ASSESSING THE PERFORMANCE OF THE BLUE MUSSEL (*Mytilus edulis*) IN AN INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) SETTING

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Assessing the performance of the blue mussel (*Mytilus edulis*) in an integrated multi-trophic aquaculture (IMTA) setting

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Abstract

Integrated multi-trophic aquaculture (IMTA) uses extractive organisms such as mussels to reduce wastes of fed organisms such as finfish and provide additional product for the growers. The physical and biochemical properties of Atlantic cod (*Gadus morhua*) wastes (feces and uneaten feed) were analyzed and the waste remediation potential and performance (growth and biochemical composition) of blue mussels (*Mytilus edulis*), were assessed. Waste generated daily by Atlantic cod represented 24.9% of the feed added to the system. Effluent was composed of particles <70 μm (36%), 70-500 μm (31%) and particles >500 μm (33%). Particles <70 μm had significantly less organic matter, lipids and fatty acids and were expected to be ingested more frequently by mussels. Effluent contained the fatty acid zooplankton markers (22:1ω11 and 20:1ω9) which accumulated in mussels. Effluent fed mussels had an inferior performance and contained significantly more MUFA, 18:1ω9 and the NMID 20:2a as well as less ω3 than algae fed mussels. It is believed aquaculture wastes have potential as a diet supplement when natural seston is low.

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List of Abbreviations and symbols

ω3 – Omega 3

ω6 – Omega 6

AA – Amino acid

AAA – α-Aminoadipic acid

aILE – Allo-Isoleucine

ALA – Alanine

ALC – Alchohol

AMPL – Acetone mobile polar lipids

APA – α-Aminopinelic acid

ARG – Arginine

ASP – Aspartic acid AFDW – Ash free dry weight

ASN – Asparagine

CFIA – Canadian food inspection agency

CI – Condition index

DHA – Docosahexaenoic acid (22:6 ω3)

DAG – Diacylglycerol

DOC – Dissolved organic carbon

DON – Dissolved organic nitrogen

DPA – Docosapentaenoic acid

DW – Dry weight

EPA – Eicosapentaenoic acid (20:5ω3)

EE – Ethyl ester

EKET – Ethyl ketone

FA – Fatty acid

FAME – Fatty acid methyl ester

FFA – Free fatty acids

GE – Glycerol either

GLN – Glutamine

GLU – Glutamic acid

GLY – Glycine

GPR – Glycine-proline (dipeptide)

HC – Hydrocarbons

HIS – Histidine

HLY – Hydroxylysine

HYP – Hydroproline

IMTA – Integrated multi-trophic aquaculture

ILE – Isoleucine

LEU – Leucine

LYS – Lysine

ME – Methyl ester

MET – Methionine

MKET – Methyl ketones

MUFA – Monounsaturated fatty acid

NMID – Non methylene-interrupted diene

n-3 – Omega 3

n-6 – Omega 6

PHE – Phenylalanine

PHP – Proline-hydroxyproline (dipeptide)

PL – Phospholipids

POM – Particulate organic matter

PRO – Proline

PUFA – Polyunsaturated fatty acid

SE – Steryl ester

SER – Serine

SFA – Saturated fatty acid

SL – Shell length

ST – Sterol

TAG – Triacylglycerol

THR – Threonine

TN – Total nitrogen

TYR – Tyrosine

VAL – Valine

WW – Wet weight

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1. Introduction

The aquaculture industry has shown tremendous growth, doubling in size over the last decade from a global value of 27 million USD in 1990 to 53 million USD in 2000 (FAO 2001). Intensive growth of fish in net pens and shrimp in coastal lagoons has been economically successful; however, it does not address water treatment and could cause the nitrification of surrounding waters (Neori *et al.* 2004). This can produce many negative effects on the environment such as eutrophication, oxygen depletion, biodiversity changes and pollution (Gowen and Bradbury 1987; Braaten *et al.* 1988; Ronnberg *et al.* 1992; Beveridge *et al.* 1994; Richardson and Jorgensen 1996; Bonsdorff *et al.* 1997; Mattila and Raisanen 1998; Pitta *et al.* 1999; Hanninen *et al.* 2000; Naylor *et al.* 2000; Troell *et al.* 2003). There is growing concern that such monoculture practices are not environmentally sustainable (Chamberlaine and Rosenthal 1995, Costa-Pierce 1996, Sorgeloos 1999, Naylor *et al.* 2000, Chopin *et al.* 2001).

To cope with nitrification of waters caused by intensive culture, some form of water treatment must be done. Two main ways for this involve either bacterial dissimilation or plant assimilation (Neori *et al.* 2004). Bacterial dissimilation uses bacterial biofilters that through several oxidative and reduction reactions, reduce wastes into harmless gases such as nitrogen and carbon dioxide (Neori *et al.* 2004). These filters are very efficient (van Rijn 1996), however, they require recirculation of water which makes them only applicable to small land based operations which grow high value species due to their high cost of operation (Zucker and Anderson 1999).

Plant assimilation uses photosynthetic organisms (e.g. algae) to absorb and assimilate excess nutrients in the water which are then, through photosynthesis, added to the plant's biomass (Neori *et al.* 2004). Using this type of system photosynthetic plants will remove excess nutrients from the fish and may also be able to balance the effects of pH, oxygen and carbon dioxide from the fish (Hirata *et al.* 1994). It has been speculated that algae and seaweeds would be best used for this process because they have a very high productivity and can be economically valuable (Gao and McKinley 1994).

Others have argued that aquaculture practices should focus on the use of extractive organisms to bioremediate the wastes created from fed organisms to create a more sustainable form of aquaculture. This is the basis for integrated multi-trophic

aquaculture, examples of which include the development of integrated cultures of salmon and kelps (Chopin *et al.* 2001) or red algae (Buschmann *et al.* 2001). To make this work fish or shrimp (fed organisms) would be cultured alongside extractive organisms (e.g. shellfish) which would extract particulate wastes for food, as well as algae or seaweed which would extract dissolved inorganic nutrients for use in photosynthesis (Troel *et al.* 2003). In such a system the normal waste sources become inputs for the other systems which thus increase productivity. Integrated multi-trophic aquaculture has three benefits, increased diversity, increased profitability and increased acceptability (Ridler *et al.* 2007).

There have been some attempts to put this rationale to use in land based systems. One such system is SeaOr Marine Enterprises which is located on the Israeli Mediterranean coast which cultures gilthead seabream along with two seaweeds *Ulva* and *Gracilaria*, as well as abalone (Neori *et al.* 2004). Neori *et al.* (2000) studied an experimental land based system that involved abalone grown with the fish *Sparus aurata* along with two algae *Ulva lactuca* and *Gracilaria conferta*. The algae were able to significantly reduce the levels of ammonia in waters leaving the fish culture, and had high growth rates. Surplus algae was used to support the growth of abalone. They speculated that by using only *Ulva lactuca* in the culture, the pollution could have been further reduced and the growth of abalone could have been doubled.

From these two examples it can be seen that land based integrated aquaculture systems are feasible. There is potential for such systems to be used in open water. After a six year pilot study successfully showed proof of concept for the culture of blue mussels as well as kelp around salmon cages, a five year project was begun in 2006 to further develop a commercial IMTA in the Bay of Fundy (Reid *et al.* 2008)

Integrated aquaculture is not, however, a completely new idea; more traditional forms of integrated aquaculture have been practiced primarily in China, Japan and South Korea (Neori *et al.* 2004). These systems usually involved the growth of fish in net pens along with shellfish and seaweed, and were done in local bays or lagoons with optimization being done by trial and error (Neori *et al.* 2004). These traditional practices of integrated aquaculture should be seen as good examples of how such systems are possible and, when today's technology is applied, are able to become a more

environmentally sustainable practice while providing a safe food source (Haya *et al.* 2004; Barrington *et al.* 2009).

As mentioned above, one aspect of integrated aquaculture is an extractive organism that is commonly some form of shellfish. One shellfish that can be used is mussels, as illustrated by the use of M*ytilus edulis* in the pilot scale tests in the Bay of Fundy (Reid *et al.* 2008). Bioremediation effects associated with mussels in Sweden can be large and are thought to be cost effective (Lindahl *et al.* 2005.)

1.1 Objectives

The objective of this project was to evaluate the potential of wastes from an onshore aquaculture site growing juvenile *Gadus morhua* as a food source for *M. edulis*. To do this the physical properties of the effluent, such as the amount being generated, the size distribution of particles and settling rates were examined to determine the availability for potential mussel ingestion. Biochemical aspects (lipid content and fatty acid content) of the effluent were also examined to evaluate the nutritional value for mussels. The presence of biomarkers indicative of fish waste was determined in mussels offered waste in order to see if ingestion occurs.

The second objective of this project was to determine the performance of *M. edulis* when reared on wastes generated from an onshore *Gadus morhua* aquaculture site. To assess this, the physical characteristics of mussels (shell length, dry weight, ash-free dry weight and condition index) as well as the biochemical characteristics (C/N, protein, lipid profile, fatty acid profile, and amino acid profile) of mussels fed fish waste were compared to that of mussels fed an algal diet.

2. Physical and biochemical properties of effluent leaving an onshore Atlantic cod (*Gadus morhua*) aquaculture facility and potential use in integrated multi-trophic aquaculture (IMTA).

2.1 Introduction

Reid *et al.* (2008a) highlighted the need for data on the physical properties of salmonid faeces such as particle distribution, settling rates and mass fraction for use in IMTA settings. Troell *et al.* (2009) reported that particulate organic concentrations are the most important factor to consider in determining the growth rates of mussels in an IMTA setting. Four main constraints associated with using mussels to reduce fish farm wastes have been identified (Troell and Norberg 1998; Troel *et al* 2009) which include: dilution of suspended solids, settling of particulates from cages, variable effects on feeding duration and intensity, and finally total particle retention by mussels. It is therefore important to determine the physical properties of particulate matter leaving finfish aquaculture sites in order to deal with these various issues.

Absorption efficiency of mussels fed salmon feed and faeces was affected by the organic content of the particulates (Reid *et al.* 2008a). Another factor that should be considered is the biochemical composition of aquaculture wastes. If the nutritional requirements are not met within a system, growth will be impaired. Not only should the nutritional requirements of organisms be taken into account but also how the nutritional values of organisms will be affected in such systems. The ultimate goal of aquaculture is to provide a marketable source of food or other biological products for humans. Therefore, it is important that such sources be nutritionally sound for people ingesting them.

Blue mussels (*Mytilus edulis*) require large amounts of polyunsaturated fatty acids (PUFA) in their diets (Budge *et al.* 2001) with emphasis on the n-6 and n-3 long chain fatty acids (Berntsson *et al.* 1997; Utting and Millican 1997). Mussel fatty acids are comprised of 30% eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (Budge *et al.* 2001). The optimal ratio of n-3/n-6 long chain PUFA is between 5 and 15 for bivalve growth (Joseph 1982, 1989; Whyte *et al.* 1991; Napolitano *et al.* 1992; Thompson *et al.* 1993; Pazos *et al.* 1997; Budge *et al.* 2001). Mussels also have the ability to selectively retain and accumulate certain essential fatty acids such as

arachidonic acid (20:4n-6) which was 5 times higher in mussels than the food given (Budge *et al.* 2001).

A study on the mussel *Mytilus galloprovincialis* showed that diet and temperature could play potential roles in the fatty acid profile of mussels (Freites *et al.* 2002). There are seasonal effects on the composition and quality of blue mussel meat, both farmed and wild (Slabyj *et al.* 1977), and seasonal variations in meat composition affects the quality of the final cooked product (Krzynowek and Wiggin 1979). Khan *et al.* (2006a) found that the fatty acid profile of an algal diet directly affected the fatty acid profile of mussels in different seasons. During April-June mussels were rich in EPA and from August-October they were rich in DHA; the two had opposite trends, as one increased the other would decrease.

If the fatty acid profile of mussels is reflected by their diet it is important to know the fatty acid profile of the diet being fed. Therefore in the context of an integrated aquaculture setting the biochemical profile of the wastes being generated and used as a food source for other organisms should be described and better understood.

It is known that large proportions of solids filtered by *M. edulis* can be rejected as pseudofeces (Foster-Smith 1975). The rate at which particles are rejected as pseudofeces can be affected by diet. For example, in response to particles with a low particulate organic matter (POM) content, mussels increased their filtration rate as well as changed the proportion of particles that were rejected as pseudofeces (Bayne *et al.* 1993). It is therefore important to establish that mussels will actively ingest particulates in fish waste and not reject it as pseudofeces while ingesting more appealing particles.

Some fatty acids synthesized at low trophic levels can be transferred up the food web and be used as biomarkers (Napolitano *et al.* 1997). Three fatty acids associated with zooplankton (20:1ω9, 22:1ω9 and 22:1ω11) were found to be useful biomarkers for the dispersion of wastes from fish farms (Biesen and Parrish 2005). The presence of these fatty acids can potentially be used to assess if mussels ingest fish wastes or if they are simply rejected as pseudofeces. Herring which can be used to formulate feed contain 20:1ω9, 22:1ω11 and 22:1ω9 which comprise 15.3±6.0%, 23.1±9.9% and 1.4±0.8% of total FA (Biesen and Parrish 2005).

Based on the above, understanding the physical properties of effluent leaving finfish farms is important to understand what fraction of effluent will be available for mussel ingestion and determine what mussels will ingest/reject. The biochemical properties are also crucial to understand how mussels will perform with a fish effluent diet and understanding what possible biochemical changes within the mussels themselves we can expect as a result. The objective of this study is to quantify the amount of wastes leaving a aquaculture facility and to determine the biochemical composition of said wastes.

2.2 Methods

2.2.1 Sampling

Effluent from an onshore aquaculture facility growing juvenile Atlantic cod was collected over a two week period from six different tanks. Each of the six tanks contained on average 667 juvenile (1 year old) cod that had an average weight of 88.5 g and represented an average biomass of 59.6 kg. Tanks drained from the bottom so that any particulate waste from fish and any uneaten feed particles were collected. The tanks had stand-pipes which restricted the amount of particulates capable of passing through the drain (Fig. 2.1). The stand-pipes were pulled on a daily basis to dislodge accumulated waste, which resulted in the expulsion of a large amount of particulate matter over a very short period. To account for this, sampling was done at three different times of day, one hour prior to pulling the stand-pipe (pre-flush, T-1h), 1 hour after the pipe had been pulled (post-flush, T+1h), 12 hours after the pipe had been pulled (mid-way between flushes, T+12h) and while the pipe was pulled (flush, T0h). These time periods were sampled to account for the total amount of wastes produced daily, and also to determine if there were any qualitative differences among the wastes generated.

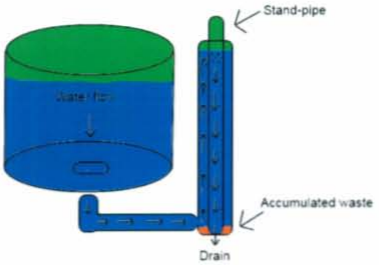

Fig. 2.1. Diagram of water flow through the stand-pipe of tanks that held juvenile Atlantic cod in an onshore aquaculture facility.

The tanks were supplied natural sea water from Logy Bay which was passed through a 50 μm sand-bed filter. In order to fully quantify the amount of waste generated by fish the influent was also sampled. Due to the sand-bed filter all particles present in the influent should be <50 μm, therefore the amount of solids (as a weight) present in the influent was removed from the <70 μm size fraction total weight. Fish were fed, on average, 391.1 g of feed daily which was taken into account when determining the amount of solids entering and leaving the tanks.

Samples were collected by attaching a flexible hose to the drain of the tanks which was then used to fill up plastic buckets. Effluent was passed through two screens (500 μm and 70 μm) to create three size fractions >500 μm, 70-500 μm and <70 μm. Each of these fractions were sampled for their dry weight content, ash free dry weight, particle size distribution, settling rate, dissolved organic carbon (DOC), total nitrogen (TN), lipid class profile and fatty acid profile.

2.2.2 Particle analysis and weight determination

Dry weights were obtained by passing a known quantity of effluent through a pre-combusted and weighed 1.2 μm GF/C filter. Ammonium formate was then passed through the filter to remove salts. Samples were dried overnight at 80°C, weighed and then combusted at 400°C overnight and weighed again to determine ash free dry weights. Particle sizes and distributions were determined using a Coulter Multisizer with a 100 μm aperture tube as well as image analysis with Image ProPlus for particles too large to be analyzed via the Coulter Multisizer.

2.2.3 Settling rates

Settling rates were determined by placing a small amount of effluent into graduated cylinders (36 cm for particles >500 μm and 18 cm for <70 μm) containing sea water at a constant temperature. The time for the first particle to settle to the bottom of the cylinder (visible with naked eye) was recorded to determine the most rapid settling rate. The effluent was left to settle for known periods of time (5, 10 and 15 min for >500 μm and 2, 3, 5 and 22.5 hr for <70 μm), and any remaining suspended particles were filtered onto a GF/C filter. Particulates that settled on the bottom were also collected. The proportion of settled particles to unsettled was used to determine variable settling rates within a given size fraction.

2.2.4 Lipid analysis

Procedures used to extract and determine lipid content were based on Parrish (1999). Samples were placed into 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 minutes. The sample was then centrifuged at 5000 rpm for two minutes and the bottom organic layer removed by double pipetting technique. Chloroform was added and the entire procedure repeated three more times, pooling the organic layers together in a lipid cleaned vial. Samples were then concentrated using a flash-evaporator (Buchler Instruments, Fort Lee, N.J.).

Lipid composition was determined using an Iatroscan Mark V TLC-FID and silica coated Chromarods using a three step development method. Lipid extracts were applied to silica rods and focused into a narrow band using 100% acetone. In the first development system, rods were developed twice for 25 and 20 minutes respectively in hexane:diethyl ether:formic acid (98.95:1:0.05) The second development system consisted of a 40 minute development in hexane:diethyl ether:formic acid (79:20:1). The third development system involved two 15 minute developments in 100% acetone followed by two 10 minute developments in chloroform:methanol:chloroform-extracted water (5:4:1). The rods were dried in a constant humidity chamber before each development and were dried and scanned after each development system. Peak data was

analyzed using PeakSimple 3.72 (SRI Inc.). Standards from Sigma Chemicals (Sigma Chemicals, St. Louis, Mo., USA) were used to calibrate the Chromarods.

Lipid extracts were transesterified into fatty acid methyl esters (FAME) in 14% BF_3/MeOH at 85°C for 1.5 hours. FAME composition was determined using a HP 6890 Series GC FID equipped with a 7683 autosampler and a 30 m (0.25 µm internal diameter) ZB wax+ column (Phenomenex, USA) using hydrogen as the carrier gas at 2ml/min. Column temperature began at 65°C for 0.5 minutes then ramped to 195°C at a rate of 40°C/min and held for 15 minutes. Temperature then ramped to 220°C at a rate of 2°C/min and held for 3.25 minutes. Injector temperature started at 150°C and ramped at a rate of 200°C/min until reaching a final temperature of 250°C, while the detector remained a constant 260°C. Fatty acid retention times were determined with Supelco, 37 component FAME mix (Product number 47885-U).

DOC and TN values were obtained from a Shimadzu TOC-VCPH. Samples were acidified to pH 2 with 2M HCl (ACS grade with organic carbon <0.05%) prior to analysis to remove inorganic carbon. CHN content was determined using a Perkin Elmer Series II. Samples were dried overnight at 80°C, weighed and then fumed in an HCl bath for 24 hours and redried for 24 hours. Samples were then pelletized and placed back in the oven until being run on the analyzer.

2.2.5 Statistical analysis

Significance was determined using one way ANOVAs followed by Holm-Sidak tests to determine where those significances laid. Kruskal-Wallis one way analysis of variance on ranks and a Dunn`s Method test was performed when data failed the assumption of equal variance or normality. No transformations were done to data prior to analysis. Statistical analysis was performed using SigmaStat 2.03 (SPSS Inc.). All results are given as mean ± SD.

2.3 Results

2.3.1 Dry weight

The influent which fed all tanks delivered 97.8±19.3 g/day of solid matter. The amount of <70 µm particulates leaving the cod tanks did not vary among the four sampling periods (Table 2.1). Although the flush generally generated much less waste daily the difference was not statistically significant due to large standard deviations.

Table 2.1. Amount of solid waste (g DW/day) leaving cod tanks at various sampling times (n = 6 tanks). Different letters (abc) denote significant differences among sampling times and (xyz) significant differences among size fractions (Holm-Sidak p <0.05).

Sampling period	<70 μm	70-500 μm	>500 μm
Flush	4.90±0.94	0.16±0.03^{ax}	8.04±2.26^{ay}
Pre-flush	29.7±16.7	24.3±7.9	21.3±9.1
Post-flush	31.8±23.9	37.4±14.2^{b}	25.4±10.5^{b}
Mid-way between flushes	24.6±37.9	26.0±8.6	27.6±16.9^{b}

The passive flow sampling periods (pre, post and mid-way between flushes) were averaged to create a single passive flow value to compare against the flush (Fig. 2.2). When the amount of particulates collected in the passive flow was compared to the amount that left the tanks during the flush period, it was found that the flush comprises only a small fraction of the particulates leaving the tanks daily for all size fractions. Of the 97.4 g of total solids (calculated from the passive flow and flush combined) leaving the tanks daily only 13.2±2.5 g (13.6%) was released during the flush.

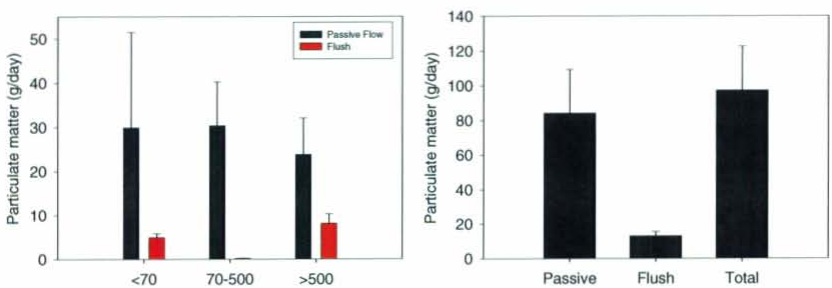

Fig. 2.2. Amount of particulate matter (g/day) leaving each tank in an onshore cod aquaculture facility via passive flow and the flush period for three different size fractions (n = 6 tanks). The <70 μm fraction was corrected for influent. Error bars are + 1 SD.

A total of 97.4±13.7 g of dry matter left the aquaculture tanks daily after correction for the influent. Of this, 34.8±21.6 g (36%) was particles <70 μm while 30.5±9.9 g (31%) was present as particles of 70-500 μm, and finally 31.9±8.5 g (33%)

was particles of >500 μm (Fig. 2.3). This means that 24.9% of the 391±116 g fed to the fish left as waste.

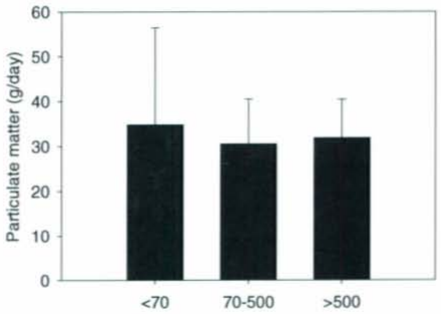

Fig. 2.3. Total particulate matter (g/day) for three size fractions of particles in effluent leaving each tank in an onshore aquaculture facility (n = 6 tanks). The <70 μm fraction was corrected for influent. Error bars are + 1 SD.

The organic content of particles had significant variations among sampling periods and size fractions (Table 2.2). Particles <70 μm had the lowest organic content in the flush and particles 70-500 μm had the greatest organic content in the passive flow. The only significant difference between sampling periods was particles obtained from the period mid-way between flushes had a significantly higher organic content than the other time periods for particles <70 μm. There were no other significant differences among the sampling periods for any size fraction.

Table 2.2. Organic content (% DW) for three size fractions of particles obtained from effluent leaving cod tanks (n = 4 days, n = 6 tanks for mid-way between flushes). Different letters (abc) denote significant differences among sampling times and (xyz) significant differences among size fractions (Holm-Sidak p <0.05).

Sampling period	<70 μm	70-500 μm	>500 μm
Flush	50.1 ± 4.0^{ax}	69.1 ± 7.0^{y}	61.0 ± 2.7^{y}
Pre-flush	38.7 ± 6.2^{ax}	58.6 ± 9.2^{y}	42.0±9.9
Post-flush	42.8 ± 7.6^{ax}	60.1 ± 4.2^{y}	51.2±9.1
Mid-way between flushes	67.8 ± 10.4^{b}	68.7±15.3	63.3±14.6

The three passive flow sampling periods were averaged together in order to compare to the flush (Fig. 2.4). There was only one significant difference between the passive flow and flush period, that being an increased organic content for particles >500 μm in the flush.

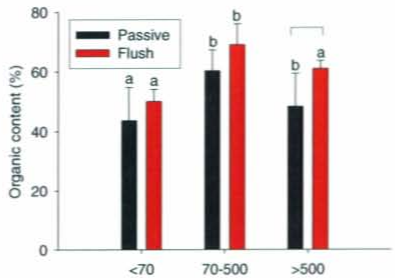

Fig. 2.4 Organic content (% DW) for three size fractions of particles obtained from the passive flow (n = 9 days) and the flush (n = 4 days). Significant differences among size fractions are indicated by different letters while brackets denote significant differences between sampling periods (Holm-Sidak p <0.05). Error bars are + 1 SD.

In order to give an overall comparison among size fractions, the organic contents of passive flow samples were averaged with that of the flush period (Fig. 2.5). Particles of 70-500 μm were found to have the highest organic content (64.5±25.2% DW) followed by particles >500 μm (54.4±35.3% DW) and finally by particles <70 μm (48.0±19.8% DW).

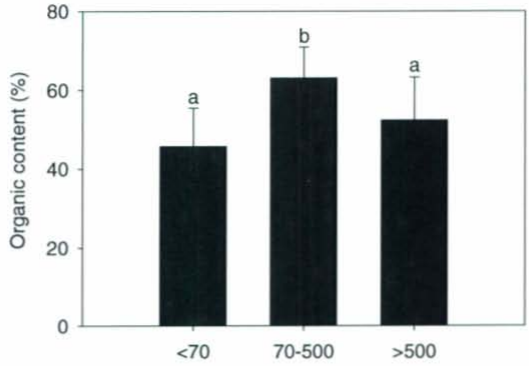

Fig. 2.5. Organic content (%) for three different size fractions of particles obtained in effluent leaving cod tanks (n = 4 sampling times). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

2.3.2 Particle characteristics

Particle distributions given by the Coulter Multisizer for particles <70 μm showed that the majority of particles were in the smaller sizes (Fig. 2.6). In terms of volume occupied by particles, larger particles comprised a larger percentage of the total volume for all sampling periods except for the flush.

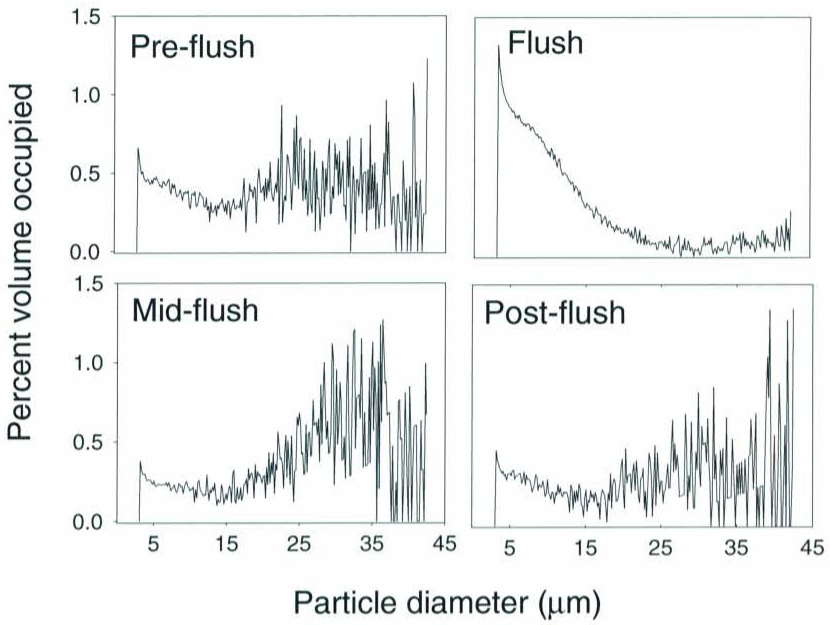

Fig. 2.6. Percent of total volume occupied by different sized particles taken for the four sampling periods obtained via Coulter counter.

Image analysis of effluent gave similar results (Fig. 2.7). Smaller particles by far outnumbered the larger ones; however, in terms of the volume occupied by particles, larger particles again comprised the larger percentage.

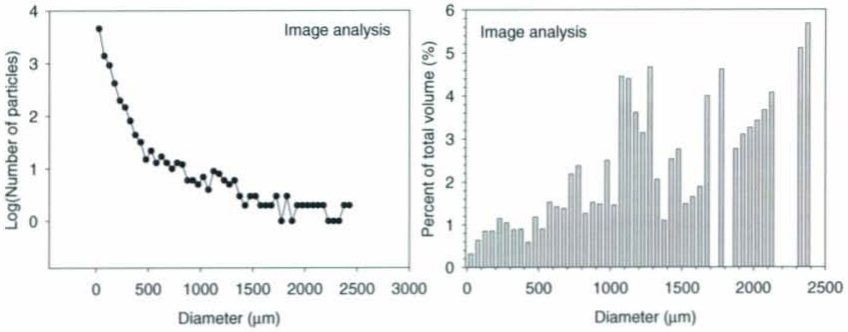

Fig. 2.7. Log (particle count) and percentage of total volume occupied by different sized particles (in 50 µm bins) present in effluent leaving all tanks. Distributions are generated from the sum of all individual samples (pooled sample).

Settling experiments revealed that particles >500 µm, which could easily be tracked with the naked eye which settled immediately, had a maximum settling rate of 2.6 cm/s. To estimate the settling rate of the slower particles the % of particles settled (based on weight) was determined after known periods of time. If left for 5 min, 75.2±6.8% of the particles would settle and after 15 min there was no increase in the amount of settled particles (Fig. 2.8). This would give the majority (75%) of particles >500 µm a settling rate between 2.6 (maximum settling rate) and 0.12 cm/s (determined from the 5 min settlement). Due to their small size, individual particles <70 µm could not be tracked to determine a maximum settling rate. After 2 h, 50.9±4.0% of particles <70 µm had settled and this increased to 64.5±8.2% after 22.5 h (Fig. 2.8). This implies that 50% of particles <70 µm have a minimum settling rate of 0.2 mm/s and 35% of particles have a maximum settling rate of 0.002 mm/s, and the remaining 15% a settling rate that falls between the two.

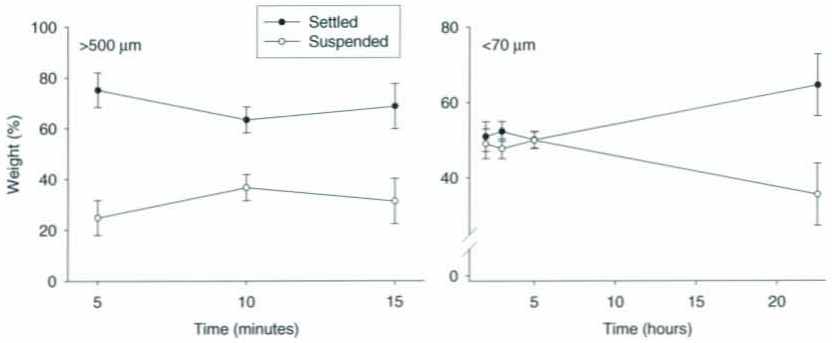

Fig. 2.8. Mass (% total) of settled and suspended particulates over time for particles >500 µm and <70 µm (n = 4). Error bars are + 1 SD.

2.3.3 Lipids

Lipid content of particles did not vary among sampling times with the exception of flush particles <70 µm, which had a significantly greater amount of total lipid than particles obtained mid-way between flush periods (Table 2.3). Particles of 70-500 µm had significantly more lipid than particles <70 µm for the pre-flush and mid-way between flush periods.

Table 2.3. Lipid content (% DW) of three size fractions of particles obtained from effluent leaving cod tanks (n = 4 days, n = 6 tanks for mid-way between flushes). Different letters (abc) denote significant differences among sampling times and (xyz) significant differences among size fractions (Holm-Sidak p <0.05).

Sampling period	<70 µm	70-500 µm	>500 µm
Flush	8.83 ± 4.48^{a}	13.02±4.19	9.40±3.53
Pre-flush	2.22 ± 1.48^{x}	13.10 ± 6.22^{y}	5.24±3.03
Post-flush	2.64±1.22	11.91±9.17	5.53±2.73
Mid-way between flushes	0.55 ± 0.31^{bx}	16.64 ± 2.67^{y}	11.56±8.15

Although there was some statistically significant variation among sampling periods (largest difference 8% DW), the three passive flow sampling times were averaged to give a single passive flow to compare against the flush (Fig. 2.9). The percent lipid content of particles obtained during the flush period was not statistically different from that obtained from the passive flow with the exception of particles <70 µm which had

less lipid in the passive flow. There was no difference in the amount of lipid across the three size fractions for particles obtained during the flush which had an average lipid content of 10.4±2.3% DW. Particles of 70-500 μm obtained during the passive flow had a significantly higher lipid content than particles <70 μm. Lipid content of the three size fractions in the passive flow were 1.8±1.1%, 13.9±2.5% and 7.4±3.6% DW for particles <70 μm, of 70-500 μm and >500 μm respectively.

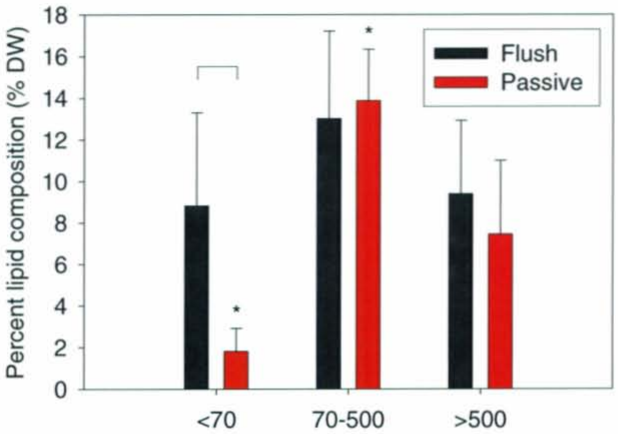

Fig. 2.9. Lipid content (% DW) for three size fractions of particles obtained from effluent leaving cod tanks during the passive flow (n = 3 sampling periods) and flushing (n = 4 days). Significant differences between sampling periods are indicated by brackets while significant differences among size fractions are indicated by * (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

In order to provide an overall comparison among size fractions of particles leaving cod tanks, the passive and flush periods were averaged (Fig. 2.10). The only significant difference observed was less lipid present in particles <70 μm compared to particles 70-500 μm.

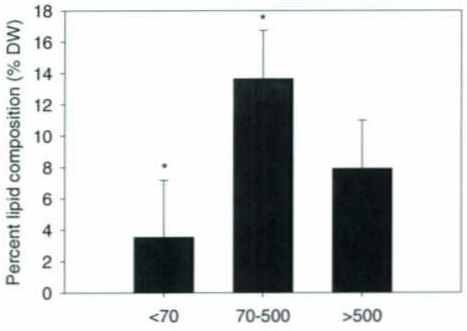

Fig. 2.10. Lipid content of three different size fractions of particles obtained from effluent leaving cod tanks (n = 4 sampling periods). Significant differences among size fractions are indicated by * (Holm-Sidak p <0.05). Error bars are + 1 SD.

Lipid class composition of particles collected in the different time periods showed some significant differences, usually <9% total lipid (Table 2.4). One notable exception was particles >500 μm from the mid-way between flushes sampling, which had a very large free fatty acid (FFA) content (82% total lipid) that was significantly larger than all other sampling times, and a significantly smaller acetone mobile polar lipid (AMPL) content (3% total lipid).

Table 2.4. Lipid composition (% total lipid) of the three size fractions of particles obtained from cod tanks during different sampling periods (n = 4 days, n = 6 tanks for mid-way between flushes). Different letters (abc) denote significant differences among sampling times and (xyz) significant differences among size fractions (Holm-Sidak p <0.05).

Lipid class	Sampling period	<70 µm % composition	70-500 µm % composition	>500 µm % composition
Hydrocarbon	Flush	3.44±2.84	2.52±3.41	2.83±2.89
	Pre-flush	0.82±0.57	3.46±1.54	5.76±5.10[a]
	Post-flush	0.57±0.52	9.19±8.76	3.16±3.53
	Mid-way between flushes	1.03±0.25	4.04±3.43	0.00±0.00[b]
Triacylglycerol	Flush	13.60±7.75	13.30±3.64	14.18±7.78
	Pre-flush	14.99±2.54	14.98±5.32	4.45±3.29
	Post-flush	15.30±3.46	14.33±6.23	9.75±3.19
	Mid-way between flushes	16.38±3.05	17.37±1.80	5.23±6.88
Free fatty acids	Flush	37.12±19.77	59.02±3.64[a]	38.63±21.85[a]
	Pre-flush	31.91±1.48	44.30±2.09[b]	35.62±20.06[a]
	Post-flush	36.54±6.98	43.08±6.92[b]	45.61±13.08[a]
	Mid-way between flushes	44.83±3.66	49.38±8.81	81.56±24.12[b]
Sterol	Flush	7.88±5.60	6.25±4.26	8.65±2.45
	Pre-flush	10.12±1.70	8.58±2.89	10.47±10.19
	Post-flush	8.28±5.96	5.94±2.12	9.91±6.49
	Mid-way between flushes	8.43±1.18	6.95±1.15	3.81±5.87
Acetone mobile polar lipids	Flush	9.31±9.72	5.41±3.27	6.45±2.02[a]
	Pre-flush	10.13±0.63	11.59±3.54	19.45±8.74[b]
	Post-flush	10.98±5.34	8.32±3.37	13.26±7.08
	Mid-way between flushes	9.83±1.96	8.67±4.45	3.49±4.68[a]
Phospholipids	Flush	4.66±2.97[a]	7.40±3.37	13.01±7.57
	Pre-flush	24.00±2.96[b]	12.78±4.49	21.99±15.12
	Post-flush	21.25±9.10[b]	13.60±4.40	13.77±5.81
	Mid-way between flushes	15.58±3.11[b]	8.98±2.21	5.41±9.04
Lipid content (% DW)	Flush	8.83±4.48[a]	13.02±4.19	9.40±3.53
	Pre-flush	2.22±1.48[x]	13.10±6.22[y]	5.24±3.03
	Post-flush	2.64±1.22	11.91±9.17	5.53±2.73
	Mid-way between flushes	0.55±0.31[b]	16.64±2.67	11.56±8.15

When the three passive flows were combined and compared against the flush period there were several significant differences (Fig. 2.11). Particles <70 μm had larger proportions of hydrocarbons (HC) and methyl ketones (MKET) in the flush than the passive flow; however, they had less phospholipids (PL). Particles of 70-500 μm obtained during the flush had a larger FFA composition; however, they had less diacylglycerol (DAG), acetone mobile polar lipids (AMPL) and PL than the passive flow. The flush period also had a larger proportion of triacylglycerol (TAG) and DAG than >500 μm particles obtained in the passive flow. In summary, most differences between the passive and flush periods were <7% total lipid; however there was an increased proportion of PL in the passive flow for particles <70 μm (17% total lipid) and an increased amount of FFA in the flush for particles 70-500 μm (15% total lipid).

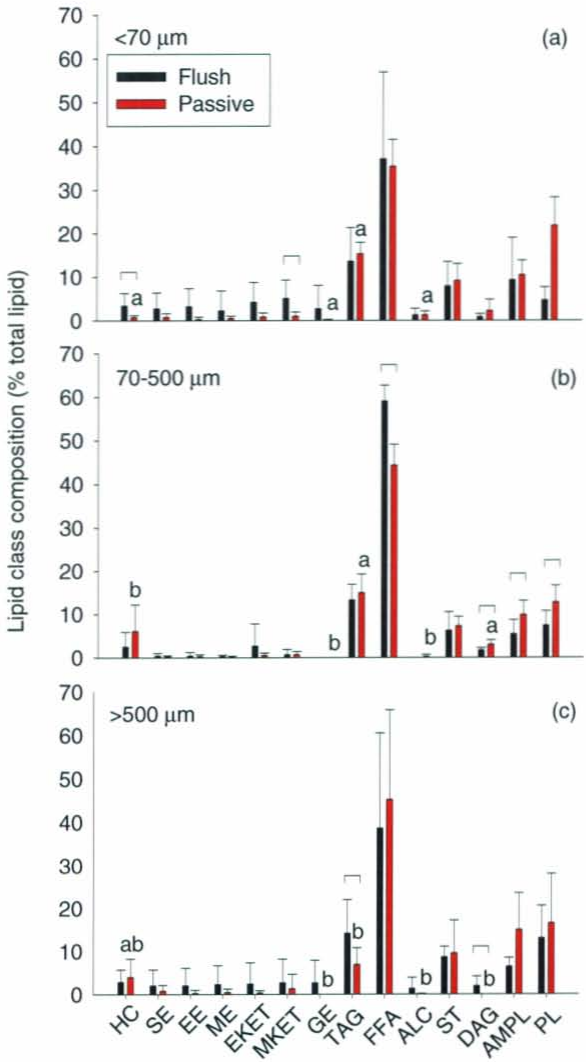

Fig. 2.11. Lipid class profile for three different size fractions of particles obtained from the passive flow as well as the flush period for tanks containing Atlantic cod (flush n = 4 days; passive flow n = 9 days). Different letters denote significant differences among size fractions while brackets denote significant differences between sampling periods (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

Quantitatively there was no difference between the lipid class content (mg/g DW) of particles obtained during the flush and the passive flow with the exception of TAG and sterol for particles >500 μm which had less of both in passive flow particles (Fig. 2.12). When the lipid class amounts were compared across size fractions it was found that particles of 70-500 μm had generally more of six lipid classes in passive flow particles than the other two size fractions, while particles <70 μm generally had less of each lipid class.

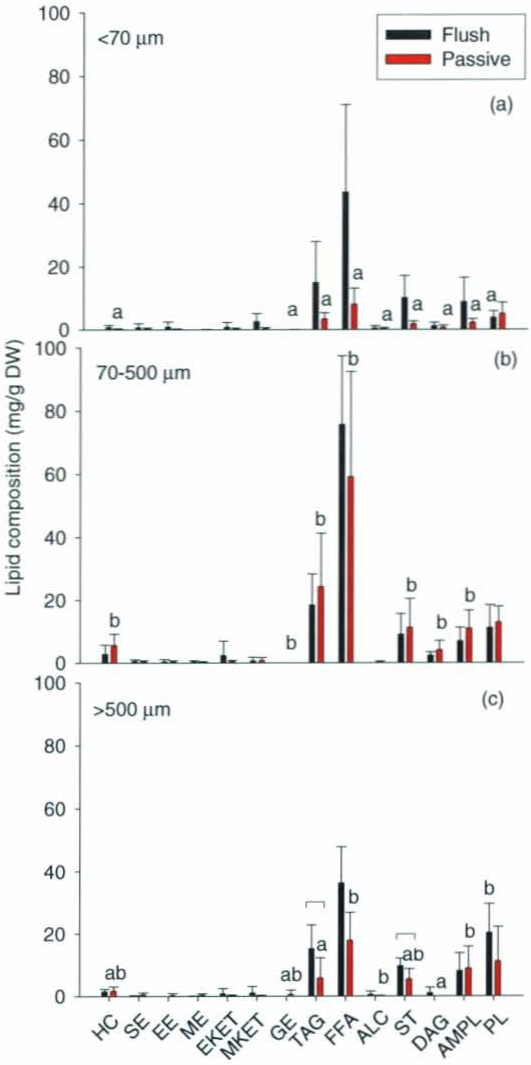

Fig. 2.12. Lipid class content (mg/g DW) of particles for three different size fractions obtained from the passive flow and the flush period for tanks containing juvenile Atlantic cod (flush n = 4 days; passive flow n = 9 days). Different letters denote significant differences among size fractions while brackets denote significant differences between sampling periods (Holm-Sidak p <0.05). Error bars are + 1 SD.

When the two sampling periods were averaged to give an overall lipid class composition for all three size fractions (Fig. 2.13), the only significant difference among particle sizes was a decreased proportion of TAG in particles >500 μm compared with the other two size fractions.

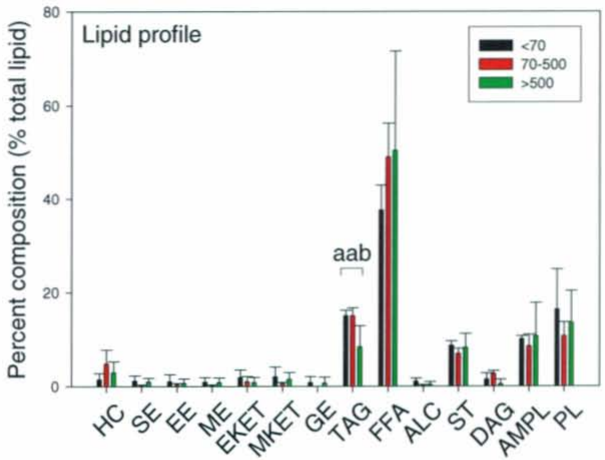

Fig. 2.13. Lipid class profile for three different size fractions of particles obtained from effluent leaving cod tanks (n = 4 sampling periods). Groups with different letters are significantly different from each other (Holm-Sidak p <0.05). Error bars are + 1 SD.

2.3.4 Fatty acids

There were some significant differences in the FA composition between the flush period and the three passive periods (largest difference 9% total FA) (Table 2.5). There were also some significant differences between size fractions for all sampling periods which were usually <7% total FA. There was one exception to this, 16:0 was present in much smaller percentages in particles >500 μm than <70 (difference 17% total FA).

Table 2.5. The FA composition (% total FA) of three different size fractions of particles obtained from cod tanks at different sampling times (n = 4 days, n = 6 tanks for mid-way between flushes). Different letters (abc) denote significant differences among sampling times and (xyz) significant differences among size fractions (Holm-Sidak p <0.05). Only FA consisting of >0.9% total FA are shown.

Fatty acid	Sampling period	<70 µm % composition	70-500 µm % composition	>500 µm % composition
14:0	Flush	8.61 ± 0.70^{x}	7.25 ± 0.17^{y}	7.35 ± 0.34^{y}
	Pre-flush	6.66 ± 2.38	7.95 ± 1.46	6.24 ± 1.65
	Post-flush	7.09 ± 1.19	7.02 ± 1.26	4.36 ± 3.42
	Mid-way between flushes	8.16 ± 0.24	7.34 ± 1.27	4.98 ± 2.77
16:0	Flush	40.63 ± 1.34^{ax}	35.35 ± 2.43^{y}	33.17 ± 1.63^{y}
	Pre-flush	33.96 ± 2.48^{bx}	39.09 ± 3.03^{y}	34.99 ± 1.96
	Post-flush	37.36 ± 2.15^{ab}	35.09 ± 4.14	20.68 ± 19.02
	Mid-way between flushes	42.64 ± 0.42^{ax}	38.47 ± 2.83	25.79 ± 14.78^{y}
16:1ω7	Flush	3.08 ± 0.57^{a}	3.51 ± 0.70	3.76 ± 0.27
	Pre-flush	6.68 ± 1.57^{x}	4.42 ± 0.28^{a}	3.87 ± 1.66^{y}
	Post-flush	7.20 ± 3.53^{b}	5.49 ± 2.83^{a}	3.32 ± 1.47
	Mid-way between flushes	3.32 ± 0.37^{ax}	2.98 ± 0.19^{by}	3.38 ± 3.19^{y}
18:0	Flush	11.34 ± 0.84^{a}	9.85 ± 1.40^{a}	10.06 ± 0.89
	Pre-flush	12.67 ± 2.13	12.08 ± 1.36^{b}	17.65 ± 4.67
	Post-flush	12.54 ± 1.64	11.00 ± 0.41	8.07 ± 5.67
	Mid-way between flushes	15.13 ± 0.72^{bx}	12.05 ± 0.61^{by}	8.90 ± 5.96^{y}
18:1ω7	Flush	2.61 ± 0.11	3.05 ± 0.28	3.30 ± 0.87
	Pre-flush	2.98 ± 0.49^{x}	3.10 ± 0.39^{x}	4.51 ± 0.54^{y}
	Post-flush	2.96 ± 0.41	3.19 ± 0.03	3.35 ± 1.20
	Mid-way between flushes	2.51 ± 0.17	2.74 ± 0.11	2.65 ± 2.35
18:1ω9	Flush	10.77 ± 0.61	11.87 ± 0.18	11.80 ± 2.12
	Pre-flush	9.10 ± 1.33^{x}	11.52 ± 0.73^{y}	8.44 ± 1.33^{x}
	Post-flush	9.15 ± 1.18^{x}	12.12 ± 1.18^{y}	5.84 ± 4.24^{x}
	Mid-way between flushes	10.42 ± 1.03^{x}	13.21 ± 1.10^{x}	8.78 ± 5.56^{y}
18:2ω6	Flush	2.53 ± 0.14^{x}	3.38 ± 0.18^{y}	3.45 ± 0.39^{y}
	Pre-flush	2.21 ± 0.30	3.25 ± 0.40	2.34 ± 0.79
	Post-flush	2.06 ± 0.35^{x}	3.87 ± 0.76^{y}	1.82 ± 0.81^{x}
	Mid-way between flushes	2.26 ± 0.22^{x}	3.89 ± 0.37^{y}	2.45 ± 1.56^{x}
20:1ω9	Flush	2.41 ± 0.42	2.75 ± 0.74	2.90 ± 0.51
	Pre-flush	2.34 ± 0.70	2.57 ± 0.94	1.53 ± 0.95
	Post-flush	1.94 ± 0.61	2.39 ± 0.97	1.98 ± 0.86

	Mid-way between flushes	1.73±0.16	1.86±0.22	1.43±0.95
20:5ω3	Flush	2.20±0.43^{ax}	2.98±0.53^{y}	3.55±0.53^{y}
	Pre-flush	3.99±0.49^{ax}	2.52±0.30^{y}	2.54±1.15^{y}
	Post-flush	2.97±0.65^{abx}	2.60±0.44^{y}	1.92±0.55^{y}
	Mid-way between flushes	1.48±0.16^{b}	2.09±0.54	2.60±2.01
22:1ω11	Flush	3.82±0.77	3.98±1.43	4.56±1.34
	Pre-flush	3.08±1.42	3.60±1.54	2.63±1.38
	Post-flush	2.59±1.69	3.18±1.74	3.29±1.07
	Mid-way between flushes	2.29±0.42	2.81±0.53	2.34±1.49
22:6ω3	Flush	3.12±0.22^{ax}	4.15±0.87^{a}	4.53±0.27^{y}
	Pre-flush	2.87±0.40	2.60±0.55^{b}	3.32±1.00
	Post-flush	2.32±0.96	2.75±0.42^{a}	2.22±0.79
	Mid-way between flushes	1.53±0.29^{bx}	2.75±0.57^{aby}	2.72±2.05

When the passive flows were averaged and compared to the flush there were several significant differences, which usually amounted to <4% of total FA (Fig. 2.14). There were many significant differences among size fractions. These were usually <7% total FA while the largest difference was a 9% total FA difference for 18:0 which was present in larger proportions in particles >500 μm than the other two size fractions.

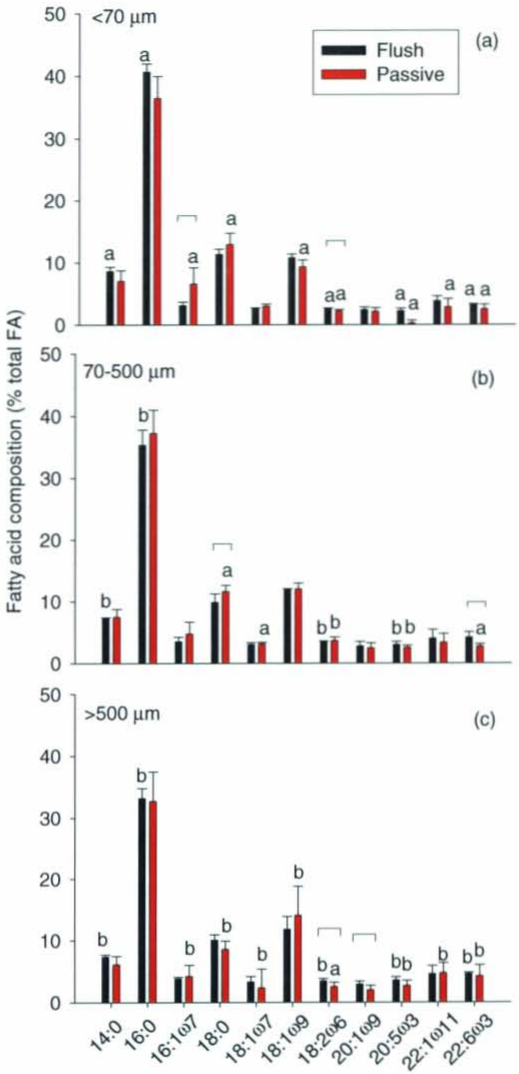

Fig. 2.14. The 11 major fatty acids (present in amounts >0.9% total FA in all sample periods) for three size fractions of particles obtained from tanks containing Atlantic cod during the passive flow (n = 9 days) and the flush (n = 4 days). Different letters denote significant differences among size fractions while brackets denote significant differences between sampling periods (Holm-Sidak p <0.05). Error bars are + 1 SD.

When the fatty acid composition of different particle sizes was looked at quantitatively many significant differences were found (Fig. 2.15). In general particles of 70-500 μm had significantly more individual FA than particles <70 μm or those >500 μm. Particles of 70-500 μm also tended to have an equivalent content of individual FAs for particles obtained from both sampling times, while particles <70 μm and >500 μm generally had larger amounts of FAs in particles obtained during the flush than the passive flow. Most differences among size fractions were <12 mg/g DW; the largest was a difference 21 mg/g DW in 16:0 for particles <70 μm. Most of the differences between sampling periods were also <12 mg/g DW; the largest was a difference of 32 mg/g DW for 16:0 which was present at much greater quantities in particles of 70-500 μm than in the other two size fractions.

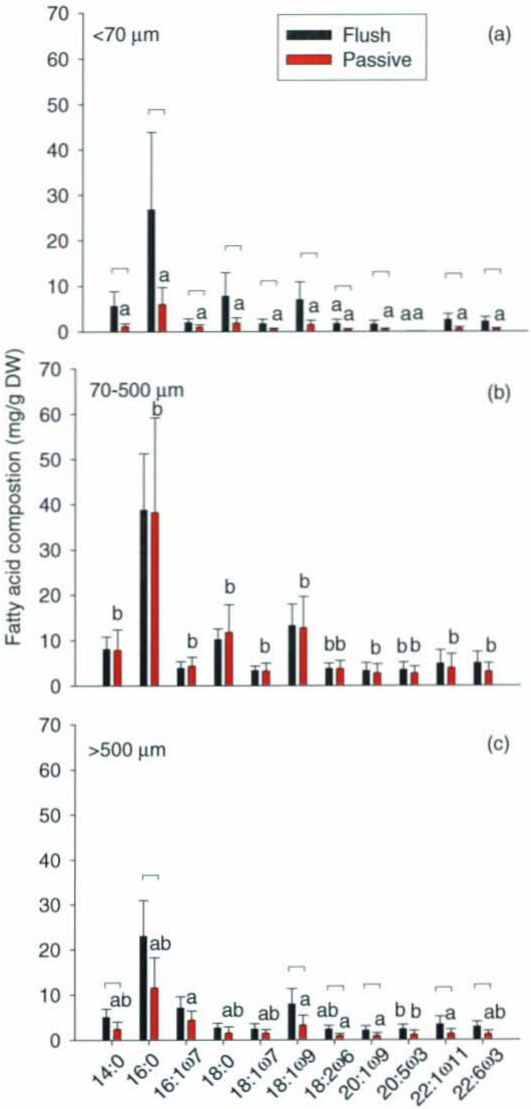

Fig. 2.15. FA composition (mg/g DW) of three size fractions for particles obtained from cod tanks during the passive flow (n = 9 days) as well as the flush (n = 4 days). Different letters denote significant differences among size fractions while brackets denote significant differences between sampling periods (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

The <70 µm fraction was further described using sums of FA groups because it is expected to be consumed the most by mussels (Fig. 2.16). There were no differences between the summed percent FA compositions of particles obtained during the flush compared to those obtained from the passive flow. Particles <70 µm were found to be comprised of 58-63% SFA, 25-27% MUFA, 10-12% PUFA and 6-7% of ω3 fatty acids. Particles obtained during the flush did, however, have a larger amount of all fatty acid groups than particles from the passive flow on an mg/g DW basis.

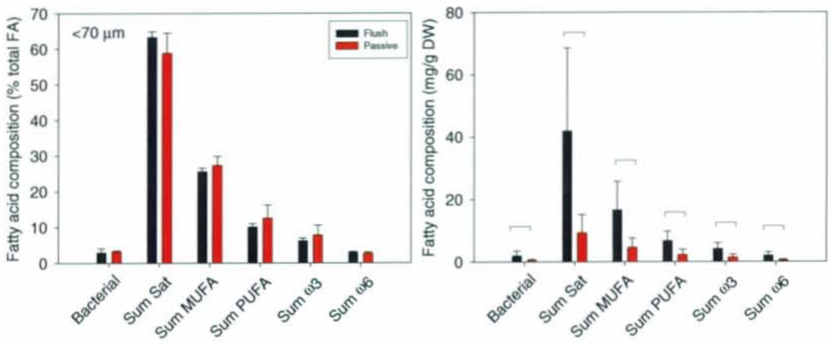

Fig. 2.16. Sum of different fatty acid groups in particles <70 µm collected from the effluent from cod tanks obtained during the flush period (n = 4 days) and passive flow (n = 9 days). Significant differences between sampling periods are indicated by brackets (Holm-Sidak p <0.05). Error bars are + 1 SD.

Particles <70 µm had a very low PUFA/SFA of 0.17±0.02 and 0.22±0.08 for particles obtained during the flush period and passive flow respectively (Fig. 2.17). Particles obtained during the flush period also had a higher ratio of DHA/EPA.

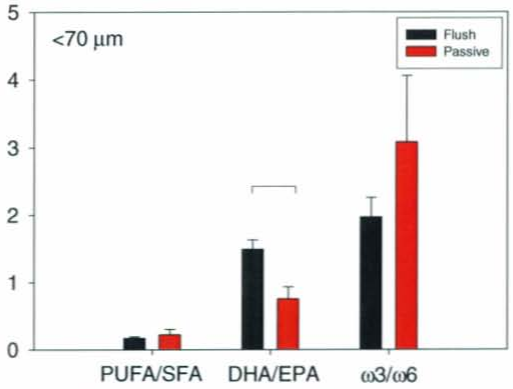

Fig. 2.17. Differences in FA ratios for particles <70 µm obtained during the flush period (n = 4 days) and passive flow (n = 9 days) from cod tanks. Significant differences between sampling periods are indicated by brackets (Holm-Sidak p <0.05). Error bars are + 1 SD.

Although there were some significant differences in the individual FA composition between the flush and the passive flow (largest difference 4% total FA), the two sampling periods were averaged in order to better summarize the three size fractions (Fig. 2.18). The FA composition of effluent contained 11 major FA, which comprised around 90% of the identified FAs. Of the 11 main FAs, two were the essential FAs DHA and EPA, a diatom marker (16:1ω7) as well as the zooplankton markers 22:1ω11 and 20:1ω9. Particles >500 µm had a smaller proportion of 14:0 and 16:0 than particles <70 µm and less 18:1ω9 than particles 70-500 µm. Particles <70 µm had a smaller proportion of 18:2ω6 than particles 70-500 µm.

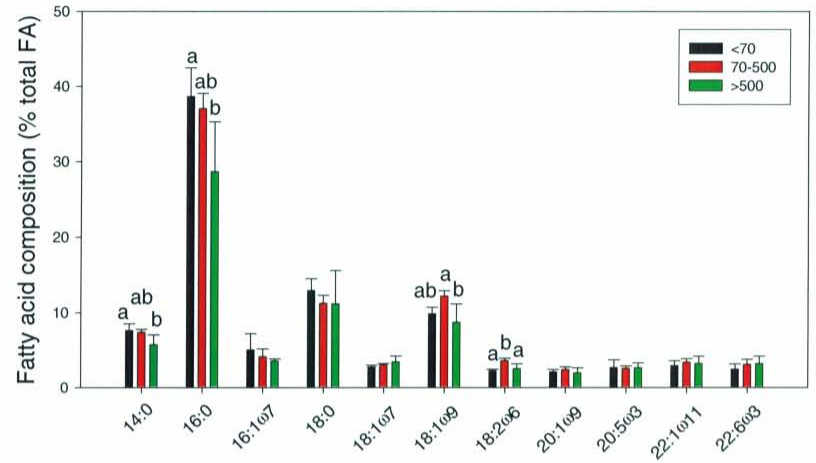

Fig. 2.18. The 11 main FA for three size fractions of particles obtained in effluent leaving cod tanks (n = 4 sampling types). Different letters denote significant differences among size fractions (Holm-Sidak p <0.05). Error bars are + 1 SD.

Fatty acid groups in particles <70 μm for the passive and flush periods were also averaged and summarized (Fig. 2.19). Saturate FAs comprised the bulk (61.9±5.7 % total FA) of FAs in particles <70 μm followed by MUFA (26.0±2.5% total FA) and PUFA (11.0±3.1% total FA).

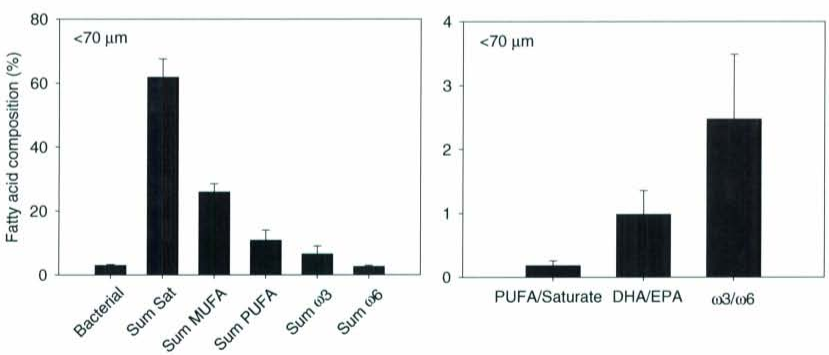

Fig. 2.19. Sum of different FA classes (% total FA) and the FA ratios for particles <70 μm from the effluent leaving cod tanks (n = 4 sampling types). Error bars are + 1 SD.

The lipid class composition of particles <70 μm was compared with that of seston collected in Charles Arm and Fortune Harbour Newfoundland with a 5 μm mesh SEA-GEAR model 900 phytoplankton net via horizontal and vertical net tows. (Alkanani *et al.* 2007) (Table 2.6). Effluent contained a much higher proportion of FFA than natural Newfoundland seston.

Table 2.6. Lipid class composition (% total lipid) of particles <70 μm obtained from tanks containing Atlantic cod in comparison to the lipid class composition of seston (Alkanani *et al.* 2007). Different letters denote significant differences among groups (Holm-Sidak p <0.05).

Lipid class	Phytoplankton (Alkanani *et al.* 2007)				Effluent
	June (n = 4)	August (n = 4)	September (n = 4)	October (n = 4)	Average (n = 4)
TAG	16.3±12.3	12.0±0.5	12.0±1.1	9.5±5.3	15.07±1.14
FFA	8.1±6.6[a]	17.0±0.03[a]	20.0±3.4[a]	6.2±4.4[a]	37.60±5.35[b]
ST	7.7±1.4	6.0±0.06[a]	6.0±0.2[a]	8.8±1.6	8.68±0.99[b]
AMPL	15.6±4.9	5.0±0.01	6.0±0.9	8.3±2.8	10.06±0.70
PL	46.1±18.9	51.0±0.4[a]	46.0±16.9	39.1±14.8	16.37±8.56[b]

The FA composition of effluent was also compared to that of natural Newfoundland seston (Alkanani *et al.* 2007) (Table 2.7). Effluent contained a larger proportion of SFA than the natural seston; however, effluent contained a smaller proportion of 20:5ω3, PUFA and ω3 than the natural seston.

Table 2.7. FA profile of effluent collected from cod tanks in comparison to the FA profile of seston (Alkanani *et al.* 2007). Different letters denote significant differences among groups (Holm-Sidak p <0.05).

Fatty acid	Phytoplankton (Alkanani *et al.* 2007)		Effluent		
	2000	2001	Flush	Passive flow	Average
	(n = 65)	(n = 66)	(n = 4)	(n = 3)	(n = 4)
20:4ω6	0.64±0.60	0.69±0.77	0.14±0.04	0.17±0.15	0.15±0.08
20:5ω3	9.28±6.48[a]	13.04±4.39[a]	2.20±0.43[a]	0.21±0.49[b]	2.66±1.08[a]
22:6ω3	14.97±11.19	14.21±9.67	3.12±0.22	2.47±0.78	2.46±0.70
ΣSFA	45.01±16.86[a]	28.39±11.79[b]	63.32±1.55[a]	58.81±5.66[a]	61.87±5.76[a]
ΣMUFA	20.15±6.18	22.79±11.36	25.66±0.89	27.29±2.52	26.00±2.52
ΣPUFA	37.74±20.23[a]	41.46±18.43[a]	10.17±0.82[b]	12.56±3.66	10.97±3.11[b]
Σω3	31.41±19.69	32.39±17.2[a]	6.30±0.68[b]	7.80±2.78	6.64±2.44[b]
Σω6	3.85±1.64	4.69±2.80	3.04±0.17	2.65±0.41	2.76±0.27
Σω3/ω6	8.04±4.85[a]	7.04±3.01	1.97±0.29[b]	3.08±0.98	2.48±1.01[b]

2.3.5 Dissolved constituents

The concentrations of dissolved organic carbon and total dissolved nitrogen were not different for the three types of passive flows; however, concentrations for the flush were much higher and significantly different from all passive flows (Table 2.8).

Table 2.8. Concentration of dissolved organic carbon and nitrogen for effluent leaving cod tanks at different sampling periods (n = 4 days, n = 6 tanks for mid-flush).

	Flush	Pre-flush	Post-flush	Mid-way between flushes
Carbon	1791±465	126.5±8.5	118.7±14.0	120.5±7.5
Nitrogen	392±105	35.5±6.6	37.1±4.0	31.0±5.4

Water from the flush period had an average concentration of 1791±465 μM of carbon and 392±105 μM of nitrogen (Fig. 2.20). However, due to the relatively small volume of water that leaves the tanks during a flush period, only 0.37±0.10 g and 0.10±0.03 g of carbon and nitrogen respectively leaves the tanks through the flush daily. In contrast, water collected during the passive flow had much smaller concentrations of carbon and nitrogen 121.9±4.1 μM and 34.5±14.5 μM respectively. However, due to the large volume of water that passes through the tanks this equates to 16.1±2.2 g of carbon and 14.5±1.3 g of nitrogen daily.

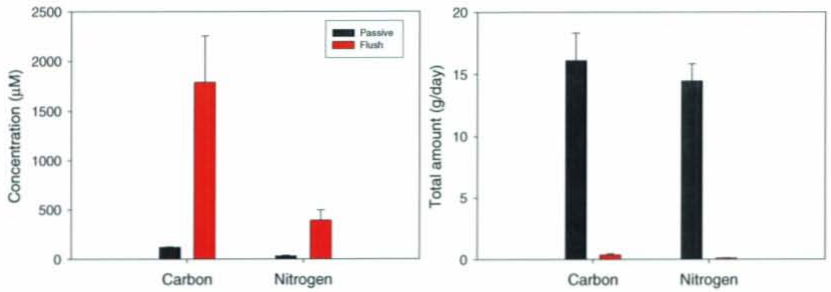

Fig. 2.20. Concentration (µM) and total amount (g/day) of dissolved carbon and nitrogen leaving cod tanks through passive flow (n = 9 days) as well as during flushing (n = 4 days). Error bars are + 1 SD.

Even though the flush period had much higher concentrations of both carbon and nitrogen due to the volume of water compared to the passive flow, the flush only comprised 2.2% and 0.7% of the total amount of dissolved carbon and nitrogen respectively leaving the tanks daily. Influent to the tanks had concentrations of 9.5±4.1 and 0.6±0.2 µM for carbon and nitrogen respectively. This equates to a total of 4.1±3.1 and 0.2±0.5 g of carbon and nitrogen entering daily. When subtracted from effluent values, a total of 12.3±3.8 and 14.3±1.4 g of carbon and nitrogen leaves the tanks daily. The total weight of dissolved carbon and nitrogen leaving the tanks daily amounts to 3.1% and 3.7% respectively of the weight fed to fish daily.

2.4 Discussion

2.4.1 Effluent output and particle characteristics

The amount of effluent leaving the cod tanks after correction for influent suggests that 24.9% of the feed added to the system leaves as solid waste, being it either feed fines or feces. This is comparable to the results obtained by Cho and Bureau (2001) who found 15-25% of the amount fed was excreted as solid waste by rainbow trout.

Particle count and volume distributions, obtained via Coulter counter and image analysis, are comparable to those reported by Cripps (1995) for unfiltered effluent leaving a freshwater hatchery containing Atlantic salmon and sea trout. Cripps found that the majority of particles were <20 µm however the volume occupied by the few larger particles was greater than the more numerous smaller particles.

M. edulis preferentially ingests smaller particles while selectively rejecting a significant proportion of particles >22.5 μm (Defossez and Hawkins 1997). This suggests that *M. edulis* will have the greatest ingestion rate of only a small proportion of the <70 μm size fraction which itself only contributes to 36% of the effluent. However the threshold diameter for rejection increases with increasing particle concentration (Defossez and Hawkins 1997). This implies that a smaller proportion of the effluent will be preferentially rejected based on size when present in larger concentrations.

M. edulis selectively rejects particles with lower organic content and increase their reaction with increasing particle concentrations (Bayne *et al.* 1993). Due to the <70 μm size fraction having less organic content than larger particles it is possible that at high effluent concentrations a larger proportion of the <70 μm size fraction will be rejected by *M. edulis* in favour of particles with a higher organic content. *M. edulis* is capable of feeding on marine snow whose diameter was >0.5 mm long (Newell *et al.* 2005), which suggests that even though mussels may preferentially select for smaller particles it is possible that they will ingest some of the larger particulate matter present in effluent. There is also evidence that mussels ingest mesozooplankton in the 100 to 1000 μm size range as well as occasional planktoners up to 3 and 6 mm in length (Davenport *et al.* 2000). Although bivalves are capable of ingesting large zooplankton they are not ingested in large numbers (Lehane and Davenport 2002). There is also evidence of size selection by mussels when ingesting zooplankton in favour of smaller zooplankton (Lehane and Davenport 2006). Another study found that oysters which ingested zooplankton would consistently reject inert particles of a similar size and concluded that ingestion was controlled by surface chemistry (Tamburri and Zimmer-Faust 1996).

Based on the above although it is possible for *M. edulis* to ingest larger particles present in the effluent the quantity of ingestion is unclear. It is expected that based on the consistent preferential selection of smaller particles/zooplankton that it is likely that the majority of consumption will occur in the smaller size ranges. More work is required to fully quantify the proportion ingested by *M. edulis* of the different sized particles present in effluent.

Based on our findings 1 kg of cod fed 1.5% their body weight daily would produce 3.7 g of waste of which 1.3 g would be particulates <70 μm. *M. edulis* require a

maintenance ration of 0.6-2.8% of their dry soft tissue mass daily (Hawkins *et al.* 1985). Water content of *M. edulis* varies between 70 and 82% (Zandee *et al.* 1980). Assuming *M. edulis* ingests only and all particles <70 µm, 1 kg of cod fed 1.5% their body weight would provide 150 g (2.8% ration and 70% water content) to 1 kg (0.6% ration and 80% water content) of mussels their maintenance ration daily. It is also important to note that the <70 µm fraction, which will presumably show the most ingestion by mussels, has the slowest settling speeds and will more easily spread to surrounding areas than the larger more rapidly settling particles.

The DOC associated with fish effluent can be a potential food source for mussels. Mussels can acquire 13% of their energy demand from sources of DOC such as dissolved free amino acids and dissolved simple sugars and may also obtain up to 10% of their required nitrogen from DON (Gorham 1988). The TN values obtained of 0.48±0.04 mg/l from this study are comparable to the 0.63±0.06 (s.e.) mg/l observed by Cripps (1995) for effluent from an Atlantic salmon hatchery.

The large FFA content of particles can be explained since FFA are produced through breakdown and have been reported to indicate the presence of cod feces (Biesen and Parrish 2005). Mussels can obtain energy from the TAG content of particles as TAG has been reported to be catalizable by several bivalve species (Gallager *et al.* 1986; Fraser 1989).

2.4.2 Temporal variation in output from cod tanks

There were several significant differences among the three sampling periods for the passive flow (Appendix I.i); however, these were usually for the mid-way between flushes period. The mid-way between flushes period had a much larger FFA content than the other two sampling times for the passive flow. This suggests that a larger portion of feces is present during this period. The mid-way between flushes period would fall between the 6 to 24 hour period after feeding reported to be when the majority of food ingested was passed by Atlantic salmon (Sveier *et al.* 1999).

The flush discharged a large amount of particulate matter in a short period of time compared to the passive flow. The particulate matter obtained from flushing was not very different in terms of lipid composition and FA composition, with no significant differences for most lipid classes and FAs (Appendix I.ii). Therefore the flush is an ideal

time to collect a large amount of waste from tanks over a short period of time when sampling. It should be noted however, that although the composition of lipids and FA are similar the amount of lipid and FA was greater in particles obtained from the flush. The increased organic and lipid content in particles of the flush is most likely due to the time spent in the stand-pipe. It is possible that the time spent settled allows the particles to aggregate into tighter packed particles. The increased composition of FFA and the decreased proportion of other lipid classes such as PL and AMPL indicate that particles in the flush are further broken down than particles from the passive flow.

There were several significant differences in the physical and biochemical properties among the three size fractions (Appendix I.iii). Based on these differences it is likely that the <70 μm fraction contains more inorganic fines from the sand-bed filter which would explain the decreased organic and lipid contents as well as the increased proportions of SFA, 14:0 and 16:0. The fish feces was most likely spread across the 70-500 μm and >500 μm fractions; however, there were some differences between these two groups. Particles of 70-500 μm had an increased organic and lipid content as well as an increased proportion of the terrestrial plant marker 18:2ω6. This marker was present in the feed which may suggest an increased amount of feed fines in the 70-500 μm fraction which would also explain the larger organic and lipid content.

Although mussels are capable of consuming some of the wastes generated from an aquaculture facility there is more waste unavailable to mussels than there is available. Not only does the amount of unavailable waste exceed the available, the quality of waste varies. Waste unavailable to mussels, that being >70 μm, has a greater lipid and FA content. This implies that the ability of mussels to remove nutrients added into a system via aquaculture feed could be quite low.

2.4.3 Evaluation of cod wastes as a diet for *Mytilus edulis*

The 46.2±28.1 mg/g AFDW of lipid present in particles <70 μm was close to the 62-79 mg/g AFDW of lipid present in natural seston that Newfoundland mussels would normally ingest.

Samples for this experiment were taken in the spring when algae blooms are known to occur. To determine what proportion of the effluent may be phytoplankton, the amount of FFA in seston was compared to that of effluent (Table 2.6). Newfoundland

seston had on average 13% FFA compared to an average of 38% FFA in effluent which leaves 25% of FFA for the waste material. This suggests that 34% of the lipids present in effluent could have come from seston in the influent. The TAG, sterol and AMPL content of effluent was comparable to that of phytoplankton; however, there were much higher levels of FFA and much lower levels of PL present in effluent as opposed to phytoplankton.

PUFA and ω3 content was much lower in effluent than seston (Table 2.7). This means in order to obtain the same amount of PUFA and ω3 FAs mussels would need to ingest 3× more lipid from effluent than seston. The proportion of ω6 FAs in effluent (2.76±0.27%) was close to that of seston (3.8-4.7%); however, the amount of 20:5ω3 (2.66±1.08%) present in effluent was much lower than phytoplankton which contained 9-13% 20:5ω3. So in order to acquire the same amount of 20:5ω3, mussels would need to ingest 2.5-45× more lipid from effluent compared to seston. Bivalves are known to require a ω3/ω6 ratio between 5 and 15 (Joseph 1982, 1989; Whyte *et al.* 1991; Napolitano *et al.* 1992; Thompson *et al.* 1993; Pazos *et al.* 1997; Budge *et al.* 2001). The ω3/ω6 ratio for particles <70 μm was only between 2 and 3.1 suggesting that the requirements for mussel growth may not be met if effluent is the sole diet. Levels of the essential FA 20:4ω6 that is a major precursor of prostaglandins which influence reproduction in molluscs (Osada *et al* 1989; Soudant *et al.* 1996) in effluent were comparable to seston.

2.4.4 Conclusions

Although mussels can play a role in the reduction of aquaculture wastes in an IMTA setting they are not and cannot be the sole solution. Mussels can be useful in reducing the amount of small particulate matter leaving an aquaculture site; however, other organisms and methods must be used in order to eliminate larger particulates which settle out of suspension more rapidly. These larger particulates also contain more lipid and FAs which will lead to loading of the benthos.

Further studies need to be done to better understand exactly which size fraction of fish effluent will be ingested by mussels and with what efficiency. Another important factor that requires more attention is potential seasonal fluctuations in utilization of finfish effluent by mussels. If mussels only use effluent as a supplemental diet when

natural food sources are scarce it could have large implications for the amount of waste reduction achieved by mussels.

2.5 Appendix I

i. Significant differences among the four sampling periods for three size fractions of particles from effluent leaving cod tanks. **Bold** denotes differences 10% (total lipid/FA/DW) or greater and back shading denotes $p < 0.01$ (Holm-Sidak). Groups with different letters are significantly different from each other. Variables not shown were not statistically significantly.

	Size (μm)	Flush	Pre-flush	Post-flush	Mid-way between flushes
Mass (g) DW/Day	70-500	**0.16±0.03**	-	**37.45±14.20**	-
	>500	8.04±2.26[a]	-	25.37±10.48[b]	27.58±16.91[b]
Organic content (% DW)	<70	50.08±4.06[a]	**38.73±6.25[abc]**	42.86±7.58[bcd]	**67.82±10.44[abd]**
Lipid content (% DW)	<70	8.83±4.48	-	-	0.55±0.31
FFA	70-500	**59.02±3.64[a]**	**44.30±2.09[b]**	**43.08±6.92[b]**	49.38±8.81[ab]
	>500	38.63±21.85[a]	**35.62±20.06[abc]**	45.61±13.08[bcd]	**81.56±24.12[abd]**
AMPL	>500	**6.45±2.02[a]**	**19.45±8.74[b]**	-	**3.49±4.68[a]**
PL	<70	13.01±7.57[a]	21.99±15.12[b]	13.77±7.08[b]	5.41±9.04[b]
16:0	<70	40.63±1.34[a]	33.96±2.48[b]	37.36±2.15[abc]	42.64±0.42[abd]
18:0	<70	11.34±0.84	-	-	15.13±0.72
	70-500	9.85±1.40[a]	12.08±1.36[b]	-	12.05±0.61[b]
16:1ω7	<70	3.08±0.57	-	7.20±3.53	3.32±0.37
	70-500	-	4.42±0.28[a]	5.49±2.83[a]	2.98±0.19[b]
20:5ω3	<70	2.20±0.43[a]	3.99±0.49[b]	2.97±0.65[ac]	2.66±1.08[abd]
22:6ω3	<70	3.12±0.22	-	-	1.53±0.29
	70-500	4.15±0.87	2.60±0.55	-	-

ii. Significant differences between the flush and passive flow for three size fractions of particles in effluent leaving cod tanks. **Bold** denotes differences 10% (total lipid/FA/DW) or greater and back shading denotes $p < 0.01$ (Holm-Sidak). Groups with different letters are significantly different from each other. Variables not shown were not statistically significantly.

	Size (µm)	Flush	Passive
Mass (g DW/ day)	<70	4.90±0.94	29.12±21.58
	70-500	**0.16±0.03**	**30.37±9.93**
	>500	8.04±2.26	23.83±8.23
Organic content (% DW)	>500	**61.03±2.74**	**48.07±10.97**
Lipid content (% DW)	<70	8.83±4.48	1.80±1.11
HC	<70	3.44±2.84	0.82±0.57
MKet	<70	5.15±4.13	1.12±0.95
FFA	70-500	**59.02±3.64**	**44.30±2.09**
DAG	70-500	1.66±0.56	2.70±1.30
AMPL	70-500	5.41±3.27	11.59±3.54
PL	<70	**4.66±2.97**	**24.00±2.96**
	70-500	7.40±3.37	12.78±4.49

iii. Significant difference among the three size fractions of particles in effluent leaving cod tanks. **Bold** denotes differences 10% (total lipid/FA/DW) or greater and back shading denote $p < 0.01$ (Holm-Sidak). Groups with different letters are significantly different from each other. Variables not shown were not statistically significantly.

	<70 µm	70-500 µm	>500 µm
Mass (g)	34.82±21.60	30.53±9.93	31.87±8.54
Organic content (% DW)	45.73±9.71[a]	63.07±7.87[b]	52.34±10.89[a]
Lipid content (% DW)	**3.56±3.62**	**13.67±2.05**	-
TAG	15.07±1.14[a]	15.00±1.73[a]	8.40±4.51[b]
14:0	7.63±0.91	-	5.73±1.33
16:0	**38.65±3.81**	-	**28.66±6.64**
18:1ω9	-	12.18±0.73	8.72±2.44
18:2ω6	2.26±0.20[a]	3.60±0.33[b]	2.52±0.68[a]

3. Performance of *Mytilus edulis* in relation to growth and biochemical composition when reared on effluent from a *Gadus morhua* aquaculture facility.

3.1 Introduction

Seafood consumption has been increasing with a recorded global consumption of 110.4 t equating to a per capita consumption of 16.7 kg/year in 2006 (FAO 2006). Seafood is a major source of omega 3 (ω3) PUFAs. Bivalves are an important source of inexpensive protein (Astorga España *et al.* 2007), among which mussels are also good sources of phytosterols (Murphy *et al.* 2002). Phytosterols have numerous health benefits including the ability to lower cholesterol and prevent some forms of tumours (Ling and Jones 1995).

Integrated multi-trophic aquaculture (IMTA) is a practice in which fed organisms (i.e. fish) are grown alongside extractive organisms (i.e. mussels) in an attempt to reduce wastes (Barrington et al. 2009). Blue mussels grown adjacent to Atlantic salmon cages in the Bay of Fundy have been reported to have increased growth rates and are currently being sold commercially (Reid *et al.* 2008b; Reid *et al.* 2008a). It is known that the fatty acid profile of the diet fed to mussels can directly affect the fatty acid profile of the mussels (Khan *et al.* 2006b). Any changes in the biochemical composition of mussels can affect their nutritional value for their human consumers. In regard to IMTA it is therefore important to understand how the biochemical composition of mussels will be changed through feeding fish waste as opposed to their normal diet.

The aim of this study was to determine the performance of *M. edulis* when reared on wastes generated from an onshore Atlantic cod (*Gadus morhua*) aquaculture site. This was done by assessing the physical characteristics of mussels (shell length, dry weight, ash-free dry weight and condition index) as well as the biochemical characteristics (C and N, lipid profile, fatty acid profile and amino acid profile) of mussels fed fish waste and comparing them to that of mussels fed a commercial shellfish diet.

3.2 Methods

There were three main components to this study, a small feeding experiment designed to determine if mussels ingest fish effluent by looking for fatty acid markers, a ten week growth trial aimed at detecting any biochemical changes in *Mytilus edulis* (from Notre Dame Bay NL) fed fish effluent when compared to mussels fed algae or starved, and a six month growth trial aimed to detect any differences in growth rate between mussels fed fish effluent *versus* an algae diet (Shellfish Diet 1800, Instant Algae® Reed Mariculture).

3.2.1 Trophic marker experiment

A small feeding experiment was undertaken to determine if mussels ingested particles in fish effluent by looking for fatty acid markers. A recirculating system was set up with a 5 L chamber in which five 2.5-3.3 cm shell length mussels were placed. It was fed by a reservoir and drained into a collecting reservoir. The collecting reservoir then circulated back into the feeding reservoir. Five individual mussels which were starved for 24 hours prior were placed into the system for 24 hour trials and fed different diets (no food, algae and fish effluent). After each trial the mussels were removed, their shell length recorded, the weight of soft tissue was measured and then the digestive gland was removed weighed and prepared for lipid and fatty acid analysis as described below.

3.2.2 Ten week biochemical trial

The goal of this experiment was to detect and biochemical changes within mussels after exposure to effluent as a diet for 10 weeks. Two A-frames each containing three racks were used for this experiment. Each A-frame was a replicate of the other; each of the three racks contained 160 (4.5-6.4 cm shell length) mussels fed three different diets (no food, algae and fish waste). The mussels were fed 1.5% of their body weight/daily.

Effluent was collected from a single tank containing Atlantic cod. The effluent was screened though a 500 µm screen followed by a 70 µm screen. Dry weight content of the <70 µm fraction was determined by filtering small amounts of effluent through a 1.2 µm 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. The algae diet was a commercial shellfish diet. Diets were

added manually to the racks daily and the water supply turned off (to prevent flushing out of diet) 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 individuals were randomly selected removed from each rack and sampled for lipid composition and fatty acid (FA) composition. Samples were also taken and tested for their *E.coli* and *Salmonella* content by the Canadian Food Inspection Agency (CFIA). Replacement mussels marked with nail polish were added to ensure the biomass of mussels remained constant. The amount fed to each tank was adjusted to maintain a 1.5% BW/day ration.

3.2.3 Six month growth trial

Six flow-through tanks were arranged into two rows of three, one row being elevated above the other. The elevated tanks were set up to drain into the lower tanks. Two of the top three tanks contained juvenile Atlantic cod (1 year old, 30 g body weight) while the other tank remained empty as a control. The lower row of tanks each contained 250 mussels (1.8-2.2 cm SL).

Fish were held in tanks with flow through circulation and fed a commercial fish feed (Skretting Europa) twice daily for a total of 1.5% of their body weight. Based on effluent concentrations found in previous experiments, the body mass of fish in each of the tanks was expected to provide mussels below with at least 1.5% of their DW daily with waste particles <70 μm. The stand-pipe for the fish tanks was pulled daily, after which the water supply was turned off for two hours in order to allow the mussels more time to filter the larger concentration of waste.

Mussels below the control tank were fed 1.5% of their DW daily with a commercial shellfish diet. Water to the tank was turned off after addition of the diet for two hours or until water clarity returned. Water samples were taken over time during this period and analyzed via a Coulter counter to determine how much algae food was removed over time.

Twenty mussels were removed from each tank on a monthly basis and sampled for shell length (SL), wet weight (WW), dry weight (DW), ash-free dry weight (AFDW), lipid composition, FA composition, C/N and amino acid composition. Protein was calculated from nitrogen content via a conversion factor of 5.8 as described by Gnaiger

and Bitterlich (1984). Condition index was calculated as (DW/SL)*100. Again samples were taken and tested for their *E. coli* and *Salmonella* content by CFIA. Replacements were added to maintain a constant biomass, so the amount fed did not inadvertently increase.

3.2.4 Biochemical analysis

Lipid composition was determined with an Iatroscan Mark V TLC-FID and silica coated Chromarods using a three step development method. The method used to prepare and analyze lipid extracts was the same as previously described (section 2.2.4).

Fatty acid methyl esters were prepared and analyzed as previously described (section 2.2.4) in order to determine fatty acid content. FAME content was determined using a HP 6890 Series GC-FID equipped with a 7683 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.

CHN content was determined using a Perkin Elmer Series II. Mussel samples were dried at 80°C, weighed and then ground using mortar and pestle. Small amounts of mussel powder (1.9-2.1 mg) were placed in tin foil capsules and returned to the oven until running on the analyzer.

Amino acid content was determined using EZ:faast amino acid analysis kits (Phenomenex, USA) and a Varian CP-3800 GC. The carrier gas was helium with a constant flow of 1.5 ml/min. Oven temperature started at 110°C and ramped to 320°C at a rate of 32°C/min. Split injection (1:15) of 2 μl was used at 250°C with a detector temperature of 320°C.

3.2.5 Statistical analysis

Significance was determined using one way ANOVAs followed by Holm-Sidak tests to determine where those significances laid. Holm-Sidak tests can be used for both pairwise comparisons and comparisons versus a control group and is more powerful than the Tukey and Bonferroni tests. Kruskal-Wallis one way analysis of variance on ranks and a Dunn`s Method test was performed when data failed the assumption of equal variance or normality. Statistical analysis was performed using SigmaStat 2.03 (SPSS Inc.). All results are given as mean ± SD.

3.3 Results

3.3.1 Trophic marker experiment

The FA profiles of dissected digestive glands from mussels fed three different diets were compared (Table 3.1). All mussels had diatom (16:1ω7 and 16:4ω1), flagellate (18:2ω6 and 18:4ω3) and bacterial (18:1ω7) markers present. Mussels fed algae or starved had no detectable amounts of the zooplankton marker 22:1ω11; digestive glands of mussels fed effluent had small amounts of the zooplankton marker 22:1ω11 (0.2±0.18%), which was significantly different ($p < 0.001$) from the starved and algal fed mussels.

Table 3.1. Fatty acid composition (% total FA) of digestive glands taken from mussels fed three different diets (control n = 6, algae and effluent n = 5). Different letters denote significant differences among treatments (Holm-Sidak $p < 0.05$).

Fatty acid	Control	Algae	Effluent
16:1ω7	4.84±0.95	4.33±1.05	4.89±0.75
16:4ω1	0.00±0.00[a]	0.40±0.19[b]	0.28±0.15
18:1ω7	2.95±0.25	2.97±0.20	2.92±0.15
18:2ω6	2.13±0.35[a]	2.26±0.35	2.77±0.48[b]
18:4ω3	2.94±0.89	2.06±0.38	3.04±1.09
22:1ω11	0.00±0.00[a]	0.00±0.00[a]	0.20±0.18[b]
20:1ω9	2.71±0.62	3.24±0.33	2.91±0.96
20:4 ω6	5.49±0.75	5.19±0.42	4.64±0.56
20:5ω3	15.67±1.14	13.98±1.72	14.19±2.56
22:5ω3	0.61±0.20	0.58±0.19	0.65±0.22
22:6 ω3	14.90±1.71	13.13±0.74	13.06±1.41
ΣBacterial	2.53±0.74[a]	11.01±1.13[b]	8.28±2.40[ab]
ΣSFA	28.79±3.98[a]	21.78±1.07[b]	25.69±2.87
ΣMUFA	14.84±1.33[a]	23.89±1.22[b]	23.51±2.40[b]
ΣPUFA	54.86±2.73[a]	51.63±2.05	48.86±4.22[b]
Σω3	39.44±1.77	35.32±2.72	35.55±5.36
Σω6	8.57±0.70	8.61±0.43	8.18±0.40
PUFA/SFA	1.95±0.42	2.38±0.21	1.93±0.32
DHA/EPA	0.96±0.16	0.95±0.12	0.93±0.08
ω3/ω6	4.64±0.59	4.12±0.44	4.34±0.51

3.3.2 Ten week biochemical trial

It should be noted that although this experiment was aimed at biochemical changes growth data was also recorded. When the growth data was examined it was found that the DW, AFDW and CI for algae fed mussels had significantly decreased throughout the experiment and was significantly different that mussels fed the other two diets.

There were no differences in lipid content (mg/g WW) among any replicates or treatments at end of the experiment (Table 3.2). There were also no significant changes throughout the experiment for mussels fed all diets After the replicates were averaged, there were still no statistical differences among diets at the end of the experiment (Fig. 3.1); however the total lipid content (mg/g WW) for starved and effluent fed mussels had significantly decreased throughout the experiment.

Table 3.2. Total lipid content (mg/g WW) of mussels in six tanks supplied different diets (no food, algae and effluent) at the start (n = 18) and end of a ten week experiment (n = 3).

		Starved		Algae		Effluent	
	Start	S1	S2	A1	A2	E1	E2
Total lipid	7.09±7.12	3.25±3.63	3.83±3.84	6.48±2.41	5.69±4.16	4.34±1.22	3.38±2.13

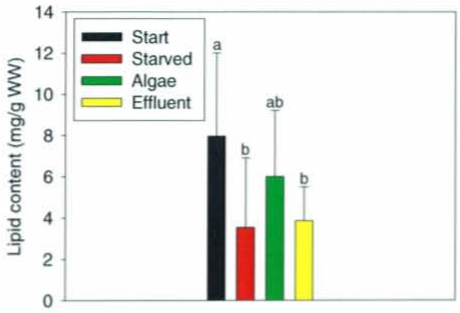

Fig. 3.1.Total lipid content (mg/g WW) at the start (n = 18) and end of the experiment for mussels feeding three different diets (algae, effluent and no food; n = 6). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

There were no significant changes in the lipid class composition (% total lipid) for mussels fed any of the three diets throughout the experiment (Table 3.3). There was one significant difference among the six mussel tanks. One of the starved mussels tanks (S1) had a significantly smaller proportion of PL than one of the effluent fed tanks (E2).

Table 3.3. Lipid class composition (% total lipid) of mussels in six tanks supplied different diets (algae, effluent and no food) at the beginning (n = 18) and end of a ten week experiment (n = 3). Different letters denote significant differences among groups (Holm-Sidak p <0.05). Ethyl ketone (EKET), triacylglycerol (TAG), free fatty acids (FFA), sterol (ST), acetone mobile polar lipids (AMPL), phospholipids (PL).

		Starved		Algae		Effluent	
Lipid class	Start	S1	S2	A1	A2	E1	E2
EKET	0.02±0.04	0.59±0.59	0.00±0.00	1.45±0.79	0.12±0.21	0.23±0.40	0.00±0.00
TAG	13.74±15.38	28.38±26.87	14.35±12.58	8.39±1.30	13.75±15.42	6.02±3.85	19.62±18.32
FFA	1.66±1.31	2.07±1.27	3.13±1.71	1.76±0.65	1.64±0.68	1.51±0.12	3.05±0.74
ST	10.20±3.97	4.71±4.17	7.43±1.14	7.39±0.33	7.00±1.29	9.67±1.72	7.44±1.97
AMPL	1.66±0.82	3.56±1.60	4.22±3.29	3.31±0.80	5.21±3.35	2.96±0.31	7.29±2.00
PL	71.58±12.69	60.64±23.65[a]	70.65±11.91	77.68±3.92	71.74±16.56	79.53±3.00	62.45±17.87[b]

There were no significant differences between the replicate tanks for each diet; the replicates were averaged to better compare the three diets (Fig. 3.2). Both mussels fed algae and those fed fish effluent increased in the proportion of acetone mobile polar lipids (AMPL).

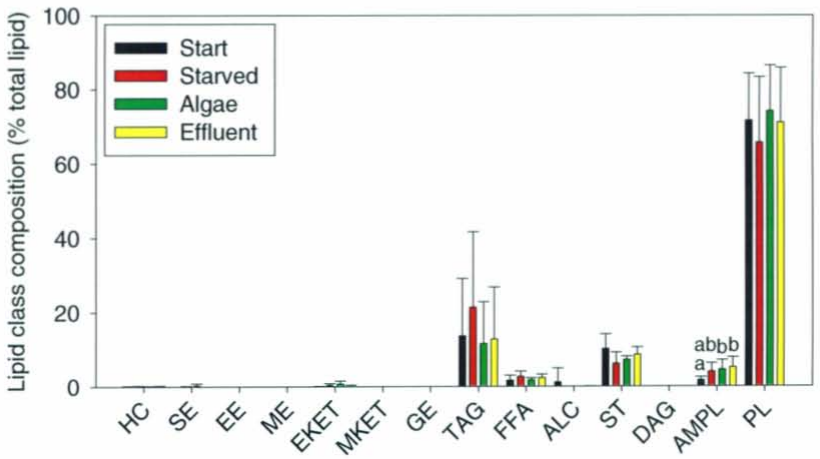

Fig. 3.2. Lipid class composition (% total lipid) for mussels at the start of the experiment (n = 18) and at the end of the experiment after feeding three different diets (algae, effluent and no food; n = 6). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

Quantitatively (mg/g WW) there were no differences in lipid classes among mussels fed any of the diets at the end of the experiment (Table 3.4). There were no significant changes in the lipid class composition (mg/g WW) of mussels fed any diet throughout the experiment.

Table 3.4. Lipid class composition (mg/g WW) of mussels in six tanks supplied different diets (algae, effluent and no food) at the beginning (n = 18) and end of a ten week experiment (n = 3). Different letters denote significant differences among groups (Holm-Sidak $p < 0.05$).

		Starved		Algae		Effluent	
Lipid class	Start	S1	S2	A1	A2	E1	E2
EKet	0.00±0.00	0.03±0.05	0.00±0.00	0.08±0.02	0.01±0.02	0.01±0.01	0.00±0.00
TAG	1.78±2.57	0.36±0.06	0.46±0.35	0.53±0.12	0.36±0.06	0.26±0.18	0.41±0.02
FFA	0.15±0.15	0.06±0.04	0.12±0.11	0.11±0.00	0.10±0.09	0.07±0.02	0.11±0.08
Sterol	0.97±1.07	0.23±0.31	0.28±0.29	0.47±0.16	0.43±0.33	0.43±0.16	0.27±0.19
AMPL	0.17±0.18	0.08±0.08	0.10±0.07	0.20±0.03	0.20±0.11	0.13±0.04	0.23±0.14
PL	6.89±6.85	2.48±3.14	2.86±3.18	5.08±2.13	4.54±3.54	3.45±0.94	2.36±1.73

When the replicate tanks for each diet were averaged, the only statistically significant difference was a decrease in the sterol content of starved and effluent fed mussels (Fig. 3.3). There were no significant differences in the amount of each lipid class across the three diets at the end of the experiment.

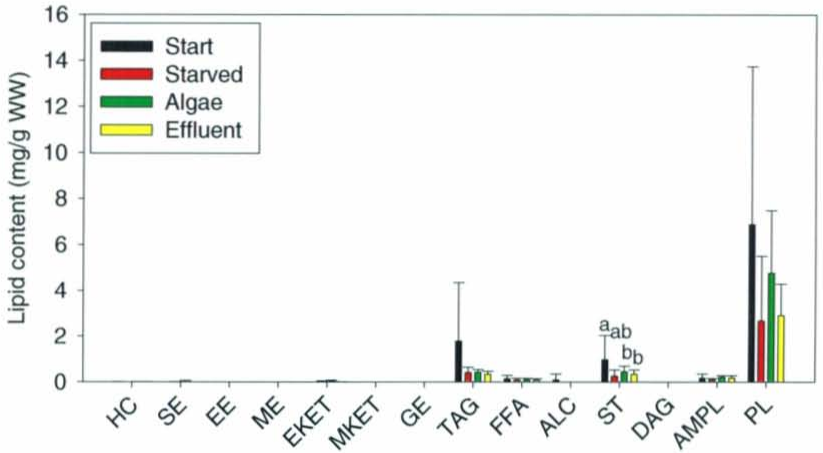

Fig. 3.3. Lipid class content (mg/g WW) of mussels at the start (n = 19) and end of the experiment after feeding three different diets (algae, effluent and no food; n = 6). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

The FA content (mg/g WW) did not statistically differ across the replicate tanks at the end of the experiment (Table 3.5). There were also no significant changes in the FA content (mg/g WW) of mussels fed any of the diets throughout the experiment.

Table 3.5. Total FA content (mg/g WW) of mussels in six tanks supplied three different diets (no food, algae and effluent) at the start (n = 18) and end of a ten week growth experiment (n = 3). Different letters denote significant differences among groups (Holm-Sidak $p < 0.05$).

		Starved		Algae		Effluent	
	Start	S1	S2	A1	A2	E1	E2
Total FA	5.51±3.23	2.24±2.37	2.68±2.64	4.37±1.66	3.84±2.76	2.87±0.80	2.30±1.40

When the replicates were averaged there were still no significant differences among diets (Fig. 3.4); however, the total FA content (mg/g WW) for starved and effluent fed mussels had significantly decreased throughout the experiment.

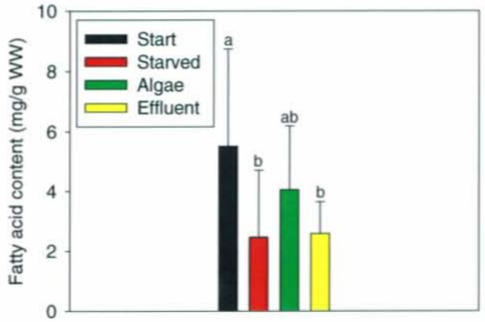

Fig. 3.4. Total fatty acid content (mg/g WW) for mussels at the start (n = 18) and end of the experiment after feeding three different diets (algae, effluent and no food; n = 6). Groups with different letters are significantly different from each other (Holm-Sidak p <0.05). Error bars are + 1 SD.

There were several differences in the composition of individual FAs among tanks at the end of the experiment as well as several significant changes from the beginning of the experiment (Table 3.6). Effluent fed mussels had a significantly smaller proportion of 20:5ω3 at the end of the experiment than at the start of the experiment as well as compared to the other diets. The proportion of 16:0, 20:4ω6, 21:5ω3 and 18:1ω9 increased at the end of the experiment for effluent fed mussels while the proportion of 18:2ω6 decreased. Effluent fed mussels had a larger proportion of 18:2ω6 than mussels fed both the other diets as well as a significantly larger proportion of 21:5ω3 than one of the starved mussel tanks. There were some significant differences between replicate tanks (largest difference 1% of total FA).

When the proportions of individual FAs for the replicate tanks were averaged there were no significant changes observed for starved mussels (Fig. 3.5); however, algae fed mussels showed two significant changes while effluent fed mussels showed six significant changes.

Table 3.6. Fatty acid composition (% total FA) of mussels in all tanks supplied three different diets at the start (n = 18) and end of the experiment (n = 3). Different letters denote significant differences among groups (Holm-Sidak p <0.05).

Fatty acid	Start	Starved		Algae		Effluent	
		S1	S2	A1	A2	E1	E2
16:0	4.20±1.26[a]	12.72±2.34	12.90±1.58	11.85±2.11	12.79±1.15	9.43±0.61[b]	11.72±1.74
16:1ω7	1.00±0.40	7.43±4.46	5.38±3.93	3.61±0.48	3.86±1.59	2.06±0.48	4.28±2.35
17:1	3.76±3.27	3.62±1.70	5.11±0.38	3.10±0.03	3.47±0.69	7.99±1.72	4.55±1.00
18:0	2.17±0.52	2.81±1.24	3.87±1.37	3.27±0.88	4.49±1.34	3.00±0.28	2.61±0.33
18:1ω7	3.40±0.58	2.32±0.54	2.07±0.78	1.95±0.17	1.98±0.21	1.52±0.21	2.06±0.52
18:1ω9	1.30±0.16[a]	1.87±0.69	1.62±0.06	2.27±0.03	1.54±0.11	4.75±1.57[b]	6.86±0.24[b]
18:2ω6	19.82±3.45[a]	1.39±0.79[ab]	1.05±0.19[a]	1.87±0.09[ab]	1.45±0.09[ab]	2.64±0.73[b]	3.58±0.42[c]
18:4ω3	1.82±0.99	2.18±1.84	1.06±0.66	0.81±0.04	0.93±0.38	0.46±0.09	0.97±0.60
20:1ω7	2.15±1.09	1.37±0.19[a]	1.09±0.23	1.03±0.27	0.69±0.28[b]	1.14±0.10	0.87±0.08
20:1ω9	1.31±0.53	3.54±0.83	2.99±0.51	3.76±0.28	2.86±0.88	4.09±0.38	3.43±0.55
20:2a	2.29±0.88	2.97±1.00	2.34±0.78	3.06±0.56	1.75±0.26	4.86±1.18	3.74±0.92
20:4ω6	1.82±0.99[a]	2.67±1.48	2.59±0.10	4.29±1.13	2.87±0.47	6.26±0.54[b]	4.64±2.26
20:5ω3	14.42±2.28[a]	19.70±3.34	22.41±2.04[ab]	17.00±2.87	26.46±5.29[a]	12.87±1.84[b]	13.82±1.94[c]
21:5ω3	1.20±0.44	1.50±0.23[a]	1.07±0.17	1.68±0.75	0.80±0.19	2.07±0.42[b]	1.62±0.25
22:2b	2.67±1.36	2.84±0.82	1.94±0.87	2.82±1.37	1.77±0.61	4.57±0.69	3.28±0.84
22:5ω3	21.28±3.62	1.21±0.12	1.37±0.05	1.05±0.01	1.09±0.23	1.52±0.24	1.50±0.14
22:6ω3	4.57±1.87	15.91±4.78	19.05±6.20	18.14±1.74	18.82±4.08	15.49±0.50	14.86±0.99

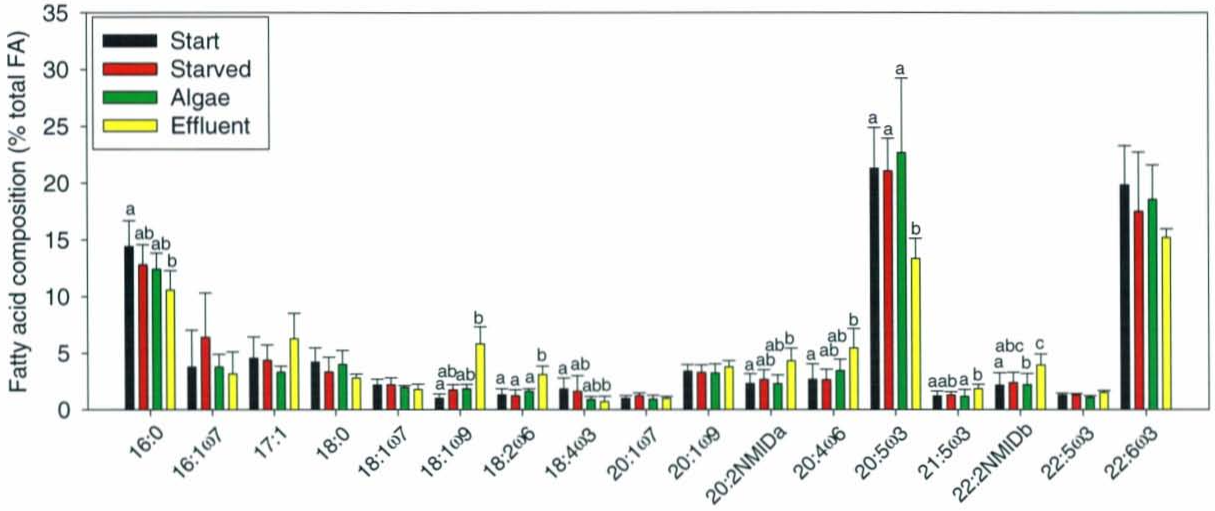

Fig. 3.5. Fatty acid composition (% of total FAs) of mussels at the start (n = 18) and end of the experiment after feeding three diets (algae, effluent and no food; n = 6). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

Effluent fed mussels decreased in the proportion of the essential FA, EPA (20:5ω3) as well as in the saturate 16:0. Effluent fed mussels also increased in the terrestrial plant marker 18:2ω6, the essential FA 20:4ω6 and the two non methylene-interrupted dienes (NMID) 20:2a and 22:2b.

To further compare differences among dietary treatments a comparison of mussels fed all three diets at the end of the experiment was undertaken (Fig. 3.6). Effluent fed mussels had a significantly larger proportion of 18:1ω9, the terrestrial plant marker 18:2ω6 and the two NMIDs 20:2a and 22:2b as well as a smaller proportion of 20:5ω3 than mussels fed both 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.

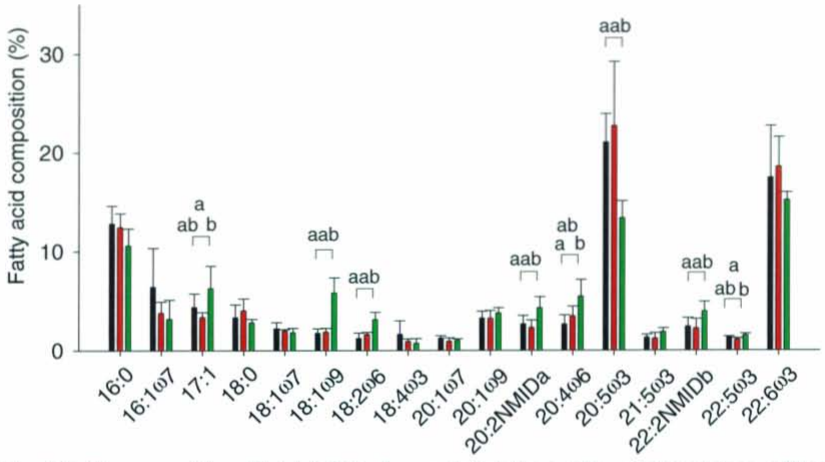

Fig. 3.6. FA composition (% total FA) of mussels fed three different diets (algae, effluent and no food) at the end of a ten week experiment (n = 6). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

The amount of individual FAs (mg/g WW) did not significantly change for mussels fed any diet throughout the experiment (Table 3.7). There were also no significant differences among mussels fed any of the diets at the end of the experiment.

Table 3.7. Fatty acid composition (mg/g WW) of mussels in six tanks supplied three different diets (no food, algae and effluent) at the start (n = 18) and end of the experiment (n = 3). Different letters denote significant differences among groups (Holm-Sidak p <0.05).

		Starved		Algae		Effluent	
Fatty acid	Start	S1	S2	A1	A2	E1	E2
16:0	0.21±0.11	0.27±0.28	0.36±0.39	0.54±0.29	0.51±0.38	0.27±0.09	0.25±0.14
16:1ω7	0.06±0.06	0.10±0.04	0.12±0.09	0.16±0.08	0.12±0.07	0.06±0.03	0.08±0.03
17:1	0.27±0.37	0.11±0.14	0.13±0.13	0.14±0.05	0.14±0.11	0.23±0.10	0.11±0.08
18:0	0.13±0.10	0.08±0.11	0.11±0.12	0.15±0.09	0.20±0.15	0.09±0.03	0.06±0.04
18:1ω7	0.18±0.10	0.05±0.04	0.05±0.05	0.08±0.03	0.07±0.05	0.04±0.02	0.04±0.02
18:1ω9	0.07±0.04	0.04±0.03	0.04±0.04	0.10±0.04	0.06±0.04	0.14±0.06	0.16±0.09
18:2ω6	1.04±0.50	0.02±0.02	0.03±0.03	0.08±0.03	0.06±0.04	0.08±0.03	0.08±0.05
18:4ω3	0.12±0.13	0.02±0.01	0.03±0.02	0.04±0.02	0.03±0.02	0.01±0.01	0.02±0.00
20:1ω7	0.12±0.09	0.03±0.03	0.03±0.02	0.05±0.01	0.03±0.03	0.03±0.01	0.02±0.01
20:1ω9	0.08±0.07	0.09±0.11	0.08±0.09	0.16±0.05	0.13±0.10	0.12±0.04	0.08±0.06
20:2a	0.13±0.08	0.08±0.10	0.06±0.06	0.13±0.03	0.07±0.05	0.14±0.04	0.09±0.07
20:4ω6	0.12±0.13	0.08±0.11	0.07±0.07	0.18±0.02	0.12±0.09	0.18±0.04	0.13±0.10
20:5ω3	0.81±0.52	0.41±0.39	0.56±0.51	0.77±0.41	0.92±0.61	0.36±0.07	0.30±0.17
21:5ω3	0.07±0.04	0.04±0.04	0.03±0.03	0.07±0.00	0.03±0.03	0.06±0.02	0.04±0.03
22:2b	0.13±0.06	0.07±0.07	0.05±0.04	0.11±0.01	0.06±0.04	0.13±0.04	0.08±0.06
22:5ω3	1.17±0.71	0.03±0.03	0.04±0.04	0.05±0.02	0.05±0.03	0.04±0.01	0.03±0.02
22:6ω3	0.24±0.14	0.43±0.54	0.55±0.65	0.81±0.38	0.79±0.61	0.44±0.11	0.35±0.22

When the replicate tanks for each diet were averaged, no significant differences in the amount of individual FAs between algae fed and effluent fed mussels were seen (Fig. 3.7). There were no significant changes in FA content (mg/g WW) of individual FAs for starved and algae fed mussels; however, there were some significant changes for effluent fed mussels. Effluent fed mussels decreased in their amount of 18:0, 18:4ω3, 18:1ω9 and the essential FAs, DHA and EPA.

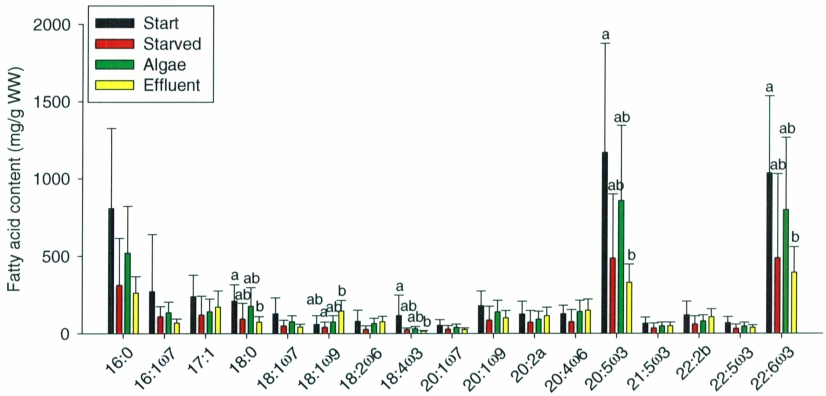

Fig. 3.7. Fatty acid content (mg/g WW) of mussels at the start (n = 18) and end of the experiment after feeding three different diets (algae, effluent and no food; n = 6). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are ± 1 SD.

There were three significant proportional changes in the different FA groups (% total FA) throughout the experiment as well as one significant differences among mussels fed different diets (Table 3.8). Both effluent fed mussel tanks had a decreased amount of ω3 at the end of the experiment and one of the effluent tanks had a decreased proportion of SFA while the other had an increased MUFA from the start of the experiment. Effluent fed mussels also had significantly less ω3 than one of the tanks fed algae as well as one of the starved mussel tanks.

Table 3.8. Sum of FA groups (% total FA) of mussels in six tanks supplied three different diets (no food, algae and effluent) at the start (n = 18) and end of a ten week growth experiment (n = 3). Different letters denote significant differences among groups (Holm-Sidak $p < 0.05$).

		Starved		Algae		Effluent	
Fatty acid	Start	S1	S2	A1	A2	E1	E2
ΣBacterial	8.04±2.62	7.06±2.72	8.62±0.49	7.62±0.82	6.80±0.71	13.29±1.81	8.87±2.22
ΣSFA	21.54±2.49[a]	18.39±1.43	19.22±2.06	19.04±2.03	19.98±2.00	15.10±1.14[b]	17.35±1.35
ΣMUFA	18.15±2.49[a]	23.25±3.07	20.75±4.17	21.98±2.88	17.56±1.21	24.92±1.17	25.49±1.80[b]
ΣPUFA	58.08±2.64	55.88±2.88	57.55±2.02	55.94±2.72	60.18±0.76	56.15±2.13	53.97±2.03
Σω3	47.02±4.42[a]	42.43±1.01[a]	46.56±5.18	41.16±3.17	50.04±0.67[a]	34.13±1.70[b]	34.98±2.02[b]

In terms of the quantities (mg/g WW) of different FA groups there were no significant changes during the experiment for mussels fed any diet (Table 3.9). There were also no significant differences in the quantity of FA groups among mussels fed any of the diets at the end of the experiment.

Table 3.9. Sum of FA groups (mg/g WW) of mussels in six tanks supplied three different diets (no food, algae and effluent) at the start (n = 18) and end of a ten week growth experiment (n = 3). Different letters denote significant differences among groups (Holm-Sidak $p < 0.05$).

		Starved		Algae		Effluent	
Fatty acid	Start	S1	S2	A1	A2	E1	E2
ΣBacterial	0.41±0.20	0.20±0.26	0.23±0.23	0.34±0.10	0.27±0.20	0.39±0.14	0.22±0.16
ΣSFA	1.19±0.70	0.41±0.44	0.53±0.56	0.83±0.42	0.80±0.59	0.44±0.15	0.39±0.23
ΣMUFA	1.06±0.82	0.47±0.45	0.53±0.46	0.88±0.28	0.65±0.46	0.72±0.23	0.57±0.33
ΣPUFA	3.15±1.73	1.29±1.42	1.55±1.55	2.51±0.94	2.29±1.64	1.60±0.38	1.26±0.78
Σω3	2.55±1.42	0.96±1.04	1.26±1.30	1.87±0.87	1.91±1.37	0.97±0.23	0.79±0.47

The replicates for each diet were averaged to better compare the treatments (Fig. 3.8). There were no significant changes for starved or algae fed mussels; effluent fed mussels decreased in the proportion of SFA, MUFA and PUFA.

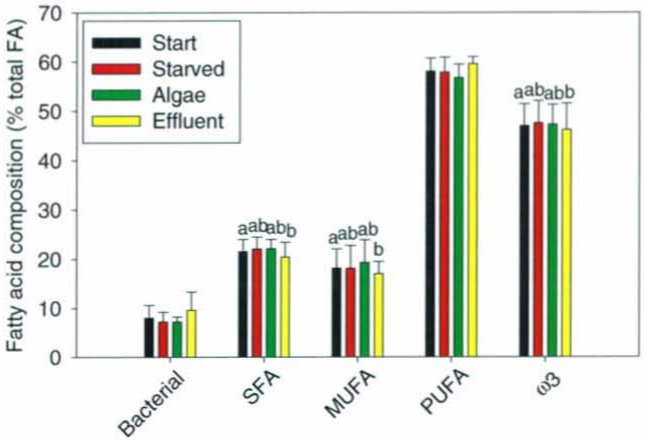

Fig. 3.8. Sum of different groups of FAs (% total FA) for mussels at the start (n = 18) and end of a ten week experiment after feeding three diets (algae, effluent and no food; n = 6). Groups with different letters are significantly different from each other (Holm-Sidak p <0.05). Error bars are + 1 SD.

When mussels fed the three diets at the end of the experiment were compared alone there were several differences in terms of the sum of different FA groups (% total FA) (Fig. 3.9). Effluent fed mussels had a larger proportion of bacterial FAs and a significantly smaller proportion of ω3 than mussels fed both other diets. Effluent fed mussels also had significantly less SFA and significantly more MUFA than algae fed mussels.

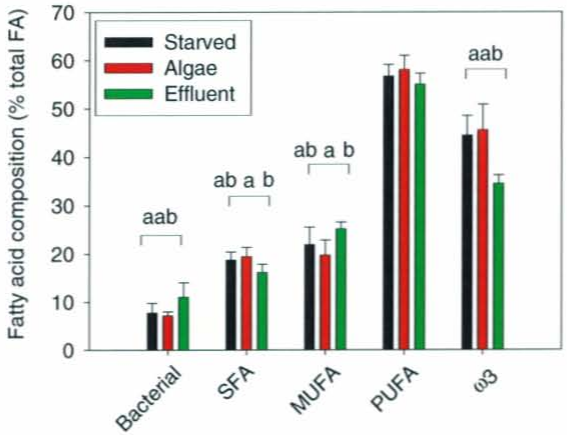

Fig. 3.9. Sum of different groups of FAs (% total FA) for mussels fed three diets (algae, effluent and no food) at the end of a ten week experiment (n = 6). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

In terms of the quantity of FA groups, the only changes between the start and end of the experiment were a decrease in the amount of SFA and ω3 in effluent fed mussels (Fig. 3.10). There were no differences among the three diets.

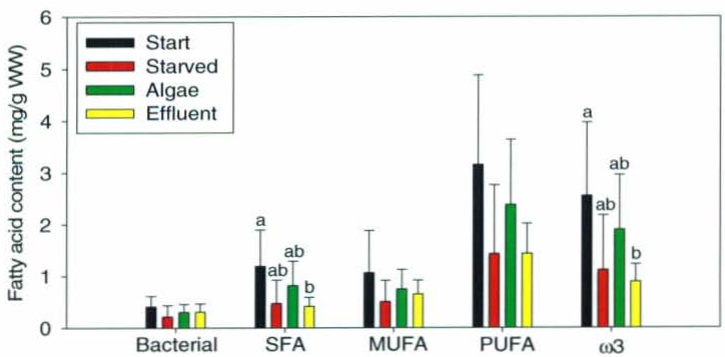

Fig. 3.10. Sums of different groups of Fas (mg/g WW) for mussels at the start (n = 18) and end of a ten week experiment after feeding three different diets (algae, effluent and no food; n = 6). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

The lipid class composition of mussels at the beginning of the experiment was compared to the lipid class composition for cultured *M. edulis* in Charles Arm and Fortune Harbour Newfoundland (Alkanani *et al.* 2007) (Table 3.10). The only significant difference between the lipid profile of mussels used in this experiment and that of cultured Newfoundland mussels was a larger proportion of AMPL in cultured mussels.

The lipid class composition of mussels fed all three diets at the end of the experiment was also compared to that of mussels from Charles Arm and Fortune Harbour (Table 3.11). The lipid content (mg/g WW) for mussels fed all three diets was lower than that of mussels collected from Charles Arm and Fortune Harbour.

Table. 3.10. Lipid class composition (% total lipid) of mussels fed three different diets (algae, effluent and no food) at the start of a ten week experiment in comparison to the lipid class composition of Newfoundland mussels at the same time of year (Alkanani *et al.* 2007). Different letters denote significant differences between groups (Holm-Sidak $p < 0.05$).

Lipid class	Alkanani *et al.* 2007.	Growth Trial Start
	n = 4	n = 18
TAG	7.8±4.7	13.7±15.4
FFA	1.0±0.7	1.6±1.3
ST	8.0±1.1	10.2±4.0
AMPL	5.0±0.9[a]	1.6±0.8[b]
PL	76.0±10.9	71.6±12.7
Total lipid (mg/g WW)	12.1±1.1	8.0±4.0

Table. 3.11. Lipid class composition (% total lipid) of mussels fed three different diets (algae, effluent and no food) at the end of a ten week experiment in comparison to the lipid class composition of Newfoundland mussels (Alkanani *et al.* 2007). Different letters denote significant differences between groups (Holm-Sidak $p < 0.05$).

Lipid class	Alkanani *et al.* 2007.				Growth Trial End		
	June	Aug	Sept	Oct	Starved	Algae	Effluent
	n = 4	n = 4	n = 4	n = 4	n = 6	n = 6	n = 6
TAG	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
FFA	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
AMPL	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
PL	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 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]

There were several differences among FA compositions of cultured mussels from Charles Arm and Fortune Harbour and the mussels used for this experiment (Table 3.12). In general the FA composition of mussels used in this experiment fell between the two values for cultured mussels; however, there was a smaller proportion of EPA and 18:0 in cultured mussels in 2000 and 2001.

Table. 3.12. Fatty acid composition (% total FA) of mussels fed three different diets (algae, effluent and no food) at the start of a ten week experiment in comparison to the FA composition of Newfoundland mussels (Alkanani *et al.* 2007). Different letters denote significant differences between groups (Holm-Sidak $p < 0.05$).

	Alkanani et al. 2007		Growth Trial Start
Fatty acid	2000	2001	Aug. 11
	n = 67	n = 75	n = 18
16:0	13.61±1.31	13.69±1.49	14.42±2.28
16:1ω7	3.96±1.97[a]	5.81±3.76[b]	3.76±3.27[a]
18:0	3.20±0.66[a]	3.00±0.85[a]	4.20±1.26[b]
18:1ω7	1.64±0.6[a]	2.25±0.64[b]	2.17±0.52[c]
18:1ω9	1.02±0.64[a]	1.33±0.44[b]	1.00±0.39[a]
18:2ω6	1.41±0.47	1.61±0.83	1.30±0.53
18:4ω3	2.12±1.47[a]	3.67±2.16[b]	1.82±0.99[a]
20:1ω9	3.05±0.73[a]	2.45±1.68[b]	3.40±0.58[a]
20:2a	3.13±0.68[a]	0.75±1.14[b]	2.29±0.88[c]
20:4ω6	2.76±0.82	2.85±1.16	2.67±1.36
20:5ω3	12.01±2.21[a]	17.02±3.49[b]	21.28±3.62[c]
21:5ω3	1.48±0.31	1.29±0.64	1.20±0.44
22:2b	3.01±0.73[a]	2.49±1.12[b]	2.15±1.09[b]
22:6ω3	21.50±2.88	19.99±4.28	19.82±3.45

When FA compositions of the three diets at the end of the experiment were compared to that of cultured mussels from Charles Arm and Fortune Harbour NL, several differences were found (Table 3.13). All three diets had a significantly larger proportion of the essential FA EPA (20:5ω3) than both averages for cultured mussels, as well as a significantly smaller proportion of 18:1ω9. Effluent fed mussels had a significantly smaller proportion of 16:0 as well as the essential FA DHA than farmed mussel values; however, effluent fed mussels also had a significantly larger proportion of the essential FA 20:4ω6 than both averages for cultivated mussels, starved and algae fed mussels.

Effluent fed mussels had a significantly larger proportion of the NMIDs 20:2a and 22:2b as well as an increased amount of the terrestrial plant marker 18:2ω6. Both algae and effluent fed mussels had significantly less of the flagellate marker 18:4ω3 than the values in the literature.

Table. 3.13. Fatty acid composition (% total FA) of mussels fed three different diets (algae, effluent and no food) at the end of a ten week experiment in comparison to the FA composition of Newfoundland mussels (Alkanani *et al.* 2007). Different letters denote significant differences between groups (Holm-Sidak $p < 0.05$).

Fatty acid	Alkanani et al. 2007		Growth Trial Start		
	2000	2001	Starved	Algae	Effluent
	n = 67	n = 75	n = 6	n = 6	n = 6
16:0	13.61±1.31[a]	13.69±1.49[a]	12.81±1.79	12.41±1.43	10.58±1.72[b]
16:1ω7	3.96±1.97	5.81±3.76	6.40±3.92	3.76±1.16	3.17±1.94
18:0	3.20±0.66	3.00±0.85	3.34±1.30	4.00±1.24	2.81±0.35
18:1ω7	1.64±0.6	2.25±0.64	2.20±0.62	1.97±0.17	1.79±0.46
18:1ω9	1.02±0.64[a]	1.33±0.44[b]	1.75±0.46[b]	1.83±0.41[b]	5.81±1.53[c]
18:2ω6	1.41±0.47[a]	1.61±0.83[a]	1.22±0.55[a]	1.61±0.24[a]	3.11±0.74[b]
18:4ω3	2.12±1.47	3.67±2.16[a]	1.62±1.38	0.88±0.28[b]	0.71±0.47[b]
20:1ω9	3.05±0.73	2.45±1.68	3.27±0.68	3.22±0.81	3.76±0.56
20:2a	3.13±0.68[a]	0.75±1.14[b]	2.66±0.87[a]	2.27±0.79[a]	4.30±1.13[c]
20:4ω6	2.76±0.82[a]	2.85±1.16[a]	2.63±0.94[a]	3.44±1.02[a]	5.45±1.71[b]
20:5ω3	12.01±2.21[a]	17.02±3.49[b]	21.05±2.88[c]	22.67±6.55[c]	13.35±1.77[d]
21:5ω3	1.48±0.31	1.29±0.64	1.29±0.30	1.16±0.62	1.85±0.39
22:2b	3.01±0.73	2.49±1.12[a]	2.39±0.90[a]	2.19±0.99[a]	3.93±0.98[b]
22:6ω3	21.50±2.88[a]	19.99±4.28[a]	17.48±5.24	18.55±3.04	15.18±0.79[b]

The amounts of FA groups for mussels used in this experiment were similar to those of cultured mussels at the beginning of the experiment (Table 3.14); however, there was a larger proportion of SFA and PUFA in cultured mussels. At the end of the experiment, mussels fed all three diets were significantly different than literature values for several FA groups (Table 3.15). Mussels fed all diets had a significantly smaller proportion of SFA than cultured mussels. The MUFA content of mussels fed all diets was significantly higher than cultured mussels; effluent fed mussels had the highest value which was significantly larger than the other diets. Starved and effluent fed mussels had a significantly lower PUFA content than literature values and effluent fed mussels had

significantly less ω3 along with a higher PUFA/SFA than the literature values and mussels fed the other two diets.

Table. 3.14. Sum of FA groups for mussels fed three different diets (algae, effluent and no food) at the start of a ten week experiment in comparison to the sum of FA groups for Newfoundland mussels (Alkanani *et al.* 2007). Different letters denote significant differences between groups (Holm-Sidak $p < 0.05$).

	Alkanani *et al.* 2007		Growth Trial Start
	2000	2001	Aug. 11
Fatty acid	n = 67	n = 75	n = 18
ΣSFA	25.4±1.8[a]	23.6±2.3[b]	21.5±2.5[c]
ΣMUFA	14.5±3.0[a]	17.7±4.1[b]	18.1±3.9[b]
ΣPUFA	61.9±3.0[a]	60.8±4.0[a]	58.1±2.6[b]
Σω3	47.8±3.7[a]	49.5±3.5[b]	47.0±4.4[a]
ΣPUFA/ΣSFA	2.4±0.2[a]	2.6±0.4[b]	2.7±0.4[b]

Table. 3.15. Sum of FA groups for mussels fed three different diets (algae, effluent and no food) at the end of a ten week experiment in comparison to the sum of FA groups for Newfoundland mussels (Alkanani *et al.* 2007). Different letters denote significant differences between groups (Holm-Sidak $p < 0.05$).

	Alkanani *et al.* 2007		Growth Trial End		
	2000	2001	Starved	Algae	Effluent
Fatty acid	n = 67	n = 75	n = 6	n = 6	n = 6
ΣSFA	25.4±1.8[a]	23.6±2.3[b]	18.8±1.6[c]	19.5±1.9[c]	16.2±1.7[d]
ΣMUFA	14.5±3.0[a]	17.7±4.1[b]	22.0±3.5[c]	19.8±3.1[c]	25.2±1.4[d]
ΣPUFA	61.9±3.0[a]	60.8±4.0[a]	56.7±2.4[b]	58.1±2.9	55.1±2.2[b]
Σω3	47.8±3.7[ab]	49.5±3.5[b]	44.5±4.0[a]	45.6±5.3[ab]	34.5±1.7[c]
ΣPUFA/ΣSFA	2.4±0.2[a]	2.6±0.4[b]	3.0±0.2[c]	3.0±0.4[c]	3.4±0.5[d]

Bacteriological tests of mussels prior to the start of the experiment found *E. coli* to be present at a level of 210 MPN (most probable number)/100 g and did not detect any *Salmonella* (Table 3.16). At the end of the experiment *Salmonella* was still undetectable for all tanks while *E. coli* levels remained low (18 – 230 MPN/100 g).

Table. 3.16. *E. coli* and *Salmonella* content of mussels fed three different diets (no food, algae, effluent) at the end of a ten week experiment. ND – not detected.

Tank	Diet	*E. coli* (MPN/100 g)	*Salmonella*
S1	Starved	<18	ND
S2	Starved	230	ND
A1	Algae	220	ND
A2	Algae	45	ND
E1	Effluent	170	ND
E2	Effluent	130	ND

3.3.3 Six month growth trial

There were numerous differences in dry weight among the three different tanks during the experiment which were usually between the algae fed tank and one or both of the effluent fed tanks (Table 3.17); however, there were no differences between the initial DW and the final DW of the mussels in each tank. Similar results were seen for AFDW and CI. Shell length was the only physical characteristic found to have significantly increased through the course of the experiment.

Table 3.17. DW (mg), AFDW (mg), SL (cm) and condition index (CI) for three tanks of mussels fed different diets (tank 18 algae, tank 19 and 20 effluent) over the course of a six month experiment (n = 5). Different letters denote significant differences among tanks while different numbers denote significant differences among sampling times (Holm-Sidak $p < 0.05$).

	Time (days)						
	0	41	73	101	145	164	193
DW							
Tank 18	101±6^{a}	104±42^{a}	100±24	103±31	134±25^{a}	104±9^{a}	110±30^{a}
Tank 19	62±18^{b1}	57±7^{b123}	102±25^{124}	60±23^{2}	66±25^{b}	78±13^{b}	72±14
Tank 20	104±13^{a}	62±17	110±27	70±23	71±23^{b}	110±18^{a}	70±24^{b}
AFDW							
Tank 18	91±7^{a}	72±25	83±19	79±27^{a}	97±14^{a}	79±7	84±23
Tank 19	51±16^{b1}	45±8^{1}	87±22^{2}	46±19^{b1}	49±23^{b1}	65±16	57±12
Tank 20	93±12^{a}	53±15	84±26	59±17^{b}	56±24^{b}	88±15	57±20
CI							
Tank 18	4.61±0.18^{a}	3.82±1.12	3.75±0.75	3.42±0.84	3.90±0.56^{a}	3.12±0.22	3.36±0.69
Tank 19	3.31±0.71^{b}	2.54±0.32	3.53±0.63	2.49±0.69	2.40±0.82^{b}	2.84±0.32	2.66±0.30
Tank 20	4.74±0.66^{a1}	2.60±0.62	6.29±5.59	2.64±0.73	2.77±0.81	3.50±0.50	2.51±0.59^{2}
SL							
Tank 18	2.18±0.08^{a1}	2.64±0.34^{a2}	2.54±0.27^{a12}	2.98±0.16^{y}	3.42±0.17^{a3}	3.32±0.08^{a3}	3.26±0.30^{a3}
Tank 19	1.86±0.15^{b12}	2.24±0.15^{b2}	2.88±0.25^{1}	2.52±0.35^{3}	2.72±0.29^{b3}	2.72±0.15^{b3}	2.68±0.25^{b3}
Tank 20	2.22±0.13^{12}	2.38±0.13^{a2}	2.92±0.11^{b3}	2.60±0.29	2.56±0.19^{b}	3.15±0.17^{b1}	2.74±0.30^{b}

There were no differences between replicate effluent tanks at the end of the experiment (Fig. 3.11); however, there were some differences between the replicates for the intermediate dates (largest differences were 0.4 cm SL, 0.04 g DW, 0.04 g AFDW and 1.4 CI). When both effluent fed tanks were averaged and compared against the algae fed tank, many differences were seen. Algae fed mussels had a significantly higher DW, AFDW, SL and CI than mussels fed fish effluent at the end of the experiment. The only significant difference between the start and end of the experiment was a significantly higher SL for mussels fed algae.

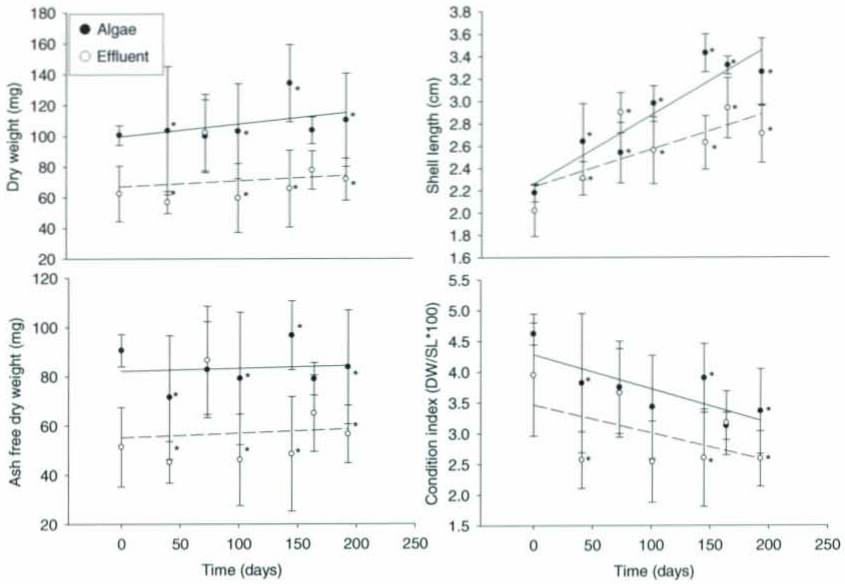

Fig. 3.11. Regressions of DW (mg), AFDW (mg), SL (cm), and CI of mussels fed an algae diet (n = 5) or fish effluent (n = 10) throughout the experiment. * indicates a significant difference between diets (Holm-Sidak $p < 0.05$). Error bars are ± 1 SD.

The C and N profile of mussels fed both diets followed a similar trend, carbon content significantly decreased and nitrogen content significantly increased in all tanks (Table 3.18). There were no differences between replicate effluent tanks or among the two effluent fed tanks and the algae fed mussel tank at the end of the experiment.

Table 3.18. Carbon and nitrogen content (mg/g DW) for three tanks fed different diets (tank 18 algae, tank 19 and 20 effluent) at the start (n = 15) and end of a six month experiment (n = 5). Different letters denote significant differences among groups (Holm-Sidak p <0.05).

	Start	Tank 18	Tank 19	Tank 20
Carbon	448 ± 10^{a}	425 ± 7^{b}	426 ± 6^{b}	426 ± 12^{b}
Nitrogen	76 ± 6^{a}	102 ± 5^{b}	110 ± 1^{b}	107 ± 3^{b}

When the averaged effluent tanks were compared against the algae fed tank there were no significant differences (Kruskal-Wallis one way ANOVA for N due to failed normality test); (Fig. 3.12). Protein content, calculated from nitrogen via a conversion factor of 5.8, of mussels fed algae and fish effluent increased from 454±42 and 435±36 mg/g DW at the start of the experiment to 592±27 and 628±17 mg/g DW respectively. Effluent fed mussels had a significantly larger protein content than algae fed mussels (Holm-Sidak test due to passed normality test).

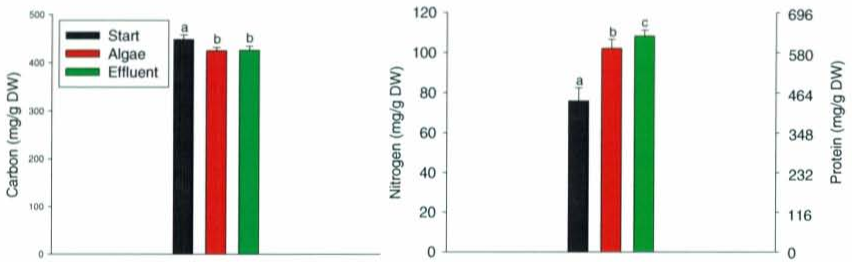

Fig. 3.12. C, N and protein content (mg/g DW) for mussels at the start (n = 15) and end of the experiment after fed algae (n = 5) and fish effluent (n = 10). Groups with different letters are significantly different from each other (Holm-Sidak p <0.05). Error bars are + 1 SD.

The C:N of mussels fed both diets significantly decreased throughout the experiment (Fig. 3.13). There was no significant difference in the C:N between mussels fed the two diets.

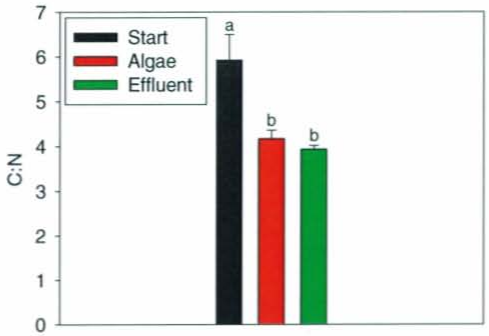

Fig. 3.13. C:N (by weight) of mussels at the start (n = 15) and end of the experiment after feeding algae (n = 5) or fish effluent (n = 10). Groups with different letters are significantly different from each other (Holm-Sidak p <0.05). Error bars are + 1 SD.

Lipid content (mg/g WW) of mussels did not statistically differ among mussels fed either diet (Table 3.19). There were no significant changes in the lipid content (mg/g WW) for mussels fed either diet throughout the experiment.

Table 3.19. Lipid content (mg/g WW) of three tanks of mussels fed different diets (tank 18 algae, tank 19 and 20 effluent) at the start (n = 15) and end of a six month experiment (n = 4). Different letters denote significant differences among groups (Holm-Sidak p <0.05).

	Start	Tank 18	Tank 19	Tank 20
Lipid content (mg/g WW)	24.34±9.84	14.71±7.91	12.60±1.32	21.11±17.73

When both effluent tanks were averaged and compared against the algae fed tank there were still no significant differences in total lipid content (mg/g WW) between mussels fed the two diets (Fig. 3.14); however, lipid content of effluent fed mussels had significantly decreased throughout the experiment.

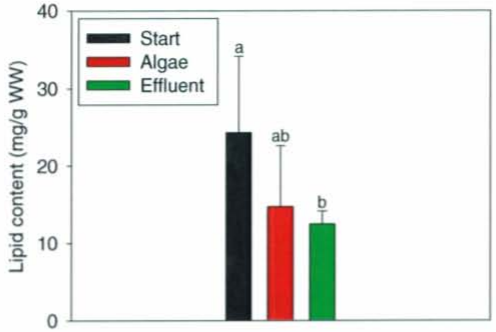

Fig. 3.14. Lipid content (mg/g WW) for mussels fed algae and fish effluent at the start (n = 14) and end (n = 4 and 8) of the experiment. Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

In terms of the lipid class composition (% total lipid) the only significant difference among the tanks at the end of the experiment was an increased proportion of AMPL in mussels in the algae fed tank compared to those in one of the effluent fed tanks (Table 3.20). The proportion of HC for all tanks decreased while the proportion of PL increased over the course of the experiment. The proportion of TAG decreased for mussels fed algae as well as for mussels in one of the effluent fed tanks throughout the experiment. There were no significant differences between replicate effluent tanks.

Table 3.20. Lipid class composition (% total lipid) for three tanks of mussels fed different diets (tank 18 algae, tank 19 and 20 effluent) at the start (n = 15) and end point of a six month experiment (n = 4). Different letters denote significant differences among groups (Holm-Sidak p <0.05).

Lipid class	Start	Tank 18	Tank 19	Tank 20
Hydrocarbon	1.94±1.18[a]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]
Steryl ester	0.43±0.64	0.09±0.18	0.30±0.37	0.45±0.89
Methyl ester	0.29±0.55	0.00±0.00	0.00±0.00	0.00±0.00
Ethyl ketone	2.89±6.71	0.06±0.12	0.05±0.09	0.24±0.29
Methyl ketone	1.08±2.57	0.00±0.00	0.00±0.00	0.00±0.00
Triacylglycerol	65.61±10.74[a]	7.64±0.91[b]	8.86±2.17[b]	9.17±6.26
Free fatty acid	2.08±5.19	3.08±3.62	2.79±2.65	2.68±2.23
Sterol	15.61±7.09	9.20±2.03	10.26±1.09	10.81±2.64
Acetone mobile polar lipid	4.34±1.67	5.94±0.99[a]	3.27±1.55	2.69±0.85[b]
Phospholipid	5.45±4.04[a]	73.98±5.25[b]	70.62±5.48[b]	73.82±5.00[b]

When the replicate effluent tanks were averaged and compared to the algae fed tank the only significant difference between mussels fed the two diets was a decreased proportion of AMPL in effluent fed mussels (Fig. 3.15). Mussels fed both diets showed a decrease in their proportion of HC. Effluent fed mussels had an increase in their proportion of TAG while the proportion of TAG decreased for algae fed mussels. Algae fed mussels increased in their proportion of PL and effluent fed mussels decreased in their proportion of PL.

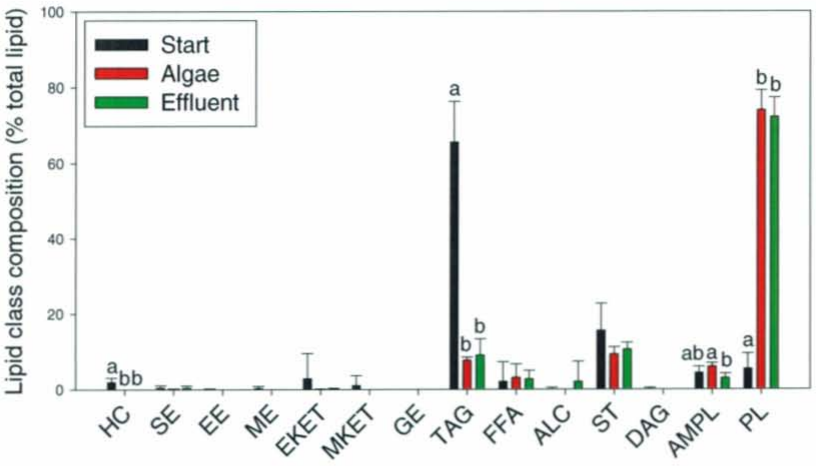

Fig. 3.15. Lipid class composition (% total lipid) for mussels at the start (n = 14) and end (n = 4 and 8) of the experiment after feeding algae and fish effluent. Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

Quantitatively there were no differences in the amount of individual lipid classes among mussels fed both diets at the end of the experiment (Table 3.21); however, there were a few significant changes during the experiment for mussels fed both diets. The amount of HC and TAG decreased for mussels in all tanks and the amount of PL increased for one of the effluent fed mussel tanks. There were no significant differences between the replicate effluent tanks.

Table 3.21. Lipid class composition (mg/g WW) for three tanks of mussels fed different diets (tank 18 algae, tank 19 and 20 effluent) at the start (n = 15) and end point of a six month experiment (n = 4). Different letters denote significant differences among groups (Holm-Sidak p <0.05).

Lipid class	Start	Tank 18	Tank 19	Tank 20
Hydrocarbon	0.49±0.47[a]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]
Steryl ester	0.10±0.17	0.01±0.02	0.04±0.05	0.06±0.12
Methyl ester	0.07±0.12	0.00±0.00	0.00±0.00	0.00±0.00
Ethyl ketone	0.73±1.99	0.01±0.01	0.01±0.01	0.03±0.04
Methyl ketone	0.22±0.48	0.00±0.00	0.00±0.00	0.00±0.00
Triacylglycerol	16.33±8.17[a]	1.13±0.60[b]	1.12±0.31[b]	1.13±0.81[b]
Free fatty acid	0.48±0.94	0.32±0.33	0.37±0.35	0.53±0.53
Sterol	3.54±1.44	1.26±0.41	1.29±0.14	2.56±2.79
Acetone mobile polar lipid	1.13±0.87	0.83±0.32	0.42±0.22	0.66±0.77
Phospholipid	1.22±0.72[a]	11.16±6.84	8.95±1.53	16.12±14.51[b]

When mussels in the replicate effluent tanks were averaged and compared to the algae fed tank there were no differences between the amount of different lipid classes for algae fed and effluent fed mussels (Fig. 3.16). Effluent fed mussels were found to have significantly decreased in their HC, ST, AMPL and increased in their TAG and PL content through the course of the experiment. Algae fed mussels had a significant decrease in the amount of HC, TAG, ST and an increase in PL during the experiment.

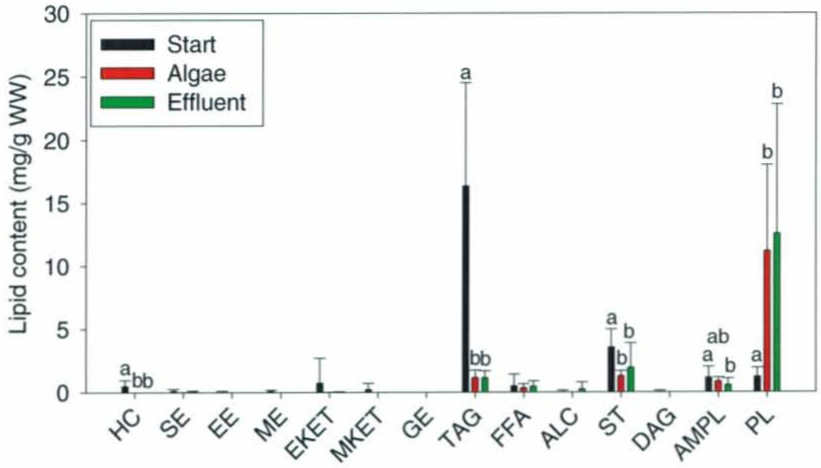

Fig. 3.16. Lipid class content (mg/g WW) for mussels at the start (n = 14) and end of the experiment after feeding algae and fish effluent (n = 4 and 8). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

There was no difference in total FA content (mg/g WW) among mussels fed either diet at the end of the experiment (Table 3.22). There were no significant changes in the total FA content (mg/g WW) for mussels fed either diet throughout the experiment.

Table 3.22. Total FA content (mg/g WW) for three tanks of mussels fed different diets (tank 18 algae, tank 19 and 20 effluent) at the start (n = 15) and end of a six month experiment (n = 4). Different letters denote significant differences among groups (Holm-Sidak $p < 0.05$).

	Start	Tank 18	Tank 19	Tank 20
FA content (mg/g WW)	17.75±8.24	9.78±5.41	8.09±1.56	13.58±10.60

When the total FA content of the replicate effluent tanks were averaged and compared to the algae fed tank there were no differences between algae fed and effluent fed mussels at the end of the experiment (Fig. 3.17). Total FA content (mg/g WW) for effluent fed mussels did however, significantly decrease throughout the experiment.

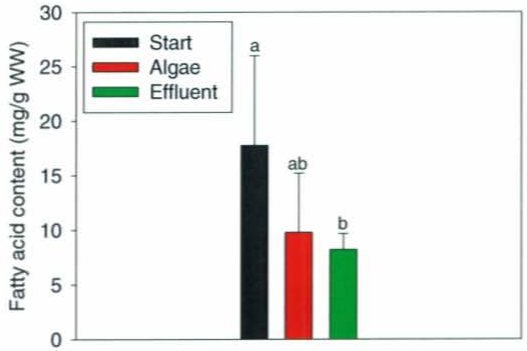

Fig. 3.17. FA content (mg/g WW) for mussels at the start (n = 14) and end of the experiment after feeding algae and fish effluent (n = 4 and 8). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

There were a few proportional differences among tanks in terms of FA composition (% total FA) at the end of the experiment (Table 3.23), all of which were between the algae fed tank and one or both of the effluent tanks. There were no differences between replicate effluent fed tanks. There were also many significant changes during the experiment for mussels fed both diets. Mussels fed both diets decreased in their proportion of 20:5ω3, 14:0 and 18:4ω3 as well as increased in their proportion of 18:0, 17:1 and the NMID 22:2b. Mussels in the two effluent fed tanks also had a significant decrease in their proportion of 16:1ω7, 16:2ω4 and 16:4ω1 as well as a significant increase in their proportion of the zooplankton marker 20:1ω9 and the NMID 20:2a during the experiment. Algae fed mussels had a significantly smaller proportion of 18:0 and the zooplankton marker 20:1ω11 than mussels in both of the effluent fed tanks as well as more 18:4ω3 than mussels in one of the effluent fed tanks.

Table 3.23. Fatty acid composition (% total FA) for three tanks of mussels fed different diets (tank 18 algae, tank 19 and 20 effluent) at the beginning (n = 15) and end of a six month experiment (n = 4). Different letters denote significant differences among groups (Holm-Sidak p <0.05).

Fatty acid	Start	Tank 18	Tank 19	Tank 20
14:0	6.38±0.55[a]	2.05±0.17[b]	1.52±0.27[b]	1.38±0.41[b]
16:0	12.82±1.13	12.48±0.31	12.80±0.53	12.85±0.59
16:1ω7	16.93±1.26[a]	4.49±0.44	3.82±0.65[b]	3.55±1.41[b]
16:2ω4	1.42±0.15[a]	0.53±0.06	0.42±0.04[b]	0.38±0.16[b]
16:4ω1	1.14±0.26[a]	0.06±0.02	0.12±0.03[b]	0.19±0.09[b]
17:1	1.42±0.64[a]	6.77±2.00[b]	7.42±1.17[b]	7.61±1.25[b]
18:0	1.62±0.36[a]	2.54±0.24[b]	3.24±0.09[c]	3.23±0.51[c]
18:1ω7	2.41±0.52[a]	2.44±0.13	2.67±0.10[b]	2.71±0.32
18:1ω9	1.15±0.45[a]	2.59±0.14	4.09±0.98[b]	3.12±2.04
18:2ω6	0.68±0.10[a]	2.68±0.16	1.38±0.30[b]	1.31±0.14
18:4ω3	2.23±0.20[a]	1.15±0.09[b]	0.76±0.16[c]	1.10±0.32[b]
20:1ω9	1.32±0.15[a]	3.25±0.27	4.77±0.06[b]	4.82±0.36[b]
20:1ω11	0.44±0.14[a]	0.98±0.06[b]	1.70±0.27[c]	1.67±0.18[c]
20:2a	0.83±0.10[a]	2.72±0.26	4.07±0.57[b]	4.63±0.44[b]
20:4ω6	0.60±0.10[a]	6.85±0.61[b]	4.52±0.34	4.96±0.84[b]
20:5ω3	30.85±2.11[a]	15.06±1.60[b]	15.31±1.08[b]	15.08±1.87[b]
21:5ω3	0.93±0.06	0.83±0.24	0.87±0.16	0.66±0.44
22:2b	1.24±0.19[a]	2.80±0.21[b]	3.44±0.71[b]	3.36±0.56[b]
22:6ω3	6.60±0.97[a]	11.86±0.46	13.78±0.53[b]	14.06±1.83

When the replicate effluent tanks were averaged and compared to the algae fed tank and the start of the experiment there were several significant changes (Fig. 3.18). Both diets increased in their proportion of 17:1, 18:0, the terrestrial plant marker 18:2ω6, the zooplankton markers 20:1ω11/20:1ω9, the NMID 22:2b, the essential FAs 20:4ω6 and 22:6ω3. As well, both treatments decreased in their proportion of 14:0, 16:1ω7, 16:2ω4, 16:4ω1 and the essential FA 20:5ω3. Effluent fed mussels also saw a significant increase in their proportion of 18:1ω7, 18:1ω9 and the NMID 20:2a as well as a significant decrease in their proportion of 18:4ω3.

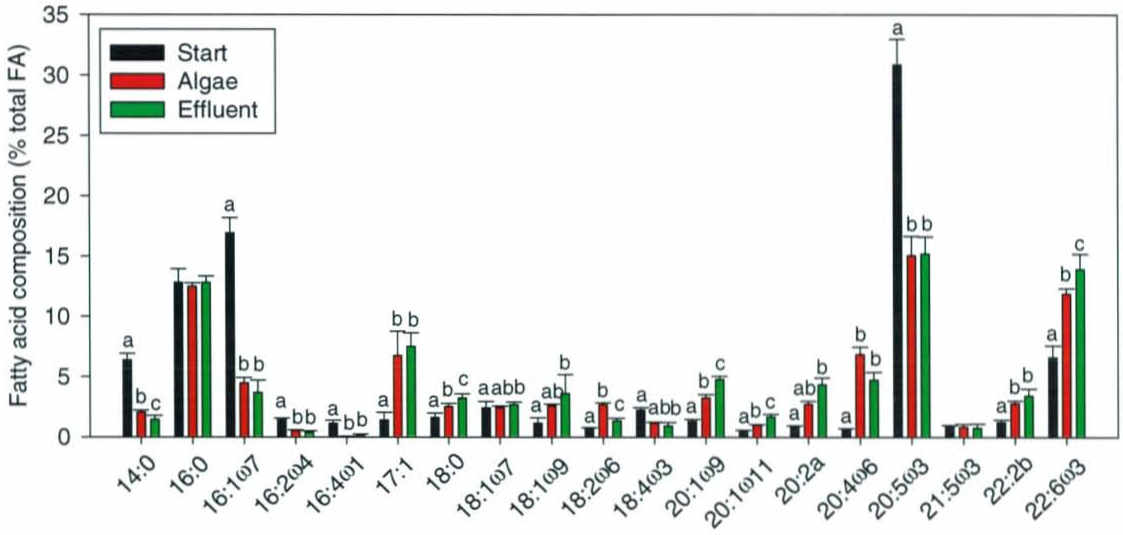

Fig. 3.18. FA composition (% total FA) of mussels at the start (n = 14) and end of experiment after feeding algae and fish effluent (n = 4 and 8). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

When the FA compositions (% total FA) of mussels fed both diets at the end of the experiment were compared alone many significant differences were found (Fig. 3.19). Effluent fed mussels had a significantly larger proportion of the zooplankton markers 20:1ω9/20:1ω11 and the NMID 20:2a as well as the essential FA 22:6ω3 but a decreased proportion of the essential 20:4ω6 compared with algae fed mussels.

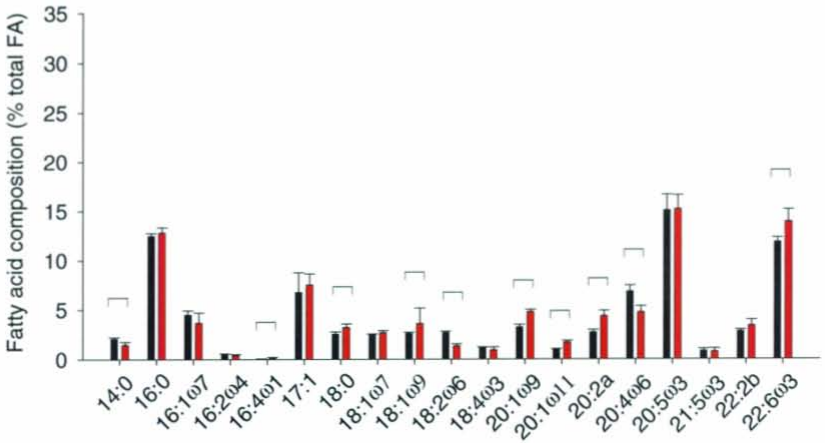

Fig. 3.19. FA composition (% total FA) for mussels fed algae (n = 4) and effluent (n = 8) at the end of a six month experiment. Brackets indicate a significant difference between groups (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

In terms of quantity of individual FAs (mg/g WW) there were no significant differences among mussels fed either diet at the end of the experiment (Table 3.24). There were several significant changes in the quantity of FAs (mg/g WW) throughout the experiment for mussels fed both diets. Mussels fed both had a decreased amount of 14:0, 16:1ω7, 16:2ω4 and 21:5ω3 at the end of the experiment.

Table 3.24. Fatty acid composition (mg/g WW) for three tanks of mussels fed different diets (tank 18 algae, tank 19 and 20 effluent) at the beginning (n = 15) and end of a six month experiment (n = 4). Different letters denote significant differences among groups (Holm-Sidak p <0.05).

Fatty acid	Start	Tank 18	Tank 19	Tank 20
14:0	1.16±0.59[a]	0.21±0.13[b]	0.12±0.04[b]	0.16±0.07[b]
16:0	2.26±1.02	1.21±0.65	1.04±0.24	1.70±1.23
16:1ω7	3.00±1.43[a]	0.45±0.30[b]	0.31±0.06[b]	0.40±0.16[b]
16:2ω4	0.26±0.13[a]	0.05±0.03[b]	0.03±0.01[b]	0.04±0.01[b]
16:4ω1	0.21±0.13[a]	0.01±0.00[b]	0.01±0.00[b]	0.03±0.04
17:1	0.26±0.18[a]	0.68±0.44	0.60±0.15	1.01±0.78[b]
18:0	0.29±0.15	0.24±0.10	0.26±0.05	0.47±0.43
18:1ω7	0.43±0.22	0.24±0.14	0.22±0.04	0.35±0.22
18:1ω9	0.20±0.10	0.26±0.16	0.34±0.12	0.27±0.18
18:2ω6	0.12±0.05	0.27±0.16	0.11±0.04	0.17±0.12
18:4ω3	0.41±0.21[a]	0.12±0.07	0.06±0.02[b]	0.17±0.19
20:1ω9	0.23±0.11[a]	0.31±0.16	0.39±0.08	0.67±0.57[b]
20:1ω11	0.07±0.03[a]	0.10±0.05	0.14±0.02	0.24±0.21[b]
20:2a	0.15±0.06[a]	0.26±0.12	0.33±0.09	0.64±0.53[b]
20:4ω6	0.10±0.04[a]	0.65±0.32[b]	0.37±0.09	0.73±0.69[b]
20:5ω3	5.48±2.69[a]	1.46±0.81[b]	1.24±0.24[b]	2.06±1.67
21:5ω3	0.16±0.08[a]	0.09±0.06	0.07±0.01[b]	0.05±0.04[b]
22:2b	0.22±0.09	0.27±0.13	0.27±0.03	0.49±0.48
22:6ω3	1.16±0.50	1.16±0.64	1.12±0.23	2.04±1.87

When the replicate effluent fed tanks were averaged and compared to the algae fed tank there were no significant differences in the quantity (mg/g WW) of FAs between mussels fed the two diets (Fig. 3.20). Most FAs were found to decrease in quantity for mussels fed both diets between the start and end of experiment; however, not all were significant and there are some exceptions that increased. There was a significant increase in the amount of the NMIDs 20:2a and 22:2b for mussels fed effluent and both diets saw a significant increase in the essential FA 20:4ω6.

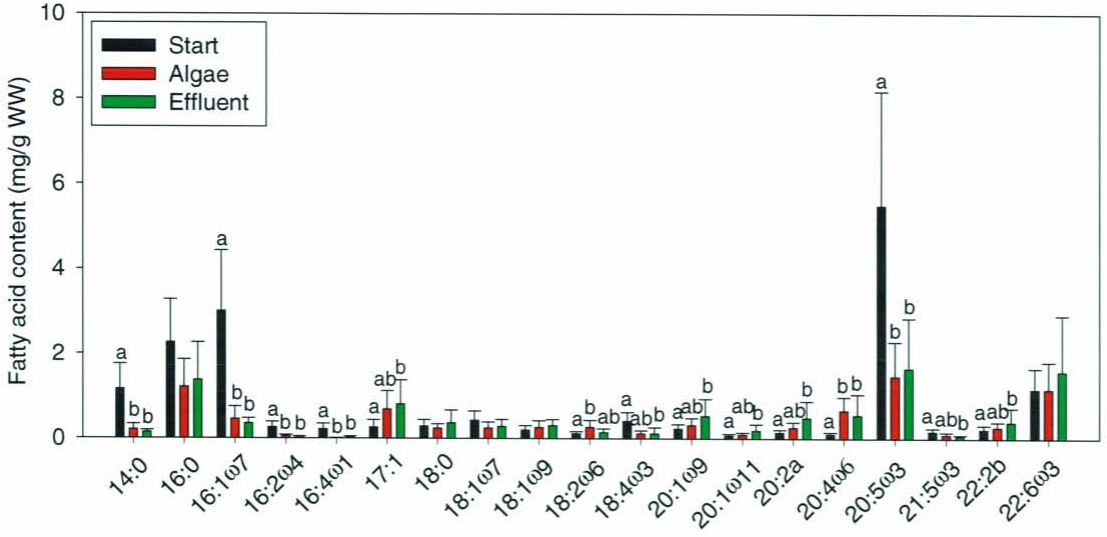

Fig. 3.20. FA content (mg/g WW) of the mussels at the start (n = 14) and end of the experiment after feeding algae and fish effluent (n = 4 and 8). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

There was only one significant difference among the three tanks at the end of the experiment based on the proportion (% total FA) of FA groups (Table 3.25). The algae fed tank had a smaller proportion of MUFA than one of the effluent fed tanks. There were also many significant changes throughout the experiment for mussels fed each diet. Algae fed mussels had an increased proportion of bacterial FAs and PUFA at the end of the experiment as well as a decreased proportion of SFA and ω3. Mussels in both effluent fed tanks also had an increased proportion of bacterial FAs and a decreased proportion of SFAs and ω3.

Table 3.25. The sum of FA groups (% total FA) for three tanks of mussels fed different diets (tank 18 algae, tank 19 and 20 effluent) at the start (n = 15) and end of a six month experiment (n = 4). Different letters denote significant differences among groups (Holm-Sidak $p < 0.05$).

Fatty acid	Start	Tank 18	Tank 19	Tank 20
ΣBacterial	3.33 ± 0.72^{a}	10.78 ± 1.64^{b}	11.21 ± 0.97^{b}	11.27 ± 1.38^{b}
ΣSFA	21.65 ± 1.55^{a}	19.52 ± 0.27^{b}	19.42 ± 0.50^{b}	19.11 ± 0.56^{b}
ΣMUFA	25.75 ± 1.12	23.13 ± 1.96^{a}	27.15 ± 0.90^{b}	26.37 ± 2.64
ΣPUFA	51.41 ± 1.95^{a}	54.62 ± 1.55^{b}	50.79 ± 0.81	51.95 ± 2.96
Σω3	42.96 ± 1.63^{a}	36.77 ± 1.92^{b}	34.33 ± 0.53^{b}	34.39 ± 1.87^{b}

When the quantity (mg/g WW) of FA groups was examined, there were no differences among mussels fed either diet at the end of the experiment (Table 3.26). There were no significant differences between the replicate effluent tanks at the end of the experiment. There were several significant changes throughout the experiment for mussels in one of the effluent fed tanks which included a decreased amount of SFA, PUFA and ω3.

Table 3.26. The sum of FA groups (mg/g WW) for three tanks of mussels fed different diets (tank 18 algae, tank 19 and 20 effluent) at the start (n = 15) and end of a six month experiment (n = 4). Different letters denote significant differences among groups (Holm-Sidak p <0.05).

Fatty acid	Date	Tank 18	Tank 19	Tank 20
ΣBacterial	0.60±0.32	1.06±0.60	0.91±0.19	1.53±1.22
ΣSFA	3.86±1.81[a]	1.90±1.04	1.58±0.33[b]	2.56±1.91
ΣMUFA	4.55±2.10	2.29±1.33	2.19±0.41	3.37±2.17
ΣPUFA	9.12±4.30[a]	5.33±2.95	4.11±0.79[b]	7.28±6.21
Σω3	7.62±3.58[a]	3.61±2.07	2.78±0.54[b]	4.80±4.03

The replicate effluent tanks were averaged and compared to the algae fed tank and the start period (Fig. 3.21). Mussels fed both diets significantly increased in the proportion of bacterial FAs between the beginning and end of the experiment. Both diets decreased in the proportion of SFA and algae fed mussels decreased in the amount of MUFA while effluent fed mussels increased in MUFA. The proportion of ω3s decreased for both diets and algae fed mussels increased in the proportion of PUFA.

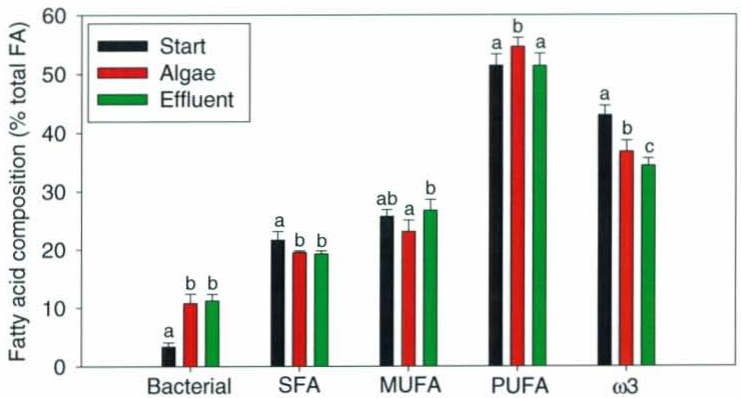

Fig. 3.21. Sum of different groups of FAs for mussels at the start (n = 14) and end of the growth experiment after feeding algae and fish effluent (n = 4 and 8). Groups with different letters are significantly different from each other (Holm-Sidak p <0.05). Error bars are + 1 SD.

In terms of the quantity (mg/g WW) of FA groups, there were no differences between both diets (Fig. 3.22); however, there were some differences between the start

and end point of the experiment. Effluent fed mussels significantly decreased in their amount of SFA and ω3 while their amount of bacterial FAs significantly increased during the experiment. Algae fed mussels were found to have significantly decreased in the amount of SFA and ω3 throughout the experiment.

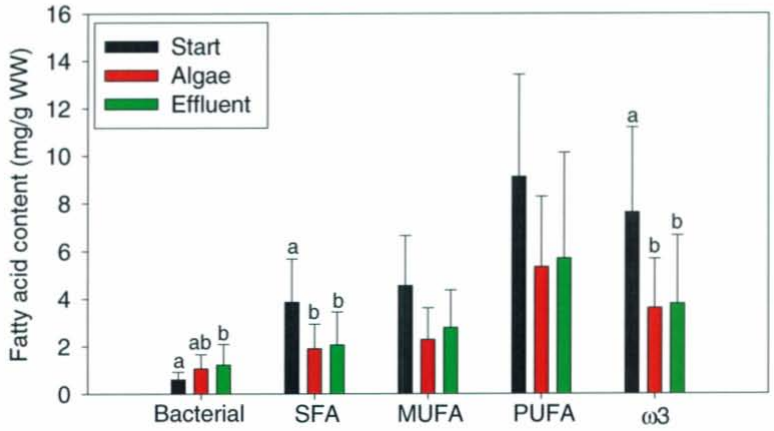

Fig. 3.22. Sum of different FA groups (mg/g WW) for mussels at the start (n = 14) and end of the experiment after feeding algae or fish effluent (n = 4 and 8). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

Amino acid composition was not found to vary qualitatively (% total AA) (Table 3.27) or quantitatively (mg/g DW) (Table 3.28) among mussels fed either diet at the end of the experiment. The amount of asparagine (ASN) in mussels fed both diets decreased both qualitatively and quantitatively throughout the experiment. The amount of glutamine (GLN) was found to have increased quantitatively for mussels in one of the effluent fed tanks during the experiment.

Table 3.27. The AA composition (% total AA) for three tanks of mussels fed different diets (tank 18 algae and tank 19 and 20 fish effluent) at the start (n = 9) and end of the six month growth experiment (n = 3). Different letters denote significant differences among groups (Holm-Sidak p <0.05).

Amino acid	Start	Tank 18	Tank 19	Tank 20
AAA	4.43±1.40	1.37±0.43	2.31±1.18	1.35±0.62
aILE	5.21±4.51	0.01±0.02	3.74±6.47	3.87±6.70
ALA	8.36±1.80	5.97±2.00	4.11±0.47	4.34±0.77
APA	1.31±1.76	1.36±2.18	1.09±1.89	1.16±1.89
ASN	2.05±0.51[a]	0.33±0.56[b]	0.00±0.00[b]	0.00±0.00[b]
ASP	6.34±1.74	6.46±3.50	4.68±0.61	4.47±0.58
GLN	0.47±0.82	16.8±14.7	30.4±12.8	25.8±21.3
GLU	5.29±0.90	5.22±2.97	3.93±0.86	4.35±0.95
GLY	10.4±2.8	10.5±3.3	10.0±0.7	12.6±3.8
GPR	0.26±0.07	2.23±1.93	2.00±1.75	2.44±2.02
HIS	2.33±0.95	1.87±1.61	2.43±1.33	2.91±1.05
HLY	2.36±1.35	1.12±1.62	0.31±0.53	0.53±0.91
HYP	6.55±4.71	5.40±8.59	4.20±5.53	3.89±6.12
ILE	6.00±1.26	5.37±2.39	4.10±0.38	4.49±0.44
LEU	7.08±6.02	8.23±2.80	2.34±2.03	2.72±2.40
LYS	2.58±2.33	0.58±1.00	3.47±3.02	0.00±0.00
MET	2.15±0.56	2.04±0.92	1.60±0.18	1.81±0.16
PHE	4.98±1.29	4.57±2.52	3.50±0.27	4.06±0.19
PHP	1.06±0.92	1.07±1.52	0.77±0.99	0.68±1.18
PRO	7.03±1.20	5.56±1.75	4.17±2.20	4.75±2.81
SER	2.58±1.27	2.55±1.41	1.52±0.48	2.93±0.78
THR	1.88±0.59	2.27±2.21	1.64±0.39	2.76±0.11
TYR	2.75±0.28	2.89±1.99	2.46±0.41	2.61±0.47
VAL	5.34±1.66	5.00±2.61	3.45±0.38	3.68±0.45

Table 3.28. AA composition (mg/g DW) for three tanks of mussels fed different diets (tank 18 algae and tank 19 and 20 fish effluent) at the start (n = 9) and end of the six month growth experiment (n = 3). Different letters denote significant differences among groups (Holm-Sidak p <0.05).

Amino acid	Start	Tank 18	Tank 19	Tank 20
AAA	15.4±9.1	4.8±1.8	7.3±2.8	4.5±1.9
aILE	21.0±25.6	0.04±0.08	16.8±29.2	16.0±27.8
ALA	30.9±9.4	21.6±11.0	14.4±5.2	15.5±5.6
APA	5.2±8.4	7.4±12.5	4.9±8.5	4.7±7.9
ASN	7.5±2.9[a]	1.8±3.2[b]	0.00±0.00[b]	0.00±0.00[b]
ASP	23.1±7.3	23.3±17.0	16.2±4.8	15.8±4.8
GLN	1.9±4.0[a]	64.2±80.8	98.4±30.5[b]	83.0±69.9
GLU	19.6±6.1	18.9±14.5	14.0±6.8	15.6±5.9
GLY	39.4±18.9	37.3±17.2	35.1±12.3	44.9±19.9
GPR	1.0±1.2	8.2±9.9	6.1±5.6	7.7±6.3
HIS	9.4±9.9	6.3±7.3	9.2±6.6	10.5±5.6
HLY	8.5±5.0	6.1±9.2	1.4±2.4	2.2±3.8
HYP	24.8±25.4	29.5±48.9	17.7±25.9	15.8±25.6
ILE	22.1±5.8	19.5±12.3	14.2±4.3	15.9±4.5
LEU	24.8±25.7	33.0±20.2	6.9±6.0	8.7±8.1
LYS	8.2±10.5	3.2±5.6	10.1±8.8	0.00±0.00
MET	8.1±3.1	7.4±4.7	5.6±2.0	6.4±1.7
PHE	18.5±6.1	15.8±11.1	12.1±3.3	14.3±3.3
PHP	4.1±4.6	5.8±8.7	3.2±4.6	2.8±4.9
PRO	26.3±9.5	23.8±17.4	15.7±12.5	17.6±13.4
SER	9.9±7.7	10.3±7.7	5.0±0.4	9.9±0.8
THR	7.3±5.9	7.7±10.1	5.8±2.8	9.6±1.5
TYR	10.4±4.6	9.7±8.6	8.8±3.9	9.3±3.4
VAL	19.8±7.0	17.5±11.9	12.0±3.9	13.1±3.9

When the AA composition (% total AA) of mussels in the replicate effluent tanks were averaged (no significant differences at end point of experiment) and compared to the algae tank and the start period there were some significant changes for effluent fed mussels (Fig. 3.23). Effluent fed mussels were found to have decreased in their proportion of ASN, GLN and hydroxylysine (HLY) while their proportion of alanine (ALA) increased during the experiment. Algae fed mussels showed no significant changes in their AA composition (% total AA) during the experiment.

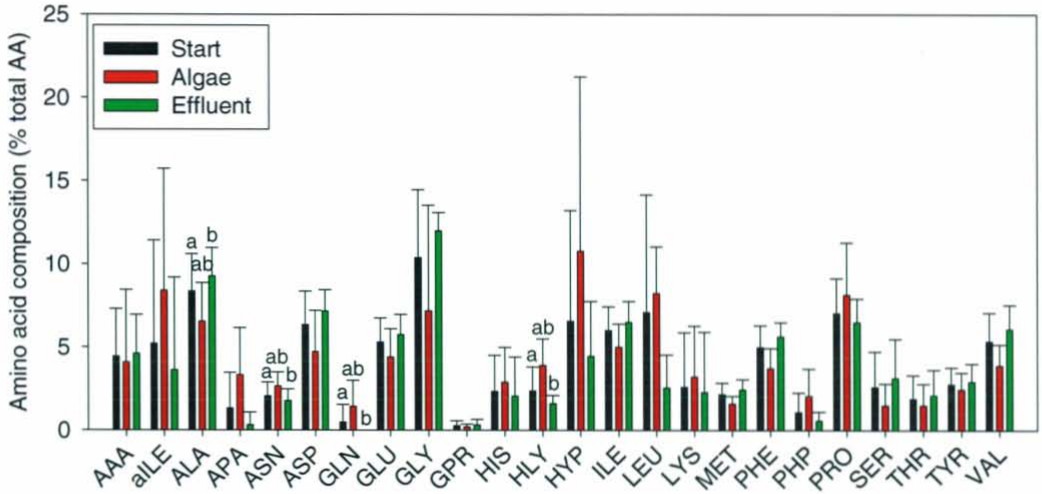

Fig. 3.23. Amino acid composition (% total AA) for mussels at the start (n = 9) and end of the experiment after feeding algae or fish effluent (n = 3 and 6). Groups with different letters are significantly different from each other (Holm-Sidak p <0.05). Error bars are + 1 SD.

When the AA composition (% total AA) for mussels fed both diets at the end of the experiment as compared alone only one significant difference was found. Mussels fed algae had a significantly larger proportion of the essential AA leucine (LEU) than effluent fed mussels.

Quantitatively (mg/g DW) there were still no significant differences between the two treatments (Fig. 3.24). Again algae fed mussels showed no significant changes between the start and end of the experiment. Effluent fed mussels however, showed an increase in the amount of ALA as well as a decrease in the amount of ASN and GLN.

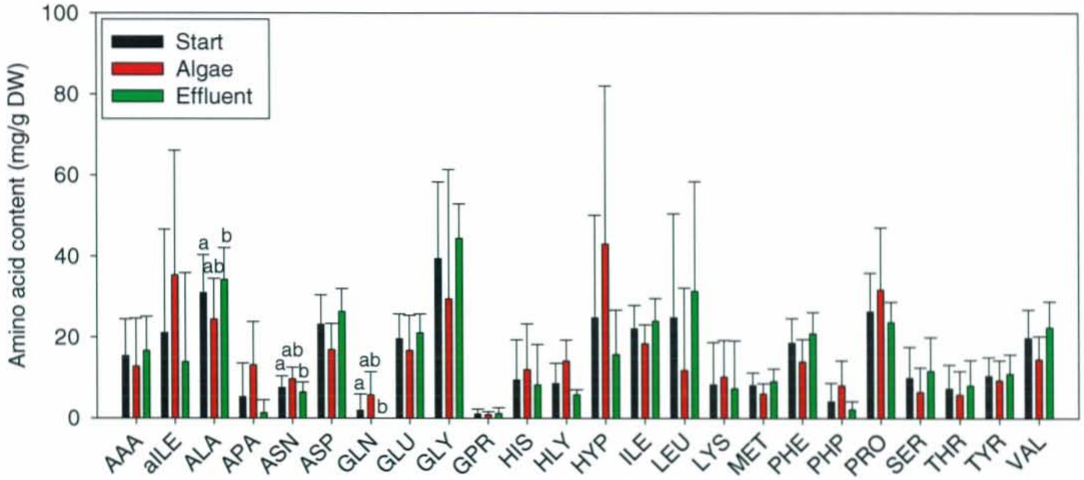

Fig. 3.24. Amino acid content (mg/g DW) for mussels at the start (n = 9) and end of the experiment after feeding algae or fish effluent (n = 3 and 6). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$). Error bars are + 1 SD.

The lipid class composition of mussels at the beginning of the experiment was compared to that for *M. edulis* found in Charles Arm and Fortune Harbour NL (Alkanani *et al.* 2007; Table 3.29). There were a few significant differences between mussels from this experiment and those previously recorded; such as a larger proportion of TAG and a smaller proportion of PL which was most likely due to the time of year sampled. When the lipid class compositions of mussels fed both diets at the end of the experiment were compared to the recorded values few significant differences were seen (Table 3.30). One significant difference observed was a decreased amount of sterol in effluent fed mussels as opposed to algae fed mussels as well the values for cultured mussels.

Table. 3.29. Lipid class composition (% total lipid) for mussels fed algae and effluent at the beginning of the growth experiment in comparison to the lipid class composition of Newfoundland mussels (Alkanani *et al.* 2007). Different letters denote significant differences between groups (Holm-Sidak $p < 0.05$).

Lipid class	Alkanani *et al.* 2007	Growth Trial Start
	June	May 22
	n = 4	n = 10
TAG	38.6±9.7[a]	65.6±10.7[b]
FFA	2.0±0.6	2.1±5.2
ST	5.7±0.7[a]	15.6±7.1[b]
AMPL	3.9±1.3	4.3±1.7
PL	48.3±2.7[a]	5.4±4.0[b]
TL (mg/g WW)	26.6±3.0	24.3±9.8

Table. 3.30. Lipid class composition (% total lipid) for mussels fed algae and effluent at the end of the growth experiment in comparison to the lipid class composition of Newfoundland mussels (Alkanani *et al.* 2007). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$).

Lipid class	Alkanani *et al.* 2007				Growth Trial End	
	June	Aug.	Sept.	Oct.	Algae	Effluent
	n = 4	n = 4	n = 4	n = 4	n = 4	n = 4
TAG	38.6±9.7[a]	7.8±4.7[b]	11.8±2.1[b]	15.7±7.4[b]	7.6±0.9[b]	9.0±4.3[b]
FFA	2.0±0.6	1.0±0.7	1.0±0.5	2.3±1.2	3.1±3.6	2.7±2.3
Sterol	5.7±0.7[a]	8.0±1.1[ab]	6.9±0.5[ab]	8.5±1.3[ab]	9.2±2.0[b]	1.5±1.9[c]
AMPL	3.9±1.3	5.0±0.9	7.2±3.1	8.1±7.8	5.9±1.0	3.0±1.2
PL	48.3±2.7[a]	76.0±10.9[b]	68.8±11.1[b]	62.8±7.6	74.0±5.2[b]	72.2±5.1[b]
TL (mg/g WW)	26.6±3.0	12.1±1.1	12.3±1.4	13.9±1.2	14.7±7.9	16.8±12.5

The FA compositions (% total FA) of mussels at the beginning of the experiment were compared to values for mussels from Charles Arm and Fortune Harbour NL (Alkanani *et al.* 2007) (Table 3.31). There were several significant differences observed, such as an increased amount of the diatom markers 16:1ω7 and 16:2ω4 as well as the essential FA EPA in this experiment as opposed to that recorded by Alkanani *et al.* (2007).

Table. 3.31. Fatty acid composition (% FA) for mussels fed algae and effluent at the beginning of the growth experiment in comparison to the FA composition of farmed Newfoundland mussels (Alkanani *et al.* 2007). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$).

	Alkanani *et al.* 2007		Growth Trial Start
	2000	2001	May 22
Fatty acid	n = 67	n = 75	n = 10
16:0	13.61±1.31[a]	13.69±1.49[a]	12.82±1.13[b]
16:1ω7	3.96±1.97[a]	5.81±3.76[a]	16.93±1.26[b]
16:2ω4	0.40±0.35[a]	0.41±0.23[b]	1.42±0.14[b]
18:0	3.20±0.66[a]	3.00±0.85[b]	1.62±0.36[c]
18:1ω7	1.64±0.6[a]	2.25±0.64[b]	2.41±0.52[ab]
18:1ω9	1.02±0.64[a]	1.33±0.44[a]	1.15±0.45[b]
18:2ω6	1.41±0.47[a]	1.61±0.83[b]	0.68±0.10[a]
18:4ω3	2.12±1.47[a]	3.67±2.16[ab]	2.23±0.19[b]
20:1ω11	1.98±0.88[a]	1.49±1.71[b]	0.44±0.14[c]
20:1ω9	3.05±0.73[a]	2.45±1.68[b]	1.31±0.15[b]
20:2a	3.13±0.68[a]	0.75±1.14[a]	0.83±0.10[b]
20:4ω6	2.76±0.82[a]	2.85±1.16[b]	0.60±0.10[c]
20:5ω3	12.0±2.2[a]	17.0±3.5[ab]	30.8±2.1[b]
21:5ω3	1.48±0.31[a]	1.29±0.64[b]	0.92±0.06[c]
22:2b	3.01±0.73[a]	2.49±1.12[b]	1.24±0.19[c]
22:6ω3	21.50±2.88[a]	19.99±4.28[b]	6.60±0.97[c]

When the FA compositions of mussels fed both diets at the end of the experiment were compared to values for mussels from Charles Arm and Fortune Harbour (Alkanani *et al.* 2007) a few significant differences were observed (Table 3.32). Again there was a decreased amount of the essential FA DHA in mussels fed both diets as opposed to the recorded values. The zooplankton marker 20:1ω9 was found to be present at higher concentrations in effluent fed mussels than the recorded values. The essential FA 20:4ω6 was also found to be present in a larger proportion for mussels fed both diets as opposed to mussels from Charles Arm and Fortune Harbour.

Table. 3.32. Fatty acid composition (% FA) for mussels fed algae and effluent at the end of the growth experiment in comparison to the FA composition of Newfoundland mussels (Alkanani *et al.* 2007). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$).

	Alkanani *et al.* 2007		Growth Trial End	
	2000	2001	Algae	Effluent
Fatty acid	n = 67	n = 75	n = 4	n = 4
16:0	13.61±1.31	13.69±1.49	12.48±0.31	12.83±0.52
16:1ω7	3.96±1.97	5.81±3.76	4.49±0.44	3.69±1.03
16:2ω4	0.40±0.35	0.41±0.23	0.53±0.06	0.40±0.11
18:0	3.20±0.66	3.00±0.85	2.54±0.24	3.23±0.34
18:1ω7	1.64±0.6	2.25±0.64	2.44±0.13	2.69±0.22
18:1ω9	1.02±0.64[a]	1.33±0.44[b]	2.59±0.14[c]	3.61±1.57[c]
18:2ω6	1.41±0.47[a]	1.61±0.83[a]	2.68±0.16[b]	1.34±0.22[a]
18:4ω3	2.12±1.47[a]	3.67±2.16[b]	1.15±0.09[a]	0.93±0.30[a]
20:1ω11	1.98±0.88	1.49±1.71	0.98±0.06	1.69±0.21
20:1ω9	3.05±0.73[a]	2.45±1.68[a]	3.25±0.27	4.80±0.24[b]
20:2a	3.13±0.68[a]	0.75±1.14[b]	2.72±0.26[a]	4.35±0.56[a]
20:4ω6	2.76±0.82[a]	2.85±1.16[a]	6.85±0.61[b]	4.74±0.64[c]
20:5ω3	12.01±2.21	17.02±3.49	15.06±1.60	15.20±1.42
21:5ω3	1.48±0.31	1.29±0.64	0.83±0.24	0.76±0.33
22:2b	3.01±0.73	2.49±1.12	2.80±0.21	3.40±0.59
22:6ω3	21.50±2.88[a]	19.99±4.28[a]	11.86±0.46[b]	13.92±1.25[b]

The proportion of FA groups for mussels at the beginning of the experiment had a few significant differences compared to those of mussels from Charles Arm and Fortune Harbour (Alkanani *et al.* 2007) (Table 3.33). There was a smaller proportion of SFA, PUFA and ω3 as well as an increased proportion of MUFA compared to cultured mussels.

Table. 3.33. Sum of FA groups (% total FA) for mussels fed algae and effluent at the beginning of the growth experiment in comparison to the FA groups of Newfoundland mussels (Alkanani *et al.* 2007). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$).

	Alkanani et al. 2007		Growth Trial Start
	2000	2001	May 22
Fatty acid	n = 67	n = 75	n = 10
ΣSFA	25.4 ± 1.8^{a}	23.6 ± 2.3^{b}	21.6 ± 1.5^{c}
ΣMUFA	14.5 ± 3.03^{a}	17.7 ± 4.1^{b}	25.7 ± 1.1^{c}
ΣPUFA	61.9 ± 3.0^{a}	60.8 ± 4.0^{a}	51.4 ± 1.9^{b}
Σω3	47.8 ± 3.7^{a}	49.5 ± 3.5^{b}	43.0 ± 1.6^{c}
ΣPUFA/ΣSFA	2.4±0.2	2.6±0.4	2.4±0.2

The proportions of FA groups for mussels fed both diets at the end of the experiment showed several significant differences when compared to mussels from Charles Arm and Fortune Harbour (Alkanani *et al.* 2007; Table 3.34). Mussels fed both diets had significantly less SFA, PUFA and ω3 as well as a larger proportion of MUFA.

Table. 3.34. Sum of FA groups (% total FA) for mussels fed algae and effluent at the end of the growth experiment in comparison to the FA groups of Newfoundland mussels (Alkanani *et al.* (2007). Groups with different letters are significantly different from each other (Holm-Sidak $p < 0.05$).

	Alkanani et al. 2007		Growth Trial End	
	2000	2001	Algae	Effluent
Fatty acid	N = 67	n = 75	n = 4	n = 4
ΣSFA	25.4 ± 1.8^{a}	23.6 ± 2.3^{b}	19.5 ± 0.3^{c}	19.3 ± 0.5^{c}
ΣMUFA	14.5 ± 3.0^{a}	17.7 ± 4.1^{b}	23.1 ± 2.0^{c}	26.8 ± 1.9^{c}
ΣPUFA	61.9 ± 3.0^{a}	60.8 ± 4.0^{a}	54.6 ± 1.5^{b}	51.4 ± 2.1^{b}
Σω3	47.8 ± 3.7^{a}	49.5 ± 3.5^{b}	36.8 ± 1.9^{c}	34.4 ± 1.3^{c}
ΣPUFA/ΣSFA	2.4±0.2	2.6±0.4	2.8±0.1	2.7±0.2

The quantity (mg/g DW) of essential amino acids in mussels fed both diets at the beginning of the experiment was compared to that of *M. galloprovinciallis* (Sengor *et al.* 2008) (Table 3.35). The amount of threonine (THR), phenylalanine (PHE), and lysine (LYS) was two times lower in mussels used for the present experiment than that of *M. galloprovinciallis*.

Table. 3.35. Essential amino acid composition (mg/g DW) of mussels fed two different diets (algae and effluent) at the beginning and end of the experiment in comparison to *M. galloprovinciallis* (Sengor *et al.* 2008).

Amino acid	Sengor *et al.* 2008 *M. galloprovinciallis*	Algae Start	Algae End	Effluent Start	Effluent End
THR	26.6	5.8±5.9	7.7±10.1	8.0±6.3	7.7±2.9
VAL	24.4	14.5±5.7	17.5±11.9	22.4±6.4	12.6±3.6
MET	9.2	6.0±2.5	7.4±4.7	9.1±3.1	6.0±1.7
ILE	21.9	18.4±4.7	19.5±12.3	24.0±5.6	15.1±4.0
LEU	35.3	11.8±20.4	33.0±20.2	31.3±27.1	7.8±6.5
PHE	31.3	13.9±5.6	15.8±11.1	20.9±5.3	13.2±3.2
HIS	15.8	11.9±11.3	6.3±7.3	8.2±10.0	9.9±5.5
LYS	38.7	10.2±9.1	3.2±5.6	7.3±11.8	5.1±7.9
ARG	32.2	-	-	-	-

When the essential amino acid composition of mussels fed the two diets was compared to those of *M. galloprovincialis* (Sengor *et al.* 2008) at the end of the experiment there were still several apparent differences (Table 3.35). Again the amount of threonine, phenylalanine and lysine were much higher (at least two times) in *M. galloprovinciallis*. The leucine content of effluent fed mussels was significantly lower than algae fed mussels which were closer to the recorded values for *M. galloprovinciallis*.

Mussels at the beginning of this experiment were not tested for contaminants due to their initial small size. When bacteriological tests were performed on mussels at the end of the experimental trial *salmonella* was not detected for mussels fed either diet (Table 3.36). The *E. coli* counts varied from 4 to 240 MPN/100 g.

Table. 3.36. *E. coli* and *Salmonella* content for three tanks of mussels fed different diets (tank 18 algae, 19 and 20 effluent) at the end of a six month growth experiment. ND – not detected.

Tank	*E. coli* (MPN/100 g)	*Salmonella*
18	110	ND
19	4	ND
20	240	ND

3.4 Discussion

3.4.1 Trophic marker experiment

The zooplankton markers 20:1ω9 and 22:1ω11 originate from the feed fed to the fish which is composed of fish meal from planktivorous fish and can be used as biomarkers for organic waste from fish farms (Biesen and Parrish 2005). These markers are important because they can be used to determine if mussels ingest solids present in cod effluent. Absence of the marker 22:1ω11 in digestive glands of mussels fed algae and its presence in glands of mussels offered effluent shows that mussels do ingest particulate matter contained in fish effluent; however, caution should be taken because mussels are capable of ingesting mesozooplankton (Davenport *et al.* 2000). Therefore this marker may not be effective in the field; however, it can be used in a lab setting when it can be assured that mussels would not have any zooplankton available for ingestion. There were significantly more bacterial FA markers in mussels fed both algae and effluent compared to the starved control. The increase in bacterial markers for mussel fed effluent was expected; however, the increase in algal fed mussels was not, but this may be explained due to the commercial shellfish diet used, which could accumulate bacteria over time. The lack of significant differences in diatom and flagellate markers among diets can be attributed to the fact that prior to the feeding experiments the mussels all shared the same tank and diet (same shellfish diet used in experiment). It is likely that any increases in these markers from the respective diets were not great enough to be detected above the background levels. The zooplankton marker 22:1ω11 was not present in mussels prior to the feeding experiment which lent to its significance.

It is likely that due to the lipid and FA profile of effluent, performance of mussels would be impaired if effluent was the sole diet available; however, Reid *et al.* (2008a) reported increased growth rates of *M. edulis* in an IMTA setting. A potential explanation for this is that particulate matter from finfish cages may be used by mussels as an alternate food source when the amount of natural seston is low. *M. edulis* grown adjacent to salmon farms in Scottish sea lochs used less energy reserves in winter compared to reference sites and it was suggested this was due to the utilization of organic wastes from the farm (Stirling and Okumus 1995).

3.4.2 Ten week biochemical trial

The decrease in DW as well as AFDW in mussels fed algae was not expected. It is possible the amount of available food was not sufficient or mussels were not ingesting enough food while it was available. Due to the use of a flow through system to feed the mussels, the water source had to be turned off to prevent flushing the diet down the drain. Water to the tanks could only remain off for a short duration without problems due to temperature and oxygen content potentially arising. It is possible that not all mussels took the opportunity to feed while food was available. Although the 1.5% soft tissue DW/day fed to the mussels was inside the 0.6-2.8% soft tissue DW/day reported to be required for maintenance of *M. edulis* (Hawkins *et al.* 1985) it is possible that this ration was inadequate to meet the requirement of the mussels. Diet amount was measured based on weight.

The significant decrease in lipid content (mg/g WW) for starved mussels was expected. The fact that there was also a significant decrease in lipid content for effluent fed mussels and not for algae fed mussels suggests that effluent is an inferior diet.

A study by Alkanani *et al.* (2007) determined the FA and lipid classes in M. edulis as well as local seston in mussel farms in northeast Newfoundland as well as the importance of FA and lipid classes in seston for the wet weight of mussels. The FA and lipid classes of mussels sampled in this experiment were compared to those observed by Alkanani *et al.* (2007).

The lack of significant differences among the three treatments suggests that effluent did not negatively affect the lipid class composition of mussels. The lipid class compositions were similar to mussels collected from Charles Arm and Fortune Harbour NL; however, total lipid content for all diets was lower than the recorded values (Table 3.13). This lower lipid content suggests that the nutritional requirement of the mussels was not being met. This is further supported for starved mussels which showed a significant decrease in lipid content over the course of the experiment.

The significant decrease in total FA (mg/g WW) for starved mussels was again expected. The significant decrease for effluent fed mussels contrasted with the lack of significant decrease for algae fed mussels again suggests that effluent is an inferior diet.

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

Based on the increase in bacterial FAs in effluent fed mussels it is likely they were exposed to a higher bacterial load than the other two diets which may also explain some of the increase in NMIDs. The increased levels of the essential FA 20:4ω6 was most likely caused by selective retention, which can occur due to stress conditions (Pirini *et al.* 2007).The terrestrial plant marker 18:2ω6 was present in the feed as well as the effluent (see section 2); its presence in mussels fed effluent suggests it may be a potential marker for aquaculture wastes.

The decreased proportion of SFAs for mussels fed all three diets (starved = 18.8±1.6%, algae = 19.5±1.9% and effluent = 16.2±1.7% total FA) compared to mussels from Charles Arm and Fortune Harbour NL (25.4±1.8% and 23.6±2.3% total FA) suggests that the amount of available food was insufficient. This is further supported by the decreased SFA and ω3 content (mg/g WW) in effluent fed mussels at the end of the experiment.

Although there is evidence that the nutritional requirements of mussels fed all three diets were lacking based on the FA groups it appears that effluent fed mussels had the poorest performance. Effluent fed mussels had the lowest levels of SFAs and ω3s of all three diets.

The results of the bacteriological tests, done by CFIA found levels of *E. coli* and *Salmonella* to be below the regulatory limits and were considered safe to eat. This is comparable to the results obtained from a six year pilot test of IMTA in the Bay of Fundy which found levels of contaminants to be below the CFIA guidelines (Reid *et al.* 2008b).

3.4.3 Six month growth trial

Although no significant growth occurred (with the exception of SL) for mussels fed either algae or effluent, based on the significantly higher DW, AFDW and CI at the end of the experiment, algae fed mussels performed better than effluent fed mussels. This indicates that effluent is either a lower quality food source or that mussels rejected a larger portion of the diet. It is important to note that algae fed mussels were given a ration of 1.5% of their soft tissue DW/day while the amount of particulates available to effluent fed mussels was calculated to be up to almost 10% of their soft tissue DW/day in particles <70 μm.

The decrease in carbon content for mussels fed both diets indicates that mussels may have been fed an inadequate ration. The increase in protein content between the start and end of the experiment for mussels fed both diets indicates that some growth did occur. The larger protein content of effluent fed mussels suggests that effluent has some potential as a diet for mussels. The C:N for mussels in this experiment were again comparable to the values recorded by Rodhouse *et al.* (1984). The significant decrease in C:N for all mussels suggests that both diets were rich in N.

The significant decrease in total lipid content (mg/g WW) in effluent fed mussels coupled with the lack of any significant decrease in algae fed mussels suggests that mussels performance was inferior when fed effluent.

Based on the lack of significant differences between mussels fed the two diets for lipid class composition it appears that effluent did not negatively affect mussel lipid composition. Mussels fed both diets underwent a decrease in their TAG content accompanied by a rise in their PL content throughout the experiment. This change from TAG to PL as the predominate lipid class can be explained; the main lipid class in *Mytilus galloprovincialis* during the spring and summer is TAG; however, during the autumn and winter, PL comprise the largest portion of lipids (Prato *et al.* 2010). The mussels used for this experiment were collected during the spring and the experiment concluded in the winter which would support this trend.

Results for total FA content (mg/g WW) were similar to those for total lipid content (mg/g WW). Mussels fed effluent showed a significant decrease in their total FA

content (mg/g WW) while algae fed mussels did not. This again suggests that effluent is an inferior diet to algae.

Some of the observed differences in FA composition between the two diets at the end of the experiment were expected, such as the increased proportion of the zooplankton markers 20:1ω9 and 20:1ω11 in effluent fed mussels. This supports the potential use of these markers as indicators of fish farm wastes. The increased amount of the NMIDs 20:2a and 22:2b in mussels fed effluent and the increase in 22:2b for algae fed mussels at the end of the experiment suggests that they were deficient in essential FAs. The larger presence of the terrestrial plant marker 18:2ω6 in mussels fed algae was unexpected and conflicts with the results found in the previous experiment. This suggests that this FA may not be ideal for use as a marker for fish farm wastes although further study is needed to determine its usefulness.

The increased amount of the diatom markers 16:1ω7 and 16:2ω4 as well as the essential FA EPA in this experiment as opposed to that recorded by Alkanani *et al.* (2007) coupled with the decreased amount of DHA for this experiment suggest that these differences are due to the diet of the mussels.

The higher level of the zooplankton marker supports the idea for their use as indicators of fish farm wastes; however, the zooplankton marker 22:1ω11 was not found to be significantly different than in mussels from Charles Arm and Fortune Harbour (Alkanani *et al.* 2007) although it was significantly higher than the algae fed diet. Caution must be taken in the use of these markers as indicators of fish farm wastes as mussels are capable of ingesting mesozooplankton (Davenport *et al.* 2000). The essential FA 20:4ω6, which was higher in both algae fed mussels and effluent fed mussels, was present in small quantities in both the commercial shellfish diet used as well as the effluent. It is likely this FA was selectively retained by the mussels. Budge *et al.* (2001) reported levels of 20:4ω6 five times greater in mussels than their phytoplankton diet and suggested mussels were capable of selectively retaining this FA.

Based on the changes in the proportions of FA groups between the start and end of the experiment it is likely that mussels fed both diets were nutritionally stressed. The loss of SFA suggests that the amount fed to the mussels was insufficient and that they were utilizing their SFA reserves for energy and the loss of ω3 suggests that mussels

were deficient in essential FAs. Although it appears mussels fed both diets were nutritionally stressed, based on the significantly lower levels of PUFA and ω3 in effluent fed mussels compared to algae fed it is likely that effluent is an inferior quality diet in terms of FAs. The increase in bacterial FAs for mussels fed both diets was expected. The effluent was known to contain bacterial FAs and the bottled commercial shellfish diet likely accumulated bacteria with age.

There is one potential explanation for the high levels of bacterial FAs found in mussels fed both diets at the end of the experiment compared to mussels from Charles Arm and Fortune harbour. It is possible that the elevated levels of bacterial FAs caused a drop in the proportion of FAs (% total FA) for the other groups. The lower proportion of SFAs and ω3s in both diets compared to the recorded values further supports the idea that mussels fed both diets were nutritionally stressed.

The only significant difference in the AA composition between mussels fed both diets was a larger amount of the essential amino acid leucine (LEU) in algae fed mussels as opposed to effluent fed mussels both qualitatively (% total AA) and quantitatively (mg/g DW). Leucine is an important precursor to sterols (Meister 1965; Rosenthal *et al.* 1974). This could explain the lower ST levels in effluent fed mussels compared to other mussels.

Arginine (ARG) which was reported to be present in *M. galloprovinciallis* was not found in any of the mussels analyzed in this experiment. The reason no arginine was found is due to the fact that arginine is not recoverable with the amino acid kit used in this experiment.

The *E. coli* and *Salmonella* counts for mussels fed effluent after a six month period were still below the guidelines set by CFIA and would be safe to eat. This again supports the findings of the pilot IMTA tests done in the Bay of Fundy (Reid *et al.* 2008b).

3.4.4 Conclusions

There were some common differences between algae fed mussels and effluent fed mussels for both experiments. Effluent fed mussels consistently had a larger proportion (% total FA) of MUFA at the end of the experiments than algae fed mussels. The same can be said for 18:1ω9 and the NMID 20:2a. Effluent fed mussels also showed a decrease

in their ω3 content for both experiments finishing with a significantly lower ω3 content than algae fed mussels.

There were also several common changes in the FA composition of mussels fed effluent between the start and end of the experiments. Effluent fed mussels significantly decreased in terms of both their total lipid and FA content (mg/g WW) during both experiments. In both experiments the proportion of SFA, PUFA and ω3 significantly decreased during the experiment while the proportion of MUFA increased for effluent fed mussels. Effluent fed mussels from both experiments had a significant decrease in the proportion of the essential FA EPA along with a significant increase in the proportion of 18:1ω9 and the terrestrial plant marker 18:2ω6.

Based on these consistent differences it is likely that the amount of PUFA as well as essential FAs present in effluent will result in poor mussel performance. The increase in NMIDs for mussels fed effluent in both experiments also suggests that the diet was lacking essential FAs. However, there was an increase in protein content of effluent fed mussels in the growth experiment, and *M. edulis* has been reported to have increased growth rates when grown in an IMTA setting (Reid *et al.* 2008b). Although the FA composition of effluent is inferior, it is likely that it may be used as a replacement food source when a better diet is unavailable as has been suggested by Stirling and Okumus (1995). This would potentially explain the increased growth reported for mussels in an IMTA setting in the Bay of Fundy (Reid *et al.* 2008b).

The increased amount of the terrestrial plant marker 18:2ω6 in effluent fed mussels at the conclusion of both experiments is an interesting result. Both the fish feed and effluent were found to have this FA present (see section 2). The accumulation of this FA in mussels fed effluent suggests it has potential to be used as a marker for fish farm waste; however, the levels in effluent fed mussels was not significantly higher than that of algae fed mussels for the growth experiment, so caution must be taken. The same can be said for the zooplankton markers 20:1ω9 and 22:1ω11 which were present in the effluent and feed but only significantly higher in effluent fed mussels for the growth experiment. Further study needs to be undertaken to determine the full use of these FAs as markers of fish farm wastes.

4. Summary

4.1 Physical and biochemical properties of effluent leaving an onshore Atlantic cod (*Gadus morhua*) aquaculture facility and potential use in integrated multi-trophic aquaculture (IMTA)

The amount of effluent found to leave tanks containing juvenile Atlantic cod (*Gadus morhua*) was equivalent to 24.9% of the amount fed daily. The period during which the stand pipe for tanks was pulled (flush) was found to generate large amounts of effluent in a very short period of time. The amount of effluent that left a tank during the flush accounted for 13.6% of the total dry mass daily. This allows for easy collection of a large amount of material from tanks in a short period of time for sampling purposes.

The lipid class composition (% total lipid) and FA composition (% total FA) did not vary greatly between particles obtained during the flush period and the passive flow (differences accounting for <9% total lipid and <4% total FA). This coupled with the large amount of effluent obtained over a short period of time makes the flush period an ideal time to sample effluent from cod tanks. It should be noted however that although the lipid and FA composition are similar there is a larger amount of lipid (% DW) in particles <70 μm obtained from the flush than those obtained during the passive flow.

Particle diameter varied from 0.1 μm to 2.4 mm. Particles <70 μm comprised 36% of the effluent followed by particles >500 μm which comprised 33% of the effluent and finally by particles 70-500 μm which comprised the remaining 31%. Particle distribution in terms of number of particles was greatly skewed towards smaller particles; however, the volume distribution of particles was skewed towards larger particles. Settling rates of particles revealed that smaller particles settled at a rate 600× slower than larger particles.

Particles <70 μm were found to have the lowest organic content (% DW) as well as lipid content (% DW). Lipid class composition (% total lipid) was similar for all particle sizes with the exception of TAG which was present at lower concentrations in particles >500 μm. The FA composition (% total FA) of the three different size fractions of particles was also similar; however, there were some small significant differences such as a decreased proportion of 14:0, 16:0 and 18:1ω9 in particles >500 μm and a larger proportion of the terrestrial plant marker 18:2ω6 in particles 70-500 μm.

Particles <70 μm are of a size most suitable for mussel ingestion; however, there is evidence that suggests there is some potential for larger particles to be ingested as well. This size fraction also has the greatest potential to spread to surrounding areas due to its low settling velocity. If it is assumed that the only size fraction ingested is that <70 μm, 1 kg of cod fed 1.5% their body weight daily could potentially provide 150 g to 1 kg of mussels for their daily maintenance requirements.

Effluent was found to contain lower levels of PUFA and ω3 than the natural seston available to Newfoundland mussels. This suggests that the performance of mussels reared on effluent will most likely be inferior to that of a natural diet. Effluent did contain some zooplankton markers 20:1ω9 and 22:1ω11 which have potential to be used as markers for aquaculture waste.

4.2 Performance of *Mytilus edulis* in relation to growth and biochemical composition when reared on effluent from a *Gadus morhua* aquaculture facility.

Digestive glands of mussels offered effluent for a 24 hour period were found to have significantly more 22:1ω11 than those of starved of algae fed mussels. This confirms that mussels do ingest cod effluent.

The total lipid and FA content (mg/g WW) significantly decreased for mussels fed effluent over a six month period. Effluent fed mussels showed an increase in the proportion of MUFA as well as a decrease in the proportion of SFA and ω3 FAs. Effluent fed mussels also significantly decreased in their proportion of EPA while the proportion of 18:1ω9 and the terrestrial plant marker 18:2ω6 significantly increased. Algae fed mussels consistently had a smaller proportion of MUFA, 18:1ω9 and the NMID 20:2a than effluent fed mussels as well as a significantly higher proportion of ω3.

The amount of PUFA and essential FAs present in the effluent was inadequate and probably resulted in poor mussel performance. There was an increase in the proportion of NMIDs in mussels fed effluent which suggests that they were lacking essential FAs. Increased protein content of mussels fed effluent for one of the experiments suggests that effluent does have some merits as a diet. It is likely effluent may be used by mussels to supplement their growth when natural diets are scarce.

The increase in proportions of the terrestrial plant marker 18:2ω6 highlights its potential as a marker of aquaculture wastes; however, caution must be taken as this FA

was only present in larger proportions for mussels fed effluent for one of the experiments. The zooplankton markers 20:1ω9 and 20:1ω11 also have potential as markers for aquaculture wastes but again caution must be taken as the proportion of these markers was only significantly higher in one of the experiments and mussels are capable of ingesting mesozooplankton.

4.3 Conclusions

The use of blue mussels in an IMTA setting has potential to reduce some of the wastes generated from the fed organisms. Mussels will ingest the wastes generated from cod but the amount of waste that mussels can ingest only represents about 36% of the particulate wastes being generated assuming they only ingest particles >70 μm.

Although mussels can only remove a fraction of the wastes being generated from an aquaculture site, the fraction that is removed is that which has the greatest potential to spread. The remaining wastes would settle very rapidly to the sea floor. This suggests if IMTA systems are to maximize their waste removal they cannot rely on mussels alone for particulate waste remediation. Other species must be used to further reduce the wastes generate from an aquaculture site, such as organisms capable of removing wastes that have fallen to the benthos beneath the cage site or other extractive organisms capable of feeding on the larger particulates while they are still in suspension.

A second drawback is the nutritional value of the waste for the mussels. While effluent does contain some essential FAs, it has much lower levels than a diet of algae. It is suggested that although effluent is less nutritional (less lipids and FA etc.) when used as a sole diet, if it is used in conjunction with a more preferable diet it could help supplement and improve growth.

This has several implications for open water IMTA systems. If the mussels cannot rely solely on the wastes generated from the IMTA site then they will be utilizing resources from the natural environment as well which will affect the nutrient loading and balancing for the IMTA system. If mussels only utilize aquaculture wastes to supplement their natural diet then it is probable that the amount of wastes removed by mussels will be dependent on environmental conditions. There will be less waste removal during periods of high natural production and more waste reduction during periods of low natural production. This assumes that phytoplankton is more inviting than cod wastes.

There are also some implications for land based IMTA systems. It should be possible to determine a limit where a minimum amount of algae is fed to mussels and the rest of the diet is composed of fish effluent. This could reduce the cost to feed mussels as well as provide some waste reduction. More research is needed to determine the correct proportion of waste to algae for optimum mussel growth. It is also possible to set up more complex system in which algae is used to recover N from effluent and then in turn used as food for mussels.

The presence of zooplankton markers and the terrestrial plant marker 18:2ω6 in cod effluent as well as their increased presence in mussels fed effluent shows potential for these markers to be used as indicators of aquaculture wastes. Although it is possible for mussels to obtain these markers from other sources besides aquaculture wastes they still have the potential if used properly to indicate if an organism has been feeding on aquaculture wastes. This will be much harder to do in a field setting, however it should not pose too much of a problem in a land based setting where the inputs can be controlled and monitored.

Although there is potential to utilize *M. edulis* in an IMTA setting, more work needs to be done to better understand its full potential in such a setting. Further research is required to determine more precisely what fraction of the effluent is utilized by mussels and what seasonal effects on utilization there may be. Determining an optimal level of effluent to supplement more preferred diets is also important to fully understand the potential for mussels in IMTA. Further studies to better understand the use of zooplankton markers and the terrestrial plant marker 18:2ω6 as indicators of aquaculture wastes is also required.

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