A word from the editor

The book series “MARINE ECOLOGY – A Comprehensive Treatise on Life in Oceans and Coastal Waters” (organized and edited by Otto Kinne and contributed to by numerous outstanding experts over years) is now freely available with online Open Access.

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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
29.04.2008

MARINE ECOLOGY

A Comprehensive, Integrated Treatise on Life in Oceans and Coastal Waters

Volume I ENVIRONMENTAL FACTORS

Volume II PHYSIOLOGICAL MECHANISMS

Volume III CULTIVATION

Volume IV DYNAMICS

Volume V OCEAN MANAGEMENT

MARINE ECOLOGY

A Comprehensive, Integrated Treatise on Life in Oceans and Coastal Waters

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VOLUME III
Cultivation
Part 3

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FOREWORD

to

VOLUME III: CULTIVATION

'Cultivation' reviews the information which has accumulated on our present capacity for supporting marine micro-organisms, plants and animals under environmental and nutritive conditions which are, to a considerable degree, controlled. The volume is subdivided into three parts, containing the following chapters*:

Part 1

Chapter 1: Introduction to Volume III
Chapter 2: Cultivation of Marine Organisms: Water-quality Management and Technology
Chapter 3: Cultivation of Micro-organisms
Chapter 4: Cultivation of Plants

Part 2

Chapter 5.1: Cultivation of Animals—Research Cultivation

Part 3

Chapter 5.11: Axenic Cultivation
Chapter 5.2: Commercial Cultivation (Aquaculture)
Chapter 6: Multispecies Culture and Microcosms
Chapter 7: Chemical Contamination of Culture Media: Assessment, Avoidance and Control

We have made every effort to present comprehensive reviews, covering essential aspects of the cultivation of marine organisms. It soon became apparent, however, that only in a few cases, comparative, critical assessments of different culture methods and technologies were possible. Many publications suffer from insufficient detail, or even total lack of information regarding source, environmental history and nutrition of the organisms cultivated or the culture method employed. Exact data on environmental factors—such as light, temperature, salinity or dissolved gases—and on diet are absolute requirements for proper evaluation of the results presented. No less important are the origin of the organisms concerned, culture-water quality and technological aspects.

Culture methods are often an outcome of empiricism and intuition. A technique is tried, and if it works, the investigator sticks with it, rationalizing only afterwards the reasons for its application and success. The factors truly critical to success have

* See Editorial Note, p. vi.

rarely been pinpointed. Some portions of the reviews presented must, therefore, remain tentative, descriptive or pragmatical.

Cultivation is not an end in itself. It serves as a means to solve specific research problems. Due to the large variety of problems and the overwhelming diversity of marine life, a multitude of different culture methods have been developed. In fact, concepts, goals and techniques applied in cultivation diverge more than in other branches of marine ecology.

Most experiments conducted on marine organisms involve elements of cultivation. Micro-organisms, crustaceans, molluscs and fishes, for example, have been maintained, reared or bred in thousands of experiments. It was neither possible nor desirable to consider all publications in detail. We have attempted to settle the conflict between our intention to present comprehensive accounts and the need to avoid undue repetition by tabulating the information at hand or by referring to pertinent books or reviews.

I acknowledge with pleasure the support, advice and criticism received from the contributors, as well as from Drs. D. F. ALDERDICE, J. R. BRETT, H. P. BULNHEIM, G. PERSOONE, A. GAERTNER and D. SIEBERS. Additional supporters are mentioned at the end of the respective chapters. The assistance of M. BLAKE, V. CLARK, J. MARSCHALL, H. L. NICHOLS, I. SCHRITT and H. WITT is deeply appreciated.

O.K.

Editorial Note

The two chapters originally envisaged to comprise Part 3 of Volume III—Diseases of Plants and Diseases of Animals—will not be published in this form. Together with a general introduction, Chapter 9 will appear in a separate two-part book:

O. KINNE (Ed.) *Diseases of Marine Animals*, Wiley, London.

The reasons for this change in our original concept are (i) the fundamental importance of animal diseases not only for cultivation, but also for proper ecological assessment of both distribution and performance of marine organisms; (ii) the large amount of information available on diseases of marine animals; (iii) the rather restricted information presently at hand on diseases of marine plants.

O.K.

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OF
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CULTIVATION

5. CULTIVATION OF ANIMALS

5.1 RESEARCH CULTIVATION

5.11 AXENIC CULTIVATION

L. PROVASOLI

(1) Introduction

Axenic cultivation originated with microbiology. The progress of this art along with the use of microbes in quantitative biology, and application of this type of cultivation to other organisms (or their parts, tissues), were due to the possibility of controlling the homogeneity and purity of biological material. Technical complexities have limited the use of axenic cultures of higher animals. Complexity of organisms, diversity of foods and of environmental conditions needed, all intensify the difficulty of providing axenic conditions suitable for growth and reproduction. The usual difficulties in devising complex artificial diets for insects, domesticated animals, and fishes are exacerbated by the necessity of asepsis and even more so when one needs to control chemical purity (i.e. chemically defined diets) as well as organismal purity.

Yet in approaching or achieving these two wholly unnatural conditions, information emerges, paradoxically, of ecological importance.

The use of chemically defined media and axenic cultures of phytoplankters, by revealing the essentiality of some vitamins, trace metals, and other nutrients, has added ecological determinants and permitted studies on rates of uptake and utilization of nutrients, and on synthesis and excretion of metabolites (p. 616). The ecological relevance of nutritional data on these producers is obvious because these organisms are osmotrophic and photosynthetic; hence their activity depends on the chemical and physical environmental variables.

The inherent phagotrophic peculiarities of animals complicate the analysis of nutritional components; growth now depends largely upon the availability, abundance, and chemical composition of the prey—a relationship that becomes more tangled as the food web becomes longer and more varied. Predation, mutualism, symbiosis, and parasitism need to be defined at the nutritional level and one can even consider the possibility that the seasonal variations in prey species, with their differences in chemical compositions, may affect growth rate, sexuality, and fertility of the predator.

The nutritional meaning of these relationships, and interdependencies of organisms can be discerned by combining axenic partners and by reconstructing defined, and increasingly complex biocenoses. Unexpected and subtler relationships between partners may then emerge, e.g., probiotic or antibiotic effects, either direct or mediated by exocrines, pheromones (p. 616), and other excreta in the medium.

The chemical definition of the essential nutritional requirements of the predator may be used to relate the nutritiousness of prey with its content of essential nutrients for the predator, and has been the basis for the exceedingly efficient diets for commercially important species (notably poultry, trout, silkworm, and other insects).

Few marine animals are in axenic culture and the results obtained are often preliminary. Because of the incompleteness of information, reference will often be made to freshwater species, or to phylogenetically related groups, in an effort to set the available information in a wider context.

(2) General Procedures

(a) Axenization

Initial axenization requires elimination of foreign organisms. This can be achieved by physical and chemical means, or their combination. Before the advent of antibiotics, axenization relied on diluting out the unwanted accompanying organisms while keeping constant or augmenting the number of organisms to be purified.

Mild, repeated centrifugation and use of nets, filter papers, and membranes of various porosity can eliminate most contaminants whose size or specific gravity substantially differ from the organism to be axenized. Following this preliminary sorting, organisms ranging from 10 to 1000 μm (protozoans, young larval forms) are collected under the dissecting microscope with a Pasteur pipette and transferred into sterile sea water. To avoid injury and to reduce to a minimum the liquid transferred with the organism from one bath to another, the bore of the pipette should be 4 to 6 times larger than the dimension of the organism to be transferred. Details on making micropipettes have been presented in DROOP (1969). The volume of sterile fluid in each bath should be proportional to the size of the organism to facilitate locating the organisms: 1 drop on a slide for 5 to 10 μm organisms (LWOFF, 1929); 0·5 to 1 ml in depression slides for 20 to 100 μm organisms; 2 to 10 ml in watch glasses, crystallizing dishes, or small Petri dishes for larger organisms. The number of sterile baths needed depends on the dilution factor (i.e. the volume of liquid carried with organisms and the volume of the bath [1 : 10–1 : 100] and the number of organisms to be diluted out [i.e. 10^6–10^{12} bacteria ml^{-1} in heavily infected cultures]).

Pipette washing is rewarding but tedious; a continuous dilution apparatus may be appealing (CLAFF, 1940). Behavioural habits or tactism may be profitably used to find easier and speedier dilution methods; e.g. 1-m-long migration tubes, V-shaped tubes for anaerobes (GLASER and CORIA, 1930), electromigration (SOLDO and VAN WAGTENDONK, 1971; p. 218).

Elimination by dilution of prey organisms larger than a few microns is easy; elimination of micro-organisms (bacteria, yeast, mould spores) is more difficult because undigested micro-organisms and the normal gut microflora of invertebrates have also to be eliminated. A high proportion of axenic protozoans can be obtained by alternating 4 series of 5 to 7 rapid baths with 3 prolonged baths. The rapid baths (5–10 mins each) serve to dilute out the contaminants; a prolonged bath (30–60 mins) allows emptying of vacuoles (ciliates) and gut. Serial washings have been used

to axenize freshwater crustaceans (STUART and co-authors, 1931) and species of *Tigriopus* (PROVASOLI and co-authors, 1959).

By combining serial washings with antibiotics the number of washings can be reduced and success increased. The effectiveness of antibiotics has often been overrated because in some cases axenization was obtained by using simply penicillin + streptomycin. Judging from many personal communications, failures far exceeded success; failures are seldom reported in print. A familiar complaint is that the same antibiotic treatments may succeed or fail under ostensibly identical conditions. This is not surprising because the bacterial flora varies widely in composition and size with seasons, localities, and biocenoses (SIEBURTH, 1967).

The uncertainty of results reflects also the properties of antibiotics: most antibiotics are bacteriostatic, inhibit a limited bacterial spectrum, were screened and hence were most effective against human pathogens. Since the marine microflora is poor in Gram-positive and rich in Gram-negative bacteria and since many Gram-negatives resist most antibiotics, mixtures of wide-spectrum antibiotics offer better prospects. Penicillin seems to inhibit quite adequately most marine Gram-positives and is generally non-toxic in high concentration (1–2·000 U ml^{-1}) to protozoans and invertebrates. Combinations of streptomycin, tetracyclins, chloramphenicol, polymixin B, and neomycin are used to inhibit the Gram-negatives; these four antibiotics are often more toxic to invertebrates than are penicillin + streptomycin and are used at 20 to 100 μg ml^{-1}. Sulpha drugs are also quite useful against Gram-negatives; KANAZAWA (1968) reported that a mixture of sulphamerazine, sulphisomidine, sulphisoxazole, and homosulphanilamide was very helpful in axenizing several seaweeds (10-min treatment at 0·02% for each sulpha drug with shaking, followed by cultivation for 15–30 days in the presence of 0·005% of each; the medium was changed every few days; see also Chapter 4.1).

Since antibacterial antibiotics are bactericidal only in high concentrations and are most active against dividing bacteria, it is advisable to use the highest concentration tolerated by the animals for a short period (overnight) in a medium favouring limited bacterial growth thanks to the addition of small quantities of peptones, yeast, or liver extracts (0·1–1 mg%) and sugars (1–5 mg%). The surviving animals are washed several times the morning after and inoculated in separate tubes of media favouring growth. Alternatively, lower doses of antibiotics are added also to the growth media.

Incomplete axenization often results in dense growth of the surviving contaminants, which are no longer restrained by competitors. Most obnoxious are yeast and fungi because the antifungal antibiotics are poorly soluble and often quite toxic. Fungizone, mycostatin (nystatin) (LEE and co-authors, 1970) and candicidin (PROVASOLI and GOLD, 1962) were successful but only after prolonged treatments. It is therefore advisable to avoid fungal or yeast contamination by washing the organisms repeatedly as soon as possible after collection from nature. Residual bacterial infections can be eliminated by testing their sensitivity to antibiotics, by plating the supernatant on nutrient agar, and using the Baltimore Biological Laboratory Sensidiscs, and so selecting the most active antibiotic or sulpha drug.

Some stages of animals are easier to axenize than others. Resting stages, such as cysts of protozoans, durable eggs of *Artemia salina*, and many eggs, are clad in poorly permeable coats, hence strongly bactericidal solutions can be used to

sterilize their surface. Short immersions in solutions of $HgCl_2$ or Merthiolate, followed immediately by ample dilution of the poison by a continuous flow of sterile sea water in closed receptacles with a filter pad at the bottom or repeated centrifugations have been successful. Motile and non-feeding stages, such as freshly hatched larvae and planulae of coelenterates, are easy to purify by washings, as are animals right after a moult because their surfaces are almost aseptic (PROVASOLI and co-authors, 1959).

(b) Sustenance Media

Only in very rare instances can one rear the axenized organism directly on artificial food, let alone chemically defined media. Obviously, direct transfer to artificial media should be attempted only when enough information is available on the food requirements of closely related species or of animals phylogenetically unrelated but feeding or preying on the same type of food. This information is rarely available for marine animals since few have been grown axenically.

A convenient approach is to aim at an intermediate step: that of growing the purified animals on living food (i.e. gnotobiotic cultures, the food organisms being mono- or dixenic, if possible). For animals feeding on micro-organisms and small algae, gnotobiotic cultures can be obtained either by eliminating the contaminants gradually or by feeding the axenized predators with axenic cultures of food organisms.

Several gnotobiotic cultures of foraminiferans and ciliates were thus obtained, even monoxenic cultures of *Allogromia* sp. (LEE and PIERCE, 1963) and of *Uronema marinum* (LEE and co-authors, 1971b) fed on one bacterial species. The availability of many axenic cultures of marine unicellular algae permitted several cultures of herbivorous crustaceans (p. 742), and should allow similar cultures of the herbivores belonging to other marine phyla. The sea squirts are good candidates for mono- or dixenic cultures since they grow well in agnotobiotic cultures and have been used for genetic investigations by SABBADIN (1971).

The advantage of this intermediate step is the availability of permanent cultures from which it is possible to obtain, with less effort, a continuous supply of axenic animals for inoculating a variety of artificial media. This goal is facilitated by selecting food organisms which are easy to eliminate: either by using an antibiotic to which they are particularly sensitive (bacteria) or by transferring the cultures to non-nutrient media kept in the dark, followed by a few washings (algae); if obligate phototrophic algae are chosen, darkness alone will suffice (DROOP, 1970; MCGINN, 1971). The intermediate step of monoxenic cultures can be avoided only in particularly favourable circumstances: the durable eggs (cysts) of *Artemia salina* are commercially available and resist bacteriocidal agents; an aseptic inoculum is thus readily obtained.

Devising an artificial medium is often an art, since representatives of many phyla or orders, even non-marine, have never been cultured. Guidance in finding crude materials rich in diverse nutrients can be derived from the literature on nutrition. Particularly useful are the symposia edited by DOUGHERTY (1959) and KINNE and BULNHEIM (1970) on cultivation of invertebrates, and the book by TAYLOR and BAKER (1968) on cultivating parasites. Inspection of the commercial

diets employed for pets, poultry, rats, trout, and the compilation of insect media (HOUSE and co-authors, 1971) will suggest a variety of nutrient sources.

The goal at this stage is to provide a 'complete' medium despite ignorance of specific requirements. In broad lines the essential nutrients are: minerals and trace elements, sources of energy and carbon (carbohydrates, fats), special building blocks (amino acids and nucleic acids), water and fat-soluble vitamins, lipids, sterols, and rarely, hormones. The raw materials often used include refined and unrefined proteins (skim milk, casein, albumen, fish protein, etc.), sugar, starch, crude RNA and DNA, blood serum, corn oil, crude lecithins, animal or vegetable sterols, blood, various yeast and liver preparations (extracts, homogenates, autolysates) and other vitamin-rich preparations.

Equally important—but often neglected—are the ratios of nutrients (proteins/carbohydrates/fats), appetite factors, the physical attributes of the diet (size, consistency) and possible toxicity of organic solutes.

(3) Gnotobiotic Cultures of Invertebrates

As mentioned, the first step toward achieving an axenic culture is to obtain continuously reproducing cultures of a predator on a restricted number of identified prey organisms. Gnotobiotic cultures and axenic species can be combined to form a planned artificial biocenosis to study the interactions between various species and to acquire information on population dynamics, predation, etc. (Chapter 6). Fortuitous, but often equally useful, are gnotobiotic cultures derived from the progressive elimination of accompanying species in the course of obtaining axenic cultures. Information on the kind of organisms that represent the actual or potential food for benthic foraminiferans was thus acquired by LEE and co-authors (1966) by tagging species of algae with ^{14}C and measuring the radio-activity of the predator—a rapid and sensitive method for sorting out the key prey species. The components of an extremely varied microflora, which were the important food organisms for foraminiferans, were thereby identified. Such an analysis leads also to simplification of the biocenosis by retaining only those prey species which contribute most to the growth of the desired species. When the bacterial components were eliminated it became apparent that the algal food, alone, failed to support continuous reproduction of 4 species of foraminiferans: 1 or 2 species of bacteria were necessary to provide essential nutrients not present in the algal food (MULLER and LEE, 1969).

The role of bacteria and other micro-organisms as food for protozoans was known long ago but hints are accumulating *that* bacteria perform other functions: e.g., some species of bacteria induce or repress sexuality in ciliates (CHATTON and CHATTON, 1925). The role of bacteria in invertebrate nutrition is largely unexplored.* In a thoughtful review on the nutrition of zooplankton EDMONDSON (1957) asks whether bacteria are utilized as food—an issue too often ignored by other ecologists. Similar gaps, in the consideration of all the significant variables involved, seem to beset mathematical modelling of the environment—brilliant mathematics can hardly compensate for poor ecology.

* Metamorphosis in the cnidarian *Hydractinia echinata* has been shown to be induced by bacteria (MÜLLER, 1969; see also p. 659).

In the process of axenizing, one often stumbles on unexpected effects caused by the elimination of one or more of the members of a natural community. In defining the nutritional value of different algae to *Tigriopus japonicus*, *Platymonas tetrathele* ('*Platymonas* No. 5') proved to be an incomplete food: it allowed only six generations of *T. japonicus*. Since *T. japonicus* had been maintained for years on *P. tetrathele* as the sole alga in bacterized cultures, the unidentified bacteria supplied the missing nutrients (no yeasts or other non-bacterial micro-organisms were present).

The nutritive value of algae for predators varies widely among algal species: some algae do not support growth of *Tigriopus* species to adulthood; some support growth to midget, infertile adults; and some to normal size, fertile adults (PROVASOLI and co-authors, 1959). Only rarely is one algal species a 'complete' food, i.e. permitting an indefinite number of generations. Similar results were obtained for a marine amoeba, *Oxyrrhis marina* and a rotifer by DROOP (1966) who gives a table on the nutritional value of 30 algae for monoxenic and agnotobiotic cultures of 12 predators. More often, two species of algae or more are needed to supply all the nutritional requirements of the predator, indicating that varied food is a safer way to satisfy all nutritional needs; algal blooms, constituted almost solely of one species, may often be an incomplete food.

However, the nutritional deficiencies of single species of algae for growing *Tigriopus japonicus* can also be relieved by adding vitamins to the culture medium (SHIRAISHI and PROVASOLI, 1959). This is apparently not an isolated phenomenon; vitamins and crude organic extracts relieved the nutritional deficiency caused by subnormal salinity stress of *Artemia salina*, fed on two algae (D'AGOSTINO and PROVASOLI, 1968). Twelve species of freshwater cladocerans could be grown indefinitely on one species of alga, *Chlamydomonas reinhardtii*, by MURPHY (1970) by adding a B vitamin mixture to the medium in which the crustacean and the alga were grown together. A definite nutritional hierarchy was found by MURPHY; some daphnids could be grown only when either the concentration or number of vitamins were higher.

Whether the vitamins and/or organic additions were beneficial directly to the crustacean or *via* algal uptake, was settled by growing *Chlamydomonas reinhardii* and *Scenedesmus obliquus*, the food organisms used for *Daphnia magna*, separately in a medium with and without vitamins and then supplying them to *D. magna* grown in a medium without vitamins. Only the algae grown in vitamins (not needed by *C. reinhardtii* and *S. obliquus*) permitted an indefinite number of generations of *D. magna*.

Hence, the nutritional value of the algae may be affected by the organic solutes present in waters (D'AGOSTINO and PROVASOLI, 1970). Conversely, organic enrichments may be very useful in obtaining continuous fertility of predators fed on algae or other prey in aseptic conditions.

These preliminary results indicate that at least some basic techniques are available to bring into gnotobiotic culture the marine organisms which can be reared in the laboratory on living prey (see also Chapter 5.1).

Newborn larvae of *Ostrea edulis* were disinfected with antibiotics and were grown monoxenically to settling stage by feeding them on *Monochrysis lutheri* (MILLAR and SCOTT, 1967). Indefinite monoxenic culture of the marine acoel turbellarian

Parotocelis luteola was obtained by KOZLOFF (1969) with either *Nitzschia dissipata* or *Navicula pavillardi* as food organisms; these diatoms were the only ones out of 12 species of diatoms tried which would support growth of the worm. Another acoel, the symbiotic *Convoluta roscoffensis*, can be grown monoxenically (PROVASOLI and co-authors, unpublished) in sterile sea water supplemented with nitrates, phosphates, trace metals, and 3 vitamins (ES enrichment; PROVASOLI, 1968) indicating that bacteria are not necessary for growth. A simple medium sufficed for *C. roscoffensis* because its symbiont, *Platymonas convolutae*, a photosynthetic chlorophyte, synthesizes and supplies *C. roscoffensis* with all nutrients needed. Some artificial symbionts belonging to the genus *Prasinocladus* performed the same feat, but several species of *Platymonas* were unable to do so (PROVASOLI and co-authors, 1968).

(4) Axenic Cultures of Protozoa

The free-living protozoans comprise an array of 'acellular' organisms of diverse origins; they range from non-photosynthetic flagellates, to Amoebae, Acantharia, Foraminifera, and ciliates. Nutritionally, most of them feed on micro-organisms and unicellular algae, but some feed on other protozoans or small larval forms of invertebrates. They share the same prey organisms with many filter-feeders and could be similarly lumped into the 'herbivores'—an ambiguous term borrowed from land food-chains which poorly defines the place of these organisms in the aquatic food web. Protozoans differ clearly from the filter-feeders in their varied ways of acquiring the same type of prey (Volume II: PANDIAN, 1975); thus, they can capture their food in locales unsuited to most filter-feeders.

Feeding on the same type of prey is not necessarily equivalent or synonymous with having the same chemical nutritional requirements. Exogenous nutritional requirements represent synthetic disabilities which seem to be mainly connected with phylogenetic position. Arranging the chapter subheadings in taxonomic order facilitates the distinction between general and group-specific nutritional requirements and comparisons between phylogenetic groups. As we will see, while phagotrophy is apparently the 'natural' way of feeding, many protozoans may display remarkable osmotrophic abilities—a physiological vestige presumably inherited from their osmotrophic, photosynthetic ancestors (see also Chapter 5.1).

(a) Colourless Flagellates

Three marine dinoflagellates have been grown axenically in defined media and two choanoflagellates on semi-defined media. Their nutritional requirements illustrate, albeit sketchily, the transition from photo-autotrophy to osmotrophic or phagotrophic heterotrophy.

Crypthecodinium cohnii (syn.: *Gyrodinium cohnii*)

Crypthecodinium cohnii is a small dinoflagellate which often abounds where species of *Fucus* decay (PRINGSHEIM, 1956). BIECHELER (1952) reports that the

swimming stage can trap *Bodo* sp., bring it near the flagellar pore and suck the cytoplasm of the prey within a few seconds. JAVORNICKY (1962) considers the swimming stage of *C. cohnii* a zoospore since no division occurs in this stage. Two or more divisions take place in the non-motile vegetative phase which lacks openings in its cell wall. Therefore, osmotrophy seems to prevail in acquiring nutrients.

Several strains of *Crypthecodinium cohnii* have been isolated and grown axenically in artificial media. The Massachusetts' strain (G.C.) (PROVASOLI and GOLD, 1962) and the Puerto Rico strain (P.R.) (GOLD and BAREN, 1966) were axenized by washings or with the help of antibiotic and antifungal mixtures and grown first in yeast digest (PRINGSHEIM, 1956) and acetate. They grow luxuriantly in simple defined media. Both are euryhaline (good but slow growth occurring even at 0·3 and 4% NaCl) and have a pH latitude unusual for a marine organism (optimum pH 5·7–7·2). They use ammonium salts as N sources; the G.C. strain—after isolation in a medium containing peptone and yeast digest—required histidine as N source (other amino acids were not utilized) but could be trained to grow in ammonium salts. The P.R. strain seems to utilize also glutamic acid and alanine at optimal temperatures (20°–28° C). Much better growth of the G.C. strain was obtained with a mixture of ammonium sulphate and histidine + betaine (other amines are also utilized). High phosphate, glycerophosphate, and nucleic acids are all a good source of phosphorous. *C. cohnii* utilizes a variety of carbon sources: glucose and glycerol are the best single C sources; ethanol, acetic and other fatty acids, succinic and fumaric were less effective when added alone; but a combination of glucose or glycerol with organic acids gave optimal growth. Biotin is an absolute requirement; thiamine is highly beneficial, but continuous low-density growth (20 transfers) was obtained in the absence of thiamine.

The only lack of versatility of *Crypthecodinium cohnii* is in response to temperature. Optimal growth for the G.C. strain occurred at 20° to 28° C; growth was inhibited at 15° to 20° and 30° C; no growth was obtained at 10° C or above 30° C. The P.R. strain had an optimum at 30° C and was inhibited at 20° and 35° C. To relieve the inhibition at 35° C, addition of tryptophan and proline and vitamin B_{12} was indispensable (GOLD and BAREN, 1966). New nutritional requirements at sublethal temperatures were similarly found in *Euglena gracilis* and *Ochromonas malhamensis* (HUTNER and co-authors, 1957) and may be of ecological importance (PROVASOLI, 1958), i.e. polluted waters may extend the geographical distribution to warmer waters.

The ability of *Crypthecodinium cohnii* to utilize several N, P, and C sources in defined media and to grow better in mixtures of nutrients, as well as its tolerance to pH, accords well for an organism thriving in an environment rich in a variety of degradation products. The PRINGSHEIM and MCLAUGHLIN strains cannot be grown in this defined medium and grow but poorly in a peptone-yeast digest-glucose-acetate medium, indicating additional undefined requirements. The sensitivity toward biotin can be used to assay this vitamin in sea water (PROVASOLI and GOLD, 1959). The ability to grow on agar (KELLER and co-authors, 1968) and the sexuality found in our strain by C. K. FRANKEL (personal communication), coupled with a good growth potential (4 millions ml^{-1} in 7 days), make of *C. cohnii* a very promising organism for biochemical research.

Oxyrrhis marina

Oxyrrhis marina is a voracious phagotrophic dinoflagellate common in the brackish supralittoral zone. It can be grown easily in the laboratory, monoxenically, on live yeast *Saccharomyces exiguus* or on the chlorophyte *Brachiomonas submarina*, (DROOP, 1953). It is extremely euryhaline (4–64‰ S) as are most supralittoral organisms. The optimal laboratory conditions are 16‰ salinity, 22·5° C and pH 8 to 10. *O. marina* does not tolerate salinities below 4‰, pH below 6·5 and temperature above 28° C (DROOP, 1959a). DROOP has unravelled its nutritional requirements in a series of papers which can serve as a model for such studies.

Oxyrrhis marina was freed of contaminants by micropipette washing and by profiting from its ability to grow on a variety of pure cultures of algae. DROOP (1959a) selected wisely *Nannochloris oculata* as the food organism; being an obligate phototroph it could be eliminated simply by inoculating *O. marina* in suitable media in the dark. This simplified greatly the finding of an artificial medium to replace the living prey—the most difficult step in axenic cultivation.

Months of failure, in which many complex supplements were tried—ranging from egg yolk to organ extracts—led to the discovery that *O. marina* needed the addition of 0·4 ml/100 of neutralized, strained but unfiltered lemon juice to a rich medium composed of Oxoid liver infusion, Bacto-Tryptone, tryptophan, glucose, soil extract, nitrate, and phosphate in sea water. Lemon juice had also been found essential for *Paramecium aurelia* by VAN WAGTENDONK and co-authors (1953). Stepwise substitution was then possible. Liver infusion and Tryptone could be substituted by vitamin-free Casaminoacids and three mixtures of B vitamins. Sea water and soil extract were replaced by artificial sea water. When it was found that the Tris buffer, and probably EDTA, were toxic, glycylglycine was used as pH buffer and histidine as metal chelator. A more defined 'untailored' maximal medium was successful; it contained minerals, trace metals, glycylglycine, histidine, 17 amino acids (supplied by 5 mixtures), 5 nucleic acid bases, 14 B vitamins (3 mixtures), 22 C sources (2 mixtures of carbohydrates and 3 mixtures of organic acids) and the lemon factor (a CCl_4 extract of lemon rind) dispersed by Na taurocholate. The essential nutrients were identified by removing from the medium all mixtures of nutrients of one type (amino acids, carbohydrates, organic acids, etc.) and testing each mixture separately and in pairs. The single components of the favourable mixes were likewise tried alone and in combination.

At the end of this protracted dissection it was found that *Oxyrrhis marina* needed as C source either acetate or ethanol (the carbon skeletons of carbohydrates and amino acids not being utilized as a C source). Valine was the most active amino acid available, alanine and proline were almost equally good, and proline acted as a growth factor when added to valine (other amino acids, NO_3 and NH_4 and urea were not utilized); vitamin B_{12} (mammalian specificity) and thiamine (or its thiazole moiety) were essential, and biotin was stimulatory. Except for the unknown lipid factors, *O. marina* is an 'acetate' flagellate not too different nutritionally from freshwater colourless flagellates (DROOP, 1959b). The surprise was the lipid factor: ubiquinone-6 (=C30) at 0·5 μg 100 ml^{-1} replaced the lemon factor. It was identified after extraction from grass juice and chromatographic separation

(DROOP and DOYLE, 1966). Once this key requirement was satisfied it was possible to demonstrate that a sterol was also needed but perhaps not absolutely (DROOP, 1970). Details of the specificity of quinones and sterols for *O. marina* have been presented by DROOP and PENNOCK (1971).

A sterol requirement is widespread among protozoans and insects; it is generally satisfied by cholesterol, except for *Paramecium aurelia* and the guinea pig which can utilize only sitosterol and stigmasterol. *Oxyrrhis marina* is more versatile, also utilizing dehydrocholesterol, cholestanol, cholestenone, cholestane, sito-, stigma- and ergosterol but not vitamins D_2 and D_3.

The absolute requirement for a ubiquinone is apparently unique for *Oxyrrhis marina*: no other organism is known to need a ubiquinone for growth. Only ubiquinone and plastoquinone are active for *O. marina*. The K vitamins, vitamin E, chromenol and chromanol forms of ubi- and plastoquinone were inactive, indicating that modifications of the nucleus or the side-chain of the molecule inactivate. The length of the unsaturated side-chain of ubiquinone, which varies according to the organism of origin, is also important: a chain of 6 to 10 prenyl residues is needed. Six to 10 prenyl residues are recorded for fungi, 8 to 9 for protozoans, 8 to 10 for bacteria, and 9 to 10 for algae and higher green plants (CRANE, 1965). Ubiquinone and plastoquinone (a normal component of chloroplasts) are present in the organisms that *O. marina* finds in the supralittoral environment and ingests. Since ubiquinone and plastoquinone are lipid soluble, and light-labile, even if liberated in the water, they are rapidly destroyed and non-accessible to an osmotroph. All the other requirements of *O. marina* can be satisfied by osmotrophy, but phagotrophy is indispensable to satisfy the ubiquinone requirement.

Loss of synthetic function *sensu* LWOFF (1943) is only compatible with survival if the organism finds in its environment, preformed, the compound it no longer synthesizes. Quite likely, therefore, quinones might be a growth factor for other filter-feeding organisms.

Noctiluca miliaris (syn.: *N. scintillans*)

Noctiluca miliaris, a unique dinoflagellate, has attracted much attention because of its large size (1 mm), bizarre morphology, and brilliant luminescence. The almost spherical body has no flagella but a long tentacle with which it sweeps the food organisms toward its oral groove; food gathering is helped by the production of mucus from the tip of the tentacle. Food organisms are engulfed and digested in food vacuoles located near the oral region (Volume II: PANDIAN, 1975). *Noctiluca* when healthy floats near the surface, eats algae voraciously, and often even eats small crustaceans and worms (PRATJE, 1921).

Noctiluca miliaris was first cultured by GROSS (1934) in Erdschreiber sea water and fed a 'brine *Chlamydomonas*' sp. (*Dunaliella parva*?). ECKERT (1965) grew *N. miliaris* on *D. tertiolecta* in artificially enriched sea water.

MCGINN and GOLD (1969) reported axenization of *Noctiluca miliaris* from Long Island Sound (USA). The initial culture was grown in sterilized sea water and fed weekly with *Dunaliella* sp. Clonal bacterized cultures were established in an artificial medium (the mineral–vitamin base of Table 5-137), favourable for the growth of *Platymonas tetrathele* which served as food. *P. tetrathele* was preferred to other algae

supporting growth of *N. miliaris* because, being an obligate photo-autotroph, it could be eliminated by darkness. Success of these monoxenic cultures depends largely on the number of food organisms—it should be less than 3000 cells ml^{-1} (an inhibitory concentration). After determining the antibiotic sensitivity of the accompanying microflora (a marine Difco nutrient agar was streaked uniformly with a loopful of the clonal culture; Difco Sensidiscs were applied on the agar; a

Table 5-137

Chemically defined medium (weight per 100 ml) for *Noctiluca miliaris* (After McGinn, 1971; reproduced by permission of Fordham University)

Mineral-Vitamin base*				
NaCl	1·8	g	Glucose	200 mg
$MgSO_4 . 7H_2O$	0·5	g	Vitamin mix 8a§	1 ml
KCl	60	mg	Diatomaceous earth¶	2 mg
Ca (as Cl^-)†	10	mg	DL-Valine	80 mg
$NaNO_3$	50	mg	L-Leucine	60 mg
K_2HPO_4	3	mg	DL-Isoleucine	40 mg
$Na_2SiO_3 . 9H_2O$	20	mg	DL-Serine	80 mg
Metals Mix‡	1	ml	DL-Threonine	80 mg
Vitamin B_{12}	0·1	μg	DL-Methionine	25 mg
Biotin	0·1	μg	L-Arginine	12 mg
Thiamine	100	μg	L-Histidine	30 mg
Tris	100	mg	L-Lysine	12 mg
			DL-Alanine	12 mg
			L-Tryptophan	15 mg
			L-Aspartic acid	30 mg
Enrichments				
Na_2 Glycerophosphate	50	mg		
RNA	1	mg		
DNA	1	mg	pH 7·5–7·8	

* This base was used without enrichments for monoxenic cultures.

† For a solution of Ca (as Cl^-), in which 1 ml contains 10 mg Ca, slowly dissolve 2·5 g $CaCO_3$ in concentrated HCl; bring final volume to 100 ml with H_2O.

‡ 1 ml contains: Na_2 EDTA, 1 mg; $FeCl_3 . 6H_2O$, 50 μg; H_3BO_3, 1 mg; $MnCl_2 . 4H_2O$, 150 μg; $ZnCl_2$, 10 μg; $CoCl_2 . 6H_2O$, 5 μg.

§ Vitamin mix 8a: 1 ml contains: thiamine HCl, 20 μg; biotin, 0·05 μg; Vitamin B_{12}, 0·005 μg; folic acid, 0·25 μg; p-aminobenzoic acid, 1 μg; nicotinic acid, 10 μg; thymine, 80 μg; choline H citrate, 50 μg; inositol, 100 μg; putrescine HCl, 4 μg; riboflavin, 0·5 μg; pyridoxamine . 2HCl, 2 μg; orotic acid, 26 μg; Ca pantothenate, 10 μg; pyridoxine . 2HCl, 4 μg.

¶ Diatomaceous earth (Fischer Sci. Co., USA) was washed several times with distilled H_2O, air-dried, and autoclaved 30 mins at 121° C.

clear zone around the disc indicated sensitivity to the antibiotic), the following antibiotic mixture was used (final concentration per ml of medium): penicillin, 100 U, streptomycin, 100 μg; erythromycin, 10 μg; chlortetracycline, 50 μg; neomycin, 100 μg. The clonal culture was treated with this mixture for 2 weeks; to obtain monoxenic cultures, the partly purified *N. miliaris* were washed in the same medium with 25 μg ml^{-1} of novobiocin (= Albamacyn, the Upjohn Co., USA) added, by alternating 6 series of 10 washes each with six 12-hr resting periods to allow emptying of the food vacuoles.

Axenic cultures of *Noctiluca miliaris* were obtained by inoculating the monoxenic cultures with *Platymonas tetrathele* in an organic particulate medium (per 100 ml of mineral–vitamin base: liver concentrate '1 : 20', 4 mg; Na acetate (anhydrous), 40 mg; RNA, 1 mg; DNA, 1 mg; soil extract, 0·2 ml; glucose, 20 mg; diatomaceous earth, 2 mg; casein, 10 mg). Two months of weekly transfers in darkness were needed to eliminate the food organisms; subcultures grown in light demonstrated complete disappearance of *P. tetrathele*.

The formula of this medium was arrived at by adding a large number of organic substances to the monoxenic cultures and selecting the ones which stimulated growth of *Noctiluca miliaris* without stimulating growth of *Platymonas tetrathele*. After finding that casein and liver concentrate were highly beneficial, many C, P and N sources were tried and the favourable ones used to compound the above medium.

A chemically defined medium was formulated after an analysis of the needs for organic P sources, lipids and lipid growth factors, trace metals, and of salinity tolerance. In this medium an amino-acid mixture substituted the liver concentrate and casein; Na acetate and soil extract were eliminated; no lipid factors were needed. Defying the expectation that *Noctiluca miliaris* might be fastidious, it was grown for a year in the chemically defined medium, and previously for 2 years in monoxenic culture (McGinn, 1971). *N. miliaris* proved to be rather euryhaline (range 0·9–3·0 g% NaCl, optimum 1·8–2·1) and tolerant, for a marine organism, to temperature (optimum 20°–25° C) and pH (6·8–7·6).

Nitrate and ammonium salts do not support growth of *Noctiluca miliaris*: an organic source of N is needed, such as casein or a complete amino-acid mixture. However, the amino acids were utilized only in the presence of particles (diatomaceous earth), probably to elicit vacuole formation (pp. 1310, 1311).

The absolute need for vitamins was not determined. It is, however, probable that vitamin B_{12}, thiamine, and biotin are needed because *Noctiluca miliaris* fed on *Platymonas tetrathele* grown in the absence of vitamins had a lower growth rate in short-term experiments, than when fed on *P. tetrathele* grown in a medium with vitamins. Additional vitamins may be stimulatory as shown by eliminating the vitamin '8a' mixture from the chemically defined medium.

In summary, *Crypthecodinium cohnii* and *Oxyrrhis marina* behave like other colourless algal flagellates (i.e. species of *Polytoma*, *Polytomella*, *Astasia*, etc.): they are osmotrophs and have a limited need for amino acids. In contrast, *Noctiluca miliaris* are more like ciliates—they are phagotrophic and need a complete assortment of the 'essential' amino acids, perhaps also RNA and DNA components.

Choanoflagellates

Diaphanoeca grandis and *Acanthoecopsis* sp., which are considered bacterial feeders and saprozoic, were axenized by serial transfers in an antibiotic mixture (Gold and co-authors, 1970). They were grown in a mineral base + acetate, liver concentrate, and several vitamins.

Diaphanoeca grandis grows moderately well in this medium but is inhibited by the richer medium used for *Acanthoecopsis* sp. (i.e. the same medium to which proteose peptone, glucose, and pyruvate were superadded). The acquisition in axenic

culture of these organisms—a great step forward—will permit identification of the minimal and optimal requirements of members of a protozoan group which has great phylogenetic importance for its similarity to sponge choanocytes and (ii) use as indicator organisms of pollution (Volume V). Interestingly, these bacteria feeders can be grown osmotrophically.

(b) Ciliates

Only a few ciliates have been grown axenically: *Miamiensis avidus*, a facultative parasite of sea horses; *Miamiensis* sp.; *Paramecium calkinsi* (brackish); *Uronema marinum*; *Uronema nigricans*; *Parauronema virginiatum*; and three unidentified species. Three media permitting good rapid growth have been developed; they are almost completely defined but the minimal essential nutritional requirements of these marine ciliates have not yet been determined (see also Chapter 5.1).

Collection and Purification

Marine ciliates are found where there is decay, pollution, and abundant microphytoplankters. They also grow in laboratory samples of marine algae and animals when decay and microbial growth develops. Following the practice of freshwater protozoologists, natural collections of water, mud, etc. may yield abundant ciliates when enriched with small quantities of organics (lettuce and hay infusions, small pieces of cheese, coagulated blood, etc., overlaid by mud).

Soldo and Merlin (1972) enriched their samples with about 1 mg ml^{-1} of powdered Cerophyl (dehydrated cereal grass leaves, Cerophyl Labs., Inc., Kansas City, Mo., USA). To free the ciliates from contaminants, they used the continuous-flow washing system of Claff (1940) which takes advantage of the negative geotropism (Volume II, Chapter 8) of many ciliates. Addition of neomycin (50 μg ml^{-1}) and adoption of a stationary period to allow digestion and emptying of vacuoles increased success (Soldo, 1960). Clonal cultures were obtained by washing single individuals in several baths of sterile sea water with penicillin G (5000 U ml^{-1}) and/or neomycin (50 μg ml^{-1}) and inoculating each individual into 0·3 ml of medium contained in wells of a 3-depression slide placed in a sterile Petri dish humidified by wet filter-paper strips. The isolates that multiplied were then transferred to screw-cap tubes with 5 ml medium.

Lee and co-authors (1971b) used a mixture of antibiotics to purify *Uronema marinum* (final concentration: penicillin, 100 U ml^{-1}; Fungizone, 2·5 μg ml^{-1}; streptomycin, 100 μg ml^{-1}; polymixin B sulphate, 100 U ml^{-1}). Six additional transfers in media containing polymixin (100 U ml^{-1}) were needed to eliminate all contaminants. Hanna and Lilly (1974) obtained pure cultures of *Uronema* by upward migration through a vertical column of sterile sea water.

Isolation Media

Ciliates so far cultured seem to adapt easily to various artificial sea waters when adjusted to the original salinity. Kaneshiro and co-authors (1969) used the Woods Hole Formula (Marine Biological Laboratory, 1964), Soldo and Merlin (1972)

employed the McCLENDON mixture, and LEE and co-authors (1971b) and HANNA and LILLY (1974) used modifications of artificial media for algae (PROVASOLI and co-authors, 1957). 'Aquamarine salts' (Aquatrol Inc., Anaheim, Calif., USA) were used by NAPOLITANO and LILLY (1972) and by SOLDO and MERLIN (1972).

A variety of crude nutrients, some novel—mostly the ones used for growing freshwater ciliates—served to compound successful isolation media: (i) lactalbumin hydrolyzate, 10% solution (Nutritional Biochemical Corp., USA), 10 ml 100^{-1}; calf serum, 5 ml 100^{-1} in filtered sea water adjusted to pH 7·5 (each component sterilized separately) for *Miamiensis avidus* (MOEWUS, 1963); (ii) yeast autolysate supernatant, 50 ml; *Ulva* homogenate in sea water, 75 ml; glucose, 5 g; Difco proteose peptone, 5 g to 1 l H_2O; pH 7·6 for *Uronema marinum* (HANNA and LILLY, 1970); the *Ulva* homogenate can be eliminated for *Paramecium calkinsi*, an estuarine form (NAPOLITANO and LILLY, 1972); (iii) marine nutrient broth, liver extract and autoclaved *Pseudomonas* sp. for *U. marinum* (LEE and co-authors, 1971b); (iv) Trypticase, 1 g; proteose peptone, 1 g; yeast nucleic acid, 0·1 g; and a mixture of 8 vitamins, 0·1 ml dissolved in 100 ml of a Cerophyl extract (0·5 g% boiled and filtered) in natural or artificial sea water used for species of *Uronema*, *Parauronema*, and *Miamiensis* and three unidentified species of ciliates (SOLDO and MERLIN, 1972). The Cerophyl extract can be substituted by a mixture of Asolectin (purified soy lecithin), cephalin, and Tween 80 (see also Chapter 5.1, pp. 601–615).

Defined Media

Three defined media allowed good growth of *Uronema marinum* (Table 5-138). As mentioned, these are not minimal media, i.e. some components may be not needed, or may be stimulatory. They have evidently been derived from successful synthetic media for freshwater ciliates. The medium of LEE and co-authors (1971b) is probably incomplete since Trypticase (10 mg%) and yeast extract (0·01%) stimulate further growth. A comparison with the other successful formulas indicates that this medium might be low in total amino acids, nucleotides, lipid factors, and in several of the 6 vitamins thought necessary for ciliates.

HANNA and LILLY isolated two strains of *Uronema marinum*: for the first strain the 1971 medium had to be supplemented with a water-soluble purified fraction of brewer's yeast. A second strain of *U. marinum*, isolated from the same environment, could be grown in a synthetic medium (H & L) of HANNA and LILLY (1972) which was quite similar in concentrations and components to the medium (S & M) of SOLDO and MERLIN (1972; see also Chapter 5.1, pp. 607, 608).

The medium of SOLDO and MERLIN (1972) at pH 7·2, and in the dark, allowed good growth of 2 strains of *Miamiensis avidus*, *Miamiensis* sp., 2 strains of *Parauronema virginianum* and *Uronema nigricans*. The similarity of the 2 media and their complexity indicate that these marine ciliates might be as exigent as those of the more fastidious freshwater species, i.e., needing nucleic acid derivatives and lipid factors, including sterols. Except for the absence of added fatty acids, stigmasterol and sodium acetate, the SOLDO-MERLIN medium is qualitatively similar to the medium used for *Paramecium aurelia*, Stock 299 (SOLDO and VAN WAGTENDONK, 1969).

Table 5-138

Synthetic media for marine ciliates (mg%, w/v) (Original)

Component	Lee and co-authors (1971b)	Hanna and Lilly (1971)	Hanna and Lilly (1972)	Soldo and Merlin (1972)
Alanine	DL-α 5	L 2·5	L 10	L 90
Arginine	L 7	L 10	L 40	L 90
Asparagine				L 27
Aspartic acid	DL 6	L 5	L 20	L 30
Glutamic acid	DL 15	L 7·5	L 30	L 120
Glycine	5	2·5	10	22·5
Histidine	L 2	L 5	L 20	L 30
Hydroxyproline	L 1			
Isoleucine	DL 4	L 15	L 60	L 37·5
Leucine	DL 12	L 15	L 60	L 37·5
Lysine HCl	L 7	L 12·5	L 50	L 75
Methionine	DL 3	L 15	L 60	L 45
Phenylalanine	DL 5	L 7·5	L 30	L 37·5
Proline	L 4	L 5	L 20	L 22·5
Serine	DL 5	L 20	L 80	DL 90
Threonine	DL 6	L 15	L 60	DL 105
Tryptophan		L 5	L 20	L 22·5
Tyrosine		L 5	L 20	L" 22·5
Valine	DL 5	L 7·5	L 30	DL 60
Glutamine	L 10			
Guanylic acid		7·5		15*
Adenylic acid		3		9*
Cytidylic acid		7·5		9**
Uridylic acid		2		9**
Purine-pyrimidine mix II	~8			¶
Na RNA (yeast)			100	
Glucose	100			
Na acetate	5	57		
Cholesterol	0·2			
Stigmasterol		0·2	0·25	
Tween 80	2		10	20
Linoleic acid		0·37		
Oleic acid		0·12		
Asolectin				20
Cephalin			10	20
Biotin	0·5 μg	0·4 μg	0·2 μg	0·05 μg
Folic acid	2·5 μg	0·4	0·5	0·25
Nicotinamide	0·1 acid	0·4	0·5	0·25
Ca pantothenate	0·1	0·6	1	0·5
Pyridoxal HCl	60 μg†	0·4	0·5	0·25
Riboflavin	5 μg	0·4		0·25
Thiamine HCl	0·2	1·13	1·5	0·75
DL-Thioctic acid		1·4 μg	10 μg	5 μg
Folinic acid	0·2 μg		0·15	
Putrescine	0.04			
Additional vitamins (mg%): PABA, 0·01; choline H citrate, 0·5; inositol, 1; thymine, 0·8; orotic acid, 0·26; B_{12}, 0·05 μg	yes	no	no	no

* Nucleoside form.
** Base only.
† Total μg of pyridoxine + pyridoxamine.
¶ Thymidine 8

A chemically defined minimal medium of *U. marinum* PW2 was reported by HANNA and LILLY (1974): it is a modified 1972 medium: the RNA was replaced by the four nucleic acids used in the 1971 medium but at 2 × the concentration. Thiamine, riboflavin, pyridoxal, nicotinamide, folic and pantothenic acids were found essential; biotin, thioctic acid and stigmasterol were disposable. Cephalin, a poor source of lipids, and Tween 80 were substituted with a mixture of oleic, linoleic, linolenic, palmitic and stearic acids. This chemically defined mixture maintained through serial transfers a population of 20,000 ciliates ml^{-1}. Phosphotidylinositol or Tween 80 at 10 mg%, as the sole lipid source sustained better growth than the fatty acid mixture, and Tween 80 the highest and most prolonged logarithmic growth.

Prospects

The availability of nearly defined media and the probability of minimal media in the near future offers great possibilities for biochemical and biophysical investigations. Maximal populations are obtained in 4 to 6 days and range from hundreds of thousands to millions of ciliates per ml, depending upon strains and species. SOLDO and MERLIN are seeking species which grow abundantly in defined media—a prerequisite for proposed studies on the biochemistry of intracellular symbioses; their 1972 paper deals with cultivating symbiont-free ciliates and a later report with symbiont-bearing species (SOLDO and co-authors, 1974). The availability of suitable purification techniques and of isolation media opens the way to the axenization of more marine species.

However, reliance on isolation media can also be restrictive in pre-selecting only organisms capable of growing on them. The paucity of ciliates in axenic culture denotes an impasse. The success of LWOFF (1932) and KIDDER and DEWEY (1951) with *Tetrahymena pyriformis*—a facultative osmotroph—has lulled protozoologists into using all solute media. But *T. pyriformis* may not be a typical ciliate. Progress in acquiring other ciliates in culture has been hampered not only by the complex nutritional requirements patiently unravelled by VAN WAGTENDONK, LILLY, HOLZ and SOLDO but, I suspect, principally by the lack of recognition that phagotrophy may be obligatory or highly advantageous to most ciliates.

Since most of the work on feeding was done on *Tetrahymena*, the data may be biased (see reviews of HOLZ, 1973 and DUNHAM and KROPP, 1973). *Tetrahymena*, like most ciliates, feeds phagotrophically on bacteria but in suitable artificial media it can be grown osmotrophically on dissolved nutrients. Recent work shows that *Tetrahymena* is very versatile and that it can use also a mixture of osmotrophy and phagotrophy; the relative dependence on, and efficiency of each of the modes of uptake depending upon culture conditions.

Tetrahymena pyriformis in sterile-filtered, particle-free 2% proteose peptone media has a low division rate, i.e., a generation time of 40 hrs at 28° C (RASMUSSEN and KLUDT, 1970). At 18° C, the generation time is 100 hrs and average food vacuole production is less than one vacuole per ciliate (RASMUSSEN, 1973). The addition of a 6 mM solution of four nucleosides (=20 × the concentration used in the normal defined medium) + 2·5% glucose shortens the generation time to about 10 hrs at 18° C with no increase in number of food vacuoles (RASMUSSEN, 1973). RASMUSSEN and ORIAS (1975) have suceeded in growing a mutant of *Tetrahymena* which, at 37° C,

is unable to form a functional oral apparatus and has no food vacuoles. Growth occurs only when to the 2% proteose peptone, or to a chemically defined medium, are added 100 mg% of each of the 9 needed vitamins and a trace metal solution at 25 × the concentration normally used in defined media. The generation time in the enriched media at 37° C is 3·5 hrs for the mutant and about 2 hrs for the wild type parental strain; the authors conclude that the food vacuole is a dispensable organelle. Since high reproduction rates can be obtained osmotrophically only when nutrients are supplied at exceedingly high concentrations, osmotrophic uptake is not too efficient.

Conversely, in low nutrient media, formation of food vacuoles becomes essential for high reproductive rates. A dramatic increase of the nutritional potential is obtained by autoclaving the 2% proteose peptone medium—a treatment causing abundant formations of particles. The generation time of 100 hrs at 18° C and 40 hrs at 28° C obtained in the same medium, when made particle free by filtration, is shortened respectively to 40 hrs and 5 hrs in the autoclaved medium. Equally short division times are obtained by the addition to the particle-free 2% proteose peptone medium of non-nutritious particles such as fine glass, clay, $CaCO_3$ and Fe and Al hydroxides (RASMUSSEN and KLUDT, 1970) and polystyrene or Sephadex particles (RASMUSSEN and MODEWEG-HANSEN, 1973).

Since the addition of particles induces vacuole formation and the average number of food vacuoles correlates with growth potential, rapid multiplication depends presumably upon the number of vacuoles. In low-nutrient media the advantage achieved by adding inert particles is obviously due to the entrapping into the vacuoles of considerable volumes of nutrient medium and to the many fold increase of the area of solute uptake. Similarly, the addition of particles of magnesium silicate was found earlier (REILLY, 1964) to enhance total growth and to eliminate the requirement for a protein factor in *Paramecium multimicronucleatum* grown axenically in a defined medium. When, in addition to the medium, the particles themselves are nutritious, vacuolar osmotrophy and phagotrophy could operate simultaneously. This transitional bipotency might have led to the exploitation of the evolutionary advantages of phagotrophy.

I postulated earlier (PROVASOLI, 1957) that phagotrophy may be indispensable, even for autotrophs, in environments where the needed trace metals (like iron) are present mostly in particulate form, as in alkaline waters and sea water. Indeed, vacuolar osmotrophy and phagotrophy co-exist in the ciliates cultured until now; they are habitual phagotrophs in nature, but can employ their varied osmotrophic abilities to grow on liquid media in the laboratory.

Nutrient liquid media, however, have to be very rich in nutrients to compensate for the limited osmotrophy occurring in the absence of the phagotrophic reaction (i.e. less vacuoles). But it is well known that very rich nutrient media are toxic to most ciliates and that non-toxic concentrations of organics support no—or exceedingly slow—growth. *Artemia salina* (p. 743) is similarly inhibited by total concentrations of amino acids above 0·6% but at, or below this concentration, no, or very slow, partial development occurs; a few milligram percent of precipitated proteins induced fast growth. Under these conditions phagotrophy becomes indispensable.

In the usual habitat of ciliates the environment has only enough nutrients to

support growth of food micro-organisms but not enough to support ciliate growth solely by osmotrophy. Even when the environment is highly polluted and rich in organic solutes the heterotrophic micro-organisms outstrip, initially, ciliate growth because their large surface/volume ratio permits a far more efficient uptake of nutrients than the vacuolar osmotrophy of ciliates. Besides, the ready availability of food organisms in such rich environments favours phagotrophy.

Conceivably, then, most ciliates depend upon, or are most efficient in dealing with nutrients as particles. Efforts to formulate particulate media might be rewarding. The complex media needed by exacting ciliates like species of *Paramecium*, *Glaucoma*, and *Tetrahymena* are in fact particulate or colloidal; the required lipid factors, sterols, fatty acids and Tweens form, at best, emulsions in liquid media.

(5) Axenic Cultures of Nematoda

Nematodes are common in soil, sand dunes, marshes, decaying seaweed, and other environments where bacteria decompose organic matter. They apparently feed on bacteria, other micro-organisms, and micro-algae. Observations of gut content are unsatisfactory to determine which prey they feed upon. Using labelled diatoms, chlorophytes, and bacteria isolated in axenic culture from the same environment, TIETJEN and co-authors (1971) found that *Chromadora axi* and *C. macrolaimoides* prefer as food algae over bacteria. On the other hand, *Rhabditis marina* grew trixenic on 3 bacteria: *Pseudomonas* sp., *Micrococcus* sp. and *Flavobacterium marinum*. By using these bacteria, C- and P- labelled, they found that *Micrococcus* sp. was not ingested. By taking advantage of the different sensitivities of these bacteria to antibiotics, a monoxenic culture of *R. marina* was established by using novobiocin 100 μg ml^{-1} + erythromycin 50 μg ml^{-1}; but *R. marina* failed to grow after the fourth or fifth transfer, indicating that *F. marinum* was not a complete food. Addition of erythromycin at 200 μg ml^{-1} eliminated *Micrococcus* sp. and *F. marinum*, leading to a monoxenic culture of *R. marina* on *Pseudomonas* sp. *Pseudomonas* sp. was a complete food, allowing 80 generations of *R. marina* (LEE and co-authors, 1970). *Nannochloris* sp., a microchlorophyte which is a good food for *Chromadora axi*, is ingested by *R. marina* in huge quantities, but it passes undigested through the gut and does not support growth of *R. marina* (TIETJEN and co-authors, 1970).

Rhabditis marina shows clear food preferences: while it ingests ten times its own weight of *Pseudomonas* sp. per day, it ingests very small quantities of other bacteria. This behaviour, as well as the fact that only *Pseudomonas* sp. can serve as a complete food, shows an unsuspected specificity toward food organisms that is perhaps not shared by other nematodes. Five species of sand-dune nematodes were cultured monoxenically on *Bacillus cereus* var. *mycoides* (YEATES, 1970).

During the purification it was found that *Rhabditis marina* was killed by Novobiocin and Coly-Mycin at 100 μg ml^{-1}; these two antibiotics did not eliminate all the bacteria either. In other attempts at purification, bacteria were eliminated but some fungi resisted; the fungi were eliminated with Mycostatin or Fungizone, which do not seem to harm *R. marina* even at concentrations as high as 5 to 50 mg ml^{-1} (LEE and co-authors, 1970).

The monoxenic medium was a sea-water nutrient agar (Difco) or a mineral-agar medium with NH_4, PO_4, and carbon sources. The physical conditions of the medium were important; liquid media were unsuitable for growth of the worms; the normal agar slopes or a 4-mm agar layer were also unsuitable because the worms penetrated the depths of the agar and died. A thin layer of agar, parallel to the walls of the test tubes, is quite convenient, and for *Rhabditis marina* a thin overlay of liquid medium was favourable; other nematode worms prefer thin agar layers but without liquid overlay (TIETJEN and co-authors, 1970).

Many media and enrichments were tried before successful artificial media for *Rhabditis marina* were obtained. In these experiments, axenic worms were used as well as monoxenic cultures on *Pseudomonas* sp., whose growth was inhibited by sublethal antibiotic doses. The initial axenic culture of *R. marina* was grown on marine nutrient agar enriched by aseptic liver chunks and whole blood. A defined synthetic organic base derived from GRACE (1962) medium for insect tissue culture and marine salts sustained growth and reproduction of axenic *R. marina* upon addition of casamino acids and filter-sterilized haemoglobin. The casamino acids can be replaced by TEM 4T (diacetyl tartaric acid ester of tallow monoglyceride, Whitco Chemical Co., New York), TWEEN 80 or a mixture of fatty acids supplemented with whole egg powder. Haemin cannot replace heamoglobin. Further definition was prevented by accidental destruction of stocks (LEE and co-authors, 1971a; TIETJEN and LEE, 1975).

Interestingly, a few labelled amino acids and carbon sources at 20 mM or lower were not taken up by *Rhabditis marina* unless these substrates were adsorbed onto 0·5- to 10-μm microspheres (Minnesota Mining and Manufacturing Co., USA). This indicates that the chitinous envelope of the worm hampers uptake of solutes through the cuticle. The substrates and building blocks are apparently absorbed only by the gut lining—phagotrophy, again, might be the preferential way of feeding (TIETJEN and LEE, 1975).

The requirements of free-living nematodes (reviewed by ROTHSTEIN and NICHOLAS, 1969) seem complex.

The last undefined component, a poorly repeatable liver extract needed by several nematodes, proved elusive and difficult to replace. It apparently supplies sterols (HIEB and ROTHSTEIN, 1968) and haemin (HIEB and co-authors, 1970) which were found essential and perhaps a small peptide (ROTHSTEIN, 1974). The liver extract can be replaced by adding to defined media haemin and human γ-globulin (BUECHER and co-authors, 1970b), haemoglobin, sterols and soy peptone (ROTHSTEIN, 1974) or sterols and haemin (VANFLETEREN, 1974). However, VANFLETEREN found that haemin was nutritionally effective only if offered as particles of lecithin-haemin or as precipitated haemin.

It had been noted that biological activity of the replacements for liver extract was associated with induction of precipitates in media (BUECHER and co-authors, 1970a) but no deliberate effort had been made to discriminate between essential requirements for specific substances and the need for particles. The difficulties encountered in replacing the liver extract were apparently two-fold, and finding the way of presenting nutrients in a most efficient way seems the next essential step.

(6) Axenic Cultures of Crustacea

Marine crustaceans have varied food habits; they comprise filter-feeders (herbivores), scavengers, and omnivores (Volume II: PANDIAN, 1975). No marine crustacean has been reared axenically. *Artemia salina*, an extremely euryhaline brine organism was selected because of the ready supply of cysts and the ease in disinfecting them. Food specificity was determined and monoxenic cultures (PROVASOLI and co-authors, 1959) served as a base for finding an artificial medium for axenic cultures. The first medium was undefined—mostly a non-toxic mélange of nutrients which permitted growth of newborn to adults (PROVASOLI and SHIRAISHI, 1959).

The main finding was that voracious particle feeders like *Artemia salina* cannot be grown in the absence of particles. The liquid part of the medium, which contained all the nutrients, in the absence of the starch particles supported development only to third stage metanauplii. Replacement of the starch particles with soluble carbon sources such as 'soluble' starch, glycogen, mono- and disaccharides, organic acids, etc., did not support more growth than the control without carbon sources. The suspicion that *A. salina* could not be induced to grow osmotrophically was confirmed by further work.

A defined medium was slowly developed. Compounding particulate media presents difficulties, one of which was defying the bacteriological indoctrination that a reproducible medium should be free of precipitates. The nutrients were supplied as solutes and particles. The building blocks (i.e. amino acids) and the carbon sources which comprise the bulk of the nutrients had to be supplied as particles of precipitated egg albumin and starch. Some growth occurred sporadically and a minimal growth rate was achieved using soluble sugars (several g%); any complete mixture of amino acids proved toxic above 0·6% total concentration and at this concentration no adults were obtained. Therefore, the requirements for indispensable amino acids or carbon sources could not be determined.

Other nutrients, such as B vitamins, trace metals, and nucleic acids—which are non-toxic at the high concentrations needed to overcome the inefficiency of the liquid uptake—could be added in solution (PROVASOLI and D'AGOSTINO, 1969). Thiamine, nicotinic, pantothenic, and folic acids, pyridoxine, riboflavin, biotin and putrescine, as well as the nucleic acids and cholesterol were indispensable. These needs resemble the requirements of insects; they diverge in the dispensability of choline and inositol which are often required by insects. The need for putrescine seemed typical of *Artemia salina* until DAVIS (1966) found it was needed for the beetle *Oryzaephilus surinamensis*.

The apparent need for phagotrophy by *Artemia salina* could be construed as an age-old adaptation to life in brines where uptake of liquids would entail intolerable physiological work for maintaining an internal osmotic pressure close to sea water in a brine environment of 15% total solids. Would a freshwater crustacean filter-feeder, facing the opposite problem, still be a phagotroph? *Daphnia magna* and *Moina macrocopa*, two freshwater daphnids, could not be grown in the absence of particles.

Since the strain of *Artemia salina* was amphigonic, the nutritional requirements were determined by the effects on growth from newborn to adults, as customary for

insect work. When the *Artemia* media were tailored to satisfy the nutritional needs of *Daphnia magna*, a parthenogenetic species, the efficiency of the media could be evaluated by the size of the progeny and the number of generations obtained. The best artificial media supported only one or two generations of *D. magna*—evidently, additional nutrients were required for continued fertility. Among the many substances tried, egg yolk enriched with vitamins E and D permitted 5 generations of *D. magna* and indefinite generations of *Moina macrocopa* (PROVASOLI and co-authors, 1970).

Recent work indicates that several lipid factors affect fertility of *Moina macrocopa*; their essentiality has not been completely determined because the medium still has an undefined component, liver infusion (Oxoid L 25), which is indispensable. However, recent change in manufacture caused loss of potency of this preparation and other crude materials are being tested to replace liver infusion.

Among the lipids favouring fertility are B-carotene, vitamins E and D, lecithin, palmitic, oleic, linoleic and linolenic acids (CONKLIN, 1972; CONKLIN and PROVASOLI, 1977).

These data have been acquired with a freshwater species and, even if not directly applicable to marine species, indicate the complexities to be unravelled; as far as we know, the chemical composition of freshwater algae does not differ basically from that of their marine counterparts.

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5. CULTIVATION OF ANIMALS

5.2 COMMERCIAL CULTIVATION (AQUACULTURE)

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(1) Introduction

For the first time in history, man's population growth is attaining dramatic, if not disastrous, dimensions. The unique domination of *Homo sapiens* over other forms of life and our exponentially increasing technological capability are adding up to a deadly potential—the potential to induce irreversible damage to individual species, ecosystems, and, in fact, to the life-supporting properties of our earth.

Although it is obvious that infinite growth is incompatible with the earth's finite size and resources, this fact begins to enter the concepts of politicians and other planners only hesitatingly. However, we are already encountering a variety of limitations—especially in regard to resources, such as food, energy, and raw materials, and with respect to the deformability of ecosystems due to man's expanding population metabolism. Limitations in natural food resources are the motor for commercial cultivation (see also Chapter 1); ecological aspects of other limitations will be considered in Volume V.

Within the last few decades, the need for commercial cultivation of marine animals has increased considerably. We must further enlarge our capacity for mariculture because there is a world-wide need for additional food, especially protein, due to an unprecedented, largely uncontrolled acceleration of human population growth, and because good fishing grounds are becoming rare, due to overfishing, extensions of national fishing limits and insufficient world-wide ocean management (Volume V). In a human population which is about to outgrow its natural endowments, search for food has a high priority.

On land, we have long passed the phase when human food was primarily secured by sampling and hunting. Sampling and hunting at the doorstep were followed by food-collection trips and migrations; as these failed to secure sufficient nutrients, the cultivation of food organisms became essential. In oceans and coastal waters, sampling and hunting persist to this day and, most likely, will continue to dominate the harvesting of food from marine waters for many years to come.

While large portions of the human population have succeeded in controlling hunger, the spectacular overall human population increase—largely due to modern medicine and sanitation—is outgrowing all attempts to eliminate food shortage. Thus, hunger is still with us. Ultimately all efforts to combat hunger are bound to fail unless we restrict birth rates. We must begin to consider human nutrition in context with world-wide food availability, energy resources, and ecosystem dynamics. Phylogenetically, food scarcity acts as population regulator and evolutionary force. Without it, the development of life on earth may have been different —in all probability, less diverse, less differentiated and with less well buffered relationships between different forms of life.

It is essential at this point to remind ourselves that, although we speak of food 'production', our food is not really produced in an industrial sense, but is the result of biological processes: inorganic matter is transformed into organic substances by plant photosynthesis, and the plant substances synthesized are, in part, resynthesized in animal bodies. At present, we can by technological means copy neither plant photosynthesis nor animal resynthesis. Our fate remains chained to biological processes which actually produce our food organisms. All we can do is to enhance these processes and to decrease the losses due to climate, predators, competitors or diseases, i.e. to cultivate our food organisms.

However, on a very large scale, cultivation *per se* may critically interfere with natural ecosystem dynamics (Volumes IV and V). If allowed to increase at the present rate, agricultural and aquacultural activities will, over decades or centuries, attain dimensions which may ultimately pose a threat to the normal functions and structures of natural ecosystems. The destructive potential of oversized, human-controlled food-production processes may become comparable to that of human waste disposal.

In recent years, political and social constellations have been conducive for supporting aquaculture research. The need for supplying additional protein for millions of malnourished people has channelled considerable amounts of money into research projects designed to explore the feasibility of mariculture. The support provided by many governments is likely to remain an essential factor for some time to come and to significantly extend our present maricultural capacity. It has produced a landslide of papers: a few landmarks, some leading the way towards a better understanding of environmental and nutritional requirements of the animals cultivated, others reporting on important technological advances, and many repetitive or outright poor without much scientific substance or innovation potential. Several authors have painted glowing pictures of flourishing underwater seafood farms—dwelling on science fiction rather than drawing from solid facts. Their optimistic predictions require correction.

At this writing, the commercial cultivation of aquatic organisms is growing into a major industry, based on interdisciplinary cooperation among fishery biologists, zoologists, botanists, microbiologists, physiologists, biochemists, geneticists and pathologists—as well as students of nutrition, engineering, sociology and economics. Success or failure of this industry depends on its ability to produce seafood at competitive prices. According to PILLAY (1976b), at least three factors have been responsible for the growing interest in aquaculture: (i) increased costs of fishing, (ii) fear of decrease in the fishery harvest by countries that depend on fishing in foreign waters, and (iii) need, in some countries, for providing alternative employments for fishermen.

In a treatise on marine ecology, commercial cultivation cannot be covered in detail. The major concern of the aquafarmer, profit making, lies outside our scope. In addition, most attempts made by biologists to analyze the profitability of aquaculture suffer from several shortcomings: (i) Objective information regarding the economics of commercial cultivation is scanty and difficult to come by (e.g. SHEPHERD, 1974). (ii) Where data are available, they often cause problems of comparison or are unsuitable for critical analyses. (iii) Most biologists seem neither sufficiently prepared nor experienced to discuss economics with much authority.

We present here a summarized overview of some ecologically significant aspects of animal mariculture.

Water-quality management and the technology employed for cultivating marine organisms are dealt with in Chapter 2. Aspects of plant mariculture are covered in Chapter 4. Details regarding the culture of aquaculture-candidate animals receive attention in Chapter 5.1; the reader is particularly referred to molluscs (p. 884), crustaceans (p. 742) and fishes (p. 986). Perspectives of marine-mammal cultivation which are related to commercial cultivation are presented on pp. 1035 to 1123. For multispecies cultures consult Chapter 6. While the pet-fish industry, the culture of sport-fish, bait animals and the production of biogenic materials useful to man belong to the field of commercial cultivation, these aspects do not receive special attention here.

To what extent can animal mariculture help to combat hunger? Are we moving in the right direction? What are the ecological implications of growing maricultural activities? This subchapter is intended as a contribution to find answers to these questions. After reviewing the principal types of animal mariculture, we briefly consider the following points: (i) the status and potential of commercial cultivation for producing more food, particularly protein, for the logarithmically expanding human population, (ii) areas and animals suitable for mariculture, (iii) major constraints of animal mariculture, (iv) general potential of animal mariculture, and (v) ecological implications and long-range perspectives.

Important books on animal aquaculture have been presented by VILLALUZ (1953), HORA and PILLAY (1962), GREENBERG (1963), SCHÄPERCLAUS (1967), IVERSEN (1968), HUET (1970, 1972), TAMURA (1970), HICKLING (1971), MCKEE (1971), BARDACH and co-authors (1972), MILNE (1972) and PILLAY (1972). Accounts and reviews on restricted fields of mariculture have been provided by COSTLOW (1969), OSHIMA and IHABA (1969), HEMPEL (1970), KINNE and BULNHEIM (1970), MCNEIL (1970), LANDIS (1971), LING (1972, 1973), NEAL (1973), PILLAY (1973, 1976b), HANSON (1974), MACCRIMMON and co-authors, (1974), USUI (1974), BRIGGS (1976), CALAPRICE (1976), CRACKNELL (1976), FORREST (1976) and WEBBER and RIORDAN (1976). A catalogue of cultivated aquatic organisms has been compiled by JHINGRAN and GOPALAKRISHNAN (1974), bibliographies on aquaculture by PILLAY (1965), ALLEN (1969), ANONYMOUS (1971a) and PURDOM (1974). For countries of the EIFAC (European Inland Fisheries Advisory Committee) region, TIEWS (1973) has reviewed present research activities. For a recent synthesis, on a global basis, of aquaculture development see Summary Report of FAO Technical Conference on Aquaculture, Kyoto, Japan (1976).

(2) Principal Types of Animal Mariculture

Animal mariculture began at the family or tribe level and was motivated by the intent to secure additional food independently of natural fluctuations. Led by far-eastern pioneers, aquatic animals have been cultivated for thousands of years (Chapter 1, p. 1). The motivation of early aquafarmers is well characterized by the old Chinese saying:

> 'Give a man a fish and he will have food for a day. Teach him to culture fish and he will have food for the rest of his life.'

Among the ancient writings on fish cultivation, the earliest paper appears to have been written, around 2100 B.C., by FO-HI (PEARSON, 1970). In 2000 B.C., the Macedonians maintained glass eels (elvers) in ponds (EALES, 1968). The first major improvement in cultivation technique was the tambak system developed for brackish-water fish culture in Japan and Indonesia, between 1200 and 1400 B.C., under the influence of the Hindu empire (TUBB, 1967).

Today, we may distinguish two basic types of animal mariculture: extensive and intensive operations. While most extensive operations are conducted in natural waters, intensive operations are performed both in natural and artificial water systems (Fig. 5-127).

Extensive culture operations are characterized by (i) a low degree of control (e.g. of environment, nutrition, predators, competitors, disease agents); (ii) low initial costs, low-level technology, and low production efficiency; (iii) high dependence on local climate and sea-water quality; (iv) use of natural water bodies (e.g. ponds, bays, embayments) and of natural, often unspecified, food organisms. Oysters, clams or herbivorous fishes are well suited for extensive culture operations.

Intensive culture operations are characterized by (i) a high degree of control; (ii) high initial costs, high-level technology, and high production efficiency; (iii) tendency toward increased independence of local climate and sea-water quality; (iv) use of man-made culture systems (e.g. tanks, artificial ponds, raceways). In intensive culture operations, a maximum of seafood is produced in a minimum of water and space. While some farmers consider high-priced animals—such as shrimp and prawn, lobster, sole or turbot—best for intensive culture operations, others concentrate on less expensive forms such as milkfish, yellow-tail or catfish. Ideally, the animals chosen should be resistant to crowding and amenable to stock improvement.

The different types of animal mariculture operations are primarily a result of the ecological and technological know-how available, the area or animal concerned, and investment considerations. Major planning items include animal selection, the biological background of the species involved; site selection; the translation of environmental and nutritional requirements into economical processes; system design and management; feed production; seedling production; control of predators, competitors and disease agents; stock improvement, and product processing and marketing. For information related to animal selection consult Chapter 5.1, especially the sections on Crustacea (p. 742), Mollusca (p. 884) and Pisces (p. 968), as well as p. 1358.

While hatching of fertilized eggs can usually be achieved rather easily, the difficulties increase when it comes to the raising of juveniles and adults. Breeding of adults and rearing of larvae usually constitute the most problematic steps in egg-to-egg cultivation. For maximum control over seedling production and stock improvement (selective breeding), egg-to-egg cultivation is a must.

Extensive operations, the oldest type of aquaculture, are still successful with animals such as oysters, mussels, clams and scallops. In developing countries with low-cost coastal land, inexpensive labour, and limited technological skill, extensive cultures usually dominate. In many subtropical and tropical areas, extensive cultures of crustaceans, molluscs or fishes may substantially supplement the protein supply of rural populations or of individual families. Community operations and

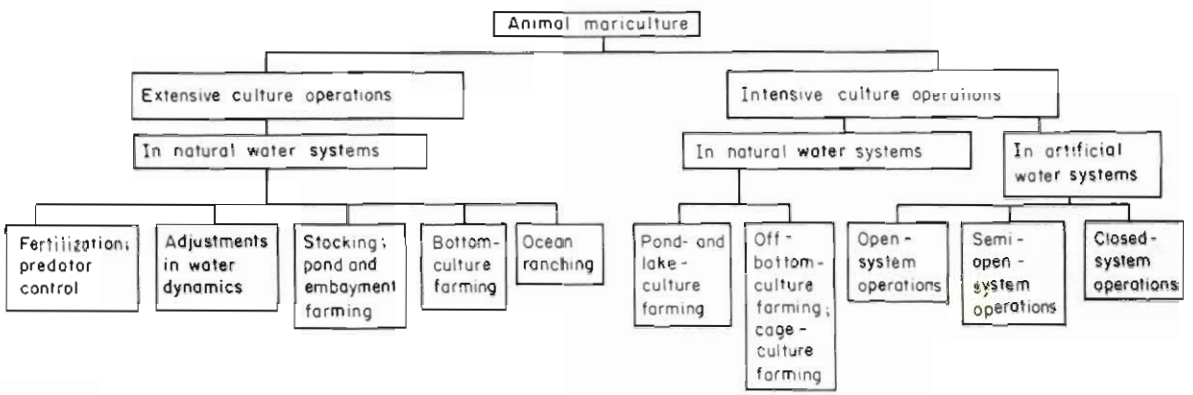

Fig. 5-127: Principal types of animal mariculture and examples of major activities. (Original.)

small near-the-house family cultures should be encouraged and supported financially as well as by making the necessary know-how available.

In highly industrialized countries, however, with rapidly increasing prices for labour and land, the economical feasibility of extensive mariculture is bound to decrease. Governmental support for large-scale, extensive culture programmes seems justified only in specific cases, e.g. where plankton feeders and herbivores are used in high-productivity waters. In the long run, intensive operations are the method of choice. In temperate regions, greenhouse techniques which parallel 'controlled-environment agriculture' are likely to play an important role, especially in connection with waste-heat release from large power plants (e.g. NASH, 1968, 1974b; COUTANT, 1971; KIRK, 1972; MARCELLO and STRAWN, 1973; REIMER and STRAWN, 1973; STEWART, 1973; KUHLMANN, 1976; TANAKA, 1976).

In extensive culture operations, examples of major activities are: fertilization, predator control, adjustments in water dynamics, stocking, pond and embayment farming, bottom-culture farming, and ocean ranching (Fig. 5-127).

Fertilization is accomplished by adding inorganic substances (e.g. phosphates, nitrates) or organic substances (e.g. manure, cottonseed cake or suitable sewage effluents) to lakes, ponds, bays or embayments (e.g. MORTIMER and HICKLING, 1954; PROWSE, 1962). Experimental phases of mass cultivation of plaice *Pleuronectes platessa* in artificially fertilized Scottish sea lochs have been pioneered by GROSS and co-authors (1944, 1946, 1950), GROSS (1947a, b, 1950) and RAYMONT (1947a, b, 1948, 1949). After addition of sodium nitrate and superphosphate, striking increases in phytoplankton and phytobenthos were followed by increased bottom-fauna development and plaice growth. The *P. platessa* released in the loch grew 2 to 4 times as fast as their counterparts in nearby unfertilized waters. Comparable experiments and results have later been obtained by several other investigators. Organic fertilizers, such as chicken manure, green manures, tobacco waste, are usually added while the culture ponds are dry (e.g. in Philippine milkfish ponds). In Taiwan, tobacco waste acts not only as organic fertilizer but, due to its nicotine content, also as biodegradable natural pesticide (BARDACH and co-authors, 1972, p. 332).

Organic fertilizers have a number of advantages (e.g. HUET, 1970; ALLEN, 1972; MILLER, 1975; ALLEN and HEPHER, 1976). They (i) tend to facilitate a shorter production cycle than inorganic fertilizers, especially for zooplankton production in fry-rearing enclosures; (ii) favourably affect pond-soil quality; and (iii) provide additional fish food. The major disadvantages of organic fertilizers include the reduction of dissolved-oxygen levels and the promotion of undesirable alga growth. Organic fertilizers may further support microbial disease agents and parasites. For details consult SCHROEDER (1974, 1975) and SCHROEDER and HEPHER (1976).

Predator control is usually achieved by preventing predators from entering the culture enclosure (e.g. by a Saran Sock Barrier, p. 66). A variety of chemical methods have been used to either completely eradicate predators or to reduce their numbers; an example is the chemical control of bivalve predators (p. 932). In bottom cultures of oysters and clams, predators (mainly sea-stars and oyster drills) have been controlled quite successfully. Predator development can also be counteracted by biological methods—i.e. by organisms or biological processes which exert detrimental effects on the predator. A simple method of predator control in pond

farming is the complete drainage of pond water prior to stocking—if necessary, in combination with chemical methods or winter freezing. Predator control has received attention from CARRIKER (1955), RABANAL and HOSILLOS (1958), SOONG and MERICAN (1958), LOOSANOFF and co-authors (1960), MACKENZIE (1961), TANG (1961), BOWBEER (1970), HOOPER and FINKELSTONE (1970) and PILLAI (1972).

Adjustments in water dynamics include such aspects as control of flow-through rate, water-level height, partial or complete water exchange, vertical water transport and splasher aeration (pp. 66, 70, 187). In coastal or open-sea areas, deep water rich in nutrients has been pumped to the surface. Such artificial upwelling has been pioneered by ROELS and associates (p. 582). Vertical transport of nutrient-rich, cool, deep water may play an increasingly important role in connection with the operation of offshore power plants. Such developments invite cooperative efforts between power-plant operation and experimental phases of commercial cultivation.

Stocking of natural water bodies has been attempted with laboratory-reared larvae or juveniles (in a few cases also with fertilized eggs), for example, of crustaceans such as the lobster *Homarus americanus* and of fishes such as herring *Clupea pallasii*, cod *Gadus macrocephalus*, *Theragra chalcogramma*, smelt *Spirinchus lanceolatus*, flounder *Paralichthys olivaceus*, and sole *Solea solea* (see also p. 1029). Most stocking programmes have yielded no significant harvest increases (e.g. TAYLOR, 1950; TAMURA, 1970; IDYLL, 1975). Major reasons for the failure of stocking programmes to contribute substantially to population recruitment appear to include the following: (i) In species with high fecundity and natural mortalities of more than 99%, even the release of several billion larvae every year may not be enough for measurable increases in population strength. (ii) The larvae or juveniles released do not seem to have been properly prepared for meeting the impact of sudden release. Abrupt changes in environmental factors such as illumination, temperature and salinity, as well as lack of sufficiently fast adjustments to natural feeding conditions and to behaviour patterns essential for *in situ* survival (e.g. escape from predators), may have prevented the larvae from establishing themselves successfully in the sea and from growing and surviving to adulthood in numbers sufficient for restocking success. (iii) In hatcheries, larval characteristics may have been favoured which are different from those required for maximum ecological success under *in situ* conditions. We are confronted here, again, with the consequences of potentially significant differences between the conditions prevailing in cultures and those representative of the animal's natural habitat. (iv) The larvae may have been released into water bodies inadequate in terms of food quality and quantity.

Release programmes of hatchery-reared larvae must take into account the best possible release conditions, e.g. in regard to sea area, water quality, season, time of day, water depth, hiding places and the presence or absence of food organisms and predators. Under unfavourable conditions, larval release may come close to feeding predators, that is, to supporting the potential 'enemies' of the population to be recruited.

Successful stocking has been reported for prawns and turtles. Natural populations of the prawn *Penaeus japonicus*, for example, have increased measurably after repeated release of laboratory-reared larvae and juveniles (KURATA and SHIGUENO,

Table 5-139

Cage-culture farming of fishes. Examples of world-wide data with particular reference to

Species	Country, type of fish culture	Stocking				Type of cage and environment
		Weight or size	Initial number	Stocking No. m^{-3}	kg m^{-3}	
Cyprinus carpio	Indonesia, West-Java, semi-traditional	8–12 cm	200–400	176	2·6	Bamboo cages (2·25 × 1·2 × 0·65 m; ~ 1·7 m^3) standing on bottom in shallow, organic enriched rivers
C. carpio	U.S.S.R.	40 g	n.i.	100–250	4·2–9·75	Floating net cages (3 m^2 × 1 m) in lakes and heated effluents
C. carpio	Japan, experimental	80–100 g	n.i.	30–35	2·0 (0·7–4·5)	Floating net cages (7 to 81 m^2 × 2 m) in lakes
C. carpio	G.D.R., experimental	n.i.	650 780 1300	100 120 200	n.i.	Floating cages (6·5 m^3) suspended from ship hulls
C. carpio	Israel, experimental	n.i.	n.i.	n.i.	61·2	Floating cages
C. carpio	Japan, commercial	70 g	3500	194	13·6	Floating net cages (9 m^2 × 2 m) in lakes
C. carpio	The Netherlands, experimental, 1974/75	n.i.	n.i.	n.i.	25	Floating net cages (6·5 m^3) in heated effluents
C. carpio	Vietnam	n.i.	n.i.	106	n.i.	Box-shaped devices of floating hard-wood plank; wire-gauze panels at shorter ends (12 × 5 × 2 m; 187 m^2)
Ictalurus punctatus	U.S.A., Texas, commercial	n.i.	n.i.	n.i.	38–99	Floating cages (1 m^3) in heated effluents
I. punctatus	U.S.A., general, commercial	30 g, 15 cm	n.i.	390	n.i.	Floating cages in ponds and lakes
I. punctatus	U.S.A., Alabama, experimental	194 g	n.i.	300–500	58–97	Floating cages (1–2·9 m^3) in ponds
I. punctatus	U.S.A., general, commercial	n.i.	n.i.	195–200	n.i.	Floating cages in ponds and lakes
I. punctatus	U.S.A., S. Nebraska, experimental	15 g	490–500	216	3–3·3	Floating cages (2·29 m^3) in reservoir
I. punctatus	U.S.A., Oklahoma	25–60 g 12·2 cm	216–360	216–360	5·4–21·6	Floating cages (1 m^3) in lakes

continental waters. n.i.: no information (Based on COCHE, 1976, and others)

Culture period	Harvesting			Feeding		Author
	Weight or size	Average fish yield (kg m^{-3})	Fish production (kg m^{-3})	Type	Daily ration (% body weight)	
4 months	180–200 g	37 in 2–3 months	n.i.	natural food	n.i.	HICKLING (1962, 1971)
3·5 months	390–480 g	48–98	40–90 (26 month^{-1})	Intensive, vegetable plus 10–15% animal protein	25–50% in several meals	GRIBANOV and co-authors (1968)
3–5 months	400–600 g	10–33	7·5–20·0	Intensive	n.i.	KURONUMA (1968)
4 months	>1000 g	450–850	70–130	Pellets	1·9–2·9	STEFFENS and co-authors (1969); STEFFENS (1970)
n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	VIOLA and BEN-ARI (1975)
6–8 months	800 g	n.i.	n.i.	Intensive	n.i.	COCHE (1976)
6·5 months	n.i.	189	164	Intensive, pellets; automatic feeding	n.i.	HUISMAN *in*: COCHE (1976)
11·5 months	n.i.	n.i.	122	Mixed rice bran, soya bean cake, etc.	n.i.	PANTULU (1976)
30 days	n.i.	69–178	30–79	Floating pellets	4, twice day^{-1}	COLLINS (1970a)
6 months	650–700	n.i.	n.i.	Floating pellets	3–11	COLLINS (1970b)
40 days	n.i.	180	57–89	Purina trout chow (floating)	1·5–4·0	SCHMITTOU (1970)
n.i.	n.i.	n.i.	n.i.	Intensive, floating pellets	n.i.	BENNET (1971)
161 days	220–285 g	35·5–57·0	32–54	Purina trout chow (floating)	n.i.	FEIT (1971)
7–9 months	380–570 g	97–197	91–175 (20 month^{-1})	Purina trout chow	1·75–6·0 once day^{-1}	COLLINS (1972)

Table 5-139—*Continued*

Species	Country, type of fish culture	Stocking: Weight or size	Stocking: Initial number	Stocking: Stocking No. m^{-3}	Stocking: kg m^{-3}	Type of cage and environment
I. punctatus	U.S.A., Alabama, experimental	20 g	n.i.	400–500	8–10	Floating cages (metal) in ponds
I. punctatus	U.S.A., S. Illinois, experimental	25–30 g 12–15 cm	200	286	7–8	Floating cages (0·7 m^3) in lakes
Oncorhynchus spp.	Canada, experimental	2000 g	71	0·04	0·08	Rigid catwalk cages
Oncorhynchus spp.	U.S.A., Washington, experimental, commercial	10–20 g	n.i.	n.i.	n.i.	Floating cages (50 × 12 × 3 m; 1800 m^3) in marine bays
Oncorhynchus spp.	Canada, experimental	n.i.	n.i.	n.i.	n.i.	Cages suspended from compartmented floating concrete structure
Pangasius spp.	Cambodia–Thailand, traditional	80–100 g	7500	46–100	4–10	Floating cages (40–75 m^2 × 2 m) in bamboo or planks along river banks
Pangasius spp.	Thailand, semi-traditional	Fingerlings 80 g	n.i.	150–300	10–30	Floating cages (1–9 m^2 × 1·5 m)
Pleuronectes platessa	U.K., Ardtoe, experimental	O-group	n.i.	n.i.	n.i.	Floating cages (6 × 3 × 1 m; 18 m^3) in brackish water heated effluent
Salmo gairdneri	U.S.A., Arkansas, experimental	56·7 g	400–700	274–480	15·5–27·2	Floating cages (1·5 m^3) in lake
S. gairdneri	U.K., commercial	n.i.	n.i.	n.i.	n.i.	Hexagonal floating cages (2–4 m diameter, 2 m deep)
S. gairdneri	U.K., commercial	n.i.	n.i.	n.i.	n.i.	Decagonal floating cages, (7·5 diameter, 3 m deep)
S. gairdneri	Chile, experimental	6–8 g	3200–6400	40–80	0·25–0·65	Floating net cages (4 × 4 × 5 m) in lakes
S. gairdneri	U.S.A., Alabama, experimental	93·8 g	n.i.	196–392	18·4–36·8	Floating cages (0·76 m^3) in marine bays
S. gairdneri	U.S.A., S. Illinois, experimental	n.i.	n.i.	242	n.i.	Floating cages in lakes

Culture period	Harvesting: Weight or size	Harvesting: Average fish yield (kg m^{-3})	Harvesting: Fish production (kg m^{-3})	Feeding: Type	Feeding: Daily ration (% body weight)	Author
5–6 months	450 g	180	n.i.	Purina trout chow (floating)	n.i.	Coche (1976)
Season	750 g	n.i.	n.i.	n.i.	n.i.	Coche (1976)
51 days	n.i.	n.i.	n.i.	n.i.	n.i.	Hunter and Fare (1970)
7 months	300–400 g	16	n.i.	Oregon pellets	n.i.	Milne (1972); Anonymous (1973)
n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	Ryan (1975)
8–10 months	1000–1200 g	80	n.i.	n.i.	n.i.	Hickling (1962, 1971)
8–10 months	~1000 g	n.i.	n.i.	Mainly: trash fish, also corn, rice bran, etc.	10–15	Bardach and co-authors (1972)
24 months	25 cm	111	n.i.	Pellets	n.i.	Richardson (1971); Milne (1972)
115 days, cold season	163 g	45–80	27·6–55·3	Intensive, pellets	n.i.	Collins (1972)
All year round	n.i.	n.i.	n.i.	Pellets	n.i.	Milne (1972)
All year round	n.i.	n.i.	n.i.	Pellets	n.i.	Milne (1972)
12–14 months	150–200 g	4·6	4·6	Intensive, pellets		Arroyo (1973)
4 months	317–355 g	103	29–65	Purina trout chow	3–5 twice day^{-1}	Tatum (1973)
Cold season	n.i.	33	30·3	Intensive, pellets	n.i.	Tatum (1973)

Table 5-139—*Continued*

Species	Country, type of fish culture	Stocking				Type of cage and environment
		Weight or size	Initial number	Stocking No. m^{-3}	kg m^{-3}	
S. gairdneri	The Netherlands, experimental	n.i.	n.i.	n.i.	21·9	Floating net cages (6·5 m^3) in heated effluents
S. gairdneri *S. salar*	Norway, commercial	n.i.	n.i.	n.i.	n.i.	Octagonal cages (12·7 m diameter, 4 m deep; ~500 m^3) in marine waters (fjords)
S. salar	F.R.G., experimental	50 g	1260	63	3·15	Rotable globe,(4·0 m diameter; 2·0 m^3) brackish water thermal effluent
Salvelinus fontinalis	Canada, Quebec, experimental	10–15 cm	1200	53	n.i.	Floating net cages (22·7 m^3) in lakes
Scophthalmus maximus	U.K., Ardtoe, experimental	35–80 g	n.i.	28–240	n.i.	Floating sea cages (1·8 × 1·8 × 1·2 m; 3 m^3)
Seriola quinqueradiata	Japan, commercial	10–50 g, 5–10 cm	1500–2500	10	0·15–0·55	Floating net cages (144 m^3), marine bays
Tilapia esculenta	Tanzania, experimental	19 g 9·8 cm	2362	19	0·36	Floating net cages (3·5 × 3·5 × 3·5 m; 43 m^3; 8 mm mesh) in Lake Victoria
T. nilotica (or/and *T. aurea*?)	U.S.A., Alabama, experimental	13·6 g	n.i.	286–857	3·9–11·7	Floating cages (0·7 m^3) in ponds
T. nilotica	Ivory Coast, experimental	9–55 g (29 g)	215–488		2–21 (9·8)	Floating cages (1 m^3) in lakes
T. zillii	Tanzania, experimental	2·6 g 5·3 cm	3538	83	0·22	Floating net cages (3·5 × 3·5 × 3·5 m; 43 m^3) in Lake Victoria
T. zillii and *T. esculenta*	Tanzania, experimental	16·3 g 9·0 cm	936	22	0·36	Floating net cages (5 × 5 × 5 m; 125 m^3) in Lake Victoria
Trachinotus carolinus	U.S.A., Florida, experimental	7–12 g	100–900	100–900	0·7–6·3	Floating cages (1 m^3) in marine bays

Culture period	Harvesting: Weight or size	Harvesting: Average fish yield (kg m^{-3})	Harvesting: Fish production (kg m^{-3})	Feeding: Type	Feeding: Daily ration (% body weight)	Author
4 months	n.i.	65	43	Intensive, pellets, automatic feeding	n.i.	Huisman *in*: Coche (1976)
All year round	n.i.	n.i.	n.i.	Pellets	n.i.	Møller (1976)
3–4 months	330 g	20	16·7	Pellets; EWOS, salmon dry feed	2	Grave personal communication)
n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	Ling (1973)
29 months	1230 g	35–41	n.i.	Moist pellets	n.i.	Hull and Edwards (1976); Milne (1976b)
7·5 months	1000–2000 g	10–15	n.i.	Intensive, trash fish (fresh)	20–25 twice day^{-1}	Tatum (1973)
6 months	46·6 g 14·2 cm	0·84	0·48	Brewery wastes and fish meal (10:1) plus leaves	50, once day^{-1}	Ibrahim and co-authors (1974)
156 days	n.i.	n.i.	n.i.	Intensive, floating pellets	n.i.	Pagan (1970)
92–164 days	157–271 g (197 g)	35–76 (61·1)	35–64 (50·8; 12·9 month^{-1})	Pellets, 25% protein content	3–6, once day^{-1}	Coche (1975)
3 months	15·6 g 9·1 cm	1·25	1·0	Brewery wastes, fish meal (10:1) plus leaves	15–30	Ibrahim and co-authors (1974)
5 months	82·2–83·1 g	1·74	1·38	Brewery wastes, fish meal (10:1) plus leaves	n.i.	Ibrahim and co-authors (1974)
11–12 months	450 g	50	23–38	Purina trout chow	5, 1–3 times day^{-1}	Anonymous (1973) Tatum (1974)

1976; see also HANAMURA, 1976; PILLAY, 1976a). Sea-turtle populations have recovered significantly after stocking (BUSTARD and TOGNETTI, 1969; EHRENFELD, 1974; HIRTH and SCHAFFER, 1974). Careful release of laboratory-reared juvenile sea turtles eliminates the high death toll normally incurred immediately after beach hatching and during the run for the water's edge due to the aggregation of large numbers of predators, especially birds, crustaceans and fishes. CARR (1967) and HIRTH (1971) reported successful release of young green turtles *Chelonia mydas* in Central and South Africa, where the natural stocks are critically depleted for a variety of reasons including overexploitation. In contrast to most other stocking programmes conducted on fishes, several release and recapture programmes employing migrating fishes such as salmonids which later return to the release site (homing) have yielded good results (p. 1335).

Translocation (transplantation) of field-born juveniles to more suitable environments can be quite successful, e.g. in bivalves. However, in motile forms such as fishes, recapture success depends on a variety of factors, several of them as yet unknown or uncontrollable. GARSTANG (1905) and BORLAY (1912) transferred young *Pleuronectes platessa* from North Sea inshore waters to the Dogger Bank and recorded 3 times higher growth rates in the translocated individuals than in their inshore-remaining counterparts. In Danish waters, BAGGE (1957) and BAGGE and BERTELSEN (1955) found translocated *P. platessa* to survive and grow well so that the value of the harvested, translocated plaice at least equalled the translocation costs. For details on transplantation of aquatic animals consult Volume V.

Pond and embayment farming has been reviewed in Chapters 2 and 5.1 (especially under *Crustacea*, p. 742, *Mollusca*, p. 884, and *Pisces*, p. 968; see also ARRIOLA, 1941; IVERSEN, 1968; MILNE, 1972; BARDACH and co-authors, 1972).

Bottom-culture farming has received attention in Chapter 5.1 (p. 928). In mollusc bottom cultures, the main measures of control involve transfer of spat and juveniles to fattening grounds, thinning, avoidance of excessive siltation and predator removal.

Ocean ranching is largely based on the predictable migratory and homing behaviour of anadromous fishes such as salmon. As is well known, salmon breed in defined areas of their home river; the juveniles migrate to the sea, utilize large marine areas for feeding and return as mature adults to the place of their birth for spawning. River-released recruits can be recaptured conveniently during their later spawning migrations. There are at least two means to enhance salmon ocean ranching: (i) by providing additional, artificial spawning channels or rivers; (ii) by releasing laboratory-reared fry or smolts.

Spawning channels must be located in pollution-free areas, and have bottom substrates suitable for spawning and egg development. Important aspects for site selection also include temperature, water quality and nutrition, as well as considerations regarding predation and competition.

Salmon release may be highly effective. Hatcheries require relatively inexpensive facilities (CALAPRICE, 1976; see also KANID'YEV and co-authors, 1970; BAKSHTANSKII and co-authors, 1973; RITTER, 1975). As in other fishes, survival and viability of hatchery-reared, released smolts tend to be lower than in field-born juveniles (BULLEID, 1973; see also p. 988). Recently, Japanese scientists have released hatchery-raised juvenile salmon *Oncorhynchus keta* after studying environ-

mental and nutritive (plankton abundance) conditions at projected release sites. As may have been expected, such precaution has paid off well and secured return rates 4 to 6 times higher than after 'blind' release of larvae. According to JHINGRAN and NATARAJAN (1976), ca 77% of the adult *Oncorhynchus keta* run on Hokkaido (Japan) have resulted from hatchery-release programmes. Since some salmonids may establish residency in limnic habitats if their migration into marine waters is significantly delayed, they can be made available to the sport fishery on a year round basis (NOVOTNY, 1975). Major obstacles to salmon migration and breeding are obstructions such as power dams or large rock slides, and river pollution.

In intensive culture operations, major activities include pond- and lake-culture farming, off-bottom and cage-culture farming, as well as culture operations in artificial open, semi-open and closed sea-water systems (Table 5-139). Intensive commercial cultivation projects invite the cooperation of ecologists and engineers (e.g. MILNE, 1976a).

Pond- and lake-culture farming has been briefly reviewed in Chapter 5.1, especially for crustaceans, molluscs and fishes (see also Chapter 2). For details consult IVERSEN (1968), HUET (1970) and BARDACH and co-authors (1972).

In off-bottom-culture farming, the animals concerned, e.g. oysters or clams, are accommodated in the free water, either on ground-rooted frames or on anchored floats. The four methods of off-bottom cultivation in oysters (rack, raft, long-line and tray methods) have been dealt with in Chapter 5.1 (pp. 929–931). Examples of fish cage-culture farming are listed in Table 5-139 (see also MILNE, 1976b). In Norway, fish net-cage culture has increased rapidly (BRAATEN, 1975). Of 79 fish farms developed during 1958 to 1968, some 44% have used net-enclosures; in 1972, about 75% of the fish farms used floating pens. Today, over 200 farmers operate more than 7000 pens. Recent papers on cage cultures have been written by HISAOKA and co-authors (1966), MILNE, (1970), TILTON and KELLY (1971), MARCELLO and STRAWN (1973), LANDLESS (1974), DAHM (1975), GRAVE (1975), KOCK (1975), MAHNKEN (1975), and MØLLER (1976).

Open-, semi-open- and closed-culture operations in artificial water systems have been reviewed in Chapter 2 (pp. 39–57). While closed-system operation provides maximum possibilities for control, it also involves high costs, a high level of technology and considerable energy requirements. For these reasons, aquaculturists have been reluctant to use closed systems. However, according to NEAL and MOCK (1976), a reasonable degree of control may also be achievable without excessive costs, by making use of solar energy, wind, tides or water power.

In intensive cultures, feed cost and water management (including thermal control) constitute the major expenses. Hence, research has concentrated on developing inexpensive, easy-to-handle, storable feeds of known quality; on exact definitions of the animals' environmental and nutritional requirements; on optimizing feeding schedules and employing automatic feeders; on 'tailor-made' water-quality management (Chapter 2); and on utilization of industrial waste heat. Progress from extensive to intensive cultivation increases the opportunity for private ownership, for controlled seed production, and for modern management; it is based on research and technological innovation (e.g. HAMLISH, 1976; MACFARLANE, 1976; PILLAY, 1976b).

(3) Status and Potential of Food Production

(a) Overall Food Requirements

According to official estimates (UNITED NATIONS, 1974), the present human world population of about 4 billion* individuals is expected to increase to about 11 billion in the year 2025 (Fig. 5-128). However, even now an estimated half a billion people receive less protein than they need; several hundred million people are critically

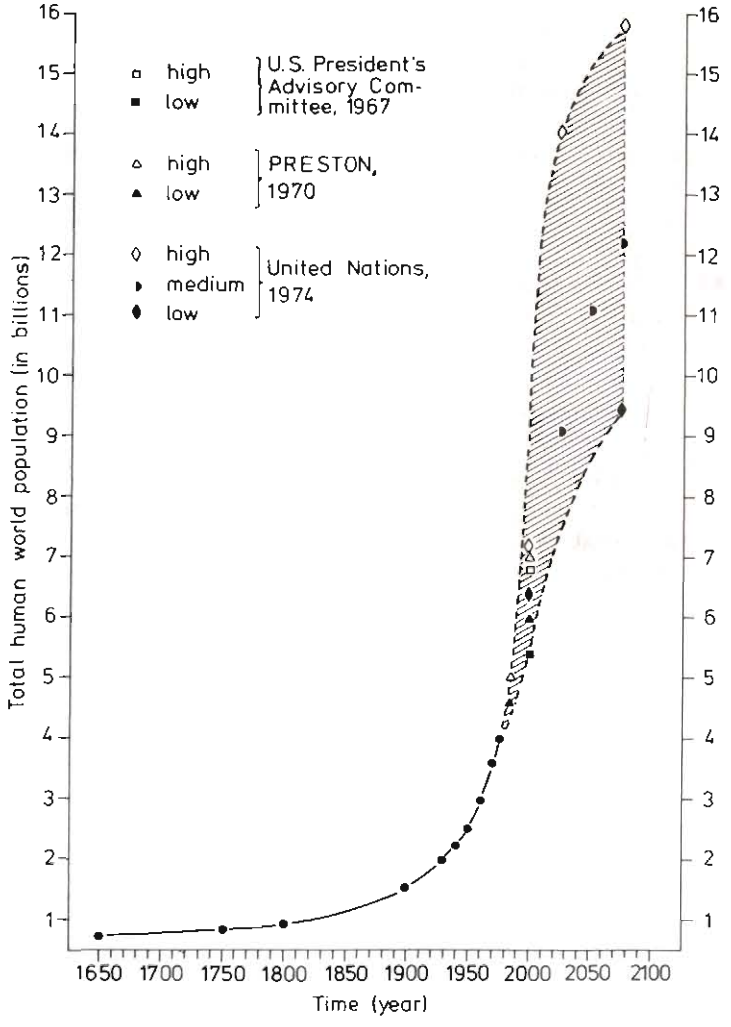

Fig. 5-128: Total human world population. Solid line: estimates based on Brockhaus Enzyklopädia, 1967; UNITED NATIONS, 1974; PIMENTEL and co-authors, 1975. Broken lines: long-range projections based on the sources listed in top of figure.

* We use here and later in the text US billions: 1 US billion = 1000 million.

protein undernourished, and millions are claimed to die every year of starvation. The major reasons for this deplorable situation are: (i) lack of control of human population growth; (ii) channelling of gigantic amounts of money, effort and energy by the leading industrial nations into military 'defence'; (iii) limitations in scientific and technological know-how and in the land and energy resources available.

At present, the daily FAO-safe allowance for a 70-kg male adult, 41 g of reference protein (egg albumin), cannot be provided for an estimated 12% of the world population, although the total world protein yield would allow 67 g protein person^{-1} day^{-1}. It is not possible to provide all people presently alive with a US diet containing 69% animal protein—even if food transportation and related problems could be eliminated. Over 70% of the protein consumed by people outside the USA is of plant origin (PIMENTEL and co-authors, 1975). As is well known, plant proteins are of lower nutritional value than are animal proteins which contain relatively large amounts of the 8 essential amino acids required by man (BURTON, 1965).

Faced with these figures, it is important to assess the total amount of food which can be made available for human needs from suitable land and water areas. To make such an assessment with a reasonable degree of reliability is very difficult, not least because of different opinions regarding the potential consequences of increasing biological knowledge and of technological progress (e.g. CROSSON, 1975; POLEMAN, 1975; SANDERSON, 1975; SPRAGUE, 1975; THOMPSON, 1975; WALTERS, 1975). In addition, uncertainty prevails in regard to the land and water areas ultimately available and to the possible effects of gigantic culture operations on natural ecosystems. Other difficult-to-assess factors include energy requirements and availability, and the degree of interference with other competing activities, including recreation.

(b) Contributions of Agriculture, Fisheries and Aquaculture

Comparisons of food production on land and in water are usually based on units of surface area, mostly hectares (1 ha = 100 ares = 10,000 m^2 = 0·01 km^2). Neither water depth nor water volume are taken into account. Hence, the productivity obtained in water bodies—even in shallow coastal water—is not strictly comparable to that recorded on land.

Primary production on well-managed European oat fields is estimated to be close to 380 g organic substances m^{-2} year^{-1} on a dry-weight basis. With about 250 g organic substances m^{-2} year^{-1}, the average primary production in oceans and coastal waters is about 1/3 lower. However, since some 71% of the earth's surface is covered by the seas, the total marine productivity exceeds that on land by far. No wonder then that the marine environment is being looked upon by many as an 'inexhaustible' protein reservoir for the growing human population.

However—in contrast to the large terrestrial plants which can be harvested without difficulty—in oceans and coastal waters, the main result of photosynthesis manifests itself in the form of microscopic, unicellular algae which are widely distributed and difficult to collect. None of the technical methods presently employed allow the harvesting of these plankters under economically feasible conditions. While it may theoretically be possible to mass-harvest plankton blooms, these occur temporarily, are restricted locally and cannot be forecasted

with a reasonable degree of reliability; in addition, plankton blooms may be poisonous. Consequently, most of the largest nutritional reservoir on earth, contained in the water masses of the World Ocean, can be used only after transformation to higher trophic levels, i.e. into the bodies of molluscs, crustaceans, fishes and other animals. This transformation involves considerable losses in terms of energy and matter.

A comparison between the principles of protein production prevailing in agriculture on the one hand and in aquaculture on the other reveals essential differences.

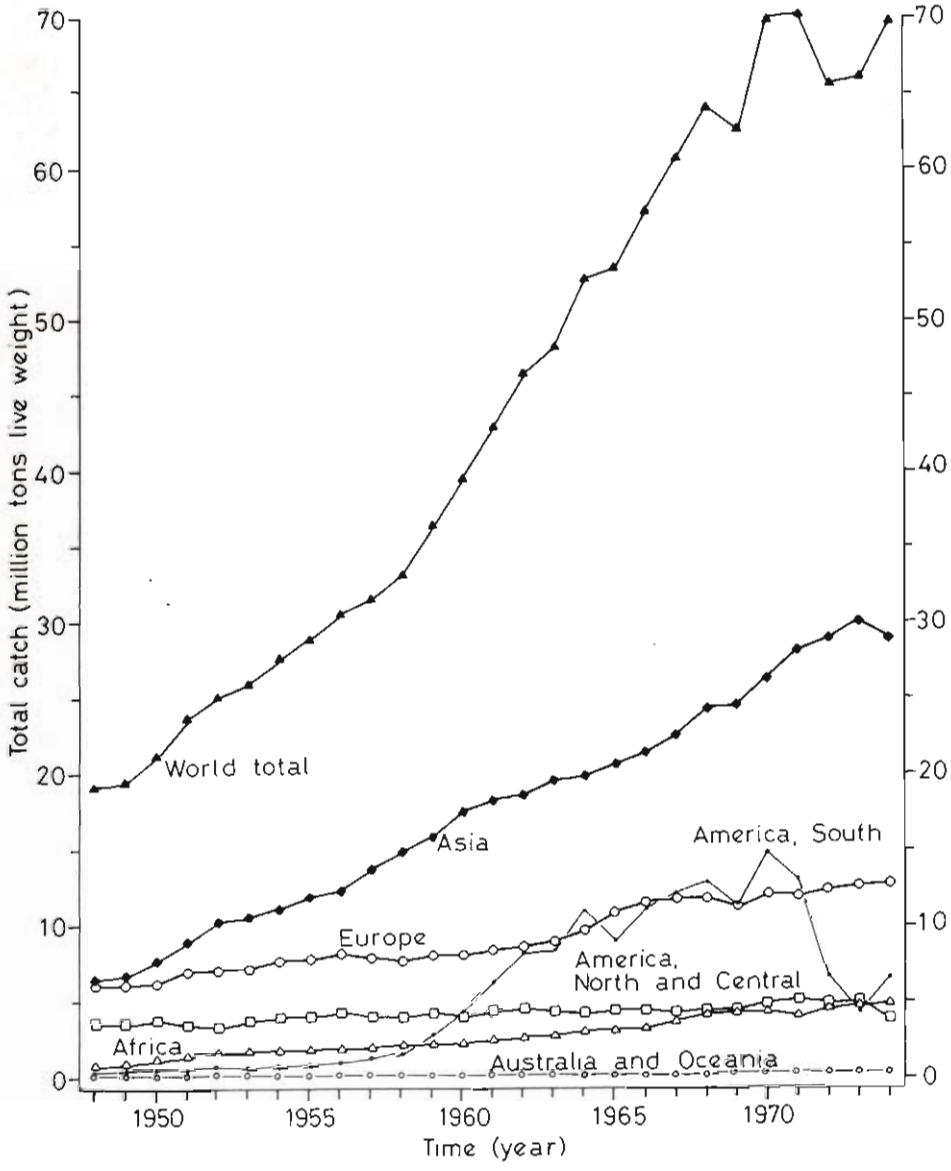

Fig. 5-129: Estimate of total world fishery catch and of subtotals for the different continents. (Based on FAO Yearbooks of Statistics, 1962 to 1973.)

In agriculture, the output of animal protein is based on the following general conditions: (i) The animals cultivated are predominantly herbivores (cattle, goats, sheep) or omnivores (poultry, hogs). The fact that they thrive on purely or primarily herbivorous feeds results in economic and immediate conversion of plant protein into animal protein. (ii) The animals have no absolute requirements for living food. Their dry feeds are readily available, inexpensive and storable. (iii) The animals reproduce under controlled conditions. This guarantees the availability of offspring and facilitates selective breeding and stock improvement. (iv) The major diseases are known and can be controlled—as can predators and most competitors. (v) There is no or very limited need for controlling or changing environmental conditions. All these facts contribute to the smoothness, efficiency and economic success of agricultural protein production. The end results of the culture process are high-quality mass products that can be afforded by many people.

In aquaculture, the output of animal protein is, to a large extent, based on: (i) The use of carnivorous crustaceans and fishes. Both aquatic carnivores and herbivores (e.g. oyster, clam, mullet or milkfish) require more procedural attention than most terrestrial animals. (ii) Several animals selected for aquaculture, especially for mariculture, have absolute requirements for living food—at least during certain life-cycle stages (e.g. larvae). Living food is expensive to raise and usually less convenient to handle than dry feeds. Storable, inexpensive diets are not yet available in many cases. (iii) In most of the marine animals commercially cultivated, controlled reproduction has not yet been achieved. Consequently, seed availability remains a major obstacle, and stock improvement is usually not yet possible. (iv) In many marine animals, our knowledge on disease agents and disease characteristics is insufficient LAUCKNER (in press), and control of disease, predation, competition and cannibalism remains to be accomplished. (v) The need for environmental control and water management (Chapter 2) is usually high. Many mariculture animals have complicated life cycles, often with several larval stages, and each stage may have specific environmental and/or nutritional requirements.

Many mariculture animals, such as oysters or shrimp can be cultivated on a commercial basis only because they command high prices. However, can we afford to invest large amounts of public money and considerable scientific manpower into producing seafood delicacies for a few gourmets? In our opinion, we must develop less expensive methods for mass production of aquatic protein and direct our attention more to low-trophic-level organisms, including unicellular animals and plants.

Within the last decade, the total world fishing catch increased considerably (Figs 5-129; 5-130). In 1973, it was close to some 66 million tons* year^{-1} comprising an estimated average of 14% protein (US Dept. of Agriculture, 1963, 1974). This impressive figure is dwarfed in comparison to terrestrial food production: it does not exceed 1% of the total amount of food harvested, and comprises only about 5% of the global annual protein yield. The annual world fishery output cannot be expected to increase at the present rate. In some areas, it may even drop below the present level because of over-fishing. The long-term effects of the tendency to extend national fishery boundaries are difficult to assess; at least for the imme-

* All measurements expressed in tons in this section refer to metric tons.

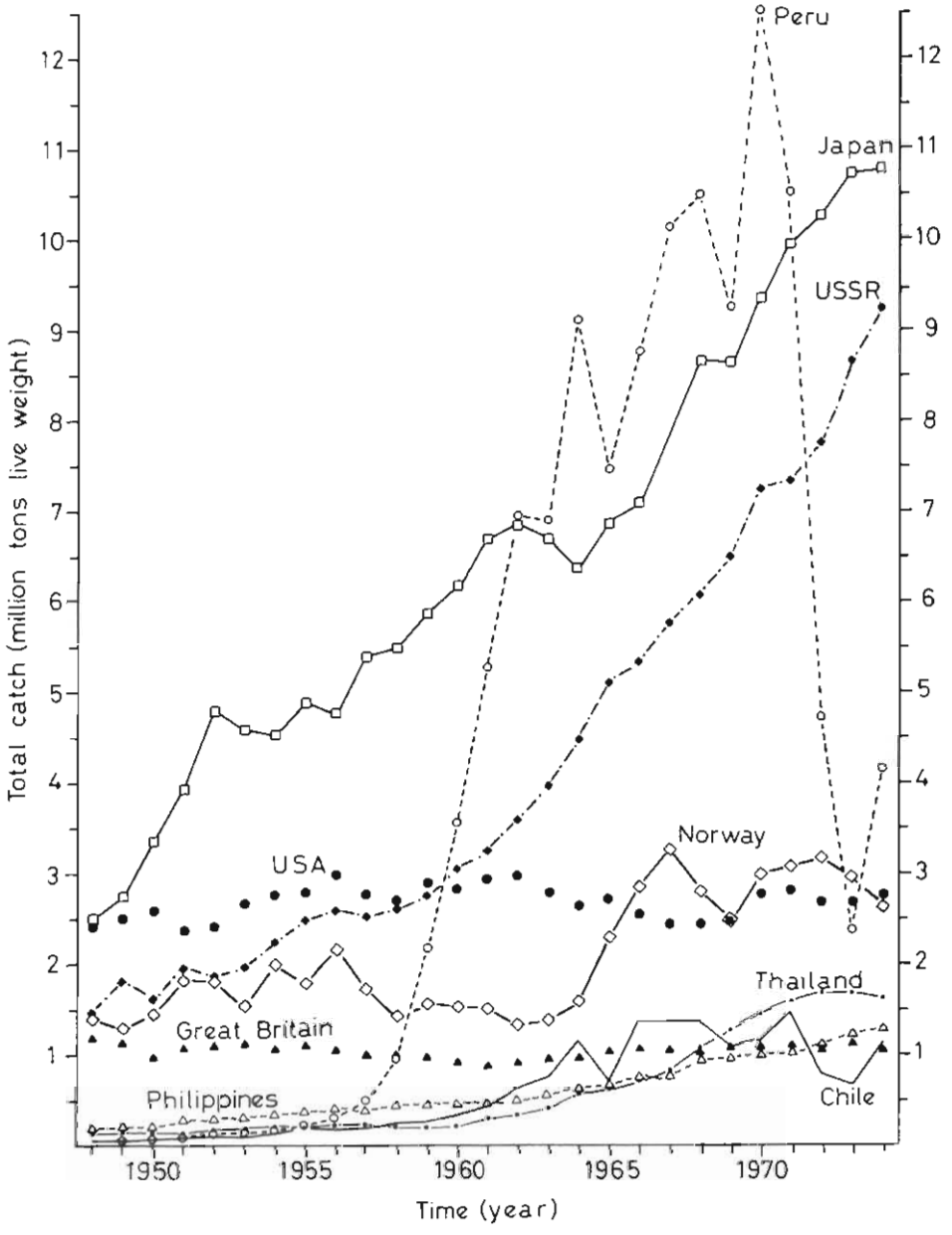

Fig. 5-130: Total fishery catch (in millions of tons live weight) for several countries. (Based on FAO Yearbooks of Statistics, 1962 to 1974.)

diate future, they may decrease the total fishery yield rather than increase it. Better international cooperation with the aim of protecting and managing the natural fishing resources is necessary and seems achievable. However, even better cooperation would most likely assure a sustainable harvest rather than allow a significant augmentation of the total annual landings.

According to PILLAY (1976a), world aquaculture production has risen from 5 to over 6 million tons year^{-1} between 1973 and 1975. Of this sum, 66% consists of fishes (freshwater, brackish-water and marine), about 16·2% of molluscs, 17·5% of seaweeds, and 0·3% of crustaceans. For 1973 and 1975, the total amount of protein obtained through aquaculture is estimated to reach 0·70 and 0·84 million tons, or 7·6% and 9·1%, respectively. In 1973, animal plus plant aquaculture products contributed less than 0·6% to the annual global protein harvest.

A critical, objective assessment of the potential of agriculture, fisheries and aquaculture for producing more human food during the next decade is difficult because of lack of comparable, reliable and meaningful data. Rough approximations have led to the following considerations and predictions.

According to FAO estimates (FAO, Rome, 1973), a total of about 11% of the world land is theoretically available for intensive plant cultivation (about 1·5 billion ha). Most of this land is already used for this purpose. Another 3 billion ha (22% of the world land) are utilized for livestock production. PIMENTEL and co-authors (1975) estimate the present world livestock to total about 1 billion cattle, 1 billion sheep, 550 million pigs, 350 million goats, 100 million buffalo, 64 million horses, 15 million mules, 40 million asses and about 11 million camels. These 3·1 billion livestock graze on an average of 1·6 ha individual^{-1}. With a human world population of 4 billion, the land available per capita amounts to only 0·38 ha.

During the decade 1974 to 1984, the annual agricultural protein harvest may increase from 122 million tons (75% plant protein, 25% animal protein) to some 170 million tons, i.e. by about 3% to 4% year^{-1}. This projected increase is based on assumed improvements in irrigation, product conservation, disease control and harvesting techniques. Considering the losses due to pests and/or waste to attain about 20%, the 1974 figure (122 million tons) would allow a theoretical daily protein ration per person of about 67 g—a value comparing favourably with the FAO safe level of 41 g person^{-1} day^{-1}.

From 1974 to 1984, the world fishery is assumed to increase its total annual protein harvest from 9·3 million tons to ca 14 million tons, i.e. by about 5% year^{-1}. During the same decade, aquaculture can be expected to increase its total annual protein harvest from 0·84 million tons to 1·7 million tons, i.e. about 10% year^{-1}. It must be pointed out, however, that the large percentage increase expected in aquacultural protein harvest would represent only 1·0% of the global protein yield predicted for the end of the 1974–1984 decade.

Considering all coastal land available and assuming significant increases in the biological and technological know-how of cultivation, a very optimistic figure for the global annual output of aquaculture-harvested protein would be 2·8 to 4·2 million tons by the end of this century. Based on this very optimistic figure, aquaculture could contribute some 1·4 to 2·1% to the global annual protein yield. This estimate is based on the assumption that agriculture-protein output continues to increase by 3% year^{-1}.

While it is difficult to make predictions for the next decade, long-range projections hardly deserve to be called predictions. KELLOG (1967) has expressed the opinion that with increased irrigation the world's agricultural potential might ultimately be doubled. At present, only about 15% of the world's cultivated land is irrigated. However, irrigation and other manipulations of environmental factors tend to

consume considerable amounts of energy, and irrigation requires, of course, large amounts of a commodity which also tends to become scarce—fresh water. According to PIMENTEL and co-authors (1975), the world animal protein harvest could be increased maximally by 30% through reduced overgrazing, use of better pasture plant species, and the application of limited amounts of fertilizers under advan-

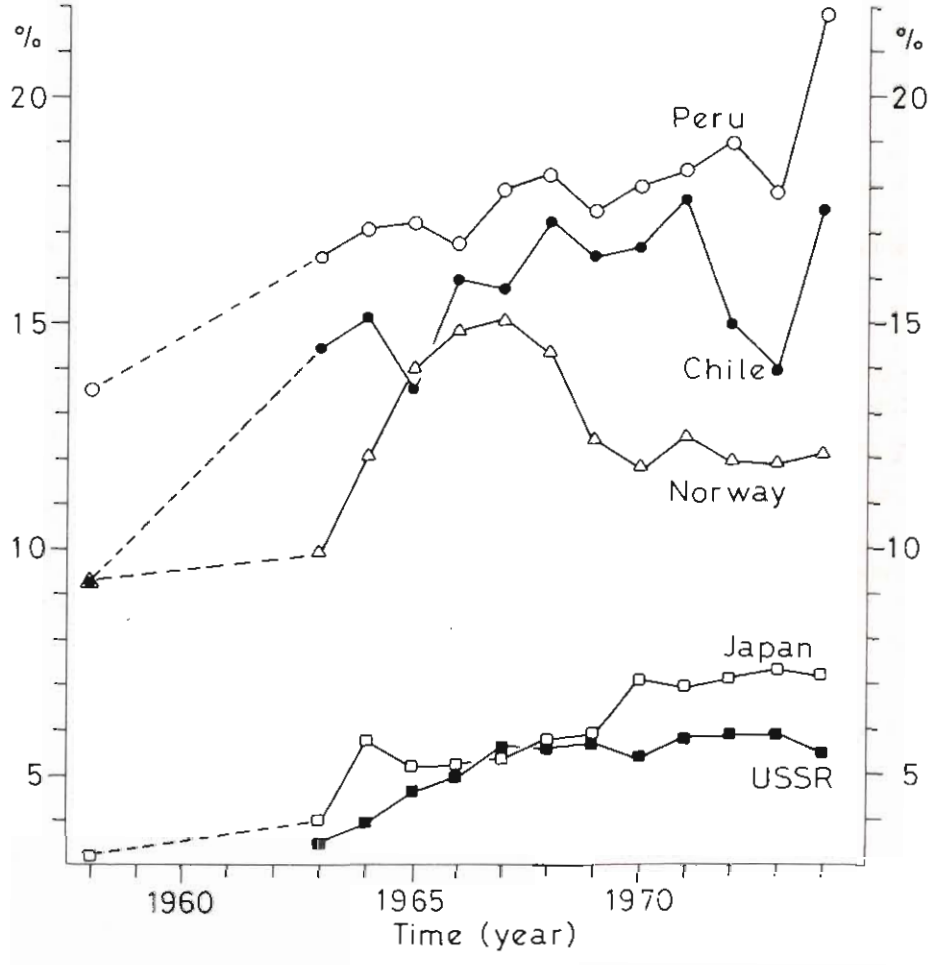

Fig. 5-131: Percentages of fish catch landed in some major fishery countries, which has been transformed into fish meal. (Based on FAO Yearbooks of Statistics, 1967 to 1974.)

tageous conditions. In order to hold the per capita protein supply in the year 2000 at the level of 1975, PIMENTEL and co-authors have calculated the following requirements: 75% increase in cereals, 66% increase in legumes and 100% increase in vegetables; they assume that such increase may be achievable during the next 25 years.

In our opinion, such long-range considerations, important as they are for

planning, tend to neglect (i) the potential impact of human-controlled food production on ecosystems; (ii) the fact that human needs, other than food, also increase as a function of human population size; (iii) the increasing living standards, i.e. increased requirement for energy, materials and recreation per capita.

The potential of new fishery resources for increasing the total protein harvest from oceans and coastal waters remains to be explored (Volume V). The large Antarctic krill populations (see also p. 809), for example, constitute marine protein reserves hitherto untapped by man. PEQUEGNAT (1958) estimates the total krill standing crop to amount to about 1·3 billion tons; KRUCKOVA and co-authors (1971) assume that some 1 million tons of krill live in the area of Georgia, and some

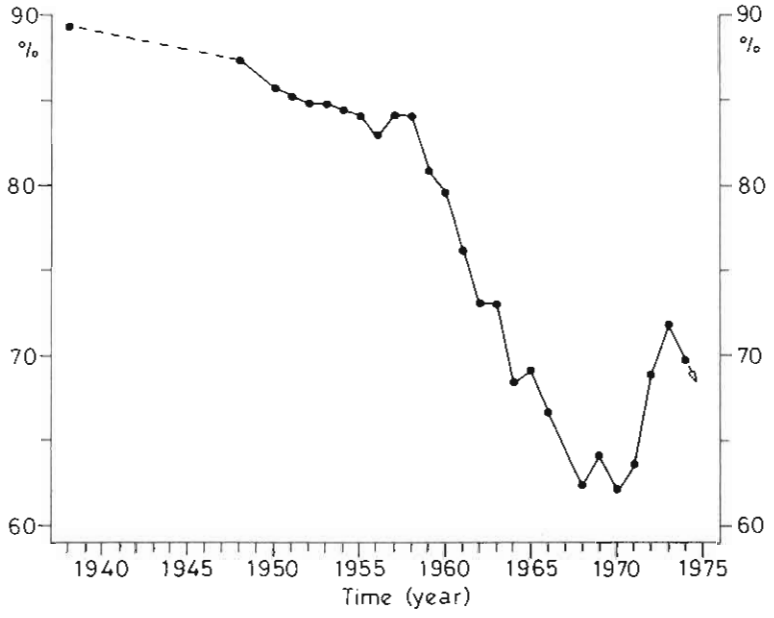

Fig. 5-132: Percentages of total world fishery catch directly utilized for human consumption. (Based on MEYER-WAARDEN, 1967, and FAO Yearbooks of Statistics, 1967 to 1974.)

12 million tons near the South Orkneys. While the preparation of krill for human consumption still poses problems (removal of the chitinous exoskeleton, transformation into an attractive final product), krill already provides for animal feeds. It seems questionable, however, whether long-term exploitation of all additional fishery resources could increase the total world ocean harvest very much above the 100 to 150 million tons year^{-1} predicted for 1985.

The total fishery harvest is not necessarily used directly for human nutrition. A large portion is transformed into fish meal, used as fertilizer, feed, or for a variety of other industrial purposes. Since the late fifties, this portion has increased substantially. Fig. 5-131 exemplifies this increase for several major fishery countries. The percentages of the total world fishery harvest directly utilized for human consumption are summarized in Fig. 5-132. The interruption of the downward trend in the

early seventies reflects primarily the sudden breakdown of the anchoveta fishery yield in South America, which is almost completely transformed into fish meal. Since 1974, the anchoveta fishery has been recovering, and the percentage total catch available as human food is decreasing again.

In the future, mariculture may attract more attention from would-be-investors for two reasons: (i) the present tendency to extend national fishing boundaries; (ii) the fact that most national and international waters are common property. Ownership security of production site and product (animals) constitute fundamental prerequisites for capital investment.

(c) Energy Requirements

Mariculture research has practically neglected considering the amount of energy required for making human food available. However, at a time when the increasing

Table 5-140

Energetic expenses of animal-protein production (After PIMENTEL and co-authors, 1975; modified; reproduced by permission of American Association for the Advancement of Science)

Animal product	Animal protein yield (kg ha^{-1})	Feed protein input (kg)	Feed energy input (10^3 Kcal)	Fossil energy input (10^3 Kcal) for the production of: Feed	Feed + animal	Animal	Kilocalorie ratio Fossil energy input to protein output
Eggs	182	672	14,406	6,070	9,560	3,490	13·1
Catfish	51	484	5,007	2,180	7,068	4,888	34·6
Pork	65	689	17,021	6,774	9,212	2,438	35·4
Milk	59	188	6,963	2,382	8,561	6,179	35·9
Beef	51	786	24,952	7,129	15,845	8,716	77·7

global demand for energy and the actual availability of fossil-fuel energy begin to affect the prices of numerous commodities and to seriously concern long-term planners, energy considerations attain more importance than ever before. The amount of energy required for making human food available depends primarily on the organism cultivated, the culture technique employed, and the local climate.

The energy requirements of food-production processes have recently been considered by PIMENTEL and co-authors (1975). The fossil-fuel energy required for obtaining a return of 1 Kcal of edible protein is about 2 Kcal for soy protein, 3·6 Kcal for corn and 10 Kcal for rice. The fossil-energy input required for obtaining 1 Kcal of animal protein is much higher: about 35 Kcal for catfish protein, 36 Kcal for milk, and 78 Kcal for beef (Table 5-140).

In the marine fisheries, large amounts of fossil-fuel energy must be expended to collect animal protein from the sea—a fact which has thus far received little, if any, consideration. Off the coast of England, for example, LEACH (1976) calculated a requirement of 7920 Kcal for harvesting 1 kg of fish; this amounts to about 20 Kcal of fossil energy required for obtaining 1 Kcal of gutted fish.

Future research in the field of animal mariculture should place more emphasis on energy requirements and pay more attention to the possibilities of converting energy obtained from solar radiation, wind and water movement. The solution of such energy-requirement problems invites cooperation between mariculturists and engineers.

(4) Areas and Animals Suitable for Mariculture

(a) Areas

The suitability of coastal areas for mariculture is not easy to assess, neither in overall terms, nor for defined areas. In many cases, the suitability depends on, and varies with, a variety of unknown or insufficiently known factors. In particular, it depends on the costs of land, labour and energy, means of transportation, availability of seedlings, and market prices of competing foods. In addition, the species to be cultivated is of importance, as well as the biological and technological know-how and the financial means of the farmer. For most maricultural purposes, only well-protected (wind, tides, wave action) shallow areas (ca 1–35 m deep), located in adequate climatic zones, can be used. Since the total shelf zone on earth amounts to less than some 3% of the global sea-surface area, and since several other commercial interests are focused on shallow-water areas and compete with the interests of the aquafarmer, the actually available total area may be much smaller than optimistic predictions would indicate.

On the basis of the usually meagre and often inadequate information available, we have summarized, for a number of countries, rough estimates of coastline length, the area presently utilized for mariculture, the area potential, and the present total annual harvest obtained from brackish and marine culture systems (Table 5-141). The table has a number of shortcomings: for many areas, the data at hand are incomplete; for others, they contain inconsistencies or seem too optimistic. Environmental and managing prerequisites have often not been sufficiently considered or have remained unknown. A few sources do not differentiate at all—others not critically enough—between fishing and aquaculture harvests or between mariculture and limniculture.

In Africa, the area potential for brackish- and marine-water commercial cultivation is particularly difficult to assess because of an almost total lack of reliable information. However, Africa seems to hold a high potential for future expansion. A detailed assessment is desirable. Africa's total area potential may be of the order of several million ha.

A recent survey of the Niger delta revealed some 730,000 ha of mangrove area, of which at least 405,000 ha may be utilizable for commercial fish culture (Pillay, 1973). Egyptian waters are suitable for rearing mullet seedlings (El-Zarka and co-authors, 1970), and for the culture of *Solea solea*. In Algeria, coastal lagoons support successful eel and mussel cultures, and along the Sudanese Red Sea coasts some 120 probate pearl-culture farms have been established.

Tunisia features a total of about 11,250 ha of coastal lagoons and some additional 85,200 ha of inland salt-water lakes (salinities between 1·2 and 40‰S), which may

Table 5-141

Area potential of brackish and marine coastal waters suitable for mariculture and present total annual mariculture harvest. Estimates on area potentials refer to the maximum estimate or indicate minimum-maximum range. n.i.: no information
(Compiled from the sources indicated)

Continent, country	Coastline (km)	Area presently utilized (ha)	Area potential (ha)	Present total annual mariculture harvest (tons)	Remarks	Author
Africa						
Egypt	n.i.	22,000△	202,000–279,000	n.i.	Brackish water basins and delta areas; partially suitable; △Lake Quarun	EL-ZARKA and co-authors (1970), LEVI and TROADEC (1974), EISAWAY and EL BOLOCK (1975)
Madagascar	n.i.	18,000	330,000□	45	□ Mangrove swamps and coastal lagoons, partially suitable	RAJAONA (1975)
Mauritius	n.i.	268	250	530	Saltwater lagoons enclosed by semi-permeable rock walls; tidal exchange via sluice gates	MINISTRY OF FISHERIES, Port Louis, Mauritius (1975)
Nigeria	n.i.	~10,000	400,000–>1,000,000	n.i.	Milkfish farming, including fresh and brackish waters	IDYLL (1973), PILLAY (1973), EZENWA (1975)
Tanzania	800	n.i.	700–1,200⊙ plus 40,000	n.i.	⊙Experimental ponds and pilot farms planned between 1975 and 1980	SINGH (1975)
Tunisia	n.i.	n.i.	~100,000	n.i.	Coastal lagoons, lakes, reservoirs	AZOUZ (1975)

America, Central and South						
Bahama Islands	n.i.	1,000	n.i.	n.i.	*Chelonia mydas*, pilot turtle ranch	CARR (1967), HIRTH (1971)
Colombia	n.i.	50	~300,000	n.i.	Coastal lagoons and estuaries; especially suitable for molluscs	MINISTERIO AGRICULTURA, Colombia (1974)
Cuba	n.i.	n.i.	130	n.i.	Coastal lagoon for mullet culture	ANONYMOUS (1974)
Ecuador	n.i.	600	n.i.	n.i.	*Penaeus vannamei*	COBO CEDEÑO (1974)
Mexico	n.i.	10,000	400,000–900,000	n.i.	*Crassostrea virginica*	ANONYMOUS (1971b), ANONYMOUS (1974)
Venezuela	n.i.	32	90,000	272	*Perna perna*, *Crassostrea rhizophorae*	SALAYA (1974)
America—North						
Canada	n.i.	4,150	>10,000	4,800	69% *Crassostrea gigas*, 30% *C. virginica*, 1% fishes	QUAYLE (1969), PILLAY (1973), STEWART (1973)
USA	22,800	75,900	5,230,000–12,173,000	351,100	99% oysters, mainly *Crassostrea virginica* unshucked, with shell, 1% *Penaeus* spp. and others	RYTHER (1968a, b), MILNE (1972), UCHIDA (1972), PILLAY (1973), SHAW (1974a), WILDMAN (1974)
Asia						
Burma	1,720	n.i.	~520,000	n.i.	Brackish and salt-water swamps and other tidal lands	PILLAY (1973)
Ceylon	n.i.	~15	140,000–190,000	n.i.	Lagoons and tidal flats, including salt-water swamps partially convertible into ponds	DEPT. FISH., Colombo (1972), LING (1972), PILLAY (1973)

Table 5-141—*Continued*

Continent, country	Coastline (km)	Area presently utilized (ha)	Area potential (ha)	Present total annual mariculture harvest (tons)	Remarks	Author
Hong Kong	~1,000 (including 198 islands)	2,500–3,000	6,500	n.i.	Coastal bays and tidal flats	BARTZ (1964), LING (1973), PILLAY (1973)
India	4,700	15,200	336,000–>2,000,000	480,000*	*Total finfish production, including fresh water	JHINGRAN and GOPALAKRISHNAN (1973, 1974), PILLAY (1973), SELVARAJ (1973)
Indonesia	~40,000 (including 350 large and 2,500 small islands)	189,000	>600,000–8,500,000●	71,500	●Salt or mangrove swamps, estuaries, brackish-water ponds and lagoons, partially suitable	SCHUSTER (1960), HORA and PILLAY (1962), PILLAY (1973), DJAJADIREDJA and POERNOMO (1972)
Japan	~27,300	343,800–450,000	686,000□	ca 900,000*	□Estimated area generally suitable, regardless of contradicting developments (e.g. industry, pollution); *37% seaweeds, 34% molluscs, 2·5% *Anguilla* spp., 12·0% *Seriola quinqueradiata*, 0·07% *Penaeus japonicus*, 14·4% others	TAMURA (1970), BARDACH and co-authors (1972), FURUKAWA (1972), SHIGUENO (1972), MILNE (1973), PILLAY (1973), KAWATSU (1974), USUI (1974), SASAKI (1975), Japan Fisheries Association (1975)
Khmer Republic	n.i.	n.i.	>50,000	n.i.	Coastal and brackish-water areas; probably 10,000 ha	LING (1972, 1973)

					for mollusc culture, ~38,000 ha for fish and shrimps	
Korea (South)	~12,000 (including several thousand islands)	27,550	169,000–232,000	86,316	~31% *Crassostrea virginica*, ~10% *Venerupis japonica*, ~2·5% *Meretrix lusoria*, 50% miscellaneous	Ling (1972), Office of Fish., Rep. Korea (1972), Pillay (1973)
Malaysia	n.i.	3,500	146,000	27,600	98% *Anadara granosa*, 1% *Penaeus* spp., *Metapenaeus* spp., 1% *Chanos chanos* and miscellaneous	Fish. Div. Malaysia (1972), Ling (1972, 1973), Pillay (1973)
Pakistan and Bangladesh	n.i.	n.i.	~166,000	n.i.	Including brackish and salt-water swamps and other tidal lands	Pillay (1973)
Philippines	~78,000 (including >7000 islands)	170,000	380,000–550,000	94,000	95% *Chanos chanos*, 2·5% *Penaeus* spp., *Metapenaeus* spp., 2·0% *Mytilus smaragdinus*, 0·5% miscellaneous	Schuster (1960), Ryther and Bardach (1968), Blanco (1972a), Ling (1972), von Westernhagen (1974)
Singapore	~150	600	3,000	n.i.	Coastal and brackish-water areas suitable mainly for mussels and cockles	Ling (1972, 1973), Pillay (1973)
Taiwan	~1,600	27,600–30,000	53,000*	31,700	*Maximum estimate; 43% *Chanos chanos*, 19·8% oysters, 17·6% eels, 17·3% clams, 2·3% others	Schuster (1960), Blanco (1972a, b), Chen (1972a), Pillay (1973), Shang (1974), Chen (1976b)
Thailand	~2,400	~11,000	166,000–>500,000	64,800	61% mussels (*Mytilus* spp.), 23% cockles (*Anadara* spp.), 9% oysters, 5% shrimps, 0·5% *Chanos chanos*	Ling (1972, 1973), Shribhibhadh (1972), Pillay (1973)
Vietnam (South)	~2,000	70,000	600,000	10,500	95% *Chanos chanos*, *Tilapia* spp., 5% shrimps, miscellaneous	Dang (1972), Ling (1972, 1973), Pillay (1973)

Continent, country	Coastline (km)	Area presently utilized (ha)	Area potential (ha)	Present total annual mariculture harvest (tons)	Remarks	Author
Australia and Oceanaria						
Australia	20,000	10,420	13,200	9,800	*Crassostrea commercialis*	Fish. Div. Austr. (1972), PILLAY (1973)
Fiji	n.i.	7	365	n.i.	Oyster culture	ANONYMOUS (1974)
New Zealand	n.i.	minimal	n.i.	10,700*	*Total fish and shellfish production	PILLAY (1973)
Europe						
Cyprus	650	n.i.	4,040	n.i.	Brackish-water basins, partially suitable	LEVI and TROADEC (1974)
Denmark	~6,000	~500	220,000**	19,200	**For low-density culture in brackish waters (e.g. stock improvement), partially suitable	ANDRÉU (1968a, b), BARDACH and co-authors (1972)
France	2,700	18,000	31,500	114,000*	~8% *Ostrea edulis*, ~45% *Crassostrea angulata*, ~47% *Mytilus edulis*, various culture methods; *unshucked, with shell, data for 1973	ANDRÉU (1968a, b), BARDACH and co-authors (1972), PILLAY (1973), LEVI and TROADEC (1974), HURLBURT and HURLBURT (1975)
FRG	470	~600	>1,000	14,000	99% *Mytilus edulis*, 1% *Cardium edule*; *unshucked, with shells	TIEWS and MEIXNER (personal communication)

Greece	~4,000 (without islands)	n.i.	400,000	n.i.	Brackish-water basins, partially suitable	LEVI and TROADEC (1974)
Iceland	n.i.	5○	n.i.	n.i.	○Pilot operation near Keflavik airport	MILNE (1975)
Italy	7,950	50,800	137,500	14,100	86% *Mytilus edulis* (unshucked), 13% eels, mullet and others	ANDRÉU (1968a, b), PILLAY (1973), LEVI and TROADEC (1974)
Netherlands	1,730	~12,000	n.i.	92,400	*Mytilus edulis* (unshucked)	ANDRÉU (1968a, b), IVERSEN (1968), HURLBURT and HURLBURT (1975)
Norway	19,300	~100	50,000–>200,000▲	n.i.	▲Rough estimate calculated from various sources	BRAATEN (1975)
Portugal	850	n.i.	n.i.	2,900	*Crassostrea angulata*	PILLAY (1973)
Romania	400	n.i.	>1,000	n.i.	Limited areas around the Donau delta	Rough estimates from various sources
Spain	3,150	>1,500	28,400	~152,000*	~89% *Mytilus edulis*, 11% *Crassostrea angulata*, bottom culture and raft culture; *unshucked, with shell	PILLAY (1973), SAN FELIU (1973), LEVI and TROADEC (1974)
Sweden	7,500	n.i.	50,000–>100,000	n.i.	Estimated area partially suitable for cage culture and hanging mussel culture	△△Rough estimates from various sources; see also BRAATEN (1975)
Yugoslavia	1,700	>100+	14,200	n.i.	+Tivat Bight; brackish-water basins, partially suitable	PILLAY (1973), LEVI and TROADEC (1974)

be suitable, at least in part, for commercial cultivation (NASFI, 1975). Along the Tanzanian coast, vast brackish-water areas, estuaries and mangrove swamps may be utilizable for mariculture (IBRAHIM, 1975); a reliable evaluation of the area potential is not yet available.

In Central and South America, only a few surveys have been made and the area potential awaits careful investigation. In several countries, feasibility studies are underway, especially on the area potential of lagoons and bays.

In Cuba, fish-pond farming in tidal flats and oyster (*Crassostrea rhizophorae*) farming have entered the experimental or pilot-scale level. Apparently, the results obtained are satisfactory. The Mexican coasts appear to be highly suitable, especially in the Gulf and Baja California regions. According to GLENN (1973) and GRANADOS (1974), mariculture may become one of Mexico's primary industries serving both domestic and export purposes. By the end of the seventies, the development of ca 400,000 ha may yield an annual harvest of some 8000 tons of prawns and 32,000 tons of oysters (with shells) (ANONYMOUS, 1974).

Several areas along the coast of Chile—especially in the Isla de Chiloe region and in the provinces of Llanquibue and Aysén—are considered suitable for mussel and oyster farming (GONZÁLEZ and co-authors, 1974; see also MERY, 1974). The total area potential in that region is yet to be evaluated. In Brazil, large areas used for extensive fish farming are located in the lower São Francisco River valley, ranging from the estuary to some 80 km upstream. Lateral lakes are utilized for extensive seed production (JENSEN, 1974). VILLEGAS (1974) mentions the possibility of prawn cultivation in the lagoons of Uruguay, but so far no estimate on area potential is available. SALAYA (1974) lists 90,000 ha as potential area for marine and brackish-water culture in Venezuela; at present, only 32 ha are being utilized. Eastern Venezuela features well protected areas, suitable for mollusc cultivation within the intense upwelling zone in the Gulf of Cariaco (MANDELLI and ACUÑA, 1975). For Argentina, a high limnicultural area potential (ca 1·01 million ha) has been estimated by MASTARRIGO (1974).

In North America, the maximum area potential may range up to some 12 million ha in the USA and to more than 10,000 ha in Canada. However, it seems safe to assume that only a relatively small portion of this area will eventually be used for mariculture. Major interests of other economically important activities concentrate on the same area. It would be unrealistic, therefore, to consider all coastal areas as potential farming sites. In highly industrialized nations such as the USA and Canada, the drive for sufficient recreational areas is bound to increase. On the other hand, lagoons and salt ponds located behind the beach, e.g. in the southern part of New England (USA), offer unique chances for mariculture farms, and these areas seem less attractive for recreation.

In Canada, the total aquaculture production reveals a rapid increase. Annual oyster harvests in British Columbia are illustrated in Fig. 5-133. In the Maritimes, oyster-production areas are restricted to shallow bays and estuaries of the southern Gulf of St. Lawrence and the coves of the Bras d'Or lake in Cape Breton. A total of about 6300 ha appears suitable for oyster culture; only 44% of this area potential is presently utilized (ROWELL, 1976).

Asia, especially the southern and eastern regions, represents the most promising area on earth for near-future extensions in mariculture. In India, brackish-water

fish farming is well established along the estuarine belt of Sundarbarns, West Bengal (SAHA and co-authors, 1964a), and extends to the tidal swampy areas of the Junput coast (SAHA and co-authors, 1964b). The Chilka lake, a brackish-water lagoon of more than 90,000 ha on the eastern seaboard of India, offers suitable sites for coastal farms, as do many other backwaters, estuaries, tidal flats and deltaic marshes. The total area available along the Indian coast is estimated to exceed 2 million ha (JHINGRAN and NATARAJAN, 1972), but only a small percentage of this area may actually be converted into aquafarms. Experimental farms in brackish and marine

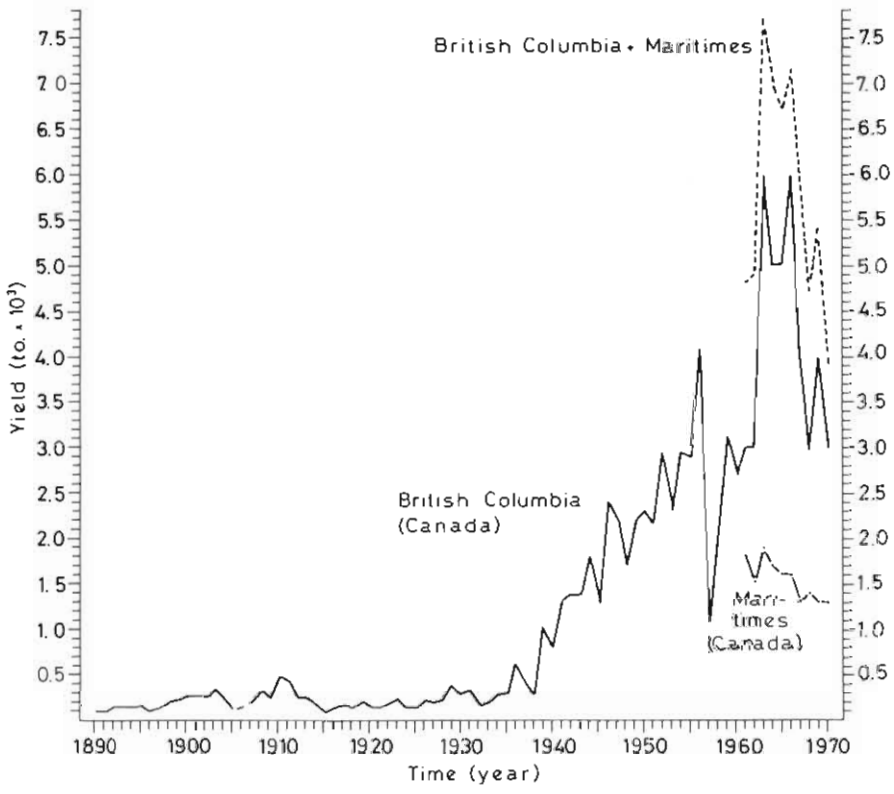

Fig. 5-133: Annual oyster harvests in British Columbia, Canada. (Based on QUAYLE, 1969.)

waters started during the early seventies along the coast of West Bengal (Midnapore), in the Province of Andhra Pradesh (Kakinada), in Tamil Nadu (Krusadai Island) and on the coast of Kerala State (Nutakkal and Ayiramthengu). Japan—the world's most successful mariculture country—has increased its total harvest from cultivated marine and brackish waters from ca 50,000 tons in 1950 to over 650,000 tons in 1972 and to ca. 880,000 tons in 1974 (Fig. 5-134). This harvest was obtained from an area occupying only 1·4% of Japan's continental shelf zone. Since ca 4·5% of the shelf (ca 6·3 million ha) are less than 50 m deep (JAPAN FISH. ASSOCIATION, 1975), the present area may be extended considerably. For the near future, an area potential of about 686,000 ha seems realistic. On the other hand, several farms which have operated successfully for many years had to be given up

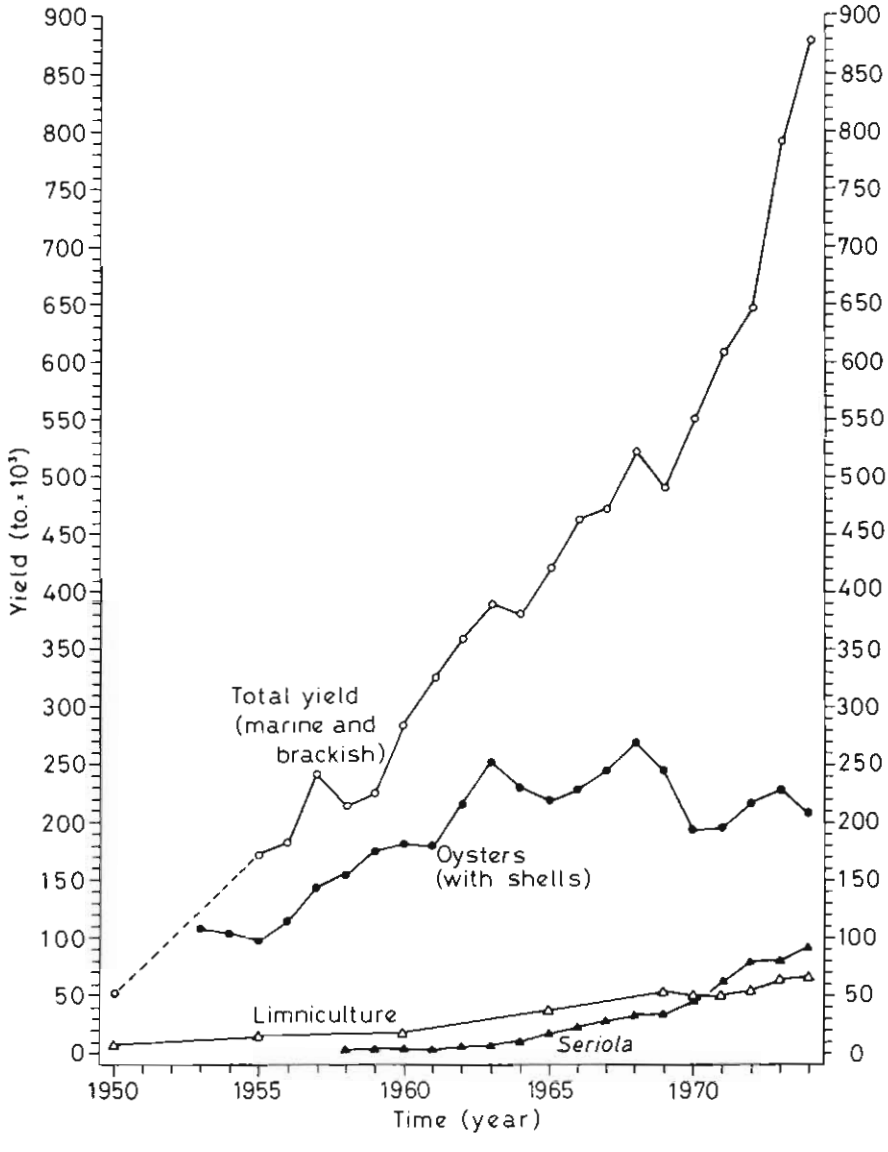

Fig. 5-134: Annual total aquaculture yield (tons) in Japan, and the contribution of the most important aquaculture branches. (Data compiled from numerous sources.)

because of increasing water pollution, especially in the Seto Inland Sea. The future development in Japan may be characterized by a keen competition between different branches of coastal-area utilization.

While a variety of marine organisms is being cultivated in Japan, the largest harvests are contributable to seaweeds (58·3% in 1973) and shellfish (41·2%); finfishes account for only 1·4% of the total yield. Although still small in overall output, shrimp and prawn cultivation (pp. 840–859) is financially particularly

rewarding. Japanese limniculture which includes highly successful eel-culture activities (p. 1364) attains only a fraction of the country's total aquaculture yield, i.e. about the same amount as yellow-tail farming. It seems that Japanese limniculture is already experiencing limitations in terms of water supply and area potential. Restrictions also prevail in Taiwan with regard to the annual *Chanos* harvest which has levelled off below the 30,000-ton mark (Fig. 5-135).

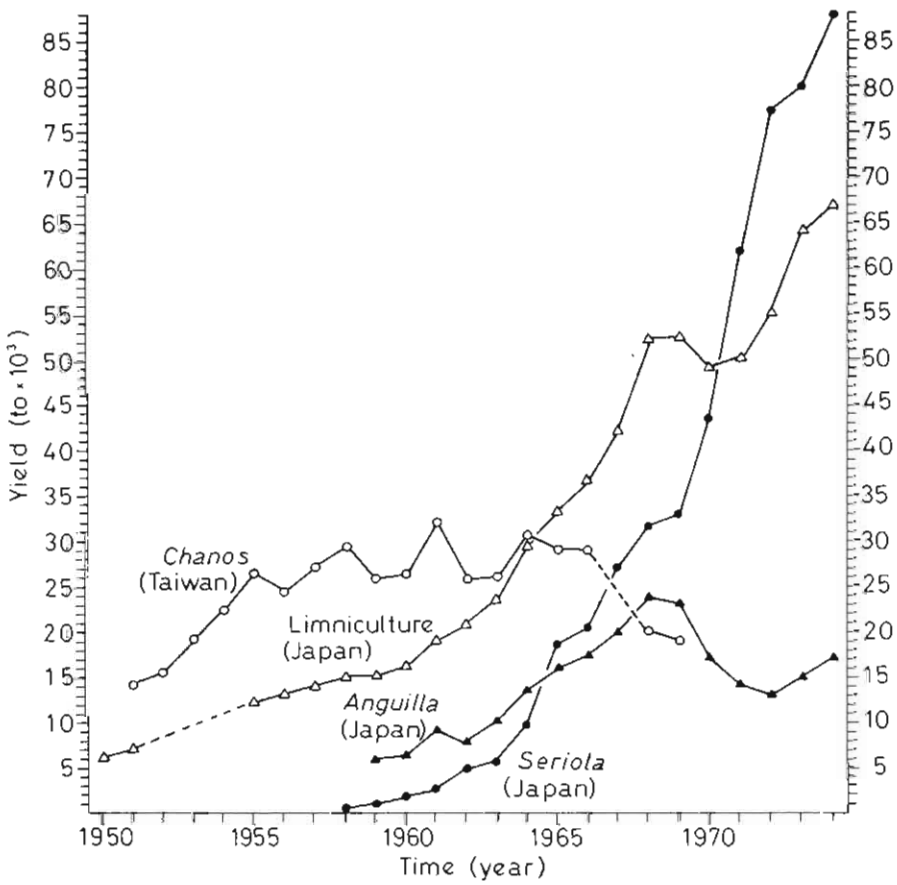

Fig. 5-135: Annual aquaculture yield (tons) of important finfish species in Japan and Taiwan. (Data compiled from numerous sources.)

In Taiwan, the total area presently available allows a relatively high output, apparently primarily due to multicropping techniques. The recent decline in total milkfish production is attributable to (i) a frequently inadequate supply of fry and (ii) high mortalities in wintering ponds (CHEN, 1976a). In 1972, the total Taiwan milkfish culture area covered about 15,624 ha. Although farming started in 1952, only 60 ha of eel ponds were established in 1966, but due to increased demand especially on the Japanese market, the area reached about 1058 ha in 1972. A total of 9550 ha is presently utilized for oyster production, whereas 3800 ha are used for

clam (*Meretrix lusoria*) culture; only 200 ha are available for cockle (*Anadara granosa*) culture (CHEN, 1976b).

For China, the information available is entirely insufficient for any reasonable judgment.

In Thailand, the area used for coastal mariculture in 1967 served for shrimp farming (82·3%; 7825 ha), cockle culture (6·6%; 626 ha), mussel culture (2·3%; 222 ha) and oyster culture (0·6%; 55 ha). In 1972, mussel and oyster culture areas had extended to 350 ha and 150 ha, respectively (LING, 1973). Although shrimp culture operations cover the largest area, they contribute only 5% to Thailand's total aquaculture harvest (SHRIBHIBHADH, 1972). The area presently used for coastal mariculture covers about 1/15 (~10,000 ha) of the total area available for future development. According to LING, at least 100,000 ha are suitable for shrimp farming and 15,000 ha may be converted into cockle, mussel and oyster farms.

In Hong Kong, oyster beds occupy at present an area of about 2000 ha, but more than 5000 ha are expected to be suitable along the coast of the New Territories.

For South Vietnam, estimates by LING (1973) indicate that approximately 20,000 ha of brackish-water ponds were utilized for finfish production in 1972, and 50,000 ha for shrimp-trapping ponds. These figures account for only 10·5% of the total area potential; swift further expansion may be expected.

In Indonesia, about 184,000 ha of brackish-water fishponds existed in 1971 (PILLAY, 1973). This represents only some 2·2% of the enormous area potential of estuarine and coastal flats, mangrove swamps and shallow coastal waters which cover about 6,000,000 ha along the coasts of Sumatra, Kalimantan, Sulawesi and West Irian. At least 600,000 ha are considered highly suitable for near-future developments (finfish and finfish–shrimp culture: 390,000 ha; shrimp culture: 100,000 ha; shellfish and others: 110,000; LING, 1973). In the Philippines, about 8 ha of culture area served in 1966 to produce 2000 tons of the mussel *Mytilus smaragdinus* (BARDACH and co-authors, 1972). In 1970, about 300 ha were used for oyster and mussel cultivation in Bacoor Bay. The potential area for mollusc cultivation in bays, coves and estuaries in Bacoor Bay, Manilla Bay and elsewhere in the Philippines totaled over 100,000 ha (BLANCO, 1972a). In Laguna de Bay, over 5000 ha of fish pens have been established within the last five years, producing 7500 to 10,000 tons of milkfish annually (PILLAY, 1976b).

The overall Asian maricultural potential may be estimated to total some 24 million ha, with India and Indonesia leading as favourites. In addition, many Asian countries have a high, not yet fully utilized, area potential for limniculture.

Australia and Oceania. In Australia, the major areas used for coastal aquaculture are located in New South Wales, southern Queensland and Tasmania. While 6400 ha are utilized in New South Wales, the potential for future extensions in this area is quite limited (ca 600–1200 ha). In southern Queensland, it seems possible to double the 4000-ha area presently utilized for oyster farming. Tasmania holds the largest promise for extension: the 20 ha presently used for oyster farming may be extendable to some 8000 ha. The potential of New Zealand for animal mariculture seems to be small; however, critical suitability screening has still to be made.

The seemingly very high area potential of Oceania's numerous atolls and thousands of islands awaits thorough evaluation. Several pilot projects and many small-scale farming operations have been started in the last few years. On Fiji, a 7-ha experimental farm project (*Crassostrea gigas, C. commercialis*) has become

operational in a 364-ha mangrove swamp reclamation scheme. On the Gilbert and Ellice islands, baitfish, milkfish and shrimp cultures seem to hold promise.

In Europe, the area potential attains maximum values in Greece (400,000 ha), Denmark (220,000 ha) and Norway (up to 200,000 ha), while the present total annual aquaculture harvest is highest in Spain (152,000 tons), France (114,000 tons) and the Netherlands (92,400 tons). Along the Spanish coast, the most important rias of Galicia used for mollusc production are the Ria de Arosa and Ria de Vigo, which cover a total water area of 230 km^2 and 183 km^2, respectively (Saiz and co-authors, 1957; Cadee, 1968). In 1968, there were almost 2800 rafts in the Galician rias; about 1800 in the Ria de Arosa; 483 in the Ria de Vigo; 210 in the Ria de Pontevedra; and smaller numbers in other nearby rias (Andréu, 1968a, *in*: Mason, 1976). In France, 8000 ha are devoted to Portuguese-oyster growing; only 5200 ha are utilized for flat-oyster culture; the scope for further extension is rather limited. In the Netherlands, the Wadden Sea accommodates about 4000 ha of mussel beds, which account for about 60 to 70% of the total harvest; the remainder is produced on the old beds in Zealand. In Norway and Scotland, sheltered fjords provide excellent opportunities for mollusc and fish farming, but environmental temperatures are rather low. On the coast of Finland, experimental production of Baltic salmon smolts *Salmo salar* (for stocking purposes) and of rainbow trout *S. gairdneri* (market-size production) started in 1975 in power plant effluents near Helsinki, using brackish water of about 6‰S. Netcage production of *S. gairdneri* in the south-west Archipelago of Finland amounted to some 250 tons in 1975; the total surface area of the cages was about 3 ha. In Scotland, more than 40 mollusc and fish farms (mostly small scale) operate along the west coast (Milne, 1975). In southern and western Iceland, low ambient temperatures can be compensated for by an almost unlimited supply of natural waste heat, thus leading to a considerable potential for heated-water cultures. However, rough weather conditions, especially during winter, lead to increased investment costs for protection. Coastal pond construction in rocks above the high-tide level may hold promise. In the northern and eastern parts of Iceland, coastal areas seem much less suitable, mainly because of extremely low winter temperatures and discontinuity layers due to melting-water runoff (Milne, 1975). Along the Mediterranean coasts, the area potential may exceed 1 million ha (Levi and Troadec, 1974), but the data thus far available are very incomplete. The total additional potential in Europe may be close to some 1·1 million ha of brackish and marine waters. In the FRG, mariculture has not yet developed on a commercial scale, except for a few bottom-culture mussel farms in the North Sea. Along the Baltic coast (Flensburg Förde) and in heated effluents of a coastal power station (Kiel Bight), salmonid experimental cage cultures appear promising.

In the USSR, more than 140 fish-rearing and acclimation facilities are in operation (Saizew, 1975). These produce some 12 billion juveniles. Employing artificial gamete-release induction, sturgeon populations have been restocked in the Caspian Sea. Fish farms along the Pacific Ocean promote local salmon stocks. Pacific salmon have been successfully translocated into the Barents and White Seas, The possibilities for rearing and reproducing several marine fishes are being studied, and the first facilities have been set up for cultivating economically valuable mollusc species.

(b) Animals

In order to assess the suitability of marine animals for mariculture, a number of factors must be considered. For convenience, the most important factors are listed here under four headings: resource availability, marketability, biological parameters and operational parameters.

The basic resources for mariculture which must be available at a reasonable price are water, land, labour (trainable workers) and energy. Since most marifarms use natural water, sufficient amounts of unpolluted sea or brackish water and coastal real estate are essential. The running costs for resources depend to a large extent on the expenses to be paid for feed, labour and energy.

Marketability includes such difficult-to-define aspects as consumer motivation and preference, and the chances for promoting aquafood consumption. Consumer motivation and preference depend more on historical and traditional trends than on health considerations or scientific aspects of nutritional requirements. Taboos, cultural experience, fashions, status seeking, aesthetic satisfaction and wholesomeness usually dominate the scene more than is realized or admitted. Aesthetic satisfaction is based on the subjective evaluation of product appearance, i.e. its flavour (taste and odour), colour, lustre, form and texture. Wholesomeness for most consumers means satisfaction in terms of vision, gustation and olfaction, and to 'feel good' during and after the meal. The chances for promoting aquafood consumption are a function of the stability of tradition, the aesthetic value and wholesomeness of the food to be sold and advertising activities.

Biological parameters comprise ecological background data of the animal to be farmed (Chapter 5.1); its requirements for culture-water treatment (Chapter 2); tolerance to crowding, domestication and disease; time to market size (grow-out time), feed requirements and feed-conversion efficiency; controlled breeding; as well as its suitability for multicrop and polyculture techniques. Production and investment optimization require detailed knowledge on the animals' environmental and nutritional requirements, rates and efficiencies of growth, reproductive biology, and organismic interactions at the individual and population levels.

Tolerance to crowding depends not only on environmental requirements, but also on mutual chemical interactions and behaviour (territoriality, aggressiveness, cannibalism). Very long grow-out periods increase both production costs and farming risks. Feed quality plays a major role in survival, growth efficiency and disease resistance.

For intensive culture operations, the suitability of an animal increases greatly if it is capable of making significant non-genetic adjustments to the culture environment and amenable to genetic stock improvement. The latter requires controlled breeding, i.e. selection and genetic recombination, with the aim of enhancing properties desired by the cultivator, for example, better growth efficiency, taste or appearance. In many species, commercially desired properties can presumably be enhanced considerably. However, in contrast to agricultural husbandry, animal mariculture cannot yet resort to selectively bred, domesticated animals. Typically, the culture material consists of wild populations or populations in which wild characteristics dominate. In all cases in which the animals cannot yet be bred under controlled conditions or in which controlled breeding would be too expensive, the

culture material must be obtained from the field. Unpredictable changes in abundance, ecological dynamics and weather contribute to a considerable degree of uncertainty in field collections of culture material.

The effort and know-how required for successful intensive mariculture operations tend to increase with the trophic level and the complexity of the animal's life cycle. Third-trophic-level crustaceans, for example, usually have several successive life-cycle stages which require changes in environment and/or nutrition. Hence, more control over environmental factors and feed becomes necessary. Some feeding schedules are complicated timetables, comprising 4, 5, 6 or even more different diets—often living organisms which must be cultured themselves and be ready for feeding at specific times.

Operational parameters include costs for facility investments, culture procedures, feed and management. Among the running costs, feed expenses usually constitute a major budget item. Management considerations include manufacturing efficiency, product processing, packaging and transportation, size and structure of the market, market stability, shelf life of the end product, and legislative protection or restriction. The fact that the cultivator may be able to determine the time of harvest and final product size constitutes a considerable advantage in comparison to seasonal fisheries activities and allows the exploitation of market dynamics. Many culture systems can be programmed in order to maximize benefits from seasonal price fluctuations and to concentrate on the most profitable sizes.

The preceding paragraphs underline the difficulties involved in analyzing in detail the suitability of a given animal for mariculture; even a well-informed cultivator cannot start a new venture without some uncertainty or risk. Of the authors who have attempted to work out keys for assessing the general suitability of different animals, we quote here NASH (1974) who has presented a basic bio-economic matrix (Table 5-142) and a matrix for assessing the overall state-of-the-art and market potential of aquaculture (Table 5-143). Some important mariculture animals and the principal methods of their cultivation are listed in Table 5-144. According to LING (1972), about 65 animal species are presently cultivated in the Indo-Pacific region (25 crustaceans, 20 molluscs and 20 finfishes). IBRAHIM (1975) lists 23 animal candidates suitable for cultivation in brackish-water areas of Tanzania (8 crustaceans, 9 molluscs and 6 finfishes). SIVALINGAM (1975) mentions about 12 finfish species which may be considered suitable candidates for commercial cultivation in African brackish waters. On the basis of their natural occurrence and abundance along the African coast and their market potential, the following species are considered most promising for coastal aquaculture: *Tilapia melanopleura*, *T. heudelotii*, *Mugil cephalus*, *M. falcipinnis*, *M. grandisquamis*, *Chrysichthys nigrodigitatus* and *Lutjanus* spp. JHINGRAN and GOPALAKRISHNAN (1974) have presented a catalogue of cultivated species, comprising some 200 entries. Additional lists of commercially cultivated animals have been presented, for example, by BLANCO (1972a) for the Philippines, by TANAKA (1972) for Japan, and by DANG (1972) for Vietnam.

Commercial mollusc cultivation is well established (Chapter 5.1, p. 901). The low trophic level of oysters, mussels, clams and scallops facilitates economic plant protein transformation (high trophic efficiency). Many representatives can be grown at high population densities and under controlled conditions. According to GLUDE

Table 5-142

Basic bioeconomic matrix for assessing the suitability of animals for aquaculture. 1: not suitable; 5: suitable; 2 to 4: in-between-scorings (After NASH, 1974; reproduced by permission of Dowden, Hutchinson and Ross)

	Oysters	Mussels	Clams	Scallops	Abalone	Crabs	Shrimps	Lobster	Krill	Brine shrimp	Salmon	Flatfish	Mullet	Rabbitfish	Dolphinfish	Pompano	Yellow-tail	Anchovy	Herrings	Eels	Milkfish	Octopus	Turtles	Bloodworm
Trophic efficiency																								
Carnivores											2	2		2	2	2		2		2				
Herbivores and browsers					3							3	3								3		3	
Plankton and filter feeders	5	5							5	5								5	5					
Scavengers and detritus feeders			4	4		4	4	4																4
Reproduction in nature																								
High fecundity	5	5	4	4	4	3	2	2	2	3	3	4	5	5	4	4	4	4	4	4	5	2	1	5
Eggs or spat easy to collect	5	5	2	1	1	1	1	1	1	5	3	1	1	2	1	1	1	3	2	1	5	4	5	1
Juveniles easy to collect	5	5	2	2	1	1	1	1	1	4	3	2	4	4	2	3	4	3	1	4	5	1	1	1
Reproduction in captivity																								
Adults easy to collect	5	5	5	5	5	5	5	5	5	5	5	5	5	4	4	5	5	5	5	5	5	5	5	5
Adults survive well	5	5	4	4	5	5	4	4	4	5	4	5	5	4	4	4	4	2	2	5	5	3	4	3
Spawning naturally	5	5	5	4	4	2	1	3	1	5	5	5	1	4	3	1	2	1	2	1	1	3	4	1
Spawning by inducement	5	5	5	4	5	2	1	1	1	1	5	3	5	3	1	1	1	3	3	1	1	1	1	1
Growth or biomass production																								
Natural fast growth rate	3	3	3	3	3	3	3	3	5	4	3	3	3	3	4	4	4	4	4	3	5	3	1	3
Growth rate can be accelerated	5	5	4	4	4	3	4	4	4	4	5	4	4	4	5	5	5	2	2	5	3	4	2	3
High survival in captivity	5	5	3	3	3	5	3	3	3	5	5	5	5	3	3	3	5	2	2	5	5	2	2	3
Early maturity	3	3	3	3	3	3	3	3	5	5	3	3	3	3	3	3	3	4	3	3	2	3	1	4
Gregarious in nature	5	5	5	5	2	2	3	2	5	5	4	3	4	4	3	3	5	5	5	5	4	1	1	4
High density in captivity	5	5	5	5	4	5	3	3	4	5	4	4	4	4	4	4	5	5	4	5	4	3	3	4
Readily available protein	3	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	3	2	4
Life cycle																								
Few development stages	4	5	4	4	4	2	4	2	4	4	5	4	5	5	5	5	5	5	5	5	5	4	5	4
Few predators	4	4	4	4	4	3	3	4	1	1	4	4	3	3	4	3	4	2	5	4	3	4	4	3
Non-cannibalistic in captivity	5	5	5	5	5	1	2	1	5	5	5	5	5	5	5	5	5	5	5	5	5	3	5	5
Matrix total	82	83	70	67	63	53	50	49	59	74	72	66	69	68	61	60	68	62	63	67	70	51	50	58

(1976), the global annual oyster consumption amounts to about 770,000 tons, i.e. more than 10% of the world aquaculture yield. The total aggregate consumption for oysters is projected to increase to more than 2·3 million tons by the year 2000; this would require a substantial annual production increase and, presumably, the inclusion of several species not yet utilized in large-scale aquaculture operations.

Among the bivalves, oysters such as *Crassostrea gigas*, *C. virginica*, *C. commercialis* and *Ostrea edulis* are cultivated in many farms with high success (p. 901). *C. gigas* represents excellent material for translocation (transplantation) into new

tropical and temperate areas where it often outgrows commercially utilized local species. Further increase in the *C. gigas* yield is expected in many localities, since seed production has become economically feasible. This fact renders *C. gigas* suitable also for temperate regions with summer temperatures high enough for rapid growth, but where natural reproduction is inhibited due to low winter temperatures (MEIXNER, 1974, 1976; PARSONS, 1974). Examples of the most promising clam and mussel species are: *Mercenaria mercenaria*, *Pecten* spp., *Venerupis* spp. and *Mytilus*

Table 5-143

Overall state-of-the-art and market potential of aquaculture. 1: no; 5: yes; 2 to 4: in-between scorings (After NASH, 1974; reproduced by permission of Dowden, Hutchinson and Ross)

	Oysters	Mussels	Clams	Scallops	Abalone	Crabs	Shrimps	Lobster	Krill	Brine shrimp	Salmon	Flatfish	Mullet	Rabbitfish	Dolphinfish	Pompano	Yellow-tail	Anchovy	Herrings	Eels	Milkfish	Octopus	Turtles	Bloodworm
Controlled spawning possible	5	5	5	4	4	2	1	3	1	5	5	4	4	1	1	1	2	4	1	3	1	2	4	1
Simple larval development achieved	5	5	5	5	5	4	5	5	2	5	5	5	2	1	1	1	3	5	3	3	5	3	4	1
Mass-produced in hatchery	5	5	5	4	3	1	4	4	1	5	5	5	1	1	1	1	1	1	1	2	1	1	4	1
Fast growth rate potential	5	5	4	4	4	3	4	4	4	5	5	4	4	4	5	5	5	4	4	5	5	4	3	3
Satisfactory feeds known	5	4	4	3	3	1	3	3	3	5	5	3	3	1	2	1	5	3	3	5	5	3	3	2
Commercial feeds available	1	1	1	1	1	1	2	1	1	1	5	1	1	1	1	1	3	1	1	2	1	1	1	1
High conversion efficiency	2	2	2	2	2	3	3	3	3	4	5	4	3	2	4	4	4	3	3	5	5	3	2	1
Hardy in captivity	5	5	3	3	3	5	3	3	3	5	5	5	5	3	3	3	5	2	3	5	5	2	3	3
High disease resistance	4	4	4	4	4	4	3	4	3	5	4	4	4	3	3	3	4	2	3	4	4	3	2	4
High density potential	5	5	5	5	4	5	3	3	5	5	4	4	4	3	4	4	4	5	5	5	4	3	3	5
Farming systems developed	5	5	3	3	3	1	4	2	1	5	4	4	4	1	1	3	5	1	1	5	5	1	3	4
High price range	5	2	4	4	4	1	4	5	1	5	5	3	2	1	4	4	5	2	2	5	1	4	5	3
High market potential U.S.	5	1	5	5	5	2	5	5	1	5	5	3	1	1	5	5	4	3	3	2	1	3	5	5
High market potential foreign	5	5	5	5	5	3	5	5	3	5	5	4	4	4	5	4	5	4	4	5	5	5	5	3
Matrix total	62	54	55	52	51	36	49	50	32	65	67	53	42	27	40	40	55	40	37	56	48	38	47	37

edulis in temperate regions, *Mytilus galloprovincialis*, *Meretrix meretrix*, *Anadara granosa* as well as *Mytilus smaragdinus* in subtropical and tropical regions.

Recently, interest has increased in the utilization of mangrove oysters such as *Crassostrea rhizophorae* (Caribbean and West Indies), *C. tulipa* (Sierra Leone), *C. brasiliana* (Brazil) and *C. belcherii* (Malaysia). According to KAMARA and co-authors (1976), many mangrove oyster areas have an over-abundance of seed—an essential prerequisite for extending farm production. The cultivation of the mangrove oyster *C. rhizophorae* has attained commercial scale in Venezuela (VÉLEZ, 1974). Among the gastropods, abalones (p. 888) rank highest as candidate

animals especially in Japan and North America, but also limpets, periwinkles and conchs may hold some promise. Although members of several cephalopod species are commercially cultivated in Japan—e.g. *Octopus vulgaris*, *Sepia esculenta* and *Loligo bleekeri* (LING, 1972)—large-scale aquaculture operations are unlikely to specialize on cephalopods. Mass cultivation of cephalopods still poses a number of problems.

In addition to the molluscs already well established in commercial cultivation, we mention here the following molluscan mariculture candidates: *Crassostrea margaritifera* and *Ostrea atherstonei* in South Africa (AFINOWI, 1975), *C. cucullata* along the Tanzanian coast (SINGH, 1975), *Mytilis platensis* in Argentina (PENCHAS-

Table 5-144

Important mariculture animals and the principal methods for their cultivation (Based on information provided by Japan Fisheries Association)

Prawn, black sea bream	Running water	Pond culture	Feeding
Prawn, blue crab, mullet, black sea bream, eel	Slowly running or still water		
Yellow-tail, red sea bream, globefish, parrot bass, amberjack, prawn	Bank partition (e.g. bay)	Partitioned *in situ* culture	
Ditto	Net partition		
Ditto	Ikesu (surface and middle layer)		
Oyster, pearl oyster, scallop	Raft (long rope type)	Unpartitioned *in situ* culture	No feeding
Oyster	Net, hibi sticks		
Short-necked clam, clam, ark shell, scallop	Sown on shallows		
Salmon, trout, prawn, blue crab, abalone, scallop, red sea bream, scorpionfish, grey rock cod, flatfishes	Release of hatchery-reared seedlings	Unpartitioned *in situ* culture	No feeding
Abalone, topshell, short-necked clam, clam, ark shell, scallop, spiny lobster, rock trout	Transplantation of nature-grown seedlings		

ZADEH, 1974), bay scallops in Japan and USA (e.g. GATES and co-authors, 1974; CASTAGNA, 1975). In Virginia, CASTAGNA grew bay scallops *Agropecten irradians* in pens to market size within 5 to 7 months, with adductor muscles much larger than in float-grown individuals (adductor-muscle size is important because this muscle is the only part of the scallop sold); DUGGAN (1973) has presented information on optimal *A. irradians* culture densities, and SHAW (1974a, b) on seed collection. In Japan, commercial scallop culture has increased rapidly after a sudden decrease of more than 80% in the production of natural stocks along the coast of Hokkaido. Japanese scallop farming started in 1963 reaching 3580 tons by 1970 and 23,000 tons by 1972. Further increase in scallop production to 39,000 tons in 1973 indicates the rapid growth of this industry, which utilizes a newcomer species to aquafarming.

In crustaceans, the often numerous larval forms, moulting and cannibalism tend

to increase the difficulties encountered by the cultivator (Chapter 5.1, p. 860). However, members of several shrimp and prawn species have been commercially cultivated in Japan with great success, notably *Penaeus japonicus* and related forms (p. 851). In spite of a high market price, *P. japonicus* contribute only 0·2% to the total Japanese aquaculture yield (JAPAN FISHERIES ASSOCIATION, 1975). RACEK (1972) has listed 40 penaeid species belonging to the genera *Penaeus*, *Metapenaeus*, *Trachypenaeus* and *Parapenaeopsis*, which are already cultivated commercially or which hold promise as candidates in the Indo-West Pacific region. BOSCHI and SCELZO (1974) have cultivated *Artemesia longinaris* in Argentina. Along the coast of Equador, the prawn *Penaeus vannamei* seems to hold promise; in localities suitable for aquafarming, this species forms 95% of the post-larval catches of culturable prawns (COBO CEDEÑO, 1974). Seed of *P. indicus*, *P. monodon* and *Metapenaeus monoceros* is also available in large quantities along the Tanzanian coast, where these species are considered mariculture candidates (IBRAHIM, 1975). In North America and Europe, the commercial cultivation of the lobsters *Homarus americanus* and *H. gammarus* (p. 859) may be possible. Additional crustacean mariculture candidates include species of the genera *Cancer*, *Callinectes*, *Menippe* and *Macrobrachium*. In commercial shrimp and prawn farming, many species can be grown conveniently in polyculture. In South East Asia, for example, almost 50% of the cultured shrimp harvested are grown together with milkfish.

In marine fishes, experiments on artificially induced breeding (Chapter 5.1, p. 1023) and on rearing of larvae (p. 968) have yielded encouraging results and major breakthroughs can be expected in the near future. Except for controlled reproduction and larval rearing, many fishes can now be cultivated without major obstacles. In cold waters and temperate regions, salmonids are the most commonly used fishes in commercial-culture operations (e.g. MACCRIMMON and MARSHALL, 1968; MACCRIMMON and CAMPBELL, 1969; MACCRIMMON, 1971, 1972; HART, 1973). For example, the commercial salt-water production of Pacific salmon *Oncorhynchus* spp., along the west coast of the United States—in addition to the yield obtained from ocean ranching (p. 1334)—reached about 880 tons in 1974–75; predictions for 1990 are 21,000 tons (MAHNKEN, 1975). In temperate and tropical areas, eels and cyprinids (e.g. common carp *Cyprinus carpio*, grass carp *Ctenopharyngodon idella*, and several Indian carp species such as *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*) provide excellent animal material for aquaculture farms. In the tropics, milkfish *Chanos chanos* (e.g. CHEN, 1952, 1976a; LIN, 1968), mullets such as *Mugil cephalus* and several species of *Tilapia* are farmed with much success. However, the commercial cultivation of eels, milkfish, and mullets depends on the availability of nature-grown seedlings. Hence, great effort is presently invested in developing suitable methods for obtaining reproduction under controlled laboratory conditions (for details see Chapter 5.1). In Indonesia, for example, the shortage of *C. chanos* seed has been estimated at 30% (BLANCO, 1972c); in Java, the annual shortage of milkfish seed exceeded 100 million fry (DJAJADIREDJA and SUHARDI, 1974). Hitherto largely unexploited milkfish-seed stocks along the coast of Sulawesi may temporarily reduce the seed-supply problem.

In Japan, the mass culture of yellow-tail *Seriola quinqueradiata* is economically very successful (e.g. FUJIYA, 1976). The yellow-tail harvest accounts for the highest single-species percentage of the annual Japanese fish-farming harvest. Much smaller

portions of the farmed finfish production are contributed by species such as the red sea bream *Chrysophrys major*, rudder fish *S. purpurescens*, porgy *Fugu rubripes* and black porgy *Mylio macrocephalus*.

Extensive eel (*Anguilla* spp.) culture is practised in Italy, Northern Ireland, Hungary and France; intensive eel culture in Japan, Taiwan, Australia, Germany and France (e.g. KOOPS, 1967; HATAYA, 1972; MESKE and CELLARIUS, 1972; CHEN, 1973; SHANG JUNG, 1973; USUI, 1974; WELLNER, 1975; FORREST, 1976).

A number of additional fishes qualify as candidates for mariculture. In temperate regions, the commercial cultivation of plaice *Pleuronectes platessa*, sole *Solea solea*, lemon sole *Microstomus kitt* and turbot *Scophthalmus maximus* has entered the experimental phase. While *P. platessa* have been raised successfully to market size, the other fishes mentioned grow faster and command higher prices (e.g. JONES, 1972). In southern Europe, gilt-head bream *Sparus auratus* and sea bass species such as *Dicentrarchus labrax* and *D. punctatus* rank high as candidates. Along the North American coast, the commercial culture of the pompano *Trachinotus carolinus* looks promising. In the US region, IVERSEN and BERRY (1968) consider a number of further species suitable, although these are not yet cultivated on a commercial scale: Atlantic permit *Trachinotus falcatus*, spotted sea trout *Cynoscion nebulosus*, red drum *Sciaenops ocellata*, red snapper *Lutjanus aya* or *L. blackfordi*, grey snapper *L. grisens* and summer flounder *Paralichthys dentatus*. In many tropical areas, the white sea bass *Lates calcarifer* and several siganid species (which, however, grow relatively slowly: BEN-TUVIA and co-authors, 1973; VON WESTERNHAGEN and ROSENTHAL, 1976) seem suitable for large-scale commercial culture operations (LAM, 1974; LICHATOWICH and POPPER, 1975). Two species of *Epinephelus* are grown in net cages in Penang (Malaysia) at a commercially viable but small scale, using trash fish as feed. For further extension, seed shortage is the primary factor (P. S. CHOO, personal communication). The threadfin *Polydactylus sexfilis* qualifies as a candidate for mariculture in the tropics. A protandrous hermaphrodite, *P. sexfilis* can be harvested at the male stage, i.e. before significant amounts of energy have been shunted from growth into egg production (MAY, 1976).

Other animals which qualify as candidates for mariculture include limnic forms which can be adapted to brackish or sea-water conditions (Volume I, Chapter 4; present chapter, p. 1375).

(5) Major Constraints of Animal Mariculture

In addition to limitations in ecological and technological knowledge, the development of animal mariculture encounters a variety of constraints. Some constraints may be overcome or reduced within the next decades, but several are likely to remain or to become even more accentuated. Major constraints facing the mariculturist include: environmental restrictions, diseases and predators, competitive economic activities, legal problems and management restrictions. These points are briefly considered in the following paragraphs.

Environmental restrictions comprise the general climate, extreme weather conditions, associated organisms (including plankton blooms) and environmental pollution. The general climate dictates the selection of culture animals and the type

of culture operation. Where animal requirements and climate differ essentially, environmental control becomes a must. This may be expensive. Extreme weather conditions affect both animals and culture constructions. Of particular importance are irregularly occurring extremes (e.g. temperatures, wind forces, wave actions) which occur only after long periods (years, decades) of average non-detrimental conditions and which are not predictable. Detrimental effects of wind and wave action on culture constructions have been reported by numerous investigators, for example, IVERSEN, 1968; MILNE, 1970, 1972; TAZAKI and co-authors, 1975; WEBB, 1975). Long-term construction-stability requirements ultimately depend on extreme conditions which prevail only once a decade or once in 25 years. Many cultivators have learned to live with the risk. They know that they cannot avoid damage from hurricanes or tropical storms and have adjusted accordingly. Some repair their insufficiently stable installations every year, rather than constructing more rigid, but very expensive facilities. MILNE (1970, 1972) provides details on how to calculate wind forces acting on net enclosures of different mesh sizes; WEBB (1975) considers extreme-weather pen design; TAZAKI and co-authors (1975) describe a new type of floating breakwater, especially designed to protect offshore fish-culture facilities against excessive wave action; KATO and co-authors (1976) report on float-structures for dissipating wave action during strong winds.

Changes in the quality and quantity of associated organisms may modify water quality or cause the accumulation of acute metabolic toxins, e.g. due to red-tide blooms. The red-tide organism *Gymnodinium breve* has killed whole stocks of cultured gaftopeil catfish *Bagre marinus* (STEWART, 1973) and rendered other cultivated animals temporarily poisonous (e.g. BANNER and co-authors, 1963; LANE, 1968; QUAYLE, 1969) thus causing marketing difficulties. In African fish ponds, molluscs have established thriving populations which act as intermediate hosts of bilharziasis and schistosomiasis agents. According to OKORIE (1975), these and related serious human diseases may threaten the future of local fish cultivation. Increased abundance of ciguatera could counteract the development of mariculture in areas with otherwise suitable conditions (BAGNIS and co-authors, 1974). Unknown changes in ecological conditions have been held responsible for the sudden decrease in abundance of the windowpane oyster *Placuna placenta* in the Philippines (BLANCO, 1972a). While the recent depletion of *Crassostrea angulata* stocks in France has been attributed to the sudden appearance of a branchial disease agent, the reasons for significant reductions in the yield of oysters in U.K. waters could not be traced with certainty (COLE, 1951; WALNE, 1974a, b).

Environmental pollution continues to reduce the area available for commercial cultivation, especially in highly industrialized areas such as Japan, the USA, but also in Europe and the USSR; examples have been documented by ESTABLIER (1969), QUAYLE (1971), FUJIYA (1972), RACEK (1972), TING and co-authors (1972), IDYLL (1973), PILLAY (1973), SAN FELIU (1973), GEORGE (1975) and MICHELE and TREFF (1975). Municipal-waste release near culture sites may introduce pathogenic viruses and bacteria which infect the animals cultivated and render them dangerous to consumers (e.g. CHOE and co-authors, 1974; VAUGHN and METCALF, 1975). ERIKSEN (1967) showed that clams *Tapes philippinarum* experimentally exposed to sea water containing spores of *Clostridium botulinum* Type E may accumulate the *C. botulinum* spores. Agricultural pesticide release into coastal waters has caused

severe losses in commercial culture systems. Excessive use of insecticides such as Thiodan and Endrin (organo-chlorine compounds highly toxic to fish) has become standard procedure in many agricultural areas, especially in tropical rice fields. A potential hazard of insecticide residues carried by river systems to brackish water and coastal aquaculture areas exists, for example, in Indonesia (HARDJAMULIA and KUSUMADINATA, 1972). On the other hand, JENG and SUN (1974) claim that the use of organo-chlorine pesticides over the past 20 years has not caused significant pesticide accumulation in the cultured molluscs and fishes of Taiwan—at least not to the extent that would render them unsuitable for human consumption. The impact of water pollution on marine ecosystems receives full attention in Volume V.

Diseases and predators can be controlled effectively only in intensive culture operations. On the other hand, diseases tend to occur more frequently in intensive than in extensive cultures. The major predators of crustaceans, molluscs and fishes have been referred to in Chapter 5.1. Diseases of marine animals are dealt with in LAUCKNER (in press).

Competitive economic activities include recreation, coastal-land construction (harbours, roads, seaside homes), navigation, sewage dumping, sand and gravel mining, and oil drilling. They restrict the total size of the area available for mariculture, and compete with commercial culture projects especially in highly industrialized countries (e.g. RYTHER, 1968a, b; UCHIDA, 1972; BRETT, 1973; BRULHET, 1975). Among the most serious competitors of the mariculturist are recreation and seaside-home construction (IDYLL, 1973; LING, 1973; LINDALL and TRENT, 1975). In Japan, the recent industrial expansion has inaugurated programmes which are forming more and more shore land and shallow sea areas (tidal flats)—often good culture grounds—into construction sites for industrial plants such as chemical refineries which, in addition, tend to reduce the local seawater quality due to pollution.

On the other hand, industrialization may sometimes also open up new aspects for mariculture; HARRIS and co-authors (1973), KILGEN and co-authors (1973) and KILGEN and HARRIS (1974) consider estuarine oil-pipeline canals in Louisiana to be potential mariculture sites. Natural standing crops of harvestable fishes ranged from 133 to 369 kg ha^{-1}. Trawl samples indicated an abundance of blue crabs *Callinectes sapidus*, and white, pink and brown shrimp *Penaeus* spp. overwintered in these canals (2·0 to 25·0‰S). The potential usefulness for mariculture of waste heat from power plants and of in-the-sea constructions (e.g. oil-drilling platforms) has already been referred to.

Many legal problems remain to be solved. While for intensive culture operations conducted in land-based farms, ownership of land, animals and facilities usually poses no problem, natural marine waters are state property or have international status. This applies to coastal regions, navigable waters, intertidal zones or open-sea areas. It is difficult to obtain the permission for sufficient control in such waters even under lease contract or after purchase, and ownership problems have largely remained unsolved.

Wild animals are usually considered national or international property. Hence, the use of field-collected, wild seedlings for stocking or the killing of wild competitors or predators poses a number of legal questions still to be answered. A complex legal situation also results from releasing laboratory-reared larvae or juveniles into their

natural environment—either for restocking local populations or for fattening of migrating 'homers'.

Harvesting procedures (time, amount, equipment, animal size, sex, etc.) require legislative regulation and protection as well as suitable measures for legal reinforcement. While it is necessary to significantly reduce the legal constraints in order to facilitate full promotion of mariculture in coastal waters, it is impossible, at present, to even guess when this may become possible. The multiple use of sea areas and the diverging interests of potential users tend to perpetuate the present situation.

Management restrictions include such factors as insufficient availability of energy and labour, education of potential employees, and lack of adequate infrastructure. Of major importance are constraints regarding equipment and technical services, seed and feed availability, transportation and marketing (e.g. unprepared potential consumers, risks due to unforeseen changes in market demand). In the Philippines, for example, milkfish culture is beginning to suffer from the limited availability of seedlings (DELMENDO, 1972), and for Indonesia, DJAJADIREDJA (1974) reports shortages of *Chanos* fry. In several African countries, fish is largely rejected as human food for a variety of historical and psychological reasons (SIMOONS, 1974).

(6) General Potential of Animal Mariculture

Most present-day efforts of animal mariculture concentrate on the production of luxury foods such as shrimps and prawns, lobsters, oysters or soles. The demand for such delicacies is high and a sufficient number of well-to-do people are able and willing to pay very high prices. The culture potential for meeting this demand is rapidly growing and the risks involved in this kind of mariculture—which is, in essence, a refining process—are calculable.

In contrast, the potential of mariculture for combating the world food crisis and for increasing the overall food harvest is rather limited. While most governments support commercial cultivation with the declared aim of reducing world-wide hunger and malnutrition, the conflict between private profit and public needs remains to be solved. It seems necessary to determine the goals of governmental support more definitely and to achieve a compromise between private and public interests acceptable both to the investing entrepreneur and social requirements.

In our opinion, mariculture will continue, for a long time, to produce less food than commercial fisheries. Even if we take the very optimistic view that the total aquaculture output will increase almost 4 times within a decade (PILLAY, 1973), i.e. to about 20 million tons year^{-1} by 1985, and assume that the total fisheries yield will increase in that time from 66 to 100 million tons year^{-1}, the share of aquaculture would amount to only $\frac{1}{5}$ of the fishery yield. Significantly, a more recent estimate by PILLAY (1976a) predicts a doubling rather than a quadrupling of the present aquaculture yield by 1985 and a fivefold increase by the end of this century.

There are, however, certain areas in which mariculture is likely to play an important, if not primary, role in the nation's food-producing activities—for example, in several countries of Asia (especially in Japan and related south eastern regions) and Africa, as well as in Israel and Mexico. Already now, in Japan and Israel, large mariculture resources and a high marketability (p. 1358) of mariculture

Table 5-145

Examples of mollusc, crustacean and fish yields (tons ha^{-1} $year^{-1}$) obtained in different regions with the culture methods indicated. Data for molluscs calculated as wet weight of pure meat (assuming average of 20% meat per oyster and 30% meat per mussel). Yields for crustaceans and fishes based on total live weight (Data compiled from the sources indicated)

Species	Country	Annual yield (tons ha^{-1})	Culture technique; location	Author
Molluscs				
Mussels				
Mytilus edulis	France	2·25	Pole culture; Mont St. Michel region	BARDACH and co-authors (1972)
		12·30	Pole culture;	HURLBURT and HURLBURT (1975)
		28·00*	String culture;	ANDRÉU (1968a, b)
		42·00*	String culture; Etang de Thau	LEVI and TROADEC (1974)
	Spain	158·00–300·00*	Raft culture, maximum yield	BRETT and co-authors (1972)
			Raft culture, Atlantic coast, maximum yield	BARDACH and co-authors (1972)
		21·00*	Raft culture; Mediterranean coast	
	Netherlands	7·7–21·0	Bottom culture, Wadden Sea and river deltas	HURLBURT and HURLBURT (1975)
Mytilus galloprovincialis	Italy	4·0	Bottom culture; Gulf of Trieste	FAVRETTO (1968)
Mytilus smaragdinus	Philippines	125·0	Bottom culture; Manila Bay	RYTHER and BARDACH (1968)
Mussels (unspecified)	Thailand	54·0	Bottom culture; Inner Gulf of Siam	SHRIBHIBHADH (1972)
Cockles and Clams				
Anadara granosa	Malaysia	6·2		
		12·5–14·0	Bottom culture; West coast of Malay Peninsula	BARDACH and RYTHER (1968) LING (1973)
Scallops	Japan	~17·0*	Long rope culture; Hokkaido	JAPAN FISHERIES ASSOCIATION (1975)
Oysters				
Ostrea edulis	France	0·4–1·7	Bottom culture; several locations along Atlantic coast	BARDACH and RYTHER (1968)
		9·0	Bottom culture; Bay of Toulon	LEVI and TROADEC (1974)

Crassostrea angulata	France	0·94–1·50	Bottom culture; several locations along Atlantic coast	Bardach and co-authors (1972)
Crassostrea gigas	Japan	8·0*	Raft culture (unit size: 32 m²) Kamoko, Niigata	Koganezawa (1976)
		29·4*	Raft culture (unit size: 166 m²); Hiroshima	Koganezawa (1976)
		16·6*	Long line culture (unit size: 54 m²); Kesennuma, Miyagi	Koganezawa (1976)
		24·4*	Long-line culture (unit size: 78 m²); Ogatsu, Miyagi	Koganezawa (1976)
		6·2*	Rack culture (unit size: 130 m²); Mangoku-ura, Miyagi	Koganezawa (1976)
		1·9	Bottom culture (unit size: 99 m²); Hiroshima	Koganezawa (1976)
		0·38	Stick culture (unit size: 99 m²); Hiroshima	Koganezawa (1976)
Crassostrea virginica	United States	0·01–0·1	Bottom culture; east coast (little or no management)	Bardach and co-authors (1972)
		0·67	Bottom culture; Cheasapeake Bay	Brett and co-authors (1972)
		1·0	Bottom culture; Long Island Sound (improper management prior to 1966)	Bardach and co-authors (1972)
		5·0	Bottom culture; Long Island Sound (improved technique: predation and siltation control)	Bardach and co-authors (1972)
		1·5	Bottom culture; leased ground (average farm)	Uchida (1972)
Crustaceans				
Macrobrachium spp.	United States	3·0	Pond culture, experimental farm; Oahu, Hawaii	Bardach and co-authors (1972)
Penaeus and *Metapenaeus* spp.	United States	0·28–0·50	*Penaeus aztecus*, experimental ponds; Texas A & M University	Wildman (1974)
		0·35	Pond culture, extensive (*P. setiferis, P. duorarum*)	Rose and co-authors (1975)
		1·12	Pond culture, intensive; Marifarm Inc.; West Bay, Gulf of Mexico	Milne (1972)

Table 5-145—*Continued*

Species	Country	Annual yield (tons ha^{-1})	Culture technique; location	Author
	Indonesia	1·0	Pond culture, experimental feeding with pelletized low protein (16%) feeds;	ANONYMOUS (1975)
	Thailand	0·25–0·90	Pond culture, mainly *P. merguiensis* and *M. monoceros*; several locations	SHRIBHIBHADH (1972)
	Japan	6·76	Pond culture, average yield for 12 companies (*P. japonicus*); several locations	FURUKAWA (1972)
	Philippines	0·9–1·0	Pond culture; Panay Island	BLANCO (1972a)
		0·15–0·2	Pond culture (supplementary stocking; in polyculture with milkfish)	LING (1973)
Fishes				
Chanos chanos	India	0·22–0·45	Pond culture, experimental; Mandapan, South India	TAMPI (1960)
		0·73	Pond polyculture with mullets and tilapias	FAO, Regional Office, Bangkok (1975)
	Indonesia	0·20–0·94	Pond culture, pond size 1·0–4·5 ha	LING (1973)
	Malaysia	0·40	Pond culture; several locations	FISHERIES DIVISION OF MALAYSIA (1972)
	Philippines	1·5–2·5	Pond culture (mainly in polyculture with shrimp)	BLANCO (1972a, b, c)
		6·33	Pond culture (maximum yield in owner-operated farm)	FAO Regional Office, Bangkok (1975)
		2·4–18·8	Pen culture; Laguna de Bay	FAO Regional Office, Bangkok (1975)
	Taiwan	1·2	Pond culture (average yield for 1969, insufficient seed supply)	CHEN (1972b)

		1·34	Pond culture, demonstration farm	LIANG and HUARY (1972)
		2·5–3·0	Pond culture (maximum yield of some well-managed ponds)	CHEN (1972b, 1976a, b)
	Thailand	0·56	Pond culture; several locations	SHRIBHIBHADH (1972)
Mugil spp.	Hong Kong	2·5	Pond culture	BARDACH and co-authors (1972)
		3·5	Pond culture (maximum yield)	
	India	4·1	Pond culture, experimental farm; Kakdwip, West Bengal	JHINGRAN and GOPALAKRISHNAN (1973)
Mugil tade	India	1·5	Pond culture, brackish water monoculture	SINHA (1976)
Seriola quinqueradiata	Japan	3·0	Pond culture (traditional method); inland sea areas	TAMURA (1970)
		280·0	Net-cage culture (rotatory cropping system); several locations	BARDACH and co-authors (1972)
Fugu rubripes	Japan	0·23	Pond and cage culture combined; average yield of 40 operators utilizing 35·4 ha in 1965; Prefectures: Kagawa, Fukui, Okayama, Yamagushi	TAMURA (1970) BARDACH and co-authors (1972)
Anguilla anguilla	Japan	5·5–7·2	Pond culture (traditional method)	KOOPS (1966)
		26·0	Pond culture (improved flow water system); Prefectures: Shizuoka, Aichi, Mie	FRITZSCHE (1970)
Polyculture *Mugil parsia* and *M. tade* together with *Penaeus monodon* and *P. indicus*	India	2·67	Pond culture; brackish-water pond with repeated tidal flushing (experimental)	SINHA (1976)

* Calculations based on actual surface area of culture enclosures.

products combine with considerable local demands for sea food. In both countries, intensive commercial cultivation of animals in fresh, brackish and marine water bodies has attained a very advanced level. Israel produces some 40% of its annual fish consumption in aquafarms. According to CROSS (1973), the per capita fish consumption in Israel was ca 10 kg in 1972. This figure exceeds by far comparable estimates for the FRG (ca 6 kg) and for the USA (ca 4 kg).

The greatest potential of mariculture lies in tropical and subtropical countries with suitable coastal areas. These countries receive a maximum of solar energy, have maximum needs for supplementary protein, but usually have minimum prices for land and labour. Mariculture could here contribute significantly to the local protein harvest and to improving the general living standard. Near-the-house cultures deserve more attention and support. The increasing demand for luxury seafoods in highly industrialized countries would provide unique chances for export.

The potential of animal mariculture is not only a function of areas (p. 1345) and animals (p. 1358), but also of the status of culture technology and management. This may be exemplified by referring to the yield of milkfish *Chanos chanos* in Taiwan. Traditionally, the annual average pond harvest of milkfish ranged from 0·2 to 0·4 tons ha^{-1}. Due to improvements in technology and management (pond fertilization, optimum stocking, multiple harvesting, i.e. more than one harvest $year^{-1}$, and polyculture, i.e. more than 1 species per culture system), this figure increased to 1·9 to 2·5 tons ha^{-1} (CHEN, 1972a) or ca 800–1000%. Maximum yields up to 4 tons ha^{-1} have been reported for several well-managed pens. The low average production of about 1·2 tons ha^{-1} during 1968 and 1969 was attributed to an insufficient supply of seed (CHEN, 1972b) which led to a suboptimal utilization of the total area capacity. A breakthrough in artificial seed production for programmed optimal stocking could possibly increase the milkfish yield for most of the pond area utilized to values close to the maximum yield obtained by some farms, thus raising the total harvest by another 200%. For several molluscs, crustaceans and fishes, examples of average annual yields (tons ha^{-1}) are listed in Table 5-145.

Of course, it is not sufficient to produce large amounts of food in a few countries. These foods must also be transported to places where they are needed. In fact, the present 'food crisis' is, to a large extent, a crisis of food distribution rather than of absolute food availability. Many countries cannot afford to pay for food plus long-distance transportation.

When we discuss the potential of animal mariculture, we must also mention the fact that this field exerts considerable stimulatory effects on neighbouring scientific fields. Much of our present knowledge on environmental tolerances, growth and reproduction, and on nutritional requirements in crustaceans, molluscs and fishes has originated from maricultural research activities.

Is there room for significant improvements? Yes, there is. Major increases in the present potential can still be gained from (i) optimization of culture environment, nutrition and culture-system design (Chapters 2 and 5.1); (ii) minimization of losses due to disease KINNE (in press), predation and cannibalism; and (iii) maximization of desirable genetic properties of the organisms cultivated (e.g. rapid growth, high resistance to crowding, high efficiency of feed conversion). Significant improvements may also result from selecting organisms and processes which reduce

the total amounts of energy and materials required for food-protein production. The pertinent potentials of micro-organisms, phytoplankters, protozoans and of plant-eating multicellular animals need to be more fully investigated. Finally, food production and marketing can be improved by increased cooperation, specialization and integration within a given country or area. Better cooperation, specialization and integration are particularly important in regard to: seedling collection or production, raising of young stages, fattening to market size, and marketing; stock improvement; disease control; feed production; and use of farm equipment.

Commercial cultivators may benefit considerably from progress in pelleted-food design and dietary encapsulation (pp. 583, 915, 979). Even small-particle feeders—such as larval natantians or fishes—have been shown to take up micro-encapsulated nutrients. In the process of microencapsulation (MEYERS and co-authors, 1971), small amounts of liquid or particulate food materials are surrounded by a wall or shell (Fig. 5-136). Microcapsules may be about 1000 to 2,500 μm in diameter. Dietary encapsulation protects nutrients against oxidative changes,

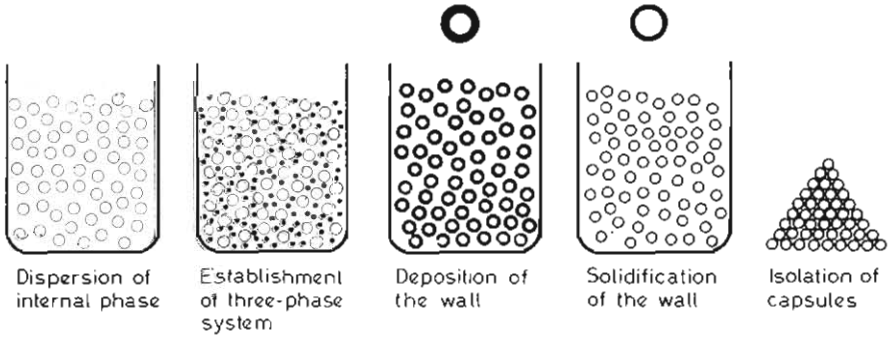

Fig. 5-136: Principal steps involved in the microencapsulation of nutritional material for particle-feeders. (After MEYERS and co-authors, 1971; modified; reproduced by permission of Miller Publishing Company, Minneapolis.)

reduces volatility, and allows a uniform distribution within a given food mixture. According to MEYERS and co-authors, the capsule walls can be natural polymers (gelatin, gums, waxes), synthetic polymers (ethyl cellulose, polyvinyl alcohol, polyethylene) or various other materials ranging from metals to sugars. If slow, controlled release of a water-soluble solid is desired, the wall material may be ethyl cellulose; if the food material is to be released upon chewing, gelatin may serve as wall material. Where possible, raw materials for dry foods should include 'waste' materials which cannot (yet) in any other way be made available for human nutrition, e.g. industrial by-products such as sewage sludge and feather meal (GROPP and co-authors, 1976). If the composition of the raw materials does not fully account for the nutritional requirements of the animal cultivated, lacking essential amino acids, vitamins or minerals must be supplemented (e.g. HALVER, 1972; TIEWS and co-authors, 1975). Additional benefits may be obtained by adding specific growth promoting substances (e.g. KOMOURDJIAN and co-authors, 1976a, b; SEN and CHATTERJEE, 1976).

Enhancement of desirable genetic properties of culture animals represents a largely untapped source for enlarging the ultimate potential of mariculture.

Genetic properties enhanced by selective breeding involve (i) maximum adjustment to culture conditions; (ii) increase of rates and efficiencies of growth; (iii) maximum disease resistance and tolerance to domestication stress; (iv) optimization of product quality, i.e. taste, odour, appearance and size. Improvements in properties desired by the cultivator are possible by hybridization (e.g. MOAV and co-authors, 1975, 1976; BAKOS, 1976), parthenogenesis, induction of polyploidy (e.g. SWARUP, 1959; PURDOM, 1972a; VALENTI, 1975), and control of sex determination. In all cases, desirable characteristics are selectively enhanced.

In molluscs, genetic studies related to mariculture have been reviewed by LONGWELL (1976). Hybridization is often possible in molluscs such as oysters and clams without much difficulty; however, offspring survival may be impaired. Examples of hybridization include crosses between *Crassostrea gigas* × *C. angulata* (IMAI and SAKAI, 1961); *C. gigas* × *C. virginica* (hybridization was followed by total larval mortality before umbo stage; DAVIS, 1950; IMAI and SAKAI, 1961); *C. gigas* × *C. echinata* (slow fertilization and cleavage; abnormal development; total larval mortality; IMAI and SAKAI, 1961). All combinations between *C. virginica* and *C. gigas*, *C. angulata*, *C. rhizophorae*, *C. eradelie*—obtained by MENZEL (1968)—resulted in embryo cleavage; those with *C. commercialis* failed to yield cleavage due to gamete incompatibility. Morphologically distinct races and geographically separated populations of *C. gigas* or *C. virginica* readily yielded fertile offspring (IMAI and SAKAI, 1961; LONGWELL and STILES *in*: LONGWELL, 1976). LOOSANOFF and DAVIS (1963) successfully crossed *Mercenaria mercenaria* and *M. campechiensis*.

In fishes, the principles of genetics, selection and hybridization have been reviewed, for example, by KIRPICHNIKOV (1961, 1972)*, CALAPRICE (1970), HUBBS (1970), CHERFAS (1972), PURDOM (1972a, 1974, 1976) and UTTER and co-authors (1974). Papers on salmon hybrids have been published by, e.g. PAVLOV (1960), KAMYSHNAIA (1961), SMIRNOV (1965), GJEDREM (1975, 1976) and NAEVDAL and co-authors (1975). For details consult the annotated bibliography of interspecific hybridization of Salmoninae by DANGEL and co-authors (1973); see also Chapter 5.1, p. 1031.

Hybridization is a common phenomenon in fishes and may result in 'hybrid vigour'. Hybridizations have been reported in *Mugil cephalus* and *Liza ramada* (*M. capito*) (YASHOUV and co-authors, 1969), sunfishes of the family Centrachidae (KEENLEYSIDE and co-authors, 1973; WHEAT and co-authors, 1974), *Tilapia* species (e.g. LOVSHIN and co-authors, 1974; PRUGININ and co-authors, 1975), *Pleuronectes platessa* and *Platichthys flesus* (e.g. PURDOM, 1972a, b), and cyprinodonts (e.g. HUBBS and DREWRY, 1959; MAKEEVA and VERIGIN, 1974). Control of sex determination facilitates the selective cultivation of the sex most amenable to the culture goal (mono-sex cultures; see also Chapter 5.1, p. 1031).

PURDOM (1976) considers selection for increased growth rate unfeasible in flatfishes because growth is very plastic and highly dependent on environmental factors. Inbred lines may be useful in F_1-hybridization, if only as a means of avoiding fitness reduction due to inbreeding. The inbreeding rate can possibly be augmented by gynogenesis (e.g. PURDOM, 1969; PURDOM and LINCOLN, 1974). Induced triploidy is seen by PURDOM as a potentially useful technique in commercial fish cultivation,

*In the 1961 paper the author's name is spelled KIRPITSCHNIKOW.

since it exceeds sexual maturation and hence limits the depression of growth associated with maturation.

Cross breeding may be facilitated by manipulating environmental factors such as temperature, salinity or pH. Conceivably, gamete incompatibility could also be reduced or eliminated by chemical or electrical means. Finally 'bridging' may be employed, i.e. the introduction of a third species—closely related to one of the selected forms. A hybrid is then obtained first with the third species and thereafter with the remaining form. These and related means for cross-breeding facilitation require more thorough investigation.

Additional opportunities for improving the present potential of animal mariculture include: (i) increased application of polyculture techniques, i.e. the accommodation of members of several different species in one culture enclosure, resulting in a more efficient use of water, labour, energy, facilities and food; (ii) adaptation of freshwater forms to increased salinities; and (iii) extension and refinement of *in situ* cultures.

Polycultures can provide increased returns per increment of investment (e.g. WEBBER and RIORDAN, 1976). The culture partners to be accommodated in polyculture must have comparable or complementary ecological requirements and be capable of adjusting to the niches available in the culture environment (SARIG and MAREK, 1975). In some cases, a culture partner may serve a useful purpose, in others it may even be essential. In tropical regions, for example, oyster cultivation often suffers from heavy algal growth which tends to cover submersed culture equipment and oysters. The introduction of siganid fishes as culture partner eliminates this problem. Siganids feed on algae and other aufwuchs organisms and, hence, keep the cultures clean (HASSE, 1976). Different systems of polycultures have been discussed by JHINGRAN (1976). A more complete picture of crustacean, mollusc and fish cultivation, including aspects of polyculture, has been presented in Chapter 5.1.

A number of freshwater and oligohaline forms adapt to and grow well in increased salinities (Volume I, Chapter 4). Among the fishes, such forms include eel *Anguilla anguilla*, pike *Esox lucius*, pike perch *Lucioperca lucioperca*, rainbow trout *Salmo gairdneri*, sea trout *S. trutta trutta*, brown trout *S. trutta fario* and common whitefish *Coregonus lavaretus* (e.g. FALK, 1970a, b; SEDGWICK, 1970; KOOPS, 1972). Young *E. lucius* have been raised successfully in brackish waters along the Baltic coast (FALK, 1970a). Common carp *Cyprinus carpio* tolerate salinities up to some 8‰; Indian carp have been grown successfully in low salinities (e.g. SAHA and co-authors, 1964a), as have several Russian sturgeons (e.g. FALK, 1970c); channel catfish *Ictalurus punctatus* grow well in salinities ranging from 3·5 to 4·9‰ (BURNSIDE and co-authors, 1975), but suffer from weight loss and high mortality in 14‰S; blue catfish *I. furcatus* are even more euryhaline than *I. punctatus* (ALLEN, 1971). Buffalo fish *Ictiobus* sp. tolerate salinities up to 9‰S and may be grown in polyculture together with channel catfish. Several *Tilapia* species have been raised successfully in brackish water, e.g. *T. mossambica* and *T. nilotica* in the Far East, and *T. aurea*, *T. nilotica* and *T. galilea* in Israel (CHERVINSKI, 1961a, b, 1966; CHERVINSKI and YASHOUV, 1971; CHERVINSKY and ZORN, 1974; see also FISHELSON and POPPER, 1958, LOYA and FISHELSON, 1969 and MARUYAMA, 1975). Possibly, *Tilapia* species can be raised together

with other fishes, crustaceans or molluscs in polyculture. The suitability of *Tilapia* species and related forms for polyculture deserves special attention. *T. nilotica* × *T. aurea* hybrids may be cultivatable in the arid-climate brackish-water ponds of Israel (FISHELSON, 1962; FISHELSON and LOYA, 1969). Finally, limnic Indian carps, such as *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* can also be grown in brackish water (SAHA and co-authors, 1964a, b, c).

In salmonids such as *Oncorhynchus* spp. and *Salmo salar*, development and growth in brackish water can be accelerated, especially when combining the salinity increase with proper photoperiods and temperature levels (HOUSTON, 1961; SAUNDERS and HENDERSON, 1970; ZAUGG and WAGNER, 1973; WAGNER, 1974; KNUTSSON and GRAY, 1975; BASULTO, 1976; BRETT, personal communication). Non-genetic adaptation to increased salinity levels in salmonids and trout has received attention from PARRY (1966), CONTE (1969), FALK (1970a, b), EPLER and CEDROWSKI (1971), IVANOV and co-authors (1971), ANDREWS and co-authors (1973) and LANDLESS (1976). MURAI and ANDREWS (1973) obtained high rates of survival and growth and high food-conversion efficiencies in brackish-water-reared *S. gairdneri* kept at an average temperature of 13·5° C. *S. gairdneri* exhibited higher smoltification and growth rates in brackish water; as other fishes, they should be transferred to the increased salinity as early as possible: 2- to 3-g fish into 4 to 12‰S; yearlings into 15 to 16‰S (SPESHILOV, 1974).

Extension and refinement of *in situ* cultures holds considerable promise. In general, *in situ* cultivation means reduced or no cost of land and of facilities (installations, pumps, filters), and low or no expenses for feed. Disadvantages of *in situ* cultures are: less control, pilferage, possible need for ships and/or divers, and insufficient legal protection.

In situ cultures comprise (i) the farming of substrate-attached organisms such as benthic algae (p. 467) or bivalves (p. 900; see also Chapter 2, pp. 69–79); (ii) the raising of motile animals which are released into the sea and return (home) to certain places for spawning such as salmon (p. 1334), or which are trained or forced to remain in certain areas (e.g. by feeding or behavioural re-enforcement; Chapter 2, p. 41); (iii) the provision of additional solid-substrate habitats, e.g. in the form of artificial reefs, artificial seaweeds, rafts, drilling platforms or harbour structures; and (iv) a variety of open-sea mariculture activities.

The provision of additional solid-substrate habitats for 'enriching' a free-water ecosystem and for increasing the mariculture potential of a certain sea area has received attention from numerous authors (e.g. ARVE, 1960; RANDALL, 1963; STROUD and JENKINS, 1961a, b; STROUD and MASSMANN, 1966; TURNER and co-authors, 1969; STONE, 1971; BUCHANAN, 1973). Increasingly, the solid-substrate surfaces become overgrown with micro-organisms, plants and attached animals. This aufwuchs, in turn, provides new microhabitats for a variety of additional organisms. Gradually, the organisms attracted begin to form a natural hard-bottom or reef community which exhibits the same or similar ecological dynamics as natural habitats of comparable size and quality. The number and diversity of organisms present and the rate of production and transformation of organic matter (the community 'metabolism') increase many times above the original level, as does the potential for harvesting human food (OGAWA, 1966, 1967, 1968; OGAWA and AOYAMA, 1966; OGAWA and TAKEMURA, 1966a, b). In the USA, several hundred

artificial reefs have been constructed and have often significantly increased the catches both in fisheries and sport fishing. Detrimental effects of artificial reefs have not become known as long as the material used was non-toxic. Especially in Japan, investigations are being carried out with the aim of optimizing artificial reef construction in terms of material and structure. The major life-supporting features of artificial reefs and of related solid substrates are the provision of surfaces for attachment of micro-organisms, plants and animals, and of hiding places, which are of vital importance for many animals, especially their offspring.

The potential of open-sea mariculture is still difficult to assess. The benefits of almost unlimited space and sea-water supply are opposed by several disadvantages such as lack of legal protection and considerable technological difficulties, costs and risks. HANSON (1974) lists as the most attractive immediate possibility atoll-based operations and the development of mariculture systems synergistic with other forms of offshore industry such as oil drilling. In the USA, open-sea mariculture is presently seriously considered as a possible means to increase the overall potential of mariculture. However, even if sufficiently supported financially, commercial-scale open-sea mariculture in North America may still be 2 to 4 decades away. In Japan, offshore mariculture in national waters has entered the experimentation phase and, according to HANSON, commercial activities appear likely within the next few years.

The difficulties encountered in open-sea mariculture are, in most sea areas, formidable. Although within reach of present-day technology, the costs may be prohibitive, and protection from heavy winds and extensive wave action is likely to remain problematic unless the farms are located sufficiently deep beneath the sea's surface. This, however, would require much larger technological efforts than many advertisers of subsurface open-sea mariculture farms realize. In international waters, legal and political problems are likely to dampen or even inhibit large-scale, open-sea mariculture projects for years to come. The sea is 'free', and the laws and agreements necessary to protect ownership and to regulate mariculture activities in international waters are not yet in sight (see also p. 1366).

For the next few decades, the potential of mariculture to produce more protein may depend more on technological and methodological progress and on finding suitable mariculture organisms than on increasing the size of the area utilized.

(7) Ecological Implications and Long-range Perspectives

Large-scale commercial culture operations have four major ecological implications that may create severe problems: (i) environmental pollution; (ii) translocation of organisms; (iii) reduction in aesthetic and recreational value of coastal land; and (iv) overexploitation of natural seed stocks.

Environmental pollution in the form of eutrophication, odour nuisance or water-colour change may be the result of releasing large amounts of culture-water effluent into nearby rivers or coastal areas (e.g. SZLUHA, 1974). The BOD (Chapter 2, pp. 108–112) produced in a raceway containing 100,000 catfish *Ictalurus punctatus* is equivalent to that of a flock of 150,000 chickens or a herd of 480 steers (MURPHY and LIPPER, 1970; see also BARKER and co-authors, 1974, and p. 177). Such large

amounts of waste water require water treatment (LIAO, 1970; YEE, 1972; HINSHAW, 1973; ODUM, 1974; WEBB, 1975; see also Chapter 2). Recycling of wastes, e.g. reuse as fertilizer, in closed culture systems as suggested on p. 1379 would reduce the pollution potential, the need for feed, and the conflict with other users of coastal land.

Cultures of oysters and mussels often represent such large and dense aggregations of filter feeders that their faecal products have led to local progradation of coastlines (DAVIS, 1956), and their metabolic activities to local reductions in oxygen and phytoplankton concentrations (UYENO and co-authors, 1970). Large oyster raft cultures operated in areas with limited water circulation release such large amounts of excretory products that they must be moved from time to time to different places in order to avoid self inhibition, i.e. reductions in growth rate. The major steps of self pollution in large estuarine aquaculture operations include (ODUM, 1974): release of metabolic end products and of toxic chemicals which are routinely employed to control unwanted plants and animals, and to combat disease agents; increased sedimentation; changes in estuarine water circulation; destruction of productive land peripheral to the estuary; and destruction of the normal production potential of the estuary itself.

Translocation of organisms due to large-scale transportation of aquaculture animals from their home area to the culture site can result in severe and not yet fully appreciable changes in the local ecosystem. Unless great care is exercised (e.g. sterilization of effluent culture water), a continuous flow of organisms (e.g. larvae, parasites, microbial disease agents) may enter the receiving water body and develop there in unpredictable ways. Translocation of marine organisms receives full attention in Volume V (see also WALFORD and WICKLUND, 1973; COURTENAY and ROBINS, 1975; ROSENTHAL, 1976).

Reduction in aesthetic and recreational value of coastal land goes hand in hand with pollution (Volume V). Large aquaculture farms may also contribute to the degrading of coastal environments in terms of constructions and significant changes in the original landscape.

Overexploitation of natural seed stocks may endanger the wild populations of those aquaculture animals which cannot yet be bred under controlled conditions, or in which controlled breeding is less convenient (more expensive) than the catching of wild seedlings. In Japan, for example, seedlings of several commercial shrimp, prawn and fish species are critically overexploited (e.g. FUJINAGA, personal communication).

Some of the most successful mariculture operations must be considered a waste of labour, energy and material. The economically highly successful cultivation of yellow-tail *Seriola quinqueradiata* in Japan, for example, makes at present about 77,000 tons of cultured fish available each year, but must use some 450,000 to 500,000 tons of animal protein (e.g. 'trash' fish, fish meal) as fish food. At the end of a laborious culture process, only about $\frac{1}{8}$ of the originally available fish protein remains; the rest has been sacrificed for transforming perfectly healthy protein into an expensive luxury product. Similar, if less obvious considerations pertain to much of the present effort invested into animal mariculture. Obviously, we are too much concerned with increasing the yield of expensive gourmet foods—often organisms with complicated life histories and high demands for environmental

control and for protein-rich feeds. Animal protein resources are transformed at relatively low conversion efficiencies into other forms of animal protein for the sole reason of obtaining high-priced commodities. The transformation process requires considerable amounts of energy and labour and produces large amounts of waste materials. At the end of the process, much less protein is available than at the beginning. As long as mariculturists concern themselves primarily with such refining procedures, they cannot significantly contribute to solving the global food crisis and cannot help to provide an ecologically sound concept for man's long-term survival.

What then are the basic ecological prerequisites for man's long-term survival, i.e. for hundreds or thousands of years—the 'near future' in geological terms? The survival of civilized human societies over hundreds or thousands of years requires first of all a drastic reduction in human population growth and a world-wide control of population size. Fig. 5-128 emphasizes the need for such measures. Practically all aspects of life are affected by the increase in the number of people and by the structure of human populations. The maximum sustainable human population size depends on the standard of living (quality of life), desired per individual and the extent to which the natural ecosystems can be deformed in order to support man. Estimates on the tolerable degree of deformation require much more information on the essential functions and structures of natural ecosystems and their resistance to man-made deformation than is presently available.

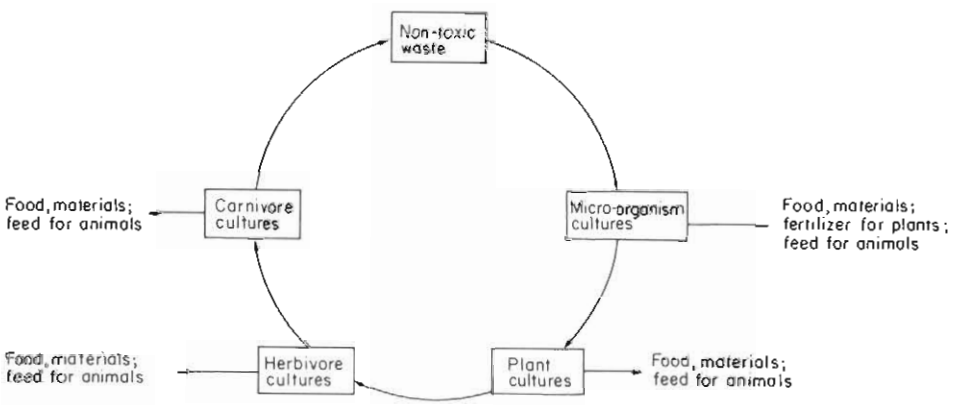

Fig. 5-137: Recycling of waste and food with minimum impact on natural ecosystems. (Original.)

The functions and structures of an ecosystem depend to a large degree on the flow of energy and matter through the system, i.e. on nutritional interrelationships between system members. Distortions of the flow of nutrients in favour of a single system component (i.e. *Homo sapiens*) beyond a critical point are bound to modify, damage or ultimately even destroy the system, including its deformer. It is our firm belief that, for man's long-term survival, there is nothing more important on earth than to reduce man's impact on nature, to analyze and comprehend essential functions and structures of natural ecosystems, and to apply the knowledge obtained in order to protect and to manage these systems with the aim of avoiding

irreversible damage. Healthy natural ecosystems have allowed man to evolve and they remain the prime prerequisite for his continued existence.

In order to increase the chances for man's long-term survival, commercial cultivation projects should direct more attention towards (i) processing vital human dietary components from low-trophic-level organisms, i.e. micro-organisms, protozoans and plants; and (ii) economic use of fossil energy resources and in-

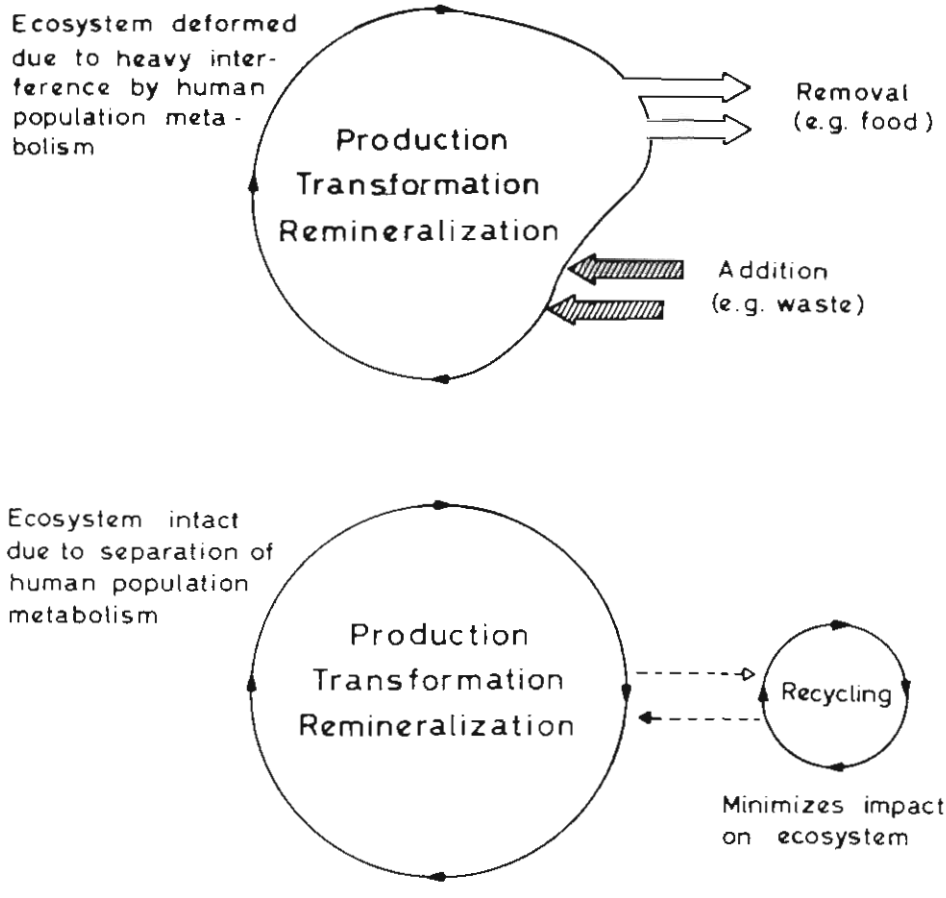

Fig. 5-138: Reduction of man's impact on the ecosystem by controlled recycling. (Original.)

creasing use of energy obtained from solar radiation, wind and water movement. In addition, a basic reorganization seems unavoidable of the pathways of human population metabolism, aiming increasingly at the use of materials and processes suitable for recycling and with the ultimate goal of establishing a cycle of production, transformation and reutilization for human needs which is as independent as possible of the recycling processes in natural ecosystems (see also Volume V).

The need for processing vital dietary components from low-trophic-level organisms seems a logical consequence if we want to reduce the gigantic amounts of food and fuel presently wasted every year by transforming primary-producer

protein into second- or third-level-producer protein. Hence, research projects should be encouraged which explore the feasibility of transforming micro-organism, protozoan and plant proteins directly into a raw product suitable for human consumption. The raw-product composition could then be supplemented in terms of amino-acid, carbohydrate, fat, mineral and vitamin composition with the aim of composing tailor-made diets to meet specific human requirements.

Optimization of food-organism farming under protein- and energy-saving conditions should be followed by the development of healthy and tasty diets. It seems surprising that many present-day food-processing activities disregard medical considerations, i.e. the processing of diets for supporting optimum health and for correcting health deficiencies. It should be possible to develop taste substances and binders that would allow transformation of the raw food obtained into a large variety of palatable, healthy diets. Of course, the acceptance of such new foods by the public would require adequate preparation and publicity. These admittedly major difficulties stand against the disastrous consequences which a continuation of present trends would ultimately produce.

While man has increasingly ignored or modified basic ecological principles, rules and dynamics, he has nutritionally remained a captive of natural productivity. With an estimated population growth from about 4 billion people in 1975 to about 12 billion people in 2075 (Fig. 5-128), such dependence may have fatal consequences, critically deforming the natural flow of energy and matter. We must separate the human population metabolism as much as possible from the natural cycle, i.e. establish a small parallel cycle of production, transformation and reutilization, fully man-controlled and specifically designed to satisfy human needs (Figs 5-137, 5-138). This would allow a significant reduction of our growing impact on natural ecosystems: less substance useful to us would be removed and less substance considered waste by us would be added. We could combine and manage anabolic and catabolic portions of human population metabolism and begin to re-harmonize our world-wide activities with basic ecological principles.

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6. MULTISPECIES CULTURES AND MICROCOSMS

M. LEVANDOWSKY

(1) Introduction

The study and application of multispecies (mixed) cultures and of microcosms is a new and important field in marine ecology. Most marine exploitation has drawn from uncultivated natural populations, and synecological studies have usually been field investigations. But there is growing evidence of the need for work with multispecies cultures. According to the mathematician VON NEUMANN, the study of complex systems with many parts can be profitably separated into complementary halves: study of mechanisms of individual units, and analysis of the organization of the units into a system (KAUFFMAN, 1971). In this chapter we are mainly concerned with the second of these.

Since the first Treatise on Marine Ecology (HEDGPETH, 1957) appeared, theories have arisen that attempt to deal with the problems of energy flow and species diversity in natural communities (Volume IV: MARGALEF, in press). Testing such theories in the natural setting is difficult and suffers from lack of controls. However, there have been some heroic ventures, such as the observation of succession and productivity in giant floating plastic bags at sea (ANTIA and co-authors, 1963) and in huge plastic domes on land (ODUM and JORDAN, 1970), the defaunating of entire islands (SIMBERLOFF and WILSON, 1970), and the work of a new school of field experimentalists represented by CASTENHOLZ (1961), CONNELL (1961), PAINE (1966) and others. Cultivation experiments with microcosmic analogues of natural communities will become more important, as culture techniques develop. An example is the work of DI SALVO (1971) with cultured microcosms of a coral reef, in which the role of labelled bacteria and substrates in the food web was observed in a manner not easily done in the field.

Beside their use as analogues of natural communities, multispecies cultures are of interest in their own right as proving grounds for theory. This is an area of much promise, and much of the discussion that follows deals with the problems that can be studied in this way.

From a practical point of view, an understanding of the properties of multispecies cultures will be useful in developing maricultural methods (Chapters 1, 4.2, 5.2). As we are beginning to learn with terrestrial agriculture, some forms of intensive monoculture are inherently unstable. The properties of multispecies systems, conveniently studied in microcosm cultures, may suggest different approaches to mariculture.

The probable effects of perturbation of marine habitats due to human activities such as water pollution (Volume V) can also be investigated conveniently with microcosms. Thus ODUM and CHESTNUT (1970) and DUNSTAN and MENZEL (1971)

have studied the effects of dilute sewage effluent on marine ponds and continuous culture systems, and several observations have been made of the effects and fate of various pesticides in model freshwater systems (COLER and GUNNER, 1970; METCALF and co-authors, 1971). Such investigations are of considerable ecological importance If we could predictively evoke, say, red tides in model systems, it would be easier to study and understand their dynamics, and hence to cope with them in nature.

But because of the newness of multispecies cultures, there exist as yet very few published studies of microcosm cultures, and most of them deal with non-marine systems. For this reason, and because many theoretical and practical problems are not restricted to marine systems, this chapter considers all microcosm studies available, whether marine, freshwater or terrestrial. In the world of theory, many of the models proposed are conceived quite generally, and in attempting to apply them to marine and estuarine systems we may come upon some special features of the latter.

For similar reasons we shall have to be more concerned with prospects than with accomplishments and facts and include basic ecological considerations pertaining to both laboratory and field work.

The chapter begins with a brief discussion of pertinent ecological theory. Following this, special features arising from interactions of species cultivated together will be discussed from two perspectives: (i) Nutritional and other chemical interactions which involve exchange of nutrients (syntrophy), production of inhibitory (antibiotic) or stimulatory (probiotic) substances, and various sorts of chemical 'messages' (pheromones). Such interactions probably give rise to holocoenotic effects in nature and have important implications in culture systems (see also Chapters 3, 4, 5). (ii) Mathematical interactions which comprise 'system' properties, involving competitive and prey–predator relations dependent on birth, growth, and death rates (the demographic variables), feeding rates, etc. (see also Volume IV). In connection with these, some results from control theory and other mathematical fields will be discussed. Of course, the two perspectives are not independent or exclusive, and most phenomena have aspects of both (LEVANDOWSKY, 1972b); but they are convenient for discussion, and such dichotomies are far from trivial or devoid of theoretical interest, as noted by DIX (1968).

Next, pertinent studies of agnotobiotic systems are described, in particular the work of ODUM and his colleagues. This is followed by a description of the relatively undeveloped field of mixed gnotobiotic cultures. The final section discusses problems and prospects.

(2) Basic Ecological Considerations

Though other chapters of the Treatise deal more extensively with the theoretical background of marine ecology (consult especially Volume II: GOOCH, 1975; Volume IV: MARGALEF, in press), it is useful to present here a rather specialized treatment of some recent theory, particularly that centering around the notions of diversity and stability.

(a) Diversity

We may conveniently and logically think of the species diversity of a system as having two components:

(i) A qualitative component: the number of different species present (while the number of species is of course a quantity, it is customary to consider presence–absence data of species as qualitative parameters, since the quantity of any given species is ignored). This is generally less in cultures than in natural communities; in gnotobiotic systems it is known precisely. In a system closed to immigration, the number of species tends to decline with extinctions. If some immigration from outside occurs, we may have a balancing of extinction and immigration, as treated in the theory of island biogeography by MACARTHUR and WILSON (1967).

(ii). A quantitative component: the equitability (LLOYD and GHELARDI, 1964) or shape of the frequency distribution of species. Some diversity indices used in field studies assume a particular distribution (logarithmic, log-normal, etc.) and simply measure species number, or some function of it. These will in general be less useful in cultivation studies, where the quantitative component is a variable of interest in its own right, responding in various ways to perturbations.

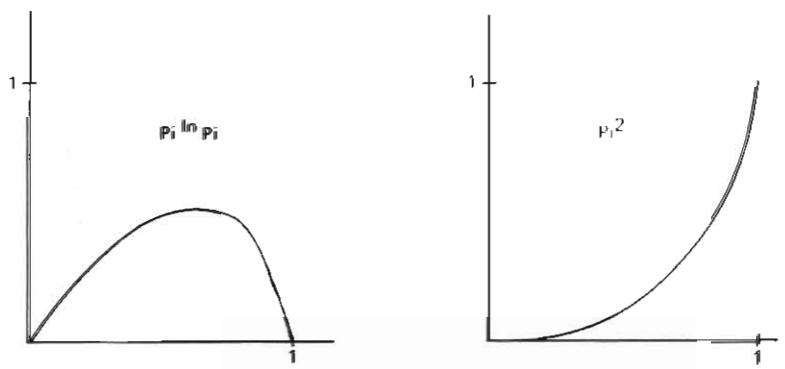

Fig. 6-1: Comparison of individual terms in the diversity expressions of SIMPSON (1949) and MARGALEF (1956). (Original.)

Of diversity measures that do not assume a given frequency distribution, the two most important are the information-theory statistic introduced by MARGALEF (1956) and its close relatives (see PIELOU, 1969):

$$H = \frac{1}{N} \ln \frac{N!}{N_1! N_2! \ldots N_n!} = \sum_{i=1}^{n} P_i \ln P_i \qquad (1)$$

and the concentration index of SIMPSON (1949):

$$C = \sum_{i=1}^{n} \frac{N_i(N_i - 1)}{N^2} = \sum_{i=1}^{n} P_i^2 \qquad (2)$$

where $P_i = N_i/N$ is the proportion of individuals of the i-th species in a total population of N organisms, of n different species.

If we examine graphs of the functions $P_i \ln P_i$ and P_i^2 in the range illustrated in Fig. 6-1, it is immediately clear that H and D respond differently to fluctuations of the underlying distribution or 'equitability'. SIMPSON's index is affected more by the very abundant species and the information index more by the moderately abundant. If these differences are kept in mind both have useful heuristic interpretations. The information index is a measure of how much new information

about a sample (in both the technical and the informal sense) is obtained on the average for each new specimen examined. SIMPSON's index measures the likelihood that two organisms taken randomly from a sample will belong to the same species. A detailed discussion of these indices from the point of view of field ecology is given in a recent review by WHITTAKER (1972) and also in Volume IV: MARGALEF (in press).

(b) Stability

Similarly, stability can be thought of as having two components. We shall say a system is qualitatively stable if all the component species persist with time, and qualitatively unstable if some species tend to extinction. But even though all species co-exist we shall think of a system as quantitatively unstable when populations fluctuate a great deal, cyclically or erratically. It will be noted that these two components are related to those of diversity. HOLLING (in press) has recently studied this distinction in detail with regard to a number of natural systems of economic importance. He concludes that the two components of stability may at times have opposite effects. In cases such as the interacting system of spruce budworm *Choristoneura fumiferana*, its predators, and several species of tree in Canada, quantitative instability is evidently needed to maintain the qualitative stability or persistence of the system. HOLLING refers to these two components as stability and resilience, respectively. Components of stability are further discussed in section 4 of this chapter, and in Volume IV: MARGALEF (in press).

(c) Stability and Diversity

We now turn to the relation between stability and diversity (see also Volume II: GOOCH, 1975 and Volume IV: MARGALEF, in press). Following early papers by MACARTHUR (1955), MARGALEF (1956), and others, it was thought plausible by many (and proven by some) that more diverse assemblages of species would be more stable. MACARTHUR (1955), PAINE (1966) and others reasoned that a predator preying on a number of species is more likely to survive in the face of fluctuations in supply of individual prey species; conversely, to the extent prey species are limited by predators, competition among them is lessened (HAIRSTON and co-authors, 1960), and hence a more numerous set of prey species can co-exist. From the point of view of energy and efficiency, MARGALEF (1956, 1963), H. T. ODUM (1956; see also reviews by E. P. ODUM, 1968, 1969) and others argue that there is a natural ecological succession from less diverse, less stable systems, through which energy flows more rapidly to diverse stable systems in which energy is used more efficiently. The latter type of system is supposed to contain more structure or 'information'.

Though these ideas have some intuitive appeal and partly arise from field observations (e.g. the plankton studies of MARGALEF), they have proven surprisingly difficult to formulate precisely and hence to test in the field. The usual textbook example of diversity, the tropical rain forest, is apparently rather fragile to human perturbation, sometimes tending irreversibly to grassland or depauperate lateritic conditions. The marine paradigm of diversity, the coral reef, has been shown in recent years to be vulnerable to the activities of a single species of sea-star

(PAINE, 1969; PORTER, 1972). In short, evidence on relationships between diversity and stability needs re-examining and extension; it may fairly be said to be an open question (WOODWELL and SMITH, 1969).

In the world of computers and mathematical models, SMITH (1969, 1972) studied simulated multispecies systems in an intuitive approach, and found that generally stability tends to decrease as new branches are added to the simulated food web (VOLUME IV: MARGALEF, in press). PARRISH and SAILA (1970) simulated a simple linearized mathematical model (see below) of a two-prey—one-predator system, and were unable to verify PAINE's (1966) conjecture that a predator might permit co-existence of otherwise competitively exclusive prey species. CRAMER and MAY (1972) studied the same model analytically, and found that with appropriate values of the parameters it is possible for the 3 species to co-exist.

More generally, MAY (1971) analyzed the local stability properties (distinction between local and global properties of mathematical systems and related matters are discussed in section 4 of this chapter) of models of trophic webs with various levels and numbers of species. In his model, stable multispecies systems could be constructed, but the likelihood of stability in general decreased, and never increased, as more species were added to the food web. The additional effects of adding energy and mass constraints to such models have been considered by ULANOWICZ (1972a, b).

Finally, there has been some interest in the local stability properties of large model systems of n species interacting in various ways, following a simulation study by GARDNER and ASHBY (1970). Though in a realistic model interactions would in general be complicated non-linear expressions making the global behaviour of such a system difficult to study (SOMORJAI and GOSWAMI, 1972), the local stability sufficiently near a point of equilibrium depends on the first (linear) terms of series expansions of (non-linear) interaction terms, and one may study stability in the neighbourhood of such points by examining the matrix equation

$$\frac{\mathrm{d}\bar{x}}{\mathrm{d}t} = \bar{A}\bar{x} \tag{3}$$

where $\bar{x}$ is the $n \times 1$ column vector of the population sizes x_i of the n different species, and $\bar{A}$ is the $n \times n$ matrix of linear interaction terms, a_{ij}. MAY (1972a) has studied this problem. His conclusions are in line with the simulation results of GARDNER and ASHBY (1970) and raise an interesting ecological question. A quantity C, the connectance, is defined as the number of non-zero elements a_{ij} of $\bar{A}$, and a quantity α, the interaction magnitude, as the mean square value of the a_{ij}. Then, if the a_{ij} and initial values of the x_i are randomly chosen, MAY concluded, by adapting a theory of random matrices from physics, that such a system is almost certainly stable if $\alpha < (n)^{-1/2}$ and almost certainly unstable if $\alpha > (n)^{-1/2}$. That is, for a given α, the probability of stability decreases sharply at some point as more species are added to the system. Again, it should be stressed that the analysis is quite idealized and ignores non-linear effects (see comments following the paper by LEWONTIN, 1969, at the Brookhaven Symposium), and so tells nothing of the global properties of the system. Some non-linear effects are discussed in section 4 of this chapter.

Nevertheless, some simple corollaries of MAY's (1972b) conclusion are of interest for ecologists. First, due to the relation between C and α, it is found that stable

systems are likely to have few but strong interactions between species, or many weak interactions. Similarly, it is possible to have large numbers of species in a (quantitatively) stable system if they are organized into subsystems of species that only interact among themselves: i.e., if the matrix $\overline{A}$ consists of submatrices arranged in blocks along its diagonal.

Thus from the world of abstract models there is a suggestion that some ecosystems may be better understood as modular collections of loosely interacting subunits, each subunit containing strongly interacting species of various trophic levels. Such an assumption is roughly consistent with multivariate field studies (ordinations, factor analysis, etc.) which usually fail to find mutually exclusive communities when only one trophic level is studied (e.g. LEVANDOWSKY, 1972a; but see VENRICK, 1971), whereas when members of several trophic levels are included, well-defined subunits are sometimes found (WILLIAMSON, 1961; FAGER and MCGOWAN, 1963; CASSIE, 1967). On the other hand ROBERTS (1974) finds that much of the instability in the random matrix models arises from the presence of 'ghost' species that take on negative values. If these are eliminated the resulting 'feasible' model has much greater stability even for larger systems. This corresponds to MAY's (1971) remark that real ecosystems are not random, but the results of long evolution.

The preceding excursion into the realm of general theory is justified by noting that multispecies cultures can play an important role in the experimental study of such questions. In such cultures, one can control both numbers of species and variability of at least some environmental factors. Thus in a somewhat preliminary study of a gnotobiotic culture system by HAIRSTON and co-authors (1968), with just this question in mind, it was found that increased diversity did not always lead to greater stability. Conversely, the answers to such questions should greatly improve our ability to predict the properties of microcosms.

(d) Natural Microcosms or Islands

The ecology of naturally occurring microcosms is probably best considered under the rubric of island ecology (MACARTHUR and WILSON, 1967). In this context the term island has a functional *rather than* geographical meaning, referring to some relatively isolated habitat that has its own biota, different from that of the surrounding 'sea'. Thus, islands are islands, but so are mountaintops, many caves, isolated ocean deeps, hot springs, ponds, aquaria, the standing water in epiphytic bromeliads, and the hair on the backs of sloths, to give a few examples. As one thinks in this vein, nature appears to abound in islands. From his parasites' point of view every man is an island. So far, the theory of island ecology has been largely statistical, intent on explaining observed diversities of biota in terms of rates of inoculation (isolation) and extinction, energy and space (carrying capacity of the island), and variety of habitats encompassed within the island ('niche volume'). But these quantities are more or less measurable and constructible, and so parts of the theory could be tested by mixed-culture techniques. Some such preliminary work has been done by MAGUIRE (1963), who studied rates of invasion and persistence of various invertebrates in isolated containers of sterile water. Also pertinent is work by CAIRNS and PATRICK (many papers, e.g. CAIRNS and RUTHVEN, 1970; CAIRNS and

co-authors, 1971; PATRICK, 1968) on settlement of sterile substrates by protists, and by KEVERN and co-authors (1966) on periphyton development.

Such studies are rather indirectly connected with dynamical theories of multispecies cultures. Nevertheless, workers with mixed cultures should cultivate an interest in the natural history of islands *sensu latu*. Assemblages of species co-existing in such situations are presumably co-adapted through evolution, and may have properties lacking in laboratory cultures assembled by convenience. A recent review (MAGUIRE, 1971) surveys work on communities in the standing water of leaf axils, hollow stems, etc. (phytotelmata). VANDERMEER and co-authors (1972) studied the exclusion of *Paramecium* spp. from bromeliad phytotelmata by biological interaction in a set of ingenious field experiments. This is in the tradition of work on population dynamics of this ciliate in various microcosms: stump waters, ponds, and the like (HAIRSTON, 1967), which has also led to multispecies culture studies (HAIRSTON and co-authors, 1968). Practical implications of this work are found in the fact that such microcosms are breeding grounds for mosquitoes and other organisms, and mosquito larval ecology is intimately related to that of the micro-organisms (see the review by MAGUIRE, 1971, especially the discussion of studies by Japanese workers: KURIHARA, 1960, and references therein).

Another kind of well-defined island is the gut of an animal with its specialized microbial community. A whole field of applied microbiology is devoted to this type of microcosm, especially the rumen microbiota of certain herbivores (HOBSON, 1969; LEWIS and SWAN, 1971; MCBEE, 1971). Such work has implications for recycling paper (cellulose) and other wastes to produce food, fertilizer, or other products. Much pertinent research lies in the future, for the study of the microbiota of most animal guts is, beyond the descriptive level (BUCHNER, 1965), a rather new field (KOCH, 1967). One may look on these microbial systems as so many continuous flow reactors, specialized in their degradative and synthetic powers.

Other types of islands might be discussed (e.g., the biota living on the hairy backs of certain large Papuan weevils; GRESSITT and co-authors, 1965), but the point has been made: investigators involved with multispecies cultures should become attuned to these areas; here, natural history can inform the experimentalist.

(e) Holistic Properties

H. T. ODUM and his colleagues have pioneered in studying the properties of undefined (agnotobiotic) culture systems ranging from test-tubes to moderate-sized lagoons. The theory developed by this school is closely related to MARGALEF's point of view (Volume IV; MARGALEF, in press), emphasizing holistic properties of ecosystems. Though individual species are sometimes monitored, what are usually measured in an experiment are overall quantities such as total biomass, change in ambient pH or mixed potentials ('Eh') at an electrode, oxygen concentration, or a function of these thought to be related to 'community metabolism'. Ecosystems are viewed as circuits for the flow of energy and matter, and the units in such systems are various trophic elements (not necessarily individual species). They are characterized by circuit diagrams adapted from those used for electrical circuits. ODUM's theory lays great stress on similarity of circuit patterns in such disparate biological systems as, say, marine plankton and a forest community, or

even in biological and economic, sociological, theological, or any other 'system'. Systems abound. Thus ODUM seriously compares 'regenerative processes' of a coral-reef community to urban renewal projects in a city. The reader may consult a recent book (H. T. ODUM, 1971) for a late, perhaps elementary, exposition of these ideas. Though they have been criticized as vague, or speculative, or in more vehement terms, they are often intriguing and seminal, and have raised numerous questions that need to be examined. The experiments and some of the theories of this school will be discussed in later sections of this chapter.

An important contribution, among others, is the emphasis on the concept of flow. ODUM noted that when communities are 'stressed' in various ways, there is less species diversity, and matter and energy may pile up instead of cycling, because certain kinds of organism are absent (e.g., in peat formation). In sewage studies, ciliates and other invertebrate filter-feeders are easily destroyed by anaerobiosis, heat, and other stresses, which slows the processing of sludge. Periodic seeding with ciliates counteracts this and speeds the treatment (MCKINNEY and GRAM, 1956; CURDS and co-authors, 1968). This situation resembles observations of JOHANNES (1965) with marine microcosms, where the phosphorus cycle was considerably slowed in the absence of protozoans and phosphorus accumulated in the bacterial decomposers.

The practical importance of such questions is obvious. In ecological theory, the question of the holistic properties in communities is central and moot, and well suited to experimental study with culture microcosms.

(3) Chemical Interactions

Water being (almost) a universal solvent, we expect that organisms coexisting in an aqueous milieu will influence each other through chemicals: secreted or leaked metabolites, waste products, and other substances. The importance in nature of such influences, variously termed 'telemediateurs' (AUBERT, 1971; PINCEMIN, 1971) and allelochemicals (WHITTAKER and FEENY, 1971), was stressed years ago by LUCAS (1947), BURKHOLDER (1952), and others, whose predictions are largely supported by subsequent research. It may not be desirable or indeed possible to classify all chemical interactions, but some major categories are fairly clear, and it will help to list them here (see also p. 616, and Volume I: FOGG, 1972; WILBER, 1972).

(i) Syntrophy, in which one organism produces a substance required by another. This may be some micronutrient such as a vitamin or chelating agent, or a major source of energy, carbon, nitrogen, etc. The substance produced may be a waste product or a leaked metabolite, or a specially synthesized secretion, from the 'viewpoint' of the donor.

(ii) Antibiosis or inhibition, in which the product of one organism inhibits another. This may occur by some direct chemical effect, or indirectly by complexing and rendering unavailable a trace metal or vitamin needed by the inhibited organism.

(iii) A more subtle effect, the triggering of various responses by chemical cues or ectocrines. Chemotactic responses, and the evocation of stages in the life cycle by

chemical changes of the milieu exemplify responses in which the chemical in question is purely a messenger, and does not directly cause the effect observed.

Examples are easily found of all three interactions. The first, syntrophy, has been treated comprehensively for marine organisms by PROVASOLI (1972; see also Chapter 5.11) the second and third have been reviewed for organisms in general by WHITTAKER and FEENY (1971). We shall mention here particular cases only to exemplify important points.

In the course of evolution, co-existing species become adapted to each others' chemical products, and organisms may become linked by an interdependence for macro- or micronutrients. This is suggested by experiments such as those of CARLUCCI and BOWES (1970) with planktonic algae, in which two species are grown together in a medium that supports neither alone, due to exchange of products. Similar studies were done with marine bacteria by BURKHOLDER (1963) as well as with marine phytoplankton and associated bacteria by HAINES (1972). LILLY (1967) and his colleagues studied a number of cases of mutual stimulation of ciliates in mixed cultures.

On the other hand, potential competitors can engage in 'chemical warfare', evolving antibiotics and inhibitors, as seen in work with phytoplankton by LEFEVRE (1964), AUBERT (1971), PINCEMIN (1971), and others, showing inhibition of one species by products of another.

A variation on the latter type is the auto-inhibition of a species by its own products. This case may be underestimated in ecological studies. For example, the high diversity of competing phytoplankton species in some relatively constant environments, such as tropical seas, is considered paradoxical (HUTCHINSON, 1961), though a number of possible explanations have been suggested (HUTCHINSON, 1967; RICHERSON and co-authors, 1970; LEVANDOWSKY, 1972b; GRENNEY and co-authors, 1973b). Here is yet another: in a relatively constant environment where limited resources are partitioned with little overlap by specialized sympatric species, most competition is intraspecific, and auto-inhibitory ectocrines might be adaptive. By a negative feed-back process, the population of each species would remain below a level at which intraspecific competition would occur. Such a mechanism would be an example of the third, 'messenger' type of interaction, and has been suggested in the case of some freshwater populations (ROSE, 1965).

The various relationships are interchangeable in the course of evolution. Among soil microbes, some antibiotics are closely related chemically and, presumably, evolutionarily to certain chelator substances, and some play different roles in different species (NEILANDS, 1972). The secretions of some marine seaweeds, which in general have antibiotic properties (PRATT and co-authors, 1951; SIEBURTH, 1968; Volume I: FOGG, 1972), form a substrate for certain bacteria, which in turn appear necessary for the alga's normal growth (PROVASOLI, 1972; Chapter 5.1). A number of such examples appear in the literature of intermicrobial symbiosis (ORENSKI, 1966; Volume I: GUNKEL, 1972).

All of these phenomena, interesting in their own right, have implications for the technology of multispecies cultures, reviewed for continuous cultures by BUNGAY and BUNGAY (1968; see also PAYNTER and BUNGAY, 1971). Recent interest in mass cultures of algae as a possible food source and gas-exchange device for space travel has focussed attention on the role of bacterial contaminants of such cultures. These

appear to be specially adapted, and laboratory strains do not usually survive in such environments (VELA and GUERRA, 1966), nor do bacterial isolates grow in standard media without algal extracts (BLASCO, 1965). Often the bacteria are found to enhance productivity through CO_2 production (e.g. NAKAMURA, 1963) though they act sometimes as inhibitors. In sewage treatments, mixed sludge and algae can be induced to form a symbiotic mat through gas exchange ('activated algae'), speeding flocculation and clearing (HUMENIK and HANNA, 1970; Chapters 2, 3, 4, and Volume V.

The various relations can be combined in different ways. YEOH and co-authors (1967) studied mixed cultures of the bacteria *Proteus vulgaris* and *Bacillus polymyxa*, where each supplied a vitamin required by the other, so that they grew mutualistically in the absence of biotin and niacin, but only in continuous culture, since *P. vulgaris* produced an inhibitor which accumulated in batch cultures. CHAO and REILLY (1972) grew the yeast *Saccharomyces carlsbergensis* and the bacterium *Acetobacter suboxidans* together chemostatically with mannitol as sole carbon source. The mannitol was oxidized by the bacterium to fructose; the yeast, which cannot use mannitol, then broke down the fructose completely. MEGEE and co-authors (1972) grew *S. cerevisiae* and *Lactobacillus casei* together and observed competition, or various types of symbiotic interaction, depending on experimental conditions. Many further examples of this sort can be found in the reviews by BUNGAY and BUNGAY (1968), HOBSON (1969) and PROVASOLI (1972).

Experiments with gnotobiotic mice and other animals have shown chemical interactions between inoculated bacteria, and, in particular, inhibition of some invading pathogens by normal gut flora species (FREITER, 1962). However, *in vivo* and *in vitro* interactions often lead to different results (HENDERSON, 1964; WAGNER and STARR, 1969), indicating that the host is involved in the interactions (ABRAMS, 1969; GORDON and PESTI, 1971), as one might expect.

A peripheral area that is partly a chemical interaction and also is important for the models discussed in following sections is that of food selectivity. Models of food webs, focussing on energy flow and the like, generally (and understandably) avoid this additional complexity. Actually there are apparently few truly non-selective feeders (Volume II: PANDIAN, 1975). Even among protozoans, soil amoebae exhibit definite hierarchies of food preferences (SINGH, 1946), as does the filter-feeder ciliate *Stentor* sp. (RAPPORT and co-authors, 1972); the rumen ciliate *Entodinium caudatum*, however, appears to ingest bacteria only according to size and abundance (COLEMAN, 1964). Part of the growing literature on prey-predator theory concerns the various types of selectivity (HOLLING, 1969; MURDOCH, 1969; RAPPORT and TURNER, 1970; Volume II: PANDIAN, 1975; Volume IV: CONOVER, MARGALEF, in press). Eventually such relations will be incorporated in the simpler food-web models now studied.

(4) Mathematical Interactions

The relations discussed in this section are mathematical in the sense that they stem from essentially mathematical aspects of the biology of a species. These are variables such as birth, growth, and death rates, feeding, assimilation, rates and patterns of turnovers, and probabilities of success in competitive situations (Volume IV: MARGALEF, in press). They are not necessarily mathematical in

the sense of implying the possibility of a well-developed specifically predictive mathematical theory based on them. Except in the simplest situations—such as the continuous culture of some micro-organisms—we do not have, and perhaps should not expect to have, elegant mathematical theories of high predictive value in ecology. Rather, many (most?) ecologists would probably agree that up to now the most mathematically ambitious and elegant theories have been the least useful. But the relative simplicity and controllability of cultured microcosms suggest that here useful models may be constructed, and what is more, tested. In turn, theories that work for microcosms should give insight into more general situations. With all this in mind, some recent results will be discussed.

(a) Single-species Models

Models of multispecies systems are built by combining models for the components, and in some cases (sanitary engineering, fisheries) heterogeneous populations are treated as though they were a single species. Therefore we briefly consider some single-species models first. More extensive treatment will be found in Volume IV of the Treatise: 'Dynamics'. We start with models which have been much studied experimentally, from microbiology.

Microbial Populations

As a first step, ignoring death, the growth rate of a population of size N may be written

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \mu N \tag{4}$$

Various expressions for the function μ have been proposed. By far the most frequently used is that of MONOD (1942), who assumed that under favourable conditions growth would be limited by uptake or transport of a limiting substance in a process obeying MICHAELIS-MENTEN kinetics (discussed in most biochemistry texts; a recent extensive treatment is PLOWMAN, 1972).

The basic growth equation is then

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \mu_m \frac{S}{K_s + S} N, \tag{5}$$

where μ_m = maximum specific growth rate, S = limiting substrate concentration, and K_s = a saturation constant. Despite the idealized nature of this model, it apparently is a reasonably good description of a wide range of nutrient-limited growth processes, and is used routinely in many fields (PEARSON, 1968). Adjustment of the model to complexities such as competitive inhibition and diffusion is reviewed by VAN UDEN (1969). ANDREWS (1968) modified Equation (5) to take account of inhibition by high concentrations of substrate, important in sewage treatment and some industrial fields, by using HALDANE's enzyme kinetics to obtain the kinetic equation

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \mu_m \frac{S}{K_s + S + S^2/K_i} N \tag{6}$$

where K_i = an inhibition constant. A generalized version of Equation (6) was independently derived and studied by YANO and KOGA (1969).

CHIU and co-authors (1972a) studied Equation (5) and other kinetic models empirically and concluded that their usefulness was improved by subtracting a decay term to account for cell death, so that (5) becomes

$$\frac{dN}{dt} = \mu_m \frac{S}{K_s + S} N - K_a N \tag{5'}$$

where K_a = a decay constant.

Another modification was formulated by AIBA and co-authors (1969) for the case of product inhibition of growth (as in brewing) giving a growth rate

$$\mu = \mu_0 \, e^{-K_1 P} \left(\frac{S}{K_s + S} \right) \tag{7}$$

where K_1 = empirical constant, μ_0 = specific growth rate at $p = 0$, p_0 = concentration of inhibiting product.

In general the models described above, and related variants, work fairly well for log-phase growth and for a chemostat culture that is not close to the wash-out point; but not for the log phase of a stationary culture or near the wash-out point of a chemostat culture. That is, they fail when populations are small. These and related problems were observed by many workers (HERBERT and co-authors, 1956); they suggest that better models would have to include some more detailed physiological assumptions. From a purely mathematical point of view, of course, the more parameters a model has, the easier it is to fit any set of data. Thus, all models are 'improved' by adding terms.

A set of models for continuous and batch cultures, some of which depend on some general assumptions about the coupling of nutrient uptake and growth, was constructed by RAMKRISHNA and co-authors (1967). They used linearized stability analysis (pp. 44 ff.) to discover steady states. KONO (1968) and KONO and ASAI (1969a, b) developed a model depending on more detailed assumptions about uptake kinetics which gives a log phase. They start with the scaled equation

$$\frac{dC_x}{d\theta} = K_0 C_m^M C_s^S - K_3 C_R, \tag{8}$$

where C_x = cell concentration, C_s^S = limiting substrate concentration, C_m^M = concentration, of a substance that reacts with the limiting substrate, θ = a time variable (scaled by the turnover time), m = a reactant representing overall activity of enzymes produced with cell growth, C_R = concentration of secretion R which accumulates in the medium and represses cell growth, M, S = orders of reaction of the reactant m and the limiting substrate s.

By introducing the concepts of critical concentration and coefficient of consumption, and some changing of variables, KONO (1968) obtained the modified equation

$$\frac{dC_x}{d\theta} = K_1^i \, K_2^j \, C_M^i \, S_S^j - K_3 C_R \tag{8'}$$

in which (i) for $C_x < C_{xc}$, the critical concentration, $i = 1, j = 0$; (ii) for $C_x \geq C_{xc}$, $i = 0, j = 1$. This appears to give a good fit to many data (Fig. 6-2).

CAPERON (1969), DROOP (1968, 1970), and WILLIAMS (1971, 1972), working with algal chemostat cultures, also developed models based on the assumption that growth rate is based on an intracellular nutrient or metabolite pool. These also are superior descriptions of the behaviour of small populations (lag period in stationary systems; near washout in chemostats). GRENNEY and co-authors (1973a) have developed this idea in a three-compartment model, studying the course of simulations with the data of CAPERON (1969).

An intriguing approach is that of WAUGH (1972), who presents a purely mathematical explanation of the lag period, based on the greater importance of stochastic

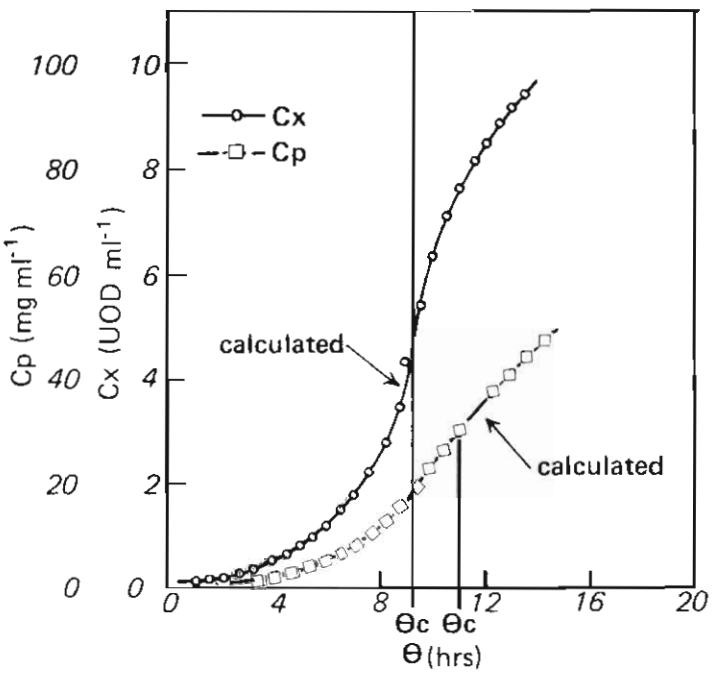

Fig. 6-2: *Lactobacillus delbrueckii*. Time course of lactic-acid fermentation from glucose. Comparison of experimental values (circles and squares) with calculated values (the curves themselves). (After KONO and ASAI, 1969b; reproduced by permission of Wiley, New York.)

effects at low population sizes. This approach would probably work near the wash-out point in chemostat cultures. Another kind of explanation of wash-out and lag period presumes the necessity of accumulating a 'conditioning' substance in the medium before growth can occur (JANNASCH 1967a, b; BUNT, 1968).

The various explanations are, of course, not mutually exclusive, and all might hold in some cases. In all cases kinetic models are improved by more detailed physiological assumptions. PAINTER and MARR (1968) discuss demographic models ('microbial demography') in which detailed assumptions about the growth rates of individual cells and their distributions are used.

In the algal models where specific growth rate is given as a function of uptake of rate-limiting nutrient from an intracellular reservoir, this, in turn, is related with

a time lag to nutrient concentration in the medium. Following CAPERON (1969), we may write the equation for the rate at time t

$$\mu_t = \mu_m \frac{S\,(t-\tau)}{K + S(t-\tau)} \tag{9}$$

where $S(t-\tau)$ = substrate concentration at time $(t-\tau)$ in the past.

More generally, we can replace $s(t-\tau)$ by the convolution integral

$$\int_0^\infty s(t-\theta)\, f(\theta)\, \mathrm{d}\theta \tag{10}$$

where $f(\theta)$ weights the importance of nutrient concentrations at various previous times. Comparison of simulations of these models with chemostat data is illustrated in Fig. 6-3. According to CAPERON (1969) the shape of the growth curve is not very sensitive to changes in the form of $f(\theta)$.

Though time-lag models have been constructed for bacterial growth (IERUSALIMSKII and co-authors, 1968; YOUNG and co-authors, 1970), and oscillatory transients are observed (AIBA and co-authors, 1967; ZINES and ROGERS, 1970), the phenomenon is apparently less pronounced in bacterial systems. Thus D'ANS and co-authors (1972) studied transient fluctuations of growth of *Escherichia coli* with the goal of maximizing yield by control-theory methods (D'ANS and co-authors, 1971), and found Monod-Haldane kinetics adequate for situations where time-lag effects, if present, should have appeared. In any case, dynamic metabolic models to explain oscillations of bacterial growth have been sceptically received (SINCLAIR and co-authors, 1971).

Models of the kind discussed so far, based on enzyme kinetics of uptake and utilization, are used mainly with microbial osmotrophs. However, HAMILTON and PRESLAN (1970b; see also STRICKLAND, 1971) report that the phagotrophic marine ciliate *Uronema marinum* seems to follow Monod-type kinetics in continuous culture. But work with the ciliate *Tetrahymena pyriformis* (CURDS and COCKBURN, 1970) gives a more complicated picture in which kinetics of feeding and growth were related to mean cell volume, and varied inversely with dilution rate. The increased cell size at low food levels agrees with SALT's (1968) observations with amoeba microcosms.

Continuous and batch cultures of higher Metazoa (e.g. REEVE, 1963; HEINLE, 1970), relatively less studied mathematically, seem to have little direct relation to the kinetic models so far discussed.

The Logistic Equation

The idea of an autocatalytic, self-limiting population process goes back at least to the time of HUME (1752). Formalization of this appeared first with the logistic equation (VERHULST, 1845–7; PEARL, 1927):

$$\frac{\mathrm{d}N}{\mathrm{d}\,t} = rN\left(\frac{K-N}{K}\right) = rN - \frac{r}{k}N^2, \tag{11}$$

which has the solution

$$N(t) = \frac{K}{1 + \dfrac{e^{-rt}}{C(r/k)}} \tag{11'}$$

where C = a constant. One may interpret this equation heuristically as representing growth of a population with 'intrinsic growth rate' r in an environment with a 'carrying capacity' K, and there is an extensive literature on these concepts in ecology and evolutionary theory (e.g. PIANKA, 1972, and references therein).

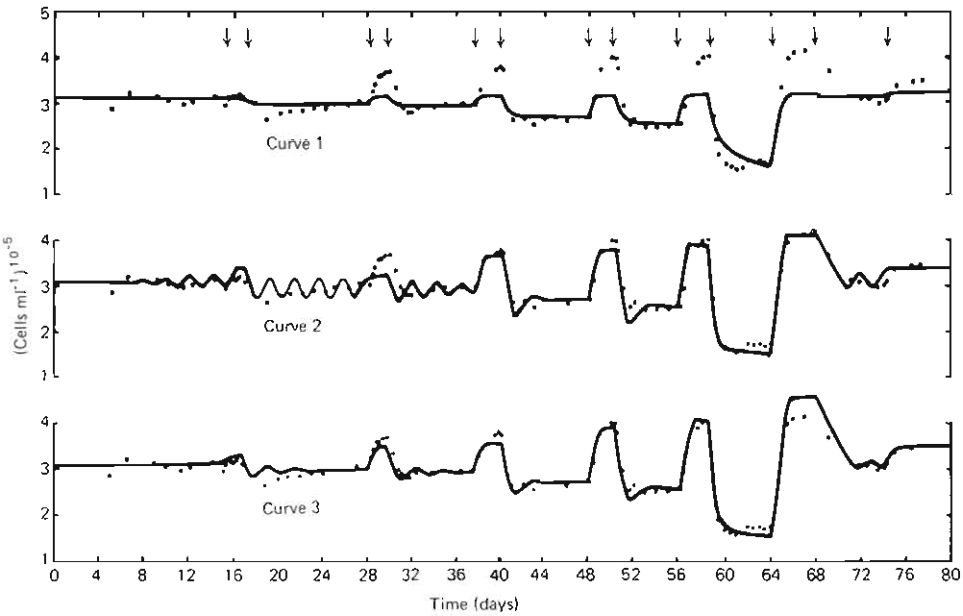

Fig. 6-3: *Isochrysis galbana*. History of population density of the marine chrysophycean in a chemostat. The three curves represent no time lag, point time lag (Equation 9, with $\tau = 14$ hrs), and an averaging time effect over past 24 hrs (Equation 10), respectively. Pairs of arrows indicate perturbation by increase or decrease of nutrient supply. (After CAPERON, 1969; reprinted by permission of Duke University Press. Copyright 1969.)

Alternatively, one can simply interpret Equation (11') as a fairly simple function that gives rise to an S-shaped curve approximating what is observed in batch cultures. From this point of view it can be thought of as an approximation of the true growth function, the first two terms in a power-series expansion of the latter. Then if more accuracy is needed a higher order polynomial can be used. This approach is explored by EDWARDS and WILKE (1968), who derive an equation that can be fitted to microbial batch-culture data—showing the classical lag, exponential, stationary, and death phases in various ways—as well as to diauxic growth in which two substrates are used successively, with sometimes an intervening lag period.

Metazoan Populations

With holozoic metazoans, the complications of various feeding mechanisms and life-cycle stages, and statistical noise associated with smaller population sizes (compared with microbial cultures) make construction of mathematical models for population growth more difficult, and appropriate models for different species may differ qualitatively. We may distinguish two approaches to this problem.

One approach derives from study of the general biology and life cycle of the organism, and results in a model in which all the components and parameters have explicit meaning. This approach, seen for instance in SMITH's (1963) model for *Daphnia magna*, seems more satisfying intellectually. It has some problems, namely: (i) it requires a rather full knowledge of the life cycle and biology of the organism; (ii) it may produce an analytically inconvenient mathematical expression (which, however, can usually be studied by computer simulation); (iii) if there are many undetermined parameters, as usually, it can be fitted to almost any set of data, and the model is hard to test.

Another approach is simply to express the available data with the simplest necessary mathematical relation, being quite cautious about interpreting the needed parameters. This seems less satisfying, and some of the philosophical and other problems have been discussed in a paper by SLOBODKIN (1965), but probably this will remain the usual approach. As an example, CHRISTIAN and co-authors (1971) have reported an intriguing, empirical, non-logistic relationship for wild and captive vertebrate populations, in which the logarithms of cumulative births and cumulative deaths are linearly related to the logarithm of time. The data displayed are striking, and perhaps will call forth a theoretical model that will increase understanding of vertebrate population dynamics. A recent discussion of various empirical and semi-empirical growth models has been presented by WATT (1968).

An example of the heuristic approach is work by GALLOPIN (1971a, b), who developed a generalized model of a resource-limited population, in which parameters have biological interpretations, and which includes exponential and logistic models, and others, as special cases. GALLOPIN's papers contain extensive mathematical analyses of stability and other qualities, using methods similar to those described in the next section. This generalized model might be a useful building block for multispecies models.

(b) General Properties of Multispecies Models

When several species are combined, in the world of models as in the real world, the results can be surprising. Given the present state of knowledge and the relative scarcity of evidence regarding various specific models of species interactions in microcosms, it seems most useful to first discuss some recent work on mathematical properties of large classes of models, with a few examples rather than describing all the various specific models of competition and predation (see also Volume IV). These are described in several recent texts (SLOBODKIN 1962; WATT, 1968; PIELOU, 1969; E. P. ODUM, 1971; KREBS, 1972). This will serve to focus attention on the question of what precisely we should like to know about the properties of microcosms.

These appear to be the main questions with respect to models of multispecies cultural systems:

(i) Is the system stable? i.e., (a) Do all the initial species continue to co-exist as the system evolves? (b) If so, do their abundances, N_i, approach a set of limiting values with time, or (c) do they oscillate periodically or vary irregularly in some range? (d) How are the results affected by minor changes of the starting conditions: does the system remain stable in the sense of (ia), or how are the properties (ib) affected by small (in some context) perturbations of initial or side conditions? These questions are also considered by HOLLING (in press), who makes a distinction between **resilience** (persistence, in the sense of (ia)), and **stability** (as in (ib), (id)) of natural systems.

Questions (ia) to (id) are oriented to the biological problem and are broader than the usual technical definition of stability in the mathematical theory of differential equations. If desired, however, they can be made quite precise mathematically. They can also be generalized for systems in which boundary conditions or system parameters that respond to environmental changes are variable in some (regular or irregular) way. This last is important in considering natural communities (PRESTON, 1969), but perhaps less so for culture systems where conditions are controlled to some extent.

(ii) How are energy and matter partitioned among the elements of the system? With what efficiencies? These questions are a main goal of the experimental studies of ODUM and his colleagues. One can also enquire, in the senses of question (i), how stable is the flow pattern of energy and matter?

(iii) Ultimately, we should like to know the effects upon the system of subtracting or adding various kinds of component species. We should like to develop a calculus of species that would enable us to put together components and adjust culture conditions so as to obtain a microcosm with desired qualities, much as an electronic engineer constructs a hi-fi set or the like. Presumably, that is one goal of our models.

Although discrete stochastic models are a better description of real populations, they are more difficult mathematically, and so less well understood than the analogous continuous deterministic models. In the following I shall discuss mainly the latter. A treatment of some particular discrete stochastic models at an intermediate level is found in the book by PIELOU (1969).

In the following paragraphs an attempt is made to provide an accurate but intuitive picture of some useful facts from the qualitative theory of ordinary differential equations, avoiding technicalities and concentrating on concepts. Readers for whom the discussion is too elementary or too advanced may consult the original papers or skim over this part. Some useful texts are those by PETROVSKII (1966), BIRKHOFF and ROTA (1969), and (more advanced) CODDINGTON and LEVINSON (1955). A useful text at the beginner's level, with orientation to biology, is the recent book by ROSEN (1970). The standard works by MINORSKY (1962, 1969) are particularly useful for questions of interest here.

We consider a system of n differential equations (in general non-linear) in the population sizes N_i of n co-existing species:

$$\frac{dN_i}{dt} = F_i\left(N_1, \ldots, N_n, \frac{dN_1}{dt}, \ldots, \frac{dN_n}{dt}\right), \tag{12}$$

where $1 \leqslant i \leqslant n$. In this context, questions (ia–d) translate into well-known problems of the theory of differential equations. Since Equation (12) determines the change with time of all n variables, we may conveniently regard them as representing the motion with time of points in an n-dimensional space having the co-ordinates N_1, $N_2, \ldots, N_n$. This is termed the phase space. If we start at a given point $p(t_0)$ at time t_0, the system (12) determines a curve or trajectory $p(t)$ which is traced out in the phase space (given that the system 12 satisfies some technical requirements which are not discussed here). Then questions of stability, oscillation, etc., become topological problems regarding the nature of various trajectories. In this way the topological notions of local and global properties become important. A local property is one which holds in some neighbourhood of a point and is thus a point property. The size of the neighbourhood where the property holds may vary with the defining point. A global property, however, is not pointwise attributable, but is a property of a set of points. For example, the fact that a circle is a closed curve, or that the sum of interior angles of a triangle on a plane is 180° are global properties. More examples and discussion can be found in books on topology and real analysis. Synonymous to this usage are the terms 'in the small' and 'in the large'.

The functions F_i (12), in general quite non-linear, can be approximated to any degree of accuracy (given a number of technical conditions) by the first, linear term of a power series expansion, in a sufficiently small neighbourhood of a given point. Thus we can, in principle, find a system of linear differential equations

$$\frac{\mathrm{d}N_i}{\mathrm{d}t} = a_i + \sum_{j=1}^{n} b_j N_j + \sum_{j=1}^{n} c_j \frac{\mathrm{d}N_j}{\mathrm{d}t}, \qquad (12')$$

where a_i, b_j, c_j are constants, which approximates (12) arbitrarily closely in sufficiently small neighbourhoods of a point. That is (12′) approximates (12) locally. Equation (12′) is called the linearized version of (12).

Though questions (i) to (iii) are quite difficult for the general system (Equation 12), they become more accessible in the linearized systems (12′). In particular, the problems of stability of the system (12′) are well understood. With regard to question (ib), if a trajectory of (12′) approaches a limit point P_1 as time goes to infinity, then the system (12′) is stable in the sense of (ib) at P_1, and Equation (12) is locally stable in that sense at P_1.

However, if the trajectories of Equation (12′) oscillate or otherwise fail to approach a limit point, we cannot say much about (12), since these are global properties. Furthermore, even if (12′)—and hence (12)—is locally stable at a point, the approximation to (12′) may be valid within such a small neighbourhood of the point that small random perturbations (as in real systems) will easily shift the trajectory out of the region of stability, and in this way the point would not be a very good equilibrium for the non-linear system. These and other problems (there may be intrinsically non-linear stabilities, from the interaction of non-linear terms, which do not appear in the linearized version) caution against arguing too strongly from properties of Equation (12′) to those of (12). Nevertheless, this seems a useful first step in the analysis, and in many cases gives an adequate idea of qualitative stability properties of the system.

Stimulated by an inconclusive computer simulation of a 3-species model system by Parrish and Saila (1970), May (1971) and Cramer and May (1972) investi-

gated analytically (in the above manner) the stability properties of a multispecies model with several trophic levels. The results are intriguing: stability proves to be not a simple mathematical consequence of diversity, but the 3-species (1 predator, 2 prey) problem of PARRISH and SAILA is shown to have stable solutions. In general, it is shown that stability properties of a given trophic level and of the entire food web, though tending to be similar, do not always coincide.

Thus, in a preliminary way, some aspects of question (i), i.e. stability, are beginning to be understood for some abstract models. An approach to question (ii), i.e., energy and matter, has been analyzed in a similar way in a paper by ULANOWICZ (1972a), already referred to.

With regard to question (iii), i.e., addition and subtraction of component species, one critical problem in the co-existence of prey and predator species involves the time-lag properties of growth-rate responses to changes in population sizes, as noted by HUTCHINSON (1948). MAY (1973) analyzed this question in a recent study for 2- and 3-member straight food chains. In general, as is well known, time lags have a destabilizing effect in model systems of the type studied. A very general class of time-delay effects is considered, including as special cases the simple-point-time-lag of HUTCHINSON (1948) and the convolution integral of CAPERON (1969), already discussed. In the simplest case (HUTCHINSON's), we write

$$\frac{dN(t)}{dt} = r\,N(t)\left[1 - \frac{N(t-T)}{k}\right]. \tag{13}$$

It can then be shown that stability or non-stability of such systems is determined by the relative magnitude of the time-delay effect and a natural time scale of the system. That is, a two-member system (e.g. herbivore and plant, say) has the possibilities:

$$T_1 > T \text{ (stable)}$$

or

$$T_1 < T \text{ (unstable)},$$

where T = the time delay effect referred to; $T_1 = 1/r$, where r = intrinsic growth rate of the herbivore.

If such a system is unstable, it can be stabilized by adding a carnivore for which

$$T_1 < T < T_2,$$

where T_2 = geometric mean of herbivore-birth-rate-time and carnivore-death-rate-time:

$$T_2 = (rb)^{-1/2},$$

b = intrinsic death rate for the carnivore, and r is as before. Thus, there are values of T_1, T, T_2 such that both herbivore–plant and carnivore–herbivore systems are unstable, but the three-member system is stable. MAY (1973) generalizes this problem to include a very broad class of equations, containing various models from fisheries research, economic entomology, etc. (WATT, 1968; WILLIAMSON, 1972) as special cases and obtains, essentially, the same results.

ROSENZWEIG (1973) has analyzed properties of a generalized system with three trophic levels, especially with regard to qualitative implications of some non-linear properties, and evolutionary problems.

The Lotka–Volterra Equations

At this point it is appropriate to mention another important paper by MAY (1972c). The well-known species interaction equations of LOTKA and VOLTERRA (see SLOBODKIN, 1961) may be written, for an assemblage of n species,

$$\frac{dN_i(t)}{dt} = N_i(t)\left[r_i - \sum_{k=1}^{n} \alpha_{ik} N_k(t)\right] \tag{14}$$

where the α_{ik} are real numbers, the interaction coefficients. Essentially, the Lotka–Volterra equations are a multispecies version of the logistic equation and many of the comments already made about the latter can be applied here with suitable modification. In particular, one may think of the Lotka–Volterra system as comprising the first few terms of a Taylor expansion. Inclusion of further terms involving higher derivatives will then improve the model, as in the 1-species case. HUTCHINSON (1947) did this for 2-species systems to include some higher order interactions involving social phenomena. Discussions of Equation 14, particularly for the case of 2 species, are found in elementary ecology texts (SLOBODKIN, 1961; for details consult Volume IV; MARGALEF, in press). The α_{ik} in this model have the antisymmetric property:

$$\alpha_{ij} = -\alpha_{ji}$$

corresponding to the overly idealized notion that interacting species influence each other's growth rate equally and oppositely. Because of the antisymmetry the system (14) has a purely neutral stability, corresponding to that of a frictionless pendulum. That is, a perturbation of the system leads to endless unchanging oscillations about an equilibrium point with amplitude determined by the perturbation. This may be expressed by saying that all the trajectories of (14) in phase space are simple closed curves. These properties, deriving from the antisymmetry of the a_{ij}, correspond mathematically to what a physicist calls a conservative system, and several investigators have pursued rather improbable analogies with statistical mechanics (KERNER, 1969; GOEL, and co-authors, 1971; LEIGH, 1971). This forbidding work, inaccessible to most biologists, has been analyzed by MAY (1972c) and others. In the paper under discussion, he observes that the neutral stability is an extremely fragile one. As noted earlier, for any continuous model expressed as a system of differential equations, there is an homologous discrete system of difference equations. The latter is closer to the reality of biological systems, where generation times are finite and population changes occur in discrete steps. The discrete system is, in general, known to be less stable then the continuous one. MAY shows that if one examines the stability properties of the system of discrete difference equations that are homologous to (14), the (neutral) stable system (14) is not approached in the limit by a series of stable discrete systems: that is, the slightest deviation of (14)

from the continuous quality makes it unstable.* There are many other objections that can be made, on biological and mathematical grounds, to analogies with statistical mechanical models (GOLDSTEIN, 1972).

This leads us to the question: what type of oscillatory behaviour can be expected in appropriate non-linear models of interacting populations. Here we turn to another paper by MAY (1972b) in which prey–predator systems are considered. This is based on a hitherto little-known paper by KOLMOGOROFF (1936; see also SCUDO, 1971) and is an application of the Poincare–Bendixson theorem (see cited

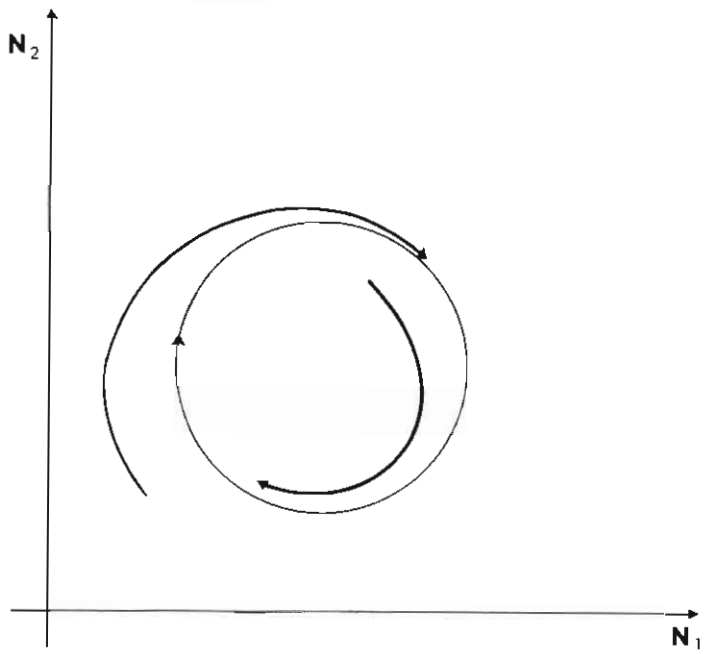

Fig. 6-4: A limit cycle: a simple closed curve in phase space to which trajectories converge with time. (Original.)

texts) to ecological models. MAY observes that for a wide class of non-linear systems of the form

$$\frac{dN_1}{dt} = N_1\, g\,(N_1, N_2)$$
$$\frac{dN_2}{dt} = N_2\, h\,(N_1, N_2), \qquad (15)$$

which includes all models developed in applied entomology and fisheries, there is either a stable equilibrium or a stable limit cycle. A limit cycle (see cited texts) is a simple closed curve in phase space to which trajectories in a region converge with time. In this region, trajectories in the interior of the curve spiral outward with time, whereas trajectories outside the curve spiral inward toward it (Fig. 6-4).

* This is a technical argument depending on the nature of the eigenvalues of (14). These all lie on the imaginary axis of the complex plane (the limiting case of the Routh-Hurwitz criterion), whereas the eigenvalues of stable homologous difference equations are in a region bounded away from the axis.

If a system of the form (15) does not have an equilibrium point, it will not appear stable in a linear stability analysis of the type described earlier, and frequently will not seem stable in computer simulations. Nevertheless, as MAY and KOLMOGOROFF pointed out, the Poincare–Bendixson theorem (in turn based on the Jordan curve theorem of pure mathematics) tells us that even if a stable equilibrium point is missing there is a stable limit cycle to which trajectories converge, set by the model parameters. As MAY argues, stably oscillating populations observed in the laboratory (e.g. NICHOLSON, 1957) or in nature (e.g. possibly the well-known lagomorph oscillations of the Arctic), are probably examples of limit cycles. HOLLING (in press) has cogently discussed the evidence for limit cycle-like effects in natural communities. Such cycles are stable under perturbations, though if the cycle passes very close to the N_2 or N_1 axis (as it may), then stochastic effects of small populations lead eventually to extinction (BARTLETT, 1960). Another fact worth noting is that the limit cycle is a 2-dimensional (2-species) phenomenon, depending mathematically on topological properties of the plane, and for larger systems the theory is much less understood (the n-body problem of physics).

RESCIGNO and his colleagues (RESCIGNO and RICHARDSON, 1965, 1967; RESCIGNO, 1968; RESCIGNO and JONES, 1972) have also studied the application of these tools in models of interacting populations, and have classified some of the qualitative possibilities in certain 3- and n-member systems. In the latter case, the generalized approach of KOLMOGOROFF is adopted.

Biochemists have recently become quite aware of these ideas, and an extensive literature, theoretical and experimental, has appeared on the oscillatory properties of metabolic systems (see SAVAGEAU, 1971; WALTER, 1972; WINFREE, 1972; and references in these). It is time for population ecologists to learn such methods of analysis, which is partly why reference has been made so extensively to recent work of MAY and others. ROSENZWEIG and MACARTHUR (1963; ROSENZWEIG, 1969, 1972) have independently developed some of the results mentioned from intuitive arguments about trajectories in phase space ('graphical analysis'), without using the well-established mathematical theory. The microcosm experiments of MALY (1969) with rotifers were directly inspired by this theory.

The problem of the properties of a system with a variable environment—hence of variable parameters—clearly important for ecological systems, is probably quite difficult. An approach to it is seen in a paper by MAY and MACARTHUR (1972) that studies the relation of such variability to diversity of sympatric species of one trophic level (or 'niche overlap' of such species). This approach appears to depend in an essential way on a theorem in MACARTHUR's niche theory (MACARTHUR, 1970). Given this context, then, it is shown that in a randomly varying environment the average food sizes for 'adjacent' species utilizing a continuum of food sizes (or some other convenient parameter) must differ by approximately the standard deviation in food size taken by each species. The result holds for a wide range of variation, and so it is claimed that there is an effective limit to the amount of niche overlap in fluctuating environments, relatively insensitive to the amount of fluctuation, unless that is severe. Such a conjecture seems well suited for testing with mixed cultures, where some kinds of fluctuations are controllable. More theoretical results along these lines are described in detail in a recent review by MAY (in press). A view of the entire area is provided by his book (MAY, 1972d).

Most ecological models to date have ignored age structure and deal with the *total* population size N as a variable. Several workers are now exploring the effects of age-specific interactions. OSTER and TAKAHASHI (in press) and AUSLANDER and co-authors (1974) have observed a certain stabilizing effect from these in a model of NICHOLSON's (1957) observations of oscillations in blow-fly populations.

(c) Some Particular Models

WATT (1968) discusses many of the particular models for multispecies systems. Usually they are applied to field phenomena: natural communities, fisheries or agriculture, or the study of pest outbreaks, parasitological interactions, and epidemics. There is little explicit modelling of controlled multispecies laboratory systems, with the exception of several similar models of microbial predator–prey systems in continuous cultures (BUNGAY, 1968; CANALE, 1969; CURDS, 1971; SUDO and co-authors, 1972). These are oriented to problems of sewage treatment, and their general nature is seen from CANALES's treatment.

Verbally, this model has the form:

(1)	Rate of change of limiting nutrient concentration	= Rate of input	− Rate of output	− Rate consumed by bacteria
(2)	Rate of change of bacterial density	= Rate of growth	− Rate of output	− Rate of consumption by protozoa
(3)	Rate of change of protozoan concentration	= Rate of growth	− Rate of output	

With certain assumptions about the mathematical form of the various terms, and convenient changes of variables, one obtains the equations

$$\frac{dx}{d\theta} = 1 - x - A\frac{x}{a+x}y \tag{16}$$

$$\frac{dy}{d\theta} = A\frac{x}{a+x}y - y - B\frac{y}{d+y}z \tag{17}$$

$$\frac{dz}{d\theta} = B\frac{y}{d+y}z - z \tag{18}$$

In this and other similar models, computer simulations yielded cyclical solutions which resemble some observations in cultures. In this area, mathematical and experimental studies of multispecies systems promise to converge.

(d) Automaton Theory

This subsection is purely speculative. Models so far considered are constructed with regard to statistical properties of interacting species, in which the units are populations. As WATT (1968) observes, a computer programme is in some ways like

an organism. Confronted with data it can make choices of action according to given rules. It can even change the rules or 'learn', to some extent, to adjust to changing stimuli. Mathematicians and others interested in the properties of such 'automata' are developing the field of automaton theory (PATTEE and co-authors, 1966). One might then be able to model the important aspects of an organism's adaptive responses, and observe statistically (with simulations, and perhaps eventually analytically) responses to various environments. In this way the properties of systems of interacting organisms with complex life cycles might be modelled. Such detailed models could include sub-theories, such as the theory of predator search strategy (PALOHEIMO, 1971), or of switching and choice in feeding (RAPPORT and TURNER, 1970), or of social interactions, more conveniently than conventional models. This field seems to lie mainly in the future (see, however, PASK, 1969) and is intimately linked to the properties of digital computers.

(5) Agnotobiotic Cultures

We deal here with incompletely defined systems of the sort found naturally, where the complete number of species present is known. Such systems can be studied from two somewhat different viewpoints.

The first concentrates on one or a small number of species in an incompletely defined system. The system is agnotobiotic simply for convenience, because of technical problems of maintaining gnotobiosis or axenicity. Nevertheless, we deal separately with these here to emphasize the distinction from the gnotobiotic systems treated in the next section, and the importance of using gnotobiotic systems for such studies when possible. A number of continuous and batch culture studies of various phagotrophic protozoans (e.g. GOLD, 1970, 1971; HAMILTON and PRESLAN, 1970b) and invertebrates (e.g. REEVE, 1963; HEINLE, 1970; MULLIN and BROOKS, 1970; ZILLIOUX and LACKIE, 1970) are primarily autecological, with emphasis on feeding rate, efficiency, life cycle, etc., of the phagotroph (Chapter 5.1). Bacterial or algal prey are usually provided in a resting state and are not growing much while serving as food. Dynamic interactions are not usually studied in such experiments; they are treated in Volume IV. Fish-pond culture (BENNETT, 1962; HICKLING, 1970), commercial aquarium techniques (SPOTTE, 1970) and laboratory-stream research (WARREN and DAVIS, 1971), though relevant here in varying degree, are covered in Chapter 2. The beginnings of aquacultural enterprise with various species (COSTLOW, 1969*; MCNEIL, 1970*; BARDACH and co-authors, 1972) are treated in Chapter 5. Studies on competition and other interactions usually deal with two species (e.g. the classic work with species of *Drosophila*, *Tribolium*, *Lemna*, *Paramecium*, *Didinium*, etc.) in an agnotobiotic setting.

The second viewpoint involves studies more concerned with the general properties of an undefined, entire assemblage of species from some habitat. This is the typical approach in studies by H. T. ODUM and his colleagues. The two viewpoints are usually rather distinct, and the distinction is used to organize the following discussion.

(a) Studies of Several Species, Usually in an Agnotobiotic Milieu

These studies centre on theoretical evolutionary problems about the amount and

* See Literature Cited of Chapter 5.1 for references.

manner of competitive and predatory relations between sympatric species. The exclusion principle and the determinants of species diversity are central problems, and in that sense we are dealing with experimental natural history (if such a phrase is possible), and the ideas go back at least to DARWIN and WALLACE. On the other hand, many of these studies have an intensely practical side, stemming from problems of resource management (Volume V), agriculture, pest control, parasitology (Chapters, 8 and 9), and the like. A complete review of such work is not called for here, but a brief discussion will be useful as a logical prelude to the later discussion of gnotobiotic systems.

Agnotobiotic cultures of *Daphnia magna* (SLOBODKIN, 1954), *Lucilia cuprina* (NICHOLSON, 1957) and other metazoans often show a tendency to oscillate about a size determined by the supply of resources, though the effect can be reduced or absent in vertebrates and other organisms with considerable social interaction (see discussion by SLOBODKIN, 1961). These and similar experiments, interpreted as models of natural populations, generated a controversy on the importance of density-dependent effects in determining natural population sizes. The controversy reached its full extent at the 1957 Cold Spring Harbor Symposium, and has persisted in various ways until today. This is an important point, since theories of community structure and diversity are based partly on the notion of species competing for limiting resources, a density-dependent effect, and also partly on models of prey–predator relations in which the dynamic interaction of density-dependent effects with population parameters is assumed.

Experimental studies of competition in an agnotobiotic setting are numerous, dealing with yeast (GAUSE, 1934), ciliated protozoans (GAUSE, 1934; VANDERMEER, 1969), hydrozoans (SLOBODKIN, 1961), *Daphnia magna* (FRANK, 1952), flour beetles (CROMBIE, 1947; PARK, 1962), bean weevils (UTIDA, 1953), house-flies (PIMENTEL, 1968), fruit-flies (MOORE, 1952; AYALA, 1972), various higher plants (DE WIT, 1960; HARPER, 1961; DONALD, 1963; VAN DEN BERGH and ENNIK, in press), and others (see ecology texts cited earlier).

Generally, such studies have more or less confirmed the qualitative conclusion of the Lotka–Volterra theory: if two competing species persist in a culture it is because (i) they use a somewhat different portion of the resources, (ii) a stabilizing predator or 'rarifying factor' is present, or (iii) environmental variability prevails that prevents competition from proceeding to a conclusion. Thus, in mixed populations of *Hydra* species, co-existence occurred because one species could utilize resources from an autotrophic symbiont (SLOBODKIN, 1961). Two species of flour beetle were able to co-exist in a culture medium of fine flour mixed with small glass tubing which only one species could penetrate (CROMBIE, 1947). The predator effect is seen in bean weevils (UTIDA, 1957), where two species persisted in the presence of an unselective parasitic wasp, and was produced experimentally by SLOBODKIN (1961), who acted as unselective predator on two species of hydroids. Observations by AYALA (1969) of co-existence of two *Drosophila* species are probably explained by the non-equilibrium nature of the serial culture technique (BOROWSKY, 1971; see discussion of LEVIN's gnotobiotic experiments with 2 strains of *Escherichia coli* in a later section) and the presence of more than one niche in the milieu (GAUSE, 1970), though they also may possibly indicate an interesting phenomenon of mutual suppression. GILPIN and JUSTICE (1972) derive a non-linear dynamic relation with this assumption (see further discussion of the various possibilities by ANTONOVICS and FORD,

1972). Extensive studies by PARK (1962) showed a certain indeterminacy of the end result of competition in mixed cultures of *Tribolium* spp. and this stimulated the development of stochastic models (NEYMAN and co-authors, 1956). On the other hand, the detailed data on various parts of the life cycle and the relative simplicity of experimental milieu led to derivation of a detailed deterministic model (TAYLOR, 1967) of the single-species growth curve. Such experiments also sometimes reveal unexpected self-regulatory phenomena, such as cannibalism (LLOYD, 1968).

VANDERMEER (1969) grew four species of ciliate protozoans individually and in mixed (agnotobiotic) culture and found a reasonably good agreement in mixed culture to the simple Gause–Lotka–Volterra model. GILL (1972), studying single and combined cultures of closely related *Paramecium* species, discovered that such models bore little relation to the outcome of competition, which instead took the form of inhibition or interference of one species by another. It was suggested that this may be caused by the bacterial endo-symbionts of *Paramecium* spp. (Kappa particles, etc.), whose ecological role was hitherto unexplained (GILL and HAIRSTON, 1972).

Similar agnotobiotic studies of prey-predator systems are also numerous, including work with protozoans (GAUSE, 1934; SALT, 1967, 1968), rotifers (MALY, 1969), weevils (UTIDA, 1957), mites (HUFFAKER, 1958), and many others. These provide data for the development of relatively detailed ecological theories about strategies of predation (HOLLING, 1969; MURDOCH, 1969) and the role of predators in the community (HAIRSTON and co-authors, 1960; HARPER, 1969; etc.). But most impressive is the field of applied ecology that has grown out of them: the biological control of pest populations by their predators. HUFFAKER (1971; see also SWEETMAN, 1958) gives an idea of the vigour and wide scope of this field—critical now that the dangers of pesticides are becoming known.

Related to these are various recent observations of the effects of a single predator or herbivore upon a whole (agnotobiotic) microcosm. Thus HURLBERT and co-authors (1972) studied artificial ponds, some containing mosquito fish *Gambusia affinis* and some lacking them. *G. affinis* greatly reduced the planktonic herbivore population (rotifers, crustaceans, insects), and ponds with *G. affinis* had dense populations of phytoplankton compared to those without. This was accompanied by a series of other ecological effects such as lower dissolved phosphate, less benthic algae, and higher temperature (greater absorption of sunlight) in ponds with *G. affinis*. These elegant experiments are reminiscent of DARWIN's (1859, p. 74) anecdotal account of the probable causal relation between the supply of cats and the abundance of clover in the neighbourhood of towns and villages in England.*

Similar effects, at a lower level, are seen in the clearing of bacteria and eliminating of protozoans and micrometazoans by mosquito larvae in the standing water of *Heliconia* spp. bracts and similar microcosms (phytotelmata) (MAGUIRE and co-authors 1968; MAGUIRE, 1971 and references therein), and in the clearing of periphyton by tadpoles (DICKMAN, 1968), both of which have major reverberations on other elements of the system. DART (1972) suggested that recolonizing of some coral

* Cats eat field mice, which otherwise destroy the combs and nests of the ground-dwelling humblebees, which pollinate the clover. T. H. HUXLEY is said (I cannot find the reference) to have extended the sequence in both directions: backward to the number of old ladies who keep cats, and forward to grazing cattle and the cost of supplying beef for Her Majesty's navy.

reefs may be facilitated by the grazing of echinoids on algal 'lawns', coral planula larvae being more likely to settle on the bare spots.

A different effect is the exclusion of 'alien' species from the community by predators, as shown with bacteria and algae by MITCHELL (1971; see also CANTER and LUND, 1968; DAFT and STEWART, 1971). Continuing in this vein we may note again the role of marine protozoans in recycling phosphorus (JOHANNES, 1965) and the accelerating effects of protozoans in sewage treatment (CURDS and co-authors, 1968). So we come to consider the interactions of various functional elements of entire ecosystems, the flow-web of matter and energy, and their holistic properties (Volume IV: MARGALEF, in press). This is the second point of view referred to at the start of this section, favoured by MARGALEF, ODUM, and others, to which we now turn.

(b) Holistic Properties of Agnotobiotic Systems

The desire to study, in a controlled setting, patterns of gross productivity, ecological succession, and biogeochemical cycling observed in the field led to early ventures in the culture of ecosystems in aquarium microcosms (ODUM and HOSKIN, 1957; WHITTAKER, 1961; BEYERS, 1963). In particular, BEYERS (1962, 1963, 1965) cultured communities derived from a number of aquatic habitats and measured CO_2 uptake and release (via pH change) and noted general similarities in qualitative patterns. These have been the subject of much discussion, but remain unexplained (COOKE, 1971a).

After extensive study of bays along the coast of Texas, H. T. ODUM and his colleagues constructed moderate-sized concrete ponds in which they sought to recreate and maintain various kinds of natural bay communities in controlled conditions (ODUM and co-authors, 1963b). This venture was strikingly successful. Communities resembling the natural ones in many (but not all) measured aspects (e.g., species content and distribution pattern, pigments, productivity measurements, concentration and distribution of various elements) were maintained, and the effects of controlled perturbations on these were observed. The three natural communities modelled were: low-salinity oyster bars; shallow grassy-bottom bay communities (30‰ S); blue-green algal mats of the type that occurs in very shallow 'pans', often hypersaline (Volume 1, Chapter 4). Adding inorganic nutrients led to a sequence of events similar to those seen in nearby polluted bays. Differences developed in the communities in the 3 replicate oyster reef ponds after 6 weeks without intermixing, and remained in the face of subsequent interpumping. It became clear that these experiments in 'ecological engineering' are a fruitful approach, providing a means of studying the measurement of such things as gross productivity of a community without such added complexities as variable currents and water masses.

An important question is the replicability of such culture experiments. How predictable are the results of seeding a given body of water—characterized by particular conditions of light, temperature, salinity, etc.—with a given agnotobiotic inoculum? This question is related to the profound problem of the relative importance and interaction of biotic and abiotic environmental factors (CONNELL and ORIAS, 1964; Volume I) and stands in need of study. Some observations were made by ABBOTT (1966), who monitored 18 parallel carboys filled with water and sediment

from a brackish (17‰S) inlet to Biloxi Bay (USA). After a week of stabilization in experimental conditions (stirring, light cycle, aeration, etc.), the carboys were inoculated with local net plankton. After another month to allow some initial ecological succession to occur, the diurnal sequence of dissolved oxygen and levels of several forms of nitrogen and phosphate were studied. These were comparable, within statistical limits (not analyzed in detail), in the 18 microcosms. This appears to be the most ambitious attempt to check variability of microcosms, and it is generally reassuring, but the area clearly needs more study.

In particular, the predictability of ecological succession should be looked at in detail. Field ecologists have been quite active lately in devising ways to compare communities by species content and abundance, and these parameters could be used to compare the end products of parallel microcosmic evolutions. Such comparison, correlated with chemical comparisons, would bear on the question: to what extent do different assemblages of species have comparable overall properties with regard to chemical cycles, etc.? This question is central and will be further discussed (or posed anew) in later sections. In this connection, the study of replicate terrestrial microcosms, started from soil and kept in closed plastic culture containers in a tropical rain forest for a year by ODUM and LUGO (1970) is pertinent. They found that different higher plants developed and dominated in each case. Diurnal patterns of O_2 and CO_2 were also different, but seemingly less so. DUNSTAN and MENZEL (1971) studied continuous cultures of natural populations of marine phytoplankton, with a standard enrichment and with dilute sewage, and found comparable developments of species assemblages and several chemical parameters. Similar results were apparently obtained in replicate cultures, but are not presented in detail or analyzed.

A thorny problem is the measurement of community productivity and community metabolism. This problem is discussed in Volume IV. A recent study by VERDUIN (1971) of energetics of a freshwater sewage-treatment lagoon contains a discussion of discrepancies arising from standard ways of computing productivity from different data in this and other examples. COOKE (1971a) also discusses some anomalies. The present reviewer hesitates to enter such a controversial area, but wonders if it might be more useful and less confusing to simply abandon such terms as community 'productivity' (net, gross, or other), 'respiration', and 'metabolism', and refer instead to diurnal patterns of dissolved oxygen, CO_2, pH, ^{14}C fixation, harvested biomass, calories, etc. But perhaps that is a cowardly way out of the problem.

An area of potential importance is the use of microcosms to study models of biogeochemical cycles. CONFER (1972) investigated steady-state circulation of labelled phosphorus in 200-l freshwater aquaria with continuous in- and out-flow, as a model of the cycle in certain types of lakes. Most incoming phosphorus was taken up by attached *algae*, and a steady state was obtained that suggested the utility of a two-compartment model for such systems. This kind of study could be used for other cycles as well.

(c) Maturity, Diversity and Stress

An important part of the theoretical framework of these experiments concerns

the effects of various stresses on the ecosystem. H. T. ODUM (1956), MARGALEF (Volume IV; MARGALEF, in press), and SANDERS (1968, 1969) have emphasized the plausibly inverse relation between environmental 'stress' and fluctuation, and diversity of organisms. ODUM has emphasized that, in stressed communities, the various cycles are slowed due to lack of appropriate species at critical points in the food web, and material and energy accumulate in sediments or other inert forms instead of being cycled. Such hypotheses seem well suited to experimental study with microcosms, as are the effects of perturbations in general.

FERENS and BEYERS (1972) recently observed adaptation of overall productivity-related parameters in an aquatic (almost gnotobiotic) laboratory micro-ecosystem subjected to gamma radiation stress. ALLEN and BROCK (1968), using mixed inocula from many sources, found that mixed heterotrophic microbial populations developing at different temperatures in a defined medium, with glucose and L-glutamic acid as carbon and nitrogen sources, became optimally adapted (in terms of glucose assimilation) to their incubation temperature over the range 25° to 75° C, in spite of cross-mixing and fresh inoculations. This indicates the powerful selective effect of temperature (Volume I, Chapter 3) on species composition in such communities, and suggests that temperature change does diminish the number of species and efficiency of cycling at this trophic level. More such work is needed.

Sanitary engineers have to be concerned with effects of chemical and other perturbations on the natural communities of sewage-treatment systems (Volume V). KINCANNON and GAUDY (1966) and others have studied the effects of salt shock on continuous heterogeneous cultures of sewage organisms, and found severe disruption of the system with sudden salinity changes, followed by adaptation after a period of time. The adapted population was severely affected by a sudden return to the original salinity (Volume I, Chapter 4). In general it appeared that the resulting adaptation was due mainly to the selection of new dominant species, rather than to biochemical adaptation of original populations. Perturbation of a marine carboy microcosm by adding large doses of phosphate and nitrate (ABBOTT, 1969) caused development of heavy blue-green algal growth. Thus various preliminary studies suggest a pattern in which microcosms adapt to perturbations by changes of species composition. In the absence of re-inoculation, continued perturbation would presumably produce depauperate communities (Volume IV; MARGALEF, in press).

This bears on the important question of the homeostatic properties of heterogeneous microcosms under perturbation. A major point in the argument for using mixed rather than pure cultures for life-support systems in space-ships (COOKE and co-authors, 1968; COOKE, 1971b) was that heterogeneous cultures systems, though less efficient, are more stable. Presumably, what's meant is more than selection of species that can survive the perturbations, although such response comprises homeostatic elements. To what extent have species interactions supportive holistic effects, which tend to preserve community structure in the face of perturbations? LEVANDOWSKY and GOLD (unpublished) tried to gauge the effects on dominant species composition of adding large inocula of single species to continuous and batch cultures of natural marine phytoplankton grown in filtered sea water in the laboratory and in plastic bags in outdoor ponds. Addition of *Thalassiosira pseudonana*, *Platymonas* sp. and *Glenodinium foliaceum* to summer water and *Isochrysis galbana* and *Prorocentrum micans* to winter water were studied in replicate cul-

tures. Some added species (*Platymonas* sp., *I. galbana*) were abundant at first, then 'collapsed' and disappeared. Others (*G. foliaceum*, *P. micans*) had a tendency to persist in moderate numbers. Some (*I. galbana*) had little detectable effect on the dominant flora, while others (*Platymonas* sp.) were associated with striking changes in succession.

Such results suggest that a main problem in modern marine ecology is to understand the various kinds of chemical and mathematical interactions of species in terms of their effects within a system, and their possible role in its homeostatic properties. Microcosms, where the possibly interacting species are at least spatially and logistically delimited, would seem to be the tool of choice in such analysis.

(d) Mathematical Simulations

Inextricably entwined with experiments and theories of the 'ODUM school' is a system of mathematical simulation deriving partly from circuit analysis of electrical and mechanical engineers (ODUM and PINKERTON, 1955) and partly from compartment analysis (WATT, 1968). This will not be described in detail here; the interested reader may consult H. T. ODUM (1971), the discussion of Texas bay systems (ODUM, 1967), the detailed description and application of the method by BURNS (1970), ODUM and co-authors, (1971), and Volume IV. A useful elementary exposition is found in a chapter by C. J. WALTERS in the text by E. P. ODUM (WALTERS, 1971). A good detailed text, though oriented completely to examples from physiology instead of ecology, is BLESSER (1969). This type of mathematics is associated with the techniques of analogue computation and simulation (DENMEAD, 1972), which has been used extensively by this group. Other recent discussion is found in the second volume of PATTEN's (1972) series on systems analysis and ecology, especially the chapter by CASWELL and co-authors (1972). Examples given are mainly from field ecology, but are clearly relevant for microcosm studies.

(e) Succession

Experiments on species succession in microcosms go back to early observations of protozoans in hay infusions (WOODRUFF, 1912), but are surprisingly scarce and tend to be purely descriptive. An exception is the study by MCINTIRE (1968), who observed benthic algal succession in parallel laboratory streams fed from a natural stream, with 6 different combinations of light (150 ft. c, 700 ft. c) and stream velocity (0, 14, 35 cm sec^{-1}). Biomass and chlorophyll *a* concentration were related by a regression formula to community age, current velocity, and illumination. Several characteristic communities appeared: a brown, felt-like growth dominated by diatoms (150 ft. c, 14 cm sec^{-1}; 150, 35); a dark green membraneous mat dominated by cyanophyceans (700, 14; 700, 35); a filamentous mat growth at the surface dominated by the xanthophycean *Tribonema minor* (700, 0). The (150, 0) stream was less predictable, and all patterns varied somewhat seasonally. A variety of interesting questions are raised by this study and it is hoped that such factorial experiments will become more common in microcosm studies.

MARGALEF (1967; Volume IV; MARGALEF, in press) conducted preliminary experiments with heterogenous marine phytoplankton in continuous cultures ar-

ranged in series and in parallel with respect to medium flow. This was an attempt to follow changes of diversity (measured as the ratio of two pigment wavelengths, D430/D665), but technical problems were experienced (algae sticking to the sides, etc.).

Cooke (1967) reported preliminary studies of succession in replicate freshwater stationary cultures inoculated with aged pond water. During 90 days, total biomass increased and both chlorophyll and gross photosynthesis (pH change) became reduced after initial increase. Measurements of chlorophyll and biomass were made by 'sacrificing' replicate aquaria periodically, so that the results depend on the replicability of such systems. Similar but more extensive experiments were conducted by Wilhm and Long (1969), in which succession of various parameters was studied in algal mat communities of 192 1-l pond-water aquaria subjected to 3 nutrient levels. These were sampled over a period of 109 days. Dominant species differed at the 3 nutrient levels. Biomass and pigments were relatively high at high nutrient levels, whereas the ratio of production to respiration (via pH measurements) approached unity in all microcosms studied.

Bick (1972) studied succession of freshwater protozoans in agnotobiotic batch cultures grown in 30-l glass aquaria, as a model for sewage treatment. Basic substrates used were peptone and cellulose, and successions were monitored for 5 weeks after inoculation with mixed samples from many sources. Succeeding states of 'purification' or substrate mineralization were characterized by distinct protozoan associations.

(f) Some Particular Holistic Properties

Observations of the diurnal oxygen cycle of cyclically illuminated brines (Nixon, 1969a)—considered a stressed community—led to the suggestion that in such communities solar energies may take the place of missing types of organisms in aiding recycling processes. Photo-respiration and photo-heterotrophy that have been observed in some species are adduced as two possible mechanisms in a speculative paper by Odum and co-authors (1971). Unstressed coral communities, where recycling is accomplished by bacteria and detritus feeders (Di Salvo 1971; Di Salvo and Gundersen, 1971; Chapter 3.1) are compared to algal-mat communities where pigment amounts and ratios were apparently controlled by ambient nutrient level (Odum and Hoskin, 1957).

A rather startling holistic effect, possibly related to the last, is the apparent maintenance of an electric potential across algal mats in daylight (Armstrong and Odum, 1964). This phenomenon may be related to the 'natural redox cells' studied by Whitfield (1972).

(g) Applications of Agnotobiotic Cultures

Sewage-treatment Systems

Useful reviews of the natural history and ecology of these microbial systems are provided by Hawkes (1965), Gaudy and Gaudy (1966), Pipes (1966), and Curds and Cockburn (1970); see also Volume V. Some interactions of components of

these communities have already been discussed. At this point it is appropriate to comment on properties of the system as a whole, and the use of kinetic models such as the Monod equation and its relatives (CHIU and co-authors, 1972a) to describe them.

PEARSON (1968) states that use is routinely made of the Monod equation, treating a heterogeneous population as though it were one species, with adequate predictability. However, different dilution rates select for different species, as proved by JANNASCH (1967a, b; see also CASSELL and co-authors, 1966; CHIU and co-authors, 1972b) who used varying dilution rates to separate species. This causes a hysteresis or inertial effect as new population balances are reached at each dilution rate. The same effect is seen when perturbations of the incoming substrate are studied (STORER and GAUDY, 1969). In general it is found that parameters such as μ_m and K_s vary with dilution rate (GAUDY and co-authors, 1967; RAMANATHAN and GAUDY, 1969), and this is usually related to shifts in species composition (LAWRENCE and MCCARTY, 1969). This is not so surprising and will no doubt occur with any model that attempts to predict gross quantities (biomass, nutrient utilization) of heterogenous populations. Hence semi-empirical models of natural phytoplankton populations, developed by STEELE (1956), RILEY (1963) and others (see DI TORO and co-authors, 1971 for discussion), have parameters representing general entities such as zooplankton, which must be re-estimated locally when the model is used.

These comments bear on the controversy among field ecologists studying natural uptake rates of substances. Thus WRIGHT and HOBBIE (1966) and MACISAAC and DUGDALE (1969) found Michaelis–Menten kinetics adequate to describe uptake of various substrates by natural populations, but VACCARO and JANNASCH (1967), MUNRO and BROCK (1968), and HAMILTON and PRESLAN (1970a) did not.

Other Applications

There are various technologies in which agnotobiotic cultures play a central role. Some are quite old, the product of centuries of trial and error, and should be studied for clues to general principles and hints of suitable techniques. For example, brewing and fermentation are ancient arts; AMERINE and KUNKEE (1968) have presented an ecological description of wine-making. Numerous examples pertaining to research cultivation (invertebrate and fish culture), commercial cultivation (e.g. fish-pond culture), and laboratory-stream research are dealt with in Chapters 2 and 5.

(h) Conclusions

We have considered agnotobiotic microcosms and their relations to some general ecological theories and their accompanying experiments. The theory, derived from H. T. ODUM's work and elsewhere, tends to be all-encompassing; it arises from numerous field and laboratory observations. Major difficulty exists in defining (operationally) the notions 'productivity', 'diversity' and 'stability'; these are not single concepts at all. The major testing ground for all these theories is likely to be work with microcosm cultures rather than field work, for in the former reproducibility or non-reproducibility will be less ambiguous.

(6) Gnotobiotic Cultures

This section considers scattered information that forms a starting point for a promising area of research: the experimental synthesis of ecosystems. Technical problems that have appeared, perhaps unexpectedly for some investigators, are indicators of the potential richness of this field, since they demonstrate our lack of understanding.

The agnotobiotic work of SALT (1967, 1968) with protozoan systems, for instance, impresses by the insight gained into certain prey–predator relations, yet the large amoebae and holotrichous ciliates studied have not been cultivated axenically, and even gnotobiotic cultures may not be easy to achieve. So there are many things we do not know yet about getting gnotobiotic cultures to grow, not to speak of predicting their properties.

(a) Continuous Cultures

Bacteria

PARKER (1966) studied interactions of 3 bacteria species in a continuous system. Cultures were fed into a common reservoir which turned over continuously. In this system competitive interactions are damped and cannot cause extinction of a population since additions are continuously provided from pure cultures. PARKER observed both stimulation and inhibition of species, and changes of growth rates; however, his results are difficult to interpret since the resulting medium of the combined cultures was a mixture of the different media of pure cultures, plus waste products. Still, the concept is interesting, since it possibly resembles natural situations in which organisms from distinct breeding reservoirs mingle and interact in a region.

Of greater interest in our context are experiments with one growth chamber containing several bacteria species, supplied continuously with sterile medium. JANNASCH (1967a, b, 1968a, b) studied competition of marine and other bacteria in continuous culture, using filtered sea water with added carbon sources. It proved possible to select for species differing mainly in population growth rate by changing the dilution rate. Such species introduced in (gnotobiotic) mixed cultures could also be separated once more in this way. Thus the outcome of competition for a limiting substrate was based on parameters of growth and reproduction of individual species under given conditions rather than on different specific nutritional requirements. This work has emphasized the importance of growth rates of competing marine bacteria populations at low nutrient levels in a way that could not have occurred from classical batch-culture studies (JANNASCH, 1969). The theoretical conclusion (POWELL, 1958) that, in competition for a limiting nutrient in continuous culture, the organism growing at the lowest nutrient level at a given dilution rate will displace the others, is now thought to be quite important in some marine habitats.

TEMPEST and co-authors (1967), MEERS (1971), and others (see review by VELDKAMP and JANNASCH, 1972) have confirmed this effect with several pairs of bacterial species. LEWIS (1967) reported the stable co-existence over a wide dilution range of two non-interacting thermophilic strains of *Lactobacillus* sp. and *Streptococcus* sp. that were limited by different (unknown) factors. By now there are many

reports of mutualistic or commensal co-existence of pairs of micro-organisms in continuous culture, in which one species derives a needed substance from the other (ORENSKI, 1966; BUNGAY and BUNGAY, 1968; VELDKAMP and JANNASCH, 1972). These interactions can be complicated, involving inhibitory effects as well.

BRUNNER and co-authors (1969) observed co-existence of *Escherichia coli* and *Serratia marcescens* over a range of dilution rates when both were limited by glucose. They interpreted this as being due to a range of variation in growth rates in the two populations, so that at various dilution rates different subpopulations with growth rates precisely right for co-existence persisted. A mathematical model along these lines was constructed. However, BRUNNER and co-authors did not look for syner-

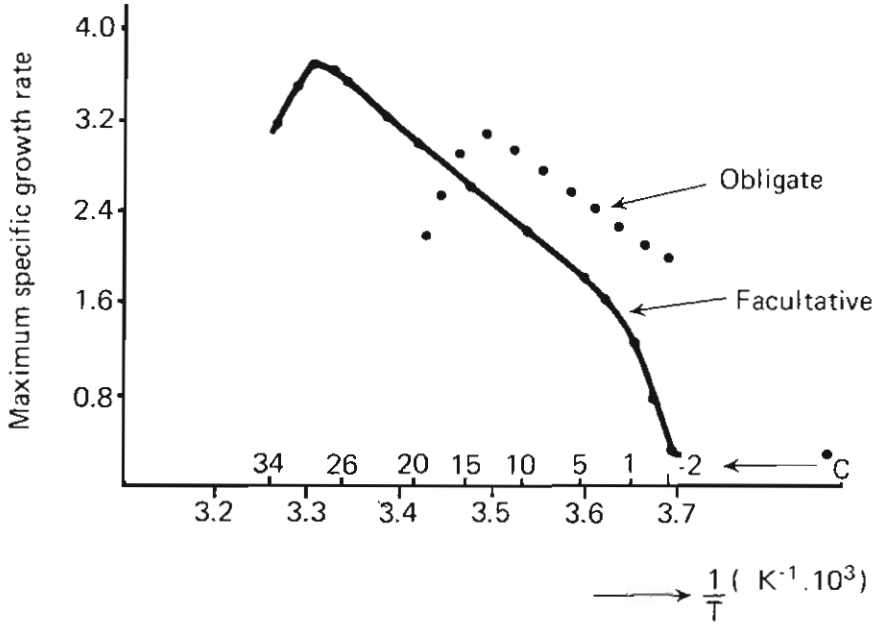

Fig. 6-5: Arrhenius plot of maximum specific growth rates of obligately and facultatively psychrophilic *Pseudomonas* species at various temperatures. (After HARDER and VELDKAMP, 1971; modified; reproduced by permission of 'Antonie van Leeuwenhoek'.)

gistic effects. It will be interesting to see if such effects can be found in other combinations. Certainly, variations of growth rates (genetic and other) occur in natural populations.

Since enzymatic reactions, and hence growth rates, vary with chemical and physical conditions, they may effect the course of competition. HARDER and VELDKAMP (1971) studied mixed continuous cultures of two species of marine psychrophilic bacteria, in which the outcome of competition depended on temperature (Figs 6–5, 6–6).

Systems Containing Algae, Protozoa and Metazoa

Few studies appear to have been conducted on continuous cultures of gnotobiotic

algal–bacterial or algal–algal mixtures, though there are many batch studies. BURKE (1962) and BURKE and co-authors (1961) studied competition between the marine dinoflagellates *Peridinium trochoideum* and *Gyrodinium* sp. in continuous and batch cultures, noting differential effects of light intensity, nutrient levels, and temperature on the course of competition. Growth ranges were different in pure and mixed culture, indicating some interaction beyond that of simple competition for nutrients.

TSUCHIYA and co-authors (1972) studied interactions of the cellular slime mould

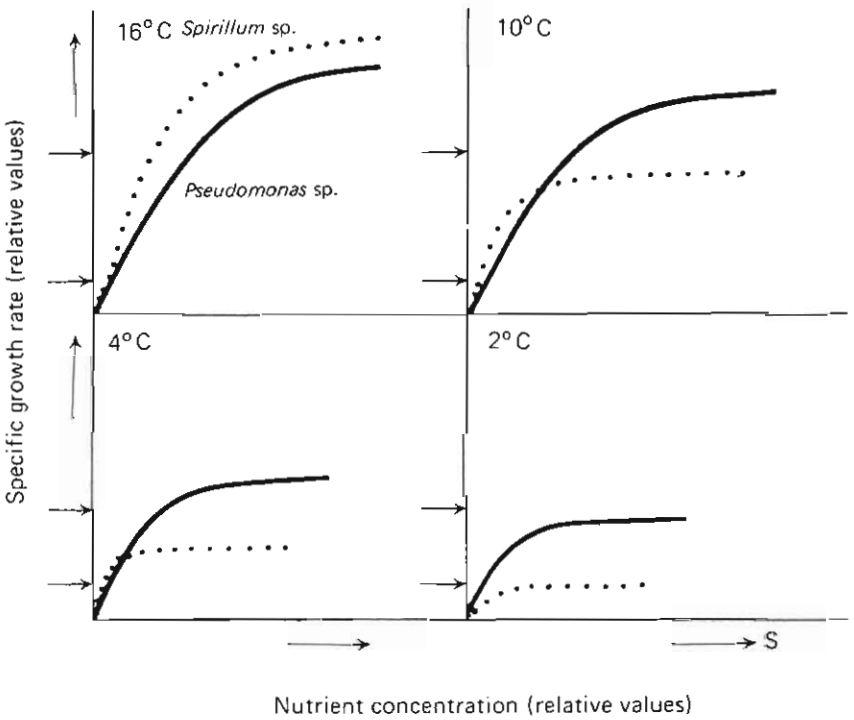

Fig. 6-6: Specific growth rate of an obligately psychrophilic bacterium *Pseudomonas* sp. and a facultatively psychrophilic *Spirillum* sp. as a function of nutrient concentration and temperature. Schematic. (After HARDER and VELDKAMP, 1971; modified; reproduced by permission of 'Antonie van Leeuwenhoek'.)

amoeba *Dictyostelium discoideum* and its prey *Escherichia coli* in continuous and batch cultures. In continuous culture, a lag period for the amoeba was followed by oscillations of the type one might expect from theory. After 2 to 4 weeks the oscillations became irregular and damped out as the amoebae aggregated on the wall of the culture container just above the medium surface (Figs 6–7, 6–8, 6–9). Efforts to resuscitate them failed. The oscillatory effect is of extreme interest, and it is to be hoped that ecologists and nutritionists will pay more attention to such research. Further studies are reported by this laboratory (JOST and co-authors, 1973) with a continuous gnotobiotic system containing *Tetrahymena pyriformis* (predator), *Escherichia coli* and *Azotobacter vinelandii* (prey), and glucose (limiting substrate) in a minimal medium. Because of non-overlapping size ranges of the three species,

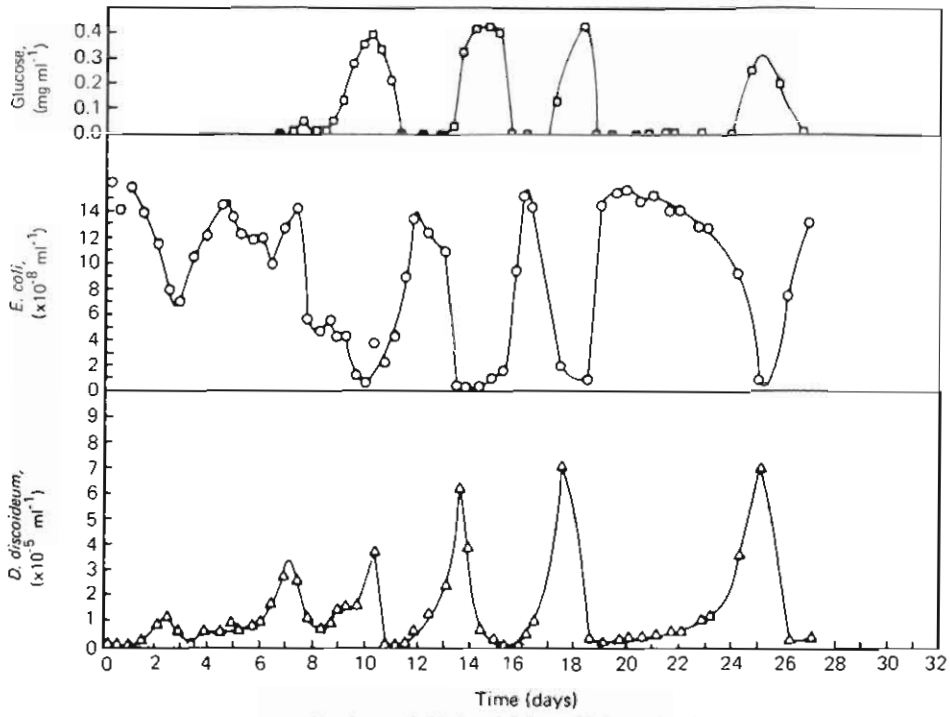

Fig. 6-7: Quantitative oscillation of *Escherichia coli* (prey), *Dictyostelium discoideum* (predator), and glucose (limiting nutrient) in continuous culture. Holding time: 8 hrs; 25° C. (After TSUCHIYA and co-authors, 1972; modified; reproduced by permission of the American Society for Microbiology.)

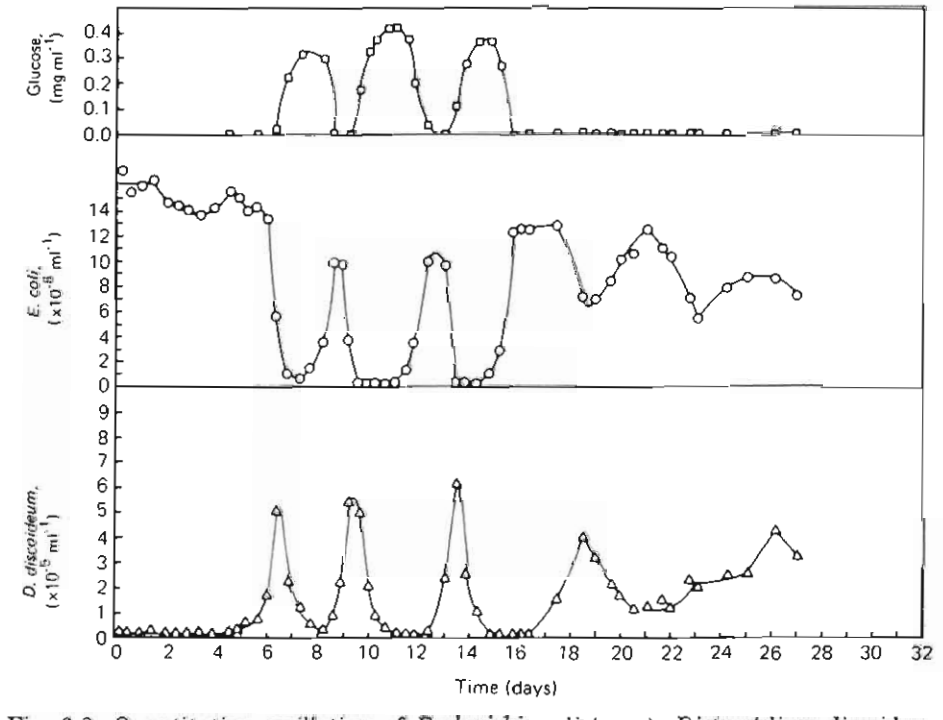

Fig. 6-8: Quantitative oscillation of *Escherichia coli* (prey), *Dictyostelium discoideum* (predator), and glucose (limiting nutrient) in continuous culture. Holding time: 16 hrs; 25° C. (After TSUCHIYA and co-authors, 1972; modified; reproduced by permission of the American Society for Microbiology.)

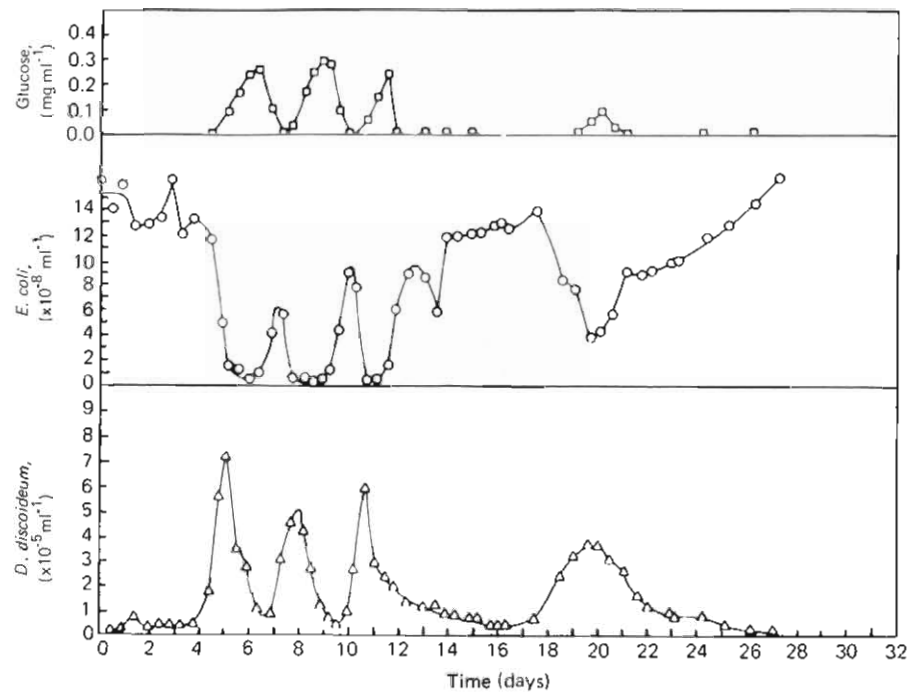

Fig. 6-9: Quantitative oscillation of *Escheria coli* (prey), *Dictyostelium discoideum* (predator), and glucose (limiting nutrient) in continuous culture. Holding time: 32 hrs; 25° C. (After TSUCHIYA and co-authors, 1972; modified; reproduced by permission of the American Society for Microbiology.)

populations were easily monitored with Coulter counters. Without the predator, competition always led to elimination of one bacterial species, but the presence of *Tetrahymena* permitted co-existence. Sustained or damped oscillations of species and substrate occurred in cultures with the ciliate and one or both bacteria. This depended on the holding time (turnover rate) and the incoming substrate concentration. The authors propose a modification of the simple Monod model to explain their data, based on the assumption of two important intermediate states in the assimilation of nutrient by *E. coli* and *Tetrahymena*.

DROOP has developed a two-stage monoxenic culture system of the marine rotifer *Brachionus plicatilis* feeding on a single algal species. A preliminary account of this system and a brief theoretical discussion of aims is given in CONOVER (1970; Chapter 2, p. 214). Further experimental data have been obtained with the system and will be reported at a future date (DROOP, personal communication). It will be interesting to see whether original theoretical expectations, based on the Monod model, are borne out.

Dr. AIBA and his colleagues (SUDO and co-authors, 1972) have also studied oscillations in monoxenic continuous cultures of the freshwater ciliate *Colpidium campylum* and the food bacterium *Alkaligenes faecalis*. The data were compared to the models of Lotka–Volterra (SLOBODKIN, 1962), BUNGAY and BUNGAY (1968) and CANALE (1969). None of these models was adequate in the original form. It was

found that reasonable simulation required incorporation of the concept of bacterial-floc formation. The formation of such flocs is an important and incompletely understood phenomenon in sewage treatments, possibly related to production of adhesive substances by protozoans (HARRIS and MITCHELL, in press).

TAUB (in press) reports observations with a two-stage (plus nutrient and yield reservoirs) gnotobiotic freshwater microbial system (apparatus described by TAUB and DOLLAR, 1968). This contained three trophic levels, represented by *Tetrahymena vorax* (herbivore), *Chlamydomonas reinhardii* (producer), *Pseudomonas*

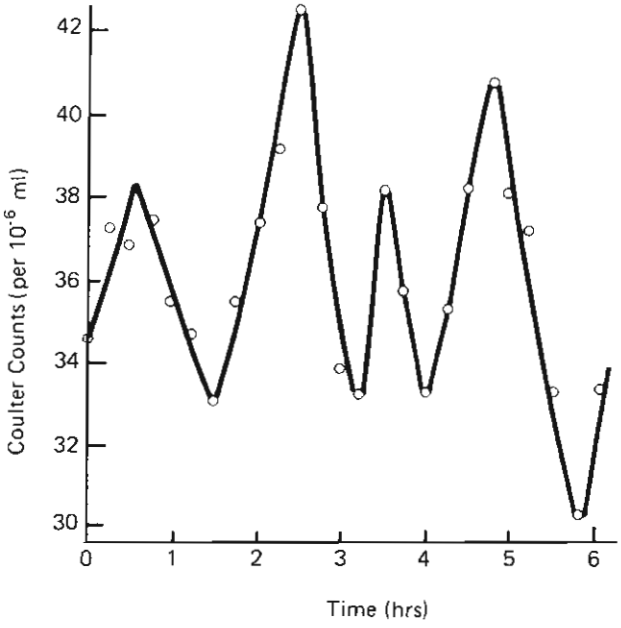

Fig. 6-10: Total cell counts in a continuous mixed culture of *Proteus vulgaris* and *Bacillus polymyxa*. Dilution rate: 0·25 hr^{-1}; 37° C. Mutualism in a minimal defined medium, where each organism requires a substance release by the other. Accumulation of inhibitor substance causes population growth to cease until pumping of medium is continued and dilutes the inhibitor. (After YEOH and co-authors, 1968; reproduced by permission of the National Research Council of Canada.)

fluorescens and *Escherichia coli* (decomposers). The experiment lasted 70 days. A mineral medium with bubbled CO_2 was used. Light (fluorescent and incandescent) was maintained at 2000 ft. c for 43 days and 1000 ft. c thereafter. Cultures were rocked (20 cycles min^{-1}), and glass beads in the flask were used to dislodge cells adhering to the sides. The limiting nutrient of the whole system was thought to be nitrate, though this could not be proved, and light limitation may also have occurred. Two replicate systems were monitored. Samples could be drawn aseptically from each chamber. Growth and production rates of algal populations were estimated using HERBERT's (1964) multi-stage model. The main results obtained

were qualitative. At high dilution rates (1·0 and 1·4 day^{-1}) *T. vorax* and *E. coli* washed out, whereas at lower rates (0·57–0·73 day^{-1}) all forms persisted. The downstream and yield communities were progressively denser and more stable with changing conditions. One of the most striking results was the great similarity of the sequence of events in the two replicate systems. This work appears to be the most ambitious gnotobiotic continuous culture reported to date, and it is hoped that the use of these techniques will become more widespread now that they have been employed successfully.

Oscillations in Multispecies Cultures

Oscillations in mixed continuous cultures have sometimes been seen in cases of mutual dependence (e.g. SHINDALA and co-authors, 1965), sometimes accompanied by inhibition (YEOH and co-authors, 1967; see HOBSON, 1969, for more examples,

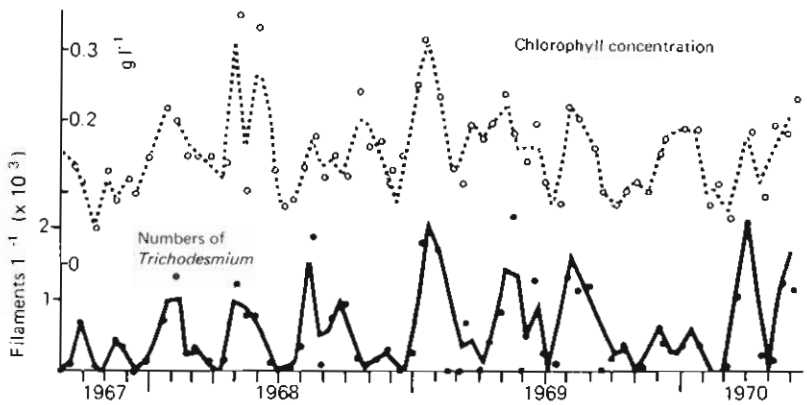

Fig. 6-11: *Trichodesmium* sp. Chlorophyll concentration and numbers at 5 m water depth in the Western Atlantic Ocean. Lines are interpolated 15-day interval values based on data points. (After STEVEN and GLOMBITZA, 1972; reproduced by permission of *Nature*.)

such as cyclic variation of pH) as shown in Fig. 6–10, or in predator–prey interactions (TSUCHIYA and co-authors, 1972). SAUNDERS and STORCH (1971) propose a scheme whereby chemical interactions between phytoplankton (leaking substrate) and bacteria (providing CO_2 and perhaps vitamins; NAKAMURA, 1963) might be coupled in an oscillatory manner. As previously noted, predator–prey theory (BUNGAY, 1968; CANALE, 1969; MAY, 1972b; etc.) tends to predict oscillatory binary relations. These observations lend interest to a 3-year field study by STEVEN and GLOMBITZA (1972) of oscillations in a natural tropical oceanic bloom of *Trichodesmium* sp. (Figs 6-11, 6-12). This harmonizes with JANNASCH's (1969) suggestion that oceanic plankton has the properties of an open system and is more like a continuous than a batch culture. More such detailed quantitative studies over long time-periods are needed to see how general the phenomenon is. We also need, of course, more mixed chemostat culture studies.

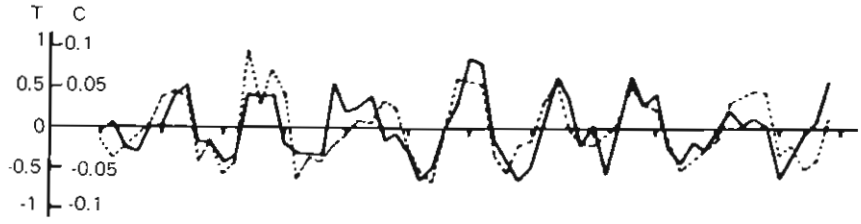

Fig. 6-12: In phase variation of chlorophyll and *Trichodesmium* sp. after smoothing by digital filter. Horizontal axis is marked at 75-day intervals. T: thousands of filaments l^{-1}; C: $\mu g\ l^{-1}$ of chlorophyll. (After STEVEN and GLOMBITZA, 1972; reproduced by permission of *Nature*.)

(b) Serially Renewed Cultures

The technically more difficult continuous culture is in some ways more revealing than batch cultures when it comes to analyzing competitive interactions. Physiological states of organisms and nutrient levels can be better controlled and competition at that level observed. However, in natural populations there is a temporal variation of physiological state, nutrient level, etc., and the full understanding of natural competition must include the effects of temporal inhomogeneities in such factors.

LEVIN (1972) studied competition between two similar strains of *Escherichia coli* in a glucose minimal medium in continuous cultures and serially renewed batch cultures. Continuous cultures led to extinction of one or another strain (LEVIN, personal communication) but the serially renewed cultures led to strikingly stable equilibria (Fig. 6-13) at ratios of population levels depending on renewal period and nutrient level. Because of the relative ease and efficiency of bacteriological plate-counting methods, it was possible to detect such equilibria at very low (or high) population ratios, which would have gone unnoticed in work with higher organisms. LEVIN suggests that competition goes in different directions at different times in the growth cycle, and so these tendencies cancel each other in a way that produces a stable equilibrium.

A mathematical model of these intuitive notions is presented by STEWART and LEVIN (1973). They distinguish equable (continuous culture) and seasonal (serially renewed culture) modes of competition, and study the various possibilities of stable co-existence.

JENSEN (1969) and JENSEN and BALL (1970) observed the fluctuations of a mixed protozoan–bacterial system to which nutrients were added at various time intervals. Longer intervals produced more or less cyclic fluctuations; shorter intervals caused lesser, non-cyclic fluctuations.

Careful studies of serially renewed cultures would clearly be useful in understanding natural communities, and would probably help in developing pertinent culture techniques. Organisms that will not co-exist in continuous or batch cultures might do so under serially renewed regimes.

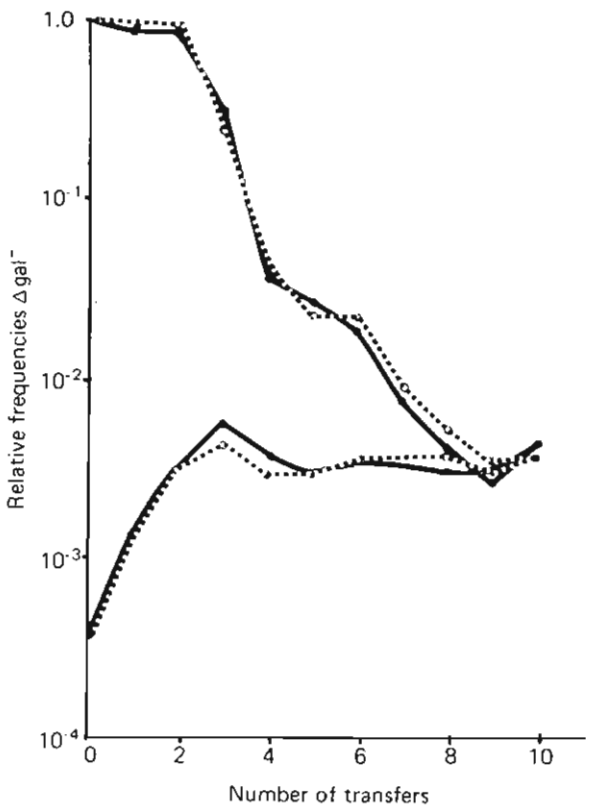

Fig. 6-13: *Escherichia coli*. Stable equilibrium between two genetic strains (δ gal- and ara-) in a glucose minimal medium. Four experiments are shown: two with initial relative frequencies of δ gal- of 0·999739 and two with initial relative frequencies of δ gal- of 0·000338. After 10 successive transfers all have reached a relative δ gal-frequency of about 0·003. (After LEVIN, 1972; reproduced by permission of American Association for the Advancement of Science.)

(c) Batch Cultures

Almost all studies of chemical interaction were done of necessity in gnotobiotic cultures, usually batch or stationary cultures. Some of the complexities of such interactions are seen in the study by SWIFT and MCLAUGHLIN (1970) on growth and interaction of the marine phytoplankters *Melosira juergensii*, *Heterocapsa kollmeriana*, *Peridinium trochoideum*, *Gyrodinium astraefissum*, and *Monochrysis lutheri*. When the diatom *M. juergensii* and the dinoflagellate *G. astraefissum* are competing with a large initial dinoflagellate inoculum, the diatoms are stimulated and grow rapidly, bleaching quickly, whereas the somewhat inhibited dinoflagellate continues to survive long after. If the initial diatom population is high the dinoflagellates are killed off rapidly. A number of such variable interactions were seen.

In the microcosmic tradition, GORDEN and co-authors (1969) constructed a heterotrophic—apparently almost gnotobiotic—system consisting of a dominant *Chlorella* sp. strain, *Scenedesmus* sp, several ciliates and invertebrates, and 11 bacterial isolates that would appear on standard media, all orginating from a waste stabilization pond. In this system, reminiscent of earlier studies by NAKAMURA (1963) and LANGE (1967), bacteria provided CO_2, thiamine and probably other subtances (in NAKAMURA's study, nitrogen sources; in LANGE's, chelator substances); in turn, they consumed algal and other products and wastes. The system was maintained in a defined medium for several years. Succession was observed for 75 days in 130 flasks, representing 4 different inoculation times. Community metabolism (pH measurements), dissolved and particulate organic matter, dissolved carbon, thiamine, and glyoxylate uptake were monitored, and bacterial diversity (assuming logarithmic distribution) was calculated for the 75 days. A bimodal pattern emerged in the composite data with early and late peaks in most variables, while mid-succession showed less diversity and activity. The authors compared the successional trends observed to those called typical of natural ecosystems, concluding that their system is a useful model of the latter. Strictly speaking, there were probably some undetected micro-organisms growing in this system but much effort went into isolations, and sterile techniques were used in manipulations, so possibly most of the dominant species were known.

The studies by SWIFT, and MCLAUGHLIN (1970) and by GORDEN and co-authors (1969), though dissimilar in perspective, illustrate a problem of mixed culture work: it can be laborious. There are many variables one would like to monitor, and variations in species combinations, and perturbations or variations in growing conditions that one would like to pursue. As a consolation, it can be said that since there are so few data as yet in this field, almost any result from a well-planned experiment will probably be of interest, and may be surprising. Thus MOSSER and co-authors (1972) found that polychlorinated biphenyls and DDT had a strong effect on the ratio of species in mixed cultures of the phytoplankters *Thalassiosira pseudonana* and *Dunaliella tertiolecta* at levels where there was no discernible effect on pure cultures.

Another peculiarity of mixed culture work is that one is often interested in long-range effects of competition and succession, and experiments may last a long time. Thus TRIBE and WILLIAMS (1967) studied competition of 3 soil bacteria in model systems for periods of up to a year, in attempts to see interactions due to the characteristically low level of activity of the autochthonous species (CONN, 1948). Inhibition of *Azotobacter chroococcum* by two slow-growing diptheroids was shown, which may explain the rarity of the former in many soils.

Predation Effects

Gnotobiotic cultures, when possible, permit a clearer observation of chemical (nutritional) as well as mathematical interaction of prey and predator. DROOP (1966) studied the ability of 30 strains of unicellular marine algae to support growth of the marine amoeba *Heteramoeba clara* and of 14 to support the holotrophic dinoflagellate *Oxyrrhis marina* and the rotifer *Philodina roseola* under monoxenic conditions. Striking differences appeared along phylogenetic lines: *O. marina* grew on all algae tested, whereas *P. roseola* survived only on the Chlorophyceae, and *H.*

clara grew on most of the Volvocales and diatoms, but on none of the Chrysophyceae or Chlorococcales tested. Reasons for inability to support growth included size and toxic effects, and undetermined nutritional inadequacies. This study has implications for questions about the maintenance of diversity in nature and the existence of 'taxocenes', sympatric assemblages of species of one taxonomic category (HUTCHINSON, 1967; Volume V).

BUNT (1970) studied the feeding of a marine amoeba (*Vexillifera*-type) which grew on a small diatom of the genus *Cocconeis*, but not on other green or red algae, or diatoms tested. Food-conversion efficiency and other quantitative features were measured in batch cultures. Efficiencies (measured as carbon content) were high, averaging 47%.

Soil microbiologists have studied gnotobiotic systems of amoebae and other protozoans with their bacterial prey since the days of BEIJERINCK (1897). The many gnotobiotic experiments of SINGH (1946) and ANSCOMBE and SINGH (1948) established the importance of these selective micropredators in controlling the species composition and level of soil bacterial populations.

HORN (1971) used these methods in an elegant study of the competitive feeding strategies that permit co-existence of four species of cellular slime mould. The hierarchy of competitive success, when feeding on 5 different food bacteria, was such as to partly explain the co-existence when all 4 are present. One slime-mould species, *Dictyostelium purpureum*, was out-competed by all others when any one bacterial food was used. Experiments with mixed food species suggested that the strength of *D. purpureum* might be that of a Jack-of-all-trades; it may be a 'fugitive species' in the sense of HUTCHINSON (1951), shifting about and surviving in changing regions.

Other extensive observations of protozoan-bacterial predation are those by COLEMAN (1964) and CURDS and COCKBURN (1968). CURDS and COCKBURN compared axenic and monoxenic cultures of *Tetrahymena pyriformis* quantitatively and obtained efficiencies of carbon incorporation comparable to those reported by BUNT (1970) and other protozoologists. As in SALT's (1967) study, individual feeding rate partly depended on concentration of feeders as well as that of prey. COLEMAN (1964) grew the rumen ciliate *Entodinium caudatum* dixenically on *Escherichia coli* in competition with 12 different bacterial species. Bacteria were used only in proportion to size and number so that this ciliate appears to be non-selective in feeding. Carbon efficiencies of 50% were reported.

Among metazoan invertebrates, gnotobiotic studies by PROVASOLI and his colleagues (PROVASOLI and co-authors, 1959, 1970; SHIRAISHI and PROVASOLI, 1959; D'AGOSTINO and PROVASOLI, 1968) of the filter-feeding crustaceans *Artemia salina* and *Tigriopus japonicus* have shown the delicacy of nutritional requirements of such organisms, leading to the necessity of a balanced diet of more than one food species (in the absence of added artificial supplements). This no doubt explains reports of failure of a number of rather crude attempts at monoxenic culture of such organisms. MURPHY (1970), on the other hand, has developed methods of monoxenic culture for a large number of freshwater daphnids, using supplements of trace metal mixes and growth factors based on the work of HUTNER and PROVASOLI with protists.

LEE and co-authors (1966, 1970) have isolated axenic or gnotobiotic cultures of many organisms from a salt marsh at Southampton, Long Island, New York (USA).

The cultures include macrophytes *Ulva lactuca*, *Enteromorpha intestinalis*, 6 nematode species, 6 foraminiferan species and about 70 species of unicellular algae, as well as several ciliate species. Many other species were maintained agnotobiotically. The food-web pathways, studied by tracer techniques with labelled food organisms, revealed much specificity in feeding of foraminiferans and other organisms. From competition experiments between phagotrophic protozoans, using various food bacteria, a picture of competitive hierarchies is emerging (LEE, personal communication; MULLER and LEE, 1972).

These investigations appear to be the most extensive and successful development of gnotobiotic culture techniques to date. One goal is to construct a gnotobiotic community in the laboratory that will have essential attributes of the natural benthic assemblage associated with species of *Ulva*, *Enteromorpha*, and other seaweeds in a salt marsh. Techniques of isolation and culture are detailed in the two papers cited. One point that emerges from this work is the importance of techniques developed by nutritional biologists working with axenic cultures in the last three decades; these should be studied by would-be microcosmologists (Chapter 5.11).

NIXON (1969a, b) attempted unsuccessfully to maintain *Artemia salina* and strains of *Dunaliella viridis* and aerial contaminant bacteria (undescribed: the system was not really gnotobiotic) from a tropical salt pan. TAUB (1969a, b) worked with several freshwater batch systems having various combinations of the rotifer *Philodina* sp., the protozoans *Tetrahymena* sp., *Chlamydomonas* sp., and several bacterial species in a defined mineral medium with 10-hr light cycle. Nitrate and possibly phosphate were thought to limit the system as a whole. No oscillations were seen, but a large bacterial population was maintained on the products of the autotrophs. The phagotrophs tended to disappear and the algae remained largely unconsumed, so there was probably little recycling.

(7) Conclusions: Toward a Calculus of Species

Some general observations are now possible. The first concerns the world of models. As suggested earlier, we must develop systems in which species as well as environmental factors are treated as primary variables: that is, we need to study the effects of adding, subtracting or exchanging species in our formal models—a calculus of species.

More effort is required for developing techniques of measuring population parameters. Measurements of basic quantities like population growth rates of individual species (especially micro-organisms) of a mixed culture may present great difficulty. As the problems slowly gain in popularity, more experimental and mathematical tools appear. Examples are the radio-autographic techniques of BROCK (1971) and others, and a recent computer algorithm of ULANOWICZ (1972b) for fitting mixed population data to second-order kinetic models. MOBLEY (1973) has extended and applied the approach of ULANOWICZ to develop a general method of fitting models to ecological data (the inverse problem of modelling) and of choosing between several such models.

In regard to the experimental approach to our calculus of species, there is a pressing need for more axenic or gnotobiotic cultures, and for a better tradition of

supplying them on request. We need to cultivate metazoans including vertebrates, if we are to resynthesize reasonable models of natural systems.

The second general conclusion concerns the real world. Many biologists have discovered (the hard way—e.g. MULLER and LEE, 1969) that it is quite difficult to grow most organisms in the absence of bacteria. This must partly be due to the great biochemical versatility of bacteria (VALLENTYNE, 1963), which in the hands of the master microbiologists who 'take pleasure in contemplating the odd tricks performed by microbes' (HUTNER, 1958, p. 864) has generated the wide-ranging field of biochemical analysis by microbial assay. Protozoa, Metaphyta, and Metazoa—mere evolutionary offshoots of the bacteria—have lost many of these odd tricks and remain ecologically dependent on the ancestral group (HUTNER, 1961; HUTNER and co-authors, 1972).

There are probably also mathematical ways in which bacteria serve to cement a system, filling up chinks between the dynamics of larger, longer-lived creatures. This involves the notion of scale in time and space. HUTCHINSON (1951, 1971) has speculated on the critical problem, little studied by others, of understanding the mathematical connexions between co-existing species with disparate sizes and life spans. Here belong too the ideas of ODUM (1956; ODUM and co-authors, 1963) on the effects of coupling various scales of biochemical cycling rates on the overall flow of matter and energy through a community (Volume IV, MARGALEF, in press). These questions will become experimentally available as the techniques of gnotobiotic culture develop. Theoretically, problems of this sort occur in the emerging field of automata theory, which deals with programmes or 'automata' (POST, 1936; TURING, 1937; DAVIS, 1958) that can form weakly coupled modular elements in higher order hierarchical automata (WHYTE and co-authors, 1969). Some of the pervasive conundrums of mathematical biology have to do with such problems (WEAVER, 1948), and perhaps experimental microcosms will provide a concrete source for this presently rather abstract subject.

The last general comment concerns the interaction of the real world and the world of models. GOLD (1970, 1971) has developed relatively well-controlled (agnotobiotic) techniques of continuous and batch culture of marine ciliates and other holotrophs (Chapter 5.1). A barrier to complete control, however, lies in the tendency of these organisms to 'swarm', and migrate up and down diurnally (GOLD, personal communication). LOMBARD and CAPON (1971) studied this phenomenon in tidepool and aquarium populations of the dinoflagellate *Peridinium gregarium*, which form aggregations in sticky plaques in sheltered benthic areas, from which they emerge daily in a swarm, returning in due course to the plaque. In one aquarium experiment they adopted a submerged bottle as home, returning faithfully to the rocks inside it every night. Leaving aside the navigational problems raised (Volume II: Chapters 7, 8, 9), what does this do to the distribution models of plankton organisms! Another example of this kind of problem, already mentioned, concerns the effects of predatory ciliates on sewage-waste treatment. PIRT and BAZIN (1972) note that the usual mathematical models predict an adverse effect due to the protozoans keeping the bacteria at a low level, slowing the degradation process. In fact, however, as noted, Protozoa in some instances appear to have an accelerating effect on treatment. This is not completely understood, but is partly related to a 'flocculating' effect of the protozoans (SUDO and co-authors, 1972) and also possibly to stimulation of bac-

terial growth by products of Protozoa (STRASKRABOVA and LEGNER, 1966, 1969; LEGNER, 1972).

Organisms have a regrettable tendency to behave, and sometimes to socialize. They are non-random when randomness is needed for stochastic models, and yet sufficiently unpredictable that deterministic models are unsatisfactory. Furthermore, when one has established their properties, more or less, they will change them by breeding or evolving (hence the adage of population geneticists: 'mathematics and sex don't mix').

These considerations suggest some inadequacies in our point of view which, perhaps, could be made clearer and smaller by asking the right questions of well-defined artificial microcosms.

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7. CHEMICAL CONTAMINATION OF CULTURE MEDIA: ASSESSMENT, AVOIDANCE AND CONTROL

M. BERNHARD

(1) Introduction

Research efforts made in the cultivation of marine organisms and in ecological experimentation have often been jeopardized because not enough attention has been paid to possible contamination. In cultivation of marine organisms many substances and materials come into contact with the culture medium and hence with the aquatic organisms, but in most cases it is tacitly assumed that these substances have no effect.

Contamination hazards arise from the moment the sea water is collected and remain a potential danger throughout the experiment. The ship from which the sea water is collected, the sampling device, the storage vessel all contaminate the sea water. In the laboratory, the culture container, the instruments used during experiment, the chemicals employed and the impurities contained in them and even the organisms used in the experiment will further contaminate the medium.

Although gross contamination hazards of the culture medium can be eliminated relatively easily, subtle contamination will always occur. Even if the culture medium consists only of natural sea water it will come in contact with the container material; since neither the container material nor the sea water are inert, reactions between the two will occur, resulting in medium contamination, however small it may be.

Since contamination is unavoidable, its effect must be minimized and kept under control so that it will not significantly alter the experimental results obtained. The question is, therefore, how can we minimize and control contamination?

As a first approximation, contamination of the culture medium may be defined as addition of unwanted substances—an addition which, in the majority of cases, is unknown to the experimenter. A more specific definition would include all alterations which the culture medium has undergone from the moment the sea water was collected all through the actual experimental procedure.

Discussing the contamination hazard of chemical microanalysis, THIERS (1957) distinguishes three types of contamination:

(i) Positive contamination, i.e. addition of extraneous matter and substances;

(ii) negative contamination, i.e. expected and unexpected losses of materials and substances;

(iii) pseudocontamination, i.e. expected and unexpected changes which are intrinsic to the system and are caused neither by addition nor by loss.

The examples given above are all concerned with positive contamination. The more unusual term 'negative contamination' refers to losses such as adsorption of substances to container walls, losses due to evaporation of volatile substances, etc. Examples of pseudocontamination are changes in the culture medium due to the continuation of chemical reactions in the sea water, the interaction of marine organisms (for example the natural population of marine bacteria) with the sea-water medium and so on. In order to limit the scope of this chapter, the problems regarding pseudocontamination are not discussed.

It is impossible, of course, to foresee all possible contamination hazards an experimentor can encounter. It is left, therefore, to his ingenuity to incorporate into his experimental design enough 'blanks' and controls so that he will be able to identify possible contamination effects. The study of contamination effects has, with few exceptions, dealt with gross and easily recognizable effects such as death, growth inhibition, morphological deformations, etc. While these effects can block the advancement of the investigation of ecologically important marine organisms, the more subtle effects which alter the physiological and behavioural responses of organisms to experimental conditions seem to be of greater importance because their detection, identification and control are far more difficult, and thus lead to a wrong interpretation of experimental results. In fact, gross contamination effects are easily noticeable even by a relatively inexperienced worker. To detect subtle effects of contamination, for example the change of a physico-chemical state of a trace element and hence the alteration of the availability of this element, will tax even the abilities of a very experienced scientist.

The contamination may not even have an effect on the organism itself but interfere with the determination of parameters the experimentor must measure. It is often overlooked that contamination can also cause enhancement of certain parameters studied. Although this may be considered positive for someone who is trying to cultivate an organism for the first time, it seems obvious that these beneficial effects should cause the same concern to the experimental ecologist as the toxic and inhibitory effects of contamination.

(2) Sources of Contamination

Of the materials used in cultivation, glass is the most widely used, especially when smaller organisms are studied. Plastics and rubbers are employed for flexible connections. Metals, alloys and porcelain (stoneware) have found applications especially in pumps serving sea-water circulation systems.

In recent years glass, metals and natural rubbers have been increasingly replaced by plastics and artificial rubbers. In the preparation of culture media and in experimentation with marine organisms, various materials—such as ion-exchange resins, filters and filter apparatus, etc.—come in contact with the culture medium. Since all these materials are not inert they will react with the sea-water medium. Additional contamination hazards may be sought in 'pure' water, chemicals, radio-isotopes, etc., used at various steps in the experimentation. Finally, natural sea water—still one of the most widely employed basic solutions for the preparation of culture media—is often contaminated from various sources prior to and during collection, especially if certain precautions are neglected.

(a) Glass, Quartz and Porcelain

Owing to its high chemical resistance and its transparency glass has long been the favourite container material in chemistry and has found early application in the cultivation of marine organisms.

In well-designed experiments, high-resistance glasses such as the well known borosilicate glasses 'Pyrex', 'Kimax', 'Jena-20', etc., have been used. Probably the borosilicate glass, produced under the tradename 'Pyrex' and other synonyms, is the most widely used laboratory glass in cultivation experiments. In the USA this type of glass is produced under the tradenames 'Pyrex' (Corning code 7740) and 'Kimax' (K-33); in England, as 'Pyrex', 'Hysil' and 'Phoenix'; in Argentina, France and the USSR, as 'Pyrex'; in Germany, as 'Duran-50' (Corning code 8330) and 'Razotherm'; in Sweden, Czechoslovakia and Poland, as 'Nife', 'Simax' and 'Termisil', respectively (HUTCHINS and HARRINGTON, 1966; ADAMS, 1972). The

Table 7-1

Major components of commercial silicate glasses in %(w/w). Approximate composition (Compiled from the sources indicated)

Glasses	SiO_2	Al_2O_3	B_2O_3	Na_2O	K_2O	CaO	Others	Author
G-20 lab. ware Jena	75·7	5·1	6·9	6·2	1·2	1·3	3·6% BaO	(a)
N-51 lab. ware Owens Illinois	74·7	5·6	9·6	6·4	0·5	0·9	2·2% BaO	(a)
7740 'Pyrex' Corning	81	2	13	4	—	—	—	(a)
8330 Jena 50 Durax	81	2	13	3	1	—	—	(b)
Kimble	74·7	5·6	—	6·4	0·5	0·9	0·1% ZnO	(c)
Vitreous silica (Quartz)	100	—	—	—	—	—	—	(a)

[a] HUTCHINS and HARRINGTON (1966).
[b] PETERS (1969).
[c] WALKER and SMITHER (1918).

Schotten G-20 glass has been used much in the past but its production will probably be discontinued since the 'Pyrex-type' glasses are much easier to work. The 'Kimble' glass N-51, another borosilicate laboratory glass, is also used in the USA.

All 'Pyrex-type' glasses are similar in their major constituents but, of course, differ widely in trace elements, since the sources of the raw materials are not the same. The different code numbers distinguish the various 'Pyrex' glasses. The major constituents and some minor constituents are listed in Tables 7-1 and 7-2.

Since we are interested to know the extent to which ions dissolved from glass may contaminate culture media, we shall briefly discuss how a glass surface can be affected by solutions.

Two processes of attack can be distinguished (PETERS, 1969 and ADAMS, 1972):

(i) Leaching is characteristic of acid and water attack. It comprises a diffusion-controlled ion exchange of H^+ against the alkali ions of the glass. This ion-exchange process is selective and the major constituents of glass are exchanged according to the following preferential order: Li, Na, K, Mg, Ca. Since leaching of ions leaves a layer of less soluble SiO_2-network the glass surface is now enriched in SiO_2. The

Table 7-2

Trace-element concentration ($\mu g\ kg^{-1}$) in sea water and selected materials (Compiled

Materials	Zn	Cu	Cr	Co
Natural sea water	10	10	0·5	0·05
Pyrex glass	730	—	—	81
Vycor glass	—	—	—	—
Polyethylene I	28	6·6	76	0·07
II (hose)	55	—	254	140
III	25	15	19	0·31
Polyethylene high pressure	90	4–30	15–300	5
low pressure	300	1,700	180–1,500	10–370
White plastic tape	2,940,000	—	—	<1
Scotch magic mending tape	1,410	—	<10	6·1
Millipore filter	2,370	—	17,600	13
Teflon	9·3	22	<30	1·7
Plexiglas (≃ Perspex)	<10	<9·5	<10	<0·05
PVC	7,120	630	2	45
Surgical rubber tubing I	3,080,000	<6	—	<30
II	41,000,000	—	420,000	7,500
III	5,350,000	—	—	—
Neoprene rubber	18,200,000	—	—	2,300
Nylon (block)	—	—	—	1,430,000
Steel hydrowire	—	19,700	—	57,000
Quartz tubing, range of 4 samples	20–33	0·3–0·16	225–602	0·64–1·7
Synth. prod. Spectrosil	1·5	2·0	6·5	0·44
Suprasil	<1	0·04	2·5	12
Water, quartz distilled I	~1	—	~2	~0·04
II	9·5	—	<10	~0·20
double distilled	~1	—	~2	<0·02
triple distilled	~0·5	—	12	<0·02

	B	Na	Mg	Al
Polyethylene, high pressure	90	170–10,000	80–1,500	80–3,100
low pressure	—	1,700–2,200	—	5,400–7,000

layer of leached silica network becomes thicker with time, hindering the diffusion of H^+ and hence causing the leaching rate to decrease.

(ii) In alkaline solution the glass surface in contact with the solution is etched. Etching involves hydration and the total dissolution of the silica network. During the etching process all components (major components and traces) present in the surface layer are released into the attacking solution. If these components are soluble in the attacking solution, etching will leave a smooth surface; otherwise the insoluble products will become deposited on the surface leaving a rough glass surface.

The pH has a strong effect on glass corrosion, especially at values higher than pH 9 (Fig. 7-1). The rate of corrosion may be altered through the presence of various elements in the attacking solution. Na, Ca and Mg—present in relatively large amounts

from THIERS, 1957; SORANTIN and PATEK, 1965; ROBERTSON, 1968a)

Fe	Sb	Cs	Ag	Hf	Sc
10	0·3	0·3	0·2	—	0·04
28,000	2,900	<100	<0·001	597	106
—	1,090,000	—	—	—	—
10,400	0·18	<0·5	<0·1	<0·5	0·008
7·4	9,000	<100	<300	<100	11
10,600	0·83	<0·15	<0·1	<0·5	0·36
600–2,100	<5	—	20	—	—
—	<10	—	<10	<10	—
—	67,100	<50	<10	⩽10	84
5,130	33	<0·3	<10	—	0·48
330	39	1·5	<0·5	<0·5	0·79
35	0·4	<0·01	<0·3	—	<0·004
<140	<0·1	<0·06	<0·3	—	<0·002
270,000	2,690	<1	<5	—	4·5
—	<100	<100	1,240	—	<8
<100	360	580	<700	—	185
—	—	—	—	—	—
—	290	—	<1,000	—	3,090
—	—	—	<300	—	—
—	54,300	—	—	—	<50
—	38–1,940	<0.1–1,390	<0·1	<0·01–23	0·10–0·18
395	0·05	1·1	0·05	<0·005	0·03
—	<0·01	<0·1	<0·01	<0·005	0·39
~1	~0·06	<0·1	<0·02	<0·005	0·0022
<0·2	0·10	0·12	<0·02	<0·005	0·0025
<0·2	<0·01	<0·1	<0·02	<0·001	<0·0001
~1	<0·02	<0·1	<0·02	<0·001	~0·0002

Si	Ca	Mn	Sr	Ba	Pb
2,000	200–20,000	<10	800	50	200
—	—	—	—	—	—

in sea water (Table 7-3)—increase the rate of attack; and so do Ni and Co, although the concentrations of these elements are very small in natural sea water. On the other hand, ions of elements such as Zn, Fe, Pb, Al and, especially, Cu even in traces decrease the rate of glass corrosion (ADAMS, 1972). The corrosion of glass caused by a saline solution such as sea water with a pH around 8 is a very slow process, involving both leaching of interstitial ions and degradation of the silica network. Although not much is known about the release of materials from glass surfaces into ambient sea water, one may anticipate that the elements present in the glass will dissolve into the sea water in part by leaching and in part by the dissolution of the SiO_2 network.

CAMPBELL (personal communication) estimated that from a borosilicate Pyrex glass (Corning code 7740) exposed to sea water at room temperature, a 0·25 to

2·5 μm thick layer of glass dissolves into sea water within a year. Since the layer of leached silica-network will increase in thickness with time, it will cause the leaching rate to decrease and thus the layer which dissolves in 1 day will be about 1/10 to 1/100 of the layer dissolved in 1 year.

In order to estimate the possible contamination to a sea-water culture medium let us assume (i) that 10 ml of sea water are present in a 12-mm-diameter test tube (which may present one of worst cases of surface to volume ratio in the cultivation of marine organisms), and (ii) that the glass surface is exposed for 1 month to sea water; from the discussion above, a layer about 1 mμm thick would then be involved in leaching and etching. The amount of elements (arranged in decreasing concentration in glass) present in the 1 mμm thick layer of a 'Pyrex' glass is compared with

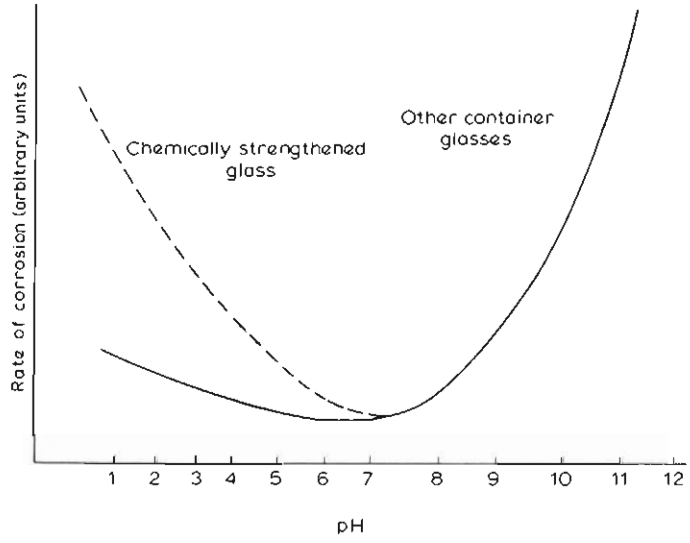


Fig. 7-1 : Rate of corrosion on two types of glass as a function of pH. (After ADAMS, 1972; modified; reproduced by permission of Marcel Dekker Inc.)

the quantities of the same elements present in the 10 ml of sea water in Table 7-3. As can be seen from that table, glass corrosion does not contribute significantly to the concentration of the major constituents (Na, K, Ca, Mg, Cl) of the sea water. However, some of the major constituents of the glass (Si, Al and Zr) could significantly increase the concentration of these elements in the sea water. In fact 30 μg Si l^{-1} were found in sea water stored in a borosilicate glass for 1 week at pH 7·5, by comparison only 0·008 μg Si l^{-1} in sea water stored for 1 week at pH 2·5 (THIERS, 1957). Especially the amount of zirconium (which has an extremely low concentration in sea water) could be easily raised by contamination from glass containers. Table 7-3 further indicates that several other elements are present in relatively high concentrations in glass, but in very low concentrations in sea water. Hence these are potential hazards for contamination and may influence the outcome of experiments designed to study these elements.

Table 7-3

Maximum amounts of elements present in borosilicate glass (Pyrex) and quartz glass, and the possible amounts which may be released from the glass into the sea water contained in a test tube (Compiled from GOLDBERG, 1965; ADAMS, 1972; Table 7-1)

Element	Borosilicate Glass (g element 100 g^{-1} glass)	(μg element cm^{-2} contained in 1-mμm thick glass surface layer)	Contamination (μg element released ml^{-1} sea water)	Sea water ($\mu g\ ml^{-1}$)	Quartz Glass (g element 100 g^{-1})
Si	37	0·15	0·5	3·0	46·6
B	4·2	0·016	0·057	4·6	
Na	1·5	0·0060	0·02	10,500	0·001
Al	1·1	0·0044	0·015	0·01	0·001
K	1·0	0·004	0·014	380	0·001
Cl	1·0	0·004	0·014	19,000	0·001
Zr	1·0	0·004	0·014	—	0·001
Li				0·17	0·001
Mg	0·1	0·0004	0·0014	1,350	0·01
Ca				400	0·01
Sr				8·0	
Hf				—	0·001
Fe				0·01	0·01
As				0·003	0·001
F				1·3	
Ti	0·01	0·00004	0·00014	0·001	0·001
V				0·002	
Mn				0·002	0·001
Ni				0·002	0·01
Zn				0·01	0·001
Ga				0·00003	
Sb				0·0005	
S				885	
Ba	0·001	0·000004	0·000014	0·03	
Y				0·0003	
Cr				0·00005	
Cu				0·003	0·001
Pb				0·00003	
P				0·07	
Bi				0·00002	
Se				0·0004	
Te				—	
Br				65	
Ag				0·00004	0·001

For glass and quartz, concentrations lower than 0·0001 are not given.

If sea water is heated in an autoclave to 120° C, a corrosion increase of about 1½ times per 10 C° can be assumed (ADAMS, 1972; CAMPBELL, personal communication). This means that the release rate of elements from glass given in Table 7-3 would increase by a factor of 10 upon a temperature increase from 20° to 120° C and exposure time would decrease from 1 year to about 4 hrs. Of course, all these estimations refer to new glass. In leached glass, the concentrations of elements in the surface layer, other than the Si, are smaller.

Considerations similar to those for borosilicate glass pertain to quartz glass (vetrius silica) except that quartz glass is fused from pure silica and, therefore, no other materials (fluxes, etc., such as the oxides of aluminium, boron, sodium, potassium, calcium, magnesium) are added (Table 7-1). Consequently, quartz glass contains these elements only as impurities (Tables 7-2 and 7-3).

As in the leaching and etching processes described for borosilicate glass, corrosion of the quartz surface will release the traces of elements present. However, since the surface layer of quartz is practically a SiO_2 network, corrosion rate will be about 10 times slower than in glass (CAMPBELL, personal communication), and since many of the elements purposely added to make the various glass types are present in much lower concentrations in quartz glass, the dissolved quantities of Na, K, B, Al, Ca, etc., will be much smaller.

ROBERTSON (1968a) has analyzed, with neutron activation, different types of quartz tubing produced synthetically and nonsynthetically (Table 7-2). With very few exceptions, synthetically produced quartz types contain fewer traces of impurities than the borosilicate glasses. Hence quartz glasses should contaminate sea water very little.

Chemical porcelain or stoneware is employed in closed (recirculating) sea-water systems for pipes, valves and pumps. Porous ceramics find application as filters of sea water and culture media.

The contamination hazards of chemical porcelain are similar to those of glass, since the sea water will act on the glaze or enamel surface which has been produced during vitrification. This glass surface has been obtained by an eutectic melting with, for example, silica and an alkali or from glazing the ceramic with materials such as feldspar (HACKLER, 1964). The release of elements from the glass surface in contact with the sea water will be governed by the same processes described for glass surface (p. 1461) and will depend on the concentration of major and minor constituents of the raw materials used. Chemical ceramics contain higher concentrations of aluminium than borosilicate glasses.

Al_2O_3 concentration ranges from 20·2% in heavy chemical porcelain to 85% in high alumina ceramics. The latter are especially suitable for the fabrication of parts used in chemical pumps. Chemical ceramics contain also relatively high concentrations of TiO_2 (0·6 to 1%) (RYLAND, 1964). Aluminium and titanium contaminations should, therefore, be given special attention.

Filters from porous ceramics are produced by mixing organic particles of a certain size with the ceramic raw material. During filtering the organic particles will be destroyed leaving a porous substance. Underfiring a regular ceramic composition may also produce pores as well as mixing graded size ceramic particles (RYLAND, 1964). Passing sea water through filters made of porous ceramics with pore sizes down to 0·5 μg will expose a large surface to sea water. Depending on the raw ma-

terials (including impurities) and the process used for the production of the filter, the amount of materials released will vary. Since no data concerning the action of sea water on ceramics have come to the reviewer's attention, it is very difficult to make an assessment of probable contaminations.

Another important problem is the opposite of release, i.e. sorption or negative contamination according to THIERS (1957). Considerable alterations of the culture medium can occur, especially in the concentrations of minor sea-water constituents, if these elements are adsorbed to the walls of containers.

Glasses are cooled liquids possessing distorted and broken bonds. Because of these bonds, glasses have a higher surface energy than crystalline substances causing sorption of ions to the walls of glass containers and the formation of bonds between

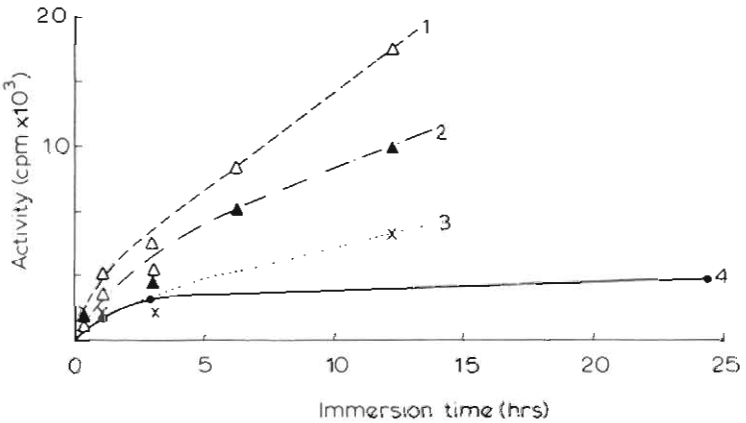


Fig. 7-2: Sorption of Ba and La ions on borosilicate glass from a neutral solution (carrier-free, pH 7·7). 1: Recleaned with NaOH, then heated to 600° C. 2: Recleaned in chromic acid, steam cleaned, then heated at 600° C. 3: Recleaned with NaOH, steam cleaned, then heated at 600° C. 4: Recleaned in chromic acid, then heated at 600° C. (After OTANI and co-authors, 1958; modified; reproduced by permission of University of Hiroshima.)

adsorbed ions and glass surface. Both anion-exchange and cation-exchange processes can occur (HASSENTEUFEL and co-authors, 1963; RILEY, 1965). For example, HASSENTEUFEL and co-authors found that 2 to 17% of the phosphorus in a 20 ml solution (50 μg P l^{-1}) can be adsorbed to Pyrex and Corda glass (surface 85 cm^2) within 24 hrs at pH 7·5 to 8. On the other hand, SCHUTZ and TUREKIAN (1965) reported no significant adsorption of Se, Ag, Co, Ce, Zn, Cr, and Sb from filtered sea-water samples after 2 months of storage in Pyrex glass containers. The sorption processes are quite complex and depend on many parameters, such as pH, and nature and pretreatment of the surface.

OTANI and co-authors (1958) documented the importance of precleaning the glass surfaces for the adsorption of barium and lanthanum ions to borosilicate glass. As can be seen from Fig. 7-2, ions are least adsorbed to surfaces precleaned with chromic acid and then heated to 600° C.

In this connection it must, however, be mentioned, that when glassware is cleaned

with the traditional chromic acid solution it is very difficult to remove residual chromium from the glass surface (THIERS, 1957).

VERZI (personal communication) investigated the effect of pH on the sorption of Zn on the surface of Pyrex glass. In the presence of EDTA, a complexing agent, Zn sorption increased with increasing pH (Fig. 7-3).

ROBERTSON (1968b) has studied the sorption of different elements to glass surfaces and plastic surfaces (Fig. 7-4); he found considerable adsorption on Pyrex glass at the normal pH of sea water. PERONI and LAVARELLO (1977) supplied indirect evidence of adsorption of organic materials to glass surfaces, most probably detergent residues from cleaning procedures. These authors observed that the spots on autoradiographs produced by marine bacteria after inoculation with ^{32}P in artificial sea water free of organic matter diminish greatly in size and number if the glassware (and chemicals used) were previously heated to 600° C in order to destroy

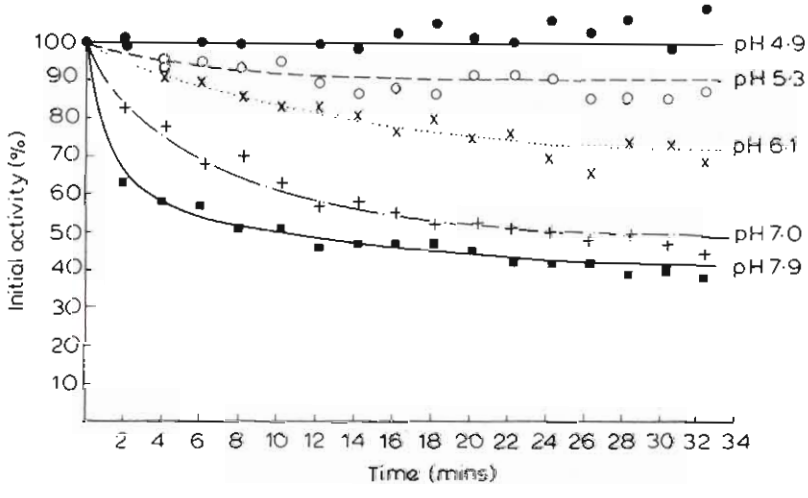


Fig. 7-3: Adsorption of radioactive zinc on the walls of a Pyrex container at different pH values in the presence of the chelation agent EDTA. (After VERZI, personal communication.)

the organic matter (Fig. 7-5). Apparently, the organic matter adsorbed to the glass surface (and in the chemicals used for preparing the artificial sea water) is responsible for the increased metabolic activity of the bacteria and hence for a greater uptake of phosphorus.

Contamination with organic substances has gained special importance since PERONI and LAVARELLO (1975) reported similar differences in the number of colony-forming units when comparing the bacterial activity in surface-water samples with deep-water samples (Fig. 7-6). These few examples show how important adsorption can be, especially when trace elements or radio-active tracers are used.

(b) Plastics and Rubbers

Plastics have found increasing application as containers for small and large volumes of sea water, as aquaria and as cultivation containers. Films of plastic

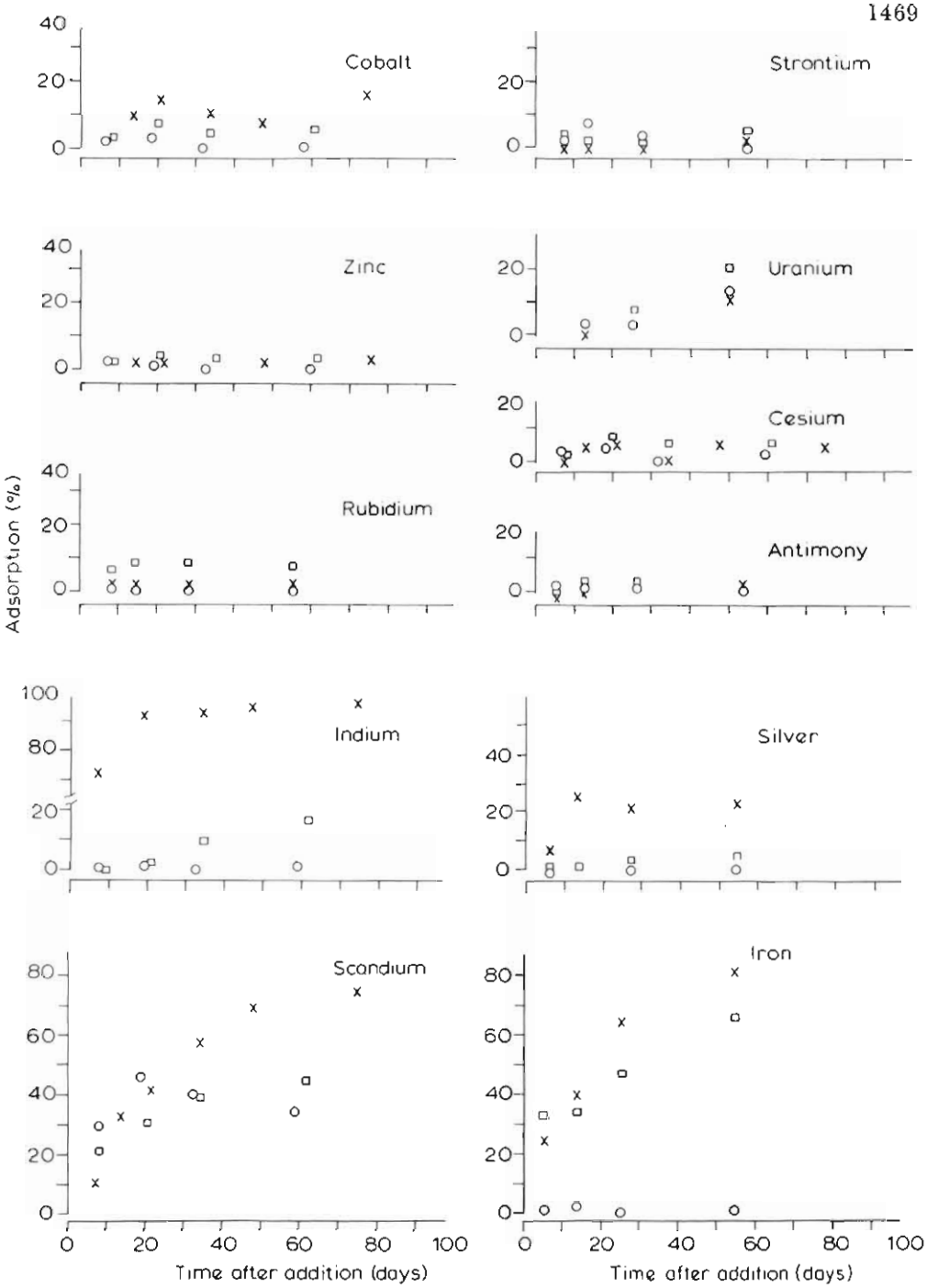
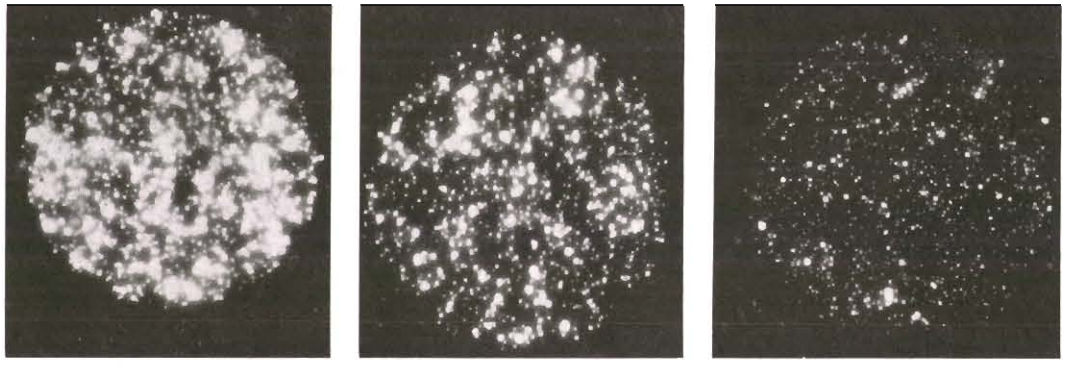


Fig. 7·4: Adsorption of trace elements in sea water to various container surfaces (spiked with radiotracers). ×: Polyethylene bottle; ○: polyethylene bottle with sea water brought to pH 1·5; □: Pyrex glass bottle. (After ROBERTSON, 1968b; modified; reproduced by permission of the author and Elsevier Publishing Co., Amsterdam.)

are used as linings for concrete aquaria, and pipes of plastic material carry sea water; flexible tubes from polyvinyl chloride (PVC) and from silicone rubber are replacing natural caoutchouc tubing, etc.

Of all these materials, probably polyethylene, PVC, 'nylon' (polyamides), silicone rubber and 'Plexiglas' or 'Perspex' (methylmetacrylate resin) are the most widely used, followed by Teflon (tetrafluoroethylene), polyester resin, and epoxy resin.

In the plastics, besides the original chemical substance characteristic of each type of plastic, we may find other materials which have been added for various purposes. Plasticizers are employed to increase flexibility and workability. Filler substances are added in order to reduce the price of the plastic or to increase its resistance; catalysts that remained enclosed after polymerization can be found. Stabilizers are added to decrease chemical reactivity. Flame retarders, colours, antistatic agents, and impurities are introduced with raw materials and hence may be found in plastics (DORBY and SEARS, 1968).



(a) (b) (c)

Fig. 7-5: Enhancement of bacterial population growth, revealed by autoradiography, due to organic impurities of chemicals used for preparing artificial sea water (ASW) and due to contamination of glass-container walls by organic matter. Bacterial strain λ was incubated in ASW: (a) Neither glassware nor chemicals heated to destroy organic impurities; (b) chemicals heated at 600° C for 24 hrs; (c) chemicals not heated, but glass test tubes heated at 600° C for 24 hrs. (After PERONI and LAVARELLO, 1977; reproduced by permission of authors.)

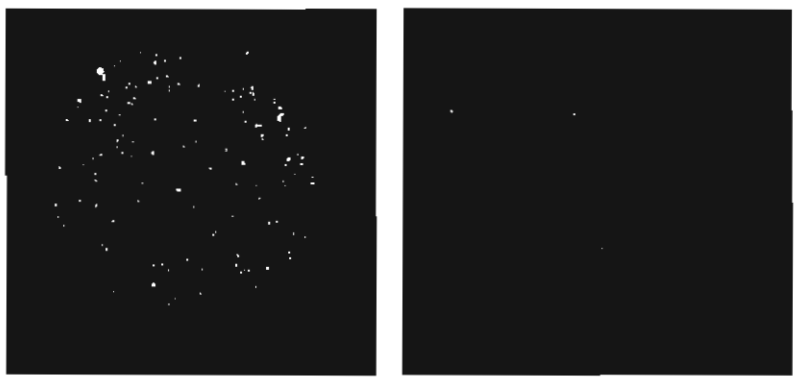
The great variety of possible products employed as 'production aids' and the even greater variety of possible substances added for various reasons does not allow us to preview possible sources of contamination for a given plastic product; especially because of the increasing efforts of the plastic industry continuously to produce better and cheaper plastics. The use of plastifier, filter and other additives are trade secrets and vary with the products (some firms change quite often and, of course, without specifying the additives). However, some general remarks on plastics will help to identify contamination hazards.

The great breakthrough in the use of plastics was achieved when it was discovered that plasticizers can improve the workability of the plastics by lowering the melting viscosity and increasing the flexibility and elasticity of the end-product (DORBY and SEARS, 1968). The plasticizer can be used as 'processing aid' during the pro-

duction and moulding of the plastic into containers, etc. After processing, the plasticizer is removed from the plastic by volatilization or, if this is not possible, the plasticizer is inactivated, e.g. by crystallization or chemical change (polymerization). If plasticizer is used as an 'end-product plasticizer', for converting rigid PVC, for example, into the flexible and elastic PVC hoses and tubes, contrary to its application as 'processing aid', the plasticizer remains at its point of action.

Plasticizers are esters of carboxylic and phosphoric acids. Hydrocarbons, halogenated hydrocarbons, polyglycols and sulphonamides are also used. By far the most frequent types of plasticizers are phthalates, followed by polymerias, phosphates and adipates. In plastified polyvinyl chloride these esters may contribute as much as 50% of the weight of the plastic.

Since plasticizers are not bound chemically to the matrix of the plastic they may leach out. At a recent conference on the environmental impact of phthalic acid esters (PAEs), evidence was presented that plasticizer can leach out of PVC tubing and



(a) (b)

Fig. 7-6: Difference in bacterial population growth. Strain was incubated in sea water from surface (a) or from great depth (b). (After PERONI and LAVARELLO, 1975; reproduced by permission of Springer-Verlag.)

PVC bags (MARX, 1972). Unfortunately none of these experiments were carried out with sea water or sea-water media. WILDBRETT (1973) found that milk and to a lesser extent detergent cleaning solutions commonly used to clean milking machines can extract considerable amounts of plasticizer from the PVC tubing. PVC tubing plasticized with 47·2% of dinonyl phthalates (DOP) lost about 5 times as much phthalates as PVC tubing containing 26·8% of polyadipate and only 5·5% di-2-ethylhexyl phthalates (DEHP). After letting 100 ml of 3 different detergent solutions rotated at 40° C in 1 m of PVC tubing, WILDBRETT found about 2·4 mg of DOP in 100 ml of the washing solution. A rough calculation—based on linear relationship between loss of plasticizer and time, and a 50% reduction or loss for every 10 C°—reveals a loss of 4 μg DOP into 1 l of cleaning solution, after the solution had been in contact with the PVC for 1 min at 20° C. JAEGER and RUBIN (1973a, b) compared the loss of DEHP from medical grade PVC tubing into 3 different perfusion solutions; a saline solution, a 4% bovine serum albumin and a perfusate (blood and albumin). They found that the amount of DEHP in the saline solution was the same as in the reagent blank, but the perfusate extracted about

3 times higher amounts of DEHP than the albumin solution. Cells, lipids and proteins of the blood perfusate enhanced the extraction of DEHP. In a perfusion apparatus the same authors circulated a solution containing 70 ml whole rate blood and 35 ml of a Krebs–Ringer bicarbonate buffer composed of 4% bovine serum, albumin and 80 mg glucose 100 ml^{-1}. After 4 hrs they found about 0·25 mg of glycolyl phthalate in the perfusate. An approximated estimation would yield 200 μg of glycolyl phthalate in 1 l of perfusate after 1 min of contact at 20° C.

From the above, one may anticipate pure sea water, similar to the saline solution, to extract only very small PVC amounts; but if a medium—especially one enriched with lipids, proteins, etc.—is passed through plasticized PVC tubing or is stored in PVC bags, contamination is likely to occur. Amounts released from the plastic which are similar to those mentioned above have a biological effect (pp. 1482–1483).

Fillers (clay, silicates, oxides, degenerated plastics, etc.) are employed to substitute part of the plastic by cheaper substances. Fillers used for reinforcement can be found in plastics, glass fibres, metal or textile fibre tissues and the like.

Catalysts comprise various inorganic and organic compounds. For the production of polyethylene, for example, the oxides of the V and VI transitional metals, salts of Ti, Zr, U, Th, Cr, etc., are used, as well as halides of Ti (III) and Ti (IV) (BUCKLEY, 1968). Other additives function as stabilizers, e.g. long-chain alkyl esters of thiodipropionic acid, phenols, alkaline-earth compounds, and u.v. absorbing substances. Special attention should be given to an additive commonly used in protecting plastics from photocatalytic oxidation resulting from exposure to sunlight. Generally, 2% of carbon black is added to the plastic. Since carbon black is toxic, plastics stabilized in this way should not be used for cultivation of marine organisms.

ROBERTSON (1968a) examined several plastics (Table 7-2) and reported that the purest materials with regard to metal contamination are Plexiglas and Teflon. Significant amounts, especially of iron, but also of zinc, antimony, and, to a lesser extent, copper and cobalt are contained in polyvinylchloride. Nylon contains relatively large amounts of cobalt. Polyethylene in block form is contaminated mainly with iron, while polyethylene (hose) has higher concentrations of antimony than block polyethylene. High-pressure polyethylene contains somewhat fewer impurities than low-pressure polyethylene. How much of these elements can leach out during storage has not been tested by ROBERTSON; he only states that sea water pumped from 1600 m depth through an antimony-containing polyethylene hose had not been contaminated with antimony.

In our laboratory, sea water passing through a plastified PVC tube, however, resulted in significant contamination. This was revealed by sea-water samples which were passed successively through a Chelex-100 column for extraction of trace elements (MACCHI, personal communication).

ROBERTSON (1972) identified another common contamination hazard. Many suppliers of pure plastic containers and tanks furnish these with coloured screw caps or other closing devices. In 1 g of a poly-seal bottle cap, ROBERTSON found among other substances: 3 mg Zn; 0·3 mg Fe and 0·02 mg Mn.

Adsorption or release of zinc by various plastics and rubbers which have been in contact with sea water for at least 2 weeks (Table 7-4) indicate the extent to which a sea-water medium may be altered (BERNHARD and PIRO, 1971). A number of materials—such as PVC (white), polyethylene, grey bottle and, especially, rubber

sheets—release zinc. In some cases concomitant release of Pb and Cu was noted. ROBERTSON (1968a) found considerable quantities of zinc in surgical rubber tubing and neoprene rubber (Table 7-2); this may explain the large release of zinc from rubber sheets indicated in Table 7-4.

Similar to glass, also plastic surfaces adsorb substances. BERNHARD and PIRO (1971) showed that silicone rubber, Moplen (polypropylene), polyethylene, Perspex and other surfaces adsorb zinc (Table 7-4). ROBERTSON (1968b) investigated the adsorption of various elements added as spikes to sea water at its normal pH 8 and after acidification (pH 1·5). At pH 8 many elements are adsorbed; but at pH 1·5 adsorption is greatly reduced, in many instances to negligible amounts. According to HASSENTEUFEL and co-authors (1963), considerable amounts of phosphate ions

Table 7-4

Adsorption or release of ionic zinc by various materials which had been in contact with sea water for at least 2 weeks (After BERNHARD and PIRO, 1971; reproduced by permission of Thalassia Jugoslavica)

Materials	Adsorption (10^{-2} µg Zn cm^{-2} surface)	Release (10^{-2} µg Zn cm^{-2} surface)	Other elements released
Silicone rubber, stoppers	1·10–1·71		
Moplen, beakers	1·60–2·56		
Polyethylene, white bottle	1·77–1·81		
Perspex, sheets	1·84–4·93		
Polyethylene, beakers	1·87		
Vipla, sheets	2·19–3·94		Pb
Silicone rubber, sheets	2·28–2·65		
Algoflon, sheets	2·59–6·79		Pb
Rubbers, for Van Dorn bottle	3·96–7·73		Pb
PVC, white		0·25– 3·00	Pb, Cu
Polyethylene, grey bottle		0·29– 0·77	
Electric cable, external cover		6·37– 9·25	Pb
Gabraster + glass wool		8·46–28·68	Pb
PVC, red		9·38–11·84	Pb
Rubber para, sheets		69·40	

Ionic zinc in natural sea water used: 2·85 µg l^{-1}, determined with anodic stripping voltametry at pH 8.

(25 to 50% of the phosphate present) can be adsorbed by polyethylene and polyvinylchloride tubes. These few examples document that sorption of substances to the surfaces is a phenomenon which deserves special attention in regard to the cultivation of marine organisms (see also Volume I: ZOBELL, 1972).

(c) Metals and Alloys

It is well known that many metals readily dissolve in sea water and thus contaminate it. Since many of the dissolved ions are also highly toxic (e.g. copper, zinc, chromium) metals and alloys must be avoided in the cultivation of marine organisms (Table 7-7). In order to avoid direct contact with the sea-water medium

and with the organisms cultured, metals are sometimes enclosed in plastics or covered with plastic paints; yet, contamination can nevertheless occur since plastics are often permeable for certain substances. The contamination hazards resulting from organic coatings are similar to those discussed in the preceding section *Plastics and Rubbers*. Catalysts, cure promotor, flow agents, driers and stabilizers are added to plastic coatings which may dissolve into the culture medium.

ROBERTSON (1972) found 'Unichrom' to contain relatively large quantities of chromium, hafnium, cobalt, antimony and zinc. Leakages have not been studied, but it may be anticipated that they occur, and that weathering and physical abrasion will contaminate the surrounding sea water with particles of these paints.

The common use of metal forceps, scissors and needles in the laboratory facilitates the contamination of sea-water media with metal ions when these instruments are introduced into the media, for example during sampling. Apparatus containing metals is also often used in collecting marine organisms at sea and thus may contaminate the organism before it is used in the experiment.

(d) Chemicals and Radio-isotopes

In cultivation, the natural sea water is often enriched with nutrients or artificial sea water is prepared (Chapters 2, 3 and 4). For different purity grades of chemicals, maximum levels of element contamination are given. In general, chemicals of the 'per analysis' grade will be sufficient for use in cultivation. However, in special cases (e.g. for requirement experiments) 'ultrapure' grades are needed. If 'per analysis' NaCl is used for the preparation of artificial sea water, 28 g of NaCl are needed to prepare 1 l of sea water (approximately 36‰S). In this amount we might find up to 80 μg Fe, while the normal iron concentration of sea water is about 10 μg Fe l^{-1} (Table 7-3). In 'ultrapure' reagents the iron contamination is about 100 times less.

Similar considerations are valid for other elements. However, contamination is not limited to metal impurities. Chelating agents, most probably the ubiquitous detergents, are also contaminating chemicals. BERNHARD and ZATTERA (1969) found that of 9·52 μg-at of zinc present in 1 l of artificial sea water only 0·04 μg-at Zn were in ionic state. The rest was chelated by substances present as impurities in the chemicals used for the preparation of the artificial sea water. If artificial sea water is prepared from chemicals heated to 600° C in order to destroy the organic matter, none of the zinc present in the chemicals will be chelated.

In general, all substances which come in contact with the sea-water medium must be suspected to contribute to its contamination. WILSON and ARMSTRONG's experiments (1954) may serve as an illustration. These authors passed sea water through a column of active carbon and thereby increased the concentration of copper in their sea water from 6 μg l^{-1} to 60 μg l^{-1}. Since they did not know at that time that the carbon was contaminated with copper they could not understand why sea water became more toxic to their sea-urchin larvae after active-carbon treatment (WILSON and ARMSTRONG, 1958).

Ion exchanger or chelating resins are often employed to eliminate certain ions from sea water (e.g. DAVEY and co-authors, 1970; DAVIES, 1973). When using this technique one must take into consideration that organic substances may leach out

of the resin and contaminate the sea water. Furthermore, the ion with which the resin in the column is charged will exchange with ions in the sea water which possess a higher selectivity to the resin. Since most resins have a lower selectivity for alkaline than for earth–alkaline ions, one should pass the sea water through a calcium regenerated column instead of a sodium generated one. Because in a Na-charged column also alkaline earth ions are exchanged against the sodium ion, the original composition of the sea water is significantly changed, not only with regard to its minor, but also to its major constituents.

Radio-isotopes are often used in experiments with marine organisms. We found radio-isotopes to be contaminated with other substances which may influence experimental results. For example, the high-activity zinc supplied by the Radiochemical Centre (Amersham, Great Britain) contained an organic chelating and complexing agent which transforms part of the radio-active zinc present in ionic form into a complexed physico–chemical state, thus altering the availability of the radio-active zinc to marine phytoplankton algae. Destruction of this substance, which most probably leaked during ion exchange (purification of the radio-active zinc), made all zinc from the radio source available to the phytoplankton (BERNHARD and co-authors, 1971). Similarly, a radiophosphorus source contained particles which interfered with autoradiography and thus had to be eliminated by centrifugation (PERONI and LAVARELLO, 1975).

The experimental ecologist should be aware of the fact that radio sources can be contaminated during production and preparation by many different substances. For example, it is likely that radio-active zinc prepared by neutron activation of a copper target will be contaminated with significant amounts of stable copper.

STEEMAN NIELSEN and WIUM-ANDERSON, (1970) found 250 μg Cu l^{-1} in commercially supplied ^{14}C ampoules. According to these authors the supplying firm had used distilled water for the dilution of the ^{14}C source, which had been prepared in a copper still.

(e) Distilled and De-ionized Water

For preparation of the various solutions used in cultivation of marine organisms pure water is needed. Two techniques are commonly used to obtain pure water: distillation and ion exchange.

Distilled water may be contaminated in various ways. Dissolved solids present in the original water may be carried over as spray or by spurting. If the distillate is not stored properly, dissolved gases such as CO_2 and ammonia may contaminate the water. Dust can enter the apparatus from the atmosphere. In fact faultily constructed stills are liable to contaminate the distillate in such a way that it becomes more impure than the original water before distillation. Commercially available distilled water may contain metal ions as impurities since the water is sometimes produced in metals stills. ROBERTSON (1968a) compared double-glass-distilled water with triple and with quartz-distilled water. He found that the impurities due to trace metals are about the same for all three water types analyzed (Table 7-2).

De-ionized water can be of great purity, but as already mentioned, organic materials leach out of the ion-exchange resin and pass into the de-ionized water.

Instructions for the preparation of pure water have been provided by THIERS (1957) and SMITH (1972).

(f) Sea Water

Small quantities of sea water for cultivation are generally collected in open, unpolluted waters. Under these conditions the ship is the main potential source of contamination. Ships, with very few exceptions, continuously discharge wastes from lavatories, kitchens, etc., into the surrounding waters. Cooling water for the engine and for other heat sources are also discharged, polluting the ambient sea water with corrosion products from the heat exchanger as well as with other wastes such as oil, greases, and petrol. In addition, run offs from various parts of the ship may be sources of contamination.

The ship's hull is usually painted with toxic anti-fouling substances. Nevertheless, after some time algae and animals settle on the hull; they may be torn loose by the manoeuvering ship. Zinc sticks used to reduce propeller corrosion and engine exhausts are additional sources of contamination.

When collecting the sea water, all gear employed—ranging from the commonly used greasy steel cable to the container and the weight—present potential contamination hazards. If the water is pumped aboard in plastic hoses, plastic contents may leach out (p. 1471). The pump and the accessories needed to lower the hose can contaminate the water.

To the reviewer's knowledge, no critical assessments have been made regarding potential contamination hazards of a ship. FONSELIUS and KOROLEFF (1963) found high zinc and copper contents in surface waters and suspected ship contamination. BERNHARD and MACCHI (1966) postulated ship contamination to be responsible for high NO_2/NO_3 concentrations in the ship's wake.

There is some evidence that hydrographic equipment may contaminate sea water. BETZER and PILSON (1970), for example, showed that Nansen bottles can contaminate sea water with particulate iron. Although sterile water samplers soiled with an oil emulsion containing a red bacteria strain did not significantly contaminate the samples (JANNASCH and MADDUX, 1967; BERNHARD and co-authors, 1974), micro-organisms attached to the surface of apparatus represent a contamination hazard.

Many of COOPER's (1958) recommendations for clean collection of sea water for chemical determinations also pertain to the sampling of uncontaminated sea water for use in cultivation experiments.

Although sea water can be contaminated from a multitude of sources, several investigators have found off-shore water more or less suitable for cultivation purposes. Summarizing previous literature, PROVASOLI (1963) concluded that 'bad sea water' for supporting phytoplankton can be improved by adding nutrients, vitamins and trace elements in chelated form. Of course, not all sea waters are alike and hence different combinations of additives are favourable. BARBER and RYTHER (1969) showed that phytoplankton growth in upwelling water can be similarly improved, when a chelator is added (see also Chapters 2, 3 and 4).

There is evidence that marine organisms enrich the surrounding water with organic substances which act as effective chelator. Organic chelators seem to be

missing in deep water. According to PERONI and LAVARELLO (1975) sea water below 300 m supports the growth of bacteria to a much lesser extent than surface water. Results similar to those yielded with phytoplankton have been obtained with animals. CLELAND (1953) and TYLER (1953) could significantly improve the fertilizability of sea-urchin eggs after adding various chelators to the sea water. According to BERNHARD (1955), the sensitive larvae of *Arbacia lixula* can be grown easily when an ion-exchange resin is added to the sea water. On the other hand, WILSON (1951) and WILSON and ARMSTRONG (1952, 1954, 1958) tested the suitability of different sea waters for supporting larval development of *Echinus esculentus* larvae, and concluded that the sea water used was deficient in some substances. Later, ALEEM (1970) repeated some of the sea-water experiments carried out by WILSON and ARMSTRONG (1958) with phytoplankton and also came to the conclusion that some trace elements (Cu) were missing.

These examples document different capacities of off-shore sea waters to support life. Whether these differences are due to contamination is not clear; some data indicate deficiencies of nutrients, vitamins and trace metals; others point to excess of trace elements.

Sea water can be contaminated easily with chelating substances. Detergents are nowadays so widely used that one will find them virtually everywhere and many chemicals are contaminated with an unknown chelator (p. 1474). If relatively large amounts of chemicals are used, these organic substances may significantly alter the availability of metal ions. Soil extract (FØYN, 1934), EDTA and other chelators (TYLER, 1953; PROVASOLI, 1963; JOHNSTON, 1964) are frequently used as 'cultivation aid'. These substances influence markedly the availability of trace metals, as has been shown in numerous experiments on uptake and loss of radioisotopes by marine organisms. In the presence of EDTA the concentration factor is reduced by one to two orders of magnitude (HIYAMA and SHIMIZU, 1964; POLIKARPOV, 1966).

Where large amounts of sea water are required for cultivation, collection of water far out to sea becomes impracticable; sea water from near the laboratory must be used. Such coastal waters are usually polluted due to waste release from nearby industrial installations or sewage plants.

All materials utilized in a sea-water system can contaminate the water. The sea-water system itself can become a source of contamination if its outlet is near the water intake. Especially in closed systems, accumulative contamination can become detrimental. In addition to substances leaching out from construction materials, waste products of the living organisms and substances added during experimentation may pollute the sea water.

(g) Miscellaneous Sources of Contamination

Contamination by filter materials (Table 7-5) has been considered by ROBERTSON (1972). In many filter materials, significant amounts of Al, Cl, Cr, Cu, Fe, Mn, Na and Zn are found. Quantitative filter paper may contain Ba, Ca, Ce, K, Mg, Ni, Pb, Si, Sr, Ti and Zr. Membrane filters have contaminated sea-water samples with zinc; but in our laboratory we found that these filters may adsorb up to 20% radio-

Table 7-5

Typical trace-element concentrations in filters (After ROBERTSON, 1972;

Filter	Element (ppm)							
	Na	Al	S	Cl	K	Sc	V	Cr
Millipore HA						0·0008		17·6
Millipore WS	55	2		140			<0·2	
Millipore AA	77	4		300			<0·2	
Nuclepore	32					0·003		2
Whatman 1	160	2		100			<0·05	
Whatman 41	30	5		20			<0·01	
Whatman 541	13	1		60			<0·03	
Whatman 42	15	3		110			<0·02	
Wiggin Teape 6615	130	200		1900			<0·3	
Four Stones A	50	30		360			<0·06	
Esparto	45	60		900			<0·3	
Charcoal paper, Wiggin Teape	110	200		4000			<2	
Glass (Whatman GF/A)	5800	1800		<100			140	
Mitef (Teflon)	3	8		3			<0·03	
Microsorban	2	10		1400			<3	
Delbag polystyrene								0·3
Delbag polystyrene	3·1		11,600					0·19
Polystyrene	1·7		457					1·1
IPC	67							0·03
IPC (W/O DBP)	72	4·6		38	<20			0·13
Whatman chromatography paper #3	0·9	7		6				1
Tissue paper					0·014			0·5
Blue paper towel	140				0·022			0·68

active zinc. Unless filters are used with care, the 'filtered' sea water may be more contaminated than the original unfiltered water. Working with a celloscope (similar to Coulter counter), we found that sea water stored for 2 months contained fewer particles than membrane-filtered sea water. JONES and DENT (1970) report that not all sediments in their closed system could be removed by filtration.

Aeration (Chapter 2) may also contribute to the contamination of the culture medium. Apart from oil and grease spills originating in the air compressor, uncleaned and unfiltered air may pollute the sea water.

(3) Effects on Organisms

Although a fast-growing body of information has become available on toxicity of environmental pollutants in marine organisms (Volume V; Water Quality Criteria —1972 (1973)), little information is at hand on the biological effects of materials used in cultivation and experimentation.

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Element (ppm)											
Mn	Fe	Co	Cu	Zn	Br	Ag	Cd	Sb	Cs	Hf	Au
	0·33	0·013		2·4		$<5\times10^{-5}$		0·030	0·002	<0·0005	
1·5			6		<1						
0·5			10		<1						
0·13	28	0·025	1·8	2·3				<0·02	<0·003		
0·3			<1		<1						
0·2			<1		2						
0·3			<0·5		0·5						
0·1			2		<1						
60			40		<5						
6			10		<1						
10			5		<0·5						
5			150		<10						
3			<50		<100						
0·3			2		<0·5						
0·1			3		2						
	12	0·01	0·57	4			0·67	0·0077			$<1\times10^{-5}$
0·14	3·9	0·007	7·1								0·0058
0·26	5	0·028	1·2								0·0061
0·03	11	0·027	1·7								0·0045
1·2	4·7	0·003				<0·001		0·0015	0·0003		
0·2		1	7	27				3			0·003
	1·0	0·024		49		0·0008		0·016	<0·0001		
4·5	650	0·052	0·14	2·8	9·8			<0·0002	<0·009		

Contaminants may not only inhibit biological activities but also enhance them. The latter possibility is often overlooked. Growth enhancement may be due, for example, to trace elements from impure chemicals, to chelation of toxic ions by ubiquitous detergents, or to particles (faeces, micro-organisms, etc.) which may serve as food.

(a) Plants

Little information is at hand on chemical contamination effects on cultivated phytoplankton. DYER and RICHARDSON (1962) studied the effects of various materials on growth in 2 freshwater species, the blue-green alga *Synechoccus lividus* and the green alga *Chlorella pyrenoidosa*. Several materials acted as inhibitors, especially some plastics. However, despite the well-known toxicity of copper, some copper alloys did not inhibit growth. BERNHARD and ZATTERA (1970) and ZATTERA and KOSSUT (personal communication) tested contamination effects on the marine phytoplankters *Leptocylindrus danicus*, *Chaetoceros danicus*, *C. curvisetum*, *Coccolithus huxleyi* and *Prorocentrum micans*. *L. danicus* and *C. huxleyi* seem

Table 7-6

Materials which have not been shown to produce inhibitory or toxic effects

Freshwater algae: *Synechoccus lividus*, *Chlorella pyrenoidosa*	Author
Metals	D & R
Cd	
Mg	
Ni	
Sb	
Ti	
Alloys	
Cu 82%, Sb 16%, Zn 2% (bearing bronze)	
Cu 67%, Zn 33% (yellow brass)	
Al alloys: 1100–0 (99% Al); 2014; 2024; 1230; 5052; 6061; 7075	
Pb 50%, Sb 50% (soft solder)	
Ag 90%, Cu 10% (Ag coinage)	
Ag solder, hard and soft	
Stainless steels: types 301; 321; 347; 17–7 PH	
Plastics	
Polyvinyl chloride (Tygon B-44-3 and R-3603; Tygoscal)	
Polyethylene (black pipe)	
Teflon	
Acrylic	
Polypropylene (white tubing)	
Penton	
Nylon (white)	
Polyester	
Epoxy (Scotchcast no. 5; Epon 828)	
Rubbers	
Silicone (silicone rubber, Medical Grade X-30146; Stopcock grease; cement Q-3-0149, fresh or cured)	
Synthetic rubber (Viton A)	
Marine algae: *Leptocylindrus danicus*, *Chaetoceros danicus*, *C. curvisetum*, *Prorocentrum micans*, *Coccolithus huxleyi*	B & Z
Plastics	
*'Pyrex-type' glass	
*Algoflon (polytetrafluorethylene)	
*Perspex (methyl methacrylate)	
*Polyethylene, natural colour	
Nylon	
*Polypropylene, T. 18 screw caps (Bio-Tech, New York)	
Polycarbonates	
Polyamide	
Polyurethane	
*Polyester (gabraster)	
*Tygon (PVC plastified)	
Rubbers	
*Artificial silicone rubber	
Natural caoutchouc	
Surgeon's gloves, smoked natural rubber	

on freshwater and marine algae (Compiled from the sources indicated)

Detergents (maximum non-inhibitory concentrations in $\mu g\ l^{-1}$)	
Autobucato 1	B & Z
Alconox 1	
Decan 1	
Ava 0·1	
Omo 0·1	
Others	
Graphite for heat exchanger	Z & K

*Materials which did not become inhibitory even after autoclaving (0·5 atm, 20 mins).
D & R: DYER and RICHARDSON (1962).
B & Z: BERNHARD and ZATTERA (1970).
Z & K: ZATTERA and KOSSUT (personal communication).

to be slightly more sensitive than the other algae tested. Most likely, some stenoplastic algae are even more sensitive, and thus materials labelled as being non-toxic may well be inhibitory for sensitive forms.

The results obtained with non-toxic materials are summarized in Table 7-6. Non-toxic materials include borosilicate glasses, polytetrafluorethylene, methylmetacrylate, polyethylene and some artificial silicone rubber. Several of these materials, such as Algoflon (Teflon), Perspex, artificial silicone rubber, Polyester, Tygon and Pyrex glass did not exert inhibitory effects even after autoclaving at temperatures at which some of the plastics melted. Materials which have produced inhibitory or toxic effects are listed in Table 7-7. The toxicity of the various PVC's to the algae may be caused by plasticizers which have leached out from the plastics (p. 1471). All natural and some artificial rubbers tested were also highly toxic. These include rubber gloves often used for washing glassware.

According to BERNHARD and ZATTERA (1970) and THOMAS (personal communication), artificial silicone rubber may, under certain circumstances, be toxic. 'Pyrex' test tubes are often supplied with black Bakelite screw caps. PROVASOLI (personal communication) found them to be toxic: Phenol was released. They must be substituted by non-toxic caps (for example, T.18 screw caps from Bio-Tech., New York).

It is surprising that the filter plates used in the Seitz filtration apparatus are toxic to algae, since this apparatus is used for sterilization of culture media. This exemplifies, however, that one must be very careful in choosing apparatus for cultivation (Chapter 2). Another example of an unexpected effect was observed in the Plymouth Laboratory (Great Britain). A formalin-based adhesive, used for veneering and chip-board shelving, seemed to have killed sensitive algal species of Dr. PARKE's culture collection (ANONYMOUS, 1964). Apparently a toxic substance had volatilized and contaminated the cultures which were growing in the room.

(b) Animals

Effects due to the Medium

The effects of several materials used in cultivation on the copepod *Euterpina acutifrons* and larvae of the sea-urchin *Arbacia lixula* have been tested by BERN-

Table 7-7

Materials which produced inhibitory or toxic effects in marine (SW) or freshwater (FW) organisms (Compiled from the sources indicated)

	Test organisms	Author
Metals		
Cu	FW algae	D & R
Fe (powder)	FW algae	D & R
Zn (powder)	FW algae	D & R
Alloys		
Cu 75%, Ni 25%	FW algae	D & R
Fe with Cu 7·5%, Ni 17·5%, Cr 2·5%, Mn 1·5%	FW algae	D & R
Stainless Steel, Avesta 842 SK	SW algae	B & Z
Rubbers		
Silicone rubber, room-temperature vulcanized	SW algae	B & Z
Neoprene (Buna N)	SW algae	B & Z
Natural rubber without blacking soot	SW algae	B & Z
Natural rubber treated with blacking soot	SW copepods	N
Rubber gloves (Marigold, Pirelli, Erista spec., Stanzoil red, Nimble fingers yellow)	SW algae	B & Z
Natural rubber, black, brown, white for stoppers	SW algae	B & Z
Rubber vacuum tubing without lining	SW algae	B & Z
Rubber vacuum tubing with lining	SW copepods	N
Rubber vacuum tubing reinforced	SW algae	B & Z
Silicone rubber tubing	SW algae	T
Caoutchouc, stoppers	SW algae	B & Z
Grey and brown rubber (Seitz filter app.)	SW algae	B & Z
Plastics		
PVC, of various types	SW algae	B & Z
PVC, of various types	SW copepods	N
PVC, of various types	FW algae	D & R
Polypropylene	FW algae	B & Z
Polypropylene (Moplene)	SW algae	B & Z
Polypropylene, black	SW algae	B & Z
Nylon, transparent film	SW algae	B & Z
Araldit, epoxyresin	SW algae	B & Z
Bakelite screw caps for 'Pyrex' test tubes	SW algae	P
Miscellaneous Materials		
Flotronics membrane	SW algae	B & Z
Membrane filter	SW algae	B & Z
Filter plates, Seitz filtration apparatus	SW algae	B & Z

D & R: DYER and RICHARDSON (1962).
B & Z: BERNHARD and ZATTERA (1970).
N: NEUNES (*in*: BERNHARD and ZATTERA, 1970).
P: PROVASOLI (personal communication).
T: THOMAS (personal communication).

HARD and ZATTERA (1970) and NEUNES (*in*: BERNHARD and ZATTERA, 1970). Natural rubber—treated with blacking soot or used for vacuum tubing—and some of the PVC's examined were toxic (Table 7-7). The materials found to be non-toxic are listed in Table 7-8. The table includes some detergents and gives their upper concentration limits which do not yet cause recognizable inhibition. Phytoplankton species seemed to be more sensitive than the *A. lixula* larvae: 'Natural rubber without blacking soot' and 'natural rubber vacuum with lining' were toxic to the algae (Table 7-7), but not to the sea-urchin larvae (Table 7-8). Also the PVC 'Viplaclear ruled', toxic to algae, did not affect the sea-urchins. Some PVC's tested were

Table 7-8

Materials which produced no toxic effects on marine test animals (Compiled from the sources indicated)

		Author
Copepod: *Euterpina acutifrons*		
'Pyrex' type glass (Ignis)		N
Tygon		N
Natural caoutchouc		N
Artificial silicone rubber		N
Polyethylene		N
Echinoderm: *Arbacia lixula*		
'Pyrex' type glass (Ignis)		B & Z
PVC plastified and not plastified		B & Z
Natural rubber without blacking soot		B & Z
Perspex (metamethylacrylate), natural		B & Z
Rubber vacuum tubing without lining		B & Z
Detergents (upper non-inhibitory concentration limit in mg l^{-1})		
Autobucato	0·1	B & Z
Omo	0·5	B & Z
Ava	0·1	B & Z
Last	0·1	B & Z & R
Bref	0·1	B & Z & R
Ariel 2	0·5	B & Z & R
Vim	0·01	B & Z & R
Calinda extra	0·01	B & Z & R

N: NEUNES (*in*: BERNHARD and ZATTERA, 1970).
B & Z: BERNHARD and ZATTERA (1970).
B & Z & R: BERNHARD and co-authors (1972).

toxic to sea-urchin larvae, others were not. This may be due to the fact that the various plasticizers leach out to various extents (p. 1471) and that they differ in toxicity.

Short-term (96 hrs) bioassays showed that the toxicity of di-n-buthyl phthalates is low (MAYER and SANDERS, 1973). The LC_{50}* range from 730 $\mu g\ l^{-1}$ for the bluegill *Lepomis macrochirus*, to 6470 $\mu g\ l^{-1}$ for the rainbow trout *Salmo gairdnerii* and

* LC_{50}: lethal concentrations for 50% of the test population.

more than 10,000 μg l^{-1} for the crayfish *Orconectes nais*. The corresponding LC_{50} (96 hrs) of di-2-ethylhexyl phthalate (DEHP) were higher than 10,000 μg l^{-1}. For comparison, the LC_{50} range of DDT lies between 7 and 19 μg l^{-1}; however, when *Daphnia magna* was exposed to lower concentrations for a complete life cycle of 21 days its reproduction was impaired; all 3 treatment levels (3, 10, and 30 μg l^{-1}) were effective. Since the lowest concentration examined produced a 60% inhibition the concentration of 'no effect' must be lower than 3 μg l^{-1}. This value is of the same order of magnitude as contaminations estimated for the possible release of PAE's from PVC tubing (p. 1471).

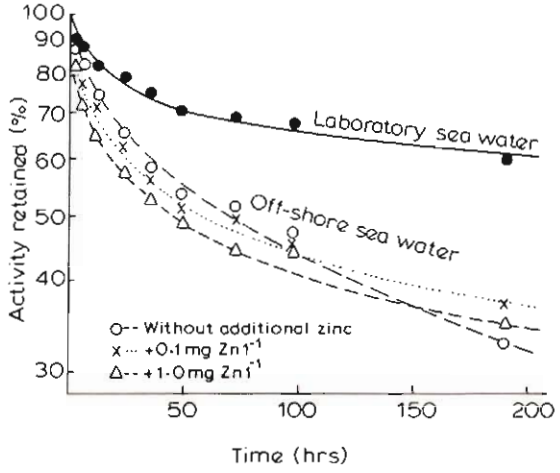

Fig. 7-7: *Pachygrapsus marmoratus*. Loss of ^{65}Zn from crabs kept in laboratory sea water and off-shore sea water with natural and increased stable zinc concentrations. (After KANE and co-authors, 1972; modified; reproduced by permission of Conseil International pour l'Exploration de la Mer.)

Subtle effects have been reported from radio-isotope experiments carried out in sea water taken from an open sea-water system and in sea water collected nearby off-shore and stored in polyethylene carboys (KANE and co-authors, 1972). The results showed that the loss of ^{65}Zn from the crab *Pachygrapsus marmoratus* was significantly faster in sea water collected at sea than the loss in sea water taken from the culture system (Fig. 7-7). KANE and co-authors concluded that the water taken from the system contained some unknown factor which inhibited or slowed zinc-exchange processes taking place on the surfaces of the animals.

HEYRAUD and FOWLER (1973), using the water from the same circulation system and employing the same techniques, repeated these experiments also adding different amounts of stable zinc to the sea water. While they found no significant difference between the water from their closed system and off-shore sea water, they also observed an increase in ^{65}Zn loss at high (100 μg Zn l^{-1}) stable zinc concentra-

tions. Increase of turn-over rates with increase of stable element concentration found by BRYAN (1966) indicates that augmenting the concentration of minor seawater constituents through contamination may considerably affect experimental results.

Effects due to Food Organisms

An example of contamination via food organisms is the use of *Artemia salina* eggs containing DDT. BOOKHOUT and COSTLOW (1970) found great differences in the survival and development of the crabs *Rhithropanopeus harrisii*, *Hexapanopeus*

Table 7-9

Effect of DDT contaminated food (*Artemia salinia* nauplii from Utah, USA) on development to megalopa and first crab of four crabs (*Rhithropanopeus harrisii*, *Hexapanopeus angustifrons*, *Libinia emarginata*, *Callinectes sapidus*). *A. salina* nauplii from California (USA) were less contaminated (After BOOKHOUT and COSTLOW, 1970; modified; reproduced by permission of Biologische Anstalt Helgoland)

Crabs	Origin of food organisms	Survival of 100 zoeae to			
		megalopa		crab	
		normal	deformed	normal	deformed
Rhithropanopeus harrisii	California	97–100	0	94	—
	Utah	0–1	95–98	0	—
Hexapanopeus angustifrons	California	74	0	69	—
	Utah	8	2	0	—
Libinia emarginata	California	95	0	63	—
	Utah	82	0	5	—
Callinectes sapidus	California	50	0	34	0
	Utah	31	0	23	1

angustifrons, *Libinia emarginata* and *Callinectes sapidus* fed with nauplii raised from eggs collected in the Great Salt Lake (Utah, USA) and with nauplii raised from San Francisco Bay eggs (Table 7-9). Crab larvae supplied with *A. salina* nauplii from Utah eggs exhibited reduced survival compared with larvae fed on nauplii hatched from California eggs. Deformed megalopa occurred only when offered nauplii from Utah. BOOKHOUT and COSTLOW analyzed brine shrimp nauplii from the two sources and found that nauplii from California contained 2300 ppb DDT, while those from Utah contained about 3 times as much. Similar toxic effects were observed when plaice larvae were fed with *A. salina* from Utah (SLOBODKIN, 1968).

NASSOGNE (1972) compared the survival of *Euterpina acutifrons* in culture media containing Multanin with and without food. He found a marked decrease in survival of the copepod when food algae (*Platymonas suecica*) were present (Fig. 7-8). This indicates the importance of the food algae as transport vehicle of toxic organic substances.

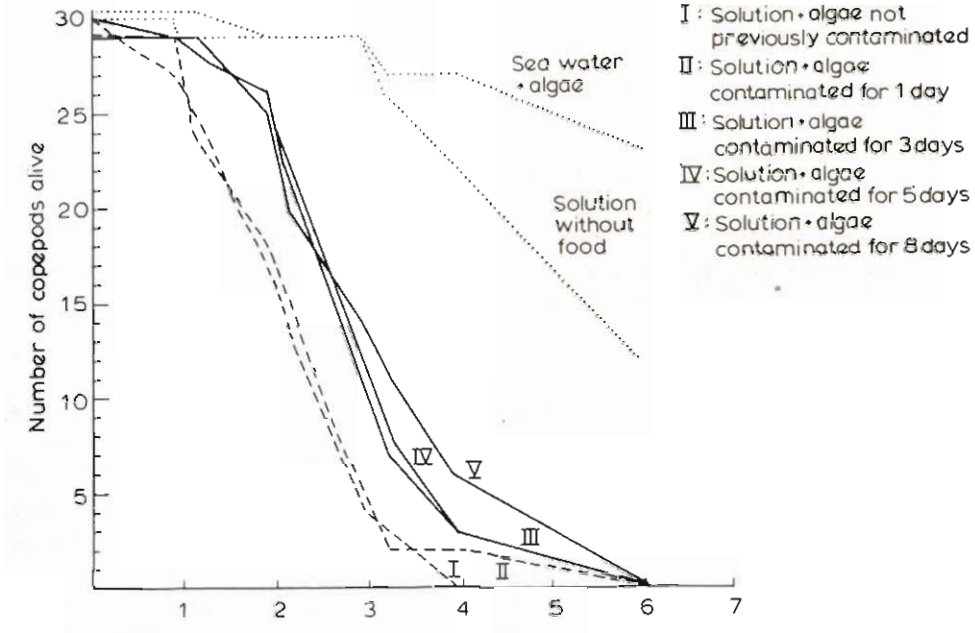

Fig. 7-8: *Euterpina acutifrons*. Survival in solution of Multanin (10^3 µg l^{-1}) without food and with *Platymonas suecica*. (After NASSOGNE, 1972; reproduced by permission of CNEN-Euratom, Italy.)

(4) Detection, Avoidance and Control of Contamination Hazards

The detection of contamination is very difficult. Often only lucky circumstances allow the experimentor to identify causes and effects of contamination. In most cases, the effects of chemical contamination are so subtle and so difficult to detect that the only efficient method seems to be to run, simultaneously with the cultivation experiment, a parallel series employing a different set of containers, laboratory ware and chemicals. Comparison of results may then provide indications as to whether or not chemical contamination presents problems.

However, taking into consideration the most probable contamination sources, it is possible to eliminate major obvious contamination hazards by avoiding the use of certain materials and substances. Chemical analyses of sea water and of experimental organisms assist in identifying the most likely contaminants. It would be ideal, of course, to test all materials and substances beforehand, using the chosen plants or animals as assay organisms; but often it is the cultivation of these organisms *per se* which represents the dominating initial problem. Hence, in such cases first gross evaluations must be carried out with easy-to-handle and readily available assay organisms. Assay organisms can help to detect and avoid all substances which exert marked toxic effects. Only in this way can the ground be prepared for cultivating more sensitive organisms.

(a) Substances and Materials Suitable for Use in Cultivation

Especially for smaller organisms, borosilicon glass from a known source (p. 1461) constitutes a most suitable container material, despite the many different kinds of plastic containers now available on the market. Since the chemical composition of a trademark borosilicate glass is known, contamination caused by leaching of ions from the glass can be predicted. Table 7-3 may help in identifying possible hazards. Glass of unknown chemical composition such as all non-standardized glasses should be avoided. For a thorough treatment of possible contamination consult the excellent article by ADAMS (1972).

Glass and of course also quartz-glass have one great advantage as compared with plastics: They can be exposed to high temperatures. This allows dry sterilization at 200° C and the burning off of any organic materials attached to their surface at 600° C. Furthermore glass containers can be used to sterilize culture media, nutrients solutions, etc., in an autoclave.

If the organic impurities released by glass are considered too high, quartz (vitreous silica) or pure plastics may be used. Plastic laboratory ware is now produced from pure plastics by many firms. Among the plastics, Teflon, polyethylene, polypropylene and polymethylmethacrylates (Plexiglas, Perspex), without additives and colours, should be given preference. Nylon is slightly active, but after several autoclavations its activity diminishes (BERNHARD and ZATTERA, 1970).

Products obtained from a known source, should warrant reasonable non-toxicity and constancy in chemical composition. Unfortunately, some containers are supplied with black or coloured screw caps which contain many impurities. Containers with such caps should be avoided since they represent a high contamination risk. In case pure plastic products are not available, extensive tests with sensitive organisms must be carried out before selecting a certain material type. If replacement is needed at a later date, the experimentor should keep in mind that the supplier may have changed the composition of the additives; hence a new test is necessary to guarantee that the replacement will not jeopardize the experiments.

Many types of polyvinyl chloride are toxic to marine organisms (p. 1481). In the selection of a non-toxic type known makes—such as Tygon, or polyvinyl chlorides used in surgery or other medical fields—should be considered. All materials must be tested since marine organisms may be more sensitive to certain substances than man.

For use in closed sea-water systems pipes, connections, and valves made from 'hard glass', chemical porcelain and pure plastics are recommended.

Metals and alloys should be eliminated from experimental cultures of marine organisms. Obviously, there is also no place for chromated and galvanised instruments, apparatus or tools. Many different plastics are now available that can substitute metals and alloys.

Although sea-water-resistant stainless steels are available, their application should be limited to the unavoidable use for scissors and knives. Lead, as weight in sampling sea water, must be securely enclosed in plastics such as polyester or better, polyethylene.

At sea, cables, collecting apparatus, etc., constructed from metals are commonly used. In the reviewer's laboratory, since 1958 biological and chemical work at sea

has been performed exclusively with nylon or polypropylene cables of different diameters and breaking strengths; all samplers (Van Dorn samplers, aseptic water samplers, plankton nets, etc.) used for collecting live specimens are either constructed exclusively from plastic materials or from stainless steel covered with polyethylene (BERNHARD and co-authors, 1974, 1977).

In cultivation, temperature control of the sea-water supply is often necessary. Metal heat exchangers will obviously corrode and thus contaminate the sea water. Heat exchangers made of graphite or Teflon should be used.

Pumps nowadays can be easily made of non-toxic porcelain or plastics; metal pumps or pumps where metal and grease come in contact with the sea water should not be used. If membrane pumps are employed the membrane must be made of non-toxic materials (silicone rubber, etc.). Natural rubber is a great contamination hazard and hence should be avoided. Membrane pumps are often supplied with neoprene rubber membranes. Since neoprene is toxic (Table 7-7) these membranes must be substituted by silicone rubber membranes. However, even silicone rubbers must be checked, since some silicone rubbers were found to release contaminating substances (p. 1481). In peristaltic pumps, non-toxic plastified PVC such as Tygon can be used.

Chemicals should be of 'per analysis' purity grade or better. When working with artificial sea water and radio-isotopes, contamination of organic chelators should be taken into consideration. In some chemicals, organic substances can be destroyed by heating them prior to preparing solutions; others have to be made up from substances free from organic matter (PERONI and LAVARELLO, 1977). If chemicals in solution are purified by passage through ion-exchange columns, leakage of organic substances from the resin pollutes the solution; if these complexing agents interfere with experimentation, special provisions must be made to destroy these organic contaminants.

Experiments employing trace elements and radiotracers are subject to interference caused by contamination with the same element from external sources. Often 'specific activities' in radiotracer work are much lower than assumed. Therefore, chemical analysis must confirm that the desired concentration level is not exceeded.

(b) Chemical Determination of the Most Common Contamination Hazards

Heavy metals are probably the most important and most easily detectable contaminants. The common use of metals, alloys, etc., in laboratories makes it extremely difficult to avoid contamination due to Fe, Cu, Zn, Ni, Pb, Cr, and, to a lesser extent, to Co and Cd.

The determination of some of the above-mentioned elements in sea water may be carried out according to procedures described by STRICKLAND and PARSONS (1968) and ANONYMOUS (1970). Nickel can be determined according to FORSTER and ZEITLIN (1966) or AFGHAN and RYAN (1968); lead, after LOVERIDGE and co-authors (1960); zinc after BARIC and BRANICA (1967) or ZIRINO and co-authors (1972).

Next to heavy metals, the so-called nutrient salts—i.e. the various forms of phosphorus and nitrogen—should be analyzed. Determination of total phosphorus

can be carried out according to STRICKLAND and PARSONS (1968). Nitrogen determinations are more elaborate since nitrogen must be determined in various forms (STRICKLAND and PARSONS, 1968; see also Chapter 2). The values obtained may be compared with those of Table 7-3.

In general, the determinations are carried out in samples of sea-water medium; but often it may be more advantageous to determine the chemical composition of marine organisms which lived in the sea-water medium; many marine organisms accumulate constituents of their ambient medium at internal concentrations which exceed by far those in the surrounding water. In ecology, great emphasis has been placed on the determination of the so-called concentration factors. The concentration factor is the ratio between the concentration of an element in an organism (g element per g fresh weight) and the concentration in the ambient sea water (g element per g sea water).

LOWMAN and co-authors (1971) provided a summary of concentration factors in different groups of marine organisms. Table 7-10 may help in selecting a potential assay organism; at the same time it provides information on the accumulation capacity of marine organisms. Some of the organisms listed may easily become a source of contamination when kept in polluted sea water prior to their use as food.

(c) Assaying with Marine Organisms for Contamination

Readily available organisms, such as stock cultures of marine bacteria, phytoplankton algae and marine copepods—but also marine organisms collected in the field—can be used as assay organisms for assessing contamination (see also Chapters 3, 4 and 5). Tests with stenoplastic organisms from stock cultures have an advantage over tests with organisms taken directly from the environment: their developmental history under laboratory conditions is known; hence abnormalities in functions and structures can be attributed to the test substance; they are not caused by unknown culture requirements. On the other hand, stock cultures may have adapted to high contamination levels and thus become insensitive. If one suspects that this has happened, a fresh isolation from nature must be used for the tests. Since organisms belonging to different trophic levels may vary in sensitivity to contaminants, and since some substances increase their effectiveness when passing through the food chain (p. 1485), the tests should include organisms from different levels. Sea-water media with and without food organisms must be assayed. Organisms belonging to the same trophic level but to different taxonomic groups may respond differently to a contaminant; therefore, it is advisable to compare the sensitivity of several species of each trophic level. Hardy species such as *Artemia salina* or various members of the genus *Tigriopus* should only be used if more sensitive organisms are not available, since these organisms are likely to indicate only severe contaminations.

The experiments themselves may be divided into short-term and long-term tests. Short-term tests are used for a first screening because many substances can be assayed with relatively little effort; but only long-term tests over at least one generation can provide reasonable assurance that contamination does not interfere significantly with cultivation. A recent study committee on pollution effects on

Table 7-10

Average concentration factors* for benthic algae, plankton, and mollusc, crustacean and fish muscle (After LOWMAN and co-authors, 1971)

Chemical group		Benthic algae	Phytoplankton	Zooplankton	Mollusc muscle or soft parts	Crustacean muscle	Fish muscle
IA	Li	—	—	—	0·28	1·2	0·47
	Na	—	—	—	0·2	0·3	0·13
	K	—	—	—	8	13	13
	Rb	—	—	—	16	13	17
	Cs	—	—	—	8	23	15
	Fr	—	—	—	—	—	—
IB	Cu	100	30,000	6,000	5,000	—	1,000
	Ag	—	23,000	9,000	7,100	7	—
	Au	470	—	—	400	400	60
IIA	Be	110	1,000	15	—	—	—
	Mg	2	2	4	1	—	0·2
	Ca	2	2·5	5	0·4	120	1·5
	Sr	96	—	—	1	3	0·1
	Ba	—	17,000	900	—	—	8
	Ra	1,400	12,000	190	1,300	140	130
IIB	Zn	410	15,000	8,000	11,000	2,000	500
	Cd	200	—	—	—	—	1,000
	Hg	—	—	—	—	—	—
IIIA	B	<1	—	1·6	—	—	2
	Al	15,000	100,000	100,000	9,000	12,000	10,000
	Ga	1,300	8,000	7,000	2,000	2,000	—
	In	—	—	—	—	—	—
	Tl	—	—	—	—	—	—

IIIB	Sc	2,000	2,000	1,000	—	300	750
	Y	480	1,000	105	12	—	250
	La	—	—	—	—	—	—
	Ce	670	90,000	1,000	360	2	0·3
	Pu	1,300	2,600	2,600	260	3	3
IVA	C	~4,000	3,600	2,800	4,700	3,600	5,400
	Si	100	2,000	300	50	—	20
	Ge	50	—	—	—	—	—
	Sn	—	6,000	450	—	—	—
	Pb	700	40,000	3,000	40	—	—
IVB	Ti	4,100	25,000	17,000	—	—	—
	Zr	2,200	60,000	25,000	2	2	<1
	Hf	—	—	—	—	—	—
VA	N	—	36,000	24,000	47,500	44,000	65,000
	P	10,000	34,000	13,000	6,000	24,000	33,000
	As	2,000	—	—	650	400	700
	Sb	—	—	—	—	—	—
	Bi	—	—	—	—	—	—
VB	V	600	600	700	1,700	330	110
	Nb	1,000	1,000	—	7	3	100
	Ta	—	—	—	—	—	—
VIA	S	1	—	—	0·3	—	1
	Se	1	—	—	—	—	—
	Te	—	—	—	—	—	—
	Po	1,000	—	—	—	—	—
VIB	Cr	1,600	2,400	1,900	440	100	70
	Mo	8	—	26	60	10	10
	W	5	—	—	20	2	3

Table 7-10—*Continued*

Chemical group		Benthic algae	Phytoplankton	Zooplankton	Mollusc muscle or soft parts	Crustacean muscle	Fish muscle
VIIA	F	1	—	—	—	—	—
	Cl	1	1	1	1	1	1
	Br	—	—	—	—	—	—
	I	5,000	—	3,000	50	30	12
	At	—	—	—	—	—	—
VIIIB	Mn	2,300	4,000	1,500	12,000	1,900	80
	Tc	—	—	—	—	—	—
	Re	—	—	—	—	—	—
VIII	Fe	4,800	45,000	25,000	9,600	2,400	1,600
	Co	800	1,500	700	600	500	10
	Ni	1,000	5,000	3,000	—	—	—
	Ru	390	200,000	34,000	3	100	0·05
	Rh	—	—	—	—	—	—
	Pd	—	—	—	—	—	—
	Os	—	—	—	—	—	—
	Ir	—	—	—	—	—	—
	Pt	—	—	—	—	—	—

*Concentration factor $= \frac{\text{g element in organism g}^{-1}\text{ fresh weight}}{\text{g element g}^{-1}\text{ sea water}}$.

marine organisms (BARBER and co-authors, 1971) has suggested that metabolic rates (e.g. ^{14}C uptake, respiration measurements), activity levels, sensory physiology, behaviour, fertilization rates, hatching success and larval development, etc., represent sensitive indicators of contamination. Selection of a suitable short-term assay depends on the availability of a proper laboratory method or on the ease with which such a method can be set up. Long-term bioassays are concerned with survival and normal development over one or more generations, reproductive potential, growth rates, etc. Certain developmental stages (e.g. fertilization, morula, gastrula, moulting) are especially sensitive; they provide good criteria for assessing kind and degree of contamination.

In the following paragraphs, we consider a few assay tests in more detail. In order to reduce the number of variables to a minimum, a simple medium should be used for initial tests, if possible with only one container material. Natural sea water and a borosilicate glass should be given preference. Also collection and transportation of natural sea water and the preparation of the sea-water medium should be carried out entirely in borosilicate glass containers. Once other materials have been checked they can substitute this glass. If one suspects the natural sea water to be contaminated, artificial sea water may be used.

Sea-urchin larvae and phytoplankton stock cultures are probably the assay organisms most readily available for most workers. The sea-urchin test (WILSON, 1951; BERNHARD, 1955) requires only natural sea water or artificial sea water prepared from the basic components NaCl, KCl, $CaCl_2$, $MgCl_2$ and $MgSO_4$ plus some $NaHCO_2$ (0·020 g l^{-1}) as buffer for a test medium. Possible contamination with transitional elements is examined in media with and without chelation agents (e.g. EDTA). Since in high concentration a chelator complexes also major ions, EDTA should not be used in concentrations higher than 10^{-3} M. For the actual sea-urchin test, ripe eggs of several females and spermatozoa of several males are collected and mixed in a beaker. The extent and uniformity of the fertilization-membrane elevation, easily observable under a microscope in polarized light, provides a first indication regarding the quality of the sea-water medium: in good-quality sea water, 90 to 100% of the fertilized eggs exhibit symmetrical membrane elevations (TYLER, 1953). Then the fertilized eggs are washed with clean sea water to remove excess sperma and added to culture containers (Boveri or crystallization dishes) with medium or substances to be assayed. After a period sufficient to reach the pluteus (for *Arbacia lixula* about 72 hrs at 20° C) the larvae are fixed with neutral formalin (10 ml of a 5% formalin solution containing about 7% borax) and inspected. Since the larval skeleton shows up very well under polarized light, the development of the skeleton may be used as indicator. BERNHARD (1955) distinguishes 6 stages of skeleton development (Fig. 7-9) and differentiates between normal and deformed skeletons. Examination under polarized light is an advantage in the presence of heavy pigmentation.

Bioassays with phytoplankton are likewise easy to conduct. In most cases cultures are available from marine laboratories. They can also be obtained easily by fresh isolation from sea-water samples and cultured in sea-water medium containing soil extract enriched with nitrates and phosphates (Chapter 4). However, phytoplankton species which require for growth only addition of nitrate and phosphate to sea water should be given preference. The material to be examined is cleaned and

introduced into appropriate culture containers (e.g. Pyrex test tubes closed with non-toxic screw caps or inverted larger test tubes) filled with culture medium. Then the containers are inoculated with a known number of phytoplankton cells. The change in number of unicellular algae from inoculation to a given time thereafter, usually 2 or 3 weeks, is determined either manually (e.g. SMAYDA, 1970) or automatically (e.g. with a Coulter counter, STRICKLAND and PARSONS, 1968). Effects of contaminants are assessed by comparing population growth rates or structural properties of experimentals and controls.

Also behaviour may be used as criterion. Changes in behaviour, however, can

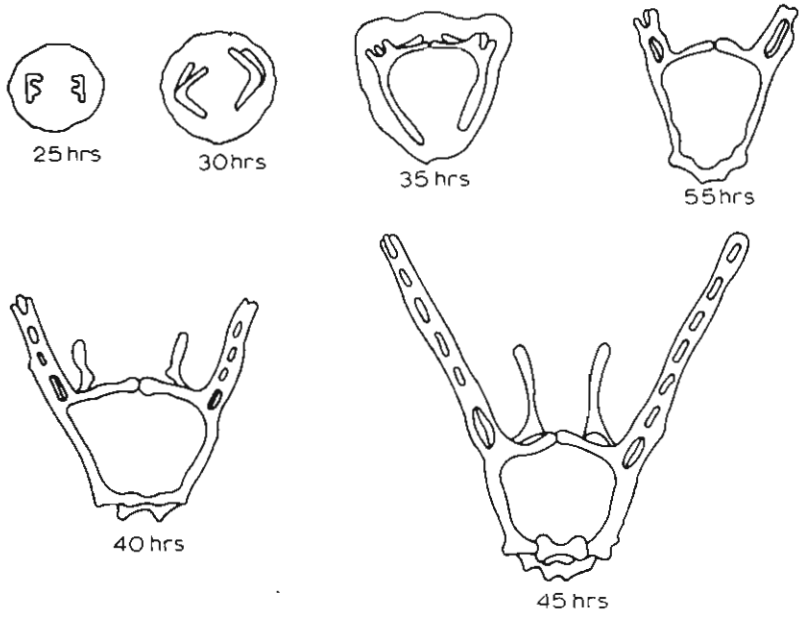

Fig. 7-9: *Arbacia lixula*. Six developmental stages used as criteria for assessment of water contamination. The number of hours indicate normal incubation periods (time after fertilization). (After BERNHARD, 1955; redrawn; reproduced by permission of the Stazione Zoologica di Napoli).

only be expected if the substance to be tested is perceived by sensory organs or exerts effects on the nervous system. Simple preference-test chambers of non-toxic plastics or glass have been described, for example by COOK and BOYD (1965) and HARFNER (1971) (Fig. 7-10). One side (A) of the chamber receives the medium containing the soluble substance to be examined; the other (A_1), equal amounts of non-contaminated medium (control). The locomotory responses of the assay animal (avoidance reaction) serve as indicators. If a solid contaminant has to be tested it is introduced into side (A) of the chamber and clean sea water is run through both inlets (A) and (A_1).

Outlines of methods for mainly routine bioassays are given in TARAS and co-authors (1971) and in Water Quality Criteria-1972 (1973). SPRAGUE (1969, 1970, 1971) has reviewed research carried out to develop bioassays for fish.

(d) Cleaning

It is not possible to suggest a general cleaning procedure which will be effective for all purposes. Any procedure adapted to remove contaminants will leave other substances in their place. The experimentor must therefore decide in each case if a given cleaning procedure is actually effective or likely to increase or modify the contamination. For ultra-micro analysis of trace elements and other substances, different cleaning procedures have been recommended (e.g. ZIEF and SPEIGHTS, 1972).

In the following, cleaning procedures sufficient for most cultivation work will be discussed.

Alkaline solutions etch glass and dissolve the glass-surface layer. If the contaminants adsorbed to the glass surface are soluble in the attacking solution a glass surface practically identical to uncontaminated glass will remain.

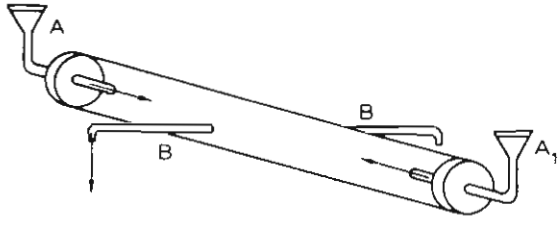

Fig. 7-10: Simple preference chamber for examining locomotory responses to contamination in culture media. Equal amounts of non-contaminated medium and test medium enters the cylinder at opposite ends via funnels (A) and (A_1) and are discharged at central (B). Not drawn to scale. (After HAEFNER, 1971; reproduced by permission of the National Resources Institute, University of Maryland.)

Water or acid solution, on the other hand, will remove the soluble ions from the surface layer. Acids are effective 'desorbers': at low pHs, ions adsorb to a lesser extent onto surfaces. Thus, etching with an alkaline solution, followed by acid attack and then rinsing in pure water would be a good cleaning procedure for glass surfaces. Since detergents act like alkaline solutions, a wash with detergents can substitute the alkaline treatment. Glass can, therefore, be cleaned with detergents followed by acid treatment and then rinsing with pure water.

To remove organic substances attached to glass surfaces the glassware should be heated to 600° C (bake out). The acid step and the bake can be omitted if small contaminations of trace elements and organic substances can be tolerated in the experimental set up; for example, in cases where trace elements had already been added together with a chelating agent to the culture medium.

The sulphuric-acid–alkaline–dichromate mixture which, in the past, has been used extensively for cleaning glassware should not be used. Apart from the fact that it is a dangerous solution to work with, chromate ions adsorbed to the glass

surface are very difficult to remove, and remaining amounts have been shown to be toxic (THIERS, 1957).

Plastics deteriorate when exposed to mineral alkaline solutions; hence alkalines cannot be used for cleaning, but detergents are suitable. Hence plastics can be cleaned efficiently with detergents followed by acid treatment (facultative) and rinsing with pure water.

If adsorbed organic matter presents a problem, glassware should be used for the experiment, or a special treatment be applied to remove the interfering organic substance. Rinsing of glass and plastic laboratory ware can either be carried out with de-ionized water or with distilled water. If water is properly distilled it should be given preference in experiments where even small amounts of organic matter (which always leach out from resins) may interfere, but in all general cultivation work de-ionized water can be safely used. Organic matter leached out from the resins may also have a beneficial effect (enhancement) by complexing trace elements.

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