Incorporation of fish feed and growth of blue mussels (Mytilus edulis) in close proximity to salmon (Salmo salar) aquaculture: Implications for integrated multi-trophic aquaculture in Norwegian coastal waters

Aleksander Handå a,b,⁎, Højune Min a, Xinxin Wang a, Ole Jacob Broch b, Kjell Inge Reitan b, Helge Reinertsen a, Yngvar Olsen a

a Norwegian University of Science and Technology (NTNU), Department of Biology, Centre of Fisheries and Aquaculture, N-7491 Trondheim, Norway
b SINTEF Fisheries and Aquaculture, N-7465 Trondheim, Norway

Abstract

The incorporation of fish feed wastes in digestive gland and mantle tissue and average growth in length and standardized dry weight of soft tissue matter (DW) of blue mussels (Mytilus edulis L.) were measured for one year (June 2010–June 2011) at three experimental stations in close proximity to a salmon (Salmo salar) farm at Tristein (63° 52’ N, 9° 37’ E) in Central Norway, with one on the west side (FW), one on the east side (FE) and one 100 m east of the farm (FE100), in addition to one reference station 4 km south of the farm.

A principal component analysis of fatty acid profiles clearly demonstrated the incorporation of fatty acids from salmon fish feed in digestive gland and mantle tissue, identified by an increased content of 18:1 (n-9). The incorporation, and consequently the separation of mussels at stations close to the fish farm from mussels at the reference station, was more pronounced in February compared to August, while no clear differences were found in June.

The growth in length correlated significantly to feed use at the fish farm (r=0.89) and to the concentration of suspended particulate matter (SPM) (r=0.53) in the autumn–winter period (Oct–Feb) (p<0.05). The mussels at the reference station showed a significantly faster growth in length compared to the mussels at all stations at the fish farm during the summer, while mussels at the FW station grew faster than the mussels at the reference station during the spring (p<0.05). The length growth was faster for mussels at the reference station than for mussels at the FW and FE100 stations (p<0.05), while no significant differences were found between mussels at the reference and the FE stations for the entire year.

The DW was significantly positively correlated to the feed use at the fish farm stations (r=0.53) (p<0.05), and the DW of mussels at stations at the fish farm was significantly higher compared to the DW of mussels at the reference station in five months during autumn and winter (p<0.05). The results suggest that the combined production of mussels and salmon can be seen as a strategy to maintain a higher soft tissue content of mussels during autumn and winter. Quantification of the mussel’s assimilation capacities of farm-derived wastes at realistic scale and under different environmental conditions is needed.

Keywords: Mytilus edulis Salmon fish feed Fatty acids Bivalve growth Integrated multi-trophic aquaculture

1. Introduction

The global salmonid production increased by around 60% from 1999 to 2009 (1.26 to 2.17 million tons), and further growth is expected (FAO, 2011). Atlantic salmon (Salmo salar) aquaculture accounts for the majority of the salmonid production (1.44 million tons), with Norway, which doubled its production from 1999 to 2009 (0.43 to 0.86 million tons), being the leading producer (FAO, 2011). The mean nutrient release from Norwegian salmon aquaculture has been estimated at 61% of feed-N and 69% of feed-P. Out of this, 41% N and 19% P are released in a dissolved form, while 20% N and 50% P are released in particulate form (Olsen et al., 2008). The theoretical mean nutrient dispersal from a Norwegian salmon farm producing 5000 t fish (FCR=1.15, 6% N and 0.9% P content in feed) is accordingly: 141 t dissolved inorganic N, 10 t dissolved inorganic P, 69 t particulate organic N and 26 t particulate organic P.

There is an increasing concern regarding the potentially negative environmental impacts that nutrient wastes from marine aquaculture may cause (Amberg and Hall, 2008; Braaten, 2007; FAO, 2009; Tett, 2008), and one of the major challenges for the sustainable development

Abbreviations: FW, Farm west (Station 1); FE, Farm east (Station 2); FE100, 100 m east of farm (Station 3); AGRb, Average growth rate in length; SGRDWb, Specific growth rate in soft tissue dry weight.

⁎ Corresponding author at: Norwegian University of Science and Technology (NTNU), Department of Biology, Centre of Fisheries and Aquaculture, Brattøra 17B, N-7491 Trondheim, Norway. Tel.: +47 91577232; fax: +47 93270701.

E-mail address: aleksander.handa@sintef.no (A. Handå).

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of salmonid cage mariculture is to minimize discharge of wastes that potentially can lead to degradation of the marine environment (Cheshuk et al., 2003). For example, the sedimentation of particulate matter may cause the organic enrichment of sediments (Carroll et al., 2003; Jusup et al., 2007; Kutti et al., 2007; Stigebrandt et al., 2004), which may have a negative effect on the benthic community if sedimentation rates exceed the turnover rate of the community (Holmer et al., 2005; Kalantzis and Karakassis, 2006), while dissolved nutrients may cause eutrophication (Cloern, 2001; Folke et al., 1994; Nixon, 1995; Skogen et al., 2009).

For the purpose of minimizing the potentially negative effects of waste discharges it has been suggested to cultivate extractive and filter feeding species at lower trophic levels in close vicinity to the fish farms, a strategy termed IMTA, or integrated multi-trophic aquaculture. IMTA is used as a means of obtaining increased biomass production, thus adding to the value of feed investments, and at the same time contributing to a more sustainable aquaculture production (Chopin et al., 2001; Chopin et al., 2008; Neori, 2008; Neori et al., 2004; Troell et al., 2003, 2009).

The dissolved inorganic nutrient wastes can be taken up by inorganic extractive species such as seaweeds (Buschmann et al., 2001; Chopin et al., 2001; Chopin et al., 2004), while released particulate organic nutrients can be consumed by filter feeding species such as mussels. Several studies have suggested that bivalve filter feeders can provide bioremediative services when co-cultivated with fish aquaculture (Folke and Kautsky, 1989; Folke et al., 1994; Gao et al., 2008; Mazolla and Sara, 2001; Peharda et al., 2007; Soto and Mena, 1999; Troell and Norberg, 1998; Whitmarsh et al., 2006), hence reducing the environmental impact associated with a great release of particulate organic matter from marine cage aquaculture (Cheshuk et al., 2003 and references therein). However, little is known about how particulate wastes from salmon farming may affect shellfish growth (MacDonald et al., 2011).

While many studies have reported a better growth for mussels grown adjacent to cage fish farms (Lander et al., 2004; Peharda et al., 2007; Sara et al., 2009; Striling and Okumus, 1995; Wallace, 1980), others have failed to demonstrate such an effect (Cheshuk et al., 2003; Navarrete-Mier et al., 2010; Taylor et al., 1992). These authors instead suggest that the distance from the farms does not substantially influence food availability and growth of mussels. Previous research has suggested several possible explanations for the lack of a distinct growth response in mussels co-cultivated with fish cage aquaculture. The explanations given are that: a) the particulate wastes of the fish farms do not increase the seston concentrations significantly above ambient levels, b) that the ambient seston concentrations remain consistently above the pseudofeces threshold level, thereby limiting the potential of mussels to increase their growth by feeding upon fish farm wastes (Cheshuk et al., 2003), c) that the mussels’ filtering response is slow to adapt to pulsed feeding regimes accompanied by d) non-uniform effluents from salmon farms leaving mussels to only ingest farm particulate wastes when natural seston concentrations are scarce, and e) that spatial and temporal differences in hydrodynamic conditions between sites, in addition to the experimental design, differ in ways which make it difficult to obtain univocal conclusions for the IMTA concept (Troell and Norberg, 1998; Troell et al., 2009, 2011). Conflicting results bring some uncertainty as to how efficiently blue mussels can reduce the organic load from fish cage aquaculture; there is hence a need to further investigate on the ability of mussels to incorporate and grow based on salmon fish feed and feces particles.

Blue mussels have been shown to clear salmon fish feed and feces quite efficiently (Handå et al., submitted for publication; MacDonald et al., 2011; Reid et al., 2010). Additionally, changes in fatty acid composition in the direction of the food source profile have demonstrated the assimilation of salmon feed and feces in bivalve tissues (Gao et al., 2006; Handå et al., submitted for publication; Redmond et al., 2010). Fish feed has traditionally contained a high percentage of the fatty acids 20:1 (n-9) and 22:1 (n-11). There has been an increasing use of terrestrial lipid sources with high proportions of, e.g. 18:1 (n-9) and 18:2 (n-6) in fish feed in recent years (Dalsgaard et al., 2003; Narváez et al., 2008; Skog et al., 2003) which can be used as tracer fatty acids for the incorporation of salmon feed. Significant changes in the proportions of these fatty acids have been found to take place within 28 days in the digestive gland (Handå et al., submitted for publication; Redmond et al., 2010) and within 90 days in mantle tissue (Fukumori et al., 2008; Post, 2002) of mussels. However, little is known about the seasonal-dependent incorporation of salmon fish feed wastes and the corresponding growth in bivalves under natural farming conditions.

The primary objectives of this study were to investigate the annual and the seasonal-dependent incorporation of fish feed waste and growth of blue mussels (Mytilus edulis) in close proximity to salmon (S. salar) aquaculture in coastal waters of Central Norway.

2. Material and methods

2.1. Location and sampling program

Seston variables and mussel growth were measured monthly for one year (June 2010–June 2011) at three experimental stations in close proximity to a salmon farm at Tristeinen (63° 52’ N, 9° 37’ E) outside the Trondheimsfjord, at the coast off Central Norway, with one station on the west side (FW), one on the east side (FE), one 100 m east of the farm (FE100), and a reference station 4 km south of the farm (Fig. 1). The distance between the fish cages and the mussels was approximately 60 m for the FW and the FE station and 120 m for the FE100 station. Hydrodynamic simulations were undertaken to determine the best location of the sampling stations (see Section 2.7). Fatty acids were analyzed for salmon fish feed sampled from the feed barge in December 2010 and June 2011, and from mussels’ digestive glands and mantle tissue in June and August 2011 and February and June 2011.

2.2. Salmon farm and area description

The fish farm consisted of eight Polar circle plastic cages (Fig. 1), each with a circumference of 157 m, with 15 m deep net pens and a volume of 36,000 m³. The total salmon production was 4,705 t, and the corresponding use of feed was 5,216 t during the sampling period (Fig. 2A). The cage on the west side, where station FW was located, contained only mussels (no fish) during the experiment. The western side of the farm was situated above the 50 m isobath, with the bottom sloping steeply down to approximately 100 m on the eastern side. Although the farm was situated approximately 4 km off shore of the main land, it was sheltered from the ocean by the skerries of Tristeinen on the western and northern sides. The area surrounding the Tristeinen skerries is dominated by marine waters (salinity <34.5‰) from the Norwegian coastal current, with temporary outflows of fresh surface water from the Trondheimsfjord.

2.3. Environmental and seston variables

The temperature and salinity were measured at a depth of 2 m with a CTD (SD 204, SAIV LTD, Norway), while integrated water samples for the analysis of suspended particulate matter (SPM), particulate organic carbon (POC), nitrogen (PON) and chlorophyll a (Chl a) were taken from a depth of 0–8 m by mixing four consecutive samples (0–2, 2–4, 4–6 and 6–8 m) taken with a Ramberg water collector (L = 2 m, V = 5 L) in a bucket prior to filtration. The samples were pre-filtered with a 200 μm net prior to a second filtration of 2 L with pre-combusted and weighed Whatman GF/C filters. The SPM was measured in parallel after drying the filters for 48 h at 70 °C, while 1/16 of two other filters was punched out in parallel and stored at −81 °C for analysis of Chl a, POC and PON. The Chl a was extracted with methanol and placed in a refrigerator for 2 h prior to measurement of in vitro fluorescence with a Turner Design Fluorometer according to Strickland and Parsons.
POC and PON were analyzed by a Carlo Erba analyzer, model NA 1106 CHN.

2.4. Growth measurements

The shell length (L) (n = 25) was measured with a digital caliper on individually marked mussels kept in rectangular net pots (0.7 × 0.4 × 0.2 m) at a depth of 2 m. Changes in soft tissue dry weight (DW) (n = 30) were represented using a condition index standardized to a certain length L' according to Bayne and Worrall (1980) and Bonardelli and Himmelman (1995). The DW was measured with an electronic weight (Mettler Toledo Precisa 180A) after drying the tissue at 60 °C in a Termaks heat cabinet for 48 h, which was then calculated as a standardized dry weight (DW') by the following equation:

\[ DW' = DW \left( \frac{L'^b}{L^b} \right) \]  

Fig. 1. Geographical location of the salmon farm and the experimental stations at the west (FW) and east (FE) side of the farm, 100 m east (FE100) and at the reference station (RS) 4 km south of the farm at Tristein in Central Norway.

Fig. 2. A) Fish biomass (right axis) and feed use (left axis) from June 2010 to June 2011, and B) temperature and salinity at 2 m depth at the west (FW) and east (FE) side of the farm, 100 m east (FE100) and at the reference station (RS) 4 km south of the farm from June 2010 to June 2011.
where DW is the weight in mg, L the length in mm and b the slope of the log_{10} DW plotted as a function of log_{10} L. The DW' corresponds to the condition index, and was scaled so it equaled the DW when L equals L'. L' was set to 40 mm based on an average shell length of 40.4 ± 0.1 mm (n = 1200 measurements) during the sample period.

The specific growth rate (μ day^-1) in DW' (SGR_{DW'}) was calculated by the equation:

\[ μ = \left( \ln DW' - \ln DW_{0}\right)/t \]  

(2)

where DW_0 and DW_t are the DW' at the start and end of each period, respectively, and t is the time in days. The percentage growth per day (P) was calculated by the equation:

\[ P = 100 \times \left( \exp(μ) - 1 \right) \]  

(3)

The average rate of length increase (μm, day^-1) (AGR_L) was calculated by the equation:

\[ μm = (L_t - L_0)/t \]  

(4)

where L_0 and L_t are L at the start and end of each period, respectively, and t is the time in days. The initial shell length was 30.5–32.5 mm (mean 31 ± 0.07 mm, n = 100).

The mussels originated from a suspended longline farm in the Åfjord (63° 56' N, 10° 11' E) 30 km away.

2.5. Fatty acid analysis

The total lipids were extracted according to Bligh and Dyer (1959), followed by an analysis of fatty acids after handling the samples according to Metcalfe et al. (1966) (see Handå et al., submitted for publication).

2.6. Data analysis

Data for the specific growth in DW' (SGR_{DW'}) and the average growth in length (AGR_L) were tested for normality using a Kolmogorov–Smirnov test, and for the homogeneity of variance using a Levene's test. The equality of means for total fatty acid content (mg g^-1 dry weight) and the relative content of identified fatty acids (% of total fatty acids) of digestive gland and mantle tissue between June 2010 (Month 1), August (Month 3), February (Month 8) and June 2011 (Month 12), in addition to between the different stations on each sampling day were tested using a Kolmogorov–Smirnov test. The equality of means for SGR_{DW'} and AGR_L between sampling stations and sampling days, as well as between summer (June–September), autumn (October–November), winter (December–February) and spring (March–May), were tested using a one-way ANOVA followed by post hoc comparisons by Tamhane’s T2, not assuming equal variances. The significance limit was set at 0.05 and the means were given with the standard error. A Spearman’s correlation analysis was performed on seston data and mussel growth for the entire year and for the five-month autumn–winter period from October to February.

Statistical analyses were performed using SPSS (rel. 17.0, Chicago, SPSS Inc.), while a principal component analysis (PCA) for fatty acid composition was performed with an Unscrambler, version 9.8 2008 (Camos software AS). Data was analyzed without weighing, leaving the PCA for each tissue to be dominated by the fatty acids that dominated the fatty acid composition. Missing measurement points in the time plots were estimated by linear interpolation between the two closest measurement points.

2.7. Hydrodynamical model

The coupled 3D hydrodynamic–biological model system SINMOD was used for the hydrodynamic modeling. The hydrodynamic part of the model is based on the primitive Navier–Stokes equations, which are solved by a finite difference scheme. Detailed model descriptions are given in Stele-Hansen and Slagstad (1991) and Slagstad and Mcclimans (2005).

Boundary conditions are generated through a four step nesting procedure using consecutively finer grids from 20,000 m to 4000 m to 800 m to 160 m and finally to 32 m. A model of 32 m horizontal resolution was set up for the area surrounding the ACE facility (Fig. 1). A total of 21 vertical layers were used in the 32 m model domain, with the surface layer up to 3 m deep, and further depths of: 3–3.5, 3.5–4, 4–5, 5–6, 6–7, 7–8, 8–10, 10–12, 12–15, 15–20, 20–25, 30–35, 35–40, 40–50, 50–75, 75–100, 100–150, 150–200, 200–250 and 250–300 m. The greatest depth in the model domain was 279.54 m. The coarser models use up to 42 vertical layers.

Atmospheric forcing data were extracted from the eKlima database (Norwegian Meteorological Institute). Data for the station at Ørlantet (63° 42' N, 9° 36' E; elevation: 10 m) for 2010 and 2011 were used.

3. Results

3.1. Environmental and seston variables

The water temperature ranged between 4 °C in February and 13.6 °C in August, with an average of 7.9 ± 0.5 °C for the entire year (Fig. 2B). On average, the salinity decreased from 28.5% down to 21.7% in July, followed by a rapid increase before leveling off and remaining stable >32% from October over the winter (Fig. 2B).

The hydrodynamic simulations indicated that the current speed of the surface water at the FE100 and the reference stations generally was between 0 and 0.2 m s^-1 (in June 2010, August 2010 and February 2011) (Fig. 3A), with the exception of FE100 in August, when the current speeds were between 0.2 and 0.5 m s^-1 almost 20% of the time. The direction of the surface current was generally from the farm to the FE100 station (Fig. 3B).

The concentration of suspended particulate matter ranged between 5.2 and 11.2 mg L^-1, with an average of 8.4 ± 0.2 mg L^-1 for the entire year (Fig. 4A) and demonstrated a similar pattern of variation as the use of feed at the fish farm, with minimum concentrations in December–January and occasional peaks in September and February. The concentration was significantly positively correlated to the feed use at the fish farm during the autumn–winter period (October–February) (r = 0.53) (p < 0.05). Coincidently, high values of SPM and Chl a during spring (March–May) resulted in a moderate correlation with Chl a for the entire year (r = 0.34) (p < 0.05).

The POC concentrations ranged between 70 and 740 μg L^{-1}, with an average of 266 ± 23 μg L^{-1}, and were negatively correlated to feed use at the fish farm (r = −0.36) for the entire year while, in contrast, the POC concentrations were positively correlated to feed use during the autumn–winter period (r = 0.40) (p < 0.05) (Fig. 4B).

The Chl a concentrations ranged between 0.06 and 18.6 μg L^{-1}, but were generally low, with an average of 2.2 ± 0.5 μg L^{-1} for the entire year, values < 0.5 μg L^{-1} during the autumn–winter period (Fig. 4C), and peak values in May. The Chl a values were positively correlated to POC (r = 0.73) and PON (r = 0.82), and negatively correlated to the feed use at the fish farm (r = −0.32) (p < 0.05). The POM: Chl a ratio (μg/μg) ranged between 0.5 and 107, with peak values during winter when the Chl a concentrations were at a minimum (Fig. 4D). The ratio was moderately correlated to feed use at the fish farm over the year (r = 0.32) (p < 0.05).

3.2. Growth in length

The mussels showed a rapid average growth in length for the five-month period from June to October before the growth leveled off and remained low in winter, followed by a steady increase in the
spring (Fig. 5A). The average growth rate ranged between 0.1 and 125 μm day⁻¹ within single months and the growth was generally high during summer (June–September) and low in autumn and winter (October–February) (Fig. 5A). The growth was significantly higher at the reference station compared to the stations at the fish farm in June–July, whereas the opposite was evident in May–June when the mussels at all stations at the fish farm grew faster than the mussels at the reference station (p < 0.05). The mussels at the reference station grew faster than the mussels at the FE100 station in the autumn–winter period and in April–May, while mussels at the FW and FE stations inside the farm grew faster than the mussels at the FE100 station in November–January and April–May (p < 0.05). A comparison of the different seasons revealed that the mussels at the reference station showed a faster growth in length compared to the mussels at all stations at the fish farm during the summer, while mussels at the FW station grew faster than the mussels at the reference station during the spring (p < 0.05) (Fig. 5B). A comparison of the average growth rates for the entire year revealed that mussels at the reference station grew faster than the mussels at the FW and FE100 stations, while no significant differences were found between mussels at the reference and the FE stations. At the fish farm, mussels at the FW station grew faster than the mussels at the FE section during autumn and at the FE100 station during winter and spring (p < 0.05).

The average growth in length for all stations was strongly correlated to temperature (r = 0.73), moderately to POC (r = 0.53), PON (r = 0.6) and Chl a (r = 0.43) and weakly to SPM (0.37) (p < 0.059), over the entire year. While no correlation was found between the growth in length and feed use over the entire year, there was a significant positive correlation in the autumn–winter period (r = 0.89), when a correspondingly high correlation to SPM was also found (r = 0.53) (p < 0.05).

3.3. Growth in soft tissue

The standardized dry weight of soft tissue matter (DW') ranged between 280 and 722 mg ind⁻¹ with an average of 517 ± 15 mg, and it exhibited a rapid increase in the summer, reaching peak values at the FW and the FE100 stations in September and at the FE and the
reference stations in October, followed by weight losses in November and December (Fig. 6A,B). Occasionally, increases were found in the period from January to May and a major decrease associated with spawning was evident at the FW, FE and the FE100 stations in June. This decrease left the mussels at the reference station with a significantly higher DW at stations at the salmon farm compared to the reference station. ** indicates a significantly higher AGRL at the reference station compared to stations at the salmon farm within months (p<0.05). The average SGRDW was positively correlated to the use of feed at the fish farm (r=0.53) (p<0.05), and the DW of mussels at one or more of the stations at the fish farm was higher compared to the DW of mussels at the reference station in August (at the FW station), September (at the FW, FE and F100 stations), October (at the FE station), December (at the FW, FE and F100 stations) and February (at the FE station) (p<0.05). The average SGRDW was positive at all the stations in summer and negative during autumn. The mussels at the fish farm stations displayed a negative SGRDW in winter and spring while a positive SGRDW was found for mussels at the reference station.

3.4. Incorporation of fatty acids in mussel tissues

3.4.1. PCA

The PCA of fatty acid (FA) profiles indicated a seasonal-dependent incorporation of salmon fish feed in digestive gland and mantle tissue in mussels, identified by an increased content of percent 18:1 (n-9) of total fatty acids. The PCA also revealed an increase in 20:1 (n-9) that could be partly related to the incorporation of fish feed. Mussels at the fish farm was weakly separated from mussels at the reference station in August and strongly in February (Fig. 7A), while no systematic pattern of variation or separation of stations at the fish farm from the reference station was found in June (Fig. 7B). The score plot (Fig. 7A, upper panel) for the digestive gland tissue showed that 92% of the variance in the data was explained by the two first principal components. A similar pattern of variation as in the digestive gland tissue was also found for the mantle tissue. The fatty acid profile changed in the direction of the salmon feed profile from the start in June to August and September (Fig. 7C), whereas no systematic pattern of variation or separation of stations at the fish farm from the reference station was found in June (Fig. 7D). The changes were more pronounced in the direction of the salmon feed signature in February compared to August, although no clear separation was found between the reference station and the stations at the fish farm. The score plot (Fig. 7C, upper panel) showed that 83% of the variance in the data was explained by the two first principal components.

In the digestive gland samples, the loading plots (Fig. 7A, lower panel) confirmed the incorporation of 18:1 (n-9) and 20:1 (n-9) in mussels at the fish farm from the start in June to August and September, 20:5 (n-3) (eicosapentaenoic acid, EPA) and 16:1 (n-7) were the dominant fatty acids at the start, while 18:4 (n-3) and 14:0 contributed more to the total FA profile in mussels in August.
22:6 (n-3) (docosahexaenoic acid, DHA) contributed more to the total FA profile in mussels at the reference station in February, thereby separating the mussels in February from the mussels in August together with 20:1 (n-9), 16:0 and 18:2 (n-6), which also contributed to changing the profile in mussels in August and February from the mussels at the start.

The loading plots also confirmed the incorporation of 18:1 (n-9) and 20:1 (n-9) in August and February in the mantle tissue (Fig. 7C, lower panel), but the pattern was not as evident as for the digestive gland samples (Fig. 7). The FA profile was dominated by EPA and 16:0 at the start, while 20:1 (n-9), 18:2 (n-6) and 18:4 (n-3) contributed more to the total FA profile in mussels in August. As for the digestive gland samples, DHA contributed more to the total FA profile in mussels in February, separating mussels in February from mussels in August in combination with 16:1 (n-7) and 18:1 (n-9).

3.4.2. Digestive gland

The general pattern of variation of the total FA content (mg/g), which reflects the content of total lipids, demonstrated an increase from the start in June until August, before a decrease was found in February and at the end of the sampling period in June (Fig. 8A). The seasonal changes were significant at the FE, FE100 and the reference stations (p<0.05). No differences were found for the total FA content in mussels at any of the stations in August and February. The total FA content was also higher at the FW compared to the FE and the FE100 stations (p<0.05), while no statistical differences were found between the total FA content of the digestive gland tissue of mussels at the FW and the reference station at the end of the sampling period.

The PCA particularly identified 18:1 (n-9), 20:1 (n-9), DHA, EPA and 18:4 (n-3) as the single fatty acids that were most responsible for the difference between the digestive gland tissue of mussels at the start in June and in August and February. The content of 18:1 (n-9) and 20:1 (n-9), as well as 18:2 (n-6), which was present in high amounts in fish feed, exhibited a similar pattern of variation as the total FA (or lipid) content of the digestive gland tissue over the year (Fig. 8A). The content of 18:1 (n-9) in digestive gland tissue was significantly higher in mussels at the FE station compared to the reference station in February, and significantly higher in mussels at the FW station compared to mussels at the FE, FE100 and the reference stations at the end of the sampling period in June (p<0.05). In contrast, the content of 20:1 (n-9) was significantly higher in the digestive gland tissue of mussels at the FW and the FE100 stations compared to mussels at the FW and the reference stations in June (p=0.05), while the content of 18:2 (n-6) was significantly higher in mussels at the FW station compared to mussels at the reference station in February (p<0.05) (Fig. 8A).

The content of 18:1 (n-9) of the total FA in the digestive gland tissue increased from 1.7±0.1% at the start to a maximum of 10±0.3% in mussels at the FE station, which reflected the high content of this FA in the fish feed (39±0.2%), while the contribution of 18:2 (n-6) increased from 1.8±0% to a maximum of 3.4±0.1% at the FW station.
in February, 20:1 (n-9) increased from 1.3 ± 0.0% to an average of 4.5 ± 2.2% for all stations in February, exceeding that of the fish feed (3.1 ± 0%).

3.4.3. Mantle

A similar pattern of variation of 18:1 (n-9), 20:1 (n-9) and 18:2 (n-6) as was found for the digestive gland tissue was also found for the mantle tissue, though with some greater variation (Fig. 8B). The total FA content of mussels at the FW station remained stable over the year, while an increase was found for mussels at the FE and FE100 stations, which resulted in a significantly higher FA content compared to that of mussels at the FW and the reference stations at the end of the sampling period (p<0.05).

4. Discussion

4.1. Food availability

The concentrations of suspended particulate matter at all stations ranged consistently above the threshold level of 4 mg L\(^{-1}\) for pseudo-feces production in mussels of 1 g of soft tissue dry matter (Widdows et al., 1979), suggesting that the SPM concentrations did not restrict mussel growth at any time of the year. Furthermore, the significant correlation between SPM and the feed use at the fish farm during the autumn–winter (October–February) period suggested that the mussels' food availability could be associated with the release of fish feed wastes during this period. The organic content of the SPM, particularly the carbon, is a feed variable that largely determines the amount of surplus energy available for growth (Bayne et al., 1987; Hawkins et al., 1997; Navarro et al., 2003), and because mussels have shown the same absorption efficiency for particulate organic C, N and P, respectively, their growth will depend on the nutritional composition of the absorbed organic matter and how this meets the mussels' nutritional requirements (Hawkins et al., 1997).

As was found for SPM, the POC concentrations correlated well to the use of feed in winter. According to an estimated minimum feed requirement of 240 µg C ind\(^{-1}\) h\(^{-1}\) at 7°C and 570 µg C ind\(^{-1}\) h\(^{-1}\) at 14°C for the weight maintenance of mussels with 500 mg DW of soft tissue matter from this population (Handå et al., in press), the POC concentrations were found to support weight maintenance and/or growth over the entire year. Even the minimum POC concentrations in January (95±11 µg C L\(^{-1}\)) would leave the mussels with a feed intake of 247 µg C ind\(^{-1}\) h\(^{-1}\), given a clearance rate of 2.6 L ind\(^{-1}\) h\(^{-1}\), which has been found for mussels of this size (40 mm) from this population (Handå et al., submitted for publication). Indeed, the temperature in January was 5°C, suggesting a 30% lower temperature-dependent feed requirement of only 168 µg C ind\(^{-1}\) h\(^{-1}\), allowing the mussels to increase their soft tissue weight during winter.

In contrast, the Chl a concentrations remained below 0.5 µg L\(^{-1}\) from October to February, suggesting that the food availability in terms of Chl a might not have met the mussels' energy requirements as a zero net energy balance is found to be sustained in M. edulis by Chl a values between 0.67 and 1.02 µg L\(^{-1}\) (Hawkins et al., 1999). Filtering activity has previously been observed to cease below a Chl a concentration of 0.3–0.6 µg L\(^{-1}\) (Dolmer, 2000; Norén et al., 1999; Røisgård and Larsen, 2001), whereas in a recent study by Stroehmeier et al. (2009), it was demonstrated that M. edulis is capable of clearing particles out of suspension at Chl a concentrations down to 0.01 µg Chl a L\(^{-1}\),
Fig. 8. A) Fatty acid content (mg g⁻¹ dry weight tissue) and contribution of selected fatty acids (% of total FA) to the total fatty acid composition of mantle and digestive gland of mussels at the west (FW) and east (FE) side of the farm, 100 m east (FE100) and at the reference station (RS) 4 km south of the farm in June 2010 and August, February and June 2011 (mean±se, n=3–5). Letters and numbers denote significant differences within months. * indicates seasonal variation between months (p<0.05).
suggesting that mussels can maintain their filtering activity at low phytoplankton concentrations in winter, thereby also maintaining bio-remediative services on fish farm wastes.

4.2. Incorporation of fatty acids in mussel tissues

The PCA suggested a seasonal-dependent incorporation of salmon fish feed in digestive gland and mantle tissues. This was evident from the increased relative content of 18:1 (n-9) in August and February. The response was significantly higher in the digestive gland tissue of mussels at the stations located at the fish farm compared to that at the reference station in February and June. Surprisingly, the content of 20:1 (n-9) was only higher in mussels at the fish farm than at the reference station in June, whereas a similar contribution to the total FA profile in mussels at the fish farm and the reference station was found in August (also for 18:1n-9) and February. This suggests that fish feed fines were filtered out and incorporated more efficiently in August and February, when phytoplankton concentrations were low, compared to after the spring bloom of phytoplankton in June which provided mussels with their primary food source.

Phytoplankton is a natural part of seston that is selected for by M. edulis prior to ingestion (Kjærboe and Møhlenberg, 1981), and mussels exhibit clearance and selected incorporation among various phytoplankton species and other organic and inorganic particles (Bougrier et al., 1997; Defossez and Hawkins, 1997; Kjærboe et al., 1980; Newell et al., 1989; Prins et al., 1991, 1994; Rouillon and Navarro, 2003). The criteria for selection are not fully known, although chemical composition, shape and size have all been suggested to play a role (Jørgensen, 1996; Ward and Targett, 1989).

The selection of phytoplankton over other organic matter in this study can be identified from the incorporation of certain fatty acids that are found in high amounts in e.g. diatoms and/or dinoflagellates. The high content of EPA and 16:1 (n-7) in digestive gland tissue and 16:0 and EPA in mantle tissue at start in June and the high content of 18:4 (n-3) in August, reflected the typical phytoplankton composition of the spring and an autumn bloom in the Trondheimsfjord area (Sakshaug and Myklestad, 1973). Based on the measured Chl α concentrations in the present study, blooms occurred in March and August, while the spring bloom was prolonged and reached its peak values in May in connection with the spring flood of river discharges that were evident from the significant decrease in salinity.

The spring bloom is based on stored nutrients in deep water, as a result of vertical mixing and low nutrient consumption over the winter, and is dominated by diatoms, while the autumn bloom is related to freshwater discharge and vertical mixing and tends to be dominated by dinoflagellates and other small flagellates (Sakshaug and Myklestad, 1973; Sakshaug and Olsen, 1986). EPA, 16:1 (n-7) and 16:0 are typical fatty acids in diatoms (Kates and Volcani, 1966; Reitan et al., 1994), whereas the contents of 18:4 (n-3), 16:0, C18:5 (n-3), EPA and DHA is high in dinoflagellates (Harrington et al., 1970). Accordingly, the high content of 16:1 (n-7) in digestive gland tissue, 16:0 in mantle tissue and EPA in digestive gland and mantle tissue at the start suggested that diatoms accounted for the larger part of the diet in spring, while the increased content of 18:4 (n-3) in digestive gland tissue in August suggested a shift towards a dinoflagellate-dominated diet during summer. The significantly higher content of 18:1 (n-9) in digestive gland tissue of mussels at the FE and of 18:2 (n-6) at the FW station compared to the reference station in February suggested an incorporation of fish feed in mussels at the fish farm in winter, whereas the general increase of 18:2 (n-6) as well as 18:4 (n-3) in August could be related to mussels feeding on flagellates, which has a high content of these FA (Hamn et al., 2001; Reitan et al., 1994) and which has been found in high densities in the Trondheimsfjord (Haug et al., 1973).

The high content of DHA in the digestive gland tissue of mussels at the reference station in February suggested that DHA did not originate from the fish feed. The high content of 18:1 (n-9) and 20:1 (n-9) in August could also originate from phytoplankton and even zooplankton, in which these FA can be present in high amounts (Sargent et al., 1981 and references therein). However, although mussels have been found to filter out various sizes of nauplii and copepodite stages of zooplankton (Davenport et al., 2000; Lehane and Davenport, 2002; Lehane and Davenport, 2004, 2006; Molloy et al., 2011), it should be questioned and further investigated if early stages of copepods could be a temporary food source of mussels.

The results of our study were consistent with previous findings that phytoplankton is the main component of the mussels’ diet (e.g. Fernández-Reiriz et al., 1996; Garen et al., 2004; Smaal and Stralen, 1990; Strohmeier et al., 2005, 2008; Wildish and Miyares, 1990) and that other organic particles may also constitute an important part of the diet, particularly when phytoplankton concentrations are low (Arimin and Bendell-Young, 1997; Bayne et al., 1993; Grant and Bacher, 1998; Handå et al., 2011). In a low seston environment, e.g. in winter, mussels can alternate between two different strategies to take maximum advantage of the available organic matter (Arimin and Bendell-Young, 1997; Bayne et al., 1993). When the SPM is high, the mussels select for particles with a high organic content (e.g. phytoplankton > fish feed > feces), resulting in an increased organic content in the consumed feed relative to that of SPM. Inorganic particles are likely rejected (Iglesias et al., 1996). When the particle concentration is low, however, there is no such strong selection of food items even if the food has a poor organic content, which leaves mussels feeding on both organic and inorganic materials. Although feces from fish has been suggested to be less suitable for supporting mussel growth compared to fish feed (Handå et al., submitted for publication), mussels are likely to have a low selection coefficient in winter, leaving both fish feed and feces to be filtered out with a high efficiency.

4.3. Growth in length and soft tissue

The maximum growth in length was found when the water temperature peaked in August–September and was accompanied by strong vertical mixing and a corresponding autumn bloom of phytoplankton, after which the growth decreased in autumn and remained low in winter. A mean peak in average length growth of 120–125 μm day⁻¹ at the reference station in June–July and at the reference station and the stations at the west side of the farm (FE and FE100) in August–September is considered high, while the average growth rates in spring (25 μm day⁻¹) and summer (79 μm day⁻¹) were comparable to the previously reported growth rate for farmed mussels of a similar length (38–65 μm day⁻¹) during this period of the year in the landlocked Koeit Bay, located close to the study area (Handå et al., 2011).

The growth in length was strongly correlated to temperature and weakly to SPM over the entire year, whereas a significant correlation was found to the feed use at the fish farm in the autumn–winter period (October–February), hence supporting the results from the PCA of the fatty acid composition showing that fish feed contributed most to the mussels' diet during autumn and winter, when phytoplankton concentrations were low, in agreement with Troell and Norberg (1998).

The growth in length was significantly higher for mussels growing on the west side (FW) of the farm in spring compared to the other stations close to the farm (FE and FE100) and at the reference station, while mussels at the reference station grew faster than mussels at the fish farm during the summer. A comparison of growth in length over the entire year revealed that mussels at the FE station exhibited equal growth rates compared to the mussels at the reference station, while mussels at the reference station grew faster than the mussels at the FW and the FE100 stations, which was mainly due to the significantly faster growth at the reference station in June–July.

Mussels at the reference station grew significantly faster than the mussels 100 m away from the salmon farm (FE100), though not significantly faster than the mussels at the east side of the farm (FE) over the year. Moreover, mussels at the west side (FW) grew faster than mussels
at the FE100 station in autumn, winter and spring. The results suggest that the placement of mussels should be firmly located in IMTA systems and that the distance between the fish and mussels should probably be less than the 120 m, as was the distance to the FE100 station, to enable mussels to perform bioremediative services on salmon farm wastes under environmental conditions such as the ones studied.

In agreement with the growth in length, the specific growth rate of DW′ peaked in August–September followed by a period with decreasing DW′ in autumn and winter, although with quite a bit of variation. The DW′ was significantly correlated to feed use at the fish farm and the general pattern of variation found showed a higher DW′ for mussels at the fish farm in autumn and winter, while the DW′ of mussels at the fish farm and the reference station was more equal in spring and highest at the reference station in June, most likely because of a more distinct spawning in mussels at the fish farm and thus a more negative SGRDW′ compared to mussels at the reference station in spring. Furthermore, the significantly higher DW′ at the FE at the beginning of the winter period resulted in a negative SGRDW′ for mussels at this station in winter, although the DW′ of mussels at the FE station was significantly higher than that of the mussels at the reference station both in December and February. This suggested that the monthly measured DW′ was more representative for growth in weight than the average of the four seasons (SGRdw′) due to large fluctuations within each period, while the four seasons represented the growth in length in a good way. The DW′ of mussels at the fish farm was particularly higher compared to that of mussels at the reference station at the FW station in August, the FW, FE and FE100 stations in September and December and at the FE station in October and February, thereby suggesting that the mussels at these stations at the fish farm used less of their energy storage in the autumn and winter months compared to mussels at the reference station.

4.4. Implications for integrated multi-trophic aquaculture in Norwegian coastal waters

The results of our study suggested a higher soft tissue content of mussels cultivated in integration with salmon farming than in monocultures during autumn and winter when ambient food availability in terms of Chl a are low. This is in agreement with Stirling and Okumus (1995) who examined blue mussels in two lochs in western Scotland and found that growth in both soft tissue and shell length of mussels kept in close proximity to salmon farms were slightly, but significantly higher compared to control mussels located further away from the farms, and that the mussels at the farms used less of their energy storages during winter. A faster growth in length of mussels in close proximity to fish farming has also been reported by Wallace (1980), Peharda et al. (2007), Lander et al. (2004) and Sara et al. (2009). Wallace found that mussels at a salmon farm in northern Norway grew faster and did not show marked growth-stoppage rings during winter compared to mussels cultivated without any influence from salmon farming, while Sara et al. examined Mytilus galloprovincialis grown up- and downstream a sea bass (Dicentrarchus labrax) and a sea bream (Sparus aurata) farm in the Mediterranean and found a faster growth in shell length and an increase in total biomass of the mussels situated downstream of the farm compared to mussels at upstream locations associated with significantly higher organic matter and Chl a concentrations downstream the farm. In contrast, Taylor et al. (1992) studied the influence of salmon farms on growth of M. edulis in British Columbia and found that the distance from the salmon farm did not have any significant influence on the mussel’s food availability. Except for that the incorporation of 18:1 (n-9), which was found in high amounts in fish feed, suggested that salmon fish feed constituted a larger part of the mussel’s diet in winter than during spring and summer, no increased concentrations of suspended particulate matter or Chl a at the fish farm compared to the reference station was neither found in our study. In another study of co-cultivation of salmon and mussels (M. edulis) in Australia, Cheshuk et al. (2003) found only some minor and not significant differences in growth of mussels situated in 70 and 100 m distance from salmon cages compared to mussels cultivated 500 and 1200 m away. Navarrete-Mier et al. (2010) studied co-cultivation of oyster (Ostrea edulis) and mussels (M. galloprovincialis) cultivated in close proximity to a sea bass (D. labrax) and sea bream (S. aurata) farm in the Mediterranean and found no differences in growth in length or soft tissue of mussels cultivated at a distance of 0 to 1800 m away from the farm. They did neither find any increase in food availability nor incorporation of components of fish feed in mussel tissue when using stable isotope ratios as tracers. Some possible explanations for this lack of measureable interactions of the co-cultivation have been addressed by Troell et al. (2011). Notwithstanding, despite the many case studies of the combined cultivation of fish and bivalves, there seem to be more differences than generalities making up the state of the art for using bivalves in open ocean IMTA.

Varying results and the lacking quantification of the assimilative capacities of farm-derived wastes by filter feeders under ambient culture conditions may explain why, although IMTA has been practiced for centuries in Asian countries to increase the carrying capacity of intensively cultured sea areas, there is still a wait-and-see attitude to this concept of ecological engineering for the purpose of reducing the environmental impact of intensive fish farming in open water in the western world (Chopin et al., 2008). The reluctance is reflected by the absence of continuous research and technological development aiming at designing sophisticated systems for integration of species at lower trophic levels with today’s intensively fed monocultures of fish. Based on the existing literature it seems that up-scaling of pilot experiments is a prerequisite for the quantification of the potential for bioremediative services of co-cultivation under various environmental conditions. Blue mussels have been shown to filter small particles of salmon fish feed (MacDonald et al., 2011; Reid et al., 2010) and feces (Reid et al., 2010), while changes in fatty acid composition have been used to demonstrate an incorporation of fish feed components in bivalves (Gao et al., 2006; Redmond et al., 2010). Redmond et al. (2010) showed, by using stable isotopes and fatty acids as tracers, that blue mussels can incorporate components of salmon fish feed, and several studies have identified the incorporation of fish farm-derived waste products in bivalves, thereby suggesting that bivalves can perform bioremediating services when integrated with fish farming (e.g. Gao et al., 2006; Mazzola and Sara, 2001; Peharda et al., 2007; Soto and Mena, 1999). Accordingly, the capability of bivalves to incorporate components of fish feed has been well documented, while, on the other hand, little is known about the incorporation of components of fish feces. The waste particulate food source for mussels to feed on in IMTA with salmon has been seen as the particular part of both feed and feces. In this study we only focused on the incorporation of components of the fish feed and not of feces. Considering that feed wastes probably account for less than 5% of the feed use in modern cage aquaculture of salmon (Mente et al., 2006), and that most of the particles probably sinks rapidly to the seafloor, the possibility of growing mussels on this part of the wastes seems challenging. However, the results from the present work indicated a seasonal incorporation of particulate wastes of salmon fish feed in M. edulis kept in close proximity to the fish suggesting that a part of the feed waste is available for mussels to feed on. The largest salmon farms are currently producing 12,000 t of fish per year, with a corresponding feed use of 13,800 t (feed conversion ratio = 1.15). Given a theoretical 5% feed loss constitutes 690 t of particles or 345 t particulate organic carbon from a single farm from which a part can be utilized by mussels. Moreover, a 5% feed loss from the Norwegian salmon production of 0.84 million tons in 2009 (FAO, 2011) comprises 49,500 t of particles, which have the possibility to be utilized by filter- and deposit feeders in IMTA. Furthermore, on a single-farm scale, the bioremediative capacities of mussels must be considered according to their capability of filtering out fish feed and feces. On a regional scale, mussels can still contribute to balancing the nutrient concentrations in, e.g. a fjord system, by filtering
out phytoplankton that has accumulated anthropogenic N from fish farming. Sheltered sites, e.g. fjords with a low current velocity, uniform currents and a long water retention time, have the potential for increased phytoplankton growth within the IMTA system. However, a low current speed is a disadvantage regarding feed wastes in that it will sink rapidly below the fish cages, thus constituting a negligible contribution to mussel growth at such sites. On the other hand, the feed particles at exposed sites will form a larger part of the food availability for mussels, whereas the currents will dilute and transport waste nutrients away so quickly that phytoplankton growth will take place outside the IMTA system. In any case one has to also take the seasonality of the mussels nutrient removal and biodeposit rates into account (see Newell, 2004 and references therein). Deposit rates of e.g. nitrogen have been estimated at equal amounts to that of the harvested biomass from mussel farms (Lindahl et al., 2005). The careful monitoring of Chl α levels, in combination with modeling of the local current conditions and the corresponding nutrient dispersal patterns downstream from salmon farms, can be a useful tool to localize possibly high productive areas with increased phytoplankton growth at a distance from the fish farm. Mussel production in such areas has the potential to contribute in equal terms to traditional IMTA in terms of ecosystem services, taking into consideration the indirect removal of anthropogenic nutrients from fish farming.

5. Conclusions

The incorporation of 18:1 (n-9), which was found in high amounts in fish feed, suggested that salmon fish feed constituted a larger part of the mussel’s diet in winter than during spring and summer. The growth in length and soft tissue matter of the mussels was closely related to season while the localization of mussels at the fish farm versus at the reference station was of minor importance to the result. Meanwhile, five months during autumn and winter with a higher soft tissue weight for mussels at the fish farm supported a seasonally-dependent utilization of salmon farm wastes for maintenance and growth of soft tissue matter. The incorporation of components of salmon fish feed and feces and the growth of mussels under different environmental conditions should be further assessed to elaborate the possibility for integrating salmon-mussel production along the Norwegian coast.

6. Concluding remarks

The results from the present study are based on an experimental design involving only a limited amount of mussels not allowing for a general consideration of the potential contribution of a combined production of mussels and salmon to mitigate a potential environmental effect of particulate nutrient wastes from salmon farming. Accordingly, the upscaling of cultures at lower trophic levels in IMTA with salmon is essential to further assess the potential for mitigating the environmental effects of farm-derived nutrient wastes, in addition to obtaining increased growth of species at lower trophic levels in IMTA in e.g. cool temperate North Atlantic waters. For example, the upscaling can take place at existing salmon farms: anchoring frames for fish cages are typically 100 × 100 m, and provide a 1 ha submerged frame that can easily be modified to provide anchoring at desired depths from which a floating structure, e.g. a collar for fish nets, can be used to produce mussels or seaweed in IMTA with salmon.

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References

Dolmer, P., 2000. Feeding activity of mussels Mytilus edulis in different temperature, e.g. a collar for fish nets, can be used to produce mussels or seaweed in IMTA with salmon. The incorporation of 18:1 (n-9), which was found in high amounts in fish feed, suggested that salmon fish feed constituted a larger part of the mussel’s diet in winter than during spring and summer. The growth in length and soft tissue matter of the mussels was closely related to season while the localization of mussels at the fish farm versus at the reference station was of minor importance to the result. Meanwhile, five months during autumn and winter with a higher soft tissue weight for mussels at the fish farm supported a seasonally-dependent utilization of salmon farm wastes for maintenance and growth of soft tissue matter. The incorporation of components of salmon fish feed and feces and the growth of mussels under different environmental conditions should be further assessed to elaborate the possibility for integrating salmon-mussel production along the Norwegian coast.


