LIPID CLASS AND FATTY ACID COMPOSITION OF PLANKTON AND SETTLING PARTICLES AT A FISH ENCLOSURE, AND OF COD (Gadus morhua) IN GILBERT BAY, LABRADOR

GEERT VAN BIESEN

Lipid Class and Fatty Acid Composition of Plankton and Settling Particles at a Fish Enclosure, and of Cod (*Gadus morhua*) in Gilbert Bay, Labrador

by ©Geert Van Biesen

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Abstract

A small cod enclosure was set up in Gilbert Bay, Labrador. The lipid class and fatty acid composition of plankton and settling particles on a transect away from the net pens were determined in order to see if biomarkers for the dispersal of organic waste could be identified. Free fatty acid levels and the proportions of the long-chain monounsaturated fatty acids 20:1ω9, 22:1ω9 and 22:1ω11 in the settling particles close to the pens (5 m) were significantly higher ($p < 0.05$) than before the fish enclosure was in operation, and than further away from the pens. This is consistent with a higher input of feed and/or feces at this location. Free fatty acids are the major lipid class in fish feces, while the long-chain monounsaturated fatty acids are present in high proportions in the feed and in fish feces.

Gilbert Bay cod (*Gadus morhua*) muscle and liver were analyzed for lipid content and composition and compared interannually and with offshore cod. This study could not detect any clear differences between Gilbert Bay cod from 2001, offshore cod from 2001 and offshore cod from 2002. However, Gilbert Bay cod caught in 2002 just after the winter had different muscle and liver fatty acid profiles than the three aforementioned groups of cod, which were caught in midsummer. The differences can be related to starvation during the winter, as evidenced by an average lower hepatosomatic index and a higher water content of muscle and liver.

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List of Abbreviations

ALC: Alcohols

amu: atomic mass units

AMPL: Acetone Mobile Polar Lipids

ANOVA: Analysis Of Variance

CTD: Conductivity-Temperature-Depth

DAG: Diacylglycerols

DFO: Department of Fisheries and Oceans

FAME: Fatty Acid Methyl Esters

FFA: Free Fatty Acids

GC: Gas Chromatography

GC – FID: Gas Chromatography with Flame Ionization Detection

GC – MS: Gas Chromatography with Mass Spectrometry Detection

HC: Hydrocarbons

HSI: Hepatosomatic Index

KET: Ketones

MAG: Monoacylglycerols

MPA: Marine Protected Area

MUFA: Monounsaturated Fatty Acids

N: Nitrogen

P: Phosphorus

PC: Principal Component

PCA: Principal Components Analysis

PL: Phospholipid

PM: Particulate Matter

POM: Particulate Organic Matter

psu: practical salinity units

PUFA: Polyunsaturated Fatty Acids

Ref: Reference Location

RSD: Relative Standard Deviation

SFA: Saturated Fatty Acids

SD: Standard Deviation

SE: Steryl Esters

ST: Sterols

Sx, with x = 1 to 4: Station x

TAG: Triacylglycerols

TLC-FID: Thin Layer Chromatography – Flame Ionization Detection

WE: Wax Esters

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CHAPTER 1

INTRODUCTION

1.1. General Introduction

Aquaculture, the farming of fish, shellfish, crustaceans and aquatic plants has become a large-scale commercial industry in many parts of the world. Climbing from 13 million tons of fish and shellfish produced in 1990 to 31 million tonnes in 1998 (85% of which in developing countries), fish farming is poised to overtake cattle ranching as a food source by the end of this decade (Brown, 2000). Canada's contribution is rather modest with 91,000 tons in 1998, but growing rapidly (124,000 tons in 2000) (DFO, 2002a). The main species are salmon, trout, mussels and oysters. In Newfoundland and Labrador however, the decline of Atlantic cod (*Gadus morhua*) stocks, with a moratorium declared in 1992, encouraged research into the possibilities of grow-out cod farming (Wroblewski *et al.*, 1998). This is likely to have excellent potential since it is a low-technology approach and gear construction, handling skills and the required equipment are already present in local communities (DFO, 1999; AMEC, 2002).

The worldwide growth in aquaculture production has increased the awareness of its environmental impacts (Iwama, 1991; Silvert, 1992; Wu, 1995; Ervik *et al.*, 1997; Stewart, 1997; Hansen *et al*, 2001). This has already led to moratoria on new developments and tighter control in several countries (Wu, 1995). The potential environmental impacts of aquaculture cover a wide array of topics. Depletion of fish stocks used as fish feed (Naylor *et al.*, 2000); the destruction of coastal ecosystems which are often important nursery grounds for marine species (Iwama, 1991); eutrophication caused by nutrients emitted directly from the fish and from the decomposition of excess

food and feces (Aure and Stigebrandt, 1990); anoxic and reducing conditions in the sediment caused by organic enrichment and affecting benthic communities (Tsutsumi *et al.*, 1991), the use of antibiotics (Bjorklund *et al.*, 1989) and antifoulants (Davies and McKie, 1987) are some of the many possible concerns.

The focus here will be on the dispersal of particulate matter originating from uneaten feed and feces, which is of direct relevance to organic enrichment in the sediment underneath and in the vicinity of the fish cages.

The 'Coasts Under Stress' project (http://www.coastsunderstress.ca/), a major interdisciplinary research project uniting natural and social scientists from Canada's East and West coasts, gave us a unique opportunity to do the fieldwork in Gilbert Bay, Southern Labrador. This bay caught the attention of biologists because of the presence of an Atlantic cod stock that is genetically distinct from offshore cod (Ruzzante *et al.*, 2000; Green and Wroblewski, 2000; Morris and Green, 2002). There is a lot of interest in characterizing this population both genotypically and phenotypically, since this can be relevant to the conservation of this particular resource. For instance, Ruzzante *et al.* (2000) found that weight and length for age classes of Gilbert Bay cod are significantly lower than for offshore cod or for inshore cod further south, which is probably related to their overwintering in sub-zero inshore waters. Their productivity and recruitment potential may thus be lower, which means that a more conservative management strategy may be required. For this reason, this bay was officially declared an Area of Interest in the Marine Protected Area (MPA) programme by DFO Canada in October 2000 (DFO, 2002b). Identification of a site as an Area of Interest is the first step in a comprehensive

MPA evaluation process. MPAs are established to conserve and protect living marine resources and their habitats, areas of high biodiversity or productivity, endangered or threatened species, and unique habitats. Management plans for individual MPAs are developed with involvement of local resource users and interested and affected parties.

Copeman and Parrish (2003) recently gave the very first description of some basic components of the food web in Gilbert Bay, contributing to the existing data on this ecosystem. Plankton and 16 species of macroinvertebrates were analyzed for lipid composition and invariably showed high levels of polyunsaturated fatty acids, indicating their physiological importance. The lipid analysis of inshore and offshore cod in this thesis gives a description of organisms higher up the food web. There is also a link with the environmental aspects, since the fatty acid composition of the cod is predominantly determined by the fatty acids it extracts from its food, and this in turn determines what fatty acids are left behind in the feces and end up in the environment.

1.2. The Output of Marine Fish Farms: Uneaten Feed and Feces

1.2.1. The Environmental Loading of a Fish Farm

The magnitude and nature of particulate matter generated by a fish farm – the environmental loading - depends on a number of variables, such as the biomass of the farm, the species being cultivated and the kind of feed used (fish of low commercial value or pellets). The size of the individual organisms is also important since this determines feed and feces particle size. Small particles (high surface to volume ratio) with a high moisture content will have the highest dispersion rate. Automatic feeding methods tend to waste more feed than feeding by hand, because with the latter method the rate at which the feed is administered can be adjusted to the rate at which the fish are feeding, and this can vary on a daily basis. Thus, it is not surprising that the literature reports a wide range for the amount of feed that is uncaptured: 1% - 38% (Wu, 1995; Stewart, 1997). The values for the amount of fecal waste are more consistent, with a range of 20% - 30% relative to the amount of feed consumed (Iwama, 1991; Stewart, 1997). It has to be stressed though, that most of the available data is on salmonids.

Typically, feed for salmonid culture is composed of 46% - 51% protein, 18% - 20% carbohydrate, 14% - 17% lipid, and varying amounts of water and minor components such as antioxidants, vitamins, pigments and therapeutic agents (Iwama, 1991). Although there have been some estimates of carbon, nitrogen (N) and phosphorus (P) fluxes underneath fish cages (Hall *et al.*, 1990), the fate of individual proteins, carbohydrates

and lipids has not been studied extensively. However, Johnsen *et al.* (1993) and Henderson *et al.* (1997) analyzed lipids in the sediments underlying salmon cages in Scotland and Norway respectively, and were able to relate them to the feed. For instance, the fatty acid 22:1ω11 was the principal fatty acid in the fish feed and its proportion in sediments underneath the cages decreased on a transect away from the cages. Henderson *et al.* (1997) observed the same trend for total lipid and individual lipid classes.

Lipids are an interesting class of compounds to analyze for several reasons. They are relatively easily separated from other components in an aqueous sample matrix and since they may contain up to 16 different subclasses, they can give valuable information upon their origin (Parrish, 1999). This is discussed in some detail in section 1.3. Some of the fatty acids in lipids are essential for normal cell functioning (e.g. ω3 and ω6 polyunsaturated fatty acids - PUFA) and these fatty acids or their precursors have to be acquired from the diet (Arts *et al.*, 2001). In particular, benthic organisms living in subzero water temperatures for part of the year, such as in Newfoundland and Labrador, require high levels of PUFA for normal membrane fluidity and these PUFA have been shown to be efficiently recycled (Budge and Parrish, 1998).

1.2.2. Impacts of Aquaculture Activities

The impacts of fish farming are highly dependent on local conditions, which means that for a given environmental loading, the impact can be quite different for different systems. A higher current velocity allows for settling material to be dispersed to a greater extent. Similarly, a greater depth under the cages allows prevailing currents to dilute and

disperse the settling material to a greater extent than in shallower depths (Iwama, 1991). Common sense dictates that fish cages should be put in well-flushed and not too shallow locations, but this can conflict with the requirements of the species being cultivated. Also, fishermen seem to prefer sheltered bays, where the cages are better protected from heavy weather.

Most authors seem to agree that the effects on the dissolved fraction are small and confined to the immediate surroundings of the fish cages, except in shallow or confined water bodies (Wu, 1995; Ervik *et al.*, 1997; Hansen *et al.*, 2001), but decreases in dissolved oxygen, and increases in biological oxygen demand and nutrients (P, organic and inorganic N) have generally been found in the water column around fish farms (Iwama, 1991; Wu, 1995). The approximate 7:1 w/w N/P ratio of fish feed and fecal matter provides well-balanced nutrients for phytoplankton (except for diatoms, which also need silicate), which could cause eutrophication (Aure and Stigebrandt, 1990). Again, this is highly dependent on local conditions.

The impact of the sedimentation of food particles and fecal pellets on the benthos is probably more important. A high input of organic detritus in the sediment can lead to anoxic and reducing conditions, which in turn can lead to the production of toxic gases such as ammonia, hydrogen sulfide and methane (Hall *et al.*, 1990; Wu, 1995; Ervik *et al.*, 1997). These chemical changes often go hand in hand with a shift in the benthic community (Tsutsumi *et al.*, 1991; Hansen *et al.*, 2001). For example, Hargrave *et al.* (1997) found a significantly higher biomass of suspension feeders in the sediment under

marine cages than in the sediment at reference locations. Typically, in undisturbed conditions the competitively dominant species are those generally classified as K-selected: large-bodied, long-lived species with a relatively stable population size close to the carrying capacity of the environment. Smaller r-selected species (small body size and short life-span) may be numerically dominant, but they contribute a relatively small proportion of the biomass. If severe or frequent disturbances occur, the conservative K-strategists are the first to succumb while the opportunistic r-species are favoured and diversity decreases. In time, the opportunists may become both biomass and numerically dominant (Ritz *et al.*, 1989).

These effects are not only important from an environmental point of view, but have direct relevance to the fish farm itself since deterioration of water and sediment quality can negatively influence its production. It is therefore important to have some idea about the extent of the area that is affected by the output of the aquaculture facility. This is where the biomarker concept proves to be very useful, as discussed in the next section.

1.3. Lipids and the Biomarker Concept

The term "lipids" as used throughout this thesis is operationally defined: it includes all compounds that are extracted using one of the procedures outlined in Section 2.3 and encompasses a wide variety of compound classes such as hydrocarbons, wax esters, sterols and steryl esters, ketones, triacylglycerols, free fatty acids, long chain alcohols, glycolipids, phospholipids and pigments. Examples of chemical structures are depicted in Fig. 1.1. Fatty acids are briefly discussed below because some will be used as biomarkers: signatures of individual organisms (or groups of organisms) or of certain environmental processes (Parrish *et al.*, 2000). As such, they can give insight into the sources and alterations affecting particulate and dissolved material.

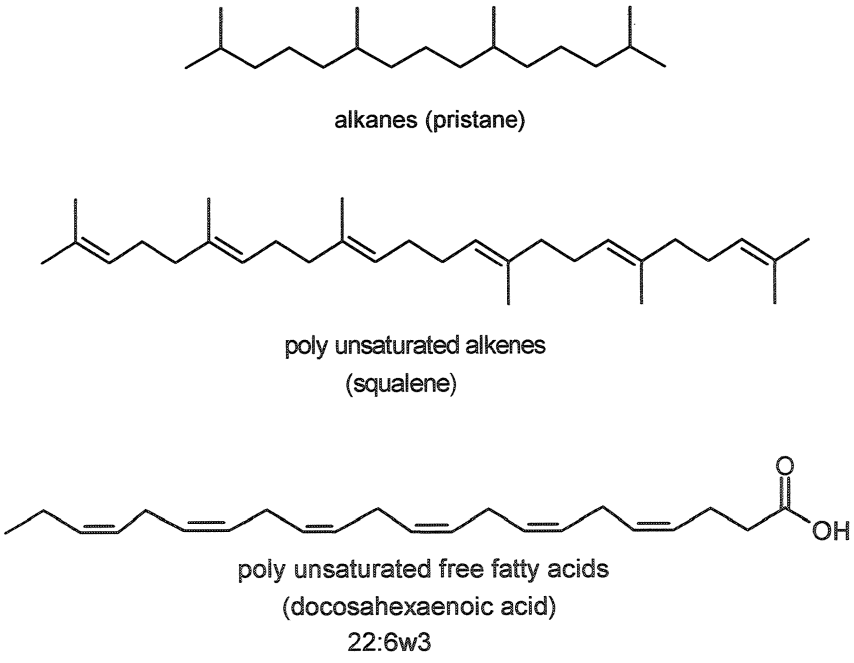
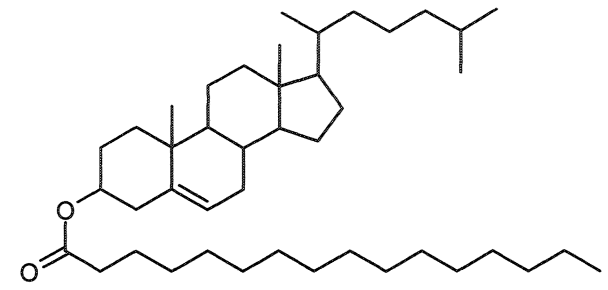
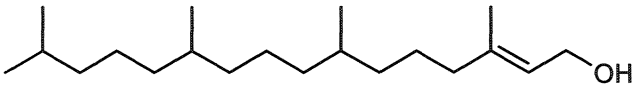


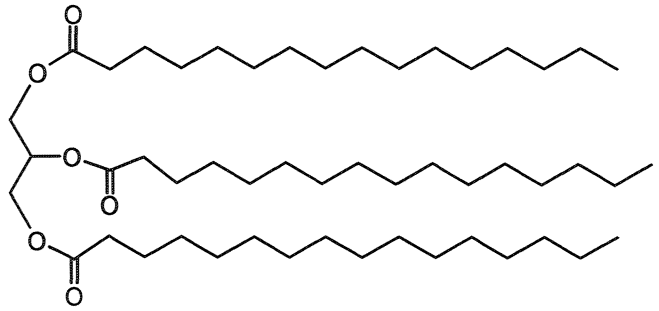
Fig. 1.1: Examples of commonly encountered lipid classes



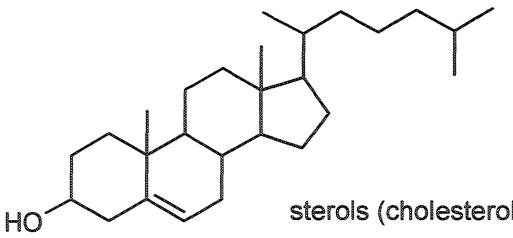
sterol esters (cholesteryl palmitate)



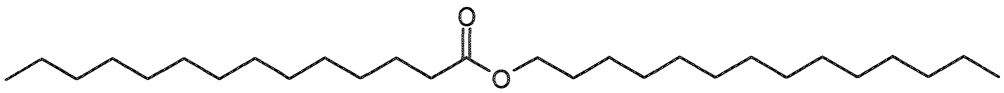
free aliphatic alcohols (phytol)



triacylglycerols (tripalmitin)

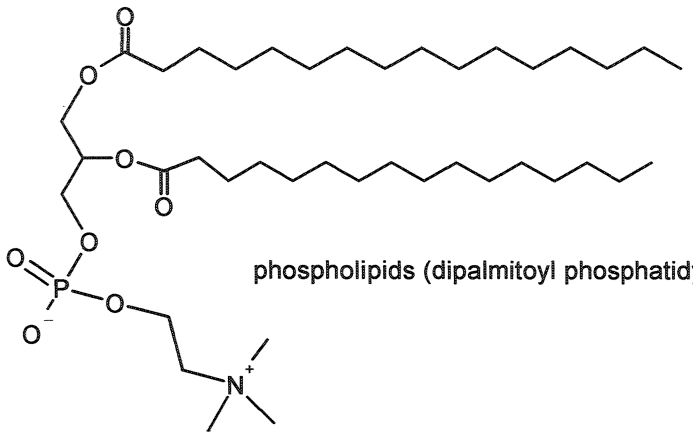


sterols (cholesterol)

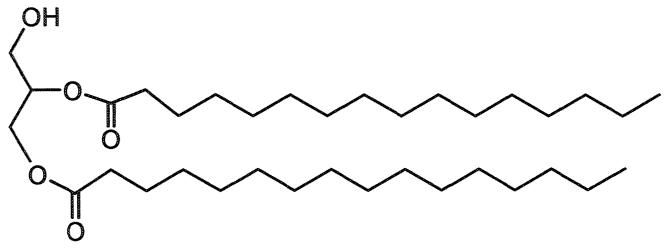


wax esters (myristoyl myristate)

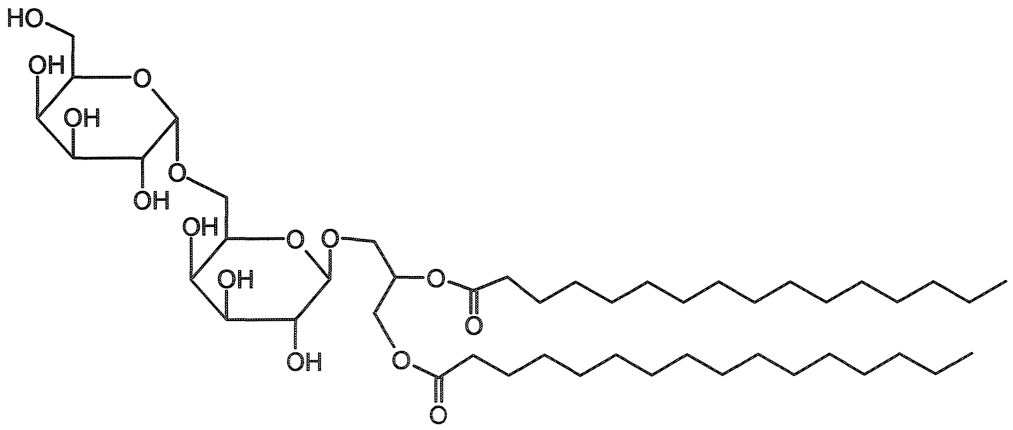
Fig. 1.1 (cont'd): Examples of commonly encountered lipid classes



phospholipids (dipalmitoyl phosphatidyl choline)



diacylglycerols (1,2-dipalmitoyl glycerol)



glycolipids (digalactosyl dipalmitoyl glycerol)

Fig. 1.1 (cont'd): Examples of commonly encountered lipid classes

Most biogenic lipid classes contain the acyl group (R-C(=O)-) because a fatty acid is incorporated into the molecule. The fatty acid moieties are usually analyzed by gas chromatography (GC) as fatty acid methyl esters (FAME) after transesterification (see Section 2.5.1). This allows for more than 50 fatty acids to be analyzed routinely. A convenient shorthand notation is often used to refer to fatty acids. In the designation A:BωC, A is the number of carbon atoms, B is the number of double bonds and C is the position of the double bond closest to the terminal methyl group. In this nomenclature system, all double bonds are assumed to be methylene-interrupted and *cis* in configuration. *Iso*- and *anteiso*-branched chain fatty acids are referred to as A:BωCi and A:BωCai respectively.

The identification and quantification of fatty acids is helpful in determining different kinds of sources. However, since there is probably no fatty acid that can be attributed to just one specific source, caution must be exercised in the use of fatty acids as biomarkers, particularly where multiple inputs (terrestrial, different species of phyto- and zooplankton, bacterial) are expected, such as in sediment traps that are deployed for a period of time. Furthermore, different fatty acids have different reactivity and biological, chemical and physical processes transform the original source signals. The use of relative and absolute concentrations instead of presence/absence, and the quantification of several biomarker fatty acids or biomarkers from different lipid classes are recommended to minimize these problems (Colombo *et al.*, 1996).

Long chain (> 24 C-atoms) fatty acids are often used as terrestrial plant indicators (Haddad *et al.*, 1992; Harvey, 1994; Colombo *et al.*, 1997). Alternatively, the sum of

18:2ω6 and 18:3ω3 can also be used as a terrestrial plant indicator. Samples exceeding the 2.5 % arbitrary limit are considered to have a significant source of terrestrial organic matter (Budge and Parrish, 1998; Parrish *et al.*, 2000).

In general, the fatty acids 14:0, 16:0, 16:1ω7, 20:5ω3, 22:5ω3 and 22:6ω3 are abundant in phytoplankton, while 16:0, 18:1ω9, 18:0, 20:5ω3 and 22:6ω3 often predominate in zooplankton (Colombo *et al.*, 1996).

The ratios 16:1/16:0 and Σ16/Σ18 can be used for discriminating diatoms from other phytoplankton (Reuss and Poulsen, 2002), with higher values indicating increased proportions of diatoms. In addition, 16:4ω1 is a useful biomarker for diatoms since it is rarely encountered in other phytoplankton (Budge and Parrish, 1998). In certain environments, diatoms are also the main producers of 20:5ω3, while dinoflagellates have higher proportions of 22:6ω3. Thus, if other microalgae do not contribute significantly to the 22:6ω3 pool, the 22:6ω3/ 20:5ω3 ratio is a marker which reflects the dominance of dinoflagellates over diatoms (Budge and Parrish, 1998; Parrish *et al.*, 2000).

Fatty acids are one of the few biomarkers that give an indication of bacterial contributions to a lipid extract (Colombo *et al.*, 1996). Odd-carbon numbered and branched-chain fatty acids are commonly produced by bacteria and can be used as bacterial biomarkers (Haddad *et al.*, 1992; Harvey, 1994; Henderson *et al.*, 1997). Budge and Parrish (1998) used the sum of unbranched 15:0, 17:0 and *iso*- and *anteiso*-branched fatty acids to determine the presence of bacteria in sediments and sediment traps. However, there is some conflicting evidence about the usefulness of these biomarkers. For instance, Harvey and Macko (1997) did not find a correlation between total fatty

acids attributed to bacteria and bacterial carbon, while other authors believe that fatty acids of common oceanic bacteria may not be compositionally different from planktonic fatty acids (Wakeham, 1995). It seems that bacterial fatty acids should only be used to determine bacterial levels relative to other samples in the same study (Parrish *et al.*, 2000).

Herbivorous copepods contain elevated amounts of the long-chain monounsaturated fatty acids 20:1 and 22:1 within the wax ester fraction (Graeve *et al.*, 1994; Albers *et al.*, 1996), and the sum of these fatty acids may be used as a zooplankton marker (Parrish *et al.*, 2000). The presence of wax esters as such is already an indication for the presence of calanoid copepods, since they are not widely distributed among the non-copepod zooplankton of the epipelagic zone. There is some evidence however that at least some marine phytoplankton species contain substantial amounts of wax esters under very specific (e.g. growth-limiting) conditions (Sargent *et al.*, 1977).

An overview of the biomarkers used in appendices 9 – 16 is shown in Table 1.1.

Table 1.1: Biomarkers used in this study

Source	**Biomarker**	**Remarks**
Terrestrial (Terr.)	18:2ω6 + 18:3ω3	> 2.5% means significant terrestrial input
Diatoms	Σ16:1/16:0	Higher ratio indicates higher diatom proportions
	Σ16/Σ18	Higher ratio indicates higher diatom proportions
	16:4ω1	
Dinoflagellates	22:6ω3/20:5ω3	Ratios > 1 indicate dinoflagellates are predominant
Bacteria (Bact.)	15:0+15:1+15:0i+15:0ai+16:0i+ 16:0ai+17:0+17:1+17:0i+17:0ai	
Zooplankton	20:1 + 22:1	

1.4. Objectives

The main objectives of this thesis were two-fold.

1) Find out if there are compounds (lipids) in that are 'typical' for a fish enclosure that can be traced back to the pens. Such markers could provide an early warning signal that organic enrichment is taking place. To investigate lipids from this source, particulate matter was collected by sediment traps on a transect away from the fish pens and analyzed for lipids. Specifically, the focus was on the fatty acid composition since some fatty acids have already been proven successful as biomarkers i.e. signatures of groups of organisms or of certain environmental processes (Parrish *et al.*, 2000). Since the cold water environment of coastal Newfoundland and Labrador during much of the year slows down chemical and possibly also bacterial decomposition and recycling of cultured fish wastes, these pristine waters could be especially susceptible to the pollution that accompanies aquaculture activities.

2) It has already been mentioned that the study area (Gilbert Bay, Labrador) is an Area of Interest in the Marine Protected Area programme because of its unique cod stock. More data that are helpful in further characterizing this ecosystem and especially this vulnerable fish resource are required. For this reason, some basic physical (temperature and salinity) and chemical (nutrient levels) data were collected. We were particularly interested to see if there would be a difference in the lipid / fatty acid signature of these Gilbert Bay cod (inshore cod) with offshore cod, since the available data suggest that unlike offshore cod they feed mainly on invertebrates (Morris and Green, 2002).

Therefore, the lipid content and composition of Gilbert Bay cod flesh and liver was determined and compared both interannually and with offshore cod. An experiment was also conducted in which inshore and offshore cod received either fish pellets or herring to see if this would change their lipid / fatty acid profiles.

There is an inherent relationship between the physical, chemical and biological data collected in this study. Temperature and salinity are among the key variables that restrict the kind of organisms that are able to survive and reproduce in any aquatic ecosystem. Given sufficient light and dissolved inorganic carbon, temperature and the nutrients nitrate, phosphate and silicate largely determine the productivity of the phytoplankton, which is the basis of the aquatic food web. This in turn influences the productivity of all higher trophic levels, from zooplankton to fish and marine mammals. Prey species composition and availability for cod, and consequently lipid composition and content of cod, are thus indirectly linked to these basic physical and chemical variables. This is also the case for the composition of the particulate matter in the water column (phyto- and zooplankton and decaying organic matter), which is collected in net tows and sediment traps. Close to the fish pens, it can be expected that the composition of the particulate matter reflects both a natural and a fish enclosure generated input. The composition of the particulate matter generated by the fish enclosure depends on the composition of the feed and the action of the cod's digestive system on the feed, and this determines the nature of the environmental signal that can be detected from the fish enclosure.

CHAPTER 2

MATERIALS AND METHODS

2.1. Sampling Area

In August, September and October 2001, field trips were made to Gilbert Bay, Labrador. Gilbert Bay is a narrow inlet, approximately 20 km long with an area of 60 km^2 (Fig. 2.1). The maximum water depth is ~80 m, with most of the bay shallower than 30 m. Two major rivers empty into the bay and contribute to the inflow of freshwater. The surface water temperature is sub-zero for about 6 months per year, from December until May, but during the summer, near-surface water (0-1 m) can exceed 15 °C (Morris and Green, 2002). Except for some boating activity, there are very few anthropogenic influences. The fishermen in the small community (~70 people) of Williams Harbour catch their fish mostly offshore, but there is also some fishing activity and aquaculture of scallops in the bay itself.

The fish pens used in our experiment were located in Captain Jacks Cove (52° 34' N, 55° 49' W), a relatively sheltered site. The reference location (52° 35' N, 55° 53' W) was a small cove just west of Bald Island, approximately 4 km northwest of the fish pens (Fig. 2.1).

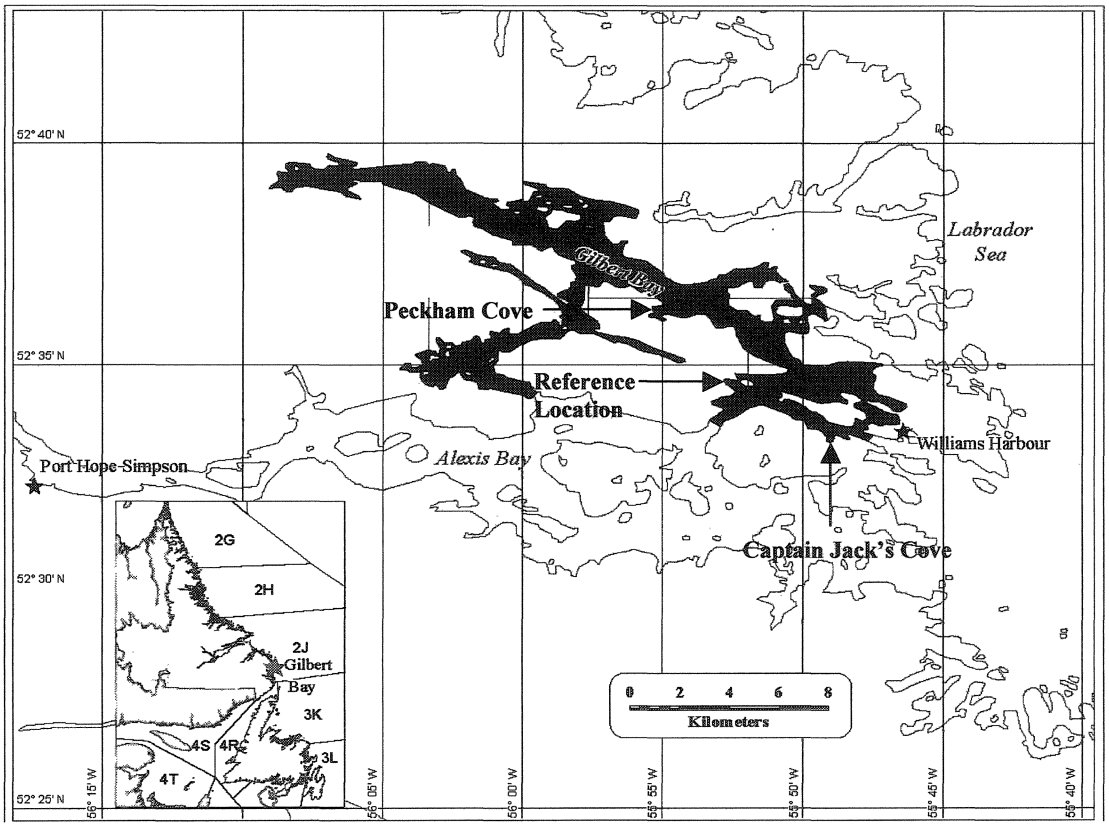


Fig. 2.1: Location map of Gilbert Bay

2.2. Sample Collection

2.2.1. Temperature and Salinity

The seawater temperature was monitored with a Minilog-T data sensor (Vemco Ltd.), attached to one of the fish pens at 5 m depth, which measured the temperature every 30 min from August 25th until October 22nd. Salinity and temperature depth profiles were taken at the locations of the sediment traps (see Section 2.2.4) using a Seabird Conductivity-Temperature-Depth (CTD) Instrument (Electronics Inc. Seacat SBE 19-03).

2.2.2. Nutrients

A Niskin bottle was used to collect water samples at a depth of 5 m in August and October at the locations of the sediment traps.

Aliquots for nutrient analysis were filtered on site using a plastic syringe and 0.45 µm filters (Corning Incorporated), and stored at –20 °C in sealed plastic test tubes until analysis. Analyses of nitrate + nitrite, phosphate and silicate were kindly performed by DFO (Department of Fisheries and Oceans) in St. John's, Newfoundland, with a Technicon Autoanalyzer II (Pulse Instrumentation Ltd., SK Canada). They used the NAP (New Analyzer Program) version 3.0 to process their data.

2.2.3. Fish

With the help of local fishermen, 238 cod (*Gadus morhua*) were caught by angling during the first two weeks of August 2001 in and just outside of Gilbert Bay and put in

two pens close to the shore in Captain Jack's Cove (Fig. 2.1). The pens were buoyed net cages of 4.5 x 4.5 x 4.5 m with a cover made of netting, and always have the top at sea level. The fish were measured, weighed, tagged, colour coded and photographed as part of another project in which the colour change of a local cod population in relation to their diet was being investigated (Gosse, 2002). Inshore or 'bay' cod typically are brown-reddish due to a high level of carotenoids in their food, which consists for a large part of invertebrates (Dr. J. Wroblewski, pers. com., 2002). Offshore cod on the other hand are black/grey and mainly feed on small fish.

Regardless of their colour, fish smaller than 35 cm were put in one pen and were initially fed capelin (*Mallotus villosus*) and fish pellets (Haddock Grower 46-14 – 6.5 mm, Zeigler Bros, Inc, Gardners, PA, USA), while bigger fish in the other pen were kept on a diet of capelin. About three weeks into the experiment however, the freezer in which the capelin was stored broke down and the capelin started to decay, so herring (*Clupea harengus*) was used instead. In total, 281 lbs of herring, 73 lbs of capelin and 18 lbs of fish pellets were used; the fish were fed every second or third day.

The reason for separating big fish from smaller ones was twofold. First, cod are cannibalistic and separation of size classes prevented the smaller fish from being eaten by the bigger ones. Secondly, we were originally interested in the effect of different kinds of feed on the lipid composition of the fish (but see Section 3.6.1). The intention was to accustom the small fish to pellets by gradually decreasing the proportion of capelin/herring and increasing the proportion of fish pellets until they would feed exclusively on the fish pellets.

At the end of the season (late October 2001), 90 fish (= 38% of total) were missing although the nets were intact. The remaining fish were weighed, measured, colour coded and photographed again and most of the big fish (larger than 35 cm at the beginning of the season) were killed for collection of otoliths to determine their age. The remaining fish (approximately 40) were transferred to Peckham Cove and kept there without feeding until the beginning of June 2002. When the pen was hauled up, it was empty except for the remains of five fish. To date, it remains unclear what happened to them. Three cod were collected again from Gilbert Bay in June 2002 and also from Hamilton Bank in August 2002, just east of the coast of Labrador at a slightly higher latitude (~ 54° N, 55° W) than Gilbert Bay. The fish from Hamilton Bank definitely belong to a different population than the ones from Gilbert Bay.

Fish (cod and herring) and fish pellets were frozen at –20 °C and transported (for about 5 - 6 hours) in coolers with ice. Upon arrival in the lab, they were stored at –20 °C until analysis.

2.2.4. Sediment Traps

While the fish were being put in the pens, sediment traps were deployed approximately 1.5 m from the seafloor at distances of approximately 5, 10, 30 and 100 m from the cages (Fig. 2.2). These locations are referred to as S1, S2, S3 and S4 respectively (S = Station). The depth of the water column at these locations was approximately 5 m, 8 m, 13 m and 25 m respectively. A sediment trap was also deployed at the reference location, where the

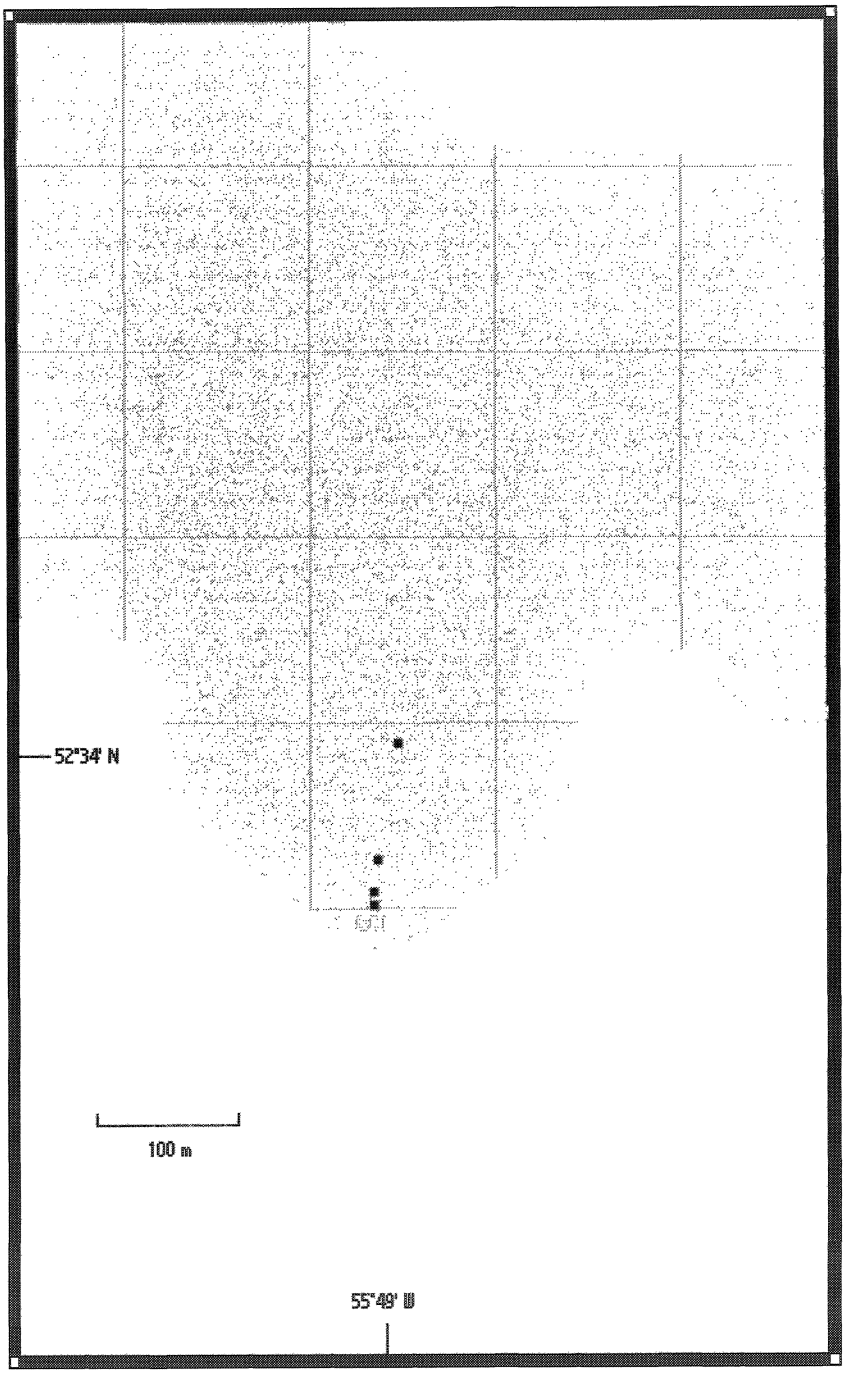


Fig. 2.2: Location of the sediment traps (black dots) and fish cages (white rectangle) in Captain Jack's Cove.

depth was 6 m. The sediment traps consisted of an aluminium frame with four PVC collection tubes (diameter of 10 cm, length of 60 cm) filled with a 4% NaCl solution. The higher density of this solution relative to seawater prevents any collected material from drifting away and prevents mixing. Each tube of the frame is considered a replicate and is treated as such in the data analysis, although one might argue that in fact the four tubes of a frame are pseudoreplicates since they are not completely independent. The alternative of deploying more sediment traps was not feasible, given the limited manpower and the logistic problems associated with the remote location of the sampling area.

There are many complications associated with particle flux and composition measurements using sediment traps, such as loss of organic matter because of leaching or grazing, collection of zooplankton 'swimmers', and resuspension into the traps of particulate matter that had already settled on the seafloor (Körtzinger *et al.*, 1994; Lee *et al.*, 1992). Nevertheless, this approach has been very valuable in estimating fluxes of organic carbon to the seafloor, and the changes in organic composition during passage of particles through the water column (Lee *et al.*, 1992). To slow down or prevent bacterial decomposition of the material collected by the sediment traps, biocides such as $HgCl_2$ (Budge and Parrish, 1998) or NaN_3 (Körtzinger *et al.*, 1994) are often used. However, the close proximity of the traps to the fish cages precluded the use of any biocide.

It also has to be stressed here that this experimental design (one transect) does not allow determination of the exact distribution of organic waste around the cages, since this depends on prevailing currents and can be asymmetrical (e.g. Henderson *et al.*, 1997). In

order to get a good estimate of this distribution, sediment traps on several transects would have to be deployed and many more samples would have to be analyzed. For the same reasons as stated before (limited manpower and the remote location of the sampling area), this was not possible within a reasonable timeframe. However, the objective was mainly to determine which biomarkers could be useful to trace back the influence of the fish enclosure on the environment. In this respect, we were looking for compounds or compound classes for which the concentration would decline moving away from the pens. A single transect was deemed sufficient for this purpose.

The protocol for sampling the trap contents for lipid analysis and dry weight determination is given in Appendix 1. Aliquots were also taken for plankton analysis; samples were preserved by adding 1 ml of Lugol's iodine and 1 ml of 10% formaldehyde to 10 ml of sample. An overview of deployment and collection times is given in Table 2.1. For convenience, the first deployment period will be referred to as 'August', the second as 'September' (although part of the deployment period was in August) and the third as 'October' (although part of the deployment period was in September).

2.2.5. Net Tows

Net tow samples were collected by towing a plankton net (20 μm) at a depth of approximately 5 m behind a small motorboat close to the positions of the sediment traps and steering back and forth parallel to the fish pens. One net tow was performed at each location (duration: ~ 30 – 40 min) and three subsamples were taken for analysis (see

below). We acknowledge the fact that these samples are not true replicates because they were not collected independently. The alternative is spending more time at each location to perform three separate net tows. However, samples at the different locations have to be collected within the shortest time frame possible to reduce the influence of temporal variations (e.g. tide) on the composition of the water column, and to minimize changes occurring between sampling and sample processing. Ideally, samples would have to be collected at the same moment in time at each station. For our situation, this would require five boats, five plankton nets, etc. which was impossible. Given these limitations, subdividing each net tow into three subsamples is a reasonable alternative. At the location closest to the fish pens (S1), horizontal net tows were impossible because of a large number of moorings, so vertical net tows had to be taken.

The material collected in the cod end was poured into a container and kept on ice until it was filtered not more than a few hours later. The protocol for taking samples for lipid analysis and dry weight determination is essentially the same as the last two points of Appendix 1, although the aliquots may be somewhat larger since these kinds of samples generally are more dilute than sediment trap samples. As for the sediment traps, 10 ml aliquots were preserved for plankton analysis by adding 1 ml of Lugol's iodine and 1 ml of 10% formaldehyde. An overview of the samples that were collected is given in Table 2.1.

Table 2.1: An overview of samples collected over the course of 2 years

Sample	Location and Date
Net tows and Nutrients	S1: Aug. 06 & Oct. 20 2001 S2: Aug. 05 & Oct. 20 2001 S3: Aug. 05 & Oct. 20 2001 S4: Aug. 06 & Oct. 20 2001 Ref: Aug. 06 & Oct. 20 2001
Sediment traps	S1: Aug. 02-Aug. 22, Aug. 22-Sep. 26 & Sep. 26-Oct. 19 2001 S2: Aug. 02-Aug. 22, Aug. 22-Sep. 27 & Sep. 27-Oct. 19 2001 S3: Aug. 02-Aug. 22, Aug. 22-Sep. 27 & Sep. 27-Oct. 21 2001 S4: Aug. 02-Aug. 22, Aug. 22-Sep. 26 & Sep. 26-Oct. 21 2001 Ref: Aug. 03-Aug. 21, Aug. 21-Sep. 27 & Sep. 26-Oct. 21 2001
Cod	In and around Gilbert Bay: Aug./Sep. 2001 & May 2002 Hamilton Bank: August 2002
Herring and Pellets	Aug./Sep. 2001

2.3. Lipid Extractions

Samples were extracted using a modified Folch *et al.* method (1957). In this procedure, outlined in Appendix 2, the samples are homogenized in chloroform:methanol 2:1. This solvent mixture is sufficiently polar to remove lipids from their association with cell constituents and to dissolve polar lipids such as free fatty acids and phospholipids. It is also sufficiently non-polar to dissolve neutral lipids such as triacylglycerols and wax esters. Further steps involve the addition of water, sonicating and centrifuging, which results in a biphasic system with a lower chloroform–phase and an upper water/methanol-phase. Lipids and non-lipids are partitioned between both phases in such a way that any co-extracted non-lipids are back-extracted to the water/methanol-phase while lipids remain in the chloroform-layer (Smedes and Thomasen, 1996). The proportions of sample, water and solvents are critical for maximal recovery. In our procedure (Parrish, 1999), samples are extracted in a solvent-to-sample-ratio of at least 3 ml per 150 mg dry weight of actual sample. This volume includes the chloroform and the methanol and the weight does not include any filters or water associated with the filter or the sample. In the water washing step, the ratio of chloroform:methanol:water is 8:4:3. This amount of water includes that in the sample and filter if present and is equivalent to a solvent-to-water ratio of 4:1.

2.4. Derivatization with Boron Trifluoride/Methanol

The purpose of this derivatization is to convert free fatty acids and fatty acid moieties of acyl lipids (mono-, di- and triacylglycerols, acylated glyceryl ethers, steryl esters, glycolipids, phospholipids) to the relatively volatile fatty acid methyl esters (FAMEs). This allows for subsequent GC-analysis (Parrish, 1999). We used 1.5 ml of a 10% (w/w) BF_3 solution in methanol (Supelco BF_3-Methanol kit) at 85 °C for 1.5 hours to derivatize the samples. This method produces equivalent or superior yields to other acid catalyzed techniques (e.g. HCl or H_2SO_4 in methanol) with no evidence of polyunsaturated fatty acid loss or artefact formation (Parrish *et al.*, 2000). The derivatization protocol is described in Appendix 3.

2.5. Analysis

2.5.1. Lipid classes

The Iatroscan® thin-layer chromatography – flame ionization detector (TLC – FID) was used to quantify lipid classes. Detailed reviews of this instrumental technique can be found in the literature (Ackman *et al.*, 1990; Ohshima and Ackman, 1991; Sebedio and Juanedo, 1991; Shantha, 1992; Parrish, 1999). The technique combines the efficiency of TLC separations and the sensitivity of the FID. Partial scanning and multiple developments allow for an extensive analysis of a single sample on a rod; up to 16 lipid classes have been identified in marine samples (Parrish, 1999). The sensitivity for any given compound depends on the proportions of ionisable carbon atoms (Ackman *et al.*, 1990; Jorgensen *et al.*, 1990) and to a certain extent on the rate of vaporization from the silica gel (Ackman *et al.*, 1990), while other factors such as planarity of the compound and molecular complexity (e.g. compounds with saturated and/or polyunsaturated acyl side chains within a lipid class) also play a part (Shantha, 1992). For lipid classes such as sterols, steryl esters, triacylglycerols, phospholipids and free fatty acids, amounts of 20 ng or less can be detected (Sebedio and Juanedo, 1991). Quantitative analysis, however, requires a careful calibration with standards that are representative of the different lipid classes of interest. This may not always be possible, so that response factors for the samples can be slightly different than for the standards. For instance, marine samples can have high concentrations of polyunsaturated lipids but only saturated standards are routinely used since suitable polyunsaturated standards are currently unavailable (Bergen *et al.*, 2000). The FID-response can also vary between sets of Chromarods and from rod

to rod within a set, although the rod-to-rod variation seems to be minimal for the Chromarods-SIII (Shantha, 1992). Care also has to be taken that the same developments are performed for the same length of time since differences in peak shapes can change the response of the FID (Ackman *et al.*, 1990). Taking all complicating factors into account, average relative standard deviations (RSDs) of about 10% for replicate analyses are considered quite good (Ackman *et al.*, 1990).

Standards and samples were manually spotted on Chromarods-SIII (blank-scanned three times) with a 25 µl Hamilton syringe (Hamilton Supplies, Reno, NV, USA) fitted with a repeating dispenser. The development was as described in Parrish (1999) and is outlined in Appendix 4. 'T Data Scan' software (1990 - RSS, Inc., version 3.10) was used to combine the three chromatograms for each rod into a single chromatogram (Fig. 2.2) and to calculate the peak areas.

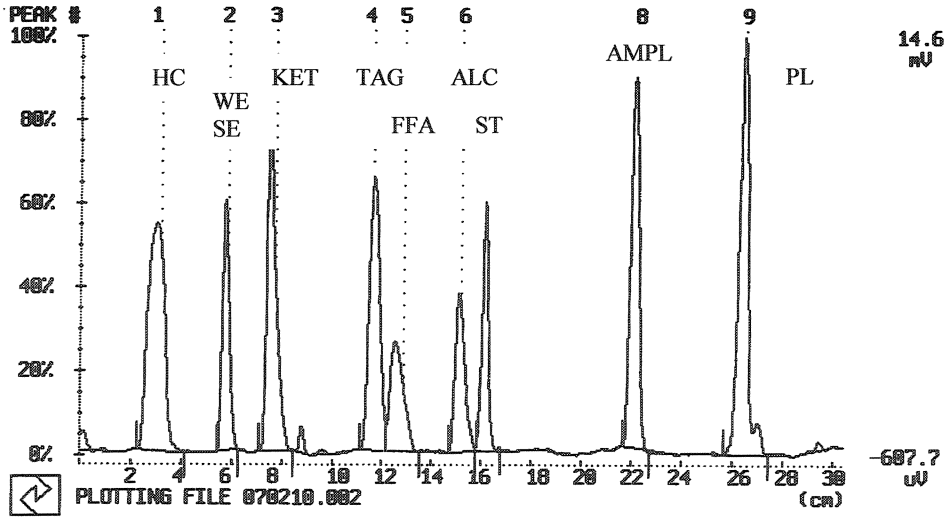


Fig. 2.3: Example of an Iatroscan® chromatogram of a 9-component standard. See Table 2.2 for abbreviations (WE: Wax Ester).

Standards (Table 2.2) were purchased from Sigma and were approximately 99% pure, except cholesteryl palmitate, which was 95% pure.

Table 2.2: Standards used for Iatroscan® calibration

Compound	**Compound class and abbreviation**
nonadecane	hydrocarbon – HC
cholesteryl palmitate	steryl ester – SE
3-hexadecanone	ketone – KET
tripalmitin	triacylglycerol – TAG
palmitic acid	free fatty acid – FFA
1-hexadecanol	alcohol – ALC
cholesterol	sterol – ST
monopalmitoyl glycerol	acetone mobile polar lipid – AMPL
phosphatidyl choline dipalmitoyl glycerol	phospholipid – PL

For each rack of 10 rods, 6 or 7-point calibration curves were prepared by applying increasing amounts (0.5 – 3.0 µl) of a standard solution containing these 9 standards in concentrations of 1 – 2 mg/ml in chloroform. Each amount was spotted on at least 3 different rods and after development and Iatroscanning, the mean of the peak areas was calculated to prepare the calibration curves. In general, linear calibration curves ($y = a + bx$) gave good correlations ($R^2 > 0.98$). However, in some instances a power curve was used ($y = ax^b$) because R^2 was slightly better. A few examples of calibration curves are presented in Appendix 5.

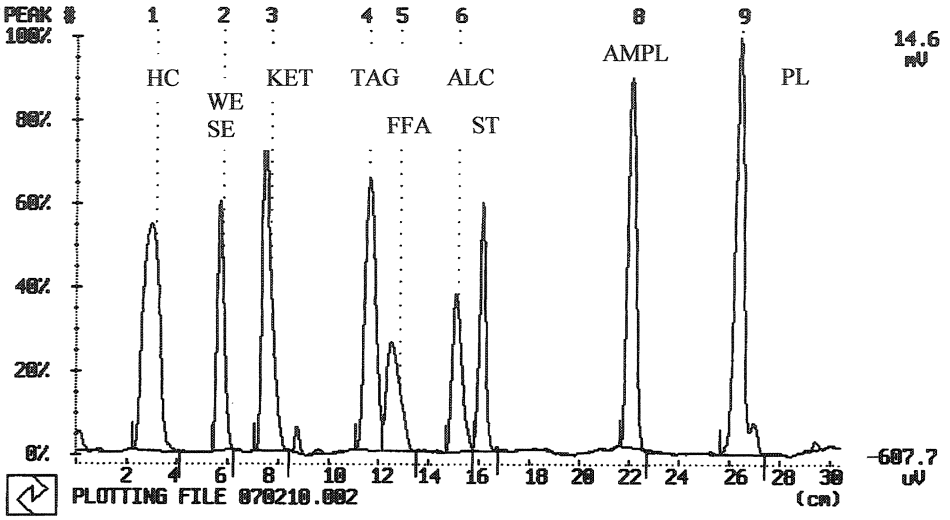
2.5.2. Fatty Acid Methyl Esters

2.5.2.1. Separation on Silica Gel

In order to clean up the samples for fatty acid determination and to allow for the possibility of hydrocarbon and sterol analyses, all the transesterified samples were subjected to column chromatography.

A small amount of lipid-cleaned glass wool was pushed from the top down into the narrow end of a Pasteur pipette and the pipette was filled with 6-7 cm (~ 0.8 g) of lipid-cleaned silica gel which had been activated for one hour at 105°C. The silica gel was added as a slurry in hexane to ensure homogenous packing. A small amount (~0.05 g) of anhydrous lipid-cleaned Na_2SO_4 was added on top of the silica gel to remove any remaining water in the sample. The transesterified lipid extract was applied and eluted with 7 ml of hexane:methylene chloride 90:10 (F1 fraction), 10 ml of hexane:diethyl ether 95:5 (F2 fraction) and 10 ml of hexane:diethyl ether 50:50 plus an additional 5 ml of diethyl ether (F3 fraction) respectively. The fractions were reduced to near dryness under a stream of N_2, transferred to 2 ml vials with hexane and reduced again to the 0.5 ml mark. They were stored at –20 °C until analysis.

Iatroscan chromatograms of the 9 component standard before and after derivatization, and after separation by a silica gel column are shown in Figs. 2.4 and 2.5 respectively.



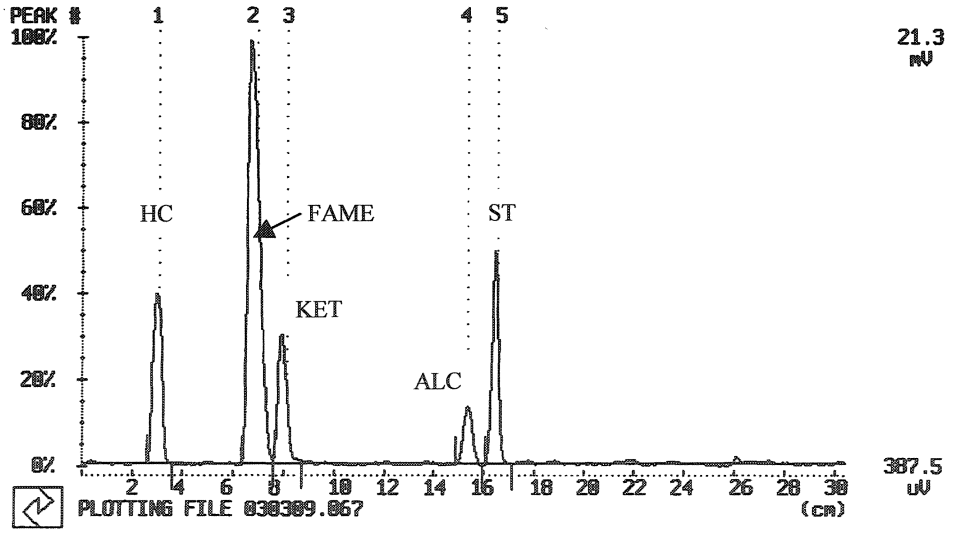


Fig. 2.4: Iatroscan® - chromatograms of a 9-component standard before (top) and after derivatization (bottom). See Table 2.2 for abbreviations.

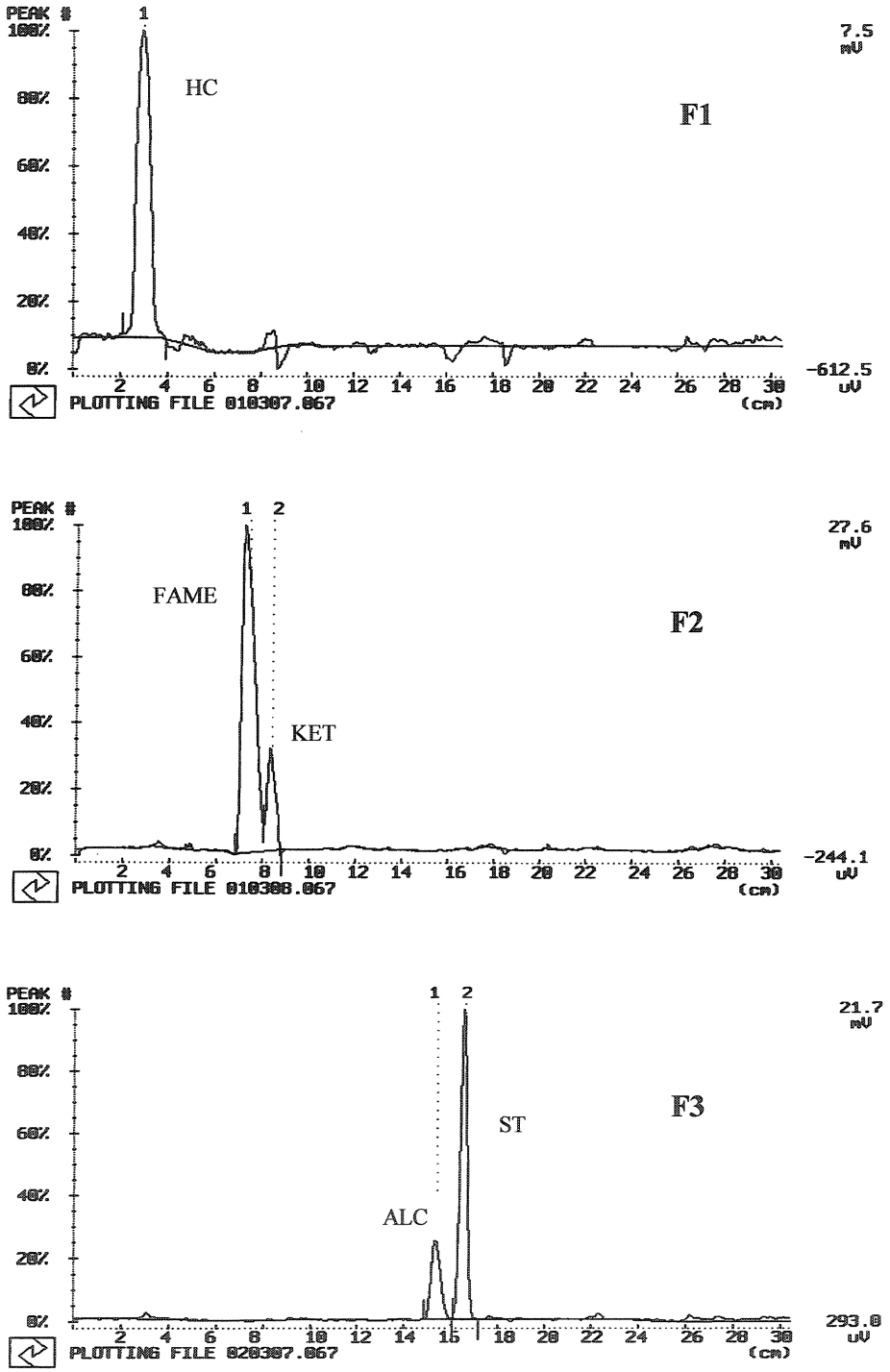


Fig. 2.5: Iatroscan® chromatograms of fractions F1, F2 and F3 of a derivatized standard, showing the complete separation of HC in F1 (top), FAME and KET in F2 (middle) and ST and ALC in F3 (bottom).

It is evident from Fig. 2.4 that the derivatization is complete: no cholesteryl palmitate, tripalmitin, palmitic acid, monopalmitoyl glycerol or phosphatidyl choline dipalmitoyl glycerol is detected in the chromatogram of the derivatized sample. The three fractions each contain the intended compound (Fig. 2.5). Any ketones and/or alcohols present will elute in F2 and F3 respectively, but this is of minor importance here.

The recovery after derivatization and fractionation was checked with a standard solution and was almost 95% for the FAME (Appendix 6).

2.5.2.2. GC-analysis

A Varian model 3400 GC equipped with a Varian 8100 autosampler was used for the fatty acid methyl ester analysis. The column was an Omegawax 320 column (cross-linked 5% phenyl methyl siloxane) with a length of 30 m, a 0.32 mm internal diameter and a 0.25 μm film thickness (Supelco, Ind., Bellefonte, PA, USA). The carrier gas was hydrogen and the flow was set at 2 ml/min. The column temperature profile was as follows: 65 °C for 0.5 min, ramping at 40 °C/min to 195 °C and hold for 15 min, ramping at 2 °C/min and holding at 220 °C for 0.75 min. The injector temperature was increased from 150 °C to 250 °C at 200 °C/min. Peaks were detected by flame ionization and the detector was held at 260 °C. The peaks were integrated using Varian Star Chromatography Software (Version 5.50) and identification was based upon retention times from Supelco standards PUFA 1 (Prod. Number 47033), PUFA 3 (Prod. Number

47085-U), Bacterial Acid Methyl Esters Mix (Prod. Number 47080-U) and 37 component FAME mix (Prod Number 47885-U).

A limited number of samples were also analyzed with a Varian Saturn 2000R 3800 gas chromatograph equipped with a mass spectrometer to confirm identified peaks. The column was a Varian CP-SIL 8 CB column with a length of 30 m, a 0.25 mm internal diameter and a 0.25 μm film thickness. The carrier gas was hydrogen and the flow was set at 1 ml/min. The injector temperature was 250 °C. The column temperature profile was as follows: 80 °C for 0.5 min, ramping at 80 °C/min to 140 °C, then ramping at 4°C/min to 200 °C, ramping at 2 °C/min to 245 °C and holding for 6.25 min. The mass range was 40 – 650 amu and electrons from the ionization source were accelerated by a potential of 70 V.

2.5.3. Plankton Analysis

Plankton analysis was performed on 2 ml of the preserved samples from August and October (one from each net tow and one from each sediment trap). The samples were observed under 200x magnification with an inverted Zeiss compound microscope using the Utermohl method (Utermohl, 1958). Biovolumes were calculated using an ocular micrometer and appropriate geometric shapes; conversion to biomass was performed as described by Booth (1993) and Strathmann (1967).

2.6. Quality Assurance and Quality Control

The ubiquity of lipids in the environment necessitates extensive cleaning procedures for all materials used in the analyses to prevent contamination. All glassware used was cleaned by rinsing three times in methanol and three times in chloroform, except for Pasteur pipettes and glass fibre filters, which were held at 450 °C for at least 3 hours. Rubber gloves (N-DEX, Nitrille) were worn during sample manipulations. Procedural blanks were performed on several occasions.

Net tow samples were subdivided for triplicate analysis. Other samples were collected in triplicate (cod, feed) or quadruplicate (sediment traps: 4 tubes per trap). For the Iatroscan® analysis, each of these samples was spotted on at least two different rods and the results were averaged. A standard containing the 9 lipid classes previously mentioned was run with every rack of 10 rods to check retention times.

For the GC-analysis, every sample was injected once and the three or four replicates of each sample were averaged.

2.7. Data Analysis

Means and standard deviations were calculated using Excel 2000 (9.0.2720). Analysis of Variance (ANOVA) was performed using SigmaStat 2.03. In some cases, the assumptions underlying ANOVA (i.e. homogeneity of variance and normal error distribution) were not met, and a non-parametric test (Kruskal-Wallis test) was performed. Groups that differed from one another were identified by a Tukey test for ANOVA and a Dunn's test for Kruskal-Wallis. They are indicated in the plots by different letters. However, it is also important to note that where no differences were observed, the results have to be interpreted cautiously, since, unfortunately, the power of our analyses (i.e. the probability of detecting a difference if there really is a difference) was usually low. This was due to small sample sizes (3 or 4 replicates) and often relatively large standard deviations.

Principal Components Analysis (PCA) and Cluster Analysis were performed using Minitab 13.31. PCA is a graphical approach to examine large databases (Meglen, 1992). It is a qualitative technique that is not a substitute for the more robust numerical procedures such as ANOVAs, but allows for a lot of information to be presented in a single graph. The aim of this procedure is to determine a few linear combinations of the original variables to summarize the data set without losing too much information. The steps involved are:

- Construction of a correlation matrix of the variables
- Eigenanalysis of the correlation matrix, which results in a column matrix of

eigenvalues (scores matrix) and a matrix of eigenvectors (coefficients or loadings matrix)

- Eigenvalues are the relative lengths of the new axes, their sum equals the total variance in the data set
- Eigenvectors determine the directions of the new axes; the coefficients are the cosines of the angles between the new and the original axes (Cliff, 1987) and are also the correlation coefficients between the variables and the principal components (Meglen, 1992)

➢ Graphical representation of the results: a plot of principal component 1 *vs* principal component 2 (PC1 *vs* PC2) is often sufficient to capture most of the information in the data set, but more plots (PC1 *vs* PC3; PC2 *vs* PC3…) can be useful. If scores are plotted, information on how well cases resemble one another is obtained: cases that plot close together have a similar composition. If coefficients are plotted, this gives information on what part of the diagram is correlated with which variables. Both pieces of information are necessary to get a complete picture of the data set.

Cluster Analysis is performed on the loadings to classify them into groups. This procedure is more objective than a classification by eye, although it is still the analyst who has to decide on the number of clusters. This is usually at the point where the similarity levels show an abrupt change. The similarity level at any step is the percent of the minimum distance at that step relative to the maximum inter-observation distance in the data.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Temperature and Salinity

The water temperature at a depth of approximately 5 m as recorded from August 25th 2001 until October 22nd 2001 by a Minilog-Tdata sensor attached to one of the fish pens is depicted in Fig. 3.1. The Minilog sensor recorded the temperature every 30 minutes and daily averages were calculated to construct the chart. The profile is almost identical to the one recorded by Green and Wroblewski (2000) in Peckham Cove (Fig. 2.1) in 1996, which was also at a depth of 5 m. Their data from the middle of August till the end of October show a gradual decrease in water temperature from 9.5 °C to 4.5 °C. By the end of December, the temperature dropped below zero and stayed below zero until the beginning of June of the following year, confirming this to be a truly 'cold water' environment.

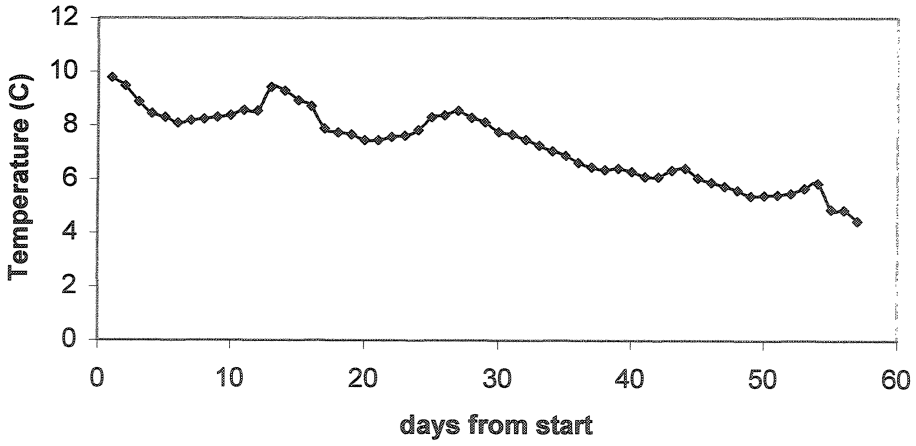


Fig. 3.1: Temperature as recorded by a Minilog sensor from late August until late October 2001

A limited number of CTD casts were performed in August 2001 at the locations of the sediment traps, giving us information about the vertical temperature profile and the salinity. Fig. 3.2 shows an example of a cast at Station 4 taken on August 3rd.

The surface water temperature was 10 °C, while the bottom water temperature was 5 °C. There was a weak thermocline at a depth of approximately 5 m. The salinity of the surface water was 26.3 psu (practical salinity units) and increased gradually to 29.8 at a depth of 22.5 m.

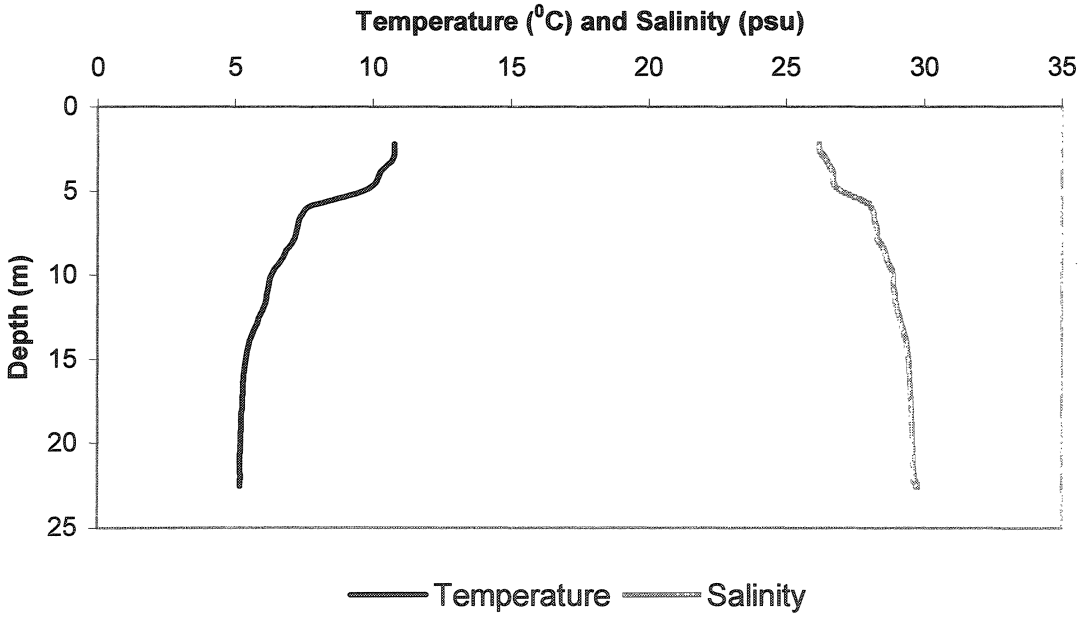


Fig. 3.2: Example of a CTD cast (Station 4)

Morris and Green (2002) did CTD casts in the Shinneys, one of the 'arms' of Gilbert Bay at the beginning of June 1997. Depending on the distance from the mouth of the Shinneys River, they found salinities of 7 to 13 psu for surface water and close to 30 psu at all

locations at a depth of more than 8 m, with a steep gradient in the upper 4-5 m of the water column. During that time of the year, there is a considerable inflow of fresh water because of melting snow. By the end of July however, river flow decreases significantly and surface waters in the bay have a salinity of more than 20 psu.

In its 'Growers Guide to Small Scale Cod Grow-Out Operations", DFO Canada (1999) recommends avoiding areas for cod aquaculture where the water temperature rises above 16 °C for prolonged periods. This would not be too much of a problem in Gilbert Bay, although the surface layer (0-1 m) can reach a temperature as high as 15 °C during the summer (Morris and Green, 2002). They also recommend 'full seawater' (32-35 ppt) salinity, which could be more problematic since the available data suggest that 30 ppt is the maximum salinity for deeper waters in Gilbert Bay. However, there is a resident cod population which seems to be doing very well under these conditions. Any location for cod aquaculture should avoid the vicinity of river mouths, however, since the increased inflow of fresh water creates a surface layer with a low salinity (< 20 psu), especially during the spring.

3.2. Nutrients

Nutrient analyses were performed on water samples collected at 5 m depth at the locations of the sediment traps on two occasions: once in August and once in October. To get a reasonable estimate of the influence of the fish pens on nutrient levels in the water column, a much more elaborate sampling protocol would have been necessary, taking into account feeding events, tides and season. For practical reasons, this was not feasible and the data presented here in Table 3.1 merely give a general indication of nutrient levels in this area at two specific occasions. The data for the five stations were pooled since there were no statistical differences between the stations ($p > 0.05$), except for the phosphate concentrations in October for which a small difference was found between S3 and S4 (Tukey-test).

Table 3.1: Summary of dissolved nutrient concentrations in Gilbert Bay. The reported values are the means ± SD of the 5 stations.

	August 2001	**October 2001**
	mean (µmol/L) ± SD	mean (µmol/L) ± SD
Phosphate	0.48 ± 0.18	0.75 ± 0.25
Silicate	4.90 ± 0.67	3.89 ± 0.84
Nitrate + Nitrite*	0.76 ± 0.26	1.14 ± 0.55

*Nitrite levels are usually small compared to nitrate levels; for instance Parrish (1998) found nitrite levels of approximately 1% relative to the sum of nitrate and nitrite.

The (nitrate + nitrite) / phosphate ratio is approximately 1.5 on both occasions, suggesting this to be a nitrogen-limited environment for phytoplankton since this figure deviates strongly from the ideal 16/1 Redfield-Richards ratio. However, as mentioned before, the sampling protocol was not elaborate enough to draw really hard general conclusions.

A correlation between distance from the cages and nutrient levels is only significant for the phosphate levels in October ($p = 0.025$), but they increase with distance from the cages. Interestingly, there is a strong positive correlation between nitrate levels and silicate levels, which is apparent both in August ($p = 0.015$) and in October ($p = 0.001$).

With the exception of some low-salinity coastal environments, dissolved inorganic nitrogen is the most important growth-limiting nutrient for phytoplankton in marine coastal waters (Gowen and Bradbury, 1987; Silvert, 1990) and the scarce available data suggest that this is also the case in Gilbert Bay during at least some parts of the year (Redfield-Richards ratio of 1.5 or less). This will restrict possible summer/fall algal blooms, even if other environmental factors such as temperature and light intensity are optimal. Fish farms are known to release considerable amounts of nitrogen compounds, mostly ammonium and some urea, both of which can be used by phytoplankton (Iwama, 1991; Gowen and Bradbury, 1987). Given a farm of big enough size, this could have an effect on plankton productivity in enclosed bays with limited flushing activity.

Fish farms do not have any known effects on silicate levels in the environment, but in any case silicate in Gilbert Bay is probably not a limiting factor in the possible development of diatom blooms. In a laboratory study, Taguchi *et al.* (1987) found no differences in

growth rates of *Hantzschia* and *Cyclotella* at silicate levels ranging from 3.1 to 49.3 µmol/L and 2.1 to 45.7 µmol/L respectively. These levels encompass our 2000 and 2001 data and if these findings are representative for other diatom species, then indeed silicate levels in Gilbert Bay will not constrain diatom development and dissolved inorganic nitrogen will be the limiting nutrient.

Nutrient availability affects the lipid composition and content of various phytoplankton (Roessler, 1990). This ability to alter lipid metabolism in response to environmental stimuli may be critical with for growth optimization and survival.

Nitrogen deficiency induces an increase in the lipid content, especially neutral lipids (triacylglycerols). This can probably at least in part be explained by the fact that storage lipids and some membrane lipids do not contain nitrogen and continue to be synthesized, while the synthesis of nitrogen containing compounds such as proteins and nucleic acids is constrained. Changes in enzymatic activity levels are also evident, however (Roessler, 1990).

Silicon is an essential nutrient for diatoms and its deficiency induces lipid accumulation in diatoms (Smith *et al.*, 1997). Laboratory studies on *Cyclotella cryptica* showed that the bulk of the lipid produced was triacylglycerol (Roessler, 1990). The activity of acetyl-Coenzyme A carboxylase, which catalyzes the rate-limiting step in fatty acid biosynthesis, increased sharply under silicon limitation, which contributed to a higher capacity for lipid synthesis.

Several field studies have reported the same correlation between lipid content and nutrient concentrations. For instance, Smith *et al.* (1997) measured nitrate + nitrite

concentrations between 4 µmol/L and < 0.5 µmol/L and silicate concentrations between 12 µmol/L and < 1 µmol/L on the continental shelf of northeast Greenland over a period of two months. Phytoplankton analysis showed a clear increase in lipid content, with the synthesis of neutral lipids and glycolipids favoured over phospholipids as nutrient concentrations declined to their minimum values.

Our data, however, do not allow us to relate any differences in lipid class composition of net tows (Section 3.4) to changes in lipid metabolism of phytoplankton, since the particulate matter collected by a net tow not only consists of phytoplankton, but also of zooplankton and detrital matter with associated bacteria. These components can be present at varying levels at different times of the year strongly influencing the lipid class composition.

3.3. Flux of Particulate Matter, Particulate Organic Matter and Total Lipids

3.3.1. Flux of Particulate Matter and Particulate Organic Matter

The flux of particulate matter per day and per unit area can be calculated from the amount of material collected in the sediment traps (Appendix 7 and Fig. 3.3).

In principle, these data can be compared either for a given station from month to month, or for a given month from station to station. Both approaches have their merits and their limitations. For instance, there can be seasonal differences in the variables we measure, so comparing the data for each station from month to month can reveal effects that have nothing to do with the influence of the fish pens. However, these effects will most likely be the same at every station, especially the 4 stations at the fish pens, since they are within only 100 m of each other. In an ideal situation with little or no seasonal influences, observed differences between the 3 months can be attributed to the presence of the fish pens, especially if they are attenuated further away from the pens (this means that we also have to compare the results from station to station). On the other hand, if data are compared for a given month from station to station, there could have been differences between the stations at the outset of the experiment. Depending on the magnitude of these differences and the way they are influenced by the fish farm activities, responses to the disturbance may not be detectable.

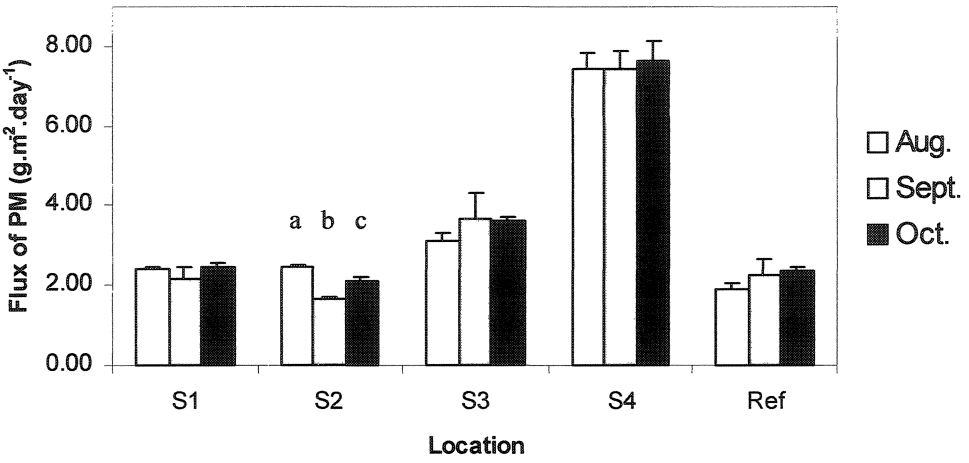


Fig. 3.3: Flux of particulate matter (PM) on a transect away from the fish pens during August, September and October. Error bars are 1 SD; n = 3 or 4; Different letters indicate $p < 0.05$.

The flux of particulate organic matter can be calculated from the ash-free dry weight of the material collected in the sediment traps by multiplying the values of Fig. 3.3 by [1– (ash-free dry weight / dry weight)] (Fig. 3.4).

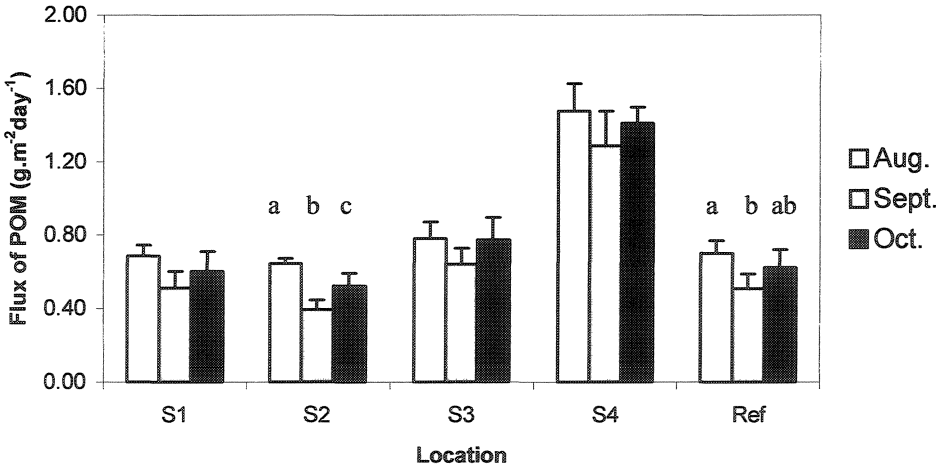


Fig. 3.4: Flux of particulate organic matter (POM) on a transect away from the fish pens during August, September and October. Error bars are 1 SD; n = 3 or 4; Different letters indicate $p < 0.05$.

The focus here will be on the flux of particulate organic matter (Fig. 3.4), since qualitatively the plot looks very similar to the flux of particulate matter. During August, the fish were being put in the pens, but were not yet fed. One could expect a higher flux during September and October because of the input of fish feed and feces, but this is not the case. This means that any influence of the fish pens is masked by the natural (seasonal) variability. A One-way ANOVA with a 5% significance level reveals statistical differences between the 3 months at S2 and at the Ref station (S1: $p = 0.056$; S2: $p < 0.001$; S3: $p = 0.132$; S4: $p = 0.251$; Ref: $p = 0.040$). The September flux is consistently lower at all stations, suggesting a bay wide effect.

It is noteworthy that there is a trend towards an increased flux moving away from the fish pens (S1→S4), which is evident for every month. This is probably related to resuspension caused by a stronger current further out in the open water, although we cannot substantiate this by current velocity measurements. The fluxes for S4 are approximately twice as high as for the other locations and the results of other analyses (e.g. lipid and fatty content and composition) further indicate that this station is very different from the others (see next sections).

There are few publications on the amount of particulate matter collected under marine fish cages and any data of course heavily depend on the biomass of fish present, the depth under the cages at which the particulate matter is collected, and the currents. Most authors estimate values between 5 and 50 $g.m^{-2}.day^{-1}$ (Iwama, 1991). For instance, Gowen and Bradbury (1987) estimated a flux of 28 $g.m^{-2}.day^{-1}$ directly underneath (input

of both feces and uneaten feed) and 8 $g.m^{-2}.day^{-1}$ in the vicinity (input of only feces) for a 50,000 kg salmonid farm. To put this in perspective: our fish enclosure had less than 150 kg of fish, which is only 0.3% of the amount of fish in the fish farm investigated by Gowen and Bradbury. If we extrapolate their flux of 8 $g.m^{-2}.day^{-1}$ to our situation, the value would be 8 x 0.003 = 0.024 $g.m^{-2}.day^{-1}$. This is only a small fraction of the natural flux of approximately 2 $g.m^{-2}.day^{-1}$ (Fig. 3.3) and it is not surprising that we cannot see any influence of the fish enclosure on the flux of particulate (organic) matter.

It is worthwhile mentioning that after a couple of weeks the pens were completely covered with brown filamentous algae (*Ectocarpus siliculosus* and *Pilayella littoralis)*, which could have acted as a filter, preventing the escape of particulate matter from the pens. Both species are common on rocks throughout Newfoundland and Labrador and are considered common fouling organisms on nets and other introduced substrates.

3.3.2. Flux of Total Lipids

Iatroscan analysis of the particulate matter collected in the sediment traps (Section 3.5) allows the calculation of the total lipid content of the organic material in the traps by summing all lipid classes and taking into account the ash-free dry weight[1] (Fig. 3.5). Combining this with the flux of particulate organic matter (Fig. 3.4), the flux of total lipids can be calculated (Fig. 3.6). See Appendix 8 for an example.

[1] A plot of the lipid content relative to the dry weight qualitatively looks similar to Fig. 3.5 and is omitted.

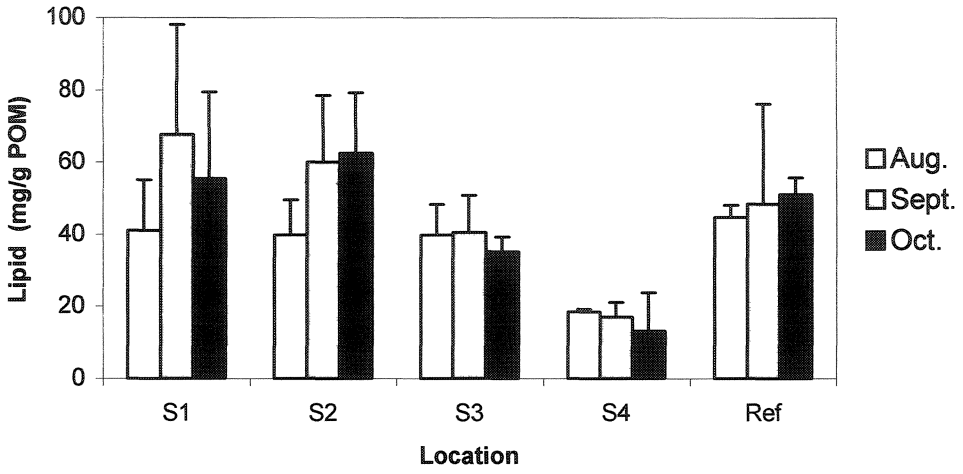


Fig. 3.5: Lipid content of particulate organic matter (POM) collected by the sediment traps. Error bars are 1 SD, n = 3 or 4.

There are no significant differences between months with respect to the lipid content of the particulate organic matter collected by the sediment traps at any of the stations (Fig. 3.5). There is, however, a highly significant correlation ($p < 0.01$) between distance from the cages on the transect (S1, S2, S3 and S4), and lipid content of organic matter during all three months. Since this includes the reference situation (August - no feeding), this suggests an influence independent of the fish pens.

Henderson *et al.* (1997) analyzed lipids in sediments underneath and on transects away from a salmon fish farm (600,000 kg of fish) that was in use for approximately 2 years. They found higher levels of total lipid within 15 m to 20 m on either side of the cages, especially in the top 5 mm of sediment. This is consistent with lipids being present as a component of waste from the fish farm that has settled on the seabed. Although they analyzed sediments and not particulate matter collected by sediment traps, which makes their data not directly comparable to ours, it does mean that for some cases it is possible

to relate higher proportions of total lipid in sediments to the presence of a fish farm. Determining factors are obviously the size of the fish farm, depth underneath the cages and current velocities.

The difference between S4 and the other stations is smaller for the flux of total lipids (Fig. 3.6) because the flux of organic material is higher at S4 than at any other station. Because of the relatively high standard deviations, no statistical differences are found in lipid flux between different months at any of the stations ($p > 0.05$) and there is also no correlation between distance from the cages and the flux of lipids ($p > 0.05$). However, because of the high variance in the data, the power of the analysis was low.

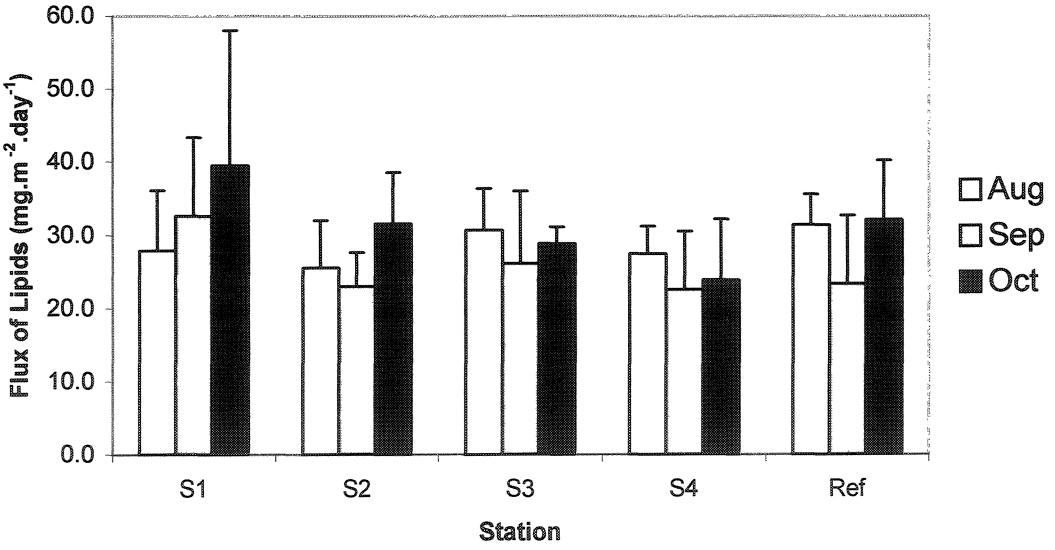


Fig. 3.6: Flux of total lipids at different stations. Error bars are 1 SD, n = 3 or 4.

There are few indications that the fish enclosure has an effect on the total lipid content of the material collected by the sediment traps or on total lipid fluxes, suggesting that these data are dominated by natural fluxes. Total lipid fluxes are of similar magnitude as those measured by Parrish (1998) at near shore locations in Trinity Bay, which ranged from approximately 10 – 30 $mg.m^{-2}.day^{-1}$.

3.4. Net Tows

The purpose of collecting the net tow samples was to get an idea of the composition of the particulate matter (plankton and detrital matter) in the water column. The advantage over sediment traps is that they are 'fresh' samples, while in sediment traps (depending on how long they are deployed) decomposition can occur. This sometimes does not allow for identification of plankton. A trade-off is that net tow samples are 'snapshots': they are data collected at a particular moment in time, unlike sediment traps, which integrate data over a period of time.

3.4.1. Plankton Analysis

For practical reasons, net tow samples were only collected in August and October (Table 2.1). Their composition shows some clear seasonal differences (Table 3.2).

Table 3.2: Composition of August and October net tows as a percentage of total biomass. Dino: dinoflagellates; Zoo: zooplankton; FecPel: fecal pellets

Station	August				October			
	Diatoms	Dino	Zoo	FecPel	Diatoms	Dino	Zoo	FecPel
S1	65	34	1	0	8	36	0	56
S2	60	14	0	26	0	1	96	3
S3	90	10	0	0	0	9	89	2
S4	66	23	1	10	0	23	53	24
Ref	81	9	0	10	1	5	93	1

In August, diatoms dominate at all stations, but to varying degrees. For instance, at S1 their biomass is approximately twice as high, while at S3 and at the reference location they are 9 times more abundant. Zooplankton is a very minor component at S1 and S4 and fecal pellets are found at S2, S4 and at the reference location.

In October, zooplankton is abundant at stations S2, S3, the reference location and to a lesser degree S4. Stations S1 and S4 also have important proportions of dinoflagellates and fecal pellets.

The general conclusion is that August net tows are dominated by diatoms, while the October net tows are dominated by zooplankton (except for S1 for which fecal pellets have the biggest contribution).

3.4.2. Total Lipid and Lipid Classes

Total lipid in August net tows ranged between 13.9 and 20.9 mg/g dry weight, which was substantially less than the 53 to 75.3 mg/g dry weight in October (Fig. 3.7). Although for both months there was a statistically significant difference between stations (One-way ANOVA, $p = 0.022$ for August and 0.027 for October), the differences were small. In August, the mean lipid content of the net tow at S2 was somewhat higher, which makes this station different from S3 and the reference location. In October, only S3 and the reference location were different from each other.

When expressed per gram organic matter, the total lipid content varied between 29.8 and 43.1 mg for August and 105 and 283 mg for October (Fig. 3.8). There was no significant

difference between the stations in August ($p = 0.45$), but there was one in October between S1 and the reference station ($p = 0.038$).

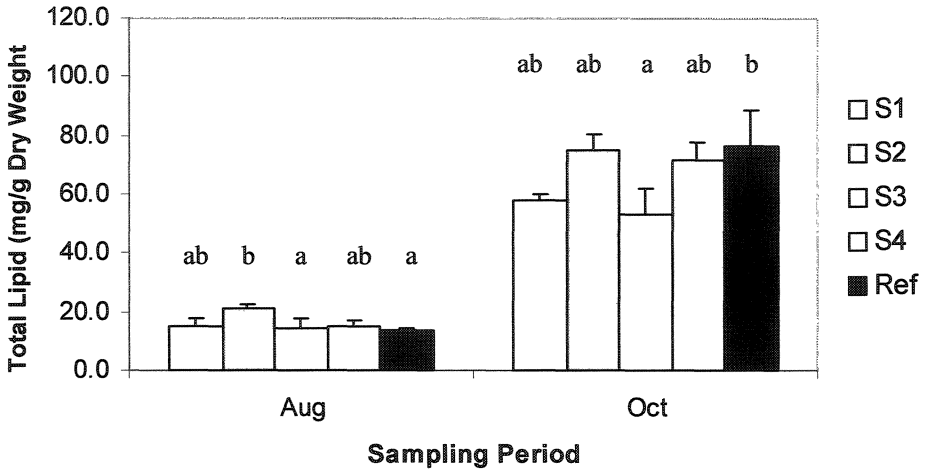


Fig. 3.7: Total lipid in mg per gram dry weight in August and October net tows. Error bars are 1 SD, n = 3. Different letters indicate $p < 0.05$.

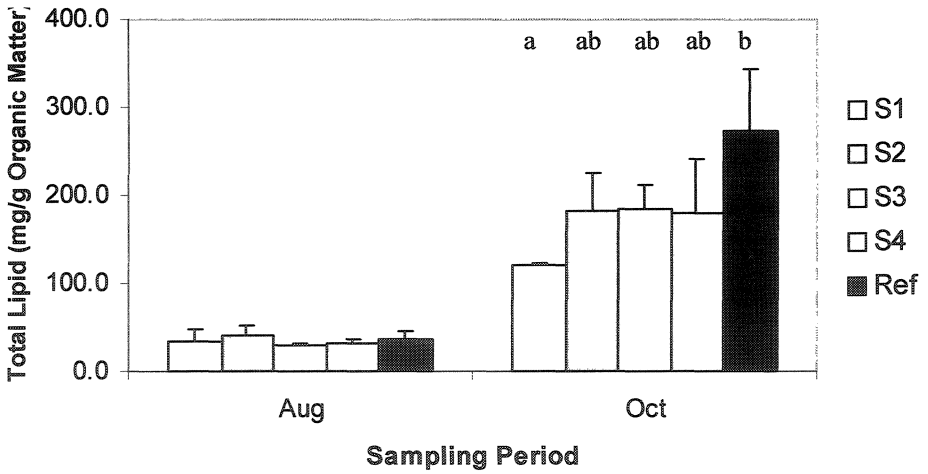


Fig. 3.8: Total Lipid in mg per gram organic matter in August and October net tows. Error bars are 1 SD, n = 3 (2 for S1). Different letters indicate $p < 0.05$.

With a few exceptions, the differences in the lipid class compositions between stations for a given month were minimal (Fig. 3.9).

The main differences in August were that the percentage of free fatty acids was somewhat higher at stations S2 and S3, that alcohols were only detected at S1 and that the percentage of AMPL was higher at the reference location.

For October, the situation was somewhat more complicated, but except for the higher percentage of wax esters/steryl esters and the lower percentage of phospholipids at stations S3 and S4, and the lack of alcohols at station S2 and at the reference station, the differences were minimal.

Blanks (filtered seawater) showed negligible amounts of all lipid classes, except for hydrocarbons, which at one occasion were present at levels comparable to the ones measured in the October net tows. It is likely that at least some of the hydrocarbons detected originated from fuel from outboard motors. This may even have been from the boat used for sampling, since sometimes fuel was observed on the water surface and could have been intercepted when hauling in the plankton net, although precautions were taken to avoid this.

A comparison of the lipid class composition of the net tows between August and October immediately shows the complete absence of wax esters/steryl esters in August and the relatively high amounts (20 - 33%) in October (Table 3.2). This is related to the presence of zooplankton during this period. Zooplankton biomass is dominated by copepods in most parts of the oceans and they can have as much as 80% of their dry

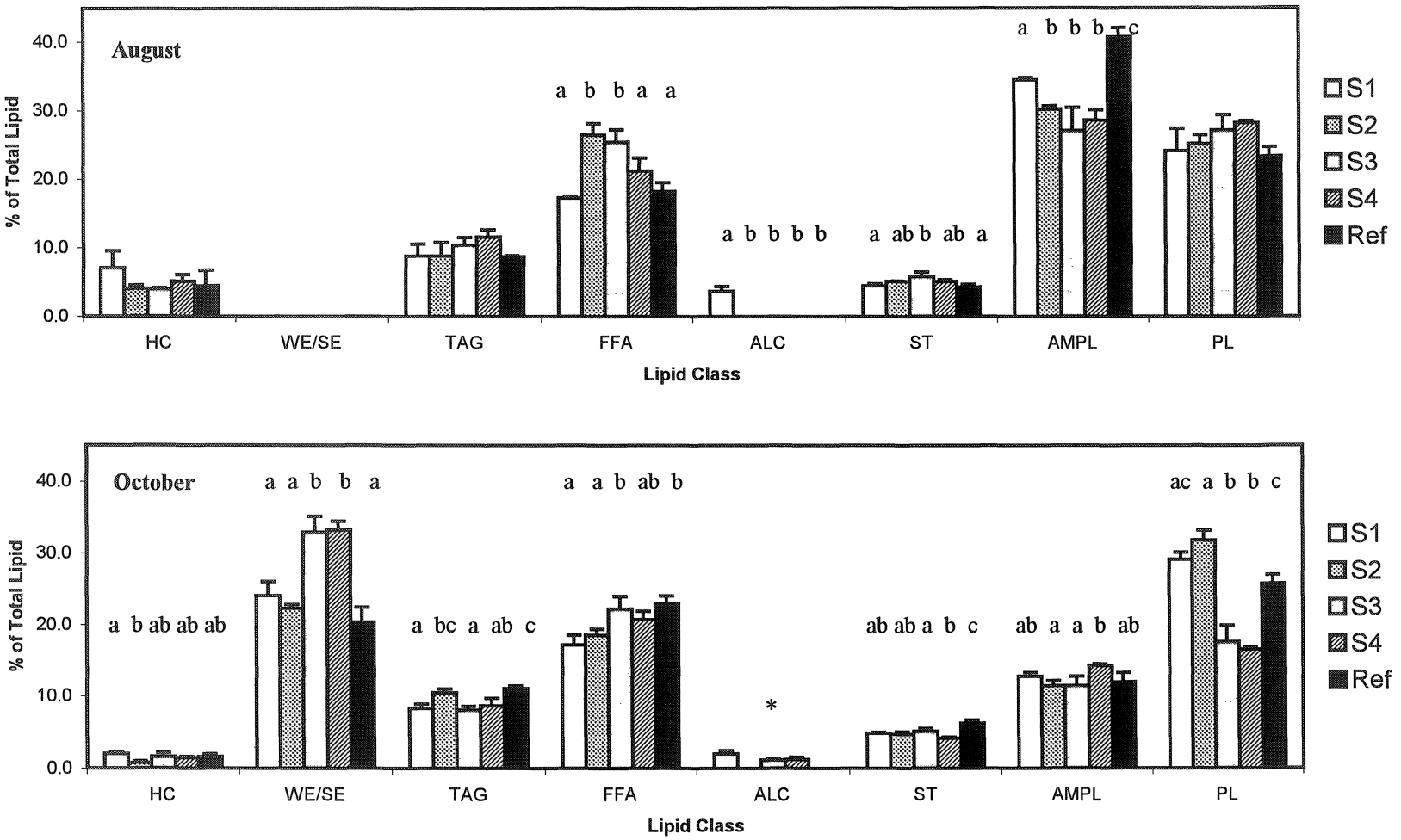


Fig. 3.9: Lipid composition of August (top) and October (bottom) net tows. Error bars are 1 SD, n = 2 or 3. Missing bars indicate < 1% or not present. Different letters indicate $p < 0.05$. *: $p = 0.018$ but Dunn's test fails to identify groups that differ from one another.

weight as wax esters (Sargent *et al.*, 1977; Albers *et al.*, 1996). Although no attempt was made to identify zooplankton species, copepod nauplii and juveniles dominated the zooplankton biomass (Dr. C. McKenzie, pers. com., 2003). Although no zooplankton was found in the sample collected at S1, significant amounts of wax esters can be released into the environment in the form of fecal pellets (56% of the biomass at S1) from animals that consume wax ester rich diets (Sargent *et al.*, 1977; Scott *et al.*, 2002).

Free fatty acid levels are relatively high, with values of 17 - 26%. Such high levels indicate acyl lipid hydrolysis. Net tow samples taken in the same area in 2000 show free fatty acid levels between 12 -19%, which are just slightly lower (Copeman and Parrish, 2003). It is not unusual to find lipid degradation products in a natural environment, indicating lipid turnover. However, these values are probably maximal ones, as the remote sampling location precluded taking the entire range of precautions to avoid lipolysis after sample handling: treatment with boiling water to deactivate lipolytic enzymes (Budge and Parrish, 1999), followed by immediate extraction and storage at –80 °C.

3.4.3. Fatty Acid Composition

The fatty acid composition of the net tows is shown in Appendices 9 (August) and 10 (October). For ease of comparison, a selection of the quantitatively more important fatty acids and sums or ratios of fatty acids that are used as biomarkers are shown in Figs. 3.10, 3.11 and 3.12 (see also Table 1.1).

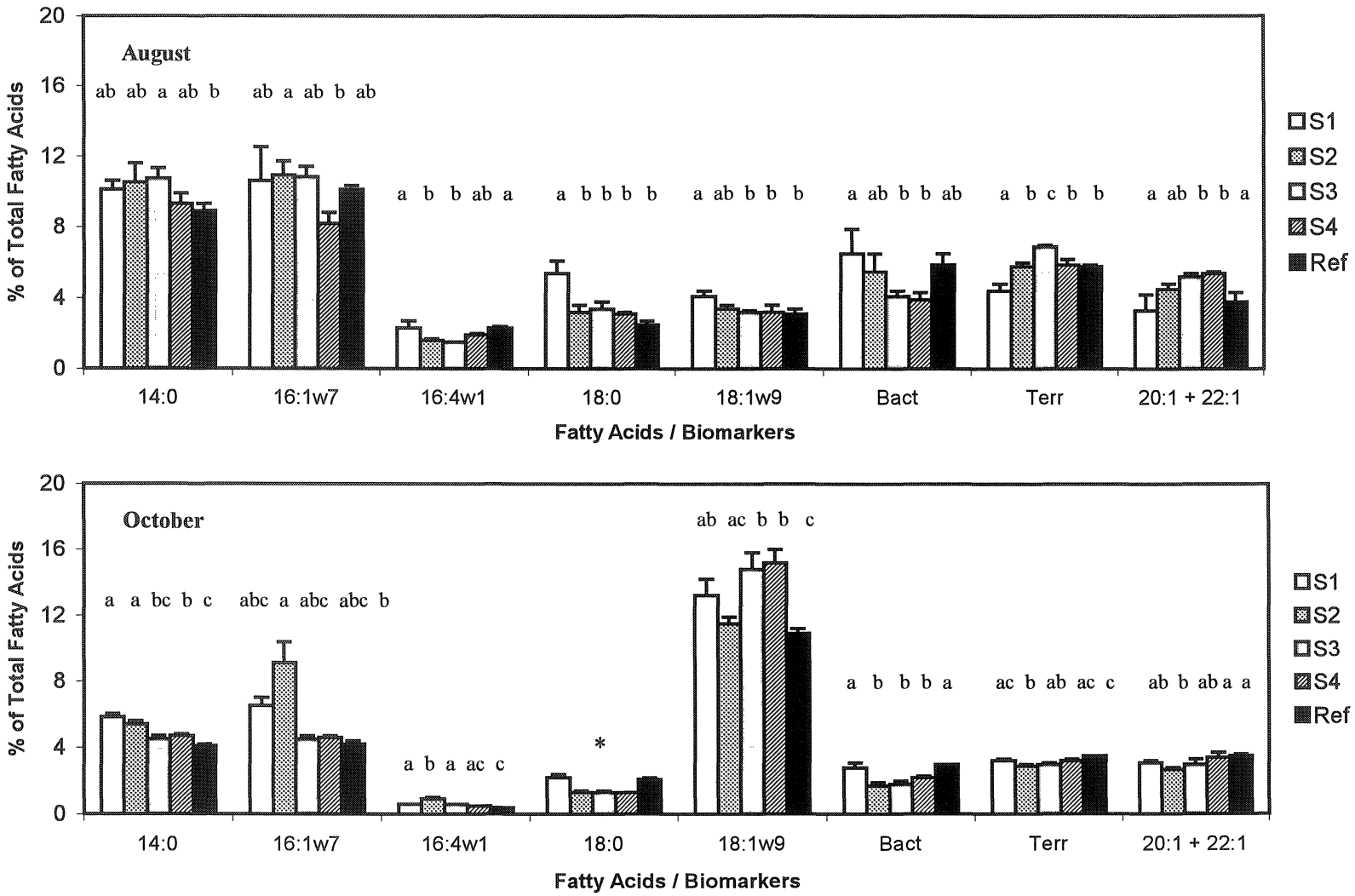


Fig. 3.10: Selected fatty acids / biomarkers in August (top) and October (bottom) net tows. n = 2 or 3, error bars indicate 1 SD. Different letters indicate $p < 0.05$. * $p = 0.041$ but Dunn's test fails to identify groups that differ from one another.

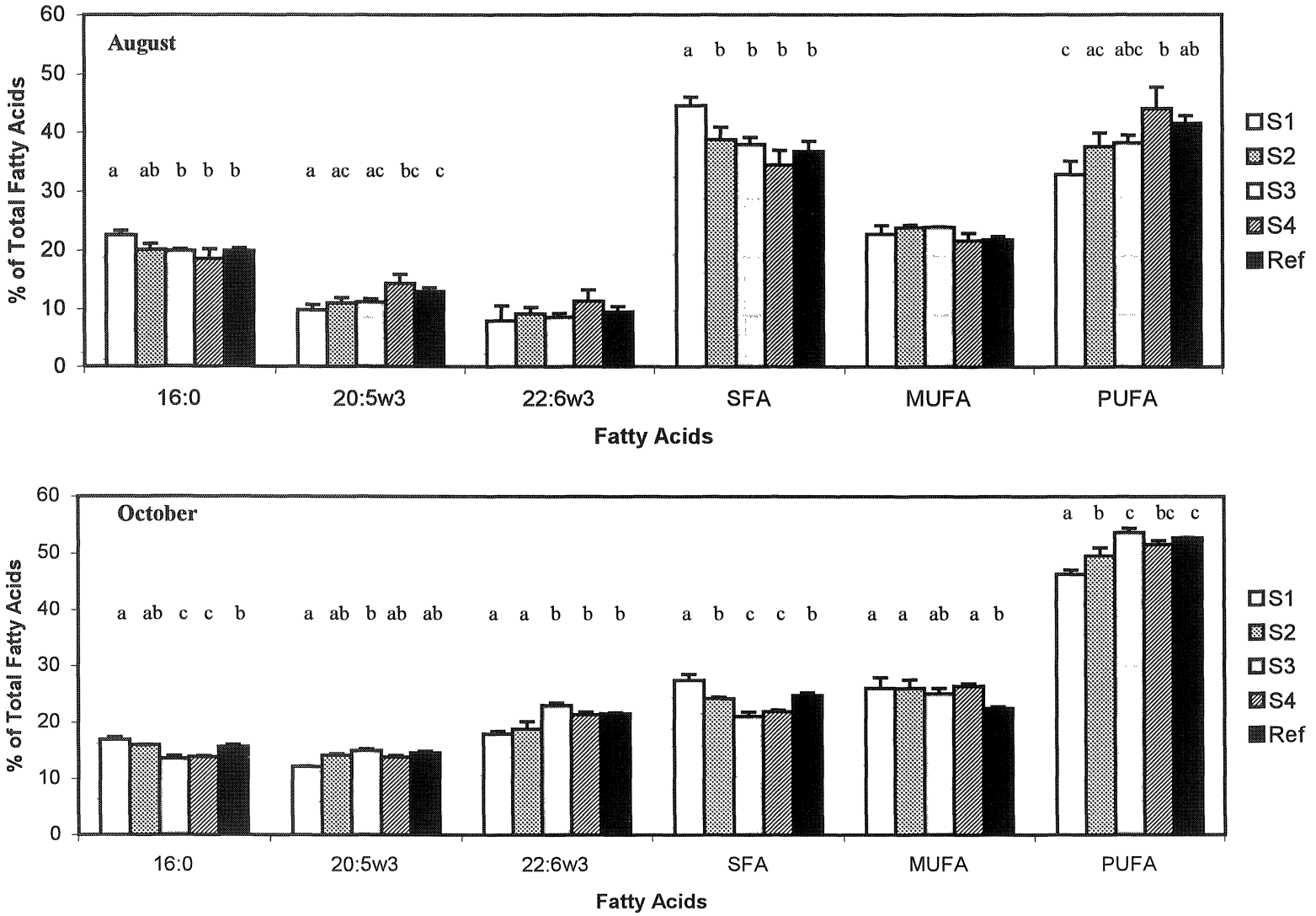


Fig. 3.11: Selected fatty acids in August (top) and October (bottom) net tows. n = 2 or 3, error bars indicate 1 SD. Different letters indicate $p < 0.05$. SFA: sum of saturated fatty acids; MUFA: sum of monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids.

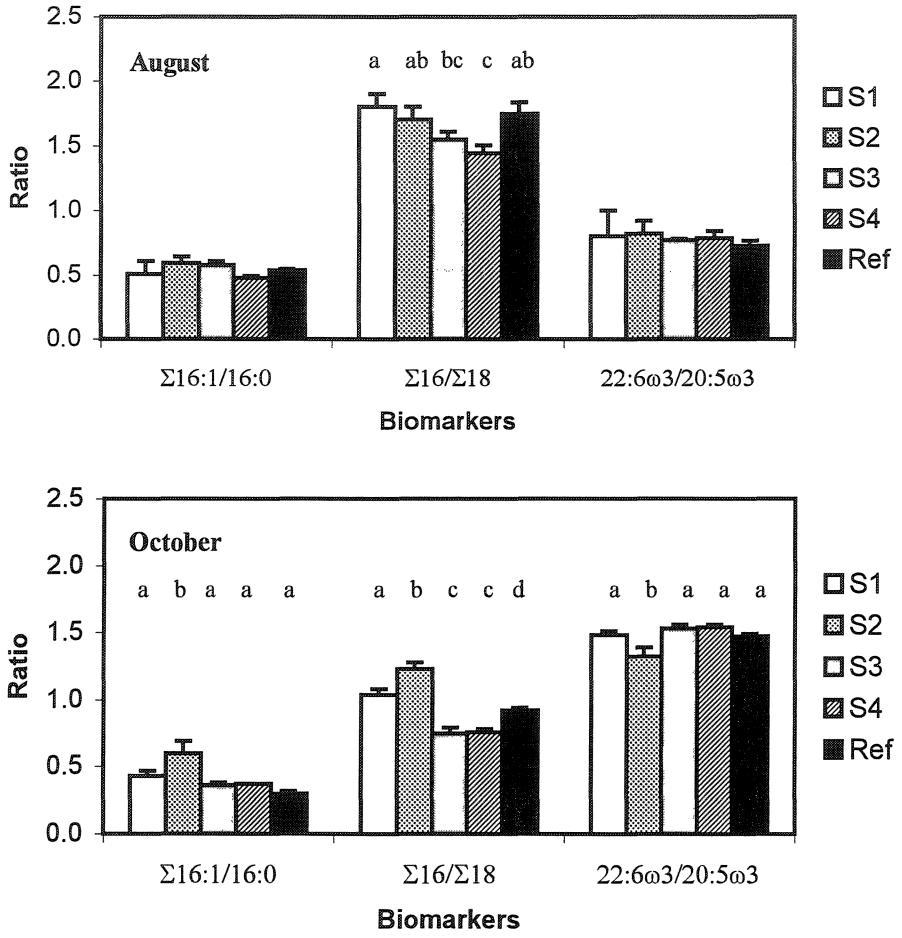


Fig. 3.12: Fatty acid ratios used as biomarkers for August (top) and October (bottom) net tows. n = 2 or 3, error bars indicate 1 SD. Different letters indicate $p < 0.05$.

For both months, there are differences between the stations for most of the fatty acids. For instance, in August the proportion of 18:0 is significantly higher at S1 than at the other stations, and in October this is the case for 16:1ω7 at S2. However, differences between the two months are more important and can be related to the composition of the net tows (Table 3.2).

In August, diatoms are abundant (Table 3.2) and this is reflected in higher values at all stations for Σ16/Σ18 (mean for the 5 stations: 1.65 ± 0.15[1] as opposed to October: 0.93 ± 0.20; $p < 0.001$), 16:4ω1 (1.93 ± 0.38 and 0.61 ± 0.20 respectively; $p < 0.001$) and Σ16:1/16:0 (0.54 ± 0.06 and 0.41 ± 0.12 respectively; $p = 0.001$).

Several studies report rather wide ranges for these biomarkers, even when one plankton group dominates the biomass. For instance, Budge *et al.* (2001) found maximum values for Σ16/Σ18, 16:4ω1 and Σ16:1/16:0 during a diatom bloom of 4.5, 4.0 and ~2, respectively. Reuss and Poulsen (2002), on the other hand, report values between 0.5 and 1.0 for the first two markers for a diatom bloom (the value for 16:4ω1 is not mentioned). These large variations can be caused by different dominating diatom species and by different environmental conditions (e.g. nutrient availability, temperature, light). As an example, *Thalassiosira pseudonana* cultures grown in different light regimes had Σ16:1/16:0 ratios ranging from 1.06 to 2.30 (Reuss and Poulsen, 2002). This means that a fixed limit for any of these biomarkers as an indicator for the dominance of one plankton group is difficult to determine and emphasises the need for a microscopic evaluation.

The 22:6ω3/20:5ω3 ratio has been used as a biomarker for the dominance of dinoflagellates over diatoms (Budge and Parrish, 1998). In this study, the value of this marker is 0.78 ± 0.03 in August and 1.47 ± 0.09 in October ($p < 0.001$), which could lead to the conclusion that dinoflagellates are more abundant in October than they are in August. This is indeed the case, but in stations S2 and S3 and in the reference location,

[1] Values for fatty acids or biomarkers mentioned in the remainder of this section are the mean ± standard deviation of the 5 stations.

dinoflagellates comprise less than 10% of the total biomass. In these stations, zooplankton dominates and could significantly contribute to the 22:6ω3 pool, increasing the 22:6ω3/20:5ω3 ratio. For instance, Lee *et al.* (1971) found that 22:6 fatty acids in the phospholipids of the copepod *Calanus helgolandicus* are synthesised from shorter unsaturated precursors, and made up 36.5% of the phospholipid fatty acid pool, which itself comprised 44% of the total lipid pool.

The proportion of 20:1 + 22:1 fatty acids is surprisingly lower in October (3.1 ± 0.3%) than in August (4.4 ± 0.9%) ($p < 0.001$), since this sum has been used as an indicator of zooplankton, especially copepods. However, not every copepod species has large amounts of these fatty acids, as shown by Saito and Kotano (2000), who report proportions between 1.0% and 3.4% for the sum of 20:1 and 22:1 isomers in four species of boreal calanoid copepods. Albers *et al.* (1996) also found species of copepods with as little as 1% for the sum of 20:1 and 22:1 fatty acids. In fact, it is often the alcohol part of the wax ester that has a large proportion of a 20:1 or 22:1 chain. Upon digestion by zooplankton consumers such as herring, sardines etc., the alcohol moiety is oxidized to the corresponding fatty acid and this accounts for the preponderance of 20:1 and 22:1 fatty acids in these fish (Sargent *et al.*, 1977).

An indication that the wax esters in this study contributed a significant part of PUFA (22:6ω3?) comes from the Iatroscan® chromatograms. The wax ester peak is split into two peaks (Fig. 3.13 top), which is often observed when a lipid class consists of both saturated / monounsaturated and polyunsaturated species (Parrish *et al.*, 1992). Upon

hydrogenation of the sample, a simple procedure which consists of adding a small amount of platinum (IV) oxide as a catalyst and bubbling hydrogen gas through the sample, the second peak merges with the first one (Fig. 3.13, bottom).

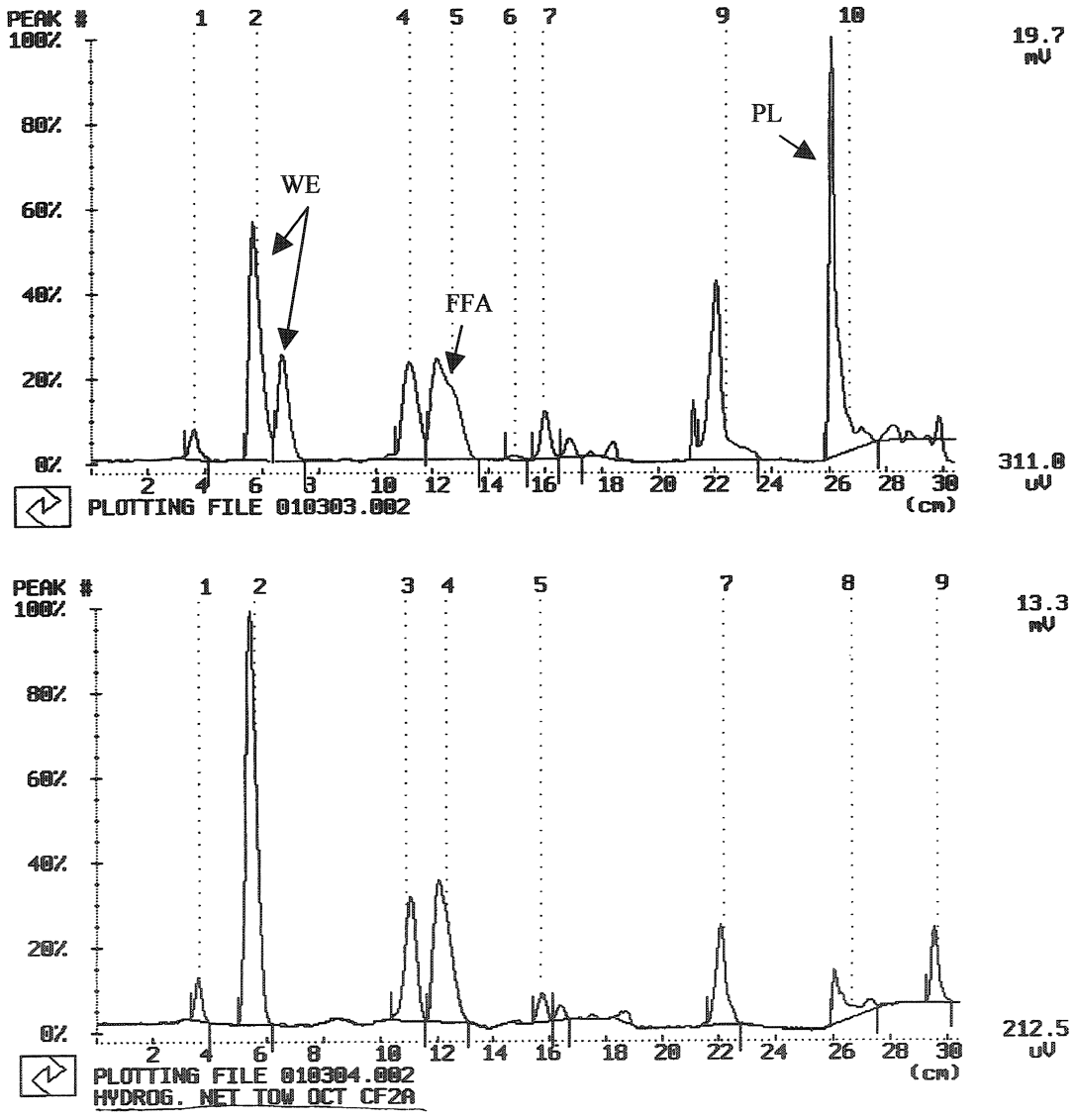


Fig. 3.13: Iatroscan® chromatogram of an October net tow at S2, with a split wax ester (WE) peak (top). After hydrogenation, there is only one WE peak (bottom). Notice also the shoulder in the FFA peak, indicating polyunsaturated FFA (top), which disappears after hydrogenation. The polar PL is almost completely lost during the procedure, probably because of the lower solubility of hydrogenated PL in chloroform.

The proportion of 18:1ω9 is 3.4 ± 0.4% in August, increasing to 13.1 ± 1.9% in October ($p < 0.001$). Its presence has been shown to correlate well with small autotrophic flagellates (< 10 μm) (Reuss and Poulsen, 2002), but they cannot be the source here since the mesh size of the plankton net was 20 μm and such small organisms would not be retained. Other authors suggest that high values of this fatty acid are caused by detrital matter (Scott *et al.*, 2002) or may reflect an animal input into the fatty acid pool (Falk-Petersen *et al.*, 2000). Detrital matter is probably not the main cause for such a large increase in 18:1ω9 here, since bacterial levels, expected to be positively correlated with decaying matter, are lower in October than they are in August (see below). Furthermore, a concomitant increase in 18:0 and 18:2ω6, which Scott *et al.* (2002) also associate with detrital matter, is not observed here. Zooplankton (and zooplankton fecal matter) however, abundant in the October net tows, is a likely candidate as an 18:1ω9 source. Several species of copepods can have large proportions of this fatty acid in their wax esters. *Metridia longa* for instance, has a proportion of 31.6% of 18:1ω9 relative to other fatty acids in its wax esters (Falk-Petersen *et al.*, 1990).

Bacterial markers are higher in August than in October (5.2 ± 1.3% and 2.3 ± 0.6% respectively, $p < 0.001$) and this also holds true for terrestrial markers (5.8 ± 0.8% and 3.2 ± 0.2% respectively, $p < 0.001$). The values for August are high compared to other studies. For instance, in net tow samples from Trinity Bay (Newfoundland), the sum of bacterial fatty acids never exceeded 2% (Budge and Parrish, 1998). Since the maximum depth of the water column at the stations where net tow samples were collected was only

25 m, there was probably some resuspension of bottom sediments and the plankton net was also collecting some degraded material from the sediments. Furthermore, none of the stations was further than 120 m away from the densely forested shore with a small stream emptying into the cove, which makes some terrestrial input likely. Budge *et al.* (2001) observed the same phenomenon in their net tow samples from Barred Island Cove, which had 3 - 6% of bacterial and 1 - 4% of terrestrial markers. The lower values for October found here are probably related to the fact that the high lipid content of these samples (Figs. 3.7 and 3.8), originating from the zooplankton, decreased the relative contribution of other fatty acid sources.

The sum of SAT, MUFA and PUFA is also different for both months, with lower proportions of SAT and higher proportions of MUFA and PUFA in October ($p < 0.001$ in all cases). This can for the most part be attributed to the much higher levels of 18:1ω9 and 22:6ω3 (see above).

A PCA was performed on 28 individual fatty acids from Appendices 7 and 8 and the composite variable 'bact', which sums all the bacterial fatty acids using all the replicates at each station (29 cases and 29 variables). This allowed for 69.4% of the total variation in the dataset to be accounted for by PC1 and PC2. Fig. 3.14 clearly shows the difference between the samples taken in August, which plot on the right side of the PC1 axis, and the ones taken in October, which plot on the left side (Fig. 3.14, top). The left side of the PC1 axis is correlated with higher levels of fatty acids that are more abundant in the October net tows and can be associated with zooplankton (e.g. 18:1ω9, 22:6ω3, 20:4ω3

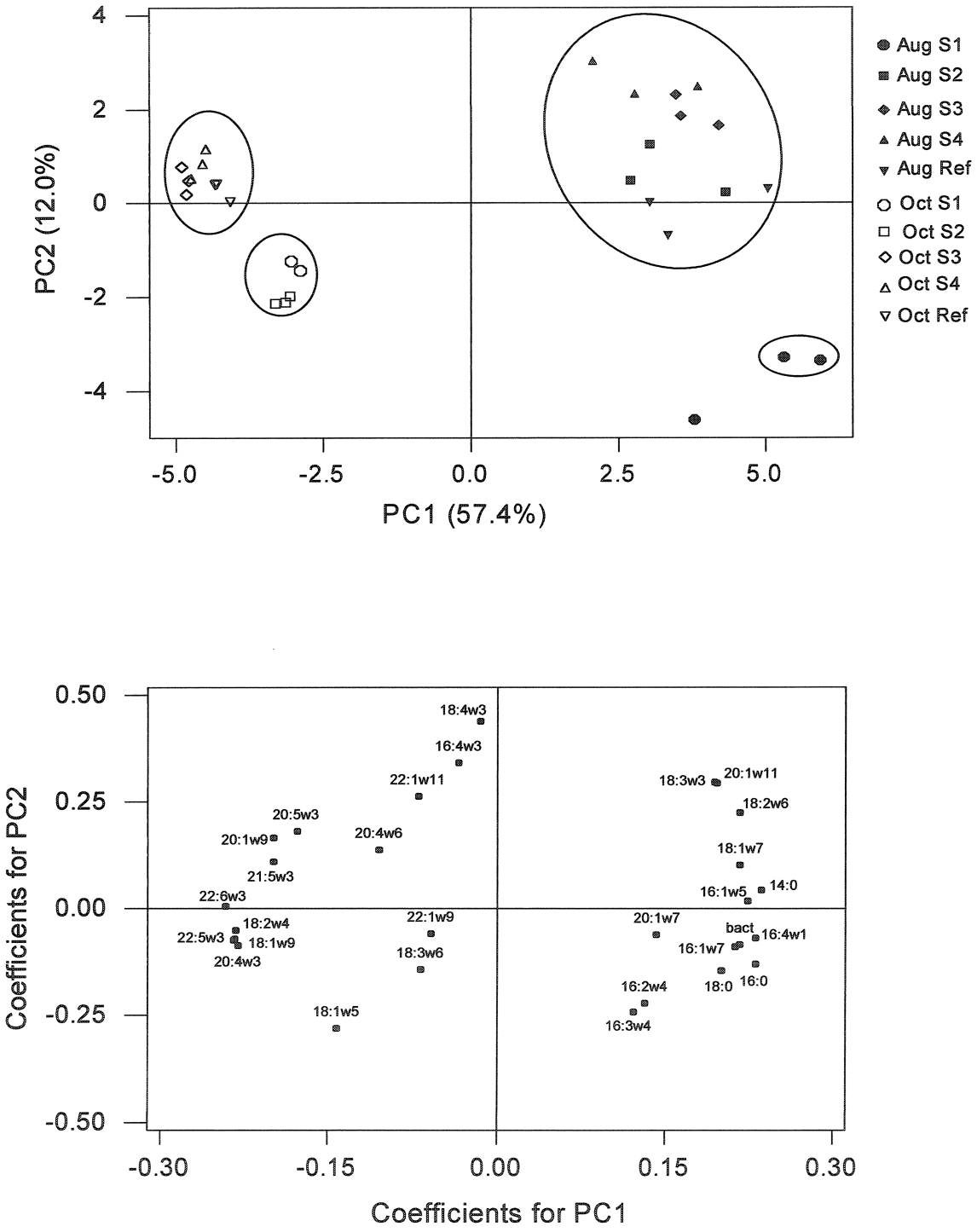


Fig. 3.14: PCA of net tow fatty acids with loadings (top) and coefficients (bottom)

and 18:2ω4 – the latter is found at levels of only 0.2% in October but is not present at all in August) (Fig. 3.14, bottom). The right side of the PC1 axis is correlated with higher levels of 16:0, 14:0, 16:4ω1, 18:1ω7 (phytoplankton / diatom fatty acids) and bacterial fatty acids, which are found at higher proportions in August net tows. This side of the PC1 axis also has less PUFA.

A cluster analysis performed on the loadings indicates that 5 clusters can be distinguished (1 of them consisting of just one data point). This means that there is some variation in the data within each month, which was also evident from Fig. 3.10 – 3.12. In particular, the net tow at S1 in August seems to be somewhat different from the other stations during that month because it plots at the negative side of the PC2 axis while the other samples all plot close together at the positive side. This could be related to the fact that this was a vertical net tow, although the net tow for S1 in October (also a vertical net tow) plots very close to S2, a horizontal net tow. The negative side of this axis is correlated with somewhat higher levels of especially 16:2ω4, 16:3ω4 and 18:1ω5. The C16 PUFA can be found at relatively high proportions in diatoms (Scott *et al.*, 2002), which dominate at all stations in August. However, from Appendix 7 it can be seen that the reference location has the same percentage of 16:2ω4 and an even higher percentage of 16:3ω4 than S1 (1.5% *vs* 1.2% at S1). It is possible that it is the lower percentage for 18:1ω5 that prevents this station from plotting closer to S1, but the origin of this fatty acid is not clear. It has to be mentioned though that the variation along the PC2 axis is only 12.0% of the total variation in the data set, which is substantially less than the 57.4% along the PC1 axis, and thus is less important.

3.5. Sediment Traps

3.5.1. Plankton Analysis

Unlike the net tow samples, a complete floristic analysis was not performed, since all the sediment trap samples consisted mainly of detrital matter. All of the October samples, but especially S4, also contained resuspended benthic diatoms (pennates), indicating a significant input from resuspended matter.

3.5.2. Lipid Classes

The lipid class composition of the particulate matter collected by the sediment traps is not that different at S1, S2, S3 and the reference location during the month of August, except that the percentage of TAG is approximately twice as high at the reference location than at the other stations (Fig. 3.15). Station S4 is clearly different, with no WE/SE and ALC, lower FFA levels and on average somewhat higher AMPL and PL levels. It has already been mentioned in Section 3.3.1 that there are many differences in variables measured at this station compared to the other stations.

The data for September show a similar pattern to those for August, with one major exception: the percentage of FFA at S1 is almost twice as high as at the other stations. The large standard deviations of S2 and S3 are caused by one of the tubes of the sediment traps at these locations having a FFA content comparable to S1 (~ 40%), while the other 3 tubes had much lower FFA levels (~ 15%).

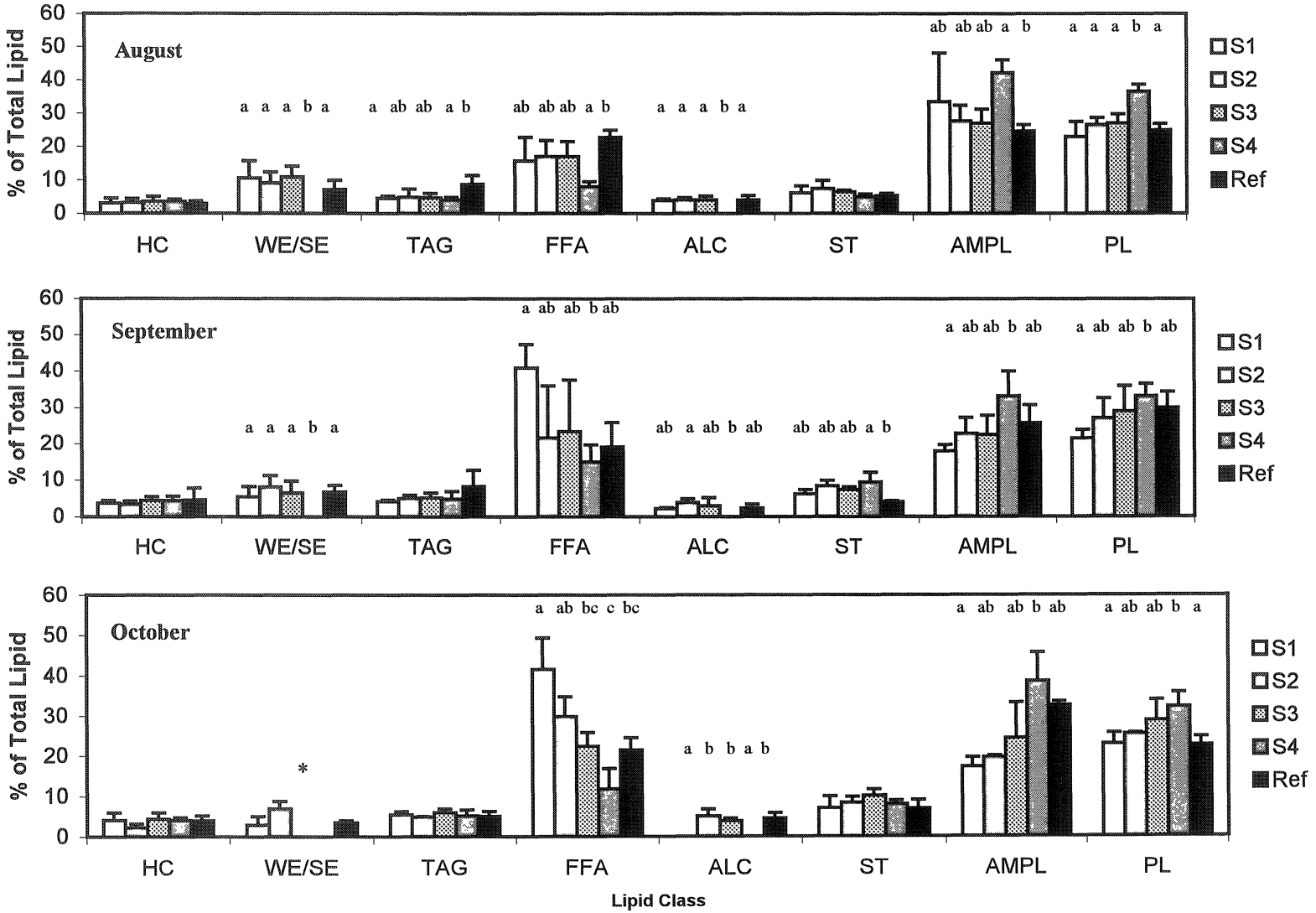


Fig. 3.15: Lipid class composition of August (top), September (middle) and October (bottom) sediment traps. Error bars indicate 1 SD, n = 3 or 4. Missing bars indicate < 1% or not present. Different letters indicate $p < 0.05$. *: $p = 0.010$ but Dunn's test fails to identify groups that differ from one another.

The October data are very similar to the September data, except that WE/SE and ALC are not present at S3 and S1 respectively. FFA levels again are very high at S1 and to a lesser extent also at S2.

Since any effect of the fish pens would be most obvious at S1 and would be attenuated moving away from the pens, it seems that the only discernable effect is an increase in FFA levels, which does not clearly extend beyond S1. This is most obvious for the October data, where there is a significant difference between FFA levels at S1 and all the other stations except for S2. In September, the unusually high standard deviations of FFA levels at S2 and S3 prevent the detection of any statistically significant differences. Although for AMPL and PL there seems to be a trend for decreasing proportions closer to the fish pens in September and October, there are no statistical differences between S1, S2 and S3[1] at any month. The same holds true for HC, TAG and ST, which are present at (much) lower levels. For WE/SE and ALC, there are differences in percentages at S1, S2 and S3 in October, but there is no real trend (levels are highest at S2).

If the data at each station are compared from month to month, the increase in FFA levels in September and October at S1 is highly significant ($p < 0.001$) (Fig. 3.16). There are only a few other lipid classes for which there is a significant difference at some stations. For instance, there is a difference in WE/SE levels between August and October at S1 and S3 ($p = 0.042$ and 0.010 respectively) and in PL levels between September and

[1] For ease of comparison, we disregard S4 here since this station has already been proven to be somewhat of a special case. Furthermore, all the available literature suggests that even for the biggest fish farms the influence on the sediment does not extent further than 50 m from the cages (S4 is at 100 m).

October at the reference location ($p = 0.019$). These differences however seem not to be related to the fish farm.

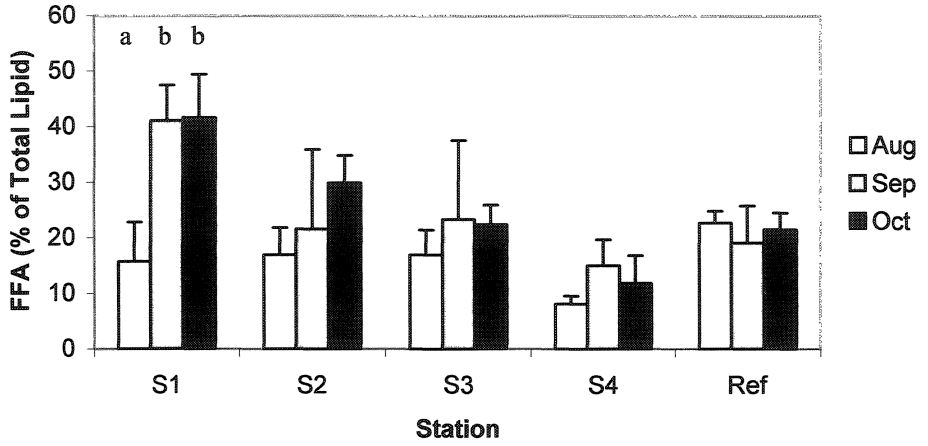


Fig. 3.16: FFA levels as % of total lipid in August, September and October at the different stations. n = 3 or 4, different letters indicate $p < 0.05$.

In conclusion, the only clear difference is higher FFA levels at S1 during September and October. This makes a causal relationship with the fish pens very likely. Two possible explanations can be put forward to explain this increase in FFA levels. First, they can arise from the hydrolysis of lipids in the particulate matter collected by the sediment traps (uncaptured fish feed, fish feces and the 'natural' input consisting of plankton and detrital matter). The fish feed consisted mainly of TAG (Table 3.3) and cod feces can have up to 30% relative to total lipid as the sum of TAG, diacylglycerols (DAG) and monoacylglycerols (MAG) (Lie *et al.*, 1987). Hydrolysis of uncaptured feed lipids and feces would indeed yield substantial amounts of FFA. However, the intermediate degradation products DAG and MAG (the latter being AMPL) were either not detected or were not present at elevated levels compared to other stations. Second, FFA can also arise

directly from the fish feces, since approximately 70% of the lipids in cod feces are FFA (Lie *et al.*, 1987). It is not possible here to distinguish between these sources, but it seems a reasonable assumption that both contribute.

Henderson *et al.* (1997), in a study on the lipid composition of sediments on a transect away from a salmon fish farm, found elevated levels of FFA, TAG, HC + WE/SE, ST and PL closer to the fish farm. They found maximum amounts for all lipid classes around 0.4 mg/g wet sediment. For comparison, Fig. 3.17 shows the amount of FFA at the different locations in this study expressed in mg/g dry weight (particulate matter), instead of as a percentage of total lipids (Fig. 3.16). Qualitatively, the plot looks very much like

Table 3.3: Total lipid and lipid class composition of fish feed (mean ± SD).

	Fish Pellets Haddock Grower (n = 3)	**Herring *Clupea harengus* (n = 3)**
Total Lipid (% of wet weight)	11.8 ± 0.6	6.1 ± 1.8
Total Lipid (% of dry weight)	12.8 ± 0.6	23.4 ± 6.7
Lipid composition (% of total lipid)		
Hydrocarbons	1.1 ± 0.4	0.7 ± 0.5
Triacylglycerols	73.2 ± 2.7	82.5 ± 8.6
Free Fatty Acids	6.6 ± 0.6	8.9 ± 4.0
Sterols	4.3 ± 0.3	-
Diacylglycerols	-	0.9 ± 1.4
Acetone Mobile Polar Lipids	5.0 ± 1.0	2.4 ± 1.5
Phospholipids	10 ± 1.9	4.6 ± 2.0

Fig. 3.16. The FFA levels during August at S1, S2 and S3 are remarkably similar around 1.8 mg/g dry weight. In September and October, their levels increase by a factor of almost 3.5 at S1 and there is also an increase (though not significant because of the high standard deviations) at S2, while they remain the same at S3, S4 and the reference location. It has to be stressed though that a comparison with their data is hard to make since their values are expressed per wet weight and they did not use sediment traps but analyzed the sediment directly. Furthermore, the farm they investigated had a biomass of 600,000 kg (~ 2,000 times the size of our fish farm), which is probably why they found differences in the levels of all lipid classes investigated. The point here is that no matter how FFA levels are expressed, they are significantly higher at S1 as a result of the fish enclosure activities.

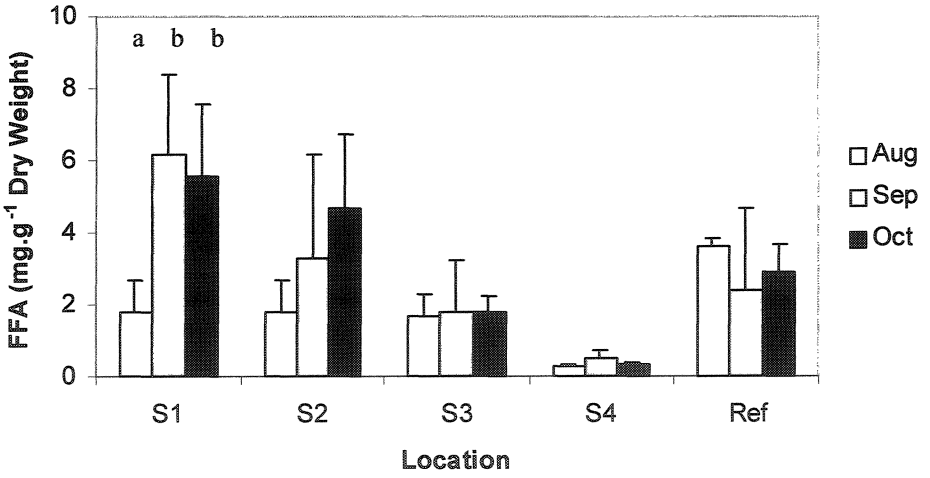


Fig. 3.17: FFA levels in mg/g dry weight in August, September and October at the different stations. n = 3 or 4, different letters indicate $p < 0.05$.

3.5.3. Fatty Acid Composition

The fatty acid composition of the sediment traps is given in Appendices 11-13. For ease of comparison, some of the quantitatively more important fatty acids and sums or ratios of fatty acids that are used as biomarkers are shown in Figs. 3.18 - 3.20.

As before with the lipid class composition, some differences can be observed between the stations for each month. In August, the differences between the stations are small, except for the fatty acids 18:1ω9, 20:3a (location of double bonds not known) and 22:6ω3. The fatty acid 20:3a was tentatively identified based upon its molecular ion (320 amu) and retention time on an Omegawax 320 column (~ 13.6 min, which is between the retention times of 20:3ω6 and 20:3ω3). Several major fragments with the same amu as 20:3ω3 were observed (e.g. 67, 79, 121, 135, 163 and 177). Its proportion is somewhat erratic, as can be seen from the relatively high standard deviations. With a few exceptions, other fatty acid levels are within the range for nearshore sediment traps deployed in Trinity Bay, Newfoundland in 1994 and 1995 (Budge and Parrish, 1998). At S4, the biomarkers Σ16:1/16:0 and Σ16/Σ18 are significantly higher than at the other stations, indicating a higher proportion of diatoms.

The September and October data in general are very similar, with the exception of 20:3a, which is present at higher proportions at some of the stations in October. Consistent throughout September and October, however, are the increased proportions of 20:1ω9, 22:1ω9 and 22:1ω11 at S1. The sum of these fatty acids makes up over 50% of the total

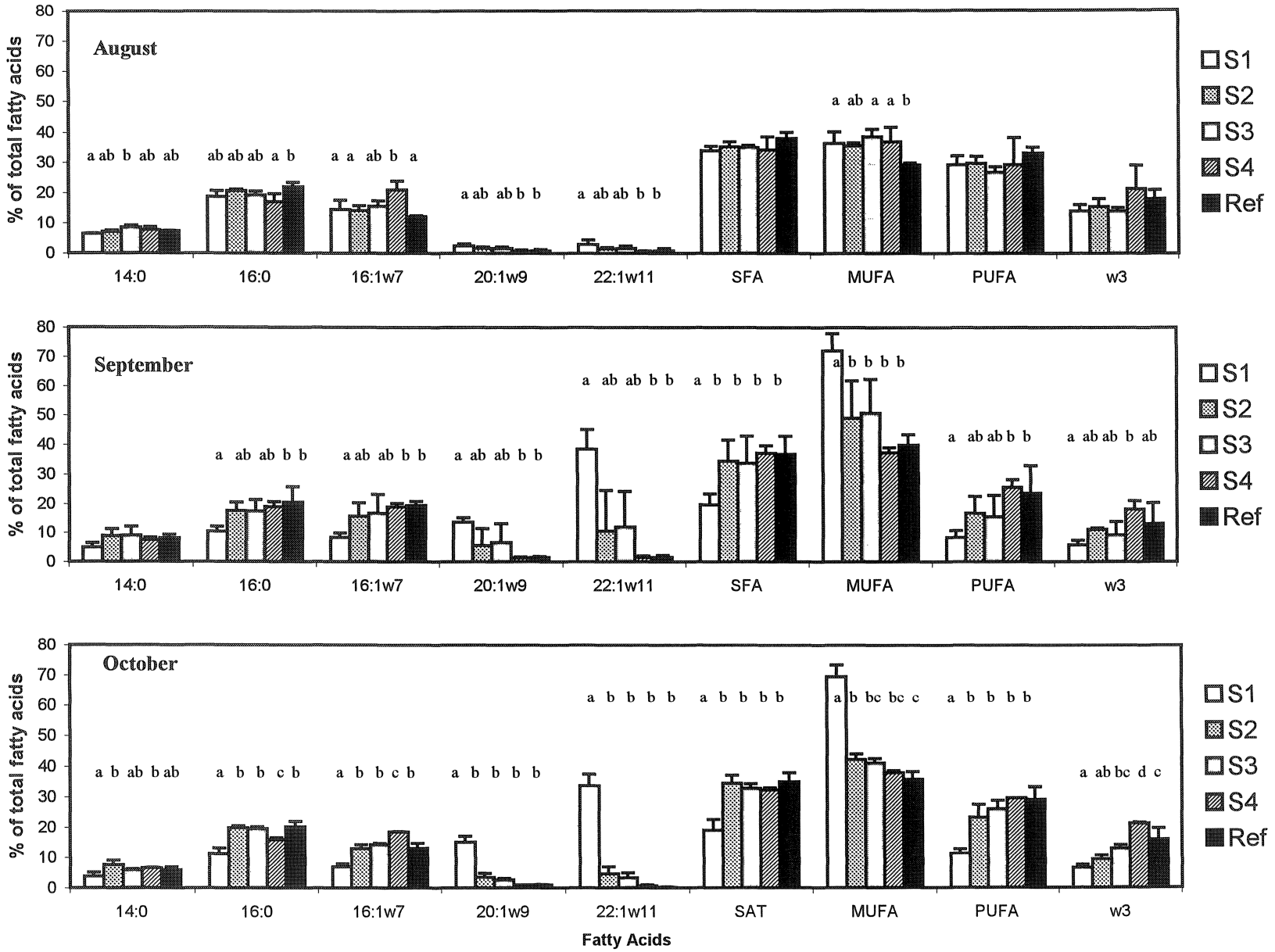


Fig. 3.18: Selected fatty acids in August (top), September (middle) and October (bottom) sediment traps. n = 3 or 4, error bars indicate 1 SD. w3: sum of ω3 fatty acids. Different letters indicate $p < 0.05$.

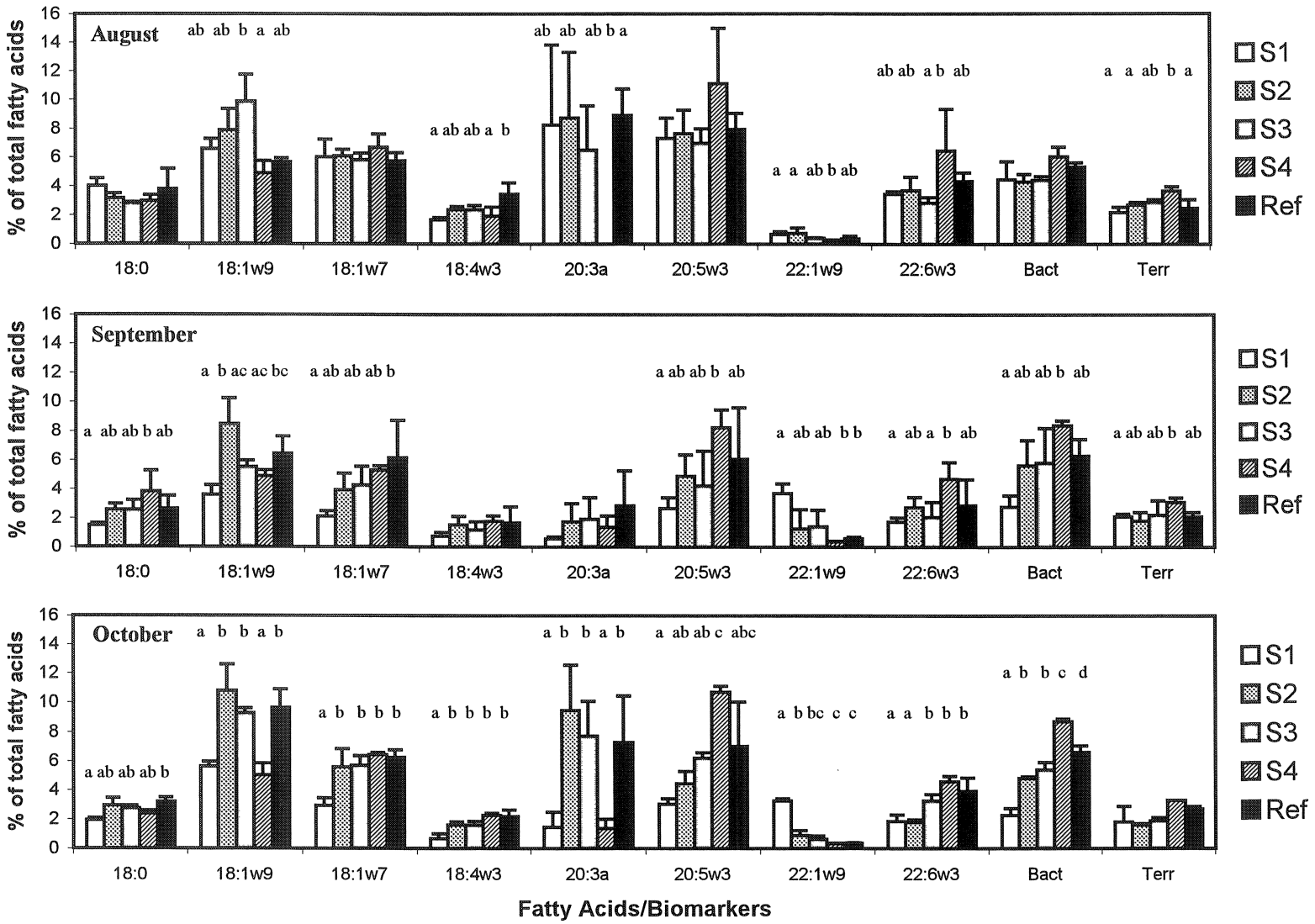


Fig. 3.19: Selected fatty acids/biomarkers in August (top), September (middle) and October (bottom) sediment traps. n = 3 or 4, error bars indicate 1 SD. Different letters indicate $p < 0.05$.

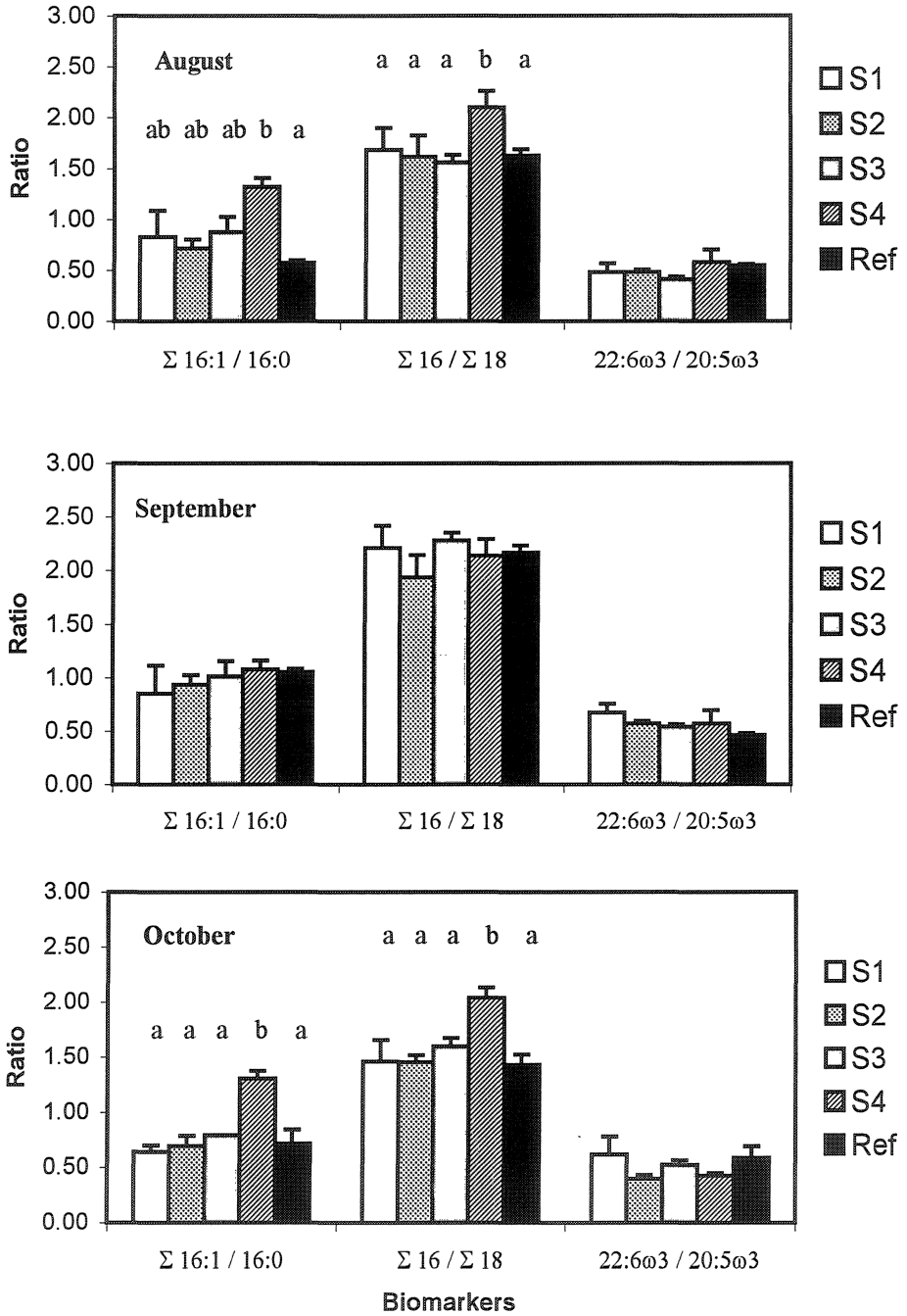


Fig. 3.20: Fatty acid ratios used as biomarkers for August (top), September (middle) and October (bottom) sediment traps. n = 3 or 4, error bars indicate 1 SD. Different letters indicate $p < 0.05$.

amount of fatty acids at this station. Such a high percentage evidently has a large influence on all other fatty acid proportions, which are usually lower at S1, except for the sum of MUFA which of course is increased. As was discussed in Section 3.5.2, FFA levels at S2 and S3 in September had relatively high standard deviations. The same phenomenon is observed here for 20:1ω9, 22:1ω9 and 22:1ω11, which suggests that these fatty acids were present as FFA in the environment and not as part of an acyl lipid.

Another way of looking at the data is the comparison between the different months. This makes the exceptionally high values of the aforementioned fatty acids at S1 stand out even more clearly (Fig. 3.21). They occur when the fish enclosure is in operation and only at S1, the location closest to the fish pens. This makes a causal relationship with the fish farm very plausible and their origin is most likely the herring that was used as feed. The focus is on herring since it comprised 75% of the feed, whereas the artificial feed was only 5% of the total, which is negligible in this context. The remaining 20% of the feed was capelin, which was not available for analysis. However, capelin is a planktivorous species, like herring, and has a fatty acid composition very similar to herring (Table 2 in Budge *et al.*, 2002). The most abundant fatty acids in herring are 22:1ω11 (23.1 ± 9.9%) and 20:1ω9 (15.3 ± 6.0%), while 22:1ω9 (1.4 ± 0.8%) is a minor component (Appendix 14). Two of the specimens had levels of 22:1ω11 and 20:1ω9 of 28% and 18% respectively, while for the other one this was only 11% and 8% respectively, indicating that the lipid signature of the feed can be highly variable.

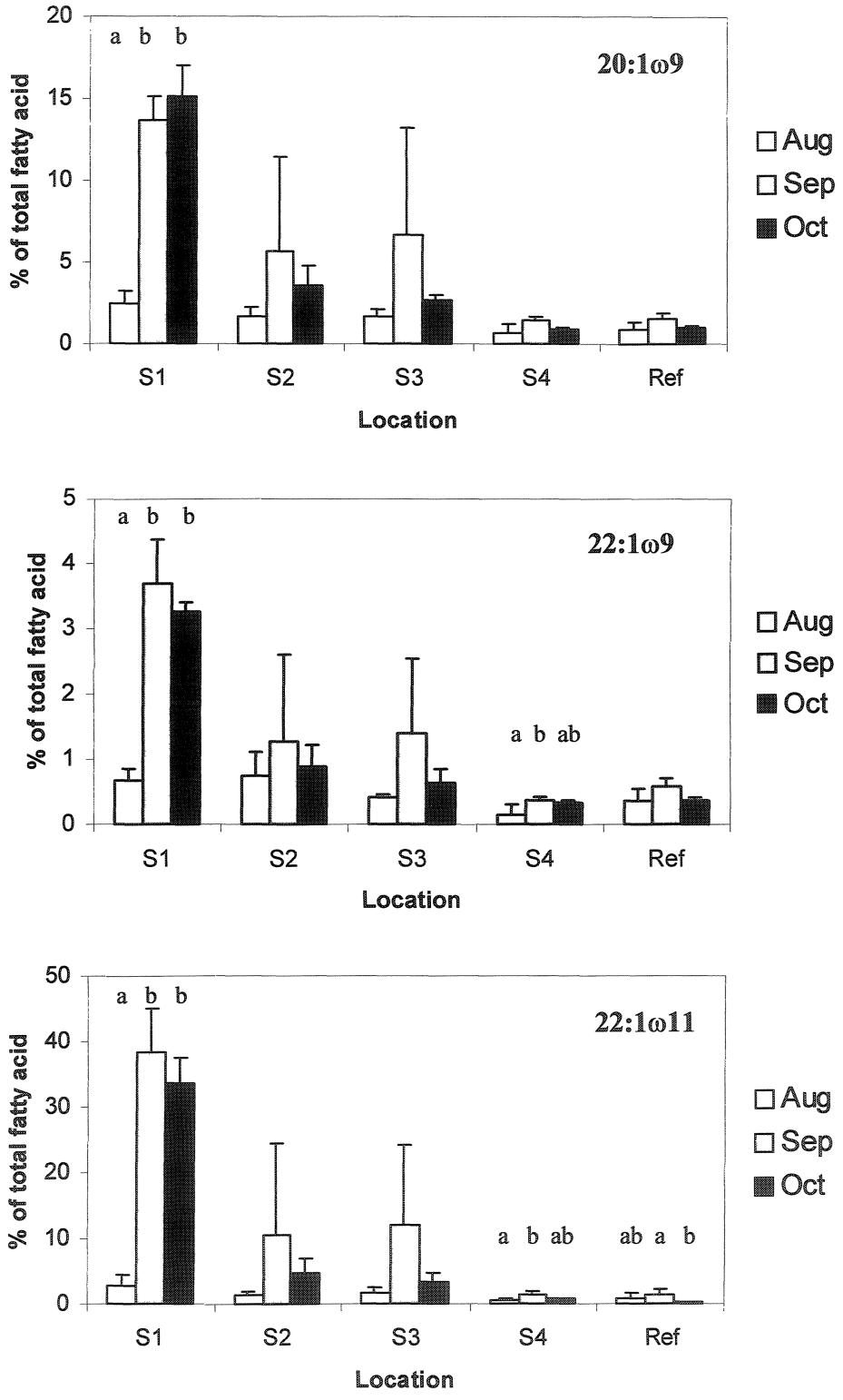


Fig. 3.21: The proportions of 20:1ω9 (top), 22:1ω9 (middle) and 22:1ω11 (bottom) during August, September and October at the different stations. n = 3 or 4, error bars are 1 SD.

It is well known that herring and other zooplankton-eating fish have high levels of 20:1 and 22:1 fatty acids, which originate from the wax esters of copepods (Sargent *et al.*, 1977). Both the alcohol moiety (which is oxidized to the corresponding fatty acid) and the fatty acid moiety of the wax ester are incorporated in triacylglycerols, the main lipid class in herring (Table 3.4).

Lie *et al.* (1987) examined lipid digestion in cod and their findings can explain why these 20:1 and 22:1 fatty acids are found in higher proportions near fish cages. Lipolytic enzymes in cod are fatty acid specific, with a preference for TAG with PUFA as substrates. This means that TAG in the feces are predominantly those containing saturated and monounsaturated fatty acids. TAG make up a small fraction of the total lipid content of feces (~10%), but each TAG molecule has 3 fatty acids. The majority (~70%) of lipids in the feces are FFA, especially saturated and long chain monounsaturated fatty acids (C20 and longer) since they are not well absorbed by cod. Both processes – less easy lipolysis and subsequent absorption – contribute to the presence of higher or approximately equal proportions of saturated and long chain monounsaturated fatty acids in the feces compared with the feed. This is illustrated in Table 3.4 by some results from Johnsen *et al.* (1993), who analyzed diet and feces of salmon (for comparison, the composition of the herring used as feed in this study is also shown). All the saturated fatty acids are present at higher levels in the feces than in the diet, while the PUFA and the short chain MUFA (up to C18) are present at lower levels. The fatty acid 20:1ω9 is found at approximately equal and 22:1ω11 at higher proportions in feces compared with the diet.

Table 3.4: Fatty acid composition (% of total fatty acid ± SD) of the diet and feces of salmon in Johnsen's study (Johnsen *et al.*, 1993). Not all fatty acids are shown.

Fatty Acids	**Diet** (Edel 7 pellets) n = 4	**Feces** n = 4
14:0	6.8 ± 0.04	9.8 ± 1.7
16:0	16.8 ± 0.2	28.9 ± 5.2
16:1ω7	7.1 ± 0.01	3.5 ± 1.3
18:0	2.1 ± 0.04	4.2 ± 0.8
18:1ω9	13.5 ± 0.1	8.7 ± 2.1
18:1ω7	3.7 ± 0.01	2.9 ± 0.5
20:1ω9	9.9 ± 0.1	10.0 ± 0.9
20:5ω3	7.3 ± 0.1	2.0 ± 1.3
22:1ω11	13.6 ± 0.1	16.3 ± 0.8
22:1ω9	1.2 ± 0.02	1.9 ± 0.2
22:6ω3	7.7 ± 0.1	3.8 ± 1.5

Johnsen *et al.* (1993) and Henderson *et al.* (1997) also analyzed the fatty acids in the sediments underneath the cages (Table 3.5).

Table 3.5: Fatty acid composition (% of total fatty acids ± SD) of sediments/sediment traps in different studies. Data for which an increase compared to a reference site was found are highlighted. Adapted from Johnsen *et al.* (1993) and Henderson *et al.* (1997). Not all fatty acids are shown.

Fatty Acids	**This study Labrador** Oct. Sed. Trap at S1	**Johnsen *et al.* (1993) Scotland** Sediment	**Henderson *et al.* (1997)* Norway** Sediment South East at 5m
14:0	3.7 ± 1.4	5.6 ± 0.4	6.2
16:0	11.2 ± 1.9	17.8 ± 1.8	21.2
16:1ω7	6.9 ± 1.0	5.9 ± 0.6	10.1
18:0	1.9 ± 0.1	4.2 ± 0.6	4.2
18:1ω9	5.6 ± 0.4	**8.8 ± 0.9**	5.8
18:1ω7	2.9 ± 0.5	3.6 ± 0.6	5.6
20:1ω9	**15.1 ± 1.9**	**10.4 ± 0.4**	**6.6**
20:5ω3	3.0 ± 0.4	4.5 ± 0.6	**2.4****
22:1ω11	**33.6 ± 3.9**	**20.8 ± 0.5**	**8.7**
22:1ω9	**3.3 ± 0.1**	**2.3 ± 0.2**	**0.3**
22:6ω3	1.9 ± 0.4	3.9 ± 0.8	**2.3****

* standard deviations not reported **except for the top 5 mm

A quantitative comparison between the three studies is difficult because of differences in samples (sediment trap samples *vs* sediment samples), the kinds and amounts of feed used, locations (Labrador, Scotland, Norway) and fish densities. Nevertheless, in all three studies the fatty acids 20:1ω9 and 22:1ω11 stand out as biomarkers for the dispersal of organic waste from fish farms. To a lesser extent this is also the case for 22:1ω9, but the proportions of this fatty acid in sediments can be quite low.

It is puzzling however that none of these studies report increased levels of the saturated fatty acids 14:0, 16:0 and 18:0 since these are not well digested by cod (and salmon). Both 14:0 and 16:0 can make up a significant proportion of the fatty acid composition of feces (Table 3.5) and could be expected to be present at much higher levels than those reported in Table 3.6. A possible explanation is the higher solubility of these shorter chain fatty acids compared to the longer chain C20 and C22 fatty acids. Unfortunately, data on the solubility of lipids in salt water are not readily available. Budge and Parrish (1998), however, found that fatty acids in the dissolved fraction of sediment traps from Trinity Bay had a shorter chain length and were more saturated than the particulate fraction, which is in accordance with findings by Körtzinger *et al.* (1994).

A PCA was performed on 28 individual fatty acids from Appendices 9 - 12 and the composite variable 'bact', which sums all the bacterial fatty acids, using all the replicates at each station (55 cases and 29 variables). This allowed for 54.1% of the total variation in the dataset to be accounted for by PC1 and PC2 (data not shown). A graphical presentation of this data set requires 16 different symbols (5 stations x 3 months + feed),

which results in a rather complicated plot. Therefore, the PCA was run again using the average values for each station (16 cases and 29 variables). This allowed for 60.6% of the total variation in the dataset to be accounted for by PC1 and PC2 (Fig. 3.22, top) and the conclusions drawn from this plot are exactly the same as when all the replicates are used. A cluster analysis on the loadings indicates that 4 clusters can be distinguished (1 of them consisting of just one data point).

The fatty acid composition of S1, the sediment trap closest to the fish pens, is very similar in September and October and plots with the herring data in one cluster on the far left. This region is strongly correlated with higher levels of the fatty acids 20:1ω7, 20:1ω9, 22:1ω9 and 22:1ω11 (Fig. 3.22, bottom). These fatty acids have a very strong influence on PC1, since all the other fatty acids plot much further to the right.

Station S4 has already proven to be somewhat of an outlier on several occasions and this is illustrated by the fact that its fatty acid composition during every sampling period is relatively constant and different from all other stations. In general, this station has higher levels of the PUFA 20:4ω6, 21:5ω3 and 22:6ω3 and of the MUFA 16:1ω5.

The rest of the data points cluster together in the centre, with the exception of S2 in October, which has somewhat higher values for 18:1ω9 and 18:2ω4. The variation along the PC2 axis for these data points is not very large. Note however that the September traps S2 and S3, for which somewhat higher values and relatively high standard deviations were recorded for the biomarker fatty acids, lie slightly apart from the other data points in the cluster.

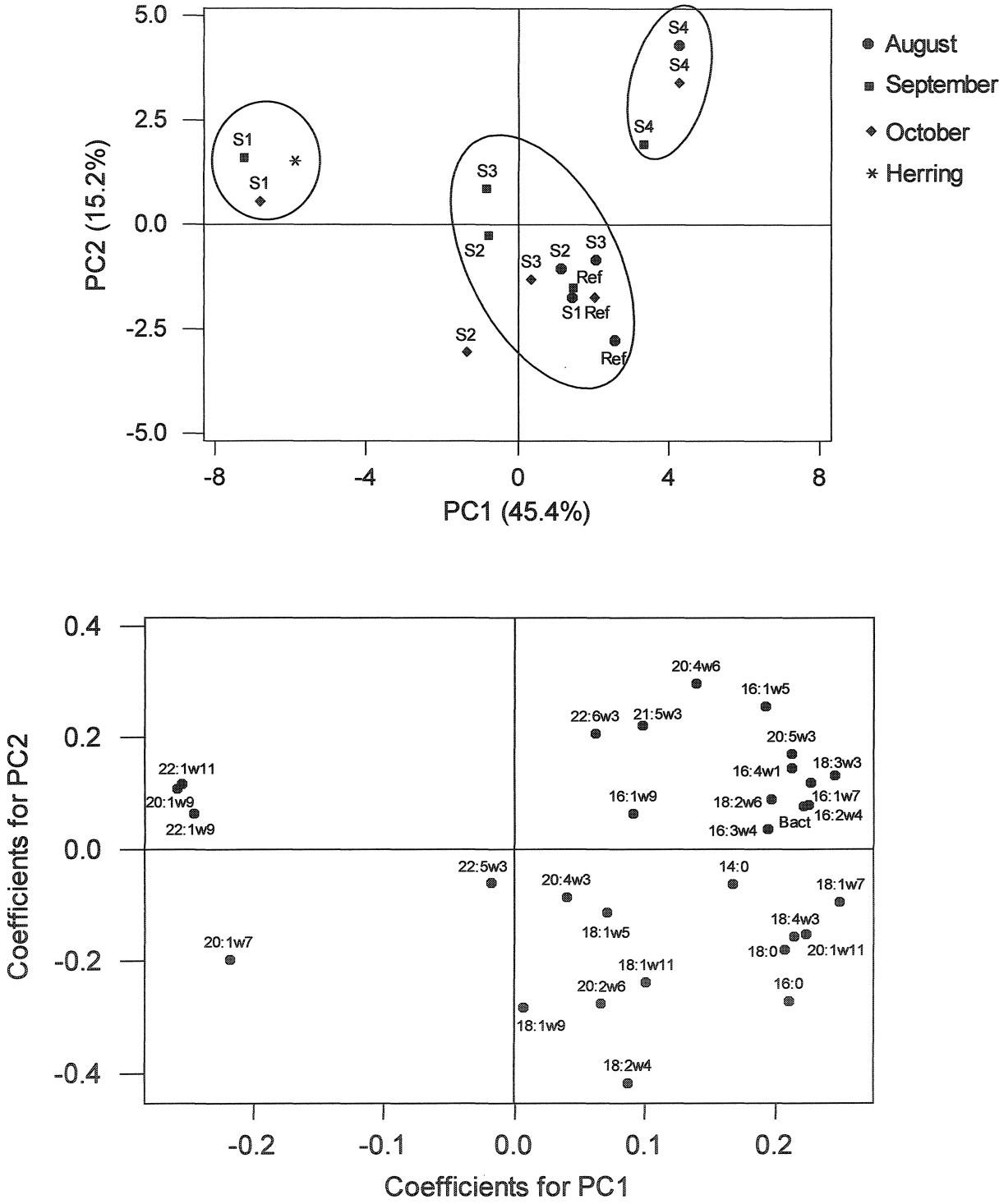


Fig. 3.22: PCA of sediment trap and herring fatty acids with loadings (top) and coefficients (bottom).

Some authors scale their data by log-transformation to level out differences in concentrations (e.g. Johnsen *et al.*, 1993). Since the fatty acids 20:1ω9 and 22:1ω11 were present at such high proportions, it was worthwhile doing this with our data as well. Since some of the data points were zero, they were arbitrary assigned a value of 0.05 so that they could be log-transformed. However, the plot of (the coefficients for) PC2 *vs* (the coefficients for) PC1 looked similar to the original plots and no additional information was gained by this transformation.

3.5.4. Lipid Class and Fatty Acid Composition of Settling Particles near a Cod Enclosure: Input of Uneaten Feed or Feces?

The above data clearly show that the lipid composition of settling particles near a cod enclosure is influenced by the input of waste from the cages. Free fatty acid levels and the proportions of the fatty acids 20:1ω9, 22:1ω9 and 22:1ω11 are significantly higher close to the pens (at ~ 5 m depth) than before the fish enclosure was in operation and also compared to settling particles further away from the pens. Since the organic waste generated by a fish farm consists of uneaten feed and feces, is it possible to distinguish between these sources? This could be helpful in managing the fish farm since if the input would be mainly from uneaten feed, a different feeding technique could prevent wastage of feed and put less stress on the ecosystem.

The high proportion of free fatty acids points towards a direct input of feces, since they are the dominant lipid class in feces (Lie *et al*, 1987). However, extensive hydrolysis of uneaten feed and/or remaining acyl lipids in feces, most likely by bacteria, would also

increase the proportion of free fatty acids. Neither Johnsen *et al.* (1993) nor Henderson *et al.*, (1997) found evidence for increased bacterial numbers under fish pens, although other authors report changing bacterial communities (a shift towards sulphide oxidizing and methanogenic bacteria) in severely impacted areas (Hansen *et al.*, 2001). In this study, no increase in bacterial fatty acids was found close to the fish pens, which favours the idea that feces are the main contributor to the lipid composition of settling particles close to the fish pens. It is noteworthy that Johnsen *et al.* (1993) did not find any bacterial fatty acids at all in cod feces, which means that a higher input of feces does not necessarily mean a higher input of bacterial fatty acids.

There is, however, another indication that at least in this study feces were the main source of free fatty acids. A comparison of TAG levels between stations and across the different months shows that, with the exception of the reference location, for which somewhat higher levels are observed during August and September (8.7 and 8.2% respectively), the proportions are always between 4.1 and 6.0% (Fig. 3.15). If uneaten feed was an important part of settling particles near the fish pens, increased TAG levels would be expected since the lipids in the herring used as feed consisted for more than 80% of TAG (Table 3.4). A suggestion for further research would be to separate TAG from other lipid classes and determine their fatty acid composition. If uneaten feed is the main source of these TAG, then their composition should be very similar to the feed. If feces are the main source, then the proportions of SFA and MUFA should be higher than in the feed, since TAG with PUFA are preferentially digested by cod as discussed before.

3.6. Cod (*Gadus morhua*)

3.6.1. Limitations of the Project

The investigation of fish stocks proved to be the most problematic part of this research. Several complications were encountered that either prevented us from collecting the data we wanted to collect or limited the applicability of our analyses. An overview of these complications is listed below.

It came to our attention only after finishing the field season that it is not possible to distinguish inshore or bay cod from offshore cod solely based upon their colour. The cod we caught were scored from 1 to 5, with 1 being a counter shaded (grey/black) cod and 5 being a cod with significant red pigmentation (bay cod). The average scoring based upon the colour for the 'offshore' cod was 2.1, while this was 3.6 for the bay cod. However, Gosse's research (Gosse, 2002) demonstrates that the variability in coloration only allows for general conclusions to be drawn regarding the time cod spent in the inshore environment. Genetic analysis is necessary to determine whether the brown/red and counter shaded cod in this study are from the same population. This means that we cannot be sure that the cod we collected in August/September 2001 just outside Gilbert Bay are truly from the offshore. Nevertheless, since their colour was different from the cod inside the bay, they must have had a different diet so it was still worthwhile to look into possible differences in lipid class and fatty acid composition. For convenience however, these cod

will still be referred to as 'offshore 2001' since they were collected outside of Gilbert Bay.

Originally, we also wanted to investigate the effect of different kinds of feed on the lipid composition of the fish, but this could not be done for two reasons. First of all, according to the feeding data from our employee in Gilbert Bay, the fish that were supposed to get the artificial feed were still being fed a mixed diet by the end of the season. Apparently, we underestimated the difficulty associated with getting the fish used to eating pelleted food. Secondly, fish that were brought back for analysis in October 2001 lost their tags during transport and it was impossible to determine which pen they came from, so they were not analyzed.

Of the 238 fish that were put in the pens in August 2001, only 148 remained at the end of October 2001. To date, we have no clear picture of what happened to them. Some could have died, but no remains were found. Escape or predation by seals or minks is also unlikely since the nets were intact. Most of the remaining fish were killed for Ms. Gosse's project but approximately 40 were transferred to Peckham Cove and kept there without feeding until June 2002. We planned to analyze their flesh and livers for lipids after overwintering. However, when the pen was hauled up, it was empty except for the remains of five fish. Again, it is unclear what happened to them.

We encountered some problems in preserving fish that were brought back for analysis. Ideally, fish would have been dissected in the field and samples of flesh and liver would have been stored in vials in a few ml of chloroform. The vials would then have been

flushed with nitrogen and stored at – 20 °C until transport to the lab in St. John's in a cooler with ice (which takes about 5 – 6 hours). Unfortunately, the remote location was only accessible by air or by boat and regulations for transporting chemicals were very strict, so we could not ship chloroform or nitrogen.

We then decided to bring back whole fish, frozen at – 20 °C, in coolers with ice. Upon arrival in the lab, they were stored at – 20 °C until analysis. Unfortunately, because of other commitments, inshore and offshore cod from 2001 were only analyzed after 4 months. This proved to be a mistake (see Section 3.6.3.1) for this kind of samples. In addition, on one occasion, I received fish that were transported without ice in the cooler (offshore 2002 cod), and they could have partially thawed. Lipid analysis showed considerable and variable amounts of free fatty acids in flesh and liver of almost all fish analyzed, indicating lipid hydrolysis. The implications are discussed in Section 3.6.3.1.

3.6.2. Some General Characteristics of the Analyzed Cod

The inshore cod caught in June 2002 were expected to have seriously depleted energy reserves because they very likely had just started eating again after several months of starvation during winter. Cod store their fat in the liver and during winter this is used as a source of energy and for the development of the gonads (Lie *et al.*, 1986; Jobling, 1988). Therefore, the hepatosomatic index (HSI: liver weight x 100 /fish weight) can be used as a crude indicator of the condition of the fish. From Table 3.7, it is evident that the hepatosomatic index of the inshore 2002 fish is quite low, with values between 1.45 and

Table 3.6: Some basic data on the cod used in this project. The sex was not determined.
Fork length: length between the tip of the snout and the fork of the tail. Hepatosomatic index: liver weight x 100 / fish weight.
Dry weights are averages of 3 samples of flesh or liver and were determined after at least 22 h at 75 °C.

		Fork Length (cm)	Wet Weight (g)	Liver Weight (g)	Hepatosomatic Index (%)	% Dry Weight of Flesh ± SD	%Dry Weight of Liver ± SD	Remarks
Inshore	1	40	815	43.5	5.34	19.2 ± 0.3	65.8 ± 0.7	Caught in Gilbert Bay in August
2001	2	44	1211	76.7	6.33	19.5 ± 0.0	65.7 ± 1.5	
	3	36	558	9.2	1.65	18.3 ± 0.2	42.7 ± 1.0	
Inshore	1	45	1260	20.2	1.60	17.2 ± 0.6	35.3 ± 1.3	Caught in Gilbert Bay in June, had probably not been eating over the winter and could have just started eating again
2002	2	46	1803	32	1.78	17.3 ± 0.1	43.0 ± 0.8	
	3	50	1859	26.9	1.45	15.6 ± 0.2	37.2 ± 5.3	
Offshore	1	26	199	5.6	2.80	18.8 ± 0.1	47.8 ± 1.9	Caught just offshore of Gilbert Bay, it is not certain if they are truly offshore and from a different population than Inshore 2001
2001	2	45	922	15.1	1.64	17.5 ± 0.8	35.8 ± 1.4	
	3	30	430	10.0	2.33	19.2 ± 0.4	51.1 ± 1.1	
Offshore	1	34	572	24.4	4.27	20.2 ± 0.1	57.5 ± 0.6	Caught in Hamilton Bank, definitely offshore and from a different population than all the other cod analyzed in this study
2002	2	38	766	24.5	3.20	19.9 ± 0.3	63.0 ± 1.9	
	3	22	121	2.3	1.90	19.6 ± 0.4	47.0 ± 0.7	

1.78. There is however one fish in each of the other groups with a HSI < 2 and there are no statistical differences between the four groups ($p = 0.155$). A HSI between 2 and 6 is considered normal and does not mean poor nutritional state (Jobling, 1988). It is not uncommon for farmed cod to have a HSI >12 when fed formulated feeds (wet pastes and pellets), but this is probably because these feeds are more rapidly emptied from the stomach. This can result in an overloading of the digestive capacity, leading to a reduction in absorption efficiency and to changes in rates of nutrient supply, which can cause increased lipid synthesis and deposition (Jobling, 1988).

Black and Love (1986) found that the water content of muscle and liver of cod increase during starvation. The typical pattern is an initial sharp increase in the water content of the liver with little change in the water content of the flesh. The water content of the liver then reaches a plateau and further starvation causes a substantial increase in the water content of the flesh. Their interpretation was that lipid reserves in the liver are used up before the protein of muscle starts to decrease. Converting the data for dry weight of flesh and lipid from Table 3.7 to water content[1] for ease of comparison with Black and Love's data, Fig. 3.23 is obtained. A number of different equations were used to fit the data points (sigmoid, logistic, Weibull, Gompertz, Hill and Chapman) but none gave a satisfactory result and the trendline is fitted by eye.

[1] % Water = 100 - % Dry Weight

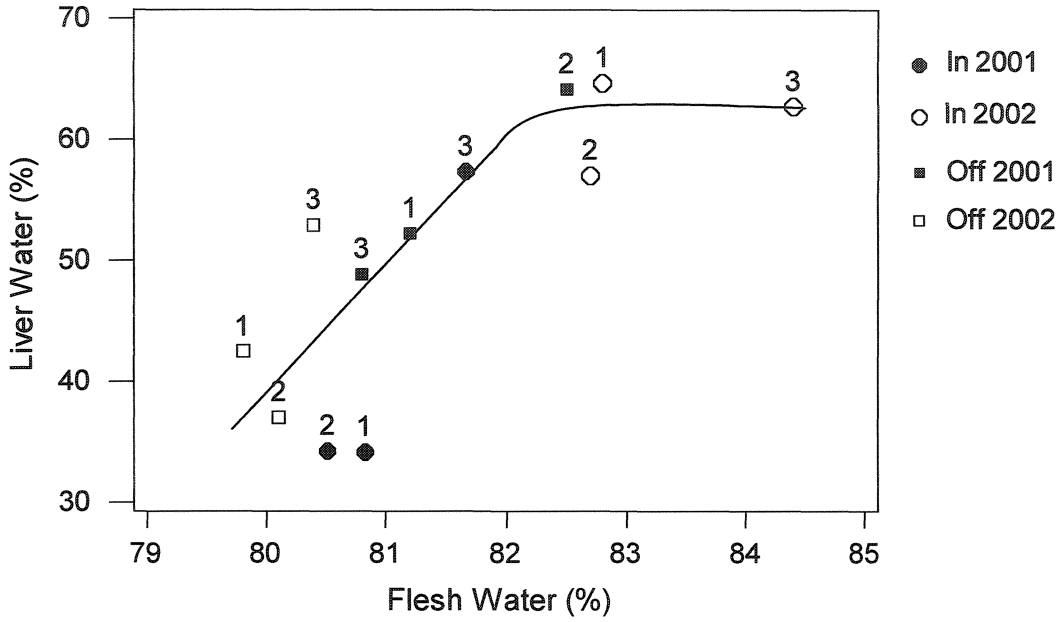


Fig. 3.23: Water content of muscle *vs* liver of cod. The trendline is fitted by eye and is not assumed to have a statistical meaning. Numbers refer to individual cod in Table 3.7.

The fish we analyzed represent a rather heterogeneous group because they were collected at different locations and at different times and of course we had no control over what and how much they were eating. They are therefore expected to exhibit some variation in water content of flesh and lipid. This is indeed the case and because of this variation, we are able to see the same trend as the one observed by Black and Love (1986). In their data, the water content of the liver levels off at approximately 75%, for our data this is at approximately 62%, but the water content of the flesh where this happens is the same (~ 82%). It is evident from Fig. 3.23 that all the inshore cod from 2002 have depleted energy reserves because they all three plot on the plateau of the trendline (high water content of both liver and flesh). This confirms the results about the condition of the fish based upon

the hepatosomatic index, since they all have a HSI < 2. However, there is one cod in each of the other 3 groups with a HSI < 2. Fig. 3.23 shows that indeed offshore cod 2 from 2001 (HSI = 1.64) also plots on the plateau and inshore cod 3 from 2001 (HSI = 1.65) is a borderline case. Offshore cod 3 from 2002 (HSI = 1.90) is an outlier, but has the highest HSI of all the fish with depleted energy reserves.

3.6.3. Lipid Content and Lipid Class Composition of Cod Muscle and Liver

3.6.3.1. Cod Muscle

The muscle of the offshore cod caught in 2002 has a slightly higher lipid content per wet weight than the muscle of the inshore cod from 2001 (Table 3.8), although there are no differences between the different groups of cod in lipid per unit dry weight, probably because the power of the analysis is too low.

Table 3.7: Total lipid content of cod muscle. In: Inshore; Off: Offshore. Different letters indicate significant difference ($p < 0.05$).

	Total Lipid % Wet Weight ± SD n = 3		**Total Lipid** % Dry Weight ± SD n = 3
In 2001	0.57 ± 0.04	(a)	2.98 ± 0.23
In 2002	0.62 ± 0.04	(ab)	3.61 ± 0.30
Off 2001	0.59 ± 0.08	(ab)	3.16 ± 0.30
Off 2002	0.71 ± 0.01	(b)	3.57 ± 0.03

The lipid content of cod muscle rarely exceeds 1% of the wet weight (Lie *et al.*, 1986; Jobling, 1988) and for instance dos Santos *et al.* (1993) in a feeding experiment report values between 0.8% and 1.0%. The lipid content of the cod muscle analyzed here is between 0.56% and 0.71%. This is similar to the values reported by Copeman and Parrish (unpublished data) of 0.5% to 0.6% for cod caught in Gilbert Bay in 2000, and Jangaard *et al.* (1967) as far back as 1967 found levels between 0.57% and 0.74%.

The lipid class composition of the muscle shows some remarkable differences between the groups (Fig. 3.24).

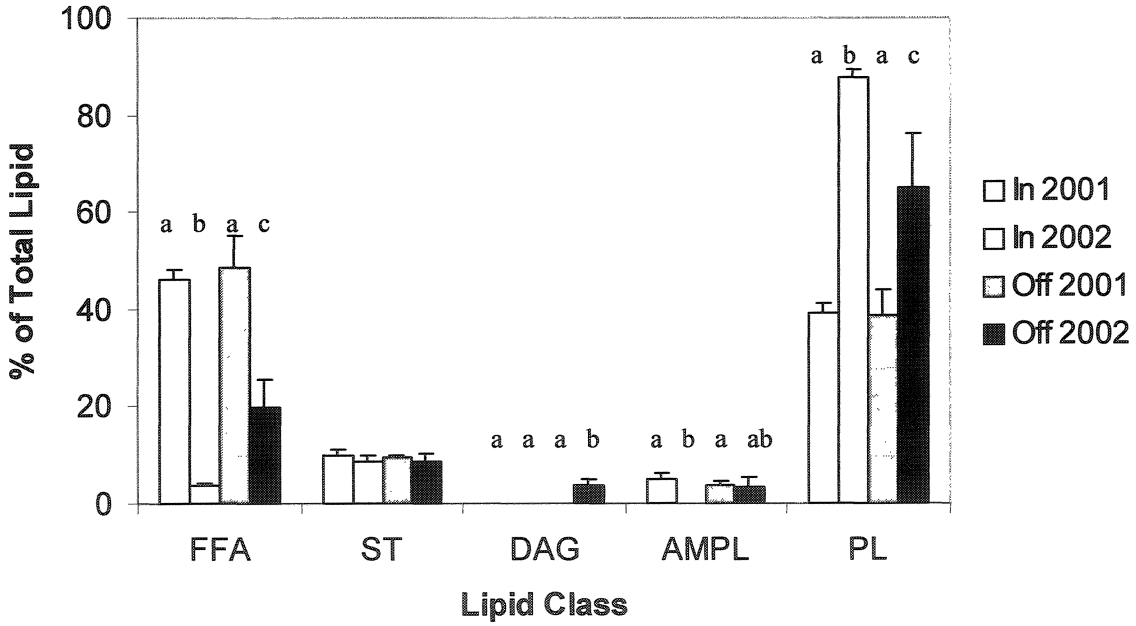


Fig. 3.24: Lipid class composition of inshore (In) and offshore (Off) cod muscle. Error bars are 1 SD, n = 3. Missing bars indicate < 1% or not present. DAG: Diacylglycerols. Different letters indicate $p < 0.05$.

The major lipid class in cod muscle was reported to be phospholipids by Lie (1986) and dos Santos *et al.* (1993), but this is only the case here for the cod caught in 2002. Sterols

are present in all cod analyzed here at levels of 9% to 10% and there are no statistical differences between the groups ($p = 0.683$). Diacylglycerols are only found in the offshore fish caught in 2002. AMPL levels are low, between < 1% and 4.9%. Free fatty acid (FFA) values range from 3.5% to 49% and most likely reflect how well the fish were preserved before analysis. The inshore cod from 2002 have the lowest FFA levels (3.5%). They were frozen and transported in a cooler with ice, stored at -20 °C upon arrival in the lab and analyzed the next day. Offshore 2002 cod were frozen but transported in a cooler without ice, and could have started to thaw. They were frozen overnight and also analyzed the day after they arrived at the lab. They have intermediate levels of FFA (19.6%). Finally, the fish with the highest levels of FFA (45.9% and 48.6%) are the ones that were frozen, transported in a cooler with ice and stored at – 20 °C but dissected approximately 4 months later.

A strong indication that these FFA originate from lipid hydrolysis comes from the correlation with PL levels (Fig. 3.25): the higher the FFA proportion, the lower the PL proportion.

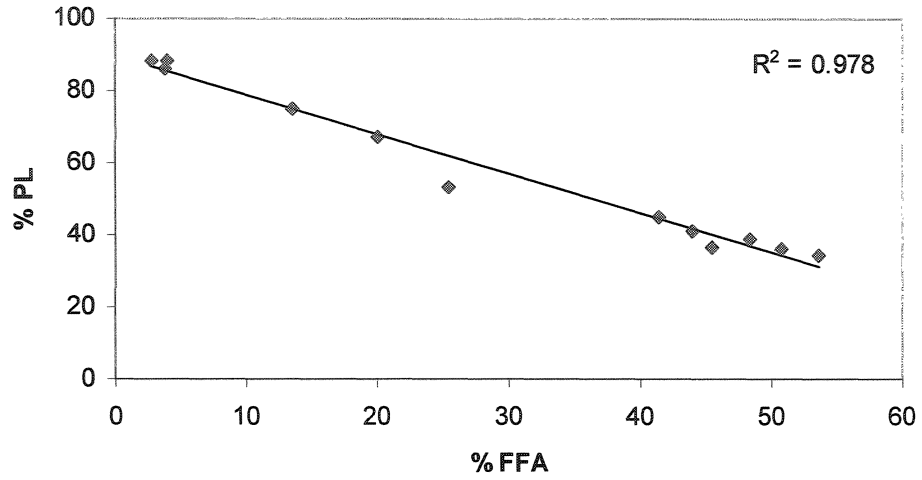


Fig. 3.25: The relationship between FFA and PL levels in muscle for the 12 cod in this study

Additional proof comes from an experiment conducted after being confronted with these findings. A cod from the aquaculture facility at the Ocean Sciences Centre was sacrificed and dissected immediately. Two samples of muscle were put in vials with 5 ml of chloroform. One of the samples was extracted and analyzed the same day, the other sample was kept in the freezer at –20 °C and extracted and analyzed after 16 weeks. The fish was then stored at –20 °C and samples of muscle were removed (without thawing the fish), extracted and analyzed after 6 and 11 weeks. All samples were removed within an area of a few cm^2.

It is evident from Fig. 3.26 that there is a dramatic change in the lipid class composition over time. At the start, only PL (87%) and ST (13%) are present. After 6 weeks, FFA levels have gone up from < 1 % to almost 20%. At the same time, DAG and AMPL appear, indicating partially degraded PL. ST levels are unaffected (14% after 6 weeks).

After 11 weeks, there does not seem to be any major change compared to the situation after 6 weeks (see also section 3.6.4.1 for the stability of fatty acids).

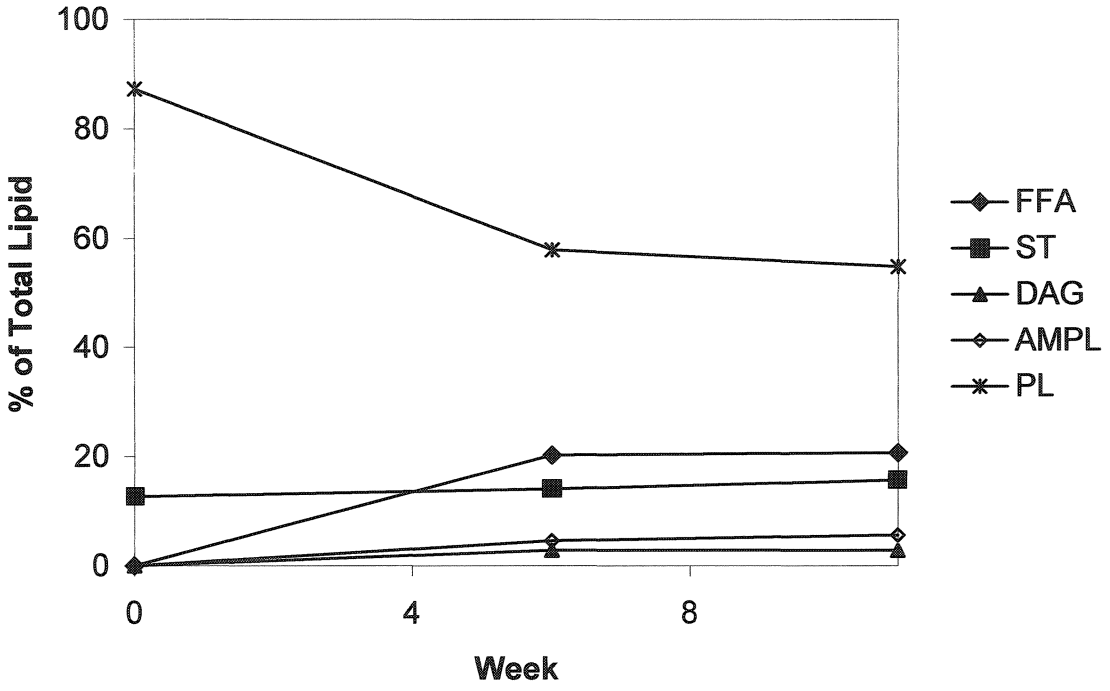


Fig. 3.26: Change over time of cod muscle lipid class composition. The whole carcass was stored at – 20 °C.

The muscle sample that was stored in chloroform but not extracted until 16 weeks later did not show any degradation and had a similar composition as the original muslce sample that was extracted and analyzed the same day (~13% ST and 87% PL). The same holds true for the chloroform extract from the original muscle sample that was also analyzed again after 16 weeks. The implication is that tissue samples should be taken as

soon as possible and stored at -20 °C in chloroform, even if they cannot be extracted and analyzed immediately.

These results are consistent with those of Bligh and Scott (1966), who showed that cod frozen for 9 months at –12 °C had an increase in FFA from 0.05% to 32.6%, which could be attributed to the hydrolysis of phosphatidylethanolamine and phosphatidylcholine.

3.6.3.2. Cod Liver

Table 3.8: Total lipid content of cod liver. In: Inshore; Off: Offshore.

	Total Lipid % Wet Weight ± SD n = 3	**Total Lipid** % Dry Weight ± SD n = 3
In 2001	35.2 ± 11.6	59.6 ± 7.4
In 2002	17.1 ± 4.6	43.8 ± 7.5
Off 2001	23.0 ± 6.9	50.5 ± 7.0
Off 2002	33.0 ± 7.8	59.0 ± 4.9

There are no statistically significant differences between the different groups of cod in total lipid content of the liver (Table 3.9), although their means can differ by as much as a factor of two (inshore 2001 *vs.* inshore 2002). This can probably partially be explained by the large variation within the groups and the small sample size. Liver lipid content is of course heavily influenced by the diet, with high-fat diets given ad libitum resulting in fish with livers that have a higher lipid content (dos Santos *et al.*, 1986). There is a very good correlation between liver lipid content and dry weight (Fig. 3.27).

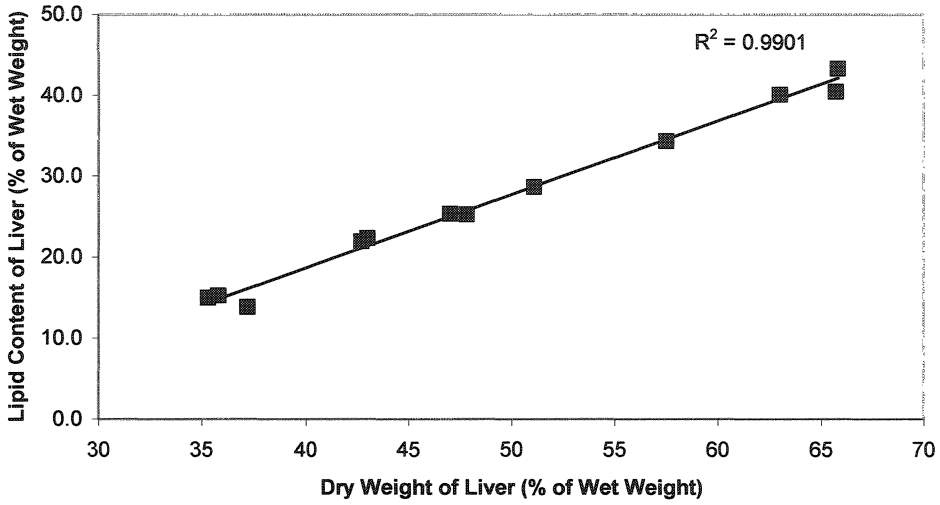


Fig. 3.27: Relationship between dry weight and lipid content of the liver for the cod in this study.

In contrast to the muscle, TAG are the main lipid class in cod liver (Fig. 3.28).

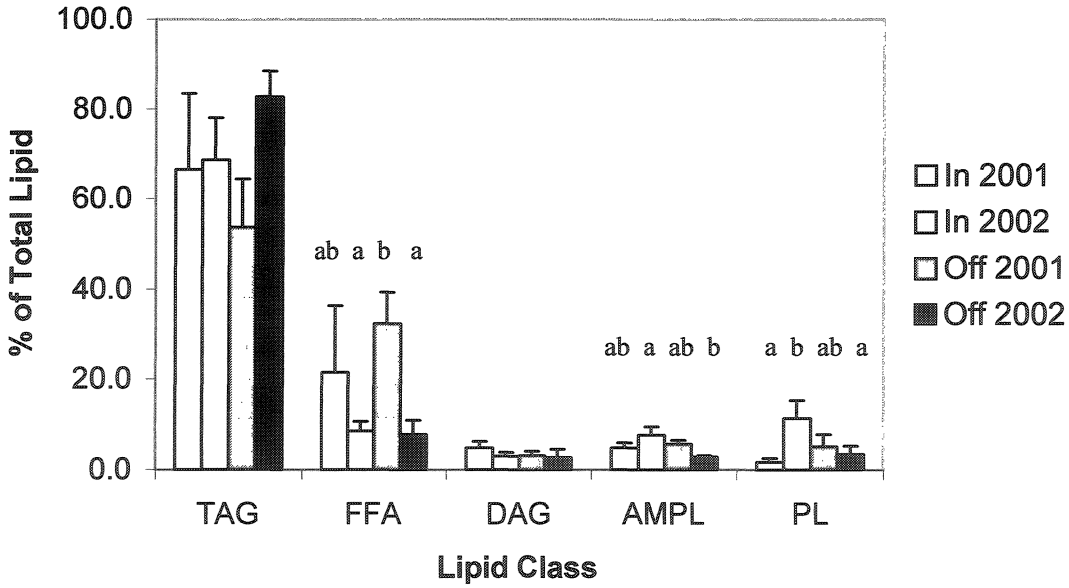


Fig. 3.28: Lipid class composition of inshore (In) and offshore (Off) cod liver. Error bars are 1 SD, n = 3. DAG: Diacylglycerols. Different letters indicate $p < 0.05$.

There are no differences in TAG proportions between the different groups, but referring to the previous section, lipid hydrolysis cannot be excluded. For instance, the offshore cod caught in 2001 have the lowest liver TAG levels, but they also have the highest FFA levels. DAG, AMPL and PL are present in all groups in relatively small proportions. The proportion of PL in the inshore cod from 2002 is relatively high, which could be related to the fact that overall, these fish had the most depleted energy reserves (Fig. 3.23). Since PL are membrane components and are not immediately used as an energy source, the proportion of PL relative to the total lipid pool should increase during starvation. A plot of % PL vs. HSI clearly shows this relationship (Fig. 3.29). It is interesting to see that the % PL rapidly increases when the HSI drops below 2%, the cut-off value for which Jobling (1988) suggests a poor nutritional state.

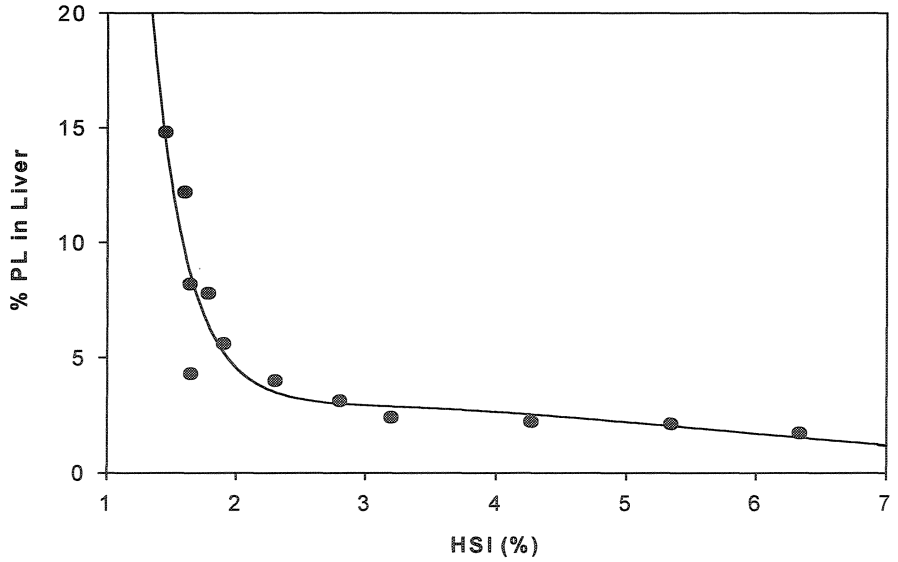


Fig. 3.29: The relationship between the HSI and the % PL in the liver. The results for the individual fish are shown. The trendline is an inverse 3^{d} order equation with an R^2 of 0.86.

Dos Santos *et al.* (1986) found substantial differences in lipid class composition of the liver depending on diet. For instance, TAG levels varied between 76% and 97% for cod fed either prawn or herring. FFA in their study were between 1% and 12%, and they also found minor amounts of DAG (1% to 5%), ST (1%) and PL (0% to 4%). Except for the offshore cod from 2001 for which probably extensive hydrolysis occurred, these values are comparable to the ones in this study. Since cod in the wild (such as the ones in this study) feed on a variety of prey (see next section), it is not surprising that they exhibit some variation in liver lipid class composition.

3.6.4. Fatty Acids in Cod Muscle and Liver

The fatty acid content of fish body lipid is a combination of both fatty acids derived from the diet and those synthesized endogenously (Henderson *et al.*, 1982).

Cod are carnivorous and feed on a variety of prey. As juveniles, they feed mainly on crustaceans such as mysids, shrimp and crab. When they reach a length of about 50 cm, fish become the predominant source of food. Depending on locality and availability, capelin, sand lace, redfish and herring are important foods but many other species of fish, molluscs, tunicates etc. are also eaten (Scott and Scott, 1988).

Cod in Gilbert Bay feed predominantly on benthic invertebrates. Morris and Green (2002) analyzed the stomach contents of 229 cod in Gilbert Bay and found that shrimp, mysids, crabs and brittle stars comprised approximately 50% of the diet. Other invertebrates found in cod stomachs included scallops, polychaetes, sea cucumbers and

sea urchins. The proportion of fish in the diet was only 6-7%. They did not report the size of the fish whose stomachs were analyzed.

As mentioned before, the cod in this study came from in and around Gilbert Bay (Inshore 2001 and 2002, Offshore 2001) and from Hamilton Bank (Offshore 2002). We can assume that a major part of their diet consisted of invertebrates because of the location where they were caught (Gilbert Bay) and their size (all fish ≤ 50 cm) although this study did not look into this.

The pathways of fatty acid metabolism in fish are well known (Castell, 1979). Fish can synthesize *de novo* from acetate the even-chain saturated and the most common mono-unsaturated fatty acids. They are unable however to synthesize any fatty acids of the ω3 or ω6 series, unless a precursor with this ω structure is present in the diet, which can be desaturated and/or elongated.

3.6.4.1. Cod Muscle

Given the problems encountered with preserving the fish, it was useful to check if the fatty acid composition of fish stored at – 20 °C would also change over time. The samples of muscle of the fish that was used in Section 3.6.3.1 were derivatized after Iatroscanning and analyzed by GC-FID. Fig. 3.30 shows the proportions of some selected fatty acids (mostly PUFA since they are especially susceptible to oxidation) over time. Since only one sample was analyzed on each occasion, no statistics can be performed but

it is evident from Fig. 3.30 that no (downward) trend for any of the fatty acids is observed.

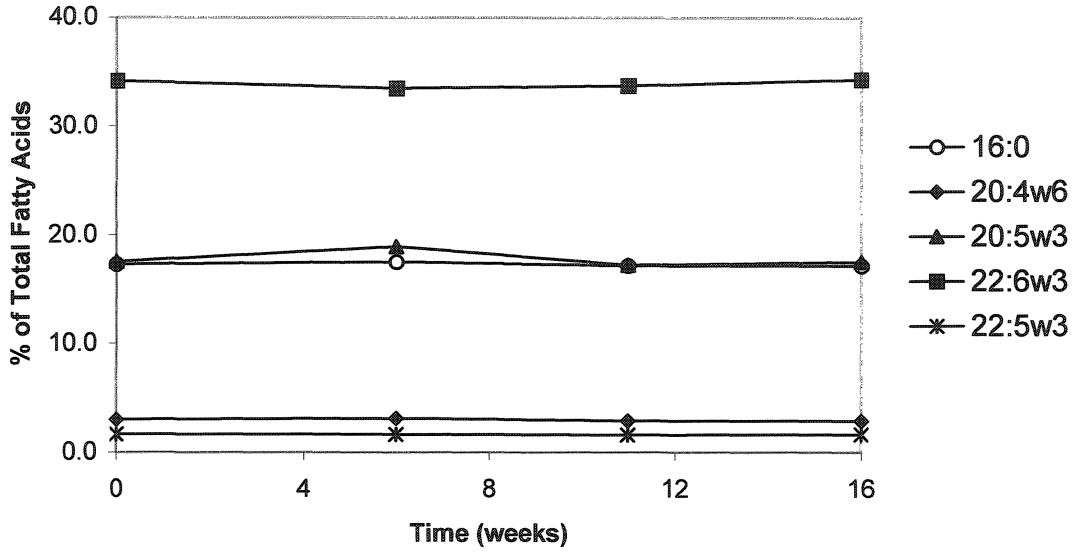


Fig. 3.30: Change in time in the percentage of some selected fatty acids of cod muscle. The whole carcass cod was stored at – 20 °C.

The fatty acid content of cod flesh can be found in Appendix 15. For ease of comparison, a selection of the quantitatively more important fatty acids is shown in Fig. 3.31.

In general, differences between the inshore cod from 2001 and the offshore cod from 2001 and 2002 are very small, although for some fatty acids the differences are statistically significant (e.g. 20:5ω3 and 16:1ω7). The proportions of the fatty acids are almost identical to the ones found by Copeman and Parrish for Gilbert Bay cod in 2000 (unpublished data) and within the range reported by Lie *et al.* (1986), dos Santos *et al.* (1993) and Jangaard *et al.* (1967).

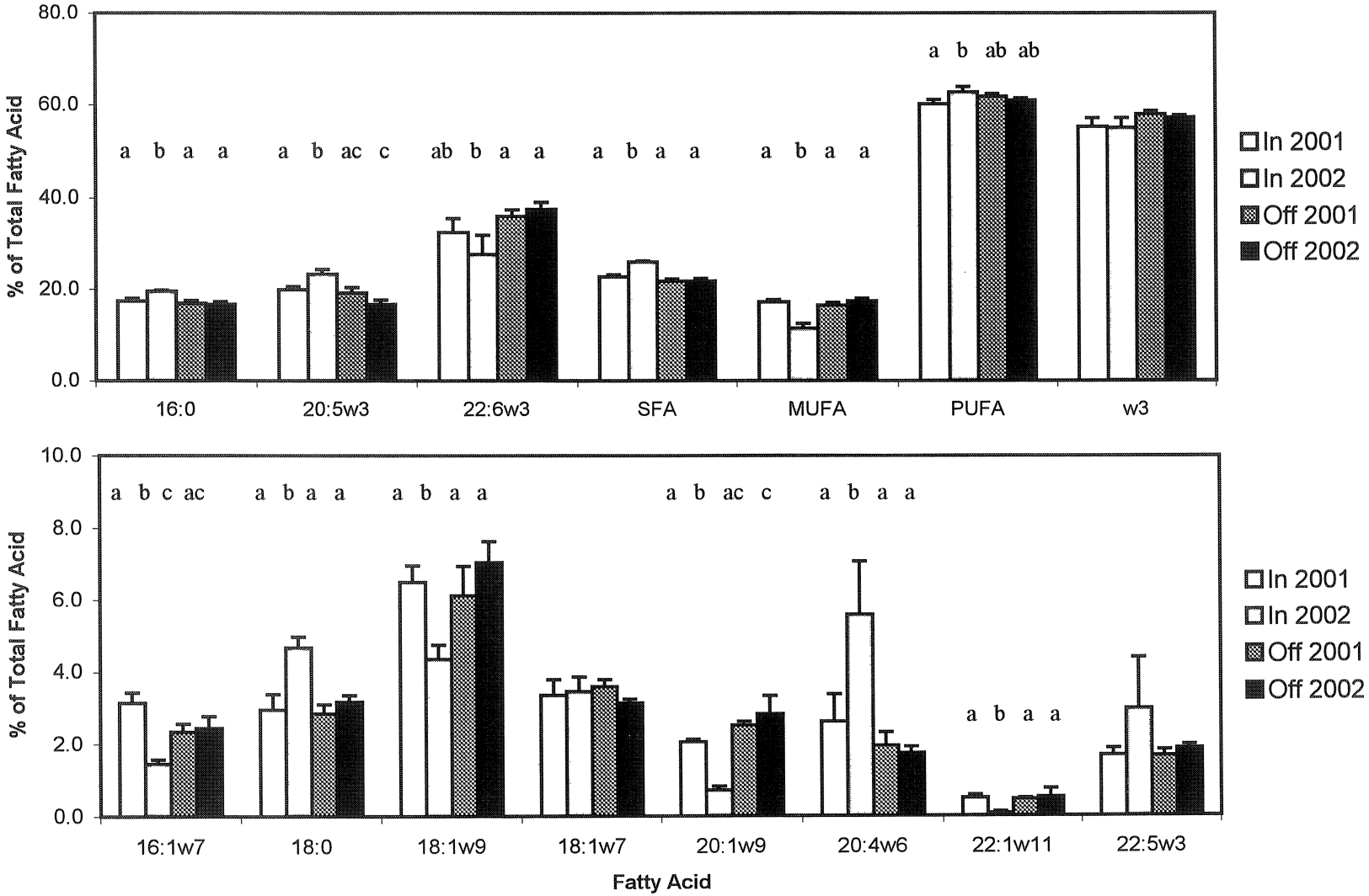


Fig. 3.31: Selected fatty acids found in cod muscle; see Appendix 13 for a complete list. Error bars are 1 SD, n = 3. Different letters indicate $p < 0.05$.

The inshore cod caught in 2002 on the other hand show some distinct differences from the other groups. These cod have higher levels of 16:0, 18:0, 20:4ω6, 20:5ω3 and SFA and lower levels of 16:1ω7, 18:1ω9, 20:1ω9, 22:1ω11, 22:6ω3 and MUFA (Fig. 3.31). It is well known that the fatty acid composition of fish can be influenced by seasonal effects such as availability of food, and temperature (Castell, 1979). The MUFA 16:1ω7, 18:1ω9, 20:1ω9 and 22:1ω11 all have significantly lower proportions in these fish and are possibly used as an energy source. This is in contrast to the SFA 16:0 and 18:0, which are enriched. The fatty acids 16:1ω7 and 18:1ω9 are abundant in phyto- and zooplankton respectively, whereas 20:1ω9 and 22:1ω11 originate from wax esters of calanoid copepods, an abundant genus of zooplankton (Sargent *et al.*, 1977). They are carried over to higher trophic levels and are found in cod prey. Herring, for instance, has considerable proportions of these MUFA (Appendix 12). When cod stop eating over the winter, the levels of these fatty acids in their muscle and liver decline if they are used as an energy source.

The PUFA 20:5ω3 and 22:5ω3 are present at higher proportions in the inshore cod from 2002, while 22:6ω3 is somewhat depleted (although for the latter there is no significant difference with the inshore cod from 2001). This balances the proportion of ω3 fatty acids such that there is no significant difference ($p = 0.145$) among the 4 groups. The standard deviation for the proportions of 22:5ω3 (and 20:4ω6) is relatively high, because one specimen in this group has exceptionally high values for these fatty acids, although the other two cod in this group also have clearly higher levels of these fatty acids than

cod from the other groups (for instance for 20:4ω6 the values for the 3 cod are 4.5%, 4.4% and 7.4%, with the highest value for the cod with the lowest HSI).

The ratio of 22:6ω3/20:5ω3,which are the two dominant PUFA, is significantly lower ($p = 0.004$) for the inshore cod from 2002 (1.2) than for the other groups (range: 1.6 – 2.3) (Appendix 15).

Jangaard *et al.* (1967) found very little seasonal changes in fatty acids of muscle lipids, except for 20:1ω9 and 22:1ω11 for which the maximum values coincided with the period of 'best condition' of the fish (August to November). They also acknowledged that large variations can occur in the fatty acid content of lipids from individual fish.

Lie *et al.* (1986) and dos Santos *et al.* (1993) in feeding experiments on cod clearly showed that the fatty acid composition of dietary lipids strongly influences muscle (and liver) lipid fatty acids. Since the differences between the inshore cod from 2001 and the offshore cod from 2001 and 2002 are minimal, it is tempting to conclude that they were all feeding on similar prey or on different prey with a similar fatty acid composition.

A PCA was performed using 28 fatty acids and the sum of bacterial fatty acids (Fig. 3.32 – 12 cases, 29 variables). A few fatty acids for which a majority of the values was ≤ 0.2% were not included, and the sum of bacterial fatty acids rather than the individual bacterial fatty acids was used because their individual values were always very low (≤ 0.3%).

PC1 and PC2 together account for 63.5% of the total variation in the data set. A cluster analysis on the loadings shows that 3 clusters can be distinguished. There is a clear

separation between the inshore cod from 2002, which plot on the far right side of PC1 and cod from the other groups. The right side of this axis is of course correlated with the same fatty acids that were previously found to be present in higher proportions in these fish while the left side of this axis is correlated with fatty acids that were found to be present in lower proportions in these fish (Fig. 3.32, bottom). Two of the inshore cod from 2001 are found near the top of the plot, which is correlated with higher levels of for instance 20:1ω7, 18:3ω3 and 16:2ω4. These two fish were the ones with the highest HSI of all fish analyzed in this study (Table 3.7). However, these fatty acids are only present in minor quantities (≤ 0.4%) (Appendix 15). The variation along this axis is also less important than along PC1 (19.4% as opposed to 44.1%).

Although the number of cod in this study (12) is smaller than the number of variables (29), PCA can still be used. According to Grahl-Nielsen (1999), the number of variables can exceed the number of samples. The only limitation is that the number of principal components in such a case cannot be higher than the number of samples. Colombo *et al.* (1996) performed PCA on a dataset which they considered too small to draw definite conclusions, but they found it useful for summarizing their data without losing much information. This is exactly the way PCA is used here: as a technique, complementary to the ANOVAs, that summarizes a lot of information in a single graph and that gives a graphical representation of which samples and variables are correlated.

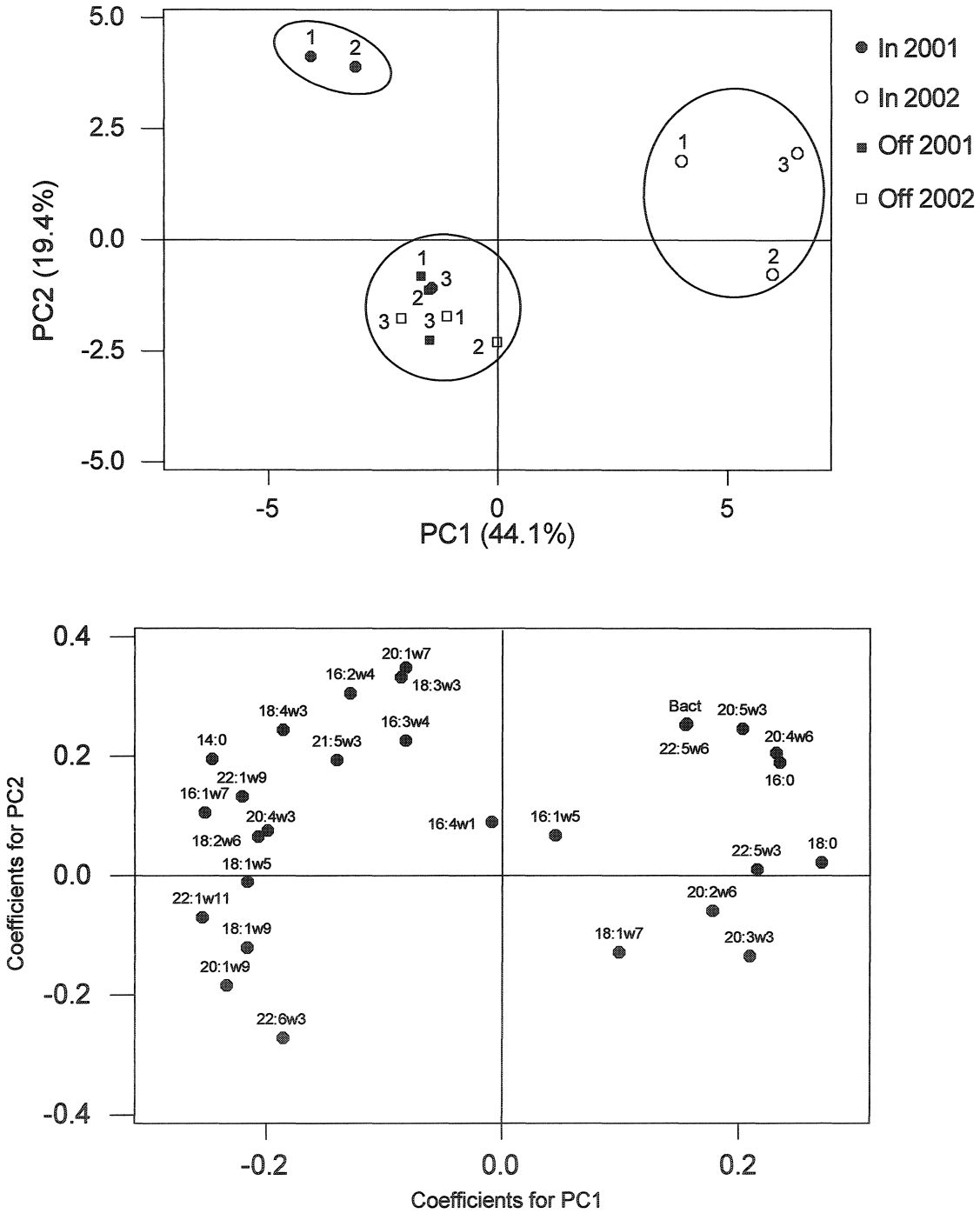


Fig. 3.32: PCA of cod muscle fatty acids, with loadings (top) and coefficients (bottom).

3.6.4.2. Cod Liver

The fatty acid composition of cod liver is presented in Appendix 16. For ease of comparison, a selection of the quantitatively more important fatty acids is shown in Fig. 3.33 and 3.34.

Although the main difference again is between the inshore cod from 2001 and the offshore cod from 2001 and 2002 on the one hand, and the inshore cod from 2002 on the other hand, the situation is less clear-cut than for the muscle fatty acids.

The fatty acids 18:3ω3 and 20:4ω6 are present at higher proportions in the liver of inshore cod from 2002 (Fig. 3.33) and this is also the case for 22:5ω3, 18:1ω7, 21:5ω3, PUFA and the sum of ω3 fatty acids (Fig. 3.33 and 3.34), although their is not always a significant difference from all other groups. 20:4ω6 and 22:5ω3 are also present at higher proportions in the muscle and have relatively high standard deviations, which is also the case for the liver.

The fatty acid 16:1ω7 is the only one with a significantly lower proportion in the inshore cod from 2002 compared to the other groups. Because of some relatively high standard variations, there are no statistical differences between the groups for 22:1ω9 and 22:1ω11 ($p = 0.108$ and 0.054 respectively) although from Fig. 3.33 it can be seen that their mean value is clearly lower for the inshore cod from 2002. Overall however, there is a significantly lower proportion of MUFA in the livers of these fish, which was also the case for the muscle. This suggests that at least some of the MUFA are used as an energy source, which is in accordance with Henderson *et al.* (1982) who propose that 22:1ω11 is

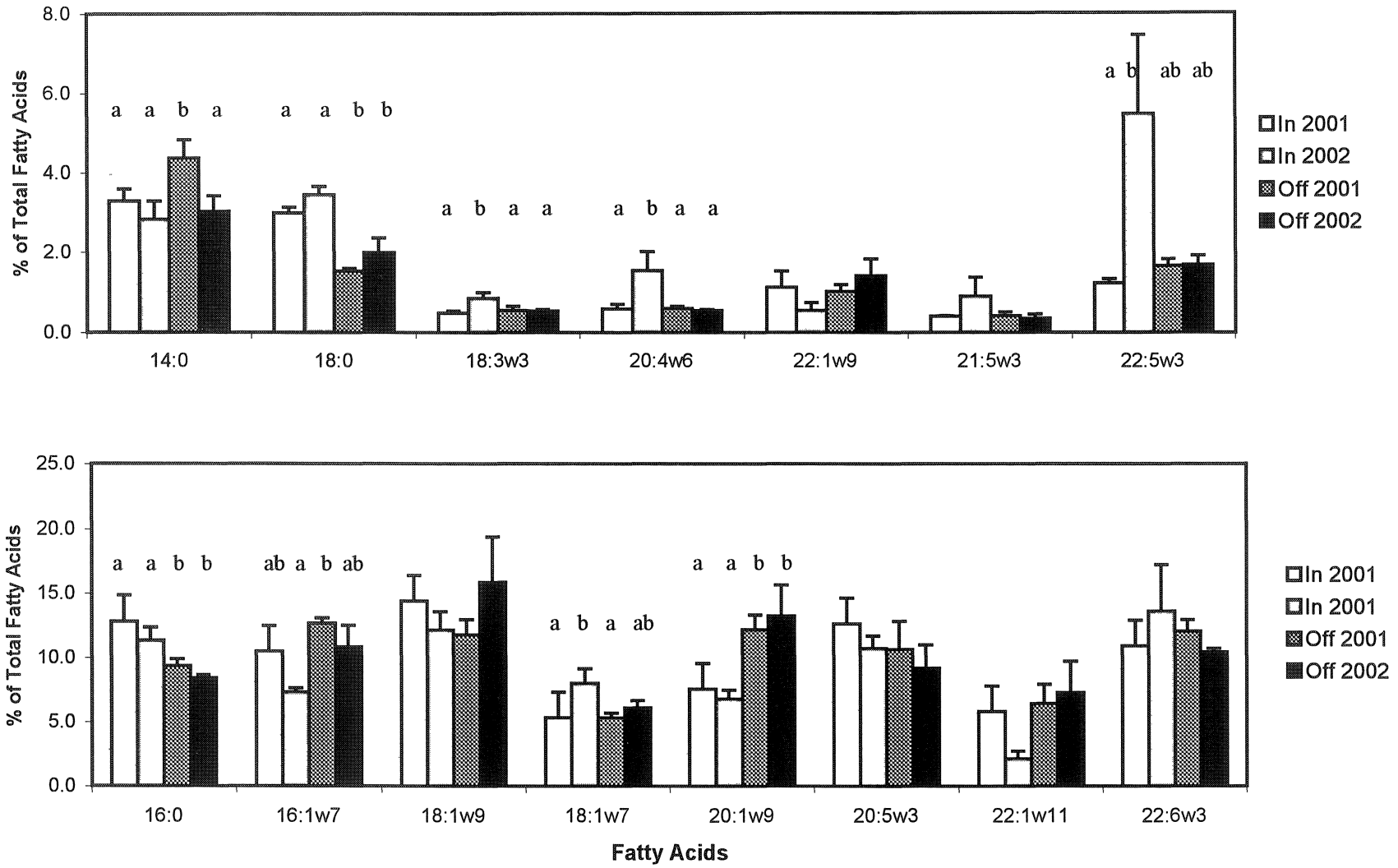


Fig. 3.33: Selected fatty acids found in cod liver; see Appendix 14 for a complete list. Error bars are 1 SD, n = 3. Different letters indicate $p < 0.05$.

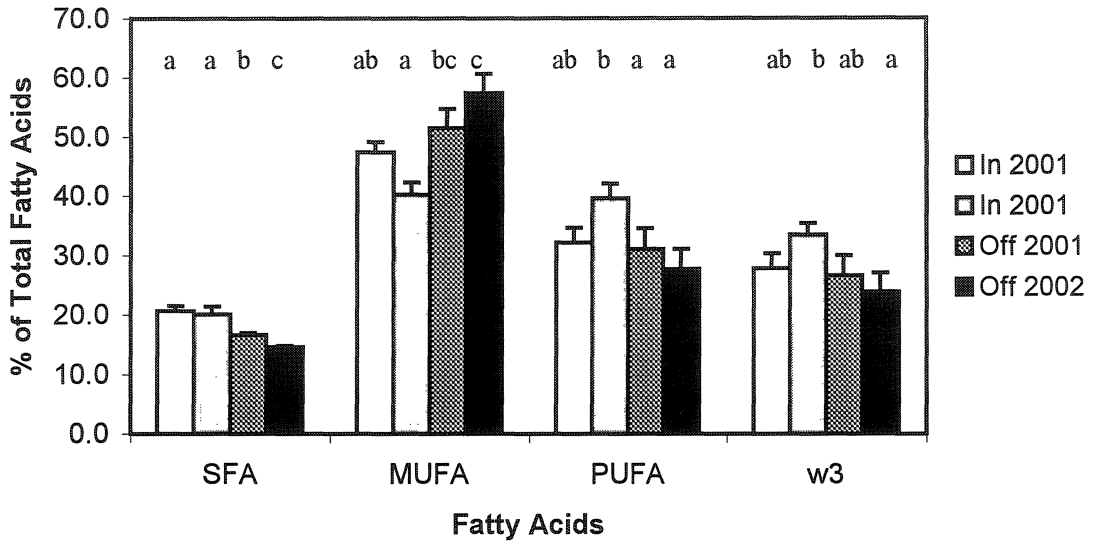


Fig. 3.34: Sum of saturated, monounsaturated, polyunsaturated and ω3 fatty acids in cod liver. Different letters indicate $p < 0.05$.

selectively used as an energy source in rainbow trout (*Salmo gairdnerii*) livers. Jangaard *et al.* (1967) found maximum values for 20:1ω9 (range: 4.0% - 14.6%) and 22:1ω11 (range: 0.8% - 13.1%) for fish in 'the best condition', which was in autumn, and consider these to be accessible metabolic reserves. The two other major MUFA, 16:1ω7 and 18:1ω9 did not vary as much.

The proportion of MUFA in the liver (~50%) is noticeably higher than in the muscle (~15%), regardless of whether or not the fish were starved. This points to MUFA either being used in metabolic processes in the muscle or being preferentially distributed to other organs than the muscle. There is also a considerable shift in the PUFA levels; the cod livers in this study have ~30% of PUFA while for the muscle this is ~60%. This is expected since PUFA are a major component of phospholipids (dos Santos *et al.*, 1993;

Arts *et al.*, 2001) and this is the major lipid class in the muscle. For both the muscle and the liver, the vast majority of the PUFA are ω3 fatty acids (Fig. 3.31 and 3.34).

There are no differences in the ratio 22:6ω3/20:5ω3 in the liver and with values of 0.9 to 1.3, this ratio is somewhat lower than for the flesh (1.6 to 2.2), with the exception of the inshore cod from 2002 which had a ratio of 1.2. This could mean that cod selectively utilise 20:5ω3 or elongate 20:5ω3 to 22:6ω3 (dos Santos *et al.*, 1993).

A PCA was performed using 28 fatty acids and the sum of bacterial fatty acids (Fig. 3.35 – 12 cases, 29 variables). As before, fatty acids for which a majority of the values was ≤ 0.2% were not included, and the sum of bacterial fatty acids rather than the individual bacterial fatty acids was used because their values were always very low (≤ 0.6%).

PC1 and PC2 together account for 61.3% of the total variation in the data set (Fig. 3.35, top). A cluster analysis on the loadings shows 4 clusters, with each of the inshore cod from 2002 different from one another and from all the other cod, which are grouped together in one cluster. The inshore cod from 2002 plot more to the left side of the PC1 axis, where there is a stronger correlation with 18:3ω3, 20:4ω6, 22:5ω3, 18:1ω7 and 21:5ω3 (Fig. 3.35, bottom). Cod from the other groups are relatively well centred on the PC1 axis, but are somewhat spread out on the PC2 axis. The negative PC2 axis corresponds with higher levels of 16:0, 18:1ω5, 18:1ω9, 18:2ω4 and 20:5ω3, while the positive PC2 axis corresponds with higher levels of 14:0, 16:2ω4, 18:2ω6 and 20:1ω9. The variation along this axis is only 17.5%, which is substantially less than the 43.8% of the PC1 axis. Nevertheless, it is interesting to see that within the cluster the 3 inshore cod

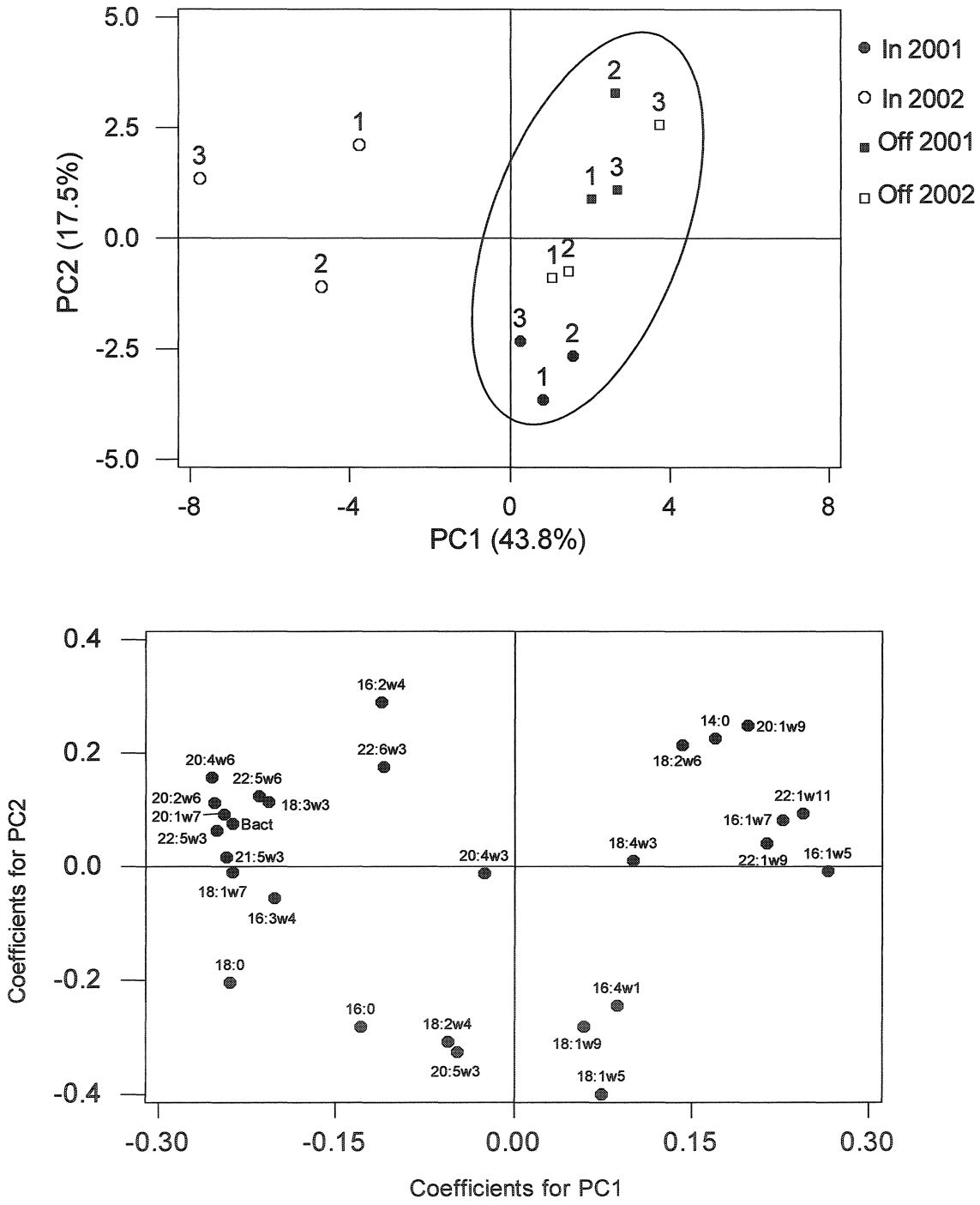


Fig. 3.35: PCA of cod liver fatty acids, with loadings (top) and coefficients (bottom).

from 2001 plot very close together on the negative side, while the 3 offshore cod from 2001 plot close together on the positive side; the offshore cod from 2002 plot in an intermediate position (except for 1 specimen). A larger sample size would certainly have been helpful in assessing if this difference is consistent. In contrast to the small difference that was found in the PCA of the flesh fatty acids between 2 inshore cod from 2001 and all the other cod, there is no relationship with the HSI (see Table 3.7).

3.6.5. Summary: Lipid Classes and Fatty Acids in Cod Muscle and Liver

The cod in this study came from in and around Gilbert Bay and from Hamilton Bank and were captured over the course of two years. The literature on feeding experiments suggests that the lipid class and fatty acid composition of muscle and liver are strongly influenced by the diet (Lie *et al.*, 1986; dos Santos *et al.*, 1993); no reference was found to the influence of possible genetic differences between cod stocks on the lipid class and fatty acid composition of their tissues. This means that the lipid class and fatty acid composition of cod muscle and liver can vary seasonally, geographically and interannually, reflecting differences in availability of prey (Budge *et al.*, 2002). Do the data gathered in this study allow distinguishing any such differences?

Where differences in the lipid class composition of cod muscle and liver were found in feeding experiments, this was after being fed diets with a very different lipid content and composition for a prolonged period of time. For instance, cod fed prawn (3.5% lipid, of which TAG was 44%) for 45 days had 76% and 3% of TAG in the liver and the muscle respectively. In contrast, cod fed enriched herring (21.1% lipid, of which TAG was 80%)

for 45 days had 97% and 9% of TAG in the liver and the muscle respectively (dos Santos *et al.*, 1993). It is very unlikely that cod in a natural situation feed on a single prey species for an extended period of time. For this reason, the lipid class composition of muscle and liver probably has limited value in distinguishing between cod stocks. Unfortunately, the poor preservation of some of the specimens restricts us from drawing hard conclusions regarding this matter. However, there is evidence that besides indicators such as the HSI and the percentage water of the liver *vs* the muscle, the lipid class composition of the liver can be helpful in determining the condition of the fish. Fish that are in a poor nutritional condition have depleted energy reserves in the liver. This is evident from a greater proportion of phospholipids than in well-fed fish, since TAG (which make up the bulk of the lipids in the liver) are preferentially used as an energy source.

Fatty acids have been used in several studies to investigate the diet and spatial scales of foraging (Iverson *et al.*, 1997, Kirsch *et al.*, 1998) and trophic relationships (St. John and Lund, 1996). This is based upon the existence of 'indicator' fatty acids used as biomarkers (Section 1.2) or upon a multivariate approach, in which information is obtained from all fatty acids simultaneously.

The fatty acid data from this study show that one of the 4 groups of cod investigated really stands out; both the muscle and the liver of the inshore cod of 2002 have a different fatty acid profile from the other 3 groups. This is related to these fish having spent several months without feeding (and at very low temperatures), as discussed in previous sections. The differences are clearly shown on the PCA plots; it is also evident from these

plots that there is some variation within the groups. There are indications based upon the PCA of the liver fatty acids that there could be a difference between the inshore cod from 2001 and the offshore cod from 2001, but this has to be substantiated with a larger sample size. If so, it would be an indication that they are feeding on different prey, although it has to be stressed that this does not necessarily mean that these two groups of cod would have a different fatty acid composition all the time. For instance, the effect of starvation on the fatty acid composition of both muscle and liver is obvious and it is likely that if all specimens had been collected immediately after the winter, the small differences we found in this study would have been levelled out. Likewise, the same prey items may not be available in equal quantities (or not at all) from year to year. This means that cod stocks can have different fatty acid profiles from year to year and depending on the time of the year they are captured. This kind of variability is very useful to study finer-scale within-species variation (Budge *et al.*, 2002) and has to be taken into account when comparing different stocks of the same species.

CHAPTER 4

CONCLUSION

The data collected in this study clearly show that the lipid composition of settling particles at a cod enclosure is influenced by the input of waste from the cages. Free fatty acid levels and the proportions of the fatty acids 20:1ω9, 22:1ω9 and 22:1ω11 are significantly higher close to the pens (at ~ 5 m) compared to before the fish enclosure was in operation and also compared to settling particles further away from the pens. These findings can be explained by a higher contribution of feces and/or uneaten feed to the settling particles at this location. Which of the two is more important could not be unequivocally demonstrated, although the data suggest that the main input is from feces. The individual fatty acids are more specific biomarkers than total free fatty acids, since their presence can be directly linked to the feed, of which they are an important component. The presence of these fatty acids at higher proportions could be an indication that organic enrichment is taking place, although a year-round study of the sediments would be necessary to evaluate this.

It has to be stressed again that specific values mentioned in this study are very dependent on site-specific conditions. Current velocity, depth under the cages, number of fish in the cages and amount of feed administered all determine how far the influence of the fish pens can be traced back in the environment, which for our pilot-scale project was not further than 5 m away from the cages along the transect chosen.

This study could not detect any clear differences between inshore cod from 2001, offshore cod from 2001 and offshore cod from 2002, based upon the lipid and fatty acid

composition of muscle and liver. However, a principal components analysis of the liver fatty acids gave some indications of a small difference between inshore cod from 2001 and offshore cod from 2001. This has to be confirmed with a larger sample size.

A very clear difference was found between the Gilbert Bay cod that were caught before the summer of 2002 (inshore 2002) and the three other groups of cod which were all caught in mid to late summer. The differences are related to starvation, which was assessed by the hepatosomatic index and the percentage of water in muscle and liver. Starved cod had a higher percentage of phospholipids in the liver, indicating depletion of triacylglycerols, which are used as an energy reserve. The fatty acids of the muscle lipids had lower levels of MUFA. Interestingly, this was especially the case for 20:1ω9 and 22:1ω11, two of the biomarkers for the environmental distribution of organic waste from the cages. For the liver fatty acids, the differences were less clear-cut, although here too a decrease in MUFA was observed and an increase in some of the PUFA. In general, principal components analysis was very helpful in visualizing these differences in the fatty acid profiles, although it contributed little additional information.

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APPENDICES

Appendix 1: Preparation of Sediment Trap Samples for Lipid Analysis and Dry Weight Determination

- Haul up the sediment trap and cap the tubes. Remove the tubes from the frame and put them in a cooler filled with ice. If more than one sediment trap is retrieved, make sure to label the tubes accordingly.
- Upon returning to the lab, allow the suspended matter to settle for 30 minutes.
- Label the tubes of each sediment trap A, B, C and D. Remove the caps of the tubes. Drain the upper 3 litres by connecting the small plastic tube at the lower end to a filter unit and loosening the screw clamp. The filtered water is waste and can be collected in a bucket.
- Disconnect the plastic tube and tighten the screw clamp again. Pour the remaining contents of the tube in a 4 litre plastic container rinsed with filtered seawater. Rinse the tube at least twice with a small amount (100 – 200 ml) of filtered seawater, making sure that all of the sediment is collected. Rinse the filtered material from the first 3 litres into the container.
- Pour the contents of the 4 litre container in a 1000 ml graduated cylinder. Make up to 1000 ml with filtered seawater. If the initial volume exceeds 1000 ml, make sure to record the exact volume by pouring the excess volume in another graduated cylinder.
- Pour the contents of the graduated cylinder(s) back into the 4 litre container. Cap and shake well.
- Take a 25 ml aliquot for dry weight determination.

Filter over a precombusted, pre-weighed glass fibre filter (diameter of 20 mm – in a labelled plastic Petri disk, with the small filtration unit, using the vacuum pump. Rinse the graduated cylinder once or twice with a small amount of filtered seawater, making sure that all of the sediment is collected on the filter. Add 10 – 15 ml of a 3% ammonium formate solution when there is about 1 ml of water left on the filter, then filter till dry. Remove any obvious swimmers. Remove the filter with a pair of tweezers, fold in half and put the filter back in the labelled plastic Petri-disk. Make a note of which filter corresponds to which sediment trap and which tube. Store at –20 °C until analysis.

- Shake the 4 litre container well and take a 100 ml aliquot for lipid analysis.

  Filter over a precombusted glass fibre filter (diameter 47 mm) with the large filtration unit, using the vacuum pump. Rinse the graduated cylinder once or twice with a small amount of filtered seawater, making sure that all of the sediment is collected on the filter, filter till dry. Remove any obvious swimmers. Fold the filter in half, fold in half again and fold in half again, using two pairs of tweezers. Put the filter in a glass tube, label the tube with a marker (making sure to put tape over the label). Store at –20 °C until analysis.

Appendix 2: Lipid Extractions

Net Tow and Sediment Trap Samples

During the entire extraction procedure, samples and solvents are kept in an ice bath to reduce oxidation of unsaturated bonds as much as possible. The procedure is for a maximum of 150 mg of dry sample, if the sample is larger, the amounts may be increased accordingly. All glassware used is lipid-cleaned.

- Put the sample in a centrifuge tube and add 2 ml of $CHCl_3$ and 1 ml of CH_3OH.
- Grind quickly into a pulp with a metal-ended rod. Wash the rod with 1 ml of $CHCl_3$:CH_3OH 2:1 and then with 0.5 ml of $CHCl_3$-extracted water (sample and filter are supposed to contain 0.5 ml of water). Cap the tube and sonicate (sonicator filled with water and ice) for 4 minutes.
- Centrifuge for 2 minutes at 3000 rpm.
- Remove the organic (bottom) layer using the double pipetting technique involving placing a long Pasteur pipette inside a short one. Transfer the organic layer into a 15 ml vial. Wash the inside pipette into the vial containing the organic layer with 3 ml of $CHCl_3$ (1.5 ml washing down the outside of the pipette and 1.5 ml washing down the inside). Wash the outside pipette into the tube containing the aqueous layer with 3 ml of $CHCl_3$ (again 1.5 ml for the inside and 1.5 ml for the outside). Re-sonicate, centrifuge and remove the organic layer using new pipettes. Repeat this procedure at least three times and pool the organic layers (the extract should be colourless, if not the procedure has to be repeated till colourless). After removing the organic layer for the last time, wash both pipettes into the vial containing the organic layer.

- Concentrate down to volume under a gentle stream of nitrogen, cap, seal with Teflon tape and store at –20 °C until further processing.

Fish Muscle and Liver

The procedure is essentially the same as for net tow and sediment trap samples, except that the volume of the solvents used is 3.5x as much (since the samples are larger and contain more lipid) and that the sample is mechanically ground with a blender (Polytron – Kinematica GmbH, Switzerland). The volume of water added is calculated from the dry weight of the sample as:

volume to be added = 3.5 ml – wet weight of sample x (100 - % dry weight)/100.

Appendix 3: Derivatization of Lipid Extracts with BF_3/CH_3OH

- Transfer the lipid extract into a lipid-cleaned, 7 ml vial and evaporate under nitrogen to dryness. The amount of lipid should not exceed 20 mg.
- Add approximately 0.5 ml of hexane and 1.5 ml of BF_3/CH_3OH, flush with nitrogen, cap with a Teflon-lined cap, seal with Teflon tape and vortex briefly. Sonicate for 4 min. and then heat in an oven at 85 °C for 1.5 hours.
- Allow to cool down, add 0.5 ml of $CHCl_3$-extracted water and 2 ml of hexane and vortex briefly (2 x 5 seconds).
- Transfer the hexane layer (upper layer) to a lipid-cleaned 15 ml vial. Do not remove any of the bottom layer; it is better to leave some of the organic phase behind.
- Add another 2 ml of hexane, vortex briefly (2 x 5 seconds) and transfer the hexane layer into the same 15 ml vial. Repeat this once more.
- Blow the pooled hexane fractions down to approximately 0.5 ml, transfer into a lipid-cleaned 2 ml vial, and rinse the 15 ml vial twice more with approximately 0.5 ml of hexane. Blow down to dryness and add 0.5 ml of hexane, flush with nitrogen, cap, seal with Teflon tape and store at – 20 °C until further processing.

Appendix 4: Iatroscan® Analysis

Sequence Leading to the First Chromatogram (HC to KET)

- Apply samples and standards with a Hamilton syringe fitted with a repeating dispenser.
- Focus twice in acetone to produce a narrow band of lipid material near the lower end of the rods.
- Condition in a constant humidity chamber (over saturated $CaCl_2$) for 5 minutes and develop twice in hexane:diethyl ether:formic acid (99:1:0.05). The first development is for 25 minutes; the rods are dried in the constant humidity chamber for 5 minutes and redeveloped for 20 minutes.
- Dry for 5 minutes in the Iatroscan® and scan to the lowest point behind the KET peak (pps scan 22 on the Iatroscan®). Make sure the air flow is 20 ml/min, the hydrogen flow is 200 ml/min and the scan speed is 30. These instrumental parameters are maintained for every scan.

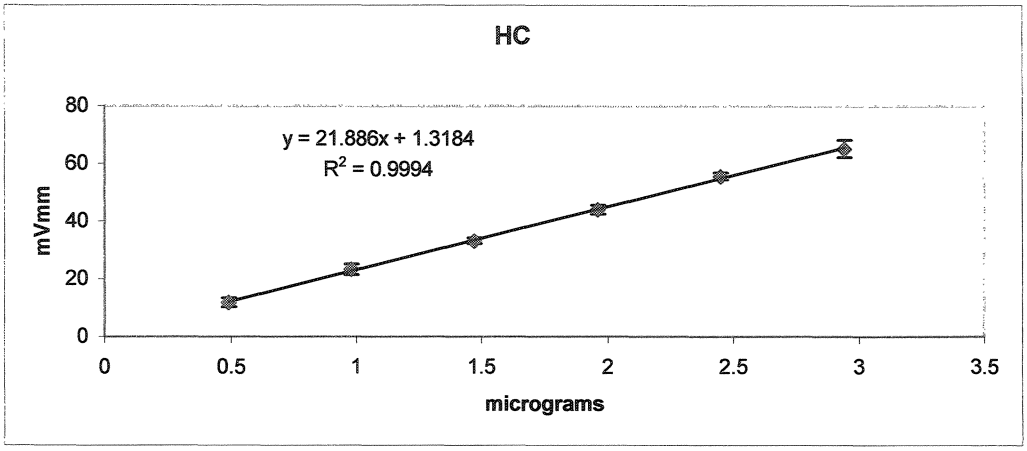
Sequence Leading to the Second Chromatogram (TAG to DAG)

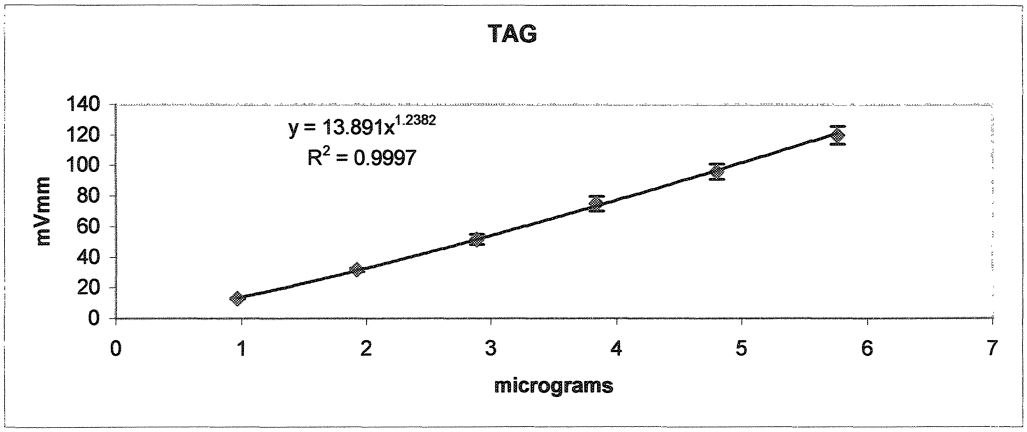
- Condition the rods for 5 minutes in the constant-humidity chamber and develop for 40 minutes in hexane:diethyl ether:formic acid (79:20:1).
- Dry for 5 minutes and scan to just behind the position of the diacylglycerol peak (pps scan 11 on the Iatroscan®).

Sequence Leading to the Third Chromatogram (AMPL and PL)

- Condition the rods for 5 minutes in the constant-humidity chamber and develop twice for 15 minutes in acetone (dry for 5 minutes in the constant humidity chamber between both developments).
- Condition for 5 minutes in the constant humidity chamber and develop twice for 10 minutes in chloroform:methanol:chloroform-extracted water (50:40:10) (dry for 5 minutes in the constant humidity chamber between both developments).
- Dry for 5 minutes and scan the entire length of the rods.

Appendix 5: Some Examples of Iatroscan® Calibration Curves





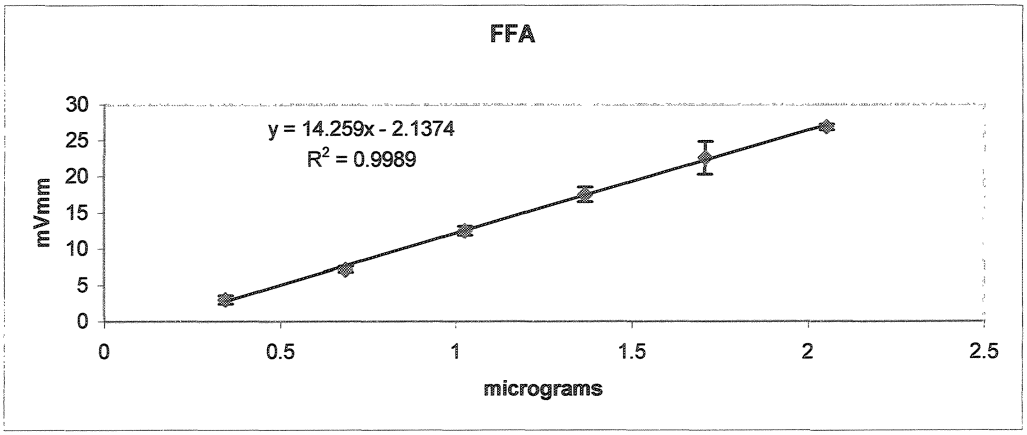


Fig. A 5.1: Three examples of calibration curves for the Iatroscan®: Hydrocarbons (top), Triacylglycerols (middle) and Free Fatty Acids (bottom)

Appendix 6: Recovery of Derivatization and Fractionation

To assess the recovery after derivatization and fractionation as discussed in Sections 2.4.1 and 2.5.2.1, a solution containing the lipids in Table A 6.1 was prepared.

Table A 6.1: Composition of the solution prepared to assess the recovery and the fractionation

Compound	Molecular Mass	Conc. (mg/L)	Conc. (mmol/L)
Pristane	268	618	2.31
Squalene	410	628	1.53
Cholesteryl palmitate	625	509	0.814
Tripalmitin	807	225	0.279
Phosphatidyl choline dipalmitoyl glycerol	733	462	0.630

After derivatization, the solution is expected to contain the compounds listed in Table A 6.2.

Table A 6.2: Expected composition of the solution after derivatization

Compound	Molecular Mass	Conc. (mmol/L)	Conc. (mg/L)
Pristane	268	2.31	618
Squalene	410	1.53	628
Cholesterol	387	0.814	315
Methyl palmitate	271	2.91	789

The number of mmoles/L of pristane and squalene should be the same before and after the derivatization. The number of mmoles of cholesterol formed is the same as the number of mmoles of cholesteryl palmitate that was originally present. The number of mmoles of methyl palmitate equals the sum of the number of mmoles of cholesteryl

palmitate plus 3x the number of mmoles of tripalmitin plus 2x the number of mmoles of phosphatidyl choline dipalmitoyl glycerol.

Three aliquots of the standard solution were derivatized. After fractionation, the actual concentrations were determined by GC-FID. This was done by comparing peak areas with the peak areas of standard solutions which contained pristane and squalene, cholesterol and methyl palmitate in approximately the expected concentrations (one-point calibration). All samples and standards were injected 3 times and the means and standard deviations were calculated. See Table 6.3 for an overview.

Table A 6.3: Overview of recovery experiment

Compound	Concentration in standard solution (mg/L)	Expected concentration after derivatization (mg/L)	Measured concentration after derivatization (mg/ml ± SD)	% Recovery ± SD (n=3)
Pristane	618	618	632 ± 11	102.3 ± 3.8
Squalene	628	628	Tr	Tr
Cholesteryl palmitate	509	--	---	---
Cholesterol	-	315	295	93.7± 3.1
Tripalmitin	225	--	---	---
Methyl palmitate	-	789	750 ± 11	94.9 ± 3.9
Phosphatidyl choline dipalmitoyl glycerol	462	--	---	---

-: not added; --: below detection limit; ---: not applicable (not measured with GC)

Only traces of squalene were recovered in the derivatized samples. This was completely unexpected, but since hydrocarbons were not analyzed after all, no attempt was made to find out the reason for this. However, Nichols *et al.* (2001) report the same phenomenon

in their study of fish lipids. They used CH_3OH / HCl / $CHCl_3$ 10:1:1 at 80 °C for 2 hours for their derivatizations and observed 'virtually complete degradation of squalene', but offer no explanation.

Appendix 7: Calculation of the Flux of Particulate Matter

The flux of particulate matter per day and per unit area can be calculated from the amount of material collected in the sediment traps by first calculating the total dry weight (DW) of the material collected in one tube of a sediment trap:

$$DW = \frac{dw \times V}{v}$$

where: dw = dry weight (g) of a v ml aliquot
V = volume (ml) the v ml aliquot was taken from (usually ~ 1000 ml)
v = volume (ml) filtered to determine the dry weight

The flux in $g.m^{-2}.day^{-1}$ is calculated from the DW by taking into account the surface area of the tube and the number of days the trap was deployed:

$$Flux = \frac{DW}{d \times (0.05^2) \times \pi}$$

where: DW = total dry weight (g) of material collected in one tube of a sediment trap
d = number of days the sediment trap was deployed
$(0.05^2) \times \pi$ = surface area of sediment tube (m^2)

Each sediment trap has four tubes and the average is calculated to obtain the values of Fig. 3.3.

Appendix 8: Calculation of the Flux of Total Lipids

This calculation is explained with an example: we will use data from one tube of sediment trap S1 in August. The lipid composition is shown in Table A 7.1.

Table A 7.1: Lipid composition of one tube of sediment trap S1 in August.

Lipid Class	mg in extract (0.5 ml)
HC	0.00993
WE/SE	0.0313
TAG	0.0211
FFA	0.112
ALC	0.0166
ST	0.0258
AMPL	0.110
PL	0.103
Total Lipid	**0.429**

The dry weight of a 25 ml aliquot was 9.39 mg; since a 100 ml aliquot was analyzed for lipids, this has a dry weight of 4 x 9.39 mg = 37.56 mg. Therefore, the amount of lipid in mg/g dry weight = 0.429 / 0.03756 = 11.43 mg/g dry weight.

The ash-free dry weight of the 25 ml aliquot was 6.66 mg, therefore the amount of organic matter was 9.39 – 6.66 = 2.73 mg. This means that (2.73 x 100%) / 9.39 = 29.07% of the sample was organic matter.

The amount of lipid in mg/g organic matter thus is: 11.43 / 0.2907 = 39.3 mg/g organic matter. Multiplying this number by the flux of organic matter in g/m^2.day gives us the flux of lipids in mg/m^2.day.

Appendix 9: Fatty Acid Composition of August Net Tows

Fatty Acids	S1 (n = 3)	S2 (n = 3)	S3 (n = 3)	S4 (n = 3)	Ref (n = 3)
14:0	10.1 ± 0.5	10.5 ± 1.1	10.7 ± 0.6	9.3 ± 0.6	8.9 ± 0.4
15:0	0.9 ± 0.0	0.7 ± 0.1	0.8 ± 0.0	0.7 ± 0.1	0.9 ± 0.1
15:0i	1.1 ± 0.1	0.9 ± 0.0	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.0
15:0ai	1.0 ± 0.6	0.6 ± 0.3	0.3 ± 0.1	0.4 ± 0.0	0.6 ± 0.2
16:0	22.6 ± 0.7	20.1 ± 1.0	19.9 ± 0.3	18.5 ± 1.7	19.9 ± 0.5
16:0i	0.3 ± 0.4	0.5 ± 0.0	-	-	0.5 ± 0.2
17:0	0.5 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.2	0.1 ± 0.2
17:0i	2.4 ± 0.4	1.8 ± 0.4	1.3 ± 0.1	1.3 ± 0.2	1.8 ± 0.3
17:0ai	0.2 ± 0.2	0.2 ± 0.1	-	-	0.5 ± 0.0
18:0	5.4 ± 0.7	3.2 ± 0.4	3.4 ± 0.4	3.1 ± 0.1	2.5 ± 0.2
Σ SAT	**44.5 ± 1.5**	**38.8 ± 2.1**	**38.0 ± 1.2**	**34.2 ± 2.5**	**36.6 ± 1.7**
15:1	-	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.0
16:1ω9	0.1 ± 0.2	0.1 ± 0.1	-	-	-
16:1ω7	10.6 ± 1.9	10.9 ± 0.8	10.8 ± 0.6	8.2 ± 0.6	10.1 ± 0.2
16:1ω5	0.8 ± 0.1	0.8 ± 0.0	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.0
18:1ω11	0.1 ± 0.2	0.4 ± 0.0	0.4 ± 0.0	0.1 ± 0.2	0.1 ± 0.2
18:1ω9	4.1 ± 0.3	3.4 ± 0.2	3.2 ± 0.1	3.2 ± 0.4	3.1 ± 0.3
18:1ω7	3.2 ± 0.2	3.0 ± 0.2	3.0 ± 0.2	3.6 ± 0.4	3.2 ± 0.1
18:1ω5	0.5 ± 0.3	0.2 ± 0.2	0.2 ± 0.1	-	0.2 ± 0.2
20:1ω11	2.3 ± 0.4	3.0 ± 0.3	3.6 ± 0.1	3.8 ± 0.3	2.8 ± 0.1
20:1ω9	0.1 ± 0.2	0.5 ± 0.1	0.5 ± 0.0	0.7 ± 0.1	0.3 ± 0.3
20:1ω7	0.7 ± 0.5	0.6 ± 0.0	0.5 ± 0.0	0.6 ± 0.1	0.5 ± 0.1
22:1ω11	-	0.2 ± 0.2	0.3 ± 0.0	0.3 ± 0.3	0.2 ± 0.2
22:1ω9	0.1 ± 0.2	0.2 ± 0.0	0.3 ± 0.0	-	0.1 ± 0.1
Σ MUFA	**22.6 ± 1.5**	**23.7 ± 0.5**	**24.0 ± 0.1**	**21.7 ± 1.3**	**21.8 ± 0.6**
16:2ω4	0.9 ± 0.2	0.8 ± 0.0	0.6 ± 0.1	0.7 ± 0.1	0.9 ± 0.1
16:3ω4	1.2 ± 0.2	0.7 ± 0.2	0.9 ± 0.0	0.7 ± 0.0	1.5 ± 0.3
16:4ω3	0.5 ± 0.1	0.7 ± 0.1	0.8 ± 0.0	0.6 ± 0.0	0.5 ± 0.1
16:4ω1	2.3 ± 0.4	1.6 ± 0.1	1.5 ± 0.0	1.9 ± 0.1	2.3 ± 0.1
18:2ω6	2.8 ± 0.3	3.5 ± 0.1	4.1 ± 0.1	3.5 ± 0.3	3.4 ± 0.1
18:3ω6	0.1 ± 0.2	0.3 ± 0.0	0.3 ± 0.0	-	0.2 ± 0.2
18:3ω3	1.6 ± 0.1	2.3 ± 0.1	2.8 ± 0.1	2.4 ± 0.0	2.4 ± 0.1
18:4ω3	4.0 ± 0.4	5.1 ± 0.2	5.6 ± 0.1	5.7 ± 0.3	5.7 ± 0.1
20:2ω6	0.2 ± 0.3	0.4 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.6 ± 0.1
20:3ω3	-	0.1 ± 0.1	0.1 ± 0.2	0.3 ± 0.2	-
20:4ω6	0.2 ± 0.2	0.6 ± 0.0	0.4 ± 0.1	0.7 ± 0.1	0.6 ± 0.0
20:4ω3	1.0 ± 0.0	1.0 ± 0.0	0.8 ± 0.0	1.0 ± 0.1	0.7 ± 0.1
20:5ω3	9.8 ± 0.8	11.0 ± 0.9	11.1 ± 0.6	14.3 ± 1.6	13.0 ± 0.6
21:5ω3	-	0.3 ± 0.0	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.2
22:5ω3	-	-	-	-	-
22:6ω3	7.9 ± 2.6	9.1 ± 1.1	8.6 ± 0.6	11.3 ± 1.9	9.5 ± 0.9
Σ PUFA	**32.6 ± 2.3**	**37.5 ± 2.3**	**38.0 ± 1.3**	**43.6 ± 3.7**	**41.5 ± 1.3**
Bact.	**6.5 ± 1.4**	**5.5 ± 1.0**	**4.1 ± 0.3**	**3.9 ± 0.4**	**5.9 ± 0.6**
Terr.	**4.4 ± 0.4**	**5.8 ± 0.2**	**6.9 ± 0.1**	**5.9 ± 0.3**	**5.8 ± 0.1**
Σ ω3	**24.8 ± 0.3**	**29.7 ± 2.3**	**30.0 ± 1.2**	**35.9 ± 3.9**	**32.1 ± 1.5**
Σ 16:1 / 16:0	**0.51 ± 0.10**	**0.59 ± 0.05**	**0.58 ± 0.03**	**0.48 ± 0.01**	**0.54 ± 0.01**
Σ 16 / Σ 18	**1.80 ± 0.11**	**1.70 ± 0.09**	**1.55 ± 0.06**	**1.44 ± 0.06**	**1.75 ± 0.09**
20:1 + 22:1	**3.3 ± 0.9**	**4.5 ± 0.3**	**5.2 ± 0.2**	**5.4 ± 0.1**	**3.8 ± 0.5**
22:6ω3 / 20:5ω3	**0.80 ± 0.19**	**0.82 ± 0.05**	**0.77 ± 0.01**	**0.79 ± 0.05**	**0.73 ± 0.04**

Appendix 10: Fatty Acid Composition of October Net Tows

Fatty Acids	S1 (n = 2)	S2 (n = 3)	S3 (n = 3)	S4 (n = 3)	Ref (n = 3)
14:0	5.8 ± 0.2	5.4 ± 0.2	4.5 ± 0.2	4.7 ± 0.1	4.1 ± 0.1
15:0	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.6 ± 0.0
15:0i	0.4 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
15:0ai	0.2 ± 0.0	-	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.0
16:0	16.9 ± 0.5	16.0 ± 0.1	13.6 ± 0.5	13.9 ± 0.2	15.7 ± 0.4
16:0i	0.3 ± 0.0	-	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
17:0	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.1
17:0i	0.7 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.8 ± 0.0
17:0ai	0.2 ± 0.0	-	0.1 ± 0.1	0.1 ± 0.0	0.3 ± 0.0
18:0	2.2 ± 0.2	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.0	2.1 ± 0.1
Σ SAT	**27.5 ± 1.1**	**24.2 ± 0.3**	**21.2 ± 0.8**	**21.9 ± 0.3**	**24.7 ± 0.4**
15:1	0.1 ± 0.0	0.1 ± 0.1	-	-	-
16:1ω9	0.2 ± 0.1	-	-	-	-
16:1ω7	6.5 ± 0.5	9.1 ± 1.3	4.5 ± 0.2	4.6 ± 0.1	4.2 ± 0.2
16:1ω5	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
18:1ω11	-	-	-	-	0.3 ± 0.0
18:1ω9	13.2 ± 1.0	11.5 ± 0.4	14.8 ± 1.0	15.2 ± 0.8	10.9 ± 0.3
18:1ω7	2.0 ± 0.3	2.0 ± 0.1	1.9 ± 0.0	1.9 ± 0.0	2.4 ± 0.0
18:1ω5	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
20:1ω11	1.5 ± 0.2	1.2 ± 0.1	1.6 ± 0.0	1.7 ± 0.1	1.6 ± 0.0
20:1ω9	0.7 ± 0.0	0.8 ± 0.1	0.8 ± 0.2	1.0 ± 0.2	0.9 ± 0.0
20:1ω7	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.5 ± 0.0
22:1ω11	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
22:1ω9	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Σ MUFA	**25.9 ± 1.9**	**26.2 ± 1.5**	**25.2 ± 0.9**	**26.4 0.5**	**22.3 ± 0.3**
16:2ω4	0.6 ± 0.0	1.1 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.1
16:3ω4	1.0 ± 0.2	1.2 ± 0.1	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.1
16:4ω3	0.6 ± 0.1	0.5 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.7 ± 0.0
16:4ω1	0.6 ± 0.0	0.9 ± 0.1	0.6 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
18:2ω6	2.0 ± 0.1	1.8 ± 0.1	1.7 ± 0.0	1.8 ± 0.0	2.0 ± 0.0
18:2ω4	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
18:3ω6	0.3 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
18:3ω4	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
18:3ω3	1.3 ± 0.0	1.2 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.0
18:4ω3	4.6 ± 0.2	4.9 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	4.7 ± 0.0
20:2ω6	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.2	0.4 ± 0.1	0.5 ± 0.0
20:3ω6	0.1 ± 0.1	-	-	-	-
20:3ω3	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
20:4ω6	0.5 ± 0.0	0.7 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	1.1 ± 0.0
20:4ω3	2.3 ± 0.1	1.9 ± 0.0	1.9 ± 0.1	2.1 ± 0.1	2.3 ± 0.0
20:5ω3	12.2 ± 0.1	14.2 ± 0.3	15.0 ± 0.3	13.9 ± 0.3	14.7 ± 0.2
21:5ω3	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
22:5ω3	1.0 ± 0.1	0.9 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	1.2 ± 0.0
22:6ω3	18.0 ± 0.5	18.8 ± 1.4	23.0 ± 0.5	21.4 ± 0.5	21.6 ± 0.2
Σ PUFA	**46.5 ± 0.8**	**49.8 ± 1.4**	**53.7 ± 0.7**	**51.5 ± 0.6**	**52.7 ± 0.1**
Bact.	**2.8 ± 0.3**	**1.7 ± 0.2**	**1.8 ± 0.2**	**2.2 ± 0.1**	**3.0 ± 0.0**
Terr.	**3.2 ± 0.1**	**2.9 ± 0.1**	**3.0 ± 0.1**	**3.2 ± 0.1**	**3.5 ± 0.0**
Σ ω3	**40.5 ± 0.6**	**42.9 ± 1.7**	**49.0 ± 0.5**	**46.8 ± 0.7**	**47.2 ± 0.2**
Σ 16:1 / 16:0	**0.43 ± 0.04**	**0.60 ± 0.09**	**0.36 ± 0.02**	**0.37 ± 0.00**	**0.30 ± 0.02**
Σ 16 / Σ 18	**1.04 ± 0.04**	**1.23 ± 0.05**	**0.75 ± 0.04**	**0.76 ± 0.02**	**0.92 ± 0.02**
20:1 + 22:1	**3.1 ± 0.1**	**2.7 ± 0.1**	**3.0 ± 0.3**	**3.4 ± 0.3**	**3.5 ± 0.1**
22:6ω3 / 20:5ω3	**1.48 ± 0.03**	**1.32 ± 0.07**	**1.53 ± 0.03**	**1.54 ± 0.02**	**1.47 ± 0.02**

Appendix 11: Fatty Acid Composition of August Sediment Traps

Fatty Acids	S1 (n = 4)	S2 (n = 3)	S3 (n = 3)	S4 (n = 4)	Ref (n = 4)
14:0	6.4 ± 0.2	7.0 ± 0.6	8.5 ± 0.7	7.7 ± 1.0	7.3 ± 0.1
15:0	0.8 ± 0.1	0.9 ± 0.0	0.9 ± 0.0	0.8 ± 0.1	0.9 ± 0.0
15:0i	1.0 ± 0.3	1.3 ± 0.3	1.2 ± 0.2	1.8 ± 0.3	0.8 ± 0.0
15:0ai	0.7 ± 0.1	0.5 ± 0.3	0.5 ± 0.1	1.5 ± 0.2	1.1 ± 0.1
16:0	18.6 ± 2.1	20.7 ± 0.4	19.1 ± 1.2	17.1 ± 2.6	22.0 ± 1.4
16:0i	0.2 ± 0.4	-	-	-	0.4 ± 0.1
16:0ai	0.2 ± 0.4	-	-	-	-
17:0	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.0	-	0.2 ± 0.3
17:0i	1.1 ± 0.5	0.8 ± 0.1	0.8 ± 0.1	1.0 ± 0.2	1.4 ± 0.1
17:0ai	-	0.1 ± 0.2	0.1 ± 0.2	0.7 ± 0.1	-
18:0	4.0 ± 0.5	3.1 ± 0.3	2.8 ± 0.1	3.0 ± 0.4	3.8 ± 1.4
Σ SAT	**33.6 ± 1.6**	**35.0 ± 1.9**	**34.9 ± 0.6**	**34.1 ± 4.4**	**37.9 ± 2.0**
14:1	-	-	0.3 ± 0.4	-	0.2 ± 0.0
15:1	-	0.1 ± 0.2	0.2 ± 0.2	-	0.5 ± 0.0
16:1ω9	-	0.1 ± 0.2	0.5 ± 0.0	-	-
16:1ω7	14.3 ± 3.2	14.1 ± 1.7	15.5 ± 1.8	20.9 ± 2.9	12.1 ± 0.3
16:1ω5	0.6 ± 0.1	0.6 ± 0.3	0.6 ± 0.1	1.5 ± 0.3	0.4 ± 0.1
18:1ω11	0.3 ± 0.1	-	0.1 ± 0.2	-	0.2 ± 0.2
18:1ω9	6.5 ± 0.8	7.9 ± 1.5	9.9 ± 1.9	4.9 ± 0.9	5.6 ± 0.3
18:1ω7	6.0 ± 1.3	6.1 ± 0.4	5.8 ± 0.4	6.7 ± 0.9	5.7 ± 0.5
18:1ω5	0.7 ± 0.3	0.7 ± 0.2	0.5 ± 0.5	0.6 ± 0.6	0.5 ± 0.1
20:1ω11	1.0 ± 0.1	1.1± 0.1	1.0 ± 0.1	0.5 ± 0.4	1.0 ± 0.2
20:1ω9	2.4 ± 0.8	1.7 ± 0.5	1.6 ± 0.4	0.7 ± 0.5	0.9 ± 0.4
20:1ω7	0.9 ± 0.1	0.9 ± 0.3	0.5 ± 0.0	0.3 0±.3	0.8 ± 0.2
22:1ω11	2.9 ± 1.5	1.4 ± 0.5	1.6 ± 0.8	0.4 ± 0.4	0.8 ± 0.8
22:1ω9	0.7 ± 0.2	0.7 ± 0.4	0.4 ± 0.0	0.1 ± 0.2	0.4 ± 0.2
Σ MUFA	**36.3 ± 3.7**	**35.4 ± 1.1**	**38.5 ± 2.4**	**36.8 ± 4.7**	**29.1 ± 0.7**
16:2ω4	1.1 ± 0.4	0.7 ± 0.2	0.8 ± 0.1	1.1 ± 0.2	0.9 ± 0.2
16:3ω4	1.1 ± 0.4	0.9 ± 0.1	1.3 ± 0.4	1.5 ± 0.6	1.2 ± 0.1
16:4ω3	-	-	0.2 ± 0.2	-	0.3 ± 0.1
16:4ω1	0.9 0.3	0.8 ± 0.2	1.0 ± 0.1	1.2 ± 0.4	0.9 ± 0.1
18:2ω6	1.2 ± 0.0	1.5 ± 0.1	1.7 ± 0.1	2.1 ± 0.1	1.6 ± 0.2
18:2ω4	0.8 ± 0.5	0.8 ± 0.2	0.7 ± 0.2	-	1.2 ± 0.5
18:3ω3	1.0 ± 0.3	1.2 ± 0.1	1.2 ± 0.1	1.6 ± 0.3	1.4 ± 0.3
18:4ω3	1.7 ± 0.1	2.3 ± 0.3	2.4 ± 0.3	1.9 ± 0.6	3.4 ± 0.8
20:2ω6	0.3 ± 0.3	0.1 ± 0.2	0.1 ± 0.2	-	0.4 ± 0.1
20:3a*	8.2 ± 5.6	8.7 ± 4.6	6.5 ± 3.1	-	8.9 ± 1.8
20:3ω6	-	0.2 ± 0.3	-	-	-
20:4ω6	0.4 ± 0.2	0.4 ± 0.0	0.2 ± 0.2	1.2 ± 0.4	-
20:4ω3	-	0.1 ± 0.2	0.1 ± 0.2	-	0.2 ± 0.2
20:5ω3	7.3 1.4	7.7 ± 1.6	7.0 ± 1.0	11.1 ± 3.8	8.0 ± 1.1
21:5ω3	-	-	-	0.2 ± 0.5	-
22:5ω3	0.5 ± 0.3	0.4 ± 0.0	0.1 ± 02	-	0.3 ± 0.2
22:6ω3	3.4 ± 0.2	3.7 ± 0.9	2.8 ± 0.4	6.4 ± 2.9	4.3 ± 0.6
Σ PUFA	**29.1 ± 2.9**	**29.7 ± 2.3**	**26.6 ± 1.8**	**29.1 ± 9.0**	**32.9 ± 1.9**
Bact	**4.4 ± 1.3**	**4.3 ± 0.5**	**4.4 ± 0.2**	**6.0 ± 0.7**	**5.3 ± 0.3**
Terr	**2.2 ± 0.4**	**2.7 ± 0.2**	**2.9 ± 0.2**	**3.7 ± 0.3**	**2.5 ± 0.6**
Σ ω3	**13.9 ± 2.1**	**15.4 ± 2.6**	**13.8 ± 1.3**	**21.3 ± 7.6**	**17.9 ± 0.3**
Σ 16:1 / 16:0	**0.83 ± 0.26**	**0.71 ± 0.09**	**0.87 ± 0.15**	**1.32 ± 0.08**	**0.57 ± 0.03**
Σ 16 / Σ 18	**1.68 ± 0.21**	**1.61 ± 0.21**	**1.56 ± 0.07**	**2.10 ± 0.16**	**1.62 ± 0.06**
20:1 + 22:1	**7.8 ± 2.5**	**5.9 ± 0.5**	**5.1 ± 1.1**	**2.1 ± 1.4**	**3.9 ± 1.4**
22:6ω3 / 20:5ω3	**0.48 ± 0.08**	**0.48 ± 0.03**	**0.41 ± 0.03**	**0.58 ± 0.12**	**0.55 ± 0.02**

*: position of double bonds not known

Appendix 12: Fatty Acid Composition of September Sediment Traps

Fatty Acids	S1 (n = 4)	S2 (n = 4)	S3 (n = 4)	S4 (n = 4)	Ref (n = 4)
14:0	5.0 ± 1.4	8.9 ± 2.4	9.0 ± 3.2	7.5 ± 0.8	8.2 ± 1.1
15:0	0.6 ± 0.1	0.9 ± 0.3	1.0 ± 0.4	1.1 ± 0.0	1.1 ± 0.1
15:0i	0.7 ± 0.1	1.4 ± 0.5	1.3 ± 0.6	1.8 ± 0.1	1.1 ± 0.2
15:0ai	0.4 ± 0.1	0.7 ± 0.2	0.9 ± 0.5	1.3 ± 0.1	0.7 ± 0.2
16:0	10.3 ± 1.8	17.5 ± 2.9	17.4 ± 3.8	18.8 ± 1.7	20.6 ± 5.7
16:0i	0.3 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.0	0.4 ± 0.1
16:0ai	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.2
17:0	0.2 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.7 ± 0.1	0.3 ± 0.3
17:0i	0.4 ± 0.1	0.9 ± 0.2	0.8 ± 0.3	1.0 ± 0.1	0.9 ± 0.1
17:0ai	0.1 ± 0.1	0.2 ± 0.0	-	0.4 ± 0.0	0.2 ± 0.2
18:0	1.5 ± 0.2	2.6 ± 0.4	2.5 ± 0.7	3.8 ± 1.4	2.7 ± 0.9
Σ SAT	**19.6 ± 3.8**	**34.4 ± 7.2**	**33.8 ± 9.1**	**37.1 ± 2.5**	**36.3 ± 5.4**
14:1	0.1 ± 0.2	0.4 ± 0.3	0.2 ± 0.2	0.4 ± 0.3	0.3 ± 0.3
15:1	0.1 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.4 ± 0.1	0.3 ± 0.1
16:1ω9	0.1 ± 0.1	0.3 ± 0.2	0.4 ± 0.2	-	0.2 ± 0.2
16:1ω7	8.3 ± 1.5	15.6 ± 4.7	16.8 ± 6.5	18.9 ± 1.1	19.5 ± 1.5
16:1ω5	0.4 ± 0.2	0.7 ± 0.2	1.0 ± 0.5	1.2 ± 0.1	0.8 ± 0.2
18:1ω11	-	0.2 ± 0.1	0.1 ± 0.2	0.2 ± 0.1	0.2 ± 0.2
18:1ω9	3.6 ± 0.7	8.5 ± 1.8	5.5 ± 0.5	4.9 ± 0.4	6.5 ± 1.2
18:1ω7	2.1 ± 0.4	3.9 ± 1.2	4.2 ± 1.3	5.3 ± 0.3	6.2 ± 2.7
18:1ω5	0.3 ± 0.0	0.3 ± 0.1	0.5 ± 0.2	0.8 ± 0.2	0.4 ± 0.3
20:1ω11	-	0.6 ± 0.7	0.5 ± 0.5	0.7 ± 0.1	0.8 ± 0.3
20:1ω9	13.7 ± 1.4	5.6 ± 5.8	6.7 ± 6.5	1.4 ± 0.3	1.5 ± 0.3
20:1ω7	1.0 ± 0.2	0.7 ± 0.4	0.7 ± 0.5	0.3 ± 0.2	0.9 ± 0.8
22:1ω11	38.4 ± 6.7	10.6 ± 13.9	11.9 ± 12.3	1.5 ± 0.5	1.5 ± 0.7
22:1ω9	3.7 ± 0.7	1.3 ± 1.3	1.4 ± 1.2	0.4 ± 0.0	0.6 ± 0.1
Σ MUFA	**72.0 ± 5.9**	**48.9 ± 12.9**	**50.8 ± 11.6**	**37.4 ± 1.7**	**40.2 ± 4.0**
16:2ω4	0.4 ± 0.1	0.7 ± 0.2	0.7 ± 0.3	0.9 ± 0.1	0.9 ± 0.4
16:3ω4	0.4 ± 0.1	0.7 ± 0.3	0.7 ± 0.4	0.9 ± 0.5	1.0 ± 0.6
16:4ω3	-	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.0	0.1 ± 0.2
16:4ω1	0.3 ± 0.1	0.6 ± 0.2	0.5 ± 0.2	0.8 ± 0.2	0.6 ± 0.3
18:2ω6	0.6 ± 0.1	1.0 ± 0.3	1.1 ± 0.4	1.5 ± 0.1	1.1 ± 0.4
18:2ω4	-	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.9 ± 1.0
18:3ω3	0.5 ± 0.1	0.8 ± 0.3	1.1 ± 0.6	1.6 ± 0.2	1.0 ± 0.5
18:4ω3	0.7 ± 0.3	1.5 ± 0.6	1.2 ± 0.5	1.8 ± 0.4	1.7 ± 1.1
20:2ω6	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.4 ± 0.0
20:3ω3	-	-	-	-	0.5 ± 0.4
20:3a*	0.5 ± 0.2	1.7 ± 1.3	1.9 ± 1.5	1.4 ± 0.8	2.9 ± 2.5
20:4ω6	0.2 ± 0.1	0.3 ± 0.0	0.3 ± 0.3	0.8 ± 0.1	0.4 ± 0.2
20:4ω3	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.2
20:5ω3	2.7 ± 0.7	4.9 ± 1.5	4.2 ± 2.4	8.2 ± 1.2	6.1 ± 3.6
21:5ω3	-	0.3 0±.4	0.2 ± 0.3	0.7 ± 0.5	0.2 ± 0.2
22:5ω3	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.2
22:6ω3	1.7 ± 0.3	2.7 ± 0.7	2.1 ± 1.0	4.7 ± 1.2	2.9 ± 1.8
Σ PUFA	**8.4 ± 2.3**	**16.7 ± 5.8**	**15.5 ± 7.4**	**25.5 ± 2.6**	**23.5 ± 9.3**
Bact	**2.8 ± 0.8**	**5.6 ± 1.7**	**5.8 ± 2.4**	**8.4 ± 0.3**	**5.5 ± 0.6**
Terr	**1.1 ± 0.2**	**1.8 ± 0.6**	**2.2 ± 1.0**	**3.1 ± 0.3**	**2.1 ± 0.9**
Σ ω3	**5.9 ± 1.6**	**11.0 ± 3.5**	**9.1 ± 4.6**	**18.0 ± 2.8**	**13.1 ± 7.2**
Σ 16:1 / 16:0	**0.85 ± 0.06**	**0.93 ± 0.16**	**1.01 ± 0.23**	**1.07 ± 0.14**	**1.05 ± 0.25**
Σ 16 / Σ 18	**2.21 ± 0.07**	**1.93 ± 0.08**	**2.28 ± 0.42**	**2.14 ± 0.16**	**2.17 ± 0.26**
20:1 + 22:1	**56.8 ± 8.7**	**18.7 ± 20.9**	**21.2 ± 20.0**	**4.3 ± 0.8**	**5.4 ± 0.4**
22:6ω3 / 20:5ω3	**0.67 ± 0.08**	**0.57 ± 0.04**	**0.54 ± 0.19**	**0.57 ± 0.11**	**0.47 ± 0.06**

*: position of double bonds not known

Appendix 13: Fatty Acid Composition of October Sediment Traps

Fatty Acids	S1 (n = 4)	S2 (n = 3)	S3 (n = 3)	S4 (n = 3)	Ref (n = 4)
14:0	3.7 ± 1.4	7.6 ± 1.4	6.0 ± 0.2	6.6 ± 0.2	5.9 ± 0.9
15:0	0.4 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	0.9 ± 0.1	1.1 ± 0.0
15:0i	0.5 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	2.0 ± 0.1	1.1 ± 0.2
15:0ai	0.2 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	1.5 ± 0.1	0.7 ± 0.2
16:0	11.2 ± 1.9	19.8 ± 0.8	19.5 ± 0.7	16.0 ± 0.7	20.2 ± 1.7
16:0i	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.3 ± 0.0
16:0ai	0.2 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.3 ± 0.2
17:0	0.2 ± 0.1	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.8 ± 0.1
17:0i	0.3 ± 0.1	0.7 ± 0.1	0.8 ± 0.2	1.0 ± 0.1	1.0 ± 0.1
17:0ai	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.3 ± 0.1
18:0	1.9 ± 0.1	2.9 ± 0.5	2.7 ± 0.2	2.4 ± 0.2	3.2 ± 0.3
Σ SAT	**19.1 ± 3.6**	**34.5 ± 2.6**	**32.9 ± 1.5**	**32.4 ± 0.6**	**34.9 ± 3.1**
14:1	-	0.2 ± 0.1	0.2 ± 0.1	0.7 ± 0.1	0.3 ± 0.1
15:1	0.1 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	0.3 ± 0.1
16:1ω9	-	0.2 ± 0.0	0.2 ± 0.1	0.9 ± 0.3	0.4 ± 0.0
16:1ω7	6.9 ± 1.0	13.0 ± 1.3	14.3 ± 0.4	18.6 ± 0.1	13.1 ± 1.6
16:1ω5	0.3 ± 0.0	0.4 ± 0.1	1.0 ± 0.2	1.3 ± 0.0	0.8 ± 0.2
18:1ω11	-	0.1 ± 0.1	-	-	0.2 ± 0.2
18:1ω9	5.6 ± 0.4	10.8 ± 1.8	9.3 ± 0.3	5.0 ± 0.8	9.7 ± 1.3
18:1ω7	2.9 ± 0.5	5.6 ± 1.2	5.7 ± 0.7	6.4 ± 0.1	6.2 ± 0.5
18:1ω5	0.4 ± 0.2	0.7 ± 0.0	1.6 ± 0.3	0.5 ± 0.1	1.0 ± 0.2
20:1ω11	-	0.2 ± 0.4	0.8 ± 0.3	0.9 ± 0.0	0.9 ± 0.3
20:1ω9	15.1 ± 1.9	3.5 ± 1.3	2.6 ± 0.4	0.9 ± 0.1	1.0 ± 0.1
20:1ω7	1.3 ± 0.1	1.2 ± 0.2	0.7 ± 0.1	0.3 ± 0.2	0.6 ± 0.1
22:1ω11	33.6 ± 3.9	4.6 ± 2.3	3.2 ± 1.6	0.7 ± 0.2	0.2 ± 0.2
22:1ω9	3.3 ± 0.1	0.9 ± 0.3	0.6 ± 0.2	0.3 ± 0.0	0.4 ± 0.0
Σ MUFA	**69.5 ± 3.9**	**42.2 ± 1.8**	**41.0 ± 1.6**	**37.9 ± 0.7**	**35.8 ± 2.5**
16:2ω4	0.3 ± 0.2	0.6 ± 0.1	0.6 ± 0.0	1.0 ± 0.0	0.6 ± 0.1
16:3ω4	0.4 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0.7 ± 0.0	0.7 ± 0.1
16:4ω3	-	-	0.1 ± 0.1	0.5 ± 0.1	0.1 ± 0.1
16:4ω1	0.3 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	1.4 ± 0.1	0.5 ± 0.1
18:2ω6	1.3 ± 1.1	0.9 ± 0.0	1.0 ± 0.1	1.4 ± 0.0	1.5 ± 0.2
18:2ω4	0.2 ± 0.1	1.2 ± 0.6	0.7 ± 0.3	0.3 ± 0.0	0.8 ± 0.2
18:3ω3	0.5 ± 0.1	0.7 ± 0.1	0.9 ± 0.2	1.9 ± 0.2	1.2 ± 0.1
18:4ω3	0.7 ± 0.3	1.6 ± 0.2	1.6 ± 0.2	2.2 ± 0.1	2.2 ± 0.5
20:2ω6	0.3 ± 0.2	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.1
20:3ω3	-	0.1 ± 0.1	0.1 ± 0.1	-	0.3 ± 0.4
20:3a*	1.4 ± 1.0	9.5 ± 3.1	7.7 ± 2.4	1.4 ± 0.6	7.3 ± 3.2
20:4ω6	0.4 ± 0.3	0.1 ± 0.1	0.4 ± 0.1	1.2 ± 0.0	1.0 ± 1.1
20:4ω3	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
20:5ω3	3.0 ± 0.4	4.4 ± 0.8	6.2 ± 0.4	10.7 ± 0.4	7.0 ± 3.0
21:5ω3	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.6 ± 0.5	0.3 ± 0.3
22:5ω3	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.5 ± 0.1
22:6ω3	1.9 ± 0.4	1.8 ± 0.2	3.3 ± 0.4	4.6 ± 0.2	3.9 ± 0.9
Σ PUFA	**11.4 ± 1.4**	**23.3 ± 4.3**	**26.1 ± 2.8**	**29.7 ± 0.1**	**29.3 ± 4.1**
Bact	**2.3 ± 0.5**	**4.8 ± 0.1**	**5.4 ± 0.5**	**8.7 ± 0.4**	**6.6 ± 0.4**
Terr	**1.8 ± 1.1**	**1.6 ± 0.1**	**1.9 ± 0.2**	**3.3 ± 0.2**	**2.7 ± 0.2**
Σ ω3	**6.6 ± 0.9**	**9.3 ± 1.4**	**12.9 ± 1.1**	**21.3 ± 0.4**	**15.9 ± 3.9**
Σ 16:1/16:0	**0.64 ± 0.06**	**0.69 ± 0.09**	**0.79 ± 0.00**	**1.30 ± 0.07**	**0.71 ± 0.13**
Σ 16 / Σ 18	**1.46 ± 0.19**	**1.45 0±.07**	**1.60 ± 0.08**	**2.04 ± 0.09**	**1.43 ± 0.09**
20:1 + 22:1	**53.3 ± 5.8**	**10.5 ± 3.6**	**8.0 ± 1.9**	**3.1 ± 0.6**	**3.1 ± 0.6**
22:6ω3/20:5ω3	**0.62 ± 0.16**	**0.40 ± 0.03**	**0.53 ± 0.04**	**0.43 ± 0.02**	**0.59 ± 0.10**

*: position of double bonds not known

Appendix 14: Fatty Acid Composition of Cod Feed

	Herring *(Clupea harengus)* (n=3)	**Haddock Grower 46-14** (n=3)
14:0	4.3 ± 0.2	5.0 ± 0.1
15:0	0.3 ± 0.0	0.4 ± 0.0
15:0i	0.1 ± 0.0	0.1 ± 0.0
16:0	11.7 ± 3.4	17.8 ± 0.3
17:0	0.1 ± 0.1	0.4 ± 0.0
17:0i	0.2 ± 0.1	0.2 ± 0.1
17:0ai	0.1 ± 0.0	0.3 ± 0.1
18:0	1.1 ± 0.4	4.4 ± 0.1
Σ SAT	**17.9 ± 3.7**	**29.4 ± 0.4**
16:1ω7	8.1 ± 0.9	7.8 ± 0.0
16:1ω5	0.2 ± 0.1	-
18:1ω9	8.7 ± 5.1	16.6 ± 0.2
18:1ω7	2.4 ± 0.4	2.8 ± 0.1
20:1ω9	15.3 ± 6.0	5.8 ± 0.1
20:1ω7	1.3 ± 0.6	0.5 ± 0.0
22:1ω11	23.1 ± 9.9	7.6 ± 0.1
22:1ω9	1.4 ± 0.8	0.9 ± 0.0
Σ MUFA	**61.1 ± 10.1**	**42.5 ± 0.5**
16:2ω4	0.6 ± 0.1	0.9 ± 0.0
16:3ω4	0.5 ± 0.0	-
16:4ω1	0.6 ± 0.1	0.6 ± 0.0
18:2ω6	0.7 ± 0.3	9.3 ± 0.1
18:2ω4	0.1 ± 0.0	0.3 ± 0.3
18:3ω6	-	0.1 ± 0.1
18:3ω4	-	0.2 ± 0.1
18:3ω3	0.3 ± 0.1	1.1 ± 0.0
18:4ω3	0.8 ± 0.2	1.3 ± 0.0
20:4ω6	0.3 ± 0.1	0.8 ± 0.6
20:4ω3	0.2 ± 0.1	0.6 ± 0.0
20:5ω3	7.2 ± 2.5	5.8 ± 0.1
22:5ω3	0.8 ± 0.1	1.0 ± 0.0
22:6ω3	8.2 ± 3.2	5.8 ± 0.2
Σ PUFA	**20.9 ± 6.5**	**28.1 ± 0.6**
Bact	**0.8 ± 0.2**	**1.5 ± 0.2**
Terr	**1.0 ± 0.4**	**10.3 ± 0.1**
Σ ω3	**17.8 ± 6.2**	**15.8 ± 0.4**
Σ 16:1 / 16:0	**0.75 ± 0.12**	**0.44 ± 0.01**
Σ 16 / Σ 18	**1.59 ± 0.34**	**0.74 ± 0.01**
20:1 + 22:1	**41.0 ± 16.6**	**14.8 ± 0.2**
22:6ω3 / 20:5ω3	**1.12 ± 0.12**	**1.00 ± 0.03**

Appendix 15: Fatty Acid Composition of Cod Muscle (n = 3 for all)

Fatty Acids	Inshore 2001	Inshore 2002	Offshore 2001	Offshore 2002
14:0	1.5 ± 0.3	0.6 ± 0.1	1.1 ± 0.0	0.9 ± 0.0
15:0	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
15:0i	0.1 ± 0.1	-	0.1 ± 0.1	-
16:0	17.4 ± 0.6	19.5 ± 0.3	17.0 ± 0.5	16.7 ± 0.5
17:0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
17:0i	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.0
17:0ai	-	0.1 ± 0.0	-	0.1 ± 0.0
18:0	3.0 ± 0.4	4.7 ± 0.3	2.9 ± 0.2	3.2 ± 0.2
22:0	-	0.1 ± 0.1	0.1 ± 0.1	-
Σ SAT	**22.6 ± 0.5**	**25.9 ± 0.3**	**21.7 ±0.5**	**21.7 ± 0.6**
16:1ω7	3.2 ± 0.3	1.4 ± 0.1	2.4 ± 0.2	2.4 ± 0.3
16:1ω5	0.2 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
18:1ω11	-	0.2 ± 0.2	-	-
18:1ω9	6.5 ± 0.5	4.3 ± 0.4	6.1 ± 0.8	7.0 ± 0.6
18:1ω7	3.4 ± 0.4	3.5 ± 0.4	3.6 ± 0.2	3.2 ± 0.1
18:1ω5	0.4 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
20:1ω9	2.1 ± 0.1	0.7 ± 0.1	2.5 ± 0.1	2.8 ± 0.5
20:1ω7	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
22:1ω11	0.5 ± 0.1	0.1 ± 0.1	0.5 ± 0.0	0.5 ± 0.1
22:1ω9	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
24:1	0.4 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
Σ MUFA	**17.1 ± 0.5**	**11.4 ± 1.0**	**16.4 ± 0.7**	**17.4 ± 0.6**
16:2ω4	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.1
16:3ω4	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
16:4ω1	0.2 ± 0.2	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.2
18:2ω6	0.8 ± 0.2	0.5 ± 0.1	0.8 ± 0.1	0.6 ± 0.0
18:2ω4	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18:3ω4	-	-	-	0.1 ± 0.0
18:3ω3	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
18:4ω3	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
20:2ω6	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
20:3ω6	-	-	0.1 ± 0.0	0.1 ± 0.0
20:3ω3	-	0.2 ± 0.2	0.1 ± 0.2	0.1 ± 0.1
20:4ω6	2.6 ± 0.8	5.6 ± 1.5	2.0 ± 0.4	1.8 ± 0.2
20:4ω3	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.0	0.4 ± 0.1
20:5ω3	19.8 ± 0.8	23.3 ± 1.0	19.1 ± 1.2	16.7 ± 1.0
21:5ω3	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0
22:5ω6	0.4 ± 0.3	0.6 ± 0.2	0.3 ± 0.0	0.3 ± 0.0
22:5ω3	1.7 ± 0.2	3.0 ± 1.4	1.7 ± 0.2	1.9 ± 0.2
22:6ω3	32.3 ± 3.1	27.6 ± 4.1	35.9 ± 1.5	37.5 ± 1.5
Σ PUFA	**60.1 ± 1.0**	**62.7 ± 1.2**	**61.8 ± 0.6**	**60.9 ± 0.5**
Σ ω3	**55.2 ± 1.9**	**55.0 ± 2.1**	**57.9 ± 0.7**	**57.2 ± 0.5**
22:6ω3/20:5ω3	**1.6 ± 0.2**	**1.2 ± 0.2**	**1.9 ± 0.2**	**2.3 ± 0.2**
Σω3/Σω6	**14.8 ± 3.9**	**8.2 ± 1.9**	**17.7 ± 2.8**	**19.6 ± 1.4**

Appendix 16: Fatty Acid Composition of Cod Liver (n = 3 for all)

Fatty Acids	In 2001	In 2002	Off 2001	Off 2002
14:0	3.3 ± 0.3	2.8 ± 0.4	4.4 ± 0.5	3.0 ± 0.4
15:0	0.3 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
15:0i	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.0
16:0	12.8 ± 0.5	11.4 ±1.0	9.3 ± 0.6	8.4 ± 0.2
16:0i	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.0
17:0	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
17:0i	0.3 ± 0.1	0.6 ± 0.2	0.3 ± 0.0	0.3 ± 0.0
17:0ai	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
18:0	3.0 ± 0.2	3.5 ± 0.2	1.5 ± 0.1	2.0 ± 0.4
20:0	0.1 ± 0.1	-	0.1 ± 0.1	-
22:0	0.1 ± 0.1	-	-	-
Σ SAT	**20.7 ± 0.8**	**20.1 ± 1.3**	**16.7 ± 0.3**	**14.6 ± 0.2**
14:1	0.1 ± 0.1	-	-	-
15:1	0.1 ± 0.1	-	-	-
16:1ω9	0.1 ± 0.1	-	-	0.2 ± 0.0
16:1ω7	10.5 ± 1.8	7.3 ± 0.3	12.7 ± 0.4	10.8 ± 1.7
16:1ω5	0.2 ± 0.0	-	0.3 0.0	0.2 ± 0.0
18:1ω11	-	0.2 ± 0.4	-	-
18:1ω9	14.4 ± 0.6	12.1 ± 1.5	11.8 ± 1.2	15.9 ± 3.5
18:1ω7	5.3 ± 0.9	8.0 ± 1.1	5.3 ± 0.4	6.1 ± 0.6
18:1ω5	0.7 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.6 ± 0.1
20:1ω9/11	7.6 ± 1.2	6.8 ± 0.7	12.2 ± 1.1	13.3 ± 2.4
20:1ω7	1.1 ± 0.1	2.2 ± 0.6	0.9 ± 0.2	1.4 ± 0.1
22:1ω11	5.8 ± 1.7	2.1 ± 0.6	6.4 ± 1.5	7.3 ± 2.4
22:1ω9	1.1 ± 0.4	0.5 ± 0.2	1.0 ± 0.2	1.4 ± 0.4
24:1	0.4 ± 0.1	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.4
Σ MUFA	**47.3 ± 1.6**	**40.3 ± 1.8**	**51.5 ± 3.1**	**57.5 ± 2.8**
16:2ω4	0.7 ± 0.1	1.0 ± 0.3	0.9 ± 0.1	0.2 ± 0.0
16:3ω4	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.0	0.5 ± 0.1
18:2ω6	1.0 ± 0.2	1.1 ± 0.2	1.4 ± 0.0	1.3 ± 0.1
18:2ω4	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
18:3ω6	0.2 ± 0.0	0.1 ± 0.1	0.2 ± 0.0	0.1 ± 0.0
18:3ω4	0.1 ± 0.1	0.1 ± 0.1	-	-
18:3ω3	0.5 ± 0.1	0.9 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
18:4ω3	1.2 ± 0.1	1.0 ± 0.6	1.3 ± 0.2	1.0 ± 0.1
20:2ω6	0.2 ± 0.0	0.8 ± 0.2	0.3 ± 0.0	0.3 ± 0.1
20:3ω3	0.1 ± 0.0	0.3 ± 0.2	-	0.1 ± 0.1
20:4ω6	0.6 ± 0.1	1.5 ± 0.5	0.6 ± 0.0	0.6 ± 0.0
20:4ω3	0.4 ± 0.0	0.5 ± 0.2	0.6 ± 0.1	0.5 ± 0.1
20:5ω3	12.6 ± 1.4	10.7 ± 1.0	10.7 ± 2.2	9.2 ± 1.9
21:5ω3	0.4 ± 0.0	0.9 ± 0.5	0.4 ± 0.1	0.4 ± 0.1
22:5ω3	1.2 ± 0.1	5.5 ± 2.0	1.7 ± 0.2	1.7 ± 0.2
22:6ω3	10.9 ± 0.7	13.6 ± 3.6	12.1 ± 0.9	10.4 ± 0.3
Σ PUFA	**31.8 ± 2.0**	**39.6 ± 2.2**	**31.7 ± 3.2**	**27.7 ±2.9**
Σ ω3	**27.5 ± 2.1**	**33.4 ± 1.6**	**27.2 ± 3.1**	**23.9 ± 2.7**
22:6ω3/20:5ω3	**0.87 ± 0.04**	**1.29 ± 0.39**	**1.16 ± 0.22**	**1.18 ± 0.24**
Σω3/Σω6	**13.1 ± 1.7**	**8.50 ± 1.3**	**10.5 ± 1.5**	**10.2 ± 0.9**