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Assimilation of salmon feed and salmon feces by the great scallop (*Pecten maximus*)

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Marine Coastal Development
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Trondheim, September 2014
Lene Stensås

Abstract

The primary objective of this Master's thesis was to study if organic waste particles released from cage fish farms could be assimilated by the great scallop (*P. maximus*) through a laboratory trial and a field trial. In the laboratory experiment, scallops were co-fed salmon feed or salmon feces with algae at two different concentrations for 25 days. In the field study, scallops were installed in the sea for 3 months at four experimental stations close to a fish farm at the coast of western Norway (Florø).

The laboratory trial showed that phytoplankton was the most important part of the scallop's diet, resulting in better growth and condition. Scallops co-fed salmon feed or feces showed higher concentrations of 18:1 (n-9) in the digestive gland tissues compared to scallops fed algae alone. This suggested that scallops have utilized salmon feed and feces, due to the high amounts of 18:1 (n-9) in the supplemented feed. The scallops in the laboratory trial were found to incorporate phytoplankton over salmon feed and feces as long as algae were present in high amount. Less selectivity was observed when phytoplankton concentration was low.

The field study showed that the fatty acid signature in scallop digestive gland tissues, and to some degree gonad tissues, had high fractions of specific fatty acids found in high concentrations in diatoms (16:0, 16:1 n-7, EPA) and dinoflagellates (16:0, 18:4 n-3, EPA, DHA). This suggested that phytoplankton was an important part of the scallop's diet. Scallops cultivated under the influence of waste particles from the fish farm incorporated a higher fraction of 18:1 (n-9) compared to the Reference stations. The incorporation of 18:1 (n-9) showed that scallops were able to exploit the fish farm waste at least 200 meters away from the fish farm at 5 meters depth. The scallops utilized the extra energy from the farm effluents for growth, resulting in a higher growth rate at the Farm station compared to the Reference station. This suggests that scallops can be a relevant species in integrated multi-trophic aquaculture.

Contents

1 Introduction

FAO (2014) has reported that global food fish production has grown with an annual rate of 3.2% for the last fifty years. In 2012 the total aquaculture production was 66.6 million tons, of which China accounted for 61.7%. Further, finfish production including inland aquaculture and mariculture was 44.2 million tons worldwide, of which Norway is established as a world leading producer of finfish in the marine environment (1.3 million tons, 2.0% of total production). Norway's long coastline provides numerous areas suitable for aquaculture. Atlantic salmon (*Salmo salar*) is the major farmed species making Norway the dominant producer and exporter of Atlantic salmon (FAO, 2014).

It is estimated that there will be 9.2 billion human on Earth by 2050. Increasing the food production is necessary to meet the growing demand of animal protein. Considering that the lack of freshwater will limit production on land and that overfishing may lead to depletion of the natural fish stock, increasing aquaculture production can be an option (Duarte et al., 2009; Péron et al., 2010). However, salmon aquaculture faces some major environmental challenges. Salmon lice, spreading of diseases, escapes and nutrient wastes from the fish farm are the major concerns (Taranger et al., 2014). The open-water fish cages used in Norway releases large amounts of nutrient wastes in the form of salmon feed and salmon feces (Carroll et al., 2003; Wang et al., 2012). The mean losses to the environment from Norwegian salmon aquaculture is estimated to 70% carbon, 62% nitrogen and 72% phosphorous of the total feed input (Wang et al., 2012). This enrichment can result in an increased primary and benthic production, especially in areas with a low exchange of water to the surroundings (Taranger et al., 2014; Skogen et al., 2009).

One strategy to reduce the ecological effects of nutrient loading from the aquaculture to the environment is integrated multi-trophic aquaculture (IMTA). In IMTA, species from different trophic levels are cultured in close proximity to each other to fully utilize the wastes from the fed culture (Troell et al., 2009). There are economic benefits of IMTA, as well as environmental, since the biomass produced is expected to increase and the feed is better utilized (Chopin et al., 2001; Troell et al, 2009). China and Canada are among the countries that are already using IMTA system at a commercial scale (Troell et al., 2003, 2009), whereas Norway with its great potential for salmon-driven IMTA is still in the research phase.

Cultivation of inorganic extractive species as seaweed and organic extractive species as filter-feeders in close proximity to a salmon farm could increase the sustainability of aquaculture by using and recycling the effluents from the farm (Troell et al., 2009; Chopin et al., 2001; Abreu et al., 2009, 2011; Handå et al., 2012a,b). Earlier studies have shown positive effects by cultivating extractive species as oysters (Jones and Iwama, 1991; Lefebvre et al., 2000), blue mussels (Redmond et al., 2010, Handå et al., 2012b; MacDonald et al., 2011) and seaweed close to fish farms (Abreu et al., 2009, 2011; Handå et al., 2013).

A great deal of research is done considering the blue mussel (*Mytilus* sp.) in IMTA system. MacDonald et al., (2011) found that the feeding activity of mussels increased when installed in close proximity to a fish farm due to increased food availability. This resulted in increased growth and condition for mussels exposed to the nutrient waste (Perharda et al., 2007; Sarà et al., 2009). Troell and Norberg (1998) suggested that blue mussels would only incorporate farm wastes if overall seston concentrations are low, like in late autumn and winter in cool temperate North Atlantic waters. This was confirmed by Handå et al. (2012a, b) who showed that the mussels incorporated fatty acids which the salmon feed and salmon feces showed high concentrations of, in particular when the food supply was low.

Bivalves have shown good results when it comes to reducing eutrophication processes caused by fish farm effluents (Soto and Mena, 1999; Jones et al., 2001). Strohmeier et al. (2009) found that blue mussels (*M. edulis*) and great scallops (*Pecten maximus*) showed a steady clearance of particulate matter even the seston quantities varied. This makes these shellfish good candidates to reduce potential negative environmental influence from fish farms through bioremediation.

The great scallop is a highly valued and exclusive seafood product in Europe. *P. maximus* is located from the Spanish coast to the north of Norway, mainly at 15-30 meters depth. They live on the ocean floor partially buried in gravel and sand, preferably at exposed areas. Scallops reach the commercial size at 100 mm after three to four years, but can be as large as 170 mm in diameter (Moen and Svensen, 2008; Bergh and Strand, 2001). Scallops are filter-feeders and clear the water for seston where phytoplankton is a most important component (Moen and Svensen, 2008; Chauvaud et al., 2001; Delaunay et al., 1993; Shumway et al., 1997). During periods of low phytoplankton abundance, detritus can be a larger part of the diet (Cranford and Grant, 1990). It is observed that scallops orientate themselves against the direction of the water flow to increase the efficiency of water filtration (Moen and Svensen, 2008; Mathers, 1976).

The global landings of *P. maximus* in 2012 was 63 681 tons (FAO, 2012), where Norway accounted for 678 tons (Strand, 2014). At present, scallops exported to the European market are mainly harvested from wild populations, in which 80% of the landings are from the Hitra and Frøya area in Central Norway (Bergh and Strand, 2001; Strand, 2014). High market values combined with the risk for depletion of natural populations of *P. maximus* have encouraged the cultivation of scallop in controlled aquaculture production. This resulted in the Norwegian Scallop Program in 1994, established by farmers, regional authorities and research institutes. The cultivation of scallops in Norway today is based on spawning in hatcheries, where they are raised to appropriate size before deployment in the sea to grow-out (Bergh and Strand, 2001).

1.1 Objectives and hypothesis of the study

The primary objective of this Master's thesis was to study if organic waste particles released from cage fish farms could be assimilated by the great scallop (*P. maximus*). The experiments undertaken to answer this question included a field study where scallops were cultivated in salmon-driven IMTA and a laboratory study. Fatty acid analysis was performed on scallops installed in close proximity to a fish farm and in reference stations in the field trial. In the laboratory trial, different scallops were fed diets consisting of algae alone and algae supplemented by salmon feed or salmon feces. The project will deliver new knowledge regarding the potential use of this high valued filter feeder in IMTA.

2 Materials and methods

2.1 Laboratory experiment

Scallops were fed a diet consisting of pure algae alone and supplemented by small particulate wastes of salmon feces or salmon feed at two different algae concentrations to determine if they were capable to assimilate organic waste particles released from fish farms.

2.1.1 Experimental design

Scallops were given six different treatments as shown in Table 1. The control groups were fed algae while the other groups were fed salmon feed or salmon feces in addition to algae. The algae concentration was set to 50 µg C/L and 300 µg C/L representing a winter situation and a spring bloom (typical values for the Trondheim fjord, Leiknes, Ø. Pers. comm).

The algal diet included *Rhodomonas baltica* and *Chaetoceros muelleri* which were provided in proportions of 90 % *R. baltica* and 10 % *C. muelleri* relative to their carbon content. Semi-continuous cultures of *R. baltica* and *C. muelleri* were cultured in polycarbonate tubes (200 L) and glass bulbs (20 L), respectively, using autoclaved seawater enriched with a Conwy medium (Anderson, 2005) and a continuous supply of CO_2. *C. muelleri* was also supplied with silicate. The algae density was counted every day prior to feeding with a Beckman Coulter-Counter Multisizer 3 and feeding suspensions were mixed with 1µm-filtered seawater to obtain the correct concentration (Table 1). The feeding tanks (25 L) were aerated to avoid sedimentation.

The salmon feed in the diet was Optiline Premium 2500 from Skretting. Salmon feces were collected from 6 kg fish anesthetized with benzoak prior to the stripping at the fish farm Farmannsøya owned by Marine Harvest located outside Roan at the coast of Norway.Salmon feed and salmon feces were homogenized and freeze-dried for 24 hour before measuring the carbon content (Carlo Erba CHN model 1106 elemental analyzer). The carbon content of the salmon feed and feces was 50 % and 30%, respectively. Every day feed pellets and salmon feces were weighed (Mettler Toledo XA204), crushed for 60 seconds in a kitchen blender (Elektrolux ASB 2600) with 100 mL 1µm-filtered seawater separately, and diluted to obtain the correct concentration (Table 1).

Table 1. Experimental treatments. Algae (R. baltica and C. muelleri) were given as control and in combination with salmon feed or salmon feces. It was 3 trays per treatment, giving a total of 18 trays.

Treatments	Food concentration (µg C/L)		
	Algae	Salmon feed	Salmon feces
Control	300	-	-
Salmon feed	300	30	-
Salmon feces	300	-	30
Control	50	-	-
Salmon feed	50	30	-
Salmon feces	50	-	30

Scallops (33.0±0.3 mm) were installed in plastic trays (40 x 12 x 70 cm, 30 L volume) with flowing seawater (Figure 1). A perforated plastic plate (2 mm holes) was placed across the tray to create a mixing zone by the water and feed inflow. This provided an even feed distribution and prevented turbulence in the water stream. The water exchange was 6 L h^{-1} which along with aquarium pumps (New-Jet 1) generated a stream of water along the tray. The aquarium pumps pumped water from the distal end to the mixing zone at a rate of 3 l min^{-1}, leaving the water to be recirculated 6 times per hour. Water level regulation was achieved by placing a vertical discharge pipe (12 cm) at the distal end. Food was added continuously at a rate of 1.5 mL food suspended per minute with peristaltic pumps (Watson-Marlow 505U) into the mixing zone. See more details in Handå et al. (2012a). Temperature was measured every day and average temperature was found to differ from 12.4 °C to 13.0 °C between the various trays.

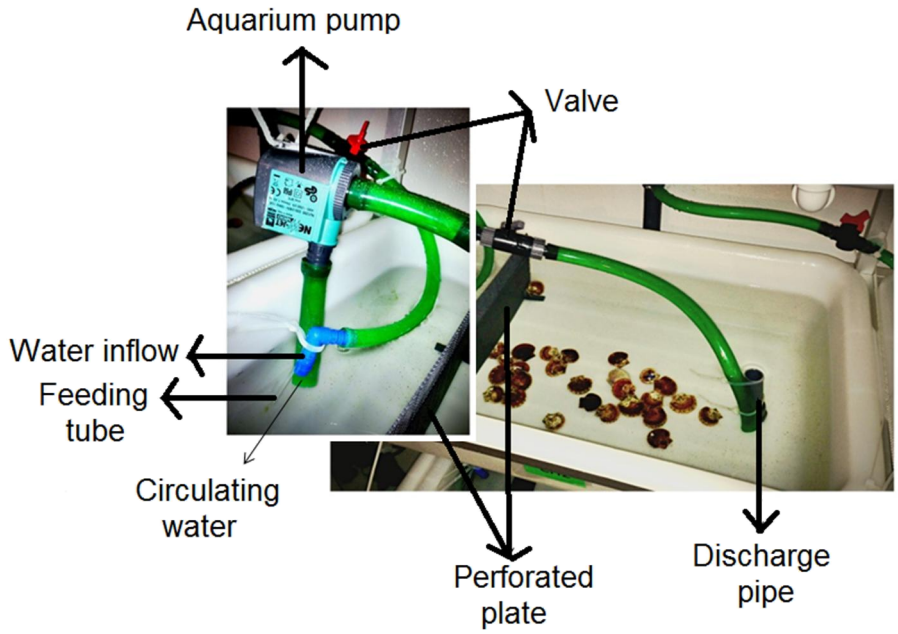

Figure 1: Experimental design

Scallops were sampled at ScalPro in Bergen in February and arrived at NTNU Center of Fisheries and Aquaculture (SeaLab) the 11th of February 2013. After arrival they were immediately placed in flow-through raceways (40 x 20 x 400 cm, 320 l volume, 1.6 L min^{-1} exchange rate) with12 ˚C for acclimatization through 14 days prior to the experiment. *Rhodomonas baltica* was continuously added in the raceway with peristaltic pumps (Watson-Marlow 505U) keeping the algal concentration at 15 µg C/L.

Seawater (35ppt) was collected from 70 meters depth in the Trondheim fjord. Water was sand-filtered and UV-treated before entering aerated reservoirs for temperature regulation and storage. Remaining impurities was successively removed with two aerially coupled CUNO AquaPure filters with nominal retention of particles >10 µm and >1 µm, respectively.

2.2 Field experiment

Scallops were deployed in the sea close to a fish farm to determine if they were capable to assimilate organic waste particles released from fish farms.

2.2.1 Experimental design

The experiment was carried out in close proximity to Marine Harvest's salmon farm Flåtegrunnen at Rognaldsvåg (N $61^0$34.586, E $4^0$48.942) just outside of Florø in Sogn og Fjordane (Figure 2). Flåtegrunnen had eight Polar circle plastic cages (Ø80 meter) placed in a row (Figure 2). This location was chosen because of its shallow depth (75-200 meter) and good water flow. The production cycle started in August 2012 and lasted to October-December 2013 with 200 000 fish each cage.

Four experimental stations were established as displayed in Figure 2 and Table 2. The Farm station was positioned in an empty cage on the eastern side of the fish farm and 200 meter to the east of that was the Farm 200 station located. The Ref East station was positioned 1.0 km to the east for the Farm station and Ref West was found 1.2 km to the west. Scallops (87.8±1.5 mm) were deployed in cages at 5 and 20 meters depth at each station, attached to the same rope 15 meters apart as illustrated in Figure 3. The rope was anchored at the bottom with buoys placed above each cage to reduce wave action. This do not apply for the Ref East station however, as it was too deep to anchor up at the bottom. Instead it was attached to a rope with buoys at the surface. The scallops were kept in the sea from the 11th of June to the 10th of September.

2.2.2 Environmental monitoring

Environmental measurements were performed by the Institute of Marine Research. Sampling to measure the concentration of nutrients at the fish farm was undertaken in September 2012, April and September 2013. Temperature and salinity was measured with STD/CTD (SAIV A/S, Norway) and hydrodynamics with current meters (SD6000 Sensor Data AS).

Table 2 Position of the experimental stations in relation to the fish farm and the number of scallops deployed.

Experimental stations		
	n	Position
Farm	75	Empty cage in the fish farm
Farm 200	75	200 meters east of fish farm
Ref East	75	1 km east of fish farm
Ref West	27	1.2 km west of fish farm

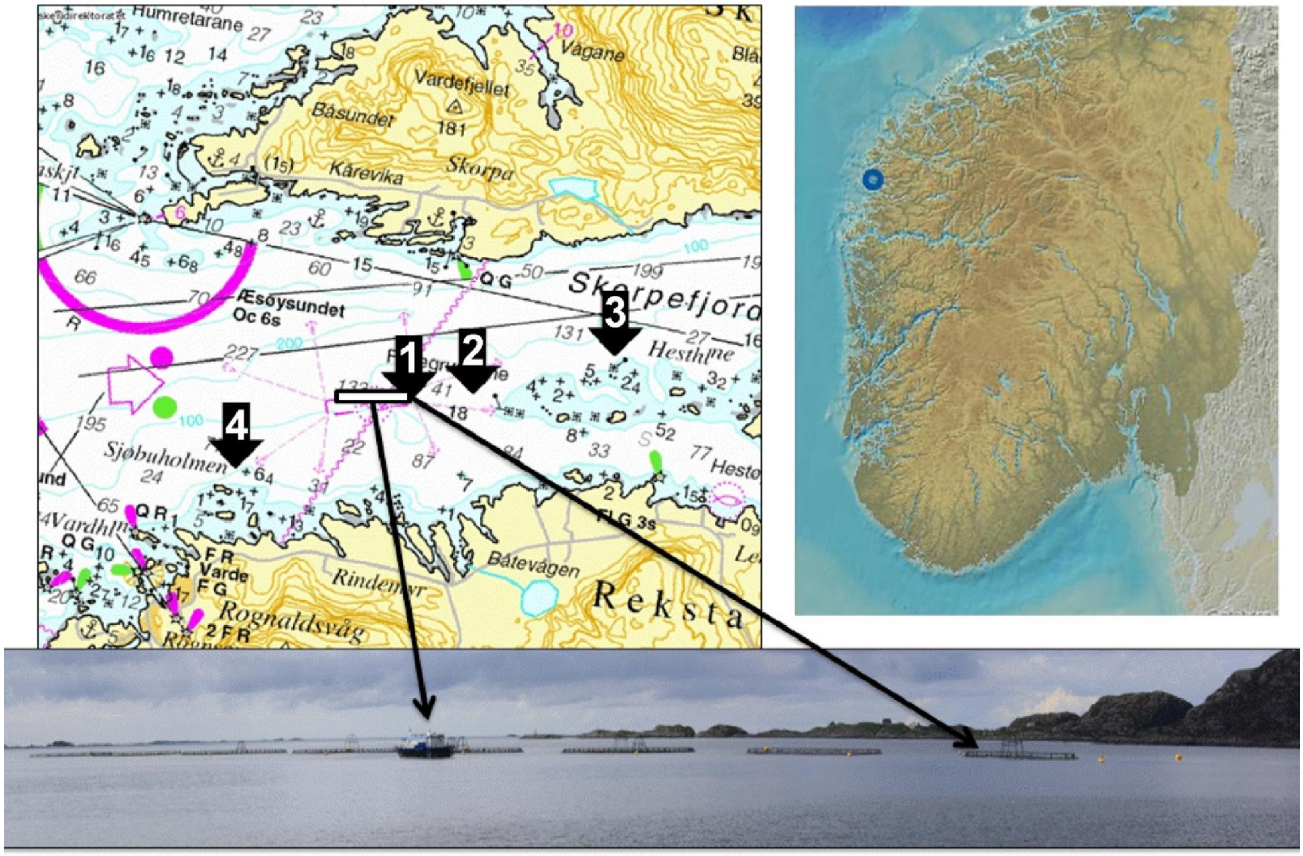

Figure 2 Position of fish farm and sampling stations. Arrows point at the experimental stations Farm (1), Farm 200 (2), Ref East (3) and Ref West (4).

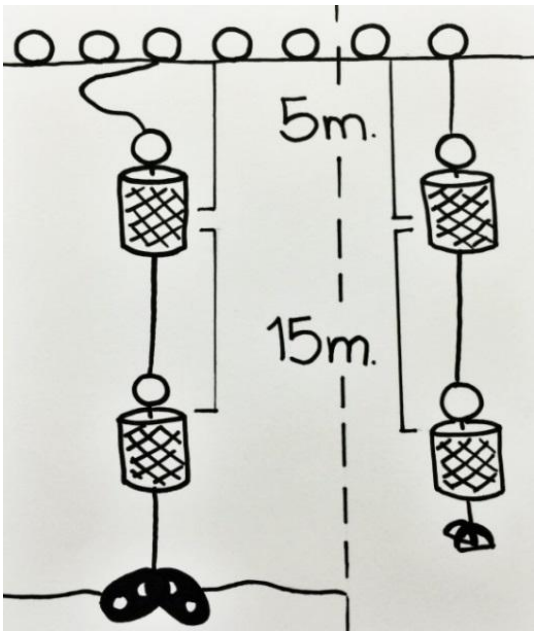

Figure 3 Attachment of cages at the Farm, Farm 200 and Ref West stations to the left of the dashed line and the Ref East station to the right.

2.3 Growth measurements and Condition index

Shell height was measured at the start and end of both field and laboratory experiment using a digital caliper (Vernier 150 mm). It was measured perpendicular from umbo. For each group, the average height was calculated as well as standard deviation and standard error. The Ref West station in the field study was excluded from growth and condition measurements due to its small size.

Average growth rate (AGR) was calculated to see increase in µm per day:

$$AGR = \frac{L_t - L_0}{t}, \qquad \text{where}$$

L_t = length at time *t*

L_o = length at time zero

T = timed passed (days)

Condition index (CI) is a method to assess the health of the organism and can be calculated in many different ways. In the field experiment CI was calculated by the relationship between the dry weight of the shell and entrails (Walne, 1976):

$$CI = \frac{Dry\ weight\ entrails}{Dry\ weight\ shell}$$

For the laboratory experiment the CI was calculated by the relationship between shell height and the dry weight (Cataldo et al., 2001 and the reference therein):

$$CI = \frac{Dry\ weight}{Shell\ height} x100$$

Shell height was measured. Scallops from the field experiment were separated into shell, muscle, gonads and remaining tissue and transferred to aluminum beakers. Tissue was dried at 105°C for 48 hours for scallops from the field trial and 24 hours for the laboratory trial, respectively.

2.4 Lipid Analysis

10 scallops from each tray were dissected to extract the digestive gland and muscle in the laboratory trial (n=3). Digestive glands and gonads were dissected from scallops from field experiment. 6 scallops was analyzed for each cage (n=6).

2.4.1 Sample preparation

In the field experiment, digestive glands and gonads of dissected scallops, filters with filtrated seawater, salmon feed and salmon feces were put directly in a freezer (-18°C) with dry ice (-78°C) and later shipped off to Trondheim with Nor Lines in a freezing room (-18°C). Digestive glands and muscles from the laboratory trial were frozen in liquid nitrogen directly after dissection. All samples were freeze-dried and flushed with nitrogen gas before storage at -80°C.

2.4.2 Total lipid

Total lipid was measured according to Bligh and Dyer (1959). Samples (22 mg) were weighed with Mettler Toledo (XA204). Distilled water (dH_2O) (0.8 mL), methanol (2 mL) and chloroform added an internal fatty acid standard (C19:0)(1 mL) were added before the samples were homogenized for 1 minute. Chloroform (1 mL) and dH_2O (1 mL) were added separately with subsequent homogenization for 20 seconds. The samples were centrifuged for 10 minutes at 5 °C and 4000 RPM.

The bottom phase was transferred to new tubes, whereas 0.5 mL was used for measuring the total lipid, and 1 mL was used for further analysis through fatty acid methylation. Samples were heated to 40 °C and flushed with nitrogen in a concentrator (Techne Dri-Block DB-3D) to evaporate the chloroform. Total lipid was determined by weighing (Mettler Toledo UMX2).

2.4.3 Fatty acid composition

Two methods were used to analyze fatty acid composition. For scallops from the laboratory experiment, fatty acid methylation was carried out according to Matcalfe (1966) after extracting the lipid as described above. This method was also used for salmon feed, salmon feces and algae from both laboratory and field experiments. Sodium hydroxide (NaOH) methanol (0,5 M, 1 mL) was mixed with the sample and heated at 100 °C for 15 minutes. Bromine trifluoride (BF_3) methanol (2 mL) and isooctane (1 mL) was added separately before heating at 100 °C for 5 and 1 minutes respectively before saturated Sodium chloride in distilled H_2O (3 mL) was added to the samples. Isooctane (0.5 mL) was mixed with the samples and centrifuged for 3 minutes and 4000 RPM. This was repeated 3 times. The top phase was transferred to vials and analyzed for fatty acids using a gas chromatograph (Perkin Elmer AutoSystem XL).

A modified version of Abdulkadir's one-step method (2008) described by Bergvik et al (2012a) was used in the field trial. This is a direct methylation without extracting the lipid first. Isooctane with internal standard (C19:0) (0.5 mL) and bromine trifluoride (BF_3) methanol (0.2 mL) were added to each sample and heated at 100 °C for 2 hours. The samples were cooled to room temperature before isooctane (0.1 mL) and distilled water (0.2 mL) were added and mixed thoroughly with a Vortex-mixer. After 4 minutes in the centrifuge at 4000 RPM and 4 °C, the top phase was transferred to vials and analyzed in the gas chromatograph.

Analysis of material collected on filters was performed as described above with a few adjustments. Isooctane with internal standard (C19:0) (0.5 mL) and isooctane (4.5 mL) was added to the samples. Thereafter all the solution volumes were multiplied by 10 relative to Bergvik et al (2012a). After 4 minutes centrifugation, the top phase was transferred to a new tube and set in a sample concentrator (Techne Dri-Block DB-3D) at 30°C with N_2 gas. When all fluid was evaporated, isooctane (0.2 mL) was added before analyzing.

Fatty acid methyl esters was analysed by a gas chromatograph (AutoSystem XL, Perkin Elmer, Waltham, MA) with TotalChrom Version 6.3.1 software as described by Bergvik et al (2012b). It was equipped by an auto-injector (injection volume of 1 µL, on-column injection, inlet temperature 250 °C) and a flame ionization detector (FID, 280 °C). A fused silica capillary column coated with a chemically bonded polyethylene glycol (CP-Wax 52CB, 25 m x 0.25 mm i.d.; Varian, Palo Alto, CA) was used. The start temperature for the oven was 90 °C for 1 minute. Temperature was increased 30 °C/ min until 150 °C and then raised 3 °C/ min until 225 °C and held for 7 minutes. Helium was used as the carrier gas. The retention times of the fatty acid methyl esters were compared to commercial standards (Nu-Chek Prep, Tokyo, Japan) and quantified by the use of C19:0 as an internal standard added prior to extraction in combination with external standard curves. Total lipid was expressed in terms of mg per g of dry weight (DW).

2.5 Statistical / data analysis

Microsoft Excel 2010 was used for record keeping of data and calculation of means and standard deviation. Graphs were made by SigmaPlot 12.0 to present mean values ± standard deviation.

All statistical analysis was carried out in SigmaPlot 12.0 with a level of significance set at $p<0.05$. One-Way ANOVA with Holm-Sidak tests compared the samples against the control for the lab experiment, and pairwise for the field experiment. When the samples were not normally distributed, Kruskal-Wallis ANOVA on Ranks was used with Dunn's test. Unpaired t-test was run between groups to see if a difference could be detected between algae concentration and depth for lab and field experiment respectively. Where this was not applicable, Mann-Whitney Rank Sum Test was used. Significant differences were presented in graphs and tables where lower case letter implied difference between samples.

3 Results

3.1 Laboratory experiment

During a 25 days experiment, scallops were fed different diets consisting of algae alone or supplemented by salmon feed or salmon feces in order to investigate their capacity to assimilate and incorporate fatty acids from salmon feed and feces.

3.1.1 Nutrient availability

Figure 4 A shows the total lipid content in *Rhodomonas baltica*, *Chaetoceros muelleri*, salmon feed and salmon feces. Salmon feed exhibited the highest total lipid content with 323 mg/g freeze dried weight (FDW), about 3 times higher than salmon feces which showed the second highest level with 103 mg/g FDW. Total lipid content of *R. baltica* and *C. muelleri* was 66 and 72 mg/g FDW, respectively. The same trend was evident for total fatty acid (TFA) per gram FDW illustrated in Figure 4 B. Salmon feed shows by far the highest content of fatty acid per unit feed.

Figure 4 C presents the fraction of the dominant fatty acids 18:1(n-9) and 16:0 in *R. baltica*, *C. muelleri*, salmon feed and salmon feces. The fatty acid that varied most between the different types of feed was 18:1 (n-9), constituting 39.2% and 25.8% in salmon feed and salmon feces, respectively, compared to merely 1.4% in *R. baltica* and 0.8% in *C. muelleri*. Salmon feces and *C. muelleri* showed a high level of 16:0.

The complete fatty acid composition of the feed is shown in Table 1. *C. muelleri* and *R. baltica* possessed the highest levels of 20:4 n-6 (ARA) whereas salmon feed and salmon feces showed low levels. Low contents of 20:5 n-3 (EPA) were detected in salmon feces, whereas low concentration of 22:6 n-3 (DHA) was detected in both feces and *C. muelleri*. *R. baltica* showed the highest contents of both EPA and DHA with 8.1 and 6.2% of TFA, respectively, followed by salmon feed with 4.0 and 3.9%.

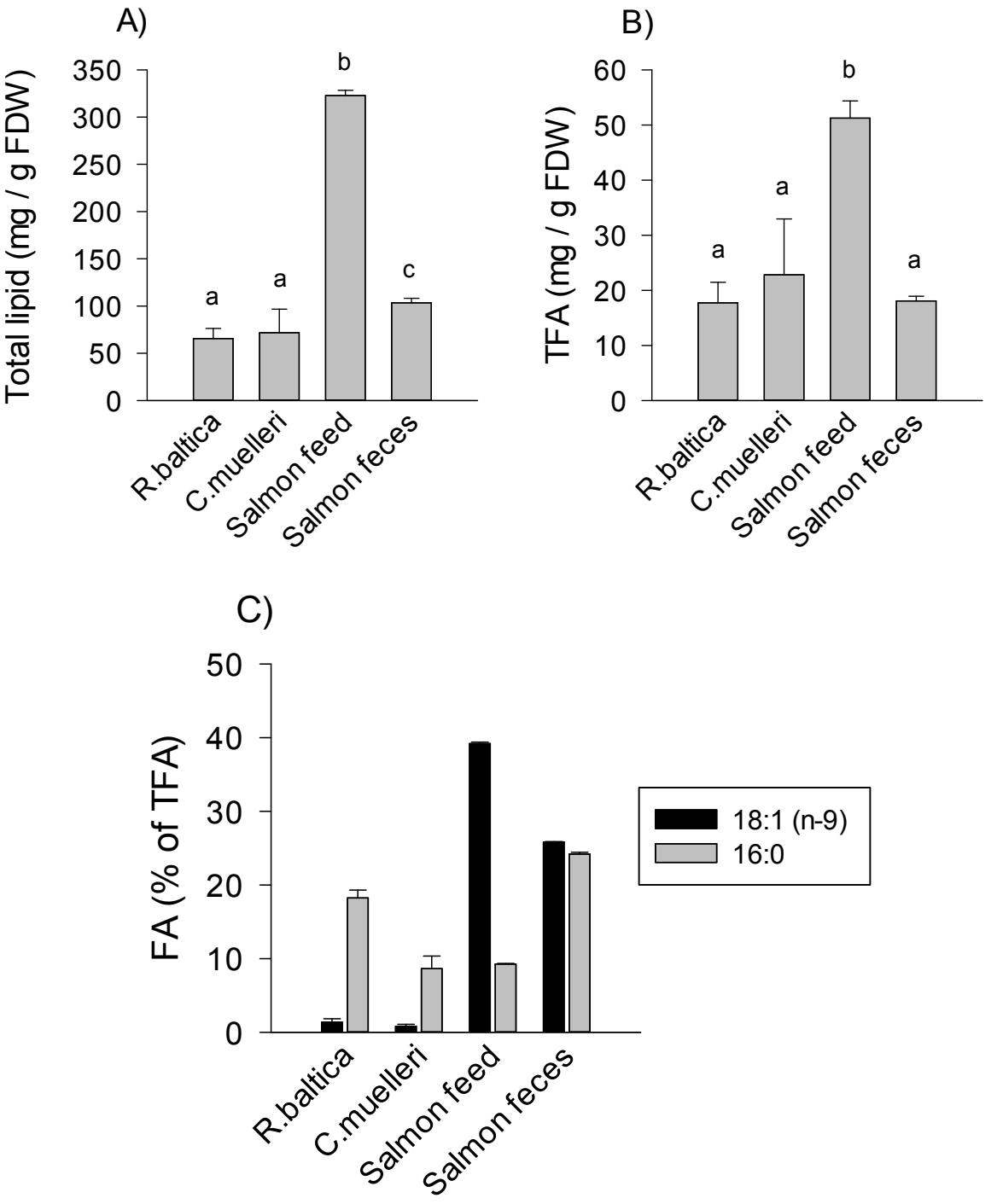

Figure 4. A) Total lipid content, B) total fatty acid (TFA) *and C) the fraction of the most abundant fatty acids* (mean ± standard deviation, n=3) to the total fatty acid content in *R. baltica*, *C. muelleri*, salmon feed and salmon feces. Different lower case letters imply significant differences (P<0.05) between the different feed components.

Table 3. Total fatty acid content and fatty acid composition (% of total fatty acid) of *Rhodomonas baltica*, *Chaetoceros muelleri*, salmon feed and salmon feces (mean ± standard deviation, n=3). ND = Not Detected.

Feed				
	Rhodomonas baltica	*Chaetoceros muelleri*	Salmon feed	Salmon feces
TFA (mg/g DW)	17.7±3.7	22.8±10.1	348.1±22.2	18.1±0.9
FA (% of TFA)				
14:0	5.0±1.0	8.8±0.5	2.9±0.0	3.9±0.0
16:0	8.6±1.7	18.2±1.1	9.3±0.1	24.2±0.2
18:0	0.6±0.2	1.2±0.3	2.4±0.1	10.9±0.1
20:0	ND	ND	0.4±0.0	1.7±0.0
22:0	ND	0.1±0.1	0.4±0.0	1.3±0.2
24:0	ND	ND	0.1±0.0	ND
14:1 (n-5)	ND	ND	ND	ND
16:1 (n-7)	0.6±0.0	37.2±4.6	2.5±0.0	1.4±0.0
18:1 (n-9)	0.8±0.3	1.4±0.5	39.2±0.2	25.8±0.0
18:1 (n-7)	3.1±0.2	2.6±1.1	2.6±0.0	2.0±0.0
20:1 (n-9)	ND	0.1±0.1	3.1±0.0	3.6±0.0
22:1 (n-11)	ND	ND	3.8±0.0	6.4±0.0
22:1 (n-9)	ND	ND	0.0±0.0	1.3±0.0
24:1	ND	ND	0.3±0.0	ND
18:3 (n-3)	17.8±0.5	1.6±1.6	6.4±0.0	2.7±0.0
18:4 (n-3)	18.7±2.0	1.5±1.3	0.5±0.8	0.3±0.0
20:3 (n-3)	0.1±0.1	ND	0.1±0.0	ND
20:4 (n-3)	0.6±0.3	0.2±0.0	0.2±0.0	0.1±0.0
20:5 (n-3)	8.1±1.4	4.5±1.0	4.0±0.0	1.6±0.0
22:5 (n-3)	0.2±0.1	0.1±0.0	ND	0.2±0.0
22:6 (n-3)	6.2±0.5	1.1±0.9	3.9±0.1	2.4±0.0
18:2 (n-6)	15.1±2.9	2.4±1.3	13.8±0.1	8.1±0.1
18:3 (n-6)	ND	ND	ND	ND
20:2 (n-6)	0.2±0.1	ND	0.1±0.0	0.1±0.1
20:3 (n-6)	0.1±0.1	0.1±0.0	ND	ND
20:4 (n-6)	2.2±0.6	7.0±0.4	0.2±0.0	0.1±0.1
22:5 (n-6)	0.4±0.1	0.4±0.2	ND	ND

3.1.2 Scallop growth and condition

Figure 5 A shows the average growth rate (AGR, µm/day) for scallops fed the control diet, and when fed salmon feed or salmon feces with high or low algae concentration. When the scallops were fed high algae concentration, the AGR was similar for all treatments. A significantly different AGR was found between high and low algae supply for the control (P=0.002) and salmon feed group (P=0.004).

Figure 5 B shows the condition index for scallops in the different groups. It was evident that high algae concentration gave scallops a higher condition index compared to low algae, supporting the enhanced growth seen in AGR. Feeding of salmon feed and salmon feces in addition to algae did not affect the condition index.

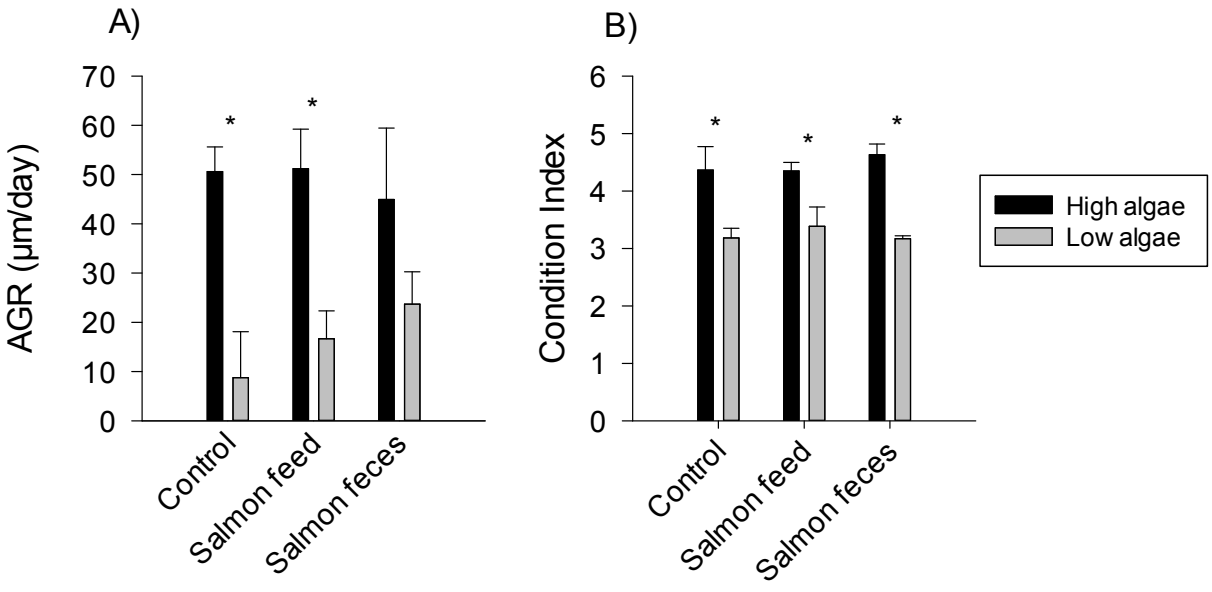

Figure 5. A) Average growth rate (AGR, µm/day) and B) Condition index (mean ± standard deviation, n=3) for scallops fed a combination of *R. baltica* and *C. muelleri* at a high (300 µg C/L) and a low algae concentration (50 µg C/L) and when supplemented with salmon feed or salmon feces (30 µg C/L). * indicates a significant difference (P<0.05) between high and low algae for the specific diet.

3.1.3 Incorporation of food components

Figure 6 shows the total lipid content (mg/g FDW) in A) digestive gland tissue and B) muscle tissue for scallops fed algae alone (control) at a high or low algae concentration, and in addition to salmon feed or salmon feces. When scallops were fed a high algae concentration, the digestive gland tissue showed a significantly higher level of lipid for all treatments; control ($P=0.004$), salmon feed ($P=0.002$) and salmon feces ($P=0.005$). The digestive gland had a higher lipid content compared to the adductor muscle. Muscle tissue did not respond to the different algae concentrations, but showed a constant total lipid content around 40 mg/g FDW for all treatments.

The total fatty acid (TFA) content in digestive gland tissue reflected the total lipid content (Figure 6 C). A higher algae supply lead to a significantly higher content of fatty acids for scallops in control ($P=0.002$), salmon feed ($P=0.002$) and salmon feces ($P<0.001$) compared to that at low algae concentration. Figure 6 D shows TFA in muscle tissue, demonstrating a very low content of fatty acids compared to digestive gland tissue. TFA in the muscles were neither affected by diet nor concentration.

Figure 7 A shows the fraction of 18:1 (n-9) in digestive gland tissue for scallops in control and when supplemented by salmon feed or feces at two different algae concentrations. Scallops fed salmon feed and salmon feces showed elevated 18:1 (n-9) levels compared to control. This reflects the high levels of 18:1(n-9) in salmon feed and feces compared to the low values detected in algae (Figure 4 C). The muscle tissues showed no significantly differences between the treatments (Figure 7 B).

The percentage of 16:0 in digestive gland tissues of scallops treated with the different diets is presented in Figure 7 C. A low algae supply led to an increased 16:0 fraction in the digestive glands for scallops fed salmon feed and feces. Scallops fed salmon feces showed significantly higher levels of 16:0 ($P=0.007$) than control when algae concentration was low. Salmon feed did not result in significantly differences from the control group ($P>0.05$), in spite of the elevated levels of the fatty acid in the digestive glands. This reflects the high 16:0 fraction found in salmon feces (Figure 4 C). Due to the low input of *C. muelleri* in the algae mix, the fraction of 16:0 was most likely similar to that of *R. baltica* and would probably not cause an increase of the fatty acid in the tissue. Muscle tissues showed slight, although significantly higher values of 16:0 for scallops fed low algae with salmon feed ($P=0.003$) and salmon feces ($P<0.001$) compared to the control group, as shown in Figure 7 D.

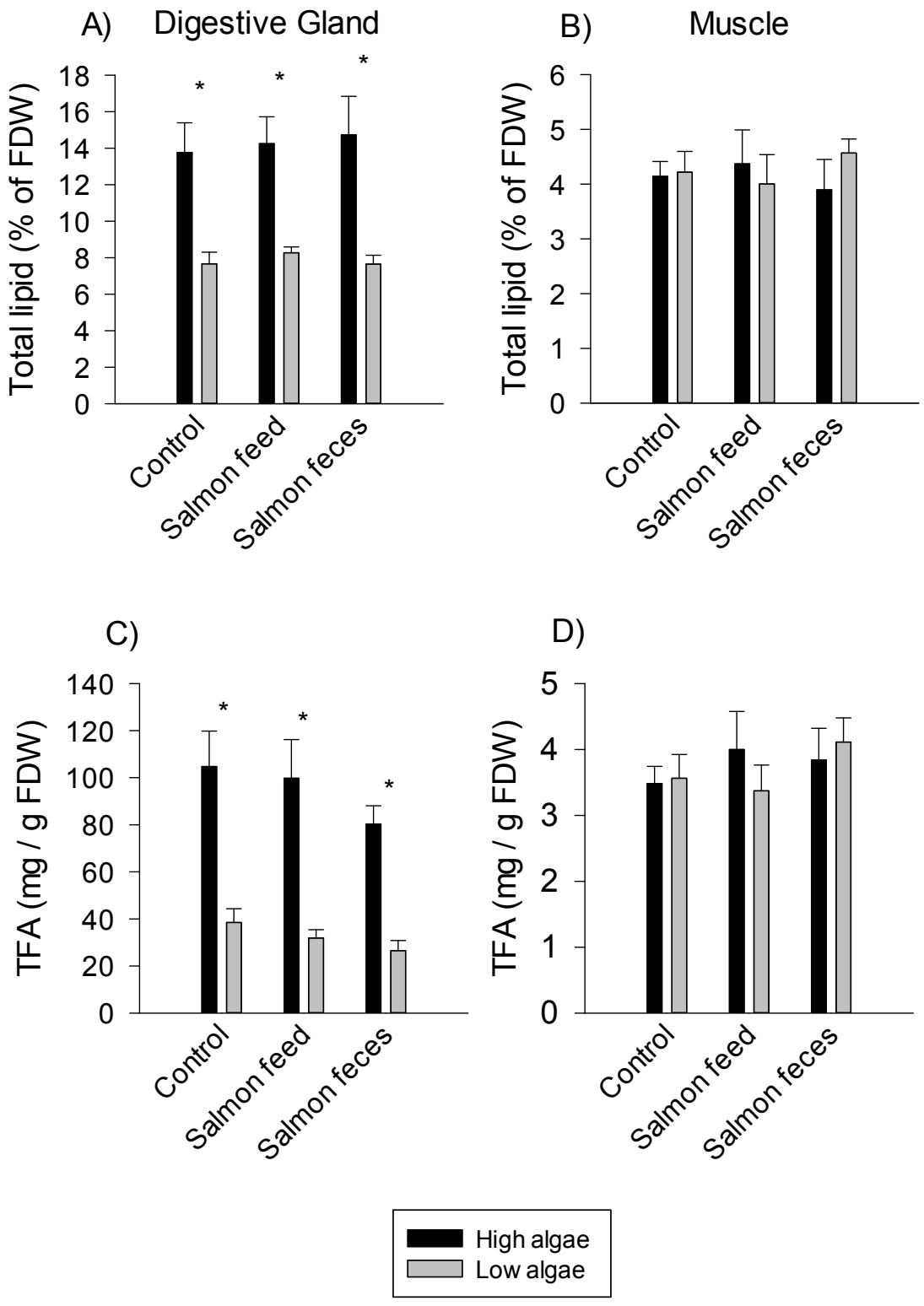

Figure 6. Total lipid content (% of freeze-dried weight, n=3) in the A) digestive gland and B) muscle, total fatty acid (TFA) content in C) digestive gland and D) muscle tissue (mean ± standard deviation, n=3) for scallops fed a combination of *R. baltica* and *C. muelleri* at a high (300 µg C/L) and a low algae concentration (50 µg C/L) alone and when supplemented with salmon feed or salmon feces (30 µg C/L). Lower case letters imply significant differences (P<0.05) from scallops fed algae alone; a and b are related to a high supply of algae, w and x to a low supply. * indicates a significant difference (P<0.05) between high and low algae for the specific diet.

Table 4 presents the total fatty acid content and the fractions of fatty acid in digestive gland tissue for scallops fed the different treatments. The fraction of EPA (20:5 n-3) was similar between the treatments. Scallop in the control and salmon feces group showed significantly higher levels of EPA when provided a low algae supply ($P=0.021$ for control, $P<0.001$ for salmon feces). The diet did not alter the level of EPA in the adductor muscle. The fraction of DHA (22:6 n-3) in both digestive gland and muscle increased substantially when scallops had a low algae supply. DHA was by far the most abundant fatty acid in the muscle tissue, and showed a substantial fraction in the digestive glands as well. The fraction of ARA (20:4 n-6) in digestive gland tissue was significantly higher for the control ($P<0.001$) and salmon feces group ($P=<0.001$) when the scallops were provided with a low algae concentration. The exact opposite was found in muscle tissue, low algae supply resulted in a significantly lower fraction of ARA for the control ($P=0.002$) and salmon feces group ($P=0.014$).

The fraction of 18:4 (n-3) increased considerably in both digestive gland and muscle tissue when scallops were provided a high algae supply. This likely reflected the high level of this fatty acid in *R. baltica* compared to the other feed components. Both digestive gland and adductor muscle remained unaffected when scallops were supplemented with salmon feed and feces in addition to algae.

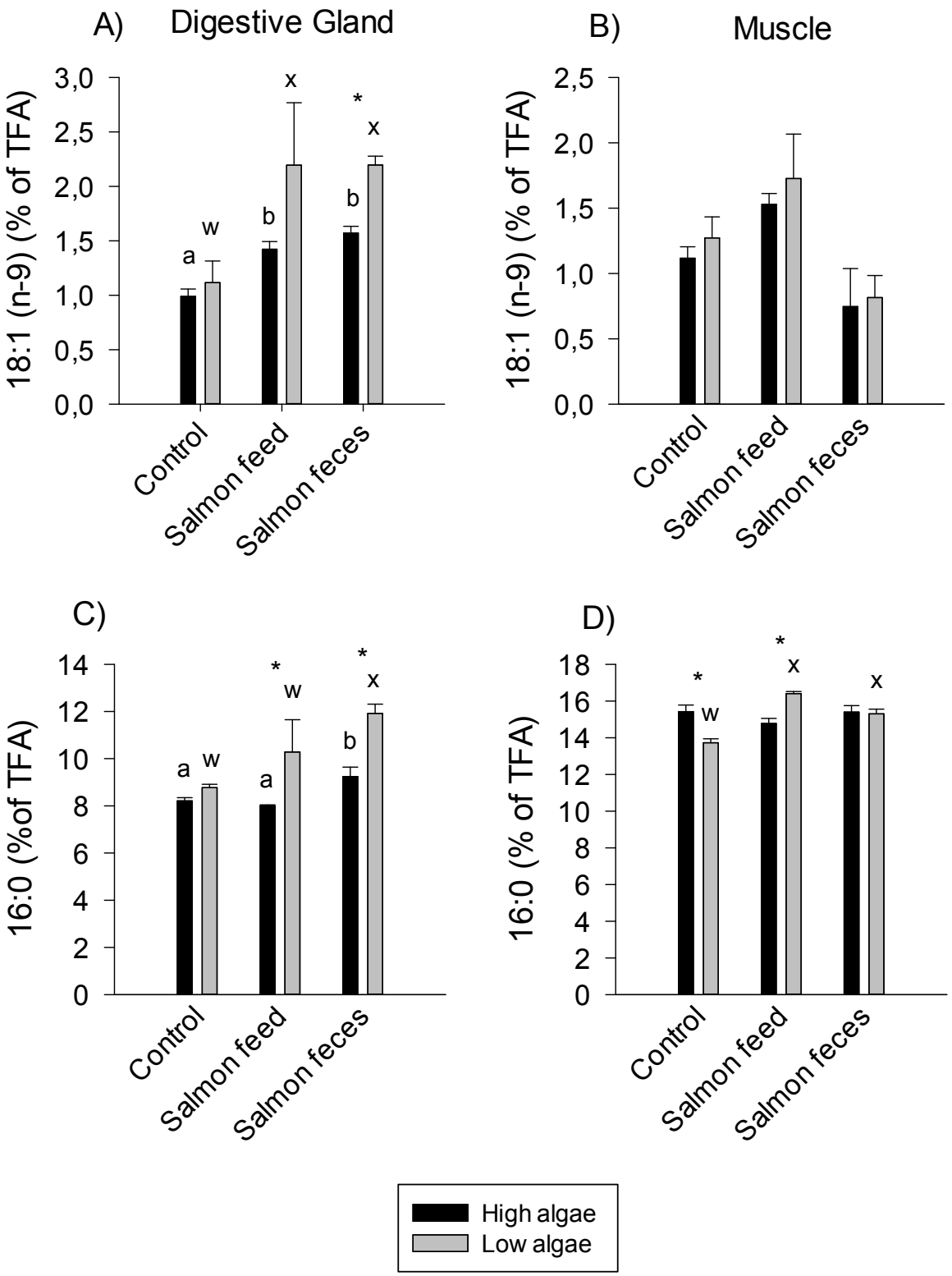

Figure 7. The fraction of 18:1 (n-9) in A) digestive glands and B) muscles, and the fraction of 16:0 in C) digestive glands and D) muscles (mean ± standard deviation, n=3) for scallops fed a combination of *R. baltica* and *C. muelleri* at a high (300 µg C/L) and a low algae concentration (50 µg C/L) and when supplemented with salmon feed or salmon feces (30 µg C/L). Different lower case letters imply significant differences (P<0.05) from scallops fed algae alone; a and b are related to a high supply of algae, w and x to a low supply. * indicates a significant difference (P<0.05) between high and low algae for the specific diet.

Table 4. Total fatty acid content and fatty acid composition (% of total fatty acid) in digestive glands for scallops fed a combination of *R. baltica* and *C. muelleri* at a high (300 µg C/L) and a low algae concentration (50 µg C/L) and when supplemented with salmon feed or salmon feces (30 µg C/L). Differences in lower case letters imply significant differences (P<0.05) from scallops fed algae alone; a and b are related to a high supply of algae, w and x to a low supply. * imply a significant difference (P<0.05) between high and low algae for the specific diet.

Digestive gland						
	Algae high			Algae low		
	Control	Salmon feed	Salmon feces	Control	Salmon feed	Salmon feces
TFA (mg/g DW)	104.8±15.1*	99.8±16.4*	80.3±7.8*	38.5±5.9*	31.9±3.5*	26.6±4.3*
FA (% of TFA)						
14:0	1.6±0.0*	1.5±0.1*	1.7±0.0*	1.0±0.0^{W}*	1.1±0.1^{W}*	1.2±0.0^{X}*
16:0	8.2±0.1^{A}	8.0±0.0^{A}*	9.2±0.4^{B}*	8.8±0.1^{W}	10.3±1.4^{W}*	11.9±0.4^{X}*
18:0	4.3±0.4*	4.4±0.6	4.4±0.3*	8.9±0.2^{W}*	8.9±0.1^{W}	9.5±0.3^{X}*
20:0	ND*	ND	ND	0.3±0.1^{W}*	0.1±0.2^{W}	ND^{X}
22:0	ND	ND	ND	ND	ND	ND
24:0	ND	ND	ND	ND	ND	ND
14:1 (n-5)	ND^{A}	0.1±0.0^{B}	ND^{A}	ND	ND	ND
16:1 (n-7)	3.5±0.1*	3.3±0.1*	3.5±0.2*	1.9±0.1*	1.8±0.1*	1.6±0.2*
18:1 (n-9)	3.5±0.1*	1.4±0.1^{B}	1.6±0.1^{B}*	1.1±0.2^{W}*	2.2±0.6^{X}	2.2±0.1^{X}*
18:1 (n-7)	4.7±0.2	4.6±0.2*	4.6±0.1*	2.4±0.0	2.2±0.6*	2.4±0.1*
20:1 (n-9)	0.3±0.0^{A}*	0.5±0.0^{B}*	0.5±0.0^{B}*	1.1±0.1*	1.3±0.2*	1.3±0.1*
22:1 (n-11)	1.0±0.0*	1.0±0,1*	0.9±0.0*	0.6±0.1*	0.5±0.1*	0.4±0.0*
22:1 (n-9)	ND	ND	ND	ND	ND	ND
24:1	ND	ND	ND	ND	ND	ND
18:3 (n-3)	10.9±1.3*	10.3±1.3*	10.7±0.9*	2.5±0.2*	2.5±0.2*	2.4±0.1*
18:4 (n-3)	9.6±1.4*	9.3±1.1*	9.4±0.8*	3.7±0.2*	3.5±0.2*	3.5±0.3*
20:3 (n-3)	0.2±0.0	0.2±0.0*	0.2±0.0*	0.2±0.0^{W}	0.2±0.0^{X}*	0.2±0.0^{X}*
20:4 (n-3)	0.4±0.1	0.3±0.0	0.3±0.0*	0.4±0.0^{W}	0.3±0.0^{X}	0.3±0.0^{X}*
20:5 (n-3)	9.4±0.7*	9.6±0.3	8.9±0.2*	11.5±0.6*	10.5±0.7	10.7±0.2*
22:5 (n-3)	0.2±0.0*	0.3±0.0*	0.3±0.0*	0.7±0.0*	0.7±0.1*	0.7±0.0*
22:6 (n-3)	10.0±0.5*	11.0±0.9*	10.7±0.5*	23.8±0.9*	22.3±0.5*	23.6±0.5*
18:2 (n-6)	8.9±0.7*	8.6±0.6*	8.5±0.5	1.4±0.2*	1.7±0.1*	1.5±0.1
18:3 (n-6)	ND	ND	ND	ND	0.4±0.3	0.5±0.0
20:2 (n-6)	1.6±0.1*	1.5±0.1*	1.5±0.1*	1.2±0.0^{W}*	1.1±0.1^{W}*	1.0±0.0^{X}*
20:3 (n-6)	0.8±0.1	0.8±0.0	0.7±0.0	0.6±0.0	0.2±0.3	ND
20:4 (n-6)	4.1±0.2*	4.1±0.3	3.7±0.2*	5.3±0.1*	4.9±0.5	4.9±0.0*
22:5 (n-6)	0.5±0.0*	0.5±0.1*	0.5±0.0*	0.9±0.0*	0.8±0.1*	0.8±0.0*

Table 5. Total fatty acid content and fatty acid composition (% of total fatty acid) in muscles for scallops fed a combination of *R. baltica* and *C. muelleri* at a high (300 µg C/L) and a low algae concentration (50 µg C/L) and when supplemented with salmon feed or salmon feces (30 µg C/L). Differences in lower case letters imply significant differences (P<0.05) from scallops fed algae alone; a and b are related to a high supply of algae, w and x to a low supply. * imply a significant difference (P<0.05) between high and low algae for the specific diet..

Muscle						
	Algae high			Algae low		
	Control	Salmon feed	Salmon feces	Control	Salmon feed	Salmon feces
TFA (mg/g DW)	3.5±0.3	4.0±0.6	3.8±0.5	3.6±0.4	3.4±0.4	4.1±0.4
FA (% of TFA)						
14:0	1.8±0.1	1.7±0.1*	1.8±0.1	1.8±0.1^{W}	2.1±0.1^{X}*	1.9±0.0^{X}
16:0	15.4±0.4*	14.8±0.3*	15.4±0.4	13.7±0.1^{W}*	16.4±0.1^{X}*	15.3±0.3^{X}
18:0	7.1±0.2*	7.1±0.2*	7.1±0.3	7.6±0.0^{W}*	8.7±0.3^{X}*	7.7±0.2^{W}
20:0	ND	ND	ND	ND	ND	ND
22:0	ND	ND	ND	ND	ND	ND
24:0	ND	ND	ND	ND	ND	ND
14:1 (n-5)	ND	ND	ND	ND	ND	ND
16:1 (n-7)	1.1±0.0^{A}*	1.0±0.0^{B}*	1.2±0.0^{B}*	0.7±0.1*	0.9±0.1*	0.7±0.1*
18:1 (n-9)	1.1±0.1	1.5±0.1	0.7±0.3	1.3±0.1	1.7±0.3	0.8±0.2
18:1 (n-7)	3.4±0.1*	3.3±0.1*	3.1±0.2*	2.2±0.1*	2.6±0.2*	2.1±0.1*
20:1 (n-9)	1.1±0.0	1.1±0.1*	1.1±0.0*	1.2±0.1^{W}	1.6±0.1^{X}*	1.4±0.1^{X}*
22:1 (n-11)	0.4±0.2	0.5±0.1	0.5±0.0	ND	ND	ND
22:1 (n-9)	ND	ND	ND	ND	ND	ND
24:1	ND	ND	ND	ND	ND	ND
18:3 (n-3)	2.2±0.1	2.0±0.2*	2.3±0.1*	0.3±0.0	0.1±0.2*	0.1±0.1*
18:4 (n-3)	3.3±0.3^{A}*	2.8±0.1^{B}*	3.2±0.1^{A}*	1.3±0.0^{W}*	1.6±0.1^{X}*	1.4±0.1^{W}*
20:3 (n-3)	0.1±0.1	0.1±0.0*	0.1±0.0*	0.1±0.0	0.1±0.0*	0.1±0.0*
20:4 (n-3)	0.5±0.0*	0.4±0.0	0.5±0.1*	0.4±0.0*	0.4±0.0	0.4±0.1*
20:5 (n-3)	12.4±0.2^{A}*	11.6±0.1^{B}*	12.4±0.3^{A}	11.4±0.4^{W}*	13.4±0.2^{X}*	12.0±0.2^{W}*
22:5 (n-3)	1.3±0.1	1.2±0.1	1.2±0.1	1.4±0.1	1.5±0.1	1.4±0.0
22:6 (n-3)	33.9±0.5*	31.8±1.1*	33.4±1.1*	39.0±0.8W*	44.5±1.6X*	41.6±0.3X*
18:2 (n-6)	2.2±0.0*	2.1±0.2*	2.3±0.1*	0.2±0.1*	0.4±0.2*	0.5±0.0*
18:3 (n-6)	ND	ND	ND	ND	ND	ND
20:2 (n-6)	1.5±0.1*	1.3±0.1*	1.5±0.2*	0.6±0.0^{W}*	0.8±0.0^{X}*	0.7±0.1^{W}*
20:3 (n-6)	0.5±0.0*	0.4±0.1*	0.5±0.0*	0.3±0.1*	0.2±0.0*	0.2±0.0*
20:4 (n-6)	3.1±0.1*	3.1±0.2	3.1±0.2*	2.4±0.1^{W}*	2.8±0.1^{X}	2.6±0.1^{W}*
22:5 (n-6)	0.8±0.0*	0.7±0.1	0.7±0.1	0.7±0.0*	0.7±0.3	0.6±0.3

3.2 Field experiment

Scallops were deployed in close proximity to a fish farm at four experimental stations for 90 days in order to investigate its capacity to assimilate and incorporate fatty acids from salmon feed and salmon feces at two different depths.

3.2.1 Environmental parameters

CTD information collected from sampling sites around the experimental stations indicated similar stratification depths for all sampling periods. The stratification depth was characterized by a quick increase of both temperature (4-6°C) and salinity (32ppt-34ppt) from 35 to 40 meters depth in April 2013. In September no such abrupt change was observed. Salinity displayed a gradual increase from 24ppt at surface water to 33ppt at 40 meters depth, whilst temperature was stable at 15°C down to 25 meter depth and decreased to 11.5°C at 40 meter depth. The stratification depth was lower than the cultivation depth. The water current direction was determined by the tidal cycle and was predominantly South-West (210-230°) and East (90-110°) at the farm.

Figure 8 shows the average weekly temperatures from the start of June to mid-September measured at the Farm station at 5 and 10 meters depth and at Ref West at 5 meter. Temperatures increased steadily from mid-June and reached a peak in August at 15°C. The Farm and Ref West stations showed similar temperature at 5 meter, and the temperature increased throughout the summer. The temperature was lower at 10 meters depth during some periods, but leveled off at similar values in mid-August.

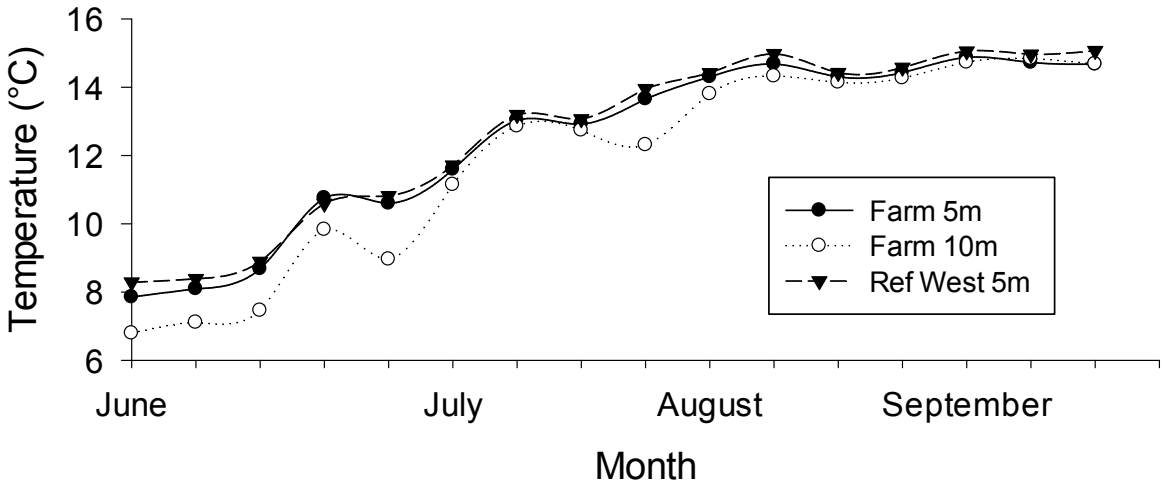

Figure 8. Weekly temperature measurements (mean) at the Farm (5 and 10 m) and Ref West station (5 m) from June to September.

3.2.2 Nutrient availability

Figure 9 illustrates the feed use and the fish biomass maintained at the farm during the production cycle from the start in August 2012 to October-December 2013. Each cage were stocked with 200 000 fish at one hundred grams size. Feed input is dependent on fish biomass as well as seawater temperature, which resulted in a reduced appetite during winter seen as a decline in feed use. In summer 2013 the feeding intensity increased substantially which led to a rapid growth in fish biomass.

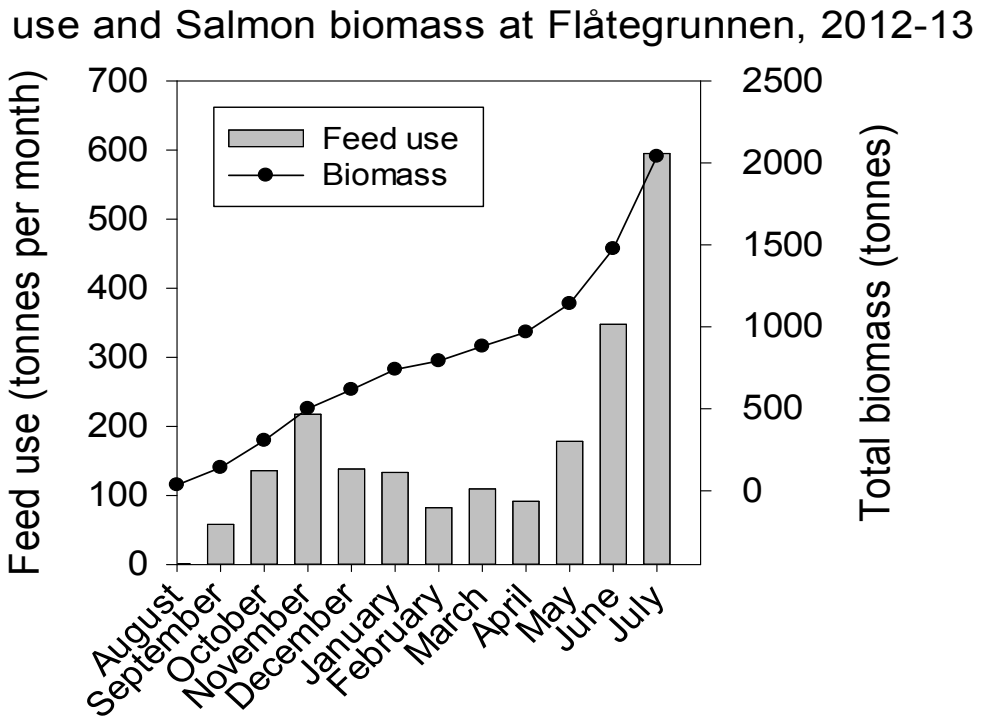

Figure 9. Food use and salmon biomass during the production cycle from August 2012 to September 2013.

The fatty acid (FA) composition of salmon feed and salmon feces was measured from all the samples collected in June-September. Figure 10 A shows the average total lipid content of salmon feed and salmon feces. Salmon feed showed higher total lipid content compared to salmon feces, showing values of 292 and 67 mg FA/g freeze-dried weight (FDW), respectively. This was also reflected by the high TFA level in salmon feed compared with salmon feces (Figure 10 B). Figure 10 C displays the fraction of 18:1 (n-9) in salmon feed and feces. The highest concentration of 18:1 was detected in salmon feed and was almost twice to that of salmon feces. The fractions of 20:5 n-3 (eicosapentaenoic fatty acid, EPA), 22:6 n-3 (docosahexaenoic fatty acid, DHA) and 20:4 n-6 (arachidonic acid, ARA) were low, particularly in feces (see Table 6).

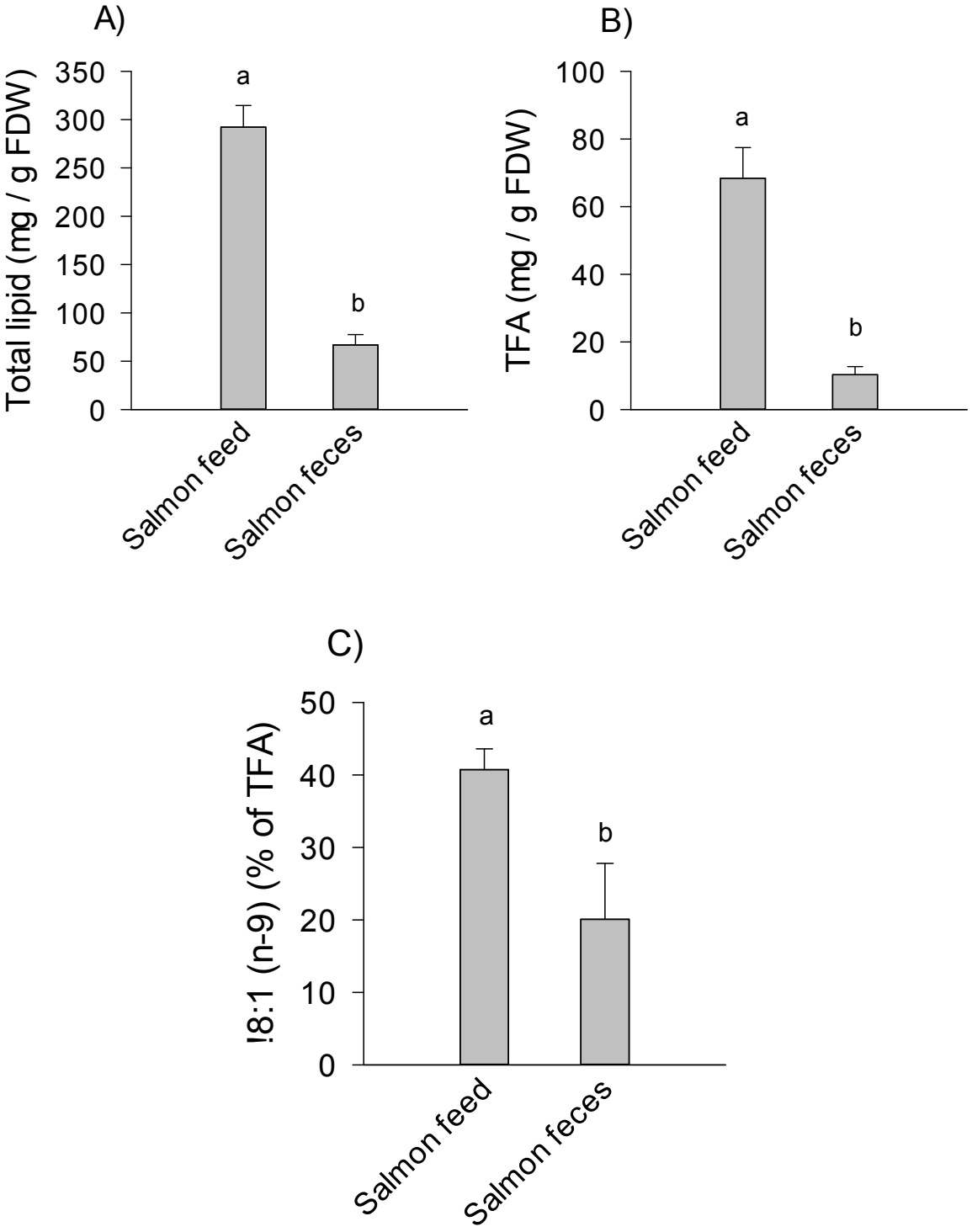

Figure 10. A) Total lipid content, B) total fatty acid and C) fraction of 18:1 (n-9) in salmon feed (% of DW, n=14) and salmon feces (% of FDW, n=8) (mean ± standard deviation) used at the farm from June to September.

Figure 11 shows particulate organic carbon content (POC < 200µm, µg C/L) in filtered seawater samples collected at the Farm station, Ref East and Ref West at 5 and 20 meters depth. The seawater at 5 meters depth possessed a slightly larger POC level compared to seawater at 20 meters depth, indicating a decline in algae abundance with increasing depth.

Figure 12 shows the fraction of selected fatty acid (% of total FA) in filtered seawater collected from the Farm, Ref East and Ref West station. The Farm station exhibited higher levels of 18:1 (n-9) than any other station at both depths. This is most likely due to waste particles of food and feces from the salmon farm, given their high concentration of this fatty acid (Table 6). The concentration of EPA and DHA was rather low in the water masses. EPA and DHA were more abundant at 5 meters depth, suggesting a higher abundance of algae in the upper water masses. The fraction of ARA was under 1.0%.

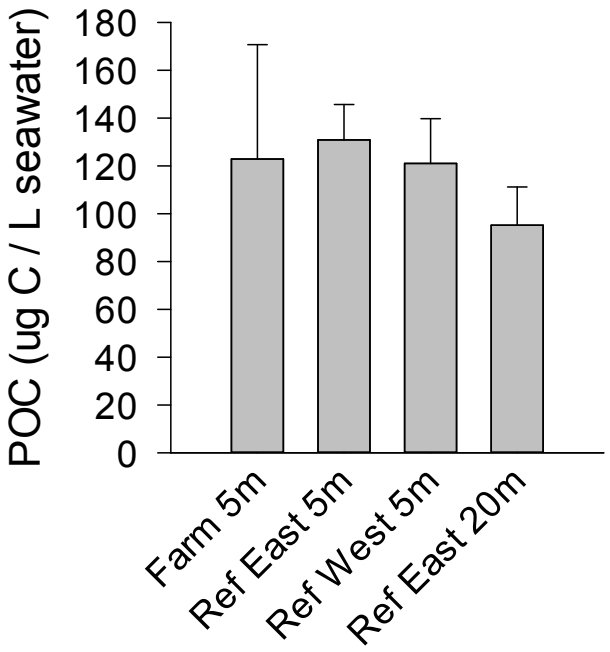

Figure 11 Particulate organic carbon content (POC, µg/L) in seawater (mean ± standard deviation, n=3) of water samples from the Farm, Ref East and Ref West stations taken in September.

Table 6 Total fatty acid content and fatty acid composition (% of total fatty acid) of salmon feed (n=14) and salmon feces (n=8) (mean ± standard deviation, n=3). ND = Not Detected.

Feed		
	Salmon feed	Salmon feces
TFA (mg/g DW)	68.4±9.1	10.3±2.4
FA (% of TFA)		
14:0	2.7±0.4	3.1±1.3
16:0	9.3±0.6	22.0±9.4
18:0	2.6±0.4	18.1±5.1
20:0	0.6±0.1	3.7±1.5
22:0	0.9±0.1	11.9±6.9
24:0	ND	ND
14:1 (n-5)	ND	ND
16:1 (n-7)	2.8±0.4	0.9±0.2
18:1 (n-9)	40.9±3.5	20.1±7.7
18:1 (n-7)	2.7±0.1	1.8±0.5
20:1 (n-9)	3.2±0.8	2.2±0.05
22:1 (n-11)	3.3±1.3	3.0±0.7
22:1 (n-9)	0.6±0.1	0.6±0.4
24:1	ND	ND
18:3 (n-3)	6.1±0.5	1.9±0.8
18:4 (n-3)	1.0±0.3	ND
20:3 (n-3)	ND	ND
20:4 (n-3)	0.2±0.0	ND
20:5 (n-3)	3.9±0.7	1.0±0.5
22:5 (n-3)	0.4±0.1	0.1±0.2
22:6 (n-3)	3.6±0.7	2.5±1.0
18:2 (n-6)	14.7±2.1	6.9±2.1
18:3 (n-6)	ND	ND
20:2 (n-6)	0.1±0.0	0.1±0.1
20:3 (n-6)	ND	ND
20:4 (n-6)	0.2±0.0	ND
22:5 (n-6)	0.1±0.0	ND

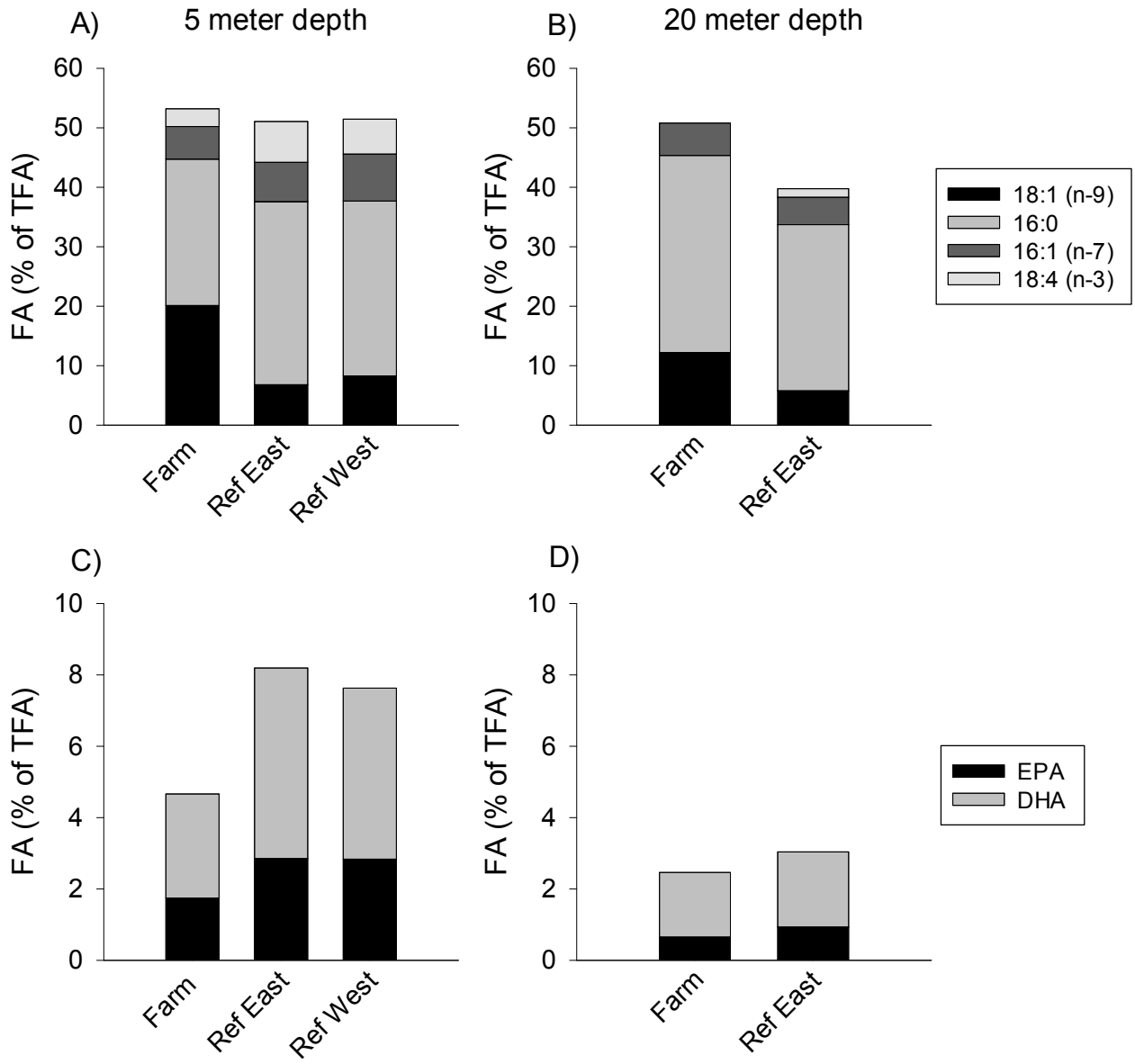

Figure 12. Average fatty acid fraction (% of TFA) of selected fatty acids in particulate matter (< 200 µm) of seawater samples (mean) taken in September at various stations at 5 and 20 meters depth.

3.2.3 Scallop growth and condition

Figure 13 A shows the average growth rate (AGR µm/day) of scallops incubated at the four experimental stations, including Farm, Farm 200 and Ref East at 5 and 20 meters depths. The AGR was significantly higher at the Farm station compared to the Ref East station (P=0.004). The scallop at Farm 200 was also high, but not significantly different from Ref East (P>0.05). The growth was poor at 20 meters depth for all stations.

Figure 13 B shows the condition index for the scallops maintained at the experimental stations. The Farm station showed the greatest condition index of the scallops at 5 meters depth, whilst scallops at 20 meter exhibited a very low condition index. This suggested that they were under inferior growth conditions, which the low AGR implied as well. Cultivation in the upper part of the water column seemed to have a positive effect on the condition index.

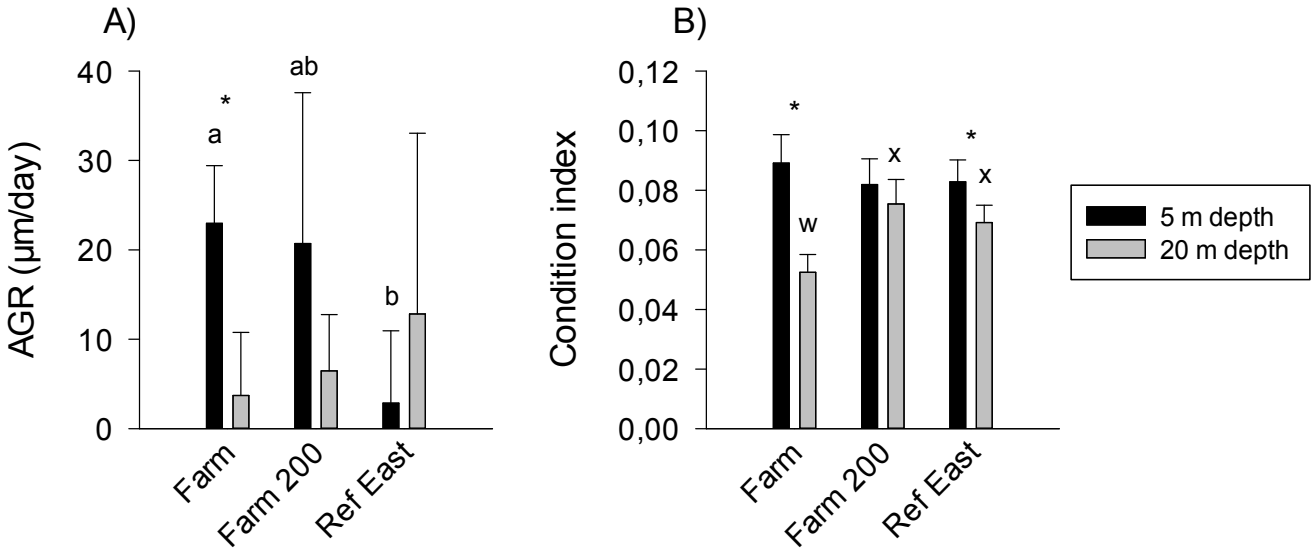

Figure 13. The average growth rate (µm/day) and B) condition index (mean ± standard deviation) to scallops cultured at 5 and 20 meters depth at three of the four experimental stations; Farm, Farm 200 and Ref East. Lower case letters *a* and *b* show significant differences (P<0.05) between samples at 5 meters depth, *w* and *x* from 20 meter. * imply a significant difference (P<0.05) between 5 and 20 meters.

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3.2.4 Incorporation of food components

Figure 14 A and B show the total fatty acid content (mg FA/ g FDW) in digestive gland and gonadic tissue for scallops deployed at the experimental stations at 5 and 20 meter depth. Scallops grown at 20 meter on the Farm station showed a significantly lower TFA content in digestive gland tissue compared to Farm 200 ($P=0.028$) and Ref West ($P=0.032$) at 20 meter depth, and the Farm station ($P=0.023$) at 5 meter. This suggested that the condition for scallops installed at 20 meters depth on the Farm station were sub-optimal. The TFA content in gonadic tissue showed no significantly variations among the stations and the two depths ($p>0.05$).

Figure 14 C shows the fraction of 18:1 (n-9) in the digestive gland tissue for scallops cultured at the experimental stations at both depths. Digestive glands from the Farm station exhibited a higher level of 18:1 at 5 meter than the other stations, and scallops from Farm 200 showed the second highest fraction. It seems like the stations under the influence from fish farm wastes exhibits a higher fraction of 18:1 (n-9). The Farm 200 station displayed the highest fraction at 20 meters. The elevated levels of 18:1 (n-9) in digestive gland tissue for scallops located in close proximity to the salmon farm are most likely due to the high fraction of this fatty acid in the seawater near the Farm station (Figure 12 A). The gonad tissue showed an overall greater concentration of 18:1 (n-9) at both 5 and 20 meter depth at the Farm station, as seen in Figure 14 D.

Table 7 present the fatty acid fraction of all known fatty acids in scallop digestive gland tissue. The fraction of 18:4 (n-3) decreased from 5 to 20 meter depths. Scallops cultured at 20 meters depth at the Farm station showed the lowest percentage of the omega-3 fatty acid. The gonad tissue indicated a decrease in fatty acids levels with increasing depth (see Table 9 and Table 10). This reflects the higher abundance of these fatty acids in the top layer in the water column as is seen in Figure 12 A.

The amount of EPA and DHA in digestive gland tissue showed no significant difference between the experimental stations. The fraction of EPA was significantly higher at 20 meters for the Farm ($P<0.001$) and Ref West stations ($P=0.001$) despite a higher abundance of EPA in the upper water body (Figure 12 B). Scallop did not show differences in the level of EPA and DHA in gonad tissue. The fraction of ARA was low in the digestive glands for all scallops, with a slightly higher fraction at 20 meter. This was also evident in gonad tissue, although it contained a higher level of ARA.

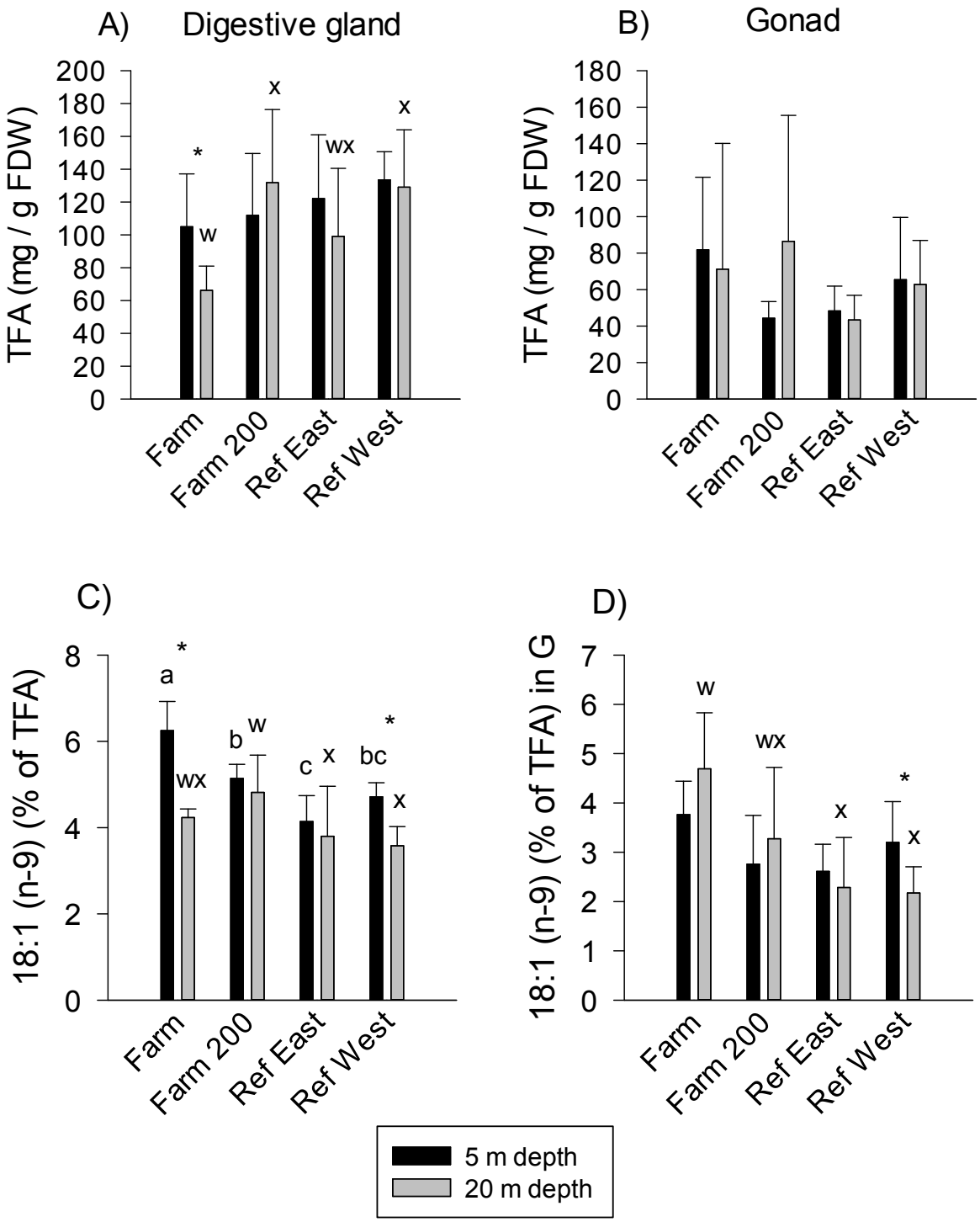

Figure 14. Total fatty acid fraction in A) digestive glands and B) gonad, and the fraction of 18:1 (n-9) in C) digestive glands and D) gonads (mean ± standard deviation, n=6) of scallops cultured at 5 and 20 meters depth at the four experimental stations; Farm, Farm 200, Ref East and Ref West. Different lower case letters *a*, *b* and *c* imply significant differences (P<0.05) between samples at 5 meters depth, *w*, *x* and *y* from 20 meter. * imply a significant difference (P<0.05) between 5 and 20 meters.

Table 7. Total fatty acid content (mg/g FDW) and fatty acid composition (% of total fatty acid) in digestive glands of scallops at 5 meters depth at the four experimental stations; Farm, Farm 200, Ref East and Ref West. Different lower case letters imply significant differences (P<0.05) between samples. * imply a significant difference (P<0.05) between 5 and 20 meters (Table 8). ND = Not Detected.

Digestive gland				
	5 meters depth			
	Farm	Farm 200	Ref East	Ref West
TFA (mg/g DW)	104.9±32.2	111.9±37,7	122.1±38.9	133.5±17.1
FA (% of TFA)				
14:0	5.4±0.4	5.6±1.0	5.3±0.3	5.6±0.5
16:0	19.2±0.9^{ab}	19.3±0.5^{ab}	18.7±0.6^{a}	20.1±0.7^{b}
18:0	5.3±0.5*	5.7±1.9	5.9±2.3	4.9±0.5
20:0	0.2±0.1^{ab}	0.1±0.1^{a}	0.2±0.1^{a}	0.3±0.1^{b}*
22:0	ND	ND	ND	ND
24:0	ND	ND	ND	ND
14:1 (n-5)	ND	ND	ND	ND
16:1 (n-7)	7.0±0.7*	7.2±2.0	6.8±1.4	7.3±0.6*
18:1 (n-9)	6.2±0.7^{a}*	5.1±0.3^{b}	4.1±0.6^{c}	4.7±0.3^{bc}*
18:1 (n-7)	3.9±0.1*	3.9±0.4*	3.8±0.3*	3.8±0.4*
20:1 (n-9)	1.8±0.2^{a}*	1.7±0.5^{ab}	1.5±0.2^{ab}	1.2±0.2^{b}*
22:1 (n-11)	0.4±0.1^{a}	0.3±0.1^{ab}	0.3±0.1^{ab}	ND^{b}
22:1 (n-9)	ND	ND	ND	ND
24:1	ND	ND	ND	ND
18:3 (n-3)	2.7±0.3*	2.7±0.6*	2.7±0.7*	2.8±0.3*
18:4 (n-3)	7.8±0.5*	8.0±1.8	8.4±2.1*	8.5±0.9
20:3 (n-3)	0.3±0.0*	0.3±0.1	0.3±0.1*	0.3±0.0*
20:4 (n-3)	0.9±0.3	1.1±0.2	1.1±0.3*	1.2±0.2*
20:5 (n-3)	17.6±1.0*	16.9±0.3	18.0±1.8	17.8±1.7*
22:5 (n-3)	0.6±0.0	0.6±0.1	0.6±0.2	0.6±0.0
22:6 (n-3)	15.3±0.7	16.3±3.7	17.3±3.4	15.8±1.2
18:2 (n-6)	3.0±0.2^{a}*	2.6±0.3^{ab}*	2.4±0.5^{b}*	2.7±0.1^{ab}*
18:3 (n-6)	ND	ND	ND	ND
20:2 (n-6)	1.0±0.1*	1,1±0,2*	1,0±0,1*	1.0±0.1*
20:3 (n-6)	0.2±0.1	0,2±0,1	0,2±0,1	0.3±0.1
20:4 (n-6)	0.9±0.1^{ab}*	1,1±0,5^{ab}	1,2±0,8^{a}	0.7±0.0^{b}*
22:5 (n-6)	0.2±0.1	0,3±0,1	0,3±0,1	0.2±0.1

Table 8. Total fatty acid content (mg/g FDW) and fatty acid composition (% of total fatty acid) in digestive glands of scallops at 20 meters depth at the four experimental stations; Farm, Farm 200, Ref East and Ref West. Different lower case letters imply significant differences (P<0.05) between samples. * imply a significant difference (P<0.05) between 5 (Table 7) and 20 meters. ND = Not Detected.

Digestive gland	20 meters depth			
	Farm	Farm 200	Ref East	Ref West
TFA (mg/g DW)	66.1±14.8	131.8±44.5	99.1±41.4	129.0±35.0
FA (% of TFA)				
14:0	6.1±1.0	5.5±0.7	5.5±0.5	6.2±0.6
16:0	19.8±1.6	18.4±1.0	18.2±1.5	18.9±2.0
18:0	6.9±0.8*	5.4±1.8	6.3±2.4	4.9±1.0
20:0	0.2±0.1	0.2±0.1	0.1±0.1	0.1±0.1*
22:0	ND	ND	ND	ND
24:0	ND	ND	ND	ND
14:1 (n-5)	ND	ND	ND	ND
16:1 (n-7)	8.2±0.8*	8.2±1.6	7.9±1.3	8.8±1.2*
18:1 (n-9)	4.2±0.2^{wx*}	4.8±0.9^{w}	3.8±1.2^{x}	3.6±0.4^{x*}
18:1 (n-7)	4.5±0.3*	4.4±0.3*	4.6±0.5*	4.5±0.6*
20:1 (n-9)	1.5±0.1^{w*}	1.5±0.6^{wx}	1.3±0.3^{wx}	1.0±0.2^{x*}
22:1 (n-11)	0.2±0.1^{wx}	0.3±0.2^{w}	0.2±0.1^{x*}	ND^{y}
22:1 (n-9)	ND	ND	ND	ND
24:1	ND	ND	ND	ND
18:3 (n-3)	1.2±0.1^{w*}	1.8±0.1^{x*}	1.3±0.4^{wx*}	1.7±0.4^{wx*}
18:4 (n-3)	4.7±0.2^{w*}	6.7±0.8^{wx}	5.2±1.6^{wx*}	7.1±1.6^{x}
20:3 (n-3)	0.1±0.0*	0.2±0.1	0.2±0.1*	0.2±0.1*
20:4 (n-3)	0.6±0.1	0.8±0.1	0.8±0.2*	0.8±0.1*
20:5 (n-3)	20,8±0,8*	21.0±4.1	22.1±3.8	22.9±2.2*
22:5 (n-3)	0,6±0,1	0.6±0.1	0.9±0.4	0.5±0.1
22:6 (n-3)	15,5±1,7	15.5±2.7	16.5±3.3	14.7±2.3
18:2 (n-6)	1,9±0,1^{wx*}	2.3±0.1^{w*}	1.7±0.3^{x*}	2.0±0.2^{wx*}
18:3 (n-6)	ND	ND	0.3±0.7	ND
20:2 (n-6)	0,8±0,1*	0.8±0.1*	0.8±0.1*	0.7±0.1*
20:3 (n-6)	0,3±0,0	0.2±0.1	0.6±0.8	0.3±0.1
20:4 (n-6)	1,4±0,2^{w*}	1.0±0.3^{wx}	1.4±0.8^{wx}	0.9±0.1^{x*}
22:5 (n-6)	0,3±0,0	0.2±0.1	0.2±0.1	0.2±0.1

Table 9. Total fatty acid content (mg/g FDW) and fatty acid composition (% of total fatty acid) in gonads of scallops at 5 meters depth at the four experimental stations; Farm, Farm 200, Ref East and Ref West. Different lower case letters imply significant differences (P<0.05) between samples. * imply a significant difference (P<0.05) between 5 and 20 meters depth (Table 10). ND = Not Detected.

Gonads				
	5 meters depth			
	Farm	Farm 200	Ref East	Ref West
TFA (mg/g FDW)	81.8±39.8	44.3±9.1	48.3±13.6	65.4±34.1
FA (% of TFA)				
14:0	3.3±0.9	3.4±0.4	3.4±0.4	3.0±0.4
16:0	24.4±4.5	25.9±3.8	22.5±1.6	22.7±0.4
18:0	9.0±2.7	11.4±3.9	9.1±1.8	9.0±1.2
20:0	ND	ND	ND	ND
22:0	ND	ND	ND	ND
24:0	ND	ND	ND	ND
14:1 (n-5)	ND	ND	ND	ND
16:1 (n-7)	2.1±0.4	2.0±0.7	2.8±0.3	2.8±0.5
18:1 (n-9)	3.8±0.7	2.8±1.0	2.6±0.6	3.2±0.8*
18:1 (n-7)	2.3±0.4	2.2±0.7	2.4±0.6	3.0±0.7
20:1 (n-9)	2.9±0.4[a]	2.6±0.3[ab]	2.4±0.1[ab]	2.1±0.4[b]
22:1 (n-11)	ND	ND*	ND*	ND
22:1 (n-9)	ND	ND	ND	ND
24:1	ND	ND	ND	ND
18:3 (n-3)	1.8±0.5*	1.4±0.5	1.8±0.4*	1.4±0.1*
18:4 (n-3)	5.0±1.8*	4.1±1.6	5.6±1.3*	4.0±0.7*
20:3 (n-3)	0.2±0.0*	0.2±0.1	0.2±0.1	0.2±0.0
20:4 (n-3)	0.7±0.2*	0.7±0.2	0.7±0.3*	0.6±0.1*
20:5 (n-3)	11.3±2.0	10.3±2.2	12.1±1.1	12.6±1.2
22:5 (n-3)	1.0±0.1	1.2±0.3	0.9±0.1	1.0±0.1
22:6 (n-3)	27.0±2.8	26.3±3.0	27.6±2.1	28.6±2.4
18:2 (n-6)	1.6±0.5	1.2±0.3	1.3±0.1*	1.3±0.3*
18:3 (n-6)	ND	ND	ND	ND
20:2 (n-6)	1.2±0.1*	1.1±0.2	1.0±0.1*	1.2±0.1*
20:3 (n-6)	0.3±0.1	0.7±0.9	0.3±0.1	0.4±0.1
20:4 (n-6)	1.7±0.4*	2.0±0.4*	2.4±0.6	2.2±0.2
22:5 (n-6)	0.6±0.1	0.7±0.2	0.6±0.4	0.6±0.1

Table 10. Total fatty acid content (mg/g FDW) and fatty acid composition (% of total fatty acid) in gonads of scallops at 20 meters depth at the four experimental stations; Farm, Farm 200, Ref East and Ref West. Different lower case letters imply significant differences (P<0.05) between samples. * imply a significant difference (P<0.05) between 5 (Table 9) and 20 meters depth. ND = Not Detected.

Gonads				
	20 meters depth			
	Farm	Farm 200	Ref East	Ref West
TFA (mg/g FDW)	71.2±68.9	86.4±69.0	43.3±13.5	62.8±24.1
FA (% of TFA)				
14:0	2.6±0.2	3.0±0.3	3.5±1.8	2.9±0.5
16:0	21.9±0.4	22.5±1.6	26.9±11.8	23.3±2.6
18:0	10.1±1.3	9.9±2.1	13.0±6.9	10.9±2.8
20:0	ND	ND	ND	ND
22:0	ND	ND	ND	ND
24:0	ND	ND	ND	ND
14:1 (n-5)	ND	ND	ND	ND
16:1 (n-7)	1.9±0.3^{w}	2.7±0.9^{wx}	2.4±1.4^{wx}	3.0±0.4^{x}
18:1 (n-9)	4.7±1.1^{w}	3.3±1.4^{wx}	2.3±1.0^{x}	2.2±0.5^{x*}
18:1 (n-7)	2.8±0.2	2.8±0.8	2.8±1.0	3.5±0.6
20:1 (n-9)	3.0±0.3^{w}	2.9±0.5^{w}	2.1±0.4^{x}	2.0±0.3^{x}
22:1 (n-11)	0.1±0.1^{wx}	0.2±0.1^{wx*}	0.3±0.2^{w*}	ND^{x}
22:1 (n-9)	ND	ND	ND	ND
24:1	ND	ND	ND	ND
18:3 (n-3)	0.9±0.3*	0.9±0.3	0.6±0.6*	0.7±0.4*
18:4 (n-3)	2.8±0.8*	3.2±1.3	2.7±1.9*	2.5±1.6*
20:3 (n-3)	0.1±0.0*	0.2±0.0	0.1±0.1	0.2±0.1
20:4 (n-3)	0.3±0.0*	0.4±0.1	0.3±0.2*	0.3±0.1*
20:5 (n-3)	12.2±0.7	12.4±1.2	12.2±5.2	13.9±2.3
22:5 (n-3)	1.0±0.2	1.0±0.2	0.9±0.4	1.2±0.3
22:6 (n-3)	29.2±0.5	28.8±2.3	25.1±9.5	28.1±2.8
18:2 (n-6)	1.4±0.3^{w}	1.2±0.3^{wx}	0.7±0.4^{x*}	0.8±0.2^{wx*}
18:3 (n-6)	ND	ND	ND	ND
20:2 (n-6)	0.9±0.1*	0.9±0.1	0.6±0.3*	0.9±0.1*
20:3 (n-6)	0.3±0.1	0.3±0.0	0.3±0.2	0.3±0.1
20:4 (n-6)	3.1±1.1*	2.8±0.8*	2.7±1.7	2.7±1.0
22:5 (n-6)	0.6±0.2	0.5±0.3	0.5±0.3	0.6±0.2

4 Discussion

The main objective of this Master's thesis was to see if organic waste particles from cage fish farms could be assimilated by the scallop *P. maximus* through a field study and a laboratory study. The results from the laboratory study showed that scallops were largely affected by the phytoplankton concentration. A high concentration of algae resulted in increased total lipid and total fatty acid (TFA) content, along with increased growth. However, the scallops incorporated salmon feed and salmon feces in the digestive gland tissue when supplied together with algae, particularly when the algae supply was low. The scallops were also found to retain essential fatty acids like 20:5 n-3 (EPA), 22:6 n-3 (DHA) and 20:4 n-6 (ARA) when the concentration of algae was low. The results from the field study showed that fatty acids known to be present in high concentrations in diatoms and dinoflagellates were found in high amounts also in the tissues of scallop. Incorporation of waste particles released from the fish farm was largely found in scallops close to the fish farm, as demonstrated by their contents of tracer fatty acids like 18:1 n-9.

Earlier studies by Redmond et al. (2010) and Handå et al. (2012b) showed that 18:1 (n-9) and 18:2 (n-6) could be used as biomarkers for assimilation of salmon feed, the latter also for *R. baltica*. In some phytoplankton and bacteria, 18:1 (n-9) can be present in high amounts (De Carvalho and Caramujo, 2014). In spite of that, 18:1 (n-9) is a very good tracer fatty acid because the high concentration of this fatty acid in the salmon feed and feces collected in the field (see Table 6) and in the laboratory trial, this compared to the contents of the algae present (see Table 3). In addition, the water samples collected at the Farm station showed elevated values of 18:1 (n-9), particularly at 5 meters depth, indicating that some salmon feed and salmon feces have been lost as waste from the fish farm (Figure 12). The water samples from the Farm station showed higher concentration of 18:2 (n-6) as well. However, *R. baltica* used in the laboratory trial showed approximately similar concentration of 18:2 (n-6) as the salmon feed and feces (see Table 3). This resulted in similar values in the scallop tissue (Table 4, Table 5) and 18:2 (n-6) was thus unsuitable as a biomarker for assimilation of salmon feed and feces.

4.1 Laboratory experiment

4.1.1 Particle selection

Phytoplankton is a part of the natural feed for *Pecten maximus* (Chauvaud et al., 2001; Delaunay et al., 1993; Shumway et al., 1997), along with zooplankton and detritus (Strand, 2014). Earlier studies by Shumway et al. (1997) and MacDonald and Ward (1994) showed a variable clearance rate for different algal species, and that this selection originated in the different size and nutrient value of the food

particles. Previous studies have shown that bivalves can control the ingestion of particulate matter by regulating the feeding rate, duration of the feeding and production of pseudofeces (Foster-Smith, 1975; Winter, 1978; Newell and Jordan 1983; Strohmeier et al., 2009). MacDonald and Ward (1994) showed that the sea scallop *Placopecten magellanicus* altered the quality of the food ingested by selectively rejecting particles poor in nutrients. Handå et al. (2012a) found that the nutrient quality of salmon feces was poor compared to the salmon feed. This was due to lower total fatty acid content and mono- and polyunsaturated fatty acids, and a higher content of saturated fatty acids in the feces. It has been demonstrated that *Mytilus edulis* does not show selective ingestion when food availability is low. It consequently ingests a larger proportion of food with reduced food quality under such conditions (Newell et al., 1989; Handå et al., 2012a,b). A selection of specific food particles was also found for scallops in this experiment. This was particularly apparent by the fraction of 18:1 (n-9) in scallop tissues, a fatty acid present in high amounts in the salmon feed and feces. Scallops co-fed by salmon feed and salmon feces showed a higher concentration of 18:1 (n-9) when the algae supply was low (Figure 7), indicating an increased ingestion of salmon feed and feces. Contrary, the scallops selected positively for phytoplankton when present in high quantities.

The digestive glands are the main storage site for lipid reserves in scallops (Barber and Blake, 1981; Lorrain et al. 2002; Gosling, 2003). A greater total lipid and total fatty acid (TFA) content was found in digestive gland tissue of scallops fed a high concentration of algae (Figure 6), likely as a direct result of better feed availability. Scallops fed salmon feed was expected to show an increased lipid content in the digestive glands since they were supplied with nutritious feed. However, this was not observed. For the adductor muscle, the total lipid content and TFA was not affected by the concentration of algae. The adductor muscle in scallops is shown to have a low content of lipids with phospholipids as the principal lipid component (Napolitano et al., 1992). The fatty acid profile in phospholipids are more species dependent than diet dependent (Napolitano et al., 1992 and the references therein), and the composition was therefore not expected to change to any great extent.

4.1.2 Incorporation of fatty acids

Scallops in the laboratory trail showed signs of incorporating phytoplankton. The shells contained high amount of 18:4 (n-3) in digestive gland tissues when the food supply was high, in contrast to the significantly lower fraction when algae was given in low concentration (Table 4). This change probably originated from *R. baltica* which contains a high content of 18:4 (n-3) compared to *C. muelleri*, salmon feed and feces, thus reflecting the feeding activity of *R. baltica* (Table 3). The n-3 fatty acids EPA and DHA displayed greatest concentrations in *R. baltica*, but salmon feed showed a high concentration of these fatty acids as well. An increase in EPA and DHA when scallops were supplemented with salmon feed in addition to algae was therefore expected. This was not the case, so the fluctuations of EPA concentration apparently reflected consumption of *R. baltica*.

The laboratory trial revealed a capacity of scallop to assimilate and incorporate salmon feed and feces. Scallops fed salmon feed or salmon feces showed elevated 18:1 (n-9) levels in the digestive gland tissue compared with the control group, indicating that the supplemented feed was incorporated into the scallop tissues. Previous studies by Handå et al. (2012a, b) demonstrated that blue mussles (*Mytilus edulis*) incorporated 18:1 (n-9) from salmon feed and, to a somewhat lesser extent, from salmon feces into digestive gland tissues. This was particularly found when the phytoplankton concentrations were low (Figure 7). Scallops co-fed by salmon feed and salmon feces did not show an increase in 18:1 (n-9) in the muscle tissue, as expected due to the high levels of phospholipids. The higher level of 16:0 in the digestive gland tissue for laboratory scallop fed salmon feces (Figure 7) could reflect that the scallops had consumed salmon feces, because feces have a high content of this fatty acid as compared to salmon feed and the algae fed the scallops (Table 3). This response was apparent in muscle tissue as well. This was expected as 16:0 is a dominant fatty acid of phospholipids. Lefebvre et al. (2000) reported that the Pacific oyster (*Crassostrea gigas*) was capable to absorb fish feces, although diatoms were found to be more efficiently absorbed.

Molluscs in general have a low capability of converting short-chain dietary n-3 and n-6 fatty acids to long-chain unsaturated n-3 and n-6 fatty acids (Pirini et al., 2007), thus depending on being supplied long-chain n-3 and n-6 fatty acids in the food. The polyunsaturated fatty acids EPA (20:5 n-3), DHA (22:6 n-3) and ARA (20:4 n-6) are regarded to be essential for growth and survival in mollusks (Trider and Castell, 1980; Soudant et al., 1996; Delaunay et al., 1993). Pirini et al. (2007) demonstrated that mollusks may adapt well to stress conditions after being fed diets lacking highly unsaturated fatty acids by retaining essential fatty acids like EPA (20:5 n-3), along with DHA (22:6 n-3) and ARA (20:4 n-6) (Trider and Castell, 1980). In the present laboratory trial, an increase in the concentration of EPA, DHA and ARA was observed in digestive gland tissue when algae concentration was low (Table 4). This indicated that scallops retain these fatty acids at the expense of other. The EPA fraction was greater in digestive gland tissue when scallops were fed at a low algae concentration. This is in agreement with Trider and Castell (1980) who showed that EPA was retained in oysters at the expense of other fatty acids. This was however not the case for the adductor muscle (Table 5), as the level of EPA was unaffected by the concentration in the diet. Most likely, this was as a result of the high levels of phospholipids since they are less susceptible to be altered by the fatty acid composition in the food (Napolitano et al., 1992 and the references therein; Torstensen et al., 2001). The fraction of DHA increased considerably in both digestive gland and muscle tissue when scallops were supplied with a low concentration of algae. It has previously been demonstrated that *P. maximus* selectively assimilated DHA (Soudant et al., 1996), and Marty et al. (1992) concluded that this reflected an important structural role of DHA in the cell membrane due to the high DHA level compared to that of other major

polyunsaturated fatty acids (PUFA). Adductor muscle tissue showed high levels of DHA, which is in accordance with Torstensen et al. (2001) who claimed that tissue with a highly active metabolism, had natural high concentrations of DHA. The n-6 fatty acid ARA showed elevated levels in digestive gland tissue when algae supply was low as well, although not to the same extent as EPA and DHA. In muscle tissue however, the response to algae concentration was opposite to that in digestive glands, with an increase in ARA with increased algae concentration.

4.1.3 Growth and condition index

Scallop showed higher average growth rate (AGR) when they were fed a high supply of algae compared to a low supply (Figure 5). Scallops fed a high concentration of algae also showed a greater condition index, giving more surplus energy that scallops can use for growth. Scallops co-fed salmon feed or feces did not show higher AGR. This finding is in accordance with Gosling (2003) and Utting (1988) who have claimed that phytoplankton is the most important factor for growth of bivalves due to larger energy stores.

Summing up results, the laboratory experiment has further confirmed that the adductor muscles would not be affected by the dietary fatty acids to the same extent as the digestive gland tissue. This was because lipids in the muscle tissue are mainly in the form of phospholipids. The digestive gland, on the other hand, is clearly a lipid storing organ, directly affected by the dietary fatty acid composition. High algae concentration led to higher lipid content in the scallop digestive gland tissue and a greater surplus of energy that could be used for growth, resulting in a higher average growth rate (AGR).The fatty acids 18:4 (n-3) and EPA was present in high amounts in *R. baltica*, and the fraction incorporated in the digestive gland of scallops tissues was largely affected by the algae concentration, indicating the importance of *R. baltica* as a food resource. Scallops incorporated a larger proportion of phytoplankton when the algae concentration was high, although some salmon feed and fatty acid was incorporated along with the phytoplankton. When the algae supply was low, scallops fed salmon feed or feces showed a higher fraction of 18:1 (n-9) in digestive gland tissues. The results indicated that the great scallops are able to utilize the salmon feed and salmon feces.

4.2 Field experiment

4.2.1 Incorporation of fatty acids

The feed use at the salmon farm increased during the spring and the summer (Figure 9). According to Wang et al. (2012) this could lead to an increase in POC in the water surrounding the fish farm because 19.0% of total carbon input to the fish farms can be lost as POC. This was not the case as the Farm station did not show elevated POC values compared to the Reference stations (Figure 11). This is based on one sampling day, however, thus failing to give a complete picture of the emission from the Farm station. Oppedal et al. (2011) showed that fish tend to crowd below 2-3 meters depth so the main discharge can be expected to be below that depth. The

filtered seawater collected at the various stations showed lower fractions of most of the fatty acids at 20 meters depth (Figure 12), making it similar to low algae concentrations in laboratory trial.

The seawater samples collected in September (Figure 12) suggests the presence of both diatoms and dinoflagellates, because of high fractions of specific fatty acids. Dinoflagellates typically contain the fatty acids 16:0, 18:4 (n-3), EPA (20:5 n-3) and DHA (22:6 n-3) (Reitan et al., 1994; Ackman et al., 1968), whereas diatoms contains 16:0, 16:1 (n-7) and EPA (Reitan et al., 1994; Ackman et al., 1968; Reuss and Poulsen, 2002; Kates and Volcani, 1965). This coincides with the findings by Sakshaug and Myklestad (1973), which discovered a dominance of dinoflagellates in Norwegian waters in the early autumn, after a diatom maximum in August. Selection of dinoflagellates and diatoms over other organic matter was evident as the scallops showed high amounts of the characteristic fatty acids mentioned above in their tissue. The high content of 16:1 (n-7) in digestive gland (Table 7, Table 8) and gonad tissue (Table 9, Table 10) in scallop from the field experiment suggested that the scallop's diet included diatoms. The high level of 18:4 (n-3) and DHA in digestive gland and gonads could be related to scallops feeding on dinoflagellates, because they may show high contents of these fatty acids. Scallops contained high amount of 16:0 and EPA as well, which are fatty acids known to be in high amounts in both diatoms and dinoflagellates.

The field trial demonstrated a higher 18:1 (n-9) fraction in the digestive gland tissue of scallops deployed at 5 meters depth at the Farm station compared to scallops at the Reference stations (Figure 14). Elevated levels of this fatty acid were detected in scallops at Farm 200 station as well. This shift towards the fatty acid that are abundant in salmon feed and salmon feces demonstrated consumption and further incorporation of fatty acids from salmon feed and feces into the digestive glands of scallops. This was only found for scallops in close proximity to the salmon farm, indicating a dispersion of wastes from the fish farm by the water currents.

Also gonad tissue of scallops from the field trial showed an overall higher fraction of 18:1 (n-9) at the Farm station (Figure 14). This suggested that the scallops had initiated gametogenesis, the first part of the bivalve reproduction cycle ending with spawning (Gosling, 2003). During the gametogenesis for scallops, energy stored in the digestive gland and the adductor muscle will be transferred to the gonads. Lipids from the digestive glands are transferred and utilized in the gonads in the early stages in gametogenesis (Barber and Blake, 1981, 1985). The enhanced levels of 18:1 (n-9) in gonad tissue for scallops at the Farm station is a sign of gametogenesis, because 18:1 (n-9) has been transferred after the scallops were deployed in the sea upon start of the experiment. Other indices that the scallops have spawned during the field experiment are poor growth and condition (Figure 13) as the scallops use their energy reserves for gonad development (Barber and Blake, 1985; Lorrain et al., 2002).

In the laboratory trial, *P. maximus* showed a preference of phytoplankton when present in high concentration (300 µg C/L). The scallops incorporated a larger fraction of 18:1 (n-9) originating from salmon feed and feces (Figure 7) when the algae supply was low (50 µg C/L). The concentration of particulate organic carbon (POC) in seawater was about 100 µg C/L (Figure 4), and would most likely result in increased incorporation of fatty acids originating from salmon feed and feces.

The amounts of EPA and DHA in the digestive glands were similar at the same depth among the experimental stations (Table 7, Table 8). The EPA concentration was higher for scallops at 20 meters depth at the Farm and Reference West station, although the concentration of EPA in the seawater was lower at 20 meter. This was in agreement with the results from the laboratory trial and Trider and Castell (1980), where it was seen that scallops retained essential fatty acids like EPA, DHA and ARA when these fatty acids not were present in high amounts in the diet. Unlike digestive gland tissue, the gonads showed similar concentration of EPA for both depths (Table 9, Table 10). The fraction of DHA was not affected by the depths for neither digestive gland nor gonad tissues. The fraction of ARA in digestive gland tissue was higher at 20 meters depth. This is in accordance with scallops fed a low algae concentration in the laboratory trial. This was also true for the gonad tissue, though the fraction of ARA was higher in gonads than the digestive glands. This further demonstrates mollusks ability to retain essential fatty acids (Pirini et al., 2007; Trider and Castell, 1980).

4.2.2 Growth and condition index

Scallops deployed at 5 meters depth at the Farm showed a higher average growth rate (AGR) compared the Ref West station (Figure 13). The AGR for scallops installed at the Farm 200 station was higher, although not significantly different from the Ref West station. The higher AGR could indicate that scallops have utilized the salmon feed and salmon feces present in the water for growth. This is consistent with previous findings by Peharda et al (2007) and Sarà et al. (2009) who showed increased growth and condition index of blue mussel (*Mytilus galloprovincialis*) when cultivated in IMTA system. Jones and Iwama (1991) on the other hand, found that Pacific Oyster (*Crassostrea gigas*) showed three times greater increase in shell height when cultivated in close proximity to the salmon farm.

The growth of *P. maximus* is minor compared to previous growth trials by Brynjelsen and Strand (1996) who showed growth resulting in AGR at 82 µm per day. Although, those scallops were of smaller size so the AGR was expected to be bigger than the scallops in the field trial. Chauvaud et al (1998) showed that daily growth decreased as the animals get older because the energy maintenance requirement increases. As the organism grows, it gets harder to gather food in excess of the threshold level thus resulting in slower growth (Broom and Mason, 1978). Strohmeier et al. (2000) demonstrated two separate periods of somatic growth and reproductive growth in *P. maximus*. Scallops transferred lipids from digestive gland tissues to the gonads after they were installed. It was therefore likely that the scallops were under a period of

reproductive growth, which is characterized by transfer of stored energy from the digestive gland and adductor muscle to the gonads (Barber and Blake, 1981, 1985). Another explanation could be fouling of the cages, particularly at the Farm station at 20 meters depth. The scallops deployed at this station showed a very low AGR and condition index (Figure 13) in combination with low total fatty acid (TFA) content in the digestive gland tissue (Figure 14), suggesting that the condition was sub-optimal. Earlier studies have shown that fouling reduces the flow of water and suspended food particles (Gosling, 2003). In addition, the scallops experience more severe competition for the food resources during autumn, because of a dominance of *Mytilus edulis* in the colonization (Claereboudt, et al., 1994; Gosling, 2003).

Summing up results, the field experiment showed similar total fatty acid (TFA) content between the two depths for both digestive gland and gonad tissues. This does not apply to digestive gland tissue in scallops at the Farm station, however, since the scallops at 20 meter were highly stressed. This resulted in a lower TFA content, low average growth rate (AGR) and low condition index for the scallops cultivated at the Farm station at 20 meter depth. Scallops installed at the Farm and Farm 200 stations would be under the influence from the emissions from the salmon farm. A higher AGR was observed for scallops at the Farm station, which could be a result of the increased quantity of food due to the emissions. The scallops showed high fractions of fatty acids typical for diatoms (16:0, 16:1 n-7, EPA) and dinoflagellates (16:0, 18:4 n-3, EPA, DHA), indicated grazing on these. The fraction of 18:1 (n-9) was higher in the digestive gland tissue in scallops cultivated in close proximity to the fish farm. Incorporation of 18:1 (n-9) was greatest for scallops at the Farm station, whereas the Farm 200 station showed the second highest concentration. This indicated that scallops could incorporate the wastes from the fish farm.

5 Conclusion

The field study showed that the fatty acid signature in scallop digestive gland tissues, and to some degree gonad tissues, was similar to seston in natural seawater. This suggests that the major part of the feed was seston. This was also confirmed by the laboratory experiment, where it was evident that scallop's condition was largely affected by the access to phytoplankton. The scallops in the laboratory trial were found to prefer phytoplankton over salmon feed and feces as long as algae were present in high amount. Less selectivity was observed when phytoplankton concentration was low. Scallops cultivated under the influence of waste particles from the fish farm incorporated a higher fraction of 18:1 (n-9), a fatty acid found in high amounts in salmon feed and feces. Scallops cultivated at 5 meter depth at the Farm station showed the largest concentration of 18:1 (n-9), whereas scallops 200 meter away from the Farm station showed high concentrations at both depths. A higher growth rate was seen for scallops cultivated at the Farm station compared to Ref East station, suggesting that scallops could utilize the extra energy from the farm effluents for growth. The results showed that scallops are able to exploit the fish farm waste at least 200 meters away from the fish farm. This suggests that scallops are a relevant species in integrated multi-trophic aquaculture.

5.1 Concluding remarks

To get a more complete understanding of scallops in integrated multi-trophic aquaculture, series of sampling of scallops should be collected over a longer time-span to investigate food incorporation when phytoplankton abundance in the seawater is low during the winter months in contrast to the higher phytoplankton concentration found through the rest of the year. Fouling of nets was a problem that needs to be solved to secure optimal growth of the scallops, particularly when cultivated at the farm. Regular mesh cages used through this field trial was not the best option, as the scallop density by far exceeded the natural maximum densities at 5-6 scallops per square meter (Bergh and Strand, 2002), and the fine mesh provided a good substrate for fouling. Another matter is the long period of time needed for scallops to reach commercial size, which is three to four years (Bergh and Strand, 2002). The Atlantic salmon are installed in the sea for a shorter time. At the fish farm where the field experiment was carried out, the fish was in the sea just below 1.5 year.

6 Litterature

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