Vol. 16: 115–131, 2024
https://doi.org/10.3354/aei00475

AQUACULTURE ENVIRONMENT INTERACTIONS
Aquacult Environ Interact

Published March 14

Assimilation of fish farm wastes by the ecosystem engineering bivalve *Atrina zelandica*

D. M. Elvines[1,2,*], G. A. Hopkins[1], C. K. MacLeod[2], D. J. Ross[2], J. A. Ericson[1], N. L. C. Ragg[1], J. S. Copedo[1], C. A. White[2]

[1]Cawthron Institute, Private Bag 2, Nelson 7010, New Zealand
[2]Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 49, Hobart, TAS 7001, Australia

ABSTRACT: As feed-additive aquaculture expands to open ocean areas, there is concern that ecologically important habitats may be adversely impacted by sedimentation of farm wastes. In this study, we investigated assimilation of salmon faecal wastes by an ecosystem engineering bivalve that occurs in open ocean environments (*Atrina zelandica*), as well as effects on physiology and fatty acid metabolism. *A. zelandica* were subjected to one of 3 treatment diets (fish faeces, 1:1 mix of algae:faeces and algae) in a 51 d laboratory trial. We found a diet-related response in fatty acid composition, including increased prevalence of oleic acid (OA) in digestive tissues of *A. zelandica* fed on both the fish faeces diet and the mixed diet, indicating fish wastes were assimilated in both treatments. Fish waste consumption was related to a more marked reduction in fatty acid content of digestive gland, as well as lower proportions of long-chain polyunsaturated fatty acids (LC-PUFA) in digestive tissues. Fatty acid composition in gonad and muscle tissues was more strongly influenced by sex. Regardless of dietary treatment, females accumulated C_{18} fatty acids in gonad tissues, particularly OA, which may preclude the use of OA as a fish waste tracer in this organ. The accumulation of specific fatty acids according to sex may indicate a capacity for preferential selection and retention or biosynthesis of biologically important fatty acids. If present, these mechanisms may increase resilience of *A. zelandica* to stress from deficiencies in LC-PUFA when using fish wastes as a trophic subsidy.

KEY WORDS: Finfish aquaculture · Trophic subsidy · Organic enrichment · Environmental effects · Fatty acids · Fish waste marker · Fatty acid metabolism

1. INTRODUCTION

Sedimentation of faecal waste and uneaten feed pellets into the marine environment from open cage finfish aquaculture can cause organic enrichment of surrounding seabed and adjacent habitats, where it generates a trophic resource for native consumer species. Enrichment effects are well studied for soft sediments and infauna communities (Beveridge 1984, Carroll et al. 2003, Kalantzi & Karakassis 2006, Keeley et al. 2012, McMullin et al. 2021), but response of epifauna and other ecosystems to this enrichment is relatively understudied (Keeley et al. 2020, McMullin 2020, Laroche et al. 2021). As the fish farming industry expands to more dynamic marine environments with higher currents, there is potential for reduced localised organic enrichment through dilution of wastes. However, stronger dispersion spreads wastes over a larger area, and in dynamic areas containing complex substrates and bathymetry, it becomes more likely that farm sedimentation will interact with functionally different ecosystems that may be less equipped to deal with this organic loading (Macleod et al. 2007, Holmer 2010). Accordingly, there is a need for more research into effects of organic enrichment and fish farm trophic subsidies on these more diverse marine

*Corresponding author: deanna.elvines@cawthron.org.nz

Publisher: Inter-Research · www.int-res.com

systems, noting that such studies may need to be site and species specific (Holmer 2010, Laroche et al. 2021).

Previous studies have shown that possible chronic effects of farm-related trophic subsidies to marine organisms can include reduced reproductive fitness in urchins (White et al. 2016, 2018), increased body condition in fish (Fernandez-Jover et al. 2007) and decreased condition in crustaceans (Drolet et al. 2022) and bivalves (Both et al. 2012), as well as potential for enhanced body condition and growth rates (Cranford et al. 2013, Irisarri et al. 2015). Other studies have shown no discernible effect (Baltadakis et al. 2020), which may be related to the abundance of alternative natural food sources (Cranford et al. 2013) or species-specific coping mechanisms. While the effects of farm-derived trophic subsidies to most organisms remain to be assessed at a population or ecosystem level (White et al. 2018, Sardenne et al. 2020), understanding how fish waste is assimilated and the potential physiological implications of this on native species would be the first step in assessing broader ecosystem risks.

Atrina zelandica, the largest bivalve and the only pinnid occurring in New Zealand, inhabits soft sediments in water depths up to 80–90 m (Hay 1990). Like other pinnid bivalves, *A. zelandica* is long lived (>12 yr) (Hay 1990, Hopkins 2002) and often occurs at bed-forming densities, where both living and dead valves provide hard, complex structures for refuge and attachment by other organisms (Hay 1990, Lohrer et al. 2008). These attributes enhance benthic biodiversity and functioning (Norkko et al. 2001) and act as nurseries for a range of organisms (Cummings et al. 1998, 2001). Like other bivalve beds, *A. zelandica* beds also provide critical ecosystem services including filtration and cycling of nutrients and water column particulates (Jackson et al. 2001, Gibbs et al. 2005), sediment entrainment (Green et al. 1998) and seabed stabilisation. Habitats comprising beds of large bivalves such as *A. zelandica* are sensitive to disturbance (MacDiarmid et al. 2013) based on habitat rarity, intolerance to damage due to external factors and long recovery timeframes. The ability of *A. zelandica* beds to influence the surrounding benthos makes this species a useful sentinel organism for monitoring the health and function of these important benthic ecosystems.

Moreover, beds of *A. zelandica* exist adjacent to a proposed open ocean fish farming area in New Zealand's Marlborough region, with a possibility of low-level organic enrichment at some of these beds. Farm-derived sedimentation is nutrient rich and can represent a readily available food source for suspension-feeding bivalves. Research performed on the closely related *A. pectinata*, which occurs in the Indo-Pacific and more widely, found that in small amounts, resuspension of seabed material may enhance food supply (Yurimoto et al. 2008). When critical thresholds of sedimentation were reached, however, mortality and energetic requirements increased. *A. zelandica* feeds on organic matter (phytoplankton, zooplankton, seston) and is thought to be selective of filtered particulates, based on food quality parameters including particle size, carbon content and morphology (Hewitt & Pilditch 2004, Safi et al. 2007). Previous studies on *A. zelandica* showed signs of stress and drop in condition with a reduction in particulate quality (Ellis et al. 2002, Gibbs et al. 2005).

While the addition of farm wastes to marine systems may boost food availability (Cranford et al. 2013, White et al. 2018), the fatty acid quality of farm subsidies will differ to that of marine seston. Due to the increasing use of terrestrial ingredients in fish feeds, farm wastes often contain lower proportions of essential omega-3 long-chain polyunsaturated fatty acids (LC-PUFA) (eicosapentaenoic acid [EPA, 20:5n3] and docosahexaenoic acid [DHA, 22:6n3]) and higher proportions of terrestrial polyunsaturated fatty acids (PUFA) of shorter chain length (C_{18}), such as oleic acid (18:1n9), linoleic acid (18:2n6) and alpha-linolenic acid (18:3n3) (OA, LA and ALA, respectively) (White et al. 2019). EPA and DHA play functional roles in important biological processes, including energy provision, growth and cell membrane structure in reproductive and larval development (Whyte et al. 1992, Caers et al. 2003, Glencross 2009, Tocher 2015), and reduced LC-PUFA in tissues may compromise both reproductive success and important biological resilience mechanisms such as membrane protection.

Fatty acid nutrition in consumer organisms typically comes directly from their diet, which is the most energy-efficient source, but some marine invertebrates, including bivalves, can modify fatty acid composition endogenously through biological processing. Most marine invertebrates have some capacity to selectively retain certain fatty acids, and some also have capacity for de novo biosynthesis (Monroig et al. 2013, Monroig & Kabeya 2018, White et al. 2019); this capacity is species dependent. The capacity for marine bivalves to biosynthesise essential LC-PUFA EPA (20:5n3) and DHA (22:6n3) is thought to be very limited (Fernández-Reiriz et al. 2006, Pirini et al. 2007, Barnathan 2009). However, marine invertebrates, including several bivalve species, can readily synthesise non-methylene-interrupted fatty acids (NMI-FA), which are a different group of PUFA (Monroig et al. 2013) that can perform similar functional roles to

EPA (20:5n3) and DHA (22:6n3) (Barnathan 2009). Thus, some marine invertebrate species receiving fish waste trophic subsidies may be able to mitigate the reduced access to dietary LC-PUFA, and associated risks, if they have the capacity to biosynthesise these or NMI PUFA.

While fatty acids are good indicators of physiological health and functioning, they also present a powerful approach for tracing organic matter through the environment and into the food web (Kelly & Scheibling 2012, Pethybridge et al. 2018, Ericson et al. 2019, Jardine et al. 2020) and, consequently, are increasingly utilised as tracers of fish waste and to examine fish farm trophic subsidies to native marine organisms (Fernandez-Jover et al. 2011, White et al. 2019). Terrestrial C_{18} fatty acids, such as OA (18:1n9), LA (18:2n6) and ALA (18:3n3), are relatively uncommon in marine primary producers, but their prevalence in modern fish feeds can provide a useful means to track assimilation of farm waste. Unfortunately, the use of these tracers can be confounded by endogenous processing of fatty acids, particularly in invertebrates (e.g. de novo biosynthesis, dietary sparing) (Kelly & Scheibling 2012), and therefore a species-specific understanding of fatty acid metabolism is often necessary to interpret results in particular species.

Here, we investigate the potential for *A. zelandica* to ingest and utilise nutrients from salmon farm organic wastes, with the aim of exploring potential effects on physiological condition and fatty acid metabolism. Through a laboratory feeding experiment, we used fatty acid composition, and OA as a fish waste marker in male and female animals, to examine waste uptake and assimilation into different body tissues. We consider the potential implications on metabolic processing of fatty acids. Using *A. zelandica* as a model organism, this study increases our understanding of interactions between fish farming and benthic consumers and provides insights into the potential physiological consequences of farm-derived trophic subsidies. Our study also illuminates sex-specific differences in fatty acid requirements of these animals and provides important insights into the efficacy of fatty acid fish waste markers in this species.

2. MATERIALS AND METHODS

2.1. Experimental setup and design

The ability of *Atrina zelandica* to filter and assimilate fish waste, and effects of this on physiological condition, were investigated over a 51 d experiment (12 Oct–1 Dec 2021). *A. zelandica* with mean (±SE) shell length 197 ± 3.2 mm were collected on 12 August 2021 by SCUBA from Pelorus Sound, New Zealand. The collection area was a relatively sheltered shallow (< 10 m depth) enclosed embayment, often with high water turbidity. After collection, individuals were placed in a damp cloth inside insulated bins to maintain humidity and prevent desiccation during transport (Delorme et al. 2021) back to Cawthron's Aquaculture Park in Nelson, New Zealand. They were then mechanically cleaned to remove epibionts (a biosecurity requirement) using fresh water and distributed among 12 continuously aerated 22 l tanks (~9 mussels per tank) with a flow-through filtered seawater system (flow rate of 1 l min^{-1}), where they were allowed to acclimatise to tank conditions for 2 mo prior to the experiment. During acclimatisation, individuals were continuously drip fed with a supply of cultured algae (∅: 3.5–12 µm, 6–10 million cells l^{-1}) comprising a 1:1 mix of *Tisochrysis lutea* and *Chaeotocerous mulleri* (supplied concentration approx. 1 million cells l^{-1}). This supply was replenished every few days. Water temperature of the tanks was incrementally increased, from 12 to 17°C, over 3 wk following collection and was then held constant at 17°C. The temperature was raised to increase metabolism and encourage reproductive development during the subsequent experimental period (Qiu et al. 2014, Rangel et al. 2017). A 12 h light:12 h dark cycle was used. Five randomly selected animals were collected from the tanks (and frozen whole) immediately prior to commencing the experiment so that baseline fatty acid composition and physiological condition could be established. Excess individuals were also removed from the tanks at this point, leaving 5 individuals per tank.

The experiment consisted of 3 different diet treatments, each with 4 replicate tanks containing 5 individuals (20 ind. per treatment). Treatments consisted of a diet comprising 100% algae (algae diet), 100% salmon faeces (faeces diet) and a 1:1 mix of algae: salmon faeces (mixed diet). The food for each diet was held in 1 slurry tank. Tanks for each treatment were dosed continuously with food slurry from the respective slurry tank via the seawater supply lines using a multi-channel peristaltic pump (Masterflex® L/S® Easy-Load® II).

Algae concentrate used for the experiment was the same as that used during acclimatisation. The salmon faeces slurry was formulated every 2 to 3 d using faeces (see description below) that had been frozen into small blocks. To mix the faeces diet slurry, the blocks were sieved into filtered seawater through

125 µm mesh, with sieving considered complete when the sieve water ran clear. The mixing rate was 1 sieved block per 5 l of filtered seawater. Once all fine particulates had been rinsed through the sieve, the larger particle fraction was discarded. Faeces for the mixed diet slurry were formulated every 2 to 3 d, at the same time as the faeces slurry was formulated and using the same method for formulation. The algae for this diet were replenished each time a new formulation was made.

Slurry tanks were continuously aerated to prevent material from settling on the bottom. However, some settlement of larger particulate material (in particular, salmon faeces) was inevitable; thus, the slurry tanks were also stirred daily to resuspend the material. The organic matter content of each slurry tank was standardised across treatments as far as was practicable to dose approximately uniform amounts of food among the different treatments throughout the experiment. To confirm total organic matter levels across treatments, inflow samples of each tank were collected, filtered and ashed (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/q016p115_supp.pdf). Mean organic matter dosing concentrations were 116 to 140 mg m^{-3}, across the 3 treatments, equating to 167–201 mg d^{-1} of organic matter delivered to each tank (seawater flow rate = 1 l min^{-1}). Haemocytometer examination of diet slurries was also undertaken to provide an idea of particulate size and shape composition (Fig. S2). As feeding requirements of *A. zelandica* are not well studied, the feeding regime and dose rates used in the experiment were established through investigation of past environmental data on organic matter concentrations at the collection site, dose rates provided to similar-sized bivalves in laboratory settings, and adjustment and monitoring of dose rates and animal responses during a laboratory pilot study.

Tanks were kept free of significant organic buildup by removing settled shellfish faeces from the tanks via siphoning several times throughout the experiment. In addition, the tanks were scrubbed midway through the experiment to remove excess algae biofilm. Pipework was flushed with clean high-pressure seawater regularly to avoid feed treatment particulates from clogging these lines.

Frozen blocks of salmon faeces used for the faeces and mixed-treatment slurries comprised settleable faecal material collected from within a seawater recirculating aquaculture system containing Chinook salmon of approximately 41 cm fork length. The fish were originally sourced from a commercial hatchery. The salmon were being fed a standard commercial pelleted diet (Skretting Orient). This diet is specifically formulated for Chinook salmon and rainbow trout, with product information specifying key ingredients as fishmeal, animal meat meals (avian, bovine, ovine), plant protein meal, wheat, fish oil, vegetable oil and poultry oil. Faeces were collected in swirl separators over a 24 h period following feeding and then gently drained (to avoid breaking up flocculated material) inside a 0.5 mm mesh bag for 30 min to remove most of the water. The resulting sludge was then distributed uniformly into muffin-tray moulds (6.4 cm diameter) and frozen overnight. The frozen blocks were then removed from the moulds and stored (frozen) until required.

2.2. Sample collection

2.2.1. Experimental animals

Feeding ceased 24 h prior to sampling at the end of the experiment, to allow for gut clearance. All animals were dissected over 2 d. Shell length, width and depth were recorded as well as sex and approximate spawning stage at gross examination; the majority of animals were ripe at the end of the experiment, but several were only at the early development stage. In one animal, the sex could not be determined. The 5 baseline animals collected at the beginning of the experiment comprised 1 partially developed male, 2 early-developed females and 2 ripe females. The gills, mantle, digestive gland, posterior adductor muscle and gonad were isolated from the rest of the soft tissue and frozen at −20°C along with the remainder of the soft body tissue.

2.2.2. Experimental diets

We also sampled 1 l of slurry from each treatment on 3 occasions during a 24 h period of the experiment for determination of food composition and fatty acid content. The first samples were taken when the slurries had been freshly mixed (and all faecal particulates were all in suspension); the next samples were taken approximately 12 h later after some settlement had occurred and then, finally, 24 h later and immediately before the slurry was mixed to resuspend the settled material. Once the samples had been collected from the slurry bins, they were left to settle during freezing and were then stored frozen until processing. Samples were later thawed, excess water was decanted (disturbing the settled layer as little as

possible) and the remaining slurry was freeze dried and homogenised. Because there was seawater remaining in the sample after decanting, salts contributed to the sample dry weights. Proportionally more salt was present in the faeces samples due to the fine particulates that were easily suspended during decanting (so less water was able to be safely decanted).

2.3. Sample analyses

Shell and all soft tissue samples were freeze dried to obtain shell weight, total soft body weight and individual organ weights for body condition indices. Indices calculated were physiological condition index (CI), muscle yield index (MYI) (both calculated as per Camacho-Mondragón et al. [2012], below), gonadosomatic index (GSI), digestive gland index (DGI) and mantle index (MI). GSI, DGI and MI were calculated in a similar way to MYI, using the weight or the respective organ against total soft body weight:

$$CI = (\text{flesh weight}/\text{total weight}) \times 100 \quad (1)$$

$$MYI = (\text{muscle weight}/\text{soft body weight}) \times 100 \quad (2)$$

Fatty acid analysis was carried out on 5 individuals from 3 randomly selected tanks of each treatment (i.e. n = 15 ind. per treatment). Dried homogenised feed slurry (30 mg), digestive gland, muscle and gonad tissue samples (50 mg) were each weighed into glass tubes for fatty acid analysis. Samples were directly methylated and analysed for fatty acid composition following the methods described in Elvines et al. (2023), except that fatty acid methyl ester was suspended in chloroform instead of hexane. Concentrations of individual fatty acids were determined using the position and area of the individual peaks relative to a known concentration of C19:0 standard in the sample. Data were analysed as percent fatty acids (>1%), and mean values are presented on both a per mass basis (µg g^{-1}) for dry weight and a percent fatty acid basis (see Tables S2–S5 in the Supplement).

2.4. Data analysis

To determine overall differences in fatty acid composition between the 3 diets and of the various tissues across the diet treatments, percent fatty acid data were analysed using a permutational MANOVA (PERMANOVA) with permutations of residuals under a reduced model (2-way crossed) based on a similarity matrix of sample data using Euclidean distance. The test used diet as a fixed factor with 3 levels (algae, mixed and faeces) and tank as a random factor (nested in diet) with 3 levels. Although the experiment was not designed to explore sex-related differences, preliminary data analysis indicated strong separation of fatty acid composition between sexes in muscle and gonad tissues. On that basis, we included sex as a term in the model for these samples (n = 16 males, n = 27 females, sex unable to be determined = 1).

Monte Carlo p-values were used for tests where the number of permutations was <150. Where terms had large (>0.25) p-values (p) and components of variation were negative or close to zero, tank and associated interaction terms were pooled. Pairwise (pw) comparisons were performed where significant differences were indicated by the global test. Fatty acids <1% in all tissue samples were excluded from the dataset. Data were visualised using principal component ordination (PCO) with vectors overlayed using Pearson correlation to show the fatty acids contributing to groupings for each tissue type. The above multivariate analyses were performed in Primer v7.0.13 with the PERMANOVA+ add-on package (Anderson et al. 2008, Clarke & Gorley 2015).

To test for significance of fatty acid content in diet slurries, body condition indices and total fatty acid content among animals from diet treatment and baseline groups, we used a generalised linear model (GLM), with a log transformation applied to non-normally distributed variables (MI, MYI, CI). For these univariate measures, we tested differences only between treatments, which included 4 levels (baseline and the 3 diet treatments [algae diet, mixed diet, faeces diet]). Tests were performed in RStudio (v2022.07.1, Build554).

We also used a GLM to test for significance of differences in individual fatty acids and fatty acid groups between diet treatments, but we used a quasibinomial family transformation (link = logit) in the models. The fatty acid groups were saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), PUFA, short-chain PUFA (SC-PUFA), LC-PUFA, summed omega-3 fatty acids (n3), summed omega-6 fatty acids (n6) and the ratio of n3:n6 fatty acids (n3:n6). Due to the strong sex-related groupings shown in the preliminary analysis and potential confounding effect this unbalanced factor may have had on statistical power, we tested for effects of treatment on males and females separately for muscle and gonad tissues. We also explored differences in individual fatty acids between male and female adductor muscle and gonad

tissues using GLMs that included the treatment, sex and associated interaction term. No significant model improvement was seen by including the tank term in any of the models, so this term was not included. Because we were mainly interested in differences between diet treatment on fatty acid composition, the baseline group data were not included in statistical comparisons, but these data are plotted alongside the main datasets for context. One individual from the algae diet treatment was immature, so gonad was unable to be collected and sex was unable to be determined; this individual was excluded from all analyses that included the sex term.

Where the model term was significant in the GLM, pairwise comparisons were performed using Tukey's test using the emmeans::pairs, with significance symbols for plotting generated using multcomp::cld. Data values presented are mean ± SE.

3. RESULTS

3.1. Diet composition

Mean (±SE) fatty acid content of the different diets was similar overall (F_2 = 0.26, $p_{(F)} > 0.5$); algae feed was 12.3 ± 0.57 mg g^{-1}, faeces feed was 15.8 ± 3.42 mg g^{-1} and mixed feed was 16.1 ± 2.39 mg g^{-1} (Fig. 1A). However, both the mixed and faeces diets had more variable fatty acid content and fatty acid composition compared to the algae treatment. Fatty acid composition was significantly different among all 3 diets ($F_{2,280}$ = 14.31, p = 0.004) (Fig. 1B).

The main fatty acids driving the differences in diet composition (Fig. 1B, Fig. S3) were the fish waste marker OA (which was relatively higher in faeces and mixed diets) and the short-chain SFA 16:0, 17:0 and 18:0, as well as 20:1. The algae diet composition was comparatively richer in 14:0, 16:1 and several PUFA including 16:2n-4, 18:3n6, EPA (20:5n3, an essential fatty acid), arachidonic acid (ARA, 20:4n6), stearidonic acid (SDA, 18:4n3) and ALA (18:3n3).

3.2. Shellfish condition

3.2.1. Condition and organ indices

Mean CI values for *Atrina zelandica* fed the algae, mixed and faeces diets were 10.56 ± 0.44, 10.43 ± 0.44 and 9.83 ± 0.33, respectively (Fig. 2A), with no differences in condition between diet treatments (Fig. 2A, Table S1) and no significant change in condition from baseline condition at the beginning of the experiment.

A significant reduction in MYI between baseline and animals from all treatments (Fig. 2C) indicates a proportional decrease in muscle mass during the experiment, irrespective of diet. There were no significant differences between treatments or baseline for

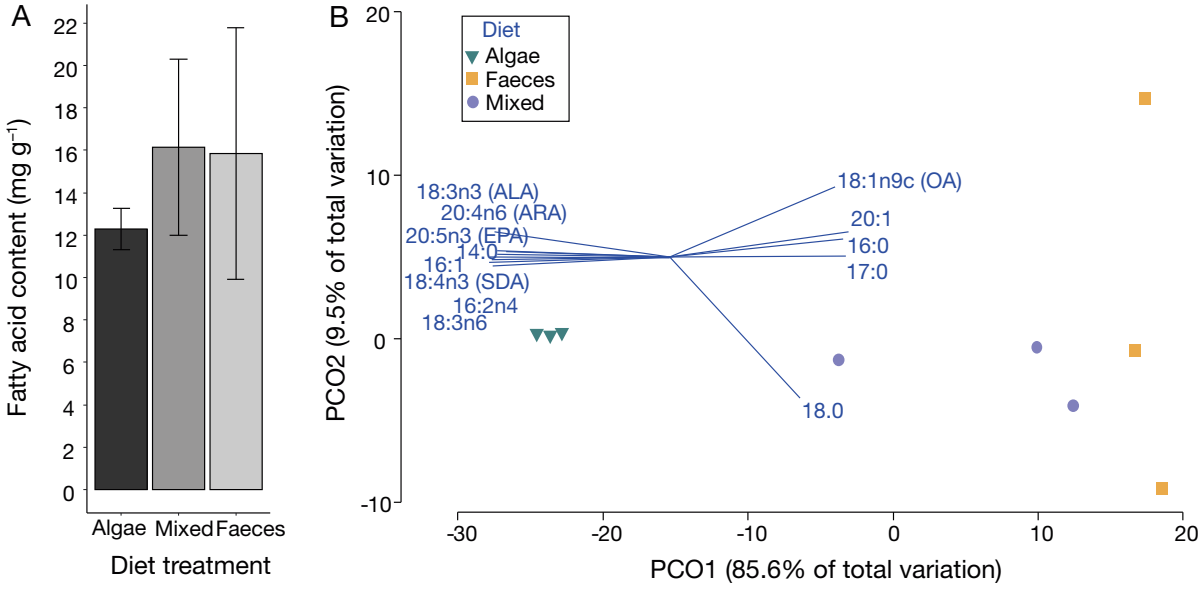

Fig. 1. (A) Mean (±SE) fatty acid content and (B) fatty acid composition of diet treatments used in the feeding trial; principal component ordination (PCO) vectors using Pearson correlation of >0.9 are overlayed. OA: oleic acid; ALA: alpha-linolenic acid; ARA: arachidonic acid; EPA: eicosapentaenoic acid; SDA: stearidonic acid

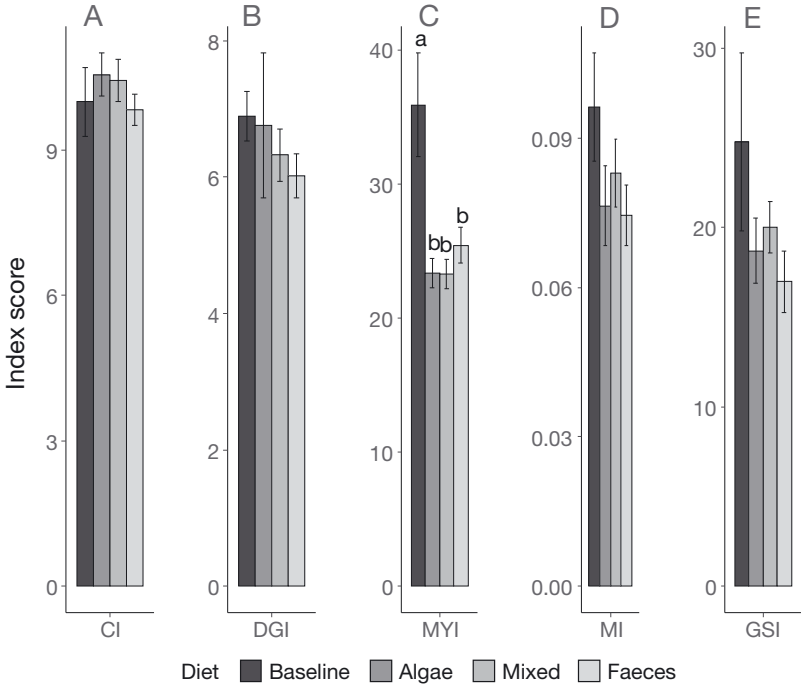

Fig. 2. Indices of overall condition and of individual organs (mean ± SE) of *Atrina zelandica* prior to the experiment (baseline, n = 5) and those fed 3 different diet treatments (n = 15 per treatment). CI: condition index (A); DGI: digestive gland index (B); MYI: muscle yield index (C); MI: mantle index (D); GSI: gonadosomatic index (E)

any of the other body condition indices (Fig. 2B,D,E). It is also worth noting that the average values of all treatments for the MI and GSI indices were lower than baseline means, with high variation in the baseline group, which had low replication (n = 5).

3.2.2. Fatty acid content

There were significant differences in fatty acid content of the digestive gland tissues between baseline and all diet treatments (Fig. 3, Table S2, global $F_3 = 63.66$, $p_{(F)} \leqslant 0.001$); fatty acid content was significantly reduced in the digestive gland at the end of the experiment for all treatments. These reductions were most marked in the faeces treatment, with fatty acid content of animals fed the faeces diet being significantly lower than that of animals fed the algae treatment. In addition, animals fed the faeces diet also showed a significant reduction in fatty acid content in gonad tissue compared to baseline (global $F_3 = 3.31$, $p_{(F)} = 0.028$; p(pw) = 0.023, Fig. 3, Table S3), while animals in other diet treatments did not. There was no difference in fatty acid content of adductor muscle tissues among any groups ($F_3 = 63.52$, $p_{(F)} = 0.96$, Table S4).

3.3. Shellfish fatty acid composition

3.3.1. Fatty acid profile

Fatty acid composition of digestive gland tissues was significantly different between all diet treatments (Table 1), despite significant among-tank variability. The difference between the algae and faeces treatments was the most highly significant. Vectors on the PCO plot (Fig. 4A) showed OA as one of the main fatty acids contributing to differences between digestive gland fatty acid composition between diets, with this difference being most prevalent in digestive tissues of animals from the faeces treatment. Two other C_{18} unsaturated fatty acids (18:3n6 and 18:4n3 [SDA]) and fatty acids 14:0 and 16:1 were elevated in animals from the algae treatment.

There were no diet-related differences in the fatty acid profile of the adductor muscle (Table 1, Fig. 4B,C) or gonad tissues, but fatty acid profiles of both tissues were different between males and females. Pearson correlations (>0.9) indicate the main fatty acids contributing to separation between male and female fatty composition in gonad

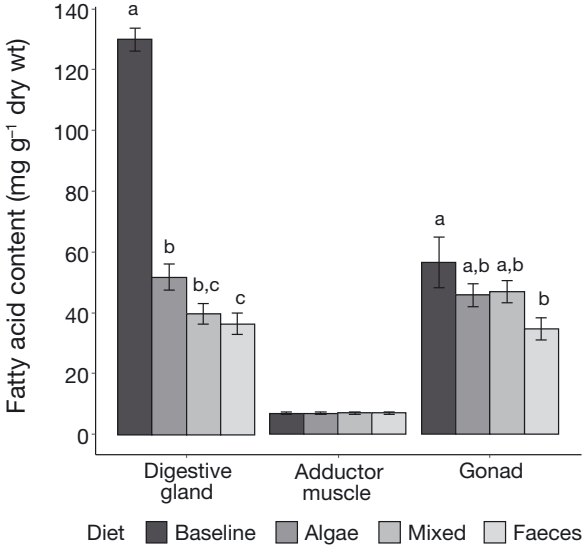

Fig. 3. Mean (±SE) fatty acid content of *Atrina zelandica* tissues at the end of the experiment across the different diet treatments. Baseline data are also shown. Letters denote significant differences between treatment levels. Group means (±SE) are provided in Tables S2–S4

Table 1. Results from permutational MANOVA tests of differences between fatty acid profiles of *Atrina zelandica* tissues between the 3 diet treatments. **Bold**: significant values ($p < 0.05$)

Fatty acid profile	df	MS	*F*(*t*)	p
Digestive gland				
Diet	2	841.41	10.47	**0.0022**
Sex	1	77.73	2.05	0.09
Tank(Diet)	6	82.476	2.18	**0.0081**
Residual	34	38.84		
Adductor muscle				
Diet	2	5.57	0.74	0.59
Sex	1	45.82	6.44	**0.002**
Tank(Diet)	6	7.61	1.07	0.38
Pooled	33	7.11		
Gonad				
Diet	2	32.69	1.38	0.28
Sex	1	782.82	44.45	**0.0001**
Tank(Diet)	6	24.00	1.36	0.20
Pooled	33	17.60		

tissues were LC-PUFA ARA (20:4n6), DHA (22:6n3), docosapentaenoic acid (22:5n3) and SFA 17:0 (Fig. 4C). These fatty acids were more prevalent in males. Meanwhile, short-chain fatty acids SDA (18:4n3), 18:3n6, 14:0 and 16:1 were more prevalent in females (Fig. 4).

In the adductor muscle samples, the male–female separation was not as strong (Fig. 4B), with 16:0, EPA (20:5n3) and DHA (22:6n3) as the main contributors to the sex-related differences in this tissue and DHA being more prevalent in male muscle tissue.

3.3.2. Individual fatty acids

Diet-related differences. *Individual fish waste markers.* OA (18:1n9) and LA (18:1n6) in digestive gland tissues differed significantly between diet treatments (Fig. 5, Table S2). OA was more prevalent in the digestive gland of shellfish fed the faeces diet (16.8 ± 1.36%) compared to the algae diet (5.05 ± 0.21%), with the mixed diet having an intermediary loading (12.4 ± 0.53%). LA was significantly higher in shellfish fed the mixed diet (7.81 ± 0.34%) compared to those fed both faeces (4.97 ± 0.36%) and algae (4.81 ± 0.19%) diets. Proportions of both OA and LA were lower in the digestive gland in the baseline animals (OA: 2.27 ± 0.37%, LA: 3.84 ± 0.39%), indicating that these fatty acids were accumulated during the experiment in all treatments.

The same diet-related trend was not observed in the adductor muscle tissue; no significant difference was observed in OA (18:1n9) proportions among diets (Fig. 6, Table S4). However, the mean proportion of OA in female adductor muscle was higher in the faeces treatment compared to other diets (Fig. 6A, Table S5), and there was relatively high variability in this treatment (3.28 ± 0.93% compared to <2.5 ± $\leqslant 0.2$% for other treatments).

As with the digestive gland, OA (18:1n9) was also accumulated in gonad tissues during the experiment, with generally higher OA levels at the end of the experiment compared to baseline for both sexes (Fig. 7A,B; baseline female = 4.67 ± 1.03%, baseline male = 1.65% [n = 1]; Fig. S4, Table S2). However, the effect of treatment differed between sexes (Tables S5 & S6). Female gonads had significantly higher proportions of OA in the mixed diet (7.87 ± 0.30%) compared to both algae and faeces diets (6.09 ± 0.27% and 6.81 ± 0.06%, respectively; Fig. 7, Table S7), while male gonads had higher OA in both mixed (3.6 ± 0.20%) and faeces (3.54 ± 0.38%) diets compared to the algae diet (2.03 ± 0.09%). Female gonads also had higher levels of OA than males across all treatments, as well as in the baseline group (Fig. S4, Table S5).

Other fatty acid indicators. Compared to shellfish fed the algae treatment (and compared to baseline), there was a reduction in SC- and LC-PUFA in digestive gland of those fed the faeces treatment, with concurrent increases in MUFA (Fig. 8A, Tables S2–S4). These reductions in PUFA were attributable to lower proportions of SC-PUFA SDA (18:4n3), ALA (18:3n3) and 18:3n6 and LC-PUFA ARA (20:4n6), EPA (20:5n3) and DHA (22:6n3) in the faeces treatment (Fig. 5). Similarly, the n3 indicator (EPA, DHA, SDA) was also lower in the faeces treatment compared to the algae treatment (and baseline levels) (Fig. 8A, Table S7), but reductions in EPA and DHA fatty acids were not significant among treatments. In addition, EPA showed marked reductions across all treatments compared to baseline (Fig. 5). Fatty acids 14:0, 16:1 and 18:1n7 were also significantly lower in digestive tissues of animals fed the faeces diet compared to the algae diet and also the mixed diet (14:0 and 16:1). As well as OA (18:1n9), 16:0 and 18:0 were higher across all treatments compared to baseline and were significantly higher in digestive tissues of animals given the faeces diet, when compared with those given the algae (16:0) and mixed (16:0 and 18:0) diets.

In adductor muscle, males fed the faeces diet showed lower proportions of 16:1, SDA (18:4n3), LA (18:2n6) and ALA (18:3n3) compared to the algae treatment and for LA, also the mixed treatment (Fig. 6). Proportions of LA were also higher than baseline, suggesting they were accumulated by animals in the algae and mixed

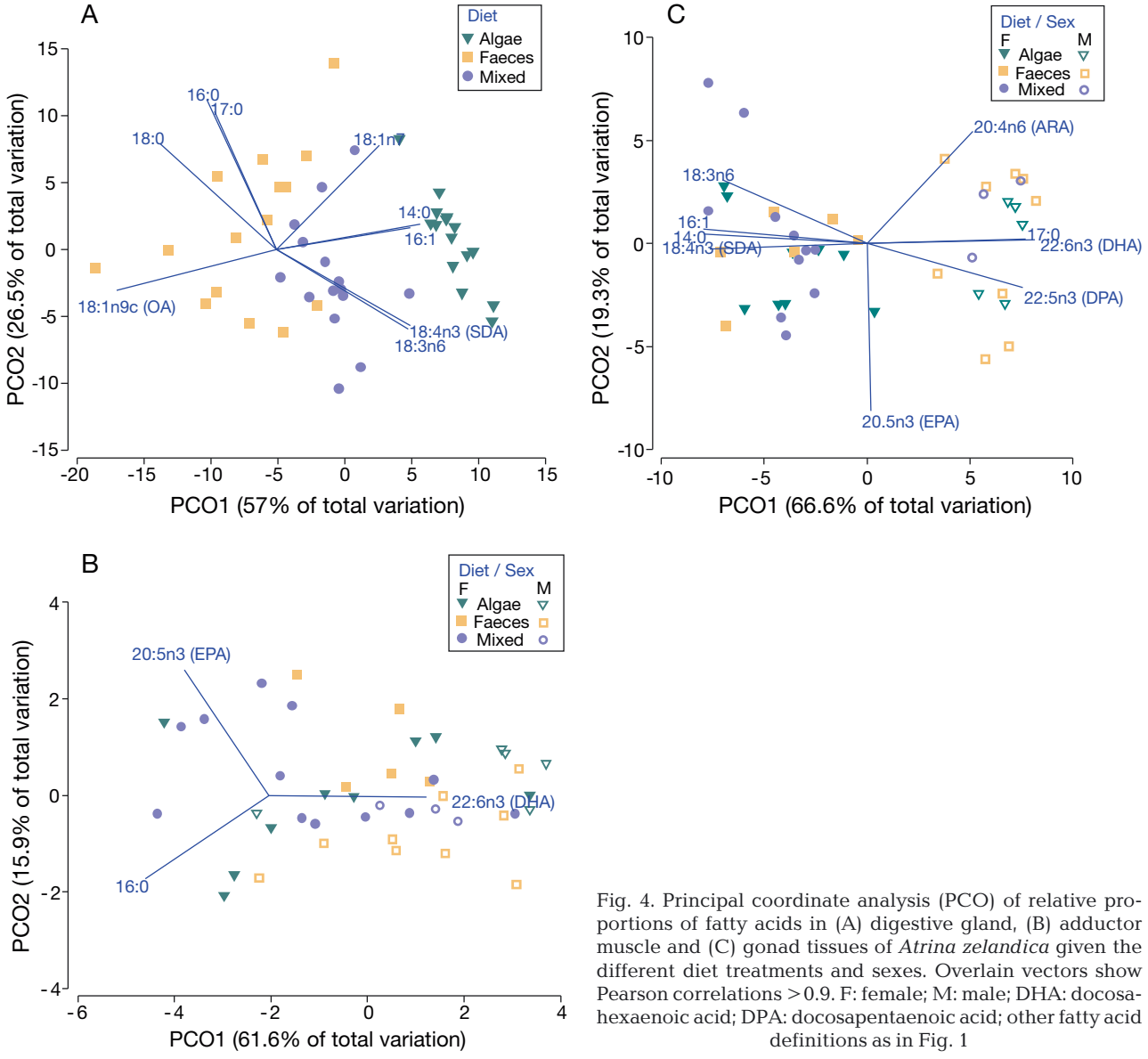

Fig. 4. Principal coordinate analysis (PCO) of relative proportions of fatty acids in (A) digestive gland, (B) adductor muscle and (C) gonad tissues of *Atrina zelandica* given the different diet treatments and sexes. Overlain vectors show Pearson correlations >0.9. F: female; M: male; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; other fatty acid definitions as in Fig. 1

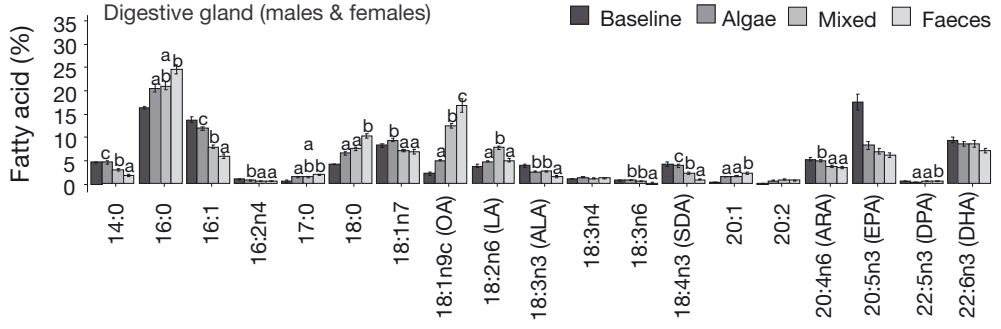

Fig. 5. Percent composition of individual fatty acids >1% (mean ± SE) in digestive gland of *Atrina zelandica* across the diet treatments. Letters denote where significant differences were found between treatments in the generalised linear model (GLM) pairwise comparisons. Baseline data are also shown, but these were not included in the GLMs. Group means (±SE) are provided in Table S2. LA: linoleic acid; other fatty acid definitions as in Figs. 1 & 4

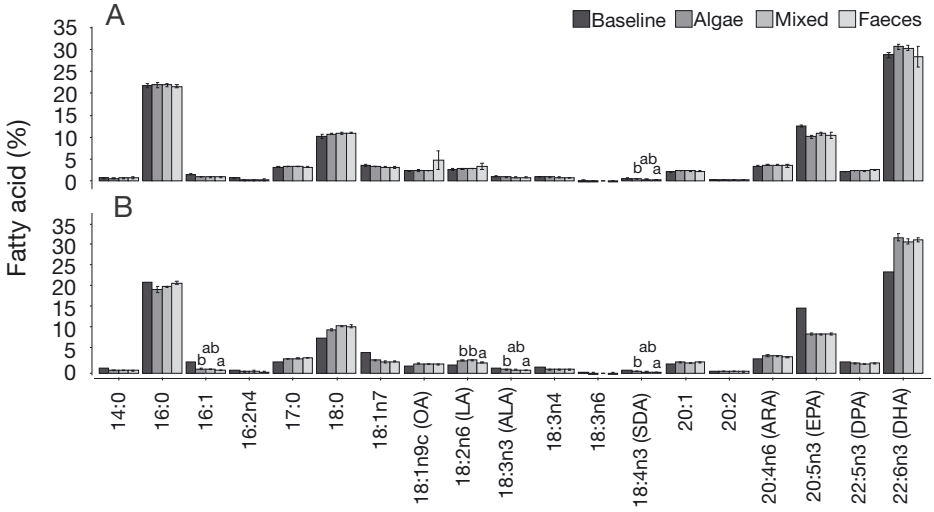

Fig. 6. Percent composition of individual fatty acids >1% (mean ± SE) in adductor muscle of (A) female and (B) male *Atrina zelandica* across the diet treatments. Letters denote where significant differences were found between treatments in the generalised linear model (GLM) pairwise comparisons. Baseline data are also shown, but these were not included in the GLMs. Group means (±SE) are provided in Table S5. Fatty acid definitions as in Figs. 1, 4 & 5

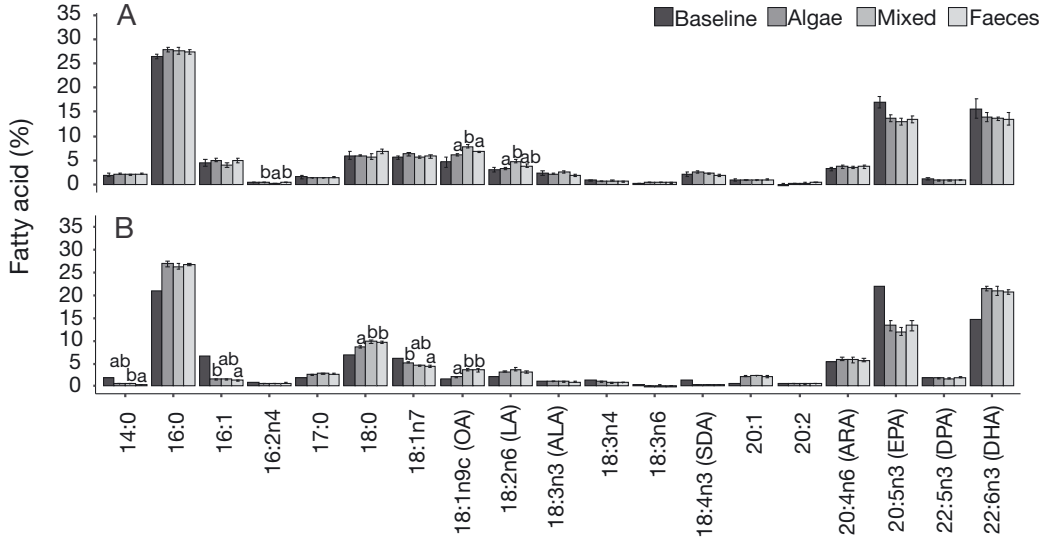

Fig. 7. Same as Fig. 6, but for gonad of (A) female and (B) male

treatments during the experiment. Female muscle tissue showed the same shifts as male muscle tissue in SDA (18:4n3) among treatments, but there were no other significant diet-related differences in this tissue.

In gonad tissues, as well as shifts in OA (18:1n9) described earlier, females showed significantly higher proportions of LA (18:2n6) in the mixed diet compared to the algae diet (Fig. 7). In contrast, males showed significantly higher levels of 18:0 in mixed and faeces diets compared to the algae diet and baseline, as well as significantly lower 18:1n7 in faeces compared to algae. Unlike females, there was an increase in LA in male gonads compared to baseline across all diets. There were also significant reductions in 14:0 and 16:1 in male gonads compared to baseline.

Sex-related differences. In addition to the diet-related shifts in fatty acids, there were strong differences in fatty acid levels between males and females, most notably in the gonads (Fig. S4, Table S7), as already noted with respect to OA (18:1n9). Across treatments, females had significantly higher proportions of many C_{18} MUFA and C_{18} PUFA (18:1n7, OA, LA [18:2n6], ALA [18:3n3] and SDA [18:4n3]) than males, and males had significantly higher proportions of DHA (22:6n3) and ARA (20:4n6). This is despite

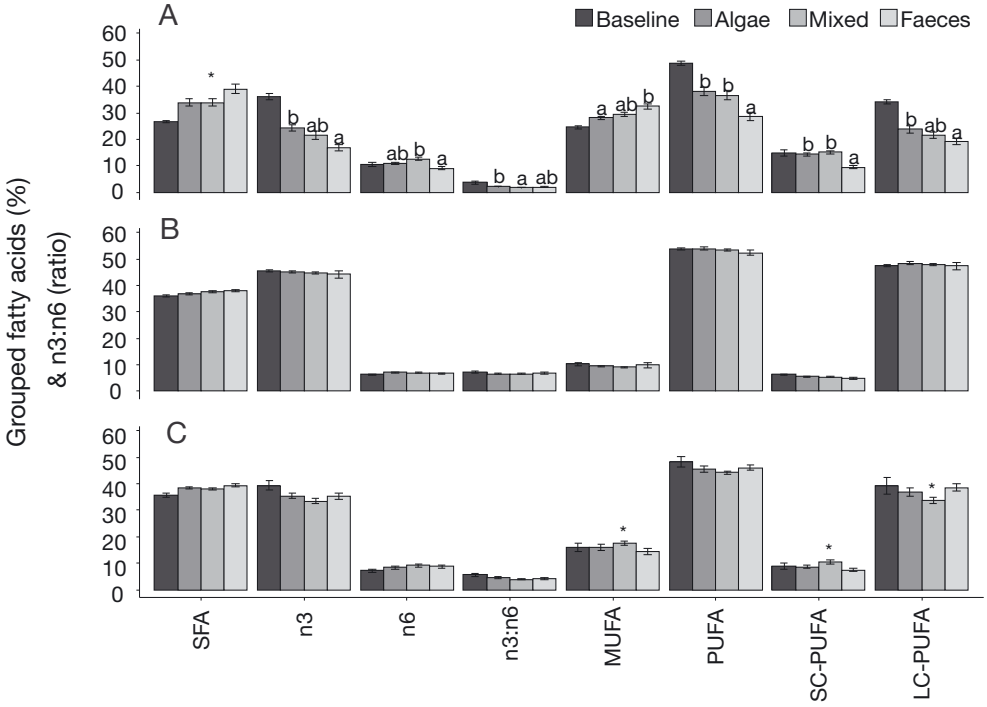

Fig. 8. Fatty acid percent composition of major fatty acid groups in (A) digestive gland, (B) adductor muscle and (C) gonad of *Atrina zelandica* across the diet treatments. Letters denote significant pairwise differences between treatments (adjusted $p < 0.05$). Asterisks denote where the global p-value for treatment was significant but where no pairwise comparisons were $p < 0.05$. Baseline data are also shown for context, but these were not included in statistical testing. Group means (±SE) are provided in Tables S2–S4. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SC-PUFA: short-chain polyunsaturated fatty acids; LC-PUFA: long-chain polyunsaturated fatty acids

similar levels of these C_{18} fatty acids in digestive gland tissues of both sexes and higher levels of DHA in female digestive gland. Sex-related differences seen in the gonad were weakly mirrored in adductor muscle.

4. DISCUSSION

Our findings show that *Atrina zelandica* assimilated fish wastes, even when an alternative algal food source was available. Due to their terrestrial origins, fatty acids OA (18:1n9), LA (18:2n6) and ALA (18:3n3) prevalent in fish feeds have been used as fish waste markers to demonstrate assimilation of fish wastes by a range of marine invertebrates (White et al. 2019). The trend observed in OA in digestive tissues across the diet treatments was consistent with the proportion of this fatty acid in the respective diets, which confirms OA as an effective fish waste marker for detecting waste assimilation by *A. zelandica*. The biochemical signature of assimilated nutrients is typically reflected in digestive gland tissues rapidly, in contrast to slower-turnover tissues, such as muscle, which more gradually incorporate dietary nutrients. While LA and ALA are often suggested as being effective tracers for aquaculture waste (Arechavala-Lopez et al. 2015, Woodcock et al. 2017, White et al. 2019), LA was present in similar amounts across the different feeds supplied to *A. zelandica*, and ALA was higher in the algae diet, precluding the use of these 2 fatty acids as fish waste markers in our study. A similar limitation has been noted in Bergvik et al. (2019), who provided scallops with fish waste and algae diets to examine waste assimilation by these shellfish but found LA to be confounded by algae sources of this fatty acid. Nonetheless, the strong reflection of dietary signatures in digestive tissue, including OA and the overall fatty acid compositional shifts towards that of fish waste for the mixed and faeces diets, provides strong evidence that even when *A. zelandica* are provided with an alternative food source, they will still assimilate fish waste.

Assimilation of fish waste nutrients into muscle and gonad tissues of *A. zelandica* was less clear. We found no significant difference related to diet in the multivariate analysis for these tissues, and while OA (18:1n9) in adductor muscles was higher for the faeces treatment, observations were highly variable and were not significantly different to other treatments. Other

studies have also noted relatively conservative timeframes for integration of OA and overall compositional shifts in fatty acids in muscle tissues in relation to diet changes and digestive gland composition (Fernández-Reiriz et al. 2015a,b, Ricardo et al. 2021). However, diet-related fatty acid shifts have been seen in muscle tissue of scallops within just 27 d (Bergvik et al. 2019) and in similar timeframes for other slow tissues (e.g. blue mussel mantle: 27 d [Redmond et al. 2010] and gill tissue: 28 d [Both et al. 2011, 2012]), so there are likely to be other factors responsible for the lack of biochemical shifts in these tissues.

During gonad development and maturation in bivalves, lipids are allocated strongly toward gonad tissues, sourced either from stored lipids or glycogen (e.g. in gonads, digestive gland or mantle) or directly from the diet (Thompson et al. 1974, Lodeiros et al. 2001, Cartier et al. 2004, Gosling 2008, Zheng et al. 2012, Lee et al. 2015). The high fatty acid content in the digestive gland at the start of our experiment compared to that of other tissues implicates this organ as a storage site for lipid energy also in *A. zelandica*. Our baseline animals had gonads that were a mixture of early to late developed, indicating many had already undergone significant development prior to the experiment. Although not directly tested, it is likely that this development occurred during the acclimatisation period when water temperature was increased, as increased temperatures trigger gonad development of other pinnid bivalves (Qiu et al. 2014, Rangel et al. 2017). If that was the case, fatty acid composition of the algae diet fed during acclimatisation would have been the main source of lipid energy used for gonad development. Indeed, the fatty acid composition of females sampled at the end of the experiment is consistent with this; key fatty acids in the digestive gland of baseline animals and those fed the algae treatment diet (SDA [18:4n3], 18:3n6, 16:1 and 14:0) were also more prevalent in gonad tissues across all treatments.

Exposure to waste over a longer timeframe and during the full gonad development period is also important to consider regarding farmed fish waste consumption by this species. Our experiment was short term and did not fully capture gonad development. The production cycle duration for coastal cage culture is 12 to 18 mo, much longer than our 51 d experiment and long enough to encompass seasonal gonad development. Under these conditions, gonad and muscle tissues may show discernible shifts in fatty acid composition. Accordingly, investigating the response to fish waste exposure over a longer timeframe and during the gonad development period should be focus for future research.

The reduction in fatty acid content of the digestive gland, with no concurrent increase in other organs, also suggests that animals depleted their lipid reserves over the duration of the feeding trial. The depletion of lipid reserves could have several explanations. Reproductive development is energetically demanding (Sastry 1963, Hassan et al. 2018), and in addition to dietary sources, endogenous reserves (protein, carbohydrate) from other organs (e.g. muscle) can be used to fuel gonad development (Gaspar-Soria et al. 2002, Lee et al. 2015, Gómez-Valdez et al. 2021). As some of the females appeared ripe at the beginning of the experiment, and *A. zelandica* are suggested to trickle spawn outside of peak spawning periods (Booth 1979), there is also a possibility that some animals had already entered the spawning stage. Even if not directly observed, any spawning activity would likely have obscured evidence of lipid reserves being re-allocated from digestive gland to gonad tissues during the experiment. Digestive gland lipid reserves may also have been catabolised to maintain normal metabolism, rather than being used to further gonad development. Moreover, some species will undergo oocyte atresia (reabsorption and autolysing of gametes) under poor conditions (e.g. insufficient food supply) to reallocate energy to physiological tasks (Camacho-Mondragón et al. 2012, Beninger 2017). If feeding ratios were not high enough to sustain the increased energy demands of reproduction, these factors could also explain reductions in *A. zelandica* gonad fatty acid content.

The adductor muscle is also a major energy storage site in pinnid bivalves (Baik et al. 2001, Lee et al. 2015, Deudero et al. 2017), and the proportional muscle mass decrease seen at the end of our experiment indicates metabolic reserves in this organ were also being utilised, possibly being catabolised for maintenance. In other pinnid bivalves, metabolic reserves in the adductor muscle are catabolised for maintenance during and post-spawning (Lee et al. 2015, Deudero et al. 2017), which is associated with a sharp decrease in muscle condition (Gaspar-Soria et al. 2002, Angel-Pérez et al. 2007). The depletion of energy reserves may also have been compounded by stress related to being in a laboratory setting (Bayne & Thompson 1970, Ericson et al. 2023), increased metabolic rate due to higher temperatures (Gómez-Valdez et al. (2021) and/or inadequate feeding rations given during the experiment.

Importantly, significantly lower fatty acid digestive gland content in the faeces treatment compared to the algae treatment suggests that the algae diet may also have been energetically superior. This is sup-

ported by significantly lower gonad fatty acid content for animals fed the faeces diet and the intermediary reduction in fatty acid content in digestive gland from animals fed the mixed diet. We note the possibility that gradual settlement of faecal particles between daily stirring events affected food availability in treatments containing faeces, but because the mean fatty acid content of the feed slurries was still similar across treatments (Fig. 1), particle settlement is unlikely to adequately explain disproportional loss in condition among treatments. It is more likely that these effects are from seston quality. *A. zelandica* have exhibited preferential particle selection based on size (e.g. algal cells of 2–20 µm) as well as carbon content and morphology (Safi et al. 2007). In addition to comprising particles in this range, fish waste typically also includes larger non-uniformly shaped particles (Fig. S2; Reid et al. 2009, Law et al. 2014). Decreasing particle quality has an energetic cost through increased filtering and particle rejection and subsequently decreased feeding efficiency. Bergvik et al. (2019) found reduced clearance rate of salmon wastes in scallops compared to algae clearance rates, and such a difference in energetic cost could be reflected in patterns of *A. zelandica* condition among treatments, most notably the faeces diet.

The faeces diet also had lower proportions of PUFA, particularly n3 fatty acids, which are essential for biological processes such as reproductive development and growth (Soudant et al. 1996, Baik et al. 2001), and such reductions were reflected in the digestive gland fatty acid composition of *A. zelandica* fed the faeces diet, along with increases in MUFA. Other work has shown reduced overall fatty acid content in blue mussels fed effluent from a cod aquaculture facility (Both et al. 2012), and while this same species showed enhanced growth on a diet of crushed fish feed pellets, consumption of fish faeces provided no such benefits (Handå et al. 2012). In contrast, our results are consistent with effects of diet quality on *A. zelandica* condition, due to either a shift in energetic requirements or nutritional composition associated with fish waste consumption. However, as we cannot rule out potential effects from diet rationing in the absence of more comprehensive food supply data, further work should be undertaken to confirm this result.

Rather than a diet-related shift, we found a significant difference in fatty acid composition between male and female gonad tissue as well as adductor muscle. This difference in fatty acid composition between male and females was strongest in gonad tissues. Fatty acid composition, especially of PUFA in the gonad and to a lesser extent adductor muscle, has been shown to shift during reproductive cycling in female pinnid bivalves (Baik et al. 2001, Qiu et al. 2014), with the strong sex-related differences seen in our study also likely related to reproductive development. The substantial reduction in EPA (20:5n3) across tissues in both males and females during the experiment indicates that this essential fatty acid was catabolised, consistent with catabolism of EPA during embryonic development in several scallop species (Marty et al. 1992, Whyte et al. 1992, Caers et al. 2003). Our study highlights the metabolic importance of EPA to both sexes; however, the significant difference in fatty acid composition between male and female gonad and muscle tissues shows that different sexes also have unique fatty acid metabolism and requirements. The accumulation of DHA (22:6n3) in males in our study suggests that this fatty acid may be more important for male than female reproductive development. This is similar to other bivalve species, where DHA was accumulated in gonad tissue of male clams *Ruditapes philippanarum* (Fernández-Reiriz et al. 2015b), as well as the mantle of *Mytilus galloprovincialis* (Fernández-Reiriz et al. 2015a), but was relatively absent from the respective organs of females in these studies. In contrast, higher proportions of many C_{18} fatty acids in female *A. zelandica* in our study suggest that these fatty acids may be important for female gonad development and/or maturation. The association of C_{18} fatty acids in our female animals is consistent with positive correlations of OA (18:1n9) and LA (18:2n6) (as well as 15:0) with gonad development in female *A. pectinata* found by Qiu et al. (2014), who also suggested that OA may be a useful indicator of female gonad maturation.

The sex-related differences in fatty acid composition of adductor muscle also implicates this tissue in reproductive development. Although primarily a storage site for protein and carbohydrate reserves (Baik et al. 2001, Deudero et al. 2017), the adductor muscle of other pinnid bivalves has been identified as an important energy store to fuel reproduction. Our study potentially supports the hypothesis of a capital breeding strategy in *A. zelandica*, where energy is mobilised from other tissues instead of directly from dietary sources. This could explain why gonad and muscle tissues did not take on a dietary signature during our experiment. The strong sex-related differences in fatty acid metabolism may also have confounded diet-related shifts in gonads, particularly in females if OA (18:1n9) is associated with female reproductive development, as it is in *A. pectinata* (Qiu et al. 2014). Nonetheless, the absence of diet-related shifts in gonad tissue indicates that short-term (i.e.

51 d per our experiment) fish waste assimilation during mid- to late-stage gonad development had negligible effects on fatty acid metabolism in these tissues, despite clear evidence of waste assimilation in digestive tissue. Certainly, if the dietary sparing and biomodification pathways involve OA, the paired use of a source tracer such as compound-specific carbon isotope analysis may help to clarify and separate diet-related effects from the effects of other physiological processes in fish waste tracing applications.

An ability to endogenously modify the fatty acid composition can allow organisms to meet different fatty acid requirements, even when subjected to the same, or suboptimal, dietary nutrient source. This modification can occur via (1) selectively retaining important fatty acids in tissues while others are catabolised or (2) de novo biosynthesis (Monroig et al. 2013). The disproportional accumulation of several C_{18} fatty acids in gonad tissue of our females and of DHA in males irrespective of diet may suggest that *A. zelandica* can biomodify fatty acids (Miller et al. 2014). Molluscs have enzymes that are required for biosynthesising OA from stearic acid (18:0) (Monroig & Kabeya 2018), and there is evidence suggesting biosynthesis of longer-chain fatty acids from C_{18} fatty acids OA (18:1n9), LA (18:2n9) and ALA (18:3n3) in other bivalve species (Caers et al. 2003, Monroig et al. 2013, Ventrella et al. 2013). The C_{18} fatty acids that we saw accumulated in females are biosynthetic precursors of LC-PUFA (20:2n6, 20:3n3) and NMI-FA, which can perform similar functional, biologically important roles to EPA (20:5n3) and DHA (22:6n3) (Barnathan 2009). Thus, depletions in EPA across all tissues during our experiment may have signalled a need for longer-chain PUFA production, activating biosynthesis through elongation of C_{18} shorter-chain fatty acids OA (18:1n9) and LA (18:2n6). Indeed, accumulation of C_{18} fatty acids as observed in female gonads has been shown to be associated with production of NMI-FA in digestive tissues of *M. galloprovincialis* (Ventrella et al. 2013). Equally, the presence of DHA and associated biosynthetic precursors in male gonad and muscle tissues could be related to an ability to selectively retain, or biosynthesise, these essential fatty acids in these organs.

Biosynthetic capabilities are not known in *A. zelandica*, and additional work would be required to determine if this species does have capacity to selectively retain and/or biosynthesise certain fatty acids, including the production of NMI-FA. Further exploration of fatty acid biomodification in *A. zelandica* is likely to have implications for understanding fish waste assimilation and its potential consequences in this species. While biological modification of tissue fatty acid composition may obscure the signature of fish waste in tissues where fatty acids are biomodified, an ability for *A. zelandica* to preferentially retain or biosynthesise fatty acids required for biological functions could reduce the consequences of lower dietary proportions of essential LC-PUFA (White et al. 2017).

5. CONCLUSIONS

Our study highlights that fish waste feeding trials and fatty acid analysis of tissues are a useful approach for examining potential interactions between benthic consumers and fish farm organic inputs. We found diet-related shifts in fatty acid composition of digestive gland tissues in *Atrina zelandica*, which provided clear evidence that *A. zelandica* assimilates fish waste even in the presence of an algal food source. This suggests that digestive gland OA and fatty acid composition of *A. zelandica* may be useful for identifying the presence of diffuse fish farm wastes in coastal areas. Fish waste consumption was related to a more marked reduction in digestive gland and gonad fatty acid content, likely associated with stronger utilisation of lipid reserves for these animals. We also saw a reduction in proportions of LC-PUFA in digestive tissues. In contrast, gonad and muscle tissues showed no diet-related shift in composition, possibly owing to reproductive development that may have occurred during the acclimatisation stage when the animals were provided with an algae diet. Moreover, fatty acid profiles were unique between males and females, highlighting that the 2 sexes have differing fatty acid requirements. The accumulation of specific fatty acids according to sex potentially indicates that *A. zelandica* has the capacity to preferentially select and retain biologically important fatty acids or may signal a capacity to biosynthesise them to meet reproductive requirements. If present, these biomodification mechanisms may increase resilience of *A. zelandica* to physiological stress related to deficiencies in LC-PUFA when using fish wastes as a trophic subsidy. However, the ability to endogenously biomodify fatty acid composition in gonad tissues, including accumulation of C_{18} fatty acids (particularly OA) in females, may also confound fatty acids as fish waste tracers in this organ.

Acknowledgements. Thanks to Anna Berthelsen, Emma Newcombe, Dan Crosset and Simon Madill (Cawthron Institute) for assistance with field collection and sample dissection. We also thank Gareth Nicholson, Michael Scott, Chris

Chamberlain, Ellie Watts, Dave Read, Hayden Cole and Hannah Appleton (Cawthron Institute) for their role in technical support for setup and running of the experiment. Finally, thanks to Zoe Hilton, Matt Miller, Navneet Kaur and Aaron Brownlow (Cawthron Institute) for their advice and assistance for sample preparation and analysis. This work was funded by Cawthron Institute, the Sustainable Marine Research Collaborative Agreement through the University of Tasmania, Ministry for Primary Industries Postgraduate Science Scholarship Award (21358), and contributes to the Ministry of Business, Innovation and Employment (MBIE) Endeavour Fund (CAWX1606). All fish manipulations were done with approval from the Nelson Marlborough Institute of Technology Animal Ethics Committee (AEC 2018 CAW01).

LITERATURE CITED

Anderson M, Gorley R, Clark K (2008) PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth

Angel-Pérez C, Serrano-Guzmán SJ, Ahumada-Sempoal MA (2007) Reproductive cycle of the mollusk *Atrina maura* (Pterioidea: Pinnidae) in a coastal lagoon system of the Mexican South Pacific. Rev Biol Trop 55:839–852

Arechavala-Lopez P, Sæther BS, Marhuenda-Egea F, Sanchez-Jerez P, Uglem I (2015) Assessing the influence of salmon farming through total lipids, fatty acids, and trace elements in the liver and muscle of wild saithe *Pollachius virens.* Mar Coast Fish 7:59–67

Baik SH, Kim KJ, Choo JJ, Choe SN, Chung EY, Park KH (2001) Seasonal variation in fatty acid composition in female pen shell (*Atrina pectinata*). Fish Aquat Sci 4: 261–264

Baltadakis A, Casserly J, Falconer L, Sprague M, Telfer TC (2020) European lobsters utilise Atlantic salmon wastes in coastal integrated multi-trophic aquaculture systems. Aquacult Environ Interact 12:485–494

Barnathan G (2009) Non-methylene-interrupted fatty acids from marine invertebrates: occurrence, characterization and biological properties. Biochimie 91:671–678

Bayne B, Thompson R (1970) Some physiological consequences of keeping *Mytilus edulis* in the laboratory. Helgol Wiss Meeresunters 20:526–552

Beninger PG (2017) Caveat observator: the many faces of pre-spawning atresia in marine bivalve reproductive cycles. Mar Biol 164:163

Bergvik M, Stensås L, Handå A, Reitan KI, Strand Ø, Olsen Y (2019) Incorporation of feed and fecal waste from salmon aquaculture in great scallops (*Pecten maximus*) co-fed by different algal concentrations. Front Mar Sci 5:524

Beveridge MCM (1984) The environmental impact of freshwater cage and pen fish farming and the use of simple models to predict carrying capacity. FAO Fish Tech Pap 255

Booth JD (1979) Common bivalve larvae from New Zealand: Pteriacea, Anomiacea, Ostreacea. NZ J Mar Freshw Res 13:131–139

Both A, Parrish CC, Penney RW, Thompson RJ (2011) Lipid composition of *Mytilus edulis* reared on organic waste from a *Gadus morhua* aquaculture facility. Aquat Living Resour 24:295–301

Both A, Parrish CC, Penney RW (2012) Growth and biochemical composition of *Mytilus edulis* when reared on effluent from a cod, *Gadus morhua*, aquaculture facility. J Shellfish Res 31:79–86

Caers M, Coutteau P, Sorgeloos P, Gajardo G (2003) Impact of algal diets and emulsions on the fatty acid composition and content of selected tissues of adult broodstock of the Chilean scallop *Argopecten pupuratus* (Lamarck, 1819). Aquaculture 217:437–452

Camacho-Mondragón MA, Arellano-Martínez M, Ceballos-Vázquez BP (2012) Particular features of gonadal maturation and size at first maturity in *Atrina maura* (Bivalvia: Pinnidae). Sci Mar 76:539–548

Carroll ML, Cochrane S, Fieler R, Velvin R, White P (2003) Organic enrichment of sediments from salmon farming in Norway: environmental factors, management practices, and monitoring techniques. Aquaculture 226: 165–180

Cartier S, Pellerin J, Fournier M, Tamigneaux E, Girault L, Lemaire N (2004) Use of an index based on the blue mussel (*Mytilus edulis* and *Mytilus trossulus*) digestive gland weight to assess the nutritional quality of mussel farm sites. Aquaculture 241:633–654

Clarke KR, Gorley RN (2015) PRIMER v7: user manual/tutorial. PRIMER-E, Plymouth

Cranford PJ, Reid GK, Robinson SMC (2013) Open water integrated multi-trophic aquaculture: constraints on the effectiveness of mussels as an organic extractive component. Aquacult Environ Interact 4:163–173

Cummings V, Thrush S, Hewitt J, Turner S (1998) The influence of the pinnid bivalve *Atrina zelandica* (Gray) on benthic macroinvertebrate communities in soft-sediment habitats. J Exp Mar Biol Ecol 228:227–240

Cummings VJ, Thrush SF, Hewitt JE, Funnell GA (2001) Variable effect of a large suspension-feeding bivalve on infauna: experimenting in a complex system. Mar Ecol Prog Ser 209:159–175

Delorme NJ, Burritt DJ, Ragg NLC, South PM (2021) Emersion and relative humidity modulate stress response and recovery dynamics in juvenile mussels (*Perna canaliculus*). Metabolites 11:580

Deudero S, Grau A, Vázquez-Luis M, Álvarez E, Alomar C, Hendriks IE (2017) Reproductive investment of the pen shell *Pinna nobilis* Linnaeus, 1758 in Cabrera National Park (Spain). Mediterr Mar Sci 18:271–284

Drolet D, Riley C, Robert S, Estrada R, Gianasi BL, McKindsey CW (2022) Effect of aquaculture-related diets on the long-term performance and condition of the rock crab, *Cancer irroratus.* Front Mar Sci 9:865390

Ellis J, Cummings V, Hewitt J, Thrush S, Norkko A (2002) Determining effects of suspended sediment on condition of a suspension feeding bivalve (*Atrina zelandica*): results of a survey, a laboratory experiment and a field transplant experiment. J Exp Mar Biol Ecol 267:147–174

Elvines DM, MacLeod C, Ross D, Sabadel A, Bury S, Brown J, White C (2023) Composition of Chinook salmon faecal wastes with implications for environmental management. Aquaculture 569:739358

Ericson JA, Hellessey N, Nichols PD, Nicol S, Kawaguchi S, Hoem N, Virtue P (2019) New insights into the seasonal diet of Antarctic krill using triacylglycerol and phospholipid fatty acids, and sterol composition. Polar Biol 42: 1985–1996

Ericson JA, Venter L, Copedo JS, Nguyen VT, Alfaro AC, Ragg NLC (2023) Chronic heat stress as a predisposing factor in summer mortality of mussels, *Perna canaliculus.* Aquaculture 564:738986

Fernandez-Jover D, Jimenez JAL, Sanchez-Jerez P, Bayle-Sempere J, Casalduero FG, Lopez FJM, Dempster T (2007) Changes in body condition and fatty acid composition of wild Mediterranean horse mackerel (*Trachurus mediterraneus*, Steindachner, 1868) associated to sea cage fish farms. Mar Environ Res 63:1–18

Fernandez-Jover D, Arechavala-Lopez P, Martinez-Rubio L, Tocher DR and others (2011) Monitoring the influence of marine aquaculture on wild fish communities: benefits and limitations of fatty acid profiles. Aquacult Environ Interact 2:39–47

Fernández-Reiriz MJ, Labarta U, Albentosa M, Pérez-Camacho A (2006) Lipid composition of *Ruditapes philippinarum* spat: effect of ration and diet quality. Comp Biochem Physiol B Biochem Mol Biol 144:229–237

Fernández-Reiriz MJ, Garrido JL, Irisarri J (2015a) Fatty acid composition in *Mytilus galloprovincialis* organs: trophic interactions, sexual differences and differential anatomical distribution. Mar Ecol Prog Ser 528:221–234

Fernández-Reiriz MJ, Irisarri J, Labarta U (2015b) Fatty acid composition of female and male clams (*Ruditapes philippinarum*): energy intake and temperature reliance. Aquacult Nutr 23:119–128

Gaspar-Soria R, Pascual MS, Fernández-Cartes V (2002) Reproductive cycle of the cholga paleta, *Atrina seminuda* Lamarck, 1819 (Bivalvia: Pinnidae) from northern Patagonia, Argentina. J Shellfish Res 21:479–488

Gibbs M, Funnell G, Pickmere S, Norkko A, Hewitt J (2005) Benthic nutrient fluxes along an estuarine gradient: influence of the pinnid bivalve *Atrina zelandica* in summer. Mar Ecol Prog Ser 288:151–164

Glencross BD (2009) Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev Aquacult 1:71–124

Gómez-Valdez MM, Ocampo L, Carvalho-Saucedo L, Gutiérrez-González JL (2021) Reproductive activity and seasonal variability in the biochemical composition of a pen shell, *Atrina maura.* Mar Ecol Prog Ser 663:99–113

Gosling E (2008) Bivalve molluscs: biology, ecology and culture. John Wiley & Sons, New York, NY

Green MO, Hewitt JE, Thrush SF (1998) Seabed drag coefficient over natural beds of horse mussels (*Atrina zelandica*). J Mar Res 56:613–637

Handå A, Min H, Wang X, Broch OJ, Reitan KI, Reinertsen H, Olsen YJA (2012) Incorporation of fish feed and growth of blue mussels (*Mytilus edulis*) in close proximity to salmon (*Salmo salar*) aquaculture: implications for integrated multi-trophic aquaculture in Norwegian coastal waters. Aquaculture 356–357:328–341

Hassan MM, Qin JG, Li X (2018) Gametogenesis, sex ratio and energy metabolism in *Ostrea angasi*: implications for the reproductive strategy of spermcasting marine bivalves. J Molluscan Stud 84:38–45

Hay CH (1990) The ecological importance of the horse mussel *Atrina zelandica* with special reference to the Marlborough Sounds. Prepared for Nelson/Marlborough Regional Office, Department of Conservation

Hewitt JE, Pilditch CA (2004) Environmental history and physiological state influence feeding responses of *Atrina zelandica* to suspended sediment concentrations. J Exp Mar Biol Ecol 306:95–112

Holmer M (2010) Environmental issues of fish farming in offshore waters: perspectives, concerns and research needs. Aquacult Environ Interact 1:57–70

Hopkins GA (2002) Aspects of the biology of the horse mussel *Atrina zelandica* Gray in Doubtful Sound and off the Otago Coast, New Zealand. MSc thesis, University of Otago, Dunedin

Irisarri J, Fernández-Reiriz MJ, Labarta U, Cranford PJ, Robinson SM (2015) Availability and utilization of waste fish feed by mussels *Mytilus edulis* in a commercial integrated multi-trophic aquaculture (IMTA) system: a multi-indicator assessment approach. Ecol Indic 48:673–686

Jackson JB, Kirby MX, Berger WH, Bjorndal KA and others (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293:629–637

Jardine TD, Galloway AW, Kainz MJ (2020) Unlocking the power of fatty acids as dietary tracers and metabolic signals in fishes and aquatic invertebrates. Philos Trans R Soc B 375:20190639

Kalantzi I, Karakassis I (2006) Benthic impacts of fish farming: meta-analysis of community and geochemical data. Mar Pollut Bull 52:484–493

Keeley NB, Macleod CK, Forrest BM (2012) Combining best professional judgement and quantile regression splines to improve characterisation of macrofaunal responses to enrichment. Ecol Indic 12:154–166

Keeley N, Valdemarsen T, Strohmeier T, Pochon X, Dahlgren T, Bannister R (2020) Mixed-habitat assimilation of organic waste in coastal environments — It's all about synergy! Sci Total Environ 699:134281

Kelly JR, Scheibling RE (2012) Fatty acids as dietary tracers in benthic food webs. Mar Ecol Prog Ser 446:1–22

Laroche O, Meier S, Mjøs SA, Keeley N (2021) Effects of fish farm activities on the sponge *Weberella bursa*, and its associated microbiota. Ecol Indic 129:107879

Law BA, Hill PS, Maier I, Milligan TG, Page F (2014) Size, settling velocity and density of small suspended particles at an active salmon aquaculture site. Aquacult Environ Interact 6:29–42

Lee YJ, Choi KS, Lee DS, Lee WC and others (2015) The role of the adductor muscle as an energy storage organ in the pen shell *Atrina japonica* (Reeve, 1858). J Molluscan Stud 81:502–511

Lodeiros CJ, Rengel JJ, Guderley HE, Nusetti O, Himmelman JH (2001) Biochemical composition and energy allocation in the tropical scallop *Lyropecten* (*Nodipecten*) *nodosus* during the months leading up to and following the development of gonads. Aquaculture 199:63–72

Lohrer D, Townsend M, Morrison M, Hewitt J (2008) Change in the benthic assemblages of the Waitemata Harbour: invasion risk as a function of community structure. Biosecurity New Zealand Tech Pap 2008/17. NIWA Rep HAM2008-101. Prepared for BNZ Pre-clearance Directorate. National Institute of Water & Atmospheric Research, Hamilton

MacDiarmid A, Bowden D, Cummings V, Morrison M and others (2013) Sensitive marine benthic habitats defined. NIWA Client Rep WLG2013-18. Prepared for Ministry for the Environment. National Institute of Water & Atmospheric Research, Wellington

Macleod CK, Moltschaniwskyj NA, Crawford CM, Forbes SE (2007) Biological recovery from organic enrichment: some systems cope better than others. Mar Ecol Prog Ser 342:41–53

Marty Y, Delaunay F, Moal J, Samain JF (1992) Changes in the fatty acid composition of *Pecten maximus* (L.) during larval development. J Exp Mar Biol Ecol 163:221–234

McMullin RM (2020) Tracking flow and fate of organic matter and contaminants within native food webs sur-

rounding finfish aquaculture. In: A quantitative analysis of organic matter inputs to rocky reef communities. PhD thesis, University of Otago, Dunedin
McMullin RM, Sabadel AJ, Hageman KJ, Wing SR (2021) A quantitative analysis of organic matter inputs to soft sediment communities surrounding salmon farms in the Marlborough Sounds region of New Zealand. Sci Total Environ 773:145146
Miller MR, Pearce L, Bettjeman BI (2014) Detailed distribution of lipids in Greenshell™ mussel (*Perna canaliculus*). Nutrients 6:1454–1474
Monroig Ó, Kabeya N (2018) Desaturases and elongases involved in polyunsaturated fatty acid biosynthesis in aquatic invertebrates: a comprehensive review. Fish Sci 84:911–928
Monroig Ó, Tocher DR, Navarro JC (2013) Biosynthesis of polyunsaturated fatty acids in marine invertebrates: recent advances in molecular mechanisms. Mar Drugs 11:3998–4018
Norkko A, Hewitt JE, Thrush SF, Funnell T (2001) Benthic–pelagic coupling and suspension-feeding bivalves: linking site-specific sediment flux and biodeposition to benthic community structure. Limnol Oceanogr 46: 2067–2072
Pethybridge HR, Choy CA, Polovina JJ, Fulton EA (2018) Improving marine ecosystem models with biochemical tracers. Annu Rev Mar Sci 10:199–228
Pirini M, Manuzzi MP, Pagliarani A, Trombetti F, Borgatti AR, Ventrella V (2007) Changes in fatty acid composition of *Mytilus galloprovincialis* (Lmk) fed on microalgal and wheat germ diets. Comp Biochem Physiol B Biochem Mol Biol 147:616–626
Qiu T, Zhang T, Bai Y, Xue D, Pan Y (2014) Gonad development of the pen shell *Atrina pectinata* from Shandong Province, China. J Shellfish Res 33:465–471
Rangel MS, Mendoza J, Freites L, Tagliafico A, Silva J, Garcia N (2017) Biometric and reproductive aspects of the pen shell *Atrina seminuda* (Bivalvia: Pinnidae) in northeastern Venezuela. Molluscan Res 37:88–97
Redmond KJ, Magnesen T, Hansen PK, Strand Ø, Meier S (2010) Stable isotopes and fatty acids as tracers of the assimilation of salmon fish feed in blue mussels (*Mytilus edulis*). Aquaculture 298:202–210
Reid GK, Liutkus M, Robinson SMC, Chopin TR and others (2009) A review of the biophysical properties of salmonid faeces: implications for aquaculture waste dispersal models and integrated multi-trophic aquaculture. Aquacult Res 40:257–273
Ricardo F, Gonçalves D, Pimentel T, Mamede R, Domingues MRM, Lillebø AI, Calado R (2021) Prevalence of phylogenetic over environmental drivers on the fatty acid profiles of the adductor muscle of marine bivalves and its relevance for traceability. Ecol Indic 129:108017
Safi KA, Hewitt JE, Talman SG (2007) The effect of high inorganic seston loads on prey selection by the suspension-feeding bivalve, *Atrina zelandica.* J Exp Mar Biol Ecol 344:136–148
Sardenne F, Simard M, Robinson SM, McKindsey CW (2020) Consumption of organic wastes from coastal salmon aquaculture by wild decapods. Sci Total Environ 711:134863
Sastry A (1963) Reproduction of the bay scallop, *Aequipecten irradians* Lamarck. Influence of temperature on maturation and spawning. Biol Bull 125:146–153
Soudant P, Marty Y, Moal J, Robert R, Quéré C, Le Coz JR, Samain JF (1996) Effect of food fatty acid and sterol quality on *Pecten maximus* gonad composition and reproduction process. Aquaculture 143:361–378
Thompson R, Ratcliffe N, Bayne B (1974) Effects of starvation on structure and function in the digestive gland of the mussel (*Mytilus edulis* L.). J Mar Biol Assoc UK 54: 699–712
Tocher DR (2015) Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. Aquaculture 449: 94–107
Ventrella V, Pagliarani A, Nesci S, Trombetti F, Pirini M (2013) Dietary enhancement of selected fatty acid biosynthesis in the digestive gland of *Mytilus galloprovincialis* Lmk. J Agric Food Chem 61:973–981
White CA, Dworjanyn SA, Nichols PD, Mos B, Dempster T (2016) Future aquafeeds may compromise reproductive fitness in a marine invertebrate. Mar Environ Res 122: 67–75
White CA, Bannister RJ, Dworjanyn SA, Husa V, Nichols PD, Kutti T, Dempster T (2017) Consumption of aquaculture waste affects the fatty acid metabolism of a benthic invertebrate. Sci Total Environ 586:1170–1181
White C, Bannister R, Dworjanyn S, Husa V, Nichols PD, Dempster T (2018) Aquaculture-derived trophic subsidy boosts populations of an ecosystem engineer. Aquacult Environ Interact 10:279–289
White CA, Woodcock SH, Bannister RJ, Nichols PD (2019) Terrestrial fatty acids as tracers of finfish aquaculture waste in the marine environment. Rev Aquacult 11:133–148
Whyte J, Bourne N, Ginther N, Hodgson C (1992) Compositional changes in the larva to juvenile development of the scallop *Crassadoma gigantea* (Gray). J Exp Mar Biol Ecol 163:13–29
Woodcock SH, Troedsson C, Strohmeier T, Balseiro P, Skaar KS, Strand Ø (2017) Combining biochemical methods to trace organic effluent from fish farms. Aquacult Environ Interact 9:429–443
Yurimoto T, Tanaka K, Nasu H, Matsuoka K (2008) Influence of resuspended sediments and their surface accumulation on a pen shell *Atrina pectinata* in Ariake Bay, west Japan. Aquat Sci 56:335–342
Zheng H, Zhang Q, Liu H, Liu W, Sun Z, Li S, Zhang T (2012) Cloning and expression of vitellogenin (Vg) gene and its correlations with total carotenoids content and total antioxidant capacity in noble scallop *Chlamys nobilis* (Bivalve: Pectinidae). Aquaculture 366–367:46–53

Editorial responsibility: Adam Hughes, Oban, UK
Reviewed by: 2 anonymous referees

Submitted: June 11, 2023
Accepted: December 5, 2023
Proofs received from author(s): March 4, 2024