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Seasonal- and depth-dependent growth of cultivated kelp (*Saccharina latissima*) in close proximity to salmon (*Salmo salar*) aquaculture in Norway

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ABSTRACT

The interest to develop an industrialized cultivation of several macroalgae in Europe is growing rapidly. Here, we demonstrate cultivation of the sugar kelp *Saccharina latissima* in integration with salmon (*Salmo salar*) aquaculture in a Norwegian coastal area. Sporophytes of *S. latissima* were deployed at 2, 5 and 8 m depths at a salmon farm and at a reference station 4 km away in August, November, February and June (2010–2011). The growth was good in late autumn and in spring, with peak lengths of the sporophytes in June, while being poor in winter and summer. As a result of a faster initial growth at the fish farm than at the reference station from August to November, the August-sporophytes reached a significantly longer length than those at the reference station in 5 out of 10 sampling months at 2 m depth, and in 9 out of 10 months at 5 m depth ($p < 0.05$) over the year, while no significantly different lengths were found at 8 m depth. The November-sporophytes showed similar growth rates and lengths at the fish farm and at the reference station, while the February-sporophytes grew faster at 5 and 8 m depths at the fish farm than at the reference station, with significantly longer blades at 5 m depth at the fish farm than at the reference station at peak lengths in June ($p < 0.05$). The sporophytes deployed in June did not survive the summer. Holding the rapid growth of *S. latissima* in spring and early summer together with the increase in salmon biomass and feed use in late summer and early autumn suggested a seasonal mismatch considering direct recycling of the nutrient input from salmon farming by macroalgae in Norwegian coastal waters.

1. Introduction

While macroalgae traditionally have been cultivated at large scale for food and other purposes in Asian countries (Murata and Nakazoe, 2001; Nisizawa et al., 1987), the interest in European countries has been low. However, new trends and possibilities for multiple uses such as food and bioactive components of functional foods, phycocolloid production and biofuels, among other applications, in addition to bioremediation services (Bixler and Porse, 2012; Buschmann et al., 2008; Holdt and Kraan, 2011; Kraan, 2010; Troell et al., 2009) have increased the interest of industrializing the cultivation of macroalgae also in Europe. In Norway, a year-through production of zoospores and juvenile sporophytes of the sugar kelp *Saccharina latissima* on culture ropes has been demonstrated by Forbord et al. (2012), and attention is now directed at developing efficient farming techniques and new technologies for cultivation of macroalgae in the sea.

The brown algae *S. latissima* is a short-lived perennial species with a seasonal development that includes a period of maximum growth in the first half of the year followed by a period of reduced growth during summer, with sorus (sporangia) formation from late autumn to early winter (Parke, 1948; Kain, 1979; Lüning, 1979; Bartsch et al., 2008). Adult sporophytes typically have a life span of 2 to 4 years, although plants may occur as annuals. *S. latissima* is distributed circumpolar in the northern hemisphere, and the natural growth sites are clear and turbid coastal waters (Borum et al., 2002). It occurs from the intertidal down to the bottom of the photic zone; as a result *S. latissima* is exposed to a wide range of temperature and light conditions (Gerard, 1988), suggesting plasticity for photoinhibition responses (Bruhn and Gerard, 1996). Accordingly, *S. latissima* holds qualities that make it an attractive candidate species for an industrialized cultivation in Norway.

In temperate marine ecosystems, inorganic nutrients are abundant mainly during winter and early spring, before the phytoplankton depletes the nutrients in the surface layer from late spring leading to

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nutrient limitation all through the summer period (Frette et al., 2004; Paasche and Erga, 1988). However, in areas with intensive fish farming, inorganic nutrients may become available in higher amounts as a result of an increased nutrient emission rate from fish farms during the warm season (Mente et al., 2006; Wang et al., 2012). Ammonium, which is the principal excretory product from protein metabolism in fish, can thus represent a significant nitrogen source for macroalgae in close proximity to fish cages at this time of the year if ambient nitrate concentrations are low (Ahn et al., 1998; Sanderson et al., 2008). Furthermore, ammonium may be a more efficient source of nitrogen for macroalgae than nitrate, especially under light limited conditions, since it may be taken up passively and does not have to be reduced to nitrite in the cytoplasm (Harrison and Hurd, 2001).

In 2011 the Norwegian production of Atlantic salmon (*Salmo salar*) and trout (*Oncorhynchus mykiss*) amounted to 1,118,341 tons (Norwegian Directorate of Fisheries, 2011), with an estimated use of 1,286,092 tons of fish feed (feed conversion ratio = 1.15). The mean nutrient release to the environment from Norwegian salmon aquaculture is estimated to 70% C, 62% N and 70% P of the total C, N and P input of feed, respectively (Wang et al., 2012). About 44% of the P and 15% of the N in the feed are released in particulate form, while 21% P and 45% N are released in a dissolved inorganic form. Parts of the particulate nutrients originating from feed losses and feces can be consumed by organic extractive filter feeding species such as mussels (Handå et al., 2012; Redmond et al., 2010), while parts of the dissolved inorganic nutrients can be taken up by inorganic extractive species such as seaweeds (Buschmann et al., 1994; Chopin et al, 2001). Thus, there is likely a potential for a combined production of mussels and macroalgae in integrated multi-trophic aquaculture (IMTA) with salmon and trout in Norway.

The main objective of this study was to compare the growth responses of *S. latissima* cultivated in close proximity to Atlantic salmon (*S. salar*) aquaculture with that of sporophytes kept at a control site in coastal waters off the coast of Central Norway. Secondary objectives were to study seasonal- and depth-dependent growth and the seasonal variation of the chemical composition of *S. latissima*.

2. Material and methods

2.1. Sampling program and site description

Temperature, salinity, chlorophyll *a* (Chl *a*), photosynthetically active radiation (PAR), Secchi depth, ammonium and nitrate were measured monthly (12 sampling months), while the length and width of the blade of cultivated *S. latissima* sporophytes were measured monthly except for in November and July (10 sampling months), from the 19th of August 2010 to the 11th of August 2011 at a salmon farm at Tristein (63° 52′ N, 9° 37′ E), off the coast of Central Norway and at a reference station 4 km south of the farm (Fig. 1A). The fish farm consisted of eight Polarcirkel plastic cages (Fig. 1A), each with a circumference of 157 m, with 15 m deep net pens and a volume of 36,000 m^3. The total salmon production in the sampling period was 4.705 tons, with a corresponding feed use of 5.216 tons (Fig. 1B). The macroalgae were cultivated in an empty sea cage on the west side of the farm with a distance of approximately 60 m to the nearest cage with salmon. The western side of the farm was situated above the 50 m isobath, with the bottom sloping steeply down to approximately 100 m depth on the eastern side. Although the farm was situated approximately 4 km off shore of the main land, it was partly sheltered from the open ocean by the skerries of Tristeinen on the western and northern sides. The study area surrounding the Tristeinen skerries and the reference station is dominated by marine waters (salinity <34.5‰) from the Norwegian coastal current, with temporary outflows of slightly fresher surface water from the Trondheimsfjord after the snow melt in the spring and as a result of stratifications in autumn.

2.2. Environmental conditions, ammonium, nitrate and Chl a

The temperature, salinity and Chl *a* were measured at 2, 5 and 8 m depths using a CTD with an external fluorometer (SD 204, SAIV Environmental Sensors and Systems LTD, Norway), while single-point measurements of PAR were taken at midday at the surface and at 2, 5 and 8 m depths with a LI-192 Underwater Quantum Sensor and a LI-1400 DataLogger (LI-COR, Inc.). Daylength was calculated using the standard formula (e.g. Sakshaug et al., 2009). Secchi depth was measured by lowering a circular white plate (Secchi disk) until it was not visible.

Samples for the analysis of ammonium (NH_4^+-N) and nitrate (NO_3^--N) were taken from integrated water samples of 0–8 m depth by mixing four consecutive samples (0–2, 2–4, 4–6 and 6–8 m) taken with a Ramberg water collector (length 2 m, volume 5 L) in a bucket prior to subsampling and further treatments. The water samples were pre-filtered with a 200 μm net prior to a second filtration on pre-combusted, acid washed Whatman GF/F filters. The concentration of NH_4^+-N and NO_3^--N was determined in parallel with a Fluorescence Detector (DFL-10) (Kerouel and Aminot, 1997) and a flow analyser with O.I. Analytical cartridge Part A002603, respectively.

Currents were not measured, and water samples for nutrient analyses were collected on a monthly basis only. In order to predict the ambient nitrate concentrations and the average distribution of nitrogen from the fish farm we therefore used the coupled 3D hydrodynamic–ecological model system SINMOD (Slagstad and McClimans, 2005; Wassmann et al., 2006). The model was used to indicate data on nitrate and ammonium over the sampling period for comparison with the measured concentrations, and thereby to, as far as possible, meet the difficulties of measuring the dynamics of 'Ghost nutrients' from fish farms in marine coastal waters (Pitta et al., 2009). The ecological component includes state variables for phytoplankton, nitrate and ammonium concentration. The 3D hydrodynamic–ecological model was run from August 15, 2010 to June 15, 2011, using a model domain of 160 m horizontal resolution in the region around the study area. Details for the simulations are given in Broch et al. (2013).

Figures for dissolved inorganic nitrogen (DIN) effluents from the fish farm were calculated from monthly reports on feed usage and corrected biomass production using a mass balance model (Wang et al., 2012) (Fig. 1B). In the simulations, a constant DIN effluent for each month was assumed. The vertical behavior of salmon kept in sea cages is driven by trade-offs between multiple environmental variables typically in the order feeding > temperature > light at moderate levels of dissolved oxygen (>85%) (Oppedal et al., 2011a), leaving the fish to crowd below 2–3 m depth (Oppedal et al., 2011b). Based on this, DIN was released from four model grid cells between 5 and 15 m depths and added to the NH_4^+-N model component (Fig. 2).

2.3. Sorus induction and seeding of ropes

Non-sporogenous individuals of *S. latissima*, first- or second- year sporophytes, were sampled at Vanvikan in Trondheimsfjorden (63° 52′ N, 9° 37′ E) in the sublittoral zone in April, July, and October 2010 and in February 2011. The lowermost portion of the sporophyte with stipe and haptera was cut off, and the blade was transferred to tanks for sorus induction according to Forbord et al. (2012). Induced sorus portions were used for release of zoospores and seeding of 6-mm ropes for cultivation of juvenile sporophytes. The ropes were incubated on horizontal plates in 30 L tanks at 10 °C with continuous water exchange of nutrient-rich deep water (0.5 L min^{-1}) from day 2 after seeding under a 12:12 light/dark cycle. The surface PAR was 30 μmol photons m^{-2} s^{-1}, and the ropes were situated 10 cm below the surface.

2.4. Cultivation at sea

When the juvenile sporophytes had reached a length of around 5 mm after 7–8 weeks, the seeded ropes were deployed at the fish

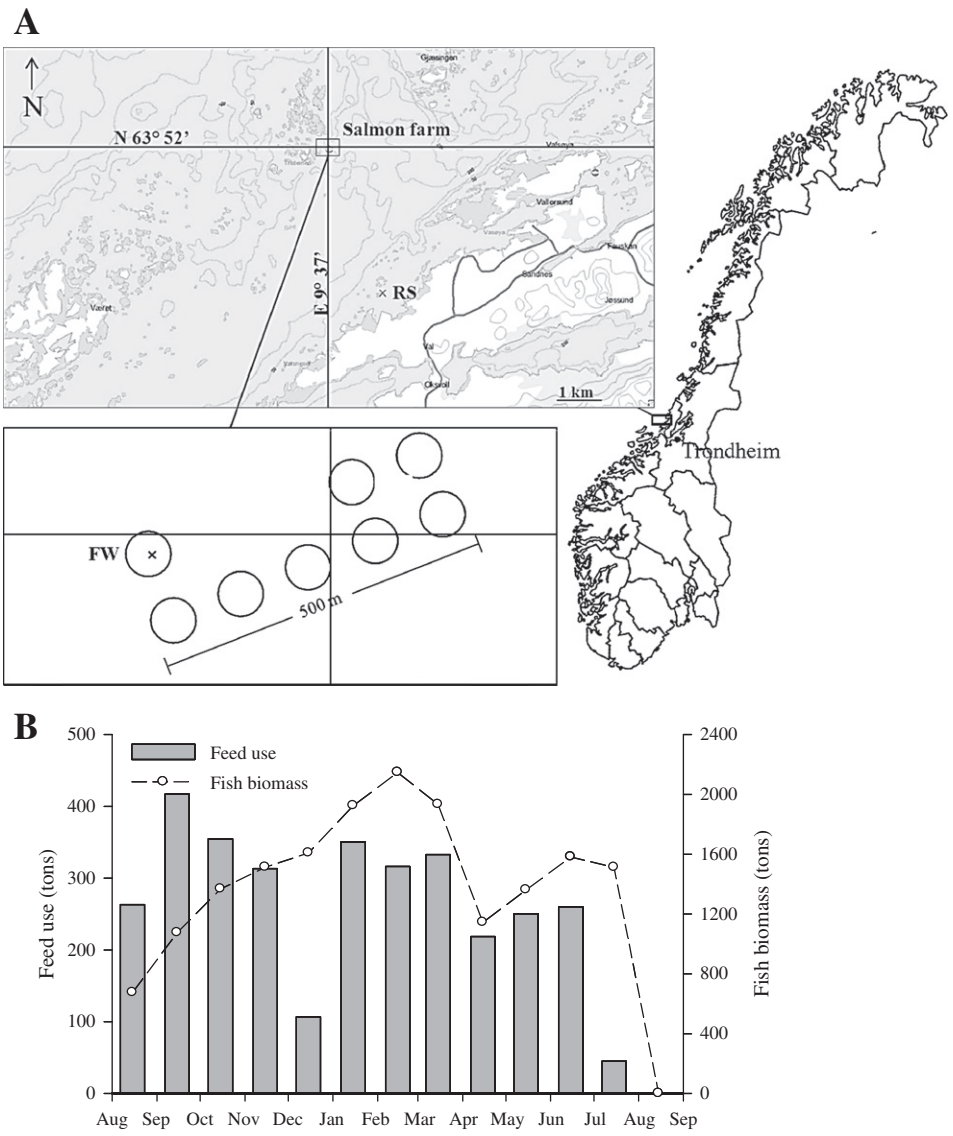

Fig. 1. A) Geographical location of the salmon farm and the experimental stations at the west side of the farm (FW), and at the reference station (RS) 4 km south of the farm at Tristein in Central Norway. B) Fish biomass (right axis) and feed use (left axis) from August 2010 to August 2011. The low feed use in December was due to delousing of the salmon.

farm and at the reference station. Sporophytes were deployed in August 2010, November 2010, February 2011 and June 2011. The August-sporophytes were cultivated on 1 m long ropes (n = 3) attached at 2, 5, and 8 m depths on a vertical 10-mm carrier line, while the November-, February-, and June-sporophytes were cultivated vertically down to 8 m depth using only the 6 mm rope without carrier line (n = 3). The length (L) and width (W) of the blade of all the sporophytes on a part of the rope (n = 10–20) at 2, 5, and 8 m depths on 3 ropes were measured in situ each month with an accuracy of 0.5 cm with a metres. The specific growth rate (μ, d^{-1}) in L ($SGR_{L'}$) was calculated by the equation:

$$\mu = (\ln L_t - \ln L_0)/t \quad (1)$$

where L_0 and L_t are the length 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:

$$P = 100 \times (e^{\mu} - 1). \quad (2)$$

2.5. Chemical composition

Carbon and nitrogen contents and carbohydrate composition of alginate, laminaran and mannitol were analyzed for the whole blade from April to September. Mixed samples of sporophytes (n = 3) from 2 to 5 m depths were stored at −80 °C until freeze drying. The dry kelp was milled (MFC mill, Janke & Kunkel) into a fine powder which was used for further analysis. Samples of freeze dried kelp were transferred to tin capsules and carbon and nitrogen was analyzed in parallels with a Carlo Erba element analyzer (model 1106).

Alginate and laminaran were determined in parallels using a plate reader at 230 nm for alginate and 540 nm for laminaran (Epoch, Bergman) according to Østgaard (1992) and Adams et al. (2011), respectively. To determine the content of mannitol, grounded kelp (20 mg) was first extracted in a $CaCl_2$ in D_2O solution (2% w/v, 1 mL, with 2 mM 2,2,3,3-tetradeutero-3-trimethylsilylpropionic acid as an internal reference) at 60 °C. The kelp powder was then weighed into Precellys24 homogenizing tubes (2 mL) with ceramic beads (1.4 mm, ~500 mg) and added preheated $CaCl_2$ solution (60 °C), homogenized in a Precellys24 bead homogenizer, kept at 60 °C for 15 min and

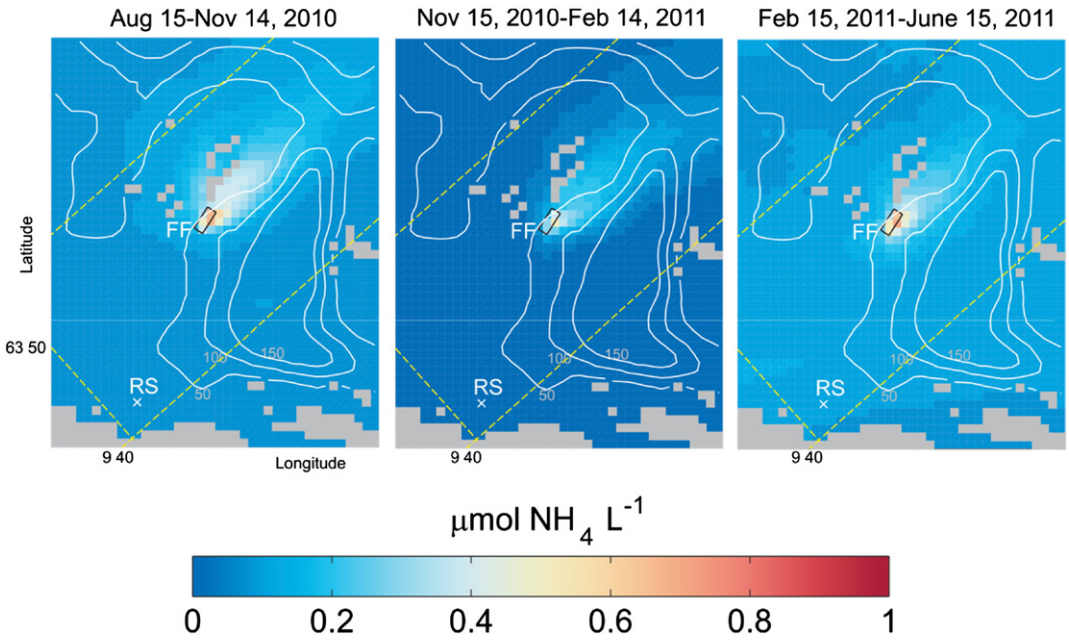

Fig. 2. Simulated concentrations of NH_4-N, including the effluent from the fish farm, in the area around the fish farm: averages over three periods (columns) at three depths (rows). The white curves are 50, 100 and 150 m isobaths. The dashed yellow lines indicate latitude and longitude (lower left corner). FF: fish farm. RS: reference station. The black rectangle outlines the fish farm mooring frame.

homogenized again. The resulting homogenate was centrifuged, and 500 μL of the supernatant was transferred to a nuclear magnetic resonance (NMR) tube (5 mm). NMR analysis was undertaken using a Bruker DRU 600 spectrometer (Bruker GMBH, Rheinstetten), using either BBO or QCI probes. Spectra were recorded with water suppression using a pulse program with 8 scans per sample before mannitol was quantified using Chenomx (Chenomx, Edmonton) software.

2.6. Data analysis

Data for length and width 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 the sporophytes length and width between sampling depths and between the station at the fish farm and the reference station were tested using a one-way ANOVA followed by post hoc comparisons by Tamhane's T2, not assuming equal variances. A one-way ANOVA was also used to test the equality of means for PAR between the different depths. The significance limit was set at 0.05 and the means are given with the standard error. A Pearson's correlation analysis was performed on ammonium-N concentration versus feed use and for growth in length and length:width ratio versus daylength, respectively, for the August sporophytes. Statistical analyses were performed using SPSS (rel. 19.0, SPSS Inc.).

3. Results

3.1. Environmental conditions, ammonium, nitrate and Chl a

Mean temperature and salinity were the same at the fish farm and at the reference station. The water temperature ranged between 4 °C at all depths in February and 13.6 °C at 2 m depth in August, with an average of 7.1 ± 0.9 °C at 2 m depth, 7.0 ± 0.9 °C at 5 m depth and 6.7 ± 0.8 °C at 8 m depth for the entire year (Fig. 3). No depth-dependent differences were measured in temperature during autumn, winter and spring. On average, the salinity increased from 27.3‰ at all depths in August to 32.4‰ in October and remained between 32.0 and 33.6‰ over the winter. In May, a more pronounced dip was measured at 2 and 5 m depths compared to 8 m depth before a peak value of 34.2‰ was found at all depths in June.

Monthly single-point measurements of the surface irradiance at the fish farm ranged between ~13 μmol m^{-2} s^{-1} in December and ~1370 μmol m^{-2} s^{-1} in May and June, with average PAR being significantly higher at 2 m depth (60 ± 4%) compared to 5 m (33 ± 4%) and 8 m depths (20 ± 3%), and significantly higher at 5 m depth compared to 8 m depth over the entire year ($p < 0.05$). The daylength decreased from 17 h at the start of the experiment in August to a minimum of 4 h in December before it increased in winter and spring to a maximum of 20 h in late June. The Secchi depth transparence at the farm was similar to that at the reference station and showed an increase from 7 m depth in August to 17 m depth in January followed by a decrease in late winter and spring, reaching a minimum level of 6 m in May.

The measured ammonium-N concentrations ranged between 0 and 1.1 μmol L^{-1}, with an average of 0.42 ± 0.1 μmol L^{-1} at the fish farm and 0.36 ± 0.1 μmol L^{-1} at the reference station (Fig. 4A). While no correlation was found between the feed use and the ammonium-N concentrations at the reference station, there was a significantly positive correlation at the fish farm ($r = 0.64$; $p < 0.05$). The model simulations indicated higher average ammonium concentrations at the fish farm than in the surrounding waters at 5 m depth (Fig. 2) for the three periods indicated. Simulated depth integrated ammonium concentrations

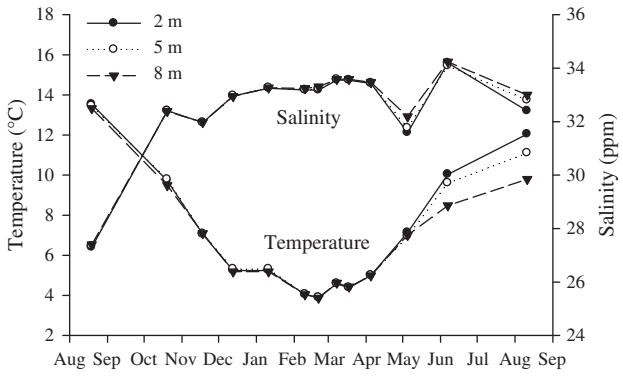

Fig. 3. Temperature and salinity at the salmon farm.

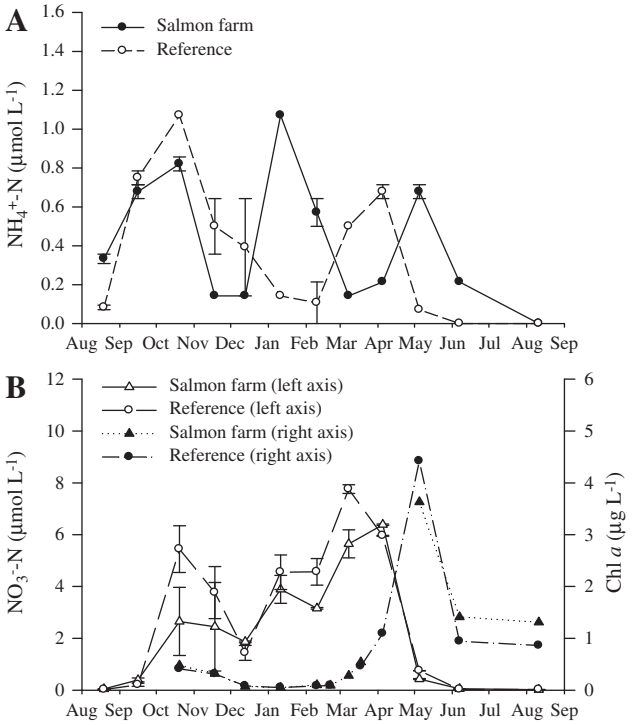

Fig. 4. A–B) Ammonia and nitrate (0–8 m mixed samples) and Chl *a* (average of in situ measurements at 2, 5 and 8 m depths) at the salmon farm and at the reference station 4 km south of the farm from August 2010 to August 2011.

at the fish farm rarely exceeded 1 µmol L^{-1}. This indicates that measured and simulated values were of similar magnitude. The concentrations were highest within or just outside the farm, and decreased rapidly away from the farm. In the simulations, the NH_4^+-N released from the fish farm was diluted by tidal and local currents and transported mainly northwards, as well as taken up by phytoplankton. Although the model shows a main current direction over the year, and that the discharge did not influence the concentrations at the reference station, local hydrodynamic conditions will transport the nutrient discharge back and forth on a local farm-scale also reaching the cultivated macroalgae in the most southern cage.

The nitrate-N concentrations ranged between 0.05 and 6.4 µmol L^{-1} at the salmon farm, with an average of 2.3 ± 0.7 µmol L^{-1}, and between 0.02 and 7.8 µmol L^{-1}, with an average of 2.9 ± 0.8 µmol L^{-1} at the reference station over the year (Fig. 4B). Simulated nitrate concentrations during November–March were higher than the measured ones, but otherwise followed the same pattern. Except for a low nitrate value measured for December, the concentrations increased during autumn and winter followed by a pronounced decrease from April to May which coincided with a distinct spring peak in Chl *a*. The Chl *a* concentrations ranged between 0 and 3.6 µg L^{-1} at the salmon farm and between 0 and 4.4 µg L^{-1} at the reference station, but were generally low with an average of 0.7 ± 0.4 µg L^{-1} at both stations over the year (Fig. 4B).

3.2. Integration with salmon aquaculture

The August-sporophytes grew faster at the fish farm than at the reference station at all depths from August to November, and at 2 and 8 m depths from February to June, while a faster growth was seen at all depths at the reference station from November to February and at 5 m depth from February to June (Table 1). The November-sporophytes showed similar growth rates at the fish farm and at the reference station, while the February-sporophytes showed a faster growth at 5 and 8 m, but not at 2 m depth, at the fish farm than at the reference station.

Table 1
Percentage growth per day in length of the blade of *S. latissima* sporophytes deployed at the salmon farm (IMTA) and at the reference station 4 km away in August and November 2010 and February 2011.

August-sporophytes	August–November		November–February		February–June	
	IMTA	Reference	IMTA	Reference	IMTA	Reference
2 m	4.0	3.2	0.5	1.0	1.4	1.3
5 m	3.7	2.7	0.7	0.8	1.6	1.9
8 m	2.5	1.9	0.1	0.6	2.8	2.4

November-sporophytes	November–June	
	IMTA	Reference
2 m	2.5	2.4
5 m	2.5	2.5
8 m	2.5	No growth

February-sporophytes	February–June	
	IMTA	Reference
2 m	4.6	4.6
5 m	4.9	4.7
8 m	4.8	4.6

As a result of the faster initial growth at the fish farm from August to November, the August-sporophytes at the fish farm reached a significantly longer length than those at the reference station in 5 out of 10 months at 2 m depth, and in 9 out of 10 months at 5 m depth ($p < 0.05$), while no significant differences were found at 8 m depth (Fig. 5A–C). The November-sporophytes reached similar lengths at the farm and at the reference station. The February-sporophytes were significantly longer at the fish farm than at the reference station at 2, 5 and 8 m depths in May, at 5 m depth in June and at 2 and 5 m depths in August ($p < 0.05$) (Fig. 5D–F). The sporophytes that were deployed in June did not survive the summer.

3.3. Seasonal- and depth-dependent growth

The August-sporophytes showed a rapid initial growth from August to November, before a period with slow growth and stagnation from November to February, followed by fast growth from February to June (Fig. 6A). The sporophytes that were deployed at the salmon farm in August were significantly longer at 2 and 5 m depths than those at 8 m depth in autumn and spring, while a significant depth-dependent length was found in the order 2 > 5 > 8 m in December and January, 5 > 2 > 8 m in May and 5 > 8 m in June ($p < 0.05$). The sporophytes that were deployed in November, like the ones from August, grew rapidly during spring and were significantly longer at 2 and 5 m depths than at 8 m depth in April and May ($p < 0.05$), whereas no significant differences were found between the lengths at the different depths at peak lengths in June (Fig. 6B). The sporophytes that were deployed in February showed a similar growth pattern as the August- and November-sporophytes, but with peak lengths in June being significantly longer at 5 and 8 m depths than at 2 m depth ($p < 0.05$) (Fig. 6C).

At peak lengths in June, the August-sporophytes were significantly longer than the November- and February-sporophytes at 2 and 5 m depths and the February-sporophytes at 8 m depth ($p < 0.05$), while the November-sporophytes were significantly longer than the February-sporophytes at 2 m depth ($p < 0.05$), but not at 5 and 8 m depths (Fig. 6D–F). From June to over the summer, the sporophytes were covered by epiphytic fouling, and much of the biomass was lost from above the stem or the meristem, with re-growth of fresh tissue being observed on some of the sporophytes in August (Fig. 7A–F).

Significant positive correlations were found for the average length of August-sporophytes versus daylength at each sampling date (r =

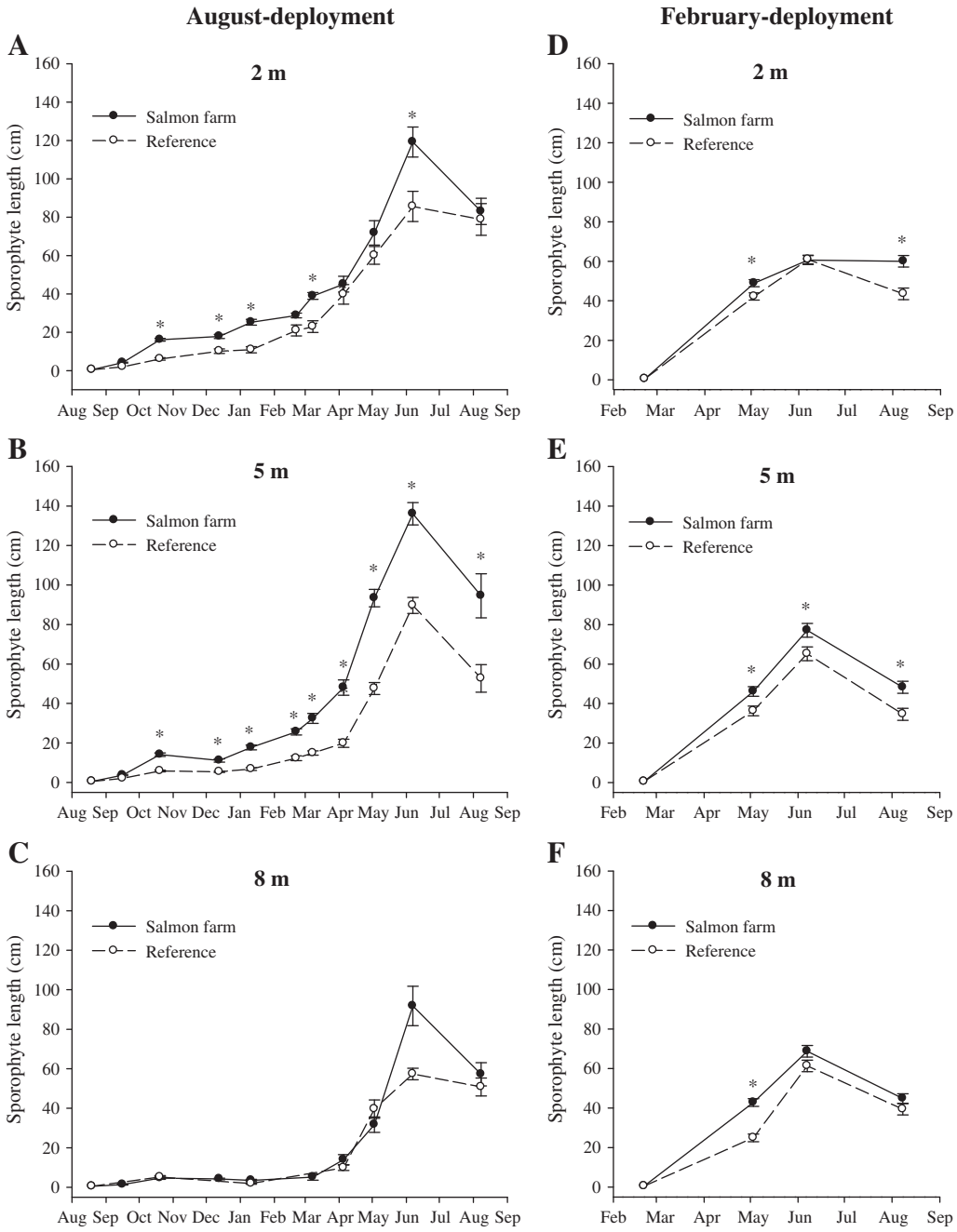

Fig. 5. A–C) Length of juvenile sporophytes of *S. latissima* deployed at 2, 5 and 8 m depths at the salmon farm and at the reference station 4 km south of the farm in August 2010, and D–F) February 2011 (mean ± se, n = 10–60). * indicates a significantly higher length at the salmon farm compared to that at the reference station within months ($p < 0.05$).

0.68 at 2 m, r = 0.72 at 5 m and r = 0.71 at 8 m depth; $p < 0.05$). The mean length:width ratio of August-sporophytes doubled from August to January at 2 and 5 m depths, while, in contrast, a decrease was seen at 8 m depth from October to March, which resulted in a significantly lower ratio at 8 m depth than at 2 and 5 m depths in winter and spring ($p < 0.05$) (Fig. 8).

3.4. Chemical composition

The nitrogen content of the August-sporophytes decreased from 3.7 to 3.9% of dry matter in spring and reached a minimum average value of 1.6% at both stations in June. After this, an increase in nitrogen was evident in the autumn which resulted in peak measurements of 5.0 ± 0.2% at the fish farm and 4.0 ± 0.5% at the reference station in September (Fig. 9A). The carbon content ranged between 23.3 ± 0.1% and 28.4 ± 0.8%, with an average of 26.2 ± 0.5% of dry matter at the fish farm and between 25.4 ± 1.0% and 28.1 ± 0.9%, with an average of 26.8 ± 0.7% at the reference station. The carbon to nitrogen ratio increased from around 7 (by weight) at both stations in spring and peaked in June at 21 at the reference station and at 16 at the fish farm, before a decrease was seen in autumn (Fig. 9A). Having about the same N-content, the higher increase in C:N ratio at the reference station than at the salmon farm in the spring suggested a higher accumulation of carbohydrates in the sporophytes at the reference station in this period. For November- and February-sporophytes, the sampling started in June, and as for the older August-sporophytes, the N-content was low in June followed by an increase until August, with a corresponding decrease in the C:N ratio indicating a low accumulation of carbohydrates in this period (Fig. 9B). In June, the February-sporophytes showed a higher N-content at the salmon farm (2.2%) than at the

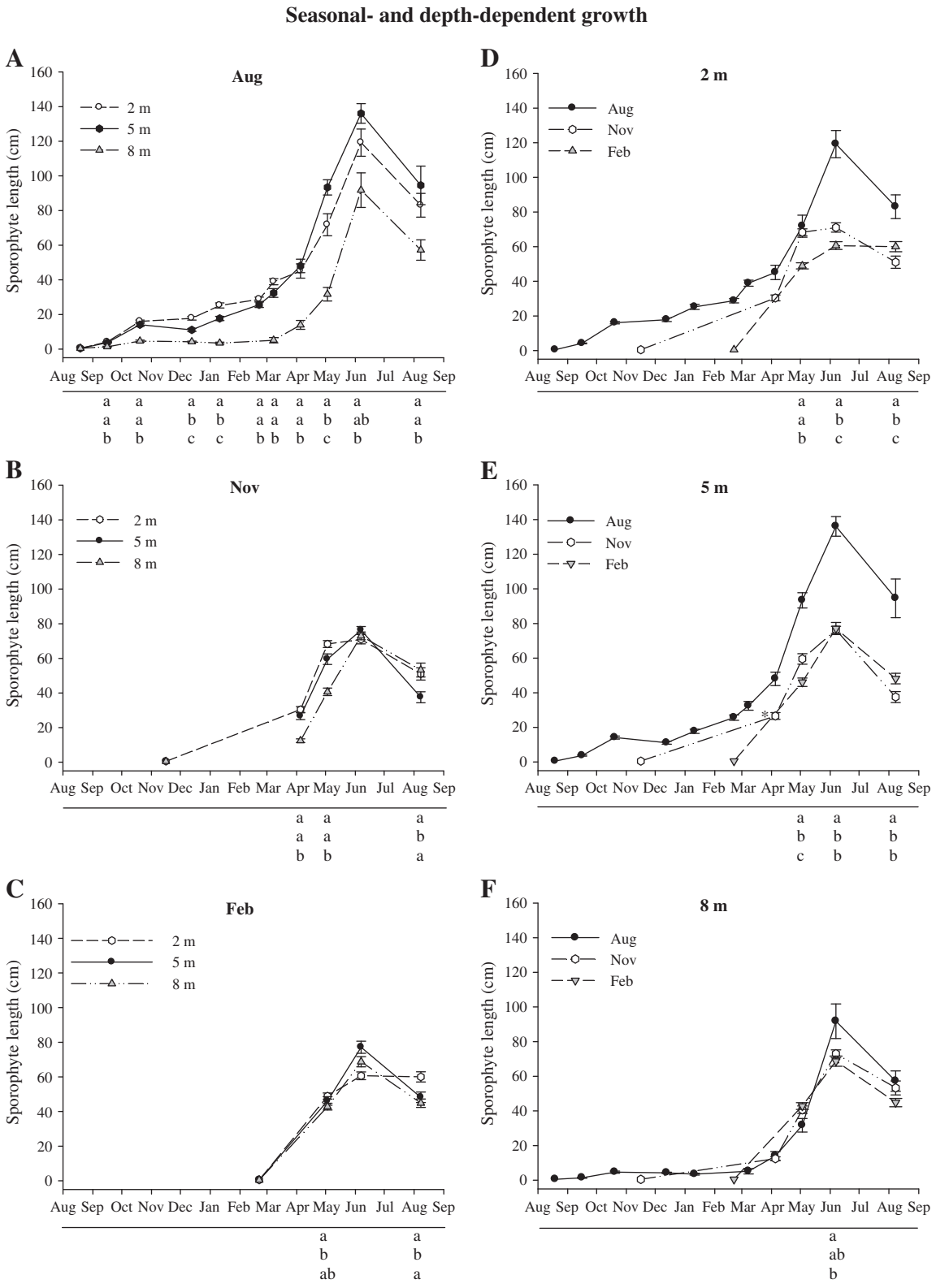

Fig. 6. A–F) Seasonal-, and depth-dependent length of juvenile sporophytes of *S. latissima* deployed at 2, 5 and 8 m depths at the salmon farm in August 2010, November 2010 and February 2011 (mean ± se, n = 10–60). Significant differences in length within months depending on the cultivation depth (A–C) and deployment time (D–F) are denoted by letters (a–c) ($p < 0.05$).

reference station (1.5%). November-sporophytes were not sampled at the salmon farm in June.

While the carbohydrate content in August-sporophytes ranged between 25 and 35% from April to June at the salmon farm, the content of the sporophytes at the reference station remained stable at 35% in April and May and peaked at 48% in June, followed by a decrease reaching minimum values of around 8% at both stations in autumn (Fig. 10A–B). Alginate is the structural compound while laminaran and mannitol are storage compounds in kelp. The alginate content ranged between 6 and 27% and was the dominating carbohydrate

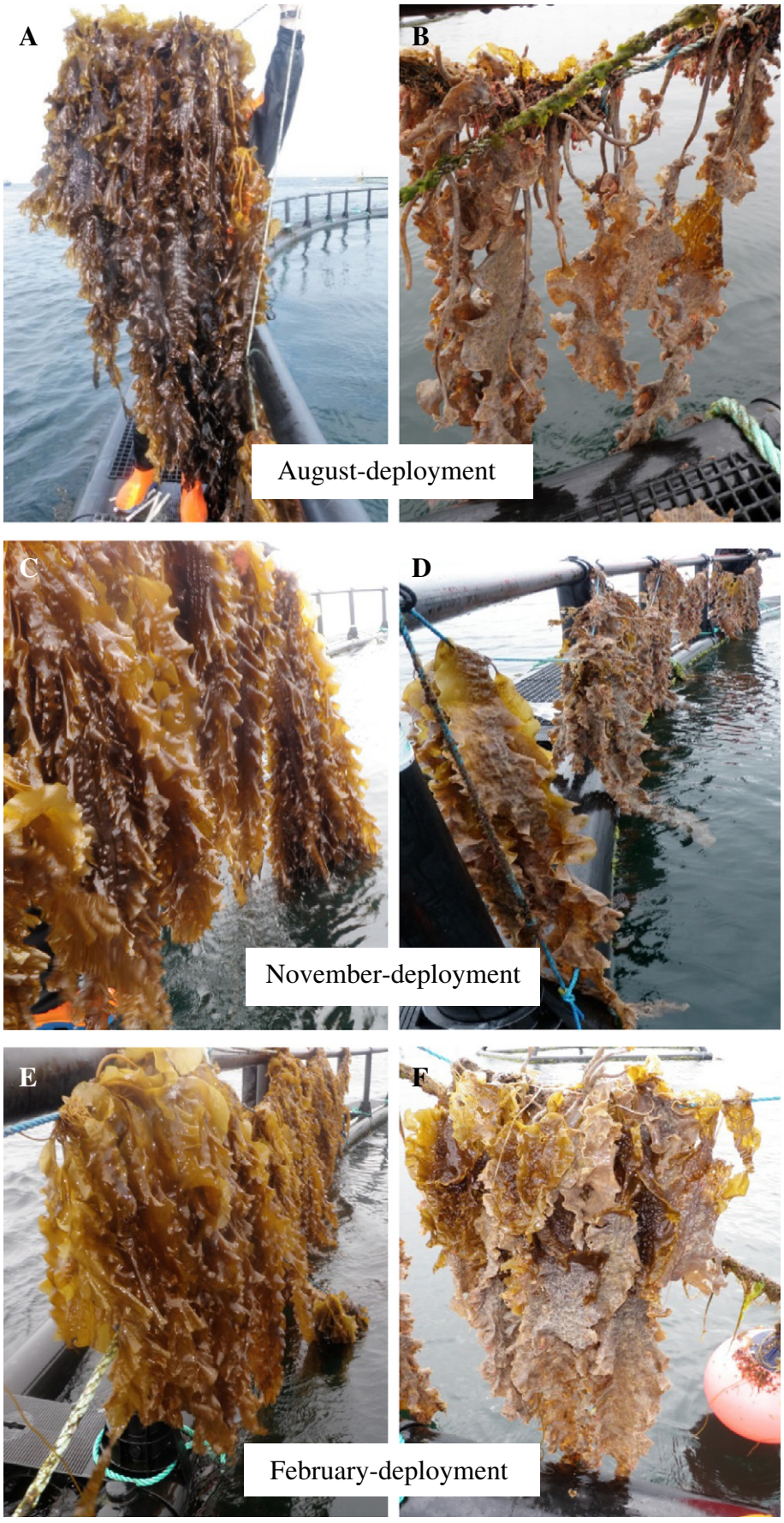

Fig. 7. A–F) Sporophytes of *S. latissima* deployed at 5 m depth at the salmon farm in August and November 2010 and February 2011 in June and August 2011, respectively.

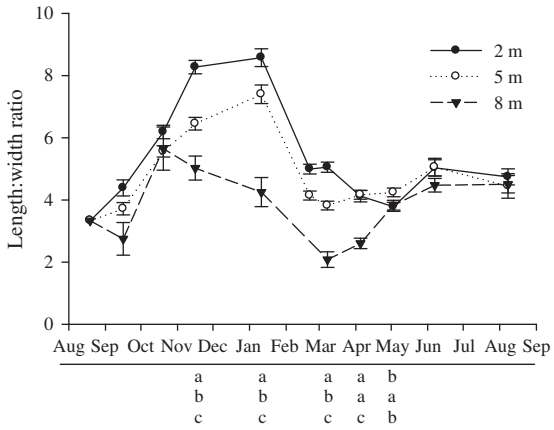

Fig. 8. Length:width ratio of juvenile sporophytes of *S. latissima* deployed at 2, 5 and 8 m depths at the salmon farm in August 2010 (mean ± se, n = 10–60). Significant differences in L:W ratio within months depending on the cultivation depth are denoted by letters (a–c) ($p < 0.05$).

followed by mannitol, which ranged from 2 to 18%, while laminaran contributed less than 3% to the total carbohydrate content, except for in June, when it accounted for 4% at the fish farm and 9% of the total at the reference station. While the alginate content peaked at 26–27% in June, the mannitol content peaked at 17% at both stations in April. The dry matter content ranged between 9% in May and 18% in August (Fig. 10A–B). As for the August-sporophytes, the carbohydrate content of the November- and February-sporophytes was relatively high in June (37–42%) and low in autumn (2–16%), with the lowest values measured for the sporophytes at the salmon farm.

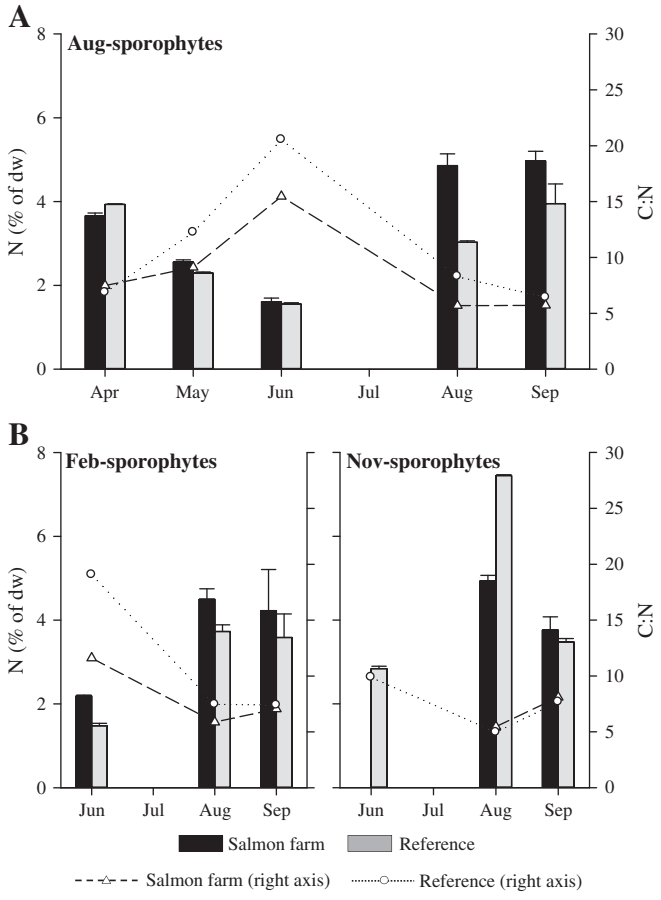

Fig. 9. A–B) Nitrogen content (% of dry weight) and C:N ratio of whole sporophytes of juvenile *S. latissima* deployed at the salmon farm and at the reference station 4 km south of the farm in August 2010, November 2010 and February 2011 (mean ± sd, n = 3).

4. Discussion

4.1. Integration with salmon farming

While nutrient recycling and increased growth of macroalgae in integration with fish aquaculture have been thoroughly documented for landbased systems (e.g. Abreu et al., 2011; Buschmann et al., 1996; Hernandez et al., 2002; Matos et al., 2006; Neori et al., 2000; Troell et al., 1999), fewer studies have been performed in the sea (e.g. Troell et al., 1997, 1999; Chopin et al., 1999, 2004; Zhou et al., 2006; Buschmann et al., 2008; Sanderson et al., 2012). The present study is the first to examine cultivation of macroalgae in integration with salmon farming in cages in Norway. A faster growth of sporophytes of *S. latissima* at the fish farm than at the reference station from August to November and February to June, with significantly longer August- and February-sporophytes at the fish farm at peak lengths in June, suggested a faster growth of macroalgae in IMTA with salmon over the sampling period. These results are consistent with similar studies showing a faster growth of macroalgae in IMTA with salmon in Canada (*Alaria esculenta* and *S. latissima*; Chopin et al., 2004), Scotland (*S. latissima*; Sanderson et al., 2012) and in Chile (*Gracilaria chilensis*; Troell et al., 1999; *G. chilensis* and *Macrosystis pyrifera*; Buschmann et al., 2008). Meanwhile, a faster growth of the August-sporophytes at the reference station than at the fish farm from November to February, and similar growth rates of November sporophytes at the farm and at the reference from November to June, suggested a seasonal-dependent effect with a more positive growth response of macroalgae deployed in August and February than in November. Furthermore, the significantly longer August-sporophytes at 2 and 5 m, but not at 8 m depth, at the fish farm than at the reference station at peak lengths in June, with the sporophytes at 5 m depth being significantly longer than those at 8 m depth, suggested a depth-dependent growth response in IMTA in the order 5 m > 2 m > 8 m over the year, although the sporophytes at 8 m depth grew fastest from February to June, and faster at the fish farm than at the reference station. This depth-dependent growth response is in agreement with Buschmann et al. (2008) who studied growth of *M. pyrifera* from 1 to 10 m depth in IMTA with salmon in southern Chile and found an optimal cultivation depth at 3 m.

One the one hand, our results suggest that IMTA with salmon can be a sound strategy to obtain enhanced growth in length of macroalgae in Norwegian coastal waters. One the other hand, the depth- and seasonal-dependent growth response emphasizes that the potential for IMTA with salmon and macroalgae as well as the potential for bioremediation services needs to be assessed holding the seasonality of the macroalgae, with a rapid spring growth, up against the salmon production pattern with higher fish biomass and feed use with a corresponding increase in nutrient discharge in late summer and autumn. In our study, both the measured and the simulated ammonium concentrations rarely exceeded 1.1 μM and remained far below the suggested threshold level of 10 μM for saturation of assimilation in *S. latissima* (Ahn et al., 1998). We therefore suggest that such low ammonium concentrations reflect typical values under semi-exposed coastal conditions with high dilution rates. Still, a significant positive correlation was found between the feed use and the ammonium concentrations at the fish farm, suggesting that the farm-derived ammonium contributed to a fertilization of the sporophytes at the farm in addition to the ambient nitrate concentrations which resulted in a positive growth response of the sporophytes deployed ≥5 m depth in August and February. This was supported by the measured higher nitrogen contents in the sporophytes at the fish farm compared to that in the sporophytes at the reference station for the August-sporophytes in August and September and for the February-sporophytes in June, August and September, shortly after the fish was slaughtered. The results are in consistency with

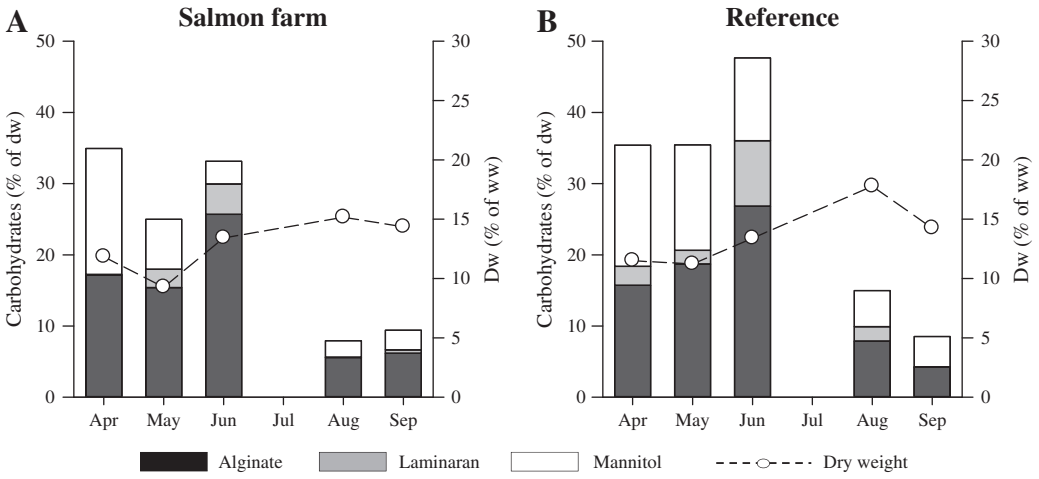

Fig. 10. A–B) Content of alginate, laminaran and mannitol and dry weight (Dw; right axis) of whole sporophytes of juvenile *S. latissima* deployed at the salmon farm and at the reference station 4 km south of the farm in August 2010 (mean ± sd, n = 3).

Sanderson et al. (2012) who found a higher nitrogen content of sporophytes kept in close proximity to salmon farms compared to controls during summer, and Chopin et al. (1999) who found higher N and P contents in *Porphyra* cultivated in close proximity to salmon and scallop aquaculture compared to that at control sites. Accordingly, a part of the nutrient fluxes can be taken up and accumulated by macroalgae in competition with phytoplankton, thereby supporting a potential for IMTA with salmon and macroalgae in such environments. Meanwhile, holding the rapid growth of *S. latissima* in spring and early summer in Central Norway together with a typical increase in fish biomass and feed use in late summer and early autumn (Wang et al., 2012) suggests a seasonal mismatch regarding *direct* recycling of the anthropogenic nutrient input from salmon farming at a local spatial scale in Norwegian coastal waters (Broch et al., 2013). Anyway, bioremediation by macroalgae will contribute equally to balancing the total input to the system independently of whether the nutrients taken up are of anthropogenic origin or not. Accumulation of nutrients by macroalgae during the spring growth may therefore still contribute to balance the nutrient input from fed aquaculture at a larger spatial scale. In Norway, the largest salmon farms are currently producing up to 12,000 tons of fish per year, with a corresponding feed use of 13,800 tons (FCR; 1.15). A theoretical loss of 45% dissolved N from such a farm (Wang et al., 2012) constitutes 373 tons (6% N in feed), from which a theoretical incorporation of e.g. 10%, directly or indirectly, would yield a production potential of 12,400 tons fresh weight (fw) macroalgae with a surface area requirement of 56 ha (given 15% dry weight and 2% N-content in June and 220 ton fw ha^{-1}; Sanderson et al., 2012).

4.2. Seasonal- and depth-dependent growth

The results revealed a seasonal- and depth-dependent growth pattern with faster growth in length at 2 and 5 m than at 8 m depth in autumn and winter and rapid growth at all depths from February to June. From June to over the summer, heavy fouling and necrosis of the distal end of the blade coincided with a decrease in length. This is in agreement with Andersen et al. (2011) who studied growth of *S. latissima* on the Skagerrak coast and found that high kelp mortality and lack of recovery over the summer coincided with heavy fouling by epiphytes and high water temperatures. Water temperatures between 10 and 15 °C have been found to give optimum growth of *S. latissima* (Bolton and Lüning, 1982; Fortes and Lüning, 1980). In our study, the temperature remained below 10 °C from February to June, suggesting somewhat sub-optimal temperature conditions during the main growth period. Meanwhile, a near linear relationship has been found between nitrate concentrations and growth rate of *S. latissima* with concentration between 0 and 10 μM (Chapman et al., 1978), indicating that the measured nitrate concentrations between 0.05 μM and 7.8 μM were well within the range for efficient uptake in *S. latissima*. The ambient concentrations did apparently not limit the growth in spring while there was an evident nutrient deficiency in summer. While the poor growth at all depths in summer was associated with nutrient deficiency and heavy fouling, the slow growth at 8 m depth in autumn and late winter and at all depths in the early winter was associated with low light conditions. This was reflected by the depth-dependent growth response seen from the significant increase in the length:width ratio of sporophytes with decreasing depth in this period. Based on the measured seasonal- and depth dependent growth of *S. latissima* in this study we suggest that this species should be cultivated ≥5 m depth and that harvesting should take place in early summer in Central Norway to avoid destruction of the crop and loss of biomass.

Our results also suggested that juveniles should be deployed in early autumn to be as long as possible before harvesting in early summer, while deployments from November to February will yield smaller but more equal sized plants. Deployments in periods with low light and short daylength will likely not have any negative impact on the spring growth of *S. latissima*, which even has been shown to survive dark periods for up to one year (Wiencke, 1990). Accordingly, sporophytes of *S. latissima* can be deployed in autumn and winter as a strategy to maximize the amount of seeded ropes at sea before the main growth season begins.

5. Conclusions

Sporophytes that were deployed ≥5 m depth in August and February reached significantly greater lengths at the fish farm than at the reference station, while no significant differences were found for the August-sporophytes deployed at 8 m depth, or for the November-sporophytes at the fish farm and at the reference station. In general, our results suggested that in Central Norway *S. latissima* should be cultivated ≥5 m depth, with deployments from August to February followed by harvesting in June to avoid destruction of the crop and loss of biomass in late summer. Holding the rapid growth of *S. latissima* in spring and early summer together with the increase in salmon biomass and feed use in late summer and early autumn suggested a seasonal mismatch regarding direct recycling of the nutrient effluents from salmon aquaculture by macroalgae. Accordingly, the capability of macroalgae to perform bioremediation services in integrated aquaculture should be considered taking the seasonal growth patterns of the multi-trophic species carefully into account.

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References

Abreu, M.H., Pereira, R., Yarish, C., Buschmann, A.H., Sousa-Pinto, I., 2011. IMTA with *Gracilaria vermiculophylla*: productivity and nutrient removal performance of the seaweed in a land-based pilot scale system. Aquaculture 312, 77–87.

Adams, J.M.M., Ross, A.B., Anastasakis, E.M., Hodgson, J.A., Gallagher, J.M., Jones, J.M., Donnison, I.S., 2011. Seasonal variation in the chemical composition of the bioenergy feedstock *Laminaria digitata* for thermochemical conversion. Bioresource Technology 102, 226–234.

Ahn, O., Petrell, R.J., Harrison, P.J., 1998. Ammonium and nitrate uptake by *Laminaria saccharina* and *Nereocystis luetkeana* originating from a salmon sea cage farm. Journal of Applied Phycology 10, 333–340.

Andersen, G.S., Steen, H., Christie, H., Fredriksen, S., Moy, F.E., 2011. Seasonal patterns of sporophyte growth, fertility, fouling, and mortality of *Saccharina latissima* in Skagerrak, Norway: implications for forest recovery. Journal of Marine Biology. http://dx.doi.org/10.1155/2011/690375.

Bartsch, I., Wiencke, C., Bischof, K., Buchholz, C.M., Buck, B.H., Eggert, A., Feuerpfeil, P., Hanelt, D., Jacobsen, S., Karez, R., Karsten, U., Molis, M., Roleda, M.Y., Schubert, H., Schumann, R., Valentin, K., Weinberger, F., Wiese, J., 2008. The genus *Laminaria* sensu lato: recent insights and developments. European Journal of Phycology 43, 1–86.

Bixler, H.J., Porse, H., 2012. A decade of change in the seaweed hydrocolloids industry. Journal of Applied Phycology 23 (3), 321–335.

Bolton, J.J., Lüning, K., 1982. Optimal growth and maximal survival temperatures of Atlantic *Laminaria* species (Phaeophyta) in culture. Marine Biology 66, 89–94.

Borum, J., Pedersen, M., Krause Jensen, D., Christensen, P., Nielsen, K., 2002. Biomass, photosynthesis and growth of *Laminaria saccharina* in a high-arctic fjord, NE Greenland. Marine Biology 141, 11–19.

Broch, O.J., Ellingsen, I.H., Forbord, S., Wang, X., Volent, Z., Alver, M.O., Handå, A., Andresen, K., Slagstad, D., Reitan, K.I., Olsen, Y., Skjermo, J., 2013. Modelling the cultivation and bioremediation potential of the kelp *Saccharina latissima* in close proximity to an exposed salmon farm in Norway. Aquaculture Environment Interactions 4, 186–206.

Bruhn, J., Gerard, V.A., 1996. Photoinhibition and recovery of the kelp *Laminaria saccharina* at optimal and superoptimal temperatures. Marine Biology 125, 639–648.

Buschmann, A.H., Mora, O.A., Gómez, P., Böttger, M., Buitano, S., Retamales, C., Vergara, P.A., Gutierrez, A., 1994. *Gracilaria chilensis* outdoor tank cultivation in Chile: use of land-based salmon culture effluents. Aquacultural Engineering 13, 283–300.

Buschmann, A.H., Troell, M., Kautsky, N., Kautsky, L., 1996. Integrated tank cultivation of salmonids and *Gracilaria chilensis* (Gracilariales, Rhodophyta). Hydrobiologia 327, 75–82.

Buschmann, A.H., Varela, D.A., Hernández-González, M.C., Huovinen, P., 2008. Opportunities and challenges for the development of an integrated seaweed-based aquaculture activity in Chile: determining the physiological capabilities of *Macrocystis* and *Gracilaria* as biofilters. Journal of Applied Phycology 20, 571–577.

Chapman, A.R.O., Markham, J.W., Lüning, K., 1978. Effects of nitrate concentration on growth and physiology of *Laminaria saccharina* (Phaeophyta) in culture. Journal of Phycology 14, 195–198.

Chopin, T., Yarish, C., Wilkes, R., Belyea, E., Lu, S., Mathieson, A., 1999. Developing *Porphyra*/salmon integrated aquaculture for bioremediation and diversification of the aquaculture industry. Journal of Applied Phycology 11, 463–472.

Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G.P., Zertuche-Gonzalez, J.A., Yarish, C., Neefus, C., 2001. Integrating seaweeds into marine aquaculture systems: a key toward sustainability. Journal of Phycology 37, 975–986.

Chopin, T., Robinson, S., Sawhney, M., Bastarache, S., Belyea, E., Shea, R., Armstrong, W., Stewart, I., Fitzgerald, P., 2004. The AquaNet integrated multi-trophic aquaculture project: rationale of the project and development as kelp cultivation as the inorganic extractive component of the system. Bulletin of the Aquaculture Association of Canada 104, 11–18.

Forbord, S., Skjermo, J., Arff, J., Handå, A., Reitan, K.I., Bjerregaard, R., Lüning, K., 2012. Development of *Saccharina latissima* (Phaeophyceae) kelp hatcheries with year-round production of zoospores and juvenile sporophytes on culture ropes for kelp aquaculture. Journal of Applied Phycology 24, 393–399.

Fortes, M.D., Lüning, K., 1980. Growth rates of North Sea macroalgae in relation to temperature, PAR and photoperiod. Helgoländer Meeresuntersuchungen 34, 15–29.

Frette, O., Erga, S.R., Hamre, B., Aure, J., Stamnes, J.J., 2004. Seasonal variability in inherent optical properties in a western Norwegian fjord. Sarsia 89, 276–291.

Gerard, V.A., 1988. Ecotypic differentiation in light-related traits of the kelp *Laminaria saccharina*. Marine Biology 97, 25–36.

Handå, A., Min, H., Wang, X., Broch, O.J., Reitan, K.I., Reinertsen, H., Olsen, Y., 2012. 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. Aquaculture 356–357, 328–341.

Harrison, P.J., Hurd, C.L., 2001. Nutrient physiology of seaweeds: application of concepts to aquaculture. Cahiers de Biologie Marine 42, 71–82.

Hernandez, I., Martinez-Aragon, J.F., Tovar, A., Perez-Llorens, J.L., Vergara, J.J., 2002. Biofiltering efficiency in removal of dissolved nutrients by three species of estuarine macroalgae cultivated with sea bass (*Dicentrarchus labrax*) waste waters 2. Ammonium. Journal of Applied Phycology 14, 375–384.

Holdt, S., Kraan, S., 2011. Bioactive compounds in seaweed: functional food applications and legislation. Journal of Applied Phycology 23, 543–597.

Kain, J., 1979. A view of the genus *Laminaria*. Oceanography Marine Biology Annual Review 17, 101–161.

Kerouel, R., Aminot, A., 1997. Fluorometric determination of ammonium in sea and estuarine waters by direct segmented flow analysis. Marine Chemistry 57, 255–274.

Kraan, S., 2010. Mass-cultivation of carbohydrate rich macroalgae, a possible solution for sustainable biofuel production. Mitigation and Adaptation Strategies for Global Change 1–20.

Lüning, K., 1979. Growth strategies of three *Laminaria species* (Phaeophyceae) inhabiting different depth zones in the sublittoral region of Helgoland (North Sea). Marine Ecology Progress Series 1, 195–207.

Matos, J., Costa, S., Rodrigues, A., Pereira, R., Sousa Pinto, I., 2006. Experimental integrated aquaculture of fish and red seaweeds in Northern Portugal. Aquaculture 252, 31–42.

Mente, E., Pierce, G.J., Santos, M.B., Neofitou, C., 2006. Effect of feed and feeding in the culture of salmonids on the marine aquatic environment: a synthesis for European aquaculture. Aquaculture International 14, 499–522.

Murata, M., Nakazoe, J., 2001. Production and use of marine algae in Japan. Japan Agricultural Research Quarterly 35, 281–290.

Neori, A., Shpigel, M., Ben-Ezra, D., 2000. A sustainable integrated system for culture of fish, seaweed and abalone. Aquaculture 186, 279–291.

Nisizawa, K., Noda, H., Kikuchi, R., Watanabe, T., 1987. The main seaweed foods in Japan. Hydrobiologia 151, 5–29.

Norwegian Directorate of Fisheries, 2011. Aquaculture statistics, key figures from aquaculture industry. http://www.fiskeridir.no/english/statistics/booklets/aquacultural-booklets.

Oppedal, F., Dempster, T., Stien, L.H., 2011a. Environmental drivers of Atlantic salmon behaviour in sea-cages: a review. Aquaculture 311, 1–18.

Oppedal, F., Vågseth, T., Dempster, T., Juell, J.E., Johansson, D., 2011b. Fluctuating sea-cage environments modify the effects of stocking densities on production and welfare parameters of Atlantic salmon (*Salmo salar* L.). Aquaculture 315, 361–368.

Østgaard, K., 1992. Enzymatic microassay for the determination and characterization of alginates. Carbohydrate Polymers 19, 51–59.

Paasche, E., Erga, S.R., 1988. Phosphorus and nitrogen limitation of phytoplankton in the inner Oslofjord (Norway). Sarsia 73, 229–243.

Parke, M., 1948. Studies on the British Laminariaceae. I. Growth in *Laminaria saccharina* (L.) Lamour. Journal of the Marine Biological Association of the United Kingdom 27, 651–709.

Pitta, P., Tsapakis, M., Apostolaki, E.T., Tsagaraki, T., Holmer, M., Karakassis, I., 2009. 'Ghost nutrients' from fish farms are transferred up the food web by phytoplankton grazers. Marine Ecology Progress Series 374, 1–6.

Redmond, K.J., Magnesen, T., Hansen, P.K., Strand, Ø., Meier, S., 2010. Stable isotopes and fatty acids as tracers of the assimilation of salmon fish feed in blue mussels (*Mytilus edulis*). Aquaculture 298, 202–210.

Sakshaug, E., Johnsen, G., Kovacs, K. (Eds.), 2009. Ecosystem Barents Sea. Tapir Akademisk Forlag, Trondheim, Norway (587 pp.).

Sanderson, J.C., Cromey, C.J., Dring, M.J., Kelly, M.S., 2008. Distribution of nutrients for seaweed cultivation around salmon cages at farm sites in north–west Scotland. Aquaculture 278, 60–68.

Sanderson, J.C., Dring, M.J., Davidson, K., Kelly, M.S., 2012. Culture, yield and bioremediation potential of *Palmaria palmata* (Linnaeus) Weber & Mohr and *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders adjacent to fish farm cages in northwest Scotland. Aquaculture 354–355, 128–135.

Slagstad, D., McClimans, T.A., 2005. Modeling the ecosystem dynamics of the Barents Sea including the marginal ice zone: I. Physical and chemical oceanography. Journal of Marine Systems 58, 1–18.

Troell, M., Halling, C., Nilsson, A., Buschmann, A.H., Kautsky, N., Kautsky, L., 1997. Integrated marine cultivation of *Gracilaria chilensis* Gracilariales Rhodophyta and salmon cages for reduced environmental impact and increased economic output. Aquaculture 156, 45–61.

Troell, M., Ronnback, P., Halling, C., Kautsky, N., Buschmann, A., 1999. Ecological engineering in aquaculture: use of seaweeds for removing nutrients from intensive mariculture. Journal of Applied Phycology 11, 89–97.

Troell, M., Joyce, A., Chopin, T., Neori, A., Buschmann, A.H., Fang, J.G., 2009. Ecological engineering in aquaculture — potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. Aquaculture 297, 1–9.

Wang, X., Olsen, L.M., Reitan, K.I., Olsen, Y., 2012. Discharge of nutrient wastes from salmon farms: environmental effects, and potential for integrated multi-trophic aquaculture. Aquaculture Environment Interactions 2, 267–283.

Wassmann, P., Slagstad, D., Riser, C.W., Reigstad, M., 2006. Modelling the ecosystem dynamics of the Barents Sea including the marginal ice zone: II. Carbon flux and interannual variability. Journal of Marine Systems 59, 1–24.

Wiencke, C., 1990. Seasonality of brown macroalgae from Antarctica—a long-term culture study under fluctuating Antarctic daylengths. Polar Biology 10, 589–600.

Zhou, Y., Yang, H., Hu, H., Liu, Y., Mao, Y., Zhou, H., Xu, X., Zhang, F., 2006. Bioremediation potential of the macroalga *Gracilaria lemaneiformis* (Rhodophyta) integrated into fed fish culture in coastal waters of north China. Aquaculture 252, 264–276.