ORGANIC FOOTPRINT AND COMPOSITION OF PARTICLES FROM MARINE FINFISH AQUACULTURE OPERATIONS

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ORGANIC FOOTPRINT AND COMPOSITION OF PARTICLES FROM MARINE FINFISH AQUACULTURE OPERATIONS

By

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Abstract

Aquaculture provides the means to meet the growing seafood demand, while enriching the local ecosystems through excess feed and faecal matter outputs. Analyzing the quality and quantity of material exiting the farms and its uptake by surrounding invertebrates highlights the effectiveness of muti-trophic, co-culturing systems where waste from one species is recycled as food for adjacent species. This thesis describes organic throughput of juvenile Atlantic cod (*Gadus morhua*) land-based tanks and uptake of organic constituents by invertebrates surrounding Atlantic salmon (*Salmo salar*) farms.

The land-based tank output showed significant lipid and fatty acid increases in outflow compared to inflow ($p<0.020$) for the breakdown indicator free fatty acid (FFA), the markers of zooplankton and subsequent indicators of the feed, 20:1ω9 and 22:1ω11, and the essential fatty acid DHA(docosahexaenoic acid, 22:6ω3). Scaling to an 1880 tonne Atlantic cod farm showed 3170±870 kg/day particulate dry weight and 4.7±1.7 kg/day DHA exiting the farms. Based on the amount of DHA required per mussel, the scaled amount of DHA could theoretically support 1400 tonnes of mussels assuming optimal consumption of the available DHA. This relates to mussels' role in multi-tropic co-culturing systems as they could assimilate valuable compounds that would otherwise be lost.

Regression analysis of particle uptake by invertebrates surrounding multiple aquaculture sites in coastal British Columbia showed a decrease in wet weight for mussels with increasing distance from the farm. There was also an increase in DHA in molluscs combined as well as mussels alone. Principal components analysis showed a

similar trend with DHA being higher in molluscs further away from the farm. In addition, bacterial fatty acid markers were higher in molluscs further from the farm except for the individual bacterial fatty acid, 18:1ω7, which remained higher closer to the farm for molluscs combined, mussels and whelks, and mussels alone; however, not for limpets alone. Additionally, a breakpoint was found for DHA in mussels at 339 m with lower DHA proportions closer to the farm. However, of the DHA present, mussels had significantly higher amounts compared to other molluscs again relating to their use in multi-trophic aquaculture.

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List of Abbreviations

ALC	Fatty alcohol
AMPL	Acetone-mobile polar lipids
ARA	Arachidonic acid (20:4ω6)
CV	Coefficient of variation
DAG	Diacylglycerol
DFO	The Department of Fisheries and Oceans
DHA	Docosahexaenoic acid (22:6ω3)
DOC	Dissolved organic carbon
DW	Dry weight
EE	Ethyl ester
EKET	Ethyl ketone
EPA	Eicosapentaenoic acid (20:5ω3)
FA	Fatty acid
FAME	Fatty acid methyl esters
FFA	Free fatty acid
FID	Flame ionization detector
GC	Gas chromatograph
GE	Glyceryl ether
GF/F	Glass fiber filters
HC	Hydrocarbon
IMTA	Integrated multi-trophic aquaculture

JBARB	The Joe Brown Aquatic Research Building
KET	Ketone
MAG	Monoacylglycerol
ME	Methyl ester
MKET	Methyl ketone
MS	Mass spectrometer
MUFA	Monounsaturated fatty acids
N	Nitrogen
PCA	Principal components analysis
PL	Phospholipid
PUFA	Polyunsaturated fatty acids
SD	Standard deviation
SE	Steryl ester
ST	Sterol
TAG	Triacylglycerol
TL	Total lipid
TLC	Thin-layer chromatography
TN	Total nitrogen
tr	Trace
WE	Wax ester
WW	Wet weight

Chapter 1

Introduction

1.1 Aquaculture

The fishing industry is reaching the maximum amount of seafood products that can be harvested from the oceans and yet there is pressure to provide more (Troell et al., 2003). The Department of Fisheries and Oceans Canada (DFO, 2008) reported 1.1 million tonnes total landings from marine commercial fishing in Canada valued at approximately 2 billion dollars in 2006. The intense stress on Canada's fisheries to produce seafood means another method is required to meet the growing demand. An alternate method of supplying the resource requires farming seafood with aquaculture.

DFO defines aquaculture as the farming of fish and shellfish for food and economic gain and it may consist of fed (e.g. salmon) or extractive (e.g. mussels) type operations on land, in coastal areas, or deep water. Globally, finfish farming ranges from small herring size fish to large tuna. Other methods of aquaculture include farming mussels and oysters or algal species such as kelp where they are seeded and allowed to grow on socks or rope lines underwater.

Currently the most prevalent marine finfish being farmed in Canada is Atlantic salmon (*Salmo salar*); however, other species include Chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*) as well as steelhead trout (*Oncorhynchus mykiss*). Non-salmonid species include Atlantic cod (*Gadus morhua*) black cod (*Notothenia microlepidota*), Atlantic halibut (*Hippoglossus hippoglossus*), haddock (*Melanogrammus aeglefinus*), and wolffish (*Anarhichas lupus*); however, they

are still being tested for their commercial potential. Freshwater finfish species include rainbow trout (*Onorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), Arctic char (*Salvelinus alpinus*), and the tilapia species *Oreochromis niloticus*, *O. mossambica,* and *O. aureus* (DFO, 2005).

Finfish aquaculture requires the use of feed for fish growth as well as their additives for health and quality maintenance. Like farming on land, farming fish places many individuals in one location. This abundance affects the local environment and ecosystems by increased inputs due to the farm.

1.2 Environmental interactions

During aquaculture operations of farmed finfish, output from the farms interact with the environment. A major input to the local ecosystem includes increased organic loading from excess feed pellets, particulate feed pellets or fines, and faecal matter (Holmer and Kristensen, 1992; Wu et al., 1995; Henderson et al., 1997). In sediments, when aerobic decomposition exceeds oxygen supply, these pollutants have been found to cause anoxia (Hall et al., 1990; Findlay and Watling, 1994), increase pathogenic bacteria, and cause methane and hydrogen sulfide production in anoxic marine waters (Samuelsen et al., 1988; Enger et al., 1989; Hargrave et al., 1997). With the close proximity of the fish there is an increased likelihood of disease such as sea-lice and viral infection to not only the cultured fish, but also the wild populations (Krkosek et al., 2007). These conditions may require the use of pesticides and disinfectants for control and prevention, leading to chemical pollution from medications and feed additives for fish maintenance (Coyne et al., 1994; Haya et al. 2001). In addition, escaped cultured fish pose a threat to wild stocks by competing for resources and outbreeding depression (Iwama, 1991; Ervik et al., 1997).

In contrast to the negative effects, the increased input from supplemented feed pellets and fish waste may enrich the environment providing more food to surrounding species. Such species may also be cultured. Integrated multi-trophic aquaculture (IMTA) is the use of organisms from multiple trophic levels for aquaculture mitigation (Chopin et al., 2001). It employs the waste from one organism as the food for another to mimic the relationships found in nature. Its design allows a fed culture to be placed alongside organic and inorganic extractive aquaculture. With these factors considered, the overall benefit of aquaculture is improved; however, the degree of improvement is subject to debate. IMTA utilizes the organic input, including wastes, from fed aquaculture species by adjacent farmed species. Given the waste recycling structure, the description of organic outputs from finfish highlights important details of the eventual food sources. Examining multiple species, fractionating effluent, and examining organic markers including uptake of lipids and fatty acids, the nutritional value of finfish output can be determined.

1.3 Lipids and fatty acids

For this research, the lipids and fatty acids produced and taken up around aquaculture sites were examined. Lipids are energetic molecules that play a role in energy storage and the structure of membranes along with intracellular signaling. There are numerous lipid structures, however their roles and sources are relatively specific in nature (Colombo et al., 1996). Within this molecular diversity it is possible to distinguish energy storage or membrane classes as well as indicators of lipid degradation and pollution. Lipids can provide evidence of an organism's condition and activity as well as be a biological indicator of deteriorating water quality (Parrish, 1988).

With their large content in membranes, fatty acids in lipids (specifically phospholipids; PL) are responsible for the level of membrane fluidity. PL contain a glycerol molecule with a phosphate group at position *sn*-3 and two esterfied fatty acids at *sn*-1 and *sn*-2. The amount of saturation in those two fatty acids determines the fluidity of the membrane. Position *sn*-1 may contain saturated or monounsaturated fatty acids and position *sn*-2 may contain polyunsaturated fatty acids. With more saturation, the fatty acids and subsequent phospholipids are more tightly packed together and have less fluidity, while more double bonds allow for space between lipids and therefore more movement (Sargent and Whittle, 1981).

Acyl lipids are composed of fatty acids that are combined to make up other molecules such as TAG (triacylglycerol) and PL and they are a major determining factor in nutritional quality of seafood where less saturation (or more double bonds) relates to a more nutritionally valuable product (Simopoulos, 1991). Fatty acids with multiple double bonds, or polyunsaturated fatty acids (PUFA), are responsible for many beneficial mammalian health effects. PUFA with a double bond starting at the third carbon from the methyl group represent the omega-3 fatty acids (ω3). They have anti-inflammatory properties along with beneficial effects on cancer and cardiovascular disease (Simopoulos, 2002).

The extraction of lipids from collected samples and the separation of individual lipid classes allows for a quantitative description of the lipid profile of samples with the Chromarod-Iatroscan system. Using increasingly polar solutions, the lipid classes migrate up the Chromarods separating due to their varying polarity. Following this thin layer separation, lipids are quantified using an Iatroscan. The lipid classes include

hydrocarbons (HC), esters, ketones, TAG, free fatty acids (FFA), fatty alcohols (ALC), sterols (ST), acetone mobile polar lipids (AMPL), and PL as well as their subgroups (Parrish, 1987).

Individual lipid classes have unique attributes. For example, TAG is a condition index and represents storage (Hømer, 1989), while the concentrations of methyl and ethyl ketones can depict nitrogen limitations of microalgae. With these reference tools, a lipid profile allows for a nutritional description of the species being sampled. The geographic location and food availability also gives rise to unique lipid signatures. An example of these unique lipid profiles includes the adaptation of Arctic zooplankton to accumulate long-term energy stores in wax esters allowing them to withstand periods of low food availability (Lee et al., 2006).

In addition to lipid classes, fatty acids may act as markers for the diet of an organism. Fatty acids consist of a carboxyl group and a hydrocarbon chain (Fig. 1.1). The chain length may range from four to 36 carbons, and it may be saturated or contain one to multiple double bonds. Here, fatty acids are reported as the number of carbons followed by the number of double bonds separated by a colon. For those unsaturated fatty acids, the location of the first double bond from the terminal methyl group is indicated following an omega symbol. For example 22:6ω3 has 22 carbons with six double bonds and the first double bond is located at the third carbon from the methyl group.

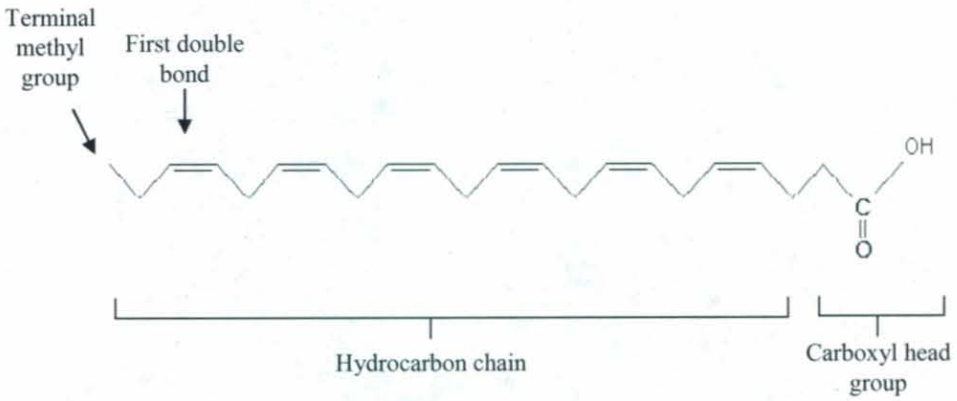

Figure 1.1: Fatty acid with 22 carbons and 6 double bonds: docosahexaneoic acid (DHA; 22:6ω3)

Fatty acid methyl esters (FAME) can be run through a gas chromatograph separating them by chain length and saturation. With this information, an organism's diet can be determined. In order for a trophic marker to function it must be consumed and conserved by a predator and then remain largely unmodified. It is important to note that no individual fatty acid can be assigned to one species alone, however groups of fatty acids can be representative of a prey (Dalsgaard et al., 2003).

Fatty acids of copepods and phytoplankton are examples of good biomarkers. Diatoms contain high amounts of 20:5ω3 (eicosapentaenoic acid; EPA) while dinoflagellates contain high levels of DHA. Along with this, green algae are rich in 18:4ω3 while red algae are rich in 20:4ω6 (arachidonic acid; ARA) (Sargent 1989). In addition, copepods are rich in the fatty acids 20:1 and 22:1 (Dalsgaard et al., 2003). This specificity within species allows for a marker that reflects a dominant species or the prey of a consumer.

An essential fatty acid (e.g. DHA, EPA, or ARA) acts as a good trophic biomarker because it is not produced at any significant rate in a consumer and it is preferentially retained. The essential fatty acids remain in the tissues of the consumer with little modification because catabolism occurs at such a slow rate (Sargent and Whittle, 1981).

Lipid classes and fatty acids act as biomarkers in the environment and can show enhanced or deteriorating quality of ecosystems, changes in predation, or a shift in the lipid or species quantity (Pohl and Zurheide 1979). Using lipids and fatty acids in addition to dry mass and dissolved constituent determinations, this research examines the environmental interactions of aquaculture with the local environment.

1.4 Objectives:

Firstly, this research describes the throughput of land-based aquaculture tanks by measuring the inflow and outflow as well as the retention by cultured fish. Content and compositional analysis of finfish farm outputs is a major requirement in understanding their interactions with the local ecosystem. Additionally, analyzing this output describes its potential as a food source in the context of IMTA, which allows the output of a fed aquaculture species to be used as feed for an adjacently cultured species, aiming to mitigate environmental implications associated with the organic and inorganic loadings due to aquaculture operations.

Following the description of the land-based tank effluent, the next objective is to determine trophic connections in coastal food webs in British Columbia using organic markers. Using trophic biomarkers, the nutritional interactions among marine species as well as potential cultured and co-cultured species of a coastal food web are compared statistically.

With descriptions of effluent and coastal food web interactions, the final objective is the spatial description of Atlantic salmon aquaculture effects on organic content and composition of adjacent invertebrate samples in coastal British Columbia.

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Chapter 2

Output of organic material from land based juvenile Atlantic cod (*Gadus morhua*) tanks

2.1 Abstract

Given aquaculture's ability to provide seafood and the growing demand for seafood production and the environmental implications associated with aquaculture operations, the quantification of finfish farm outputs, such as the enriched effluent, is a major requirement to understand the effects on the local ecosystem. A mass balance experiment was conducted in which the dry mass, dissolved organic carbon, total nitrogen, lipid classes, and fatty acids were quantified for the inflow and outflow of land-based juvenile Atlantic cod (*Gadus morhua*) tanks. Mass determination showed 89.9±15.4 g/day of dry weight material in the inflow, increasing to 96.8±15.4 g/day in the outflow due to excess feed and faeces. This, along with input from the feed, input gives a 24% output over input.

Lipid class and fatty acid analysis showed significant increases in the outflow compared to inflow ($p<0.020$). Specifically, the breakdown indicator free fatty acid (FFA) (43.1±9.5% total lipid), the markers of zooplankton and subsequent indicators of the feed, 20:1ω9 and 22:1ω11, and the essential fatty acid DHA were all higher in the outflow compared to the inflow.

The 96.8±15.5 g/day of dry material exiting the land-based tanks due to the presence of the fish alone computed to 3170±870 kg/day exiting an 1880 tonne Atlantic cod farm. Along with this, there was 0.14±0.04 g/day of DHA exiting the tanks computing to 4.7±1.7 kg/day at farm size, which could theoretically support 1400 tonnes

of mussels assuming an average whole live weight of 5.31 g/mussel and consumption of all available DHA. This relates to mussels role in multi-tropic co-culturing systems where they would assimilate valuable compounds that would otherwise be lost.

2.2 Introduction

Aquaculture provides a means to meet growing seafood demands without further depleting food grade fish stocks; however, there is increasing pressure on feed grade fish stocks and a realization that there are significant local environmental impacts. Economically, the Department of Fisheries and Oceans (DFO) reported that 155,000 tonnes of product was valued at approximately $715 million in 2005 (DFO, 2008) and shows aquaculture are one of the fastest growing industries.

Along with national growth, aquaculture is also increasing in Newfoundland where DFO reported 83,000 tonnes valued at $40 million (DFO, 2008). In 2006, the Newfoundland and Labrador Provincial government along with the Federal government contributed $10 million to a larger $155 million industry based project geared towards salmon farming expansion. This rapidly growing industry necessitates research regarding species native to the area and a means to maintain an ecologically sustainable industry. Initially because of the number of people employed and then due to the moratorium on cod fishing in 1992, the Atlantic cod (*Gadus morhua*) fishing industry in Newfoundland has been a topic of much interest. Aquaculture allows for independence from the natural cod stocks that are still recovering.

In order to mitigate effects on the environment (Chapter 1), new methods of culturing fish are being developed. One prominent method is termed integrated multi-trophic aquaculture. IMTA uses organisms from multiple trophic levels for aquaculture

mitigation. It employs the waste from one organism as the food for another, so that a fed culture is placed alongside organic and inorganic extractive aquaculture. A research group in New Brunswick headed by Thierry Chopin is conducting ongoing experiments using finfish, blue mussels (*Mytilus edulis*), and kelp (*Laminaria saccharina* and *Alaria esculenta*) (Bhardwaj, 2003). By placing the seaweed and mussel socks near the finfish cages, the effluent from the farm can be filtered, removing some of the lost nutrients. In addition, the New Brunswick group is experimenting with species of sea cucumber cultured on the seabed.

Aquaculture techniques using multiple species have been in place for some time with records of mariculture using rice and shrimp or fish dating back a thousand years in China. However, these techniques are considered to have uncontrollable environmental impacts and therefore less sustainable (Neori et al., 2004). More experimentation is needed to demonstrate the ability of IMTA to mitigate impacts at a commercial scale.

In order to implement integrated aquaculture, it must be economically viable and there is a substantial market for the co-cultured species. Seaweed accounted for over 20% of the total global aquaculture production in 1998 equaling 5.9 billion USD. It is used in animal feed, pharmaceuticals, and human consumption (FAO, 2000). For the most part, mussels are harvested for consumption. Environmentally, mussel and seaweed farming also cause changes to their local ecosystems. For example, although mussel crops do not require exogenous feeding, they increase sedimentation and excrete dissolved inorganic nutrients (Dahlbäck and Gunnarsson, 1981; Grant et al., 1995; Christensen et al., 2003)

Seaweed is a natural extractor of inorganic compounds such as phosphorus and nitrogen. Inorganic nitrogenous waste from netpens is excreted as ammonium, which is

efficiently taken up by seaweed (Neori et al., 2004). Based on a study done in Sweden on nitrogen and phosphorus removal by seaweed near a fish farm, there was a greater uptake of both nutrients closer to salmon aquaculture sites (Troell et al., 1997). They found 1.9-2.1 mmol $g^{-1}dw^{-1}$ difference in nitrogen between the algae at 1 km and 150 km from the fish farms as well as 0.28 – 0.34 mmol $g^{-1}dw^{-1}$ difference in phosphorus between the algae at the same distances (Troell et al., 1997). Integrating seaweeds into fish aquaculture may counterbalance nutrient inputs and other metabolic aspects such as dissolved oxygen, acidity, and CO_2 levels (Neori et al 2004).

The mussel component of the IMTA design is responsible for organic extraction. The ongoing research in New Brunswick, Canada shows small organic particle concentrations decreasing with distance from the farm site. Along with this, mussels (specifically *Mytilus edulis*) cultured next to a farm site showed increased growth rates by 50% and kelps (*Saccharina latissima* and *Alaria esculenta*) had an increased growth rate of 46% compared to a reference site (Chopin et al., 2004), which is presumably due to the increased input of faecal matter from the adjacent finfish cages. In addition, Troell et al. (2003) reported seaweeds and shellfish harvested from coastal waters (mostly non-IMTA derived) removed almost a million tons of protein and about 150,000 metric tons of nitrogen per year.

Details such as the long-term effects and retention of chemicals including therapeutants used for finfish culture are currently being studied. To date, therapeutants used in salmon aquaculture have not been detected in kelps collected from the IMTA sites (Haya et al., 2004). In addition to this, Chopin et al. (2004) reported that levels of heavy metals, arsenic, PCBs, and pesticides were below regulatory limits over a five-year span.

Given IMTA requires the use of organic input, including wastes, from one aquaculture species to be used by another, the description of the organic output from finfish provides important details of the food sources being recycled. In addition, waste produced by the fish depends on the digestibility of feed constituents. Apparent digestibilities in cod range from 69-83% for dry matter, 76-90% for protein, 65-98% for starch, and 99% for lipids (Hemre et al., 2003).

More investigation into the nutritional quality of the mussels harvested around finfish aquaculture sites is required. In addition, seafood is prized for its fatty acid composition and therefore in order to retain its value, both nutritionally and economically, the quality of the mussels cultured in IMTA must be maintained.

For this research, the inflow and outflow contents and composition of a land-based system were examined, quantified and modeled to determine nutrient retention and production. The closed system nature of the land-based facility allows for the output from the tanks to be directly related to the inflow, the output from the fish, and the excess feed.

This study aims to interpret the throughput of land-based tanks using lipid and fatty acid data. As mentioned in Chapter 1, lipids and fatty acids act as biomarkers for trophic interactions. Table 2.1 shows a list of the lipids and fatty acids predominantly used as biomarkers throughout this section.

Table 2.1: Lipid and fatty acid biomarkers

Significance	Lipid	Unit	Reference
Storage condition indicator	TAG	% TL	Hølmer 1989
Breakdown indicator and faeces marker	FFA	% TL	Van Biesen and Parrish 2005
Zooplankton/Copepod & fish feed marker	20:1ω9	% TFA	Dalsgaard et al., 2003
	22:1ω9	% TFA	Mayzaud et al., 2007
	22:1ω11	% TFA	Dalsgaard et al., 2003
EPA, diatoms marker	20:5ω3	% TFA	Dalsgaard et al., 2003
DHA, dinoflagellates marker	22:6ω3	% TFA	Graeve et al., 1994
Bacterial fatty acid marker	18:1ω7	% TFA	Morris et al., 1985
	*i*15:0	% TFA	Morris et al., 1985
	*ai*15:0	% TFA	Morris et al., 1985
	*i*17:0	% TFA	Kaneda, 1991
	*ai*17:0	% TFA	Kaneda, 1991

TAG: triacylglycerol
FFA: free fatty acid
TL: total lipid
TFA: total fatty acid
EPA: eicosapentaenoic acid
DHA: docosahexaenoic acid
i: iso
ai: anteiso

2.3 Methods:

2.3.1 Sampling methods for tank modeling

Sampling took place in the Dr. Joe Brown Aquatic Research Building (JBARB) under supervision of those employed on site. Samples were taken from six, 6000 L tanks containing juvenile Atlantic cod (*Gadus morhua*) maintained by the JBARB staff. The tanks' maintenance included daily feeding, temperature and dissolved oxygen measurements, and pulling of the standpipe to remove settled material. In addition, the number of fish stocked in each tank and their bulk weight measurements was recorded daily. The tanks contained from 488 to 970 fish ranging from 74.1 to 103.4 g average weight (Table 2.2).

Table 2.2: Fish data for tanks in the Joe Brown Aquatic Research Building (JBARB)

Tank Number	Number of Fish	Average Weight (g)	Tank Biomass (kg)	Feed Weight/ Day (g)	Feed Size (mm)
1	768	93.3	73.2	366.0	4.0/6.0
2	970	82.0	79.5	596.6	4.0/6.0
3	529	77.8	41.2	308.7	4.0
4	700	103.4	72.4	361.9	4.0/6.0
5	550	100.5	55.3	442.2	4.0/6.0
6	488	74.1	36.2	271.2	4.0

Samples were collected over 12 consecutive days as well as on one additional day (Table 2.3). Samples from the supply water and the wastewater were collected in clean 20 L plastic buckets. The supply water was sampled from the valve located next to the tanks and the wastewater was sampled from the pipes leaving each tank. Sampling the inflow took place following 5 min of flushing to ensure a representative sample that was free of settled matter. Approximately 20 L was collected at each sampling period. The wastewater was taken from the drainpipe directly under each tank, using a flexible tube which was attached to the drainpipes and fed into a labeled bucket.

Table 2.3: Schedule for sampling in Joe Brown Aquatic Research Building (JBARB) juvenile Atlantic cod tanks

Date	Description	Time of Sampling
April 30	Preflush and Inflow	10:30 am
May 1	Flush	11:30 am
May 2	Postflush	12:30 pm
May 3	Preflush and Inflow	10:30 am
May 4	Flush	11:30 am
May 5	Postflush	12:30 pm
May 6	Preflush and Inflow	10:30 am
May 7	Flush	11:30 am
May 8	Preflush and Inflow	10:30 am
May 9	Postflush	12:30 pm
May 10	Flush	11:30 am
May 11	Postflush	12:30 pm
May 20	Midway between flushes	8:00 am*

*Standpipe pulled (flush) at 3:30 pm previous day

Figure 2.1: Land based tanks where samples were collected in the Joe Brown Aquatic Research Building (JBARB)

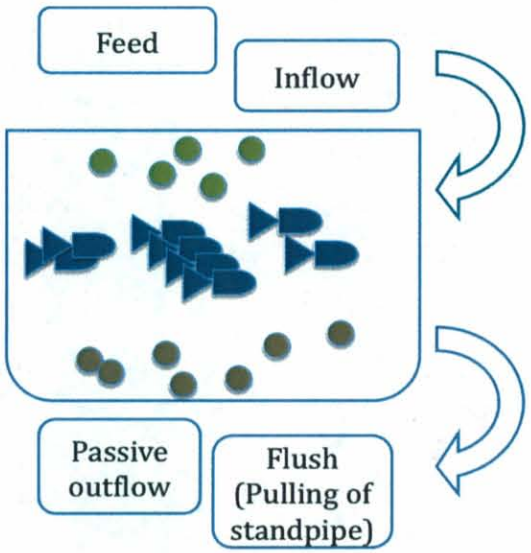

Figure 2.2: Schematic diagram of Joe Brown Aquatic Research Building (JBARB) tank inputs and outputs

In order to determine the total amount of material exiting each tank, samples were taken at various times during the regular maintenance schedule in the building (Table 2.3). Samples were taken from the tanks one hour prior to pulling the standpipe, while pulling the standpipe, and one hour after pulling the standpipe. The one additional day included sampling 17.5 hours after pulling the standpipe, representing a sample midway between flushes. When the standpipes on the tanks are pulled the largest amount of water and settled particles exits the tanks at one time. The fish were fed daily, approximately an hour before pulling the standpipe, with a mixture of Europa 15 (4.0 mm) and Europa 18 (6.0 mm) feed pellets made by Skretting.

The wastewater samples, the flush (from pulling of standpipes) and passive flow out of the tanks, were fractionated by screening and sub-sampled for analysis. The size fractions used were >500 μm, 70 - 500 μm, and <70 μm. Analysis included quantifying

dissolved organic carbon (DOC), total nitrogen (TN= DON + DIN), dry weight, ash weight, ash free dry weight, total lipids, lipid classes, and fatty acids.

2.3.2 Dry weight

Samples were filtered though a glass fibers filter (1.2 µm GFC). Ammonium formate (3.5%) was added to remove the salts. The filters were then dried in an oven at 100°C overnight and the dry weights were determined by the difference in filter weights. For ash-free dry weights the filters were burned in a muffle furnace at 450°C overnight and reweighed. The loss of weight was used to calculate the percentage organic weight and the remainder accounted for ash-weight.

2.3.3 DOC and TN

To sample dissolved organic carbon and total nitrogen, 30 ml 1.2 µm GFC-filtered effluent was placed into a 40 ml clear glass DOC vial. Samples were stored at -20°C and analyzed with a total organic carbon analyzer, which also analyzed both organic and inorganic nitrogen (Shimadzu TOC-V_{CPH} equipped with a TNM-1 Total nitrogen measuring unit and an ASI-V autosampler).

2.3.4 Lipid determination

Particulate lipid samples were filtered though a 47 mm GF/F filter. The lipids were extracted from the retentate according to Parrish (1999). They were homogenized in a mixture of 2:1 ice cold chloroform and methanol manually with a metal rod and in some cases with a Polytron homogenizer. Following this, chloroform extracted water was added so that the ratio of the mixture was 8:4:3 cholorform:methanol:water. After this, the samples were then vortexed, sonicated for four minutes in an ice bath, and centrifuged

at 5000 rpm for two minutes. Following this, the bottom organic layer was removed using the double pipetteing technique where a longer lipid cleaned pasteur pipette is placed inside a shorter one so as to not disturb the top aqueous layer. Chloroform was added to the sample and the process was repeated three times to ensure complete extraction of the lipids. The organic layers were pooled in a lipid-cleaned vial and concentrated using a flash evaporator (Buchler Instruments, Fort Lee, N.J.).

A three-step TLC development system (Parrish, 1987) was used to determine the lipid class composition. The method uses silica coated Chromarods and an Iatroscan Mark VI TLC-FID. The rods were spotted with the samples that were focused using 100% acetone. The first development system was hexane:diethyl ether:formic acid (98.95:1:0.05) where the rods were developed for 25 minutes. They were then removed for five minutes and redeveloped for 20 minutes. The second development system involved hexane:diethyl ether:formic acid at a ratio of 79:20:1. The rods were developed in this system for 40 minutes. The last development system first involved developing the rods in 100% acetone for two 15 minute intervals then there were two 10 minute developments in chloroform:methanol:chloroform-extracted water (5:4:1). The rods were scanned in the Iatroscan after each development system and then placed in a constant humidity chamber. The chromatograms were analyzed using PeakSimple version 3.72. Standards used for calibration were obtained from Sigma Chemicals, St. Louis, Mo., USA.

Further analysis included fatty acid separation and analysis. The fatty acid methyl esters (FAME) were obtained with 14% BF_3/MeOH incubation for 1.5 hours at 85°C including agitation half way. They were analyzed on an HP 6890 GC-FID equipped with

an HP 7683 autosampler. The chromatograms were integrated using chromatography software (Varian Galaxie Chromatography Data System Version 1.9.3.2) and identified from retention times obtained with standard FAME mix (Supelco: 37 component FAME mix) in addition to a bacterial acid fatty ester and PUFA mix.

The length of the gas chromatography (GC) column was 30 m with an internal diameter of 0.25 µm. It used carbowax polyethyleneglycol and had a 1 m guard column on the front end (ZB wax+, Phenomenex, U.S.A). The column temperature began at 65°C where it was held for 0.5 min.. The temperature was ramped to 195 °C at a rate of 40 °C/min., where it held for 15 min. then ramped to a final temperature of 220 °C at a rate of 2 °C/min. This final temperature was held for 0.75 min. The carrier gas was hydrogen which flowed at a rate of 2 ml/min. The injector temperature started at 150 °C and ramped to a final temperature of 250 °C at a rate of 200 °C/min. The detector temperature stayed constant at 260 °C. The conversion of the acyl lipids into their FAME had an average efficiency of derivatization of 88.0% determined by Iatroscan.

2.3.5 Statistics

Data were reported as mean ± standard deviation (unless otherwise indicated). T-tests (two-sample) and ANOVA (one-way) were conducted where the criterion of significance was 5% and included regression analysis with Sigmastat and Minitab.

2.4 Results and discussion

2.4.1 Mass Balance

The results from the dry weight filter analyses (all size fractions as well as the flush and passive flow) are shown in Fig 2.3. The total amount of dry weight material exiting the tanks was 187±39 g/day (n=6). The majority of material exited the tanks with

the passive flow and less came out when the standpipe was pulled. The difference in the pre-, post-, and midway between flushes was not found to be statistically significant (one-way ANOVA, p=0.710, df=13) allowing for the calculation of an average passive flow. Along with this, the amount in the flush accounted for 7% of the output when considering the entire outflow for the day. The flush would presumably include settled material from the previous 24 hours.

The samples were fractionated (<70, 70-500, and >500 μm) as shown in Fig. 2.3. Most material was present in <70 μm, 121±13 g/day, compared to 27.1±3.4 g/day and 24.2±3.2 g/day in the 70-500 and > 500 μm fractions, respectively. From the flush samples, 7.9±2.0 g/day of >500 μm was exiting representing the greatest contribution. The < 70 μm contained 5.1±1.3 g/day and the 70-500 μm contained 0.07±0.02 g/day. The larger particles, which are usually excess feed and faecal matter settle during the course of the day and are released when the standpipe is pulled, which causes an abundance of material >500 μm. The process of fractionation can breakdown larger material effecting particle size distribution; however, samples were processed in a way to minimize this effect.

The sum of the outputs (passive flow and flush) from every size fraction is shown in Fig 2.3. The distribution of the particles remained the same with the majority of material in the <70 μm fraction. In addition, Table 2.4 shows the amount of material exiting the tanks when corrected for the inflow contribution. The sand-bed filter removes material >50 μm from the water supply therefore the correction requires subtracting this material from the <70 μm. The inflow contained 89.9±15.4 g/day, leaving a total, including the 70-500 μm and >500 μm fraction, of 96.8±15.5 g/day as a corrected output

from the tanks. This output relates only to the presence of faecal matter and excess feed pellets and fines.

The feed to waste conversion ratio was computed by dividing the outflow of the tanks by the amount fed to the fish. In order to accurately compute this, the corrected output from the fish was used. Given the outflow (96.8 g/day) and the average input from the feed (391 g/day) this represents 24.8% output which is similar to the 15-25% feed to waste conversion ratio reported by Cho and Bureau (2001). The appendix (Table A-2.1) gives a complete list of dry weight results.

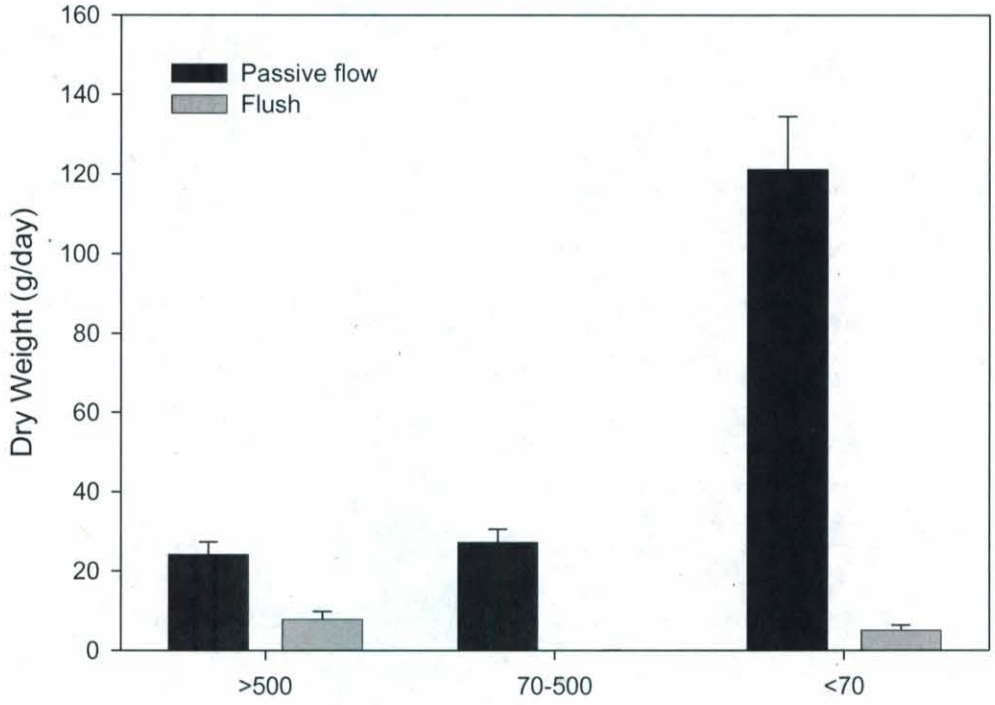

Figure 2.3: Daily mass (dry weight) in effluent from JBARB tanks from <70 µm, 70-500 µm and 500 µm size fractions. Bars: average + SD

Table 2.4: Throughput of particulate material in land-based cod tanks

Size fraction (µm)	Weight in effluent (g/day)	Feed (g/day) n=6	Inflow (g/day) n=9	Balance (g/day)
<70	124±37	-	89.9±15.4	34.4±10.0
70-500	30.5±8.3	-	-	30.5±8.3
>500	31.9±8.5	391±116	-	31.9±8.5
Total	187±39	391±116	89.9±15.4	96.8±15.5

n=6 for each size fraction (<70 µm, 70-500 µm, >500 µm) as well as for balance

To further describe the output from the tanks, the organic content of the dry material was determined. The average organic content in the outflow was 54.3±3.0% (Table 2.5). The 70-500 μm size fraction contained the highest percentage of organic material with 63.8±3.8% which was significantly higher than both the <70 μm with 46.2±5.6% and the >500 μm with 51.9±3.8% (t-test, $p \leq 0.001$, df=10 for both). The >500 μm and <70 μm were not significantly different from one another (t-test, p=0.065 df=10).

Holmer and Kristensen (1992) reported the percentage of organic weight (represented as percentage loss-on-ignition) in sediment under farm cages to range from 18.2% to 23.5% at depths of 0-1 cm. Another study showed similar results where the percentage of organic material in sediment beneath salmon cages off Norway was 15.0%-28.2% (Johnsen et al., 1993). The sediment beneath the cages receives much of the material that is lost from the farms; however, a portion of it will be biogeochemically lost during and after sedimentation due to leaching effects (Reid et al. 2008). The land-based tanks provide fresh material and therefore have fewer loses to the environment. This could account for a higher percentage of organic material exiting the juvenile cod tanks. In addition, the larger material had a lower organic content than the 70-500 μm and the <70 μm fractions suggesting the lighter, more organically enriched material could be settling further away from the cage sites.

Along with this, ash weight was determined (Table 2.5) and mirrors the organic weight distribution. The majority of the inorganic material was in the <70 μm with an average value of 53.8±5.6%. This relates to the input from the supply water, which has an inorganic input of 58.4±10.4%.

Table 2.5: Organic and ash content in outflow size fractions (<70 μm, 70-500 μm, >500 μm) from 6 tanks

Size fraction (μm)	n	Average organic (%)	Average Ash (%)
<70	6	46.2±5.6	53.8±5.6
70-500	6	63.8±3.8	35.7±4.5
>500	6	51.9±3.8	47.7±3.8
Average	6	54.0±3.0	45.7±3.1
Inflow	4	41.6±10.4	58.4±10.4

2.4.2 Dissolved organic carbon and total nitrogen

Dissolved organic matter (DOC) is an important component of the global carbon cycle and the exchange of gases in the ocean (Hedges, 1992) and supports heterotrophic bacteria (Pomeroy, 1974). Total nitrogen (TN) is a measure of total dissolved organic and inorganic nitrogen. Nitrogen plays an important biological role in marine life for phytoplankton and nitrogen fixing bacteria where it migrates through the nitrogen cycle changing its oxidation state.

DOC and TN were analyzed here to determine if the effluent of the fish tanks contained an altered level of these components. Due to the input from the feed and faecal matter from the fish, an increase in one or both DOC and TN would be expected (Fig. 2.4).

The amount of material in the preflush, postflush and midway between flushes was not significantly different (one-way ANOVA, $p=0.190$, $df=17$) and therefore averaged for a passive flow concentration. It is clear that higher concentrations of DOC and TN are exiting the tank when the standpipe is pulled to flush the tanks (Fig. 2.4). Given the flush represents the build up of particles from the previous 24 hours a high

concentration of DOC and TN seems reasonable. Conversely, the flush represents very little of the total mass of carbon and nitrogen exiting the tanks per day (Fig. 2.4). The flush is contained in a 20 L daily pulse while the passive flow continues throughout the day so there is substantially more on a daily basis: 16.1 g of DOC in the passive flow compared to 0.369 g in the flush and 14.5 g TN in the passive flow and 0.097 g TN in the flush. The appendix, Table A-2.2 shows tabulated results for DOC and TN.

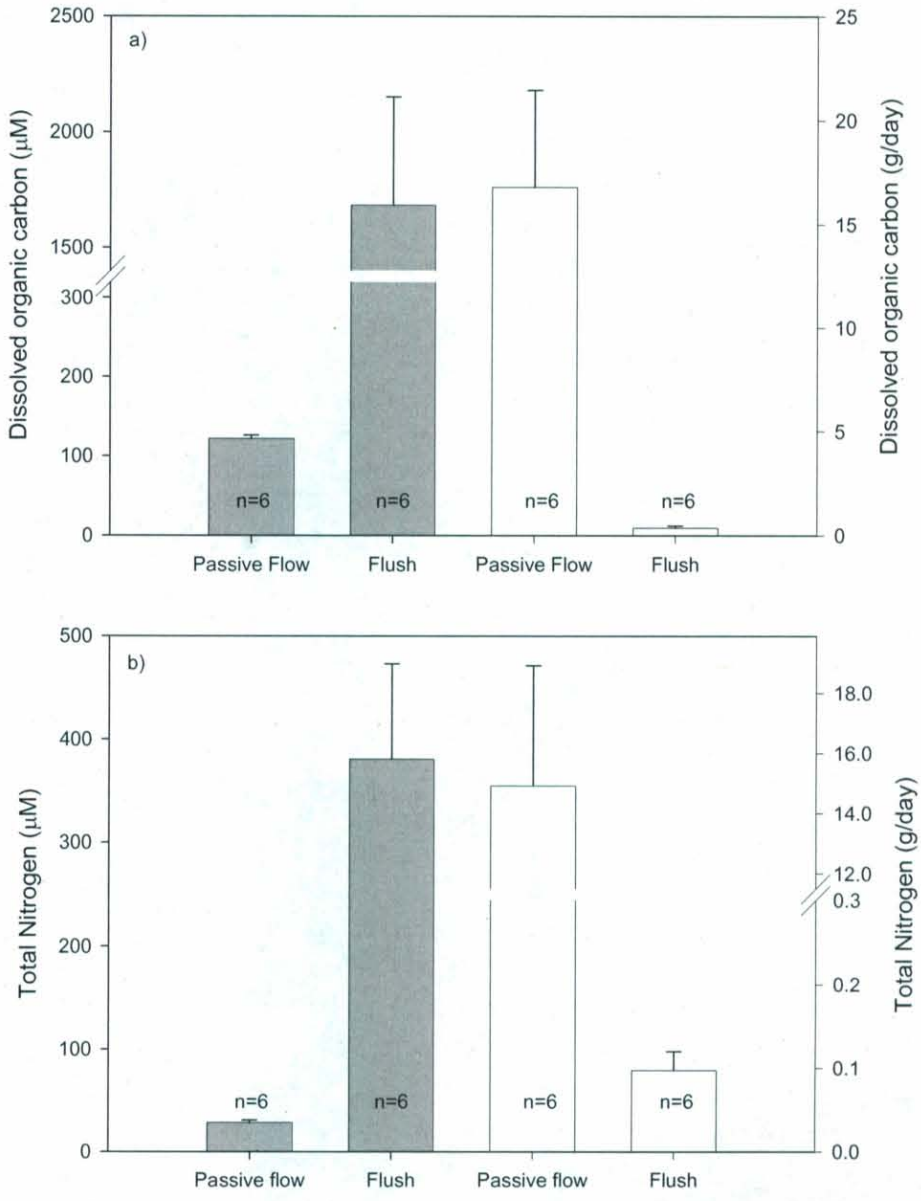

Figure 2.4: Dissolved organic carbon (a) and total nitrogen (b) for the flush and passive flow
The axis on the left relates to the grey bars; the axis on the right to the white bars
Bars: average + 1 SD

The DOC exiting the tanks at 122 μM is higher than the 40-105 μM reported for spring seawater south of Greenland (Duursma, 1962) as well as those reported for seawater by Fry et al. (1996), who gave a range from 43 to 114 μM. However, Millero et al. (1996) reported a wide range of 60-210 μM for coastal ocean water. This range includes the value reported here for the tank effluent indicating feed and faecal matter input may not have a large effect on DOC levels.

The level of TN in the effluent exiting from the tanks, 28.5 μM per day, is within the range reported by Millero (1996) of 4-60 μM; however, it is below that reported by Suzuki et al. (1985): 37.8 to 43.3 μM for the total nitrogen of surface water. The levels of TN are not elevated in the tank effluent suggesting TN may not be affected by the input of feed and faecal matter.

2.4.3 Lipids

The total lipid of the influent and effluent particles, feed, and fish are shown in Fig. 2.5. The inflow source to the tanks was variable with an average of about 7.2±6.9 mg/g DW (dry weight). A sand filter was used for the supply water filtering to approximately 50 μm which would permit the passage of many small particles including primary producers to contribute to the total lipid entering the tanks.

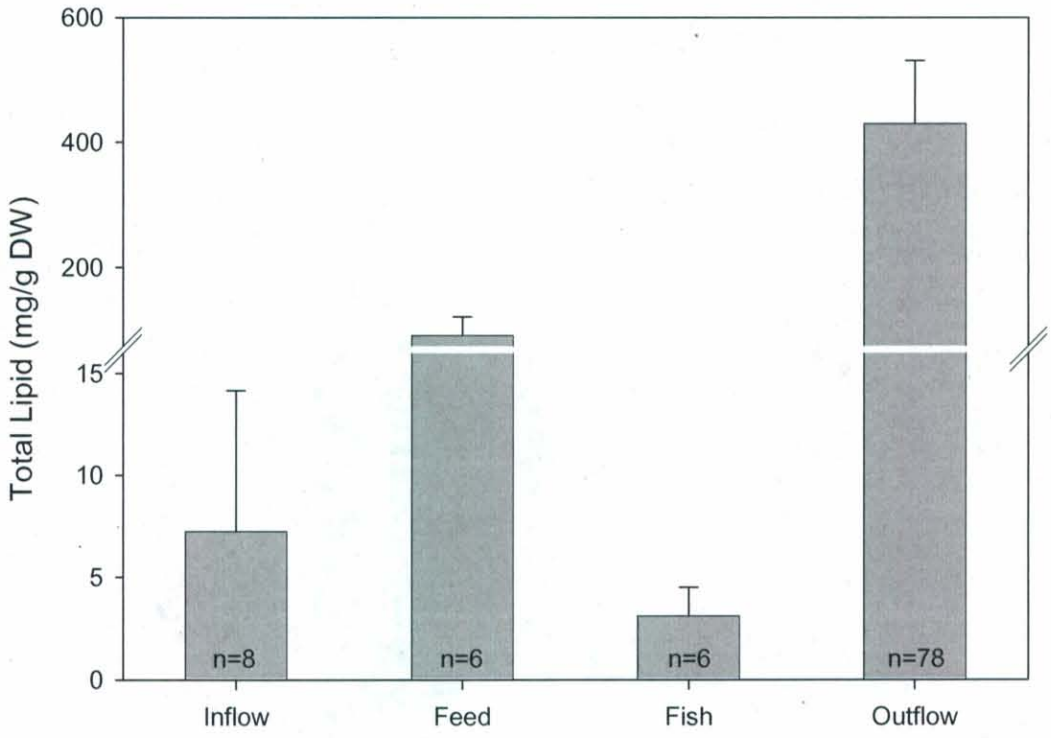

Figure 2.5: Total lipid in inflow, small feed, large feed, fish and outflow
DW: dry weight
Bars: average + 1 SD

Total lipids in feed samples showed 91.7±29.6 mg/g dry weight (DW) or 9.2% DW consistent with the minimum 15 and 18% oil content reported for the 4.0 mm and 6.0 mm Europa feed by Skretting. This also compares to lipid contents in other commercial finfish feed used in experiments, which had 10-26% lipid (Björnsson et al., 2000; Pérez-Casanova et al., 2009).

One fish from each of the six tanks was selected randomly and lipid extracted using the same method described previously. The total average lipid for the fish was 3.1±1.4 mg/g DW or 0.31±0.14% DW. Parrish et al. (2007) reported total lipid composition for Atlantic cod (*Gadus morhua*) to be 0.4±0.01% DW.

The effluent had the highest average lipid content with about 430 mg/g DW, and contributed significantly more lipid than the inflow water supply. The higher values in the effluent must be due to the waste and excess feed from the fish.

Of the 430 mg/g DW lipid exiting the tanks, about 25% was in <70 μm, 50% in the 70-500 μm, and 25% in >500 μm (Table 2.6). Most of the lipid material fell in the 70-500 μm size range.

Table 2.6: Total lipid of influent and different size fractions of effluent

Size fraction (μm)	Inflow (mg/g)	Outflow (mg/g)
<70	-	108±47
70-500	-	213±74
>500	-	109±49
total	7.23±6.93	430±100

n=6 for each size fraction (<70 μm, 70-500 μm, >500 μm)

Particulate lipid classes and fatty acids were also analyzed in the tank inflow, feed, fish and outflow. The water entering the tanks provided multiple lipid sources (grey bars in Fig. 2.5 (a)). The most abundant lipid class is acetone mobile polar lipids. AMPL (acetone-mobile polar lipids) includes a group of lipids that migrate up the Chromarod in the polar solvent, acetone. The group includes glycolipids along with their associated pigments and chloroplasts indicating plant-like material (Parrish et al. 2000). Its presence in the inflow highlights contributions from algae and plant material to the supply water. Another contributor to the inflow is free fatty acids (FFA) at 12.9±10.8%. As FFA is an indicator of breakdown (Van Biesen and Parrish, 2005), its presence in the inflow shows the contribution of material from digestion and other breakdown processes in the ocean supply water as well as in the sand bed filters. At 6.9±21.4% total lipid, triacylglycerol (TAG) was also an important contributor of lipid to the inflow. TAG contains a glycerol backbone to which three fatty acids are esterified. It is a condition index and relates to lipid storage (Hømer, 1989). It can be broken down by removing the three fatty acids from the glycerol backbone when energy is required at times of low food availability or when functions that are more prominent are required such as developing the gonads (Parrish et al., 2007). This process removes one of the fatty acids at a time converting TAG to diacylglycerol (DAG) then to monoacylglycerol (MAG). Parrish et al. (2000) found a high flux of TAG or storage in organisms falling to the benthos in the spring months. Given the sampling period occurred from April 30th to May 20th, the high level of TAG seems reasonable. Other contributors to the inflow lipids are phospholipid (PL) and sterol. PL contains two fatty acids esterified to a glycerol backbone and, along with sterol,

plays a role in cell structure. PL is a major component of membranes and can indicate newly synthesized material.

The lipid profile of the inflow was consistent with values reported for other Atlantic coastal water. Parrish et al. (1988) described suspended particulate matter in spring samples taken from Bedford Basin, Nova Scotia at 70 m. When converting values found from the tank inflow to the comparable µg/l, they were similar to those reported from Bedford Basin (Table 2.7). Parrish et al. (1988) found HC at 8.1 µg/l that is within the range found here. Average TAG and FFA were both higher than values reported from the Bedford Basin, however the tank inflow was highly variable with large standard deviations relating to the changing conditions in the supply water. The range from the JBARB includes the values reported by Parrish et al. showing their similarity to other east coast Canadian coastal waters in spring. ST, AMPL and PL were all similar to the reference values.

Table 2.7: Influent from JBARB compared to particulate lipid class values from Bedford Basin, Nova Scotia reported by Parrish et al. (1988)

	JBARB Inflow (µg/l)	Parrish et al. (1988) (µg/l)
HC	4.9±5.4	8.1
TAG	32.3±59.9	11.2
FFA	28.8±45.9	6.0
ST	2.2±4.1	2.0
AMPL	14.1±7.9	19
PL	6.3±4.8	3.0

Two sizes of feed, 4.0 mm and 6.0 mm, fed to the cod were also analyzed (Fig 2.6 (b)-grey bars). There was an abundance of TAG in the feed samples comprising 74.4±13.5% total lipid. Along with this PL, AMPL, and sterol were also large

contributors. The contribution of FFA was very small at $1.67x10^{-3}\pm4.08x10^{-3}$%. Given FFA is an indication of breakdown and contains less nutritional value a trace amount being supplied through the feed is logical. The appendix gives a tabulation of the amount of lipids in the feed (Table A-2.3) as well as the proximate composition of both feeds from the product packaging (Table A-2.4).

Total lipids and lipid classes were also determined in a small number of fish. The small sample size led to higher variance; however, the major lipid class in the fish was PL (35.2±31.1%) again indicative of membrane material. TAG was also abundant in the fish at 36.8±25.2% representing storage of consumed lipids. Parrish et al. (2007) reported PL at 62.7±5.8% TL and TAG at 3.5±2.7% TL for Atlantic cod sampled in Bonne Bay, Newfoundland. Compared to the juvenile Atlantic cod from the land-based tanks the PL was higher while the TAG was lower; however, the study attributed the low TAG values to the depleted energy reserves from the winter months during which time stored energy is used up to develop the gonads (Parrish et al., 2007). For the fish from the land-based tanks, feeding occurs daily and therefore there is no time when stores of TAG are used due to low food availability. In addition, the fish from the land-based tanks have lower PL values, which is likely due to maintenance diet as opposed to one that would promote growth. PL relates to development of membrane material and subsequent growth which was not a goal for the fish during this period as operators were waiting for optimal marine conditions to transport the fish to coastal netpens. Table A-2.3 tabulates the amount of lipids present in the fish.

The lipid profile of material coming out of the tanks showed FFA with 43.1±9.5% total lipid as the major contributor. This is much higher than the 25% total lipid which is

considered a maximal proportion in seawater (Parrish, 1988) reflecting FFA as an indicator of breakdown as well as a marker for faeces (Van Biesen and Parrish, 2005). Along with this, PL (15.0±5.4%), TAG (12.8±2.0%), AMPL (10.6±3.4%), and sterol (8.5±3.0%) contributed to the lipid output. The black bars in Fig. 2.6 show this graphically. The large contribution from faeces in the outflow water is shown here with the abundance of the breakdown lipid, FFA. Acyl lipids, PL, TAG, and AMPL were all present in the feed supplied to the fish.

Lipid profiles shown in Fig 2.6 indicate more complexity in the outflow than the inflow with representation from every lipid class in the outflow. In addition, FFA in the outflow was significantly higher ($df=20$, $p<0.001$) than in the inflow. There was, however, significantly more AMPL in the inflow than in the outflow (t-test, $df=9$, $p=0.022$). The outflow also contained significantly more HC (t-test, $df=16$, $p=0.004$), FFA (t-test, $df=12$, $p<0.001$), sterol (t-test, $df=9$, $p=0.040$) and AMPL (t-test, $df=9$, $p=0.009$) than the feed (Table A-2.4). Along with this, there was significantly less TAG in the outflow than the feed (t-test, $df=5$, $p<0.001$).

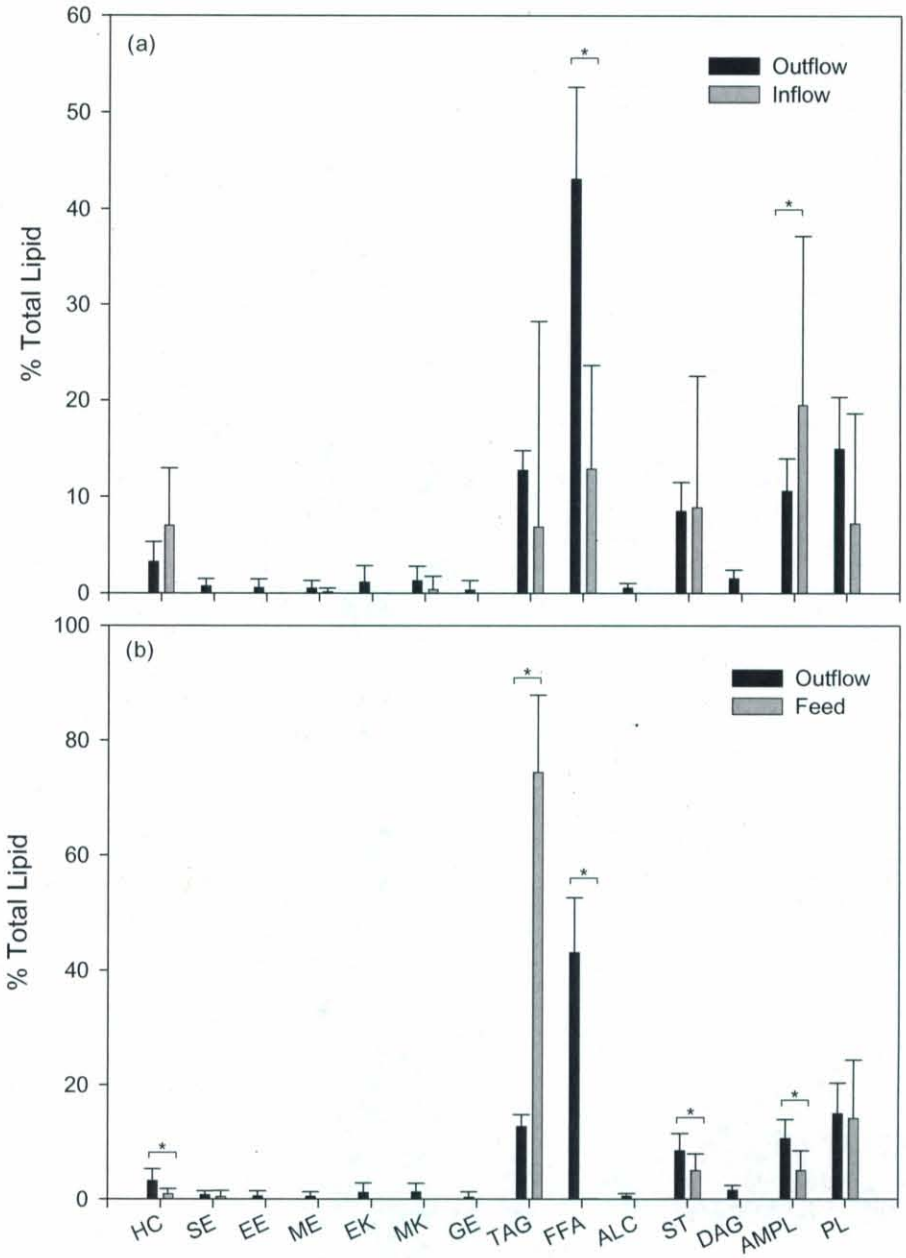

Figure 2.6: Lipid profiles of (a) outflow and inflow as well as (b) outflow and feed
*Significantly different ($p<0.05$). Bars: average + SD, n=6-78
HC: hydrocarbon; SE: steryl ester; EE: ethyl ester; ME: methyl ester; EKET: ethyl ketone; MKET: methyl ketone; GE: glyceryl ether; TAG: triacylglycerol; FFA: free fatty acid; ALC: fatty alcohol; ST: sterol; DAG: diacylglycerol; AMPL: acetone mobile polar lipid; PL: phospholipid

In order to further describe material exiting the tanks the outflow was fractionated into <70 μm, 70-500 μm, and >500 μm (Fig. 2.7). This fractionation describes what is available for consumption by surrounding organisms (e.g. mussels) according to preferred size ranges of particles.

Comparing with t-tests showed significantly less TAG in the >500 μm size fraction than the <70 μm and 70-500 μm size fractions (df=22, p=0.011 and df= 21, p=0.013, respectively). Along with this, FFA was significantly higher in 70-500 μm particles than the <70 μm fraction (df=22, p=0.003). This could be due to the faecal matter and subsequent FFA being mostly larger particles. The smaller <70 μm also had more TAG than the larger >500 μm, which relates to the smaller material coming from the water supply and feed fines.

The ALC (fatty alcohols) showed significant differences among <70 μm and 70-500 μm (df=15, p=0.002) and <70 μm and >500 μm (df=19, p=0.004). Although this was statistically significant there was little ALC in the entire outflow compared to the other lipid classes present.

Finally, there was significantly more DAG in <70 μm than >500 μm (df=14, p=0.036) and again more in 70-500 μm than >500 μm (df=21, p=0.000). DAG is a breakdown product of TAG in which one of the fatty acids attached to the glycerol backbone is removed therefore a similar relationship between size fractions for both TAG and DAG were logical.

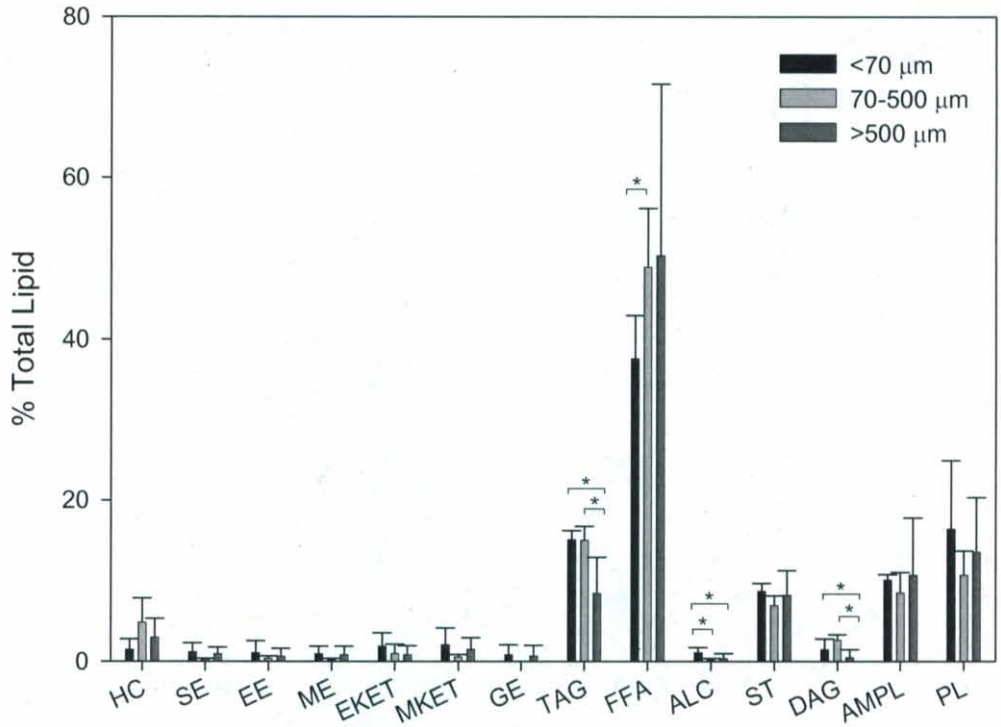

Figure 2.7: Lipid profile of the tanks daily effluent for all size fractions (<70 μm, 70-500 μm, and >500 μm)
*Significantly different ($p<0.05$). Bars: average + SD, n=13 for each size fraction
Abbreviations explained in legend to Fig. 2.6

2.4.4 Fatty Acids

Fatty acids provided another means for analyzing the throughput of the tanks. Lipid and fatty acid markers allow for descriptions of the material entering and exiting the tanks using markers listed in Table 2.1.

The most abundant fatty acids entering the tanks are shown in Fig. 2.8 (a) with the black bars. Palmitic acid (16:0), a saturated fatty acid with sixteen carbon atoms, had the highest percentage at over 20% total fatty acid. Along with this, 14:0 and 18:0 were also present, and all three are abundant saturated fatty acids found in nature and are present in most marine organisms. The fatty acid 16:1ω7 is a diatom marker (Viso and Marty, 1993) indicating diatoms in the supply water. In addition to this, the essential fatty acid 20:5ω3 (eicosapentaenoic acid; EPA) is another diatom marker (Viso and Marty, 1993) and was also found in the inflow at 2.8% total fatty acids.

The fatty acids in the feed are shown in Fig. 2.8 (b) with the black bars. They include the essential fatty acids 22:6ω3 (docosahexaenoic acid; DHA) and EPA at 11.6% and 12.9% total fatty acids, respectively added for nutritional enrichment. Along with this, 16:0 was present at 18.3% and 18:1ω9 at 13.2%. Johnsen et al. (1993) reported similar values in feed studied from a marine salmon farm in western Norway (Table 2.8) where 16:0 was present at 16.8±0.2%, 18:1ω9 with13.5±0.1%, DHA (22:6ω3) at 7.7±0.1%, and EPA (20:5ω3) at 7.3±0.1% total fatty acids. The zooplankton fatty acids 20:1ω9 and 22:1ω11 exiting the tank had 1.9±1.4% and 2.5±2.7% respectively; however, the feed examined by Johnsen et al. contained much more 20:1ω9 at 9.9±0.1% and 22:1ω11 at 13.6±0.1% possibly due to more zooplanktivorous fish meal/oil in the feed.

Henderson et al. (1997) examined feed supplied to salmon cages reporting 16:0 present at 11.4% total lipid, 18:1ω9 with 4.5%, DHA at 8.2%, and EPA at 10.1%, 20:1ω9 at 10.7% and 22:1ω11 at 14.7% (Table 2.8). Again, the zooplankton fatty acids represented more of the total lipid than the feed used for the juvenile codfish; however, both of the feeds reported were used for salmon farms, which could account for the discrepancy. The fatty acid 18:1ω9 is a major fatty acid in all animals. The composition of the feed reflects the diets of small planktivorous-feeding fish used to make the fishmeal (Iwana, 1991). The major fatty acids in the feed are presented in the appendix Table A-2.5.

Table 2.8: JBARB fatty acids from feed compared to feed values reported by Johnsen et al. (1993) and Henderson et al. (1997)

	JBARB Feed (%)	Johnsen et al. (1993) (%)	Henderson et al. (1997) (%)
16:0	18.3±0.5	16.8±0.2	11.4
18:1ω9	13.2±4.7	13.5±0.1	4.5
20:1ω9	1.9±1.4	9.9±0.1	10.7
20:5ω3	12.9±1.8	7.3±0.1	10.1
22:1ω11	2.5±2.7	13.6±0.1	14.7
22:6ω3	11.6±0.7	7.7±0.1	8.2

The fatty acid profile of the fish showed the most retention of the essentials EPA (13.6%) and DHA (15.4%). The fatty acid 16:0 was also a major fatty acid and is used in storage as well as for structural purposes such as membrane bilayers. The essential fatty acid DHA is also found in bilayers where it is preferentially conserved for use in phospholipids (Dalsgaard et al., 2003).

Kirsch et al. (1998) reported Atlantic codfish fatty acids levels of EPA and DHA at 11.98% and 21.15% total fatty acids, respectively (Table 2.9). These are similar to

levels found in cod examined here. The levels found in the fish are dependent on the amount supplied to the fish in their feed, as they cannot synthesize their own essential fatty acids. In addition, Kirsch et al. (1998) reported 20:1ω9 at 4.64% and 22:1ω11 at 3.84%, which are similar to 2.61% and 1.52% for 20:1ω9 and 22:1ω11, respectively. The major fatty acids in the fish are shown in the appendix Table A-2.5.

Table 2.9: Fatty acid proportions in JBARB cod compared to reference values reported by Kirsch et al. (1998)

	JBARB Atlantic cod (%)	Kirsch et al. (1998) (%)
16:0	14.7±2.9	14.47±0.37
18:0	3.73±1.19	3.03±0.11
18:1ω9	7.48±6.13	10.30±0.31
20:1ω9	2.61±0.66	4.64±1.83
20:5ω3	13.6±3.2	11.98±1.50
22:1ω11	1.52±0.52	3.84±1.82
22:6ω3	15.4±4.0	21.15±4.20

The fatty acids were also analyzed in the effluent from the six tanks (grey bars in Fig 2.7). There was an abundance of 16:0 at 34.8±2.5% as well as 11.8±1.7% of 18:0 that are naturally occurring, abundant fatty acids. The fatty acid 18:1ω9, was also present at 10.3%. For the essential fatty acids, 2.6±0.4% was 20:5ω3 or EPA and 2.9±0.7% was 22:6ω3 or DHA.

Johnson et al. (1993) reported faecal fatty acids in salmon from an aquaculture cage in western Norway (Table 2.10). The faecal matter was obtained by squeezing the gut after killing the fish. They found similar results with 16:0 at 28.9±5.2%, 18:1ω9 at 8.7±2.1%, and the essential fatty acids were reported with 3.8±1.5% for DHA and 2.0±1.3% for EPA. There was a discrepancy in 18:0 where they reported 4.2±0.8%

compared to the 11.8±1.7% found here. They also found an abundance of 20:1ω9 at 10.0±0.9% and 22:1ω11 at 16.3±0.8% compared to 2.2±0.4% for 20:1ω9 and 3.2±0.7% for 22:1ω11 described here, which directly reflects the diets (Table 2.8).

Table 2.10: Fatty acid proportions JBARB outflow compared to faecal and reference location values from western Norway reported by Johnsen et al. (1993)

	Johnsen et al. (1993) Faeces (%)	JBARB Output (%)	Johnsen et al. (1993) Reference location (%)
16:0	28.9±5.2	34.8±2.5	36.9
18:0	4.2±0.8	11.8±1.7	22.4
18:1ω9	8.7±2.1	10.3±1.1	3.6
20:1ω9	10.0±0.9	2.2±0.4	0.2
20:5ω3	2.0±1.3	2.6±0.4	2.0
22:1ω11	16.3±0.8	3.2±0.7	0.2
22:6ω3	3.8±1.5	2.9±0.7	2.3

The inflow and outflow were compared using two sample t-tests (Fig 2.8 a). Examining the results shows significantly more of the zooplankton fatty acids, more specifically the copepod fatty acids 20:1ω9 and 22:1ω11 in the outflow compared to the inflow ($df=15$, $p<0.001$ and $df=12$, $p=0.002$, respectively). The outflow would expectedly contain more zooplankton fatty acids as the feed pellets used contain meal from fish that consume plankton and therefore contain fatty acids of zooplankton. With excess feed pellets settling to the bottom of the tanks, the fatty acids they contain are present in the outflow. Also, long chain monounsaturated fatty acids (MUFA) are not well absorbed by cod (Lie et al., 1987).

The essential fatty acid 22:3ω6 was also significantly higher in the outflow compared to the inflow ($df=10$, $p=0.017$). The feed are supplemented with essential fatty

acids and excess feed pellets allow for their fatty acids to enter the outflow. There was also significantly more 18:0, 18:1ω7, 18:1ω9, and 18:2ω6 in the outflow than the inflow. These fatty acids were present in the feed and therefore uneaten pellets could contribute them to the outflow. The fatty acid 18:1ω9 is a major fatty acid for animals. The fatty acid 18:1ω7 is typical of sulphur-oxidizing bacteria (Volkman et al., 1998; Pond et al., 2002; Pistocchi et al., 2005) and 18:2ω6 is a precursor for the essential fatty acids (Pond et al., 2002).

Comparing the feed to the outflow using t-tests continued to show enrichment with essential fatty acids (Fig 2.8 b). DHA (22:6ω3) and EPA (20:5ω3) were significantly higher in the feed than the outflow ($df=7$, $p<0.001$ and $df=5$, $p<0.001$, respectively). There was also significantly more 14:0, 16:0 and 18:0 in the outflow compared with the feed ($df=10$, $df=42$, $df=19$, respectively and $p<0.001$ for each). These fatty acids are all naturally occurring in all organisms and are less nutritionally valuable than the PUFA which are preferentially retained by the fish.

Some of the fatty acids exiting the tanks were elevated in comparison to a non-impacted reference location reported by Johnson et al. (1993). The background levels reported off the coast of Norway showed an abundance of 16:0 and 18:0 (36.9% and 22.4%, respectively). The level of 16:0 is the same as that found exiting the tanks at 34.8±2.5%; however, there is more 18:0 in the reference location than found in the tank effluent (11.8±1.7%). This can be explained by the low input of 18:0 from the supply water. The amount of 18:0 in the inflow was 8.6±3.7% which is also lower than the reference location. The input discrepancy can be accounted for by the sensitivity of fatty acids to seasonality and oceanic events such as algal blooms.

Specifically, the level of 20:1ω9 was 1.2% total fatty acids and 22:1ω11 was 0.2% compared to the elevated 2.2±0.4% for 20:1ω9 and 3.2±0.7% for 22:1ω11 described here. In addition to this, DHA and EPA were found to be 2.3% and 2.0% in the reference location. The amount of EPA exiting the tanks was 2.91±0.73% and for DHA was 2.62±0.44%. These are only slightly elevated and most likely represent the retention of the essential fatty acids by the fish.

The outflow was fractionated into <70 μm, 70-500 μm, and >500 μm sizes. This allowed the size-specific fatty acid profiles to be compared (Fig 2.9). After fractionation, the overall trend of the fatty acid profile remained the same. Significant differences in size fractions were found for 16:0, 18:0, 18:1ω9, 18:1ω7, 18:2ω6, and 22:6ω3 ($p < 0.001$-0.039). For example, the >500 μm fraction had significantly less 16:0 than the 70-500 μm and the <70 μm fractions. The latter represented the largest difference among the fractions amounting to 10.0% of the total fatty acids. All other significant differences were much smaller (0.01-3.5%).

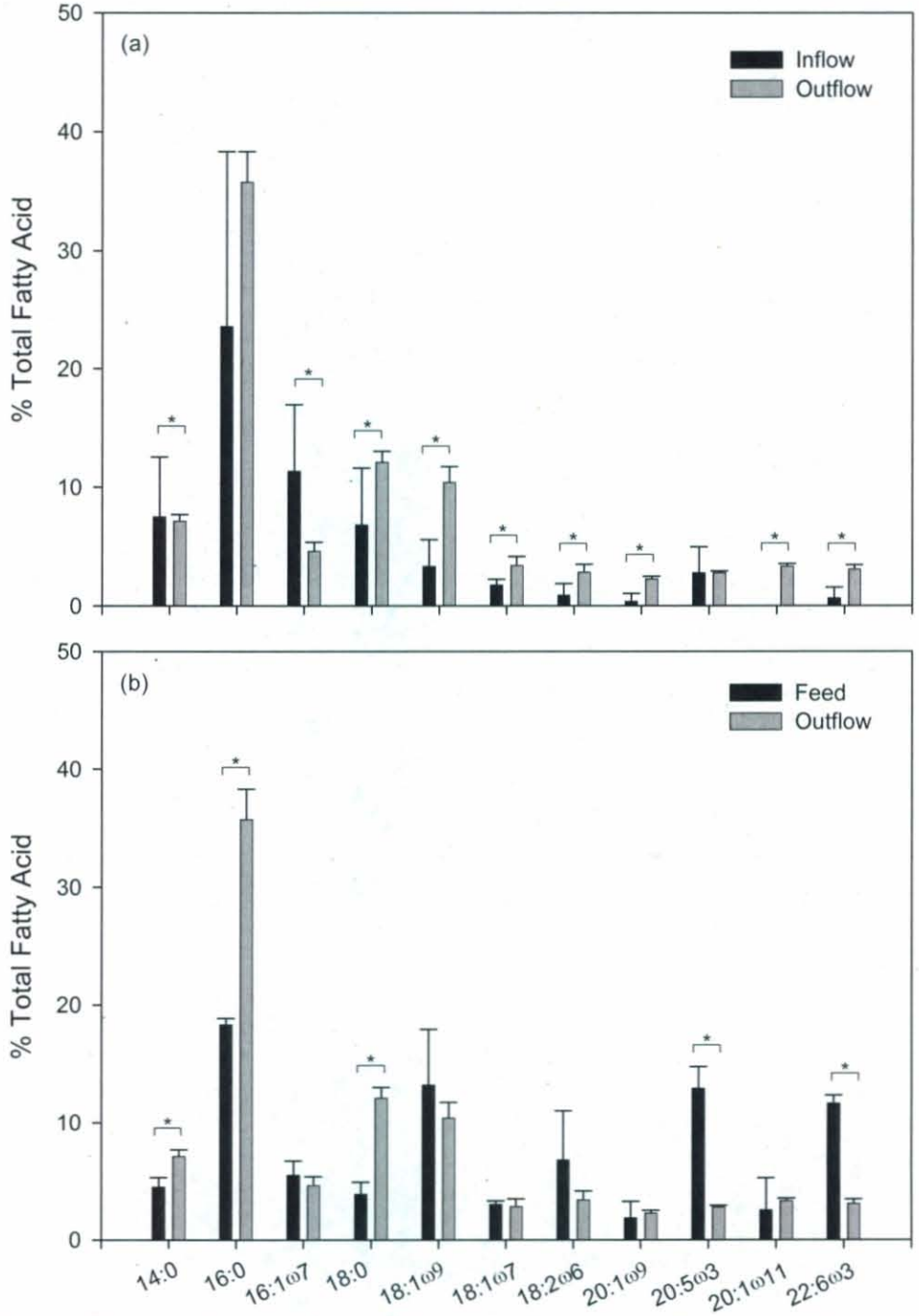

Figure 2.8: Most abundant fatty acids in (a) inflow and outflow and (b) feed and outflow
* Significantly different ($p<0.05$). Bars: average + SD

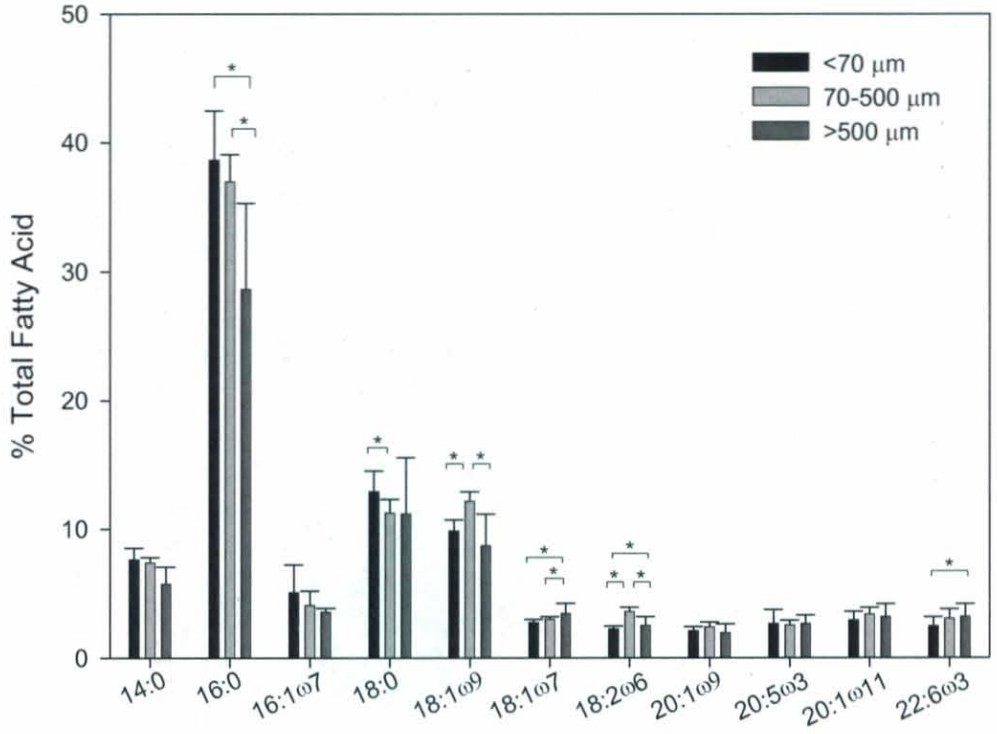

Figure 2.9: Most abundant fatty acids in the outflow for all size fractions (>500 μm, 70-500 μm, and < 70 μm)
* Significantly different ($p<0.05$). Bars: average + SD

2.5 Scaling results

In order to scale-up the output of the experimental tanks to larger scale aquaculture facilities, the data were used to calculate the amount of material per kilogram of fish biomass (Table 2.11). Along with this, the amount contributed by the presence of the fish and its feed alone was calculated by subtracting the material supplied by the inflow. The inflow was filtered through a sand filter to ≤50 μm so the <70 μm fraction was corrected for the input from the inflow material. This corrected <70 μm fraction was added to the 70-500 μm and >500 μm fractions to give a total corrected output so that, Total corrected material output = (<70 μm – inflow input) + 70-500 μm + >500 μm.

Table 2.11 shows the rates of output from the cod tanks uncorrected, corrected for the inflow, calculated per kilogram of biomass, and then scaled to operational size cod farms. The scaling is based on the 2005 Newfoundland Aquaculture Industry Association's (NAIA) report (Clift, 2005), and gives the biomass of an operational Atlantic cod farm ranging from 1,305,000 kg to 1,879,500 kg at harvest. It is important to note the calculations do not take into account the effects of currents nor other environmental parameters such as average temperature and salinity; however, they do provide an estimate of the potential particulate output from net pens at the time of harvest.

The corrected effluent was also compared to the commercial feed as a percentage of dry weight (Table 2.12). This highlights the low essential fatty acid contribution compared to the commercial feed. Here the lipid component of the effluent represents 7.15% DW compared to the 37.7% DW found with the supplied feed. In line with this lower lipid input, the essential fatty acids represent a lower percentage DW (0.29 %) compared to the feed (8.63%). Of this total essential fatty acid, DHA contributes 0.15%

DW in the effluent while DHA represents 3.38% DW in the feed reflecting the feeds nutritional value. As previously highlighted, FFA was the major lipid class contributor to the effluent making up 3.36% DW. The feed contained trace amounts of FFA. The effluent acts an enrichment to the local environment; however, closer examination shows lower nutritional quality compared to a commercial feed. Nonetheless, without aquaculture operations, no enrichment would be present. In addition, the feed examined here satisfies juvenile cod; however, larger, commercial-size cod are supplied feed that reflects their growing nutritional requirements. Dividing the total essential fatty acids contributed from the outflow (Table 1.11) by those contributed from the feed gives 3.4%. This represents an estimate of the amount of feed in the effluent, which is inline with other measurements quantifying the amount of waste feed and fines (Cromey et al., 2002; Reid et al., 2008).

Table 2.11: Output rates from cod aquaculture operations

		JBARB tanks						Farm size	
		Outflow (all size fractions, flush included)		Outflow corrected for inflow		Corrected outflow per kilogram biomass		1305 tonne farm	1880 tonne farm
	n	(g/day)	CV (%)	(g/day)	CV (%)	($g*day^{-1}kg^{-1}$)	CV (%)	(kg/day)	(kg/day)
Dry Mass	6	186.8±48.2	26	96.8±15.5	35	1.69±0.46	27	2200±610	3170±870
Organic Mass	6	93.8±19.8	21	64.7±13.0	20	1.13±0.25	22	1480±330	2130±480
Ash Weight	6	94.8±31.1	33	43.2±19.6	45	0.76±0.28	37	990±370	1420±530
Total Lipid	6	8.30±2.57	31	6.81±2.12	31	0.12±0.05	38	161±62	232±89
FFA	6	3.26±1.05	32	3.19±1.08	34	0.06±0.02	38	75±29	108±41
Total Essential FA	6	0.34±0.09	26	0.28±0.08	29	0.0050±0.0019	37	6.6±2.4	9.4±3.5
DHA	6	0.16±0.04	28	0.14±0.04	30	0.0025±0.00089	36	3.3±1.2	4.7±1.7

JBARB: Joe Brown Aquatic Research Building
FFA: free fatty acid
FA: fatty acid
DHA: 22:6ω3; docosahexaenoic acid

Table 2.12: Composition of inflow-corrected output from JBARB compared to cod feed as a percentage of dry weight

	Corrected Outflow		Feed	
	n	% DW (%)	n	% DW (%)
Organic Mass	6	70.0±15.9	-	88.58
Ash Weight	6	43.8±6.0	-	11.42*
Total Lipid	6	7.15±1.60	6	37.7±12.7
FFA	6	3.36±0.99	6	tr
Total Essential FA	6	0.29±0.08	6	8.63±3.13
DHA	6	0.15±0.04	6	3.38±1.11

JBARB: Joe Brown Aquatic Research Building
tr: trace
*: Maximum ash content from product information: Europa 15 and 18 by Skretting, sizes 4.0 mm and 6.0 mm

2.5.1 Dry Mass

To calculate the throughput of dry material, the inflow and outflow were compared (Fig. 2.9). Inflow measurements were taken on four of the 13 sampling days. Daily fluctuations in the water supply were accounted for by directly comparing the outflow to the inflow on the four days they were both measured (Fig. 2.10). This significant linear regression (df=17, p=0.006) was used to calculate the predicted inflow from measured outflows for the sampling days inflow was not collected. It is important to note the linear relationship does not represent the data near the origin; however, a non-linear relationship showed little improvement to the r^2 and p-values, and within the range of data used here, 47-290 g/day the linear model remains representative.

An additional input from the fish showed a dry mass of 96.8±15.5 g/day or 1.69±0.46 g/day/kg biomass exiting the tanks (Table 2.11). This output solely relates to the presence of the fish: i.e. excess feed and faecal material. Scaling to a large, 1880

tonne cod farm gives 3172±872 kg/day dry mass supplied to the surrounding ecosystem. It is important to note that the throughput of the land-based tanks is based on juvenile fish and the farm sizes relate to harvest size fish allowing for an estimate only. An additional caveat is that the estimates of outflow, Table 2.11, are first approximation, based on 1:1scaling by mass and therefore, may be an overestimation.

For the ash-weight, the inflow values were calculated again by comparing the inflow and outflow on days where both samples were taken (Fig. A-2.1). This significant linear relationship (df= 21, p=0.0303) was used to determine the inflow on days it was not measured. Again, closer to the origin the linear relationship was not representative of the inflow based on the outflow. The non-linear relationship increased r^2 value, however it was less significant with an increased p-value. Therefore, the linear equation was used to predict inflow on days it was not sampled as values of the outflow (20-175 g/day) used for predictions did not require extrapolation beyond this range. Once the inflow was accounted for in the <70 μm fraction, a total output showed ash-weight contributed 43.2±19.6 g/day to the outflow and 2131±477 kg/day when scaled to the large size aquaculture site.

Unlike the dry mass and ash-weight, the organic output did not show a significant linear or non-linear relationship between the inflow and outflow (Fig. A-2.2). The input from the supply water is, for the most part, inorganic material after passing through the sandbed filters therefore the majority of the organic material contributing to the outflow comes from the addition of feed. This is confirmed by the consistent abundance of

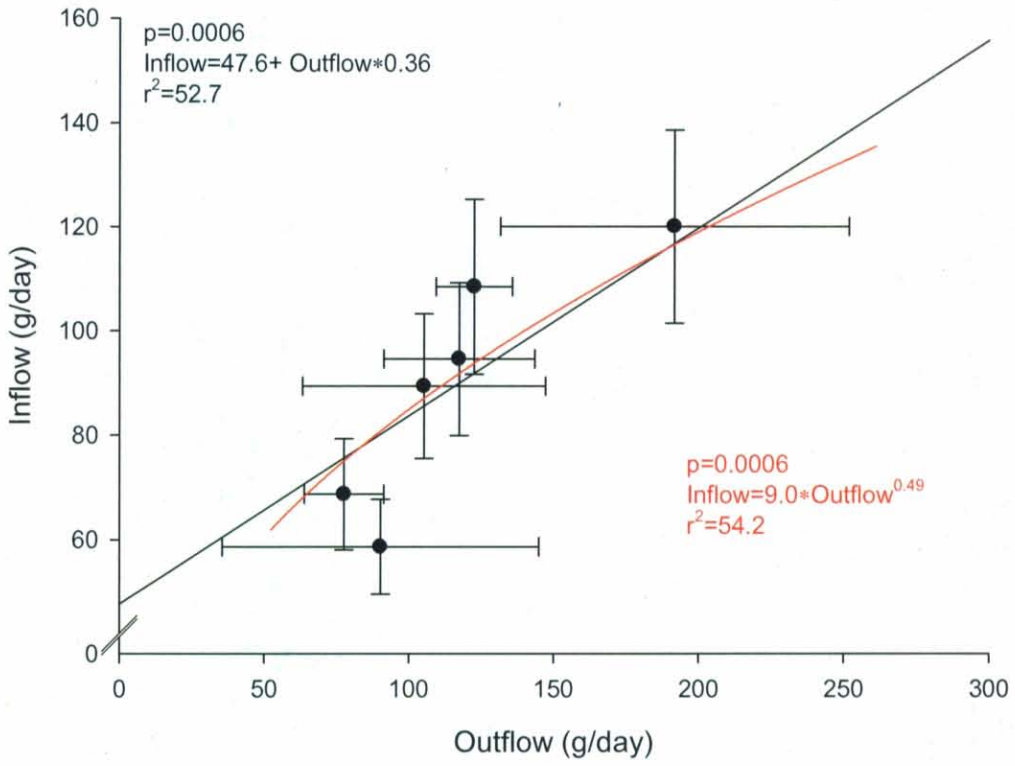

Figure 2.10: Inflow and outflow rates compared for dry mass
Regression line calculated for raw data.

organic material in the outflow and the non-significant relationship between the outflow and the inflow. Due to this, the <70 μm fraction was corrected by subtracting an average of the inflow organic material.

The land-based facility produced organic material at a rate of 64.7±13.0 g/day, which scales to 2130±480 kg/day. Organic material contains proteins and lipids that have the potential to be used by surrounding organisms, although an over abundance can overload the local ecosystem leading to a eutrophic environment.

2.5.2 Lipids

As previously reported the total lipid exiting the tanks was 460±108 mg/g DW, or 8.30±2.57 g/day. When corrected for the seawater inflow this becomes 6.81±2.12 g/day. The correction followed the same procedure as the dry mass where the inflow and outflow TL were compared to give a significant linear relationship and this equation was used to predict the inflow values on days it was not collected (Fig. A-2.3). Scaling this to an 1880 tonne cod farm gives 232±89 kg/day TL exiting the farm solely due to the presence of the fish.

FFA, the indicator of breakdown and faeces, was the most abundant particulate lipid class exiting the tanks and therefore the amount being contributed to the surrounding environment by an industrial size operation is of interest. With an 1880 tonne farm, 108±41 kg/day is being released.

Although further research is required, fatty acids in the free form have the potential to cause problems for marine animals near aquaculture sites. Elevated plasma FFA compete with glucose as an energy source reducing glucose oxidation, which can upset glucose metabolism (Boden and Shulman, 2002). Additionally, fatty acids in their

free form have been shown to be hemolytic, toxic to mice and reduce growth in marine diatoms (Yasumoto et al., 1990; Lawerence et al., 1994; Arzul et al., 1995). Nevertheless, certain individual fatty acids in the effluent of fish farm can be nutritionally enhancing.

2.5.3 Fatty acids

The essential fatty acids supplemented in the fish feed increase the nutritional quality of the surrounding water through parts of uneaten feed pellets exiting in the effluent. The sum of the essential fatty acids, 22:6ω3, 20:5ω3, and 20:4ω6, gave only 0.28±0.08 g/day exiting the tanks due to the contribution of the fish; however, when scaled to the size of an industrial cod farm there is up to 9440±3480 g/day. Essential fatty acids are required for development and as such are preferentially retained by consumers, therefore their input here indicates potential enrichment to the local ecosystem.

Of the essential fatty acids, DHA is highly valuable in terms of nutritional quality and one of the limiting factors for larval development in fish (Rainuzzo et al, 1997). Here it makes up approximately 50% of the total essential fatty acids, and provides 0.16±0.04 g/day from the land-based tanks. A working size farm with 1880 tonnes of fish would produce 4710±1680 g/day to organisms surrounding the marine net pens representing approximately 2% of the TL supplied. The availability of this DHA to surrounding organisms requires investigation. In addition, the levels of FFA as well as feed additives, pesticides, antibiotics, and other feed and faecal associated contaminants must be considered to fully understand the value of this enrichment to the environment.

2.5.4 IMTA

As previously discussed, heavy material settles to the benthos beneath the farms and lighter material disperses following current patterns. Through IMTA, strategically

placed mussels and seaweed aim to consume the excess particles and nutrients from fed aquaculture subsequently removing them, leading to increased growth in the adjacent species, but also maintaining high seaweed and mussel nutritional values.

The quantity and quality of material exiting a farm can be estimated from the JBARB cod tanks scaled to a farm size as a 1:1 first approximation. Although the size range of food consumption for mussels varies in the literature, Davenport et al. (2000) reported that mussels ingest particles and mesozooplankton larger than 500 μm suggesting that incorporation of all size fractions (the corrected <70 μm, 70-500 μm, and <500 μm) would account for all available food sources for mussels. From here, an estimated number of mussels that can be co-cultured next to an Atlantic cod farm can be calculated.

Alkanani et al. (2007) described mussel weight with the equation $y=0.31+0.01x$ (y=weight in grams and x is time in days) for mussels grown in socks in Notre Dame Bay, Newfoundland. Using this equation, the weight of mussels at the time of harvest (500 days post socking) is 5.31 g. Along with this, each mussel requires approximately 0.080 g/day at the time of harvest assuming an average daily food ration for mussels of 1.5% of their dry body weight (Thompson and Bayne, 1974; Hawkins et al., 1985). Knowing the scaled farm produces $3.17x10^6$ g/day, the number of mussels that could be sustained is $4.0x10^6$ suggesting 210 tonnes of mussels could be supported (Table 2.13). An average mussel farm in Newfoundland produces about 200 tonnes of mussels (38,000,000 mussels at 5.31g/mussel) so there is sufficient food for a mussel farm in the output from the scaled fish farm. However, for mussel development when grown adjacent to fed aquaculture, the quality of the food being supplied should also be considered.

Essential fatty acids are important in development of the mussels themselves as well as for marketing in terms of nutritional value. The sum of essentials and DHA alone was used as a quantifier of diet quality. DHA output can be compared with the data of Khan et al. (2006) who reported mussel growth, lipid and fatty acid data for cultured mussels. As the mussels grow, the amount of DHA per gram mussel is maintained at a level of 1.8 mg/g (Khan et al., 2006) so that every increased gram of mussel mass requires 1.8 mg of DHA. In addition to this, the weight increase per day at the time of harvest is 0.01 g/mussel so the DHA required per mussel is $1.8x10^{-5}$ g /mussel. The scaled cod farm produces 4.7 kg DHA so that $260x10^{6}$ mussels or 1400 tonnes (assuming an average weight of 5.31 g/mussel) of mussels could be maintained (Table 2.14). The average production of mussels for Newfoundland in 2007 was 3,390 tonnes (NAIA). This shows that a single 1880 tonne Atlantic cod farm could theoretically sustain a maximum of approximately 40% of the mussels reared in Newfoundland in terms of DHA requirements demonstrating the large capability of multi-trophic, co-culturing systems.

Table 2.13: Estimating mussels sustainable from mass output from scaled Atlantic cod fish farm

Weight equation[a]:	y=0.31+0.01x, x=500 days
Weight per mussel:	y=5.31 g/mussel
Weight produced:	3.17×10^6 g/day
Required feed:	5.31 g/mussel * 0.015 (fed) = 0.080 g/mussel
Mussels sustained:	$\frac{3.17 \times 10^6 \text{ g/day}}{0.080 \text{ g/mussel}} = 4.0 \times 10^7$ mussels
Mass of mussels sustained per day:	4.0×10^7 mussels * 5.31 g/mussel = 2.1×10^8 g
	= 210 tonnes

[a]Alkanani et al., 2007

Table 2.14: Estimating mussels sustainable from DHA output from scaled Atlantic cod fish farm

DHA required[b]:	1.8 mg/g
Weight increase:	5.32 g -5.31g = 0.01 g/day/mussel
DHA per mussel	1.8 mg/g * 0.01 g = 1.8×10^{-2} mg = 1.8×10^{-5} g /mussel
DHA produced	4.7 kg = 4.7×10^3 g/day
Mussels sustained per day:	$\frac{4.7 \times 10^3 \text{ g/day}}{1.8 \times 10^{-5} \text{ g /mussel}} = 260 \times 10^6$ mussels
Biomass sustained daily:	260×10^6 mussel * 5.31 g/mussel = 1.4×10^6
	= 1400 tonnes

[b]Khan et al., 2006

2.6 Conclusions

The dry weight, dissolved organic carbon, total dissolved nitrogen, particulate lipid classes, and their fatty acids were examined in the inflow and outflow as well as in the feed and fish in land-based juvenile Atlantic cod (*Gadus morhua*) tanks. The mass balance shows 89.9±15.4 g/day of dry weight material in the inflow, increasing to 187±49 g/day in the outflow, however decreases to 96.8 g/day when contributions from the input

are considered. This, along with input from the feed input gives a 24% output over input. The different size fractions (<70 μm, 70-500 μm, >500 μm) were found to contribute differently to the overall dry weight. The proportion of organic material (56.4±4.5% DW) was higher than literature values found in sediment beneath finfish net pens. The analysis of the total lipid showed an output of 460±108 mg/g WW. Lipid class content of this material showed significantly more FFA exiting the tanks (43.1±9.5%) compared to the input from the feed and the inflow ($p<0.001$ for both). This indicator of breakdown represents the faecal material from the fish.

There was significantly more of the zooplankton fatty acid markers 20:1ω9 and 22:1ω11 in the outflow than the inflow ($p<0.003$). These fatty acids are representative of the fish used to manufacture the feed pellets and therefore can be used as a marker for the feed. In addition, there was significantly more DHA (22:3ω6) in the outflow compared to the inflow ($p=0.017$) reflecting the input from the feed pellets. There was also significantly more 18:0, 18:1ω7, 18:1ω9, and 18:2ω6 in the outflow than the inflow.

Output was calculated per kilogram of biomass and scaled to operational-sized Atlantic cod farms for total output consideration. The dry mass released was 3170±870 kg/day for an 1880 tonne farm which has the capability to support 210 tonnes of mussels assuming mussels require 0.080 g/day per mussel at the time of harvest.

The quality of material exiting the tanks was also analyzed by calculating the DHA that would be released. Again, given an 1880 tonne farm and assuming mussels require 1.8×10^{-5} g /mussel at the time of harvest, 1400 tonnes of mussels could be supported if there was a 1:1 scaling by mass and if current effects were minimal.

Comparing to the annual mussel production in Newfoundland, this scaled farm produces enough DHA to theoretically support over 40% of the mussels grown in Newfoundland. It is important to note this calculation does not consider the local environmental factors unique to each farm and should be considered an upper theoretical limit as not all food will be delivered to surrounding mussels; however, this abundance of DHA shows the potential of IMTA systems in terms of nutritional quality where adjacent organisms could capture DHA that would otherwise be lost.

The land-based system allows a determination of the throughput of tanks and possible food sources from the tank effluent. Continuing with this, Chapter 3 will examine the lipid content and composition of species surrounding finfish cages, describing lipid and fatty acid uptake, including the uptake of the essential fatty acids by the surrounding invertebrates.

2.7 References

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2.8 Appendix 1

Table A-2.1: Daily mass (dry weight) in effluent from six JBARB tanks (mean±s.d.)

	Size fraction (μm)	n	Weight of effluent (g/day)	CV (%)
Passive Flow	<70	6	119±37	31
	70-500	6	30.4±9.9	32
	>500	6	23.8±8.2	35
Flush	<70	6	4.9±0.9	19
	70-500	6	0.16±0.03	19
	>500	6	8.0±2.3	28
Inflow		6	89.9±15.4	17
Total			186±49	26
Corrected Total			96.8±33.8	35

Table A-2.2: Dissolved organic carbon and total nitrogen for flush and passive flow in juvenile Atlantic cod tanks

	n	DOC (μM)	TN (μM)	DOC (g/day)	TN (g/day)
Passive	3	122±4	34.6±3.2	16.1±2.2	14.5±1.3
Flush	4	1790± 470	392±105	0.369±0.101	0.097±0.026
Total	-	-	-	16.5±2.2	14.6±1.3

Table A-2.3: Lipid profile for the inflow, feed, fish and outflow

Lipid Class	Inflow (% total lipid) n=11	Feed (% total lipid) n=6	Fish (% total lipid) n=6	Outflow (% total lipid) n=78
TL*	40.9±56.6	91.7±29.6	0.31±0.14	46.0±10.8
HC	7.01±5.94	0.95±0.88	0.92±1.08	3.07±1.67
SE	-	0.44±1.08	1.84±3.52	0.79±0.46
EE	-	-	-	0.68±0.33
ME	0.12±0.40	-	0.07±0.17	0.64±0.39
EKET	-	-	-	1.22±0.56
MKET	0.40±1.33	-	1.24±2.68	1.36±0.75
GE	-	-	-	0.50±0.44
TAG	6.87±21.35	74.4±13.5	36.8±25.2	12.8±3.8
FFA	12.9±10.8	0.002±0.004	7.24±4.85	45.6±7.0
ALC	-	-	-	0.53±0.50
ST	8.89±13.7	5.0±2.9	11.8±7.07	7.94±0.90
DAG	-	-	-	1.51±1.06
AMPL	19.5±17.7	5.0±3.5	5.97±4.69	9.74±1.12
PL	7.22±11.40	14.1±10.2	35.2±31.1	13.5±2.8

* : % wet weight (g/g)
HC: hydrocarbon

SE: steryl ester
EE: ethyl ester
ME: methyl ester
EKET: ethyl ketone
MKET: methyl ketone
GE: glyceryl ether
TAG: triacylglycerol
FFA: free fatty acid
ALC: fatty alcohol
ST: sterol
DAG: diacylglycerol
AMPL: acetone mobile polar lipid
PL: phospholipid

Table A-2.4: Proximate composition of juvenile Atlantic cod feed

Feed component	4.0 MM	6.0 MM
Crude Protein (Min.)	55%	50%
Crude Fat (Min.)	15%	18%
Crude Fiber (Max)	1.5%	1.5%
Calcium (Actual)	3.0%	3.0%
Phosphorus (Actual)	2.0%	1.4%
Sodium (Actual)	1.0%	1.0%
Vit A (Min.)	5000 IU/kg	5000 IU/kg
Vit D (Min)	3000 IU/kg	3000 IU/kg
Vit E (Min.)	200 IU/kg	200 IU/kg

Skretting Europa 16 and 18

Table A-2.5: Most abundant fatty acids for the inflow, feed, fish and outflow

Fatty Acid	Inflow (% total fatty acids)	Feed (% total fatty acids)	Fish (% total fatty acids)	Outflow (% total fatty acids)
	n=11	n=6	n=6	n=78
14:0	7.5±5.0	4.48±0.82	2.88±0.78	6.92±0.65
16:0	23.6±14.7	18.3±0.5	14.7±2.9	34.8±2.5
16:1ω7	11.3±5.6	5.49±1.24	5.04±1.81	4.25±1.07
18:0	6.8±4.8	3.87±1.04	3.73±1.19	11.8±1.7
18:1ω9	3.3±2.3	13.2±4.7	7.48±6.13	10.3±1.1
18:1ω7	1.7±0.5	3.01±0.31	6.84±4.87	3.08±0.37
18:2ω6	0.89±0.98	6.80±4.20	2.50±0.72	2.79±0.25
20:1ω9	0.38±0.66	1.87±1.39	2.61±0.66	2.15±0.41
20:5ω3	2.8±2.2	12.9±1.8	13.6±3.2	2.62±0.44
22:1ω11	-	2.52±2.74	1.52±0.52	3.18±0.68
22:6ω3	0.63±0.93	11.6±0.7	15.4±4.0	2.91±0.73

Table A-2.6: Most abundant fatty acids in order for inflow, feed, fish, and inflow of JBARB Tank

Fatty Acid	*Inflow* (% total fatty acids)	Fatty Acid	*Feed* (% total fatty acids)	Fatty Acid	*Fish* (% total fatty acids)	Fatty Acid	*Outflow* (% total fatty acids)
n=11		n=6		n=6		n=78	
16:0	23.6±14.7	16:0	18.3±0.5	22:6ω3	15.4±4.0	16:0	35.7±4.2
16:1ω7	23.6±14.7	18:1ω9	13.2±4.7	16:0	14.7±2.9	18:0	12.1±2.8
14:0	7.5±5.0	20:5ω3	12.9±1.8	20:5ω3	13.6±3.2	18:1ω9	10.4±1.9
20:3ω3	7.08±10.04	22:6ω3	11.6±0.7	18:1ω9	7.48±6.13	14:0	7.13±1.38
18:0	6.8±4.8	18:2ω6	6.80±4.20	18:1ω7	6.84±4.87	16:1ω7	4.61±2.13
18:1ω9	3.3±2.3	16:1ω7	5.49±1.24	16:1ω7	5.04±1.81	18:1ω7	3.38±1.12
15:0	2.82±3.99	14:0	4.48±0.82	18:0	3.73±1.19	22:1ω11	3.32±1.37
20:5ω3	2.8±2.2	18:0	3.87±1.04	14:0	2.88±0.78	22:6ω3	3.07±0.91
18:4ω3	2.02±1.25	18:1ω7	3.01±0.31	20:1ω9	2.61±0.66	18:2ω6	2.84±0.74
18:1ω7	1.7±0.5	22:1ω11(13)	2.52±2.74	18:2ω6	2.50±0.72	20:5ω3	2.77±0.80
16:1ω9	1.15±0.67	20:1ω9	1.87±1.39	22:5ω3	2.41±0.48	20:1ω9	2.27±0.77
*ai*15:0	1.09±1.12	22:5ω3	1.82±0.18	20:4ω6	2.21±0.80	20:3ω3	1.12±5.12
16:2ω4	1.07±0.45	16:4ω1	1.41±0.88	22:1ω11(13)	1.52±0.52	17:0	0.80±0.12
16:4ω1	0.95±0.67	18:4ω3	1.30±0.38	18:1ω6	1.36±1.92	24:1	0.77±0.30
18:2ω6	0.89±0.98	20:4ω6	1.14±0.08	24:1	1.12±1.11	15:0	0.70±0.11
16:1ω5	0.83±0.32	18:3ω3	1.12±0.57	18:4ω3	0.83±0.44	16:2ω4	0.56±0.17
*i*15:0	0.69±0.53	16:2ω4	1.08±0.15	16:2ω4	0.82±0.22	20:0	0.52±0.16
22:6ω3	0.63±0.93	16:3ω4	0.94±0.52	16:3ω4	0.68±0.36	16:1ω9	0.48±0.45
*ai*16:0	0.53±0.68	18:1ω11	0.62±1.51	20:4ω3	0.51±0.15	18:3ω3	0.47±0.21
*ai*17:0	0.51±0.66	21:5ω3	0.61±0.11	16:4ω1	0.49±0.30	22:1ω9	0.46±0.31

Table A-2.7: Lipid class profile exiting tanks daily per kilogram of biomass

Lipid Class	*Amount exiting* ($mg*day^{-1}kg^{-1}$)
HC	3.41±1.99
SE	0.71±0.57
EE	0.32±0.42
ME	0.23±0.21
EKET	0.87±0.80
MKET	1.36±0.83
GE	0.04±0.05
TAG	19.7±7.7
FFA	51.0±18.0
ALC	0.87±0.53
ST	11.2±3.8
DAG	2.95±1.95
AMPL	13.5±6.7
PL	22.1±8.4

n=6 for each lipid class
* : % wet weight (g/g)
HC: hydrocarbon
SE: steryl ester
EE: ethyl ester
ME: methyl ester
EKET: ethyl ketone
MKET: methyl ketone
GE: glyceryl ether
TAG: triacylglycerol
FFA: free fatty acid
ALC: fatty alcohol
ST: sterol
DAG: diacylglycerol
AMPL: acetone mobile polar lipid
PL: phospholipid

Table A-2.8: Fatty acid profile exiting tanks daily per kilogram of biomass

Fatty Acid	*Amount exiting* ($mg*day^{-1}kg^{-1}$)
14:0	8.4±4.0
16:0	44.8±26.2
16:1ω7	5.6±2.7
18:0	15.3±10.6
18:1ω9	17.0±15.9
18:1ω7	4.1±2.5
18:2ω6	4.5±4.2
20:1ω9	3.2±1.7
20:5ω3	3.0±1.0
22:1ω11	4.7±2.3
22:6ω3	2.8±0.9

n=6 for each fatty acid

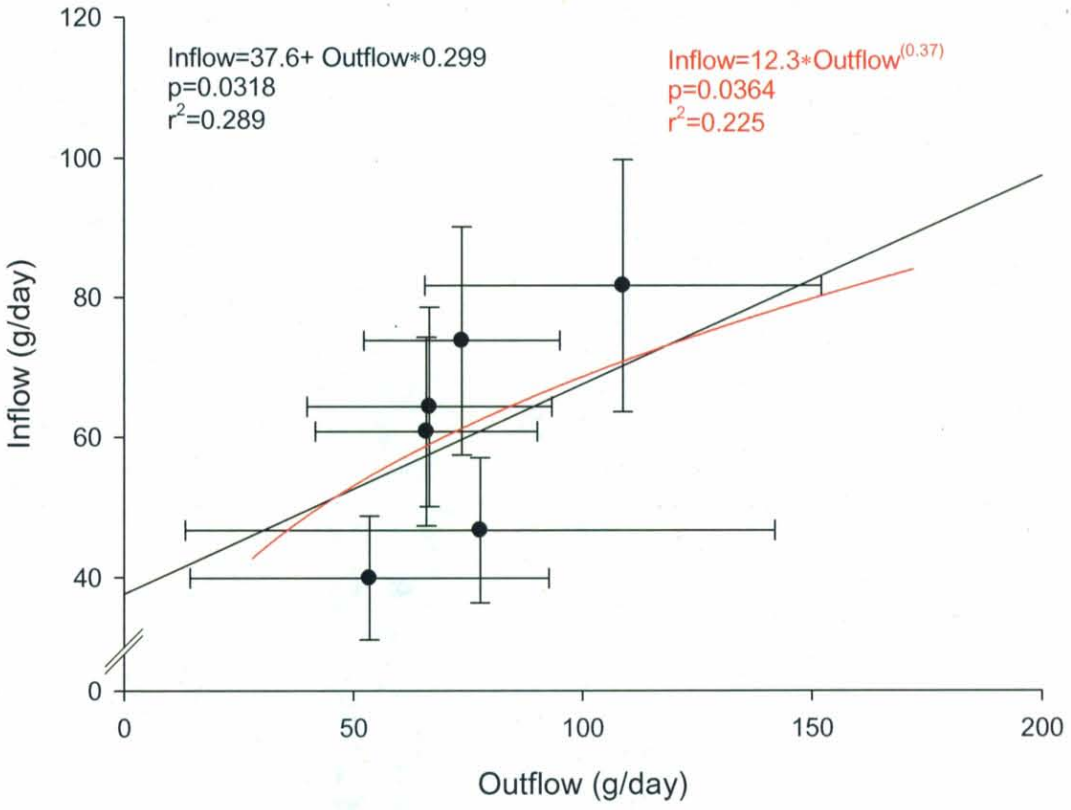

Fig A-2.1: Inflow and outflow rates compared for ash weight
Regression lines calculated for raw data.

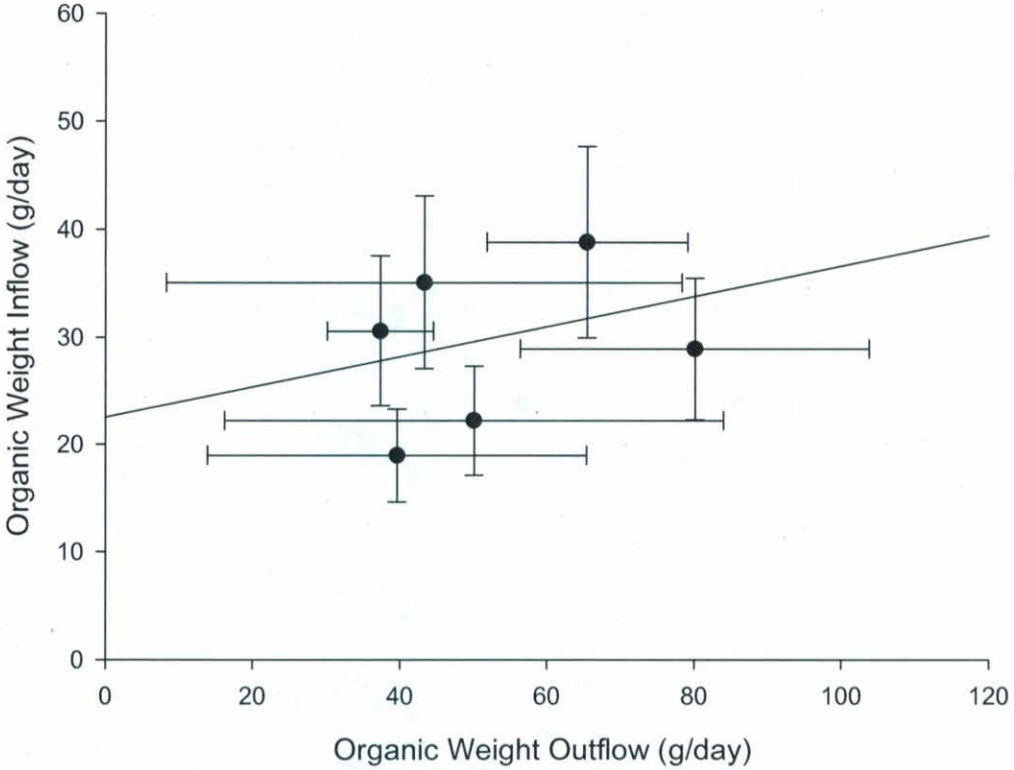

Fig A-2.2: Inflow and outflow rates compared for organic weight
Regression line calculated for raw data (p=0.117)

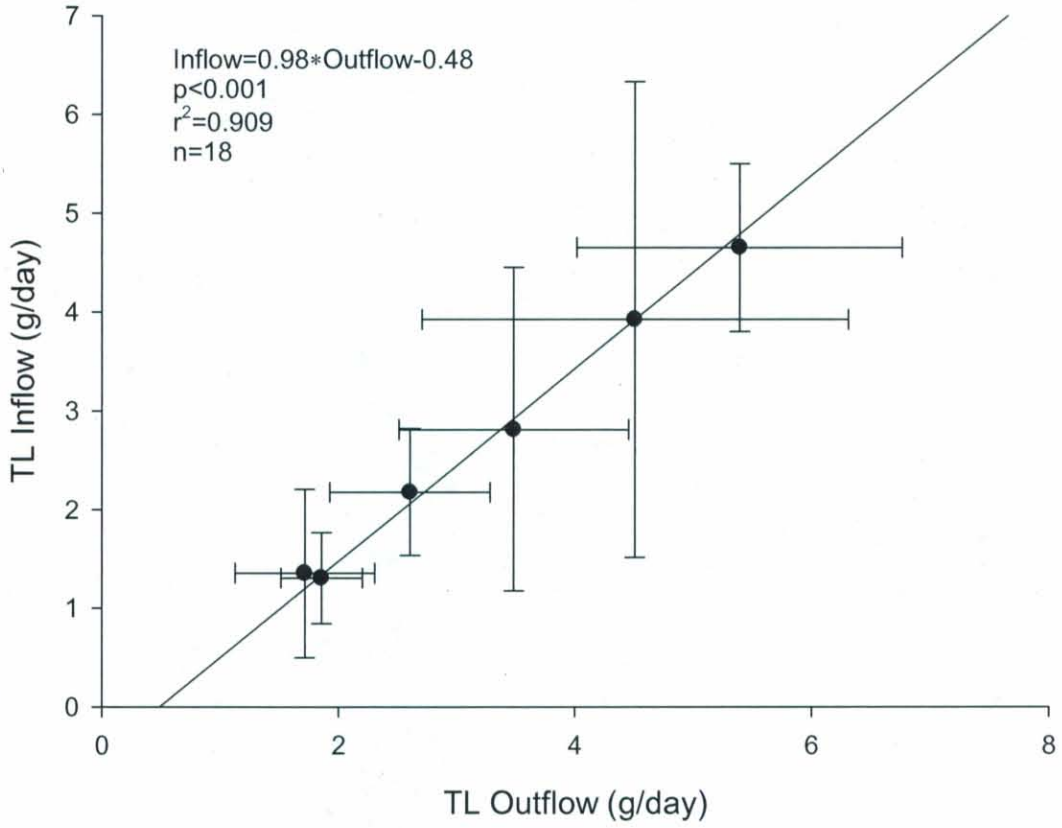

Fig A-2.3: Inflow and outflow rates compared for total lipid
Regression line calculated for raw data

Chapter 3

Invertebrate uptake of organic constituents in the vicinity of Atlantic salmon (*Salmo salar*) aquaculture sites in British Columbia

3.1 Abstract

Aquaculture is a fast growing industry that provides seafood for the growing population. Finfish aquaculture enriches the local environment through output of food particles and faecal matter to the benthos, which can be taken up by surrounding invertebrates. Samples of invertebrates, primary producers, seawater, and sediment cores were taken surrounding multiple aquaculture sites in coastal British Columbia. Dissolved organic carbon (DOC) showed a significant decrease with distance ($p=0.027$) and an overall average of 300 μM at 1 m depth. Mussel wet weight as well as the zooplankton fatty acid marker (ZFA) and 20:1ω9 individually, decreased significantly with distance from the farms ($p<0.03$). Analysis of the lipids showed an increase in 22:6ω3 (docosahexaenoic acid, DHA) with distance in molluscs as well as mussels alone ($p<0.01$). Principal components analysis analysis showed a similar trend with DHA being higher in molluscs further away from the farm. However, of the DHA present, mussels had significantly higher amounts compared to other molluscs relating to their use in multi-trophic, co-culturing systems. Bacterial fatty acid markers increased in molluscs with distance from the farm possibly due to antibiotic suppression from the farms; however, 18:1ω7, which is representative of methane oxidizing bacteria, remained higher closer to the farm. In addition, DHA proportions in mussels showed a significant breakpoint at 339 m from the farm. The increase in DHA with distance suggests co-

cultured mussels would benefit from placement further than 339 m from the farm where DHA would be optimized.

3.2 Introduction

Aquaculture is a fast growing means of seafood production. It includes the farming of shellfish, shrimp, algae, oysters, and finfish for food and economic gain (the Department Fisheries and Oceans; DFO, 2008). The aquaculture industry produced approximately 155 thousand tonnes of product valued at approximately $715 million in 2005 (DFO, 2008) and a large portion of aquaculture revenue comes from the farming of Atlantic salmon (*Salmo salar*). In 2003, 105,050 tonnes of Atlantic salmon were produced in Canada valued at $434 million. Of that, $213 million were produced on British Columbia's 131 Atlantic salmon sites (DFO, 2008). This large industry has social, political, and environmental interactions. The farming of salmon occurs in two phases. Initially, spawning takes place in freshwater, land-based tanks where the fish are grown to two years of age. Following this, a second phase involves transporting the fish to ocean netpens for further growth and maintenance until harvesting for consumption (DFO, 2008).

Industrially prepared feed pellets are used to grow and maintain the farmed salmon. They are composed of up to 50% protein, 20% carbohydrates and 15% lipids along with additives such as vitamins, colour, and therapeutic agents. Fish feed is made from a variety of sources, including smaller fish that consume plankton (Iwana, 1991). The fish consume the majority of the feed pellets distributed, yet 1-38 % of feed is not eaten and settles beneath the farm or is carried out in the farms effluent (Wu, 1995; Reid et al., 2008). However, due to feeding practice improvements the latter is likely an

overestimate. The other component of the bio-deposits beneath the farms and in the effluent is a result of the fish faecal matter. These two waste products from the fish add to the sediment accumulated beneath the farm and increase the downward flux of carbon.

The increase in such carbon-rich sediment can lead to anoxic conditions and create a reducing environment producing ammonia, hydrogen sulphide and methane (Hall et al., 1990). These conditions can cause eutrophication where an increase in nutrients accelerates aquatic plant growth, which subsequently deplete available oxygen when the plants begin to decompose. With a shifting environment below the farm, there are changes in organisms that inhabit this area, as is the case for areas of eutrophication. Excess particles have been traced in surrounding ecosystems and organisms through analysis of sediment, mussels, and fucus using organic biomarkers such as lipids and stable nitrogen and carbon isotopes (Ye et al., 1991; Van Biesen and Parrish, 2005; Yokoyama et al., 2006).

Along with these studies, salmon farming has been found to have a large impact on the local benthic community. A study in 1995 by Findlay and Watling in Maine, USA found changes in carbon flux from the farm as well as changes in sediment biogeochemistry. They found increased carbon flux at the edge of the farm from 2-fold to 6-fold, but not at a reference site 10 m from the farm. In addition, the complexity of organic matter sedimentation increased compared to the reference site with some dependence on seasonality (minimum in July and maximum in November for cage sites). There was also a shift to microbial and macrofauna communities representative of carbon enrichment (Findlay and Watling, 1995).

Another study in the Gaeta Gulf, Italy (Mazzola et al., 2000) showed the impacts to the benthic community by collecting monthly samples of meiofauna. Again, there was an accumulation of organic material. Along with this, they found a depletion of redox potential values. This reduction caused a decrease to the depth of meiofaunal penetration into the sediment (Mazzola et al., 2000). The increased organic matter present under the farm also showed an impact on the amount of meiofaunal densities that were 50% lower under the farm than at the reference site. The sediment samples collected at the farm site location showed a large concentration of copepods, nematodes, and polychaetes, which was not typically characteristic of the study area (Mazzola et al., 2000).

The particles in the effluent and bio-deposits beneath farms show environmental impact from the increase in carbon flux and organic matter sedimentation forming anoxic, reducing conditions beneath the farms. Some studies examining the recovery rates for benthic communities following a farms' decommissioning showed slow recovery rates and long-term effects (Jarp and Karlsen, 1997; Mazzola et al., 2000; Pohle et al., 2001).

A study by Henderson et al. (1997) showed the lipid and fatty acid profiles of feed and sealoch sediment surrounding salmon cages in Scotland. The surface sediment directly under the cages showed increased lipid content decreasing with distance. The major lipid class was triacylglycerols (TAG), which displayed a similar decrease. Other lipid classes in the sediment including free fatty acids, sterols, polar lipids, as well as the hydrocarbons and sterol/wax esters group showed the same decrease. Specific fatty acids also decreased with distance from the farm namely the branched chain and odd chain-length fatty acids along with the copepod fatty acid 22:1ω11.

The output analysis in Chapter 2 showed an abundance of dry weight exiting the tanks along with significant increases in total lipid including FFA and the essential fatty acid, DHA. Analyzing the uptake of constituents by organisms surrounding the farm with lipid and fatty acids biomarkers follows the description of the land based tank output in terms of food sources available for adjacent species. For this work, specific lipid biomarkers were used to determine the interactions between the farm site and the surrounding organisms as well as the organic footprint around the farm. A list of fatty acids as well as markers that were used in this study and others is shown in Table 3.1. Investigating waste recycled as food relates to integrated multi-trophic aquaculture (IMTA) as mussels are employed to take up the particles surrounding finfish sites to promote augmented growth while reducing environmental impact potential.

The data used for this research project includes lipid analyses of samples taken surrounding three salmon aquaculture sites in BC that were used to determine the aquaculture-environment interaction, uptake, and dispersion of these constituents. The BC field samples were collected at three aquaculture locations including active and fallow farms as well as farms where fish were killed by disease. The specimens were taken at increasing distances from different farm sites to examine lipid content and composition as a function of increasing distance and to gain perspective on the farms area of influence.

Table 3.1: Lipid and fatty acid biomarkers used to examine effects of Altantic salmon aquaculture effluent

Significance	Lipid	Unit	Reference
Storage condition indicator	TAG	% TL	Hølmer 1989
Breakdown and faeces indicator	FFA	% TL	Van Biesen and Parrish 2005
Dispersion of organic waste from fish farms	ZFA%FFA%WW	%TFA*%WW	This study
Indicator of mussels FA quality	NMID	%TFA	Alkanani et al., 2007
Individual zooplankton/Copepod & fish feed fatty acids	20:1ω9	% TFA	Dalsgaard et al., 2003
	22:1ω9	% TFA	Mayzaud et al., 2007
	22:1ω11	% TFA	Dalsgaard et al., 2003
Zooplankton fatty acid marker (ZFA)	20:1ω9, 22:1ω9, 22:1ω11	%TFA	This study
EPA, diatoms marker	20:5ω3	% TFA	Dalsgaard et al., 2003
DHA, dinoflagellates marker	22:6ω3	% TFA	Graeve et al., 1994
Individual bacterial fatty acids	18:1ω7	% TFA	Morris et al., 1985
	*i*15:0	% TFA	Morris et al., 1985
	*ai*15:0	% TFA	Morris et al., 1985
	*i*17:0	% TFA	Kaneda, 1991
	*ai*17:0	% TFA	Kaneda, 1991
Bacterial fatty acid marker (BFA)	*i*15:0, *ai*15:0, 15:0, 15:1, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, 17:1	%TFA	This study
Indicator of Carnivory	P/S	-	Cripps and Atkinson 2000

TAG- triacylglycerol; TL- total lipid; FFA- free fatty acids; ZFA%FFA%ww – marker for the dispersion of organic waste from the fish farm. Combines lipid and fatty acid farm indicators ZFA and FFA; estimate of ZFA present in free form. NMID- non-methylene- interrupted diene; TFA-total fatty acid; *i*- iso; *ai*- ante-iso; P/S -Ratio of polyunsaturated fatty acids (PUFA): saturated fatty acids (SFA)

3.3 Methods

3.3.1 Sampling and Analysis Methods for BC Aquaculture Sites

Samples were taken from benthic and intertidal zones and surface seawater on the periphery of three Atlantic salmon aquaculture sites in British Columbia to determine lipids in the surrounding marine invertebrates, macroalgae, sediment, and seawater. Samples included molluscs such as mussels, chitions, clams, limpets, isopod, periwinkle, sea stars, and whelks along with the plant species eelgrass and fucus (Table 3.2). Sampling took place around fish farms in the Broughton Archipelago off the north-eastern part of Vancouver Island in April/May of 2003 and again in September of 2004, and in Clayoquot Sound, on the west side of Vancouver Island, in June 2004 (Fig. 3.1 b and c, black squares; Table 3.3). The first field trip to the Broughton Archipelago in April/May 2003 included sampling from four aquaculture sites. The first site was an active farm operating for 17 years and that remains fallow every two years for two months. It was comprised of 27 pens with fish averaging 0.2 kg. The second farm was comprised of six net pens (25 m x 25 m) and was stocked with fish approximately 2.5 kg in size. Sampling took place at one station with good tidal flushing 200 m from the farm. The third location was no longer in operation being fallow for about a year prior to sampling; however, was an active site for approximately 6 or 7 years prior. Sampling took place 500 m from the aquaculture site. This location did not have good tidal flushing. The fourth site was comprised of two farms in operation for approximately eight years. Three weeks prior to sampling the fish has been killed due to disease. Sampling took place at three stations 75 m, 300 m, and 400 m from the farm sites.

The trip to Clayoquot Sound in June 2004 included sampling by hand along the seashore at seven stations at 306 m, 371 m, 518 m, 727 m, 967 m, 1686 m, and 2358 m following a transect from the farm. The final trip to the Broughton Archipelago in September 2004 included two sampling sites. The first was in operation for a year and contained 20 net pens with mature fish. Samples were taken along a transect from seven stations at 92 m, 168 m, 260 m, 775 m, 1242 m, 1483 m, and 1950 m. This site also included sampling for DOC and TN. The second site was no longer in operation and had only ever contained smolts. Samples around this site were taken from one station at 1738 m from the farm site.

Table 3.2: Species collected surrounding the periphery of British Columbia aquaculture locations

Samples	Species
mussel	*Mytilus edulis*
chiton	*Katharina tunicata*
crab	*Pagurus granosimanus*
limpet	*Acmaea testudinalis*
clams	*Prothothaca staminea*
isopod	*Acanthaspidia* sp.
periwinkles	*Littorina scutulata*
whelks	*Aeneator* sp.
sea star	*Pisaster ochraceus*
eelgrass	*Zostera marina*
fucus	*Fucus gardneri* and *Fucus spiralis*

Table 3.3: Location and sampling details for field samples from British Columbia's aquaculture sites

Farm/Location	Station number	Distance (m)	Status	Specimens collected/ Water Analysis
Broughton Archipelago April/May 2003				
1st site (Blunden Pass)	1	25	Active	Net tows
	2	150		
2nd site (Betty's Cove)	1	200	Active	Invertebrates, net tows
3rd site (Upper Retreat Pass)	1	500	Fallow	Invertebrates, macroalgae, net tows
4th site (Sir Edmund Bay)	1	75	Active*	Invertebrates and macroalgae
	2	300		
	3	400		
Clayoquot Sound June 2004				
1st site	1	306	Active	Invertebrates
	2	371		
	3	518		
	4	727		
	5	967		
	6	1686		
	7	2358		
Broughton Archipelago Sept. 2004				
1st site (Burdwood Island)	1	92	Active	Invertebrates, macroalgae, net tows,
	2	168		DOC and TN
	3	260		
	4	775		
	5	1242		
	6	1483		
	7	1950		
2nd site ('Eelgrass Cove')	1	1738	Not active	Invertebrates and macroalgae

* Fish kill three weeks prior to sampling

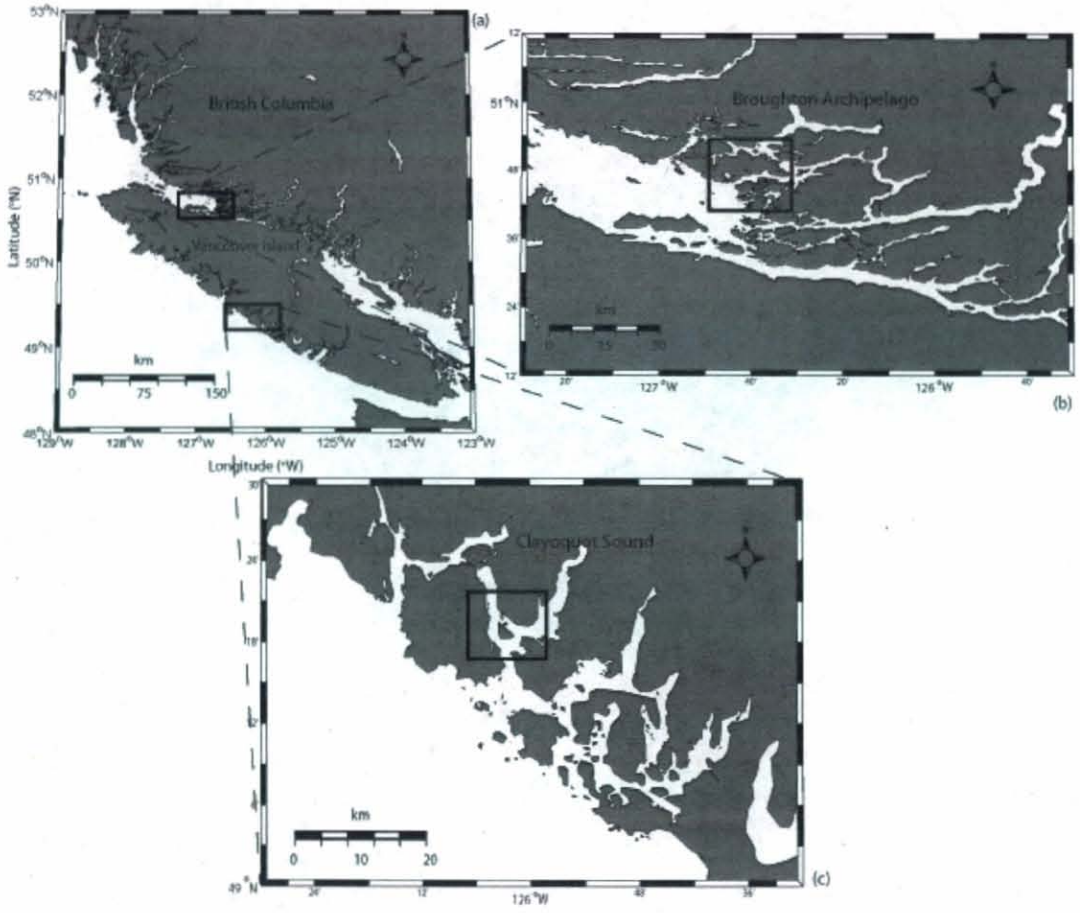

Figure 3.1: Map of (a) Vancouver Island sampling areas (b) Broughton Archipelago and (c) Clayoquot Sound indicated by black squares

3.3.2 Sample Handling

Samples were stored in large plastic bags at -20°C then shipped to Newfoundland in dry ice. Upon arrival they were stored at -80°C for a week. After this the invertebrates were removed from their shells, weighed and placed in test tubes with chloroform. They were stored at -20°C under nitrogen and their caps were sealed with Teflon tape.

3.3.3 Analysis

The samples extraction followed a method by Parrish (1999). They were homogenised manually with a metal rod or with a homogenizer. The bottom organic layer was removed using the double pipetting technique, more chloroform was added to the sample and the procedure repeated three times to ensure complete extraction of the lipids. The organic layers were all pooled in a lipid-clean vial.

Lipid class composition was determined using a three step development system method (Parrish, 1987) with silica coated Chromarods and an Iatroscan Mark V TLC-FID. The three chromatograms were joined together using The T data scan 3.10 chromatography analysis program (RSS Inc. Bennis. Tenn., USA). Calibration was done using standards from Sigma Chemicals (Sigma Chemicals, St. Louis, Mo., USA).

The fatty acid methyl esters (FAME) were obtained using 14% BF_3/MeOH for 1.5 hours at 85°C agitated at 45 minutes. They were analyzed on a Varian 3400 GC-FID equipped with an autosampler. The chromatograms were integrated using the Varian Star Chromatography Software (Version 5.50) and identified from retention times by standards from Supelco 37 component FAME mix (Product number 47885-U), Bacterial acid fatty ester mix (product number 47080-U), PUFA 1 (product number 47033) and PUFA 3 (product number 47085-U).

The GC column length was 30 m with a film thickness of 0.25 μm and it had a 1 m guard column on the front end. The column temperature began at 65°C and it was held at this temperature for 0.5 minutes. The temperature ramped to 195 °C at a rate of 40 °C/min, where it held for 15 minutes then ramped to a final temperature of 220 °C at a rate of 2 °C/min. this final temperature was held for 0.75 minutes. The carrier gas was hydrogen which flowed at a rate of 2 ml/minute. The injector temperature started at 150 °C and ramped to a final temperature of 250 °C at a rate of 200 °C/minute. The detector temperature stayed constant at 260 °C. The conversion of the acyl lipids into their FAME had an average efficiency of derivatization of 92.1% determined by Iatroscan.

3.3.4 Statistics

To analyze the data, regression as well as principal components analysis (PCA) along with SAHN (sequential, agglomerative, hierarchical, non-overlapping) cluster analysis was performed using statistical software (Minitab 15 and Sigmastat). Statistica was also used for breakpoint analysis.

3.4 Results and Discussion

3.4.1 Coastal British Columbia food web

3.4.1.1 Lipid and fatty acid data

As a first step to examine overall trophic interactions the samples from British Columbia, the average amount of the major lipid groups for each sample type (as a percent of total lipid) was determined (Table 3.4). These included the TAG (triacylglycerol), PL (phospholipid), Bacterial fatty acid marker (BFA, Table 3.1), SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), P/S (ratio PUFA/SFA), ω3 (Σomega-3 fatty acids), and zooplankton fatty

acid marker (ZFA, Table 3.1). TAG was most abundant in the hermit crabs and least abundant in the eelgrass. The clams had the most PL while the net tows had the least. The BFA was highest in cores and lowest in fucus, while the SFA was highest in the net tows and lowest in the chitons. The core samples had the highest levels of MUFA and lowest levels of PUFA. MUFA was lowest in eelgrass. The clam showed the highest PUFA; however, there was only one sample available. Other sampled species with high amounts of PUFA were the chitons and mussels. Cores also had the least amount of P/S and ω3 while chitons had the highest P/S ratio. As expected from the high amount of PUFA, the clam contained the most ω3, and mussels contained the second highest percentage. ZFA was highest in the whelks and lowest in the net tows.

Clams had the most PL, PUFA, and ω3 suggesting high amounts of membrane material and optimal retention of the essential fatty acids. Mussels, another filter feeder, also had high amounts of PUFA and ω3 indicating the selection of essential PUFA. The cores contained high BFA, SFA and MUFA along with low PUFA and ω3, which is consistent with decomposition of settled particles on the bottom (Dalsgaard et al., 2003). The plant species, fucus and eelgrass, along with their periphyton, had high amounts PUFA as well as ω3. Fucus had low amounts of BFA, however the eelgrass samples contained higher amounts of BFA ($p<0.001$; $df=34$). The plant species fucus and eelgrass had the lowest amounts of BFA as well as TAG and MUFA, respectively. The levels of SFA and PUFA found here are similar to those reported by Napolitano et al. (1990) for three cultured algae species where total SFA ranged from 22.4-27.9% and total PUFA ranged from 39.4-53.6% total fatty acid.

Latyshev et al. (2009) reported proportions of fatty acids in edible crabs of the northwestern Pacific consistent with those found in the hermit crabs sampled here. SFA and MUFA ranged from 14.4-20.3% and 24.6-49.9% TFA, respectively. In addition, PUFA and ω3 represented between 27.4-56.0% and 21.2-51.5% TFA, respectively. Although there was no bacterial fatty acid summation, the bacterial fatty acids reported were a small contributor to the total proportion of fatty acids similar to the crabs sampled in BC.

In a study by Freites et al. (2002), mussels (*Mytilus galloprovincialis*) were investigated from subtidal and rocky shore locations. They showed SFA, MUFA and PUFA levels similar to those found here. Larval scallops investigated by Delaunay et al. (1992) reported TAG levels similar to the BC mussels. TAG relates to storage and levels of TAG vary annually with ranges from 18-45% over the course of a year (Li et al., 2007).

Table 3.4: Average of major lipid groups for samples collected in coastal British Columbia

	n	TAG	PL	BFA	SFA	MUFA	PUFA	P/S	ω3	ZFA
Mussels	93	25.3±13.4	39.4±11.9	7.9±2.6	23.3±3.3	21.2±3.3	52.3±4.8	2.3±0.5	38.0±4.7	2.7±0.7
Limpets	46	11.3±13.9	44.9±11.8	5.3±1.9	20.7±4.8	26.4±3.4	50.4±7.1	2.6±0.7	25.6±6.6	2.1±1.7
Fucus	31	27.6±11.2	39.9±11.8	**2.4±0.5**	27.5±1.0	22.3±4.1	48.7±4.1	1.8±0.2	21.5±4.0	0.4±0.3
Whelks	20	28.7±22.6	37.8±19.3	4.3±2.0	23.0±3.3	27.8±5.8	47.9±6.1	2.2±0.6	34.7±3.4	**2.9±3.8**
Cores	12	14.6±0.2	35.9±0.2	**11.4±6.9**	32.3±6.1	**36.7±2.5**	**23.9±7.1**	**0.8±0.3**	**13.1±4.9**	0.5±0.4
Periwinkles	11	31.0±11.3	35.4±10.0	4.6±1.2	23.7±1.5	27.5±2.7	47.6±3.4	2.0±0.3	33.2±3.1	2.6±0.7
Net tows	6	21.3±11.2	**11.8±5.6**	3.1±0.5	**35.1±6.4**	18.0±1.1	45.2±7.8	1.4±0.5	33.2±7.2	**0.34±0.07**
Eelgrass	5	**1.5±2.0**	50.1±18.3	9.0±0.4	30.4±1.4	**14.0±1.6**	49.6±2.5	1.6±0.1	37.7±2.6	1.3±0.8
Hermit crabs	3	**42.6±16.5**	22.0±8.9	4.0±1.9	28.2±12.0	21.3±3.2	49.2±9.7	2.0±1.0	35.4±12.3	2.0±1.9
Chitons	2	18.5±0.7	45.0±12.9	6.46±0.01	**16.7±0.2**	23.2±0.6	54.7±0.3	**3.28±0.02**	24.2±1.8	2.1±0.2
Isopods	2	21.5±6.1	16.4±8.7	2.8±0.2	19.0±1.0	27.9±0.1	51.5±1.1	2.7±0.2	27.9±2.1	1.4±0.1
Clam	1	8.7	**58.2**	2.9	23.1	19.0	**55.1**	2.4	**40.5**	1.6
Sea star	1	33.2	41.8	5.1	17.0	29.1	51.3	3.0	33.1	6.5

Abbreviations in column headings similar to those in Table 3.1. Data are mean±s.d except where n=2 when they are mean±half range. Boldface indicates highest and lowest values within a column. TAG (triacylglycerols), PL (phospholipids) measured as % total lipid; BFA (bacterial fatty acids), SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), ω3 (omega-3 fatty acids), and ZFA (zooplankton fatty acids) measured as % total identified fatty acids.

3.4.1.2 Food web

Subjecting the data gathered from coastal BC to principal components analysis (PCA) allows for the reduction of multiple variables down to fewer correlated components. Fig. 3.2 shows the PCA of major lipids in the complete range of sample types along with a SAHN cluster analysis of the scores and the loadings. The first two principal components accounted for 62.1% of the variance with an increase to 81.2% with the addition of PC3. From the output, food web connections can be inferred based on lipid comparisons of all the samples taken. Organisms such as plankton produce fatty acids that are taxon-specific. They are largely unmodified in their consumers giving a history of diet prior to the time of sampling. In this way, they act as biomarkers for an organisms' diet, condition, and activity (Dalsgaard et al. 2003). Other lipids can act as a biomarker in the environment showing waste material from fish farms (Van Biesen and Parrish, 2005). From the cluster analysis of the scores, Fig. 3.3, there are three groups that include ω3 (total omega-3) and PUFA (polyunsaturated fatty acids); MUFA (monounsaturated fatty acids) and BFA; and finally ZFA, P/S (a ratio of polyunsaturated fatty acids to saturated fatty acids used as a carnivory index; Cripps et al., 2000), TAG (triacylglycerol) and PL (phospholipid). The groupings display relationships between the lipids. For example, in the case of ω3 and PUFA, ω3 is a subset of PUFA.

Zooplankton lipids are often an indicator of diet (Arts, 1999). The grouping of ZFA with P/S indicates that zooplankton lipids are linked to a high P/S ratio indicative of carnivory. TAG is a storage class (Hølmer, 1989) and PL is a major component in membrane material. Storage and membrane material may be representative of growth. In addition, BFA and SFA fall on the negative side of PC1, while PUFA and ω3 are on the

opposite side of the origin. This dichotomy reflects nutritional value in addition to saturation as ω3 has clear anti-inflammatory health benefits (Simopoulos, 2002).

The cluster analysis of species loadings showed two groups. One included the mussels, whelks, periwinkles, isopod, and limpets. The second grouping contained the marine plants fucus and eelgrass. The first group implied that the lipid composition of these invertebrates is similar. This provides insight into their feeding habits relating the similar food consumption and predation among group members. The mussels are filter feeders, and whelks are known to feed on bivalves such as mussels (Lambert and Dehnel, 1974). Most of the mussel samples collected were found on a rocky substrate. The periwinkle, which feed on plant particles and microalgae (Voltolina and Sacchi, 1990), is included in this group. Periwinkles fit logically in this group given that mussels feed on organic particles in the water column including algae. Limpets feed on periphyton and some on the plants themselves (Willcox, 1905), while isopods are omnivorous scavengers feeding on decaying substrate or plants and diatoms (Carefoot, 1973). The lipid composition of the group is consistent with their similar feeding patterns as well as predation on each other. Interestingly, there is no clustering among these algal feeders and the net tows, which would presumably include the algae from the water column. This may suggest another possible food source. However, net tows were positive on PC3 (Fig. 3.2) relating them to the mussels, whelks, periwinkles, and isopods as they carry the same positive sign on the third component. The additional cluster in the loadings groups the seagrass with the alga species. This group reflects the similarities in these primary producers' lipid composition.

The overall picture shows the invertebrate group in the loadings on the right hand side of the x-axis and therefore correlates with the ω3-PUFA and ZFA-P/S-PL and TAG groups (Fig. 3.2). Specifically the mussels align closest to the ω3-PUFA group and the periwinkle and whelks aligned closest to the ZFA-P/S-PL and TAG group. PC3 continued the relationship between PUFA and mussels as they are both negative in the third component. This suggests an abundance of PUFA in the mussels, reflected in Table 3.4.

Other relationships observed include the clams in the bottom right hand quadrant indicating their abundance of ω3 and PUFA. The fucus and eelgrass show a relationship with the saturated fatty acids (SFA) by falling in the lower left quadrant, confirmed by Table 3.4, as both have higher SFA.

Along with this, the influence of the cores bacterial fatty acids was investigated using a PCA without the core samples (Appendix Fig A3.1). Major changes included the MUFA dissociating from the BFA, which was abundant in the cores, and migrating to the lower, right-hand side. However, on PC3, the BFA and MUFA both remained positive in PC3. The BFA grouped with PL possibly indicative of a growth in bacterial fatty acids. ZFA and P/S remained grouped; however, TAG and PL are no longer included. The loadings showed smaller groupings. Mussels and clams, which were both high in PUFA, are grouped along with whelks and periwinkles, as well as fucus and hermit crabs. The whelks and periwinkles are predators feeding on available food sources (Meinkoth, 2002). The grouping of the scavenger hermit crab and the fucus suggests the crabs were consuming brown algae or associated plant species.

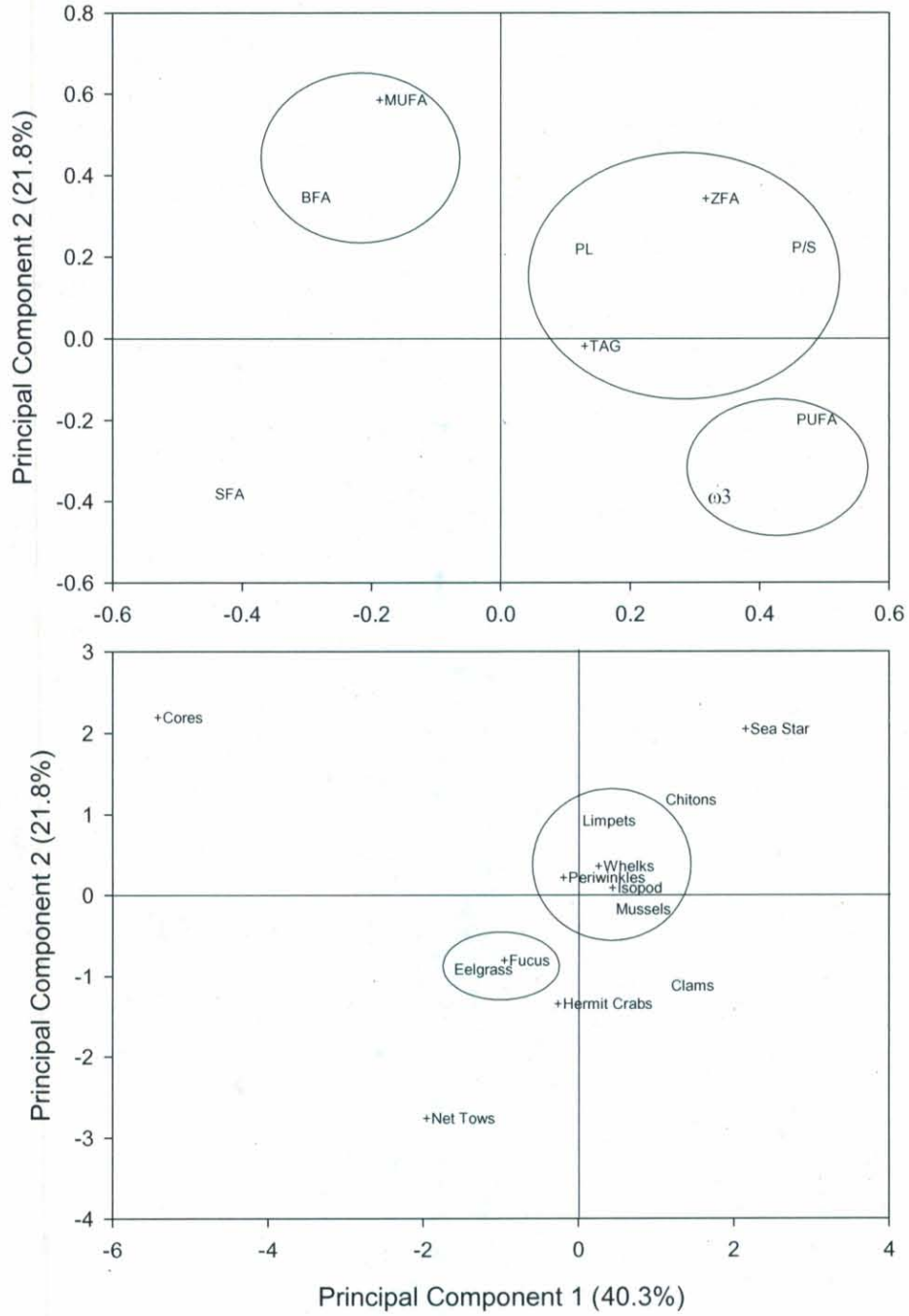

Figure 3.2: PCA of major lipids and species
Cluster analysis grouped the coefficients and scores
+ Indicates the sign of the loading on PC3, those remaining were negative

3.4.2 Effects of Aquaculture

3.4.2.1 Dissolved organic carbon and total nitrogen

Following the food web description the effects associated with the farms presence were examined. The average amount of DOC next to the farms was approximately 300 μM at 1 m depth and 85 μM at 10 m depth. These values are higher than those reported for ocean water: 43 to 114 μM (Fry et al., 1996). Millero et al. (1996) reported a range of 60-210 μM for coastal ocean water, higher than the values for Fry et al. (1996), however still below the concentrations found at the fish farm. The increased organic input from excess feed and faecal matter likely explains the elevated values. From the previous chapter the level of DOC exiting the tanks daily was 122 μM, which is lower than DOC detected in the samples taken in BC adjacent to the farms. In the previous chapter, a controlled environment was examined where the effluent from the fish tanks was measured alone. Here the measurements were taken in the field and the local environment plays a role. Also, many of the farms studied here have been active for some time and operate on a larger scale than those sampled in Chapter 2.

In the samples surrounding the farms in British Columbia there was a significant decrease ($p=0.027$, $n=14$, slope=-0.066) at 1 m depth in DOC with distance from the farm. When deeper water was sampled the decrease was not significant ($p=0.505$, $n=13$, slope=-0.00421) (Fig. 3.3). The inputs from the feed and faecal matter contribute organic material immediately near the surface of the farm therefore increasing the DOC levels. At greater depths, this input was not significant possibly due to mixing currents around the farm.

There was larger error in the 10 m depth samples possibility representing more variability in deeper samples.

The total dissolved nitrogen (TN) exiting the farm was approximately 30 μM (Fig. A-3.2). This is within the range for coastal ocean, 4-60 μM (Millero, 1996). This amount is also similar to the levels (35 μM) exiting the land-based tanks in the previous chapter. The regressions with distance from the farm were not significant for the 1 m depths ($p=0.543$, $n=14$, slope= -0.0043) or the 10 m depths where the relationship had no slope ($p=0.991$, $n=13$, slope=0) (Fig. A-3.2) indicating nitrogen levels are uniform throughout. The farms output of nitrogen was not significantly different to those levels 1200 m away.

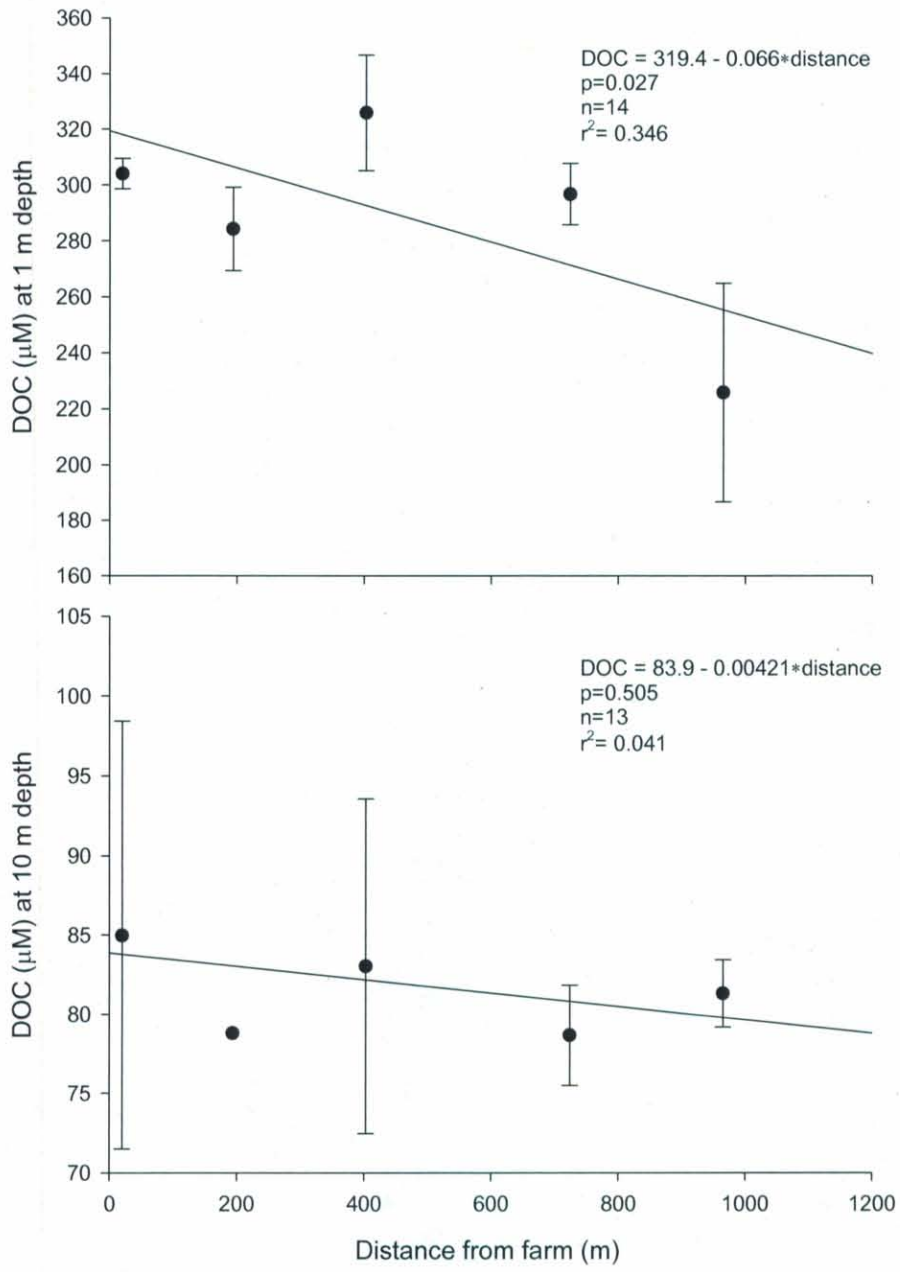

Figure 3.3: Dissolved organic carbon (DOC) for 1 m and 10 m depths
Data are shown as mean ± s.d. Regressions plotted through raw data. Samples collected from Burdwood farm, September 2004.

3.4.2.2 Regression Analysis

Regression analyses were performed investigating relationships between individual lipid classes and fatty acids for all molluscs with distance from the farm. Those lipids significantly correlated with distance, both positively and negatively, are shown in Table 3.5. These include, TL/WW, the lipid classes ethyl ketones (EK), TAG, free fatty acids (FFA) and sterols (ST) and the bacterial fatty acids *i*15:0, 15:1, *ai*16:0, 18:1ω7 as well as the overall bacterial fatty acid marker, BFA. It also includes the essential 22:6ω3 or DHA, the farm marker ZFA%*FFA%WW, which approximates the proportion of zooplankton fatty acids in the free form, and 22:2NIMD, the less nutritious supplementation for ω3 (Alkanani et al., 2007).

Table 3.5: Lipids significantly correlated with distance (m) from farms for all molluscs combined

Lipid	Unit	p-value	r-value	slope
TL	g/WW	0.026	0.172	3.02E-04
EK	%TL	0.021	0.173	3.08E-04
TAG	% TL	0.027	0.165	3.85E-03
FFA	% TL	0.018	-0.177	1.69E-03
ST	% TL	0.026	-0.167	-1.14E-03
15:0*i*	% TFA	0.013	-0.186	-5.30E-05
15:1	% TFA	0.002	0.234	1.39E-04
16:0*ai*	% TFA	0.008	-0.197	-1.20E-04
17:1	% TFA	0.002	0.234	7.56E-04
16:4ω3	% TFA	0.000	-0.260	-9.06E-04
18:1ω11	% TFA	0.002	-0.228	-8.20E-05
18:1ω7	% TFA	0.006	-0.204	-7.74E-04
18:3ω4	% TFA	0.047	-0.148	-6.10E-05
18:5ω3	% TFA	0.024	-0.168	-3.20E-05
20:3ω3	% TFA	0.022	-0.172	-2.83E-04
20:4ω3	% TFA	0.008	-0.198	-1.13E-04
22:2NIMD	% TFA	0.002	0.230	3.76E-04
22:6ω3	% TFA	0.007	0.200	1.70E-03
24:1	% TFA	0.014	0.183	4.40E-05
BFA	% TFA	0.011	0.191	7.50E-04
ZFA%*FFA%WW	%TFA*%WW	0.043	0.157	6.90E-05

TL/WW: Total lipid per wet weight; %TL: percent total lipid; %TFA: percent total fatty acid; EK: Ethyl ketone; TAG: Triacylglycerol; FFA: Free fatty acid; ST: Sterol; *i*: iso; *ai*: ante-iso; NMID: C_{22} non-methylene interrupted dienes; BFA: Bacterial fatty acid

In addition to these relationships, all molluscs along with mussels, limpets, and whelks alone were examined individually to understand their uptake of nutritional lipids and fatty acids with distance from the farm. These biomarkers described a footprint associated with the aquaculture operations. Table 3.6 shows the lipid classes and fatty acids that were examined for significance depicting a nutritional and biomarker footprint of the farms.

Table 3.6: Linear regressions of nutritional and biomarker lipids with distance (m) from farm

Grouping	All Molluscs (n=179)		Mussels (n=93)		Limpets (n=48)		Whelks (n=21)	
Lipids	slope	p-value	slope	p-value	slope	p-value	slope	p-value
TL/WW	3.02E-4	0.026*	2.20E-4	0.043*	5.85E-4	0.001**	-1.57E-4	0.824
TAG	3.85E-3	0.027*	3.80E-4	0.844	1.28E-2	0.000**	-7.57E-3	0.307
FFA	-1.69E-3	0.018**	-8.39E-4	0.300	-4.75E-3	0.016**	6.50E-4	0.810
20:1ω9	-8.00E-5	0.483	-2.53E-4	0.011**	3.53E-4	0.134	-9.27E-4	0.121
20:4ω6	-4.18E-4	0.507	2.70E-4	0.060	4.86E-3	0.001**	-2.02E-4	0.579
20:5ω3	1.45E-4	0.784	-9.40E-5	0.889	-1.31E-3	0.34	1.20E-4	0.913
22:1ω9	2.00E-6	0.866	1.60E-5	0.069	1.20E-5	0.634	-1.02E-4	0.269
22:1ω11(13)	-8.50E-5	0.308	2.00E-6	0.873	9.20E-5	0.609	-4.32E-4	0.497
22:6ω3	1.70E-3	0.007**	1.10E-3	0.006**	-1.14E-4	0.246	-6.49E-4	0.366
[a]Σ Essentials	1.42E-3	0.032*	1.28E-3	0.126	3.43E-3	0.076	-7.34E-4	0.238
[b]Σ ZFA	-1.63E-4	0.333	-2.35E-4	0.019**	4.57E-4	0.131	-1.46E-3	0.243

* Significant $p<0.05$
** Significant $p<0.020$
[a]Σ Essentials 20:4ω6, 20:5ω3, 22:6ω3
[b]Σ ZFA: 20:1ω9, 22:1ω11, 22:1ω9

From these results TL/WW, TAG, 22:6ω3 and Σ Essentials (20:4ω6, 20:5ω3, 22:6ω3) were positively correlated and FFA was negatively correlated with distance from the farm for all molluscs together. TL/WW, 20:1ω9, 22:6ω3 and Σ ZFA are significantly correlated for mussels considered alone, along with TL/WW, TAG, FFA and 20:4ω6 for limpets alone. Regression analysis of the most significant relationships ($p<0.02$) are shown in Fig. 3.4, 3.5 and 3.6. The remaining significant relationships ($p<0.05$) are shown in the Appendix Fig.A-3.3.

Free fatty acid proportions (FFA) were significantly higher closer to the farm for all molluscs along with limpets when considered alone (Fig. 3.4a and 3.6b). The abundance of FFA in the invertebrates adjacent to the farms reflects the increased amount of FFA in the particles being supplied to the surrounding water. In Chapter 2, the most abundant lipid

class exiting the tanks was FFA directly relating to the faecal input from the fish (Johnsen et al. 1993; Van Biesen and Parrish, 2005). The faecal input from the fish is therefore a lipid source for the surrounding invertebrates. This significant relationship remained when the limpets are considered alone; however, this was not the case for the mussels or whelks considered alone, reflecting the limpets increased uptake of FFA.

Along with the linear regression, a non-linear regression was also included. Some of the data showed an exponential-like increase followed by a leveling off indicating a non-linear relationship. Since most mixing in the oceans is turbulent, the non-linearity seems logical. In this way, a particle or compound concentration reaches levels equivalent to the surrounding environment with increasing distance from the point of origin (Beer, 1997). Where there was an improvement in significance, non-linear regressions were included with linear ones (Figs. 3.4-3.7 in red).

Docosahexaenoic acid (DHA; 22:6ω3) was found to be significantly positively correlated with distance for all molluscs as well as mussels alone (Fig. 3.4). The concentration of DHA increased with distance from the farm. This relationship increases the r^2 value from 0.075 to 0.144 for mussels when the nonlinear, power-law equation is fitted to the data.

In addition, the amount of DHA per mussel continued the trend and diminished the possibility that mussels were increasing in size with distance from the farm thereby diluting their DHA as a result of increased size. Fig. 3.7a and b, showed a significant increase of approximately 0.5 μg/g ($p=0.0123$, $n=89$, slope=0.248) for DHA content in mussels and a significant decrease in 1 g/mussel ($p=0.0313$, $n=89$, slope= -0.0007) over equivalent

distances. Fig 3.7c shows the amount of DHA per mussel increased significantly ($p=0.0011$, $n=45$, slope=8.87E2) with distance from farm. It is important to note the differences in the distance range for the concentration of DHA (μg/g) versus the wet weight (g) and subsequently DHA (μg) per mussel. Some smaller mussel samples were extracted together and therefore do not allow all mussel concentrations to be multiplied by their individual wet weight. Nevertheless, from those matched to their weight, a persistent increase in DHA indicates the environment immediately adjacent to the farms provides lower levels than further away. The feed given to the fish is enriched with essential fatty acids including DHA; however, it is not seen in the organisms surrounding the farm suggesting optimal retention by the fish in the sea cages, and a lower amount for the surrounding invertebrates.

Despite the increased DHA with distance, mussel weight decreases significantly with distance from the farm. The heavier mass closer to the farm relates to the increased organic output from the farm (Reid et al. 2010). This again relates to their use in IMTA where they would be cultured next to fed aquaculture sites and employed to take up a portion of the farms effluent; however, also suggests placement immediately adjacent to the farms may not be optimal in terms of essential fatty acid proportions. From the graph (Fig 3.7b) there is a set of mussels that were heavier at 500 m compared to others sampled at the same distance. They had consistent weights greater than 8 g/WW; however, if these three mussels are not considered the decrease in wet weight remains significant ($p=0.0195$, $n=85$, slope=-0.0005).

Although there was an increase in DHA with distance, mussels had a significantly higher sum of essential fatty acids (20:4ω6, 20:5ω3, 22:6ω3) overall compared to other molluscs (t-test, $p<0.001$, df=177) (Table 3.7). DHA proportions alone were also significantly higher than in other molluscs (t-test, $p<0.001$, df=177). The average level of DHA in the mussels was 5 times higher than all other molluscs. Eicosapentaenoic acid (EPA; 20:5ω3) was not significantly different (t-test, $p=0.567$, df=177) between mussels where the average was 16% for mussels and the other molluscs. Arachidonic acid (ARA; 20:4ω6) was significantly higher in the other molluscs than in mussels (t-test, $p<0.001$, n=177). The average ARA in all mussels was 3 times less than all other molluscs. There was a large error associated with ARA in the remaining mollusc grouping (Table 3.7); however, the varying amounts of ARA stored in the different species could account for this. Therefore, mussels are more effective at taking up DHA than other molluscs; however, their assimilation of other essential fatty acid (EPA and ARA) is not as competitive. The effective uptake of DHA by mussels compared to other molluscs relates to their potential use in IMTA as they concentrate available DHA from all sources.

Table 3.7: Essential fatty acids in mussels and other molluscs

	n	Σ Essentials (%)	DHA (%)	EPA (%)	ARA (%)
Mussels	94	31.65±5.83*	12.91±2.84*	15.65±4.66	3.10±1.01
Other molluscs	85	27.81±5.85	2.62±2.75	16.07±5.10	9.12±7.12*

Σ Essentials: 20:4ω6+ 20:5ω3+ 22:6ω3
DHA: 22:6ω3; EPA: 20:5ω3; ARA: 20:4ω6
* Significantly higher ($p<0.001$)

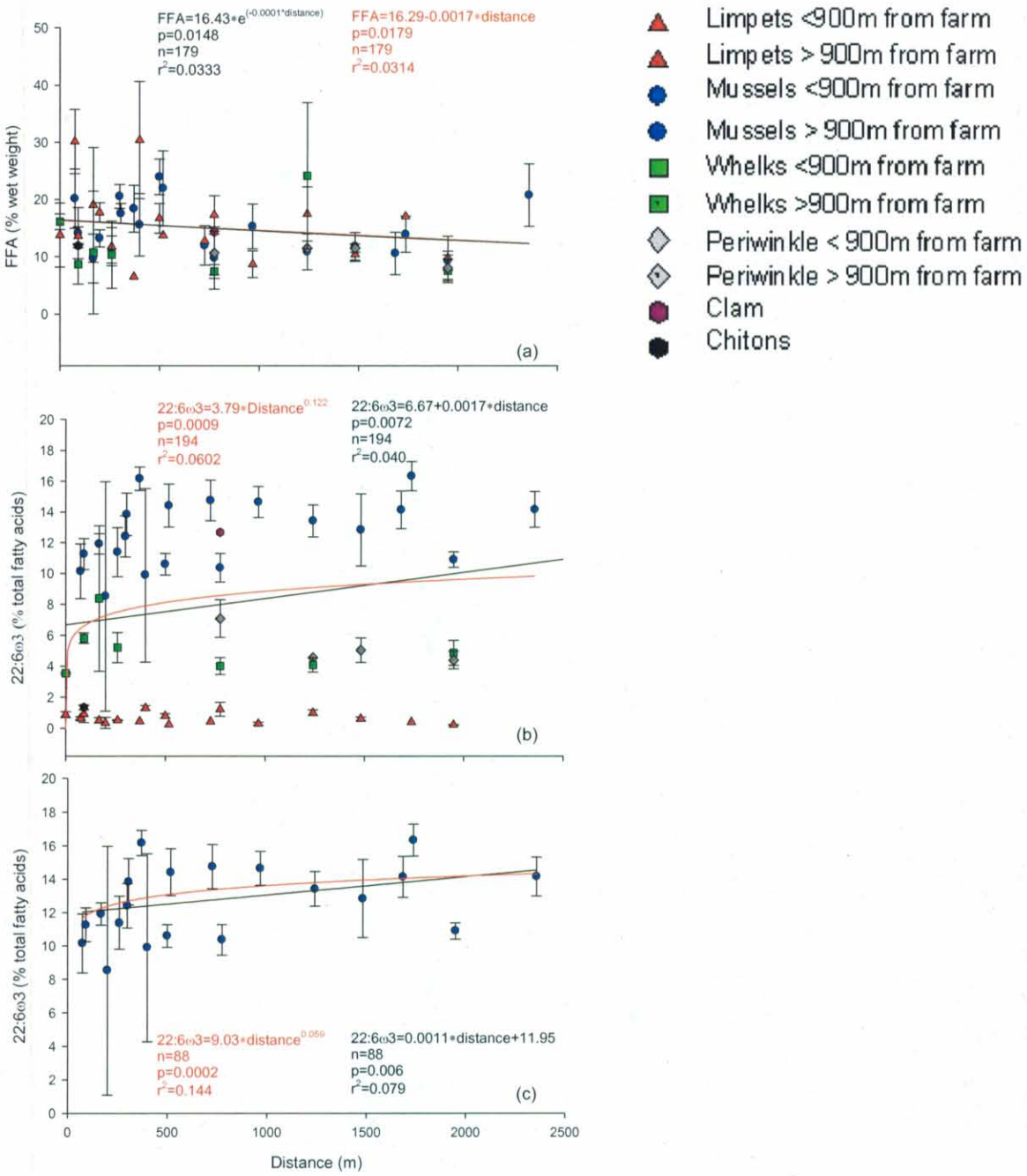

Figure 3.4: Regressions of nutritional and biomarker lipids with distance from farm (a) free fatty acid for all molluscs, (b) DHA for all molluscs, and (c) DHA for mussels alone. Fatty acids are percentage of total identified fatty acids. Data are mean ± s.d. Regression lines are plotted through the raw data.

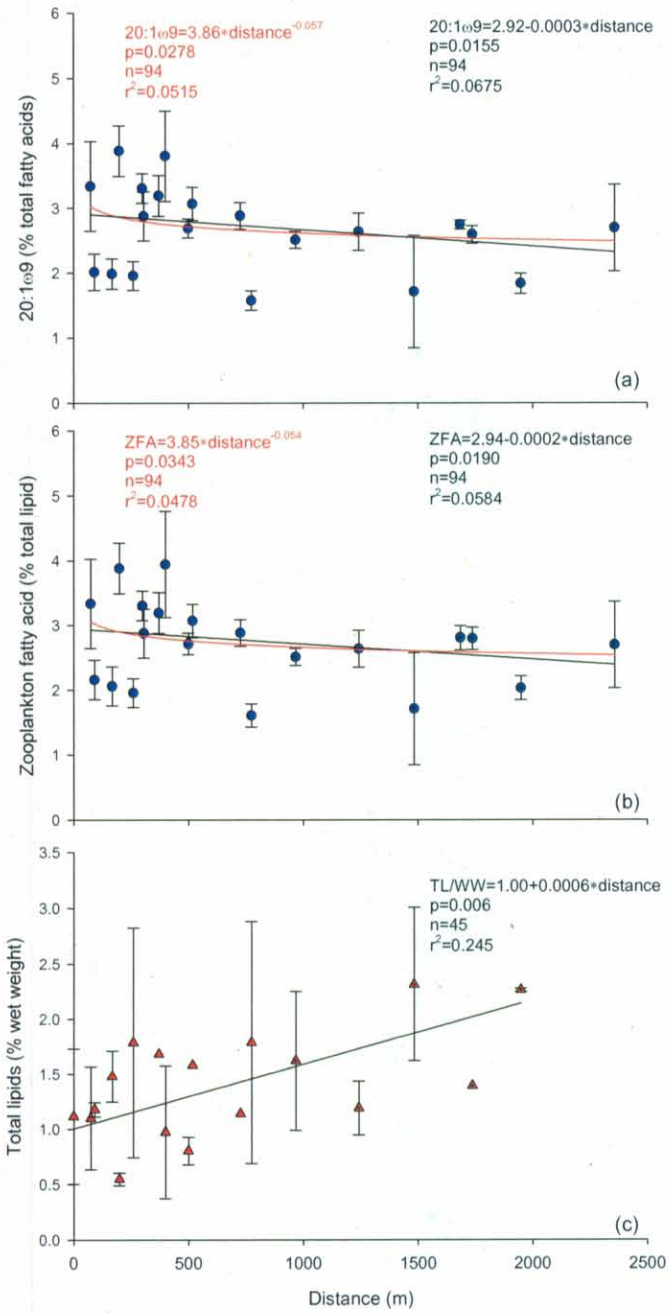

Figure 3.5: Regressions of nutritional and biomarker lipids with distance from farm (a) 20:1ω9 for mussels alone, (b) ZFA for mussels alone, and (c) TL/WW for limpets alone. Fatty acids are percentage of total identified fatty acids. Data are mean ± s.d. Regression lines are plotted through the raw data.

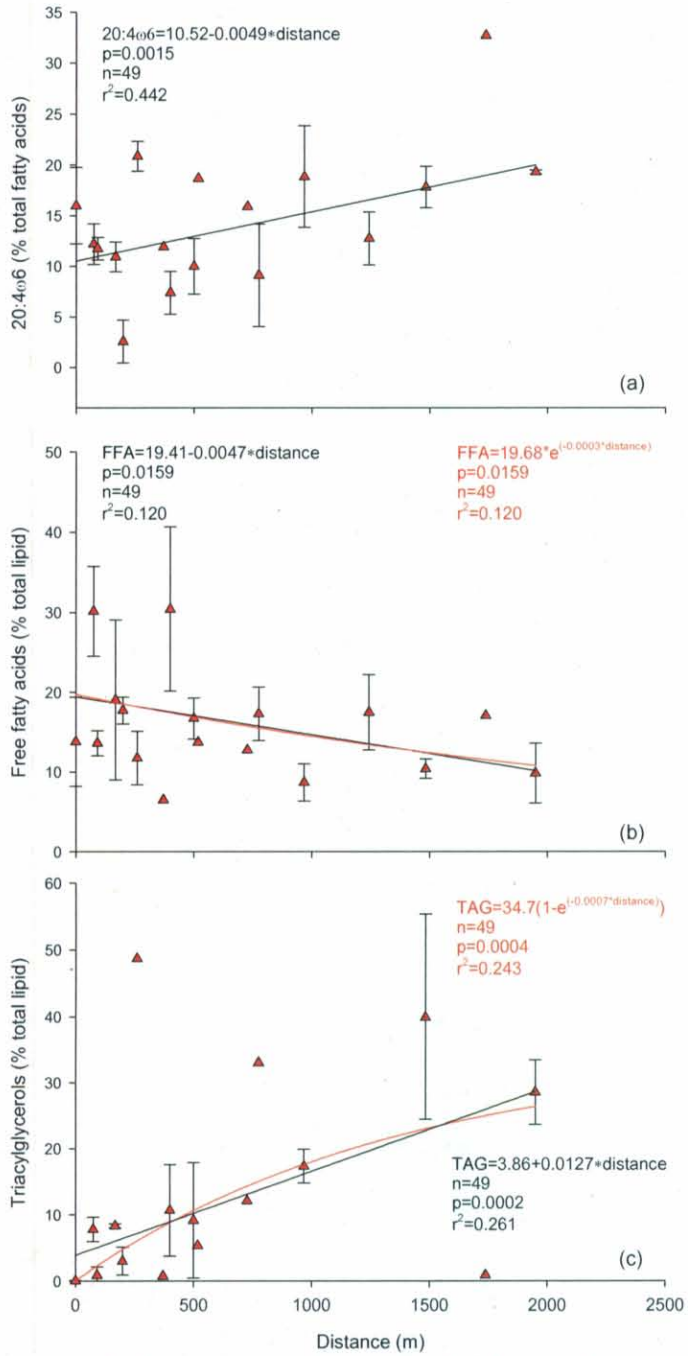

Figure 3.6: Regressions of nutritional and biomarker lipids with distance from farm (a) 20:4ω6 for limpets alone, (b) FFA for limpets alone, and (c) TAG for limpets alone. Fatty acids are percentage of total identified fatty acids. Data are mean ± s.d. Regression lines are plotted through the raw data.

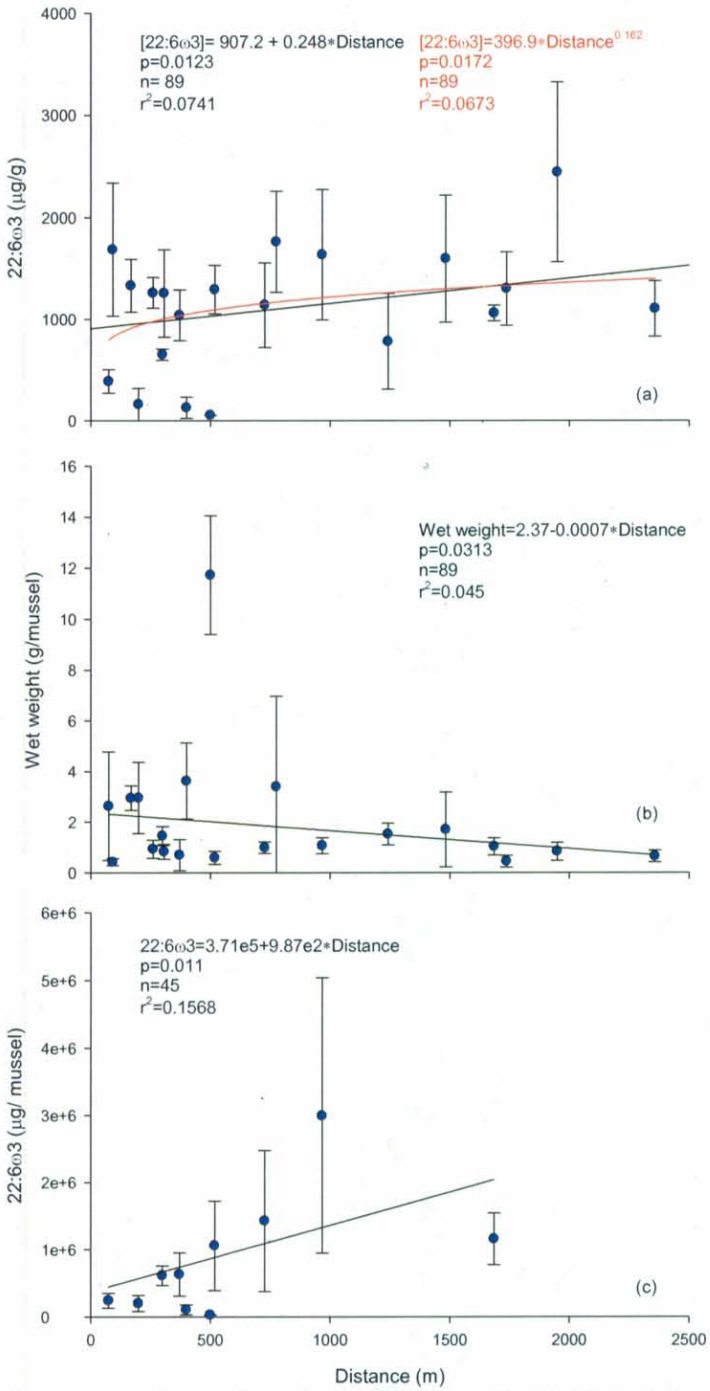

Figure 3.7: DHA and wet weight in mussels (a) 22:6ω3 (µg/g), (b) wet weight (g/mussel), and (c) 22:6ω3 (µg/mussel). Fatty acids are percentage of total identified fatty acids. Data are mean ± s.d. Regression lines are plotted through the raw data.

The regression analysis also showed ZFA (20:1ω9, 22:1ω11(13), 22:1ω9) and the fatty acid 20:1ω9 alone were significantly higher closer to the farm for all mussels (Fig. 3.5 a and b). These fatty acids can indicate the farms' influence. The herbivorous copepod markers, including 20:1ω9, are present in the fish feed and have low digestibility, making them a possible biomarker for the cages. The majority of the fishmeal is composed of meal and oil from planktivorous feeding fish like herring (Iwana, 1991; Van Biesen and Parrish, 2005). However, Davenport et al. (2000) have shown that mussels will consume mesozooplankton making it possible for them to take up these fatty acids themselves. Nevertheless, the significant decrease with distance from the farm is consistent with the farm source. For these relationships, a significant linear function was fitted to the data along with a significant power function clearly showing an influence from the fam.

For the limpets, the TL/WW and TAG were significantly higher further from the farm (Fig 3.5c and Fig 3.6c). These are indicators of the organisms' condition. TL/WW is a measure of the amount of lipid of all types that an organism has while TAG is an indicator of the proportion in storage (Hølmer 1989; Dalsgaard et al., 2003). The fatty acids are stored on a glycerol backbone to be used for energy when required.

Arachidonic acid (ARA; 20:4ω6) was significantly lower closer to the farm for limpets (Fig 3.6a). ARA is a polyunsaturated fatty acid (PUFA) and an essential fatty acid required for development (Rainuzzo et al., 1997). Similar to the DHA increase for mussels with distance from the farm, the levels of ARA also increased with distance. For limpets, however, the relationship was linear. The fatty acid analysis of the feed sampled in Chapter 2 showed the amount of ARA was 1.14±0.08%. This is lower than the levels of DHA

supplemented in the feed (11.63±0.71%). The decrease nearer to the farm may be due to the abundance of other less nutritious fatty acids. In addition, the cultured fish efficiently take up the essential fatty acids supplied in the feed (90-98% digestibility in salmon: Sigurgisladottir et al., 1992) and excess feed pellet losses can be as low as 1%.

TL/WW increased with distance from the farm for mussels alone as well as all molluscs. This indicates quantity, but relates less to quality. TAG (%TL) also increased significantly for all molluscs and mussels alone (Fig. A-3.3) and is used for storage and is an indicator of condition.

3.4.2.3 Area of Influence

Following regression analysis of the significant relationships, visual inspection of the graphed data showed possible distances at which the fatty acid proportions shifted to background values. Using statistical programming (Statistica 9.1) and following the procedure of Copeman et al. (2008), a significant break was determined for 22:6ω3 for the mussel data alone. A piece-wise non-linear fitting algorithm was used to determine the breakpoint. The analysis tested the hypothesis that two linear functions with significantly different slopes on either side of a break better represent the data than one continuous linear function. Using the model: Fatty acid= b_a + (b_b x distance) + [(b_c x distance - break)] x (distance > break)], where b_a is the intercept, b_b is the slope before the break, and b_c is the slope after the break, the parameters were estimated (Table 3.8) using a custom-loss analysis with the Rosenbrock and Quasi-Newton functions. Other non-linear relationships for the fatty acids were examined, but they showed no significant breakpoints.

Table 3.8: Non-linear, piecewise regression results between 22:6ω3 (DHA) and distance from farm (df=88)

	Estimate	p-level	Standard Error
b_a	9.77	<0.0001	0.82
b_b	0.01	0.002	0.004
b_c	-0.01	0.003	0.004
break	339 m	<0.0001	64

The 22:6ω3 breakpoint was found at 339 m for mussels alone, corresponding to 12.7% of total fatty acids. The change suggests that at distances greater than 339 m mussels shift to approximate background levels and maximize the 22:6ω3 proportions.

Although other fatty acids did not show significant breakpoints, ZFA and 20:1ω9 showed significant non-linear relationships with higher proportions of feed-related fatty acids nearer to the farms indicating a drop to background levels as distance increases. In order to better describe this shift, the distances at which the fitted models intersect the mean fatty acid proportions were calculated. Using the mean values and the non-linear functions the determined distances allow for a reference point for the transition between the farms' influence and the background values. Using the equations: ZFA = 3.85 x Distance $^{-0.054}$ and 20:1ω9 = 3.86 x Distance $^{-0.056}$, the distance at which the equations reach the mean values (2.74% and 2.70% TFA, respectively) are 544 m and 529 m for ZFA and 20:1ω9, respectively. Although the difference is not large, the change suggests culturing at distances greater than 339 m and closer than 544 m would optimize 22:6ω3 proportions thus enhancing the mussels' nutritional quality; however, there would also be an influence from the farm reflecting the remediation goal of IMTA where the co-cultured mussels would filter farm related deposits.

Visual inspection of the regression data (Figs. 3.4-3.6) also suggests a distance at which fatty acids shifted to background values. The significant non-linear relationships showed a shift in values at 306 m distance for 22:6ω3 and at 400 m for 20:1ω9 as well as ZFA. These values were apparent for the mussels considered alone and confirmed by two sample t-tests. Again, other fatty acids were examined, however no significant shifts in values were found.

Table 3.9: Average 22:6ω3, 20:1ω9 and ZFA for mussels separated by near and far field distances

	22:6ω3		20:1ω9		ZFA	
Mussels	Near (<306 m)	Far (>306 m)	Near (<400 m)	Far (>400 m)	Near (<400 m)	Far (>400 m)
average	11.71±2.80	13.50±2.70	2.96±0.78	2.49±0.58	3.00±0.79	2.54±0.57
C.V(%)	24	20	26	23	26	23

Significant differences $p<0.05$. Data are average± 1 S.D.

This again suggests distances less than 400 m maximize the fatty acid marker representative of excess feed and faecal pellets, and at the same distance, DHA levels are also maximized. Both breakpoint analysis and empirical observations show optimal placement of co-cultured organisms in B.C. to include distances between 340 – 400 m from the farm where the area of farm influence and DHA concentrations are highest.

3.4.2.4 Mollusc PCA and loading analyses

Further analysis included PCA using those fatty acids found to be significantly correlated, both positively and negatively, with distance from the farm (Table 3.5) for all mollusk taxa. These fatty acids were then used for PCAs with all molluscs combined, groupings of molluscs, and individual mollusc taxa. In addition, regression of the loadings

against distance from the farm allows for the interpretation of PC1 in a near field/far field manner.

Fig. 3.8 shows the PCA for mollusc lipids significantly correlated with distance from the farm. PC1 of the coefficients places 20:3ω3 and 18:1ω7 on the right-hand side and 22:6ω3 on the left. The fatty acid 20:3ω3 is the 'dead-end' part of the elongation of essential 18:3ω3 to 22:6ω3 (Tocher, 1993). In addition, 18:1ω7 is a bacterial fatty acid (Volkman et al., 1998; Pond et al., 2002; Pistocchi et al., 2005) and Chapter 2 showed it to be significantly higher in the outflow of land-based cod tanks therefore relating it to the farms' presence. Both 20:3ω3 and 18:1ω7 opposed DHA (22:6ω3) on the left-hand side of PC1 denoting the presence of the more highly unsaturated fatty acids towards the negative or left side and a bacteria fatty acid presence along with a possible 'dead-end' pathway towards the right.

The mussel samples (Fig. 3.8; blue circles) fell furthest to the left showing a higher amount of DHA than in the other molluscs. The limpets (red triangles) clearly fell furthest to the right associating them with 18:1ω7 and 20:3ω3. In addition, there was a significant regression of the loadings on the first principal component (PC1) versus distance ($p<0.001$; $n=166$, slope=-0.0012). The loading regression again shows DHA to be higher further away

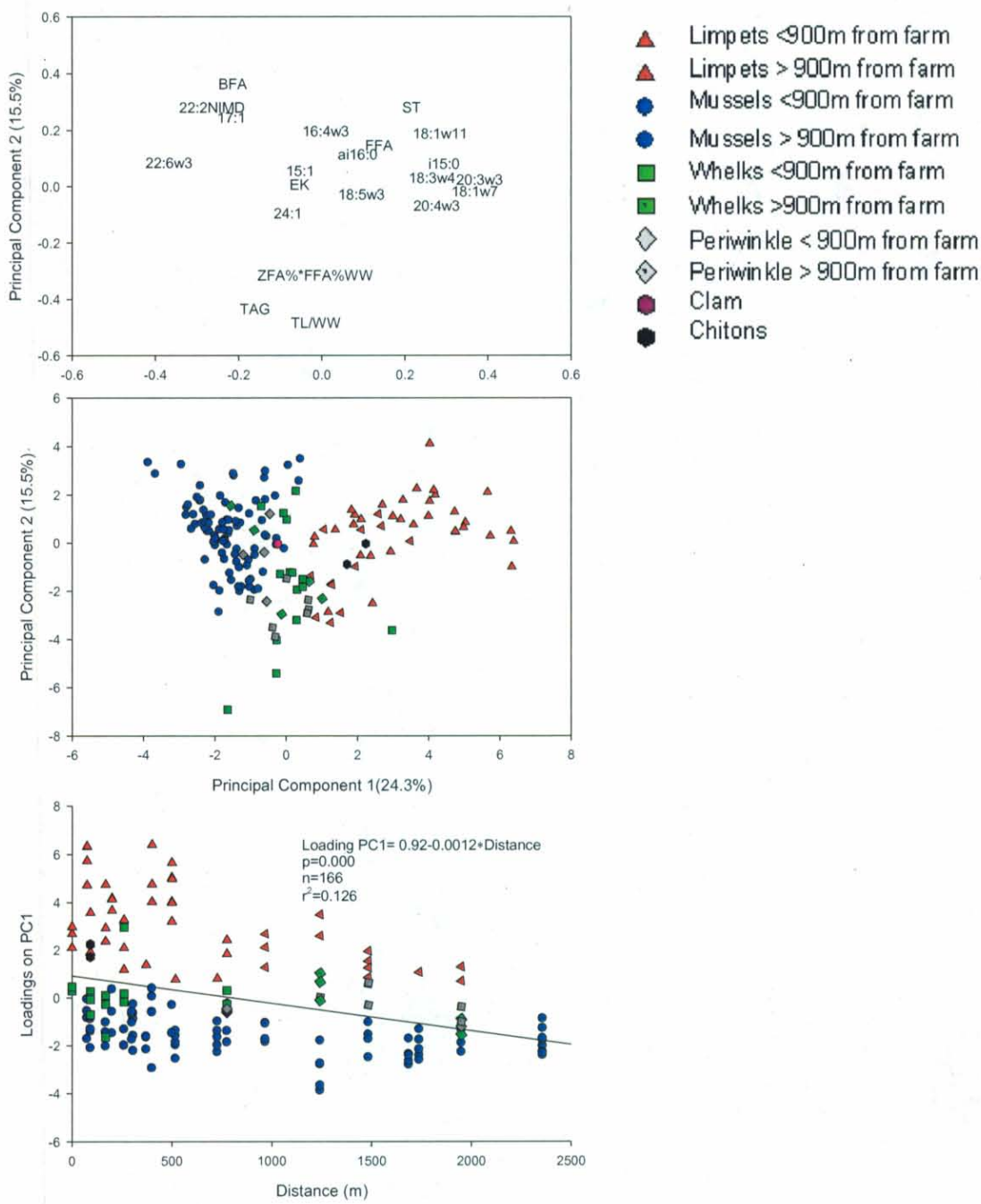

Figure 3.8: PCA of lipids significantly correlated with distance in all molluscs. PCA includes fatty acids (%TFA) significantly regressed with distance.

from the farm. DHA correlates with growth in mussels (Alkanani et al., 2007) and its depletion nearer the farm suggests a less enriched feeding location in terms of DHA. Individually, the bacterial fatty acids *i*15:0 and *ai*16:0 are higher closer to the farm. These are indicative of sulfate reducing bacteria (Sahl et al., 2008) and relate to anaerobic conditions reflecting the increased organic load on the benthic environment beneath the farms. The fatty acid 18:1ω7 was also higher closer to the farm indicative of methane oxidizing bacteria (Sargent et al., 1987; Volkman et al., 1998; Pond et al., 2002; Pistocchi et al., 2005). These bacteria thrive in higher methane conditions, which have previously been shown to result from aquaculture operations (Hall et al., 1990). The regression of the loadings also places 20:3ω3 higher closer to the farm. Although it is the 'dead-end' elongation product of the essential 18:3ω3, it has been shown that a possible Δ8 desaturation pathway along with retroconversion can utilize 20:3ω3 to produce the essential 22:6ω3 (Cook et al., 1993; Tocher, 1993).

There was also a significant regression of the loadings ($p=0.028$; $n=107$, slope=0.0006) from the PCA of mussels and whelks alone (Fig. 3.9). Here TL/WW and TAG are found closer to the farm, indicating more storage lipids and more lipid overall; however, when examining the regression analysis, the whelks and mussels display the opposite trends for both TL/WW and TAG. The whelks show more lipids closer to the farms, which is influencing the placement of TL/WW and TAG here. Further away from the farm there were higher amounts of DHA and C_{22} non-methylene interrupted dienes (NMIDs), trends that remain from the previously discussed PCA. BFA was also higher further away from the farm. Given the abundance of fish confined in one location, this may

seem counterintuitive; however, suppression of the local bacteria by antibiotic use provides a possible explanation.

There was an apparent separation of the mussels and whelks where mussels are located to the bottom right hand side of PC1 again connecting the mussels to higher amounts of DHA compared to other molluscs (Table 3.7) as DHA falls in the same region on PC1. However, this placement also relates mussels to the bacterial fatty acid 17:1 and the BFA suggesting mussels have more of these bacteria-associated fatty acids.

A PCA of mussels alone shows DHA on the right side of PC1 (Fig. 3.10; $p=0.0008$, $n=84$, slope=0.0008). From the regression, the right-hand side placement relates to distances further away from the farm. This is consistent with the previous PCAs as well as DHA proportions regressed against distance (Fig. 3.4 c). The farms' presence presumably contributes non-essential fatty acids to the local ecosystem, which then represent the majority of available lipid sources reflected in the uptake by adjacent invertebrates. However, of the available DHA, mussels contained significantly higher amounts compared to the other molluscs (Table 3.7) reflecting their efficiency in assimilating available essential fatty acids. With the PCA of mussels alone, the fatty acid 18:1ω7 again fell closer to the farm opposing the BFA, reflecting its association with methane-oxidizing bacteria.

Previously when considering multiple mollusc taxa in PCAs, the NMIDs positioned themselves further away from the farm. Examining mussels alone, NMIDs were central on PC1. They indicate substitution of ω3 fatty acids by the less nutritious NMIDs therefore correlating negatively with growth in mussels (Alkanani et al., 2007). With all mollusc taxa they appear further from the farms indicating an environment where mussels may grow

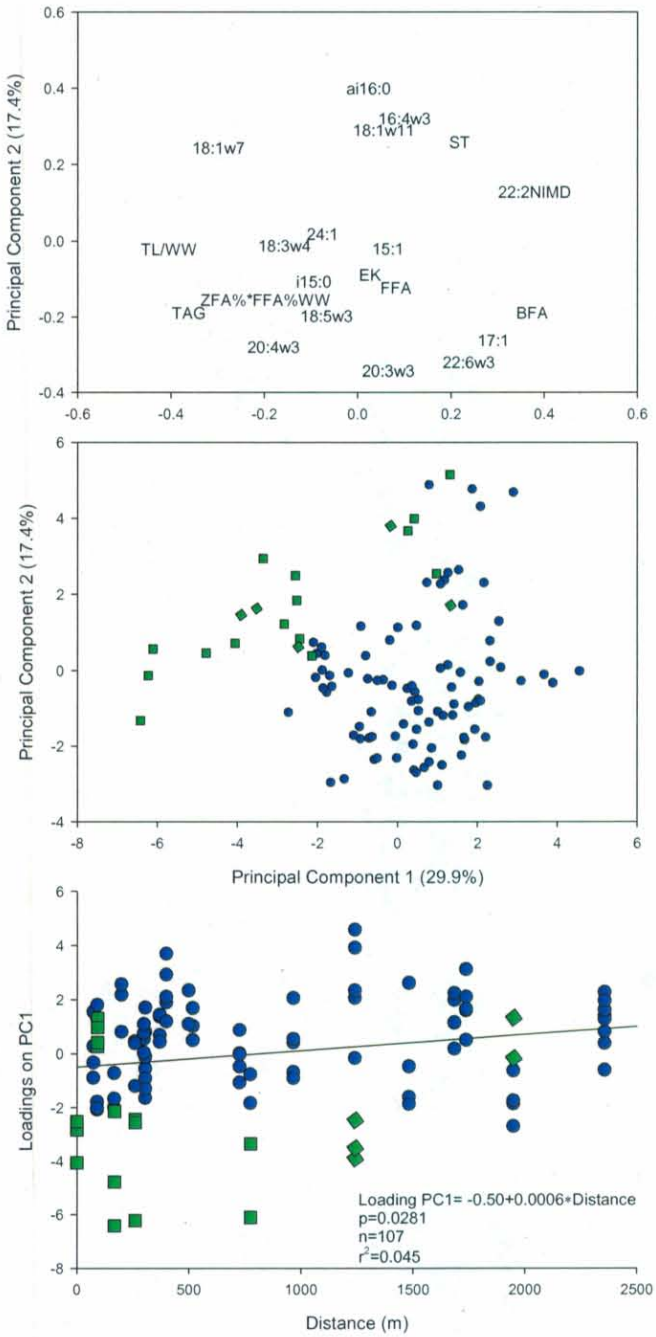

Figure 3.9: PCA of lipids significantly correlated with distance in mussels and whelks. PCA includes fatty acids (%TFA) significantly regressed with distance. See legend Fig 3.8.

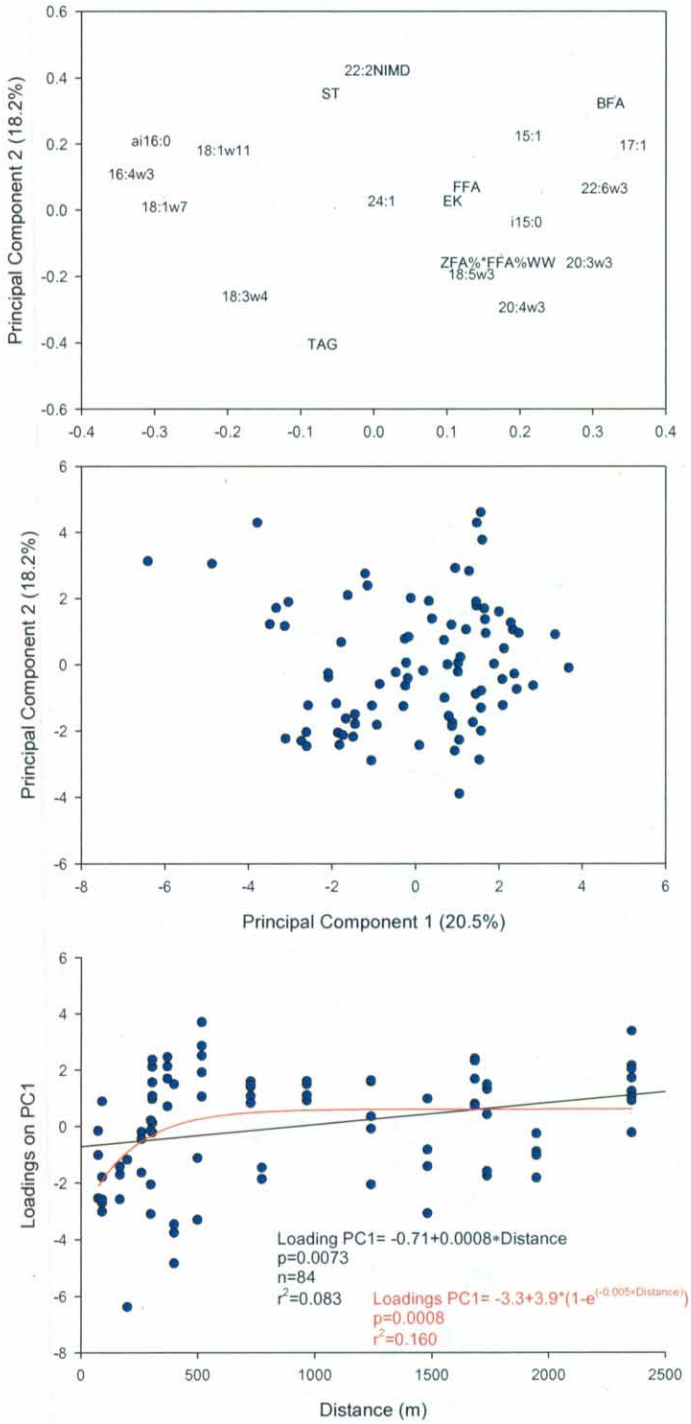

Figure 3.10: PCA of lipids significantly correlated with distance in mussels.
PCA includes fatty acids (%TFA) significantly regressed with distance (except TL/WW).

better, however when mussels alone are considered, they are located closer to the farms possibly reflecting the tradeoff between increased organic content closer to the farms and increased DHA further away.

A PCA of lipids significantly correlated with distance in limpets (Fig. 3.11; $p<0.001$; $n=44$) shows a higher TL/WW and TAG further from the farm. Closer to the farm there was again more bacterial *i*15:0 and *ai*16:0. Consistent with the previous PCAs, the bacterial fatty acid 18:1ω7 was present in a higher abundance closer to the farm. Unique to the limpets' PCA, BFA was higher closer to the farm reflecting the limpets' association with those bacterial fatty acids. Those limpets further from the farm showed higher TL/WW and TAG, which is consistent with the regressions in Fig. 3.5 (c) and 3.6 (c). However, these characteristics remained when considering limpets sampled from active farms only, not including specimens from farms that were fallow at the time of sampling suggesting less influence on the limpets from the farm.

The BC field sample analyses showed significant differences in mollusc lipid content and composition nearer to the farm compared to further away. The regressions showed fewer essential fatty acids closer to the farm for all mollusc and individual mollusc taxa, with an increase in lipid storage further from the farm for the limpets alone. The PCAs continued these trends where DHA was higher further away from the farm. In addition, bacterial fatty acid markers increased with distance from the farm; however, 18:1ω7 consistently remained higher closer to the farm for all molluscs, mollusc groupings, and mussels and limpets alone.

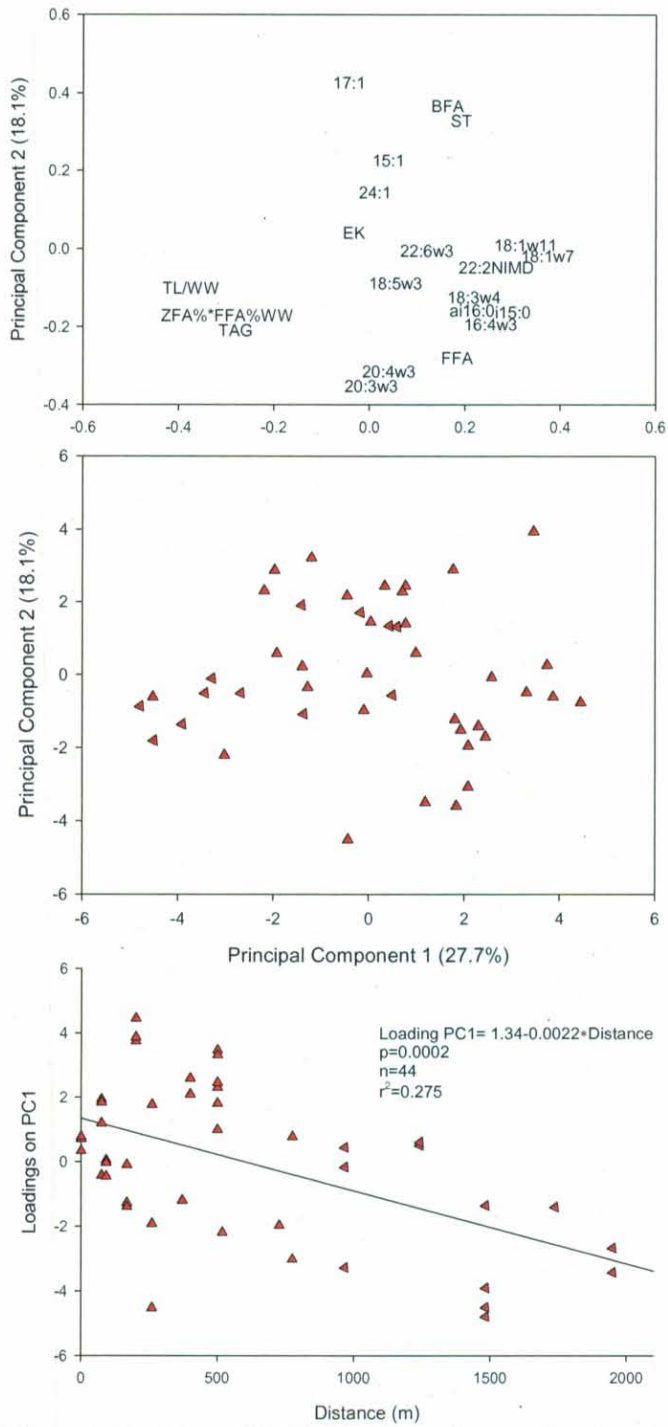

Figure 3.11: PCA of lipids significantly correlated with distance in limpets. PCA includes fatty acids (%TFA) significantly regressed with distance.

3.5 Conclusions

Based on the uptake of organic constituents in a coastal BC food web, PCAs reveal consistent groupings of algal consumers as well as their predators. In addition, filter feeders such as clams and mussels contain higher amounts of PUFA and ω3 FA compared to other invertebrates, cores, and net tow samples.

The organic composition of water and mollusc samples shows significant differences between near and far field regions around BC aquaculture sites. DOC is significantly higher closer to the farm (300 μM at 1 m depth and 85 μM at 10 m depth) reflecting the input of DOC from the farm.

Regression analysis with distance shows mussels alone are heavier closer to the farm, decreasing in wet weight with distance. This relates to the increase in organic output. Lipid regressions show significant increases in essential fatty acids with distance from the farm. DHA is significantly lower nearer to the farm sites for all molluscs and mussels alone and ARA is significantly lower closer to the farm for limpets alone. The uptake by neighbouring invertebrates reflects the contributions of non-essential fatty acids from the farms' operations subsequently limiting the uptake of essential fatty acids. Of the available DHA however, mussels contain significantly higher amounts of this essential fatty acid than other molluscs. Regression analysis also shows decreases in ZFA and 20:1ω9 individually with distance from the farm as well as a decrease in FFA. These indicate inputs from excess feed and faecal matter reflecting the farms inputs to the local ecosystem.

Breakpoint analysis of the mussel data shows distances closer than 339 m to have significantly less DHA suggesting finfish-mussel co-culturing systems would maximize DHA by placing mussels further than 339 m from the finfish aquaculture sites.

PCA analysis including the regressions of the loadings on PC1 continues the trend found with regressions alone, where higher DHA relates to areas further away from the farm. PCAs also show BFA to increase with distance; however, 18:1ω7 remains higher closer to the farm consistently for all PCAs. Its association with methane-oxidizing bacteria suggests enrichment of this bacterial type near to the farm. Although this works shows many significant differences and significant regressions of organic constituents around farms, additional research should include considerations of current data as well as further analysis of the described breakpoints.

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3.7: Appendix 2

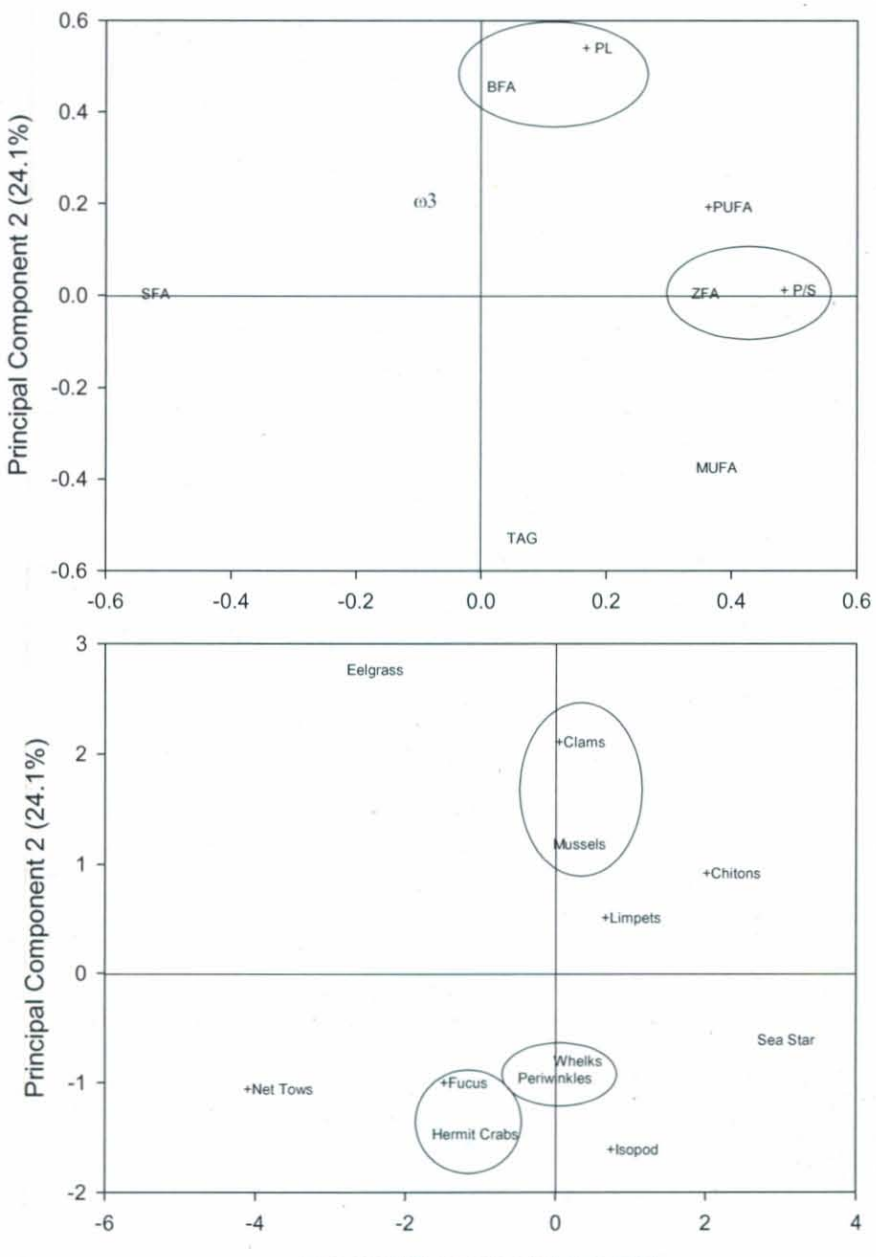

Figure A-3.1: PCA of Major lipids and Species (excluding core sediment samples) Using cluster analysis to group coefficients and scores
+ Indicates the sign of the loading on PC3, those remaining were negative

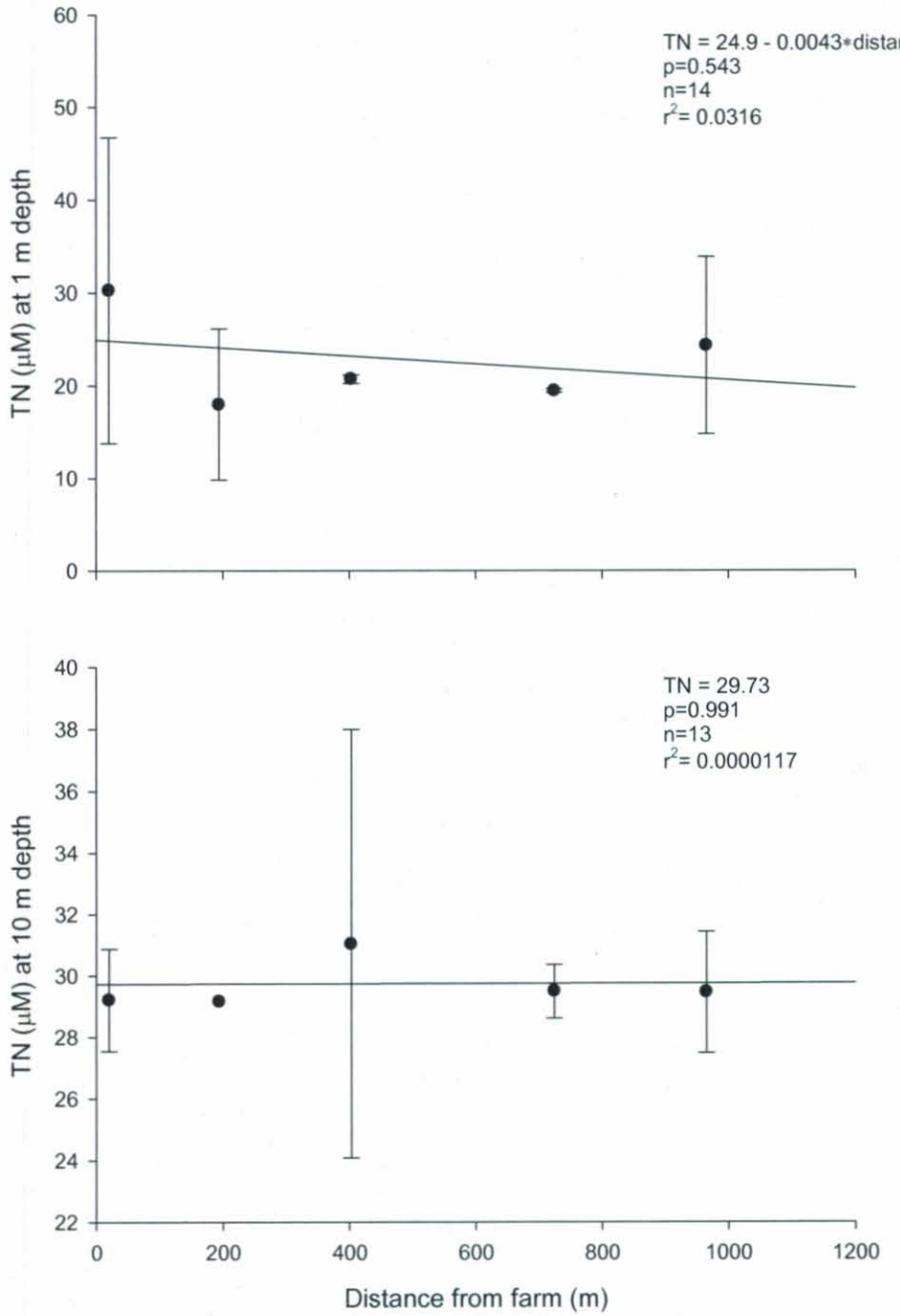

Figure A-3.2: Total nitrogen (TN) for 1 m and 10 m depths
Data are shown as mean ± s.d.
Regression lines are plotted through the raw data.

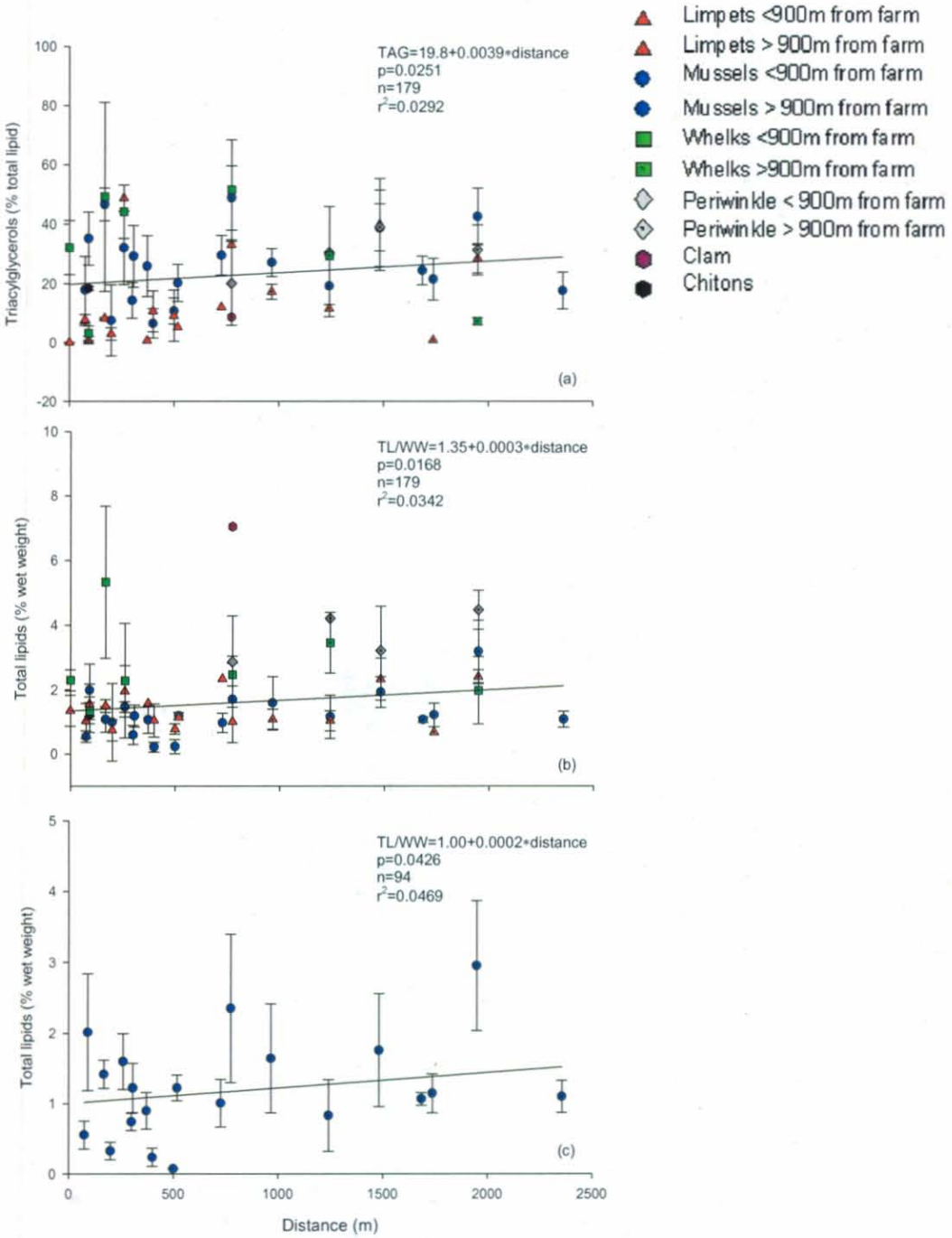

Figure A-3.3: Regressions of nutritional and biomarker lipids with distance from farm (a) TAG for all molluscs, (b) TL/WW for all molluscs, and (c) TL/WW for mussels alone. Fatty acids are percentage of total identified fatty acids. Data are mean ± s.d. Regression lines plotted through the raw data.

Chapter 4

Conclusions

4.1 Summary and implications

As the seas reach their maximum harvest potential, aquaculture provides a means to continue supplying fish to the worlds' growing population, while maintaining the food-grade wild fish stocks (Troell et al., 2003; DFO, 2008). Environmental interactions arise due to the abundance of individuals in one location. One of the largest considerations is increased organic input from excess feed particles and faecal matter and their interactions with the local ecosystem (Ervik et al., 1997). This thesis describes organic throughput of juvenile Atlantic cod (*Gadus morhua*) land-based tanks as well as uptake of organic constituents by invertebrates surrounding marine aquaculture operations.

Comparing the input and output of land-based tanks showed a significant increase in dissolved organic carbon (DOC) and dry weight material. Dry weight output increased to 187±39 g/day in the outflow giving 24% output compared to input. The output also contained significantly more free fatty acid (FFA), which reflects the faecal matter exiting the tanks. Fatty acid profiling also showed signatures of the feed and feed fines having significantly more zooplankton fatty acids 20:1ω9 and 22:1ω9 in the outflow compared to the inflow. In addition, the feed supplemented essential fatty acid, DHA, was significantly higher in the outflow than the inflow.

As anticipated, these differences reflect the additional inputs attributed to the farms presence. Understanding their roles as a by-product provides information for aquaculture's environmental interactions with the local ecosystems and details food sources for

surrounding invertebrates in line with multi-trophic, co-culturing systems where fish farm effluent is used as food for surrounding, cultured species.

In order to consider operational-size farm contributions to neighbouring species and continuing the idea of co-cultured, commercial species, the output of a land-based farm was calculated per kilogram of biomass and scaled to an operational-sized, 1880 tonne Atlantic cod farm. This allows for total output consideration. Scaling shows the production of over 3000 kg dry mass daily, which is capable of supporting 210 tonnes of mussels, assuming mussels require 0.080 g/day at the time of harvest (Alkanani et al., 2007). Furthermore, the quality of material, reflected by the DHA output from the scaled farms, could support over 40% of mussels grown in Newfoundland. This does not take into account current data and assumes total availability of the produced DHA to surrounding invertebrates as well as a 1:1 first approximation scaling; however, this highlights the potential of IMTA in utilizing this excess DHA.

Having described the output of a scaled farm in terms of food sources for multi-trophic, co-culturing systems, analysis of coastal marine food webs analyzing nutritional interactions among coastal invertebrates and potential cultured species aided in understanding their selected feed supplies. Principal components analysis (PCA) and cluster analysis of organic constituents in invertebrates, marine plants, net tows and core samples from coastal BC revealed groupings of algal consumers and their predators. Other interactions included high amounts of PUFA and ω3 in the filter feeders, clams and mussels.

Further examination included regressions of organic constituent uptake with distance from the farm by invertebrates surrounding aquaculture sites. Samples were taken on transects from Atlantic salmon (*Salmo salar*) farm locations in BC. The spatial relationships showed significant differences in near field and far field locations. DOC was significantly higher closer to the farms than further away, consistent with the land-based tank output in Chapter 2. Along with this, mussels wet weight was higher closer to the farm, which relates to aquaculture's organic output and uptake by mussel (Reid et al., 2010). In addition, the farm marker, FFA, decreased with distance for all molluscs and the feed marker, comprised of zooplankton fatty acids (ZFA), also decreased for mussels alone. Further regression analysis showed significant decreases in essential fatty acids with distance. DHA is significantly lower nearer to the farm for all mollusc taxa as well as mussels alone and ARA is significantly lower for limpets alone.

Overall, there is a significant decrease in the faecal marker, FFA, from the farm, which supports previous findings by Van Biesen and Parrish (2005) and relates to the presence of the farm. Significant changes in mussel data showed a breakpoint where DHA was significantly less closer than 339 m to the farm. This suggests a finfish-mussel co-culturing systems would maximize DHA by placing mussels further than 339 m from the finfish aquaculture sites.

PCA analysis and regression of the loadings on PC1 showed a decrease in DHA closer to the farm, consistent with the regression analysis. There was also more BFA further from the farms; however, 18:1ω7, a fatty acid associated with methane-oxidizing bacteria,

remains higher closer to the farm consistently for all PCAs suggesting this bacteria type thrives near the farms.

While effluent from the fish farms does represent an additional source of essential fatty acids, it also represents an even greater source of other fatty acids. Specifically, there is less DHA for mussels and less ARA for limpets nearer to the farm highlighted by the regressions and breakpoint analysis of DHA. The farm contributes non-essential fatty acids to the local ecosystem at levels that represent the majority of available lipid sources, reflected in the uptake and lipid profile of adjacent invertebrates. Nevertheless, mussels are more efficient at taking up available DHA as they contain significantly higher amounts compared to other mollusc taxa.

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