochloremia, leucopenia, and reduced blood clotting time are typical. In freshwater fishes, hemodilution, diuresis and blood electrolyte loss may occur as adverse side effects. In marine species, dehydration and hemoconcentration result. Liver glycogen and interrenal Vitamin C depletion occur, followed by interrenal hypertrophy and loss of cellular function (Chavin, 1973; Peters, 1979; Wedemeyer and McLeay, 1981).

(iii) Tertiary Level: Individuals and Populations. Stressors eliciting biochemical and physiological responses first affect the performance of individuals, then the population.

In individuals, major adverse performance effects include: changes in normal predator avoidance, feeding, and reproductive behavior (Goodyear, 1972; Billard and co-authors, 1981). A possible mechanism may be the depletion of biogenic amine stores in the brain which have been associated (Thomas and co-authors, 1981b) with behavioral disturbances in striped mullet *Mugil cephalus*. Other effects include decreased condition as measured by such indices as proportional stock density and relative weight (Anderson, 1980); reduced tolerance to subsequent stressors (Wedemeyer and McLeay, 1981); and impaired fish

health, such as increased morbidity and mortality rates due to infectious and non-infectious diseases (Snieszko, 1974; Wedemeyer and co-authors, 1976; Hodgins and co-authors, 1977; Ellis, 1981; G. Peters and co-authors, 1981).

Physiological responses that have altered growth, survival, longevity, or reproduction, are likely to become manifested at the population level through effects on density-dependent compensatory processes (George, 1977; Ryan and Harvey, 1977; McFarlane and Franzin, 1978; Esch and Hazen, 1980; Goodyear, 1980). The effects of stress on fish health are particularly important since poor health tends to reduce survival, growth, and reproductive success, and thus to reduce the intrinsic growth rate of the population. The resulting strain on population renewal processes will eventually reduce the capacity to compensate for additional stressors (e.g., Rosenthal and Alderdice, 1976; Goodyear, 1980). Thus, the long-term significance of the effects of stress and disease on individual fish is a function of the degree to which the size, productivity, or persistence of fish populations are ultimately affected.

Reliable estimates of population-level responses to stress and disease are difficult to obtain. Predictive methodologies to assess responses to single and multiple stressors do not yet exist, except where substantial mortalities can be expected to occur. In situations where environmental stressors affect physiology or behavior to such an extent that normal growth or mortality patterns are altered, the response of the population will be determined by the outcome of all the intra- and interspecies interactions that influence the rates of survival, growth, and reproduction of the affected individuals.

The problem centers around the basic concept in population dynamics that the status of any population at a particular time is determined by the balance between mortality and natality. Thus, mortality of individual fish due to disease instead of due to senescence only alters the timing of their inevitable death. If the population is to be stable, losses due to mortality must be offset by reproductive success. If the population is to increase, recruitment to succeeding life stages must exceed the losses from mortality. If mortality exceeds recruitment, the population will decline. An increasing population will eventually experience resource limitations and mortality rates will then increase and/or reproductive success will decline until a new equilibrium is reached. In a declining population, mortality will tend to decrease and/or reproductive success to increase until a new equilibrium occurs. To prevent eventual extinction, the average number of young produced must equal the average number of deaths. Thus, an increase in the death rate from stress and disease (that reduces the number of fish surviving to spawn) must be accompanied by a compensatory increase in the number or survival rates of larvae produced per unit spawner. The fact that populations are so regulated is well-illustrated by the continued persistence of species commercially exploited by man. However, there are limits to the ability of populations to compensate by such density-dependent processes. It is also apparent that when part of the compensatory reserve has been used, the tolerance of the affected population to subsequent stress factors will be correspondingly reduced (Sullivan and co-authors, 1978).

A number of density-dependent mechanisms can result in a compensatory response when stress has adversely affected growth, reproduction or survival of individuals (Goodyear, 1980). Thus, merely documenting a particular incidence of mortality from stress and disease does not necessarily establish that the population as a whole has been, or will be, affected. A discussion of a few direct and indirect pathways by which stress may be manifested illustrates the practical and theoretical aspects involved. If only a species is

directly affected, the ultimate effect of an increase in mortality due to diseases on population size and persistance will be little different from mortality from any other cause. Any differences would be due to changes that might occur at higher levels of biological organization if the biomass from disease mortality was channeled into different ecosystem components which themselves influenced the growth or survival of the individuals in the population in question. If no such changes occur, an increase in direct mortality from disease will correspondingly decrease the remaining compensatory reserve of the population (Goodyear, 1977). However, whether the affected population will decline or not depends on the nature of the mechanisms that control the relation between stock size and recruitment.

Interactions between a population and other community/ecosystem components can indirectly influence its growth and mortality rates. For example, if changes occur in a community that affect species composition, and because populations are normally in part controlled by interactions with other species, then it will be difficult to establish a link between population declines and particular habitat alterations.

An environmental stressor that is not directly lethal to a particular species may indirectly result in population declines by reducing the availability of food to some critical life stage (Johnson, 1968). The effect may be either on the food organisms themselves, or on their prey items. The resulting reduced ration available to the species concerned would decrease growth and increase the probability of predation, starvation and disease.

Alternatively, stress-related mortality in predators of a competitor could increase the population of the competitor species to the detriment of the population concerned. It is theoretically possible for the mortality rate of a population subjected to environmental stressors to decline. For example, a stressor may have a greater detrimental effect on a principal predator than on the species in question. Similarly, a significant reduction in a competitor population could occur which then resulted in an increased food supply to the population concerned. In such cases, the ultimate result of stress could be an increase in the abundance of a particular species.

In situations where density-independent environmental factors have a strong influence on the relative composition of the community, the relative year-class strength of the various fish species may undergo rather substantial annual fluctuations. These fluctuations may also cause the importance of species interactions to vary from year to year. In cases where such variation is important, it will cause further complications in establishing the relations between disease incidence and stressors such as contaminants. These complications arise from the natural variability in populations and, more importantly, from the fact that the response mechanism will probably vary from year to year. Thus, to achieve statistical validity, a longtime-series of data is usually required which may be impractical to obtain (McCaughran, 1977). Unfortunately, even when a population decline can be measured, it will frequently only be possible to correlate this decline with variations in environmental and biological factors rather than to establish cause and effect. If fishing pressure is simultaneously acting to depress abundance, the role of environmental factors in a decline will be still more difficult to determine (for review consult Gulland, 1983).

Pathobiology of the Stress Response: Fish Diseases

Host-Pathogen-Environment Relations

The concept that stressful environmental alterations will pre-dispose fish to infectious and non-infectious diseases which was pioneered by Snieszko and co-workers, has become well-accepted in recent years (e.g., Snieszko, 1972, 1974; Sindermann, 1980). Thus, it is now clear that diseases are commonly not single-caused events and that mere pathogen exposure will not necessarily result in epizootics (Bullock and Snieszko, 1969; Isenberg and Ballows, 1981; for details consult Volume I: Kinne, 1980a, b). In most cases, an equilibrium exists in the interactions between fish, their pathogens, and the aquatic environment which must be altered for disease to occur (Wedemeyer and co-authors, 1976; Esch and Hazen, 1980; Walters and Plumb, 1980). The fact that disease is one result of the interaction between a stimulus and the response of a biological system suggests that an understanding of fish diseases is a required aspect of an understanding of the biology of fishes *per se* (Volume I: Kinne, 1980a).

The interactions between fish, their environment, and their pathogens that can lead to fish diseases are complex, but the particular environmental conditions associated with the initiation of epizootics are becoming somewhat better defined (Christensen, 1980). Ecological aspects such as interspecific interactions and population density are also known potential stressors (Schreck, 1981; Klinger and co-authors, 1983). Behavioral factors such as social dominance may also be relevant. For example, only carp *Cyprinus carpio* prominent in the social dominance hierarchy produced antibodies against trypanosomes (Barrow, 1955). More recent research has shown that subordinate fishes in social hierarchies tend to be chronically stressed compared with more dominant individuals (Ejike and Schreck, 1980; Peters and co-authors, 1980; Scott and Currie, 1980). Thus, both abiotic and biotic stressors should be considered in assessing potential causes for disease.

It has also become apparent that the occurrence of disease in fish populations is a potential indicator of environmental pollution (Mearns and Sherwood, 1974; Sindermann, 1980; Malins and co-authors, 1982). When the host-pathogen-environment relation is favorable for the host, fish populations normally exhibit good health, growth, and survival. When this relation is impaired, the incidence of infectious and non-infectious diseases will begin to increase. When the relation is poor, health problems accompanied by reduced growth, and survival, may become chronic (Roberts, 1978; Schäperclaus, 1979).

Vibriosis *(Vibrio anguillarum),* viral erythrocytic necrosis (VEN), myxobacterial gill disease (Myxobacteria species), and bacterial hemorrhagic septicemia *(Aeromonas liquefaciens)* are stress-mediated infectious diseases which have become classic examples in aquaculture (Wedemeyer, 1970; Snieszko, 1974). Coagulated yolk, a disease of salmonid eggs and alevins, liver neoplasms, epithelial papillomas, skeletal deformities, chromosomal anomalies, and the life-threatening diuresis that can occur as a result of transporting freshwater fishes are examples of non-infectious health problems which have been associated with otherwise sublethal environmental stressors (Wedemeyer and co-authors, 1976; Sindermann, 1979, 1980; Malins and co-authors, 1982).

As mentioned, stress-mediated fish diseases have promise as biological indicators of adverse environmental quality (i.e., in biological monitoring). Of the infectious fish diseases, those due to facultative bacterial pathogens such as aeromonads, pseudomonads, and the Myxobacteria, are particularly well-suited because such microorganisms are

ubiquitous in both inland and marine waters. For example, the incidence of Myxobacterial infections in migrating anadromous salmonids in the Columbia River (USA) is a function of both water temperature and fish density in the ladders around the dams (Pacha and Ordal, 1970). Infectious fish diseases of marine species which may indicate that tolerance limits to stress have been exceeded include vibriosis *(Vibrio anguillarum),* fin rot (fin erosion, *Aeromonas* and *Pseudomonas* sp.), ulcer disease *(Vibrio anguillarum),* red disease *(Vibrio anguillarum),* Lymphocystis and papillomatosis, and Ichthyophoniasis or other fungus infestations (Mearns and Sherwood, 1974; Wellings and co-authors, 1976; Christensen, 1980). Viral erythrocytic necrosis may be a potentially useful indicator disease in estuarine habitats due to the fact that infected fish show reduced tolerance limits to altered environmental parameters such as dissolved oxygen concentrations (MacMillan and co-authors, 1980).

Non-infectious diseases that offer promise as indicators of adverse conditions in the marine environment include: chromosomal and morphological abnormalities of eggs and larvae, skeletal anomalies, and neoplasms (Sindermann, 1979, 1980).

In most instances, the epizootiological principles discussed above will be followed. Exceptions must be interpreted with caution. For example, undetected asymptomatic or latent infections may be activated by a later stressful event with the result that an epizootic may occur in the apparent absence of pathogen exposure. Also, if pathogens are present in sufficient numbers, infections may be expected regardless of a favorable environment and healthy physiological conditions (for details consult Volume I: Kinne, 1980b).

Statements on habitat requirements of fishes that do not include information on the fish-pathogen-environment relation are incomplete. More information on stress factors which can potentiate infectious processes are of particular importance both in aquaculture and in assessing the biological costs associated with proposed alterations in the aquatic environment.

A number of environmental alterations have now been associated with poor fish health. These include unfavorable or fluctuating temperatures, dissolved oxygen concentration, and salinities, as well as exposure to contaminants (Wedemeyer and co-authors, 1976; Knittel, 1981; Malins and co-authors, 1980, 1982). A summary of stress-mediated diseases, together with a description of common environmental conditions implicated in their occurrence, is presented in Table 1–30.

Infection to Disease: Mechanisms

As discussed, the response to a stressor tends to alter the functioning of organ systems in such a way as to achieve compensation and thus to increase survival. Although disease resistance (Volume I: Kinne, 1980) may involve several aspects of the host-defense system — such as tissue repair, immunosuppression, inflammation, phagocytosis — the specific mechanisms involved in teleosts are poorly understood (Fletcher, 1978, 1981; Ellis, 1981; O'Neil, 1981).

Elevated levels of glucocorticosteroid hormones which occur in response to a stressor may be involved through the suppression of non-humoral (cellular) host-defense factors, such as the inflammatory response, thus allowing latent infections to become active or new infections to spread (Parrillo and Fauci, 1979). This phenomenon has been used to facilitate the development of fungus and bacterial infections in both warm and cold-water fishes (Roth, 1972; Bullock and Stuckey, 1975). Neish (1977) suggested that fungus

Table 1-30
Summary of stress-mediated fish diseases and of the environmental factors commonly associated with their occurrence. (Compiled from Esch and Hazen, 1980; Sindermann, 1980; Wedemeyer and McLeay, 1981; Malins and co-authors, 1982)

Fish diseases	Predisposing environmental factors
Furunculosis (Agent: *Aeromonas salmonicida*)	Low oxygen (4 mg l^{-1} for salmonids); crowding; handling in presence of the pathogen
Bacterial gill disease (Agent: Myxobacteria species)	Crowding; unfavorable environmental conditions; chronic low oxygen (4 mg l^{-1} for salmonids); elevated ammonia (more than 0.02 mg l^{-1} for salmonids); excessive amounts of particulate matter in water
Columnaris (Agent: *Flexibacter columnaris*)	Crowding or handling during warm-water periods if carrier fish are present; temperature increase if pathogens are present, even if fish are not crowded or handled
Kidney disease (Agent: *Renibacterium salmoninarum*)	Water hardness less than about 100 mg l^{-1} (as $CaCO_3$); unfavorable diet composition
Hemorrhagic septicemias Red-sore disease (Agent: *Aeromonas, Pseudomonas*)	External parasite infestations; inadequate pond cleaning; handling; crowding; elevated ammonia; low oxygen; chronic exposure to trace contaminants; elevated water temperatures; handling after overwintering at low temperatures
Vibriosis (Agent: *Vibrio anguillarum*)	Handling; dissolved oxygen lower than about 6 mg l^{-1}, especially at water temperatures of 10 to 15 °C; brackish water of 10 to 15 ‰ salinity
Parasite infestations	Overcrowding of fry and fingerlings; low oxygen; excessive size variation among fish in ponds
Spring viremia of carp	Handling after overwintering at low temperatures
Fin erosion	Crowding; low dissolved oxygen; nutritional imbalances; chronic exposure to trace contaminants; high total suspended solids; secondary bacterial invasion
Epithelial tumors	Chronic, sublethal contaminant exposure
Epithelial ulceration	Chronic, sublethal contaminant exposure
Skeletal anomalies	Chronic adverse environmental quality; PCB; heavy metals; Kepone; Toxaphene exposure; Vitamin C deficiency

infestations of adult salmon returning to fresh water were related to simultaneously occurring elevated cortisol levels. In mammals, the degradative phase of the phagocytic process in macrophages has become inhibited in individuals subjected to sufficient environmental impact to elevate glucocorticoids (Lockhard and co-authors, 1973). In teleosts, stressors associated with epizootics also activate the pituitary-interrenal axis and substantially increase circulating levels of 17-hydroxycorticosteroids (Mazeaud and co-authors, 1977; Barton and co-authors, 1980; Donaldson, 1981; Wedemeyer and McLeay, 1981). However, it is not yet clear that the physiological concentrations of glucocorticosteroids produced are sufficient to account for an impaired host-denfense system.

Nevertheless, the lymphopenia which accompanies the stress response in certain teleosts might be sufficient to play a role in reduced disease resistance (Pickford and co-authors, 1971; McLeay, 1975a,b).

Skin, mucus, and other aspects of the non-antibody host-defense mechanisms of fishes which may be affected by stressors have recently been discussed by Fletcher (1981). Cutaneous defense mechanisms can evidently be affected by glucocorticosteroids because cortisol implants increase normal susceptibility to *Saprolegnia* and *Ichythyopthirious* (Pickering and Christie, 1981). Necrotic cutaneous lesions associated with stress occur at the site of tag insertion if Atlantic salmon *Salmo salar* are exercised at high water temperatures (Morgan and Roberts, 1976). Stress can affect normal mucus production, but the role of such effects in disease susceptibility is not clear (Fletcher, 1978, 1981). Other non-antibody humoral factors such as ceruloplasmin, metallothionein, and C-reactive proteins have received some attention in fish in terms of their role in non-genetic adaptation, but any role in disease susceptibility remains to be established (Ramos and Smith, 1982; Fletcher, 1981). Thus, although there is agreement that the physiological changes induced by stressors include a variety of effects on the humoral and non-humoral defense systems, the data required to establish cause-and-effect relations is not yet available.

Available data indicates that the extent to which pathogen exposure progresses into infection and then to overt disease depends on the outcome of complex interactions between the biochemical systems of the invading organism, physical and chemical conditions in the aquatic environment, and the humoral and cellular defense systems of fish. In turn, the activation of pre-existing latent or subclinical infections is evidently also explicable in terms of fundamental biochemical and physiological mechanisms. Impairment of the reticuloendothelial system, other non-antibody defense systems, and immunosuppression associated with alterations in endocrine function seem to be the significant effects involved in potentiating infectious disease processes.

Non-infectious Diseases: Special Case of Anadromous Fishes

Non-infectious fish diseases can also be useful as indicators of adverse conditions in the aquatic environment. A particularly striking example is the impact of environmental factors on the parr-smolt transformation of anadromous salmonids (Wedemeyer and co-authors, 1980). Otherwise sublethal alterations in water chemistry, temperature, and photoperiod can inhibit the development of smoltification and result in poor health as indicated by impaired migratory behavior, salinity tolerance and, ultimately, survival in seawater. For example, the development pattern of the gill Na^+K^+ATPase system in coho salmon parr *Oncorhynchus kisutch* is impaired, in a dose-dependent manner, by otherwise sublethal copper exposure. Survival in freshwater, and growth are not affected; but the ability to hypoosmoregulate is impaired, and delayed mortality in seawater is likely (Lorz and McPherson, 1976). A particularly insidious biological consequence is that the normal migratory activity of smolts is also inhibited.

The survival of anadromous salmonids in seawater can also be affected if smolts are exposed to pollutants such as polychlorinated biphenyls, fuel oil, and herbicides which are now increasingly common in surface waters because of intensive forest and range management, industrial, and agricultural practices (Lorz and co-authors, 1978; Folmar and co-authors, 1982). Exposing juvenile coho salmon to the dimethylamine salts of 2,4-

dichlorophenoxyacetic (2,4-D) acid used for brush, weed, and vine control on non-crop lands inhibits their later migratory behavior as smolts. Other formulations, such as the esters of 2,4-D used for control of aquatic vegetation, may also inhibit the seawater tolerance and migratory activity of smolts inhabiting such waters.

Water temperature alterations can also adversely affect the parr-smolt transformation. The ensuing physiological effects may express themselves as delayed or accelerated smolt development or parr-reversion. In coho this effect is particularly dramatic. ATPase development is greatly retarded at 6 °C and accelerated at 20 °C (Zaugg and McLain, 1976). Since water temperatures above 15 °C accelerate growth, smolts can be produced in less time. Unfortunately, the rate of parr-reversion is also accelerated — which shortens the period during which migratory behavior, and thus survival in seawater, will be normal. Juvenile fall chinook salmon *Oncorhynchus tshawytscha* also revert to the parr form more rapidly at elevated water temperatures but this effect is minimized if the salinity is increased to between 10 and 20‰ (Clarke and co-authors, 1981).

For other anadromous salmonids, such as steelhead trout *Salmo gairdneri,* the effect of temperature can be more severe. For this species, the upper limit for normal ATPase development, but not for growth, is approximately 13 °C (Zaugg and Wagner, 1973). The temperature sensitivity of Atlantic salmon *Salmo salar* may be similar to that of steelhead trout.

A final aspect of otherwise sublethal stress is the effect of population density during rearing on the ultimate survival of anadromous salmonids in seawater. The physiological effects are eventually manifested as reduced ocean survival, but tests have revealed only minimal abnormalities in ATPase development and other aspects of smolt functionality (Strange and co-authors, 1977; Fagerlund and co-authors, 1981; Sandercock and Stone, 1982).

Effects of Diseases at the Population Level: Problems of Monitoring

As previously discussed, experience gained from aquaculture, and pollution surveillance and monitoring programs indicates that the presence of disease in fish populations is a potential indicator of unfavorable conditions existing in the aquatic environment concerned. However, several practical and theoretical factors must be considered to provide information on the effects of such fish diseases on population renewal processes. The actual outcome of increased mortality due to diseases will depend on the degree of change in the 'normal' mortality, and its timing in relation to the density-dependent processes that control population size. It is entirely possible for a population to decline, change very little, or to increase, if the density-dependent processes are sufficiently intense. Even in cases where population declines have actually occurred, they may be time-consuming to detect because normal year-to-year variations in population abundance are sometimes quite large.

Thus, even in the simplest case where diseases result in direct mortalities and no higher level of biological organization is directly affected, assessments of the role of such diseases in population declines present imposing technological problems. Two types of biological information are required. First, timing and extent of increased mortality attributable to disease must be determined. This could, perhaps, be extrapolated from testing under controlled conditions. Second, knowledge must be obtained of the nature,

timing, and intensity of the density-dependent processes that regulate the size of the population in question. The latter requirement is not restricted to assessing effects of diseases; it is also a long-standing, largely unsolved problem in fishery-resource management in general (Cushing, 1968; Hunter, 1976; Gulland, 1983). In the case of chronic, sublethal fish diseases the problem is further complicated by the fact that such diseases can be expected to result in physiological consequences which induce indirect mortality and effects on other species in the community. Thus, it is theoretically possible for chronic diseases such as liver neoplasms, which are associated with marine pollution, to have a greater impact on population density than might be predicted on the basis of their direct effects on individuals. Disease-induced changes in physiological function that directly cause mortality are well-recognized and generally amenable to field measurement. However, such changes may also modify the interactions of diseased fishes with other trophic levels or with the aquatic environment. Any mortality resulting from such interactions will be indirect, and may be all but impossible to quantify by presently available field-sampling techniques. However, one such indirect effect that can at least be estimated is effects on predator avoidance (Goodyear, 1972; Coutant, 1973; Kania and O'Hara, 1974; Sullivan and co-authors, 1978; Coutant and co-authors, 1979). If chronic fish diseases result in altered predation rates, the indirect mortality in the population can be expected to rise correspondingly. As with directly caused mortality however, the ultimate significance of indirectly caused mortality will also depend on its timing and magnitude with respect to the density-dependent processes that regulate population size. A second information requirement is the effect of poor fish health in reducing growth because of changes in feeding behavior or food conversion efficiency (e.g., Gilderhus, 1966; Broderius and Smith, 1979). If the early life stages of marine fish species are involved, seemingly minor reductions in growth rate can profoundly increase total, indirectly caused, mortality because of size-dependent survival (Goodyear, 1980). For example, the normal daily survival rate for a larval fish may be only 0.75 during the 30 days from hatching to a particular size. If slower growth increases this time to 33 days, the remaining population size would decrease by about 58%. If the normal daily survival rate has been decreased because of other direct or indirect effects of a particular fish disease, then the total increase in mortality because of reduced growth could be much greater.

Data should also be obtained on the effect of the disease in question on reproductive success. Poor fish health can affect reproduction through a variety of mechanisms. For example, reduced growth can both diminish the number of eggs produced by spawning females (e.g., Bagenal, 1963; Jensen, 1971), and delay the age at which sexual maturity is attained (e.g., Lett and Kohler, 1976; Shuter and Koonce, 1977). Information on both effects should be provided because the total lifetime egg production by females would be decreased. For such populations to remain stable, a compensatory response such as increased survival of the fewer eggs produced would be required.

Finally, information should be provided on the extent to which poor fish health reduces normal tolerance limits to extreme environmental conditions such as temperature, oxygen and salinity (Hazel and co-authors, 1971; Elliott, 1981; Hughes, 1981; Smart, 1981). This is because spatial and temporal variations in environmental conditions may, in turn, produce spatial and temporal variations in the survival of fish with sublethal chronic diseases. If such variability exists, it must be considered in determining age-specific survival rates in the population as a whole.

In conclusion, establishing the ecological significance of diseases revealed by marine surveillance and monitoring programs requires, as a minimum, information on changes in survival, growth, and reproductive rates, together with knowledge of the density-dependent processes that control population size. In situations where the indirect effects of disease must be assessed, none of the required information is particularly amenable to measurement. The problem is complicated by the fact that environmental alterations resulting in poor health in one species will usually also result in poor health in other members of the community. Thus, quantitative information on interactive effects may also be required. In general, difficulties in designing and conducting the research needed to evaluate the population-level significance of fish diseases are substantial even if the effects are obviously catastrophic.

Situations where the objective is pollution monitoring *per se,* are currently of major interest. New and improved information is needed in 2 general areas: (i) the laboratory testing required to derive 'no effect' levels under controlled conditions; and (ii) methodology for field verification.

Information needs in the first area are grounded in the biological concept that, effects on populations are an emergent property of effects on individuals. If there are no effects on individuals (including effects on individuals of any interacting populations), there can be no effects on the population. Thus, correctly designed single-species laboratory toxicity tests to determine contaminant exposure levels that have no effect can provide very relevant information (Mount, 1980).

In cases where information on the contribution of contaminants to poor fish health in a particular population must be obtained, the research effort must be increased to reflect the multiplicity of the biological interactions discussed above. The first step is to determine the extent to which the population is being exposed to contaminants. This requires the design and conduct of a statistically adequate sampling and chemical analysis program. If contaminants are found, controlled testing is required to determine their potential as a cause of the associated fish diseases. If positive results are obtained, relevance under field conditions must still be evaluated. This requires identification of any other environmental factor that may be simultaneously stressing the population, as well as information on the processes controlling population size. If the identified contaminants are found to be nontoxic to the species in question, it is still possible that they are exerting ecological effects through actions on other populations. As discussed, the full resolution of this question would require information on the dynamics of the entire ecosystem. Clearly, a complete study of this kind is beyond the capability of most research institutions. However, incremental progress would nevertheless advance knowledge so that future problems could be solved more easily. The lack of such basic ecological information is presently hindering the adequate definition of manegement alternatives available for solving many present-day fishery resource problems.

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