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Effects of nutrients from fish farms on culture of blue mussel (Mytilus edulis)

Hojune Min

Marine Coastal Development
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Supervisor: Helge Reinertsen, IBI
Co-supervisor: Kjell Inge Reitan, Sintef

Norwegian University of Science and Technology
Department of Biology

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Hojune Min

Abstract

Intensive salmonid cage culture releases nutrients and organic matter that lead eutrophication of coastal waters. Integrated multi-trophic aquaculture (IMTA) is receiving great attention as a means of reducing organic/inorganic nutrient loads to the environment and increasing productivity by rearing viable secondary organisms.

In this study blue mussels (*Mytilus edulis*) were cultured close to a salmon (*Salmo salar*) cage farm at Tristen, Bjugn in Sør Trøndelag, Central Norway from June 2010 to February 2011. 3 experimental stations at the fish farming area and 2 reference stations (1.8 and 3.6 km away from the fish farm, respectively) were positioned to test if the integrated mussel culture reduces the environmental impact from salmon cage culture.

Highest water temperature recorded was 14.3 °C in July. Water temperature was above 10 °C until middle of October and the lowest was 3.1 °C in winter. Total particulate matter (TPM) and chlorophyll a contents varied 6.3 – 10.5 mg L^{-1} and 0.1 - 2.5 µg L^{-1}, respectively. The mussel (n=450) length increased from 31.1±0.04 mm (standard length±SE) to 41.5±0.17 mm. The mussel growth was high in summer (June – September) and was close to zero in the winter (October – February).

Multiple regression analysis showed that water temperature was the prime factor affecting condition index (CI) (meat dry weight/shell dry weight) and specific growth rate (SGR) of mussel. No differences were found in the environmental variables and food availabilities among the stations. Significantly higher CIs at the experimental stations (station 1, 2 and 3) than at the reference stations were caused by utilization of fish farm discharges by the mussels. Fatty acids 18:1 (n-9), 18:2 (n-6) and 20:5 (n-3) signatures and the ratio of n-3 to n-6 in the digestive gland tissue of mussels collected in February support the findings. SGR in shell length was highest at reference station 5 (0.29 % day^{-1} in summer and 0.02 % day^{-1} in winter). This result might be caused by slow current speed at the station.

It is concluded that, mussels may play an important role reducing environmental impact from salmon farm and it gives a possibility to integrating mussels on the salmon cage culture.

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1. Introduction

1.1. General introduction

While worldwide fishery production reached to its maximum yield in mid-80's, aquaculture production showed fastest growth among all food production sectors (FAO, 2008). With the remarkable increase in seafood consumption, aquaculture manifests its importance as an alternative food source. However, the aquaculture industry is still at an infant stage of development. Particularly mariculture has large potential for further growth because there are many unexploited coastal and offshore areas and several unexploited aquaculture candidates.

Despite the fact that aquaculture has large potential as a future food source, aquaculture industry has faced environmental concerns in recent years. Salmonid cage farming can cause a series of problems. Among possible obstacles such as natural habitat destruction, genetic pollution by escaping and introduction of chemicals (Kumar, 1995; Lucas and Southgate, 2003), excessive nutrient discharges from a fish farm are big concern. According to Wu (1995), up to 85 % of phosphorus, 52 – 95 % of nitrogen, and 80 – 89 % of carbon entering to a fish would be wasted to the environment by fish excretion, respiration and feces. Laurel and Donald (1997) cited that only 30 % of the nitrogen and 32 % of the phosphorus would be retained by a salmonid body. Together with uneaten feed, those excessive nutrients affect the sea bed of the farm sites by sediment accumulation and the water quality by reducing dissolved oxygen (DO), increasing nutrient levels and turbidity, and cause habitat destruction in the end changes in biodiversity (Wu et al., 1994; Wu, 1995; Troell and Berg, 1997).

1.2. IMTA

A number of strategies are developed to reduce excessive nutrients from fish farms, including using improved feed formulation with better digestibility, continuous monitoring and improved feeding regime, site rotation and reduced stock density (Cheshuk et al., 2003; Lucas and Southgate, 2003). Integrated multi-trophic aquaculture (IMTA) can be another solution. Rather than diluting like many of the conventional strategies, IMTA focuses on

conversion: by-products (wastes) from fed organism (e.g. finfish and shrimp) are recycled to become inputs for organic/inorganic extractive organisms (e.g. shellfish and seaweed, respectively). IMTA intends to remove and recycle metabolites from the fish farms, reduce organic/inorganic nutrient loads to the environment and increase productivity by rearing co-cultured species and thereby enhance the energy efficiency (Troell et al. 2003).

IMTA has been applied for centuries. In many Asian countries, especially in China, traditional land based aquatic polyculture methods such as fish cultivation in the rice field, farming different fish species with different ecological niches, or utilizing by-product from domestic animals to fish farming have been used with a view to utilizing all available resources in the system and maximizing the productivity and profit (Woynarovich, 1979; Ye, 2002). In recent years, there have been advances and improvements on the IMTA methods. Ryther et al. (1972) suggested to introduce extractive organisms (i.e. microalgae, shellfish and small invertebrates) to the system to treat anthropogenic wastes for a maximum aquaculture yield. Anthropogenic wastewater can be nutrient sources for phytoplankton and the phytoplankton will be consumed by shellfish. Then solid wastes from the shellfish are fed to small invertebrates such as polychaete worms or amphipods and the food chain will be closed by secondary fed organism (e.g. lobster and flounder) who fed these invertebrates (Ryther, 1972; Ryther et al., 1975). Analogous to Ryther's work, Tenore (1976) introduced abalone (*Haliotis rufescens*, *H. fulgens*, and *H. discus*) to the system to remove nutrients regenerated from bivalves. Krom (1995) demonstrated that the impact of fed organism (*Sparus aurata*) can be mitigated by integrating seaweed (*Ulva lactuca*).

In recent years, with rising environmental issues, more ecosystem based integrated systems have been developed (Fig. 1). Western countries have studied and improved IMTA systems and recently focused on alleviation of environmental impacts from intensive fish farms by raising extractive organisms. Species selection is an important key for a successful IMTA practice. There has been several studies integrating seaweed cultivation to open water aquaculture which resulted in reduced inorganic loads from a fish farm and enhanced seaweed growth (Vandermeulen and Gordin, 1990; Neori, 1991; Haglund, 1993; Chopin et al., 1999; Buschmann et al., 2001; Abreu et al., 2009; Mai et al., 2010). Other studies focused on removing organic nutrients by introducing bivalve cultivation to the IMTA system and demonstrated that bivalves can be used as a biological filter (Jones and Iwama, 1991; Stirling and Okumus, 1995; Mazzola and Sara, 2000).

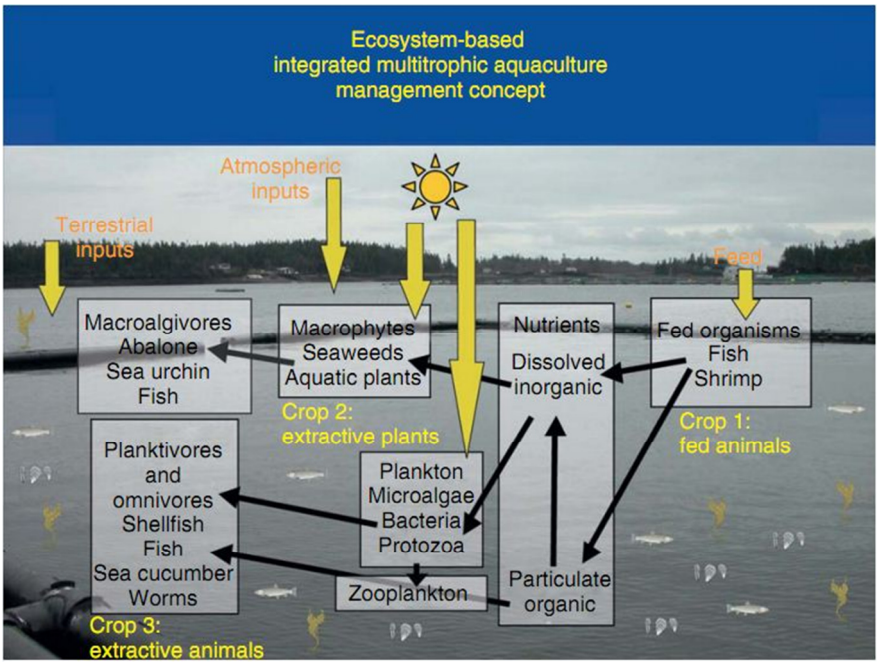

Fig. 1: Ecosystem based integrated multitrophic aquaculture system (IMTA) management concept. The wastes from fed organism are reused as, food and energy for organic / inorganic extractive organisms. Biomitigation takes place while harvesting those crops from the system (Chopin et al., 2008a).

1.3. **IMTA in Norway**

After Norway started salmonid aquaculture in 1960s, the industry has experienced rapid growth in quantity and quality. During 1970s, the industry underwent significant breakthroughs both in biological and technological aspects such as smolt rearing, improvement of dry feed and cage design (Liu et al., 2011). However, due to fast quantitative expansion of salmonid culture during 1980's to 1990's, the industry faced serious disease problems. Since then, the Norwegian government has strictly controlled and monitored the salmon industry (Maroni, 2000). In this respect IMTA can be suggested as a new approach for the further growth of Norwegian salmonid aquaculture industry.

Aquaculture in Norway is based on monoculture of salmonid species (*Salmo salar* and *Oncorhynchus mykiss*), consisting 98 % of total production (936,908 ton, in 2009) and other species i.e. Arctic char (*Salvelinus alpinus*), Atlantic cod (*Gadus morhua*) and halibut (*Hippoglossus hippoglossus*) constituting the remaining 2 % (Fiskeridirektoratet, 2010).

Therefore, Norwegian aquaculture is mainly based on cage fish farming in the sea. Since the fish cages are getting bigger and stock densities higher in recent years, the fish cage farms have been moved from sheltered areas to more opened and exposed offshore areas to ensure rapid water exchange and waste dispersal from the fish cages. In this context, the focus of IMTA in Norway is to implement IMTA in offshore cage cultures and current researches mainly concentrate on testing extractive organisms to the system (Barrington et al., 2009). A numbers of laboratory experiments (Mai et al., 2010; Redmond et al., 2010; Skriptsova and Miroshnikova, 2011) and field studies (Jones and Iwama, 1991; Taylor et al., 1992; Stirling and Okumus, 1995; Troell and Norberg, 1998; Cheshuk et al., 2003; Peharda et al., 2007; Gao et al., 2008) with different extractive species candidates have been conducted, globally.

Currently an IMTA project is running at Aquaculture engineering AS (ACE) (http://www.aceaquaculture.com) in Norway. ACE is an offshore cage fish farm located in mid-Norway, where biological and engineering researches are practiced. The project involves growing seaweed (*Saccharina latissima*), scallop (*Pecten maximus*) and blue mussel (*Mytilus edulis*) on long lines at different distances from the salmon fish cages of ACE.

1.4. **Description of blue mussel**

Blue mussel (*Mytilus edulis*) belongs to the family Mytilidae in the class Bivalvia and has a wide distribution in the northern hemisphere. The mussel are found in European waters from the White sea of Russia to the Atlantic coast of southern France, mainly due to its tolerance to environmental variables such as salinity, desiccation, temperature, and oxygen tension. By the virtue of tolerance, blue mussel can inhabit in a wide range of microhabitats. (Gosling, 1992).

Blue mussel is usually assumed to be microphagous typically live on suspended particles larger than 4 μm (Gosling, 1992), such as bacteria, phytoplankton, micro-zooplankton, detritus and dissolved organic matter (Davenport et al., 2000; Gosling, 2003). Mussels acquire nutrients by pumping water through sets of ctenidia, selecting particles from the water and transporting collected material to the mouth owing to a series of movement of ctenidia and labial palps. Although there are controversies regarding the mechanism of transportation of material (Ward et al., 1993), several researchers found that mussels are

selective feeders that filter and choose the suspension by size, concentration and nutritional value (Jørgensen, 1949; Reid et al., 2010; MacDonald et al., 2011).

Blue mussel inhabits along the Norwegian coastal line and can be found from rocky subtidal zone to completely submerged condition (Gosling, 1992). The total production of blue mussel in Norway was 1649 tons in 2009 (Fiskeridirektoratet, 2010).

1.5. Integrating mussels on salmon cage culture

The conceptual open-water integrated culture system of fish farm with filter feeding bivalves suggests that the bivalves are cultured adjacent to fish cages to reduce nutrient loadings by filtering and assimilating particulate wastes from the fish farms (Chopin et al., 2008b). It was natural to choose *Mytilus edulis* as a candidate species for the IMTA practice in Norway since blue mussel is native species in the Norwegian environment and commercially viable. If the fish farm wastes (faeces and fish feed) were broken down into particulate by waves and fish movement, mussels may be suitable for absorbing those wastes.

The effect of nutrients from fish farm on the growth of mussel is locale specific. Some studies found enhanced growth (Stirling and Okumus, 1995; Gao et al., 2006; Peharda et al., 2007), while other studies (Taylor et al., 1992; Troell and Norberg, 1998; Cheshuk et al., 2003) not. The Norwegian fjords are generally regarded as oligotrophic water in terms of chlorophyll a concentration (Aure et al., 2007). Integrating mussels with salmon cage culture could be good advantage when the natural food concentration, especially phytoplankton level is suboptimal for the mussel growth after the spring bloom.

1.6. Trophic marker

The contribution of organic load from a fish cage to the extractive organisms can be evaluated by tracing trophic markers. A trophic marker is a compound whose origin can be uniquely and easily identified, that is inert and not harmful to the organisms and that is not selectively processed during food uptake and incorporation. Also, it is metabolically stable and hence transferred from one trophic level to the next in both a qualitative and quantitative manner (Dalsgaard et al., 2003).

Fatty acid trophic markers (FATM) have been widely used to trace the predator-prey relationship of the marine ecosystem. FATM is based on the observation that marine primary producers produce certain fatty acid patterns that may transferred conservatively to the next tropic level (Gao et al., 2006). The fatty acid patterns in marine ecosystem that are commenced by primary producers such as phytoplankton and macroalgae are typically composed with saturated fatty acids (14:0, 16:0, and 18:0), mono unsaturated fatty acids (16:1 (n-7), 18:1 (n-9)) and poly unsaturated fatty acids (20:5 (n-3), 22:6 (n-3)) (Napolitano et al., 1997). Meanwhile, fish feed typically contains high percentage of marine source such as herring and capelin that has high proportions of longer chain monounsaturated fatty acids, such as 20:1 (n-9) and 22:1 (n-11) (NRC, 1993). In recent years, higher levels of terrestrial sources which have high proportions of 18:1 (n-9), 18:2 (n-6), 18:3 (n-6) and low n-3/n-6 ratio (NRC, 1993) are used in the fish feed. The difference in fatty acid patterns between marine primary producers and fish feed would be conservatively transferred to the blue mussel tissues. The characteristics of fatty acid patterns of the mussel tissues can therefore be used as an indicator of assimilation of fish farm waste.

1.7. **Aim of the study**

The aim of this thesis is to assess the effects of waste from fish cages on the culture of blue mussel. This will be achieved by analyzing mussel growth, environmental variables and chemical analyses of mussel and water samples. In this study mussels were grown in different distances from a salmon farm from June 2010 to February 2011.

To be more specific, this study focuses on:

1) Study the growth of blue mussels placed in different distances from salmon cages under exposed coastal waters.

2) Analyze environmental variables and food availability at the study site.

3) Evaluate the impact of excessive nutrient from salmon cages by analyzing chemical composition (Fatty acid analysis) of the mussel tissues and mussel growth.

4) Consider the possibility of integrating blue mussels to the salmonid cage culture under open water condition in Norway

The study is a part of project INTEGRATE founded by the Research Council of Norway, NTNU and SINTEF.

2. Materials and methods

2.1. Experimental setups

The present study was carried out from June 2010 to February 2011 at Aquaculture Engineering AS (ACE) experimental fish farming facility located at Tristein, Bjugn in Sør Trøndelag, Central Norway (Fig. 2 and 3).

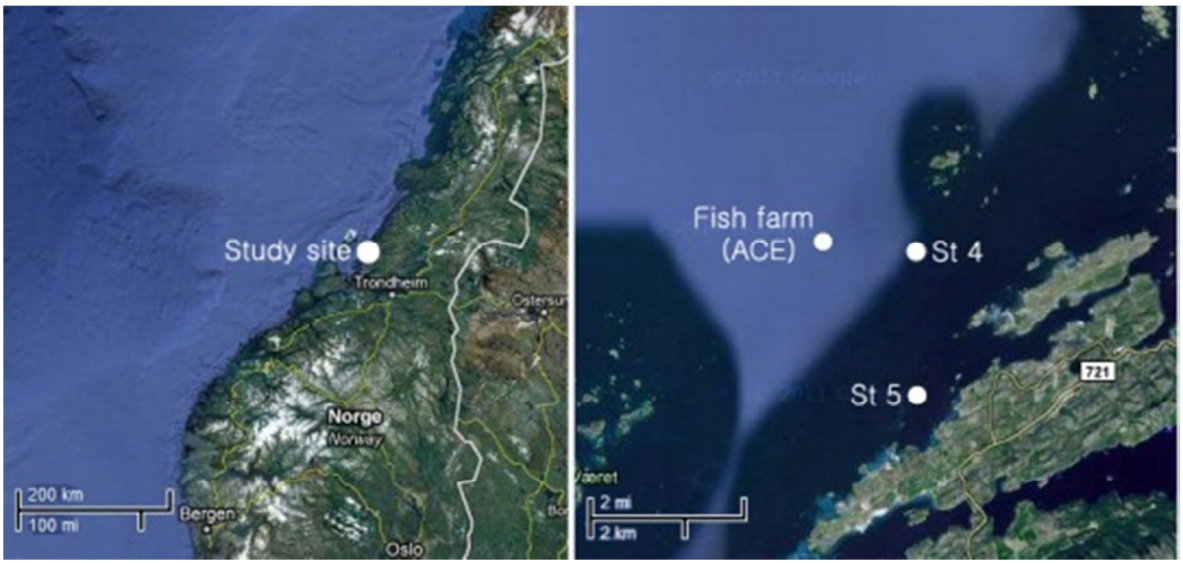

Fig.2. The location of Aquaculture engineering (left). ACE and station 4 and 5 (right).

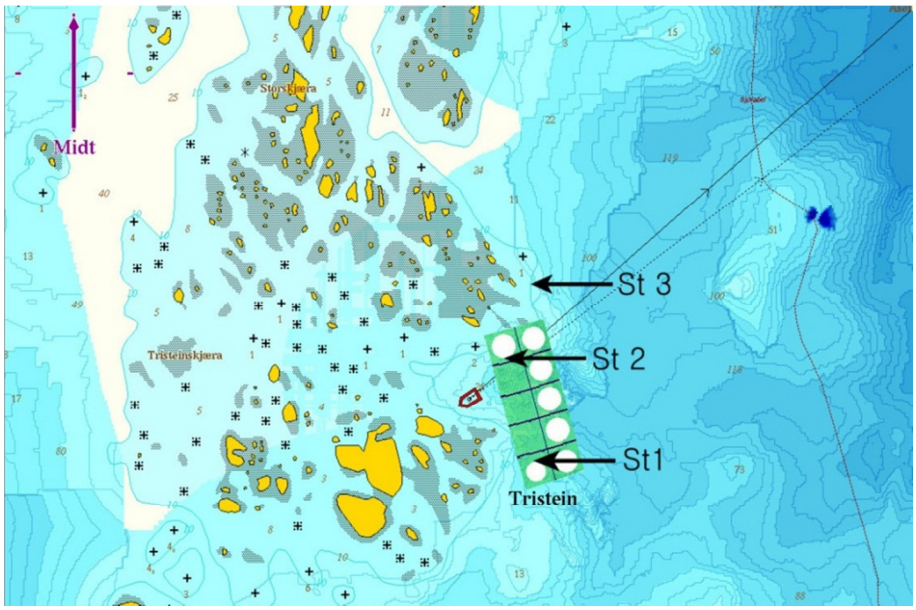

Fig.3. ACE and the location of station 1, 2 and 3, and feeding barge (red pentagon)

ACE has seven cages for salmon farming. Six cages had a diameter of 50 m and a depth of 25 m, whereas one cage at station 1 had a diameter of 30 m and a depth of 25 m. The distance between the cages was 100 m from center to center. The total biomass of the fish farm was 514 tons in June 2010 and increased to 2,810 tons in February 2011.

Five sampling stations were established around the fish farm. Three experimental stations were located at the fish farm: station 1 (63°51'0.21" N, 9°37'2.06"E) and 2 (63°32'24.50"N, 9°37'20.41"E) were located inside the fish farm and station 3 (63° 52'32.40"N, 9°37'21.60"E) was placed 200 m away from nearest cage of ACE (Fig. 3). Two reference stations were placed further away from the farm: station 4 (63°52'34.40"N. 9°37'21.60"E) was located 1.8 km from ACE, and station 5 (63°50'36.90"N, 9°39'35.20"E) 3.6 km from ACE and 500 m away from the shore (Fig. 2).

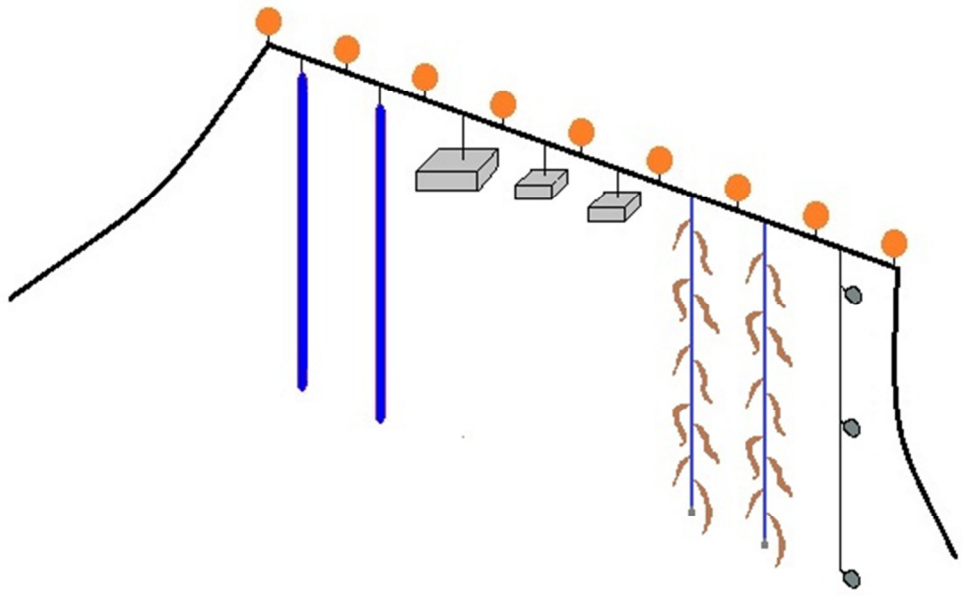

Fig. 4. The experimental setup for the field experiment at station 1 – 5. From left: two mussel collectors, one larger crab pot for scallops, two smaller crab pots for mussels, two seaweed lines and a line with temperature loggers. The crab pot for scallops and the seaweed lines were used for another experiment.

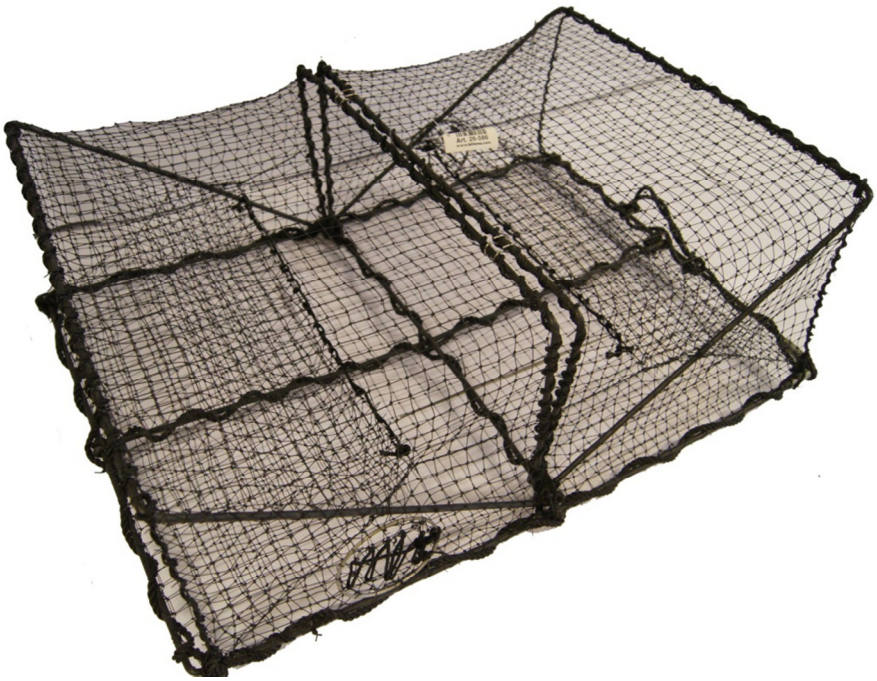

Fig. 5. A small crab pot (60 × 40 × 20 cm) used for storage of mussels

Fig. 6. A perforated basket (15 × 15 × 10 cm) used for storage of 80 tagged mussels.

At each station, two seaweed lines, two mussel collectors (8 m long), one bigger crab pot (85 × 55 × 25 cm) for scallops and two smaller crab pots (60 × 40 × 20 cm, Fig. 5) for mussels at 2 m depth were installed (Fig. 4). One of the small crab pots were filled with about 1000 individuals of mussels for condition index (CI) analysis. A perforated basket (15 × 15 × 10 cm, Fig. 6) with 80 tagged mussels for growth measurement was kept in the other small crab pot. The mussels in this experiment were provided from Åfjord Skjell AS at 25th May 2010. The seaweed and scallop data are part of another project and not used in this study.

2.2. **Water sampling procedure**

Water samples were collected for various analyses with a 2 m long water collector (4 L). During the first three months (June - August) water samples were collected from three different depths (2, 5 and 8 m). From the September sampling, integrated water samples were collected from 2 to 8 m depth. The water samples were pre-filtrated with 200 μm plankton net to remove larger particles. The filtration was carried out at the feeding barge of ACE, except the June and July samplings which took place at Sealab.

2.2.1. **Total particulate matter**

Water sample (2 L) was agitated until the sample became homogeneous and then filtrated onto pre-weighed glass microfiber filters (Whatman GF/C, 47 mm). The filters were brought back to Sealab and dried in the Termaks convection oven type TS4115 (Termaks AS) at 80 °C for 24 hours before weighing. The filters were weighed using a METTLER TOLEDO, model XA204.

2.2.2. **Chlorophyll a**

Another 2 L of water sample was filtrated for chlorophyll a, particulate organic carbon (POC) and particulate organic nitrogen (PON) analysis. The water samples were filtered on glass microfiber filters (Whatman GF/C, 47 mm) which were burned at 450 °C for 3 hours before the filtration. The filters were stored in a – 80 °C freezer (YIT Building Systems AS,

ULT freezer) at Sealab until analysis.

Chlorophyll a was analyzed with methanol as solvent (Holm-Hansen and Remann, 1978). Two subsamples with a diameter of 9 mm were taken from the GF/C filter and then put into methanol in 10 ml tubes. The samples were stored at 4 °C in a refrigerator for one hour for extracting chlorophyll a from the filters. The tubes were agitated well until the content became homogeneous and the fluorescence was measured with a Turner Designs fluorometer model 10. The amount of chlorophyll a was calculated as:

$$\mu g \text{ chlorophyll a } L^{-1} = (Avl \times F \times E \times 1000 \times K) / Fs \times S \times V$$

Avl: value of fluorescence scale

F: calibration factor, currently 0.29

E: extraction volume in ml (10 ml)

Fs: splitting factor (1.00, 3.16, 10.0 or 31.6)

S: sensitivity (1 or 100)

V: filtered volume in ml

K: whole filter area / subsample area (K=16)

2.2.3. **Particulate organic carbon and particulate organic nitrogen**

Two other subsamples of 9 mm diameter from the GF/C filter were put into pre-burnt tubes (450 °C for 3 hours). The subsamples were placed in fumes of hydrogen chloride (HCl, 38 %) for 20 minutes to remove any inorganic carbon and left for 20 minutes in an atmosphere for acid evaporation. The subsamples were kept at -20 °C freezer until analysis. POC and PON analyses were performed using Carlo-Erba model 1106 CHN analyzer. All analysis of POC and PON contents were done by Marte Schei at SINTEF Fisheries and Aquaculture.

2.2.4. **Other environmental measurement**

Conductivity and temperature from surface to 8 m were measured using a CTD model SD204 (SAVIS A/S). Later the conductivity was converted into salinity with included software (Misnisoft SD200W). Temperature loggers were placed at 2 and 5 m depth at station

1 and 5. Station 3 had temperature loggers at 2, 5 and 8 m depth. The loggers recorded temperature every 30 minutes from 22nd July 2010 to 12th January 2011. Secchi depth of the stations at the sampling days was measured using a 30 cm diameter, white secchi disk attached to a rope marked at 1 m intervals.

2.3. **Mussel sampling**

Mussel samplings were performed from June 2010 to February 2011. During the period, SGR and CIs were measured every month. For SGR analysis 80 individuals 31.5±1.5 mm in shell length were selected and labeled with laminated vinyl tags (http://www.floytag.com/) at each station. The tagged mussels were kept in perforated basket (15 × 15 × 10 cm, Fig. 6), and put into one of the crab pots (60 × 40 × 20 cm, Fig. 5). For CI and fatty acid analysis mussels were collected from the other crab pot, which contained about 1000 individuals.

Initial shell length and CI measurements in June were performed at Sealab. CI analysis of station 2 was available from September. At the August sampling, growth measurement at station 1 was delayed to 2nd September because of lack of time. The perforated box with tagged mussels at station 2 was lost after the measurement at 19th August, and replaced with 80 new mussels at 2nd September. From November, station 4 was broken and lost that no measurement could be performed. Shell length measurements for SGR in December could not be performed because of strong wind. Mussel samples for fatty acid analysis were collected at June, August, and February samplings.

Table 1: Mussel sampling for estimation of specific growth rate, condition indices and fatty acid analysis

Date	Mussel sampling				
(days)	station 1	station 2	station 3	station 4	station 5
03-Jun (0)	CI, SGR, FA	CI, FA	CI, SGR, FA	CI, SGR, FA	CI, SGR, FA
22-Jul (47)	CI, SGR	SGR	CI, SGR	CI, SGR	CI, SGR
19-Aug (76)	CI, FA	SGR	CI, SGR, FA	CI, SGR, FA	CI, SGR, FA
02-Sep (90)	SGR	SGR	-	-	-
16-Sep (104)	CI, SGR	CI, SGR	CI, SGR	CI, SGR	CI, SGR
20-Oct (138)	CI, SGR	CI, SGR	CI, SGR	CI, SGR	CI, SGR
16-Nov (167)	CI, SGR	CI, SGR	CI, SGR	-	CI, SGR
14-Dec (193)	CI	CI	CI	-	CI
11-Jan (223)	CI, SGR	CI, SGR	CI, SGR	-	CI, SGR
21-Feb (251)	CI, SGR, FA	CI, SGR, FA	CI, SGR, FA	-	CI, SGR, FA

CI, condition indices; SGR, specific growth rate; FA, fatty acid analysis

2.3.1. **Specific growth rate of shell length**

Shell lengths of 80 tagged mussels from the perforated basket (Fig. 6) were measured every month with a vernier calipers at 0.1 mm level. SGR was determined according to Chatterji et al. (1984):

$$SGR\ (\%) = ((\mathrm{Ln}\ L_2 - \mathrm{Ln}\ L_1) \,/\, (T_2 - T_1)) \times 100$$

where L_1 and L_2 are individual shell lengths at the time T_1 and T_2.

2.3.2. **Condition indices**

Every month, 30 mussels of similar size from each station were collected and then kept one night in a cold room (4 °C) at Sealab before the analysis. After the shell length measurements, the mussels were opened and separated into tissues and shells. The tissues were dried in a Termaks convection oven type TS4115 (Termaks AS) at 80 °C for 24 hours while shells were air-dried. The dried tissue and shell weight were measured using a METTLER TOLEDO, model XA204. The two different CI analysis methods were applied according to Freeman (1974) and Davenport and Chen (1987).

$$CI = \text{Tissue dry weight (DW)} / \text{shell dry weight (SW)} \times 100$$

$$CI = \text{Tissue dry weight} / \text{shell Length (SL)} \times 1000$$

2.4. **Lipid and fatty acid analysis**

Mussel samples were dissected and separated into digestive gland and mantle tissue for total lipid and fatty acid analysis. Fish feed samples were collected from ACE feeding barge at 21st February. The samples were kept in cryo tubes (2 ml) with N_2 gas and stored in a –80 °C freezer (YIT Building Systems AS, ULT freezer).

Lipids were extracted using the modified method of Bligh and Dyer (1959). Mussel and feed samples were freeze-dried and then extracted with Chloroform ($CHCl_3$), methanol (CH_3OH) and distilled water (H_2O). The chloroform and distilled water were added in sequence. A known amount of chloroform was transferred into a pre-weighted test tube and dried under N_2 atmosphere at 40 °C. Total lipid was determined gravimetrically (mg/g dry weight).

Fatty acid methyl esters (FAMEs) were determined quantitatively to establish the fatty acid composition by standard method of capillary gas chromatography(Metcalfe et al., 1966). All analysis of total lipid and lipid contents were done by Kjersti Rennan Dahl, Department of Biology, NTNU.

2.5. **Statistical analysis**

Statistical analysis was carried out by SPSS (version 18.0) except principal component analysis (PCA), which was performed with The Scrambler X (Version 10.1). For CIs and SGR, one way ANOVA (analysis of variance), and Fisher's LSD (least significant difference) method for post hoc multiple comparisons were used. Multiple regression analysis were used to find relations of environmental variables to CIs and SGR. In the analysis, environmental variables selected by stepwise method were put together with CIs or SGR. Correlation analysis was used to look into coefficient between environmental variables. TPM and chlorophyll a contents were tested with a nonparametric test to compare the environmental

condition of the stations. Since nonparametric test does not support post hoc test, two independent samples were compared with Mann-Whiteny U test method one by one. The boxplots were made with SPSS and other graphs were made with sigmaplot (version 10.0).

If necessary, figures were presented with mean values ± standard error. The level of significance was given as $p < 0.05$ for all analysis.

3. Results

3.1. Environmental conditions

3.1.1. Temperature

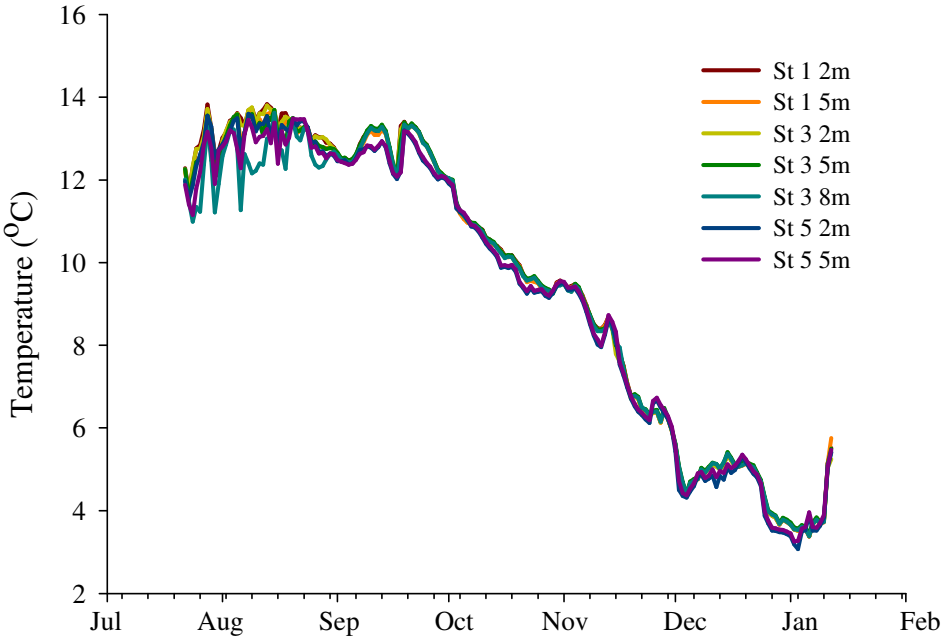

Fig. 7. Water temperature from 2 m to 8 m depth at the study site during the experiment

The water temperature at the stations during the experiment showed a homogeneous temperature distribution (Fig. 7). The highest temperature, 14.3 °C was recorded in July 28th, and the temperature remained close to that level (> 11 °C) until late September. Thereafter the water temperature declined until early January. The lowest temperature, 3.1 °C was recorded in 3rd January.

3.1.2. **Salinity**

The salinity was about 28 ‰ in June and dropped to 22 ‰ at the July sampling (Fig. 8). The salinity increased to about 27 ‰ in August. From October to February the salinity varied between 32 to 33 ‰.

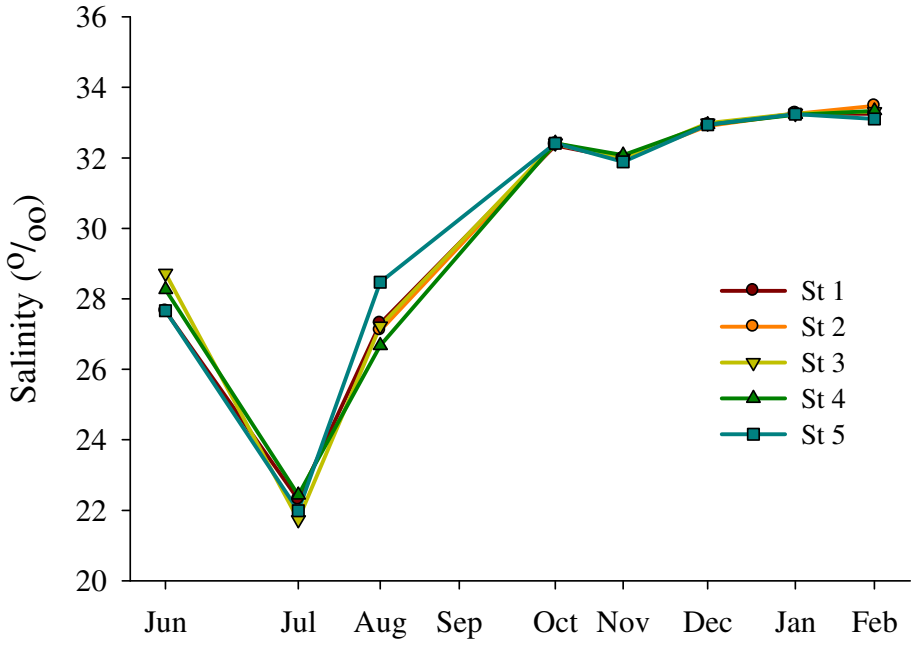

Fig. 8. Salinity (‰) at 2 m depth at the stations during the experiment.

3.1.3. **Total particulate matter**

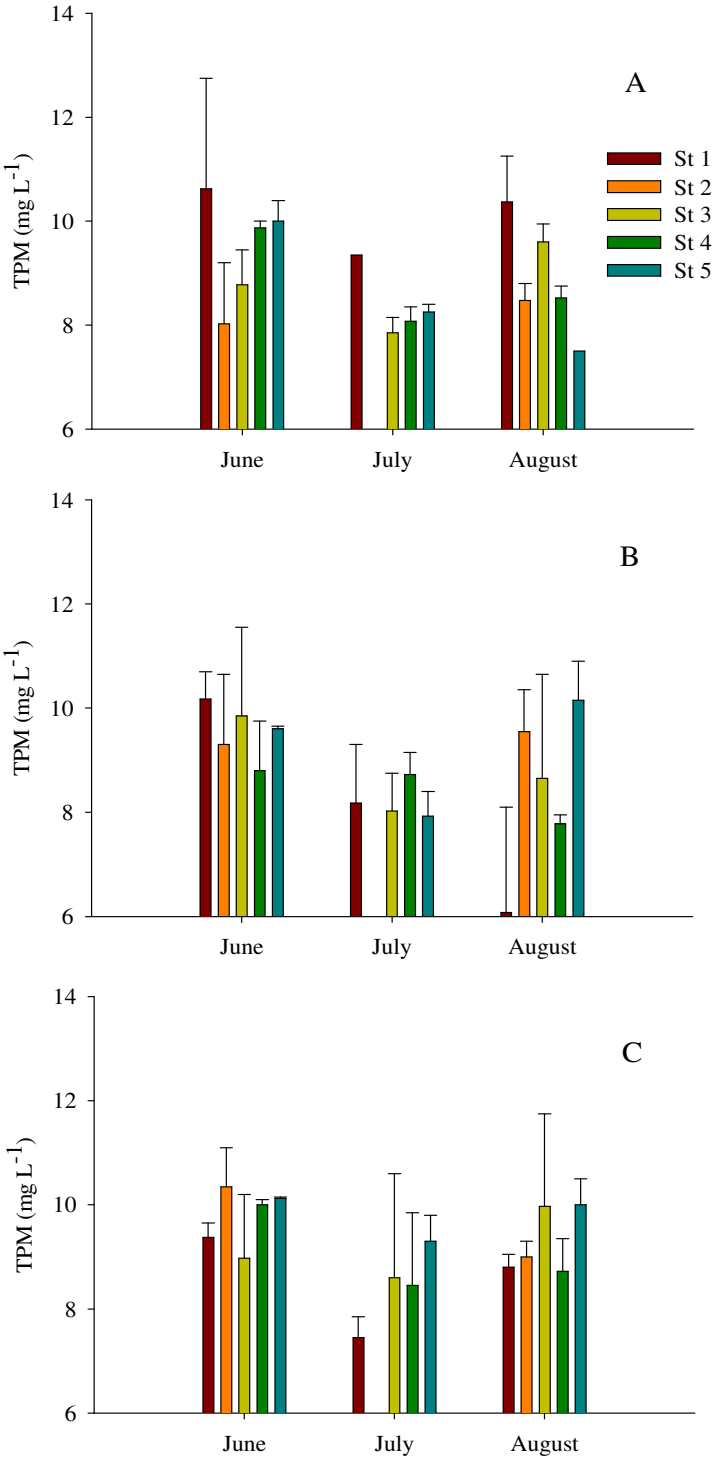

Fig. 9. Total particulate matter (mg L^{-1}) at 2 (A), 5 (B) and 8 m depth (C) at the stations from June to August 2010.

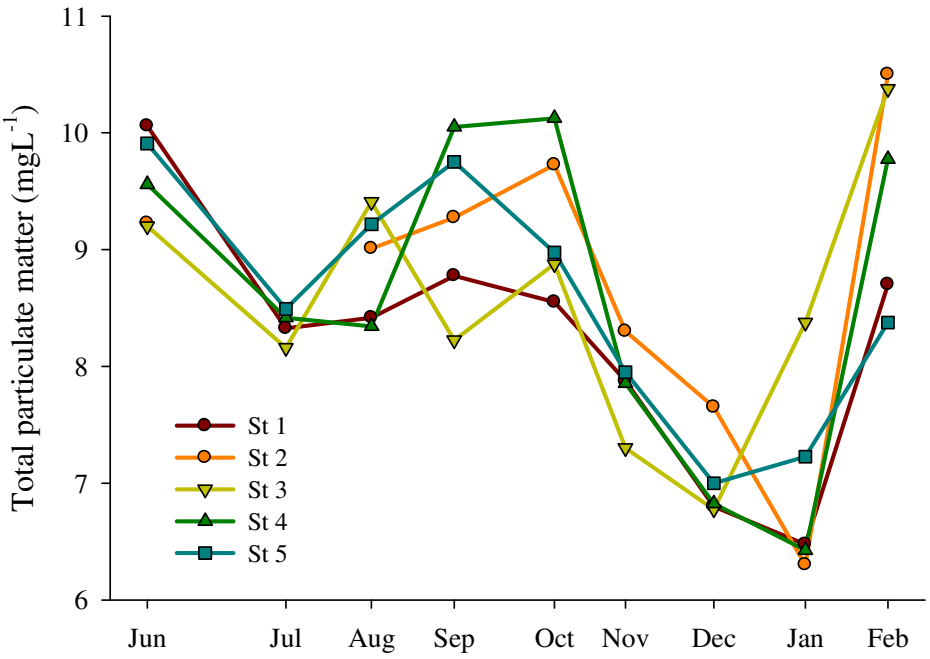

Fig. 10. Total particulate matter (mg L^{-1}) at the stations during the experiment. From September water samples were collected as integrated sampled from 2 – 8 m depth, whereas the first 3 months total particulate matter from 2, 5 and 8 m depth were averaged.

Total particulate matter (TPM) measurement at 2, 5 and 8 m depth showed no significant differences between the depths at the stations (Fig. 9). TPM at the 5 stations ranged from 10.50 mg L^{-1} (February, station 2) to 6.30 mg L^{-1} (January, station 2) (Fig. 10). TPM decreased at all stations in July but from August to October TPM showed relatively high values compare to the other months. Then TPM decreased until January and then increased again in February.

3.1.4. **Biomass of fish stock in ACE and amount of feed supplied**

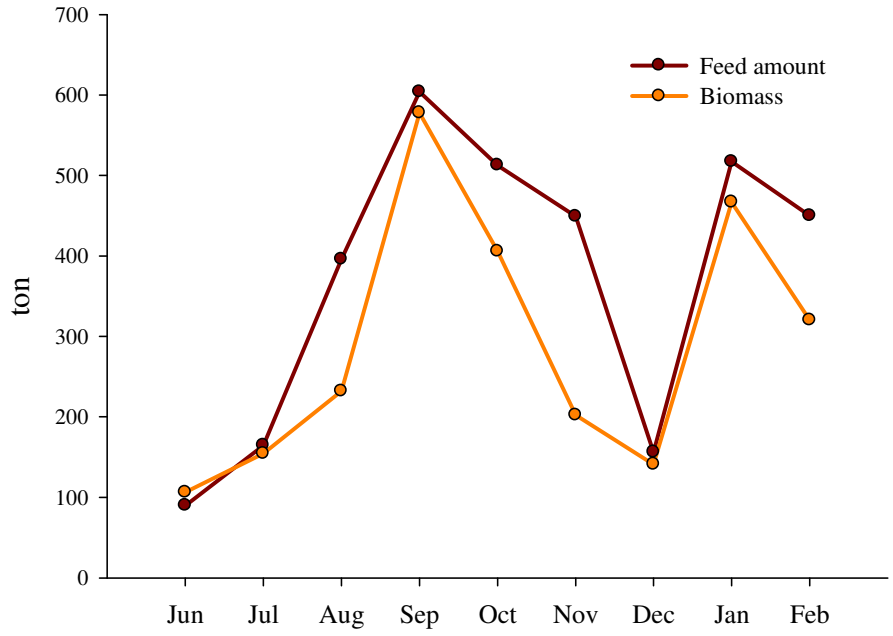

Fig. 11. Biomass of fish stock in ACE and amount of feed supplied from June 2010 to February 2011.

The fish biomass and amount of supplied feed from ACE data were provided from ACE (http://www.aceaquaculture.com/). The biomass at ACE was at its minimum in June, 106 tons, and reached 578 tons in September (Fig. 11). The biomass decreased until December, 141 tons before increasing to 450 tons in January. The amount of supplied feed followed the fish biomass curve, with a minimum addition of 90 tons in June and a maximum of 603 tons in September. Feed was supplied 5 to 6 times a day.

3.1.5. **Chlorophyll a**

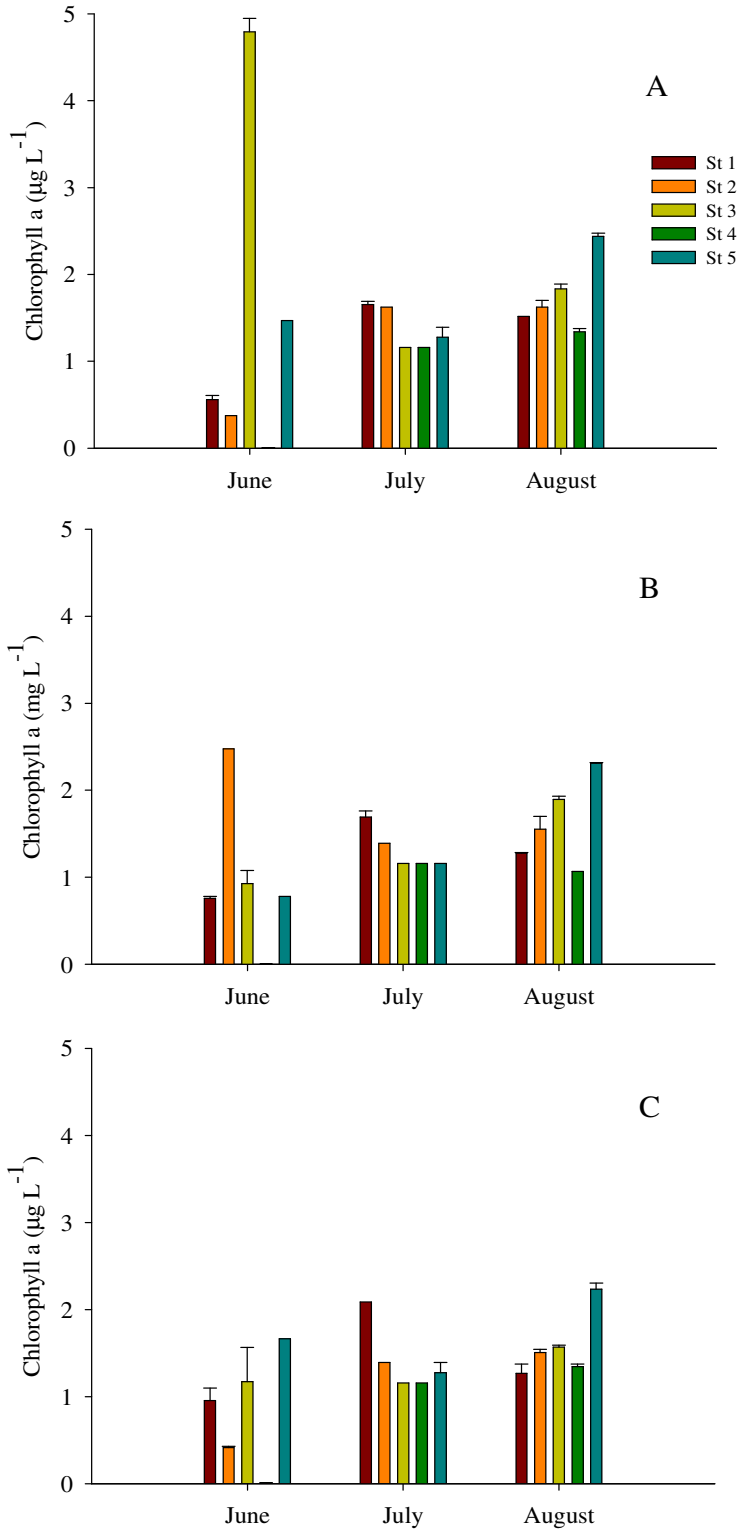

Fig. 12. Chlorophyll a values (µg L^{-1}) at 2 (A), 5 (B) and 8 m depth (C) at the stations from June to August 2010.

The chlorophyll a content at 2, 5 and 8 m depth showed significant variations by the depth and the stations in June, whereas in July and August no significant differences were recorded (Fig. 12).

The mean chlorophyll a values from 2 to 8 m depth were not significantly differed among the stations (Fig. 13). Chlorophyll a content had highest values between 1.3 to 2.5 µg L^{-1} in the summer season (July – August) and the lowest values were between 0.03 to 0.1 µg L^{-1} in the winter season (December – January). The chlorophyll a content started to increase again in February (Fig. 13).

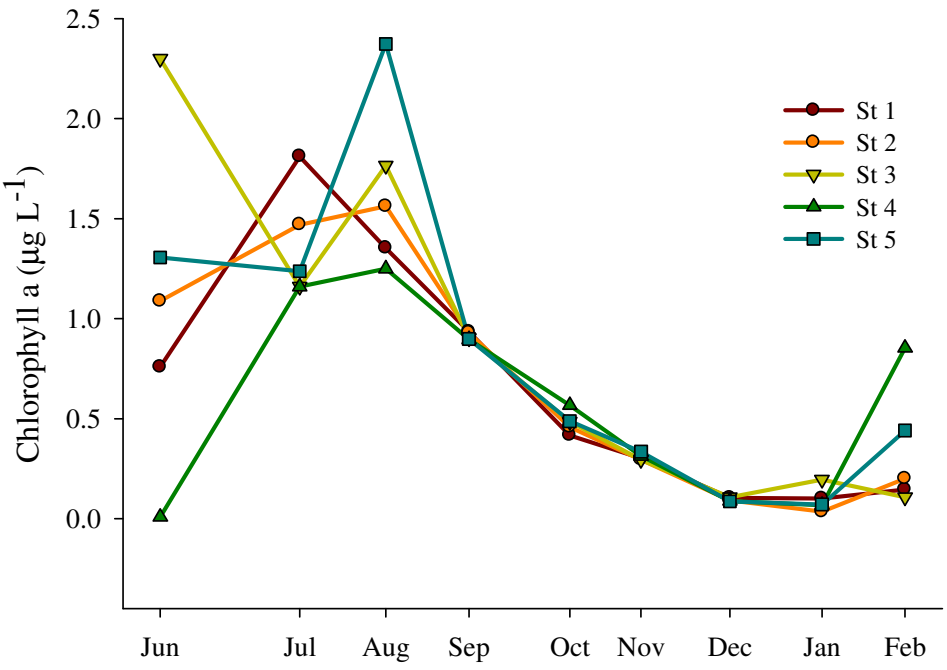

Fig. 13. Mean chlorophyll a values (2 - 8 m, µg L^{-1}) at the stations during the experimental period.

3.1.6. **Secchi depth**

Secchi depth at the stations during the experiment showed a homogeneous pattern (Table 2). Secchi depth was shallowest in July, 5 - 6 m depth and deepest in January, 16 - 18 m. Secchi depth showed an inverse correlation with chlorophyll a (r = - 0.49) and TPM (r = - 0.243) contents.

Table 2. Secchi depth (m) of the study site during the experimental period

Month	stations				
	1	2	3	4	5
June	13	13	14	13	10
July	5.5	5	6	6	5.5
August	8	8	8.5	8	7
September	10	10	12	10	12
October	11	11.5	12	11	10
November	12.5	11.5	12	13	12.5
December	13	13	13	12.5	12
January	17	17	18	18	16
February	12.5	11.5	12	13	12.5

3.1.7. **Particulate organic carbon and nitrogen**

The amount of particulate organic carbon (POC) and particulate organic nitrogen (PON) at the stations, μgC L^{-1} and μgN L^{-1}, are shown in Fig. 14A and B. The mean POC and PON levels during the experimental period were 236 and 34 μg L^{-1}, respectively, and no significant differences were recorded among the stations. Except for a lowered POC and PON contents in July, the recorded levels showed a nearly continuous decline during the experimental period (Fig. 14A and B). POC values decreased from 469 to 139 μg L^{-1} and PON decreased from 61 to 22 μg L^{-1}. POC/PON (C/N) ratio fluctuated showing the highest ratio in November (8.49) and the lowest in January (5.87) (Fig. 14C).

Significant positive correlation coefficient (r) was found between chlorophyll a to POC (r = 0.57) and to PON (r = 0.59).

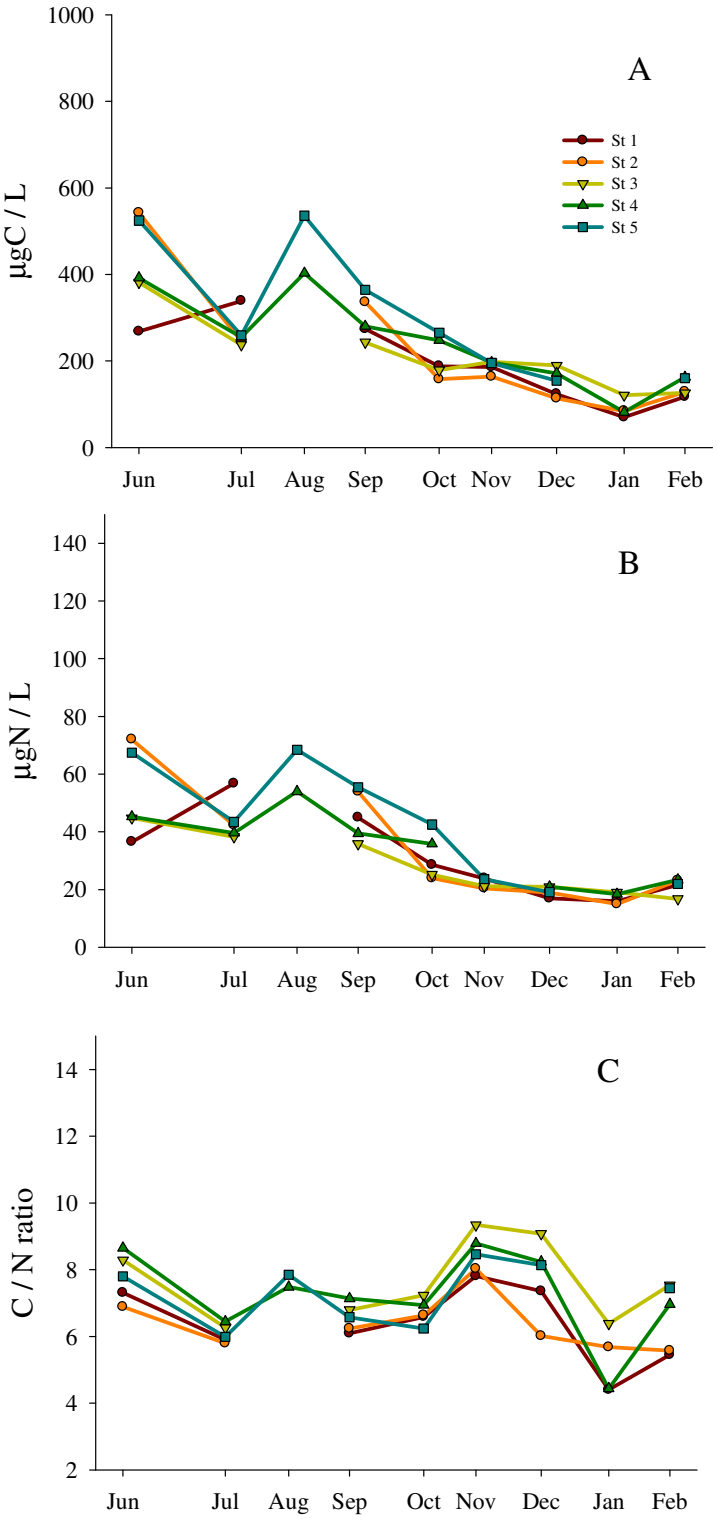

Fig. 14. Particulate organic carbon (A) and nitrogen (B) values and ratio of particulate organic carbon to nitrogen (C) in the seston during the experimental period.

3.1.8. **Water currents**

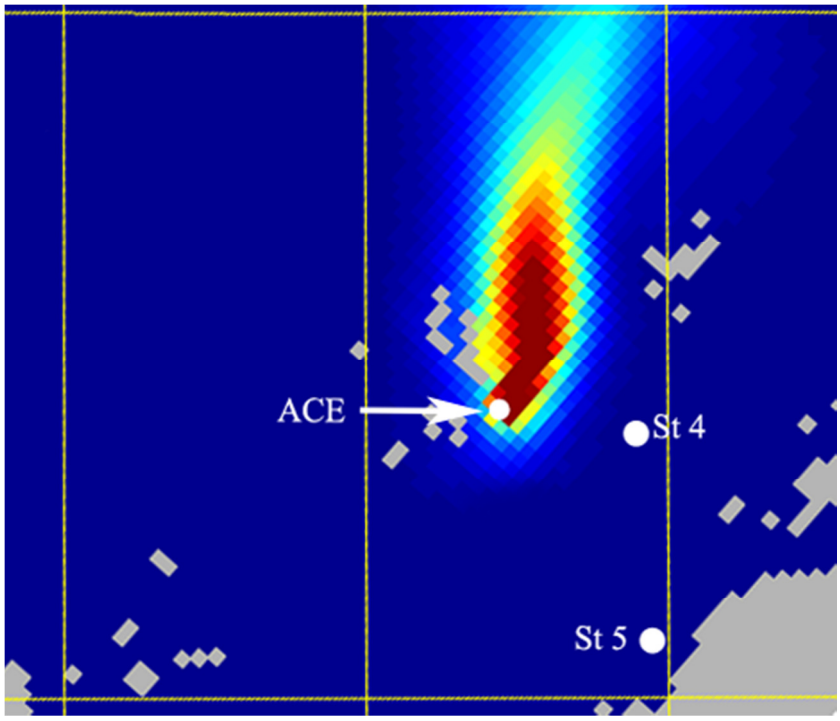

Fig. 15. Nutrient (nitrogen) emission from ACE by passive transport from 24th April to 18th May, 2008. Red color indicates high nitrogen loadings. Upside is north.

Passive nutrient emission model of ACE, made by Ole Jacob Broch, SINTEF (Fig. 15) suggests that the average nutrient discharges from 24th April to 18th May, 2008 were directed mainly to the north (Broch, 2010). The report predicted that the discharge direction can be different by seasons, but the main emission direction would not change over seasons. According to the model, station 4 and 5 were not influenced by fish farm discharges from ACE.

3.2. **Mussel growth**

3.2.1. **Specific growth rate of shell length**

A high survival rate was recorded during the experiment at all stations (Table 3). Loss of mussels happened during the handling and measurement. SGR analysis of station 4 was not available from November.

Table 3. Mortality and missing numbers of mussels used for SGR analysis during the experiment.

Station	Initial number	Mortality	Lost
1	80	1	3
2	80	0	12
3	79	0	3
4	80	1	3
5	80	3	0

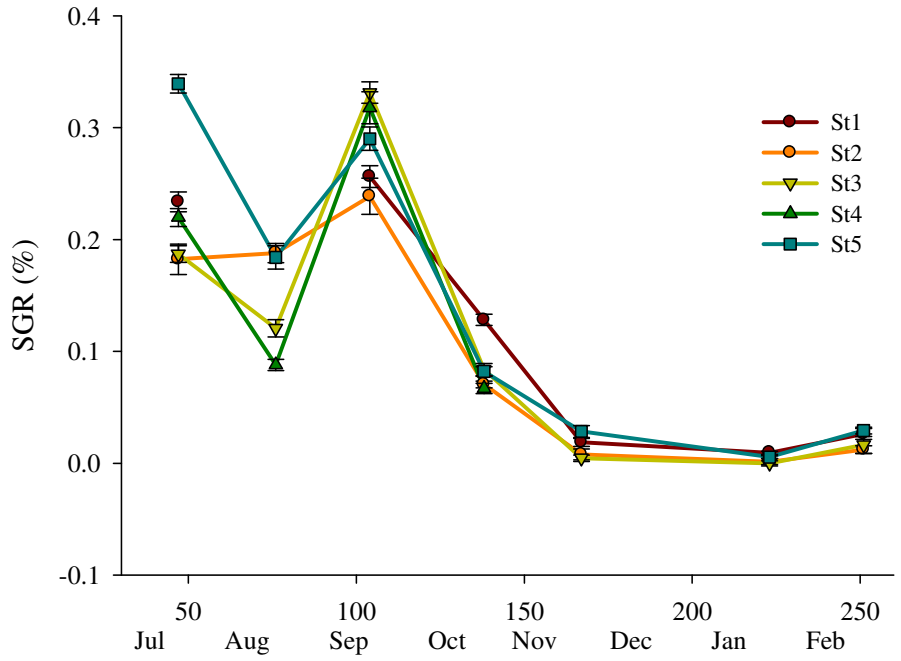

Fig. 16. Specific growth rate (% day^{-1}) of mussels (n = 80) in length during the experiment.

The SGR (% day^{-1}) in mussel shell length was 0.23 % in average during the summer season (July – September) (Fig. 16). The highest levels, 0.23 to 0.34 %, were recorded in September and declined to near zero from November until January. In August, SGR decreased at all stations, probably due to spawning. Monthly comparisons of SGR in mussel shell length among the stations are attached in Appendix 1.

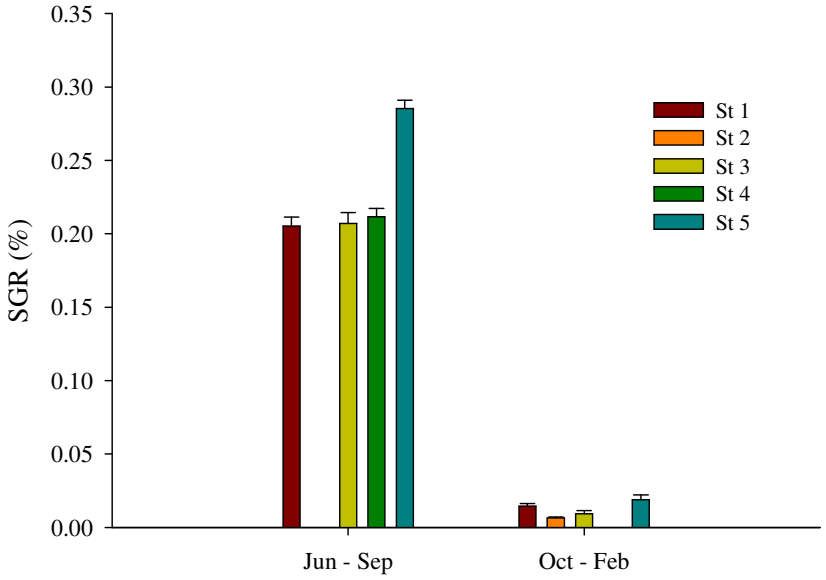

Fig. 17. Specific growth rate (% day^{-1}) of mussels (n = 80) in shell length June to September and October to February.

The SGR of mussels in shell length at station 5 showed a significantly higher growth rate (0.28 %) than at the other stations during June to September (Fig. 17). There were no significant differences among station 1, 3 and 4 during the period. From October to February, station 5 showed highest SGR, which was significantly higher than station 2 and 3.

3.2.2. **Condition index (tissue dry weight / shell weight)**

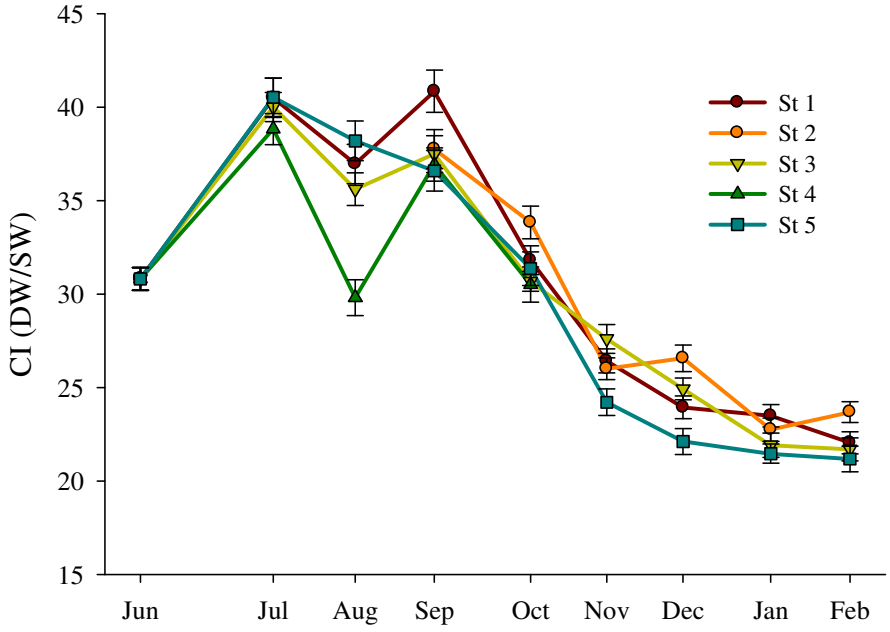

Fig. 18. Condition index (tissue dry weight / shell weight) of mussels (n = 30) at the stations during the experiment.

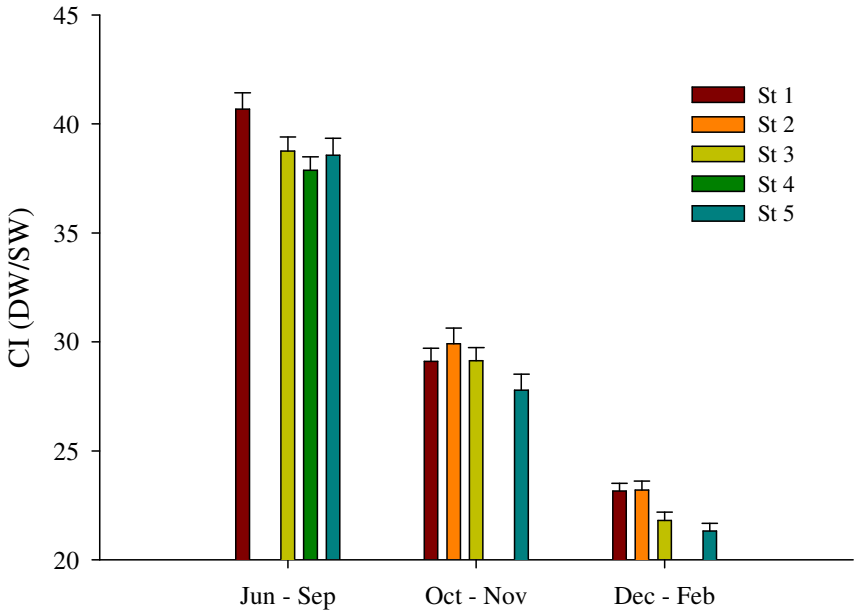

Fig. 19. Condition index (tissue dry weight / shell weight) of mussels (n = 30).

At the start of the experiment in June the CI was 30.8 (Fig. 18). The high CI (39.0) was maintained during the summer season (July – September). The CI started to decrease from October (30.5 - 33.8), and reached to lowest values from December (21.1 – 26.6). In August, all stations showed a decrease in CI, probably due to spawning. Monthly CI comparisons among the stations are attached in Appendix 2 and 3.

In the summer season (July – September), station 1 showed the highest CI value, significantly higher than station 4 and 5 (Fig. 19). No significant differences were found among station 3, 4 and 5. From October to November, the CI showed a transitional period into the winter season. During that period, station 1, 2 and 3 showed a significantly higher values than station 5. No significant differences were found among station 1, 2 and 3. During December to February, station 1, 2 and 3 had significantly higher CI values than station 5. At Station 2, the CI value was significantly higher than at the other stations.

3.2.3. **Condition index (tissue dry weight / shell length)**

The CI was as low as 6.5 in June (Fig. 20) and increased until September to 19.1 in average (Fig. 20). From November, all stations showed a decrease in the CI values (13.6 – 16.1) and reached the lowest CI in February (12.0 – 14.4).

During summer (July – August), station 1 showed a significantly higher CI than the other stations (Fig. 21). There were no significant differences among station 3, 4, and 5. From September to October, the CI values at station 1, 2, and 3 were significantly higher than at station 4 and 5. There were no statistical differences among station 1, 2, and 3. During winter (November – February), the CI was significantly higher at station 2 than at the other stations. Station 1 and 3 had a significantly higher CI values than station 5 but no difference was found between the two former stations.

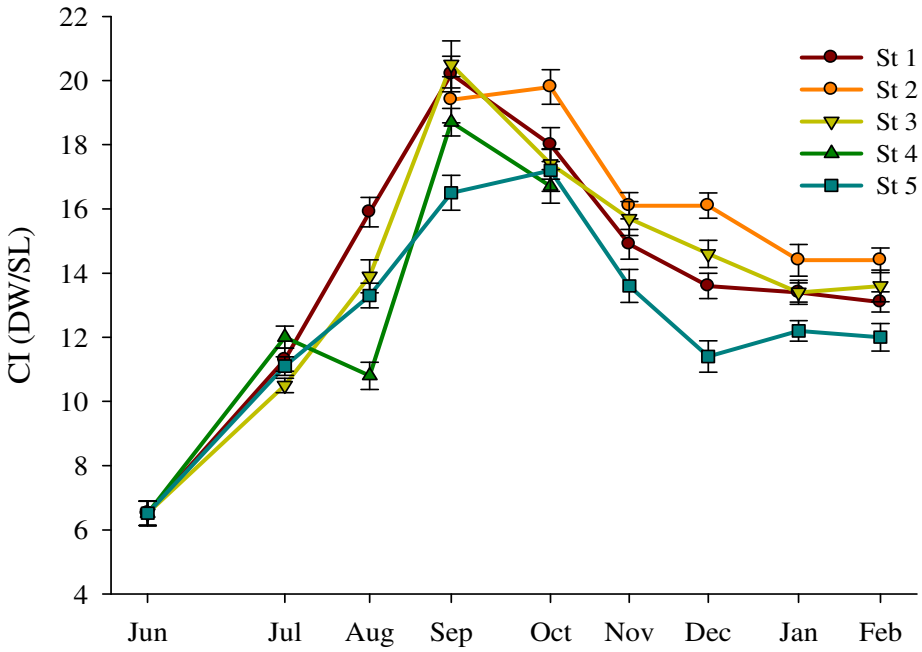

Fig. 20. Condition index (tissue dry weight / shell length) of mussels (n = 30) at the stations during the experiment.

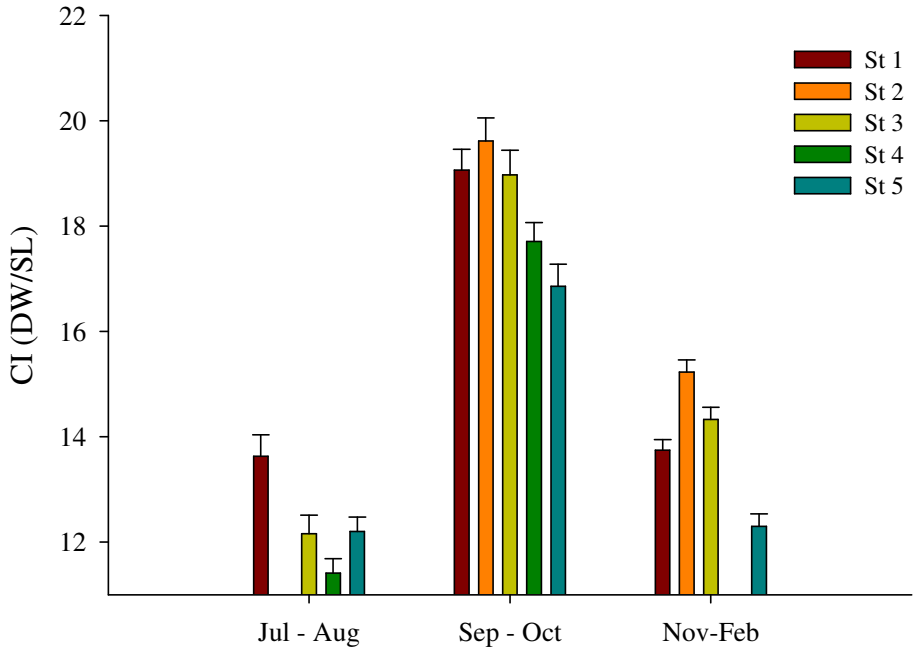

Fig. 21. Condition index (tissue dry weight / shell length) of mussels (n = 30) .

3.3. **Effect of environmental variables on growth and CIs of mussels**

Multiple regression analysis revealed that environmental variables were well related to shell length SGR (R^2 = 0.639) and CI (DW/SW) (R^2 = 0.618) (Table 3). However CI (DW/SL) was not strongly related to environmental variables (R^2 = 0.406). Water temperature was the main factor affecting mussel growth and the temperature alone affected CI (DW/SW) R^2 = 0.605, and SGR R^2 = 0.515. However relation with the CI (DW/SL) was R^2 = 0.040 which was low compare to other indicators. Spawning in August made regression analysis less significant. Exclusion of August sampling on the statistic table made R square values higher (SGR: R^2 = 0.702, CI (DW/SW): R^2 = 0.655 and CI (DW/SL): R^2 = 0.456).

Table 3. Adjusted R square levels by multiple regression analysis during experimental period

	SGR	CI (DW/SW)	CI (DW/SL)
Water Temperature	0.515	0.605	0.04
Food sources[a]	0.124	0.013	0.356
Sum of R^2	0.639	0.618	0.406

[a] includes fish feed, TPM and chlorophyll a

3.4. **Analysis of fatty acid as a tracer**

The fatty acid profiles of mussel digestive gland and mantle tissue and feed sample are attached in Appendix 4 (mussel samples, n=3, replicated; feed samples, n=3). The major fatty acids found in the mussel tissues were saturated fatty acids (SFAs) 14:0, 16:0, monounsaturated fatty acids (MUFAs) 16:1 (n-7), 18:1 (n-9) and poly unsaturated fatty acids (PUFAs) 20:5 (n-3), 22:6 (n-3). The major fatty acid found in the fish feed (pellets) was SFAs 14:0, 16:0, MUFA 18:1 (n-9) and PUFA 18:2 (n-6). The pellets showed a fatty acid composition with high MUFA 18:1 (n-9) and PUFA 18:2 (n-6) compare to mussel tissues.

The ratio of n-3 to n-6 PUFA of digestive gland tissue in June was 15.0 and the ratio of pellets was 1.3 (Fig. 22A). The n-3/n-6 ratio at station 1 in August showed lowest ratio among the stations and significantly lower than at station 5. There was no statistical difference among stations 3, 4 and 5 in August. In February the n-3/n-6 ratio at station 1 was

significantly lower than at other stations. Station 2 and 3 showed a significantly lower ratio than station 5. No significant difference was found between station 2 and 3 in February.

The n-3 to n-6 ratio PUFA of mantle tissue did not show certain patterns as shown in the digestive gland tissue (Fig. 22B). In August, the n-3/n-6 ratio was significantly higher at station 1 than at other stations meanwhile in February station 5 was significantly higher at the other stations.

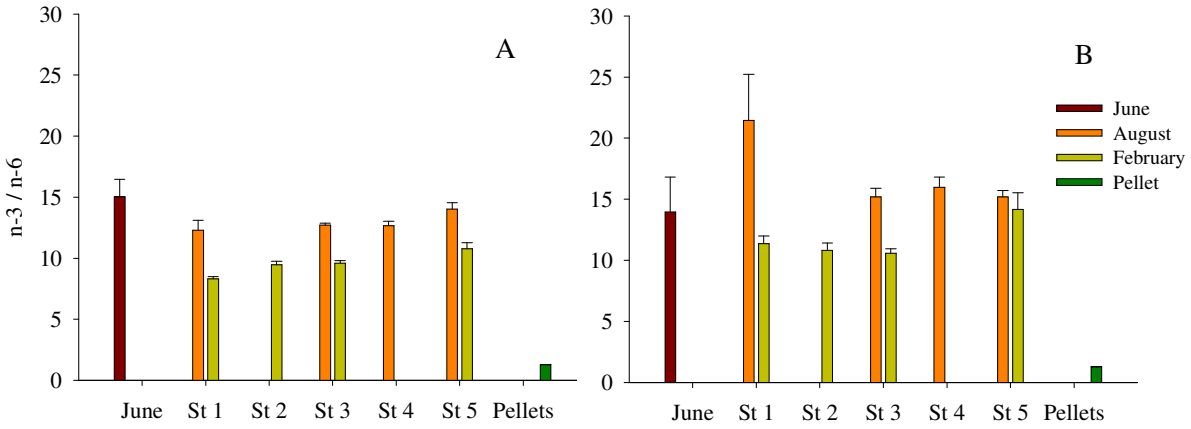

Fig. 22. The ratio of n-3 to n-6 of digestive gland tissue (A) and mantle tissue (B) of mussels in June, August and February at station 1 – 5, and pellets (n=3).

The relative contents of the fatty acids, 18:1 (n-9), 20:5 (n-3) and 18:2 (n-6) of are shown in Fig. 23. In February the percentage of 18:1 (n-9) in digestive gland tissue at station 1, 2 and 3 was significantly higher than at station 5. No significant differences were found among station 1, 2 and 3 (Fig. 23A). In August, no difference was found among stations. On the other hand, 18:1 (n-9) signature of mantle tissue had no significant differences in August (Fig. 23B). In February, station 2 and 3 showed significantly higher 18:1 (n-9) composition than at station 1 and 5.

The percentage of 20:5 (n-3) in digestive gland tissue in February was significantly higher at station 5 than the other stations (Fig. 23C). There were no significant differences among station 1, 2, and 3. In August, station 5 had highest percentage and it was significantly higher than at station 1 and 3. For mantle tissue, station 2 and 3 had significantly lower relative content of the fatty acid 20:5 (n-3) than the other stations in August and February (Fig. 23D).

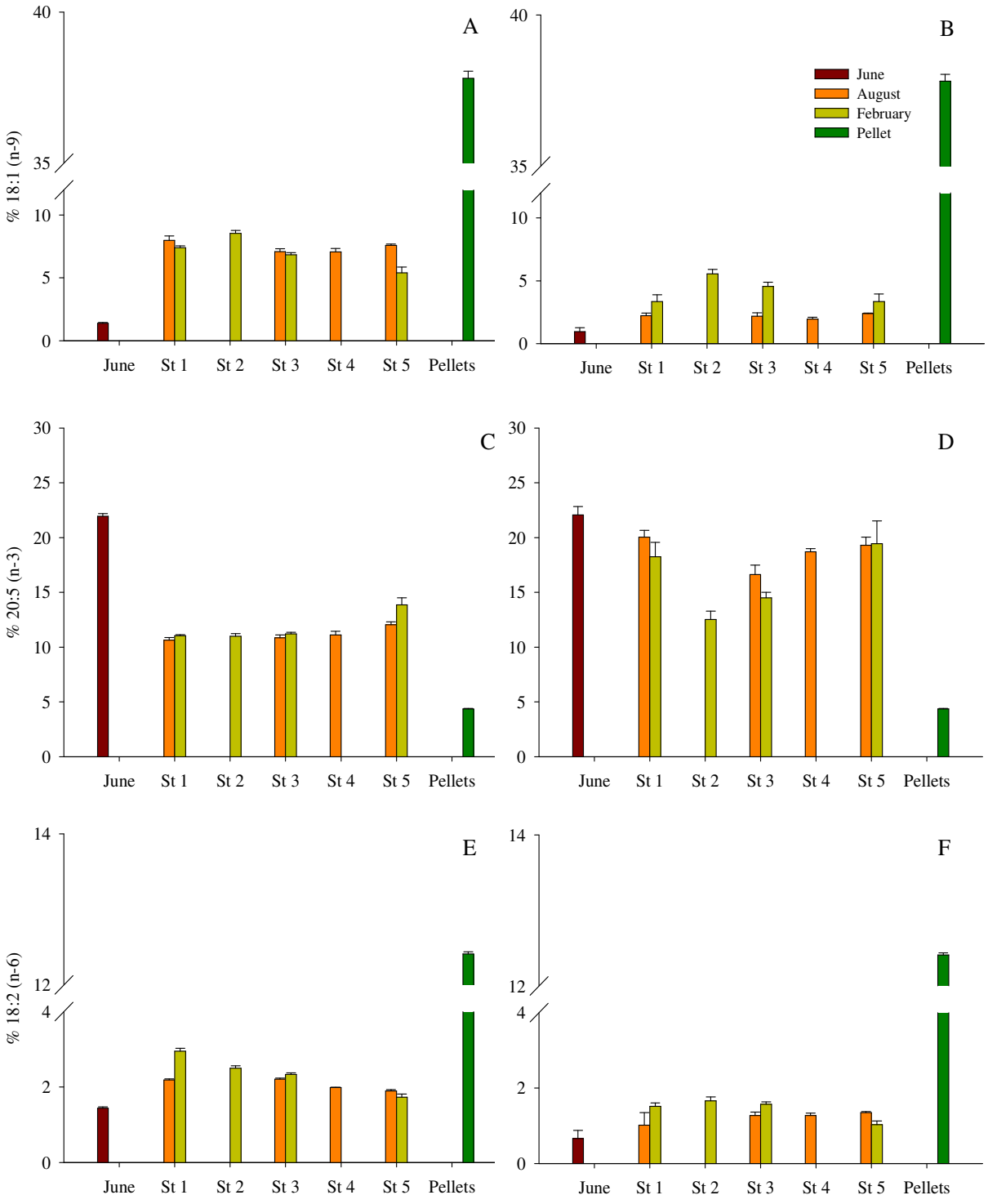

Fig. 23. Selected fatty acids composition of digestive gland tissue (A, C and E) and mantle tissue (B, D and F) of mussel in June and February at station 1 – 5, and pellets.

The fatty acid composition of 18:2 (n-6) in digestive gland tissue at station 1 in February was significantly higher than at the other stations (Fig. 23E). And station 2 and 3 showed significantly higher percentage than station 5. In August station 1 and 3 showed significantly higher 18:2 (n-6) values than station 4 and 5. No significant differences were found in 18:2 (n-6) signature of matle tissue in August (Fig. 23F). In February the composition of 18:2 (n-6) at station 1, 2 and 3 was higher than at station 5.

The PCA showed clear patterns of fatty acid profiles of digestive gland tissue in February (Fig. 24A). Since the salmon feed had substantially different fatty acid patterns, it could not be included on one plot. The distance between station 1 and 5 were mainly caused by the appearance of fatty acid 18:1 (n-9), 16:1 (n-7) and 20:5 (n-3) (Fig. 24B).

On the other hand, mantle tissue in February did not show clear separation among stations (Fig. 25).

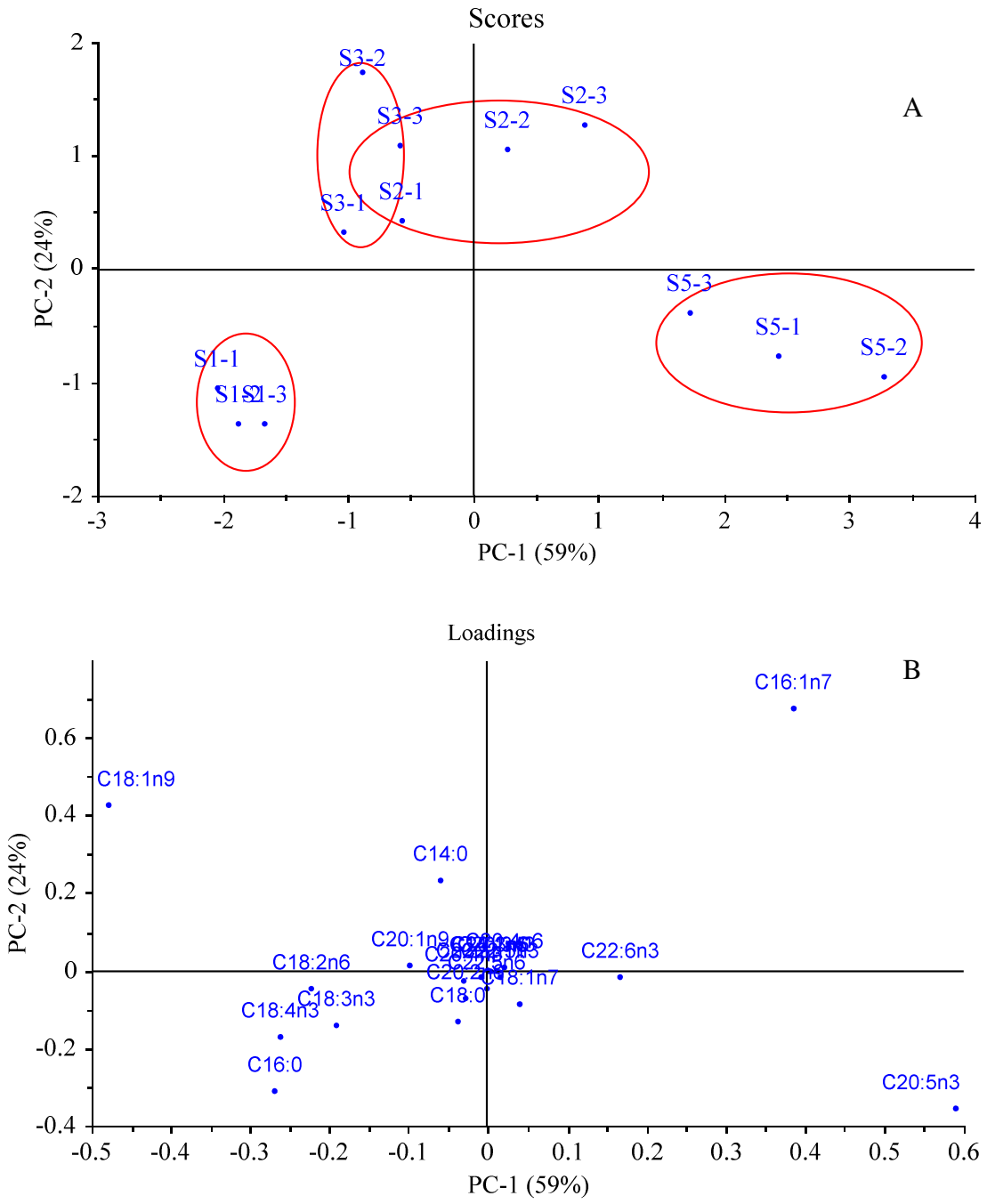

Fig. 24. Principal component analysis of fatty acid profiles in the digestive gland tissue of mussels in February. Score plot (A) showing stations and its replications (e.g. S5-3 is 3rd replication of station 5). Loading plot (B) showing the fatty acids contributing to score plot. Bracketed numbers at the axes are the percentage of variance of fatty acid explained by principal component 1 and 2.

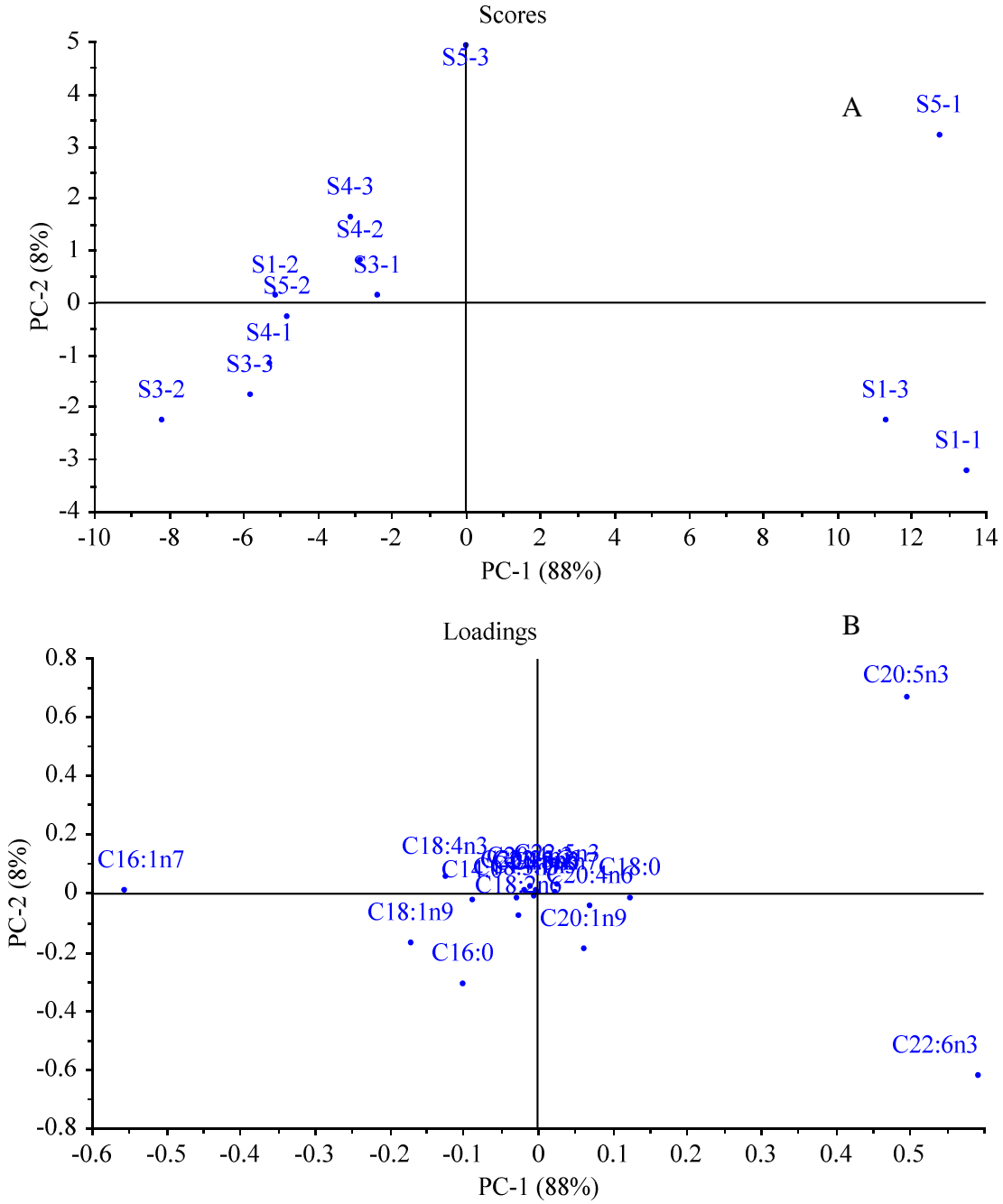

Fig. 25. Principal component analysis of fatty acid profiles in the mantle tissue of mussels in February. Score plot (A) showing stations and its replications (e.g. S5-3 is 3rd replication of station 5). Loading plot (B) showing the fatty acids contributing to score plot. Bracketed numbers at the axes are the percentage of variance of fatty acid explained by principal component 1 and 2.

4. Discussion

This study focuses on the possibility of integrating mussel (*Mytilus edulis*) culture to the salmon cage culture in open water condition in Central Norway. This is the first study in Norway where blue mussels are cultured in an IMTA system in exposed open sea. In this study mussels were grown in the different distances from the fish farm from June 2010 to February 2011. The mussel growth, CIs together with environmental conditions and food availability were evaluated in order to assess the effect of fish farm discharges on the mussel culture. The fatty acid analysis of digestive gland and mantle tissue of mussels and feed sample were performed in order to evaluate if the fish farm discharges have been taken up by the mussels.

4.1. Mussel growth

The CIs of mussels grown within the salmon farm (station 1 and 2) showed higher values than mussels grown at the reference stations (station 4 and 5). The result suggests that fish farm discharges impacted on mussel growth. This result is in consistency with previous studies showing improved mussel growth at the fish farms (Stirling and Okumus, 1995; Troell and Norberg, 1998; Peharda et al., 2007).

The two CIs showed different patterns. For example, CI (DW/SW) values peaked in July while CI (DW/SL) was highest in September. This difference might be caused by allometric mussel growth. A previous study shows that young mussel populations have a sigmoidal shell length growth curves (Theisen, 1973). Mussels might have faster shell length growth than shell weight growth when they are young. The low effect of environmental variables to CI (DW/SL) (see chapter 3.3) might be due to the allometric growth of the young mussels. This illustrates that the CI (DW/SL) might not be an ideal indicator to investigate environmental impact on mussel growth when the mussels are young.

Unlike CIs, the highest SGR was recorded at station 5 during the experimental period. The difference in shell length growth might be caused by strong current at the study site. Station 5 was located at a sheltered area near the shore. The stations in the fish farm (station 1 and 2)

and the vicinity area (station 3 and 4) was located at more exposed conditions and might face strong wave actions. This is in agreement with another study showing limited mussel shell length growth in the fast current speed (Harger, 1970).

The low SGR and CI in August can be explained by spawning. Mussels (*Mytilus edulis*) are considered to be opportunistic spawners and spawn when feeding conditions are favorable (Gosling, 1992). Favorable food availability and optimal water temperature range in August might provide the ground for spawning.

4.2. **Environmental impacts**

To specify mussel growth and CIs, water temperature, salinity, chlorophyll a, TPM, C/N contents, and fish feed provision from ACE were considered. None of these environmental variables was significantly different among the stations during the experiment.

Water temperature was the primary factor that affected mussel growth. The favorable water temperature maintained from June to September (above 10 °C) and maximum shell growth and CIs were found during that period. Decreasing water temperature strongly coincided with the declined mussel shell growth and CIs. This result corresponds with previous studies. According to Page and Hubbard (1987), growth was not affected if water temperature ranged between 10 to 18 °C. Bayne (1976) also argued that water temperature had little effect on scope of growth between 10 to 20 °C. At the study site water temperature became suboptimal from middle of October when SGR was 0.01 % day^{-1}. The long and cold winter at the study site slowed the mussel shell length growth. Similar result was found when the mussels were cultured at high latitude in Scottish sea lochs where suboptimal water temperature (below 10 °C) was maintained from November to April (Stirling and Okumus, 1995).

Salinity at the study site fluctuated during the early period of the study, but unstable salinity from June to August did not affected to SGR and CIs at the lowest salinity at July (21.7 ‰ at station 3). It is widely accepted that mussels can survive at salinity as low as 4 to 5 ‰, and the growth of mussels was not affected when mussels were gradually acclimated to lowered salinity levels (Kautsky et al., 1990).

4.3. **Food sources**

Food availability was second factor controlling mussel growth. In this study food source were assumed to be suspended particulate matter (SPM), microalgae and fish farm discharges (i.e. uneaten feed, fish metabolites and feces).

Throughout the experiment, chlorophyll a values ranged 0.03 to 2.5 μg L^{-1}. Norwegian fjords are assumed as oligotrophic environments in terms of chlorophyll a concentration, generally containing less than 2 μg L^{-1} after the spring bloom, due to low nutrient levels (Aure et al., 2007). Previous studies suggested that threshold concentration for cessation of mussel feeding is 0.4 to 0.5 μg L^{-1} (Dolmer, 2000; Riisgård et al., 2003). At the study site, the threshold chlorophyll a concentration of mussel was maintained during the winter season. During the period, other organic food sources might be utilized to sustain mussel growth.

SPM concentrations ranged from 6.5 to 10.5 mg L^{-1} which exceed the threshold level of 4.3 mg L^{-1} for pseudofaeces production in the mussels of 35 mm in shell length (Widdows et al., 1979; Bayne et al., 1989; Troell and Norberg, 1998). At these concentrations, particle ingestion rate of a mussel is saturated and the mussel choose food source by nutritional values. Organic contents of seston is important in determining food quality for filter feeders (Bayne et al., 1987). Natural seston has low organic contents per unit particle volume (Widdows et al., 1979). Mussels could have preferential selection of fish farm discharges of high organic contents. This might explain enhanced growth of mussels nearby fish farm (station 1 and 2). Mussels at station 1 and 2 could have high quality SPM than other stations in spite of statistically same TPM levels at all stations during the experiment.

Other than pseudofaeces production mussels regulate ingestion rate by controlling clearance rate (Foster-Smith, 1975; MacDonald and Ward, 1994). Strohmeier et al. (2009) and Widdows (1976) suggested that mussel clearance rate had no correlation over wide range of temperature changes (4.6 to 19.6 °C), provided sufficient food was supplied. This may imply that suppressed mussel SGR and CIs in the winter season were caused not only by lowered metabolic rate due to the low water temperature but also by limited natural food, i.e. chlorophyll a (Widdows, 1976). This could be an advantage growing mussels nearby fish farm when the natural food sources are limited in the winter season. This assumption is

supported by higher CIs of mussels at the fish farm compare to the reference stations in winter. There are evidences that mussels and other bivalves utilize non-planktonic food sources to meet the energy requirements when the phytoplankton concentration is low (Widdows et al., 1979; Rodhouse et al., 1984; Page and Hubbard, 1987).

Chlorophyll a measurements did not show noticeably increased level by salmon farm discharges. Nitrogen and phosphorus discharges might influence to the phytoplankton bloom but no influences from the fish farm were identified in this study. Cheshuk et al. (2003) and Taylor, et al. (1992) found no increase in chlorophyll a level by fish farm in their studies. The two studies explained that:

1) Phosphorus and nitrogen discharges could be flushed off before phytoplankton utilize it.

2) The increased phytoplankton could be dispersed before detect it.

3) Even if phytoplankton level increased, zooplankton might have depleted it.

TPM contents showed no significant differences by the depths. This result is assumed to be caused by great vertical water mixing. Although TPM values varied over time, the result showed no significant differences by stations. One expectation should be that fish biomass and supplied feed from ACE is correlated with TPM. If so, TPM should be higher at station 1 and 2 than other stations. However the result showed that TPM concentration at station 1 and 2 had no advantages over other stations. Previous studies (Troell and Norberg, 1998; Cheshuk et al., 2003) suggest the following reasons:

1) Geographical condition: Fast current speed at the study site reduced the nutrient concentration. Delusion by large water mass also facilitated horizontal and vertical water mixing over the study area.

2) Periodic feed supply: Feed was supplied 5 to 6 times a day. This might cause only a temporal TPM increase at the study site.

Neither chlorophyll a nor TPM can explain the different shell growth or CIs at the stations. Comparably low R square values of food sources at the mussel growth and CIs – environmental variable regression analysis (Table 3) might be caused by coarse sampling method. If the dispersion was that fast, water sampling should be performed more often.

Perhaps hourly measurement might be necessary to record the impact of periodic nutrient provision from the fish farm.

Secchi depth showed an inverse correlation with chlorophyll a and TPM values. Phytoplankton and particulate matter seemed to limit the water transparency. Generally, Secchi disk depth is consistent with the depth of approximately 10 % of surface light (Wetzel, 2001). Primary production by phytoplankton is a light related process that occurs typically down to euphotic zone where about 1 % of surface light can reach (Devlin et al., 2008). During the experiment, light was not limiting factors for the phytoplankton production between 2 – 8 m, except in July.

POC and PON levels at the study site were highest in June, ranging 268 – 542 and 37 - 72 $\mu g\ L^{-1}$, respectively. POC and PON in the open sea at a similar latitude (59°45' N, 00°30' E) have much lower levels (Head et al., 1998), 184 – 268, 27 – 42.3 $\mu g\ L^{-1}$, respectively. Slightly higher values of POC and PON have been observed in more sheltered areas in Tromsø fjord where POC and PON in June were 298 – 661 and 38 – 96 $\mu g\ L^{-1}$ (Gasparovic et al., 2005), and southern Baltic Sea, 620 and 83 $\mu g\ L^{-1}$(Dorota et al., 2005), respectively. Correlation analysis suggests that POC and PON patterns at the study site seemed to be more affected by phytoplankton level than fish farm discharge or TPM levels. Previous studies found strong correlation between POC and PON level and chlorophyll a contents (Kautsky, 1982; Page and Hubbard, 1987).

The C/N ratio in June in the study area ranged 6.9 – 8.6, similar to the study at Tromsø fjord (Gasparovic et al., 2005) and in southern Baltic Sea (Dorota et al., 2005), but was higher than in the open sea at similar latitude (Head et al., 1998). Kreeger and Langdon (1993) reported that mussel growth was lowered when the C/N ratio is over 10. Despite the ratio did not show any clear patterns during the experimental period, the favorable ratio for mussel growth was maintained throughout the experimental period.

4.4. **Fatty acid signature on mussel**

Fatty acid analysis suggests that particles from the fish farm were utilized by mussels. Three fatty acids of the digestive gland tissue sampled in February showed clear patterns of influence of fish feed on mussels. The fatty acids 18:1 (n-9) and 18:2 (n-6) are considered to

originate from terrestrial sources which are included in fish feed (Johnsen et al., 2000). The increase in the percentage of these fatty acids at the stations close to the fish farm (station 1, 2 and 3) was significantly higher than at station 5 which was located 3.6 km away from the fish farm. Fatty acid 20:5 (n-3), which originated from marine primary producers, is comparably low in fish feed (Napolitano et al., 1997). Significantly higher percentage of 20:5 (n-3) in the digestive gland tissue of mussels at station 5 than at the other stations was recorded.

Terrestrial feed sources are usually depleted in n-3 PUFA compare to marine sources and comparably high in n-6 PUFA (Menoyo et al., 2007). The n-3/n-6 ratio of the digestive gland tissue of mussels near the fish farm (station 1, 2 and 3) were lower than at station 5. Especially in February, station 1, 2 and 3 showed significantly lower ratio than station 5. The results might suggest that mussels at station 1, 2 and 3 utilized nutrients from the fish farm while mussels at station 5 depended on natural food sources. This result is supported by Redmond (2008) who showed that fatty acid 18:1 (n-9), 18:2 (n-6) and the n-3/n-6 ratio could be used as a tracer of the consumption of salmon feed.

Moreover, PCA shows a distance between station 1 and 5, which was mainly caused by fatty acid 18:1 (n-9), 18:2 (n-6) and 20:5 (n-3). This clear separation of the fatty acid profiles of digestive gland tissue of mussels in the PCA plot shows that mussels at the fish farm area acquired food sources from uneaten fish feed while mussels at reference station relied more to on natural food sources.

Mantle tissue of mussels showed less clear patterns during the experiment. This result correspond to Redmond (2008), who found limited response of mussel mantle tissue to the fatty acid contents of fish feed. This result might be caused by the fact that mantle tissue has a structural role and the fatty acids are highly selectively incorporated (Sargent et al., 1989). The fatty acid patterns of digestive gland tissue are on the other hand affected by food quality and quantity in a relatively short period (Shin et al., 2008). Furthermore Lubet et al. (1986) have shown large differences in lipid composition of female and male mantle tissue. The ambiguous analysis result of mantle tissue might be due to the mussels used in this study were not distinguished by gender.

The unclear fatty acid analysis results in August might be caused by selective feeding behavior of mussels. Previous studies confirmed preferential food selection of algae over non-living particulate matter (Widdows et al., 1979; Rodhouse et al., 1984; Page and

Hubbard, 1987). In August, the phytoplankton level was high and mussels might choose algae as their primary food source. Another possible explanation is spawning. Decrease in lipid contents in the mussel tissues due to spawning is observed in the *M. edulis* (Zandee et al., 1980) and many other bivalves (Ruiz et al., 1992; Páez-Osuna et al., 1993; Narváez et al., 2008).

5. Conclusion

Discharges from salmon farming affected mussel CIs. This suggests that mussels utilized nutrients from the fish farm as a food source. However shell length growth was higher at the reference station. Shell length growth of mussels at the experimental stations might be limited by fast current speed at the fish farm. The benefits from integrating mussels as a biofilter on the salmon cage culture are very convincing and limited but significant commercial benefits are predicted.

Water temperature was the primary factor that affected mussel growth. Favorable water temperature maintained from June to middle of October. Food availability was second important factor. Although chlorophyll a levels varied seasonally, phytoplankton appeared as an important food source. SPM was another food source for mussels and played an important role especially when the phytoplankton was depleted in winter to maintain metabolism and growth. Although TMP contents were not different by stations, higher organic contents of fish farm discharges than natural SPM might cause higher CIs of mussels near the fish farm.

Despite none of measured environmental conditions were significantly different, fatty acid analysis supports the impact of fish farm discharges to mussel growth. Fatty acid profiles of 18:1 (n-9), 20:5 (n-3) and 18:2 (n-6) and n-3/n-6 ratio on digestive gland tissue of mussels collected in February suggests that fish farm discharges were actually utilized by mussels.

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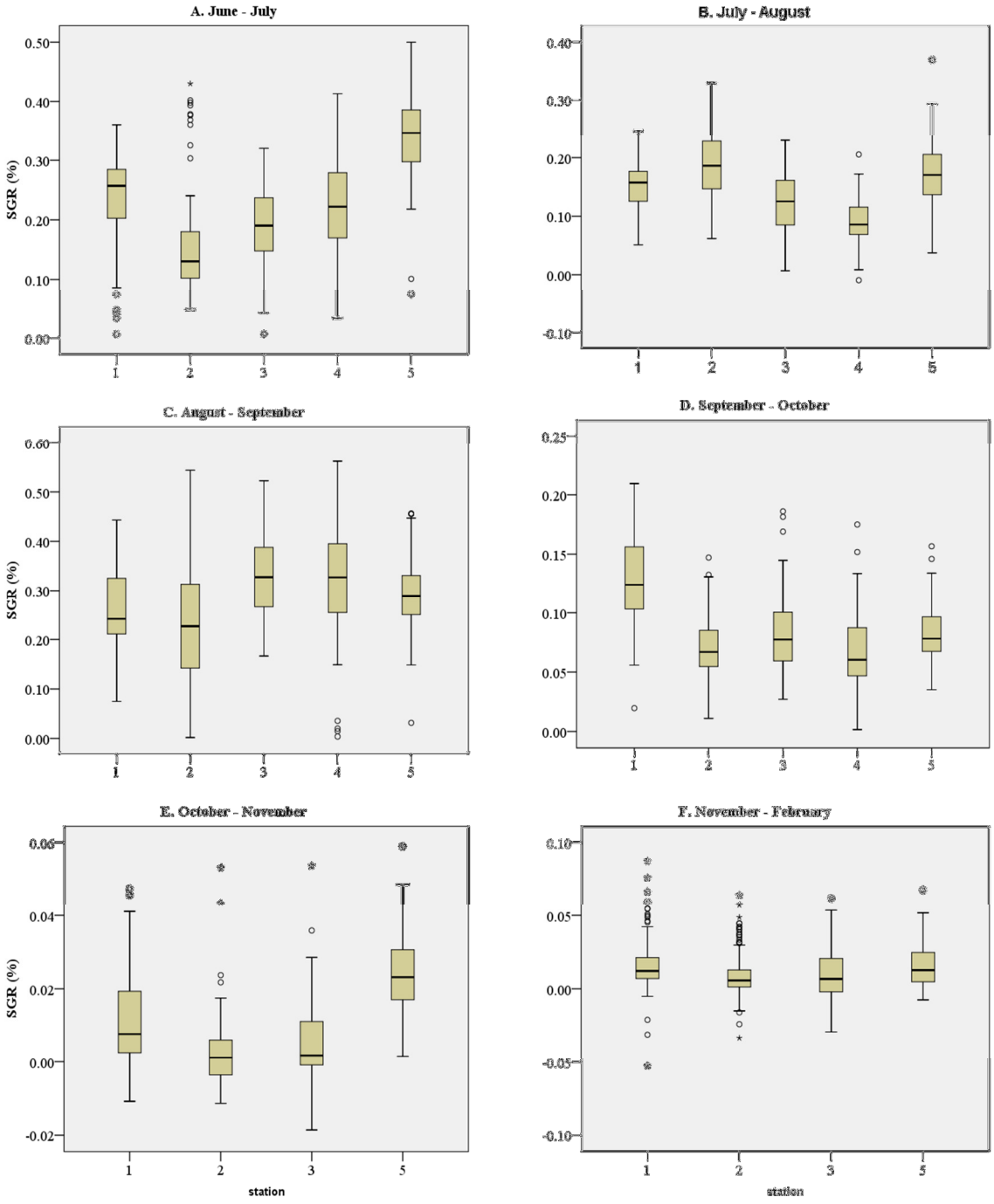
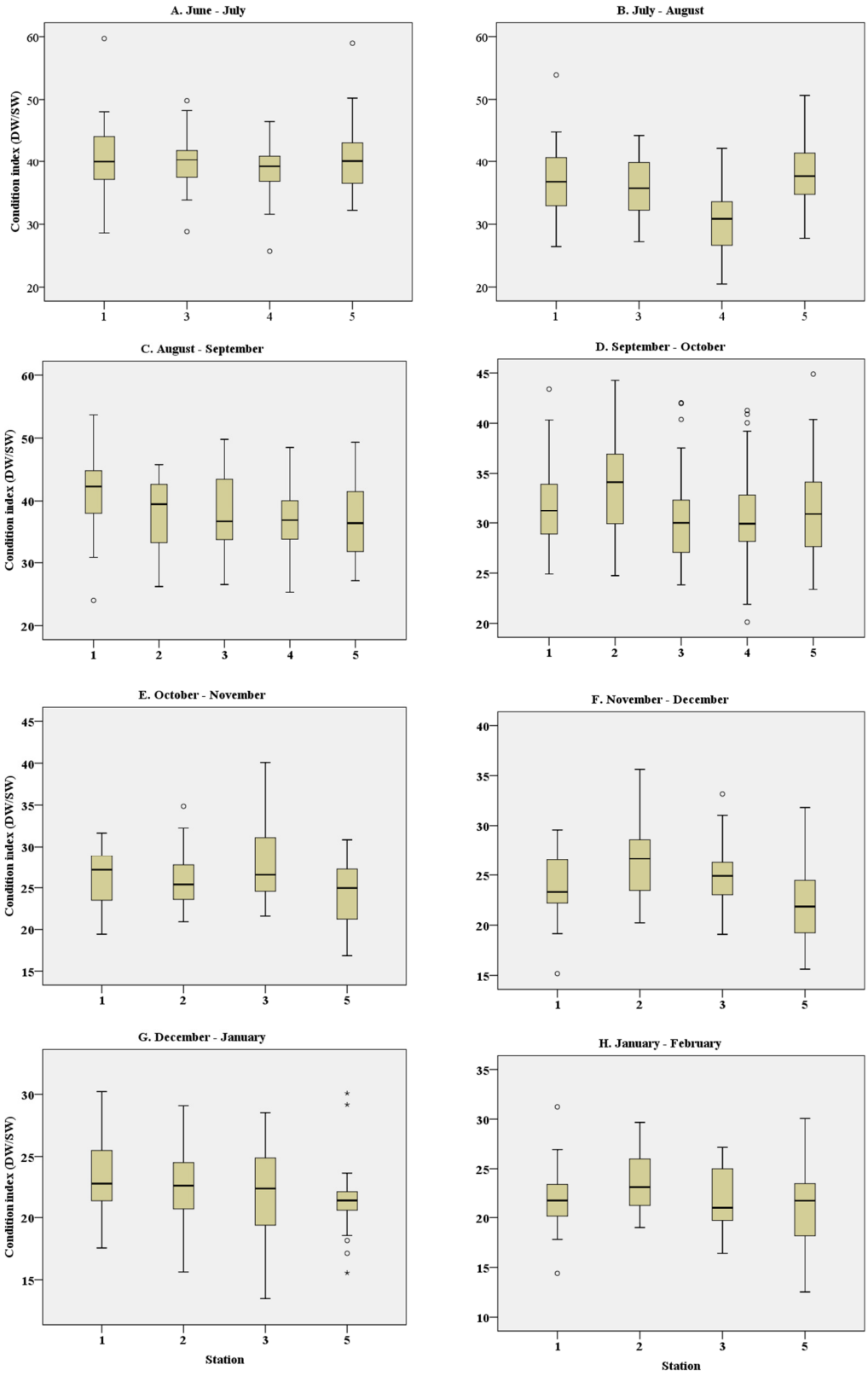
7. Appendix

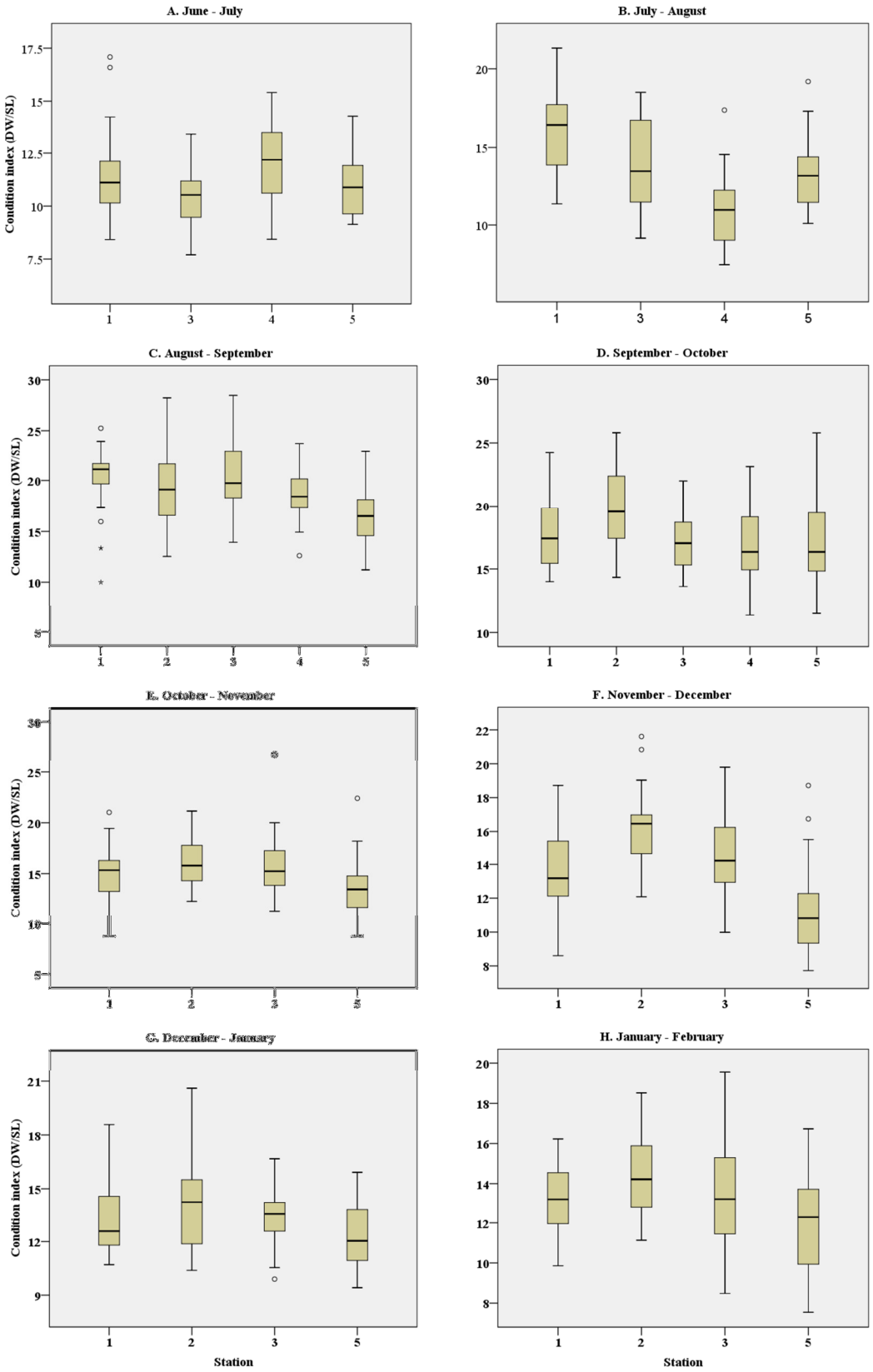
Appendix 1. Monthly specific growth rate (%, day^{-1})



Appendix 2. Monthly Condition index (meat dry weight / shell dry weight)



Appendix 3 Monthly condition index (meat dry weight / shell length)



Appendix 4.

Fatty acid composition (%) of digestive gland tissue of mussels

Fatty acid	June	august (N=6)				February (N=6)				Feed
	(N=6)	st1	st3	st4	st5	st1	st2	st3	st5	(N=6)
C14:0	3.9±0.06	6.56±0.13	6.71±0.27	6.35±0.01	6.54±0.07	3.4±0.05	4.1±0.15	3.55±0.1	3.2±0.05	2.97±0.01
C16:0	11.02±0.21	15.56±0.21	15.13±0.21	15.38±0.28	15.15±0.29	14.69±0.22	13.81±0.27	13.16±0.18	13.75±0.24	10.94±0.07
C18:0	2.41±0.13	1.97±0.02	1.77±0.08	1.78±0.11	1.69±0.09	2.13±0.06	1.66±0.06	1.78±0.12	2.16±0.17	3±0.09
C16:1n7	10.84±0.13	5.99±0.11	6.45±0.16	6.31±0.16	7.53±0.2	5.87±0.13	7.77±0.1	8.33±0.42	6.9±0.65	2.7±0.01
C18:1n9	1.39±0.06	7.98±0.36	7.08±0.23	7.07±0.25	7.59±0.09	7.4±0.15	8.54±0.22	6.83±0.16	5.4±0.46	37.81±0.23
C18:1n7	2.45±0.04	1.54±0.03	1.63±0	1.62±0.05	1.78±0.03	2.26±0.02	2.04±0.02	2.1±0.03	2.35±0.07	2.46±0.02
C20:1n9	1.04±0.04	2.27±0.15	2.19±0.1	2.37±0.15	2.3±0.14	4.04±0.18	3.76±0.19	3.78±0.2	3.67±0.33	2.99±0.01
C18:3n3	2.34±0.1	2.88±0.05	3.18±0.07	2.87±0.06	2.51±0.08	2.93±0.02	2.08±0.01	2.33±0.09	1.75±0.05	5.31±0.02
C18:4n3	7.79±0.09	9.36±0.34	8.87±0.18	7.88±0.29	8.07±0.49	6.07±0.22	4.83±0.08	5.22±0.06	3.92±0.32	1.15±0.02
C20:3n3	-	0.47±0.08	0.58±0.02	0.5±0.01	0.53±0.01	-	-	0.08±0.05	-	-
C20:4n3	0.56±0.05	0.42±0.04	0.39±0	0.38±0.02	0.37±0.03	0.57±0.02	0.4±0.04	0.47±0.03	0.26±0.08	0.27±0
C20:5n3	21.96±0.24	10.63±0.25	10.84±0.28	11.11±0.34	12.04±0.27	11.05±0.1	10.99±0.25	11.22±0.13	13.86±0.64	4.35±0.05
C22:5n3	0.66±0.05	0.54±0.04	0.59±0.02	0.62±0.01	0.59±0.01	0.8±0.02	0.77±0.02	0.77±0.04	0.91±0.05	0.5±0
C22:6n3	11.84±0.22	18.13±0.2	18.53±0.2	18.53±0.2	17.5±0.11	20.6±0.32	20.64±0.35	20.65±0.36	21.72±0.5	4.87±0.1
C18:2n6	1.44±0.03	2.19±0.03	2.21±0.03	1.98±0.01	1.9±0.03	2.95±0.08	2.5±0.06	2.33±0.04	1.73±0.08	12.41±0.03
C20:2n6	0.54±0.02	0.45±0.04	0.45±0.01	0.49±0.03	0.41±0.01	0.83±0.02	0.5±0.02	0.65±0.04	0.63±0.02	0.15±0
C20:3n6	-	-	0.02±0.01	0.06±0.02	0.01±0.01	-	-	0.02±0.02	-	-
C20:4n6	0.71±0.06	0.47±0.03	0.57±0.01	0.59±0.02	0.55±0.03	1.17±0.08	1.2±0.04	1.06±0.01	1.54±0.19	0.24±0
C22:5n6	-	0.11±0.04	0.13±0.04	0.2±0	0.11±0.04	0.15±0.07	-	0.19±0.04	0.08±0.05	0.08±0
unknown	19.11±0.41	12.49±0.39	12.65±0.69	13.87±0.34	12.81±0.25	13.03±0.39	14.41±0.52	15.4±0.24	16.17±0.43	2.73±0.04
Sum sat	27.73±1.2	59.52±0.74	57.44±2.53	55.03±0.52	60.07±2.82	37.24±4.45	27.8±0.31	36.51±0.89	23.23±3.07	60.9±1.18
Sum mono	25.19±1.27	44.03±1.27	42.35±2.21	40.81±0.41	49.51±2.64	35.93±3.96	31.52±1.39	41.67±0.58	23.21±4.51	175.62±4.45
Sum poly	76.49±3	113.03±3.59	112.91±5.23	105.97±2.64	114.63±5.33	86.12±9.15	62.53±1.9	89.06±3.49	56.82±7.78	102.09±1.6
Sum (n-6)	4.3±0.24	7.99±0.34	8.26±0.48	7.77±0.14	7.69±0.49	9.28±0.9	5.99±0.21	8.43±0.35	4.82±0.65	44.84±0.99
Sum (n-3)	72.19±2.78	105.04±3.26	104.66±4.76	98.2±2.6	106.94±4.91	77.17±8.13	56.54±1.76	80.63±3.19	52.01±7.16	57.25±0.61
(n-3)/(n-6)	15.02±1.44	12.29±0.82	12.72±0.15	12.66±0.36	14.02±0.54	8.31±0.19	9.47±0.27	9.59±0.2	10.76±0.52	1.28±0.01
DHA/EPA	0.49±0.05	1.71±0.03	1.71±0.04	1.68±0.06	1.46±0.04	1.86±0.04	1.89±0.07	1.84±0.03	1.58±0.08	1.12±0.01
lipid[mg]/FDW[g]	201.5±5.3	268.19±4.98	257.43±10.03	237.72±5.59	269.65±8.73	232.32±23.44	180.18±7.43	220.31±5.89	154.7±15.36	260.27±65.14
FA[mg]/FDW[g]	129.42±5.36	216.57±4.83	212.7±9.61	201.81±2.85	224.22±10.65	159.29±17.57	121.85±3.54	167.24±4.69	103.26±15.2	338.61±7.09

Fatty acid composition (%) of mantle tissue of mussels

Fatty acid	June	august (N=6)				February (N=6)				Feed
	(N=6)	st1	st3	st4	st5	st1	st2	st3	st5	(N=6)
C14:0	2.35±0.27	3.28±0.47	2.5±0.16	2.86±0.22	3.39±0.16	1.11±0.28	2.32±0.22	2.25±0.08	1.42±0.25	2.97±0.01
C16:0	22.41±2.32	19.29±0.07	18.19±0.18	18.23±0.54	17.5±0.21	16.34±0.42	17.52±0.28	17.37±0.52	15.64±0.52	10.94±0.07
C18:0	4.65±1	2.9±0.37	2.38±0.25	2.6±0.22	1.9±0.1	2.96±0.49	1.45±0.1	1.66±0.05	2.46±0.41	3±0.09
C16:1n7	4.82±0.95	5.02±0.8	3.61±0.2	4.34±0.33	6.78±0.26	5.94±2.39	12.24±0.8	11.03±0.26	8.47±1.81	2.7±0.01
C18:1n9	0.96±0.31	2.22±0.22	2.19±0.25	1.96±0.14	2.39±0.04	3.35±0.55	5.55±0.35	4.56±0.32	3.35±0.6	37.81±0.23
C18:1n7	2.96±0.31	1.98±0.01	2.02±0.05	2.05±0.08	2.33±0.04	2.65±0.14	2.26±0.05	2.22±0.03	2.53±0.04	2.46±0.02
C20:1n9	2.45±0.33	2.9±0.33	3.67±0.18	3.15±0.16	2.96±0.16	4.53±0.43	3.46±0.12	3.22±0.12	3.26±0.1	2.99±0.01
C18:3n3	1.05±0.33	1.73±0.25	1.39±0.08	1.55±0.07	1.39±0.12	1.28±0.11	1.37±0.01	1.5±0.07	0.93±0.21	5.31±0.02
C18:4n3	2.47±0.8	3.62±0.58	2.51±0.15	3.01±0.11	2.75±0.26	1.64±0.51	3.39±0.16	3.08±0.16	2.05±0.38	1.15±0.02
C20:3n3	-	-	-	-	-	-	-	0.02±0.02	-	-
C20:4n3	-	0.17±0.11	-	0.03±0.03	-	0.05±0.05	0.29±0.03	0.24±0.05	0.12±0.08	0.27±0
C20:5n3	22.07±0.78	20.03±0.63	16.63±0.87	18.71±0.27	19.31±0.73	18.26±1.29	12.51±0.78	14.49±0.51	19.44±2.07	4.35±0.05
C22:5n3	0.89±0.28	1.23±0.05	1.09±0.04	1.16±0.01	1.2±0.01	1.26±0.08	1±0.09	1.08±0.08	1.22±0.12	0.5±0
C22:6n3	15.54±1.34	22.61±1.86	27.34±0.8	23.47±0.61	21.59±0.78	25.6±2.42	18.05±0.3	17.77±0.17	20.68±1.93	4.87±0.1
C18:2n6	0.66±0.21	1.02±0.33	1.28±0.09	1.27±0.07	1.35±0.03	1.52±0.09	1.66±0.1	1.57±0.07	1.03±0.1	12.41±0.03
C20:2n6	0.5±0.16	0.43±0.14	0.73±0.05	0.43±0.14	0.56±0.03	0.54±0.18	0.55±0.03	0.67±0.02	0.43±0.14	0.15±0
C20:4n6	1.08±0.24	1.15±0.13	1.24±0.03	1.2±0.01	1.15±0.08	2.18±0.28	1.17±0.02	1.27±0.05	1.71±0.17	0.24±0
C22:5n6	-	-	-	0.13±0.06	-	-	0.03±0.03	0.11±0.05	-	0.08±0
unknown	15.14±1.4	10.4±0.17	13.24±0.94	13.85±1.26	13.43±0.88	10.8±0.87	15.06±0.11	15.88±0.82	15.24±1.22	2.73±0.04
Sum sat	15.59±1.87	17.98±2.98	10.12±1.12	11.99±0.63	13.1±1.79	14.52±3.22	26.06±1.36	28.15±0.65	16.56±2.58	60.9±1.18
Sum mono	6.88±1.45	8.9±1.74	4.95±0.4	5.82±0.35	8.29±1.09	13.63±4.9	28.63±0.86	27.88±1.04	16.38±3.9	175.62±4.45
Sum poly	27.86±6.35	36.27±5.65	22.94±2.65	25.84±1.4	28.14±3.53	34.65±5.04	49.09±3.25	55.52±2.72	38.27±4.09	102.09±1.6
Sum (n-6)	1.54±0.43	2±0.46	1.41±0.13	1.53±0.06	1.72±0.16	2.91±0.53	4.16±0.17	4.8±0.21	2.73±0.45	44.84±0.99
Sum (n-3)	26.32±5.92	34.26±5.2	21.53±2.53	24.31±1.37	26.43±3.37	31.75±4.51	44.94±3.15	50.72±2.56	35.55±3.64	57.25±0.61
(n-3)/(n-6)	13.97±2.84	21.44±3.8	15.19±0.72	15.97±0.85	15.18±0.53	11.37±0.63	10.81±0.59	10.58±0.35	14.15±1.37	1.28±0.01
DHA/EPA	0.7±0.05	1.14±0.11	1.67±0.11	1.25±0.03	1.13±0.08	1.39±0.04	1.46±0.07	1.23±0.04	1.09±0.08	1.12±0.01
lipid[mg]/FDW[g]	92.13±8.14	90.76±8.51	65.89±5.78	73.55±3.52	75.14±7.22	105.52±12.87	147.92±5.76	156.21±4.7	110.94±11.11	260.27±65.14
FA[mg]/FDW[g]	50.32±9.66	63.15±10.37	38±4.13	43.65±2.26	49.53±6.41	62.81±13.16	103.79±4.37	111.55±4.03	71.21±10.26	338.61±7.09