J Appl Phycol (2015) 27:1963–1973
DOI 10.1007/s10811-014-0519-8

Commercial cultivation and bioremediation potential of sugar kelp, *Saccharina latissima*, in Danish waters

Gonçalo S. Marinho · Susan L. Holdt · Mads J. Birkeland · Irini Angelidaki

Received: 16 August 2014 / Revised and accepted: 21 December 2014 / Published online: 20 January 2015

Abstract Several seaweed species have been successfully tested for their biofilter potential for integrated multi-trophic aquaculture (IMTA). In this study, *Saccharina latissima* bioremediation potential was assessed over 12 months with respect to the yield, phosphorous (P) and nitrogen (N) content and removal. The experiment took place at two commercial cultivation areas: in close proximity to a blue mussel and fish farm (IMTA) and at a reference site, both situated outside Horsens Fjord in Denmark. The maximum biomass yield over the first growing season was achieved in August (1.08±0.09 and 1.51±0.13 kg fresh weight (FW) m^{-1}) and September (0.92±0.18 and 1.49±0.16 kg FW m^{-1}). Yield was significantly higher at the IMTA compared to the reference site in August ($P<0.05$). A second growing season did not improve biofiltration efficiency. The highest N and P removal was achieved in August and September. Again, the IMTA location showed better N and P removal compared with the reference site in August: 5.02–7.02 g N and 0.86–1.23 g P m^{-1} of cultivation line ($P<0.05$). *S. latissima* shows potential for assimilation and removal of nutrients, particularly nitrogen. Seasonal variations of seaweed biofilter efficiency, condition, and potential applications should be taken into account when evaluating the best suited harvest time. For Horsens Fjord, our results showed that the harvest time should take place in August–September in order to achieve maximum biofiltration efficiency (including N and P in epiphytes). However, for human consumption, it is better to harvest in May when the seaweed is free of epiphytes.

Keywords Biofilter · Nitrogen removal · Phosphorous removal · Epiphytes · Year-round variation · N quota · IMTA

G. S. Marinho (✉) · I. Angelidaki
Department of Environmental Engineering, Technical University of Denmark, Miljøvej, Building 113, 2800 Kongens Lyngby, Denmark
e-mail: gosm@env.dtu.dk

S. L. Holdt
National Food Institute, Technical University of Denmark, Søltofts Plads 221, 2800 Kongens Lyngby, Denmark

M. J. Birkeland
DHI, Agern alle 5, 2970 Hørsholm, Denmark

Introduction

While the world's food fish supply from aquaculture has reached a new historic maximum, supplying 47 % of total fish consumed in 2010 (FAO 2012), there is rising social and political concern regarding the environmental impact of intensive monoculture production systems (e.g., finfish and shrimp). Salmon aquaculture releases around 62–63 % of feed nitrogen (N) and 70 % of feed phosphorous (P) into the environment in Norway (Olsen et al. 2008; Wang et al. 2012). According to Danish legislation, trout aquaculture is allowed to release around 60 % feed N and feed P (Olsen et al. 2008). Dissolved inorganic nutrients account for approximately 45 and 18 % of total released N and P, respectively (Wang et al. 2012), which are directly available for algae and may contribute to eutrophication (e.g., Nixon 1995; Skogen et al. 2009).

Seacage culture in Denmark is limited by an emission quota of P and in particular N, due to environmental concerns. This has been a major factor limiting the development of Danish aquaculture industry, whose production of fish has been capped for 20 years since 1987 (Holdt and Edwards 2014). However, fish production (350 t N y^{-1}) only contributes to less than 1 % of the total N contribution of the inner Danish waters (43,000 t N y^{-1}) to the Baltic Sea (652,000 t N y^{-1}; HELCOM 2012). Integrated multi-trophic aquaculture

(IMTA) has been proposed as a sustainable ecological approach for mitigation of the nutrient load into the aquatic environment. In such production systems, organisms from different trophic levels are co-produced, and waste nutrients from fed species are assimilated and converted into valuable biomass by the extractive species, providing environmental and potentially economic benefits compared with fed only aquaculture (see reviews by Neori et al. 2004; Chopin et al. 2012). Several studies have investigated the bioremediation potential of different seaweed species in IMTA (e.g., Chopin et al. 2001; Troell et al. 2003; Neori et al. 2004; Matos et al. 2006; Rodrigueza and Montaño 2007; Abreu et al. 2009, 2011; Mata et al. 2010). Of the brown seaweeds, the genus *Laminaria* sensu lato (*Laminaria* and *Saccharina*) is one of the most studied, selected on its high bioremediation potential, growth rate, and commercial application (Sanderson et al. 2012; Reid et al. 2013; Handå et al. 2013).

The potential for application of seaweed very much depends on the condition and content of the harvested biomass. This also includes the occurrence of heavy epiphytic fouling in summer, which has been associated with reduced growth and increased mortality of *Saccharina latissima* in Norway (Andersen et al. 2011; Handå et al. 2013), and is also an important factor to consider for scaled up/commercial cultivation of the species, especially with regard to bioremediation potential and application of the fouled biomass. In addition, the chemical composition of seaweeds varies throughout the year as a result of changes in environmental conditions (e.g., Ito and Hori 1989; Fleurence 1999; Holdt and Kraan 2011). Indeed, seaweeds have the ability to integrate over temporal environmental nutrient variations (Troell et al. 1997; Chopin et al. 2001). Increased nitrogen content has been reported in seaweed cultivated near salmon farms compared to those cultivated at reference sites away from nutrient emissions of aquaculture activities (Chopin et al. 2000; Abreu et al. 2009; Sanderson et al. 2012) even in locations with high N dilution (Buschmann et al. 2008a). The benefits of seaweed cultivation in close vicinity to fish farms may be particularly interesting during late spring and summer, when the ambient nitrate is often limited/depleted (Paasche and Erga 1988; Frette et al. 2004). Higher growth rates were found in *S. latissima* sporophytes cultivated close by salmon farm cages compared to those at a reference station (Handå et al. 2013); however, absence of data on the biomass yield limits the evaluation of the biofilter efficacy. Sanderson et al. (2012) reported increased biomass yield and N content in *S. latissima* cultivated in the vicinity to fish farm cages, but seasonality of these parameters was not assessed. Harvest time is predicted to have an effect on epiphytic coverage, N and P content, biomass yield, and thus biofiltration potential of *S. latissima*.

In this study, *S. latissima* bioremediation potential was monitored over 12 months in a commercial off-coast IMTA and a reference site. Monitoring focused on the yield, P and N content, and total assimilation (removal when biomass harvested) of the produced biomass (also including epiphytes). Furthermore, the seaweed biomass composition (N and P), yield, and the bioremediation potential of the two locations were compared and evaluated in conjunction with environmental data from each site. The best harvest time(s) was evaluated on basis of the highest bioremediation, best quality/condition of the seaweed and with respect to the potential application of the seaweed biomass.

Material and methods

Experimental location The experiment was run in two commercial cultivation areas by Hjarnø Havbrug A/S outside but in vicinity of Horsens Fjord, in the inner Danish waters. The IMTA site was located in the area As Vig (55° 47.529′ N, 10° 03.027′ E) approximately 100 m from a blue mussel SmartFarm^{TM} (35 tubes including nets) and 500 m from rainbow trout (*Oncorhynchus mykiss*) farm cages (175 t year^{-1}; Fig. 1). The reference site (REF) was established in a 100-ha seaweed cultivation area (55° 49.045′ N, 10° 06.824′ E) located at approximately 2,000 m from the same fish farm cages. This area is expected to be out of range of the nutrient load from the mussel and fish farms. Both locations commercially produce *S. latissima*, where 7 and 90 km of *S. latissima* cultivation lines were deployed at, respectively, IMTA and REF site during 2011–2013. The seaweed farms consist of longlines, each one containing approximately 200 seeded droppers (4 m in length, 1 m apart), keeping a distance of 8–10 m between longlines. Longlines ware buoyed at a depth of 1 m using buoys placed every 8–10 m of longline, and sinkers ware tied in the end of each dropper (seeded line) in order to keep it strait. Both ends of the longlines were tied to heavy anchors keeping the longlines stretched in a horizontal position. One longline was selected for the monitoring in this study (see below). The rainbow trout grow out season in the sea is from April to May to October to December. Mussel natural settlement occurs from spring to late summer, and harvest time is in the autumn (Hjarnø Havbrug A/S, P. Schmedes, personal communication).

Seeded line production Cultivation lines with *S. latissima* seedlings were produced at the commercial hatchery Seaweed Energy Solution (former Seaweed Seed Supply A/S). The seedlings were produced through conventional breeding technique, using spores released from large reproducers collected from the Horsens Fjord, Denmark. Sporogenesis was induced by application of a short day photoperiod (Pang and Lüning 2004). The spore solution was evenly distributed in tanks with the collectors, and spores were allowed to attach to the cultivation lines (6 mm diameter polypropylene). Further developed seedlings were grown up

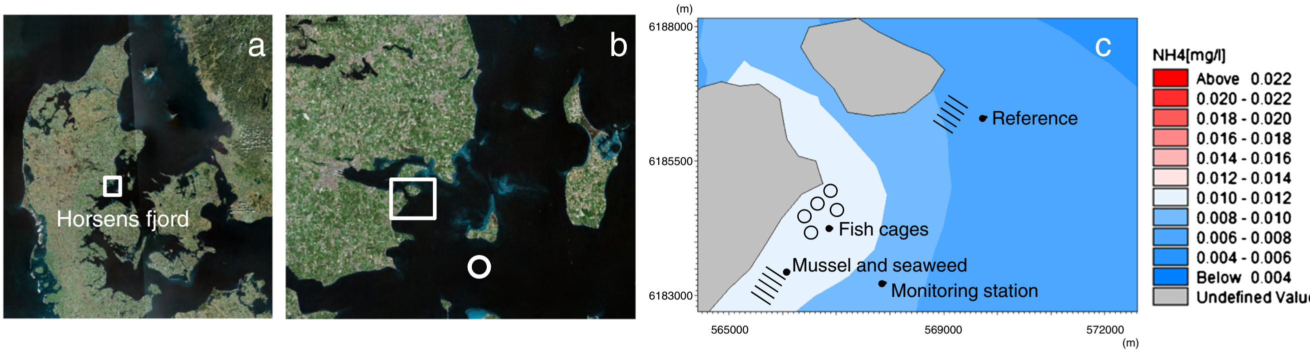

Fig. 1 **a** The location of Horsens Fjord in Denmark. **b** The location of the experimental area (*square*) outside Horsens Fjord, and the recently permitted fish farm south of Endelave island (*circle*). **c** Modeled surface ammonium concentration (mg L^{-1}; fish production season, April–November) around the fish farm cages and the location of the two seaweed cultivation areas. The *lines* represent the cultivation ropes of seaweed in the reference site and seaweed and mussels at the IMTA site, southwest of the fish cages (*circles*); however, they are not correct in proportion or number. The environmental monitoring station (st. 6883) was providing data for modeling

to approximately 3 mm (around 6 weeks) before deployment at sea.

Deployment of experimental lines Out of the 90-km commercially cultivated seaweed lines at the REF site, a longline, with 200 droppers from the same batch deployed in 15 January 2013, was selected and tagged to be used for further monitoring. On the 21 May 2013 (i.e., just prior to the start of the experiment), 64 droppers from this selected longline were randomly chosen and moved from the REF site to a new longline at the IMTA site shortly after the fish has been stocked in the farm cages.

Monitoring Sporophyte density (seedlings m^{-1} line) on the droppers (cultivation lines) at both cultivation sites was measured 20 days after deployment at the IMTA site (June), by counting all the plants growing on the first meter of the cultivation line (n=3). The cultivated biomass was sampled monthly at both experimental sites, throughout the experimental period (May 2013–May 2014), to determine the biomass production and chemical composition and the sporophyte length and weight. In each sampling, three droppers were randomly selected (triplicates) and the biomass growing in the first meter (approximately 1–2 m depth) harvested and weighed. The biomass yield was determined as the harvested biomass, in fresh weight basis (FW), per linear meter of cultivation line. At least 10 individuals were randomly selected from each one of the droppers (triplicates), and individual thallus length and weight (FW) were measured. The specific growth rate was determined by the formula: SGR (day^{-1})=Ln (FW)−Ln (IW)/T, where FW = final weight, IW = initial weight, and T = days in culture. Visible epiphytes were removed prior to measurements and analyses. All collected samples were kept frozen until chemical analysis.

Environmental conditions A three-dimensional model was applied to describe and assess the environmental impacts in connection to rainbow trout (*O. mykiss*) farms in the inner Danish waters. The model is based on the general modeling system MIKE 3 developed by the company DHI (Denmark) and defines the central environmental concerns regarding water quality and organic waste, with a flexible and fine mesh polygon system around the fish farm site, where in each polygon, the environmental parameters are examined. The MIKE 3 model has been used for both the hydrodynamic and ecological models.

Data entered into the hydrodynamic model include wind speed (10 m above sea level), atmospheric pressure, air temperature, incoming solar radiation, water levels, and water temperature and salinity at the model boundaries together with freshwater inflow from riverine sources. Furthermore, the boundary concentrations for relevant pelagic state variables, initial maps of state variables, and terrestrial nutrient loading are entered into the ecological model (MIKE 2005a). The ecological model combines the features of an eutrophication model (EU) and a sediment transport model (MT). The EU-MT model is using ECO Lab which is an integrated part of the MIKE software system. The ECO Lab model includes pelagic parameters such as phytoplankton (C, N, P), chlorophyll-*a*, zooplankton, detritus (C, N, P), and inorganic nutrients (NO_{2+3}-N, NH_4-N, and PO_4-P) and an extensive description of biogeochemical processes in the sediment including remineralization and deposition and resuspension of organic and inorganic matter to and from the bottom as a function of shear stress generated by currents and waves (MIKE 2005b).

Waste products from the cultured fish are included in the model as sources of NH_4 and PO_4, and two fractions of organic particles with varying settling velocity are used to represent feces and feed waste. The model results were calibrated against measured surface and seabed concentrations of inorganic and total nitrogen, inorganic and total phosphorus, dissolved oxygen, chlorophyll-*a*, and salinity and temperature from monitoring stations within the model domain.

Analytical methods Bimonthly samples (including August) were further analyzed. Chemical analyses were performed according to AOAC procedures (AOAC 2006), and samples were run in duplicates. All samples were freeze-dried, finely milled using a Siebtechnik Screening disc mill TS 250, and homogenized prior to analysis. Moisture content was determined after drying at 105 °C for 24 h. Samples were analyzed for ash content (550 °C for 6 h); total nitrogen, using the Kjeldahl method after acid digestion using a Kjeltec system; and total phosphorous, by inductively coupled plasma mass spectrometry (7500ce ICPMS, Agilent Technologies) using external calibration with internal standardization (^{45}Sc), after acid digestion with concentrated Suprapur nitric acid in a microwave oven (Multiwave 3000, Anton Paar, Austria).

Statistical analysis Data are expressed as mean±standard error. Data were tested for normality using Kolmogorov-Smirnov and homogeneity of variance using Leven's test. Means within groups (year-round) was analyzed using one-way ANOVA analyses of variance, at a significance level of $P<0.05$. When F values showed significance, individual means were compared using Tukey's post hoc test. Means between groups (experimental sites) were compared using a two-sample T test, at a significance level of $P<0.05$. Where homogeneity of variance was not assumed, a Welch correction was applied. Statistical analyses were performed using OriginPro 9.0 (OriginLab Corporation).

Results

Sea temperature ranged from 0 to 23 °C, and salinity ranged from 12 to 29 psu at the cultivation areas (Table 1). The current speed ranged from 0.1 to 35.7 and 0.2 to 34.1 cm s^{-1} at the IMTA and REF site, respectively. The direction of current runs northeast/southwest around 50 % of the time for each of the directions. Modeled environmental parameters showed similar temperature, salinity, irradiance, ambient nitrate and phosphate concentrations in both experimental sites. Lower nutrient concentrations were found in spring and summer time, coinciding with the peak in the chl *a* (data not shown). Modeled and measured ambient nitrate concentration in the surface water of the bay where the IMTA facility is located showed high degree of concordance, which verifies the model (Fig. 2). The ammonia concentration increased up to 0.012 mg L^{-1} at the IMTA location due to the finfish production. There was no effect on the REF site (Fig. 1c).

There were obvious changes in the appearance of the *S. latissima* plants due to seasonal coverage by fouling epiphytes which first appeared in June and had maximum coverage in September (Fig. 3). The main epiphytes not only

Table 1 Modeled environmental parameters at both reference (REF) and IMTA sites showing annual seasonal variation

	Temperature (°C)	Salinity (psu)	Irradiance (µmol photons m^{-2} s^{-1})	Current velocity (cm s^{-1})	Nutrients (mg L^{-1})		
					Nitrate + nitrite	Ammonium	Phosphate
IMTA site							
June–August	16.0 (7.0–22.7)	23.6 (18.2–27.9)	753.5 (359.7–1,068)	5.2 (0.1–23.2)	0.005 (0.0006–0.0350)	0.005 (0.0004–0.0214)	0.006 (0.0005–0.0269)
September–November	13.8 (5.9–19.4)	23.1 (19.3–28.6)	337.8 (63.4–798.5)	4.6 (0.2–25.8)	0.035 (0.0018–0.1174)	0.023 (0.0008–0.0451)	0.020 (0.0040–0.0408)
December–February	4.3 (−0.2–8.8)	23.2 (13.7–26.8)	150.2 (47.6–483.1)	7.6 (0.1–35.7)	0.113 (0.0645–0.1985)	0.013 (0.0030–0.0277)	0.017 (0.0074–0.0369)
March–May	5.6 (−0.3–12.9)	18.1 (12.2–25.3)	538.2 (218.9–920.1)	5.9 (0.2–22.7)	0.025 (0.0026–0.1043)	0.005 (0.0002–0.0224)	0.002 (0.0003–0.0080)
REF site							
June–August	16.6 (8.0–22.5)	23.2 (16.9–27.0)	761.7 (358.8–1,090)	8.9 (0.6–24.0)	0.004 (0.0008–0.0271)	0.004 (0.0005–0.0154)	0.005 (0.0007–0.0206)
September–November	13.7 (5.6–19.2)	22.5 (19.0–23.0)	343.2 (63.8–809.4)	6.6 (0.5–27.5)	0.036 (0.0020–0.1691)	0.020 (0.0009–0.0490)	0.017 (0.0040–0.0327)
December–February	4.1 (−0.1–8.1)	22.9 (19.0–26.6)	148.6 (44.9–477.4)	10.3 (0.3–34.1)	0.111 (0.0667–0.2006)	0.012 (0.0030–0.0267)	0.017 (0.0074–0.0367)
March–May	5.9 (−0.5–13.5)	17.7 (11.7–24.2)	545.8 (220.0–935.6)	9.3 (0.2–30.4)	0.027 (0.0025–0.1307)	0.004 (0.0003–0.0156)	0.002 (0.0004–0.0080)

Data expressed as mean, minimum–maximum shown in brackets

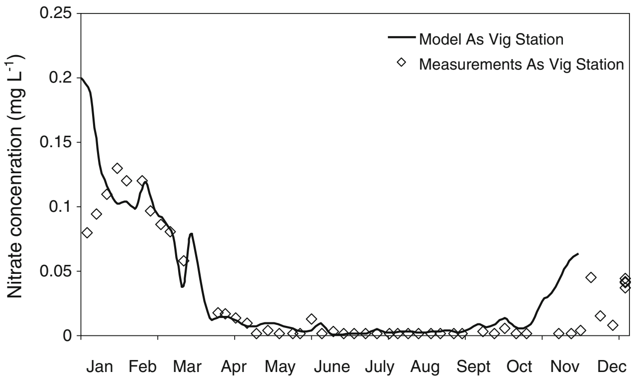

Fig. 2 Modeled (*line*) and measured (*symbols*) ambient nitrate concentration in the surface water from the monitoring station located in As Vig (st. 6883; year 2005), the bay where the IMTA facility is located. This year was a typical year regarding the environmental conditions

consisted of bryozoans but also barnacles, other filamentous seaweed, and blue mussel juveniles.

Generally, there was a tendency for *S. latissima* sporophytes to increase in length from May to June at the REF site, followed by continuous loss of biomass until November ($P<0.05$; Fig. 4a). Minimum sporophyte length was found in November (25.4±0.327 cm), while highest sporophyte length was found in March (102±7.93 cm) and May (123±5.08 cm), with no significant difference in between the two last mentioned months ($P>0.05$). The increase in length was reduced from May to September at the IMTA site, but the differences were not significant. Sporophytes increased again in length from September to February followed by higher increment from February to May. Highest sporophyte length was achieved by the end of experiment in May (101±16.8 cm), although not significantly different from March (77.6±15.1 cm). Difference in sporophyte length between cultivation sites (IMTA and REF) was significant in June, July, September, and January ($P<0.05$).

Dry matter content changed during the year with the highest values found in July–September (21.7–22.5 %), and the lowest values found in January–March (11.3–12.7 % DW), with no significant difference between cultivation sites. Generally, there was a tendency that the weight of *S. latissima* sporophyte increased from May to August, followed by loss of biomass in September ($P>0.05$; Fig. 4b). Sporophyte weight increased again between September and February and then more from February to the end of experiment in May. The highest individual weight ($P<0.05$) was found in May 2014 in both REF (67.2±8.07 g FW) and IMTA (91.7±25.7 g FW) sites, with no significant difference between sites. Difference in sporophyte weight between cultivation sites showed significance in September and November. The seaweed showed exponential growth from September to May (REF: $R^2=0.8404$ and IMTA: $R^2=0.9591$) with a similar specific growth rate for both locations ($P>0.05$) of 0.0089±0.0008 d^{-1} and 0.0121±0.0014 d^{-1} in the REF and IMTA site, respectively (Fig. 4b).

Sporophyte density on the cultivation lines, 20 days after re-deployment at the IMTA site and 146 after the first deployment at sea (REF site), was 145±20 and 162±25 seedlings m^{-1} of line at the REF and IMTA site, respectively. Biomass yield increased from May to August but not significantly at the REF site ($P>0.05$; Fig. 4c). Highest biomass yield in the first growing season was achieved in August (1.1±0.09–1.5±0.12 kg FW m^{-1} of cultivation line) and September (0.9±0.18–1.5±0.16 kg FW m^{-1} of cultivation line), with significantly higher biomass yield in the IMTA site compared to the REF site found in August. High loss of biomass was observed

Fig. 3 *Saccharina latissima* biomass harvested in **a** May representing clean seaweed and **b** September representing seaweed covered by epiphytes of, e.g., bryozoans, barnacles, other filamentous seaweed, and blue mussel juveniles

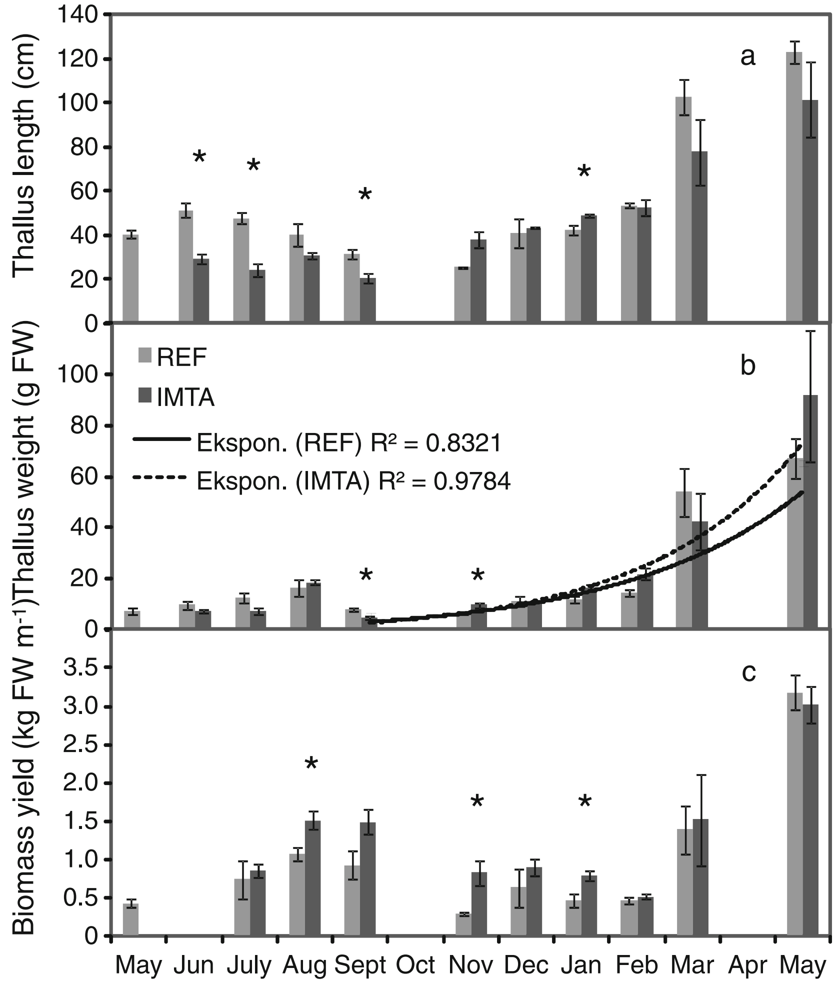

Fig. 4 Year-round variation in the **a** length (cm; n=30), **b** individual weight (g FW; n=30) including exponential fit (R^2) presented as *line* (REF) and *dotted line* (IMTA), and **c** yield (kg FW m^{-1} of cultivation line; n =3) of *Saccharina latissima* sporophytes cultivated at both reference (REF) and IMTA site near Hjarnø fish farm in 2013–2014. Deployment at sea: 15 January 2013. *Asterisks* represent significantly difference between REF and IMTA (P<0.05). Values are mean±SE

after September followed by a stable period. Biomass yield increased again between February and May at both experimental sites. Highest biomass yield over two growing seasons was found by the end of experiment in May 2014 (P<0.05) in both experimental sites (3.0±0.23 and 3.2±0.24 kg FW m^{-1} of cultivation line) with no significant difference between sites (P>0.05).

N content was significantly higher in the REF sporophytes than in the IMTA sporophytes (P<0.05) between July and November (Fig. 5a). The minimum N values were found in July in both experimental sites (0.89±0.019–0.49±0.048 % dry weight; DW), while the highest values were found in November–January in the REF site (3.09±0.107–3.12± 0.112 % DW) and January in the IMTA site (3.67±0.212 % DW). Epiphytes increased significantly the N content of biomass sampled in July and November at the IMTA site but not in September (P>0.05), whereas no significant difference was found in the biomass sampled at the REF site. P content was relatively stable from May to November (P>0.05) followed by an increase from November to January and decreasing in March (Fig. 5b). The lowest P values were found in July (0.0529±0.0003–0.0526±0.00006 % DW), while the highest values were found in January (0.823±0.029–0.791±0.021 % DW). No significant differences were found in the P content between cultivation sites throughout the sampling period (P>0.05). P content was higher in the seaweed samples including epiphytes compared with those of seaweed alone, with significant difference in the September samples (P<0.05).

There was a tendency for higher N assimilation and thereby removal when harvested (mentioned as removal hereafter) at the IMTA site compared to the REF site from July to January, being significantly different in August, November, and January (Fig. 5c). Maximum N removal was achieved in August (5.0–7.0 g N m^{-1} of cultivation line) and September (4.7–7.2 g N m^{-1} of cultivation line) in both experimental sites, being significantly higher in the IMTA site compared to the REF site in August. The minimum values were found in May (0.6 g N m^{-1} of cultivation line) in the beginning of the sampling period in 2013. P removal was similar in both cultivation sites throughout the sampling period (P>0.05) with exception of significantly higher P removal at the IMTA site in August (P<0.05; Fig. 5d). P removal increased from May to August, decreased in November, and increased from November

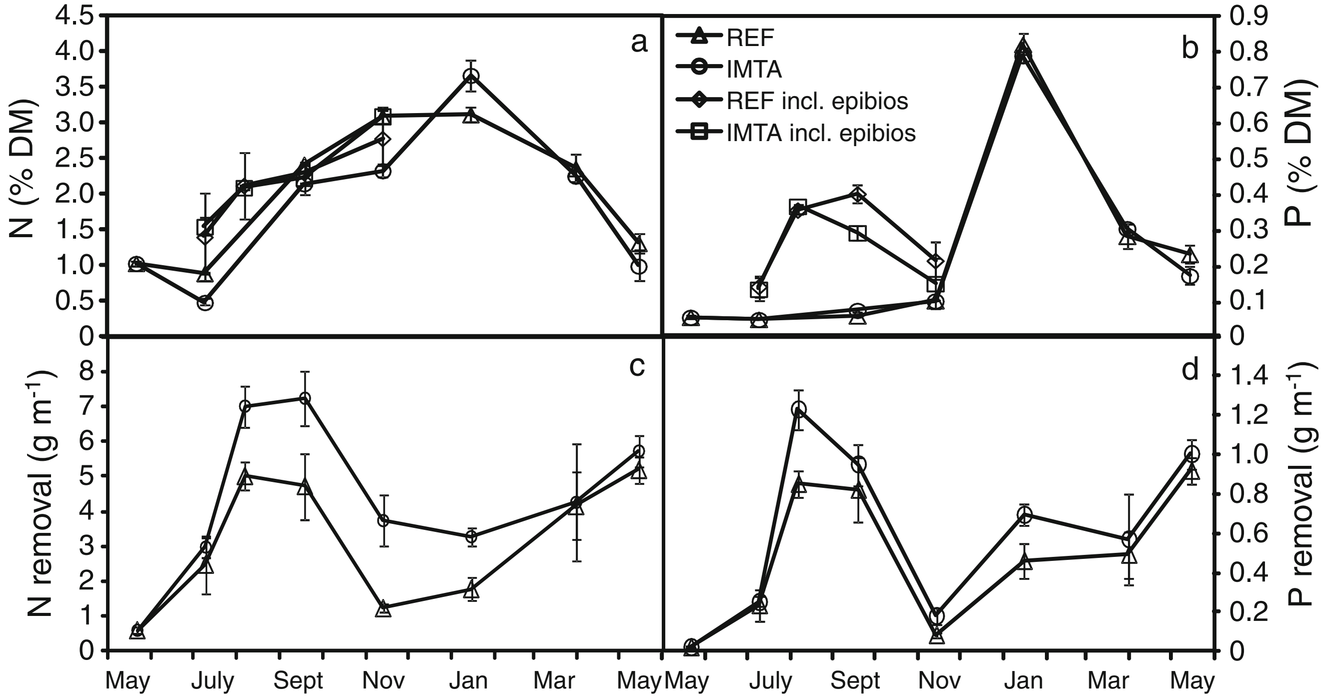

Fig. 5 Year-round variation in the **a** nitrogen (N) and **b** phosphorous (P) content (%DW) and **c** nitrogen and **d** phosphorous removal (g N or P m^{-1} of cultivation line) of *Saccharina latissima* cultivated at both reference (REF) and IMTA site near Hjarnø fish farm in 2013–2014. Deployment at sea: 15 January 2013. The N and P content of seaweed including epiphytes is also presented. Values are mean±SE (n=3)

to May (P<0.05). Maximum P removal was achieved in August and September in both REF (0.86–0.83 g P m^{-1} of cultivation line) and IMTA (1.2–0.96 g P m^{-1} of cultivation line) site but not significantly higher than that found in January, March, and May (2014) in the respective sites, with exception of the values found at the IMTA site in August (P<0.05). Minimum values were found in May (0.03 g P m^{-1} of cultivation line) in the beginning of the sampling period.

Large-scale biomass production and N removal were estimated based on the data collected in this study and the cultivation setup for *S. latissima* established at Hjarnø Havbrug A/S (Table 2). A minimum biomass production of 2.1 t FW ha^{-1} should be achieved in May corresponding to a N removal of 3 kg. Maximum biomass production should be found in September (5.1–7.1 t FW ha^{-1}) on an annual cultivation, while on a biannual cultivation, a maximum biomass production of 6.7–7.1 t FW ha^{-1} should be achieved in May corresponding to a N removal of 23.7–39.4 and 26.0–31.2 kg ha^{-1}, respectively. Full-scale cultivation of the 102-ha REF site should be able to achieve a maximum production of 506–521 t FW in August–September (annual) or 723 t FW ha^{-1} in May (biannual) corresponding to a N removal of 2,448–2,589 or 2,663 kg ha^{-1}, respectively. This is in accordance with the company prospects/goal for 2016; 700 t FW year^{-1} corresponding to a N removal of 2.8 t year^{-1} (Hjarnø Havbrug A/S, M. Mølgaard, personal communication). The commercial harvest by Hjarnø fish farm at the REF site yielded 1 kg FW m^{-1}

Table 2 Biomass yield (kg FW m^{-1} of cultivation line), estimated production (t FW ha^{-1}) and N removal (kg ha^{-1}) when biomass harvested of *Saccharina latissima* cultivated at both reference (REF) and IMTA site upon different harvest times

	Harvest time	Cultivation site	
		IMTA	REF
Harvested biomass (kg FW m^{-1} line)	May 13	–	0.41
	August 13	1.02	0.99
	September 13	1.30	1.01
	May 14	1.23	1.42
Harvested biomass (t FW ha^{-1})	May 13	–	2.1
	August 13	5.5	5.0
	September 13	7.1	5.1
	May 14	6.7	7.1
N removal (kg ha^{-1})	May 13	–	3.0
	August 13	38.2	25.1
	September 13	39.4	23.7
	May 14	31.2	26.0

All cultivation lines were from the same nursery/reproduction batch and were deployed at sea in January 2013. Data were computed using data on biomass yield measured in 4 m length cultivation lines, the N content of the biomass collected in the first meter of line (2 m depth) and meters of cultivation lines per ha based on current features of the cultivation system. In future, the fish farm will place 400 longlines at the 100 ha REF site, which means 4 longlines ha^{-1} each of 220 m length and parallel 8–10 m apart rope with a total harvest of 5,890 kg FW in January to May and with a mean biomass yield of 2–4 kg FW m^{-1} from June to July 2014 from lines deployed in 2012–2013.

Discussion

Stagnation in frond elongation and loss of apical biomass in summer followed by increase in length from September/November to May observed in this study is in accordance with the growth pattern previously reported for the *S. latissima* (Lüning 1979; Sjøtun 1993; Andersen et al. 2011). This may be explained by endogenous growth rhythms (Lüning 1993) and/or nitrate limitation (Sjøtun 1993). The biomass increase until August was probably a result of the observed increased N content (reserve N) and the storage of large amount of photosynthetic product and reserve carbohydrate (e.g., laminarin) as reported for this species (Black 1950). This most likely results in biomass increment but not necessarily in an increase of length. Fast frond growth during late winter and spring lead to gain in exponentially increase of thallus weight and increment in biomass yield. These data show that seasonal growth pattern is a main factor affecting biomass production in *S. latissima* farming. In this study, occurrence of epiphytes (mainly bryozoans) started in July (summer) and increased until August/September when apical and old biomass was lost (summer abscission of distal tissue), and epiphytes only remained present in the apical part of the fronds until November (autumn). The absence of epiphytic organisms from December to June may be related with the low average seawater temperature during this period (2–12 °C) as the infestation of kelp farms by common epiphytes such as hydroids and copepods has been attributed to increasing seawater temperature on kelps harvested in April–July (Park and Hwang 2012; Peteiro and Freire 2013a). Similarly, the epiphytic colonial bryozoan *Membranipora membranacea* is commonly found in commercial cultivation of *Saccharina japonica* during mid-summer in Asia (Kawashima 1993).

S. latissima farming in a biannual cultivation cycle seems to have high potential because maximum epiphyte-free biomass yields twice as much compared to the annual cultivation cycle (deployed at same time as the biannual), regardless the cultivation site. The biannual cultivation is without increasing operational costs, however, increasing risk by leaving the crop longer at sea. These results are opposite of that reported by Peteiro et al. (2006), due to reduced number of *S. latissima* sporophytes on the cultivation line the second year. On the other hand, no enhanced biofilter efficiency was found biannually compared to the annual cultivation in this study, because a biannual cultivation requires two growing seasons, and this makes it less attractive in a bioremediation perspective. Moreover, leaving the biomass late summer means that old frond biomass will get lost, and lost biomass is loss of potential removal of N and P and thereby value, unless the apical part of the biomass is cut/harvested and the basal part is allowed to regrow for the second season.

The maximum *S. latissima* biomass yield at both experimental sites (3–3.2 kg FW m^{-1} rope over 16 months, with only around 6 weeks in hatchery) of this study is lower than previously reported for *S. latissima* cultivated in Spain (4–16 kg FW m^{-1} rope; Peteiro et al. 2006; Peteiro and Freire 2013b) and Canada (11–17 kg FW m^{-1} rope; Chopin et al. 2004). This may be due to recorded temperatures in this study's cultivation area reached down to 0 °C during the main growth period (October–May) and up to 23 °C during summer which is outside the reported optimal growth temperature range of 10–15 °C (Fortes and Lüning 1980; Bolton and Lüning 1982). Furthermore, salinity at the cultivation area reached a minimum of 12–15 psu from February to April, while *S. latissima* presents strong reduction of growth at 16 psu and shows optimal growth at salinity limits between 23 and 31 psu (Bartsch et al. 2008). Moreover, irradiance in winter time (December–February) reaches down to 48 μmol photons m^{-2} s^{-1} which is below photosynthetic saturation level of kelps (150 μmol photons m^{-2} s^{-1}; Lüning 1979). Another important aspect to consider is that seedling production in this study was done based on the direct sowing of spores on collectors using reproducers collected from natural populations, while in some other studies, seedlings were produced from established gametophyte cultures (e.g., Peteiro et al. 2006; Peteiro and Freire 2009). These different techniques may influence the seedling density and ultimately the biomass production. Furthermore, earlier deployment at sea most likely enhances biomass yield as it has been shown in the Atlantic waters of NW Spain, possibly due to longer cultivation period during the cold season (Peteiro and Freire 2009).

The highest N content was achieved in January in both cultivation sites. This peak matches the peak of the ambient nitrate concentrations in the area. Similarly, the lowest values found in May–July match the lowest ambient nitrate concentrations (measured and modeled nitrate concentration Fig. 2). Moreover, the higher nitrogen content found in the seaweed cultivated in the REF site compared to the IMTA indicates that the release of inorganic nutrients from the fish farm (Fig. 1c) is negligible compared to the naturally occurring background concentrations of nitrogen. This especially taking into account that the IMTA site is located 500 m from the fish farm and that the IMTA site is only located downstream in 50 % of the time. Moreover, the fish farm in this study is relatively small compared with other commercial salmonid farms. While seaweeds cultivated in the vicinity of fish farms usually have an increase in their N content (Chopin et al. 2000; Abreu et al. 2009; Sanderson et al. 2012; Handå et al. 2013), utilization of waste nutrients from fish farms by the seaweed is very site specific and dependent on a number of factors including nutrient loading, current speed and direction, and distance to the nutrient source. Increased N content of *S. latissima* sporophytes grown on droppers, frames, and longlines in close proximity (10–55 m away) to salmon cages has been reported (Sanderson et al. 2012; Handå et al. 2013). However, such small distances are not realistic in commercial scale IMTA. This is due to interference with normal fish farming activities and the large areas required for seaweed farming. Moreover, cultivation of seaweed in very close proximity to the fish farm has been associated with increasing amount of organic particles, resulting in a less clean biomass and higher occurrence of epiphytes (Abreu et al. 2009), not desirable for seaweeds for human consumption.

N content of *S. latissima* following the ambient levels was also concluded in another recent Danish study on natural seaweed populations; however, the minimum N content was observed to be later (July to November) compared to this study (Nielsen et al. 2014). Another study on natural populations of *S. latissima* also showed minimum N content in summer (Black 1950). Moreover, our data are consistent with that reported by Handå et al. (2013) who found increasing N content from May to August–September in cultivated *S. latissima* in Norway. P content followed the same annual seasonal variation pattern as N with the highest concentrations found in January in both experimental sites (0.79–0.82 % DW) and the lowest concentrations found in May–September (0.06–0.08 % DW) which is, respectively, higher and lower than the mean P content of *S. latissima* cultivated in IMTA with salmon culture in Canada (3.09±0.75 mg g^{-1} DW; Reid et al. 2013).

Higher N removals (driven by higher yield) of seaweed cultivated at the IMTA site compared to the REF site in August (approximately 5–7), November (approximately 1–4), and January (approximately 2–3 g N m^{-1} of cultivation line) are in accordance with similar studies showing faster growth and higher biomass production of *S. latissima*, *Gracilaria chilensis*, and *Macrocystis pyrifera* cultivated in IMTA with salmon compared to reference sites (Troell et al. 1999; Buschmann et al. 2008b; Sanderson et al. 2012; Handå et al. 2013). The same pattern was observed for the P removal. The specific growth rates were however not significantly different between REF and IMTA in our study. The highest N removals and yields also after the fish were harvested (January) at the Danish fish farm further support that the ambient nutrient concentrations have higher impacts on the biofilter potential of the seaweed.

The yield of brown seaweeds on an annual basis (12–60 t DW ha^{-1} $year^{-1}$) is equivalent to 80–400 t FW ha^{-1} $year^{-1}$ by rope cultivation reported from worldwide experiences (Bruton et al. 2009). The highest values were found in Asia, while in Europe, there is a lack of values for yield in upscaled experiments. Sanderson et al. (2012) estimated a maximum extrapolated yield of 340 t FW ha^{-1} $year^{-1}$ in Scotland, while an estimated yield of 30.4–45.6 t FW ha^{-1} over one growing season was reported for *S. latissima* cultivated in Spain (Peteiro and Freire 2013b; Peteiro et al. 2014). In the present study, the lower extrapolation yields were made based on the yield achieved in large-scale cultivation, which should give more realistic values for commercial *S. latissima* cultivation, particularly in North European waters. Moreover, yields used for the extrapolation in this study (0.41–1.42 kg FW m^{-1} cultivation line) are comparable to the average value reported for the commercial harvest by Hjarnø Havbrug A/S fish farm at the REF site (1 kg FW m^{-1} rope with a total harvest of 5, 890 kg FW from January to May) from lines deployed in 2012–2013. Another important aspect to consider is that different cultivation systems/setups used in the different studies (e.g., longlines, droplines, frames, and distance between cultivation structures) will have an effect on the actual and estimated yields. In addition, extrapolation in this study may underestimate the yield as the cultivation lines were deployed in January, because earlier deployment time has been reported to improve biomass yield of *S. latissima* (Peteiro and Freire 2009). Moreover, N content used in the data computation was determined for *S. latissima* cultivated at 2 m depth and N content may increase with depth.

The highest yield and thereby biofilter efficiency found in August–September could be considered as the best annual cultivation harvest time. However, biomass collected during this period presented heavy biofouling that makes it unsuitable for human consumption, which is currently the main commercial application for *S. latissima* biomass (Peteiro et al. 2006; Sanderson et al. 2012). Commercial application for this biomass could then be considered such as supplement for fish feed. The epiphytes would not necessarily be a hindrance when utilized for animal feed (manuscript submitted); however, the biomass value for animal feed will be less than for human consumption (Bruton et al. 2009). The biomass should be harvest in May to be clean and utilized directly for human consumption before the fouling season begins. This will however compromise the bioremediation potential of the biomass. The REF site with seaweed cultivation was recently organic certified which should increase market price as organic products are in increasing demand.

The monitoring in this project has already been of high importance for this particular fish farm as it has provided valuable data on the effect of harvest time on biomass yield and N removal contributing to a (qualified informed) decision regarding selection of harvest time. Moreover, this project has provided bioremediation data for the Danish authorities. The seaweed harvested in the commercial IMTA system compensates the N budget or N quota that limits the Danish fish farms. Hjarnø fish farm has just recently been permitted a fully compensated (nil netto N output) fish farm 10 times the size of their existing fish farm. This facility is further away from the fjord and with more water exchange (Fig. 1b); however, the compensation/bioremediation with mussels and seaweed should be at the existing IMTA and REF facilities. The fish farm has initiated the production and will be fully operational (2,200 t of fish, 8,000 t of mussels, and 700 t of seaweed produced) in 2016. This will then not be IMTA but with separated fed and biofilter cultures. This future upscale in biofilter cultures could then lead to 80 t N net removal from the mussels by biomass harvest. Moreover, mussels remineralize nutrients, since mussels respire approximately 35–40 % of consumed N (Kiørboe and Møhlenberg 1981). This means that cultivated mussels actually contribute locally with dissolved N and that seaweed in an IMTA/polyculture system takes advantage of being close to mussel cultivation.

Further improvement of biomass production and bioremediation may include *S. latissima* domestication towards the development of an improved commercial variety (e.g., faster growth, larger plants, higher N tissue content), study/selection of better cultivation sites for optimal growth, new seeding materials for higher seedling densities, and optimized cultivation system (e.g., configuration and growth structures).

Conclusions

The recognition by the Danish authorities of the environmental service of biofilters, such as seaweed and mussels, through the establishment of N credits is an unprecedented move from direct uptake of nutrients released from fish farms towards a wider geographic ecosystem context (compensation). The use of the seaweed *S. latissima* shows potential for assimilation of nutrients, particularly nitrogen. This has potential environmental and economic benefits (e.g., wastewater management and for application of biomass) for the specific fish farm, even though the effect of N bioremediation is negligible to the major N budget of the inner Danish waters. Yields of the commercial cultivation of *S. latissima* achieved in the present study are lower than other reported and mainly extrapolated figures from smaller scale cultivations. The harvest time should be carried out in August–September in an annual cultivation in order to achieve maximum biofiltration efficiency or May in order to maximize biomass value for human consumption. Harvest time will depend not only on the biomass yield and biofilter efficiency but also the epiphyte coverage which has a significant effect on the commercial application/value of the biomass.

Acknowledgments This study was supported/financed by The Danish AgriFish Agency (GUDP)—3405-11-0375. Special thanks are due to the other project partners Danish Aquaculture Organization, Orbicon, TrippleNine, Seaweed Seed Supply/Seaweed Energy Solution, and not least Hjarnø Havbrug A/S that provided practical help and logistics at sea, Cátia Ribeiro for helping out in the laboratory, and valuable comments by Julia Overton.

References

Abreu MH, Varela DA, Henríquez L, Villarroel A, Yarish C, Sousa-Pinto I, Buschmann AH (2009) Traditional vs. integrated multi-trophic aquaculture of *Gracilaria chilensis* C. J. Bird, J. McLachlan & E. C. Oliveira: productivity and physiological performance. Aquaculture 293:211–220

Abreu MH, Pereira R, Yarish C, Buschman AH, 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

Andersen GS, Steen H, Christie H, Fredericksen S, Moy FE (2011) Seasonal patterns of sporophyte growth, fertility, fouling, and mortality of *Saccharina latissima* in Skagerrak, Norway: implications for forest recovery. J Mar Biol 2011:1–8. doi:10.1155/2011/690375

AOAC (2006) Official methods of analysis of AOAC International, 18th edn. AOAC International, Gaithersburg

Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R, Karsten U, Molis M, Roleda MY, Schubert H, Schumann R, Valentin K, Weinberger F, Wiese J (2008) The genus *Laminaria* sensu lato: recent insights and developments. Eur J Phycol 43:1–86

Black WAP (1950) The seasonal variation in weight and chemical composition of the common British Laminariaceae. J Mar Biol Assoc United Kingdom 29:45–72

Bolton JJ, Lüning K (1982) Optimal growth and maximal survival temperatures of Atlantic *Laminaria* species (Phaeophyta) in culture. Mar Biol 66:89–94

Bruton T, Lyons H, Lerat Y, et al. (2009) A review of the potential of marine algae as a source of biofuel in Ireland. Sustainable Energy Ireland. http://www.seai.ie/Publications/Renewables_Publications_/Bioenergy/Algaereport.pdf. Accessed 1 May 2014

Buschmann AH, Hernández-González MC, Aranda C, Chopin T, Neori A, Halling C, Troell M (2008a) Mariculture waste management. In: Jørgensen SE, Fath BD (eds) Encyclopedia of ecology. Elsevier, Oxford, pp 2211–2217

Buschmann AH, Varela DA, Hernández-González MC, Huovinen P (2008b) 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. J Appl Phycol 20:571–577

Chopin T, Yarish C, Wilkes R, Bleya E, Lu S, Mathieson A (2000) Developing *Porphyra*/salmon integrated aquaculture for bioremediation and diversification of the aquaculture industry. J Appl Phycol 11:463–472

Chopin T, Buschmann AH, Halling C, Troell M, Kautsky N, Neori A, Kraemer GP, Zertuche-González JA, Yarish C, Neefus C (2001) Integrating seaweeds into marine aquaculture systems: a key toward sustainability. J Phycol 37:975–986

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

Chopin T, Cooper JA, Reid G, Cross S, Moore C (2012) Open-water integrated multi-trophic aquaculture: environmental biomitigation and economic diversification of fed aquaculture by extractive aquaculture. Rev Aquac 4:209–220

FAO, Fisheries and Aquaculture Department (2012) The state of world fisheries and aquaculture 2012. Food and Agriculture Organization of the United Nations, Rome

Fleurence J (1999) Seaweed proteins: biochemical, nutritional aspects and potential uses. Trends Food Sci Technol 10:25–28

Fortes MD, Lüning K (1980) Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. Helgol Meeresunters 34:15–29

Frette O, Erga SR, Hamre B, Aure J, Stamnes JJ (2004) Seasonal variability in inherent optical properties in a western Norwegian fjord. Sarsia 89:276–291

Handå A, Forbord S, Wang X, Broch OJ, Dahle SW, Størseth TR, Reitan KI, Olsen Y, Skjermo J (2013) Seasonal- and depth-dependent growth of cultivated kelp (*Saccharina latissima*) in close proximity to salmon (*Salmo salar*) aquaculture in Norway. Aquaculture 414–415:191–201

HELCOM (2012) Fifth Baltic Sea pollution load compilation (PLC-5)—an executive summary. Baltic Sea Environment Proceedings No. 128A.

Holdt SL, Edwards MD (2014) Cost-effective IMTA: a comparison of the production efficiencies of mussels and seaweed. J Appl Phycol 26: 933–945

Holdt SL, Kraan S (2011) Bioactive compounds in seaweed: functional food applications and legislation. J Appl Phycol 23:543–597

Ito K, Hori K (1989) Seaweed: chemical composition and potential food uses. Food Rev Int 5:101–144

Kawashima S (1993) Cultivation of the brown alga, *Laminaria* "kombu". In: Ohno M, Critchley AT (eds) Seaweed cultivation and marine ranching. Japan International Cooperation Agency, Jokosuka, pp 25–40

Kiørboe T, Møhlenberg F (1981) Particle selection in suspension-feeding bivalves. Mar Ecol Prog Ser 5:291–296

Lüning K (1979) Growth strategies of three *Laminaria* species (Phaeophyceae) inhabiting different depth zones in the sublittoral region of Helgoland (North Sea). Mar Ecol Prog Ser 1:195–207

Lüning K (1993) Environmental and internal control of seasonal growth in seaweeds. Hydrobiologia 260–261:1–14

Mata L, Schuenhoff A, Santos R (2010) A direct comparison of the performance of the seaweed biofilters, *Asparagopsis armata* and *Ulva rigida*. J Appl Phycol 22:639–644

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

MIKE (2005a) MIKE 3 flow model mud transport module. User guide. DHI Water and Environment. Release 2005.

MIKE (2005b) MIKE 21/3 EU, eutrophication module. A scientific description. DHI water and environment. Release 2005.

Neori A, Chopin T, Troell M, Buschmann AH, Kraemer GP, Halling C, Shpigel M, Yarish C (2004) Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231:361–391

Nielsen MM, Krause-Jensen D, Olesen B, Thinggaard R, Christensen P, Bruhn A (2014) Growth dynamics of *Saccharina latissima* (Laminariales, Phaeophyceae) in Aarhus Bay, Denmark, and along the specie's distribution range. Mar Biol 161:2011–2022

Nixon SW (1995) Coastal marine eutrophication: a definition, social causes, and future concerns. Ophelia 41:199–219

Olsen LM, Holmer M, Olsen Y (2008) Perspectives of nutrient emission from fish aquaculture in coastal waters: literature review with evaluated state of knowledge. Fishery and Aquaculture Industry Research Fund, Norway. http://www.aquacircle.org/images/pdfdokumenter/udvikling/andre/norden/fhf-nutrients_and_aquaculture.pdf. Accessed 1 Jun 2014

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

Pang SJ, Lüning K (2004) Breaking seasonal limitation: year-round sporogenesis in the brown alga *Laminaria saccharina* by blocking the transport of putative sporulation inhibitors. Aquaculture 240: 531–541

Park CS, Hwang EK (2012) Seasonality of epiphytic development of the hydroid *Obelia geniculata* on cultivated *Saccharina japonica* (Laminariaceae, Phaeophyta) in Korea. J Appl Phycol 24:433–439

Peteiro C, Freire Ó (2009) Effect of outplanting time on commercial cultivation of kelp *Laminaria saccharina* at the southern limit in the Atlantic coast, N.W. Spain. Chinese J Oceanol Limnol 27:54–60

Peteiro C, Freire Ó (2013a) Epiphytism on blades of the edible kelps *Undaria pinnatifida* and *Saccharina latissima* farmed under different abiotic conditions. J World Aquac Soc 44:706–715

Peteiro C, Freire Ó (2013b) Biomass yield and morphological features of the seaweed *Saccharina latissima* cultivated at two different sites in a coastal bay in the Atlantic coast of Spain. J Appl Phycol 25:205–213

Peteiro C, Salinas JM, Freire Ó, Fuertes C (2006) Cultivation of the autoctonous seaweed *Laminaria saccharina* off the Galician coast (NW Spain): production and features of the sporophytes for an annual and biennial harvest. Thalassas 22:45–53

Peteiro C, Sánchez N, Dueñas-Liaño C, Martínez B (2014) Open-sea cultivation by transplanting young fronds of the kelp *Saccharina latissima*. J Appl Phycol 26:519–528

Reid GK, Chopin T, Robinson SMC, Azevedo P, Quinton M, Belyea E (2013) Weight ratios of the kelps, *Alaria esculenta* and *Saccharina latissima*, required to sequester dissolved inorganic nutrients and supply oxygen for Atlantic salmon, *Salmo salar*, in integrated multi-trophic aquaculture systems. Aquaculture 408–409:34–46

Rodrigueza MRC, Montaño MNE (2007) Bioremediation potential of three carrageenophytes cultivated in tanks with seawater from fish farms. J Appl Phycol 19:755–762

Sanderson JC, Dring MJ, Davidson K, Kelly MS (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

Sjøtun K (1993) Seasonal lamina growth in two age groups of *Laminaria saccharina* (L.) Lamour. in Western Norway. Bot Mar 36:433–441

Skogen MD, Eknes M, Asplin LC, Sandvik AD (2009) Modelling the environmental effects of fish farming in a Norwegian fjord. Aquaculture 298:70–75

Troell M, Halling C, Nilsson A, Buschmann AH, 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, Rönnbäck P, Halling C, Kautsky N, Buschmann A (1999) Ecological engineering in aquaculture: use of seaweeds for removing nutrients from intensive mariculture. J Appl Phycol 11:89–97

Troell M, Halling C, Neori A, Chopin T, Buschmann AH, Kautsky N, Yarish C (2003) Integrated mariculture: asking the right questions. Aquaculture 226:69–90

Wang X, Olsen L, Reitan K, Olsen Y (2012) Discharge of nutrient wastes from salmon farms: environmental effects, and potential for integrated multi-trophic aquaculture. Aquac Environ Interact 2:267–283