J Appl Phycol (2016) 28:1153–1165
DOI 10.1007/s10811-015-0673-7

The effect of light and nutrient availability on growth, nitrogen, and pigment contents of *Saccharina latissima* (Phaeophyceae) grown in outdoor tanks, under natural variation of sunlight and temperature, during autumn and early winter in Denmark

Teis Boderskov[1] · Peter Søndergaard Schmedes[1] · Annette Bruhn[1] · Michael Bo Rasmussen[1] · Mette Møller Nielsen[1] · Morten Foldager Pedersen[2]

Received: 11 March 2015 / Revised and accepted: 14 July 2015 / Published online: 29 July 2015

Abstract Late summer harvest of cultivated *Saccharina latissima*, prior to seasonally determined negative length growth, is considered advantageous in North Atlantic waters to optimize biomass yields. We hypothesized that seasonal increase in tissue protein and pigments over autumn and early winter would counterbalance the loss of biomass, and increase the absolute harvestable amount of protein and pigments. The hypothesis was tested in a land-based, factorial-designed, pilot-scale experiment using whole algae individuals exposed to naturally relevant high or low availability of nutrients and light. The experiment was conducted during fall/early winter in Grenaa, Denmark, in outdoor tanks, exposed to ambient light and temperature variations. With high nutrient availability, the absolute harvestable amounts of nitrogen, fucoxanthin, and chlorophyll *a* increased by 50.1–60.1, 21.7–53.7, and 47.0–73.5 %, respectively, despite a loss of biomass of 16.2–18.7 %. Under low nutrient availability, there was a net loss of biomass (8.1–9.5 %), tissue nitrogen (10.7–44.1 %), and fucoxanthin (7.1–17.2 %), and a minor increase in chlorophyll *a* (2.5–22.8 %). Nutrient availability had a significant negative impact on the biomass growth, but a positive control on the tissue concentration of nitrogen, chlorophyll *a*, and fucoxanthin. Our results, from a land-based experiment, indicate that early winter harvest of *S. latissima* biomass grown under high nutrient availability in Denmark, fulfills a higher degree of nutrient bioremediation, and has an improved biomass quality in regards of increased concentrations of pigments and nitrogen rich compounds.

Keywords Biomass quality · Bioremediation · Chlorophyll *a* · Cultivation · Ecosystem services · Fucoxanthin

✉ Teis Boderskov
teisboderskov@gmail.com

[1] Department of Bioscience, Aarhus University, Vejlsøvej 25, 8600 Silkeborg, Denmark

[2] Department of Environmental, Social and Spatial Change (ENSPAC), Roskilde University, Universitetsvej 1, 4000 Roskilde, Denmark

Introduction

Cultivation of large brown algae (Laminariales) is gaining momentum in Europe. Cultivation of algae in coastal waters is focused on large brown algae, and *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, L. Druehl and G.W. Saunders is the most commonly cultivated species across the North Atlantic area (Forbord et al. 2012; Schmedes and Boderskov 2013; Handå et al. 2013; Holdt and Edwards 2014; Peteiro and Freire 2009, 2013b). The biomass produced, and the implicit ecosystem services obtained through assimilation and immobilization of nutrients, receive great attention from environmental authorities and industry. Research and development activities seek to optimize algal biomass yields and reach a proper quality for intended purposes of the biomass such as food, feed, and energy (Adams et al. 2009; Handå et al. 2013). The mitigation effect is currently being tested using seaweed as a compensation crop for mitigation of coastal eutrophication deriving from land-based sources (Seghetta et al., unpublished), but particularly, also in relation to reducing nutrient loading from aquacultural production of fed species, such as fin fish (Sanderson et al. 2012; Handå et al. 2013; Holdt and Edwards 2014; Marinho et al. 2015).

The quality and potential yield of *S. latissima* biomass varies on temporal and spatial scales with the availability of light and nutrients being the most central controlling environmental factors (Black 1950; Gevaert et al. 2001; Nielsen et al. 2014). Thus, the desired end use of the produced biomass adds to determine optimal cultivation sites as well as the optimal timing of deployment and harvest. Summer is often considered the optimal time for harvest when production of energy or hydrocolloids is the major target, since biomass yield as well as the tissue content of carbohydrates in kelps peak in late summer (Black 1950; Adams et al. 2011). However, if bioremediation of nutrients is the major target, summer harvest is not optimal because (1) seaweeds contains less nitrogen (N) and phosphorus (P) in summer than during the rest of the year (Nielsen et al. 2014; Marinho et al. 2015) and (2) harvest in summer creates a temporal mismatch between nutrient excretion from fin fish production and seaweed nutrient assimilation in salmonid production in temporal regions, as the fish are typically deployed in early spring and not slaughtered before late autumn/early winter, as also emphasized by Handå et al. (2013).

However, leaving the algal biomass in the water during summer has proved problematic in some areas due to fouling of the biomass by filter feeders (e.g. mussels, ascidians, hydrozoans) and epiphytic algae (Handå et al. 2013; Peteiro and Freire 2013a). Some argue that harvest of the seaweed-epiphyte complex yields an improved nutrient extraction (Marinho et al. 2015); however, the quality of the biomass for food, feed, or other industrial purposes is somewhat compromised. Others argue that the seaweed will shed the biofouling organisms with the old frond tissue during autumn and winter (Lüning and Pang 2003) or recommend cultivation in areas with high water movement, as this will limit the settlement of biofouling organisms. The water movement should preferably be in the form of currents, as high wave energy can be destructive for algae crops (Sanderson et al. 2012).

In addition to the hydrocolloids, *Laminaria* biomass contains a broad range of high-value components, amino acids, polyphenols and pigments, causing a strong interest in the food, feed, and nutraceutical industry (Holdt and Kraan 2011). The brown algae-specific carotenoid, fucoxanthin, has unique bioactive properties (D'Orazio et al. 2012), both pigments and polyphenols serve as strong antioxidants (Jimenez-Escrig et al. 2001), and pigments generally constitute natural colorants that transfer through feed to flesh (Soler-Vila et al. 2009). The amino acid composition of seaweeds is original and different from that of land plants (Cerna 2011). The synthesis and tissue concentration of high-value components of industrial interest within the biomass (i.e., amino acids, pigments, and antioxidants) increase with nitrogen availability (Gerard 1988; Kopsell et al. 2007), and most photosynthetic pigments (e.g., chlorophylls) also increase with decreasing light availability (Bruhn and Gerard 1996; Gomez and Wiencke 1998; Roleda and Dethleff 2011). Concentrations of pigments, such as carotenoids, respond to light availability in a more complex manner, reflecting their accessory as well as photoprotective functions. For instance, the carotenoid content has been shown to increase with increasing light in first-year fronds of *S. latissima*, whereas the response was opposite in 2-year fronds (Henley and Dunton 1995). The natural increase in nutrients in conjunction with the decreasing light intensity during fall or early winter, would therefore potentially deliver another range of valuable products as well as increased ecosystem services when the seaweed is harvested in late fall or early winter.

When leaving the biomass in the water during autumn and early winter in areas with high nutrient availability, e.g., in close proximity to aquaculture activities, the focus would be on minimizing the natural biomass loss, but in particular, maximizing the tissue content of nutrients and other valuable compounds such as pigments. In open water cultivation, the only practically adjustable factor is the depth of cultivation and therefore, the light availability at this time of the season. A lowering of the cultivation lines of only a few meters in the water column could have profound effects on pigment concentrations since the irradiance in the water column decreases exponentially.

When harvesting, one method is "blade tip cutting," where the haptera, stipe, and frond meristem is left for regrowth (Scoggan et al. 1989). This method allows for multiple harvests per season and saves the producer from investing in new seeded lines every season (Sanderson et al. 2012). When practicing this method, the internal distribution of valuable compounds becomes important, as the position of cutting could affect the harvest yield of both photosynthates, nutrients and pigments (Henley and Dunton 1995).

The aim of this study was to investigate how environmentally realistic variations in nutrient availability and light affect growth and tissue content of nitrogen and major pigments in *S. latissima* during fall and early winter. We hypothesize that harvest in winter under naturally high ambient nutrient availability will result in negative length growth, but higher tissue concentrations—and thus absolute harvestable amounts—of nitrogen (with positive implications for bioremediation as well as protein production) and pigments (antioxidants). We expected further that decreased light availability (mimicking variations in deployment depth) would have a negative effect on growth, but a positive effect on tissue concentrations and absolute harvestable amounts of pigments (antioxidants). The results are discussed in the perspectives of production of high-value food and feed ingredients, ecosystem services and harvest methods.

Materials and methods

We cultured *S. latissima* in a large land-based outdoor cultivation system during fall and early winter, applying a factorial design with two realistic levels of nutrients (limiting and saturating) and two light regimes (corresponding to the light conditions of two cultivation depths of approximately 2 and 4 m in open Danish waters). We analyzed and tested the effects and interactions of light and nutrient concentration on growth and internal concentrations of nitrogen (N), carbon (C), and selected major pigments (chlorophyll *a* (Chl *a*) and fucoxanthin). By analyzing algal tissue from the basal, medial, and apical parts of the fronds, we also aimed to explain the dynamics of incorporation, allocation, and utilization of N and C within the frond during autumn and early winter.

Algae collection Individuals of *S. latissima* were collected at 5-m depth on the 17th of September 2012 from Fornæs, Grenaa, Denmark (56° 26′ 35.94″ N, 10° 57′ 32.42″ E), and transported directly to the research facility at AlgeCenter Denmark, Grenaa.

Tank system The tank system was made up by two separate water systems, each with six 2000-L tanks connected in parallel to a central header tank of 2000 L (Fig. 1). Each system was initially filled with sand/UV-filtered seawater from a nearby public aquarium. The water was recirculated in each system with a flow rate of approximately 400 L h^{-1} and continuously filtered through a drum filter (60 μm). Each header tank was automatically supplied with sand/UV-filtered seawater when the water level decreased due to water cleaning or evaporation. The tanks were aerated 10 min every hour, in order to generate water mixing and to circulate and shift the position of the algae in the tanks. As the tanks were located outside, temperature and light varied according to the natural climatic conditions. Insolation and air temperature were logged every 5 min by a photosynthetically available radiation (PAR) sensor (Photosynthetic Light (PAR) smart sensor S-LIA-M003) and an air temperature logger (HOBO: 12-bit temperature/RH smart sensor S-THB-M002) placed just beside the tanks.

Experimental design and conditions The experiment was set up as a 2-factorial experiment with two levels of nutrient concentrations and two levels of light (44 and 21 % of incoming light). The high concentration of nutrients was chosen to match the concentrations relevant for close proximity to fish farming, natural upwelling, and naturally high late autumn/winter concentrations in Danish coastal waters (Ahn et al. 1998; Hansen 2013). One of the two independent water systems (i.e., with six connected tanks) was set up with naturally high nutrient concentrations, while the other was set up with low nutrient concentrations.

Nutrient fertilization was carried out using industrial grade fertilizer (Min Have Næring NPK 5-1-4, Garta DK), tested in own lab to contain 3603 mM NO_3^-, 405 mM NH_4^+, and 279 mM PO_4^{3-}. The nutrient additions were balanced to add 7.34/0.17 μmol NO_3^- L^{-1} day^{-1}, 0.82/0.02 μmol NH_4^+ L^{-1} day^{-1}, 0.57/0.01 μmol PO_4^{3-} L^{-1} day^{-1} in the high and low nutrient system respectively. Nutrient stock solutions were continuously supplied to the header tanks using peristaltic pumps. Water samples for the analysis of NO_3^-, NH_4^+, and ortho-PO_4^{3-} were taken from each tank every second week. The salinity of the tanks remained stable, ranging between 24 and 26 PSU during the experiment.

The light intensity at the bottom of the tanks corresponded to 44 % of the insolation in the high light treatment and 21 % of the insolation in the low light treatment. Consequently, during the experiment, the daily average of irradiance fluctuated between 1.5 and 38 μmol photons m^{-2} s^{-1} in the low light treatment and between 2.9 and 76 μmol photons m^{-2} s^{-1} in the high light treatment. These light levels were chosen as they approximately correspond to the light levels at, respectively, 2- and 4-m depth in the open parts of Kattegat (data from the national database for marine data; DNAMAP). The light treatments were generated by covering half of the tanks (randomized) of both nutrient treatments with semi-transparent lids to create a higher light attenuation (the low light treatment), whereas tanks with no lid represented the high light treatment.

Preconditioning of algae Preconditioning of algae was initiated on the 27th of September. The algae were divided into two size classes of <70 and >70-cm length and were thereafter distributed evenly among the 12 tanks. This ensured that all tanks had the same distribution of smaller first-year algae and larger second- or third-year algae. On a weight basis, the majority of algae was second- or third-year individuals. The algae were kept in the tanks under natural light and nutrient conditions until the 11th of October, 5 days prior to the start of the experiment. On the 11th of October, nutrient additions were initiated and the semi-transparent lids mounted on the randomly selected tanks. At the start of the experiment, none of the algae were fertile and at the termination of the experiment only one algae from the high light, high nutrient treatment was found fertile.

Growth measurements The experiment was initiated on the 16th of October and terminated after 49 days on the 3rd of December, due onset of air temperatures below 0 °C. At the initiation of the experiment, seven individuals from each tank were randomly selected and removed for measuring the initial distribution of C and N within the thallus. The remaining algae represented approximately 0.8 kg fresh weight (FW) of algae in each tank (several individuals), equivalent to a biomass density of approximately 0.4 kg FW m^{-2}. Frond elongation

Fig. 1 Simplified visualization of the experimental design in the cultivation facility. The two separate water systems consisted of a header tank and six experimental tanks each with a volume of 2 m^3. The two different nutrient treatments (18 and 0.1 μM N) were generated by addition of nutrient stock solution in the header tank. Light treatments were generated by covering three random tanks within each level of nutrients with a lid

was measured using the punch hole method (Parke 1948) while biomass growth was measured by recording changes in algal FW biomass over time. At the onset of the experiment, two holes (each 5 mm in diameter) were punched in the lamina 10 cm above the meristem, in each of three large (>70 cm), randomly chosen individuals from each tank. The initial FW biomass of algae in each tank was measured. The FW biomass (of all algae) and the distance from the meristem to the punched holes were subsequently measured every second week over the course of the experiment. The water concentration of dissolved nutrients was measured at the experiment start/end and during the experiment at the same intervals as for the growth monitoring.

Another seven individuals from each tank were randomly selected by the end of the experiment to follow changes in the distribution of C and N within in the thallus. Tissue samples (each of 10–30 g FW) were cut from the basal, medial, and apical parts of the frond of each individual sampled at the start and end of the experiment, and stored at −18 °C until further analysis of tissue content of C, N, and pigments.

Biochemical analyses Water samples were analyzed for concentrations of NO_3^-–N using a NO–NO_2–NOx analyzer (Thermo Environmental Instruments Inc. 42C), and for concentrations of ortho-P and NH_4^+–N by standard spectrometry methods (Grasshoff et al. 1983). Algal samples were freeze dried to constant dry weight (DW) and finely ground. Concentrations of carbon and nitrogen were analyzed using a PerkinElmer 2400 Series II CHN Analyzer (PerkinElmer Inc., USA).

Pigments were extracted from 20 to 40 mg DW subsamples suspended in 4 mL 90 % acetone followed by 60 s. sonication. Samples were left to extract for 1 h after which they were filtered through Whatman Anodisc (0.1 μm) filters and analyzed using high-performance liquid chromatography (HPLC). The HPLC system (Waters, Milford, MA, USA) was equipped with a 600E solvent delivery system, a 717 autosampler set at 4 °C, a C18 column (dimensions=250× 4.6 mm, particle size=5 μm, Spherisorb-ODS1 Waters, USA) and a 996 photodiode array detector. The detector was set at 440 and 660 nm for carotenoid and phorbin peak integration, respectively. After sample injection (40 μL of acetone extract), pigments were eluted by linear gradient from 100 % solvent A (51:36:13 methanol/acetonitrile/MilliQ water, *v*/*v*/*v*) to 75 % A and 25 % B (70:30 ethyl acetate/acetonitrile, *v*/*v*) for 5 min followed by 5- and 20-min isocratic hold at 75 % A and 100 % B, respectively. The flow rate was 1.2 mL min^{-1}. The solvent composition was returned to initial conditions over a 5-min gradient, followed by 5 min of system equilibration before injection of the next sample. Pigments were identified by comparison with a library of pigments spectra. Standards for Chl *a* and fucoxanthin, were obtained from DHI Laboratory Products (Hørsholm, Denmark).

The area-specific dry matter content was calculated by using disks of a known diameter perforated from the individuals used to measure the length increment. The disks were punctured from the thallus, weighed fresh, freeze dried, and weighed dry.

Data analysis Repeated measures ANOVA, with one "within subjects" factor (time, with two or three levels depending on analysis) and two "between subjects" factors (nutrient and light treatment, each with two levels and both considered fixed factors), was used to test if frond elongation, growth rates, and tissue C, N, and pigment concentrations changed over the course of the experiment. Mauchly's test of sphericity was used to test if the error covariance matrix of the orthonormalized transformed dependent variables was proportional to the identity matrix. If not (i.e., when $p<0.05$), p values for tests including the repeated factor (time) were adjusted using the Greenhouse-Geisser or the Huynh-Feldt corrections (Quinn and Keogh 2002).

Three-factorial ANOVA was used to test if nutrient treatment, light treatment, and frond section (i.e., the basal, mid, and distal parts of the frond) affected the final content of C, N, chlorophyll, and fucoxanthin in *S. latissima*. Two-factorial ANOVA was used to test if the final harvestable yield of C, N, chlorophyll, and fucoxanthin differed among nutrient and light treatments. Komogorov-Smirnoff test was used to check for normality and Levenes test was used to test for

homogeneity of variances. Data were ln-transformed when necessary to obtain normally distributed residuals and/or to obtain equal variances. Post hoc comparisons for the repeated factor (time) were carried out using Sidak's adjustment for multiple comparisons. Post hoc comparisons for the factorial ANOVA analyses were conducted using Tukey's test. Tests were conducted using a significance level α=0.05 and all statistical analyses were carried out using SPSS v. 22. All data are given as means±standard error (SE), unless stated otherwise.

Results

Environmental conditions—temperature, light, and nutrients

Air temperature decreased gradually from 13 °C in late October to below zero in early December (Fig. 2). Incoming PAR decreased also gradually from ca. 15 mol photons m^{-2} day^{-1} in late October to ca. 0.6 mol photons m^{-2} day^{-1} in late November, which is equivalent to 0.13–3.15 and 0.26–6.30 mol photons m^{-2} day^{-1} for the low and high light treatments, respectively (Fig. 2). Insolation averaged 5.5 mol photons m^{-2} day^{-1} during the experiment, corresponding to an average of 1.2 and 2.4 mol photons m^{-2} day^{-1}, or 13.9 and 28.8 μmol photons m^{-2} s^{-1} at the bottom of the tanks with the low and high light treatments, respectively. The average nutrient concentrations was 17.98/0.10 μM NO_3^-, 1.64/0.00 μM NH_4^+ and 6.56/0.20 μM PO_4^{3-} in the high and low nutrient system respectively (Fig. 3). Concentrations of dissolved inorganic nitrogen (NH_4^+ and NO_3^-) ranged from <0.1 to ca. 1 μM in the low nutrient treatment and from <1 to ca. 51 μM in the high nutrient treatment, with an increasing trend during the experiment in both treatments. Phosphate concentrations was stable in the low nutrient treatment and increased from ca. 1 μM to 15 μM over the course of the experiment in the high nutrient treatment.

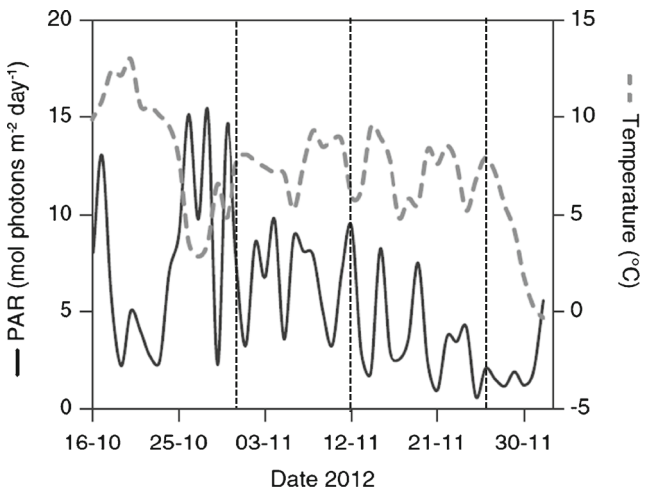

Fig. 2 Fluctuations in incoming light and air temperature during the experiment, measured continuously by a logger situated among the tanks. The sampling dates are indicated as *vertical lines*

Frond elongation, biomass changes, and growth

Algae from all treatment combinations continued to produce new frond tissue as indicated by the increasing distance between the meristem and the hole punched in the frond (Fig. 4a). The average distance between the meristem and the hole (across all treatments) increased from 10 to ca. 20 cm, corresponding to an average frond elongation rate of 0.12–0.28 cm day^{-1} depending on period and treatment (Fig. 4b). Average (across treatments) elongation rate increased over time (Table 1) being faster during the second and third periods than during the first. Frond elongation was unaffected by all time × treatment interactions and by the nutrient and light treatments including their interaction.

Although the algae continued to produce new frond tissue, they lost weight during the experiment (Fig. 4c). The loss of biomass during the experiment corresponded to ca. 13 % of

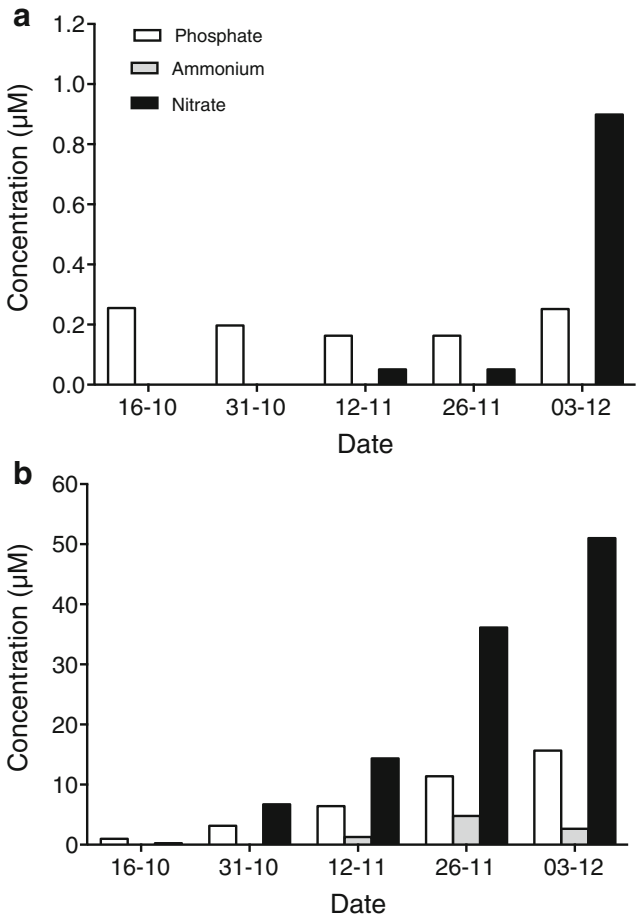

Fig. 3 Development in nutrient concentrations in the cultivation tanks during the experiment. **a** Low nutrient treatment, **b** High nutrient treatment. Values are means across three replicate tanks within each treatment

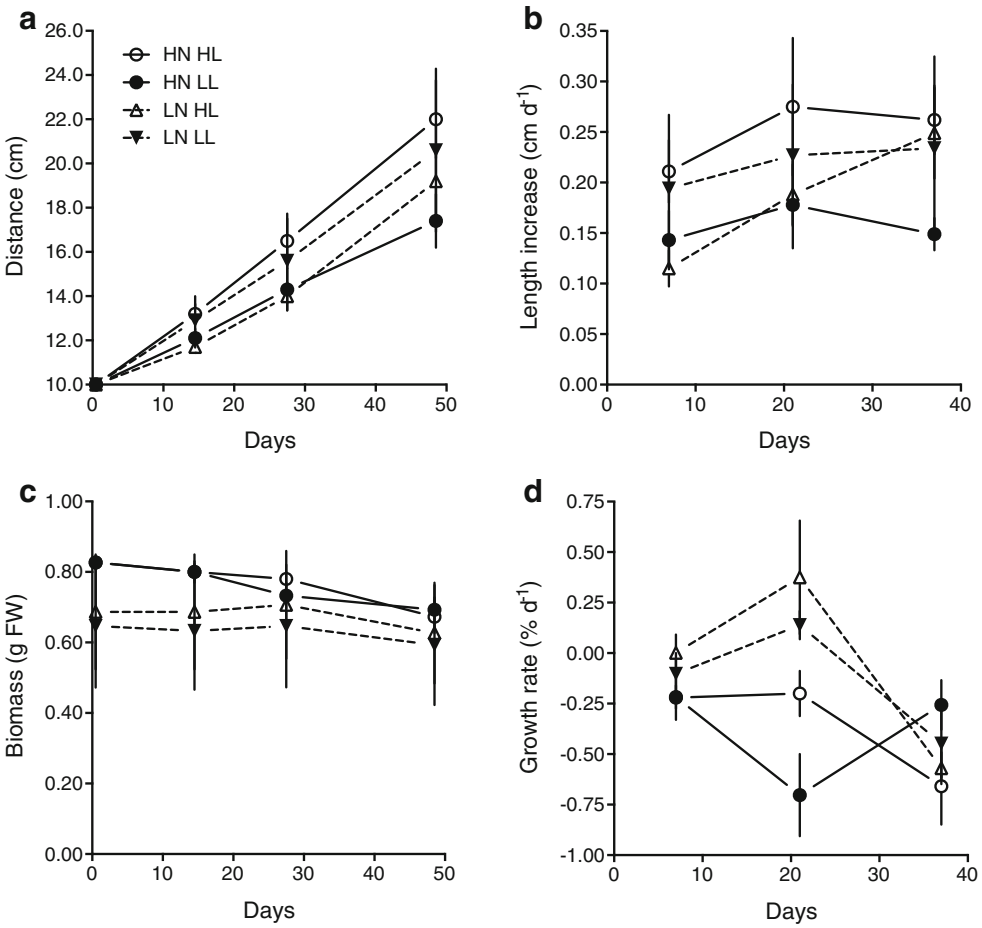

Fig. 4 *Saccharina latissima* growth during the experiment. **a** Distance between the meristem and the hole punched to measure frond extension. **b** Daily rates of meristemal length growth measured over three periods during the experiment. **c** Changes in fresh algal biomass per tank. **d** Growth rates per tank (% of FW day^{-1}) measured over three periods during the experiment. Values are means ±1 SE across three replicate tanks within each treatment

the initial biomass when averaged across all treatments. The loss of biomass over time resulted in low specific growth rates that depended on treatment as indicated from the significant time × nutrient and time × light interactions (Table 1). Growth rates of algae from the two high nutrient treatments remained negative throughout the entire experiment, whereas those from the two low nutrient treatments remained almost constant over the first period, increased during the second and decreased during the third. The time × light interaction was significant (Table 1) and driven by the fact that growth rate of algae exposed to low light remained rather constant throughout the experiment, whereas those from the high light treatments were higher and more variable over time (Fig. 4b). There was no significant time × nutrient × light interaction nor any effect of the nutrient × light interaction on relative growth rates (Table 1). The final area-specific dry weight of the *S. latissima* fronds averaged 0.027±0.004 g DW cm^{-2} (n= 36) and did not vary among treatments.

Tissue carbon, nitrogen and pigment concentrations

The initial C content averaged 36.7±0.3 % DW (across all treatments), and changed little throughout the experiment, although algae exposed to high nutrient availability experienced a small, significant decrease in C content from 36.9±0.4 to 35.3±0.4 % of DW (Table 2 and Fig 5a). The C content was neither affected by light treatment, nor by any of the interactions between time, light, and nutrients (Table 2).

The initial N content was similar across treatments (ca. 0.85±0.08 % of DW), but decreased to 0.63±0.14 % of DW in algae exposed to low nutrient levels (Fig. 5b). In contrast, the N content increased substantially in algae exposed to high nutrient concentrations (final content: 2.69±0.19 % of DW), yielding a significant interaction between time and nutrients (Table 2). Neither light, nor any of the other interactions between time, nutrients, and light had any significant effect on the N content.

The content of Chl *a* averaged initially 0.074± 0.012 mg g^{-1} DW across all treatments, but was marginally higher in algae from the low light treatment (0.084±0.008 vs. 0.064±0.006 mg g^{-1} DW, Table 2 and Fig. 5c). The content of Chl *a* remained rather constant over time in algae from the low nutrient treatment (0.068±0.013 mg g^{-1} DW), whereas it increased substantially in algae exposed to high nutrient levels, ending up averaging 0.256±0.054 mg g^{-1} DW (Fig. 5c). The

Table 1 Results of repeated measures ANOVA analyzing the effect of time, nutrient and light (depth) treatments on lengthwise growth rate and biomass-specific growth rate of *Saccharina latissima*

Variable	Source	*df*	MS	*F*	*p* value
Lengthwise growth rate	Within-subjects effects				
	Time	2	0.345	7.780	0.004
	Time × nutrients	2	0.081	1.836	0.191
	Time × light	2	0.103	2.322	0.130
	Time × nutrients × light	2	0.038	0.849	0.446
	Error (time)	16	0.044		
	Between-subjects effects				
	Nutrients	1	0.023	0.054	0.822
	Light	1	0.268	0.626	0.452
	Nutrients × light	1	0.746	1.746	0.223
	Error	8	0.428		
Biomass-specific growth rate	Within-subjects effects				
	Time	2	0.544	7.058	0.006
	Time × nutrients	2	0.459	5.961	0.012
	Time × light	2	0.300	3.888	0.042
	Time × nutrients × light	2	0.059	0.762	0.483
	Error	16	0.077		
	Between-subjects effects				
	Nutrients	1	0.679	17.340	0.003
	Light	1	0.025	0.631	0.450
	Nutrients × light	1	0.004	0.094	0.767
	Error	8	0.039		

Data for lengthwise growth rate were ln-transformed prior to analysis to obtain homogeneity of variances

Chl *a* content remained unaffected by light and any of the interactions between time, nutrients, and light (Table 2).

Concentrations of fucoxanthin followed the same pattern as Chl *a* across treatments: averaging 0.073±0.012 mg g^{-1} DW by the beginning of the experiment and increasing to 0.172± 0.018 mg g^{-1} DW in algae exposed to high nutrient levels and remaining constant (0.060±0.019 mg g^{-1} DW) in algae from the low nutrient treatment (Table 2 and Fig. 5d). The fucoxanthin content was initially slightly higher in algae from the high nutrient and high light treatment than in those from the high nutrient and low light treatment, whereas light had no effect on the content of fucoxanthin in the low nutrient treatments (Table 2).

Internal distribution of C, N, and pigments

The initial C content was highest in the intermediate part, lower in the basal part, and lowest in the apical part of the fronds (Fig. 6a). Although statistically significant (Table 3), the absolute differences in C content between different parts of the frond were relatively small. Exposure to high nutrient availability led to a decrease in C content across the entire frond and, especially so in the basal part (Fig. 6a). By the end of the experiment, the C content was still highest in the intermediate part, but lowest in the basal part. Low nutrient availability led also to a C decrease in the basal part of the frond, whereas that of the intermediate region increased slightly and no changes appeared in the apical part.

The basal part of the fronds contained initially more N than the intermediate and apical parts (1.1, 0.8, and 0.7 % DW, respectively, Fig. 6b). Exposure to high nutrient availability led to a general increase in the N content (Table 3), but did not affect the relative distribution of N along the frond; the basal part had still higher concentrations of N than the intermediate and distal parts. Exposure to low nutrient availability caused a significant decrease in N from the intermediate part of the frond, whereas the N content in the basal and apical parts remained unchanged. The intermediate part of the frond thus had the lowest N content in the nutrient-depleted algae (1.1, 0.4, and 0.6 % DW, respectively, Fig. 6b).

The initial Chl *a* concentration (ca. 0.1 mg g^{-1} DW) did not vary significantly along the frond (Fig. 6c). N enrichment caused a substantial increase in the Chl *a* content along the entire frond (Table 3) and especially so in the basal part, where the final Chl *a* concentration ended up being much larger than in the intermediate and distal parts of the fronds. The Chl *a* concentration in the basal part of the fronds of algae exposed to low nutrient availability did also increase during the experiment, whereas the Chl *a* content in the mid and apical tissues remained unaltered.

Table 2 Results of repeated measures ANOVA analyzing the effect of time, nutrient, and light (depth) treatments on the content of C, N, chlorophyll *a* and fucoxanthin in *Saccharina latissima*

	Source	*df*	MS	*F*	*p* value
Carbon	Within-subjects effects				
	Time	1	1.912	43.64	<0.001
	Time × nutrients	1	6.498	148.32	<0.001
	Time × light	1	0.028	0.63	0.450
	Time × nutrients × light	1	0.006	0.13	0.732
	Between-subjects effects				
	Nutrients	1	1.802	7.90	0.023
	Light	1	0.094	0.41	0.540
	Nutrients × light	1	0.006	0.03	0.872
	Error	8	0.228		
Nitrogen	Within-subjects effects				
	Time	1	3.930	218.64	<0.001
	Time × nutrients	1	5.656	314.66	<0.001
	Time × light	1	0.014	0.79	0.399
	Time × nutrients × light	1	0.016	0.87	0.380
	Between-subjects effects				
	Nutrients	1	7.102	594.67	<0.001
	Light	1	0.002	0.19	0.677
	Nutrients × light	1	0.046	3.83	0.086
	Error	8	0.012		
Chlorophyll	Within-subjects effects				
	Time	1	0.046	47.57	<0.001
	Time × nutrients	1	0.051	52.58	<0.001
	Time × light	1	<0.001	0.02	0.901
	Time × nutrients × light	1	0.001	0.55	0.478
	Between-subjects effects				
	Nutrients	1	0.056	72.48	<0.001
	Light	1	0.002	2.61	0.145
	Nutrients × light	1	0.001	1.09	0.327
	Error	8	0.001		
Fucoxanthin	Within-subjects effects				
	Time	1	0.011	52.54	<0.001
	Time × nutrients	1	0.013	63.07	<0.001
	Time × light	1	<0.001	0.01	0.980
	Time × nutrients × light	1	<0.001	1.72	0.226
	Between-subjects effects				
	Nutrients	1	0.026	187.93	<0.001
	Light	1	<0.001	0.02	0.884
	Nutrients × light	1	0.001	8.97	0.017
	Error	8	<0.001		

The final fucoxanthin concentrations in algae exposed to high nutrient levels were significantly higher than in N-replete algae (Table 3). Nutrient enrichment led to higher levels of fucoxanthin along the entire frond although the basal part experienced the highest increase (Fig. 6d). The significant interaction between nutrient treatment and plant part (Table 3) showed that the fucoxanthin concentration varied along the frond in nutrient enriched algae while it remained more or less even along the frond in algae from the low nutrient treatment.

Harvestable biomass and tissue components

The harvestable biomass and the tissue biochemistry summarizes into the absolute harvestable amounts of particular compounds that have a potential industrial value (pigments/antioxidants and proteins), or represent a value in relation to ecosystem services (mitigation of C, N, and P). The harvestable amounts of N, fucoxanthin, and Chl *a* increased by 50.1–60.1, 21.7–53.7, and 47.0–73.5 %, respectively, under high nutrient availability in late autumn although *S. latissima* lost 16.2–18.7 % of its biomass (Fig. 7). Nutrient-depleted algae suffered a smaller loss of harvestable biomass (8.1–9.5 %), but experienced also a net loss in harvestable N (10.7–44.1 %) and fucoxanthin (7.1–17.2 %). Only the absolute amount of Chl *a* increased in these algae (2.5–22.8 %; Fig. 7). Nutrient enrichment had thus a significant negative impact on biomass and C content, but a positive effect on the absolute harvestable amount of N, Chl *a*, and fucoxanthin (Table 4). Light had no effect on these response variables except in the case of N, where the total amount of N was slightly higher in N-enriched algae exposed to low light than in the other treatments (Table 4 and Fig. 7).

Discussion

The results demonstrated that nutrient availability is a crucial factor in the cultivation of *S. latissima* in both stimulating the shedding of the apical part of the fronds, and at the same time, increasing the buildup of valuable compounds in the remaining tissue on the cost of carbon reserves in the autumn/winter season in Denmark.

Growth physiology It has been previously described that the tissue concentrations of N in natural and cultivated kelps increase during fall and winter as ambient inorganic nutrients concentrations increase (Black 1950; Rosell and Srivastava 1985; Gevaert et al. 2001; Nielsen et al. 2014; Marinho et al. 2015). It also has been described how tissue C contents decrease, and more apical tissue is lost than meristematic tissue is produced resulting in net length and biomass decrease (Gevaert et al. 2001; Nielsen et al. 2014). This pattern was taken to reflect the life strategy of kelps, taking up and storing N as proteins, pigments, free amino acids, or inorganic nutrients under high ambient concentrations at the expense of stored carbohydrates (Conolly and Drew 1985; Gevaert et al. 2001).

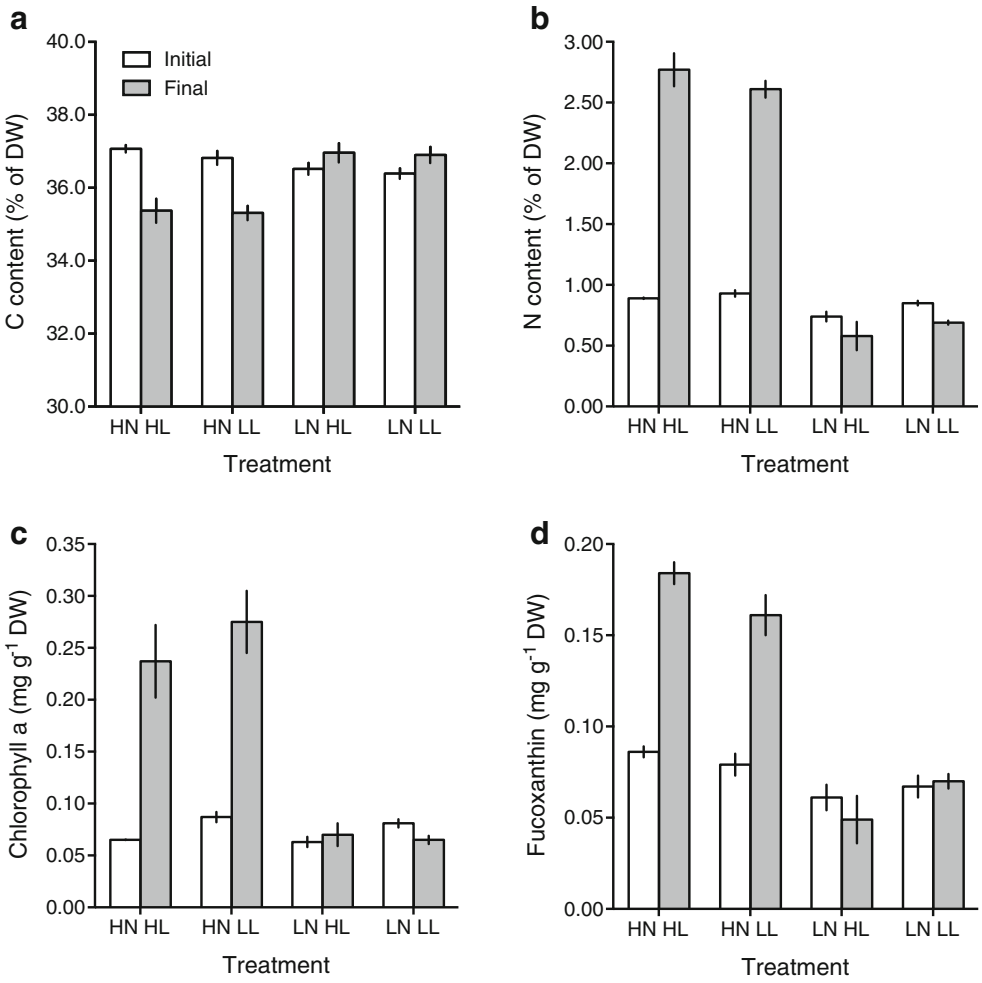

Fig. 5 Initial and final content of total C, total N, chlorophyll a, and fucoxanthin in *Saccharina latissima* from the four different experimental treatments. Values are given as means ±1 SE across three replicate tanks within each treatment. *HN* high nutrient, *LN* low nutrient, *HL* high light, *LL* low light

The metabolic activity appeared to be concentrated in the basal part of the frond near the meristem, where the loss of C indicated that stored C was respired to incorporate N and synthesize new tissue, proteins, and pigments. The loss of C and N in the medial part of the frond in nutrient replete or nutrient-limited algae, respectively, supports an internal translocation of C and N from the old frond to supply the growth at the meristem, as also described by Conolly and Drew (1985). The increase in tissue N content over the entire frond in the nutrient replete algae indicated that N uptake was active over the entire frond.

The difference between the two light treatments did not appear to have much impact on the growth nor the biochemical composition of the algae. The light compensation and saturation points (E_c and E_k) of *S. latissima* have been defined as ranging from 2 - 22 and 15–170 μmol photons m^{-2} s^{-1}, respectively (Fortes and Lüning 1980; Borum et al. 2002). As the daily average light intensities during the experiment ranged between 1.5 and 38 and 2.9–76 μmol photons m^{-2} s^{-1}, respectively, in the low and high light treatments, these light intensities were considered as ranging from limiting to saturating light conditions, and as being realistic for late autumn/early winter conditions at Danish latitudes. Both treatments support more than the described minimum requirements for growth of *S. latissima* of between 50 and 70 mol photons m^{-2} $year^{-1}$ (Borum et al. 2002). However, the meristematic growth rate in the nutrient replete algae was higher in the high light treatment, causing a higher length increase in the new frond. The same was observed by Conolly and Drew (1985), where winter growth rates were not increased by nutrients alone, whereas nutrients in combination with light (photoperiod) had a marked effect. The growth rates described in this study are in the same range as late fall/early winter growth rates described by Conolly and Drew (1985) for full plants with the old frond intact. Temperatures during the experiment were below the optimal temperatures for growth of *S. latissima* sporophytes of approximately 10–15 °C (Fortes and Lüning 1980), adding to explain the relatively low growth rates. However, the experimental temperatures reflected natural autumn and winter temperatures in Danish waters. Several authors have proposed an underlying circannual rhythm as the main factor controlling growth vs sporogenesis in laminarians, and the *Zeitgeber* for sporogenesis being short day lengths following long day lengths, and

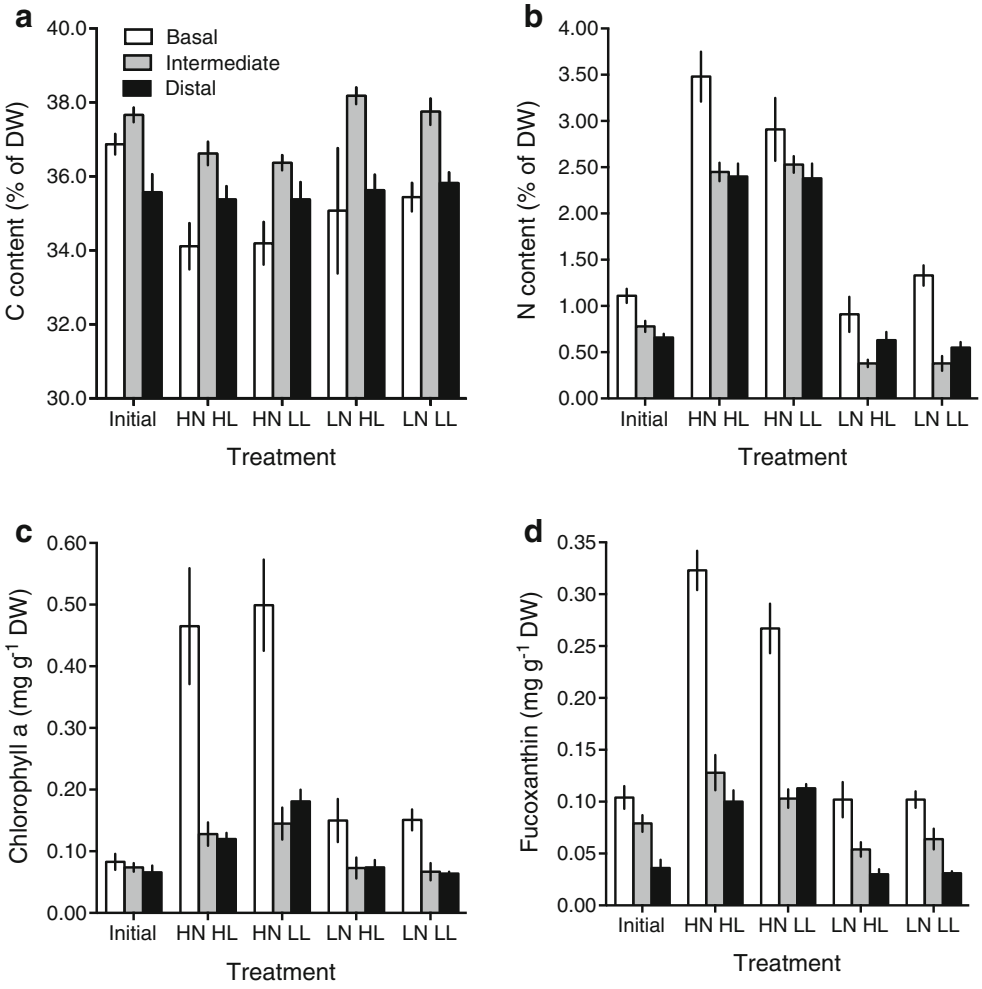

Fig. 6 Initial and final distribution of C, N, chlorophyll *a*, and fucoxanthin along the frond of *Saccharina latissima* over the course of the experiment. Values are means ±1 SD across three replicate tanks within each treatment. *HN* high nutrient, *LN* low nutrient, *HL* high light, *LL* low light

to some degree, nutrient replete conditions (Bartsch et al. 2008) and references herein. In the preset study, only one specimen proved visibly fertile at the termination of the experiment. We assume that the relatively short duration of the experiment with short day lengths did not allow time for detecting the slow formation of sori in *S. latissima* (6–8 weeks) (Pang and Lüning 2004).

Cultivation technology, ecosystem services, and production of high value compounds In seaweed cultivation practice, the main focus is a positive business case and the question of when and how to harvest the biomass to obtain the best economy is essential. For this reason, the quality, and not only the quantity, of the harvested biomass is important. During autumn, the absolute harvestable biomass will decrease due to shedding of the old fronds, and high ambient nutrient concentrations in this study only seemed to stimulate this process. However, the internal nutrient and pigment concentration were significantly positively affected by the high ambient nutrient concentrations, causing the absolute harvestable amount of N and major pigments to markedly increase during fall. Consequently, the value of the biomass increases during fall and early winter (1) as an instrument for uptake and bio-mitigation of nutrients and (2) as a source of N containing compounds (i.e., protein and amino acids) and pigments for the food and feed industry. The results suggest that the positive mitigation effect of cultivating *S. latissima* in close proximity to fish farms in Denmark persists during fall when the fish production/feeding is ongoing (Ahn et al. 1998; Handå et al. 2013).

If a late harvest is practiced, the blades of the *S. latissima* harvested would be fouled with epiphytic animals and algae which could positively affect the nutrient concentration of the harvested biomass, but also restrict the use of the biomass to other purposes than human consumption were epiphytes are unwanted (Handå et al. 2013; Marinho et al. 2015). As the algae can survive for several years, farmers could leave the biomass for the coming spring. However, the length growth in spring would result in a high rate of shedding of old and valuable biomass. A solution could be to practice blade tip cutting of the algae in late fall/early winter before the high

Table 3 Results of three-factor ANOVA testing the effects of nutrient treatment, light treatment, and plant part (i.e., basal, mid, and apical parts of the lamina) on final content of C, N, chlorophyll *a* and fucoxanthin, respectively

Variable	Factors	*df*	MS	*F* ratio	*p* value
Carbon	Nutrients (N)	1	13.47	25.98	<0.001
	Light (L)	1	0.68	1.31	0.265
	Plant part (P)	2	14.47	27.91	<0.001
	N × L	1	0.42	0.81	0.376
	N × P	2	1.91	3.68	0.041
	L × P	2	0.35	0.67	0.520
	N × L × P	2	0.46	0.88	0.428
	Error	23			
Nitrogen (ln)	Nutrients (N)	1	19.37	431.91	<0.001
	Light (L)	1	0.01	0.07	0.797
	Plant part (P)	2	1.34	29.78	<0.001
	N × L	1	0.05	1.04	0.318
	N × P	2	0.52	11.64	<0.001
	L × P	2	0.03	0.59	0.563
	N × L × P	2	0.12	2.69	0.089
	Error	23	0.05		
Chlorophyll (ln)	Nutrients (N)	1	6.88	81.98	<0.001
	Light (L)	1	0.06	0.72	0.406
	Plant part (P)	2	3.94	46.96	<0.001
	N × L	1	0.14	1.69	0.206
	N × P	2	0.19	2.30	0.122
	L × P	2	0.01	0.14	0.873
	N × L × P	2	0.05	0.54	0.589
	Error	23	0.08		
Fucoxanthin	Nutrients (N)	1	0.106	211.80	<0.001
	Light (L)	1	0.001	1.74	0.200
	Plant part (P)	2	0.059	119.33	<0.001
	N × L	1	0.002	3.15	0.089
	N × P	2	0.016	32.56	<0.001
	L × P	2	0.001	1.86	0.178
	N × L × P	2	0.001	1.77	0.192
	Error	23	<0.001		

Data for N and Chl were ln-transformed prior to analysis to obtain homogeneity of variances

growth period in spring and possibly gain a surplus in the absolute biomass harvested.

When considering the use of *S. latissima* for pigment production, the Chl *a* content significantly increased in all treatments during the experimental period. As the light intensity, and daily amount of incoming light decreased during the experimental period, the increase in this pigment was expected as a general shade acclimation, emphasizing that algae at 2–4 m depth, in Danish waters, are adapting to the decreasing ambient light intensity at this time of the year (Lobban and Harrison 1994; Lambers et al. 2008). The highest fucoxanthin

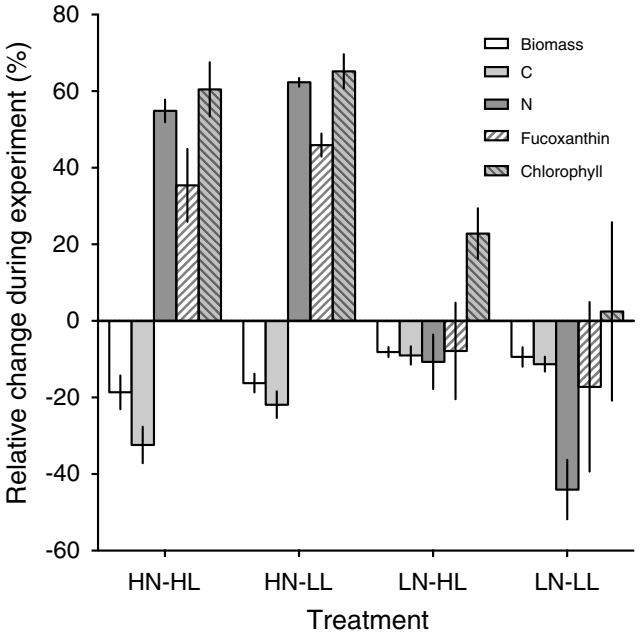

Fig. 7 Changes in percent from late summer to early winter in the absolute harvestable amounts of biomass, carbon, nitrogen, fucoxanthin, and chlorophyll *a*. Treatments: (1) High N, high light. (2) High N, low light. (3) Low N, high light. (4) Low N, low light. Results are mean values across replicate experimental tanks ± 1 SE (n=3)

and Chl *a* concentration would therefore be expected at the time were high internal nutrient concentrations and low light levels are prevailing. However, our results also clearly shows the positive effect of nutrient availability on the tissue concentrations of fucoxanthin and Chl *a*.

Returning to the value of the biomass of *S. latissima*, variations of tissue concentrations of nutrients and pigments along the frond length becomes relevant; also in a discussion of alternative harvest techniques or multiple croppings per season as suggested by Sanderson et al. (2012). When practicing a harvest of the frond above the meristem in order to reuse seeded lines, the internal distribution of valuable compounds becomes important. Nitrogen was present in higher concentrations near the meristem than elsewhere in the frond, and also the concentrations of pigments were significantly higher in the basal/meristematic part of the blade. Considering *S. latissima* solely as a mitigation tool, our results showed no vast variations of the outcome if merely harvesting the frond

Table 4 Results of two factor ANOVA analyzing the effect of nutrient and light (depth) treatments on the final harvestable yield of C, N, fucoxanthin, and chlorophyll *a* of *Saccharina latissima*, exposed to four different combination of light and nutrient availability

	Carbon	Nitrogen	Fucoxanthin	Chlorophyll *a*
Nutrients	<0.010	<0.001	0.005	0.005
Light	0.250	0.046	0.980	0.560
Light × nutrients	0.090	0.006	0.470	0.360

above the meristem; however, if pigments make a contribution to the economy, our results indicated that a significantly lower outcome could be expected if harvesting above the meristem. This variation is seasonally determined and thus, the differences in the internal distribution are most likely typical for the time of season where the experiment were conducted (Henley and Dunton 1995).

In conclusion, our results from tank experiments show that postponing the harvest of *S. latissima* cultured at Danish latitudes to early winter could, in areas with high ambient nutrient concentrations, yield a substantial increase in total harvestable tissue N and major pigments, despite a net loss of biomass. This could have implications for the use of *S. latissima* as a crop for nutrient compensation as well as for production of protein and pigments. However, these results need to be verified in open sea cultivation.

Acknowledgments This study was conducted as a part of the "MacroAlgae Biorefinery" (MAB3) project funded by The Danish Council for Strategic Research (DSF), as well as "Seaweed for polishing of waste water" funded by The Foundation for Development of Technology of the Danish Water Sector (VTU-fonden). We thank Kitte Linding Gerlich and Tanja Quottrup Egholm for their valuable guidance and assistance in the laboratory, and Peter M. Kofoed for carrying out the CN analyses.

References

Adams JM, Gallagher JA, Donnison IS (2009) Fermentation study on *Saccharina latissima* for bioethanol production considering variable pre-treatments. J Appl Phycol 21:569–574

Adams JMM, Toop TA, Donnison IS, Gallagher JA (2011) Seasonal variation in *Laminaria digitata* and its impact on biochemical conversion routes to biofuels. Bioresour Technol 102:9976–9984

Ahn O, Petrell R, Harrison P (1998) Ammonium and nitrate uptake by *Laminaria saccharina* and *Nereocystis luetkeana* originating from a salmon sea cage farm. J Appl Phycol 10:333–340

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 UK 29:45–72

Borum J, Pedersen MF, Krause-Jensen D, Christensen PB, Nielsen K (2002) Biomass, photosynthesis and growth of *Laminaria saccharina* in a high-arctic fjord, NE Greenland. Mar Biol 141:11–19

Bruhn J, Gerard VA (1996) Photoinhibition and recovery of the kelp *Laminaria saccharina* at optimal and superoptimal temperatures. Mar Biol 125:639–648

Cerna M (2011) Seaweed proteins and amino acids as nutraceuticals. Adv Food Nutr Res 64:297–312

Conolly NJ, Drew EA (1985) Physiology of *Laminaria*. III. Effect of coastal eutrophication on seasonal patterns of growth and tissue composition in *L. digitata* Lamour. and *L. saccharina* (L.) Lamour. Mar Ecol 6:181–195

D'Orazio N, Gemello E, Gammone MA, de Girolamo M, Ficoneri C, Riccioni G (2012) Fucoxantin: a treasure from the sea. Mar Drugs 10:604–616

Forbord S, Skjermo J, Arff J, Handå A, Reitan KI, 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. J Appl Phycol 24:393–399

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

Gerard VA (1988) Ecotypic differentiation in light-related traits of the kelp *Laminara saccharina*. Mar Biol 97:25–36

Gevaert F, Davoult D, Creach A, Kling R, Janquin MA, Seuront L, Lemoine Y (2001) Carbon and nitrogen content of *Laminaria saccharina* in the eastern English Channel: biometrics and seasonal variations. J Mar Biol Assoc UK 81:727–734

Gomez I, Wiencke C (1998) Seasonal changes in C, N and major organic compounds and their significance to morpho-functional processes in the endemic Antarctic brown alga *Ascoseira mirabilis*. Polar Biol 19:115–124

Grasshoff K, Erhardt M, Kremling K (1983) Methods of seawater analysis. Verlag Chemie, Weinheim

Handå A, Forbord S, Wang XX, Broch OJ, Dahle SW, Storseth 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 414:191–201

Hansen JW (2013) Marine områder 2012. NOVANA. Videnskabelig rapport fra DCE - Nationalt Center for Miljø og Energi nr. 77. (in Danish)

Henley WJ, Dunton KH (1995) A seasonal comparison of carbon, nitrogen, and pigment content in *Laminaria solidungula* and *L. saccharina* (Phaeophyta) in the Alaskan arctic. J Phycol 31:325–331

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

Jimenez-Escrig A, Jimenez-Jimenez I, Pulido R, Saura-Calixto F (2001) Antioxidant activity of fresh and processed edible seaweeds. J Sci Food Agric 81:530–534

Kopsell DA, Kopsell DE, Curran-Celentano J (2007) Carotenoid pigments in kale are influenced by nitrogen concentration and form. J Sci Food Agric 87:900–907

Lambers H, Chapin FS, Pons TL (2008) Plant physiological ecology. Springer, New York

Lobban CS, Harrison PJ (1994) Seaweed Ecology and Physiology. Cambridge university press, Cambridge

Lüning K, Pang SJ (2003) Mass cultivation of seaweeds: current aspects and approaches. J Appl Phycol 15:115–119

Marinho G, Holdt S, Birkeland M, Angelidaki I (2015) Commercial cultivation and bioremediation potential of sugar kelp, *Saccharina latissima*, in Danish waters. J Appl Phycol:1–11. doi:10.1007/s10811-014-0519-8

Nielsen M, 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 species' distribution range. Mar Biol 161:2011–2022

Pang JS, 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

Parke M (1948) Studies on British Laminariaceae. I. Growth in *Laminaria saccharina* (L.). Lamour. J Mar Biol Assoc U K 27: 651–709

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

Peteiro C, Freire O (2013a) Epiphytism on blades of the edible kelps *Undaria pinnatifida* and *Saccharina latissima* farmed under different abiotic conditions. J World Aquacult 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

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge

Roleda MY, Dethleff D (2011) Storm-generated sediment deposition on rocky shores: Simulating burial effects on the physiology and morphology of *Saccharina latissima* sporophytes. Mar Biol Res 7:213–223

Rosell K-G, Srivastava LM (1985) Seasonal variation in total nitrogen, carbon and amino acids in *Macrocystis integrifolia* and *Nereocystis luetkeana* (Phaeophyta). J Phycol 21:304–309

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

Schmedes PS, Boderskov T (2013) Cultivation of two kelp species, *Laminaria digitata* and *Saccharina latissima*, in Danish waters - geographic variation in growth and biochemical composition. Masters thesis, Århus University, Denmark

Scoggan J, Zhimeng Z, Feijiu W (1989) Culture of kelp (*Laminaria japonica*) in China. FAO Training Manual 89/5 (RAS/86/024), Electronic edition. Available at: http://www.fao.org/docrep/field/003/AB724E/AB724E00.htm

Soler-Vila A, Coughlan S, Guiry MD, Kraan S (2009) The red alga *Porphyra dioica* as a fish-feed ingredient for rainbow trout (*Oncorhynchus mykiss*): effects on growth, feed efficiency, and carcass composition. J Appl Phycol 21:617–624