



Latitudinal, seasonal and depth-dependent variation in growth, chemical composition and biofouling of cultivated *Saccharina latissima* (Phaeophyceae) along the Norwegian coast

Silje Forbord^{1,2} · Sanna Matsson^{3,4} · Guri E. Brodahl¹ · Bodil A. Bluhm⁴ · Ole Jacob Broch² · Aleksander Handå² · Anna Metaxas⁵ · Jorunn Skjermo² · Kristine Braaten Steinhovden² · Yngvar Olsen¹

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Abstract

The Norwegian coastline covers more than 10° in latitude and provides a range in abiotic and biotic conditions for seaweed farming. In this study, we compared the effects of cultivation depth and season on the increase in biomass (frond length and biomass yield), chemical composition (protein, tissue nitrogen, intracellular nitrate and ash content) and biofouling (total cover and species composition) of cultivated *Saccharina latissima* at nine locations along a latitudinal gradient from 58 to 69° N. The effects of light and temperature on frond length and biofouling were evaluated along with their relevance for selecting optimal cultivation sites. Growth was greater at 1–2 m than at 8–9 m depth and showed large differences among locations, mainly in relation to local salinity levels. Maximum frond lengths varied between 15 and 100 cm, and maximum biomass yields between 0.2 and 14 kg m⁻². Timing of maximum frond length and biomass yield varied with latitude, peaking 5 and 8 weeks later in the northern location (69° N) than in the central (63° N) and southern (58° N) locations, respectively. The nitrogen-to-protein conversion factor (averaged across all locations and depths) was 3.8, while protein content varied from 22 to 109 mg g⁻¹ DW, with seasonality and latitude having the largest effect. The onset of biofouling also followed a latitudinal pattern, with a delayed onset in northern locations and at freshwater-influenced sites. The dominant epibiont was the bryozoan *Membranipora membranacea*. Our results demonstrate the feasibility of *S. latissima* cultivation along a wide latitudinal gradient in North Atlantic waters and underscore the importance of careful site selection for seaweed aquaculture.

Keywords Phaeophyceae · Abiotic factors · Epibionts · *Membranipora membranacea* · Protein content · Seaweed aquaculture · Specific nitrogen-to-protein conversion factor

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✉ Silje Forbord
Silje.Forbord@sintef.no

✉ Sanna Matsson
sma@akvaplan.niva.no

¹ Department of Biology, Centre of Fisheries and Aquaculture, Norwegian University of Science and Technology, 7491 Trondheim, Norway

² Department of Environment and New Resources, SINTEF Ocean, 7465 Trondheim, Norway

³ Akvaplan-niva, 9296 Tromsø, Norway

⁴ UiT - The Arctic University of Norway, 9296 Tromsø, Norway

⁵ Dalhousie University, Halifax, Nova Scotia, Canada

Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), the worldwide production of seaweed is almost 30 million tonnes per year, predominantly of red and brown macroalgae produced in Asian countries such as China and Indonesia (FAO 2018). Compared to Asia, production technology and number of species in seaweed cultivation are in their infancy in Western Europe. However, there is a rapidly growing interest in seaweed cultivation, and the production of sugar kelp *Saccharina latissima* (Linnaeus) Lane, Mayes, Druehl and Saunders reached almost 1000 t in Europe in 2018 (FAO 2018) with Norway contributing to 174 t. There are presently 406 permits for macroalgal cultivation distributed over 83 locations and 23 companies in Norway (Directorate of Fisheries 2019). *Saccharina latissima*, our

focal species, is the most suited species for cultivation in North Atlantic waters due to its high growth rate (Handå et al. 2013; Peteiro and Freire 2013; Bak et al. 2018; Sharma et al. 2018), high content of valuable components (Holdt and Kraan 2011; Schiener et al. 2015; Bak et al. 2019) and well-described life cycle (Flavin et al. 2013; Redmond et al. 2014; Forbord et al. 2018). Consequently, its cultivation has been prioritized by commercial actors.

Over the last decade, expertise has been developed in cultivating and harvesting seaweed that potentially can be used for food, feed and fertilizers and for production of pharmaceuticals, cosmetics, chemicals and bioenergy (Stévant et al. 2017; Buschmann and Camus 2019). The stated overall goal has been to establish a Norwegian bio-economy based on cultivated seaweed (Skjermo et al. 2014). Therefore, comprehensive knowledge of growth potential and quality of *S. latissima* along the wide spanning coast of Norway would assist farmers in decisions on location and timing of deployment and harvest with maximized production and minimized loss. To date, this knowledge is lacking.

Saccharina latissima is widely distributed circumpolarly in the northern hemisphere (Bolton et al. 1983) and occurs on both sides of the Atlantic from the Gulf of Maine along the coasts of Europe and in the Pacific along the North American coast as well as in some areas in Japan and Arctic Russia (Druehl 1970; Druehl and Kaneko 1973; Lüning 1990; Bartsch et al. 2008). Approximately half of the world's natural kelp beds of *S. latissima* are found along the coast of Norway (Moy et al. 2006), suggesting that habitat suitability may also be high for farming along the entire coast. *Saccharina latissima* grows optimally at temperatures between 10 and 17 °C (Druehl 1967; Fortes and Lüning 1980) and salinities of 30–35 psu (Kerrison et al. 2015); conditions met along most parts of the Norwegian coastline. In addition, light and nutrient availability regulate depth distribution and productivity (Hurd et al. 2014; Xiao et al. 2019). Light intensity and day length are more variable seasonally at high than at low latitudes. In temperate regions, increasing temperature at the sea surface during spring causes stratification of the water column, varying in timing and strength along a latitudinal gradient, resulting in substantial seasonal differences in nutrient availability along the coast (Rey et al. 2007; Ibrahim et al. 2014; Broch et al. 2019). This seasonal variation in the abiotic environment (light, temperature and nutrients) will likely cause phenology differences in developmental stages and biochemical composition along the latitudinal gradient which in turn will affect the cultivated biomass and eventually the end-products (Hurd 2000; Handå et al. 2013; Peteiro and Freire 2013; Marinho et al. 2015a; Schiener et al. 2015). While there have been previous cultivation trials with *S. latissima* at several locations along the Norwegian coast, there has been no systematic study comparing the cultivation potential in different regions related to these abiotic factors to date.

Undesirable for seaweed production, the seaweed frond provides a substratum for fouling organisms to settle on and grow. Fouling by epibionts usually occurs from spring to autumn (Peteiro and Freire 2013a; Førde et al. 2016; Rolin et al. 2017; Matsson et al. 2019), depending on location (Matsson et al. 2019), latitude (Rolin et al. 2017) and interannual variation (Scheibling and Gagnon 2009). Epibionts can form a barrier inhibiting nutrient (Hurd et al. 2000) and light absorption (Andersen 2013) and may cause loss of biomass through increased drag and friction and decreased flexibility (Krumhansl et al. 2011). Biofouling results in seaweed biomass being less attractive for human consumption, affecting the commercial value of the yield (Park and Hwang 2012). Kelp with low value for human consumption may, however, still be used in other industries, for example production of animal feed (Bruton et al. 2009). To avoid biomass loss and reduced monetary value, kelp is usually harvested before the onset of epibionts (Fletcher 1995; Park and Hwang 2012). Considering the goal to optimize and survey kelp cultivation along a large latitudinal gradient, it therefore becomes necessary to establish the phenology of epifouling along this gradient.

The overall objective of our study was to examine the effects of latitude, season and cultivation depth on biomass accumulation, chemical composition (including protein content) and biofouling of *S. latissima*. Specifically, we hypothesised that a latitudinal pattern of abiotic factors would provide the potential of a progressively northward pattern in production of biomass, chemical composition and biofouling, with associated implications for the harvesting period along this latitudinal gradient. We also hypothesised that seaweed cultivated at deeper waters would exhibit lower biomass accumulation, altered chemical content and lower amount of fouling organisms than biomass cultured at shallower water.

To address these questions, we used nine locations from 58 to 69° N over a cultivating season, which varied in light regime, salinity, temperature and ambient nitrate. The effects of light, temperature and intracellular nitrate (I-DIN) on seaweed frond length and biofouling were evaluated. The study also aimed to establish specific nitrogen-to-protein conversion factors (K_p) with regard to total amino acids (AA) and total nitrogen (Q_N) to improve the protein content estimate for the region and propose a general K_p for cultivated *S. latissima* in Norway. The present study, with its systematic approach over a large spatial extent, provides valuable knowledge on opportunities and challenges associated with *S. latissima* cultivation to seaweed farmers and stakeholders along temperate and Arctic coasts of Europe.

Materials and methods

Experimental set-up To determine the effects of latitude and environmental factors (i.e. light and temperature) on seaweed

growth, chemical content and biofouling, nine study locations covering a wide latitudinal range were selected from available commercial farms with cultivation permits along a gradient from south (58° N) to north (69° N) in Norway. At each site, seaweed was cultivated at each of two depths (1–2 and 8–9 m) to compare growth performance (frond length and biomass yield), chemical composition (protein, tissue nitrogen, intracellular nitrate and ash content) and biofouling (total cover and community structure) for *Saccharina latissima* over an entire cultivation season.

Three of the locations (4–60° N, 7–67° N and 8–67° N) were situated in fjord systems, representing large sections of the Norwegian coast (Table 1). Data on freshwater discharge for the fjord sites were obtained for 2017 from simulations by the Norwegian Water Resources and Energy Directorate (2019). The 4–60° N location was influenced by highly fluctuating freshwater discharge throughout the cultivation period, with a peak in May and relatively high levels until the end of June. At location 7–67° N, freshwater discharges also fluctuated, but with a steadier increase from February through April followed by a pronounced peak in runoff from mid-May to mid-June and further relatively high discharge in July. At location 8–67° N, freshwater discharge was relatively low from the end of February to the beginning of May, followed by increasing runoff levels through May and a very pronounced peak in the beginning of June. Two depths were chosen to evaluate the effect of shallow (1–2 m) and deeper (8–9 m) cultivation and its effect on seaweed growth, chemical content and biofouling. Previous studies have shown significantly different growth and protein content between the two depths that we selected for our study (Handå et al. 2013; Sharma et al. 2018).

Seeded lines of *S. latissima* were deployed in February 2017 because at that time: (i) there were naturally occurring sori at all locations eliminating the need to establish cultures of gametophytes or to artificially induce sori (Forbord et al. 2012), (ii) light conditions were adequate at all locations to allow seedlings to grow immediately upon deployment (Handå et al. 2013), and (iii) ambient nutrient levels were high (Broch et al. 2013, 2019). It is likely advantageous for most farmers in southern Norway and temperate Europe to deploy their seed lines before February.

Production and deployment of seedlings Sporophytes of *S. latissima* with mature sori were collected near each study site in December 2016 and shipped to the seaweed laboratory (63° N; Fig. 1) for production of seed lines. This procedure is according to the recommendations of the Norwegian Environment Agency requiring that cultivated algae should be of local genetic origin, applying the precautionary principle (Fredriksen and Sjøtun 2015). Seedlings were produced concurrently in the seaweed laboratory for all nine locations, according to Forbord et al. (2018). A solution of $\sim 250,000$ spores mL⁻¹ seawater was sprayed onto 1.2-mm-diameter twine coiled around PVC spools.

The spools were then incubated for 7 weeks in nutrient-rich seawater (148 $\mu\text{g NO}_3^- \text{N L}^{-1}$, 20.6 $\mu\text{g PO}_4\text{-P L}^{-1}$) in a flow-through (120 L h⁻¹), light- and temperature-controlled system (70 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at the surface and 10 °C) in the seaweed hatchery. When the seedlings reached an average length of ~ 0.5 cm, the twines from each location were entwined onto 22 ropes, each 10 m long and 14 mm thick, packed in polystyrene boxes with cool packs and express-shipped to the location where the fertile sporophytes were collected. Fourteen of the 22 ropes had seedlings entwined at 1–2 m and 8–9 m with the gap intended to avoid self-shading of the sporophytes cultivated at 8–9 m depth. The remaining 8 of the 22 ropes had seedlings uniformly distributed along 1–9 m to use for biomass measurements and as backup in case other lines were lost. The ropes were deployed vertically approximately 6 m from one another. Deployments took place as soon as possible after the delivery of seedlings to the site and within 1 to 21 days depending on weather conditions and practicalities (Table 1). The ropes with seedlings were kept in running seawater in tanks on land until deployment.

Environmental variables Light intensity (Lux) and temperature (°C) were recorded at all locations at 2 and 8 m depth every 15 min using Onset HOBO pendant loggers (temperature accuracy ± 0.53 °C, resolution 0.14 °C). The Lux measurements were converted to PAR using the empirical relationship $\text{PAR} = 0.0291 \text{ Lux}^{1.0049}$ obtained by comparing Lux measurements with PAR sensor data (Long et al. 2012; Broch et al. 2013). Loggers were cleaned on every sampling date to minimize the impact of fouling.

Growing degree-day (GDD, °C day⁻¹) is an integrated index of the thermal history experienced by an organism, used to explain variations in biological processes (Trudgill et al. 2005). The GDD was calculated by adding the average daily water temperature measured at each location to -1.8 °C, the latter being used as the point of zero growth as in Saunders and Metaxas (2007). GDD was calculated from 13 March 2017, when all loggers had been installed at the experimental sites.

To quantify variation in light incidence over the cultivation period, the accumulated light was calculated by adding the average daily photosynthetically active radiation (PAR) measured at each depth and each location. For location 1–58° N, the loggers malfunctioned and light and temperature were instead taken from a nearby location (N58°13.3' E08°28.2') that was omitted from the study.

Growth measurements Sampling was done every 2 to 4 weeks from April to August, for a total of 8 planned sampling dates. At the northernmost location (9–69° N), one extra sampling was done in late September because of a prolonged growing season. Due to rough weather conditions and other constraints, not all locations were sampled as scheduled

Table 1 Key information about the nine experimental sites including coordinates, dates for received and deployed seed lines at the farms, type of location, salinity, dates of sampling and company/research institutes owning the cultivation permits

Station name	Coordinates (degree, decimal minutes N, E)	Received/deployed (day.month, 2017)	Type of location	Salinity (PSU)	Sampling dates (day, month, 2017)	Owners of permits
1–58°N	58° 03.325' 07° 51.220'	7.2/9.2	Sheltered	30–32 (Sætre 2007)	2.5, 18.5, 12.6, 3.7, 6.9	Norway Seaweed
2–60°N	60° 08.960' 05° 09.264'	9.2/9.2	Semi-sheltered	30–32 (Sætre 2007)	18.4, 3.5, 15.5, 2.6, 14.6, 14.7	Austevoll Seaweed Farm
3–60°N	60° 08.931' 05° 14.162'	9.2/9.2	Semi-sheltered	30–32 (Sætre 2007)	18.4, 4.5, 16.5, 1.6, 13.6, 5.7, 9.8, 4.9	Ocean Forest
4–60°N	60° 23.576' 06° 18.437'	9.2/11.2	Fjord	10–30 (Sjötun et al. 2015)	17.4, 4.5, 18.5, 1.6, 13.6, 9.7	Hardangerfjord Seaweed Farm
5–61°N	61° 00.254' 04° 42.095'	9.2/13.2	Sheltered	30–33 (Sætre 2007)	3.5, 29.5, 27.6	Hortimare
6–63°N	63° 42.279' 08° 52.232'	9.2/9.2	Semi-exposed	31–33.5 (Sætre 2007)	25.4, 4.5, 18.5, 31.5, 15.6, 3.7, 9.8	Seaweed Energy Solutions
7–67°N	67° 14.190' 14° 50.680'	8.2/10.2	Fjord	25–33 (Busch et al. 2014)	27.4, 4.5, 22.5, 30.5, 16.6, 7.7, 18.8	Salten Algae
8–67°N	67° 43.068' 15° 24.403'	8.2/10.2	Fjord	20–33 (Mykssvoll et al. 2011)	23.4, 14.5, 4.6, 17.6, 10.7	Folla Alger
9–69°N	69° 45.259' 19° 02.176'	8.2/21.2	Sheltered	33.1–33.5 (Matsson et al. 2019)	21.4, 5.5, 16.5, 31.5, 14.6, 5.7, 9.8, 5.9, 28.9	Akvaplan-niva

(Table 1). At each sampling time, the maximum length of the sporophyte fronds was measured for ten randomly selected individuals from each of five ropes at both depths, for a total of 50 individuals at each depth. The same ropes were sampled throughout the experiment. Kelp biomass (kg m^{-1}) was measured from mid-May to the end of the sampling period by scraping off the sporophytes from a 0.5-m section of 4 of the uniformly seeded ropes at each of the two sampling depths. Excess water was minimized by letting it run off for 1 min before weighing the kelp biomass to the nearest 0.1 kg with a Salter Brecknell Electro Samson 25 kg scale, with 0.02 kg precision.

Chemical analysis Ten sporophytes, each consisting of the frond, stipe and holdfast, from each of five ropes at each of the two depths, were collected for analysis of chemical composition. Sporophytes were carefully shaken to minimize excess water, and all ten from each rope and depth were placed in individual plastic zip-lock bags without removing epibionts. The samples were transported onshore in coolers where they were stored immediately at $-20\text{ }^{\circ}\text{C}$. They were shipped frozen to the laboratory (Fig. 1) at the end of the experimental period in September 2017 and stored at $-20\text{ }^{\circ}\text{C}$ until further analysis. Three samples of 10 sporophytes from each depth and each site were used for chemical analysis.

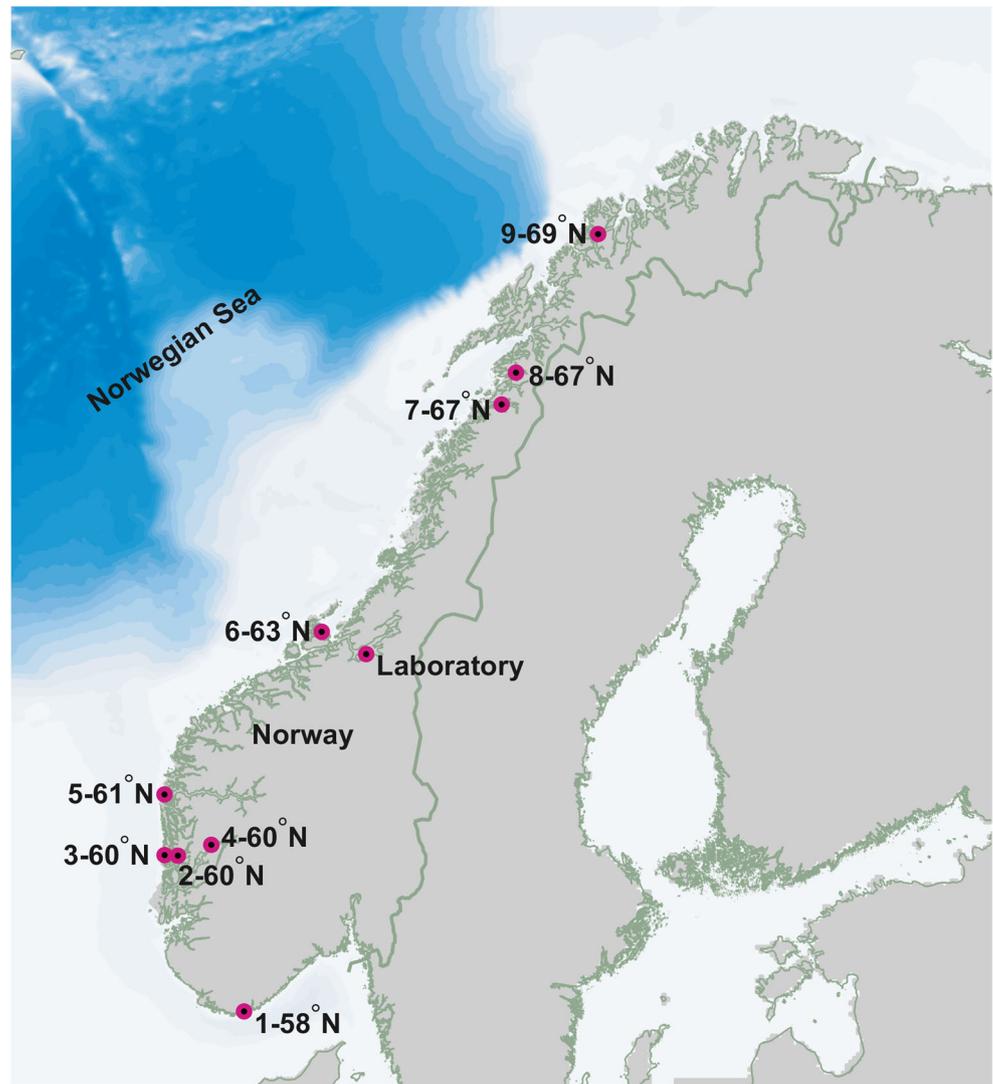
Dry weight (DW) of frozen *S. latissima* was determined by placing samples (1–2 g) in pre-weighed and pre-dried ceramic crucibles and dried at $105\text{ }^{\circ}\text{C}$ in a Termaks B8133 incubator

(Labolytic AS) for 24 h. Ash content was determined by incineration of samples in a muffle furnace at $600\text{ }^{\circ}\text{C}$ for 12 h.

For analysis of intracellular nitrate content (I-DIN), 0.06 g semi-frozen *S. latissima* material from each sample was transferred to a test tube with a cork and filled with 6 mL of distilled water. The samples were boiled for 30 min, cooled and filtered through a $0.45\text{-}\mu\text{m}$ polysulfone syringe filter to remove algal debris before diluting by mixing 0.3 mL of the solution with 9.7 mL distilled water. The test tubes were kept frozen at $-20\text{ }^{\circ}\text{C}$ and thawed prior to analysis of nitrate (I-DIN content) using an auto analyser (Flow Solution IV System, O.I Analytical, method according to Norwegian Standard 4745 (NSF 1975)).

The remaining biomass from each sample was stored at $-80\text{ }^{\circ}\text{C}$ until freeze-drying (Hetosicc CD 13–2) at $-40\text{ }^{\circ}\text{C}$ for 48 h. The freeze-dried kelp was homogenized into a fine powder and later used for carbon-nitrogen (CN) and amino acid analysis. CN was analysed using $\sim 2\text{--}3$ mg freeze-dried samples on an elemental analyser (Elementar vario EL cube, with acetanilide as standard). For analysis of amino acids, freeze-dried samples (50–100 mg) were hydrolysed in 6 M HCl containing 4% mercaptoethanol for 24 h at $110\text{ }^{\circ}\text{C}$ and neutralized to pH 1.5–3.0 by 5 M NaOH. The samples were filtered through a GF/C Whatman filter and diluted either 1:1 or 2:1 with a citrate buffer (Sodium Diluent Na220, pH 2.2). The analysis was performed by High-Performance Liquid Chromatography, HPLC (Agilent Infinity 1260, Agilent Technologies) coupled to an online post-column

Fig. 1 The locations of the experimental sites along the Norwegian coastline and the seaweed laboratory where the seed lines were produced and distributed from. The name for each site is composed of a consecutive number and the latitude



derivatization module (Pinnacle PCX, Pickering Laboratories, USA), using ninhydrin (Trione) as a reagent and a Na^+ -ion exchange column (4.6×110 mm, 5 mm). All buffers, reagents, amino acid standards and the HPLC-column were obtained from Pickering Laboratories (USA). HCl and mercaptoethanol were obtained from Sigma-Aldrich. Amino acids were analysed from locations 2–60° N, 6–63° N and 9–69° N for the entire experimental period and in addition once for each cultivation depth before the onset of clearly visible fouling from the other six locations.

Protein content was calculated as the difference between the total mass of amino acids isolated after sample hydrolysis and the mass of water bound to the amino acid unit after destruction of the peptide bond (18 g of H_2O per mole of amino acid).

The specific nitrogen-to-protein conversion factors (K_p) were calculated according to Mosse (1990):

$$K_p = \frac{(\text{AA} \times 1.1)}{N} \quad (1)$$

where AA is the sum of amino acid residues in % DW (the sum of amino acids after subtracting the molecular weight of water) and N is the total nitrogen content (% of DW). The total sum of the amino acids was multiplied by 1.1 to correct for the amino acids that were excluded from the HPLC analysis due to destruction during acid hydrolysis (Watanabe et al. 1983; Øie and Olsen 1997). The estimated protein content for each sample was determined by multiplying total % N of DW with its corresponding K_p conversion factor. The measured K_p for each sample was used in the estimation of protein content for that specific sampling day, depth and location.

Biofouling One sporophyte from each of the five ropes at each cultivation depth was collected on every sampling date from April onwards (Table 1), transported onto land and laid flat on a white background next to a ruler (1 mm accuracy). Biofouling was quantified as percentage cover on each frond, using image analysis. To image the entire frond, 1–3 images were taken depending on size, with an Olympus tough TG5 digital camera mounted on a tripod 25 cm above the frond. If the frond could not be completely imaged with three images, one image each was taken of the meristematic, middle and distal regions. Percent cover of biofouling for each taxon of epibiont was measured with the software Coral Point Count with Excel extensions (CPCe) (Kohler and Gill 2006). One hundred points per seaweed frond were randomly distributed on the images, and the biofouling organisms underneath the points were identified and recorded for each point. Mobile organisms such as amphipods (including Caprellidae), isopods and gastropods were registered but omitted from further analysis. We recorded the bryozoan species *Membranipora membranacea* and *Electra pilosa*, the classes bivalvia (most likely *Mytilus edulis*), hydrozoa (including the genera *Obelia* and *Tubularia*, indistinguishable on images) and filamentous algae/diatoms. Organisms that could not be identified from the images were marked as ‘unidentifiable’.

Statistics and data analyses Independent-samples *t* tests were used to compare K_p and protein content between the two cultivation depths after confirming the assumption of normality (Shapiro-Wilk’s test) and homogeneity of variance (Levene’s test). A non-parametric test (Kruskal-Wallis) was used where normal distribution could not be verified. Two-way analysis of variance (ANOVA) was used to examine the effects of sampling date and location (random factors) on protein content at each depth for three selected locations (2–60°N, 6–63°N and 9–69°N). Two-way ANOVA was also used to examine the effect of sampling date (random factor) and depth (fixed factor) on frond length, biomass yield, I-DIN, Q_N , DW, ash and total biofouling cover at each location. A three-way ANOVA was run to analyse the effects of depth (fixed factor), location (random factor) and sampling date (random factor) on temperature, GDD and accumulated PAR. Although the assumption of homogeneity of variance was violated for most datasets (as indicated by Levene’s tests), the two- and three-way ANOVA was run anyway because the analysis is relatively robust to heterogeneity of variance when group sizes were equal/approximately equal (Jaccard and Jaccard 1998).

Linear mixed effects models (LMM) were used to study the relationships between measured variables (GDD, light and I-DIN), seaweed frond length and total

biofouling cover. The best fitted models were chosen by comparing the alternative models using Akaike information criterion (AIC). Fixed effects that were not significant ($p > 0.05$) in likelihood ratio tests were omitted from the best fitted models. When evaluating frond length, light, temperature (as GDD), I-DIN and total biofouling were used as fixed effects. To account for variation in frond length among locations and for repeated observations within locations, we used ‘location’ and ‘sampling date’ as random intercepts. For total biofouling cover, light, temperature (as GDD), I-DIN, biomass and frond length were tested as fixed factors. To account for variation of biofouling cover between locations and repeated observations within locations, we used ‘location’ and ‘sampling date’ as random intercepts. To account for the effect of temperature on location, ‘GDD’, ‘location’ and ‘sampling date’ were used as random intercepts. To account for the effect of temperature on location, GDD was added as random slope. All factors were averaged across the ropes (n). Residual plots did not reveal any obvious deviations from homogeneity of variance or normality. p values were acquired by likelihood ratio tests of the full model against the models without the individual effects. R^2 values for the LMMs were calculated using the package r2glmm (Jaeger 2017) using the Nakagawa and Schielzeth (2013) approach.

Data are presented as mean \pm standard error (SE). Means were considered significantly different at $\alpha < 0.05$. Statistical analyses were performed using IBM SPSS Statistical software (Version 25) and R, version 3.5.1 (R Core Team 2018) through RStudio version 1.1.456 (RStudio Team 2016). LMMs were modelled by using the package lme4 (Bates et al. 2015). In addition to R, plots were made using Systat SigmaPlot software (version 14).

Results

Environmental conditions

There was a significant interaction of depth, location and sampling date on water temperature ($F_{47,3338} = 12.80$, $p < 0.001$), GDD ($F_{47,3338} = 1.807$, $p = 0.001$) and accumulated PAR ($F_{47,3324} = 35.11$, $p < 0.001$) (detailed statistics found in Table 1 in Online Resource 1). Temperature varied from 2.8 to 17.0 °C at 2 m and from 4.5 to 16.7 °C at 8 m depth with the largest and smallest ranges at low and high latitudes, respectively (Table 2). A clear latitudinal pattern in GDD was evident for the two cultivation depths with the northernmost location exhibiting the lowest GDD from mid-March until late-August and the southernmost location exhibiting the highest GDD (Fig. 1 in Online Resource 2). The differences in GDD between

depths were greater (> 200 GDD) for the freshwater-influenced locations (4–60° N, 7–67° N, 8–67° N) in the end of the cultivation period, suggesting stronger stratification than at other sites. Accumulated PAR was highest at location 2–60° N for both depths, decreasing to one-fourth from 2 to 8 m depth (Fig. 2 in Online Resource 2). Locations with freshwater influence showed the lowest PAR at 2 m (7–67° N) and 8 m depth (4–60° N and 7–67° N), decreasing almost to one-sixth at deeper waters.

Growth measurements

Changes of mean frond length and biomass yield of *S. latissima* over time varied greatly among cultivation sites (Fig. 2), and a latitudinal related pattern was apparent with locations in the south reaching their maximum length and biomass earlier in the cultivation period than locations further north.

There was a significant interaction ($p < 0.05$) between depth and sampling date on frond length for all locations except 2–60°N and 5–61°N (detailed statistics found in Table 2 in Online Resource 1), the two stations with fewest records and early onset of biofouling. At these two locations, frond length varied between depths across all dates ($p < 0.05$). Light had a significantly positive effect on seaweed frond length (LMM likelihood ratio test: $\chi^2_1 = 22.26$, $p < 0.001$), while growing degree-day (GDD), intracellular nitrate (I-DIN) and biofouling did not show a significant effect (Table 3). Across all locations, mean maximum frond length was 48.9 ± 9.5 (mean \pm SE) cm at 1–2 m cultivation depth and 43.0 ± 10.6 cm at 8–9 m depth (Fig. 2). The longest fronds were found at location 6–63°N at both depths, while the fronds were shortest at location 8–67°N.

The interaction between sampling date and location was significant ($p < 0.05$) for biomass yield for more than half the locations (2–60°N, 3–60°N, 6–63°N, 7–67°N and 9–69°N), while locations 1–58°N and 4–60°N showed significant differences

between depths across all dates ($p < 0.05$) (detailed statistics found in Table 2 in Online Resource 1). Biomass reached mean maximum yield across all locations of 4.5 ± 1.8 kg m⁻¹ at 1–2 m cultivation depth and 2.3 ± 1.0 kg m⁻¹ at 8–9 m depth (Figs. 2 and 3). Maximum biomass was reached at 1–2 m at location 6–63°N in early July and at 9–69°N in early September, and maximum yield was low at all freshwater-influenced sites, and lowest at location 8–67°N at both depths in July.

Chemical composition

Ash and dry weight content A significant interaction ($p < 0.05$) between depth and sampling date on ash content of *S. latissima* was found for the locations in the south-west (2–60°N to 4–60°N) and in the north (7–67°N and 9–69°N), while locations 1–58°N and 6–63°N showed significant differences ($p < 0.05$) between depths across all dates (detailed statistics found in Table 3 in Online Resource 1). Ash content varied greatly among locations and decreased from spring to summer until the onset of biofouling, as opposed to the freshwater-influenced site 8–67°N where ash content increased at both depths over the sampling period. Ash content ranged in average between 140 ± 27.2 and 428 ± 40.7 mg g⁻¹ DW at 1–2 m and between 212 ± 22.7 and 519 ± 8.0 mg g⁻¹ DW at 8–9 m. The two fjord locations 4–60°N and 7–67°N showed the lowest ash content at both depths. The interaction between depth and sampling date for dry weight (DW) was significant ($p < 0.05$) for four of the locations (2–60°N, 4–60°N, 6–63°N and 9–69°N) (detailed statistics found in Table 3 in Online Resource 1). DW increased throughout the sampling period and was generally higher at 1–2 m depth (9.6–27.1% of WW) compared to 8–9 m depth (6.8–23.7% of WW). Ash and DW content are displayed in Online Resource 3.

Q_N and I-DIN The interaction between depth and sampling date on total tissue nitrogen content (Q_N) was only significant ($p < 0.05$) for the locations with the poorest

Table 2 Monthly mean water temperature (°C) throughout the sampling period for all nine experimental locations at 2 m and 8 m depth

Location	1–58°N	2–60°N	3–60°N	4–60°N	5–61°N	6–63°N	7–67°N	8–67°N	9–69°N
Depth	2 m/8 m	2 m/8 m							
February	2.8/7.4	5.4/6.1	5.4/6.0	6.1/6.6	6.0/6.1	6.3/6.3	4.1/4.6	n.a	4.6/4.9
March	4.7/6.8	5.7/5.8	5.7/5.8	5.9/6.0	6.0/6.1	6.3/6.2	4.3/4.5	4.0/4.0	4.9/5.0
April	7.4/7.0	6.9/6.8	7.0/6.8	6.9/6.6	6.9/6.9	6.9/6.8	4.9/5.0	4.3/4.2	4.9/4.9
May	10.4/9.3	10.6/9.5	10.8/9.5	11.2/9.5	9.9/9.4	8.5/8.3	7.3/6.6	6.8/6.2	5.5/5.2
June	13.2/12.5	13.4/12.6	13.6/12.5	13.7/11.0	12.8/12.4	10.3/10.0	11.6/8.4	11.4/9.4	7.2/6.6
July	15.8/15.3	15.3/13.6	15.3/13.4	15.5/11.7	14.8/13.4	12.8/12.3	12.7/9.3	13.9/11.4	8.5/8.0
August	17.0/16.7	15.9/16.0	16.0/15.9	15.8/14.7	15.8/15.5	14.3/14.0	12.8/11.1	14.7/13.3	9.1/8.7

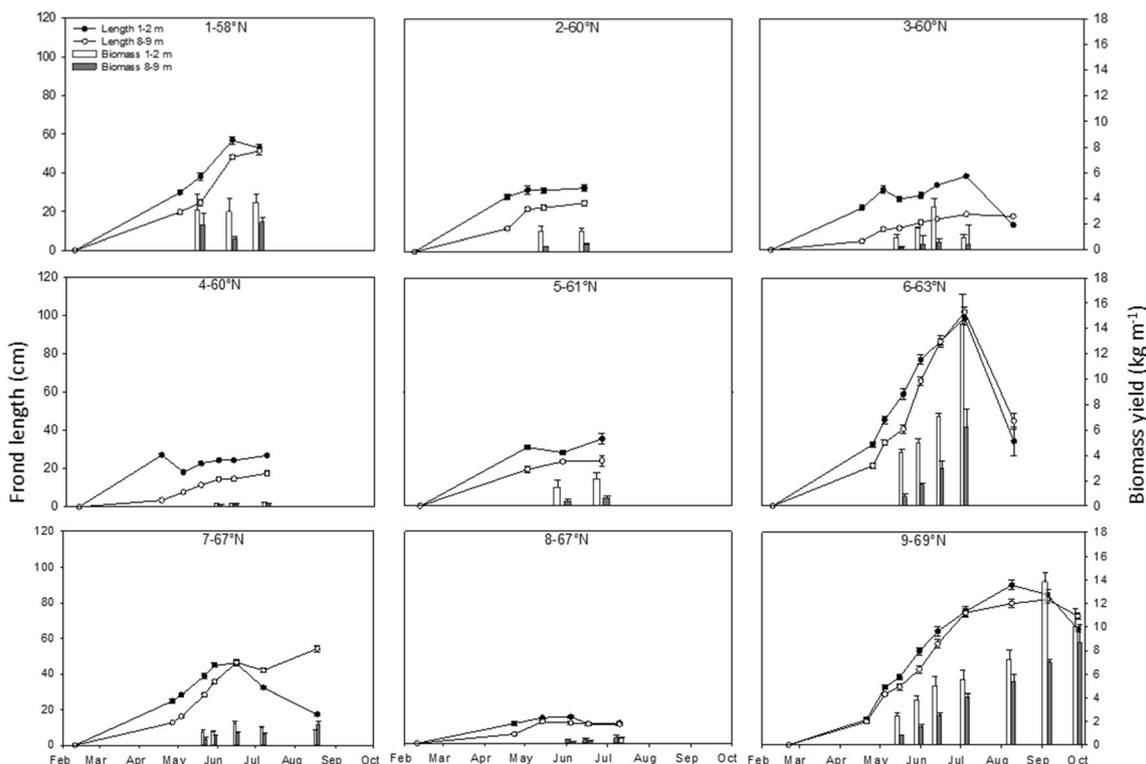


Fig. 2 Length (solid line, left y-axis) and biomass (bars, right y-axis) for both cultivation depths for all nine locations during the experimental period (February–September). Mean ± SE, $n = 50$ for length and $n = 4$ for biomass

growth (2–60°N, 4–60°N and 8–67°N). Depth differences were significant ($p < 0.05$) for locations 7–67°N and 9–69°N, while the sampling date was significant for the location with the most sampling points (1–58°N, 3–60°N, 6–63°N and 9–69°N) (detailed statistics found in Table 4 in Online Resource 1). Q_N varied from 6.2 ± 0.4 to $39.1 \pm 0.7 \text{ mg N g}^{-1} \text{ DW}$ across all sites, depths and seasons and decreased throughout the cultivation period until biofouling became dominant during summer and fall and then Q_N content increased for most locations (Fig. 4a).

There was a significant interaction ($p < 0.05$) between sampling date and depth on intracellular nitrate (I-DIN) for the south-west locations 2–60°N, 3–60°N, 4–60°N and location 7–67°N in the north (detailed statistics found in Table 4 in Online Resource 1). I-DIN content was significantly lower ($p = 0.009$) at 1–2 m than at 8–9 m at location 9–69°N. The

strongest seasonal pattern in I-DIN content was detected at the two northernmost locations 8–67°N and 9–69°N with sampling date having significant effect ($p < 0.05$). I-DIN varied from 0.001 ± 0.140 to $0.700 \pm 0.200 \text{ mg NO}_3^- \text{ g}^{-1} \text{ DW}$ across all sites, depths and seasons (Fig. 4b), showing a weak latitudinal pattern, with earlier depletion at the locations in the south.

Protein content The average nitrogen-to-protein conversion factors (K_p) for *S. latissima* did not exhibit a seasonal or latitudinal trend but varied across locations, depths and sampling dates (Table 4), with an average of 3.9 ± 0.3 for 1–2 m depth and 3.7 ± 0.2 for 8–9 m depth. An overall average value across all locations and depths was of 3.8 ± 0.1 . K_p was only significantly different between depths at two fjord locations with freshwater runoff at the surface, 4–60°N ($t_3 = 3.56$, $p = 0.038$) and 7–67°N ($t_4 = 3.31$, $p = 0.030$).

Table 3 Linear mixed effects models for the log-transformed dependent variable seaweed frond length, with and without light as fixed factor. Location and sample date are random intercepts. Models are ranked in

descending order after AIC value (i.e. the best-fitted model are presented first) with associated R^2 value

Rank	Formula	K (parameters)	AIC	Δ AIC	R^2
1	$\log(\text{Frond length}) \sim \text{Light} + (1 \text{Location}) + (1 \text{Sample date})$	5	123.7	0.0	0.06
2	$\log(\text{Frond length}) \sim 1 + (1 \text{Location}) + (1 \text{Sample date})$	4	143.7	20.1	

Fig. 3 Difference in frond size and density of *S. latissima* **a** between 1–2 m cultivation depth (top rope) and 8–9 m cultivation depth (bottom rope) after 69 days of cultivation at sea (18.04.2017) at location 2–60°N. **b** 1–2 m depth (top rope) compared to 8–9 m depth (bottom rope) after 146 days cultivation at sea (07.07.2017) at location (7–67°N) with a freshwater-influenced surface layer



There was a significant interaction of location and sampling date on protein content at both depths at three selected locations in the south-west (2–60°N), central (6–63°N) and north (9–69°N) ($p < 0.001$, detailed statistics found in Table 5 in Online Resource 1). At location 9–69°N, protein content decreased steadily between the first and the last sampling date at both depths, whereas it increased at 2–60°N from June and at 6–63°N from July as the kelp fronds became heavily fouled (Fig. 5).

Protein content increased from the southern to the northern locations and ranged from 23.0 ± 0.5 to 101 ± 4.0 mg g⁻¹ DW at 1–2 m depth and from 22.0 ± 0.1 to 110 ± 0.6 mg g⁻¹ DW at 8–9 m depth, although differences between depths were only statistically significant ($p < 0.001$) at four locations (Fig. 6; detailed statistics given in Table 6 in Online Resource 1). Again, depth differences were greatest at fjord locations with a surface freshwater layer, 4–60°N and 7–67°N, and all three freshwater-influenced sites deviated from the general

latitudinal pattern of an increase in protein content from south to north.

Biofouling The interaction between depth and sampling date was significant ($p < 0.05$) for six of the locations (detailed statistics given in Table 7 in Online Resource 1). Percentage biofouling cover on kelp fronds increased with season at all sites and depths (Fig. 7), from ~0% in April–June to a maximum of 3.8–81.4% in June–September. At both depths, the onset of biofouling occurred earlier at lower (mostly around May) than higher latitudes. At the northernmost location, biofouling cover did not exceed 20% before September. Exceptions to the latitudinal pattern, showing relatively low biofouling cover, were freshwater-influenced locations (4–60°N and 7–67°N; Table 1), and the southernmost location (1–58°N). Biofouling cover was higher at deeper depths at four locations, whereas two locations (Fig. 7; 3–60°N and 8–67°N) had more biofouling at shallow water, and three

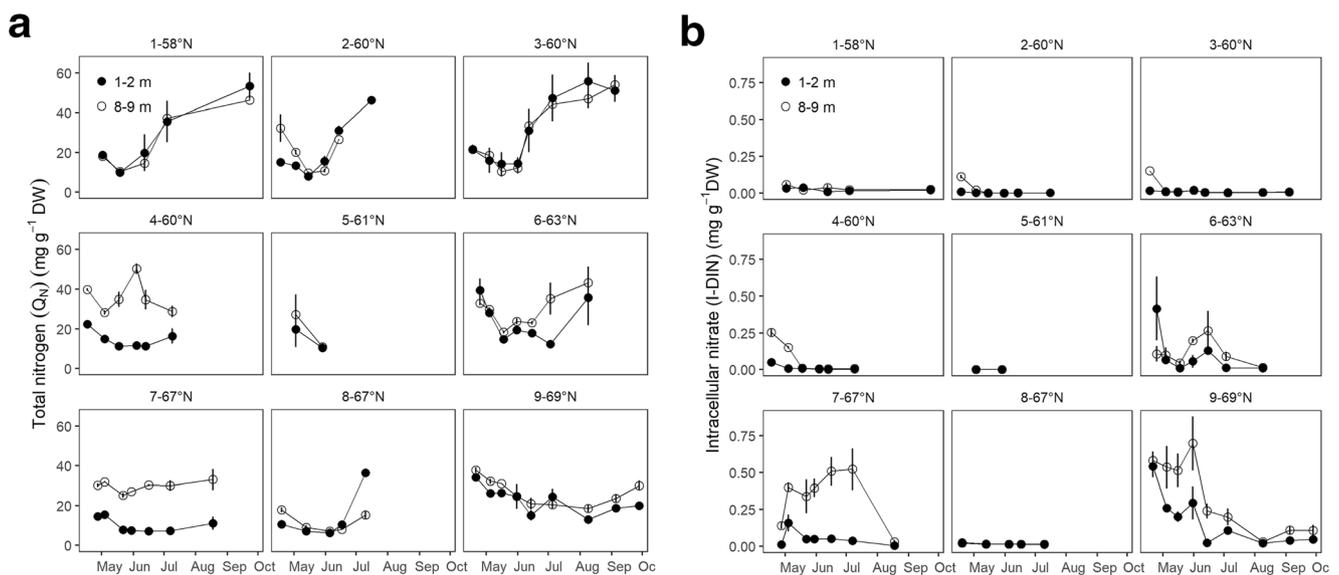


Fig. 4 Latitudinal and seasonal pattern in **a** total nitrogen content (Q_N) (mg N g⁻¹ DW) and **b** intracellular nitrate content (I-DIN) (mg NO₃⁻ g⁻¹ DW) of *S. latissima* from all nine experimental sites at 1–2 m and 8–9 m depth across the sampling period. Mean ± SE, $n = 3$

Table 4 Average nitrogen-to-protein conversion factors (K_p) for all nine location over the registration period. Asterisks indicate significant difference between depths ($p < 0.05$). Mean \pm SE, $n = 21$ (6–63°N, 9–69°N), $n = 18$ (2–60°N), $n = 3$ (1–58°N, 3–60°N, 4–60°N, 5–61°N, 7–67°N, 8–67°N)

K_p	1–58°N	2–60°N	3–60°N	4–60°N*	5–61°N	6–63°N	7–67°N*	8–67°N	9–69°N
1–2 m	3.9 \pm 0.2	4.0 \pm 0.2	3.7 \pm 1.4	4.6 \pm 0.2	2.7 \pm 0.8	3.9 \pm 0.3	4.2 \pm 0.2	3.8 \pm 0.5	3.9 \pm 0.3
8–9 m	3.8 \pm 0.1	3.8 \pm 0.3	4.6 \pm 1.4	3.1 \pm 0.5	3.9 \pm 0.8	4.0 \pm 0.4	3.6 \pm 0.1	3.1 \pm 0.1	3.7 \pm 0.6

locations had no significant differences between depths (2–60°N, 5–61°N and 6–63°N).

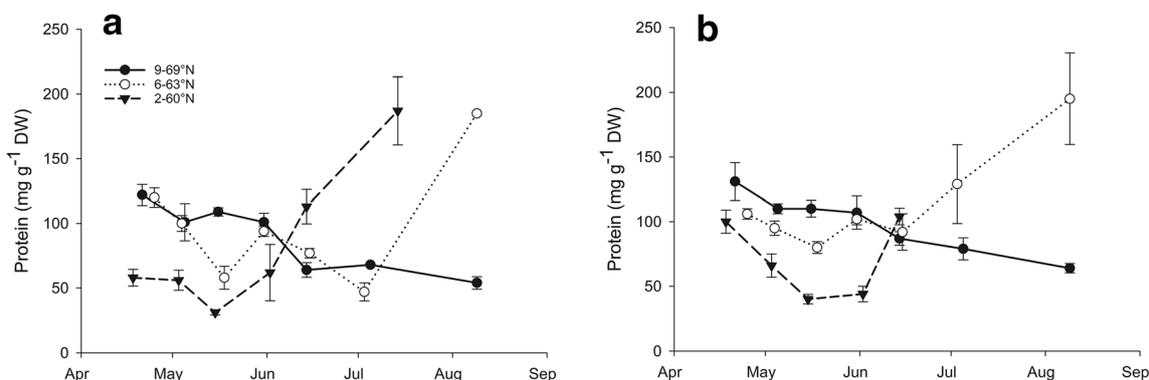
Maximum biofouling cover varied widely among locations and was highest (81.4 \pm 5.9%) at 1–2 m depth at 3–60°N and lowest (6.5 \pm 1.3%) at 4–60°N at the same depth in early July (Fig. 7). The biofouling community varied between these two locations, with *M. membranacea* dominating at 3–60°N and *Bivalvia* at 1–2 m depth at 4–60°N (Fig. 8). On the following sampling event in early July, most seaweed biomass was lost at both locations.

The biofouling community was initially dominated by filamentous algae fouling the tips of the fronds, and/or diatoms (Figs. 7, 8 and 9) at all locations except at the southernmost location (1–58°N) and all freshwater-influenced locations. Filamentous algae and diatoms were later replaced by the bryozoan *M. membranacea*, which was the dominant epibiont at most locations by the end of the experiment. The freshwater-influenced locations, though, had a higher occurrence of hydroids and bivalves compared to *M. membranacea* and to other sites, and hydroids appeared earlier than bryozoans (Online Resource 4).

The linear mixed effects model showed that temperature (as GDD) had the highest effect on biofouling of all variables (Table 5). GDD had a significantly positive effect (LMM likelihood ratio test: $\chi^2_1 = 21.48$, $p < 0.001$), and light had a significant negative effect (LMM likelihood ratio test: $\chi^2_1 = 15.27$, $p < 0.001$) on total biofouling cover, while I-DIN, frond length and biomass yield were not significant.

Discussion

Growth performance The frond length and biomass yield peaked 5 and 8 weeks later in the northern (9–69°N) than in the central (6–63°N) and southern (1–58°N) locations, respectively, likely because of seasonal differences in temperature, daylight and an earlier depletion of ambient inorganic nutrients by phytoplankton blooms in the low than high latitudes (Rey et al. 2007; Ibrahim et al. 2014). Maximum frond length and biomass yield were greatest at central (6–63°N, in summer) and northern (9–69°N, in autumn) locations, with levels comparable to *S. latissima* previously cultivated in Norway (Handå et al. 2013; Fossberg et al. 2018; Forbord et al. 2019; Matsson et al. 2019) and as high as or higher than several cultivation trials across Europe under variable conditions (Peteiro et al. 2014; Mols-Mortensen et al. 2017; Bak et al. 2018). The maximum yield of 14 kg m⁻¹ found in our study is far lower than registered for other cultivated kelp species like *Macrocystis pyrifera* in Chile (up to 22 kg m⁻¹) (Macchiavello et al. 2010) and hybrids of *Undaria pinnatifida* and *Undariopsis peterseniana* in Korea (37.5 kg m⁻¹) (Hwang et al. 2012) due to both morphology/individual biomass potential and breeding strategies. Since the use of local strains is highly recommended in several Scandinavian countries, breeding is not of current interest as a tool to increase the biomass yield of commercial cultivation of *S. latissima* (Fredriksen and Sjøtun 2015; Hasselström et al. 2018; Barbier et al. 2019). Growth in length and biomass yield was poorest at the freshwater-influenced locations as in previous trials in Denmark during periods of low salinity (Marinho et al. 2015b; Bruhn et al. 2016). A reduction in growth up to

**Fig. 5** Development in protein content (mg protein g⁻¹ DW) of cultivated *S. latissima* over the entire sampling period at locations 2–60°N, 6–63°N, and 9–69°N at **a** 1–2 m depth and **b** 8–9 m depth. Mean \pm SE, $n = 3$

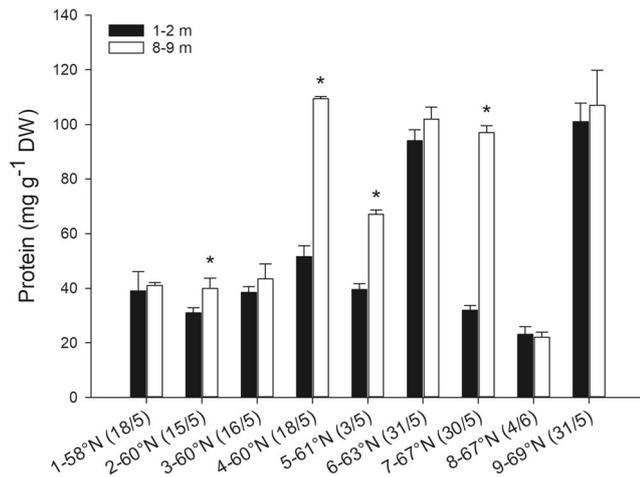


Fig. 6 Protein content (mg protein g⁻¹ DW) for all experimental sites and both depths measured before clearly visible biofouling occurred (sampling date indicated in parentheses after each location name). Asterisk on top of the bars indicates significant differences ($p < 0.001$) between depths. Mean \pm SE, $n = 3$

25‰ at a salinity of 21 psu for juvenile *S. latissima* has been observed in the NW Atlantic (Gerard et al. 1987). At the freshwater-influenced locations in this study, vertical differences in temperature suggested the presence of a fresher surface layer resulting in stronger stratification, reducing nutrient input to surface waters (Rey et al. 2007), making these locations unsuited for commercial cultivation. Cultivation locations should not exhibit seasonal or sporadic reductions in salinity much below 30–35 psu as low salinity can severely

suppress kelp growth (Spurkland and Iken 2011; Kerrison et al. 2015). Frond length and biomass measured at the southern locations (1–58°N to 5–61°N) did not reach those at central and northern locations (6–63°N, 9–69°N), probably because of more severe and long-lasting nutrient limitation in these regions during large parts of the cultivation period (Young et al. 2007; Kerrison et al. 2015; Broch et al. 2019).

Frond lengths and biomass yields were higher at 1–2 m than at 8–9 m depth for all locations during most of the cultivation period, similarly to findings from earlier studies in Central Norway (Forbord et al. 2012; Handå et al. 2013; Sharma et al. 2018). An intermediate cultivation depth of 5 m has previously been tested for *S. latissima* in Norway but did not show a significant difference in peak growth from either 2 or 8 m depth (Handå et al. 2013). This was opposite to the findings of cultivated *M. pyrifera* in Chile where the sporophytes cultivated at 3 m depth were significantly larger and heavier than the ones from 1 and 6 m depth that did not show a significant difference from each other (Varela et al. 2018). The effect of depth is not constant but depends on local environmental variations, therefore several depths should be tested for new farm locations if uniformly seeded drop lines are not used. The linear mixed effects analysis showed that light had a significant positive impact on seaweed frond length and that reduced light availability at 8 m depth was limiting sporophyte growth in *S. latissima*, as also shown for other brown algae (Cronin and Hay 1996). In summer, however, shorter frond lengths and lower biomass yields were found at 1–2 m than 8–9 m depth at several locations. This was presumably an effect of high freshwater runoff in the surface layer or of high

Fig. 7 Cover of biofouling (% of fouled frond area) as a function of time for all study locations at 1–2 m and 8–9 m depth. Mean \pm SE, $n = 4–5$

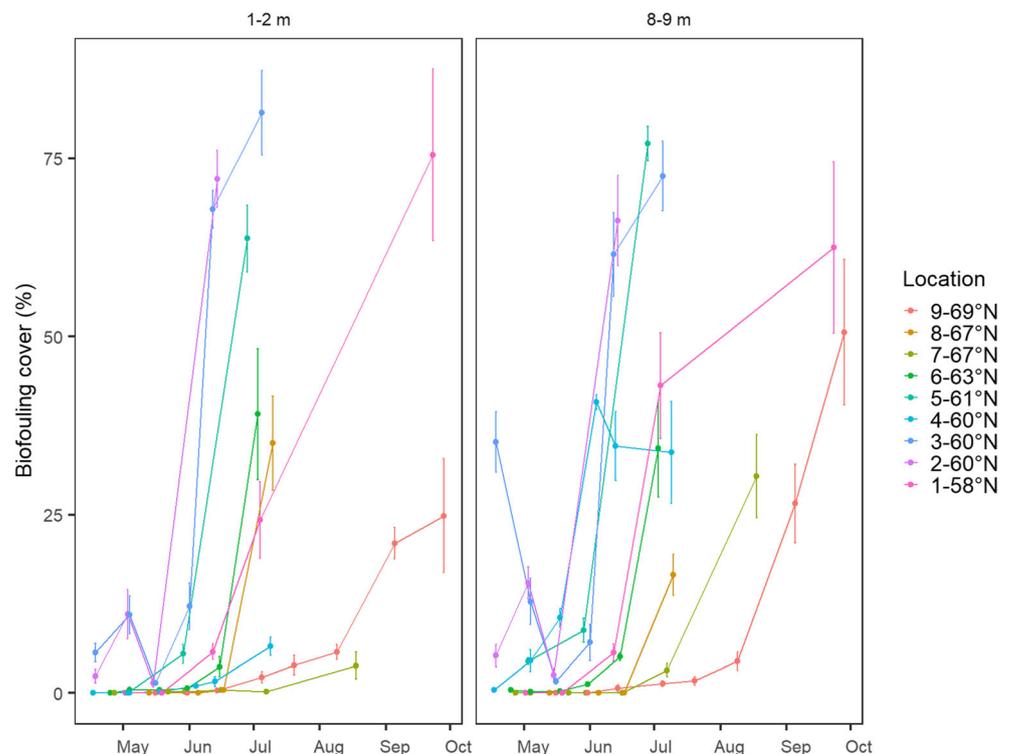
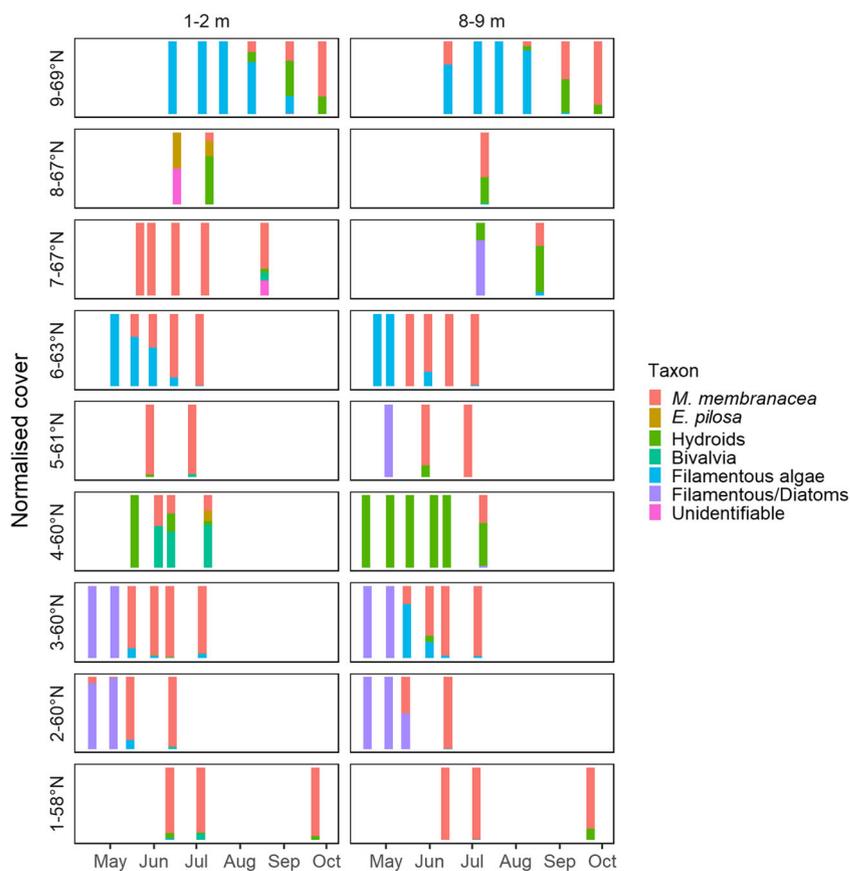


Fig. 8 Epibionts fouling *S. latissima* from all the locations at 1–2 m and 8–9 m depth. Data are shown as normalized cover, with the proportion each taxon constituted of the total cover of all epibionts. Mean, $n = 4-5$



irradiance that may suppress algal growth (Fortes and Lüning 1980; Spurkland and Iken 2011). Exposure of 1–2 h to light at 500–700 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ can lead to significant photoinhibition and photodamage in *S. latissima*, in turn causing

loss of biomass and even death of tissue (Bruhn and Gerard 1996; Hanelt et al. 1997). Because high irradiances ($> 700 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) were only measured for less than 2 h at most of our sites (data not shown), low salinity was the

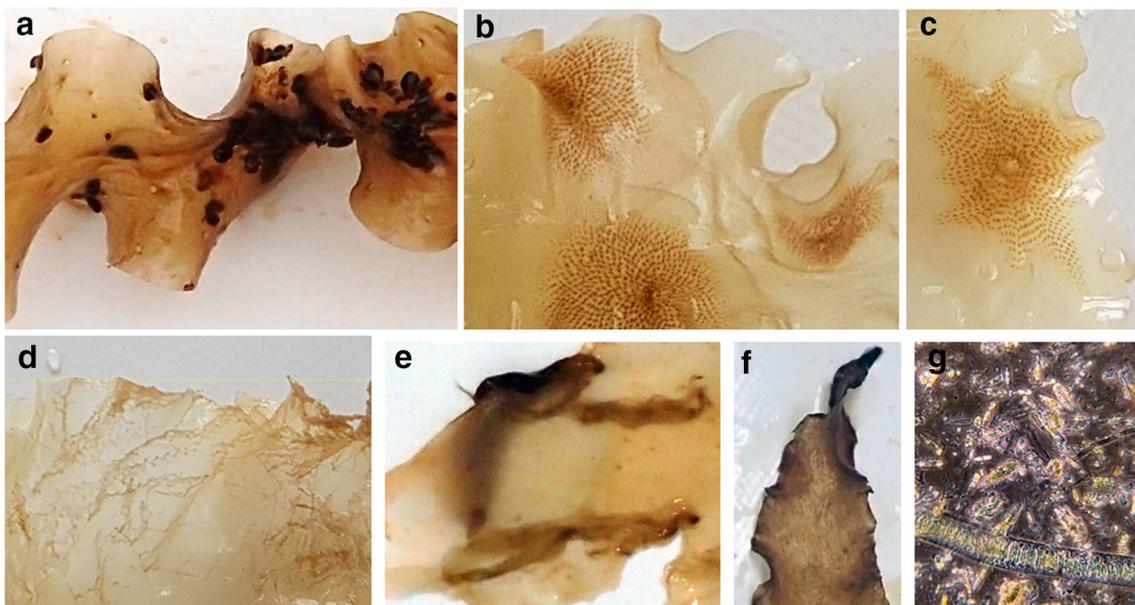


Fig. 9 Images of the epibionts found and registered in this study. **a** Bivalvia. **b** *Membranipora membranacea*. **c** *Electra pilosa*. **d** Hydroids. **e** Filamentous algae. **f** Diatoms. **g** Diatoms at $\times 40$ magnification

Table 5 Linear mixed effects models for the log-transformed dependent variable biofouling cover, with and without temperature (GDD) and light as fixed factors. Location and sample date are random intercepts andGDD as random slope. Models are ranked in descending order after AIC value (i.e. the best-fitted model are presented first) with associated R^2 value

Rank	Formula	K (parameters)	AIC	Δ AIC	R^2
1	$\log(\text{Total biofouling} + 1) \sim \text{Light} + \text{GDD} + (1 + \text{GDD} \text{Location}) + (1 \text{Sample date})$	8	256.4	0.0	0.51
2	$\log(\text{Total biofouling} + 1) \sim \text{GDD} + (1 + \text{GDD} \text{Location}) + (1 \text{Sample date})$	7	269.3	12.9	0.46
3	$\log(\text{Total biofouling} + 1) \sim \text{Light} + (1 + \text{GDD} \text{Location}) + (1 \text{Sample date})$	7	275.5	19.1	0.28

more likely cause for lower growth at 1–2 m depth during summer.

Overall, sea temperature never exceeded 17 °C, a threshold that may cause loss of tissue and death of *S. latissima* (Gerard et al. 1987). In fact, our results for the northernmost location showed that although the temperature never exceeded the optimal lower temperature of 10 °C (Druehl 1967; Fortes and Lüning 1980), the maximum biomass yield and frond length were among the highest in this study. We therefore suggest that the optimal temperature range for growth of *S. latissima* might in fact be lower than 10 °C for some ecotypes. Our findings are contrary to suggestions made by Westmeijer et al. (2019) who proposed that the low temperatures at locations north of the Arctic Circle make them unsuitable for seaweed cultivation.

Chemical composition In our study, total tissue nitrogen content (Q_N , mg N g⁻¹ DW) of cultivated *S. latissima* decreased during spring followed by an increase in autumn, in agreement with Handá et al. (2013). The increase in Q_N during late summer and autumn was likely an effect of increased biofouling. Q_N exceeded the critical concentration of 1.7% of DW for sustaining growth at maximum rates suggested for *Fucus vesiculosus* (Pedersen and Borum 1997) only in the beginning of the sampling period, and for some locations only at 8–9 m depths. Similarly, Manns et al. (2017) found a decrease in Q_N in May–July and suggested that the Q_N was a more reliable indicator of the physiological nutritional state of the seaweeds than the ambient nitrate concentration. Even though the ash content of the seaweed followed the same seasonal pattern as Q_N , the variation in nitrogen content has not been found to be related to the content of ash (Bak et al. 2019). The ash content has, however, been found to be negatively correlated with frond length (Nielsen et al. 2016), which agrees largely with our results.

Similarly to Q_N , the intracellular concentration of nitrate (I-DIN, mg NO₃⁻ g⁻¹ DW) is suggested to express the nutritional state of the alga, but unlike Q_N and protein content, it is not affected by onset of biofouling during summer. I-DIN is easily measurable and studies have revealed that there is a close and significant relationship between I-DIN and both growth rate and ambient nitrate concentrations (Wheeler and Weidner 1983; Young et al. 2007;

Jevne et al. unpublished results), which is highly variable over short time intervals and can be challenging to measure. In our study, I-DIN followed a seasonal pattern with highest content in the beginning of the sampling period similar to Sjøtun and Gunnarsson (1995), when ambient nitrate is in surplus before stratification of water layers and the phytoplankton spring bloom begin (Rey et al. 2007; Broch et al. 2013, 2019; Ibrahim et al. 2014). These conditions occurred later at high compared to low latitudes and at greater depths at high than at low latitudes.

Specific nitrogen-to-protein conversion factors (K_p) based on total amino acids are needed for *S. latissima*, because the commonly used conversion factor of 6.25 previously used for seaweed tends to overestimate the protein content (Lourenço et al. 2002; Mæhre et al. 2018), thereby misleading consumers. The overall K_p average across locations, depths and seasons of 3.8 ± 0.1 found in this study lies within the range of earlier published values of 2.0 and 6.25 (Schiener et al. 2015; Angell et al. 2016; Nielsen et al. 2016; Biancarosa et al. 2017; Manns et al. 2017; Sharma et al. 2018; Bak et al. 2019). K_p was only significantly higher at 1–2 m than at 8–9 m depths when kelp was affected by freshwater runoff, suggesting that it is acceptable to use the same K_p value of 3.8 for *S. latissima* cultivated at different depths in full marine salinity conditions.

Latitude, seasonality, local conditions and to some extent depth affected the protein content in cultivated *S. latissima* in the present study. Protein content was higher at high than at low latitudes throughout the cultivation period, following the latitudinal pattern in ambient nitrate fluctuation (Harnedy and FitzGerald 2011). Seasonally, the protein contents were higher by a factor of 3 in spring than in summer, which is in agreement with a 4- to 8-fold difference in protein content found for *S. latissima* between winter/spring and summer in Denmark and the Faroe Islands (Marinho et al. 2015a; Mols-Mortensen et al. 2017). In contrast, there was not found significant correlation between protein content and season in another experiment from the Faroe Islands, most likely the result of smaller seasonal fluctuation in nutrients (Bak et al. 2019).

The sharp increase in protein content to almost 20% of DW at two locations in our study (2–60°N and 6–63°N) from June onwards was probably due to fouled biomass, the protein originating from epibionts and not from the kelp itself. *M. membranacea*, the main fouling epibiont at these locations,

has a high protein content (> 15% of DW on cultivated *Saccharina japonica*, Getachew et al. 2015). It has also been suggested that a higher protein content found in kelp at deeper waters is a result of reduced light exposure (Cronin and Hay 1996; Ak and Yücesan 2012; Sharma et al. 2018). A significantly higher protein content in kelp cultivated at deeper than shallower waters occurred at four locations in this study. These locations had either poor seaweed growth, early onset of biofouling, and/or had a stratified freshwater layer. Statistical differences in protein content between depths (0–10 m) were not found either for cultivated *S. latissima* in the Faroe Islands (Bak et al. 2019) or for wild *S. latissima* in Denmark (Nielsen et al. 2016).

Biofouling Biofouling varied latitudinally, with a later onset northward, except for two freshwater-influenced locations and the southernmost location. Visible fouling, excluding diatoms and filamentous algae, appeared in May at 60°N and 2 months later at 69°N, allowing for delayed kelp harvest with increasing latitude. This is broadly in agreement with earlier studies on cultivated *S. latissima* in Norway, reporting that epibionts were first observed at 59°N in early May (2012) (Lüning and Mortensen 2015), at 61–63°N in mid-June (2013) (Førde et al. 2016), and at 69–70°N in mid-July (2014) (Matsson et al. 2019). Despite some possible interannual variation, the combination of all studies suggested a latitudinal pattern in biofouling phenology. However, there may be a large spatial variation in cover and species composition of epibionts fouling cultivated kelp within closely located sites (Matsson et al. 2019). Therefore, careful site selection can reduce biofouling levels and, hence, increase biomass yield at a given latitude.

The species composition of the epibionts, and thus possibly their effect on kelp biomass, varied among locations. At most locations, epibionts were dominated by the bryozoan *M. membranacea* like in many earlier studies across different regions (Lüning and Mortensen 2015; Førde et al. 2016; Rolin et al. 2017). At locations influenced by freshwater, however, hydroids and bivalves also covered the seaweed fronds to a high degree. Adults of the bivalve *Mytilus edulis* are euryhaline and larval growth is optimal in salinities from 25 to 30 psu (Brenko and Calabrese 1969). The hydroid *Obelia geniculata* also tolerates low salinities (Cornelius 1982). We suggest that one or more life stages of *M. membranacea* may be sensitive to low salinity, explaining the low occurrence of this species at freshwater-influenced locations.

We observed a succession of species inhabiting the surface of *S. latissima*, with diatoms and filamentous algae as the first visible taxa, later replaced by *M. membranacea*. The same pattern of variation was observed on cultivated *Alaria esculenta* in Ireland (Walls et al. 2017). This is in agreement with the latter of the four phases of succession proposed by Wahl (1989). The algal surface is immediately covered with a film of dissolved chemical compounds (macromolecules), hours later by bacteria

and after the second day by diatoms. Larvae and algal spores settle after one to several weeks depending on latitude and season. Wahl (1989) suggested that the initial phases are purely physically driven, and temperature (as GDD) had indeed the highest effect on total biofouling in our experiment, presumably through its effect on metabolic rates of ectothermic invertebrates. Increased temperature usually results in shorter developmental times and higher growth rates of ectotherms (Atkinson 1994) and growth, development and reproduction are also regulated by thermal history (Trudgill et al. 2005). In Nova Scotia, Canada, thermal history explained 76–81% of the variation in the abundance of settlers of *M. membranacea* (Saunders and Metaxas 2007). Additionally, changes in winter and spring temperatures had the strongest relationship with the timing of settlement and abundance, whereas changes in summer temperatures had the strongest effect on colony size and total coverage on the seaweed frond (Saunders et al. 2010). Our study also showed that biofouling decreased with increasing light availability. Lower light levels may be a consequence of increased levels of food particles in the water column, which have been linked to higher biofouling (Saunders and Metaxas 2009). Increased light may upregulate production of surface metabolites acting to deter establishment of fouling organisms (Pavia and Toth 2000; Rickert et al. 2016). In contrast, there are studies suggesting a benefit of cultivation at deeper waters, where there are lower light intensities, to delay or minimize fouling (Gendron et al. 2007; Førde et al. 2016). Lowering the seaweed to greater depths later in the cultivation season may be beneficial in some areas, but the effects appear to be location specific.

To date, there are no established standards in Norway for an acceptable amount of biofouling for human applications, but if the primary end-use is human consumption or the biochemical industry, the seaweed surface should contain as few impurities as possible and preferably no fouling (SM, personal communication with seaweed farmers). For other applications, e.g. animal feed or soil fertilizer, a prolonged growth season even with increased biofouling may be beneficial because it initially enhances seaweed biomass harvesting yield and nitrogen/protein content along with associated epibiont biomass.

Conclusions

The variation in growth performance, biochemical composition and biofouling of cultivated *S. latissima* was mainly caused by seasonality and depth, varying systematically along a latitudinal gradient. Maximum frond length and biomass yield occurred up to 2 months earlier at southern locations than at locations further north, resulting in the potential to supply the consumer market or processing industry for an extended period of time. Protein content, total tissue nitrogen (Q_N) and intracellular nitrate (I-DIN) showed a decreasing seasonal trend before onset of biofouling and the seasonal

decrease was delayed at higher latitude. The same delay with latitude was observed for biofouling organisms, suggesting that a cultivation and harvesting strategy should follow these latitudinal patterns. Production, expressed in terms of frond length and biomass yield, was higher at shallow cultivation depths than deeper, whereas protein, ash, Q_N and I-DIN were generally higher at deeper depths. Salinity appeared to have an important impact by diminishing seaweed biomass yield and frond length, ash content, biofouling cover, accumulated light and GDD at deeper cultivation depths enhancing protein content and altering biofouling species composition.

Our study is the first to compare cultivation at several seaweed farms over a large latitudinal gradient documenting that kelp farming shows great potential along all latitudes from 58 to 69°N, except in areas with high local environmental variations, as high freshwater runoff. Due to local variations, pilot investigations should be undertaken to determine the suitability of a given potential farm location, by generating knowledge on suitable cultivation depths and the optimal deployment and harvesting windows.

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Author contributions AH conceived the idea of the study, and SF, SM, JS, OJB, KBS and YO contributed to the experimental planning and design. SF, SM, GB, OJB, BB, AH, KBS and JS produced the seedlings and/or participated in field work. SF and GB prepared the samples for chemical analysis and SM generated the biofouling data. SF and SM contributed equally to the development and the design of the paper, analysed the data and co-wrote the drafts of the manuscript. All authors contributed to manuscript writing/editing and read and approved the submitted version.

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