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Lipid composition of *Mytilus edulis* reared on organic waste from a *Gadus morhua* aquaculture facility

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Abstract – The purpose of this study was to determine biochemical changes occurring in blue mussels (*Mytilus edulis*) fed effluent from an Atlantic cod (*Gadus morhua*) aquaculture facility over a period of ten weeks, compared to those in mussels fed a commercial shellfish diet and those supplied only filtered seawater. The total lipid and fatty acid content (mg g^{-1} wet weight) significantly decreased for mussels fed effluent during the experiment. The only change in the lipid class composition (% total lipid) at the end of the experiment was a significant increase in the proportion of acetone mobile polar lipids. There were several significant changes in the fatty acid composition (% total fatty acid) including an increase in the proportion of 18:1ω9, 18:2ω6, 20:4ω6, 21:5ω3 and the dienoic non-methylene-interrupted fatty acids 20:2a and 22:2b and significant decreases in the proportions of 16:0, 18:4ω3 and 20:5ω3. The increase in non-methylene interrupted dienes suggests that the amount of essential fatty acids in the effluent may be insufficient for optimal mussel growth. The presence of the terrestrial plant marker 18:2ω6 in both the fish feed and the effluent and its increased proportion in mussels fed effluent suggest that this fatty acid may have potential as a marker for aquaculture wastes.

Key words: Integrated multi-trophic aquaculture (IMTA) / Cod / Mussels / Lipid classes / Fatty acids

1 Introduction

Due to growing concern about the environmental stability of aquaculture monoculture practices (Chamberlaine and Rosenthal 1995; Costa-Pierce 1996; Sorgeloos 1999; Naylor et al. 2000; Chopin et al. 2001), integrated multi-trophic aquaculture (IMTA) is being considered as a possible solution. IMTA systems use extractive organisms such as bivalves and algae to reduce the wastes of fed organisms such as finfish. IMTA has three main benefits: increased species diversity, increased profitability and increased acceptability (Ridler et al. 2007). Currently IMTA is being practiced in the Bay of Fundy, where blue mussels (*Mytilus edulis*) are being cultivated alongside Atlantic salmon (*Salmo salar*). The mussels exhibit high growth rates, and are sold commercially (Reid et al. 2008a, 2008b).

The biochemical composition of mussels reflects that of their diet (Khan et al. 2006). Understanding how the biochemical composition of mussels may change in an IMTA setting would aid in understanding the limitations to using mussels in IMTA, such as knowing how much of the natural diet can be substituted with waste material. It could also uncover any potential detrimental or beneficial effects on the mussels or, potentially, for their consumers.

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The aim of this study was to compare the biochemical profile of blue mussels reared on Atlantic cod (*Gadus morhua*) waste with that of mussels fed an algal diet. The rationale was that any change in diet may result in changes to the biochemical composition of mussels, which may in turn change their growth rate and nutritional value for consumers. To do this, the lipid class and fatty acid composition of mussels fed fish waste for a period of ten weeks was compared with that of mussels fed a commercial shellfish diet.

2 Methods

2.1 Experimental setup

Two identical A-frames, each containing three shallow trays (each with a volume of 49 L) were used for this experiment. They were supplied with sand bed-filtered water from Logy Bay NL at a rate of 1.9 L min^{-1}. Each tray contained 160 mussels (4.5 to 6.4 cm shell length) fed three different diets (no food, algae and fish waste). The mussels were fed 1.5% of their dry weight daily.

Effluent was collected from a single tank containing 1-year-old Atlantic cod fed a commercial diet (Skretting Europa). The effluent was passed through a 500 μm screen followed by a 70 μm screen. Dry weight content of the <70 μm

fraction was determined by filtering small amounts through a 1.2 μm Whatman GF/C filter followed by 5 ml of ammonium formate and then drying at 80 °C. Dry weight was used to determine how much effluent was required to provide mussels with 1.5% of their body weight per day. The algal diet was a commercial shellfish diet (Shellfish Diet 1800, Instant Algae® Reed Mariculture) that contained a mix of *Isochrysis* (30%), *Pavlova* (20%), *Tetraselmis* (20%) and *Thalassiosira weissflogii* (30%). Food was added manually to the trays daily and the water supply turned off (to prevent flushing of food) for two hours or until any coloration of the water was removed. Starved mussels received only sand bed filtered sea water.

Every 2.5 weeks, 20 mussels were removed from each tray and sampled for lipid and fatty acid (FA) composition. Replacement mussels marked with nail polish were added to ensure the biomass of mussels remained the same. Food level was adjusted to maintain a ration of 1.5% of the mussel's body weight per day.

2.2 Biochemical analysis

Procedures used to extract and determine lipid content were based on a modified protocol of Folch et al. (1957) described by Parrish (1999). Samples were placed in a mixture of ice cold chloroform:methanol (2:1) and homogenized using a Polytron PCU-2-110 homogenizer (Brinkmann Instruments, Rexdale Ontario, Canada). Chloroform-extracted water was added creating a chloroform:methanol:water ratio of 8:4:3, after which the sample was sonicated in an ice bath for 4 to 10 min. The sample was then centrifuged at 2 000 × g for two minutes and the bottom organic layer removed by double pipetting. Chloroform was added and the entire procedure repeated three more times, pooling the organic layers in a vial previously cleaned of all lipids. Samples were concentrated with a flash-evaporator (Buchler Instruments, Fort Lee, N.J.).

Lipid composition was determined with an Iatroscan Mark V TLC-FID and silica-coated Chromarods, using a three step development. Lipid extracts were applied to the silica rods and then focused into a narrow band using 100% acetone. In the first development system, rods were developed twice, for 25 and then 20 min, in hexane:diethyl ether:formic acid (98.95 :1: 0.05) The second consisted of a 40 min development in hexane:diethyl ether:formic acid (79:20:1) and the third involved two 15 min developments in 100% acetone followed by two 10 min developments in chloroform:methanol:chloroform-extracted water (5:4:1). The rods were dried in a constant humidity chamber before each development and dried and scanned after each development. Peak data was analyzed using PeakSimple 3.72 (SRI Inc.). Calibration standards were obtained from Sigma Chemicals (Sigma Chemicals, St. Louis, Mo., USA).

Lipid extracts were transesterified into fatty acid methyl esters (FAME) in 14% BF_3/MeOH at 85 °C for 1.5 h. FAME composition was determined with an HP 6890 Series GC-FID, equipped with a 7 683 autosampler and a 30 m (0.25 μm internal diameter) ZB wax + column (Phenomenex, USA), using hydrogen as the carrier gas at 2 ml min^{-1}. Column temperature began at 65 °C for 0.5 min and was then ramped to 195 °C at a rate of 40 °C min^{-1} and held for 15 min. Temperature was then ramped to 220 °C at a rate of 2 °C min^{-1} and held for 3.25 min. Injector temperature started at 150 °C and ramped at a rate of 200 °C min^{-1} to a final temperature of 250 °C, while the detector remained constant at 260 °C. Fatty acid retention times were determined with a Supelco 37-component FAME mix (product number 47885-U).

2.3 Statistical analysis

Significance was determined using one-way ANOVAs followed by a Holm-Sidak test, which can be used for both pairwise comparisons and a comparison versus a control group and is more powerful than Tukey or Bonferroni tests. A Kruskal-Wallis one-way analysis of variance on ranks and a Dunn test were performed when data did not meet the criteria of equal variance or normality. Statistical analysis was performed with SigmaStat 2.03 (SPSS Inc.). All results are given as mean ± SD.

3 Results

There were no significant differences in lipid or fatty acid content or lipid class composition at the end of the experiment among mussels fed any of the diets; however, the total lipid content (mg g^{-1} WW) for starved and effluent-fed mussels had significantly decreased by the end of the experiment (Fig. 1). There was only one significant change in terms of lipid class composition (% total lipid) of mussels after ten weeks: both mussels fed algae and those fed fish effluent showed an increase in the proportion of acetone mobile polar lipids (AMPL) (Fig. 2). Although FA content (mg g^{-1} WW) did not differ statistically among mussels fed any of the diets after 10 weeks, the total FA content (mg g^{-1} WW) for starved and effluent fed mussels had significantly decreased by the end of the experiment (Fig. 3).

There were no significant changes in the proportions of individual FAs between starved and algae-fed mussels. Effluent-fed mussels showed several significant changes (Fig. 4), including a decrease in the proportion of the essential FA eicosapentaenoic acid (EPA, 20:5ω3) as well as in the saturate 16:0 (palmitic acid). Effluent-fed mussels also increased in the terrestrial plant marker 18:2ω6, the essential FA arachidonic acid (ARA, 20:4ω6) and the two dienoic non-methylene-interrupted fatty acids (NMID) 20:2a and 22:2b.

To further compare differences among dietary treatments a comparison of mussels fed all three diets was undertaken at the end of the experiment (Fig. 5). Effluent-fed mussels had a significantly larger proportion of 18:1ω9, 18:2ω6 and the two NMIDs 20:2a and 22:2b, as well as a smaller proportion of 20:5ω3 than mussels fed either of the other diets. Effluent-fed mussels also had a larger proportion of 17:1 and 22:5ω3 than algae-fed mussels, and a significantly higher proportion of 20:4ω6 than starved mussels.

When the FA composition was compared quantitatively (mg g^{-1} WW) there was only one difference among the treatments at the end of the experiment (Fig. 6). Starved mussels had significantly less 18:1ω9 than effluent-fed mussels. In effluent-fed mussels, the amounts of 18:0, 18:4ω3, 20:5ω3 and

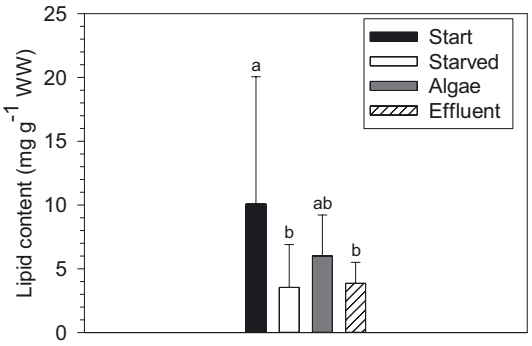

Fig. 1. Total lipid content (mg g^{-1} WW) at the start (n = 18) and end of the experiment for mussels fed three different diets (algae, effluent and no food) (n = 6). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are +1 SD.

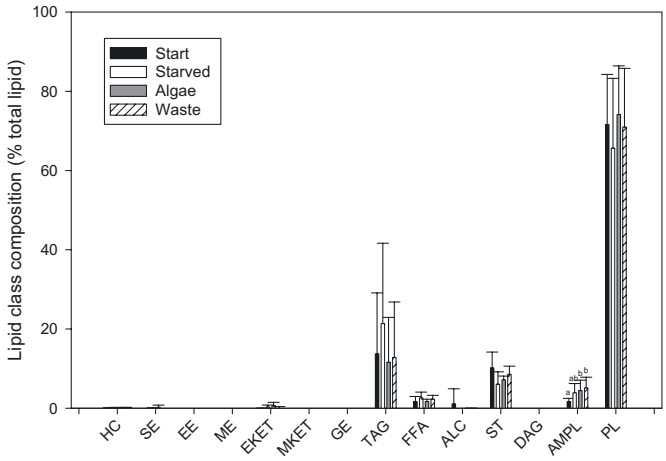

Fig. 2. Lipid class composition (% total lipid) at the start (n = 18) and end of the experiment for mussels fed three different diets (algae, effluent and no food) (n = 6). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are +1 SD.

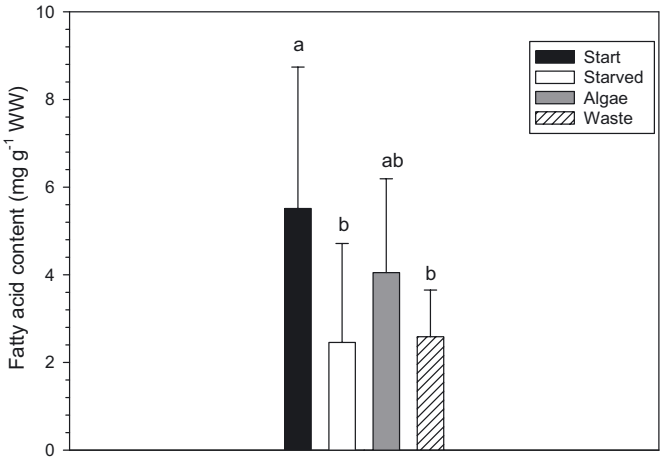

Fig. 3. Total fatty acid content (mg g^{-1} WW) at the start (n = 18) and end of the experiment for mussels fed three different diets (algae, effluent and no food) (n = 6). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are +1 SD.

Table 1. Lipid class composition (% total lipid) of mussels fed three different diets (algae, effluent and no food) at the start of the feeding experiment compared with the lipid class composition of Newfoundland mussels reported by Alkanani et al. (2007).

Lipid class	Alkanani et al. 2007	Feeding trial start
	August	August 11
	n = 4	n = 18
Triacylglycerol	7.8 ± 4.7	13.7 ± 15.4
Free fatty acid	1.0 ± 0.7	1.6 ± 1.3
Sterol	8.0 ± 1.1	10.2 ± 4.0
Acetone mobile polar lipid	5.0 ± 0.9*	1.6 ± 0.8*
Phospholipid	76.0 ± 10.9	71.6 ± 12.7
Total lipid (mg g^{-1} WW)	12.1 ± 1.1	8.0 ± 4.0

* Significant differences between the groups.

22:6ω3 had all significantly decreased by the end of the experiment while the amount of 18:1ω9 had increased.

The lipid class composition of mussels at the beginning of the experiment was compared to the lipid class composition of cultured *M. edulis* in Charles Arm and Fortune Harbour Newfoundland by Alkanani et al. (2007; Table 1). The only significant difference between the lipid profile of mussels used in this experiment and that of these cultured Newfoundland mussels was a larger proportion of AMPL in the cultured mussels.

The lipid class compositions of mussels fed all three diets were again compared to mussels from Charles Arm and Fortune Harbour NL at the end of the experiment (Table 2). The lipid class compositions were similar, although the total lipid content for all three treatments was lower than the values recorded by Alkanani et al. (2007).

The FA composition of mussels at the beginning of the experiment was also compared to that of mussels from Charles Arm and Fortune Harbour NL (Table 3). In general, the FA composition of mussels used in this experiment fell between the two values for cultured mussels, although there was a smaller proportion of EPA and 18:0 in the cultured mussels.

When, at the end of the experiment, the FA compositions of mussels fed the three diet treatments were compared to the proportions in cultured mussels from Charles Arm and Fortune Harbour NL, several differences were found (Table 3). Both starved and algae-fed mussels had a significantly larger proportion of 20:5ω3 than cultured mussels from Charles Arm and Fortune Bay, while the value for effluent-fed mussels fell between the two. Mussels fed effluent had a significantly larger proportion of 18:1ω9 than cultured mussels. Effluent fed mussels had a significantly smaller proportion of 16:0 and the essential FA docosahexaenoic acid DHA (22:6ω3) than the farmed mussels; however, effluent fed mussels also had a significantly larger proportion of 20:4ω6 than cultivated mussels and starved and algae-fed mussels. Effluent-fed mussels had a significantly larger proportion of 20:2a, 22:2b and the terrestrial plant marker 18:2ω6. Values for the flagellate marker 18:4ω3 were significantly lower in both algae-fed and effluent-fed mussels than values reported in the literature.

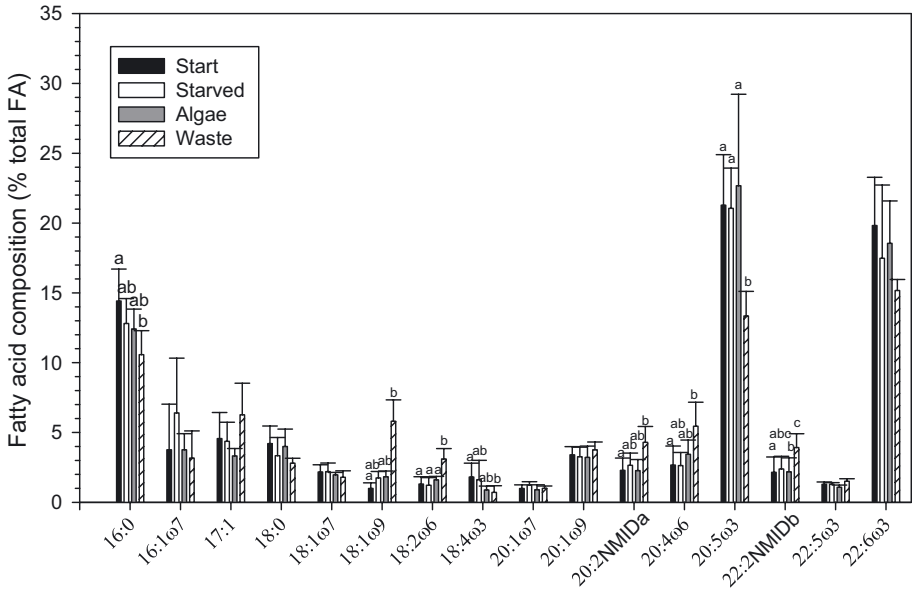

Fig. 4. Fatty acid composition (% of total FAs) at the start (n = 18) and end of the experiment for mussels fed three diets (algae, effluent and no food) (n = 6). Only FA comprising >0.9% total FA are shown. Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are +1 SD.

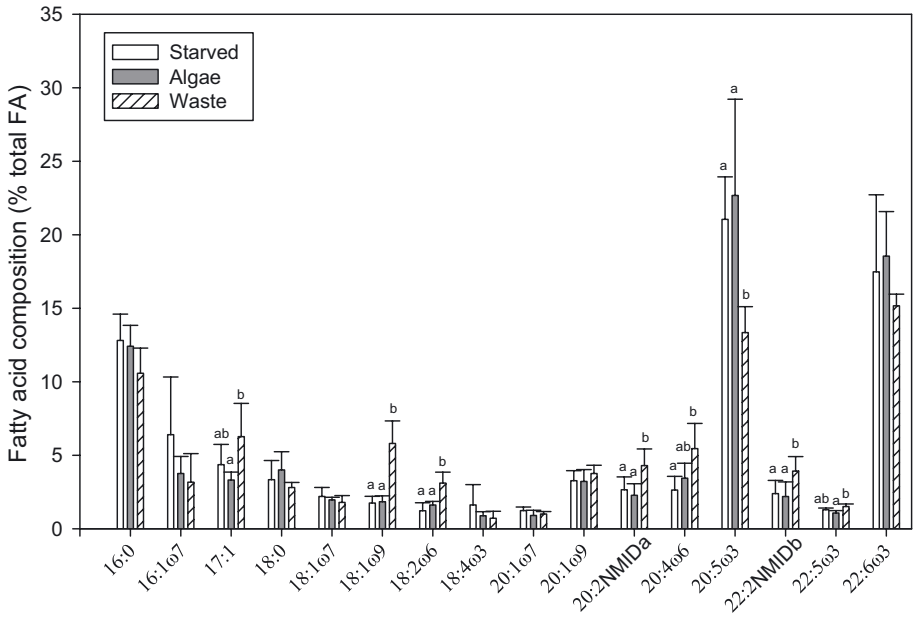

Fig. 5. Fatty acid composition (% total FA) for mussels fed three different diets (algae, effluent and no food) at the end of a ten week experiment (n = 6). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are +1 SD.

The amount of 16:0 present in mussels fed any of the diets in this experiment was lower than the values for mussels maintained on salmon feed recorded by Redmond et al. (2010; Table 4). The amount of the terrestrial marker 18:2ω6 was higher in salmon feed supplemented mussels than in those fed any of the diets except for effluent. The amount of the essential FA EPA was generally higher in mussels used in this experiment than in those of Redmond et al. (2010), with the exception of the mussels fed effluent. The amount of 20:4ω6 was greater in effluent-fed mussels than in starved mussels and those supplemented with salmon feed.

4 Discussion

The decrease in lipid content (mg g^{-1} WW) in starved mussels was unsurprising, but the fact that this occurs in effluent-fed mussels and not for algae-fed mussels suggests that effluent may be an inferior diet.

The lack of differences among the three treatments suggests that effluent did not affect the lipid class composition of mussels. The lower lipid content of mussels fed all diets in this experiment in comparison to mussels from Charles Arm and Fortune Harbour NL (Alkanani et al. 2007) suggests that the

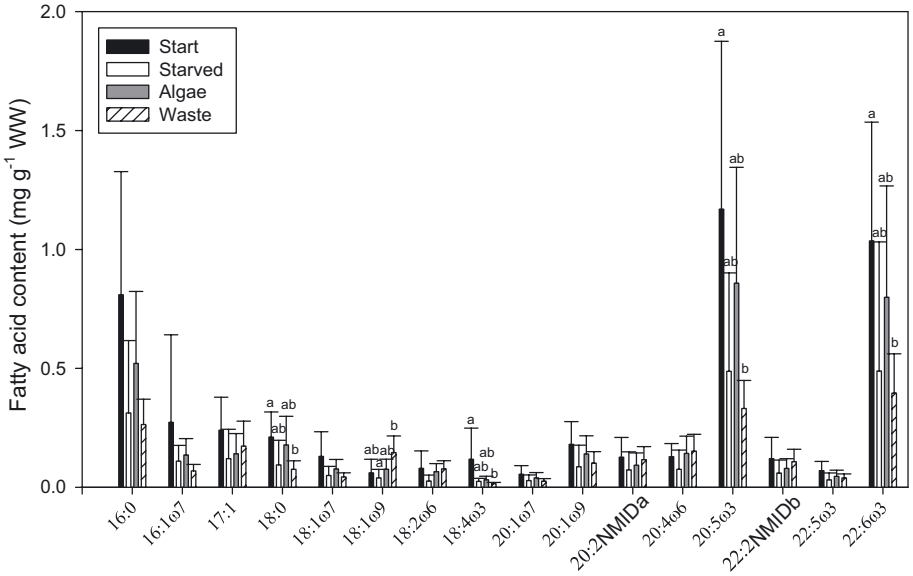

Fig. 6. Fatty acid composition (mg g^{-1} WW) at the start (n = 18) and end of the experiment after for mussels fed three diets (algae, effluent and no food) (n = 6). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are +1 SD.

Table 2. Lipid class composition (% total lipid) of mussels fed three different diets (algae, effluent and no food) at the end of the feeding experiment compared with the lipid class composition of Newfoundland mussels reported by Alkanani et al. (2007). Groups with different letters are significantly different from each other.

Lipid class	Alkanani et al. 2007				Feeding trial end		
	June	Aug.	Sept.	Oct.	Starved	Algae	Effluent
	n = 4	n = 4	n = 4	n = 4	n = 6	n = 6	n = 6
Triacylglycerol	38.6 ± 9.7[a]	7.8 ± 4.7[b]	11.8 ± 2.1	15.7 ± 7.4	21.4 ± 20.3	11.6 ± 11.3[b]	12.8 ± 14.0
Free fatty acid	2.0 ± 0.6	1.0 ± 0.7	1.0 ± 0.5	2.3 ± 1.2	2.6 ± 1.5	1.7 ± 0.6	2.3 ± 1.0
Sterol	5.7 ± 0.7	8.0 ± 1.1	6.9 ± 0.5	8.5 ± 1.3	6.1 ± 3.1	7.2 ± 0.9	8.6 ± 2.0
Acetone mobile polar lipid	3.9 ± 1.3	5.0 ± 0.9	7.2 ± 3.1	8.1 ± 7.8	3.9 ± 2.3	4.4 ± 2.6	5.1 ± 2.7
Phospholipid	48.3 ± 2.7	76.0 ± 10.9	68.8 ± 11.1	62.8 ± 7.6	65.6 ± 17.6	74.1 ± 12.3	71.0 ± 14.8
Total lipid (mg g^{-1} WW)	26.6 ± 3.0[a]	12.1 ± 1.1[b]	12.3 ± 1.4[b]	13.9 ± 1.2[b]	3.5 ± 3.4[c]	6.0 ± 3.2[c]	3.9 ± 1.6[c]

nutritional requirement of the mussels was not being met. This is further supported for the starved mussels, which showed a decrease in lipid content over the course of the experiment. Although the 1.5% soft tissue DW day^{-1} fed to the mussels was within the range (0.6 to 2.8% soft tissue DW day^{-1}) recommended for body maintenance of *M. edulis* by Hawkins et al. (1985), it is possible that this ration did not meet the nutritional requirements of the mussels.

The decrease in total FA (mg g^{-1} WW) for starved mussels was also unsurprising. The decrease for effluent-fed mussels contrasted with the lack of a similar decrease for algae-fed mussels again suggests that effluent may be an inferior diet.

The lower amount of the essential FA EPA and the higher proportions of the NMIDs 20:2a and 22:2b in effluent-fed mussels compared with those from Charles Arm and Fortune Harbour suggest that effluent-fed mussels were nutritionally stressed, since NMIDs are synthesized by bivalves to replace essential FAs (Klingensmith 1982; Pond et al. 1998; Zhukova 1991; Prato et al. 2010). NMIDs have been seen to correlate negatively with growth in *M. edulis* (Alkanani et al. 2007). Another explanation for the higher levels of NMIDs in effluent-fed mussels may be the role they play in resistance to microbial lipases (Irazu et al. 1984; Pirini et al. 2007).

The higher level of the essential FA 20:4ω6 was most likely caused by selective retention, which can occur under stress conditions (Pirini et al. 2007). The terrestrial plant marker 18:2ω6 was present in the fish feed as well as the effluent and was also found in the effluent-fed mussels. These observations are consistent with those of Redmond et al. (2010), who found that 18:2ω6 increased when mussels were fed salmon feed and suggested that 18:2ω6 may could potentially serve as a marker for aquaculture wastes. A similar trend was discovered for two fish species, *Trachurus mediterraneus* off the shore of Spain and *Pollachius virens* in fjords in northern Norway, which were both found to accumulate 18:2ω6 when exposed to aquacultural wastes such as feed pellets (Fernandez-Jover et al. 2007; Skog et al. 2003).

5 Conclusion

Significant decreases in concentrations of 20:5ω3 and 22:6ω3 in effluent-fed mussels suggest that the effluent was

Table 3. Fatty acid composition (% total FA) of mussels fed three different diets (algae, effluent and no food) at the beginning and end of the feeding experiment compared with the FA composition of Newfoundland mussels reported by Alkanani et al. (2007). Groups with different letters are significantly different from each other.

Fatty acid	Alkanani et al. 2007		Feeding trial start	Feeding trial end		
	2000	2001	Aug. 11	Starved	Algae	Effluent
	$n = 67$	$n = 75$	$n = 18$	$n = 6$	$n = 6$	$n = 6$
16:0	13.6 ± 1.31	13.7 ± 1.49	14.4 ± 2.28	12.8 ± 1.79	12.4 ± 1.43	10.6 ± 1.72^{b}
18:0	3.20 ± 0.66^{a}	3.00 ± 0.85^{a}	4.20 ± 1.26^{b}	3.34 ± 1.30	4.00 ± 1.24	2.81 ± 0.35
16:1ω7	3.96 ± 1.97^{a}	5.81 ± 3.76^{b}	3.76 ± 3.27^{a}	6.40 ± 3.92	3.76 ± 1.16	3.17 ± 1.94
18:1ω7	1.64 ± 0.6^{a}	2.25 ± 0.64^{b}	2.17 ± 0.52^{c}	2.20 ± 0.62	1.97 ± 0.17	1.79 ± 0.46
18:1ω9	1.02 ± 0.64^{a}	1.33 ± 0.44^{b}	1.00 ± 0.39^{a}	1.75 ± 0.46^{b}	1.83 ± 0.41^{b}	5.81 ± 1.53^{c}
18:2ω6	1.41 ± 0.47	1.61 ± 0.83	1.30 ± 0.53	1.22 ± 0.55^{a}	1.61 ± 0.24^{a}	3.11 ± 0.74^{b}
18:4ω3	2.12 ± 1.47^{a}	3.67 ± 2.16^{b}	1.82 ± 0.99^{a}	1.62 ± 1.38	0.88 ± 0.28^{b}	0.71 ± 0.47^{b}
20:1ω9	3.05 ± 0.73^{a}	2.45 ± 1.68^{b}	3.40 ± 0.58^{a}	3.27 ± 0.68	3.22 ± 0.81	3.76 ± 0.56
20:2a	3.13 ± 0.68^{a}	0.75 ± 1.14^{b}	2.29 ± 0.88^{c}	2.66 ± 0.87^{a}	2.27 ± 0.79^{a}	4.30 ± 1.13^{c}
20:4ω6	2.76 ± 0.82	2.85 ± 1.16	2.67 ± 1.36	2.63 ± 0.94^{a}	3.44 ± 1.02^{a}	5.45 ± 1.71^{b}
20:5ω3	12.0 ± 2.21^{a}	17.0 ± 3.49^{b}	21.3 ± 3.62^{c}	21.0 ± 2.88^{c}	22.7 ± 6.55^{c}	13.3 ± 1.77^{d}
22:2b	3.01 ± 0.73^{a}	2.49 ± 1.12^{b}	2.15 ± 1.09^{b}	2.39 ± 0.90^{a}	2.19 ± 0.99^{a}	3.93 ± 0.98^{b}
22:6ω3	21.5 ± 2.88	20.0 ± 4.28	19.8 ± 3.45	17.5 ± 5.24	18.5 ± 3.04	15.2 ± 0.79^{b}

Table 4. Fatty acid composition (% total FA) of mussels fed three different diets (algae, effluent and no food) compared with the FA composition of mussels whose diet was supplemented with salmon feed (Redmond et al. 2010).

Fatty acid	Redmond et al. 2010		Feeding experiment			
	Control	Supplemented	Start	Starved	Algae	Effluent
	$n = 7$	$n = 7$	$n = 18$	$n = 6$	$n = 6$	$n = 6$
16:0	22.4 ± 2.14^{a}	21.5 ± 0.70^{a}	14.4 ± 2.28^{b}	12.8 ± 1.79^{bc}	12.4 ± 1.43^{bc}	10.6 ± 1.72^{c}
18:0	3.49 ± 1.35	4.14 ± 1.49	4.20 ± 1.26	3.34 ± 1.30	4.00 ± 1.24	2.81 ± 0.35
16:1ω7	8.64 ± 5.61	4.15 ± 3.83	3.76 ± 3.27	6.40 ± 3.92	3.76 ± 1.16	3.17 ± 1.94
18:1ω7	2.85 ± 0.24^{ab}	3.23 ± 0.22^{a}	2.17 ± 0.52^{c}	2.20 ± 0.62^{b}	1.97 ± 0.17^{c}	1.79 ± 0.46^{c}
18:1ω9	3.09 ± 1.77^{ac}	7.19 ± 3.43^{b}	1.00 ± 0.39^{c}	1.75 ± 0.46^{c}	1.83 ± 0.41^{c}	5.81 ± 1.53^{b}
18:2ω6	2.39 ± 2.48^{ac}	4.56 ± 0.88^{bc}	1.30 ± 0.53^{a}	1.22 ± 0.55^{a}	1.61 ± 0.24^{ac}	3.11 ± 0.74^{c}
18:4ω3	1.85 ± 0.86	0.81 ± 0.27	1.82 ± 0.99	1.62 ± 1.38	0.88 ± 0.28	0.71 ± 0.47
20:1ω9	3.13 ± 0.78^{a}	6.28 ± 0.96^{b}	3.40 ± 0.58^{a}	3.27 ± 0.68^{a}	3.22 ± 0.81^{a}	3.76 ± 0.56^{a}
20:4ω6	3.12 ± 1.24^{a}	2.88 ± 0.89^{a}	2.67 ± 1.36^{a}	2.63 ± 0.94^{a}	3.44 ± 1.02^{ab}	5.45 ± 1.71^{b}
20:5ω3	16.1 ± 2.73^{ac}	16.7 ± 2.63^{abc}	21.3 ± 3.62^{b}	21.0 ± 2.88^{ab}	22.7 ± 6.55^{b}	13.3 ± 1.77^{c}
22:6ω3	16.3 ± 3.23	15.3 ± 2.64	19.8 ± 3.45	17.5 ± 5.24	18.5 ± 3.04	15.2 ± 0.79

lacking in essential FAs. It is likely that the FA profile of fish effluent is inadequate to support optimum mussel growth when used as the sole diet. In contrast *M. edulis* has been reported to have increased growth rates in an integrated aquaculture setting (Reid et al. 2008a). Although this effluent is deficient in FAs it may be used as a replacement food source when a better diet is unavailable (Stirling and Okumus 1995), which may explain in part the enhanced growth reported for mussels in an open-water setting IMTA in the Bay of Fundy, Canada (Reid et al. 2008a).

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