ISBN 978-82-326-5012-5 (printed ver.)
ISBN 978-82-326-5013-2 (electronic ver.)
ISSN 1503-8181

Doctoral thesis
Silje Forbord

Doctoral theses at NTNU, 2020:333

Silje Forbord

Cultivation of *Saccharina latissima* (Phaeophyceae) in temperate marine waters

Nitrogen uptake kinetics, Growth characteristics and Chemical composition

NTNU
Norwegian University of Science and Technology

Doctoral theses at NTNU, 2020:333

NTNU
Norwegian University of Science and Technology
Thesis for the Degree of
Philosophiae Doctor
Faculty of Natural Sciences
Department of Biology

NTNU
Norwegian University of
Science and Technology

Silje Forbord

Cultivation of *Saccharina latissima* (Phaeophyceae) in temperate marine waters

Nitrogen uptake kinetics, Growth characteristics and Chemical composition

Thesis for the Degree of Philosophiae Doctor

Trondheim, October 2020

Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

NTNU
Norwegian University of
Science and Technology

NTNU
Norwegian University of Science and Technology

Thesis for the Degree of Philosophiae Doctor

Faculty of Natural Sciences
Department of Biology

ISBN 978-82-326-5012-5 (printed ver.)
ISBN 978-82-326-5013-2 (electronic ver.)
ISSN 1503-8181

Doctoral theses at NTNU, 2020:333

Printed by NTNU Grafisk senter

TABLE OF CONTENT

ACKNOWLEDGEMENTS

The present work was accomplished at the Institute of Biology at the Norwegian University of Science and Technology (NTNU), Trondheim, Norway. The PhD research was funded by the Research Council of Norway through the grant MACROSEA (254883) coordinated by SINTEF Ocean.

Firstly, I would like to express my gratitude to my main supervisor Professor Yngvar Olsen (NTNU) for excellent help in the writing and editing phase of my work, I have learned a lot about algal physiology and scientific writing along the way. Many thanks also to my co-supervisor Senior scientist Jorunn Skjermo (SINTEF Ocean) for great help in the planning and practical phase of several experiments, for text editing and for all the fun at conferences! Thanks a lot to Research Manager Aleksander Handå (SINTEF Ocean) for being an excellent project leader of MACROSEA, making this project come together in the first place, and for always staying positive about my work when I was not.

My PhD was definitively not accomplished alone. I am very grateful to all my co-authors (Lone, Sanna, Siv, Kristine, Ole Jacob, Torfinn, Guri, Bodil, Anna, Vegard, Aleksander, Jorunn and Yngvar), the skilful technicians analysing my samples (Kjersti, Marte, Rasa and Siv) and preparing the hatchery before experiments (Åsmund and Arnstein), and to everyone participating during the field registrations. A special thanks to the seaweed farmers contributing to the registrations in Paper III. Huge thanks are due to the whole MACROSEA team, it is very valuable to be part of a bigger group sharing the same goal!

A big thank you to the staff and fellow PhD students at NTNU Sealab and TBS for being my "home away from home" during my PhD period. And a big thanks to my research group Aquatic Bioresources and to the rest of my colleagues and friends at SINTEF Ocean for still being my home during these four years.

I would like to give special thanks to the legendary Dr. Klaus Lüning for teaching me how to cultivate our dear *Saccharina* at the beautiful island of Sylt in 2008.

Thanks to my friends for keeping me distracted with nonsense and sparkling wine, and to my family for always being proud of me.

Finally, the biggest “thank you” and forever gratitude goes to my dear husband Tor for always supporting, cooking and cleaning (and still loving me through all my ups and downs…), but above all for being the most dedicated father in the world for our daughters. When one of our girls referred to my laptop as the “evil stepdaughter”, I understood that I had given it too much attention, and I am sorry for that! To Ella and Nora, remember to always believe in yourself and that the old cliché “it is never too late to follow your dreams”, has something to it! Love you guys to the moon and back!!

A happy memory from the very first cultivation experiment with *Saccharina latissima* at SINTEF back in 2009. Photo: Karl Tangen

Silje Forbord

Trondheim, June 2020

ABSTRACT

Cultivation of macroalgae is an emerging industry in Europe and the sugar kelp *Saccharina latissima* is one of the most economically and ecologically relevant species. New consumer trends, market demands and opportunities for multiple uses of macroalgae such as food, bioactive components, feed, fertilisers and biofuels has strengthened the motivation for industrial macroalgae cultivation in Western countries. The Norwegian coastline covers more than ten degrees in latitude and provides a range of abiotic and biotic conditions for successful seaweed farming. Since 2008, more than 40 companies have been established aiming for commercial farming along the coast, but the cultivation is still in a preliminary phase due to the high production costs and a premature market for bulk biomass.

The present study has increased our biological knowledge needed for the cultivation of *S. latissima* and emphasises the importance of studying growth and dynamics of biochemical content under different environmental conditions, including different latitudes, seasons and depths, but also different seedling sizes and nutritional histories. This was performed under various ecologically realistic scenarios, as well as under conditions that are not commonly found along the Norwegian coast, but which are biologically interesting to include. Furthermore, this new fundamental understanding of physiological mechanisms makes us better understand the ecological niche and nutritional requirements of cultivated *S. latissima*.

The coupling between extracellular nitrate (NO_3^-) concentration, initial NO_3^- uptake kinetics, intracellular NO_3^- concentration and specific growth rate is paramount for understanding both the nutrient and environmental conditions required for efficient cultivation of *S. latissima,* as this will have essential importance for future large-scale production. It appears that *S. latissima* requires high ambient NO_3^- concentrations for maintaining rapid growth and is not able to compete for available nutrients with the more efficient phytoplankton during the late spring and summer. This means that *S. latissima* has to take up most of the NO_3^- needed early in the season when ambient concentrations are high, and that the period after the spring bloom represents a negative shift in nutrient uptake that involves sporophyte growth mainly based on internal inorganic and organic nitrogen components. A positive relationship between both total intracellular nitrogen components and mean growth rate to the ambient nitrate concentration was confirmed.

There are several methods to influence the biomass yield and chemical composition of the harvestable *S. latissima* biomass. If the motive is to produce biomass with a high content of internal nitrogen components such as protein, cultivation during periods of high ambient nitrate is essential. This occurs during the period from the late fall to spring along the Norwegian coastline, depending on the latitude, but could also be accomplished by pumping deep water into land-based tank systems or by moving the cultivation lines to deeper depths when the nutrients are depleted in the surface layer due to phytoplankton blooms. However, if the main goal is to produce the highest harvestable yield, adequate light intensities must be provided to the sporophytes, together with high ambient nitrogen, to increase their length and weight. The hatchery phase also impacts the biomass productivity at sea. Seeding lines with spores followed by an incubation period of 42 days in the hatchery before deployment gives an advantage to reach the highest possible yield compared to seeding with gametophytes or juvenile sporophytes.

A monitoring study of cultivated *S. latissima* along the Norwegian coast revealed a south-north gradient in biomass development during the spring and summer, with the southern location reaching maximum frond length and biomass yield 2 months earlier that the northernmost location. Irrespective of latitude, which clearly has a huge influence on growth and chemical content due to large differences in essential resources such as nutrients and light, site selection in general must be considered before deployment. 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 deployment and harvesting windows that would assist farmers to maximise production and minimise loss.

The long-term goal is to use this newly gained knowledge to contribute to the future development and establishment of a new bioeconomy based on cultivated seaweed. Industrialisation of a nearly carbon-neutral, non-fed cultivation of macroalgae will contribute to an environmentally-friendly development of the aquaculture sector and to a sustainable exploitation of the ocean.

LIST OF PAPERS

I. **Forbord S**., Etter S. A., Broch O. J., Dahlen V. R., Olsen Y. Initial short-term nitrate uptake in juvenile, cultivated *Saccharina latissima* (Phaeophyceae) of variable nutritional state. Submitted manuscript.

II. Jevne L. S., **Forbord S**., Olsen Y. The effect of nutrient availability and light conditions on the growth and intracellular nitrogen components of land-based cultivated *Saccharina latissima* (Phaeophyta). Submitted manuscript.

III. **Forbord S**., Matsson S., Brodahl G. E., Bluhm B. A., Broch O. J., Handå A., Metaxas A., Skjermo J., Steinhovden K. B., Olsen Y. (2020). Latitudinal, seasonal and depth-dependent variation in growth, chemical content and biofouling of cultivated *Saccharina latissima* (Phaeophyceae) along the Norwegian coast. *Journal of Applied Phycology.* DOI:10.1007/s10811-020-02038-y

IV. **Forbord S**., Steinhovden K. B., Solvang T., Handå A., Skjermo J. (2019). Effect of seeding methods and hatchery periods on sea cultivation of *Saccharina latissima* (Phaeophyceae): a Norwegian case study. *Journal of Applied Phycology.* DOI: 10.1007/s10811-019-01936-0

ABBREVIATIONS

AA	Amino acids
C	Carbon
DIN	Dissolved inorganic nitrogen
DON	Dissolved organic nitrogen
DW	Dry weight
E-DIN	External nitrate content
GDD	Growing degree-day
I-DIN	Intracellular nitrate content
I-DON	Small molecules of intracellular organic nitrate
K_p	Specific nitrogen-to-protein conversion factor
L:D	Light:Dark
N	Nitrogen
N:C	Nitrogen:Carbon ratio
NH_4^+	Ammonium
NO_3^-	Nitrate
NO_2^-	Nitrite
OD	Optical density
PAR	Photosynthetically active radiation
PES	Provasoli's Enriched Seawater
PON	Particulate organic nitrogen
Q_N	Total tissue nitrogen
RGR	Relative daily growth rate
U	Uptake rate normalised to nitrogen content
V	Uptake rate normalised to dry weight
WW	Wet weight

1. BACKGROUND

Distribution of *Saccharina latissima*

The sugar kelp *Saccharina latissima* (Linnaeus) Lane, Mayes, Druehl and Saunders 2006 [synonym: *Laminaria saccharina* (Linnaeus) Lamouroux] is a cold water species distributed circumpolar in the northern hemisphere where it occurs from the intertidal zone down to the bottom of the photic zone (Bolton et al. 1983). The species is present on both sides of the Atlantic, from Nova Scotia along the coasts of Europe and in the Pacific along the North American cost, as well as some areas outside of Japan and in the 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 cultivation along the entire coast.

S. 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 which are met along most of the Norwegian coastline. Temperature, wave exposure and competition with other species are among the factors affecting the propagation of *S. latissima* and the southern limit is highly determined by temperature (Lüning 1990; Azevedo et al. 2019). In addition, the availability of light and nutrient resources regulates depth distribution and productivity (Hurd et al. 2014; Xiao et al. 2019). The light saturation point of growth for *S. latissima* juvenile sporophytes has been shown to be at 50-70 µmol photons m^{-2} s^{-1}, with an optimum photon flux density of 110 µmol as the sporophytes approach 6 weeks in age (Kain and Jones 1969; Egan et al. 1989). Reports for the same species indicate that 1- to 2-year-old fronds show photoinhibition at 250 µmol photons m^{-2} s^{-1} (Fortes and Lüning 1980).

In temperate North-Atlantic waters, *S. latissima* shows a seasonal dependent growth pattern with fast growth during the first half of the year. From late spring to late summer, heavy fouling and necrosis of the distal end of the lamina occurs (Handå et al. 2013; Førde et al. 2016; Matsson et al. 2019).

***Saccharina latissima* life cycle**

The sporophyte of *S. latissima* consists of a lamina (frond), stipe and haptera (Figure 1.1). The lamina grows from an intercalary meristem located between the lamina and the stipes, which is short, round and attach to the substrate with a branched haptera.

Figure 1.1: *Saccharina latissima* consisting of haptera (1), stipes (2) and lamina (3). Illustration: Lydia Torsvik Gieselmann

S. latissima has a diplo-haplontic, heteromorphic life cycle, which alternates between a microscopic haploid (n) gametophyte generation and a macroscopic diploid (2n) sporophyte generation (Kain 1979), as shown in Figure 1.2. The life cycle is common for all the different Laminariales, with minor differences. In Norway, *S. latissima* is commonly fertile during winter (October–January) when the days are short, and temperatures are low. Sorus with sporangia develops on the lamina and meiosis produces 32 motile meiospores (zoospores) per sporangium (Schreiber 1930; cited in Kain 1979). When mature, the 4-8 µm long spores are released into the surrounding water and spread with water currents until they settle at a suitable substrate. After settlement, they lose their two flagella and the initial germling growth phase starts with the formation of a germtube in the distal end of the spore. The germtubes are filled with the cell content of the spore, making a primary cell, which further develops into male and female gametophytes (Williams 1921; cited in Kain 1979). Gametophytes increase in length via cell division, while progressively developing branches. Male gametophytes are thin and heavily branched, while the female have thicker cells and are less branched (Arbona and Molla 2006). Gametogenesis in Laminariales is induced by blue light (400–512 nm), whereas the optimum temperature varies with geographical

distribution (Lüning and Dring 1975; Lüning and Neushul 1978; Lüning 1980). Once the light- and temperature conditions are suitable, the development of reproductive structures is triggered (Lüning 1980). Female gametophytes produce an oogonium, which again produces one non-motile spherical gamete (oosphere/egg) when fertile. The male gametophyte develops spermatocysts which produce spermatozoids with two flagella. A signal chemical attracts the spermatozoids, which swim to the oogonium, and fertilise the egg (Müller et al. 1979). The fertilised gametes develop into a microscopic sporophyte that grows into the full-sized sporophyte of up to 3 m in length (Su et al. 2017).

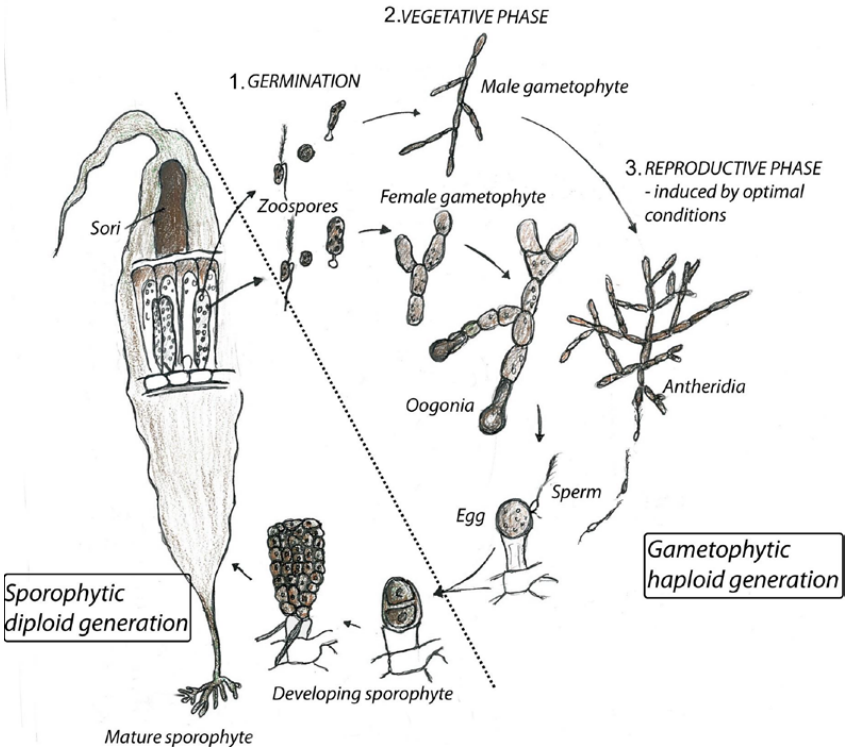

Figure 1.2: Schematic presentation of the life cycle of *Saccharina latissima*. Illustration: Sanna Matsson.

The haploid gametophyte stage can be cultivated under controlled environmental conditions in a laboratory. In weak red-light, gametophytes have vegetative growth and will increase in size, without producing gametes or spermatocysts. Fertilisation can be induced at any

moment by modifying the light regime and exposing the gametophytes to blue light. Studies have shown that the ability to produce gametes is still good after 30 years of cultivation under red-light conditions in many kelp species (Druehl et al. 2005).

Cultivation

Production and use

The cultivation of seaweed has a long tradition in Asian countries like China, Indonesia, Japan, Korea and the Philippines, and algae constitute a large and natural part of people's diet. The first attempts to cultivate the kelp *Saccharina japonica*, a close relative to *S. latissima*, has history starting from the 1930–1940s in northern China, with the breakthrough coming at the end of the 1950s with the horizontal longline method (Su et al. 2017). Approximately 220 species of seaweed are of commercial value, while the number of species that are intensively cultivated is relatively low, posing a challenge to finding new species that can offer novel products (Hafting et al. 2015). The most common seaweed taxa to cultivate are the brown algae *S. japonica* (kombu), *Undaria pinnatifida* (wakame) and *Sargassum fusiforme* (hiziki), together with the red algae *Euchema* spp. and *Kappaphycus alvarezii* (both for carrageenans), *Gracilaria* spp. (for agar) and *Porphyra/Pyropia* spp. (nori), with an annual production reaching almost 30 million metric tons (FAO 2018; Buschmann and Camus 2019).

S. latissima is one of the fastest-growing species of kelp in European waters, with annual production capacities estimated at 75–170 tons wet weight (WW) per hectare at sea (Broch et al. 2013; Holdt and Edwards 2014; Broch et al. 2019). Europe's contribution to the global macroalgae cultivation is negligible, and a production of 1000 metric tons *S. latissima* was reported in 2017 (FAO 2018). However, new consumer trends, market demands and opportunities for multiple uses of macroalgae such as food, bioactive components for functional food and feed ingredients, fertilisers and biofuels (Shahidi 2009; Holdt and Kraan 2011; Rajapakse and Kim 2011; Fleurence et al. 2012; Hafting et al. 2012; Tabarsa et al. 2012; Wargacki et al. 2012; Fernand et al. 2017) has strengthened the motivation for industrial macroalgae cultivation in Europe.

In Norway, the cultivation of macroalgae was initiated 10 years ago with small-scale experiments. The cultivation is still in a preliminary phase, even though several seaweed farmers along the Norwegian coast cultivated and harvested up to 176 tons of the two kelp species *S. latissima* (174 t) and *Alaria esculenta* (2 t) in 2018. In 2019 the production of *S. latissima* was reduced to 66 t while the production of *A. esculenta* had increased to 44 t, and the value of the two species had increased significantly (Directorate of Fisheries 2020). There are presently 475 permits for macroalgal cultivation distributed over 97 locations and 16 companies in Norway (Directorate of Fisheries 2020).

Efforts to make seaweed a part of the everyday human consumptions, by informing about seaweed health benefits and launching of new products, have thus resulted in another type of awareness among customers during the last 2–3 years. Easily accessible products such as salt, pesto, breading and dried flakes can influence people to use seaweed to enhance flavour (Mouritsen et al. 2012) and add more iodine into their daily diet (Roleda et al. 2018), and can provide opportunities for eventually trying larger portions of seaweed. To produce these kinds of products, the amount of seaweed currently cultivated is sufficient, but if the market is aiming for other types of products from this versatile biomass, e.g. feed ingredients, fertilisers and biofuels, we need to massively upscale the production (Olafsen et al. 2012; Skjermo et al. 2014). Industrial, cost effective cultivation requires novel technology of the whole production line targeting mechanisation and automation of the seedling processes, deployment at sea and harvesting operations.

Cultivation technology and strategies

The cultivation process consists of two major stages: 1) a nursery stage that provides conditions necessary for the development of microscopic gametophytes through their sexual phase and the subsequent development of small sporophytes suitable for deployment at sea (Figure 1.3, left); and 2) a sea-farming stage that involves the cultivation at sea of these juvenile sporophytes until they reach a suitable size and chemical composition for the desired end-products (Figure 1.3, right).

Figure 1.3: Juvenile sporophytes of *S. latissima* cultivated on a 1.2 mm string and ready for deployment at sea (left) and cultivated *S. latissima* biomass after approximately 3-4 months at sea (right)

Cultivation protocols have been established for the most common kelp species, like *S. latissima* (Flavin et al. 2013; Redmond et al. 2014; Forbord et al. 2018)*, A. esculenta* (Arbona and Molla 2006) and *Laminaria digitata* (Edwards and Watson 2011). In addition, other seaweed species are becoming attractive for cultivation in Europe due to their high protein content and demand for human consumption, like the red algae *Palmaria palmata* and *Porphyra* sp. (Edwards and Dring 2011; Lavik 2016; Schmedes et al. 2019; Schmedes and Nielsen 2019).

There are three main strategies for producing kelp seedlings with seeding of the growth substrate with either spores, gametophytes or small sporophytes. Seeding with spores and gametophytes is the most incorporated technique to date (Forbord et al. 2018). Seeding with spores requires fertile sporophytes and is seasonal-dependent if these are collected in natural habitats; however, fertility can also be induced by an artificial day rhythm and thus enable access to spores independent of natural seasons (Pang and Lüning 2004; Forbord et al. 2012). Gametophytes can be kept in continuous cultures and be available for year-through seeding or the production of microscopic sporophytes for direct seeding. This is advantageous as the use of incubation facilities can be shortened by several weeks or, in the case of direct seeding, omitted completely. Limited information exists on which of these three strategies yields the highest biomass at sea and how the age and developmental stage at deployment will affect the chemical content of the biomass at harvest.

Fouling by epibionts usually occurs from spring to autumn, depending on location, latitude, and inter-annual variation (Scheibling and Gagnon 2009). Epibionts can form a barrier inhibiting nutrient (Hurd 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 values for human consumption may, however, still be used in other industries, for example in the production of animal feed (Burton 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).

Biochemical content

The biochemical content of *S. latissima* varies along the thallus of individual sporophytes (Boderskov et al. 2016), making it important to analyse samples from the whole lamina, and not only at specific areas like the meristematic zone. Even more important is the strong influence of the external environment, like nutrients, light, temperature, salinity and currents that vary with season and depths (Schiener et al. 2015; Bruhn et al. 2016; Nielsen et al. 2016; Bak et al. 2019).

The moisture content of fresh *S. latissima* is very high and can account for up to 75–90% of the biomass. The remaining dry matter contains nutritional elements such as carbohydrates, proteins, lipids, vitamins and minerals in varying amounts (Holdt and Kraan 2011).

S. latissima, like other marine algae, may contain large amounts of polysaccharides, up to 70% of DW (Nielsen et al. 2016; Manns et al. 2017), notably alginate and some cellulose for cell wall structure, but also storage polysaccharides like fucoidan, laminaran and mannitol (Holdt and Kraan 2011; Schiener et al. 2015). The latter two are found to be highest in the summer and autumn and support growth during the dark winter period when the photosynthetic activity is low. In contrast, lipids accounts for a very low fraction of the dry matter, only around 0.6–3.4% of DW depending on the season (Marinho et al. 2015c; Monteiro et al. 2020), which is a lot lower than for most other marine organisms.

The internal nitrogen components like total tissue nitrogen (Q_N), intracellular nitrate (I-DIN) and protein are found to be significantly interrelated and correlated with the ambient seawater nitrogen. Q_N usually varies from 10-40 mg g^{-1} DW (1-4 % of DW) (Gerard et al. 1987), but values both below and above have been registered for nitrogen limited and saturated sporophytes of *S. latissima* (Chapman et al. 1978; Boderskov et al. 2016). Levels of Q_N are dependent on age, size and the nutritional history of the sporophytes, among other factors, and may thus vary among individuals. Critical values to maintain growth and survival are suggested for several species (Pedersen and Borum 1996), but these have not yet been determined for *S. latissima*.

I-DIN is not commonly measured for macroalgae. In general, this pool is not believed to represent a major fraction of the total nitrogen storage, at least not for *S. latissima* (Young et al. 2007). However, as the content of I-DIN has shown a close and significant relationship with growth rate and ambient nitrate concentrations (Wheeler and Weidner 1983), I-DIN could be an important indicator of the nutritional status of the algae, and quantification of this fraction is therefore relevant.

The global human population is expected to increase by over a third (2.3 billion people) by 2050, requiring an estimated 70% increase in food production (Godfray et al. 2010). In particular, protein is one of the main nutrients that will be in short supply in the future. Alternative protein sources and production methods are required to fulfil the demand of consumers and meet predicted global protein requirements (Bleakley and Hayes 2017). Seaweed is considered a viable source of protein, and some species, like the red algae *Porphyra* spp., are known to contain as much as 35–47% protein which is comparable to soybean (Fleurence 1999; Garcia-Vaquero and Hayes 2016). The protein fraction of *S. latissima* ranges from 3–26% of DW, mainly increasing during the winter and decreasing over the spring and summer period (Fleurence 1999; Marinho et al. 2015b; Schiener et al. 2015; Mols-Mortensen et al. 2017). Terrestrial agriculture already requires approximately 75% of the total global freshwater, while marine algae do not use freshwater or arable land to grow. However, the current extraction processes of algal protein and other high valuable compounds are time-consuming and not yet economically viable.

S. latissima is known to be high in minerals with an ash content of 15–45% of DW (Marinho et al. 2015a; Bak et al. 2019), and it can contribute with 10–100 times higher mineral

content than traditional vegetables when used as feed and food supplements (Holdt and Kraan 2011; Pereira 2011). Iodine is an important mineral that is used to prevent goitre; only 32 mg DW of unprocessed *S. latissima* is enough to meet the recommended daily intake levels for most healthy humans (Roleda et al. 2018). However, up to 88% reduction in iodine content can be obtained by water blanching the biomass at ≥45 °C for ≥30 sec, without compromising other valuable compounds like total protein (Nielsen et al. 2020).

Even though macroalgae in general add value to the daily diet by contributing essential nutrients such as vitamins and minerals, they can sometimes contain elevated levels of inorganic arsenic, total arsenic and cadmium, while levels of mercury and lead are generally low. High levels of inorganic arsenic and cadmium are particularly found in brown algae and may limit their use as food and feed ingredients. There are currently no regulations for macroalgae used as food (Duinker et al. 2016; Barbier et al. 2019).

Nitrogen uptake kinetics and metabolism

Nitrogen (N) plays an important role in maintaining the biogeochemical balance of the marine environment and exists in more chemical forms than most other elements. All these transformations are undertaken by marine organisms as part of their metabolism, either to synthesise structural components or to gain energy for growth (Gruber 2008). The internal concentration of a nutrient depends on the concentration of that nutrient in the surrounding environment and the algae's ability to extract this from the sea (Hurd et al. 2014).

Nitrogen is considered to be the potential limiting nutrient for macroalgal growth, both globally and in Norwegian coastal waters, especially during the summer months after the phytoplankton blooms have depleted the concentration of dissolved inorganic nitrogen (DIN) to almost zero (Hanisak 1983; Rey et al. 2007; Ibrahim et al. 2014; Broch et al. 2019). In temperate regions, upwelling brings up and mixes nutrient-rich deep water with nutrient-deficient surface water. In Norway, this takes place in the autumn-early spring period, dependent on latitude. Nutrients from greater depths are then redistributed to the surface layer, representing a nutrient reservoir which supports the phytoplankton spring bloom in the following year (Voss et al. 2013).

The major sources of nitrogen available for macroalgae are nitrate (NO_3^-), ammonia (NH_4^+), and urea (Hurd et al. 2014). The concentration of NO_3^- in surface water can vary from almost zero in the tropics and subtropics up to several tens of µmol L^{-1} (µM) in the temperate, Arctic and Antarctic oceans outside the vegetation period (Voss et al. 2013). North-East Atlantic deep water contains around 10 µmol L^{-1} of NO_3^- (Forbord et al. 2012), representing the highest concentration of nitrate that algae are exposed to in their natural environment. Nitrate assimilation capacity varies several folds seasonally in intertidal brown algae in response to nitrate availability; in the winter, nitrate concentrations are high and not limiting, but in the summer, nitrate is normally significantly depleted (Young et al. 2007). NO_3^- can be stored inside the seaweed cells at concentrations exceeding ambient concentrations, and is therefore assumed to require active uptake (Harrison and Druehl 1982; Harrison and Hurd 2001), but this has only been confirmed for a few species (Roleda and Hurd 2019). An active mechanism of uptake will exhibit saturation kinetics; at low nutrient concentrations, the uptake rate then increases linearly with increasing concentration, while at higher concentrations, a further increase does not lead to a much higher uptake rate. Uptake kinetics can therefore commonly be described by a rectangular hyperbola of the Michaelis-Menten equation type (Chapman et al. 1978; Harrison and Druehl 1982; Wallentinus 1984; Harrison and Hurd 2001; Phillips and Hurd 2004). In other cases, saturation does not occur at high nutrient concentrations, and a linear uptake with increasing nitrate concentration may be experienced. Unsaturated uptake is common for NH_4^+ (Phillips and Hurd 2004; Abreu et al. 2011; Martínez et al. 2012), but it has also been described for NO_3^- (Harrison et al. 1986; Ahn et al. 1998; Sánchez-Barredo et al. 2011; Martínez et al. 2012).

Nutrient uptake rates in seaweed are affected by, and vary considerably with, variations in chemical (concentration of nutrients in their ionic or molecular form), physical (light, salinity, temperature, desiccation and water motion) and biological factors (Thomas et al. 1987; Hurd 2000; Harrison and Hurd 2001). Biological factors include the surface area-to-volume ratio, type of tissue, age, nutritional state and inter-seaweed variability (Wallentinus 1984; Pedersen 1994; Hurd et al. 2014). Perennial algae appear to have slower uptake rates and are better adapted to a low and constant nutrient supply than ephemeral macroalgae which dominate areas with high and episodic nutrient concentrations (Pedersen and Borum 1997;

Harrison and Hurd 2001; Thornber et al. 2008), while younger tissues are found to have a higher N demand than older tissues because they are actively growing and hence have a higher uptake rate (Roleda and Hurd 2019).

When NO_3^- is taken up, it can be stored in intracellular pools (I-DIN) in the vacuoles and cytoplasm (Hurd et al. 2014) or be reduced to nitrite (NO_2^-). Incorporation in vacuoles may occur when the uptake rate of NO_3^- is greater than the rate of conversion to NO_2^-. Nitrite is further reduced to NH_4^+ in the chloroplasts and converted into biomolecules such as amino acids (Figure 1.4) (Harrison and Hurd 2001). Dependent on the size of the I-DIN pool, algae may be more capable of growing in areas where the nutrient availability varies over the seasons. High internal tissue concentrations of inorganic nitrogen suggest nutrient storage, whereas low internal tissue concentrations may indicate nutrient deficiency (Hanisak 1979). Other potential nitrogen reserve pools in macroalgae include proteins, free amino acids, nucleotides and pigments (Bird et al. 1982; Lapointe and Duke 1984; Jones et al. 1996; Naldi and Wheeler 1999; Young et al. 2007), while proteins account for the largest intracellular N-source (Pueschel and Korb 2001). High contents may also be a result of nutrient saturation for these components, whereas low levels may suggest nutrient sub-saturation.

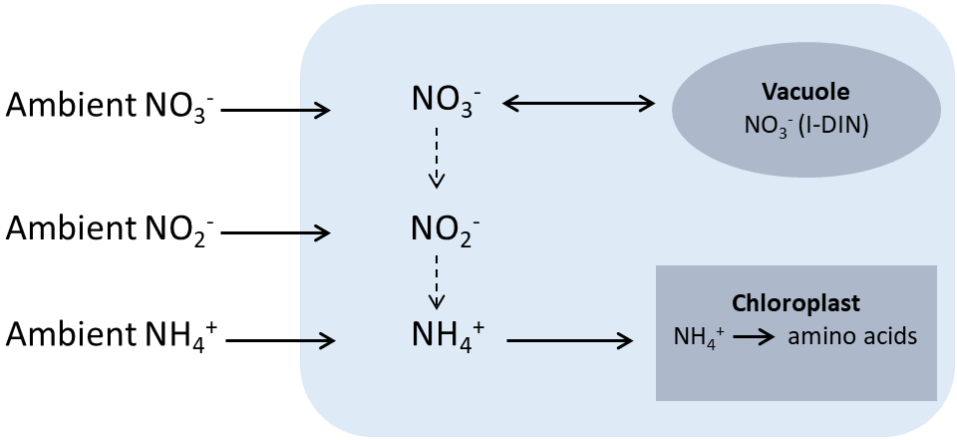

Figure 1.4: A simplified overview showing the main pathway of inorganic nitrogen inside a seaweed cell from uptake to the conversion of biomolecules like amino acids. Illustration modified from Syrett (1981).

The nitrate uptake rates for N-saturated and N-starved cultivated *S. latissima* under realistic environmental conditions are not well described in the literature. Previous experiments with this species were often performed under very high nitrate concentrations, which are not naturally found in North-Atlantic waters, and only for individuals collected from natural populations. The capability of storing I-DIN and the time kinetics of this process are also inadequately described for cultivated *S. latissima* under various nutritional states.

2. AIMS OF THE THESIS

The main aim of this thesis was to investigate nitrogen uptake kinetics, growth characteristics and chemical composition, with focus on the intracellular nitrogen components for cultivated *Saccharina latissima*. This was performed under various ecologically realistic scenarios, mostly representative for conditions along the Norwegian coast (North-East Atlantic coastal waters).
The long-term goal is to use this new fundamental knowledge of the ecological niche and nutritional requirements of *S. latissima* to contribute to the future development and establishment of a Norwegian bioeconomy based on cultivated seaweed of high quality.

To achieve the main aim, four specific objectives were defined:

1) Determine the initial short-term nitrate uptake rates and the internal nitrate and total nitrogen storage capacity for nitrogen-saturated and nitrogen-limited young *Saccharina latissima* sporophytes, and with nitrate concentrations representative of Norwegian coastal waters **(Paper I).**
2) Examine how various light conditions and nutrient availability affected growth and intracellular nitrogen components in *Saccharina latissima* by growing adult, cultivated sporophytes in land-based tanks with four different combinations of high and low light and high and low nutrient supply over an experimental period of 20 days with subsequent analysis of tissue nitrogen metabolites **(Paper II).**
3) Examine the effects of latitude, season and cultivation depth on biomass accumulation, chemical composition and biofouling of cultivated *Saccharina latissima* by using nine locations from 58 to 69°N over a cultivating season **(Paper III).**
4) Compare how three different seeding methods of *Saccharina latissima* (meiospores, gametophytes or direct seeding with juvenile sporophytes) and time of hatchery periods will affect growth and protein content during 80 and 120 days of sea cultivation **(Paper IV).**

3. METHODS

Nutrient uptake kinetics

There are several different methods that can be used to measure nutrient uptake rates in macroalgae (Harrison et al. 1989), and the method used can affect the results substantially. This includes choice of tissue parts (whole sporophyte vs. thallus discs), how the nutrient preconditioning is carried out (number of days), varying biomass in relation to volume of medium during the experiment, incubation time and normalisation of uptake rates (Lubsch and Timmermans 2019). In the experiment with *S. latissima* in **Paper I**, whole sporophytes were used instead of thallus discs for each substrate concentration, as this has several advantages, such as including interplant variations, which will give a more accurate estimate of plant population response (Harrison and Druehl 1982).

Laboratory measurements of nutrient uptake in seaweeds are normally performed by adding epiphyte-free tissue to seawater with saturating levels of all nutrients, trace metals and vitamins, except for the nutrient being studied. There are two main techniques commonly used for measuring nutrient uptake rates in the laboratory: the perturbation method and the multiple flask technique (Harrison et al. 1989). A combination of these two techniques was used in **Paper I** to study the short-term uptake of NO_3^- at seven different substrate concentrations, taking out samples over a time interval of 90 min. The short-term nitrate uptake reflects the instantaneous uptake following a perturbation in nutrient concentration. The uptake rate will be constant for a short time after perturbation before intracellular feedback from the increasing nutrient content in the algal tissues and before the ambient concentration has been substantially reduced. The uptake rate depends on the initial nutritional state of the algae. Uptake rates measured over a long period of time will become proportional to and vary with the growth rate.

Nitrate was measured in water samples analysed colorimetrically at 550 nm after the reduction of NO_3^- to NO_2^- through a copperised cadmium coil in a Flow Solution IV System, O. I. Analytical Auto Analyser following Norwegian Standard 4745 (NSF 1975).

The uptake rates were determined according to Equation 1:

$$V = \frac{(S_i - S_f) \text{ x vol}}{\text{t x DW}} \text{x } 24 \quad (1)$$

where V is the specific uptake rate (µg N g^{-1} DW day^{-1}), S_i is the initial substrate concentration (µg NO_3-N L^{-1}), S_f is the final substrate concentration (µg NO_3-N L^{-1}), vol is the volume (L), t is the time between sampling (h) and DW is the dry weight of the total biomass in the flask (g).

Growth measurements

The *S. latissima* seedlings were produced according to the cultivation protocol published by Forbord et al. (2018) using the seaweed hatchery at Seaweed Energy Solutions for **Papers I** and **II** and the hatchery at SINTEF Sealab for **Papers II** and **IV**. Some dissimilarity may occur between the hatchery procedures.

Seaweed growth can be measured in several ways; the most common is frond elongation or biomass increase between two registration points or total increase during the cultivation period. Length can be measured by either using the punch hole method (Parke 1948), following the movement over time of one point (hole) in the meristem (**Paper II)**, or measuring length of the whole lamina from above the stipe to the distal end (**Paper III** and **IV**). The advantage of measuring frond elongation is that it is an easy and fast method that does not require much equipment, and destruction of the biomass is not necessary as the measurements can be made on seaweed still attached to the cultivation lines. The drawback is that you need a large number of sporophytes to include the intraspecific variations in length that normally occur on a rope due to self-shading and individual growth performances.

The measurement of the total biomass yield (**Paper III** and **IV**) is relevant to use for large-scale cultivation when the overall production is more important than the growth of single individuals, and when comparing growth from one year to another on the same cultivation site. The drawbacks using this method are seen when weighing fresh biomass; the water included in the measurements has a large impact on the final weight, and how much water is shaken off before applying the method is variable. It is also challenging to perform the weighing while the biomass is still attached to the cultivation lines. Weighing the dry weight is much more accurate, but it requires harvesting and destruction of the biomass, which is not

always feasible. Any fouling of the biomass can also contribute substantially to the final weight, a problem that is experienced during the summer and fall. It is crucial to bear this in mind when comparing weights between different sites, as the biofouling will vary a lot depending on season, latitude and environmental factors such as salinity, as seen in **Paper III**.

The area of the sporophytes has been shown to give a good estimate of the standing biomass (Stagnol et al. 2016) and the increase in width, as with length, can vary considerably during the growth season and reflect the grade of exposure (Foldal 2018). The width will also be affected by the sporophyte density on the cultivation substrate (Peteiro and Freire 2013). The width is measured across the widest area of the lamina (**Paper IV**), and the area is calculated according to Broch et al. (2013):

Area = 0.75 * length * width

Growth can be estimated based on how fast the kelp grows relative to time, e.g. per day and length increment (μ, day^{-1}), as reported in **Paper IV**. This can be calculated as the relative growth rate (RGR, Equation 2)**:**

$$\mathrm{RGR}\ (day^{-1}) = \frac{\left(\frac{L_1 - L_0}{T}\right)}{L_0} \qquad (2)$$

where L_1 represents length, area or biomass at a given sampling date, L_0 the length, area or biomass at the previous sampling date, and T is the elapsed time (days) between these sampling days.

The RGR can be further compared to the specific initial cellular nitrogen-based uptake rate (U, Equation 3; **Paper I**), where U will mirror the capacity of *S. latissima* to sustain growth at given ambient concentrations of nitrate. Thus, under steady state conditions, when the processes of uptake and growth are balanced and Q_N remains constant, an estimated U value can potentially correspond to the steady state growth rate (μ, day^{-1}) that is achievable by the algae at that concentration.

$$U = \frac{\mathrm{V}}{\mathrm{Q_N}} \mathrm{x}\ 24 \qquad (3)$$

where U is the specific initial cellular N based uptake rate (day^{-1}), V is the specific uptake rate based on biomass (μg g^{-1} DW h^{-1}), and Q_N is the total tissue nitrogen (μg N g^{-1} DW).

To quantify the number of seedlings covering the twine substrate and how this correlates with growth performances at sea, a new method was tested by collecting images of the seeded ropes two days prior to deployment in **Paper IV**. The values presented were normalised to a percentage of the output range, where 0% is a clean, white substrate and 100% is a substrate completely covered by sporophytes (Figure 3.1).

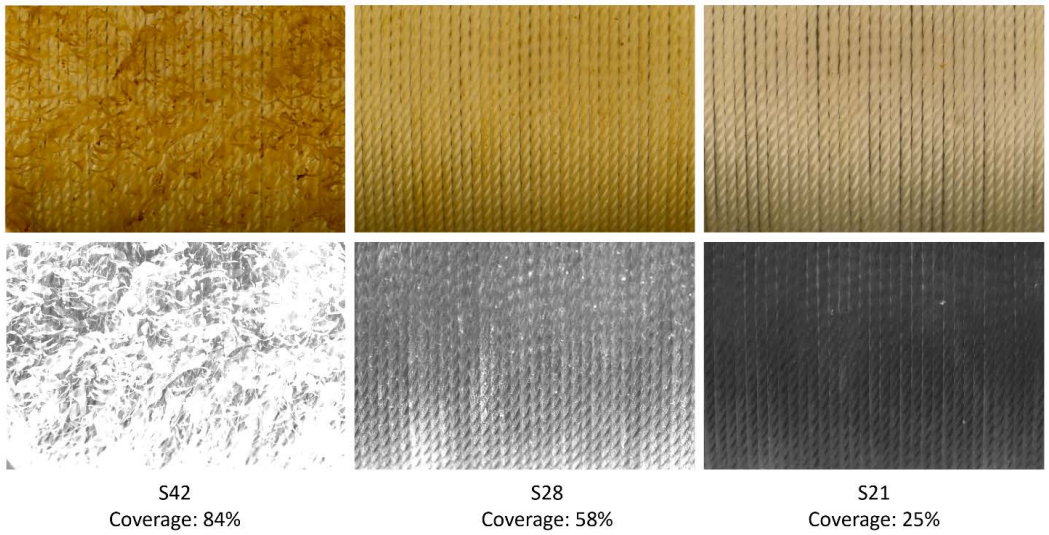

Figure 3.1: Colour images of substrate (top row) and corresponding saturation image planes (bottom row). Treatment S42 (left) had an average substrate coverage of 84%, treatment S28 (mid) with an average substrate coverage of 58% and treatment S21 (right) with an average substrate coverage of 25%.

Estimation of total tissue nitrogen and protein content

Proteins are polypeptides formed from amino acids (AA) through amide linkages (peptide-bonds). These bonds are synthesised by the carboxyl group of the AA at the end of the growing peptide chain, which reacts with the amino group of an incoming free AA, thereby releasing a water molecule (Safi et al. 2018). For extraction, seaweed proteins can be found in soluble and insoluble forms, with a ratio that is dependent on the species selected and the extraction method used. Several methods are available for the determination of total soluble proteins. These can be based on colorimetric assays, such as the Lowry method (Lowry et al. 1951), where the presence of the peptide bonds or the presence of certain AAs plays a role for

protein quantification. Another approach is the determination of the total amount of nitrogen present (Q_N) in the sample, as seen in the Kjeldahl method (Kjeldahl 1883). The total nitrogen can be converted into total protein content because each peptide bond contains a nitrogen atom, although some AAs have additional nitrogen atoms in their structure. The commonly used nitrogen-to-protein conversion factor (K_p) of 6.25 often used for seaweed tends to overestimate the protein content (Lourenço et al. 2002; Mæhre et al. 2018) because this factor presupposes that the nitrogen content of the protein is 16%, which generally applies for animal proteins (Mariotti et al. 2008). Algae commonly have high concentrations of non-protein nitrogenous substances such as pigments, nucleic acids, free amino acids and inorganic nitrogen (nitrate, nitrite and ammonia), so the nitrogen content is significantly lower than 16% (Jones 1941). Specific K_p-values for different species, seasons and locations are required to improve the protein content estimate.

The most precise method for protein determination has been suggested to be the analysis of total amino acid concentration (Angell et al. 2016; Mæhre et al. 2018). Protein content is then calculated as the difference between the total mass of AA isolated after sample hydrolysis and the mass of water bound to the AA 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) calculated are according to Mosse (1990):

$$K_p = \frac{(AA*1.1)}{N} \quad (4)$$

where AA is the sum of amino acid residues in mg g^{-1} DW (the sum of amino acids after subtracting the molecular weight of water) and N is the total nitrogen content (mg g^{-1} DW, Q_N). The total sum of the amino acids is multiplied by 1.1 to correct for the amino acids that are excluded from HPLC analysis due to destruction during acid hydrolysis (Watanabe et al. 1983; Øie and Olsen 1997). The estimated protein content for each sample is accordingly determined by multiplying total Q_N with its corresponding K_p conversion factor.

In **Paper III**, the AA for all locations, sampling points and depths were analysed and used to calculate the corresponding K_p from Equation 4 and the subsequent protein content. The overall K_p average of 3.8 ± 0.1 found in this study across locations, depths and seasons lies within the range of previously 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). In cases where the AA contents were not analysed, the mean K_p of 3.8 found in **Paper III** was used to calculate the protein content according to the Kjeldahl method in **Paper II** and season- and location-specific K_ps of 3.6 (May) and 4.3 (June) were used in **Paper IV**.

In **Paper II**, the outcome of the Kjeldahl method with a K_p of 3.8 was compared with the colorimetric Bio-Rad DC Protein Assay (BIO-RAD 2013), which is an improved version of the Lowry assay. The first method gave a slightly higher protein yield than the latter method for all treatments, except the surface water and high light treatment (S1; Figure 3.2), but the differences were not significant for any of the treatments ($p > 0.05$).

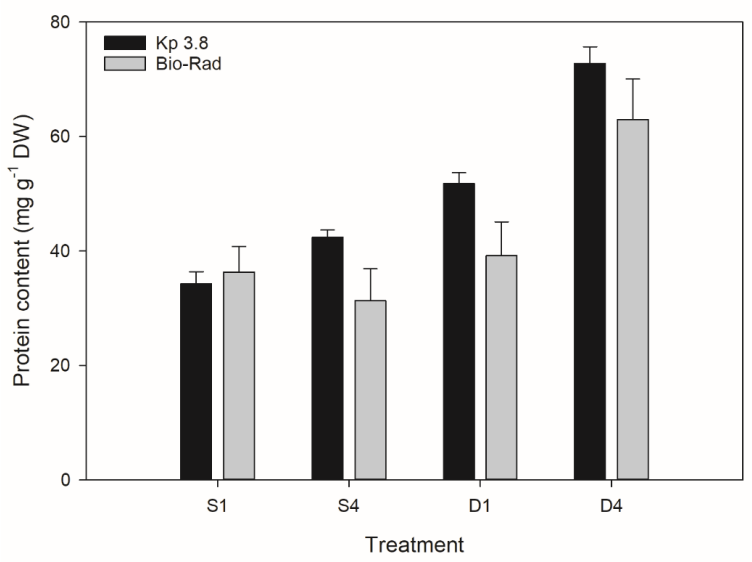

Figure 3.2: Comparison of protein content (mg g^{-1} DW) from the nitrogen-to-protein conversion factor (K_p) of 3.8 and Bio-Rad (Lowry) analysis for the four different treatments in **Paper II**. Mean ± SE, n=8.

Both the Lowry and Kjeldahl methods are indirect protein determinations; the only way to directly determine protein content is by AA analysis (Mæhre et al. 2018; Safi et al. 2018). Comparison of the outcome of different protein assays should be performed with caution as the methods are based on different principles and, as such, can result in different values.

Estimation of internal intracellular nitrate

There are different methods for analysing the internal nitrate pools (I-DIN) accumulated in the vacuoles of the macroalgae (Young et al. 2007). A common method used in **Papers I, II** and **III** was to boil fresh tissue fragments for 30 min in distilled water, causing the cells to rupture and the NO_3^- content to leak into the surrounding water (Fujita et al. 1988; Hurd et al. 1996). The concentration of I-DIN was measured in the extracts using an Auto Analyser (Flow Solution IV System, O.I Analytical) following Norwegian Standard 4745 (NSF 1975). Other common analytical methods include boiling thallus pieces in water for 10 min followed by overnight extraction at 4 °C and ethanol extraction of ground tissue overnight at room temperature (Young et al. 2007). Due to the different extraction methods, the comparison of I-DIN content with previous experiments should be performed with caution.

4. MAIN RESULTS AND DISCUSSION

Nutrient uptake kinetics

Seaweed aquaculture can potentially remove over 75,000 tons of nitrogen $year^{-1}$ by producing 2 million tons (DW) of seaweed over an area of 1250 km^2, an equivalent of 60.3 t N km^{-2} $year^{-1}$ based on an estimated Q_N content of 37.6 mg N g^{-1} DW (Xiao et al. 2017). The potential for using seaweed for bioremediation is accordingly huge. In Norway, the idea has primarily been to use for seaweed in integrated multi-trophic aquaculture (IMTA) where species from lower trophic levels are co-cultured to utilise waste nutrients from fed species. This has been suggested as a strategy to mitigate the potentially negative environmental impacts of nutrient release from fish cage aquaculture (Chopin et al. 2001; Handå et al. 2013; Fossberg et al. 2018). The seaweed cultured in this system primarily use ammonium (NH_4^+) as their main nitrogen source.

In order to understand the physiology, nutrient kinetics and successful implementation of seaweed into a sustainable bio-based economy, it is necessary to grasp fundamental concepts, such as the response to nutrient additions, nutrient uptake rates and storage capacity in relation to resource availability. The results can contribute to the optimisation of seaweed production, evaluation of the bioremediation potential, and at the same time gain a better understanding of the ecophysiology in relation to resource availability. In **Paper I**, the initial short-term uptake rates of NO_3^- were determined for variable ambient concentrations of nitrate (1.7 – 18.5 µM) for nitrogen-saturated and nitrogen-limited juvenile sporophytes of *S. latissima*. The results revealed that *S. latissima* with deficient internal nitrogen pools (Q_N and I-DIN) exhibited significantly higher ($p < 0.001$) initial uptake rates of NO_3^- than sporophytes with higher internal nitrogen pools (see Figure 2 in **Paper I**). For both nutrient-deficient ($r = 0.98$, $R^2 = 0.95$, $p < 0.001$) and -saturated ($r = 0.91$, $R^2 = 0.83$, $p < 0.001$) sporophytes, the uptake rates of nitrate showed strong positive and significant correlations to the substrate concentration within their natural range. This is an advantage which enables them to take up the nutrient more quickly when it becomes available. No clear saturation level was found for nitrate concentration exceeding double the maximum deep-water concentration potentially experienced in North-East Atlantic water, indicating

that the concentration to reach saturation must be higher than tested in the current experiment.

Large differences in nutrient uptake are found between seaweed species, with fast-growing species having larger uptake capabilities than slower growing species with differentiate tissues, like *S. latissima*. The slope of the linear portion of the curve (α) is useful when comparing the uptake abilities of two species or between experiments. A high α (steep linear slope) indicates a high affinity for nitrate at low concentrations, expressing water volume cleared for NO_3^- per time and biomass. The α for uptake rates based on DW (V; Figure 2 in **Paper I**) was significantly lower ($p < 0.001$) for the N-saturated (26 ± 0.03) then for the N-limited (0.41 ± 0.03) sporophytes. These values are comparable to findings for first year *L. groenlandica* (0.32 ± 0.04) while two and three year-old sporophytes of the same species were much lower (0.10 ± 0.01 and 0.09 ± 0.01), suggesting that the uptake rates decrease with age (Harrison et al. 1986).

Growth

The marine environment is heterogeneous, and environmental conditions (i.e. nutrient supply, temperature, light, exposure to waves and currents, etc.) can vary greatly on a small scale. Since kelps are sessile organisms, they must tolerate the physiochemical conditions in their surrounding environment to be able to grow (Kerrison et al. 2015). The environmental conditions at a particular site should be adequately known before it is used for large scale seaweed cultivation to minimise failures. Pilot investigations should be undertaken to determine the feasibility of the location, including knowledge on suitable cultivation depths and the appropriate deployment and harvesting windows.

Seaweed farms for *S. latissima* should be located where the temperature is within the range of 5–15°C for most of the year, and the temperature should not exceed 20°C for more than a few days (Kerrison et al. 2015). This temperature window makes almost the entire coast of Norway suitable for *S. latissima* cultivation, especially when the biomass is harvested before summer, as is normally the case in southern and central parts of Norway (**Paper III**). Climate change may represent a future challenge for the cultivation of a cool temperate kelp species such as *S. latissima* (Park et al. 2017). Moving cultivation operations further north or offshore and developing breeding programs for more temperature-tolerant strains, can be ways to mitigate these challenges.

Salinity should ideally be between 30–35 psu and locations should not have seasonal or sporadic reductions in salinity much below this level because abrupt, even short-term reductions in salinity can severely inhibit kelp growth, as explicitly seen in **Paper III** (Figure 4.3 b). Salinity in the surface waters in fjord locations may be significantly lower than recorded, particularly during periods of heavy snow melt or rainfall (Mortensen 2017). A solution for these locations could be to lower the seaweed to depths of 8–9 m in spring/summer, where a significant increase in sporophyte length and significantly higher protein content were detected for some locations in **Paper III**.

Growth was not measured during the experiment in **Paper I** due to the short-term design of the study, but both the N-saturated and the N-limited sporophyte groups increased their length during the 8 days of preconditioning (data not shown). The bleaching of the tissue and the low Q_N and I-DIN content for the N-limited sporophytes clearly supported high growth during the preconditioning phase when the algae consumed intracellular nitrogen from pigments and internal storage, and most likely from proteins and other N-containing components as well. The uptake rates are strongly connected to growth since the nitrogen taken up is converted into biomolecules essential for sustaining tissue accumulation and survival.

The value of the nitrogen-specific uptake rate (U, day^{-1}) during steady-state growth is equal to the carbon specific growth rate (μ) and can reflect the capacity of *S. latissima* to sustain growth at given ambient concentrations of nitrate, even though uptake and growth are not directly coupled. Thus, under steady-state conditions, when the processes of uptake and growth are in the balance and gives a constant Q_N, an estimated U value can potentially correspond to the steady-state growth rate (μ, day^{-1}) that is achievable by the algae at that nitrate concentration. The sporophytes in **Paper I** were acclimatised to nutrient-deficient and nutrient-sufficient conditions, aiming to achieve growth at steady-state conditions. Figure 4.1 (left) shows the potential maximum relative growth rates (RGR, day^{-1}), equal to U during steady-state conditions, which can be achieved for the given nitrate concentrations and nutritional states, and is reflected by Q_N. The current U values correspond well with the RGR (day^{-1}) of cultivated *S. latissima* based on an increase in length in the period from May to June in Central Norway found in **Paper IV** (Figure 4.1, right), where the RGR fluctuated between

0.02 and 0.05 day^{-1}. The ambient NO_3^- concentration was found to vary between 0.4 and 6.6 µM in that region in that period (unpublished data).

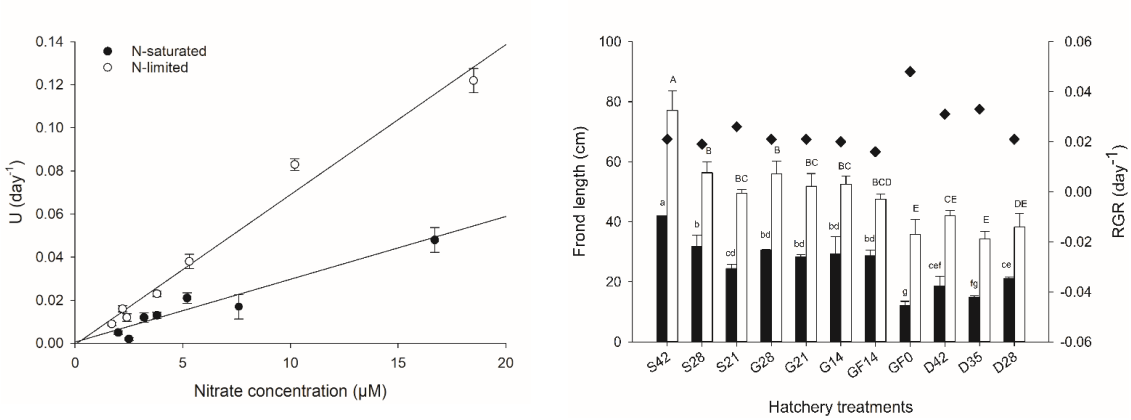

Figure 4.1: The N-specific uptake rates (U) of nitrogen saturated (●) and nitrogen limited (o) sporophytes from **Paper I** (left) corresponds well with the RGR-values (♦) in **Paper IV** (right, right axis).

Paper II was designed to have one "winter condition" with low light intensity and high nutrient availability (D4) and one "summer condition" with high light intensity and low nutrient availability (S1). In addition, two conditions seldom seen in nature were tested: high light in a combination with high nutrient (D1) and low light with low nutrient (S4).

S1: Surface water and one layer of plant cover filter ('high light, low nutrient')

S4: Surface water and four layers of plant cover filter ('low light, low nutrient')

D1: Deep water and one layer of plant cover filter ('high light, high nutrient')

D4: Deep water and four layers of plant cover filter ('low light, high nutrient')

The first of these two uncommon conditions can be experienced during summer if a storm breaks the stratified surface layer and brings nutrient rich deep-water to the surface. The latter uncommon condition can be found during summer under a surface layer that is highly affected by freshwater runoff and with particles diminishing the light intensity.

The mean growth rates for sporophytes grown in surface water were not significantly different for those grown in high light (S1, 0.27 ± 0.11 mm day^{-1}) and low light (S4, 0.22 ± 0.10 mm day^{-1}, $p > 0.05$). However, for deep water, sporophytes grown in low light showed a growth rate

that was significantly higher (D4, 0.744± 0.12 mm day^{-1}) than those grown in high light (D1, 0.48 ± 0.16 mm day^{-1}, $p < 0.01$, Figure 4.7). This may be a result of the higher nitrate (E-DIN) measured for low light treatments (D4) compared to high light treatments (D1, Figure 4.2). A possible explanation for the difference in E-DIN concentration in tanks receiving similar deep water might be that there were accidental differences in the water supply rate to the tanks or of minor variation in the biomass distribution. It may also be an effect of a differences in the community of opportunistic diatoms, which was observed growing on some of the tank walls, but not quantified. The measured increase in E-DIN concentration for all tanks during the experimental period is probably explained by the decrease in biomass, as sporophytes were removed systematically for chemical analysis. This increase was only significant ($p < 0.05$) for treatments receiving surface water (S1 and S4).

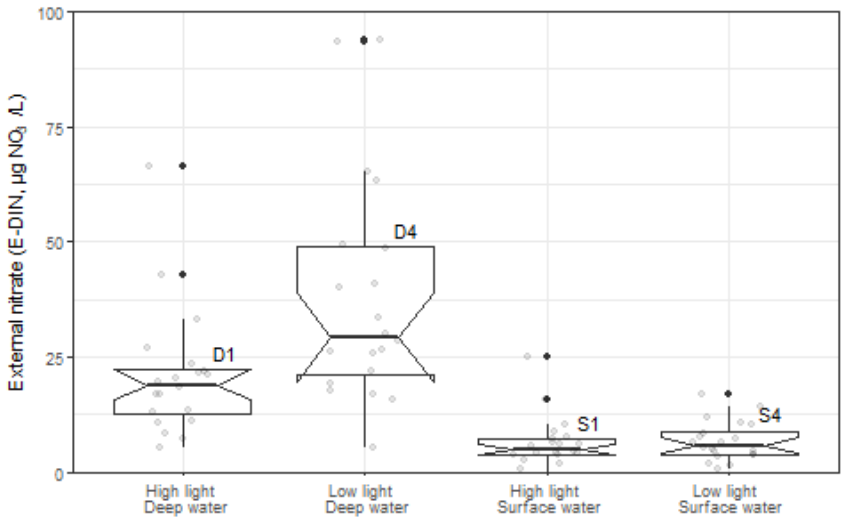

Figure 4.2: Concentration of external nitrate (E-DIN, µg NO_3^- L^{-1}) in different treatments (D1, D4, S1, S4) during the acclimation and experimental period. Boxplots show lower, middle, and upper quantiles. Notches extend to median ± 1.58 * IQR /sqrt(n) and gives a rough 95% confidence interval for the median. Whiskers extend from the hinge to the largest or smallest value within 1.5*IQR from the hinge.

The mean growth rate of the sporophytes for all treatments combined was positively related to the mean E-DIN concentration during the experimental period ($r = 0.82$, $p < 0.001$), whereas there was no clear pattern between growth rate and light level. This indicated that nitrate was the primary controlling factor for growth in both high and low light treatments. This conclusion

agrees with the general pattern of variation found in intracellular nitrogen components in the sporophytes (see section "Intracellular nitrogen components"). The significant positive relationship between growth rate and E-DIN concentration found in this study has been reported in previous experiments, both for tank cultivation and experiments at sea (Handå et al. 2013; Boderskov et al. 2016).

Another factor that may have affected the differences in growth, outlined in **Paper II**, could be the light intensity was measured to be above the tolerance limit for *S. latissima,* thus causing photoinhibition and diminishing growth for parts of the experiment. One of the 'high light, high nutrient' (D1) treatment tanks had 24 days where recorded light levels reached 250 µmol m^{-2} s^{-1} during the day (data not shown), which might have caused photoinhibition. It has been shown that *S. latissima* grown from 10–15 ˚C at a light intensity of 250 µmol m^{-2} s^{-1} showed a 50% lower growth rate compared with kelps grown at 110 µmol m^{-2} s^{-1} which was found to be the optimum for photon flux in this temperature range (Fortes and Lüning 1980).

Paper III showed that frond lengths and biomass yields were higher at a depth of 1–2 m than at 8–9 m for all nine experimental locations during most of the cultivation period (Figure 4.3). Across all locations, mean maximum frond length was 48.9 ± 9.5 cm at a cultivation depth of 1–2 m and 43.0 ± 10.6 cm at a cultivation depth of 8-9 m, while biomass reached mean maximum yield across all locations of 4.5 ± 1.8 kg m^{-1} at a cultivation depth of 1–2 m and 2.3 ± 1.0 kg m^{-1} at a cultivation depth of 8–9 m (see Figure 2 in **Paper III**). E-DIN concentrations were not measured, but we found that light availability had a significant positive impact on seaweed frond length ($p < 0.001$) and the reduced light availability at a depth of 8–9 m was probably the main reason for the poor sporophyte growth. During the summer, however, shorter frond lengths and lower biomass yields were found at a depth of 1–2 m than at 8–9 m at several locations. This was presumably an effect of high freshwater runoff in the surface layer resulting in lower E-DIN concentrations, or of high irradiance that may suppress algal growth (Fortes and Lüning 1980; Spurkland and Iken 2011). Exposure to light intensities of 500–700 µmol m^{-2} s^{-1} for 1–2 hours can lead to significant photodamage in *S. latissima* and may, in turn, cause losses of biomass and even death of tissues (Bruhn and Gerard 1996; Hanelt et al. 1997). Because high irradiances (>700 µmol m^{-2} s^{-1}) were only measured for less than 2 h at most of the cultivation sites, low salinity was a more likely cause of lower growth at a depth of 1–2 m during summer.

Figure 4.3: Differences in frond size and density of *S. latissima* A) between the 1–2 m cultivation depth (top rope) and the 8–9 m cultivation depth (bottom rope) after 69 days of cultivation at sea (18/4-2017) at location 2-60°N; B) 1–2 m depth (top rope) compared to 8–9 m depth (bottom rope) after 146 days of cultivation at sea (07/7-2017) at location 7-67°N with a freshwater-influenced surface layer.

Paper III also revealed that frond length and biomass yield of *S. latissima* 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 (Figure 4.4), respectively, probably because of seasonal differences in temperature, daylight and an earlier depletion of ambient inorganic nutrients by phytoplankton blooms in the lower than in the high latitudes (Rey et al. 2007; Ibrahim et al. 2014). This can result in the potential to supply seaweed biomass to the consumer market or processing industry for an extended period. Frond lengths and biomass yield varied greatly between the nine cultivation sites investigated (Figure 4.4). Maximum frond lengths varied between 15 and 100 cm, and maximum biomass yields between 0.2 and 14 kg m^{-2}. The highest growth was registered at central (6-63°N, in summer) and northern (9-69°N, in autumn) locations. This was probably because the central location offered the only semi-exposed conditions in the experiment, a location that is suitable for *S. latissima* growth. The northern location had a longer period of high ambient nutrient supply than the locations further south, which were also favourable for good growth. This, together with high salinity and low temperatures during the entire

cultivation period, made these two locations best suited for *S. latissima* cultivation among the sites tested when deploying seed lines in February.

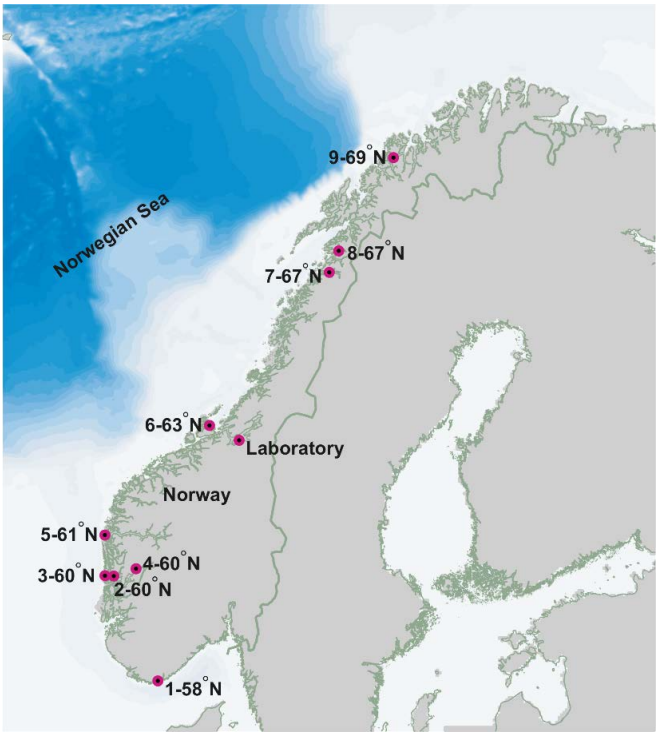

Figure 4.4: Nine stations along the Norwegian coast participating in cultivation experiments during large parts of 2017.

One important finding from **Paper IV** was that the size and age of the seedlings at deployment had a significant effect on the sporophyte frond lengths and yields after a growth period of 80 and 120 days at sea. All 11 different treatments tested were deployed randomly at the same farm on the same day, experiencing the same environmental conditions. The method of seeding lines with spores incubated in the hatchery for 42 days (S42) prior to deployment gave significantly ($p < 0.001$) better results for length, yield and area measurements than the lines seeded with gametophytes or juvenile sporophytes, and those kept in the hatchery for less than 42 days (Figure 4.1, right and Figures 7 and 8a in **Paper IV**). One possible explanation for this result could be that the long incubation period in the hatchery before deployment stimulated the development into sporophytes from a higher number of spores than the treatments with shorter incubation times. The S42

treatment was also used to produce the seedlings in **Paper III** and other previous experiments in Norway (Forbord et al. 2012; Handå et al. 2013; Fossberg et al. 2018; Sharma et al. 2018). The shortest fronds and lowest biomass yield were registered for the direct seeded treatments using a commercial glue to attach the sporophytes to the ropes on the day of deployment. This could be explained by the juvenile sporophytes grown in culture flasks having less developed hapetra compared to the sporophytes on rope in the incubators, and the fact that the first period at sea was used to develop a proper attachment to the substrate instead of frond elongation. These large differences might have levelled out by a longer cultivation period at sea by deployment in e.g. August–September instead of February.

The treatment showing the highest substrate coverage after the hatchery phase was spore treatment S42 with an average coverage of 84%. The spore treatments with 28 and 21 days of incubation had a coverage of 58% and 25%, respectively (Figure 3.1). For the gametophyte seeding, the G28 treatment with 28 days of incubation had a substrate coverage of 43% on average compared to G21 and G14 with a coverage of 25% and 9%, respectively. The GF14 treatment that was induced in white light before seeding and incubated in the hatchery for the same number of days as the G14 treatment had a coverage of 10%. The relationship between mean frond length (cm) after 90 (May) and 120 (June) days of sea cultivation and the substrate coverage (%) before deployment revealed strong positive correlations (May; $r = 0.84$, June; $r = 0.90$). Linear regression was used to fit straight lines to the data (Figure 4.5), and the linear association reached statistical significance for both May ($R^2 = 0.7$, $p = 0.018$) and June ($R^2 = 0.8$, $p = 0.006$). We demonstrate a possible first step through the measurement of the substrate coverage as a form of early-stage control of the seedling quality and expected quantity of biomass produced. The method makes processing a large number of images possible with little effort compared to manual counting/analysis of the substrate itself or images of it.

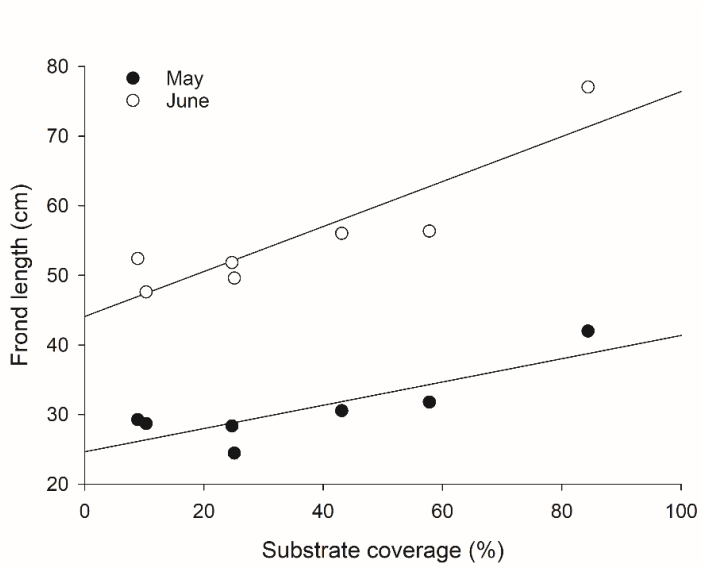

Figure 4.5: The mean frond length (cm) for May and June as a function of the substrate coverage (%) at deployment in sea in February, with regression lines showing the linear trends.

The seed lines in **Paper III and IV** were deployed at sea in February for several reasons: (i) naturally occurring sori were present, eliminating the need to establish cultures of gametophytes or to artificially induce sori (Forbord et al. 2012), which is time- and resource-demanding; (ii) light conditions were adequate to allow seedlings to grow immediately upon deployment (Handå et al. 2013); and (iii) ambient nutrient levels were high (Broch et al. 2013; Broch et al. 2019). It is likely advantageous for most farmers in southern Norway and temperate Europe to deploy their seed lines before February. Even earlier deployment in the preceding summer or fall may be advisable as the maximum growth potential of *S. latissima* recorded in commercial harvests deployed in September-October (personal communication with Norwegian seaweed farmers) was never reached south of 63°N (**Paper III**). Early deployment could also be profitable for seaweed farmers further north, as *S. latissima* deployed in August in Central Norway has previously shown significantly longer fronds the following June than sporophytes deployed in November and February (Handå et al. 2013), and deployment date has shown to be one of the most important factors affecting yields of seaweed cultivation (Camus et al. 2018). The drawback of deployments in the fall compared to the late winter could be an increased risk of losing biomass due to winter storms, the

entangling of lines and other unforeseen events over a long cultivation period in the sea. Another disadvantage of deployment in the fall is that the juvenile sporophyte stages of kelp can be very sensitive to high light levels (Augyte et al. 2019). In these cases, initial deployment at a greater depth could be beneficial.

Intracellular nitrogen components

As for growth, environmental conditions are likely to affect the chemical composition of the seaweed (Kerrison et al. 2015; Schiener et al. 2015), and, in turn, the type of products that the biomass can be used for. Seaweed for human consumption or for high quality products like cosmetics and pharmaceuticals must be harvested before the onset of fouling organisms to avoid quality deterioration. If the biomass is used as a raw material for the provision of valuable compounds like proteins, the grade of fouling is less critical.

Proteins

The average nitrogen-to-protein conversion factors (K_p) used to determine the protein content for *S. latissima* found in **Paper III** did not exhibit a seasonal or latitudinal trend, but varied across locations, depths and sampling dates with an average of 3.9 ± 0.3 for a depth of 1–2 m and 3.7 ± 0.2 for a depth of 8–9 m (see Table 4 in **Paper III**). The K_p was only significantly higher ($p > 0.05$) at 1–2 m than at 8–9 m when kelp was affected by freshwater runoff. We conclude that the overall average K_p value of 3.8 ± 0.1 found across all locations and depths is acceptable to use for *S. latissima* cultivated at different depths and at different locations in full marine salinity conditions along the Norwegian coast. This evaluated conversion factor will improve the protein content estimate and propose a new K_p for cultivated *S. latissima* in Norway.

In the tank cultivation experiment in **Paper II**, the high- and low-light conditions were mimicking different cultivation depths, and the high- and low-nitrogen availability was mimicking different seasons. The protein content (calculated from Q_N*3.8) differed significantly ($p < 0.05$) between the treatments in the following order:

D4: 72.8 ± 3.0 mg protein g^{-1} DW (low light, high nutrient)

D1: 50.4 ± 2.9 mg protein g^{-1} DW (high light, high nutrient)

S4: 41.0 ± 1.8 mg protein g^{-1} DW (low light, low nutrient)

S1: 34.7 ± 2.1 mg protein g^{-1} DW (high light, low nutrient)

The treatments given high nutrients had higher protein contents than low nutrient treatments, with the highest protein content found in a combination of high nutrient and low light (D4, Figure 4.8 lower panel). This can be understood because of the higher E-DIN concentration in the D4 tanks compared to the other treatments (Figure 4.2), already discussed in the "Growth" section. It has also been suggested that a higher protein content found in seaweed at deeper waters is the result of reduced light exposure (Ak and Yücesan 2012).

Protein content from the nine different locations in **Paper III** (Figure 4.4) ranged from 23.0 ± 0.5 to 101 ± 4.0 mg g^{-1} DW at a depth of 1–2 m and from 22.0 ± 0.1 to 110 ± 0.6 mg g^{-1} DW at 8–9 m. Differences between depths were only statistically significant ($p < 0.001$) at four of the nine locations (Figure 4.6). Depth differences were greatest at fjord locations with a surface freshwater layer (4-60°N and 7-67°N) together with two of the locations with early and high biofouling activity (2-60°N and 5-61°N). As in **Paper II**, this is likely a result of a higher E-DIN concentration at greater depths together with reduced light exposure.

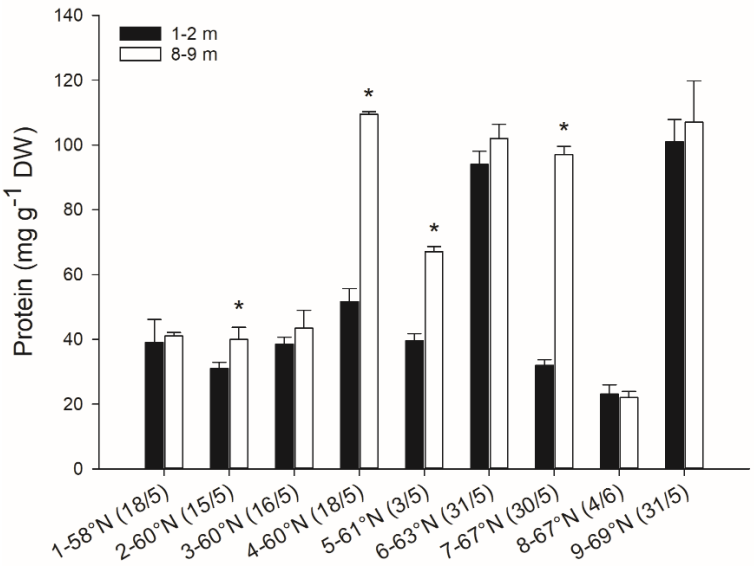

Figure 4.6: Protein content (mg protein g^{-1} DW) for nine experimental sites and two depths measured before clearly visible biofouling occurred (sampling date indicated in parentheses after each location name). Asterisk on top of the bars indicates significant differences ($p < 0.001$) between depths. Mean ± SE, n = 3.

Seasonally, the protein content measured in **Paper III** was higher by a factor of 3 in the spring than in the 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). As also seen from **Paper III,** the protein content was higher at high than at low latitudes throughout the cultivation period, following the latitudinal pattern in E-DIN fluctuation (Harnedy and FitzGerald 2011). All three freshwater-influenced sites (4-60°N, 7-67°N and 8-67°N, Figure 4.4) deviated from the general latitudinal pattern of an increase in protein content from south to north.

In **Paper IV**, a seasonally decrease in protein content was evident for all 11 treatments and the mean content was 73.3 ± 2.4 mg g^{-1} DW in May and 57.2 ± 1.5 mg g^{-1} DW in June. The content was lower than that found in May and June in the previous year in **Paper III** in the same area (6-63°N) at a depth of 1–2 m. The location used for **Paper IV** was a sheltered site compared to the semi-exposed site used in **Paper III,** which probably resulted in a higher E-DIN concentration, which again led to a higher protein content. The 11 treatments in **Paper IV**, with different ages, sizes, and densities, were deployed on the same day at the same location. The size and density of the seedlings upon deployment had a significant effect on the length and yield after 120 days at sea but did not affect the protein content substantially (no statistical analysis could be run due to n=2). Because the sea cultivation conditions were similar for all treatments, no large differences were expected to be found in the protein content between them.

Total intracellular nitrogen content

There was a strong relationship between growth and the content of tissue nitrogen (Q_N) as well as for I-DIN and E-DIN (Figure 4.7). While the growth rate showed clear saturation relationships to both E-DIN and I-DIN, the relationship between growth rate, Q_N and protein appeared linear and more scattered (see Tables 2 and 3 in **Paper II** to review the statistical coefficients obtained for the relations illustrated in Figure 4.7).

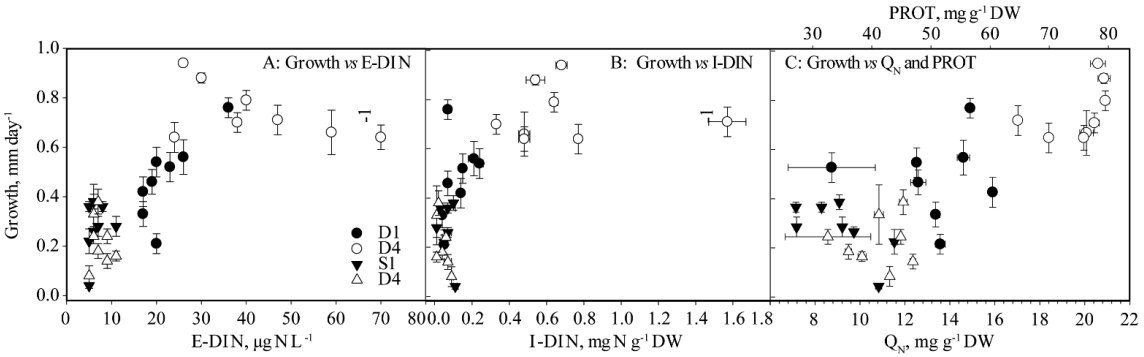

Figure 4.7: Sporophyte growth rate in length (mm day^{-1}) as a function of external and internal nitrogen components. A: Growth rate (mm day^{-1}) as a function of external nitrate concentration (E-DIN); B: Growth rate (mm day^{-1}) as a function of intracellular nitrate contents (I-DIN); C: Growth rate (mm day^{-1}) as a function of total intracellular nitrogen (Q_N, mg N g^{-1} DW) and protein (mg protein g^{-1} DW). Bars express 1 SE of the mean if exceeding symbols.

The critical level of Q_N is defined as the lowest value that will result in positive growth, referred to as Q_0 for microalgae, while Q_M defines the lowest value of Q_N at which maximum growth may occur (Droop 1968; Droop 1973). Q_N can be higher than Q_M, which will reflect the storage of I-DIN or organic N-components. The Q_N measured for juvenile sporophytes after N-starvation in **Paper I** was 19.7 mg N g^{-1} DW, while the adult sporophytes in **Paper II** were found to have a Q_N as low as 10 mg N g^{-1} DW while still sustaining positive growth. The Q_N values of 6.2 and 13.2 mg N g^{-1} DW found for sporophytes under N-limitation in **Paper III** and **IV**, respectively, underscore the suggestion that adult sporophytes can sustain growth at lower Q_N than younger plants (Table 1). The Q_N value will likely vary between species and their developmental stages.

Table 4.1: Sporophyte stage, E-DIN, Q_N and I-DIN values from the different papers under N-limited and N-saturated conditions. *min value in the season, ** max value in the season, N.A = not analysed.

	Paper I		**Paper II**		**Paper III**		**Paper IV**	
	Prior to experiment		During tank cultivation		During sea cultivation		During sea cultivation	
Nutrient status	Limited	Saturated	Limited	Saturated	Limited	Saturated	Limited	Saturated
Sporophyte stage	Juvenile	Juvenile	Adult	Adult	Adult	Young	Adult	Adult
E-DIN (µg NO_3^- L^{-1})	14	140	5.1	36.6	N.A	N.A	N.A	N.A
Q_N (mg N g^{-1} DW)	19.7	29.5	10	19.2	6.2*	39.1**	13.2*	20.5**
I-DIN (mg N g^{-1} DW)	0.002	0.46	0.05	0.69	0.001*	0.700**	N.A	N.A

In **Paper II,** the Q_N-values were found to increase with increasing ambient nitrogen concentrations (Figure 4.8, lower panel) and has been suggested to act as a reliable indicator of the physiological nutritional state of the seaweeds (**Paper I, II** and **III**).

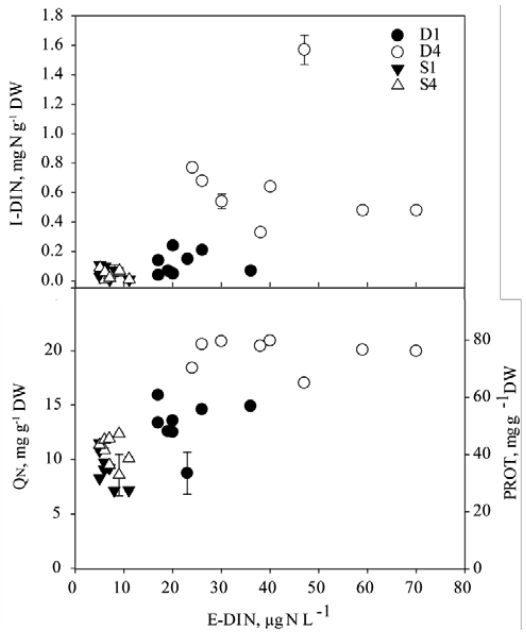

Figure 4.8: Intracellular nitrogen components as a function of mean external nitrate concentration (E-DIN, µg N L^{-1}). Upper panel: Intracellular nitrate (I-DIN, mg N g^{-1} DW), Lower panel: Total intracellular nitrogen (Q_N, mg N g^{-1} DW) and protein (PROT, mg protein g^{-1} DW). Bars express 1SE of the mean if exceeding symbols.

The feasibility of Q_N as an indicator of nutritional state for *S. latissima* was apparent in **Paper III** over longer cultivation periods along the Norwegian coast, where Q_N was highest early in the season and at greater depths and high latitudes where nitrate was not yet depleted by phytoplankton blooms (Figure 4.9). The decline in Q_N content in *S. latissima* during the spring and summer is understood as a consequence of metabolic demands exceeding nutrient uptake during growth. It has been suggested that seaweeds in general are more sensitive to environmental changes when their intracellular nitrogen reserves are exhausted (Gerard 1997; Gao et al. 2013), like biofouling settlement. This agreed with the situation at most locations in **Paper III** where the algae was heavily overgrown by epibionts during the late spring and summer at times when the Q_N content was low and the fouling organisms contributed to raise the Q_N -content to as much as 53.4 mg N g^{-1} DW before terminating the experiment (Figure 4.9).

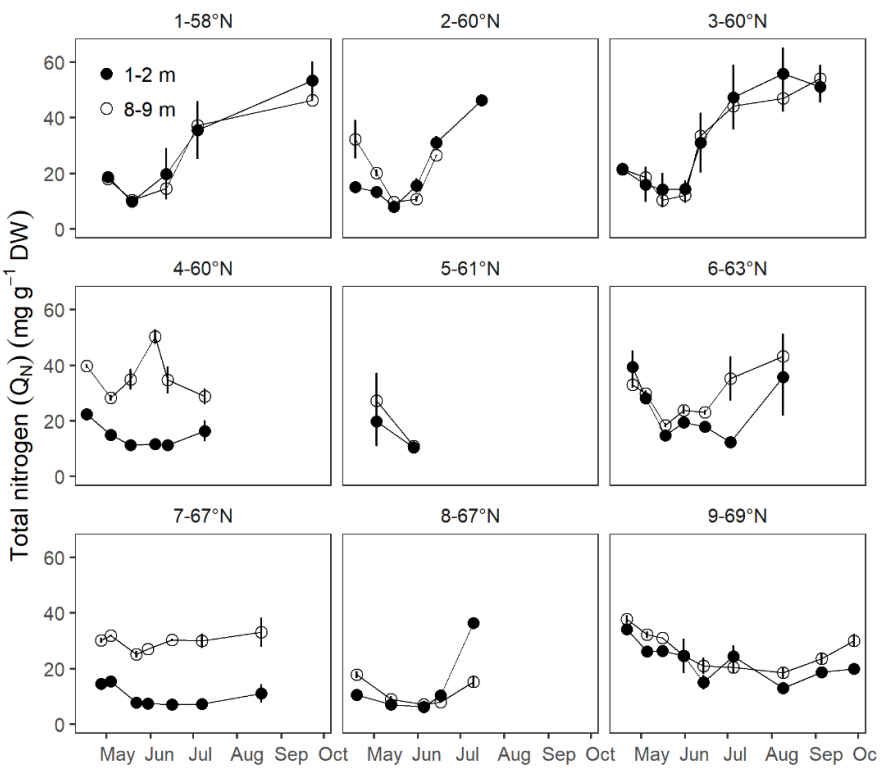

Figure 4.9: Latitudinal and seasonal pattern in total nitrogen content (Q_N, mg N g^{-1} DW) of *S. latissima* from nine experimental sites at depths of 1–2 m and 8–9 m depth across a sampling period. Mean ± SE, n = 3.

Intracellular nitrate content

It has previously been stated that seaweeds have the ability to store nitrate for use for growth in periods where the ambient nitrogen is low, e.g. during the late spring and summer, and that this storage can constitute up to 5–10% of the total tissue N (Harrison and Hurd 2001). Some species can store NO_3^- in their vacuoles when the NO_3^- concentration of the seawater is high and use it for growth when the NO_3^- concentration in the seawater is limited (Chapman et al. 1978), whereas others may have very limited storage capabilities. Correspondingly, high internal tissue concentrations indicate nutrient storage, while low internal tissue concentrations may indicate nutrient deficiency (**Paper I, II, III**). The differentiation into meristematic, photosynthetic and supportive tissues in *S. latissima* may, however, reduce the need for internal nutrient storage to sustain growth (Duarte 1995; Pedersen and Borum 1996).

Cultivated *S. latissima* did not exhibit high storage capability of internal inorganic nitrate (I-DIN) in any of the experiments in **Papers I, II** or **III**, even though the sporophytes were exposed to high ambient NO_3^- concentrations over many weeks in **Papers II** and **III** (Table 4.1). In **Paper I**, N-starved sporophytes had a maximum I-DIN concentration of 0.12 ± 0.01 mg NO_3^- g^{-1} DW (0.02 % of Q_N), and the N-saturated sporophytes had a maximum I-DIN of 0.60 ± 0.08 mg NO_3^- g^{-1} DW (1.6 % of Q_N). Linear regression did not reveal a significant relationship between I-DIN content and E-DIN concentration for either N-saturated ($r = 0.196$, $R^2 = 0.038$, $p = 0.359$) or N-limited sporophytes ($r = 0.034$, $R^2 = 0.001$, $p = 0.880$, Figure 4.10). This suggests that the exposure time kinetics of I-DIN accumulation is slower and that I-DIN because of the low contents is not an important nitrogen storage that can support algal growth for a long time.

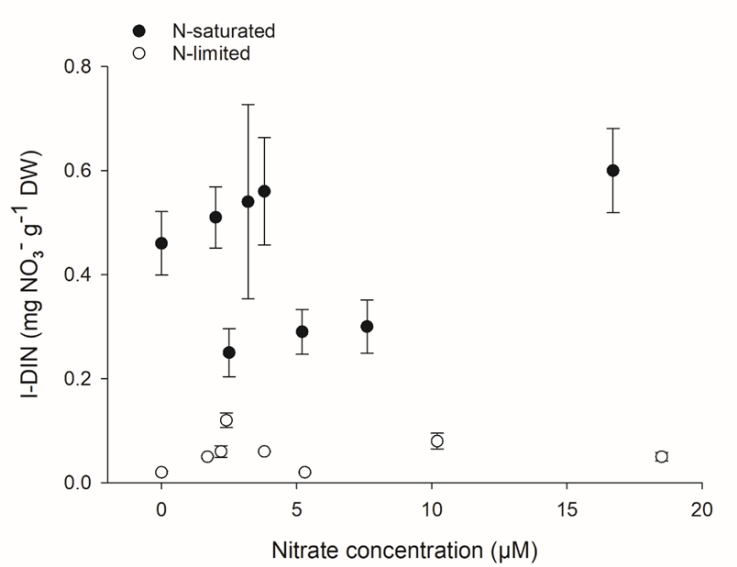

Figure 4.10: Mean intracellular nitrate concentration (I-DIN, mg g^{-1} DW) for the different initial incubation concentrations (µM) after 300 min incubation of N-saturated and N-limited sporophytes. Mean ± SE, n = 3.

In **Paper II**, the highest I-DIN content of 0.69 ± 0.14 mg NO_3^- g^{-1} DW was measured in adult, first-year sporophytes that had been supplied with nutrient-rich deep-water and acclimated for 4 weeks in tanks at low light intensities (Table 4.1). The lowest content was measured in the low nutrient treatments for both high and low light intensities (0.05-0.06 ± 0.01 mg NO_3^- g^{-1} DW), and for the D1 'high nutrient, high light' treatment (0.12 ± 0.03 mg NO_3^- g^{-1} DW). The surprising observation was the difference between high and low light exposure, which not only showed a significant difference in growth, but also significant differences in I-DIN content when both treatments were exposed to high ambient NO_3^- concentrations (Figure 4.8, upper panel). As for growth and Q_N, this was most likely caused by the higher E-DIN concentration measured in the D4 tanks compared to the D1 tanks (Figure 4.2).

Ambient nutrient concentrations were not measured in **Paper III**, but based on existing knowledge about the natural fluctuations in nutrients over the season and depths along the Norwegian coast, the latitudinal- and depth-dependent patterns found for I-DIN were found to reflect the nutritional conditions (see Figure 4b in **Paper III**). The I-DIN contents followed a seasonal pattern, with the highest values at the beginning of the sampling period when the ambient nitrate concentration was surplus before stratification and the onset of the

phytoplankton spring bloom (Rey et al. 2007; Ibrahim et al. 2014; Broch et al. 2019). These conditions occurred later at high compared to low latitudes, and at greater depths at high than at low latitudes. I-DIN varied from 0.001 ± 0.140 to 0.700 ± 0.200 mg NO_3^- g^{-1} DW across all sites, depths and seasons and was not as affected by biofouling settlement as found for the Q_N content.

Even though relatively low I-DIN contents were found for *S. latissima* in our studies, other kelp species are found to contain larger reserves. About 21 mg NO_3^- g^{-1} DW was occasionally found in *L. longricruris*, which is 28,000 times the ambient maximum winter concentration in the water column (Chapman and Craigie 1977). Different analytical methods could also contribute to the large differences between experiments (Young et al. 2007), and care should be taken when comparing results without knowing which method was used.

As for Q_N, I-DIN can be used to express the nutritional nitrogen state of the alga and is an easily measurable proxy for assessing nitrogen nutritional state of both wild and farmed macroalgae.

Quality criteria

The desired quality of cultivated kelp is usually dependent on the end product. The Pegasus-European Guidelines for a Sustainable Aquaculture of Seaweeds (Barbier et al. 2019) lists traits usually related to biomass production and quality:

1. High yields
2. Specific shape/size, texture, colour, flavour, etc.
3. High growth rates/fast transition between lifecycle stages
4. High amount of specific target compound (protein, pigments, lipids, iodine, etc.)
5. Resistance to infection by pathogens or epiphytes
6. Resilience to changing abiotic factors
7. Low accumulation of contaminants (e.g. cadmium, inorganic arsenic, etc.)
8. High nutrient uptake rates
9. Low emission of halocarbon

The present thesis has targeted several of these quality criteria, including:

Criteria 1 (**Paper II, III, IV)**

Criteria 3 (**Paper IV)**

Criteria 4 **(Paper I, II, III, IV)**

Criteria 5 **(Paper III)**

Criteria 6 **(Paper II, III)**

Criteria 8 **(Paper I)**

Many of the criteria are closely related to each other and have already been discussed in the above sections, except for criteria 5 concerning epiphytes. This had a major focus in **Paper III**, comparing the effects of cultivation depth and season on the biofouling (total cover and species composition) of cultivated *S. latissima* at nine locations along a latitudinal gradient from 58°N to 69°N. The interaction between depth and sampling date was significant ($p < 0.05$) for six of the locations. Percentage biofouling cover on kelp fronds increased with season at all sites and depths, from ~0 % in April–June to a maximum of 3.8–81.4% in June–September (see Figure 7 in **Paper III**). 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; Figure 4.4), and the southernmost location (1-58°N). The impact of freshwater had a negative effect on the sporophyte growth but reduced the settlement rate, resulting in a change in the fouling community (see Figure 8 in **Paper III**).

To date, there are no established standards in Norway for an acceptable amount of biofouling on seaweed cultivated 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. For other applications, e.g. animal feed or soil fertiliser, a prolonged growth season even with increased biofouling may be beneficial because it could initially enhance the harvesting yield of seaweed biomass and nitrogen/protein content along with associated epibiont biomass. As seen in Figure 4.11, different organisms can settle and grow on the seaweed fronds, with the bryozoan *Membranipora membranacea* (Figure 4.11, B) as the dominating species. Befouling has been

the main focus for another PhD study in the current research project MACROSEA (Matsson et al. 2019).

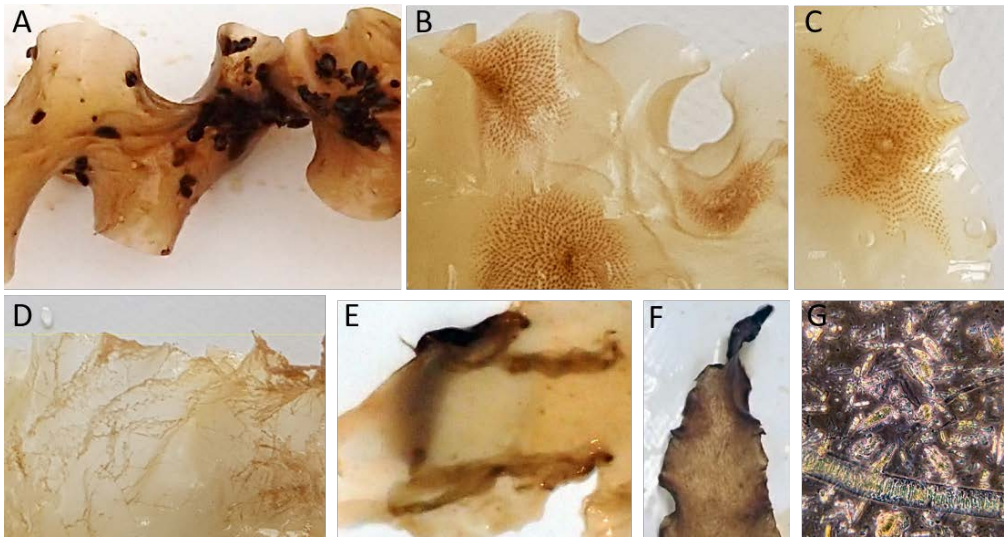

Figure 4.11: Images of the epibionts found and registered in **Paper III**, A: Bivalvia, B: *Membranipora membranacea*, C: *Electra pilosa*, D: Hydroids, E: Filamentous algae, F: Diatoms, G: Diatoms at 40x magnification.

Strategies to increase biomass yield and protein content

One major bottleneck for commercialisation and the year-round supply of high-quality seaweed biomass is the seasonal- and depth-induced variation in biomass yields and biochemical content, as well as the phenology and magnitude of biofouling cover. This information is essential for farmers when planning the deployment and harvesting time and to identify end-products and can be further utilised to allocate resources effectively.

To extract high value compounds from cultivated seaweed biomass for super food and pharmaceuticals (antioxidants like fucoxanthin and polyphenols), fish feed (proteins) or biofuels (carbohydrates), the cultivated biomass can be manipulated with variations in nutrient and light availability to increase compounds, aiming to increase the yields so that there is a greater biomass to extract from. By the time of deployment at sea in Norway (September–February), the light intensity and temperature are low and the nutrient availability in the surface waters is in excess (Broch et al. 2019), giving suitable conditions for

growth. However, during the spring blooms from March–April, the nutrients are depleted in the upper layers of the water column (Rey et al. 2007; Ibrahim et al. 2014), while the light increases and the seaweed growth decreases. Recent experiments from China have shown that nutrient concentration could be enhanced by different actions. By artificial upwelling of nutrient rich deep-water increasing the nutrient concentration in the surface waters, the average yield of kelp was increased by 55 g per plant (Fan et al. 2019). The results from tank cultivation in **Paper II** showed an increased growth rate and protein content by offering high nitrogen concentrations to the sporophytes, results which are also supported by the findings from field cultivation in **Paper III** during the first period at sea. A decrease in both length and protein content was observed towards late spring and summer, and a solution to overcome this could be to lower the seaweed cultures to depths of 8–9 m towards summer, where a significant increase in sporophyte length and significantly higher protein content was registered for some locations. **Paper I** showed an increased growth based on the N-specific uptake rates (U) for both N-saturated and N-limited sporophytes at high nitrate concentrations compared to lower ones, while **Paper IV** showed that the size, age and developmental stage of the seedlings at deployment had a significant effect on biomass production. Thus, by deploying seed lines seeded with spores incubated in the hatchery for 42 days, a higher biomass will be given at sea than for any treatments seeded with gametophytes or juvenile sporophytes, or incubated for a shorter time period in the hatchery prior to deployment. Even though there is a large cost in establishing a seaweed hatchery, the steps of laboratory conditioning account for only 5% of the total energy consumption in seaweed production, because these processes only involve the use of electricity for illumination and air and water pumping for approximately two months, while the steps of the grow-out phase (including deployment and harvesting) account for 95% of the total energy consumption due to diesel and petrol consumption (Alvarado-Morales et al. 2013).

To produce high quantities of seaweed, it is crucial to move from land-based experiments to large-scale open sea cultivation that allows for larger biomass production, but where the only practical adjustable factor is the cultivation depth and, as a consequence of that, the light and nutrient availability. On the other hand, advantages of land-based cultivation include better control of the cultivation system, easy access to the produced biomass regardless of the weather conditions, and increased potential to be used for bioremediation

for land-based fed aquaculture (IMTA). In such systems, as tested in **Paper II**, it is easy to manipulate key resources such as light intensity and nutrient loading with combinations that do not normally occur in sea and thus, have a higher control over biomass yield, chemical composition and epiphytes (Harrison and Hurd 2001; Azevedo et al. 2016). If we consider the emerging market of functional products from seaweed for human consumption, which requires traceability and security of supply, on-land cultivation can be essential for this production, allowing the highest levels of control (Hafting et al. 2012). A combination of these two cultivation techniques producing biomass for different end-products could be a sustainable solution.

5. CONCLUSIONS AND FUTURE PERSPECTIVES

Conclusions

The present study has increased our knowledge on cultivation of the kelp *Saccharina latissima* and emphasises the importance of studying growth and dynamics of biochemical content under different environmental conditions, including different latitudes, seasons and depths, but also various seedling sizes and different nutritional history. This was performed under various ecologically realistic scenarios, as well as under conditions that are not commonly found along the Norwegian coast, but which are biologically interesting to include.

It appears that *S. latissima* requires high ambient NO_3^- concentrations for maintaining rapid growth and that the alga is not able to compete for available nutrients with the more efficient phytoplankton during the late spring and summer. This means that *S. latissima* has to take up most of the NO_3^- needed early in the season when ambient concentrations are high, and that the period after the spring bloom represents a negative shift in nutrient uptake that involves sporophyte growth mainly based on internal inorganic and organic nitrogen components. We believe that this information about the coupling between extracellular nitrate concentration, initial NO_3^- uptake kinetics, intracellular NO_3^- content and specific growth rate is new fundamental knowledge, which helps us to better understand the ecological niche and the nutritional requirements of *S. latissima*. Such quantitative knowledge is paramount for understanding both the nutrient and environmental conditions required for the efficient cultivation of *S. latissima* as it will have an essential importance for future large-scale production.

For a natural range of light supply, we found that the external nitrate concentration was the primary controlling factor for growth and for the storage of internal nitrogen compounds (protein, total tissue nitrogen and internal nitrate) in our experiments with cultivated *S. latissima*. For light intensities below the range in our experiments, light or energy supply may become the primary limiting factor for growth, while for higher light supply, light inhibition of growth may become severe.

The current study has improved our understanding of careful site selection for improving growth and protein content during a cultivation season. 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 best deployment and harvesting windows that would assist farmers to maximise production and minimise loss. Our study is the first to compare cultivation at several farm locations over a large latitudinal gradient, documenting the fact that kelp farming shows great potential along all latitudes from 58 to 69°N, except in areas with high local environmental variations, such as high freshwater runoff. Additional measurements of nutrients, salinity, turbidity and degree of exposure (currents and waves) could be beneficial because they will also affect seaweed growth and production (Peteiro and Freire 2013; Matsson et al. 2019). A long-term goal is to use this newly gained knowledge to contribute to the future development and establishment of a Norwegian bioeconomy based on cultivated seaweed.

The main conclusions from the enclosed papers are:

Paper I:

- For both nutrient-deficient and saturated sporophytes, uptake rates of nitrate were linearly related to the substrate concentration within their natural range, an advantage which enables them to take up this nutrient more quickly when it becomes available.
- An increase in biomass in periods of low nitrogen availability, typically in spring and summer along the Norwegian coast, must mainly be based on intracellular nitrogen components like protein and other nitrogen metabolites.
- The pool of intracellular nitrate (I-DIN) probably needs a long time and high ambient nitrate concentration to reach high levels and is not an important storage for sustaining growth during periods of low nitrogen availability.
- Knowledge on the ability of *S. latissima* to take up available nutrients and their intracellular nitrogen content are important aspects when scaling up seaweed farms. It enables the farmers to estimate the carrying capacity of production at a cultivation sites and to predict the potential biomass production based on the nutritional history of the seaweed and the ambient nutrient concentrations.

Paper II:

- The mean growth rate of the sporophytes for all treatments combined was positively related to the external nitrate concentration during the experimental period.
- The experiment confirmed the positive relationship between total intracellular nitrogen components (Q_N, protein and I-DIN) and external nitrate concentration.
- The growth rate showed clear saturation relationships to both external nitrate concentrations and I-DIN, whereas the relationship between growth rate, Q_N, and protein appeared linear and more scattered.
- No positive correlation was found between growth rate and different light levels, suggesting that nitrate was the primary controlling factor for growth at both high- and low-light treatments. The pattern found for the contents of intracellular nitrogen components agreed with this conclusion.

Paper III:

- 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.
- The total intracellular nitrogen components (Q_N, protein and I-DIN) showed a decreasing seasonal trend before the onset of biofouling and the seasonal decrease was delayed at higher latitude, 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 at deeper, whereas protein, ash, Q_N and I-DIN were generally higher at greater depths.

Paper IV:

- Different seeding methods and hatchery periods have a high impact on the growth performance of *S. latissima* at sea.
- Twine seeded with spores pre-cultivated in the hatchery for 42 days gave significantly better growth measurements than any of the other treatments tested in this experiment and a clear coherence was found between days in the hatchery before deployment and growth performance at sea for the spore seeding method.
- All measured growth variables were poor for the direct seeded treatments during the relatively short cultivation period of 120 days at sea, but a longer cultivation period might have levelled off the differences between the seeding methods. The reduced costs resulting from skipping the hatchery phase and entwining process may make up for this from a business perspective.
- Image analysis of substrates before deployment seemed to be a useful tool when assessing frond lengths at sea, but the method needs to be further developed to include predictions about harvesting yields.

Future perspectives

The current thesis has contributed with new knowledge on the physiological, environmental, and technological aspects that will be of use in a future development and up-scaling of the seaweed industry. However, there are still some basic knowledge gaps that need to be further investigated.

The most important bottleneck to solve regarding the up-scaling of seaweed production in Norway and other Western countries is the market segment which is not yet ready to handle the biomass delivered from upcoming large-scale cultivation. Without the market, the up-scaling of production and development of automated equipment in association with this is challenging. The feedback from the e.g. fish feed companies has been that the delivery of biomass with a predictable chemical content is essential if they are going to implement the use of new sources of biomass in their production. At the moment, this is difficult due to the strong correlation between biochemical content and the variable environmental conditions during a cultivation period at sea. Site selection is crucial to this, and the optimal site is

dependent on the desired end-product. This has been one of the major aims of this thesis, but it still needs further investigation with other deployment times and an even larger range of sites along the coast. Cultivation trials further north of 69°N are especially interesting to investigate the impact of high latitude on chemical content, growth, and biofouling.

Mathematical modelling can be an important tool for evaluating the suitability of a location before starting cultivation trials and can be both time- and cost-efficient for farmers. The model system can consider the effects of sporophyte density, size distribution and mechanical interactions between individuals, and the reduction of water flow through the seaweed farm. The further development of seaweed growth models (Broch and Slagstad 2012) to include the content of valuable compounds (e.g. proteins, polyphenols, pigments, carbohydrates) in kelps and how these depend on and arise as interactions between external conditions, metabolism, and growth should be considered. Furthermore, the development of more detailed models for the content and accumulation of potentially unwanted components (e.g. iodine, heavy metals, and environmental toxins) are needed, as well as linking model parameters to genetic studies.

Developing cultivars for improved traits like high biomass production, increased content of valuable compounds and low affinity for biofouling could be of great importance for the future industry and are of current interest. Obtaining sporophytes that do not hybridise with natural populations is also essential for the survival of a future industry, an approach the agriculture industry has used for several commercial species (e.g. banana, melon, grapes). It is crucial to maintain the biodiversity of marine ecosystems; because these ecosystems are sensitive to biological invasions, care must be taken during the up-scaling of kelp cultivation to prevent damage. The use of local genetic material, as well as technologies to prevent hybridisation between cultivated and wild populations are important elements in the responsible and sustainable utilisation of seaweed. The use of local strains is highly recommended in several Scandinavian countries at the present time and breeding is not used as a tool to obtain the wanted traits of *S. latissima* (Fredriksen and Sjøtun 2015; Hasselström et al. 2018; Barbier et al. 2019; Goecke et al. 2020), but several ongoing research projects are focusing on how to use breeding for value creation.

Industrial cost-effective cultivation requires novel technology with a high degree of mechanisation and automation along the entire production chain comprising hatchery,

deployment, cultivation, monitoring and harvesting. To facilitate transitions from "low-tech" (labour intensive) to "high-tech" (cost-effective) cultivation, technology-transfer from the successful marine and maritime industries in Norway to adapt efficient methods for seeding, deployment and harvest should be facilitated. Further, model tools for the simulation and visualisation of dynamic systems to perform reliable structure analysis of efficient sea farm concepts for different species and environmental conditions should be developed.

Finally, it is a worldwide challenge to reduce CO_2 emissions, a cause of global warming. Reducing man-made CO_2 emissions, including those produced through the burning of fossil fuels, is a key element in mitigating greenhouse gas emissions and the effects of climate change. Seaweed cultivation has gained attention recently as a climate positive solution for the removal of CO_2. More insight and knowledge about this opportunity is necessary; for instance, how to store the biomass safely e.g. in deep valleys on the ocean floor. The environmental impacts, technological challenges, and societal economic aspects of this is of course important issues that need to be investigated.

In order to further facilitate a successful up-scaling of macroalgae cultivation and create a new bio-marine industry in Norway, a national research infrastructure should be established. This should serve as a centre for innovative industry and research groups targeting the new, blue bioeconomy. The industrialisation of a nearly carbon-neutral, non-fed cultivation of macroalgae will contribute to an environmentally friendly development of the Norwegian aquaculture sector and to the sustainable exploitation of the ocean.

6. REFERENCES

Abreu MH, Pereira R, Buschmann A, Sousa-Pinto I, Yarish C (2011) Nitrogen uptake responses of *Gracilaria vermiculophylla* (Ohmi) Papenfuss under combined and single addition of nitrate and ammonium. Journal of Experimental Marine Biology and Ecology 407 (2):190-199

Ahn O, Petrell RJ, Harrison PJ (1998) Ammonium and nitrate uptake by *Laminaria saccharina* and *Nereocystis luetkeana* originating from a salmon sea cage farm. Journal of Applied Phycology 10 (4):333-340

Ak İ, Yücesan M (2012) Effect of light intensity on the pigment composition of *Gracilaria verrucosa* (Rhodophyta). Fresenius Environmental Bulletin 21 (8):2126-2131

Alvarado-Morales M, Boldrin A, Karakashev DB, Holdt SL, Angelidaki I, Astrup T (2013) Life cycle assessment of biofuel production from brown seaweed in Nordic conditions. Bioresource technology 129:92-99

Andersen G (2013) Growth, survival and reproduction in the kelp *Saccharina latissima*–Seasonal patterns and the impact of epibionts. PhD thesis, University of Oslo, Oslo

Angell AR, Mata L, de Nys R, Paul NA (2016) The protein content of seaweeds: a universal nitrogen-to-protein conversion factor of five. Journal of Applied Phycology 28 (1):511-524

Arbona J, Molla M (2006) Cultivation of brown seaweed *Alaria esculenta*. Aquaculture explained, vol 21. Bord lascaigh Mhara, Dublin

Augyte S, Yarish C, Neefus CD (2019) Thermal and light impacts on the early growth stages of the kelp *Saccharina angustissima* (Laminariales, Phaeophyceae). Algae 34 (2):153-162

Azevedo IC, Duarte PM, Marinho GS, Neumann F, Sousa-Pinto I (2019) Growth of *Saccharina latissima* (Laminariales, Phaeophyceae) cultivated offshore under exposed conditions. Phycologia 58 (5):504-515

Azevedo IC, Marinho GS, Silva DM, Sousa-Pinto I (2016) Pilot scale land-based cultivation of *Saccharina latissima* Linnaeus at southern European climate conditions: Growth and nutrient uptake at high temperatures. Aquaculture 459:166-172

Bak UG, Nielsen CW, Marinho GS, Gregersen Ó, Jónsdóttir R, Holdt SL (2019) The seasonal variation in nitrogen, amino acid, protein and nitrogen-to-protein conversion factors of commercially cultivated Faroese *Saccharina latissima*. Algal Research 42:101576

Barbier M, Charrier B, Araujo R, Holdt SL, Jacquemin B, Rebours C (2019) PEGASUS - PHYCOMORPH European Guidelines for a Sustainable Aquaculture of Seaweeds. COST Action FA1406. Roscoff, France

Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R (2008) The genus *Laminaria sensu lato*: recent insights and developments. European Journal of Phycology 43 (1):1-86

Biancarosa I, Espe M, Bruckner C, Heesch S, Liland N, Waagbø R, Torstensen B, Lock E (2017) Amino acid composition, protein content, and nitrogen-to-protein conversion factors of 21 seaweed species from Norwegian waters. Journal of Applied Phycology 29 (2):1001-1009

BIO-RAD (2013) DC Protein Assay US. Accessed on: April. 30, 2020. [Online]. Available: http://www.bio-rad.com/webroot/web/pdf/lsr/literature/LIT33.pdf

Bird KT, Habig C, DeBusk T (1982) Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). Journal of Phycology 18 (3):344-348

Bleakley S, Hayes M (2017) Algal Proteins: Extraction, Application, and Challenges Concerning Production. Foods 6 (5):33

Boderskov T, Schmedes P, Bruhn A, Rasmussen M, Nielsen M, Pedersen M (2016) 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. Journal of Applied Phycology 28 (2):1153-1165

Bolton JJ, Germann I, Lüning K (1983) Hybridization between Atlantic and Pacific representatives of the Simplices section of *Laminaria* (Phaeophyta). Phycologia 22 (2):133-140

Broch OJ, Alver MO, Bekkby T, Gundersen H, Forbord S, Handå A, Skjermo J, Hancke K (2019) The kelp cultivation potential in coastal and offshore regions of Norway. Frontiers in Marine Science 5:529

Broch OJ, Ellingsen IH, Forbord S, Wang X, Volent Z, Alver MO, Handa A, Andresen K, Slagstad D, Reitan KI, Olsen Y, Skjermo J (2013) Modelling the cultivation and bioremediation potential of the kelp *Saccharina latissima* in close proximity to an exposed salmon farm in Norway. Aquaculture Environment Interactions 4 (2):187-206

Broch OJ, Slagstad D (2012) Modelling seasonal growth and composition of the kelp *Saccharina latissima*. Journal of Applied Phycology 24 (4):759-776

Bruhn A, Tørring DB, Thomsen M, Canal-Vergés P, Nielsen MM, Rasmussen MB, Eybye KL, Larsen MM, Balsby TJS, Petersen JK (2016) Impact of environmental conditions on biomass yield, quality, and bio-mitigation capacity of *Saccharina latissima*. Aquaculture Environment Interactions 8:619-636

Bruhn J, Gerard V (1996) Photoinhibition and recovery of the kelp *Laminaria saccharina* at optimal and superoptimal temperatures. Marine Biology 125 (4):639-648

Burton T, Lyons H, Lerat Y, Stanley M, Rasmussen MB (2009) A review of the potential of marine algae as a source of biofuel in Ireland. Dublin, Ireland

Buschmann AH, Camus C (2019) An introduction to farming and biomass utilisation of marine macroalgae. Phycologia 58 (5):443-445

Camus C, Infante J, Buschmann AH (2018) Overview of 3 year precommercial seafarming of *Macrocystis pyrifera* along the Chilean coast. Reviews in Aquaculture 10 (3):543-559

Chapman A, Craigie J (1977) Seasonal growth in *Laminaria longicruris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. Marine Biology 40 (3):197-205

Chapman A, Markham J, Lüning K (1978) Effects of nitrate concentration on the growth and physiology of *Laminaria saccharina* (Phaeophyta) in culture. Journal of Phycology 14 (2):195-198

Chopin T, Buschmann AH, Halling C, Troell M, Kautsky N, Neori A, Kraemer GP, Zertuche-González JA, Yarish C, Neefus C (2001) Integrating seaweeds into marine aquaculture systems: a key toward sustainability. Journal of Phycology 37 (6):975-986

Directorate of Fisheries (2020) Akvakulturstatistikk (tidsserier)-Alger (*in Norwegian*). http://www.fiskeridir.no/Akvakultur/Statistikk-akvakultur/Akvakulturstatistikk-tidsserier/Alger. Accessed 30.01 2020

Droop M (1973) Some thoughts on nutrient limitation in algae. Journal of Phycology 9 (3):264-272

Droop MR (1968) Vitamin B 12 and marine ecology. IV. The kinetics of uptake, growth and inhibition in *Monochrysis lutheri*. Journal of the Marine Biological Association of the United Kingdom 48 (3):689-733

Druehl LD (1967) Distribution of two species of *Laminaria* as related to some environmental factors. Journal of Phycology 3 (2):103-108

Druehl LD (1970) The pattern of Laminariales distribution in the northeast Pacific. Phycologia 9 (3-4):237-247

Druehl LD, Collins JD, Lane CE, Saunders GW (2005) An evaluation of methods used to assess intergeneric hybridization in kelp using Pacific Laminariales (Phaeophyceae). Journal of Phycology 41 (2):250-262

Druehl LD, Kaneko T (1973) On *Laminaria saccharina* from Hokkaido. The botanical magazine = Shokubutsu-gaku-zasshi 86 (4):323-327

Duarte CM (1995) Submerged aquatic vegetation in relation to different nutrient regimes. Ophelia 41 (1):87-112

Duinker A, Roiha IS, Amlund H, Dahl L, Lock E-J, Kögel T, Måge A, Lunestad BT (2016) Potential risks posed by macroalgae for application as feed and food-a Norwegian perspective. National Institute of Nutrition Seafood Research (NIFES), Bergen, Norway

Edwards M, Watson L (2011) Cultivating *Laminaria digitata*. Aquaculture explained, vol No. 26.

Edwards MD, Dring MJ (2011) Open-sea cultivation trial of the red alga, *Palmaria palmata* from seeded tetraspores in Strangford Lough, Northern Ireland. Aquaculture 317 (1):203-209
Egan B, Vlasto A, Yarish C (1989) Seasonal acclimation to temperature and light in *Laminaria longicruris* de la Pyl. (Phaeophyta). Journal of Experimental Marine Biology and Ecology 129 (1):1-16
Fan W, Zhao R, Yao Z, Xiao C, Pan Y, Chen Y, Jiao N, Zhang Y (2019) Nutrient Removal from Chinese Coastal Waters by Large-Scale Seaweed Aquaculture Using Artificial Upwelling. Water 11 (9):1754
FAO (2018) The global status of seaweed production, trade and utilization, vol 124. Globefish Research Programme. Rome
Fernand F, Israel A, Skjermo J, Wichard T, Timmermans KR, Golberg A (2017) Offshore macroalgae biomass for bioenergy production: Environmental aspects, technological achievements and challenges. Renewable and Sustainable Energy Reviews 75:35-45
Flavin K, Flavin N, Flahive B (2013) Kelp farming manual: a guide to the processes, techniques, and equipment for farming kelp in New England waters. Ocean Approved LLC, Saco, ME, USA.
Fletcher RL (1995) Epiphytism and fouling in *Gracilaria* cultivation: an overview. Journal of Applied Phycology 7 (3):325-333
Fleurence J (1999) Seaweed proteins: biochemical, nutritional aspects and potential uses. Trends in Food Science & Technology 10 (1):25-28
Fleurence J, Morançais M, Dumay J, Decottignies P, Turpin V, Munier M, Garcia-Bueno N, Jaouen P (2012) What are the prospects for using seaweed in human nutrition and for marine animals raised through aquaculture? Trends in Food Science & Technology 27 (1):57-61
Foldal S (2018) Morphological relations of cultivated *Saccharina latissima* at three stations along the Norwegian coast (*in Norwegian*). Master's thesis in Marine Coastal Development, Norwegian University of Science and Technology, Trondheim, Norway
Forbord S, Skjermo J, Arff J, Handå A, Reitan K, Bjerregaard R, Lüning K (2012) Development of *Saccharina latissima* (Phaeophyceae) kelp hatcheries with year-round production of zoospores and juvenile sporophytes on culture ropes for kelp aquaculture. Journal of Applied Phycology 24 (3):393-399
Forbord S, Steinhovden KB, Rød KK, Handå A, Skjermo J (2018) Cultivation protocol for *Saccharina latissima*. In: Charrier B, T W, Reddy C (eds) Protocols for Macroalgae Research,. 1st edn. CRC Press, Taylor Francis Group, Boca Raton, FL, London, New York, NY, pp 37-59
Fortes M, Lüning K (1980) Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. Helgoländer Meeresuntersuchungen 34 (1):15
Fossberg J, Forbord S, Broch OJ, Malzahn AM, Jansen H, Handå A, Førde H, Bergvik M, Fleddum AL, Skjermo J, Olsen Y (2018) The potential for upscaling kelp (*Saccharina latissima*) cultivation in salmon-driven integrated multi-trophic aquaculture (IMTA). Frontiers in Marine Science 5:418
Fredriksen S, Sjøtun I (2015) Risikovurdering ved utsetting av ikke-stedegen tare (*in Norwegian*). vol 2015. Oslo, Norway
Fujita RM, Wheeler PA, Edwards RL (1988) Metabolic regulation of ammonium uptake by *Ulva rigida* (Chlorophyta): A compartmental analysis of the rate-limiting step for uptake. Journal of Phycology 24 (4):560-566
Førde H, Forbord S, Handå A, Fossberg J, Arff J, Johnsen G, Reitan K (2016) Development of bryozoan fouling on cultivated kelp (*Saccharina latissima*) in Norway. Journal of Applied Phycology 28 (2):1225-1234
Gao X, Endo H, Taniguchi K, Agatsuma Y (2013) Combined effects of seawater temperature and nutrient condition on growth and survival of juvenile sporophytes of the kelp *Undaria pinnatifida* (Laminariales; Phaeophyta) cultivated in northern Honshu, Japan. Journal of Applied Phycology 25 (1):269-275
Garcia-Vaquero M, Hayes M (2016) Red and green macroalgae for fish and animal feed and human functional food development. Food Reviews International 32 (1):15-45

Gerard V, DuBois K, Greene R (1987) Growth responses of two *Laminaria saccharina* populations to environmental variation. Hydrobiologia 151 (1):229-232
Gerard VA (1997) The role of nitrogen nutrition in high-temperature tolerance of the kelp, *Laminaria saccharina* (Chromophyta). Journal of Phycology 33 (5):800-810
Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: the challenge of feeding 9 billion people. Science 327 (5967):812-818
Goecke F, Klemetsdal G, Ergon Å (2020) Cultivar Development of Kelps for Commercial Cultivation - Past Lessons and Future Prospects. Frontiers in Marine Science 8:17
Gruber N (2008) The marine nitrogen cycle: overview and challenges. In: Capone DG, Bronk DA, Mulholland MR, Carpenter EJ (eds) Nitrogen in the marine environment, vol 2. 2nd edn. Elsevier, pp 1-50
Hafting JT, Craigie JS, Stengel DB, Loureiro RR, Buschmann AH, Yarish C, Edwards MD, Critchley AT (2015) Prospects and challenges for industrial production of seaweed bioactives. Journal of Phycology 51 (5):821-837
Hafting JT, Critchley AT, Cornish ML, Hubley SA, Archibald AF (2012) On-land cultivation of functional seaweed products for human usage. Journal of Applied Phycology 24 (3):385-392
Handå A, Forbord S, Wang X, Broch OJ, Dahle SW, Størseth TR, Reitan KI, Olsen Y, Skjermo J (2013) Seasonal- and depth-dependent growth of cultivated kelp (*Saccharina latissima*) in close proximity to salmon (*Salmo salar*) aquaculture in Norway. Aquaculture 414-415:191-201
Hanelt D, Wiencke C, Karsten U, Nultsch W (1997) Photoinhibition and recovery after high light stress in different developmental and life-history stages of *Laminaria saccharina* (Phaeophyta). Journal of Phycology 33 (3):387-395
Hanisak M (1979) Nitrogen limitation of *Codium fragile* ssp. tomentosoides as determined by tissue analysis. Marine Biology 50 (4):333-337
Hanisak M (1983) The nitrogen relationships of marine macroalgae. Nitrogen in the Marine Environment Academic Press, New York:699-730
Harnedy PA, FitzGerald RJ (2011) Bioactive proteins, peptides and amino acids from macroalgae. Journal of Phycology 47 (2):218-232
Harrison J, Druehl LD (1982) Nutrient uptake and growth in the Laminariales and other macrophytes: consideration of methods. Degradative Processes in Marine Macrophytes:99-120
Harrison P, Druehl L, Lloyd K, Thompson P (1986) Nitrogen uptake kinetics in three year-classes of *Laminaria groenlandica* (Laminariales: Phaeophyta). Marine Biology 93 (1):29-35
Harrison P, Parslow J, Conway H (1989) Determination of nutrient uptake kinetic parameters: a comparison of methods. Marine Ecology Progress Series:301-312
Harrison PJ, Hurd CL (2001) Nutrient physiology of seaweeds: application of concepts to aquaculture. Cahiers de biologie marine 42 (1-2):71-82
Hasselström L, Visch W, Gröndahl F, Nylund GM, Pavia H (2018) The impact of seaweed cultivation on ecosystem services-a case study from the west coast of Sweden. Marine pollution bulletin 133:53-64
Holdt S, Edwards MD (2014) Cost-effective IMTA: a comparison of the production efficiencies of mussels and seaweed. Journal of Applied Phycology 26 (2):933-945
Holdt SL, Kraan S (2011) Bioactive compounds in seaweed: functional food applications and legislation. Journal of Applied Phycology 23 (3):543-597
Hurd C, Harrison P, Druehl L (1996) Effect of seawater velocity on inorganic nitrogen uptake by morphologically distinct forms of *Macrocystis integrifolia* from wave-sheltered and exposed sites. Marine Biology 126 (2):205-214
Hurd CL (2000) Water motion, marine macroalgal physiology, and production. Journal of Phycology 36 (3):453-472
Hurd CL, Harrison PJ, Bischof K, Lobban CS (2014) Seaweed ecology and physiology. 2 edn. Cambridge University Press, Cambridge

Ibrahim A, Olsen A, Lauvset S, Rey F (2014) Seasonal variations of the surface nutrients and hydrography in the Norwegian Sea. International Journal of Environmental Science and Development 5 (5):496
Jones AB, Dennison WC, Stewart GR (1996) Macroalgal responses to nitrogen source and availability: amino acid metabolic profiling as a bioindicator using *Gracilaria edulis* (Rhodophyta). Journal of Phycology 32 (5):757-766
Jones D (1941) Factors for Converting Percentages of Nitrogen in Foods and Feeds into Percentages of Protein. US Department of Agriculture, Washington DC
Kain J (1979) A view of the genus Laminaria. J Oceanogr Mar Biol Ann Rev 17:101-161
Kain JM, Jones N (1969) The biology of *Laminaria hyperborea*. V. Comparison with early stages of competitors. Journal of the Marine Biological Association of the United Kingdom 49 (2):455-473
Kerrison PD, Stanley MS, Edwards MD, Black KD, Hughes AD (2015) The cultivation of European kelp for bioenergy: Site and species selection. Biomass and Bioenergy 80:229-242
Kjeldahl J (1883) Neue methode zur bestimmung des stickstoffs in organischen körpern (New method for the determination of nitrogen in organic substances). Zeitschrift für analytische Chemie 22 (1):366-382
Krumhansl KA, Lee JM, Scheibling RE (2011) Grazing damage and encrustation by an invasive bryozoan reduce the ability of kelps to withstand breakage by waves. Journal of Experimental Marine Biology and Ecology 407 (1):12-18
Lapointe BE, Duke CS (1984) Biochemical strategies for growth of *Gracilaria tikvahiae* (Rhodophyta) in relation to light intensity and nitrogen availability. Journal of Phycology 20 (4):488-495
Lavik AQ (2016) Developing a laboratory cultivation protocol for local species of *Porphyra* spp. M. Sc., NTNU, Trondheim, Norway
Lourenço SO, Barbarino E, De-Paula JC, Pereira LOdS, Marquez UML (2002) Amino acid composition, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical seaweeds. Phycological Research 50 (3):233-241
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. Journal of biological chemistry 193:265-275
Lubsch A, Timmermans KR (2019) Uptake kinetics and storage capacity of dissolved inorganic phosphorus and corresponding dissolved inorganic nitrate uptake in *Saccharina latissima* and *Laminaria digitata* (Phaeophyceae). Journal of Phycology 55:637-650
Lüning K (1980) Critical levels of light and temperature regulating the gametogenesis of three *Laminaria* species (Phaeophyceae). Journal of Phycology 16 (1):1-15
Lüning K (1990) Seaweeds: their environment, biogeography, and ecophysiology. John Wiley & Sons, New York
Lüning K, Dring M (1975) Reproduction, growth and photosynthesis of gametophytes of *Laminaria saccharina* grown in blue and red light. Marine Biology 29 (3):195-200
Lüning K, Neushul M (1978) Light and temperature demands for growth and reproduction of laminarian gametophytes in southern and central California. Marine Biology 45 (4):297-309
Manns D, Nielsen MM, Bruhn A, Saake B, Meyer AS (2017) Compositional variations of brown seaweeds *Laminaria digitata* and *Saccharina latissima* in Danish waters. Journal of Applied Phycology:1-14
Marinho GS, Holdt SL, Angelidaki I (2015a) Seasonal variations in the amino acid profile and protein nutritional value of *Saccharina latissima* cultivated in a commercial IMTA system. Journal of Applied Phycology 27 (5):1991-2000
Marinho GS, Holdt SL, Birkeland MJ, Angelidaki I (2015b) Commercial cultivation and bioremediation potential of sugar kelp, *Saccharina latissima*, in Danish waters. Journal of Applied Phycology 27 (5):1963-1973
Marinho GS, Holdt SL, Jacobsen C, Angelidaki I, Barnathan G (2015c) Lipids and Composition of Fatty Acids of *Saccharina latissima* Cultivated Year-Round in Integrated Multi-Trophic Aquaculture. Marine Drugs 13 (7):4357-4374

Mariotti F, Tomé D, Mirand PP (2008) Converting nitrogen into protein—beyond 6.25 and Jones' factors. Critical reviews in food science and nutrition 48 (2):177-184
Martínez B, Pato LS, Rico JM (2012) Nutrient uptake and growth responses of three intertidal macroalgae with perennial, opportunistic and summer-annual strategies. Aquatic botany 96 (1):14-22
Matsson S, Christie H, Fieler R (2019) Variation in biomass and biofouling of kelp, *Saccharina latissima*, cultivated in the Arctic, Norway. Aquaculture 506:445-452
Mols-Mortensen A, Ortind EáG, Jacobsen C, Holdt SL (2017) Variation in growth, yield and protein concentration in *Saccharina latissima* (Laminariales, Phaeophyceae) cultivated with different wave and current exposures in the Faroe Islands. Journal of Applied Phycology 29 (5):2277-2286
Monteiro JP, Rey F, Melo T, Moreira AS, Arbona J-F, Skjermo J, Forbord S, Funderud J, Raposo D, Kerrison PD, Perrineau M-M, Gachon C, Domingues P, Calado R, Domingues MR (2020) The Unique Lipidomic Signatures of *Saccharina latissima* Can Be Used to Pinpoint Their Geographic Origin. Biomolecules 10 (1):107
Mortensen LM (2017) Diurnal carbon dioxide exchange rates of *Saccharina latissima* and *Laminaria digitata* as affected by salinity levels in Norwegian fjords. Journal of Applied Phycology 29 (6):3067-3075
Mosse J (1990) Nitrogen-to-protein conversion factor for ten cereals and six legumes or oilseeds. A reappraisal of its definition and determination. Variation according to species and to seed protein content. Journal of Agricultural and Food Chemistry 38 (1):18-24
Mouritsen OG, Williams L, Bjerregaard R, Duelund L (2012) Seaweeds for umami flavour in the New Nordic Cuisine. Flavour 1 (1):4
Moy F, Alve E, Bogen J, Christie H, Green N, Helland A, Steen H, Skarbøvik E, Stålnacke P (2006) Sugar Kelp Project: Status Report No 1. Norsk institutt for vannforskning - NIVA, Oslo, Norway
Müller D, Gassmann G, Lüning K (1979) Isolation of a spermatozoid-releasing and-attracting substance from female gametophytes of *Laminaria digitata*. Nature 279 (5712):430-431
Mæhre HK, Dalheim L, Edvinsen GK, Elvevoll EO, Jensen I-J (2018) Protein Determination—Method Matters. Foods 7 (1):5
Naldi M, Wheeler PA (1999) Changes in nitrogen pools in *Ulva fenestrata* (Chlorophyta) and *Gracilaria pacifica* (Rhodophyta) under nitrate and ammonium enrichment. Journal of Phycology 35 (1):70-77
Nielsen CW, Holdt SL, Sloth JJ, Marinho GS, Sæther M, Funderud J, Rustad T (2020) Reducing the High Iodine Content of *Saccharina latissima* and Improving the Profile of Other Valuable Compounds by Water Blanching. Foods 9 (5):569
Nielsen MM, Manns D, D'Este M, Krause-Jensen D, Rasmussen MB, Larsen MM, Alvarado-Morales M, Angelidaki I, Bruhn A (2016) Variation in biochemical composition of *Saccharina latissima* and *Laminaria digitata* along an estuarine salinity gradient in inner Danish waters. Algal Research 13:235-245
NSF (1975) NS 4746 Water analysis. Determination of the sum of nitrite-nitrogen and nitrate-nitrogen (*in Norwegian*). Norges Standardiseringsforbund, Oslo, Norway
Olafsen T, Winther U, Olsen Y, Skjermo J (2012) Value created from productive oceans in 2050. Report of a working group appointed by the Royal Norwegian Society of Sciences (DKNVS) and the Norwegian Academy of Technological Sciences (NTVA), Trondheim, Norway
Pang SJ, Lüning K (2004) Breaking seasonal limitation: year-round sporogenesis in the brown alga *Laminaria saccharina* by blocking the transport of putative sporulation inhibitors. Aquaculture 240 (1-4):531-541
Park CS, Hwang EK (2012) Seasonality of epiphytic development of the hydroid *Obelia geniculata* on cultivated *Saccharina japonica* (Laminariaceae, Phaeophyta) in Korea. Journal of Applied Phycology 24 (3):433-439

Park J, Kim JK, Kong J-A, Depuydt S, Brown MT, Han T (2017) Implications of rising temperatures for gametophyte performance of two kelp species from Arctic waters. Botanica marina 60 (1):39-48
Parke M (1948) Studies on British Laminariaceae. I. Growth in *Laminaria saccharina* (L.) Lamour. Journal of the Marine Biological Association of the United Kingdom 27 (3):651-709
Pedersen MF (1994) Transient ammonium uptake in the macroalga *Ulva lactuca* (Chlorophyta): Nature, regulation, and the consequences for choise of measuring technique. Journal of Phycology 30 (6):980-986
Pedersen MF, Borum J (1996) Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. Marine Ecology Progress Series:261-272
Pedersen MF, Borum J (1997) Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake. Marine Ecology Progress Series 161:155-163
Pereira L (2011) A review of the nutrient composition of selected edible seaweeds. In: Pomin VH (ed) Seaweed: Ecology, nutrient composition medicinal uses. Nova Science Publishers, Hauppauge, pp 15-47
Peteiro C, Freire Ó (2013) 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. Journal of Applied Phycology 25 (1):205-213
Phillips JC, Hurd CL (2004) Kinetics of nitrate, ammonium, and urea uptake by four intertidal seaweeds from New Zealand. Journal of Phycology 40 (3):534-545
Pueschel CM, Korb RE (2001) Storage of nitrogen in the form of protein bodies in the kelp *Laminaria solidungula*. Marine Ecology Progress Series 218:107-114
Rajapakse N, Kim S-K (2011) Nutritional and digestive health benefits of seaweed. In: Advances in food and nutrition research, vol 64. Elsevier, pp 17-28
Redmond S, Green L, Yarish C, Kim J, Neefus C (2014) New England seaweed culture handbook-nursery systems. Connecticut Sea Grant CTSG-14-01, vol 7.
Rey F, Aure J, Danielssen D (2007) Temporal and spatial distribution of nutrients. In: Sætre R (ed) The Norwegian Coastal Current- Oceanography and Climate. Tapir Academic Press, Trondheim, pp 73-88
Roleda MY, Hurd CL (2019) Seaweed nutrient physiology: application of concepts to aquaculture and bioremediation. Phycologia 58 (5):552-562
Roleda MY, Skjermo J, Marfaing H, Jónsdóttir R, Rebours C, Gietl A, Stengel DB, Nitschke U (2018) Iodine content in bulk biomass of wild-harvested and cultivated edible seaweeds: Inherent variations determine species-specific daily allowable consumption. Food chemistry 254:333-339
Safi C, van Leeuwen J, Telleman Y, Engelen-Smit N, van den Broek L, Harmsen P (2018) Quantification of proteins in seaweeds. In: Charrier B, Wichard T, Reddy C (eds) Protocols for Macroalgae Research. CRC Press, Boca Raton, London, New York, pp 211-224
Sánchez-Barredo M, Ladah LB, Zertuche-González JA (2011) Nitrate uptake and duration of internal nitrogen reserves in the kelp *Eisenia arborea*. Botanica Marina 54 (5):441-446
Scheibling RE, Gagnon P (2009) Temperature-mediated outbreak dynamics of the invasive bryozoan *Membranipora membranacea* in Nova Scotian kelp beds. Marine Ecology Progress Series 390:1-13
Schiener P, Black KD, Stanley MS, Green DH (2015) The seasonal variation in the chemical composition of the kelp species *Laminaria digitata, Laminaria hyperborea, Saccharina latissima* and *Alaria esculenta*. Journal of Applied Phycology 27 (1):363-373
Schmedes PS, Nielsen MM (2019) New hatchery methods for efficient spore use and seedling production of *Palmaria palmata* (dulse). Journal of Applied Phycology. doi:10.1007/s10811-019-01998-0

Schmedes PS, Nielsen MM, Petersen JK (2019) Improved *Palmaria palmata* hatchery methods for tetraspore release, even settlement and high seedling survival using strong water agitation and macerated propagules. Algal Research 40:101494
Schreiber E (1930) Untersuchungen über parthenogenesis, geschlechtsbestimmung und bastardierungsvermögen bei Laminarien (*in German*). Zeitschrift fur wissenschaftliche Biologie Abteilung E: Planta 12 (3):331-353
Shahidi F (2009) Nutraceuticals and functional foods: whole versus processed foods. Trends in Food Science & Technology 20 (9):376-387
Sharma S, Neves L, Funderud J, Mydland LT, Øverland M, Horn SJ (2018) Seasonal and depth variations in the chemical composition of cultivated *Saccharina latissima*. Algal Research 32:107-112
Skjermo J, Aasen IM, Arff J, Broch OJ, Carvajal A, Christie H, Forbord S, Olsen Y, Reitan KI, Rustad T (2014) A new Norwegian bioeconomy based on cultivation and processing of seaweeds: Opportunities and R&D needs. SINTEF Fisheries and Aquaculture
Spurkland T, Iken K (2011) Salinity and irradiance effects on growth and maximum photosynthetic quantum yield in subarctic *Saccharina latissima* (Laminariales, Laminariaceae). Botanica Marina 54 (4):355-365
Stagnol D, Macé M, Destombe C, Davoult D (2016) Allometric relationships for intertidal macroalgae species of commercial interest. Journal of Applied Phycology 28 (6):3407-3411
Su L, Pang SJ, Shan TF, Li X (2017) Large-scale hatchery of the kelp *Saccharina japonica*: a case study experience at Lvshun in northern China. Journal of Applied Phycology 29 (6):3003-3013
Syrett PJ (1981) Uptake and utilization of nitrogenous compounds. Canadian Bulletin of Fisheries and Aquatic Sciences 210:182-210
Tabarsa M, Rezaei M, Ramezanpour Z, Waaland JR (2012) Chemical compositions of the marine algae *Gracilaria salicornia* (Rhodophyta) and *Ulva lactuca* (Chlorophyta) as a potential food source. Journal of the Science of Food and Agriculture 92 (12):2500-2506
Thomas T, Harrison P, Turpin D (1987) Adaptations of *Gracilaria pacifica* (Rhodophyta) to nitrogen procurement at different intertidal locations. Marine Biology 93 (4):569-580
Thornber CS, DiMilla P, Nixon SW, McKinney RA (2008) Natural and anthropogenic nitrogen uptake by bloom-forming macroalgae. Marine Pollution Bulletin 56 (2):261-269
Voss M, Bange HW, Dippner JW, Middelburg JJ, Montoya JP, Ward B (2013) The marine nitrogen cycle: recent discoveries, uncertainties and the potential relevance of climate change. Phil Trans R Soc B 368:20130121
Wallentinus I (1984) Comparisons of nutrient uptake rates for Baltic macroalgae with different thallus morphologies. Marine Biology 80 (2):215-225
Wargacki AJ, Leonard E, Win MN, Regitsky DD, Santos CNS, Kim PB, Cooper SR, Raisner RM, Herman A, Sivitz AB (2012) An engineered microbial platform for direct biofuel production from brown macroalgae. Science 335 (6066):308-313
Watanabe T, Kitajima C, Fujita S (1983) Nutritional values of live organisms used in Japan for mass propagation of fish: a review. Aquaculture 34 (1-2):115-143
Wheeler W, Weidner M (1983) Effects of external inorganic nitrogen concentration on metabolism, growth and activities of key carbon and nitrogen assimilatory enzymes of *Laminaria saccharina* (Phaeophyceae) in culture. Journal of Phycology 19 (1):92-96
Williams JL (1921) The gametophytes and fertilization in *Laminaria* and *Chorda*. (Preliminary Account.). Annals of Botany 35 (140):603-607
Xiao X, Agusti S, Lin F, Li K, Pan Y, Yu Y, Zheng Y, Wu J, Duarte CM (2017) Nutrient removal from Chinese coastal waters by large-scale seaweed aquaculture. Scientific reports 7:46613
Xiao X, Agusti S, Lin F, Yu Y, Pan Y, Li K, Wu J, Duarte CM (2019) Resource (light and nitrogen) and density-dependence of seaweed growth. Frontiers in Marine Science 6:618
Young EB, Dring MJ, Savidge G, Birkett DA, Berges JA (2007) Seasonal variations in nitrate reductase activity and internal N pools in intertidal brown algae are correlated with ambient nitrate concentrations. Plant, cell & environment 30 (6):764-774

Øie G, Olsen Y (1997) Protein and lipid content of the rotifer *Brachionus plicatilis* during variable growth and feeding condition. In: Live Food in Aquaculture. Springer, pp 251-258

Paper I

Initial short-term nitrate uptake in juvenile, cultivated *Saccharina latissima* (Phaeophyceae) of variable nutritional state

Silje Forbord[a,b*], Siv Anina Etter[a], Ole Jacob Broch[b], Vegard Rønning Dahlen[a], Yngvar Olsen[a]

[a]Norwegian University of Science and Technology, Department of Biology, Centre of Fisheries and Aquaculture, N-7491 Trondheim, Norway
[b]SINTEF Ocean, Department of Environment and New Resources, N- 7465 Trondheim, Norway

*Corresponding author: Silje Forbord, SINTEF Ocean, Department of Environment and New Resources, N-7465 Trondheim, Norway, Phone 0047 97122001, silje.forbord@sintef.no

Abstract

To reach the goal of large-scale seaweed cultivation in Norway, new knowledge concerning commercially important species like the kelp *Saccharina latissima* is essential. This includes fundamental understanding of physiological mechanisms like nutrient uptake kinetics which makes us better understand its ecological niche and nutritional requirements. The initial short-term nitrate (NO_3^-) uptake kinetics in cultivated *S. latissima* juvenile sporophytes were evaluated under nitrogen-saturated and nitrogen-limited conditions. The uptake was measured for concentrations in a gradient from 2-18 µM NO_3^- which is representative of Norwegian coastal waters in the main growth season of winter and early spring. Preconditioning treatments led to internal nitrogen pools (total tissue nitrogen and intracellular nitrate) that were significantly lower for the N-limited than for the N-saturated sporophytes prior to the experiment. Nitrate uptake rates, related to biomass (V) and intracellular nitrogen content (U), were linearly related to the substrate concentrations for both N-limited and N-saturated sporophytes, indicating that *S. latissima* requires high ambient nitrate concentrations to maintain rapid growth. The sporophytes with deficient internal nitrogen pools exhibited higher uptake rates of NO_3^- than sporophytes with higher internal pools of nitrogen. Mathematical modelling was used to investigate the temporal development of total tissue nitrogen (Q_N) based on the nitrogen-specific uptake rates (U) and revealed a near linear response of U to changes in Q_N. The model also found that a maximum estimated value for Q_N was only approached after more than 20 days at external NO_3^- concentrations of 8 µM. These results expand the physiological understanding of cultivated *S. latissima* and are important for a sustainable upscaling of seaweed farm production.

Keywords

Initial nutrient uptake rates, Intracellular nitrate, Mathematical modelling, Nitrate uptake, *Saccharina latissima,* Seaweed cultivation, Sporophyte growth, Total intracellular nitrogen, Uptake kinetics

1. Introduction

Saccharina latissima (Linnaeus) Lane, Mayes, Druehl, and Saunders 2006 is a cold water kelp species that grows rapidly in late winter and early spring when dissolved inorganic nutrients like nitrate (DIN) are available in excess and other environmental factors such as temperature, light conditions and salinity are favorable (Kerrison et al. 2015). Cultivation of *S. latissima* in Norway normally involves deployments during the autumn and winter (Stévant et al. 2017) and harvesting in the period from May-August, depending on the latitude and degree of epifouling on the biomass (Forbord et al. 2020). The interest in seaweed cultivation has increased in Norway over the last decade, but the production of *S. latissima* did not reach more than 174 metric tons in 2018 (Directorate of Fisheries 2020), compared to the total world production of almost 30 million metric tons of seaweed (FAO 2018). To upscale and industrialize the seaweed production, more knowledge on how to utilize and optimize farm locations in terms of key resources such as light and nutrient availability is essential. This enables the farmers to identify suitable cultivation depths and seasons and to ideally predict the potential biomass production.

Nitrogen is considered to be the potential limiting nutrient for macroalgal growth, both globally and in Norwegian coastal waters, especially during summer months after phytoplankton blooms have depleted the concentration of DIN to almost zero (Hanisak 1983; Ibrahim et al. 2014). The major sources of inorganic nitrogen available for macroalgae are nitrate (NO_3^-), ammonium (NH_4^+), and urea (Hurd et al. 2014). North-East Atlantic deep water contains around 10 µmol L^{-1} (µM) of NO_3^-, representing the highest concentration of nitrate that the algae are exposed to in its natural environment (Forbord et al. 2012). The concentrations of NH_4^+ are normally lower, but variable and dependent on regeneration processes in the water column (Hurd et al. 2014). NO_3^- can be stored inside the seaweed cells at concentrations exceeding ambient concentrations and is therefore assumed to require active uptake (Harrison and Druehl 1982; Harrison and Hurd 2001); however, this has only been confirmed for a few species (Roleda and Hurd 2019). With an active mechanism of uptake, this will normally exhibit saturation kinetics, i.e. the rate of uptake can be described by a rectangular hyperbola like the Michaelis-Menten equation (Chapman et al. 1978; Harrison and Druehl 1982; Wallentinus 1984). In other cases, saturation does not occur at high nutrient concentrations, and a linear uptake may be experienced.

Unsaturated uptake is common for NH_4^+ (Phillips and Hurd 2004; Abreu et al. 2011; Martínez et al. 2012), but it has also been described for NO_3^- (Harrison et al. 1986; Ahn et al. 1998; Sánchez-Barredo et al. 2011; Martínez et al. 2012).

Nutrient uptake rates in seaweed are affected by, and vary considerably with, chemical (concentration of nutrients in their ionic or molecular form), physical (light, salinity, temperature, desiccation and water motion) and biological factors (Thomas et al. 1987; Harrison and Hurd 2001). The latter includes the ratio of surface-area:volume (SA:V), type of tissue, age, nutritional state and variability between species (Wallentinus 1984; Hurd et al. 2014). The nitrate taken up can be stored in intracellular pools (I-DIN) in the vacuoles (Hurd et al. 2014) or reduced to nitrite (NO_2^-). Incorporation in vacuoles may occur when the uptake rate of NO_3^- is greater than the conversion rate to NO_2^-. Nitrite is further reduced to NH_4^+ and incorporated into biomolecules such as amino acids (Harrison and Hurd 2001). Dependent on the size of this I-DIN pool, it has been suggested that the algae may be more capable of growing in areas where the nutrient availability varies over the seasons (Hanisak 1979). Other potential nitrogen reserve pools in macroalgae include proteins, nucleic acids, free amino acids and pigments (Lapointe and Duke 1984; Jones et al. 1996; Young et al. 2007; Naldi and Wheeler 1999), where proteins account for the largest N-source (Pueschel and Korb 2001). High contents of these components may be a result of nutrient saturation, whereas low levels could suggest nutrient sub-saturation.

The short-term nitrate uptake reflects the instantaneous uptake following a perturbation in nutrient concentration. The uptake rate will be constant for a short time after perturbation, before intracellular feedback from the increasing nutrient content in the algal tissues and before ambient concentration has been substantially reduced. Uptake rates measured over a long period of time will become proportional to and vary with the growth rate. Short-term uptake following a perturbation in ambient concentration is not quantitatively associated with the growth rate, but the uptake rate depends on the initial nutritional state of the algae.

The aim of this study was to determine the initial short-term nitrate uptake rates and the total nitrogen and internal nitrate storage capacity for cultivated, juvenile N-saturated and

N-limited *S. latissima* exposed to nitrate concentrations that are representative of Norwegian coastal waters. These results, under controlled laboratory conditions with ecologically relevant nutrient concentrations, provide new and valuable knowledge that expands the physiological understanding of cultivated *S. latissima* with different nutritional histories.

2. Materials and methods

2.1 Experimental design

Saccharina latissima sporophytes used in this experiment were prepared according to methods described by Forbord et al. (2018) and cultivated at sea from September 2016 to March 2017 at the seaweed farm Taraskjæret in Central Norway (63°42'N 08°52'E). The ambient nitrate concentration at this site in mid-March is typically around 6 µmol L^{-1} at a depth of 0-8 m (unpublished data). Visible epiphyte-free sporophytes with a length of 7-13 cm were chosen for the experiment. Half of the sporophytes were placed in a tank with running flow-through deep water from a depth of 90 m with a nitrate concentration of ~10 µmol L^{-1} for nitrogen saturation for 8 days and half of the sporophytes were placed in artificial seawater (Grasshoff et al. 2009) enriched with modified f/2 medium without nitrogen and silicate. The sporophytes were N-depleted for 8 days prior to the experiment. The medium was replaced once in that period. After this, the sporophytes started to bleach and decompose, suggesting severe nitrogen starvation.

The experiment had a duration of 300 min and was conducted with a combination of the perturbation- and multiple flask techniques (Harrison et al. 1989) to examine the uptake of NO_3^- as the only available nitrogen source for saturated and depleted *S. latissima* sporophytes added to a concentration gradient (Table 1). The experiment was performed over two separate days in a laboratory holding a temperature of 10 °C and a light intensity of 40 µmol m^{-2} s^{-1} above the experimental flasks to ensure sufficient energy supply and photosynthetic activity. 250 mL flasks were filled up with artificial seawater without nitrogen and silica and added nitrogen stock solution to create the target concentration. Water motion to reduce boundary layer effects that could reduce uptake rates was achieved by placing the flasks on orbital shakers. Five specimens of whole, untrimmed *S. latissima* sporophytes of approximately similar sizes were haphazardly collected from the appropriate

preconditioning tank and added to each replicate flask. The flasks were placed on the orbital shakers at 100 rpm. Deviations in the initial measured concentrations compared to the planned ones (0.25, 0.5, 1, 2, 4, 8 and 16 μM) were found for all treatments due to the fact that the artificial seawater had a background N-content of ~1 μM or more (Table 1). In addition to seven concentrations, with six replicates each (n = 6), two control samples without *S. latissima* biomass were included in both experiments (saturated and depleted) corresponding to low (2.0 and 1.7 μM) and high (16.7 and 18.5 μM) concentrations to monitor potential changes in nitrogen during the experiments due to microbiological activity.

Table 1: Seven different incubation concentrations of nitrate (μM) used for the N-saturated and N-limited experiments

Nutritional history	**Initial concentration (μM)**						
N-saturated	2.0	2.5	3.2	3.6	5.2	7.6	16.7
N-limited	1.7	2.2	2.4	3.8	5.3	10.2	18.5

Upon sampling, 2 mL water samples were collected from each flask after 5, 10, 20, 30, 50 and 90, 180 and 300 min of incubation. Samples from the controls were taken at the first and last sampling points. All samples were transferred to pre-marked 15 mL plastic tubes and frozen until analysis. After the experiment, the sporophytes from all experimental flasks were gently dried with paper towels and the total wet biomass per flask was weighed. Two sporophytes from each replicate were weighed individually, dried at 80^{o} C for 24 h, and weighed again to estimate the total dry weight biomass for each flask.

2.2 Chemical analysis of nitrate

Prior to analysis, the frozen water samples were thawed at room temperature and filtered using a 0.45 μm syringe filter to remove algal debris. Water samples were analyzed colorimetrically at 550 nm after the reduction of NO_3^- to NO_2^- through a copperized cadmium coil in a Flow Solution IV System, O. I. Analytical Auto Analyzer following Norwegian Standard 4745 (NSF 1975).

2.3 Calculation of uptake rates

Substrate concentrations of NO_3^- were measured at each sampling point to follow the change in concentration over time, reflecting the disappearance of the nutrient from the medium. The substrate concentrations were related to biomass (grams dry weight; g DW) in each flask and subsequently used to calculate uptake rates. The uptake rates were determined in each replicate flask according to Equation 1:

$$V = \frac{(S_i - S_f) \text{ x vol}}{\text{t x DW}} \text{x } 24 \quad (1)$$

where V is the specific uptake rate (µg g^{-1} DW day^{-1}), S_i is the initial substrate concentration (µg L^{-1}), S_f is the final substrate concentration (µg L^{-1}), vol is the volume (L), t is the time between sampling (h) and DW is the dry weight of the total biomass in the flask (g). This calculation was done for the six replicate flasks and a mean value ± standard error (SE) expressed the final results. The uptake rate remained constant over time in the period from 5-90 min of incubation before levelling off; therefore, the measurements made beyond 90 min were not included in the calculation of uptake rates.

The initial uptake of NO_3^- per unit of cellular N was calculated for each replicate flask according to Equation 2:

$$U = \frac{\text{V}}{Q_N} \text{x } 24 \quad (2)$$

where U is the specific initial cellular N based uptake rate (day^{-1}), V is the DW specific uptake rate calculated from Eq. 1 (µg g^{-1} DW h^{-1}), and Q_N is the total tissue nitrogen (µg N g^{-1} DW) measured from the start of the experiment.

2.4 Total intracellular nitrogen analysis (Q_N)

Total intracellular tissue nitrogen (Q_N) contents were analyzed for the whole thalli for N-saturated and N-limited sporophytes. Three sporophytes taken from each conditioning tank immediately before the uptake experiment were frozen at -20°C and later stored at −80°C until freeze-dried (Hetosicc CD 13-2) at -40°C for 48 h. The dried kelp was homogenized into

a fine powder. Samples of 0.4-1 mg freeze-dried kelp were transferred to tin capsules and analyzed for N in quadruplicate with a Carlo Erba element analyzer (model 1106).

2.5 Intracellular nitrate content (I-DIN)

After 300 min incubation, three sporophytes from three of the replicate flasks from each concentration were haphazardly collected and frozen at -20°C. For the 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-µm polysulfone syringe filter to remove algal debris. The tubes were kept frozen at -20°C, then defrosted and shaken prior to analysis. The extracts were used to determine the concentration of I-DIN using an Auto Analyzer (Flow Solution IV System, O.I Analytical) after Norwegian Standard 4745 (NSF 1975).

2.6 Data treatment and statistical analysis

Large outlying substrate concentrations were determined as extreme outliers using SPSS and were removed from the data set as they were likely to represent contamination in the flasks. This applies for one replicate at two concentrations obtained for N-saturated sporophytes and one replicate at three concentrations obtained for N-limited sporophytes (n=5). Data from the 180- and 300-min time intervals were excluded because the uptake was only constant over time up to 90 min.

To reveal correlations between reduction in nitrate concentrations in the medium *versus* time for N-saturated and N-limited sporophytes, correlations between uptake rates (V and U) *versus* substrate concentrations (µM) and internal nitrate (I-DIN) concentration *versus* substrate concentration (µM), simple linear regressions analysis were performed. The Pearson correlation coefficient (r) was calculated to measure the linear correlation between the different variables.

The mean I-DIN and Q_N values for N-saturated and N-limited treatments were compared using an independent-samples t-test after confirming the assumption of normality (Shapiro-Wilk's test) and homogeneity of variance (Levene's test). Data are presented as mean ± standard error (SE) unless otherwise stated. The significance level was set to $p = 0.05$.

Statistical analysis was performed using IBM SPSS Statistical software (Version 25) and plots were made using Systat SigmaPlot software (version 14) and MATLAB (Release 2017).

2.7 Mathematical modelling of Q_N dynamics

A simple ordinary differential equation for the dynamics of total tissue nitrogen (Q_N) was formulated based on the results from the uptake experiment described in Sections 2.1-2.4. A Monte Carlo type simulation was performed in order to analyze the variability in how the N-affinity may change with Q_N for Q_N values outside of the experimental range i.e. the functional response of the N-specific uptake rates (U) to the values of Q_N. Using the model, the implications of the uptake results on the temporal changes in Q_N were assessed for different external concentrations of NO_3^-.

3. Results

3.1 Initial uptake rates of nitrate

The uptake of nitrate in *S. latissima* was measured as the reduction in nitrate concentration over time in the water samples with different initial substrate concentrations added. The linear reduction of nitrate found in the 90 min experimental period revealed a constant uptake rate for each concentration, both for the N-saturated (Figure 1a) and N-limited (Figure 1b) sporophytes. The uptake rate levelled off after 90 min (data not shown) and nitrate was not depleted during the experimental period (up to 300 min). Linear regression showed a significant ($p < 0.05$) relationship between concentration and time for all initial concentrations tested, except for 2.5 µM for the N-saturated sporophytes (Table 2).

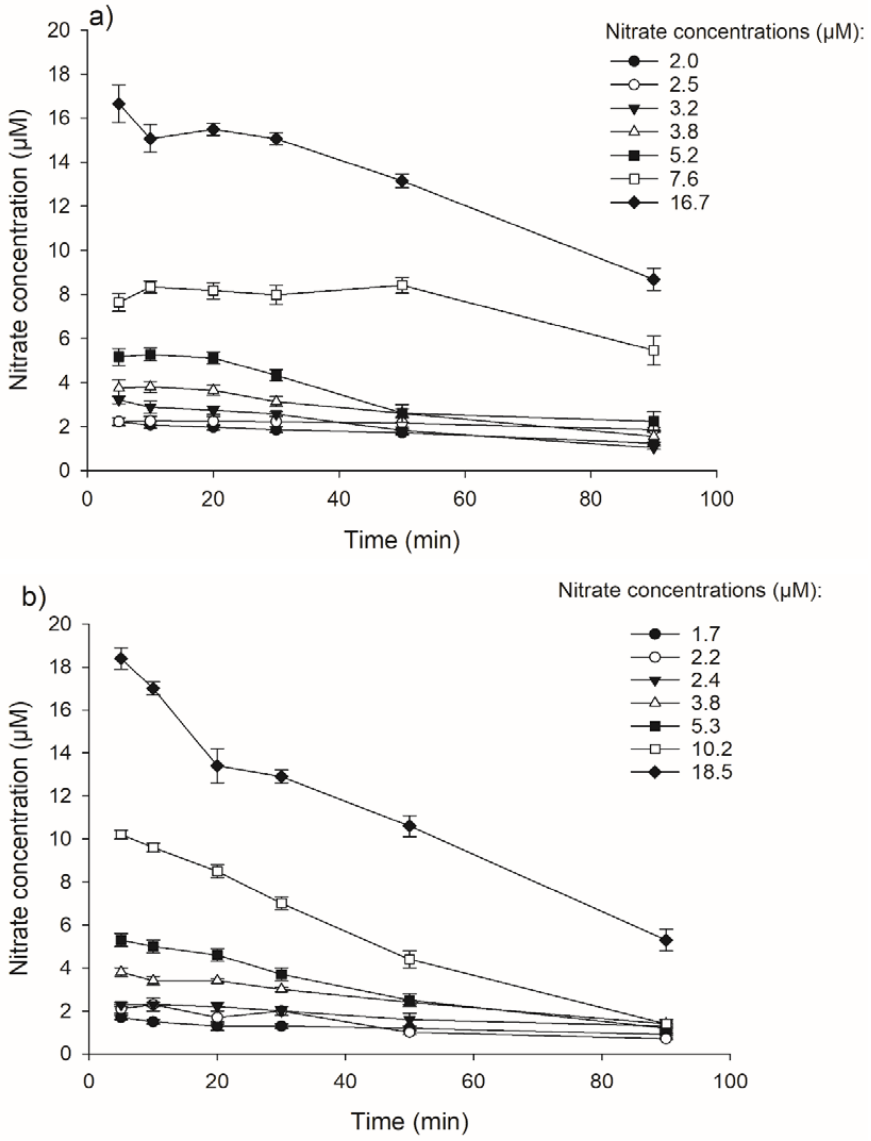

Figure 1 Nitrate (µM) removal from the medium over a time period of 90 minutes for seven different initial concentrations for N-saturated (a) and N-limited (b) sporophytes. Legends show initial nitrate concentrations. Mean ± SE, n=5-6.

Table 2: Linear regression coefficients (slope, R^2 and p-values) of nitrate concentration *versus* time of uptake for the different initial concentrations (µM) for N-saturated and N-limited sporophytes of *S. latissima*.

N-saturated sporophytes				**N-limited sporophytes**			
Initial conc.	**Slope**	**R^2**	**p**	**Initial conc.**	**Slope**	**R^2**	**p**
2.0	-48.6	0.52	<0.001	1.7	-65.5	0.56	<0.001
2.5	-16.6	0.08	**0.105**	2.2	-29.9	0.58	<0.001
3.2	-31.8	0.80	<0.001	2.4	-38.5	0.52	<0.001
3.8	-23.2	0.64	<0.001	3.8	-30.3	0.83	<0.001
5.2	-15.8	0.64	<0.001	5.3	-16.1	0.80	<0.001
7.6	-11.4	0.31	<0.001	10.2	-9.00	0.94	<0.001
16.7	-9.04	0.79	<0.001	18.5	-6.12	0.89	<0.001

3.2 Nitrate uptake rates as a function of substrate concentrations

Uptake rates of NO_3^- (V) normalized to dry weight ranged from 64.8 ± 18.5 to 1414 ± 170 µg N g^{-1} DW day^{-1} for N-saturated sporophytes (Figure 2), increasing with increasing nitrate concentration to which a strong positive (r = 0.91) and significant correlation was apparent (R^2 = 0.83, p < 0.001). The V-values for N-limited sporophytes ranged from 179 ± 14.2 to 2407 ± 111 µg N g^{-1} DW day^{-1} (Figure 2); the values increased with increasing nitrate concentration, revealing a strong positive relationship (r = 0.98, R^2 = 0.95, p < 0.001). For both experiments, there was a positive and linear relationship between uptake rate and nitrate concentration throughout. The slopes of the two regression lines (0.26 ± 0.03 for N-saturated and 0.41 ± 0.03 for N-limited sporophytes), describing the substrate specific affinity, were significantly different (p < 0.001).

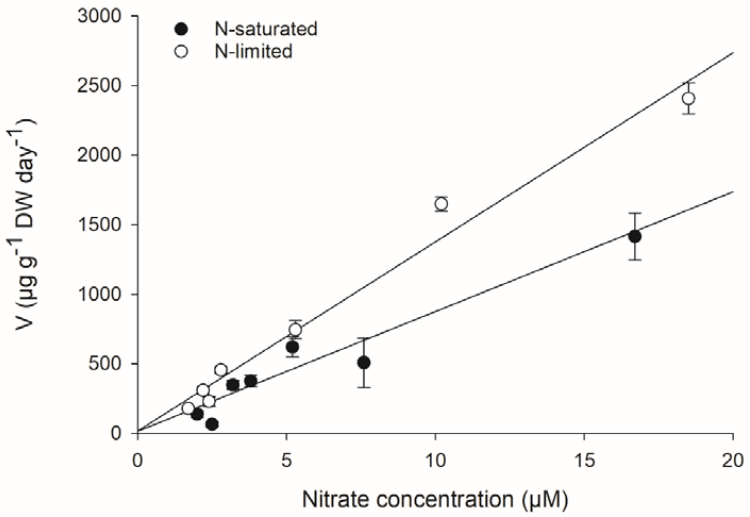

Figure 2 Mean uptake rates of NO_3^- (V, µg g^{-1} DW day^{-1}) for N-saturated and N-limited sporophytes as a function of initial ambient NO_3^- concentration. The value for N-saturated sporophytes perturbed with 7.6 µM nitrate deviated somewhat from the other values and was not included in the line fitting by linear regression. Mean ± SE, n = 5-6.

Mean nitrogen-specific uptake rates (U, day^{-1}) as a function of nitrate concentrations for N-saturated and N-limited sporophytes are shown in Figure 3. U expresses nitrogen uptake rates normalized to tissue nitrogen (Q_N) of the algae instead of biomass (DW). Nitrogen-specific uptake rates (U) for N-saturated sporophytes ranged from 0.002 ± 0.001 to 0.048 ± 0.006 day^{-1}, increasing with increasing nitrate concentrations to which a strong positive ($r = 0.87$) and significant correlation was apparent ($R^2 = 0.75$, $p < 0.001$). The values of U for N-limited sporophytes ranged from 0.009 ± 0.001 to 0.122 ± 0.006 day^{-1}, increasing with increasing nitrate concentration, revealing a strong positive relationship ($r = 0.98$, $R^2 = 0.95$, $p < 0.001$). The slopes of the two regression lines (0.003 ± 0.001 for N-saturated and 0.007 ± 0.001 for N-limited sporophytes) were significantly different ($p < 0.001$).

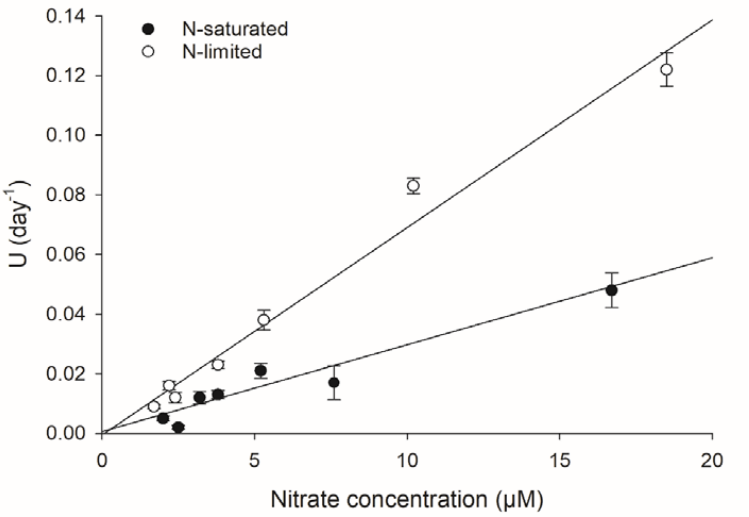

Figure 3 Mean nitrogen-specific uptake rates (U, day^{-1}) for N-saturated and N-limited sporophytes as a function of initial nitrate concentration. The value for N-saturated sporophytes perturbed with 7.6 µM nitrate deviated somewhat from the other values and were not included in the line fitting. Mean ± SE, n =5-6.

In the control samples run without seaweed, only a small increase (< 2.5%) in NO_3^- concentration was measured in the culture media during the experimental period for the highest initial concentration. The measured nitrate reduction in the seawater was therefore attributed to algal uptake.

3.3 Total intracellular nitrogen content (Q_N)

The N-saturated sporophytes had a total tissue N-content (Q_N) of 29.5 ± 0.5 mg g^{-1} DW before the start of the experiment, which was significantly higher ($p < 0.001$) than the Q_N value found for N-limited sporophytes of 19.7 ± 0.6 mg g^{-1} DW.

3.4 Mathematical modelling and simulated Q_N dynamics

In order to discuss the temporal development of Q_N, it is necessary to know or assume something about the N-uptake rates as well as for values of Q_N between and outside the experimental values for the N-saturated and N-limited sporophytes described above. Here,

we first show that under certain assumptions the choice of response of U to Q_N is relatively low, and then use this to study the temporal development of Q_N under some relevant external concentrations of NO_3^- like those in the current experiment (Figure 3).

The main assumption is that the N-specific uptake rate U (Equation 2) is a linear function of the external concentration of NO_3^- (denoted by [NO_3]). Thus, it was assumed that

$$U = U(Q_N, [\mathrm{NO3}]) = f(Q_N)[\mathrm{NO3}] \quad (3)$$

where *f* is *some* response function of the N-specific uptake rate to Q_N. For each Q_N, $f(Q_N)$ represents the N-affinity of the sporophytes, the slope of the U *versus* [NO_3] line (c.f., Figure 3). It can be assumed that the function *f* decreases with increasing Q_N. The function was assumed to have the form:

$$f(Q_N) = a - bQ_N^{\alpha} \quad (4)$$

for exponents α ranging from 0 to 2. Values of $\alpha < 1$ will give concave response curves, as found for microalgae (Olsen 1989), while $\alpha > 1$ implies convex curves. Choosing $\alpha = 1$ gives a linear response of the uptake rates to changes in Q_N.

A Monte Carlo type simulation was performed to analyze the variability and uncertainty in the choice of *f*. For each simulation, the value of α was selected pseudo-randomly from a uniform distribution (α in [0, 2]), while the values of *f*(19) and *f*(29) were selected pseudo-randomly from normal distributions with a mean and standard deviation (SD), as shown in Figure 3. For each choice of α, the parameters *a* and *b* were determined based on the selected *f*(19) and *f*(29) (known values, the slopes of the lines in Figure 3).

On average, the simulations reveal a near linear response of the N-specific uptake rate (U) to changes in Q_N (Figure 4, black curve). The SDs for Q_N values between 19 and 29 were smaller than the SDs for the slopes of the curves in Figure 3 (cf. Figure 4, red bars and dots). Outside the interval [19, 29] for Q_N, the SD increased, reflecting the increased ranges in the functional responses (Equation 4).

Figure 4 also reveals that the simulated theoretical average maximum Q_N of the algae was 36 mg N g^{-1} DW (the intersection of the black line with the horizontal axis) with a range from 32 to 48 mg N g^{-1} DW (the intersections of the boundary of the grey region with the horizontal Q_N axis).

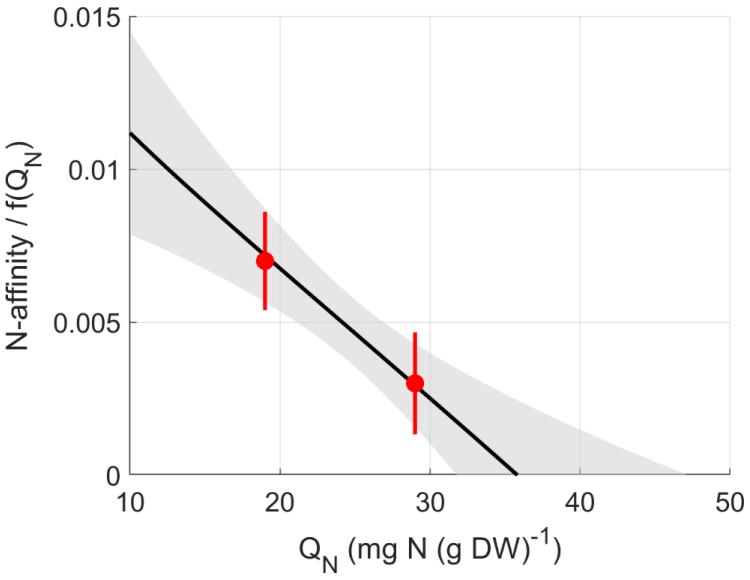

Figure 4 Plot of the theoretical affinity to NO_3^- (y-axis) as a function *f* of Q_N (x-axis). The slopes of the lines in Figure 3 for Q_N = 19 and Q_N = 29 are represented by the red dots, with corresponding standard deviations (vertical red lines). The black curve and the grey region represent the results of a Monte Carlo-type simulation (*n* = 3000 runs) of *f* (in Equation 3) as a function of Q_N. The black curve indicates the mean values of *f* for each value of Q_N and corresponding SD (grey region).

How Q_N changes with time, can be expressed through an appropriate differential equation. The relationship between dry weight (DW g), total tissue N (Q_N, mg N g^{-1} DW) and absolute nitrogen content (N_{abs} g N) (see Section 2.3 above) is

$$Q_N = N_{abs}/DW.$$

This means that:

$$\frac{1}{Q_N}\frac{dQ_N}{dt} = \frac{1}{N_{abs}}\frac{dN_{abs}}{dt} - \frac{1}{DW}\frac{dDW}{dt} = U - \mu\,, \tag{5}$$

where *U* is the nitrogen-specific uptake rate (Equations 2 and 3) and μ is the biomass specific growth rate.

Q_N must therefore satisfy the following differential equation:

$$\frac{dQ_N}{dt} = Q_N(f(Q_N)[\mathrm{NO3}] - \mu), \tag{6}$$

All solutions to Equation 6 have a sigmoid shape, and for $\alpha = 1$ in Equation 4 and $\mu = 0$ in Equation 6 we get the standard logistic equation when $[NO_3]$ is kept fixed (Murray 2002). Equation 6 was solved, corresponding to each choice of *f* by the Monte Carlo simulation, by a standard forward Euler method with short time step.

The temporal dynamics of Q_N by Equation 6 were simulated for $[NO_3^-] = 1$, 4, and 8 µmol L^{-1}. The results illustrated how Q_N over a longer time period varied with external concentrations and time (Figure 5). The uptake and following influence on Q_N were slow, a maximum value (theoretical/model) was only approached after > 20 days at external concentrations of $[NO_3^-] = 8$ µmol L^{-1}.

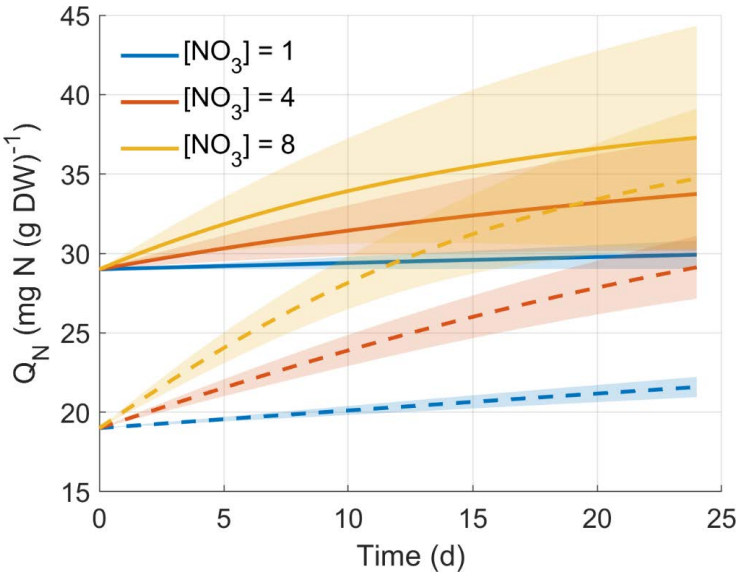

Figure 5 Simulated temporal development of Q_N from Equation 6 for two initial values of Q_N (Q_N (0) = 19 (dashed lines) and Q_N (0) = 29 (solid lines)) and three different external concentrations of NO_3^- (blue: NO_3^- = 1; red: NO_3^- = 4; yellow: NO_3^- = 8). The curves are mean values of n = 3000 simulations, while the shaded regions represent the SDs. The growth rate was assumed to be $\mu = 0$.

3.5 Intracellular nitrate content (I-DIN)

The N-saturated sporophytes showed an I-DIN content of 0.46 ± 0.06 mg g^{-1} DW prior to incubation (0 µM, Figure 6), whereas the N-limited sporophytes showed an I-DIN content of

0.020 ± 0.002 mg g^{-1} DW, which was significantly lower than that of the saturated sporophytes ($p = 0.002$). The highest I-DIN values were found for the N-saturated sporophytes for all initial nitrate concentrations throughout. Values for the N-saturated sporophytes ranged from 0.25 ± 0.05 to 0.60 ± 0.08 mg g^{-1} DW (mean 0.44 ± 0.04 mg I-DIN g^{-1} DW), while the values for N-limited sporophytes ranged from 0.02 ± 0.00 and 0.12 ± 0.01 mg g^{-1} DW (mean 0.06 ± 0.01 mg I-DIN g^{-1} DW). Linear regression did not reveal a significant relationship between I-DIN content and nitrate concentration for either N-saturated ($r = 0.196$, $R^2 = 0.038$, $p = 0.359$) or N-limited sporophytes ($r = 0.034$, $R^2 = 0.001$, $p = 0.880$).

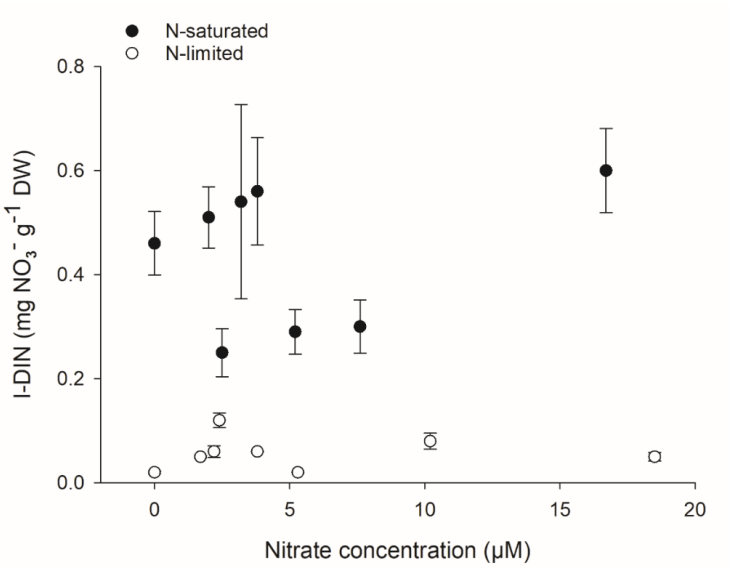

Figure 6 Mean intracellular nitrate concentration (I-DIN, mg g^{-1} DW) for the different initial incubation concentrations (µM) after 300 min incubation of N-saturated and N-limited sporophytes. Mean ± SE, n = 3.

The quantitative nitrogen amount accumulated in I-DIN pools was low compared to total Q_N of the algae. Percentage I-DIN of total intracellular N (Q_N) was 1.6% for N-saturated and 0.02% for N-limited sporophytes. I-DIN is accordingly not an important nitrogen storage that can support algal growth for a long time. However, it does reflect the nutritional nitrogen state of the algae.

4. Discussion

We found that sporophytes of *S. latissima* with deficient internal nitrogen pools (Q_N and I-DIN) exhibited higher uptake rates of NO_3^- than sporophytes with higher internal nitrogen pools. For both nutrient-deficient and -saturated sporophytes, the uptake rates of nitrate were linearly related to the substrate concentration. No clear saturation level was found for nitrate concentrations exceeding double the maximum deep-water concentration potentially experienced in natural North-East Atlantic water. A mathematical model was used to investigate the temporal development of Q_N for N-saturated and N-limited sporophytes based on the nitrogen-specific uptake rates (U), and revealed a near linear response of U to changes in Q_N. The model also found that a maximum estimated value for Q_N was only approached after more than 20 days at external NO_3^- concentrations of 8 µM.

4.1 Uptake kinetics of nitrate

The uptake of NO_3^- has generally been found to exhibit rate-saturation with increasing concentrations, suggesting an active transport mechanism (Chapman et al. 1978; Phillips and Hurd 2004). This is not supported by the findings in our study, as the uptake of NO_3^- showed an unsaturated response with a linear increase for both N-saturated and N-limited sporophytes, even for nitrate concentrations higher than the maximum concentrations found in North-East Atlantic deep-water (Voss et al. 2013; Ibrahim et al. 2014). Saturation will, however, most likely be met for higher nitrate concentrations and for a longer uptake period, and an indication of an upcoming saturation could be seen for the N-depleted sporophytes at the highest N-concentration

It appears that several kelp species do not exhibit saturation kinetics for nitrate uptake for nutrient concentrations well beyond the highest natural concentrations (Harrison et al. 1986). This has been found for *Laminaria groenlandica* (Harrison et al. 1986), *Macrosystis pyrifera* (Kopczak 1994) and *Eisenia arborea* (Sánchez-Barredo et al. 2011). It has been suggested that this characteristic kinetic response is mainly dependent on the nutritional state and/or life-history of the individuals rather than the species (Thomas et al. 1985). Moreover, for intertidal seaweeds from New Zealand, both saturated and non-saturated uptake patterns were found for different individuals of the same population (Phillips and Hurd 2004), supporting this idea. While other studies have run experiments with NO_3^- concentrations from 30-450 µM for various species (Harrison et al. 1986; Martínez and Rico

2004; Phillips and Hurd 2004; Abreu et al. 2011; Li et al. 2019), the maximum NO_3^- concentrations of 16-18 µM in our experiment were much lower, but still above 2-8 µM, which is the ecologically relevant range of concentrations along the Norwegian coast during the winter and early spring when cultivated *S. latissima* has its highest growth rates (Broch et al. 2019; Forbord et al. 2020).

The affinity and uptake capabilities were lower for N-saturated than N-limited sporophytes, in agreement with several previous studies (Druehl et al. 1989; McGlathery et al. 1996; Ahn et al. 1998). It has also been found that nitrate uptake in macroalgae generally proceeds at considerably lower rates than the uptake of ammonium (NH_4^+) for both N-saturated and N-limited sporophytes (Pedersen and Borum 1997; Corey et al. 2013; Liu et al. 2016). A recent experiment showed that when offered both NH_4^+ and NO_3^- simultaneously, the sporophytes selectively took up NH_4^+ at a higher rate until the concentration became low (~3µM). The uptake of NO_3^- then proceeded at a lower rate than when just offered alone (Etter et al., unpublished data).

It is important to consider type of tissue, age, nutrient preconditioning, biomass-to-volume of medium during incubation, incubation time and normalization of uptake rates when comparing published results from different experiments.

4.2 Nitrogen-specific uptake rates and simulated Q_N dynamics

The value of the nitrogen-specific uptake rate (U, day^{-1}) during steady-state of growth is equal to the carbon specific growth rate (µ) and can reflect the capacity of *S. latissima* to sustain growth at given ambient concentrations of nitrate even though uptake and growth are not directly coupled. Thus, under steady state conditions, when the processes of uptake and growth are in balance and gives a constant Q_N, an estimated U value can potentially correspond to the steady state growth rate (µ, day^{-1}) that is achievable by the algae at that concentration. The sporophytes in the current experiment were acclimated to nutrient deficient and nutrient sufficient conditions, aiming to achieve growth at steady state conditions. Figure 3 shows the potential maximum relative growth rates (RGR, day^{-1}), equal to U during steady state, which can be achieved for given nitrate concentrations and nutritional states, reflected by Q_N. The current U values correspond well with the RGR (day^{-1})

of cultivated *S. latissima* based on an increase in length in the period from May-June in Central Norway, where the RGR fluctuated between 0.02 and 0.05 day^{-1} (Forbord et al. 2019). The ambient NO_3^- concentration is found to vary between 0.4-6.6 µM in that region in that particular time period (Forbord et al., unpublished data).

It appears that *S. latissima* requires high ambient NO_3^- concentrations for maintaining rapid growth and are not able to compete for available nutrients with the more efficient phytoplankton during late spring and summer. This means that *S. latissima* have to take up most of the NO_3^- needed early in the season when ambient concentrations are high, and that the period after the spring bloom represents a negative shift in nutrient uptake that involves sporophyte growth mainly based on internal inorganic- and organic nitrogen components. The coupling between extracellular nitrate concentration, initial NO_3^- uptake kinetics, intracellular NO_3^- concentration and specific growth rate is as far as we understand new fundamental knowledge which makes us better understand the ecological niche and the nutritional requirements of *S. latissima.* Such quantitative knowledge is paramount for understanding both the nutrient- and environmental conditions required for efficient cultivation of *S. latissima* as it will have an essential importance for future large-scale production. This also underscore the importance of careful site selection and for finding the best suited cultivation and harvesting periods for optimal nutrient utilization.

Our experimental results did not reveal whether the value of U supporting the maximum growth rate (U_{max}) was linearly or non-linearly related to the nutritional state of the algae, expressed in terms of Q_N. Under the assumptions made, U can be assumed to be a linear function of both Q_N and the ambient nitrate concentration in the [19, 29] interval (Figure 3). It is important to note that the relations between Q_N and uptake capabilities are most uncertain for minimal and maximal values of Q_N.

4.3 Total intracellular nitrogen content (Q_N)

The N-limited sporophytes had significantly lower Q_N (19.7 mg N g^{-1} DW) than the N-saturated ones (29.5 mg N g^{-1} DW), as a result of the preconditioning treatments, and in agreement with Gerard (1997). Bleaching of tissue, as observed for our N-limited sporophytes after 8 days of starvation, suggested severe N-limitation, and was most likely due to the loss of pigment-protein complexes (Chapman et al. 1978; Hanisak 1990). The

affinity is unlikely to reach zero, reflecting zero uptake, meaning that the algae will exhibit a minimum affinity for Q_N below 36 mg N g^{-1} DW. In the same way, under steady state conditions where U = μ, it is expected that algae can maintain a maximum growth rate at a similar Q_N value (c.f., Q_M for microalgae; Droop 1968). This means that our N-saturated sporophytes were close to being nitrogen-sufficient, with capabilities of growing close to their maximum growth rate. A Q_N content of 35 mg N g^{-1} DW has been previously reported for N-saturated, juvenile *S. latissima* (Gerard 1997), which also agrees well with the results from our model.

The content of Q_N has been found to increase with increasing ambient nitrogen concentrations (Young et al. 2007; Martínez et al. 2012), and has been suggested to act as a reliable indicator of the physiological nutritional state of the seaweeds (Manns et al. 2017; Forbord et al. 2020). The feasibility of Q_N as an indicator of nutritional state for *S. latissima* has been previously shown over longer cultivation periods along the Norwegian coast where Q_N was highest early in the season and at greater depths where nitrate was not yet depleted by phytoplankton blooms (Forbord et al. 2020). The decline in Q_N content in macroalgae during the spring and summer are a consequence of metabolic demands exceeding nutrient uptake during growth.

During the course of the uptake experiment, Q_N would potentially increase only by 0.7% after 90 min of incubation given the highest uptake rates (V) for N-limited sporophytes, and even less for the N-saturated ones (0.3%). This shows that altering the value of Q_N is a slow process and that the initial steady state situation is not greatly affected during the experiment. Moreover, a maximum value for Q_N estimated from the mathematical model was only approached after more than 20 days at external NO_3^- concentrations of 8 μM. It therefore follows that the reduced nitrate uptake beyond 90 min incubation was mainly an effect of a reduced ambient nitrate concentration and not an effect of increased feedback on uptake following increased Q_N, reflecting nutritional state. Our results are in line with findings showing no significant changes in total Q_N content one week after nitrate resupply in two *Fucus* species that were N-deprived for 15 weeks (Young et al. 2009).

4.4 Intracellular nitrate content (I-DIN)

Our results revealed that young, cultivated sporophytes of *S. latissima* did not hold large

reserves of intracellular nitrate (I-DIN) at the conditions of our experiment. We also found that neither of the sporophyte-groups exhibited a significant increase in the content of I-DIN, even after 300 min exposure to more than 16 µM of ambient nitrate.

Potentially, if all nitrate taken up during the experiment at the highest concentration was accumulated directly in the I-DIN pools, the N-saturated sporophytes would increase their I-DIN content by 17%, which lies within the range of the I-DIN concentrations measured after the experiment. This suggests that the N-saturated sporophytes could store most of the surplus nitrate accumulated in the algae without any need for reduction and incorporation in biomolecules. On the other hand, if the N-limited sporophytes stored all of the nitrate taken up during the experiment at the highest concentration, their I-DIN pool would increase by over 700%, which was not the case. As a result, we can infer that the intracellular equilibration process between N-compartments is a slow process and that the sporophytes must have incorporated nitrate taken up after perturbation into small molecular N-components, amino acids and pigments rather than depositing nitrate in the vacuoles.

The highest measured I-DIN value of 0.6 mg NO_3^- g^{-1} DW for N-saturated sporophytes was comparable to the higher range of values found for nitrogen-sufficient sporophytes of *S. latissima* (Jevne et al., unpublished data), measuring the I-DIN content in adult, first-year sporophytes that had been supplied with nutrient rich deep-water and maintained in tanks at relatively low light intensities. Similarly, cultivated *S. latissima* at sea followed a seasonal I-DIN pattern with the highest content in spring at a depth of 8-9 m (0.7 mg NO_3^- g^{-1} DW) compared to barely detectable contents towards the summer when ambient nitrate was depleted (Forbord et al. 2020). As for Q_N, I-DIN can be used to express the nutritional state of the alga because 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 data). I-DIN concentration is also easily measurable.

The current study did not show a positive linear relationship between I-DIN content and increasing substrate concentration for either of the two pre-treatments during 300 min of perturbation, confirming the slow storage process. This is supported by Naldi and Wheeler (1999), who found a significant increase in I-DIN only after 8-9 days of nitrogen enrichment in *Ulva fenestrata* and *Gracilaria pacifica*, resulting in an increase from < 1% to 7% I-DIN of total

Q_N for the former species and from < 1% up to 2% of Q_N for the latter species. I-DIN contributed to only 1.6% of Q_N for N-saturated and 0.02% of Q_N for the N-limited sporophytes in the current experiment.

New and existing growth models for *S. latissima* and other commercially important seaweeds (Broch and Slagstad 2012; Hadley et al. 2015; van der Molen et al. 2018) will always require updates and further development. Information like the seaweed's nutrient uptake capabilities of ecologically relevant concentrations can support users in finding suitable cultivation sites, validate their production expectations and in the longer term predict the chemical composition like e.g. the amount of harvestable proteins. Nutrient uptake rates can also be used when calculating the seaweeds capability and efficiency for bioremediation, especially in areas with heavy nitrogen run-off from land. Experimental studies under controlled conditions are crucial to further explore the importance of nitrogen uptake and will strengthen the understanding of macroalgae's nutrient demand (Lubsch and Timmermans 2019).

5. Conclusions

Seaweed-based ecosystems are potentially very productive. However, this productivity must be sustained through the acquisition and utilization of nutrients, particularly nitrogen. *S. latissima* is abundant along the Norwegian coast and has its highest growth and nutrient uptake rates during periods with high ambient nitrate concentration, typically late autumn to early spring, dependent on latitude. The nitrate concentrations of 2-8 µM used in the current experiment were ecologically relevant for Norwegian coastal waters, but saturation kinetics were not found for these concentrations or for concentrations twice as high as the maximum values found in the natural environment of *S. latissima*. This indicates that *S. latissima* cannot compete efficiently for nitrate in the late spring and summer, where nutrient starvation is experienced and an increase in biomass in these periods is based on intracellular nitrogen components ($U < \mu_m$), cf. Equation 5. This pool of intracellular nutrients mainly consists of nitrogen-containing compounds like proteins and pigments, and stored inorganic nitrate to a smaller extent, which most likely needs a long time of increased uptake at high ambient nitrate concentrations to reach high levels. The conservative growth

strategy and low nutrient uptake of *S. latissima* is ecologically advantageous in physically stable environments with restricted but predictable nutrient resource availability, like in North-Atlantic coastal waters during winter. Knowledge of nitrate uptake kinetics and utilization capabilities in *S. latissima* are important aspects for a sustainable scaling up of seaweed farm production. This enables the farmers to identify potential locations for their cultivation, to estimate the carrying capacity of the cultivation site, and could predict the potential biomass production based on the nutritional history of the seaweed.

Acknowledgements

This work was funded by the Research Council of Norway project no. 254883 (MacroSea). We are grateful to Seaweed Energy Solutions for providing experimental biomass, to the staff at Trondhjem Biological Station for help with setting up the laboratory facilities, to Marte Schei (SINTEF Ocean) for analyzing the CN samples and to Aleksander Handå and Jorunn Skjermo (SINTEF Ocean) for their valuable input during the planning phase of the experiment.

Conflicts of Interest

Declaration of interest: none

6. References

Abreu MH, Pereira R, Buschmann A, Sousa-Pinto I, Yarish C (2011) Nitrogen uptake responses of *Gracilaria vermiculophylla* (Ohmi) Papenfuss under combined and single addition of nitrate and ammonium. J Exp Mar Biol Ecol 407 (2):190-199

Ahn O, Petrell RJ, Harrison PJ (1998) Ammonium and nitrate uptake by *Laminaria saccharina* and *Nereocystis luetkeana* originating from a salmon sea cage farm. J Appl Phycol 10 (4):333-340

Broch OJ, Alver MO, Bekkby T, Gundersen H, Forbord S, Handå A, Skjermo J, Hancke K (2019) The kelp cultivation potential in coastal and offshore regions of Norway. Front Mar Sci 5:529

Broch OJ, Slagstad D (2012) Modelling seasonal growth and composition of the kelp *Saccharina latissima*. J Appl Phycol 24 (4):759-776

Chapman A, Markham J, Lüning K (1978) Effects of nitrate concentration on the growth and physiology of *Laminaria saccharina* (Phaeophyta) in culture. J Phycol 14 (2):195-198

Corey P, Kim JK, Duston J, Garbary DJ, Prithiviraj B (2013) Bioremediation potential of *Palmaria palmata* and *Chondrus crispus* (Basin Head): effect of nitrate and ammonium ratio as nitrogen source on nutrient removal. J Appl Phycol 25 (5):1349-1358

Directorate of Fisheries (2020) Akvakulturstatistikk (tidsserier)-Alger (*in Norwegian*). http://www.fiskeridir.no/Akvakultur/Statistikk-akvakultur/Akvakulturstatistikk-tidsserier/Alger. Accessed 30.01 2020

Droop MR (1968) Vitamin B 12 and marine ecology. IV. The kinetics of uptake, growth and inhibition in *Monochrysis lutheri*. J Mar Biol Assoc UK 48 (3):689-733

Druehl L, Harrison P, Lloyd K, Thompson P (1989) Phenotypic variation in N uptake by *Laminaria groenlandica* Rosenvinge (Laminariales, Phaeophyta). J Exp Mar Biol Ecol 127 (2):155-164

FAO (2018) The global status of seaweed production, trade and utilization, vol 124. Globefish Research Programme. Rome

Forbord S, Matsson S, Brodahl GE, Bluhm BA, Broch OJ, Handå A, Metaxas A, Skjermo J, Steinhovden KB, Olsen Y (2020) Latitudinal, seasonal and depth-dependent variation in growth, chemical composition and biofouling of cultivated *Saccharina latissima* (Phaeophyceae) along the Norwegian coast. J Appl Phycol. doi:10.1007/s10811-020-02038-y

Forbord S, Skjermo J, Arff J, Handå A, Reitan K, 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 (3):393-399

Forbord S, Steinhovden KB, Rød KK, Handå A, Skjermo J (2018) Cultivation protocol for *Saccharina latissima*. In: Charrier B, T W, Reddy C (eds) Protocols for Macroalgae Research,. 1st edn. CRC Press, Taylor Francis Group, Boca Raton, FL, London, New York, NY, pp 37-59

Forbord S, Steinhovden KB, Solvang T, Handå A, Skjermo J (2019) Effect of seeding methods and hatchery periods on sea cultivation of *Saccharina latissima* (Phaeophyceae): a Norwegian case-study. J Appl Phycol. doi:10.1007/s10811-020-02042-2

Gerard VA (1997) The role of nitrogen nutrition in high-temperature tolerance of the kelp, *Laminaria saccharina* (Chromophyta). J Phycol 33 (5):800-810

Grasshoff K, Kremling K, Ehrhardt M (2009) Methods of seawater analysis. Third edn. John Wiley & Sons, Weinheim

Hadley S, Wild-Allen K, Johnson C, Macleod C (2015) Modeling macroalgae growth and nutrient dynamics for integrated multi-trophic aquaculture. J Appl Phycol 27 (2):901-916

Hanisak M (1979) Nitrogen limitation of *Codium fragile* ssp. tomentosoides as determined by tissue analysis. Mar Biol 50 (4):333-337

Hanisak M (1983) The nitrogen relationships of marine macroalgae. In: Carpenter EJ, Capone DG (eds) Nitrogen in the Marine Environment. Academic Press, New York, pp 699-730

Hanisak MD (1990) The use of *Gracilaria tikvahiae* (Gracilariales, Rhodophyta) as a model system to understand the nitrogen nutrition of cultured seaweeds. Hydrobiologia 204 (1):79-87

Harrison P, Druehl L (1982) Nutrient uptake and growth in the Laminariales and other macrophytes: A consideration of methods. In: Srivastava LM (ed) Synthetic and Degradative Process in Marine Macrophytes. Walter de Gruyter Academic Press, pp. 99-120

Harrison P, Druehl L, Lloyd K, Thompson P (1986) Nitrogen uptake kinetics in three year-classes of *Laminaria groenlandica* (Laminariales: Phaeophyta). Mar Biol 93 (1):29-35

Harrison P, Parslow J, Conway H (1989) Determination of nutrient uptake kinetic parameters: a comparison of methods. Mar Ecol Prog Ser:301-312

Harrison PJ, Hurd CL (2001) Nutrient physiology of seaweeds: application of concepts to aquaculture. Cah Biol Mar 42 (1-2):71-82

Hurd CL, Harrison PJ, Bischof K, Lobban CS (2014) Seaweed ecology and physiology. 2 edn. Cambridge University Press, Cambridge

Ibrahim A, Olsen A, Lauvset S, Rey F (2014) Seasonal variations of the surface nutrients and hydrography in the Norwegian Sea. Int J Environ Sci De 5 (5):496-505

Jones AB, Dennison WC, Stewart GR (1996) Macroalgal responses to nitrogen source and availability: amino acid metabolic profiling as a bioindicator using *Gracilaria edulis* (Rhodophyta). J Phycol 32 (5):757-766

Kerrison PD, Stanley MS, Edwards MD, Black KD, Hughes AD (2015) The cultivation of European kelp for bioenergy: Site and species selection. Biomass Bioenergy 80:229-242

Kopczak CD (1994) Variability of nitrate uptake capacity in *Macrocystis pyrifera* (Laminariales, Phaeophyta) with nitrate and light availability. J Phycol 30 (4):573-580

Lapointe BE, Duke CS (1984) Biochemical strategies for growth of *Gracilaria tikvahiae* (Rhodophyta) in relation to light intensity and nitrogen availability. J Phycol 20 (4):488-495

Li H, Zhang Y, Chen J, Zheng X, Liu F, Jiao N (2019) Nitrogen uptake and assimilation preferences of the main green tide alga *Ulva prolifera* in the Yellow Sea, China. J Appl Phycol 31 (1):625-635

Liu H, Wang F, Wang Q, Dong S, Tian X (2016) A comparative study of the nutrient uptake and growth capacities of seaweeds *Caulerpa lentillifera* and *Gracilaria lichenoides*. J Appl Phycol 28 (5):3083-3089

Lubsch A, Timmermans KR (2019) Uptake kinetics and storage capacity of dissolved inorganic phosphorus and corresponding dissolved inorganic nitrate uptake in *Saccharina latissima* and *Laminaria digitata* (Phaeophyceae). J Phycol 55:637-650

Manns D, Nielsen MM, Bruhn A, Saake B, Meyer AS (2017) Compositional variations of brown seaweeds *Laminaria digitata* and *Saccharina latissima* in Danish waters. J Appl Phycol:1-14

Martínez B, Pato LS, Rico JM (2012) Nutrient uptake and growth responses of three intertidal macroalgae with perennial, opportunistic and summer-annual strategies. Aquat Bot 96 (1):14-22

Martínez B, Rico JM (2004) Inorganic nitrogen and phosphorus uptake kinetics in *Palmaria palmata* (Rhodophyta). J Phycol 40 (4):642-650

McGlathery KJ, Pedersen MF, Borum J (1996) Changes in intracellular nitrogen pools and feedback controls on nitrogen uptake in *Chaetomorpha linum* (Chlorophyta). J Phycol 32 (3):393-401

Murray JD (2002) Mathematical Biology: I. An Introduction. 3 edn. Springer, New York

Naldi M, Wheeler PA (1999) Changes in nitrogen pools in *Ulva fenestrata* (Chlorophyta) and *Gracilaria pacifica* (Rhodophyta) under nitrate and ammonium enrichment. J Phycol 35 (1):70-77

NSF (1975) NS 4746 Water analysis. Determination of the sum of nitrite-nitrogen and nitrate-nitrogen (*in Norwegian*). Norges Standardiseringsforbund, Oslo, Norway

Olsen Y (1989) Evaluation of competitive ability of *Staurastrum luetkemuellerii* (Chlorophyceae) and *Microcystis aeruginosa* (Cyanophyceae) under P limitation. J Phycol 25 (3):486-499

Pedersen MF, Borum J (1997) Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake. Mar Ecol Prog Ser 161:155-163

Phillips JC, Hurd CL (2004) Kinetics of nitrate, ammonium, and urea uptake by four intertidal seaweeds from New Zealand. J Phycol 40 (3):534-545

Pueschel CM, Korb RE (2001) Storage of nitrogen in the form of protein bodies in the kelp *Laminaria solidungula*. Mar Ecol Prog Ser 218:107-114

Roleda MY, Hurd CL (2019) Seaweed nutrient physiology: application of concepts to aquaculture and bioremediation. Phycologia 58 (5):552-562

Sánchez-Barredo M, Ladah LB, Zertuche-González JA (2011) Nitrate uptake and duration of internal nitrogen reserves in the kelp *Eisenia arborea*. Bot Mar 54 (5):441-446

Stévant P, Rebours C, Chapman A (2017) Seaweed aquaculture in Norway: recent industrial developments and future perspectives. Aquacult Int 25 (4):1373-1390

Thomas T, Harrison P, Taylor E (1985) Nitrogen uptake and growth of the germlings and mature thalli of *Fucus distichus*. Mar Biol 84 (3):267-274

Thomas T, Harrison P, Turpin D (1987) Adaptations of *Gracilaria pacifica* (Rhodophyta) to nitrogen procurement at different intertidal locations. Mar Biol 93 (4):569-580

van der Molen J, Ruardij P, Mooney K, Kerrison P, O'Connor NE, Gorman E, Timmermans K, Wright S, Kelly M, Hughes AD (2018) Modelling potential production of macroalgae farms in UK and Dutch coastal waters. Biogeosciences 15 (4):1123-1147

Voss M, Bange HW, Dippner JW, Middelburg JJ, Montoya JP, Ward B (2013) The marine nitrogen cycle: recent discoveries, uncertainties and the potential relevance of climate change. Phil Trans R Soc B 368:20130121

Wallentinus I (1984) Comparisons of nutrient uptake rates for Baltic macroalgae with different thallus morphologies. Mar Biol 80 (2):215-225

Wheeler W, Weidner M (1983) Effects of external inorganic nitrogen concentration on metabolism, growth and activities of key carbon and nitrogen assimilatory enzymes of *Laminaria saccharina* (Phaeophyceae) in culture. J Phycol 19 (1):92-96

Young EB, Berges JA, Dring M (2009) Physiological responses of intertidal marine brown algae to nitrogen deprivation and resupply of nitrate and ammonium. Physiol Plant 135 (4):400-411

Young EB, Dring MJ, Savidge G, Birkett DA, Berges JA (2007) Seasonal variations in nitrate reductase activity and internal N pools in intertidal brown algae are correlated with ambient nitrate concentrations. Plant, Cell Environ 30 (6):764-774

Paper II

The effect of nutrient availability and light conditions on the growth and intracellular nitrogen components of land-based cultivated *Saccharina latissima* (Phaeophyta)

Lone Sunniva Jevne[1*], Silje Forbord[1,2], Yngvar Olsen[1]

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

[2] SINTEF Ocean, Department of Environment and New Resources, N- 7465 Trondheim, Norway

***** Corresponding author**:**

Lone Sunniva Jevne, lone.s.jevne@gmail.com

Abstract

The chemical composition of seaweed varies between seasons and depths as an effect of resource supply and other environmental conditions. These factors are particularly important to assess for economically feasible species like the kelp *Saccharina latissima*. This paper examines how differences in light conditions and nutrient availability affect the growth and intracellular nitrogen components in *S. latissima.* This was done through cultivating sporophytes in land-based tanks with four different combinations of high/low light and high/low nutrient supply over an experimental period of 20 days, with measurements of growth rate and subsequent analysis of tissue nitrogen metabolites. The low light treatments had a significantly lower median light intensity than the high light treatments, and the tanks receiving deep water (high nutrient) had a significantly higher concentration of external nitrate than the tanks receiving surface water (low nutrient). The results revealed that the mean growth rate and the intracellular nitrogen components of the sporophytes were positively related to the external nitrate concentration during the experimental period. Further, the growth rates were higher for the sporophytes grown under 'low light, high nutrient' conditions than those grown under 'high light, high nutrient' conditions. No positive correlation was found between growth rate and different light levels. This indicates that nitrate was the primary controlling factor for growth at both high and low light treatments. The pattern found for the contents of intracellular nitrogen components agreed with this conclusion.

Keywords

Growth rates, Intracellular nitrogen components, Light intensity, Nutrient availability, Protein content, *Saccharina latissima*, Seaweed aquaculture

1 Introduction

Seaweed cultivation is increasing rapidly along the North Atlantic coasts (Stévant et al. 2017; Grebe et al. 2019; Forbord et al. 2020), while farming of seaweed has been an established industry for decades in many Asian countries, producing almost 30 million metric tons per year (FAO 2018). Seaweed biomass is mainly used for human consumption and in the phycocolloid industry, but it also has a wide range of applications as feed ingredients, fertilizers, biofuels, and biochemicals (Skjermo et al. 2014; Barbier et al. 2019; Buschmann and Camus 2019). Deposition of seaweed biomass has even been considered for future carbon capture and storage (Bio-CCS, Krause-Jensen et al. 2018).

One of the most common and promising species for large scale cultivation in Norway is the kelp *Saccharina latissima* (Linnaeus) with a production of 174 metric tons in 2018 (Directorate of Fisheries 2020). It has a high potential of growth (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 a well described life cycle (Flavin et al. 2013; Redmond et al. 2014; Forbord et al. 2018). The growth rate of *S. latissima* varies over the seasons and the important factors for controlling growth are believed to be light intensity, ambient nutrient concentration, temperature, salinity, and seawater currents (Broch and Slagstad 2012; Bartsch et al. 2008; Xiao et al. 2019). In the same way that macroalgae have adapted to various light and temperature regimes, they have also adapted to different ambient nutrient levels. Because *S. latissima* can store nutrients as internal nitrate in the vacuoles (Hurd et al. 2014) and grow at relatively low temperatures, they benefit from growing in temperate areas where the nutrient availability varies over the seasons (Pedersen and Borum 1996; Gordillo 2012).

Nitrogen (N) can exist in different forms in seawater; as an inorganic form like nitrate (NO_3^-), nitrite (NO_2^-), or ammonium (NH_4^+); or as part of organic substances like dissolved (DON) or particulate (PON) nitrogen (Capone et al. 2008; Hurd et al. 2014). In temperate regions, the ambient concentration of NO_3^- will vary with depth and season, and will normally show an inverse trend to temperature, with a reduction in spring and summer due to uptake by phytoplankton (Rey et al. 2007; Young et al. 2007; Ibrahim et al. 2014; Broch et al. 2019). The opposite pattern is common for DON and PON (Sipler and Bronk 2015). For most

temperate areas, NO_3^- concentration will vary from undetectable to around 420 µg L^{-1} (Hurd et al. 2014). In the Trondheimsfjord (Central Norway) the concentration of NO_3^- is typically around 140 µg L^{-1} at 70 m depth across all seasons (Forbord et al. 2012), and remain at this level for deeper depths. This is a typical feature of Norwegian North East Atlantic deep-water, and 140 µg L^{-1} represent the highest natural nitrate concentration experienced by macroalgae.

Light intensity will affect the N uptake indirectly because the demanded energy for the active uptake and fixating of N to molecules and proteins comes from photosynthesis (Lapointe and Tenore 1981; Hurd et al. 2014). It has been shown that *S. latissima* grown between 10–15 ˚C at a high light intensity of 250 µmol m^{-2} s^{-1} showed a 50% lower growth rate compared with kelps grown at 110 µmol m^{-2} s^{-1}, which was found to be the optimum for photon flux in this temperature interval (Fortes and Lüning 1980). The degree of photo inhibition under conditions of high light exposure was shown to be proportional to light intensity and exposure time (Bruhn and Gerard 1996). Chapman et al. (1978) found that photosynthesis in *S. latissima* increased with increasing internal NO_3^- pools. Moreover, Chapman and Craigie (1977) found that the growth of *Laminaria longicruris* in temperate seawater increased during spring as a result of the high ambient NO_3^- concentrations during winter, assuming storage of NO_3^- in the tissues followed by depletion of NO_3^- reserves and decreased growth during the summer months.

The aim of this experiment was to examine how various light conditions and nutrient availability affected the growth and intracellular nitrogen components in cultivated *S. latissima*. This was carried out by growing sporophytes in land-based tanks with four different combinations of high/low light intensities and high/low nutrient supply over an experimental period of 20 days with subsequent analysis of tissue nitrogen metabolites. The chemical composition of macroalgae varies between seasons, environmental conditions, and depths, and these factors are particularly important to assess for economically feasible species like *S. latissima*.

2 Materials and methods

The experiment with *S. latissima* was carried out in the outdoor research facility of Seaweed Energy Solution AS (63°44'N, 10°34'E) in Trondheim, between May and June 2014.

2.1 Experimental design and sampling

2.1.1 Collection of sporophytes and experimental set up

The sporophytes used in the experiment were cultivated according to Forbord et al. (2018). The incubation time in the laboratory was 8 weeks and the seeded lines were deployed at sea in the outer coastal area of Central Norway (63°42'N, 8°52'E) in October–November 2013. The visible epiphyte-free sporophytes were harvested in May 2014, at an average frond length of 45.8 ± 2.4 cm (n = 40), and transported, cold and moist, to the experimental site. Immediately after arrival, the sporophytes were transferred to seawater filled tanks (r = 0.75 m, h = 0.75 m, volume = 1325 L) and were later fixed to ropes on PVC frames (100 x 50 cm) with cable ties and placed in the bottom of the tanks. The adult sporophytes used in the experiment were collected from the same location within a few days, assuming that the differences between them were minimal. The sporophytes that had visible mechanical damage from the sampling were discarded.

The experiment included four treatments, each with two replicate tanks. Two different sources of water were used: surface water to simulate growth in a nutrient depleted environment and deep water to simulate growth in a nutrient saturated environment. Plant cover filters (Axley) were used on top of the experimental tanks to simulate growth at high or low light levels and to ensure that the sporophytes did not suffer from photo inhibition. One layer of plant cover diminished light to 30% compared to no cover and represented high light intensity, while four layers of plant cover represented low light intensity. The treatments combined either surface water or deep water (low and high nutrient concentrations, respectively) and either one or four layers of plant cover filter (high and low light intensity, respectively). This gave four treatments with the following combinations of light intensity and water quality:

S1: Surface water and one layer of plant cover filter (‘high light, low nutrient’)

S4: Surface water and four layers of plant cover filter (‘low light, low nutrient’)

D1: Deep water and one layer of plant cover filter (‘high light, high nutrient’)

D4: Deep water and four layers of plant cover filter (‘low light, high nutrient’)

The environmental conditions of S1 represented the low nutrient situation in surface waters during late spring and summer, whereas D4 represented the high nutrient situation in deeper waters. The environmental conditions of S4 and D1 are more uncommon in nature, but were biologically interesting to include.

The surface water was pumped from 1 m and the deep water from 100 m depth from the fjord outside the experimental location. The flow through rate in the tanks was set to 3 L min^{-1} and gave a water exchange rate in the tanks of 3 times per 24 h. To even out the variation in temperature that occurred due to the different depth of the water sources, the surface water was pumped through a cooling system placed in the deep-water basin before transported to the experimental tanks. Each tank had an aeration system at the bottom to ensure water circulation. The experimental tanks were placed in an order of 3 × 3 on a pier with direct temporal exposure sunlight from south and east (Figure 1). One of the replicate tanks for each treatment was placed on the edge of the pier facing east or south and the rest in the middle to spread the light exposure among the replicas.

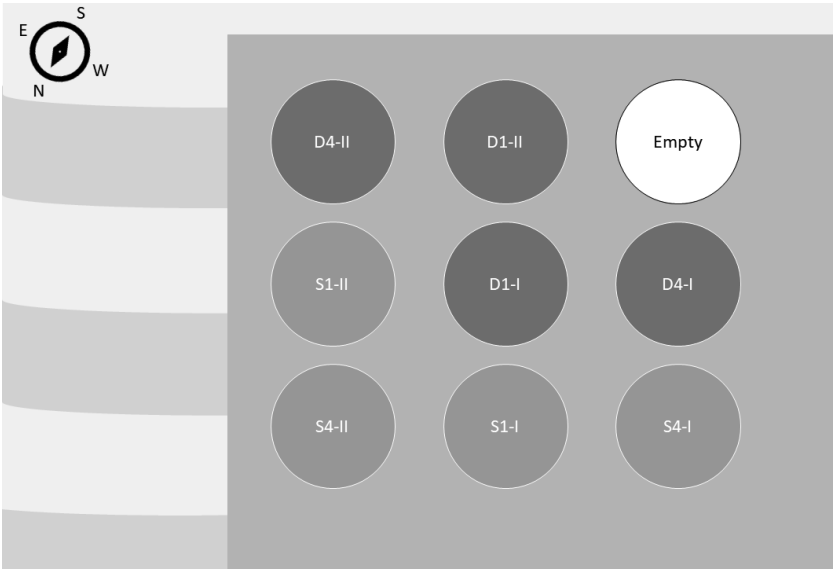

Figure 1 Experimental tank placement. High nutrient treatments (deep water; D) are shown in dark grey circles and low nutrient treatments (surface water; S) are shown in light grey circles. High light treatments are marked with 1, representing one layer of plant cover. Low light treatments are marked with 4. The two replicate tanks are marked with I and II. Stripes illustrate the sea.

2.1.2 Sampling and measurements

The sporophytes were acclimatized to the new culture conditions for 4 weeks after harvesting and transfer to the experimental tanks. Subsequently, five individuals from each tank were collected every fifth day for chemical analysis (four times over 20 days in total). The sporophytes were placed in pre-labelled zip-lock bags and frozen at -18 °C until analyzed. Frond elongation was measured using the punch hole method at each sampling day (Parke 1948). The growth rate in terms of length (mm day^{-1}) in periods between sampling was calculated based on length measurements.

2.1.3 Environmental conditions

Onset Application HOBO loggers (temperature accuracy ± 0.53 °C, resolution 0.14 °C) placed in the bottom of the tanks were used to log temperature (°C) and light intensity (LUX) every 15 min throughout the experimental period. Light was converted from LUX to PAR with the

conversion factor for blue sky only (Lux × 52^{-1}) (Thimijan and Heins 1983). Light was then averaged for light received between 08:00–20:00 to exclude night-time values. One of the loggers malfunctioned during the experiment and light levels were not captured in that tank (S4-I). The light intensity was in addition measured before start in one of the tanks with one layer of plant cover filter with a spectrophotometer (JAZ, Ocean Optics). The temperature at the surface of the tanks was measured manually once a week as a control. Water samples were taken for nutrient analysis twice a week from the tanks and filtered through a 0.2 µm polysulfone filter before being frozen at -18°C until analyzed.

2.2 Chemical analysis.

Five semi-frozen individual sporophytes from each sampling were milled with a kitchen grinder (Kenwood PRO 1500) or a hand-blender and put back in the freezer.

2.2.1 Dry weight

The dry weight (DW) was determined by weighing samples of 1.19 ± 0.01 g (mean ± SE) algal material before and after drying for 24 h at 70 °C in a Fermaks oven.

2.2.2 Carbon and nitrogen analysis

Carbon and nitrogen (CN) contents were analyzed for the whole thalli. The samples were dried for 24 h at 70 °C in a Fermaks oven and milled (MFC mill, Janke & Kunkel) into a fine powder that was used for further analysis. Samples of 0.4–1.0 mg dried kelp powder were transferred to tin capsules and CN was analyzed in quadruplicate with a Carlo Erba Element Analyzer (model 1106).

2.2.3 Protein content

The nitrogen component was used to calculate the protein content of the samples by using a nitrogen-to-protein conversion factor (K_p) of 3.8. This factor was determined based on measurements of total amino acids for locally cultivated *S. latissima* sporophytes (Forbord et al. 2020). Protein results that are further discussed in this paper are based on this method. Protein content was also analyzed with the colorimetric Bio-Rad DC Protein Assay according to the manufacturer's specification (BIO-RAD 2013). This Bio-Rad method is an improved version of the Lowry assay (Lowry et al. 1951), and the results were compared with the

protein estimates based on nitrogen and the K_p factor. In the Bio-Rad protein extraction procedure, semi-thawed algae material (1.16 ± 0.01 g, mean ± SE) were measured and transferred to 15 mL centrifuge tubes (Cellstar) with triplicates from each sample. Sodium hydroxide (NaOH, 0.5 M, 10 mL) was added to the tubes, which were then vortexed before they were frozen in liquid nitrogen. The tubes were thawed at room temperature, boiled for 30 min, cooled to room temperature, and stored in a refrigerator for 24 h. One hour before centrifuging the samples, they were shaken and put in the refrigerator again. The samples were then centrifuged for 15 min (4000 RCF) in a table centrifuge (Kendro megafuge). The supernatants were extracted and kept at -18 ˚C. NaOH (0.5 M, 10 mL) was added to the precipitates, which were then shaken, boiled for 30 min and cooled for 24 h. This was repeated until the supernatants from 3 rounds were collected in the same tube for each parallel. From the thawed supernatant, 100 µL was pipetted into glass test tubes. There were two parallels for each collection of supernatants. BIO-RAD DC reagent A (500 µL) was added to the test tubes and they were vortexed. Then, 4.0 mL BIO-RAD DC reagent B was added and the tubes were vortexed again. After 15 min the absorbance was read at 750 nm in a spectrophotometer (JENWAY 6715 UV/Vis). Absorbance values were used to calculate the concentration of proteins in the algal material, through comparison with a standard curve of absorbance measurements from known concentrations of bovine serum albumin.

2.2.4 Intracellular dissolved nitrate (I-DIN) and small molecules of intracellular organic nitrogen (I-DON)

Semi-frozen *S. latissima* material (0.12 ± 0.00 g; mean ± SE) from each sample was transferred to a test tube with a cork and filled with 12 mL of distilled water. The samples were boiled for 30 min, cooled, and filtered through a 0.45 µm polysulfone syringe filter to remove algal debris. The concentration of intracellular dissolved nitrate (NO_3^-, NO_2^-) in 0.6 mL of extract was determined using an auto analyzer (Flow Solution IV System, O.I Analytical), according to Norwegian Standard 4745 (NSF 1975). The remaining 0.6 mL of extract was oxidized by adding sodium hydroxide (3 mL) and potassium peroxide sulphate (12 g L^{-1}), autoclaving for 20 min, and analyzing for nitrate using the auto analyzer. The small molecules of intracellular organic nitrate (I-DON) were calculated as the difference between the total dissolved nitrate and inorganic nitrate in the same sample.

2.2.5 External inorganic nitrate (E-DIN)

The water samples from the experimental tanks were analyzed for dissolved nitrate in the water (E-DIN) using the auto analyzer, as described above. In order to better capture the environment that the sporophytes were grown in, the E-DIN measures were calculated as the moving average of the last 5 measurements.

2.3 Statistics

Variables were checked for normality (using a Shapiro-Wilk test), outliers, and homogen variance between groups (using a Levene's test). Based on these tests, the variables I-DIN, E-DIN, and total intracellular nitrogen (Q_N, mg N g^{-1}DW) were subjected to transformation by the natural logarithm to conform to normality inside groups, no outliers, and homogeneity of the variances. Variances among treatments were tested with a two-way ANOVA with planned contrasts: first between water sources, then between light sources among the same water treatments. The variables tested with this two-way ANOVA were I-DON, Protein, Growth, and the logarithmically transformed variables ln(I-DIN) and ln(E-DIN +1). The assumptions of heteroscedasticity and normality of the errors were checked in residual plots. Where there was evidence of heteroscedasticity, a linear model was fitted using generalized least squares, where it is possible to allow for different variances in the grouping factors. The variables Q_N and Q_C (mg C g^{-1}DW) did not show normality in each treatment and were tested with Kruskal-Wallis tests, and post hoc multiple comparisons were checked by Dunn's test with Bonferroni adjustment. A comparison of protein yield between two different methods was tested by an independent-samples t-test.

Relationships between variables were investigated by linear regression or by correlation, where the fit did not hold the assumptions of random error and homoscedasticity for the residuals. Pearson's correlation (r) was used if the assumptions of linear relationship, no outliers, and normality among the variables held. Where the assumption about linearity and outliers were violated but the variables showed a monotonic relationship, Spearman's rank correlation (r_s) was used.

All statistical analysis were done in R (3.6.1; R Core Team 2019) except the independent-samples t-tests, which were run in IBM SPSS Statistical software (Version 25). The significant

threshold was α = 0.05. Values are given as an average of the duplicate tanks ± standard error (SE), if not otherwise specified. For values given as median, the variation is given as ± median absolute deviation (MAD). Effect sizes are given as Cohen's d (Sullivan and Feinn 2012). In addition to R, plots were made using Systat SigmaPlot software (version 14).

3 Results

3.1 Light conditions

The tanks exposed to reduced light conditions (D4 and S4) had a median PAR intensity of 13.9 ± 8.1 µmol m^{-2} s^{-1} (median ± MAD), whereas the median for tanks exposed to higher light (D1 and S1) was 34.2 ± 22.9 µmol m^{-2} s^{-1} (Figure 2). The PAR intensity was significantly different between the two treatments (H_1 = 48.9, $p < 0.001$). Multiple comparisons of the mean ranks between treatment groups showed that the light intensity only differed significantly between groups set up with different light regime, but not within these regimes (H_3 = 54.3, $p < 0.001$).

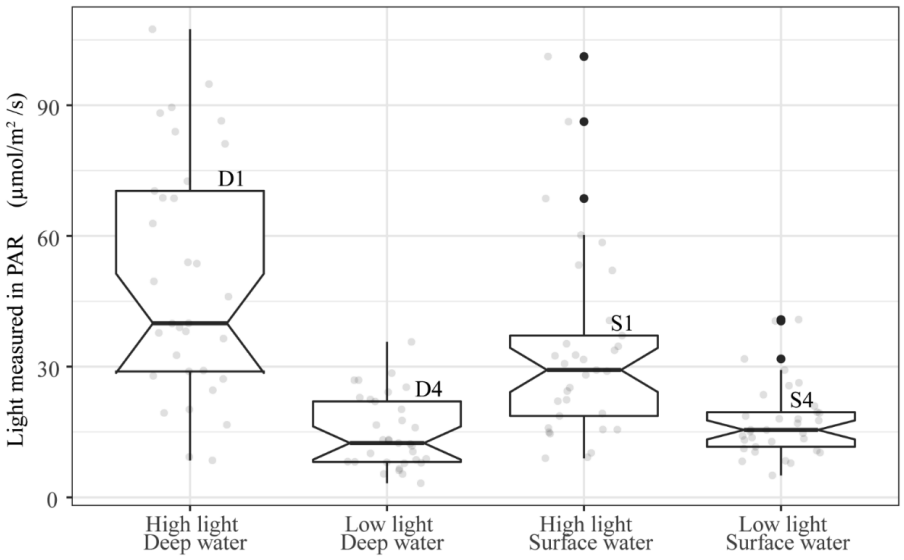

Figure 2 Mean light intensity (PAR, µmol m^{-2} s^{-1}) for the treatments in the experimental period (1st–19th of June 2014). The individual mean light intensities each day (08:00–20:00) is shown as light gray dots in the plot. Notches extend 1.58 * IQR/sqrt(n), giving roughly a 95%

CI. Whiskers extend from the hinge to the largest or smallest value within 1.5*IQR from the hinge. Values outside this are shown as a black dot.

3.2 Temperature

The mean water temperature was 10.2 ± 0.1 °C in tanks receiving deep water (D1 and D4) and 13.1 ± 0.2 °C in tanks receiving temperature acclimated surface water (S1 and S4, Figure 3). The differences in temperature were relatively small, and a significant difference was found between the water sources ($H_3 = 86.3$, $p < 0.001$), but not within water source ($p > 0.05$).

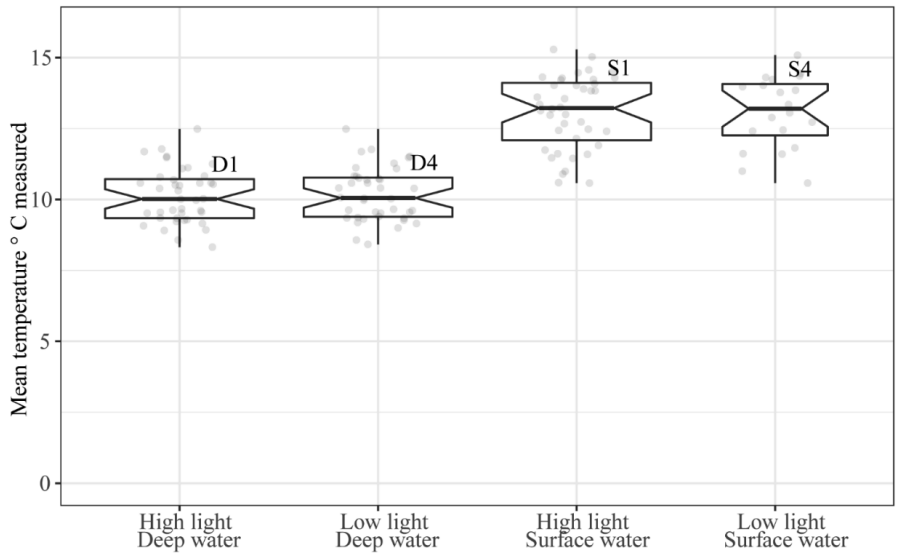

Figure 3 Mean water temperature (°C) in the experimental period (1st–19th of June 2014). Boxplot width is scaled for number of observations, and the mean individual temperature measurements are shown as light gray dots in the plot. Notches extend 1.58 * IQR/sqrt(n) giving roughly a 95% CI. Whiskers extend from the hinge to the largest or smallest value within 1.5*IQR form the hinge.

3.3 External nitrate concentration (E-DIN)

The external nitrate concentration ($NO_3 + NO_2$; E-DIN; µg NO_3-N L^{-1}), increased with time, but this increase was only significant for treatments receiving surface water ($r = 0.88$, $p < 0.01$).

Figure 4 summarizes the range of values, medians, and quartiles found for the different treatments in the experimental period. It illustrates higher E-DIN concentrations and variability in D4 compared to D1; both received deep water but were kept at different light intensities. Nitrate levels differed significantly between the tanks supplied with deep water (median ± MAD 36.6 ± 20.8) and those receiving surface water (median ± MAD 5.07 ± 3.56, $H_3 = 25.6$, $p < 0.001$). The difference found for tanks supplied with deep water was unexpected and likely a result of variable supply rate of deep water to the individual tanks. Although the difference was not statistically significant, it created a gradient in nitrate concentration in the deep-water treatments (D1 and D4) that were useful for understanding the interactions of light and nutrient supply with the sporophytes.

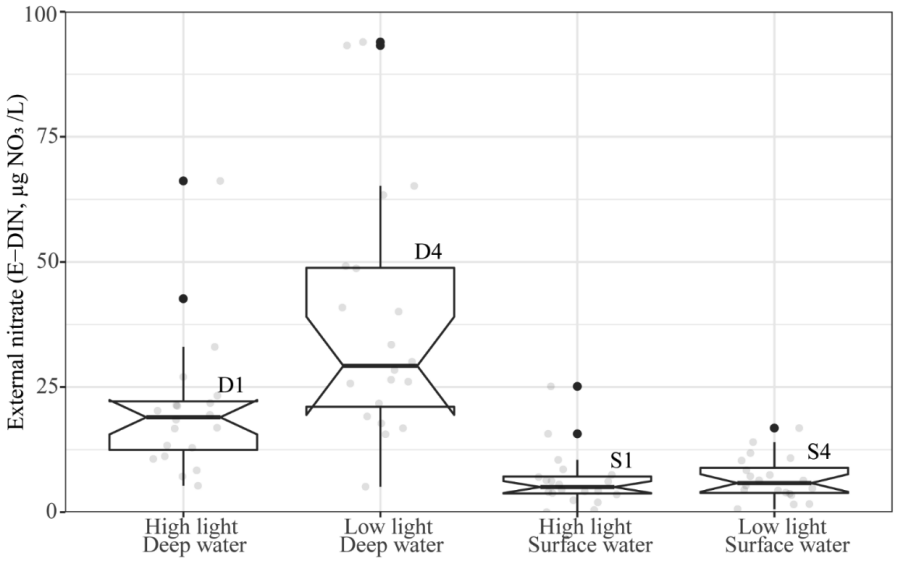

Figure 4 Concentration of external nitrate (E-DIN, µg NO_3-N L^{-1}) in different treatments (D1, D4, S1, S4) during the acclimation and experimental period (11th of May–19th of June 2014). Boxplot show lower, middle, and upper quantiles. Notches extend to median ± 1.58 * IQR /sqrt(n) and gives a rough 95% confidence interval for the median. Whiskers extend from the hinge to the largest or smallest value within 1.5*IQR form the hinge.

3.4 Growth rates

Figure 5 shows the mean growth rate in length (mm day^{-1}) of the seaweed for the different treatments. The mean growth rates in surface water (S1 and S4) were not significantly different for sporophytes grown in high light (0.273 ± 0.109 mm day^{-1}) and low light (0.220 ± 0.100 mm day^{-1}, d = 0.5, 95 % CI: -0.58 - 1.59, p > 0.05). However, for deep water (D1 and D4), sporophytes grown in low light showed a growth rate (0.744 ± 0.115 mm day^{-1}) significantly higher than those grown in high light (0.475 ± 0.164 mm day^{-1}, H_1 = 8.04, p < 0.01) and the effect size was very strong (d = -1.9, 95% CI: -3.2 - -0.61, p = 0.01). This may be a result of the higher nitrate concentrations for low light treatments compared to high light treatments (Figure 4), suggesting that nitrogen is most likely the primary limiting factor for growth in all treatments.

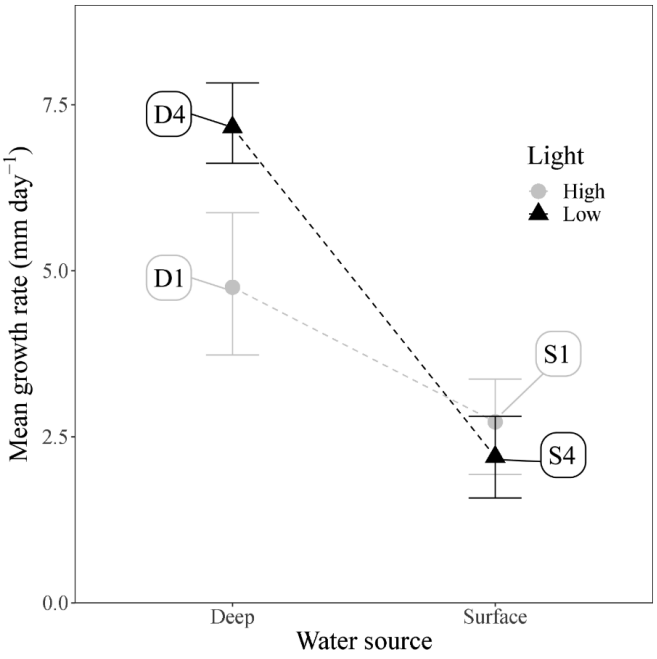

Figure 5 Mean growth rate in length (mm day^{-1}) obtained for sporophytes maintained at different light levels (high light level: black circle, low light: grey triangle) in surface and deep

water for two replicate tanks in the experimental period. Bars express 95% confidence interval based on non-parametric bootstrap (b=1000) on the different groups (n=2).

The regression of growth rate *versus* water type × light level was significant ($F_{3,28}$ = 29.3, $p < 0.001$, adjusted R^2 = 0.733). However, there was a small tendency of heteroscedasticity among the covariates and a linear model allowing for differences in variance between the variables was fitted using a generalized least square regression with maximum likelihood (see model in Table 3). The interaction term water type × light level was found to be significant ($F_{1,28}$ = 13.4, $p < 0.01$), with a small effect size of 0.17. This might suggest that the growth rate of the sporophytes was affected differently by light levels when grown in deep water, but not in surface water. It might also have been due to the nitrate gradient.

3.5 Intracellular N-components and external nitrate (E-DIN)

The measured intracellular nitrogen components showed a positive but variable relationship with E-DIN in the period before harvesting (I-DIN, Figure 6A). Sporophytes maintained in tanks supplied by surface water showed the lowest I-DIN levels (0.052 ± 0.009 mg N g^{-1} DW for S1 and S4), whereas those supplied by deep water showed highly variable contents (0.122 ± 0.027 mg N g^{-1} DW for D1 and 0.687 ± 0.136 mg N g^{-1} DW for D4). The highest values were found for sporophytes maintained in deep water at low light intensity and high E-DIN concentrations (D4, Figure 4). Regression analysis on the logarithmically transformed variables showed a significant positive relationship between ln(I-DIN) and ln(E-DIN +1) and a significant interaction between ln(E-DIN +1) and water source ($p < 0.001$, Table 1). Inclusion of the interaction term made the model significantly better ($p < 0.01$).

I-DON did not exhibit a clear relationship to E-DIN (Figure 6B). Values were scattered and sometimes very high for low E-DIN, suggesting that the N-deprived sporophytes may have undergone intracellular hydrolysis before or during analysis; it cannot be excluded that this also affected the I-DON values for less nutrient deprives sporophytes at high E-DIN. The pattern found for sporophytes maintained in deep water suggested saturation in I-DON above an E-DIN concentration of 30 µg N L^{-1}. Regression analysis on I-DON and the logarithmically transformed variable E-DIN did not show a significant positive relationship between the two (I-DON and ln(E-DIN +1), $p > 0.05$, Table 1).

Figure 6C shows Q_N (mg N g^{-1} DW) and protein contents (PROT, mg g^{-1} DW, proportional to Q_N) as a function of E-DIN, showing an overall positive correlation between the untransformed variables (r_s = 0.67, $p < 0.001$). The overall pattern suggested a saturation level for Q_N of ~20 mg N g^{-1} DW and ~80 mg protein g^{-1} DW for E-DIN concentrations above 30 µg L^{-1}. Sporophytes supplied with surface water (S1 and S4) had a mean total Q_N of 9.96 ± 0.41 mg N g^{-1} DW whereas those supplied by deep water had mean Q_N of 13.3 ± 0.77 mg N g^{-1} DW for D1 and 19.8 ± 0.48 for D4, corresponding to 37.8, 50.5, and 75.2 mg protein g^{-1} DW, respectively.

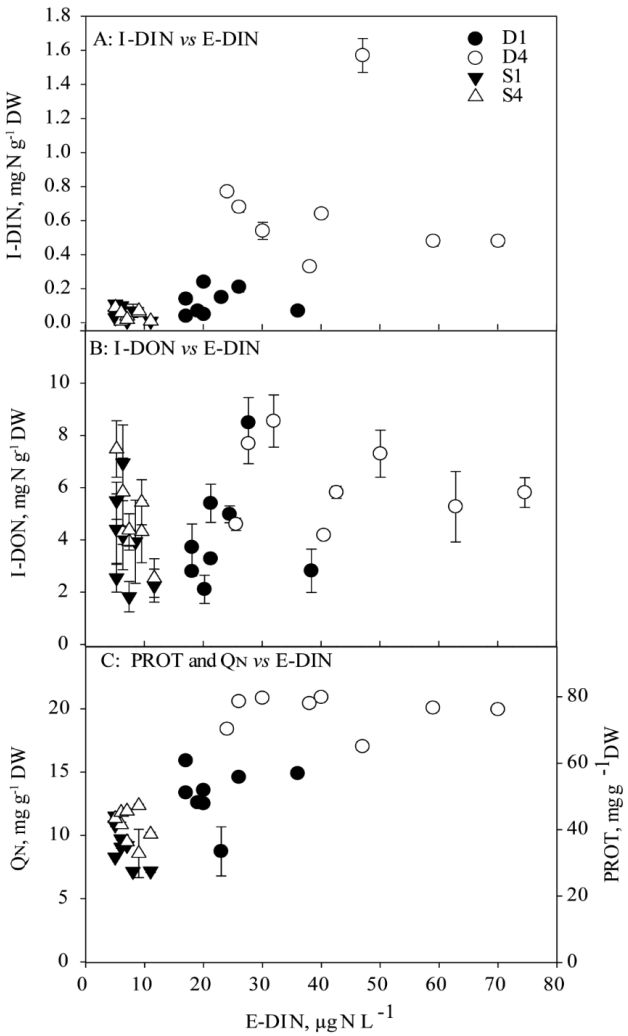

Figure 6 Intracellular nitrogen components as a function of mean external nitrate concentration (E-DIN, µg N L^{-1}). A: Intracellular nitrate (I-DIN, mg N g^{-1} DW), B: Intracellular organic small molecular nitrogen (I-DON, mg N g^{-1} DW), C: Total intracellular nitrogen (Q_N, mg N g^{-1} DW) and protein (PROT, mg protein g^{-1} DW). Mean E-DIN was estimated as the mean of the last five measurements. Bars express 1SE of the mean if exceeding symbols. Regression coefficients are given in Table 1.

Table 1 Regression coefficients derived by linear regression or Spearman correlation coefficient of internal cellular components. Intracellular nitrate (I-DIN, mg N g^{-1} DW), intracellular organic small molecular nitrogen (I-DON, mg N g^{-1} DW), protein contents (mg g^{-1} DW), total intracellular nitrogen (Q_N, mg N g^{-1} DW), and the moving average (last five measurements) of the nitrate concentration in the water (E-DIN, µg N L^{-1}), not the concentration on the day. Regressions and coefficients are marked with asterisks if significant (*: p< 0.05, ** p< 0.01, *** p< 0.001).

Relationship	Function	β_0 (±SE) β_1 (±SE) β_2 (±SE) β_3 (±SE)
ln(I-DIN) *vs* ln(E-DIN +1) *** R^2 (adjusted) = 0.65 (0.61)	f(y) = β_0+ β_1 * ln(E-DIN+1) + β_2 * water source + β_3 (water source * ln(E-DIN+1))	-6.64 (1.82)** 1.54 (0.53)** 7.42 (2.64)** -3.52 (1.06)**
I-DON *vs* ln(E-DIN+1) R^2 (adjusted) = 0.055 (0.024)	f(y) = β_0+ β_1* ln(E-DIN+1)	3.21 (1.22)* 0.569 (0.43)
Q_N (and K_p)*vs* ln(E-DIN+1)	Spearman correlation coefficient	0.73 ***
I-DON *vs* I-DIN	Spearman correlation coefficient	0.71 ***
PROT *vs* I-DIN	Spearman correlation coefficient	0.51**
Q_N *vs* I-DIN	Spearman correlation coefficient	0.68***

Because the protein content is based on Q_N, protein showed the same correlation with E-DIN as Q_N. Q_N and protein of treatments in different water sources were significantly different ($H_1 = 19.11$, $p < 0.001$). The highest values were found for sporophytes maintained in deep water at low light intensity, and comparisons between all groups ($H_3 = 24.47$, $p < 0.001$) with post hoc tests showed that D4 was significantly different from treatments supplied with surface water (S1 and S4, $p < 0.01$) and from the treatment supplied with deep water and high light (D1, $p = 0.05$).

The total carbon contents (Q_C, mg C g^{-1} DW) was 292 ± 4.4 g^{-1} DW in surface water (S) tanks and 278 ± 6.5 mg C g^{-1} DW in deep water (D) tanks, and accordingly slightly lower in the most protein rich sporophytes. The difference between S and D tanks was, however, not statistically significant ($H_1 = 2.51$, $p > 0.05$).

3.6 Intracellular N-components and intracellular nitrate (I-DIN)

The measured intracellular nitrogen components in the sporophytes showed an overall positive relationship to I-DIN (Figure 7). I-DON values for low I-DIN sporophytes were highly variable (Figure 7A) for reasons suggested above in Section 3.5.

Q_N and protein (Figure 7B) showed saturation type relationships with an increasing cellular I-DIN. The patterns of variation of both were slightly different from that seen for E-DIN (Figure 6), as D1 values grouped with S values to greater degree, whereas D4 values were higher throughout. All cellular N components in Figure 7 showed saturation levels for I-DIN content beyond 0.3 - 0.4 mg N g^{-1} DW.

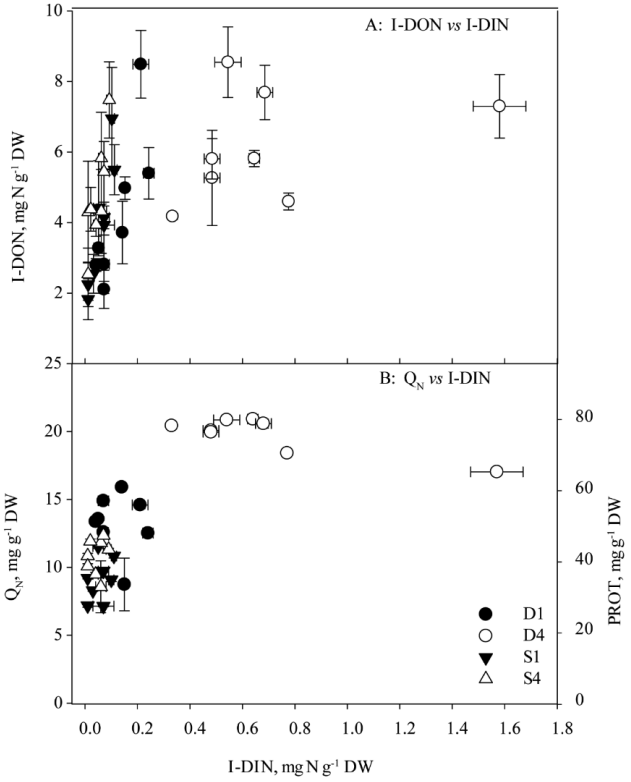

Figure 7 Intracellular nitrogen components as function of intracellular nitrate (I-DIN, mg N g^{-1} DW). A: Intracellular organic small molecular nitrogen (I-DON, mg N g^{-1} DW), B: Total intracellular nitrogen (Q_N, mg N g^{-1} DW, left axis) and protein contents (mg g^{-1} DW, right axis). Bars express 1 SE of the mean if exceeding symbols. Correlation coefficients are given in Table 1.

3.7 Relationship between algal growth rate and nitrogen components

The mean growth rate (mm day^{-1}) of the sporophytes is plotted as functions of extracellular (E-DIN) and intracellular variables in Figure 8. The growth rate of frond length showed clear saturation relationships to both E-DIN (Figure 8A) and I-DIN (Figure 8B), whereas the relationship between growth rate, $Q_{N,}$ and protein appeared linear and more scattered. Tables 2 and 3 review the statistical coefficients obtained for the relations illustrated in Figure 8.

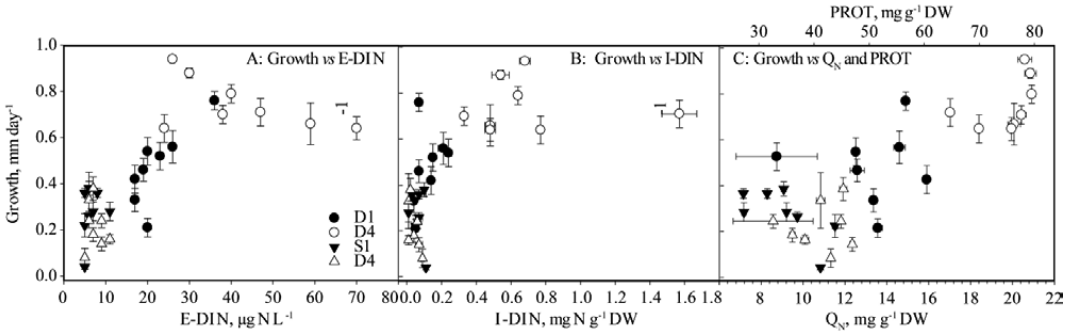

Figure 8 Sporophyte growth rate in length (mm day^{-1}) as a function of external and internal nitrogen components. A: Growth rate (mm day^{-1}) as function of external nitrate concentration (E-DIN); B: Growth rate (mm day^{-1}) as a function of intracellular nitrate contents (I-DIN); C: Growth rate (mm day^{-1}) as a function of total intracellular nitrogen (Q_N, mg N g^{-1} DW) and protein (mg protein g^{-1} DW. Bars express 1 SE of the mean if exceeding symbols. Regression coefficients are given in Table 3.

Table 2 Correlation coefficients (Pearson or Spearman) for relationships between growth rate and nitrate in the water (E-DIN, µg N L^{-1}, moving average last five measurements), Intracellular nitrate (I-DIN, mg N g^{-1} DW), total intracellular nitrogen (Q_N, mg N g^{-1} DW), intracellular organic small molecular nitrogen (I-DON, mg N g^{-1} DW) and protein contents (mg g^{-1} DW). Significant correlation coefficients are marked with asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Relationship	Correlation coefficient
Growth rate *vs* ln(E-DIN)	Pearson: 0.82 ***
Growth rate *vs* ln(I-DIN) ~| Deep water (D) ~| Surface water (S)	Pearson: 0.71 *** Pearson: 0.72 ** Pearson: -0.3
Growth rate *vs* Q_N and Protein	Pearson: 0.67 ***
Growth rate *vs* I-DON	Pearson: 0.38 *

Table 3 Least square regression of growth rate (μ) regressed on water*light (treatments), nitrate in the water (E-DIN, μg N L^{-1}, last five measurements), Intracellular nitrate (I-DIN, mg N g^{-1} DW) and total intracellular nitrogen (Q_N, mg N g^{-1} DW. Variation was allowed to vary between treatments, and estimated standard deviation is given for each treatment. Regression coefficients are marked with asterisks if significant (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

What	Function	β_0 (±SE) β_1 (±SE) β_2 (±SE) β_3 (±SE)	Estimated SD and multiplication factors
μ *vs* Water*Light AIC -33.4	$f(y) = \beta_0 +$ β_1*water + β_2 *light + β_3 *water*light	0.48 (0.058) *** -0.20 (0.069) ** 0.27 (0.071) *** -0.32 (0.088) **	D1: 1.00 * 0.15 = 0.15 D4: 0.70 * 0.15 = 0.11 S1: 0.67 * 0.15 = 0.10 S4: 0.61 * 0.15 = 0.09
μ *vs* ln(E-DIN+1) AIC - 28.3	$f(y) = \beta_0 +$ β_1*ln(E-DIN+1)	-0.236 (±0.087) * 0.239 (±0.034) ***	D1: 1.00 * 0.120 = 0.120 D4: 1.61 * 0.120 = 0.193 S1: 0.83 * 0.120 = 0.100 S4: 1.01 * 0.120 = 0.121
μ *vs* ln(I-DIN) AIC - 33.5	$f(y) = \beta_0 +$ β_1*ln(I-DIN) + β_2 * water + β_3 *ln(I-DIN) * water	0.789 (±0.054) *** 0.127 (±0.034) *** -0.673 (±0.110) *** -0.165 (±0.439) ***	D1: 1.00 * 0.143 = 0. 143 D4: 0.83 * 0.143 = 0.119 S1: 0.75 * 0.143 = 0.107 S4: 0.59 * 0.143 = 0.084
μ *vs* Q_N AIC - 36.3	$f(y) = \beta_0 +$ β_1* Q_N + β_2 *water + β_3 * Q_N *water	-0.004 (±0.183) 0.038 (±0.010) *** 0.558 (±0.234) * -0.068 (±0.018)***	D1: 1.00 * 0.170 = 0.170 D4: 0.55 * 0.170 = 0.094 S1: 0.47 * 0.170 = 0.080 S4: 0.61 * 0.170 = 0.104

The mean fraction of I-DIN associated with Q_N varied from 0.43–0.61 % of Q_N for the S4 and S1 treatments, respectively, and from 0.97–3.6% of Q_N for the D1 and D4 treatments, respectively. The sporophytes cultivated in deep water showed accordingly higher (although variable) contents of I-DIN than those cultivated in surface water, and I-DIN constituted only a minor part of the total intracellular nitrogen.

3.8 Comparison of protein estimation methods

The nitrogen-to-protein conversion factor (K_p) of 3.8 gave slightly higher and less scattered protein contents in *S. latissima* than the Bio-Rad method for all treatments, except for the S1 treatment (Figure 9). The differences were, however, not significant for any of the treatments ($p > 0.05$).

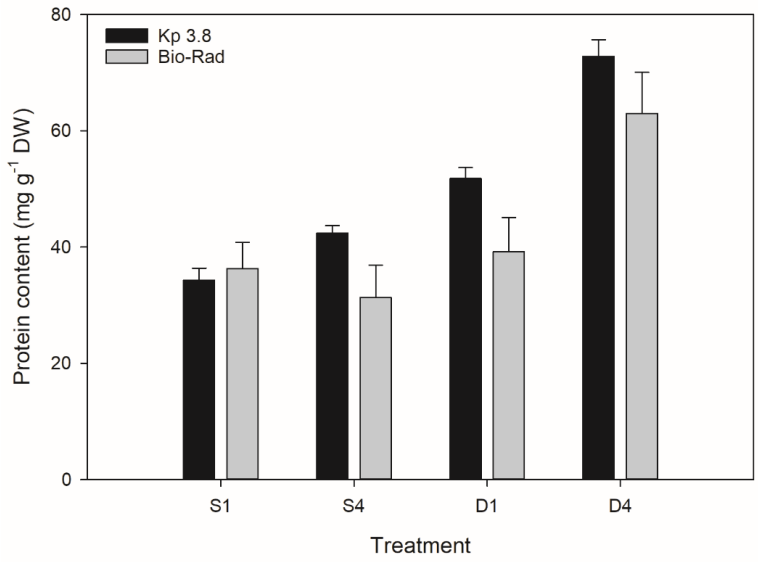

Figure 9 Comparison of protein content (mg g^{-1} DW) based on the nitrogen-to-protein conversion factor (K_p) of 3.8 mg protein mg^{-1} N and Bio-Rad analysis for the different treatments. Mean ± SE, n=8.

4 Discussion

The main objective of the present study was to assess how the growth and internal nitrogen metabolites of *S. latissima* were affected by different light and nitrate regimes. The low light treatments had a significantly lower median for light intensity than the high light treatments ($p < 0.001$) and the tanks receiving deep water had a significantly higher concentration of nitrate (E-DIN) than the tanks receiving surface water. There were pronounced variations in both light levels and E-DIN concentrations received in the tanks with the same treatment, resulting in gradients in light and nutrients across treatments. The overall conclusions on the

primary controlling resource of the sporophytes were apparent. The mean growth rate of the sporophytes for all treatments combined was positively related to the mean E-DIN concentration during the experimental period (Pearson's $r = 0.82$, $p < 0.001$), whereas there was no clear pattern between growth rate and light level. This indicated that nitrate was the primary controlling factor for growth in both high and low light treatments, according to the Liebig's law of the minimum (de Baar 1994). The significant positive relationship between growth rate and E-DIN concentration found in this study has also been found in earlier experiments (Handå et al. 2013; Kerrison et al. 2015; Boderskov et al. 2016).

The high variability in the light intensity measured in the different tanks within the same light treatments was assumed to be because of edge effects, i.e., the sides of the tanks were not lightproof, leading to higher light intensities for tanks not shaded by others. Two of the high light treatment tanks placed on the edge were exposed to light levels that temporarily reached 250 µmol m^{-2} s^{-1} during the day (D1-II 24 days, S1-II 8 days, data not shown), which could have been high enough to cause photo inhibition (Fortes and Lüning 1980). On the other hand, periodic shading of the light sensors situated at the bottom of the tanks by the growing sporophytes may have resulted in lower measured irradiance than what the sporophytes actually experienced.

Although the sporophytes' growth rates were positively related to the E-DIN concentration for all treatments combined ($r = 0.82$, $p < 0.001$, Table 2), it cannot be completely excluded that the growth measured in D1 was temporarily photo inhibited during parts of the experiment due to the high light recorded in one of the tanks over two thirds of the days. Photo inhibition may then have explained the lack in overall mean correlation between growth and light. As further discussed below, the pattern found for intracellular nitrogen components in the algae supported nitrogen limitation, as the contents of nitrogen components formed a continuous pattern with both E-DIN and growth rate.

One important finding in the current experiment was that the sporophytes grown with high nutrient and low light (D4) showed a higher growth rate than those grown in a high nutrient environment with higher light levels (D1, $p < 0.01$). This was most likely caused by the higher E-DIN concentration measured in the D4 tanks compared to the D1 tanks. The E-DIN concentration measured in the D1-I tank was significantly lower than that measured in the

D4-II tank standing right next to it, but both tanks received deep water. A possible explanation for the difference in E-DIN concentration in tanks receiving similar deep water might be that there was a difference in the community of opportunistic diatoms, which was observed growing on some of the tank walls, but not quantified. It may also be an effect of accidental differences in the water supply rate of deep water to the tanks or of minor variation in the biomass distribution. The measured increase in E-DIN concentration for all tanks during the experimental period is probably explained by the decrease in biomass as sporophytes were removed systematically for chemical analysis. This increase was only significant for treatments receiving surface water (S1 and S4).

An earlier study showed that the specific growth rate of *S. latissima* increased with the E-DIN concentration up to 70–140 µg NO_3-N L^{-1} (Wheeler and Weidner 1983). It is therefore likely that the algae of our experiment did not experience growth saturating concentrations of external NO_3^-. This supports our conclusion that NO_3^- is limiting for all treatments, including D4, which was exposed to the highest concentrations. This conclusion is also in agreement with previous results for the growth capabilities of *S. latissima*. The highest growth rates recorded in earlier studies are 8–17 mm day^{-1}, obtained in June (Parke 1948), and 10–20 mm day^{-1}, obtained in March to May (Nielsen et al. 2014). The highest growth rate found in D4 in our study was 7.7 mm day^{-1}, likely lower than the maximum growth rate that might be obtained in saturating conditions of NO_3^- and light.

The temperatures received in this experiment were within the optimal range of 10–17 °C for *S. latissima* (Druehl 1967; Fortes and Lüning 1980), and the small temperature differences between surface water and deep water treatments did not apparently affect the results very much.

Our combined results for the four treatments revealed a complete pattern of variation in intracellular nitrogen components of nutrient deprived and more nutrient sufficient *S. latissima.* Sporophytes grown in surface waters (S treatments) experienced low ambient nitrate concentrations and were characterized by low growth rates and low contents of the measured nitrogen components. Sporophytes grown in temperature acclimated deep water (D treatments) were exposed to higher, although variable, ambient nitrate concentrations and were characterized by higher growth rates and higher contents of intracellular nitrogen

components. The four treatments formed together a continuum characterizing the intracellular contents of nitrogen components for variable ambient nitrate concentrations and growth rates. It is important to note that the overall pattern of variation in intracellular components found is similar to that obtained in earlier studies of both freshwater and marine microalgae (Healey and Hendzel 1979; Sterner and Elser 2002).

The growth rates were found to increase with increasing nitrogen levels for all variables measured in the current experiment (E-DIN, I-DIN, Q_N and protein). The highest correlations were found for growth rate *versus* E-DIN and Growth rate *versus* I-DIN. These data must be carefully interpreted because the nitrogen metabolites also correlated with each other, making it somewhat difficult to extract the exact relationship between growth rate and each variable. The most obvious finding to emerge from the relationships between I-DIN and E-DIN is the increasing positive relationship between these variables, where sporophytes grown in the highest concentration of E-DIN consistently showed higher concentrations of I-DIN, Q_N and protein. The concentration of E-DIN measured in the tanks could account for 61% of the variation in the measured I-DIN concentrations. A higher concentration of I-DIN with increasing E-DIN concentrations is in accordance with *S. latissima*'s ability to store nitrate when the nutrient is available in excess (Wheeler and Weidner 1983; Young et al. 2007; Bartsch et al. 2008; Hurd et al. 2014). This storage capability is, however, found to be relatively low, constituting only a small fraction (3.6 %) of the maximum Q_N content for the D4 treatments. This level of I-DIN is in agreement with earlier field studies and an experiment made to characterize nitrate uptake kinetics of *S. latissima* (Forbord et al. 2020; Forbord et al., submitted). Even though the value of I-DIN is low, the variable correlates well with other nitrogen metabolites and growth rate.

The relationship between nutrients used for growth and uptake is not directly coupled because the algae assimilate and store nitrogen. Moreover, in times of low nitrate concentrations and uptake, growth may take place on the expense of intracellular nitrogen components. It therefore makes more sense to evaluate their nutritional status by assessing the tissue N contents like Q_N and I-DIN (Dalsgaard and Krause-Jensen 2006; Manns et al. 2017). Seaweeds are in general more sensitive to environmental changes when their Q_N reserves are exhausted (Gerard 1997; Gao et al. 2013). The Q_N content only exceeded the

critical minimum concentration of 17–20 mg N g^{-1} DW suggested for sustaining growth at maximum rates (Chapman et al. 1978; Hanisak 1983; Pedersen and Borum 1997) in the D4 tanks (19.2 mg N g^{-1} DW), whereas the other treatments had mean Q_N values of 9.2–14.7 mg N g^{-1} DW, suggesting that the sporophytes were N-deprived. The previously published critical Q_N concentrations do not necessarily regard the physiological state of the seaweed, so the Q_N concentration for sustaining growth will most likely vary between species, developmental stage, and nutritional status.

The quantity of small molecules of I-DON, calculated as the difference between the total dissolved nitrate and inorganic nitrate in the same sample, was believed to be overestimated due to its high fraction compared to, e.g., protein. A part of this fraction could be hydrolyzed proteins from the process of boiling samples to release small molecules from algal tissue. Severely nutrient starved *S. latissima* from S treatments could have contained a high share of dead cells, characterized by an increased rate of intracellular protein hydrolysis, which may be further enhanced during boiling. It cannot be excluded that this also may take place in nitrogen sufficient algae during boiling. The results obtained for I-DON are, in our view, uncertain, and the method is questionable. I-DON results are therefore omitted from further discussion.

The highest measured protein content of 73.2 ± 2.8 mg g^{-1} DW, found for the D4 treatment, was higher or similar to values registered in May–June for *S. latissima* in several other experiments using the same estimation method based on amino acids (Marinho et al. 2015; Bak et al. 2019; Forbord et al. 2019; Forbord et al. 2020). The D4 sporophytes had almost twice the protein content compared to the S1 and S4 treatments. This coincided with previous experiments, where higher protein content was found in macroalgae in deeper waters, interpreted to be a result of reduced light exposure (Cronin and Hay 1996; Ak and Yücesan 2012; Sharma et al. 2018; Forbord et al. 2020) and a higher E-DIN concentration (Harnedy and FitzGerald 2011).

Calculating the protein content from the nitrogen-to-protein conversion factor (K_p) gave a higher, but not significantly, protein content for all treatments compared to the Bio-Rad method, except for the S1 treatment. The Bio-Rad method estimates the soluble protein fraction of the sample, while the K_p method based on Q_N, estimates total protein (Safi et al.

2018). Some uncertainty is connected with the use of K_p factors, because they may change with season, depth, location and species. K_p factors have been shown to vary in the range of 2.0–6.25 (Lourenço et al. 2002; Schiener et al. 2015; Angell et al. 2016; Biancarosa et al. 2017; Manns et al. 2017; Sharma et al. 2018; Bak et al. 2019; Forbord et al. 2020). The 6.25 value is the standard factor used for pure protein and is obviously not relevant for biomass samples that contain other N-rich components like nucleic acids and pigments. One should be careful to compare the outcome of the different protein assays with each other as the methods are based on different principles and can result in different values (Barbarino and Lourenço 2005; Mæhre et al. 2018). The protein estimates from the current experiment are supported by two independent methods.

5 Conclusions

The present study was designed to determine the effect of light conditions and nutrient concentration on the growth and nitrogen metabolites of cultivated *S. latissima*. The experiment confirmed a positive relationship between total intracellular nitrogen components (Q_N, protein and I-DIN) and E-DIN, and between growth rate and E-DIN. Our combined results of the four treatments revealed a complete pattern of variation of intracellular nitrogen components, of nutrient deprived and nutrient saturated *S. latissima.* The most obvious finding to emerge from this study was that the growth rate of *S. latissima* was lower for the sporophytes grown under 'high light, high nutrient' conditions than 'low light, high nutrient' conditions. Lastly, no relationship was found between growth rate and different light levels, indicating that nitrate was the primary controlling factor for growth in both high and low light treatments. This is also supported by the pattern found in contents of intracellular nitrogen components related to E-DIN concentrations.

6 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7 Author Contributions

LJ participated in planning the experiment, carried out the practical work including field measurements and chemical analysis, most of the data treatment, and contributed to writing the manuscript. SF wrote the main part of the manuscript, contributed to analysis of samples and data treatment. YO conceived the idea of the study, acquired funding and participated in the planning and writing process and contributed to data treatment.

8 Funding

This work formed part of the Research Council of Norway project no. 254883 (MacroSea), together with internal funding from the Norwegian University of Science and Technology (NTNU).

9 Abbreviations

I-DIN, intracellular nitrate content; I-DON, small molecules of intracellular organic nitrate; E-DIN, external nitrate concentration; Kp, specific nitrogen-to-protein conversion factor; Q_N, total tissue nitrogen content

10 Acknowledgments

We are grateful to the staff at Seaweed Energy Solutions (SES) for kindly providing research facilities, experimental biomass, and support. Thanks to Kjersti Andresen and Siv Anina Etter (NTNU) for analyzing the water samples and to Marte Schei (SINTEF Ocean) for conducting the CN analysis.

11 References

Ak İ, Yücesan M (2012) Effect of light intensity on the pigment composition of *Gracilaria verrucosa* (Rhodophyta). Fresenius Environ Bull 21 (8):2126-2131

Angell AR, Mata L, de Nys R, Paul NA (2016) The protein content of seaweeds: a universal nitrogen-to-protein conversion factor of five. J Appl Phycol 28 (1):511-524

Bak UG, Mols-Mortensen A, Gregersen O (2018) Production method and cost of commercial-scale offshore cultivation of kelp in the Faroe Islands using multiple partial harvesting. Algal Res 33:36-47

Bak UG, Nielsen CW, Marinho GS, Gregersen Ó, Jónsdóttir R, Holdt SL (2019) The seasonal variation in nitrogen, amino acid, protein and nitrogen-to-protein conversion factors of commercially cultivated Faroese *Saccharina latissima*. Algal Res 42:101576

Barbarino E, Lourenço SO (2005) An evaluation of methods for extraction and quantification of protein from marine macro-and microalgae. J Appl Phycol 17 (5):447-460

Barbier M, Charrier B, Araujo R, Holdt SL, Jacquemin B, Rebours C (2019) PEGASUS - PHYCOMORPH European Guidelines for a Sustainable Aquaculture of Seaweeds. COST Action FA1406. Roscoff, France

Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R (2008) The genus Laminaria sensu lato: recent insights and developments. Eur J Phycol 43 (1):1-86

Biancarosa I, Espe M, Bruckner C, Heesch S, Liland N, Waagbø R, Torstensen B, Lock E (2017) Amino acid composition, protein content, and nitrogen-to-protein conversion factors of 21 seaweed species from Norwegian waters. J Appl Phycol 29 (2):1001-1009

BIO-RAD (2013) DC Protein Assay US. Accessed on: April. 30, 2020. [Online]. Available: http://www.bio-rad.com/webroot/web/pdf/lsr/literature/LIT33.pdf

Boderskov T, Schmedes P, Bruhn A, Rasmussen M, Nielsen M, Pedersen M (2016) 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. J Appl Phycol 28 (2):1153-1165

Broch OJ, Alver MO, Bekkby T, Gundersen H, Forbord S, Handå A, Skjermo J, Hancke K (2019) The kelp cultivation potential in coastal and offshore regions of Norway. Front Mar Sci 5:529

Broch OJ, Slagstad D (2012) Modelling seasonal growth and composition of the kelp *Saccharina latissima*. J Appl Phycol 24 (4):759-776

Bruhn J, Gerard V (1996) Photoinhibition and recovery of the kelp *Laminaria saccharina* at optimal and superoptimal temperatures. Mar Biol 125 (4):639-648

Buschmann AH, Camus C (2019) An introduction to farming and biomass utilisation of marine macroalgae. Phycologia 58 (5):443-445

Capone DG, Bronk DA, Mulholland MR, Carpenter EJ (2008) Nitrogen in the marine environment. 2 edn. Academic Press, New York

Chapman A, Craigie J (1977) Seasonal growth in *Laminaria longicruris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. Mar Biol 40 (3):197-205

Chapman A, Markham J, Lüning K (1978) Effects of nitrate concentration on the growth and physiology of *Laminaria saccharina* (Phaeophyta) in culture. J Phycol 14 (2):195-198

Cronin G, Hay ME (1996) Effects of light and nutrient availability on the growth, secondary chemistry, and resistance to herbivory of two brown seaweeds. Oikos 77 (1):93-106

Dalsgaard T, Krause-Jensen D (2006) Monitoring nutrient release from fish farms with macroalgal and phytoplankton bioassays. Aquaculture 256 (1-4):302-310

de Baar HJW (1994) von Liebig's law of the minimum and plankton ecology (1899–1991). Prog Oceanogr 33 (4):347-386

Directorate of Fisheries (2020) Akvakulturstatistikk (tidsserier)-Alger (*in Norwegian*). http://www.fiskeridir.no/Akvakultur/Statistikk-akvakultur/Akvakulturstatistikk-tidsserier/Alger. Accessed 30.01 2020

Druehl LD (1967) Distribution of two species of *Laminaria* as related to some environmental factors. J Phycol 3 (2):103-108

FAO (2018) The global status of seaweed production, trade and utilization, vol 124. Globefish Research Programme. Rome

Flavin K, Flavin N, Flahive B (2013) Kelp farming manual: a guide to the processes, techniques, and equipment for farming kelp in New England waters. Ocean Approved LLC, Saco, ME, USA.

Forbord S, Matsson S, Brodahl GE, Bluhm BA, Broch OJ, Handå A, Metaxas A, Skjermo J, Steinhovden KB, Olsen Y (2020) Latitudinal, seasonal and depth-dependent variation in growth, chemical composition and biofouling of cultivated *Saccharina latissima* (Phaeophyceae) along the Norwegian coast. J Appl Phycol. doi:10.1007/s10811-020-02038-y

Forbord S, Skjermo J, Arff J, Handå A, Reitan K, 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 (3):393-399

Forbord S, Steinhovden KB, Rød KK, Handå A, Skjermo J (2018) Cultivation protocol for *Saccharina latissima*. In: Charrier B, T W, Reddy C (eds) Protocols for Macroalgae Research,. 1st edn. CRC Press, Taylor Francis Group, Boca Raton, FL, London, New York, NY, pp 37-59

Forbord S, Steinhovden KB, Solvang T, Handå A, Skjermo J (2019) Effect of seeding methods and hatchery periods on sea cultivation of *Saccharina latissima* (Phaeophyceae): a Norwegian case-study. J Appl Phycol. doi:10.1007/s10811-020-02042-2

Fortes MD, Lüning K (1980) Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. Helgolander Meeresun 34 (1):15

Gao X, Endo H, Taniguchi K, Agatsuma Y (2013) Combined effects of seawater temperature and nutrient condition on growth and survival of juvenile sporophytes of the kelp *Undaria pinnatifida* (Laminariales; Phaeophyta) cultivated in northern Honshu, Japan. J Appl Phycol 25 (1):269-275

Gerard VA (1997) The role of nitrogen nutrition in high-temperature tolerance of the kelp, *Laminaria saccharina* (Chromophyta). J Phycol 33 (5):800-810

Gordillo FJ (2012) Environment and algal nutrition. In: Wiencke C, Bischof K (eds) Seaweed biology. Ecological Studies (Analysis and Synthesis), vol 219. Springer, Berlin, Heidelberg, pp 67-86

Grebe GS, Byron CJ, Gelais AS, Kotowicz DM, Olson TK (2019) An ecosystem approach to kelp aquaculture in the Americas and Europe. Aquaculture Reports 15:100215

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

Hanisak M (1983) The nitrogen relationships of marine macroalgae. In: Carpenter EJ, Capone DG (eds) Nitrogen in the Marine Environment. Academic Press, New York, pp 699-730

Harnedy PA, FitzGerald RJ (2011) Bioactive proteins, peptides and amino acids from macroalgae. J Phycol 47 (2):218-232

Healey F, Hendzel L (1979) Indicators of phosphorus and nitrogen deficiency in five algae in culture. J Fish Res Board Can 36 (11):1364-1369

Holdt SL, Kraan S (2011) Bioactive compounds in seaweed: functional food applications and legislation. J Appl Phycol 23 (3):543-597

Hurd CL, Harrison PJ, Bischof K, Lobban CS (2014) Seaweed ecology and physiology. 2 edn. Cambridge University Press, Cambridge

Ibrahim A, Olsen A, Lauvset S, Rey F (2014) Seasonal variations of the surface nutrients and hydrography in the Norwegian Sea. Int J Environ Sci De 5 (5):496-505

Kerrison PD, Stanley MS, Edwards MD, Black KD, Hughes AD (2015) The cultivation of European kelp for bioenergy: Site and species selection. Biomass Bioenergy 80:229-242

Krause-Jensen D, Lavery P, Serrano O, Marbà N, Masque P, Duarte CM (2018) Sequestration of macroalgal carbon: the elephant in the Blue Carbon room. Biol Lett 14 (6):20180236

Lapointe BE, Tenore KR (1981) Experimental outdoor studies with *Ulva fasciata* Delile. I. Interaction of light and nitrogen on nutrient uptake, growth, and biochemical composition. J Exp Mar Biol Ecol 53 (2-3):135-152

Lourenço SO, Barbarino E, De-Paula JC, Pereira LOdS, Marquez UML (2002) Amino acid composition, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical seaweeds. Phycol Res 50 (3):233-241

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275

Mæhre HK, Dalheim L, Edvinsen GK, Elvevoll EO, Jensen I-J (2018) Protein Determination—Method Matters. Foods 7 (1):5

Manns D, Nielsen MM, Bruhn A, Saake B, Meyer AS (2017) Compositional variations of brown seaweeds *Laminaria digitata* and *Saccharina latissima* in Danish waters. J Appl Phycol:1-14

Marinho GS, Holdt SL, Angelidaki I (2015) Seasonal variations in the amino acid profile and protein nutritional value of *Saccharina latissima* cultivated in a commercial IMTA system. J Appl Phycol 27 (5):1991-2000

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 (9):2011-2022

NSF (1975) NS 4746 Water analysis. Determination of the sum of nitrite-nitrogen and nitrate-nitrogen (*in Norwegian*). Norges Standardiseringsforbund, Oslo, Norway

Parke M (1948) Studies on British Laminariaceae. I. Growth in *Laminaria saccharina* (L.) Lamour. J Mar Biol Assoc UK 27 (3):651-709

Pedersen MF, Borum J (1996) Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. Mar Ecol Prog Ser:261-272

Pedersen MF, Borum J (1997) Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake. Mar Ecol Prog Ser 161:155-163

Peteiro C, Freire Ó (2013) 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 (1):205-213

R Core Team (2019) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

Redmond S, Green L, Yarish C, Kim J, Neefus C (2014) New England seaweed culture handbook-nursery systems. Connecticut Sea Grant CTSG-14-01, vol 7.

Rey F, Aure J, Danielssen D (2007) Temporal and spatial distribution of nutrients. In: Sætre R (ed) The Norwegian Coastal Current- Oceanography and Climate. Tapir Academic Press, Trondheim, pp 73-88

Safi C, van Leeuwen J, Telleman Y, Engelen-Smit N, van den Broek L, Harmsen P (2018) Quantification of proteins in seaweeds. In: Charrier B, Wichard T, Reddy C (eds) Protocols for Macroalgae Research. CRC Press, Boca Raton, London, New York, pp 211-224

Schiener P, Black KD, Stanley MS, Green DH (2015) The seasonal variation in the chemical composition of the kelp species *Laminaria digitata, Laminaria hyperborea, Saccharina latissima* and *Alaria esculenta*. J Appl Phycol 27 (1):363-373

Sharma S, Neves L, Funderud J, Mydland LT, Øverland M, Horn SJ (2018) Seasonal and depth variations in the chemical composition of cultivated *Saccharina latissima*. Algal Res 32:107-112

Sipler RE, Bronk DA (2015) Dynamics of dissolved organic nitrogen. In: A. HD, A. CG (eds) Biogeochemistry of marine dissolved organic matter. Second edn. Elsevier, Amsterdam, pp 127-232

Skjermo J, Aasen IM, Arff J, Broch OJ, Carvajal A, Christie H, Forbord S, Olsen Y, Reitan KI, Rustad T, Sandquist J, Solbakken R, Steinhovden KB, Wittgens B, Wolff R, Handå A (2014) A new Norwegian bioeconomy based on cultivation and processing of seaweeds: Opportunities and R&D needs. SINTEF Fisheries and Aquaculture

Sterner RW, Elser JJ (2002) Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton university press, Princeton

Stévant P, Rebours C, Chapman A (2017) Seaweed aquaculture in Norway: recent industrial developments and future perspectives. Aquacult Int 25 (4):1373-1390

Sullivan GM, Feinn R (2012) Using effect size—or why the P value is not enough. J Grad Med Educ 4 (3):279-282

Thimijan RW, Heins RD (1983) Photometric, radiometric, and quantum light units of measure: a review of procedures for interconversion. HortScience 18 (6):818-822

Wheeler W, Weidner M (1983) Effects of external inorganic nitrogen concentration on metabolism, growth and activities of key carbon and nitrogen assimilatory enzymes of *Laminaria saccharina* (Phaeophyceae) in culture. J Phycol 19 (1):92-96

Xiao X, Agusti S, Lin F, Yu Y, Pan Y, Li K, Wu J, Duarte CM (2019) Resource (light and nitrogen) and density-dependence of seaweed growth. Front Mar Sci 6:618

Young EB, Dring MJ, Savidge G, Birkett DA, Berges JA (2007) Seasonal variations in nitrate reductase activity and internal N pools in intertidal brown algae are correlated with ambient nitrate concentrations. Plant, Cell Environ 30 (6):764-774

Paper III

Journal of Applied Phycology
https://doi.org/10.1007/s10811-020-02038-y

23RD INTERNATIONAL SEAWEED SYMPOSIUM, JEJU

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. Brodahl[1] · Bodil A. Bluhm[4] · Ole Jacob Broch[2] · Aleksander Handå[2] · Anna Metaxas[5] · Jorunn Skjermo[2] · Kristine Braaten Steinhovden[2] · Yngvar Olsen[1]

Received: 29 June 2019 / Revised and accepted: 7 January 2020

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

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10811-020-02038-y) contains supplementary material, which is available to authorized users.

✉ 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

Published online: 23 January 2020

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 ~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 μg NO_3^--N L^{-1}, 20.6 μg PO_4–P L^{-1}) in a flow-through (120 L h^{-1}), light- and temperature-controlled system (70 μmol photons m^{-2} 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 $PAR = 0.0291\ Lux^{1.0049}$ 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 (Sjøtun 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 (Myksvoll 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 °C. They were shipped frozen to the laboratory (Fig. 1) at the end of the experimental period in September 2017 and stored at − 20 °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 °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 °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-μm 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 °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 °C until freeze-drying (Hetosicc CD 13–2) at − 40 °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 ~ 2–3 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 °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

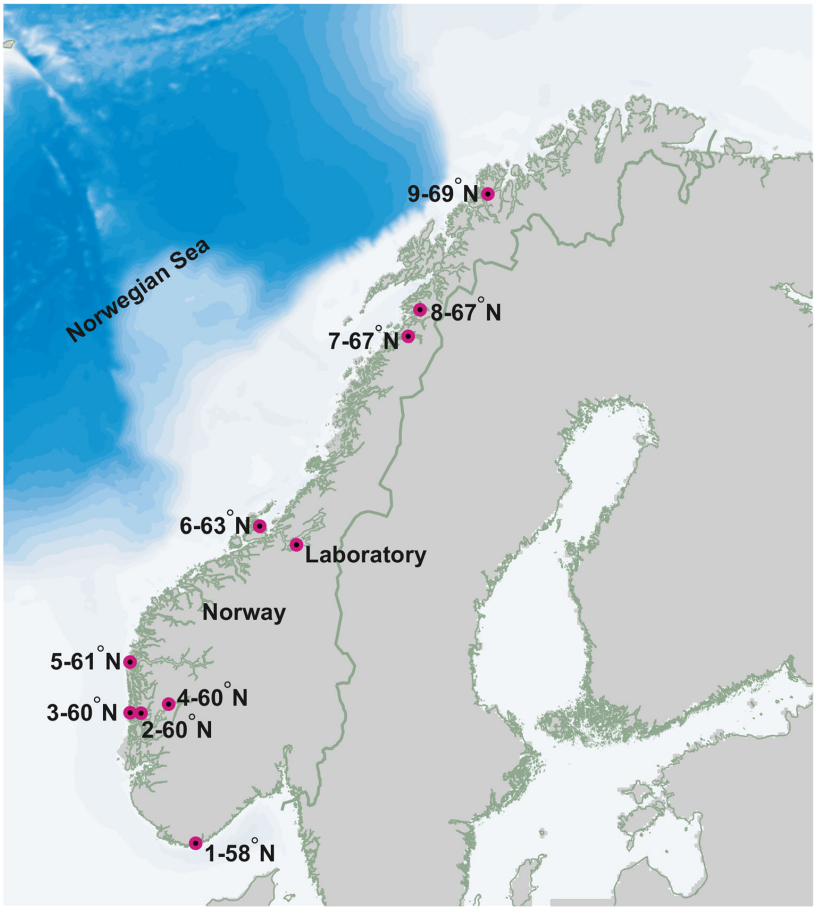

Fig. 1 The locations of the experimental sites along the Norwegian coastline and the seaweed laboratory where the seed lines were produced and distributed from. The name for each site is composed of a consecutive number and the latitude

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):

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

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 ± 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 ± 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	2 m/8 m	2 m/8 m	2 m/8 m	2 m/8 m	2 m/8 m	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

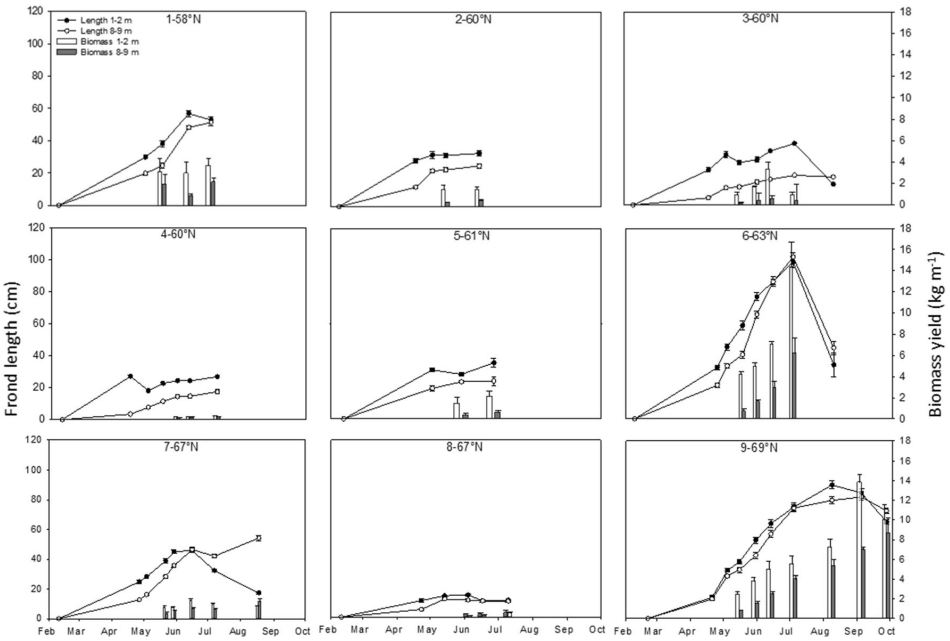

Fig. 2 Length (solid line, left *y*-axis) and biomass (bars, right *y*-axis) for both cultivation depths for all nine locations during the experimental period (February–September). Mean ± SE, $n = 50$ for length and $n = 4$ for biomass

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 ± 0.7 mg N g^{-1} 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 ± 0.200 mg NO_3^- g^{-1} 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	ΔAIC	R^2
1	log(Frond length)~Light + (1|Location) + (1|Sample date)	5	123.7	0.0	0.06
2	log(Frond length)~ 1 + (1|Location) + (1|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

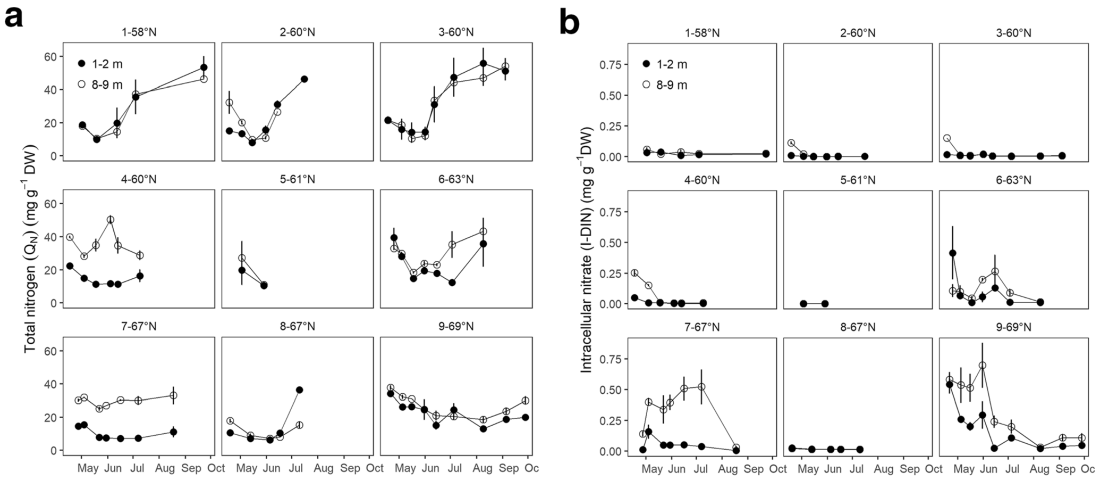

Fig. 4 Latitudinal and seasonal pattern in **a** total nitrogen content (Q_N) (mg N g^{-1} DW) and **b** intracellular nitrate content (I-DIN) (mg NO_3^- g^{-1} DW) of *S. latissima* from all nine experimental sites at 1–2 m and 8–9 m depth across the sampling period. Mean ± SE, $n = 3$

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 ± 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 ± 0.2	4.0 ± 0.2	3.7 ± 1.4	4.6 ± 0.2	2.7 ± 0.8	3.9 ± 0.3	4.2 ± 0.2	3.8 ± 0.5	3.9 ± 0.3
8–9 m	3.8 ± 0.1	3.8 ± 0.3	4.6 ± 1.4	3.1 ± 0.5	3.9 ± 0.8	4.0 ± 0.4	3.6 ± 0.1	3.1 ± 0.1	3.7 ± 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 ± 5.9%) at 1–2 m depth at 3–60°N and lowest (6.5 ± 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

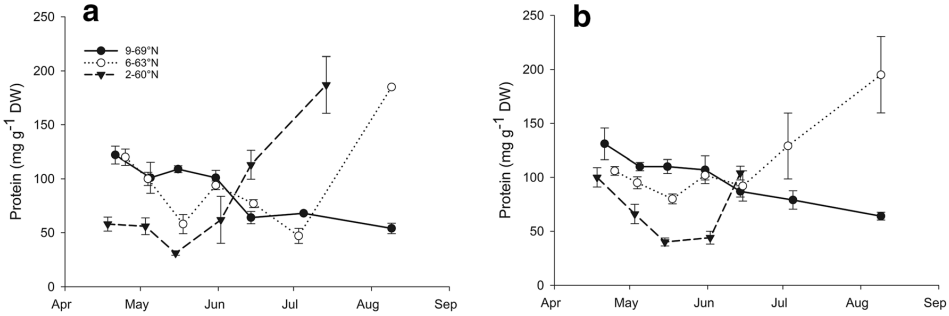

Fig. 5 Development in protein content (mg protein g^{-1} DW) of cultivated *S. latissima* over the entire sampling period at locations 2–60°N, 6–63°N, and 9–69°N at **a** 1–2 m depth and **b** 8–9 m depth. Mean ± SE, $n = 3$

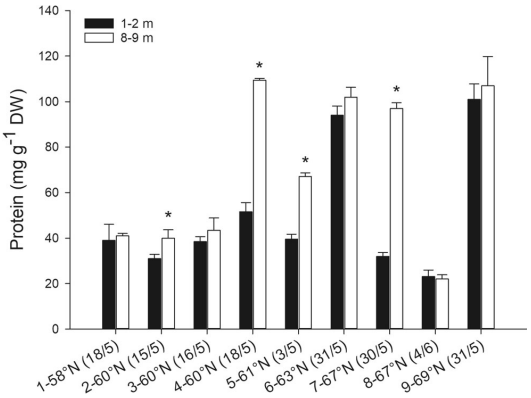

Fig. 6 Protein content (mg protein g^{-1} DW) for all experimental sites and both depths measured before clearly visible biofouling occurred (sampling date indicated in parentheses after each location name). Asterisk on top of the bars indicates significant differences ($p < 0.001$) between depths. Mean ± 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

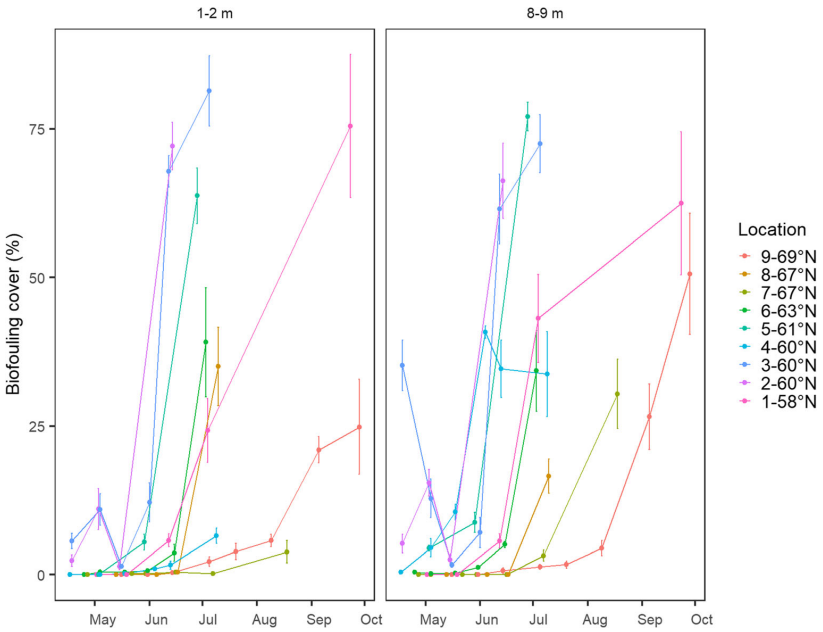

Fig. 7 Cover of biofouling (% of fouled frond area) as a function of time for all study locations at 1–2 m and 8–9 m depth. Mean ± SE, $n = 4–5$

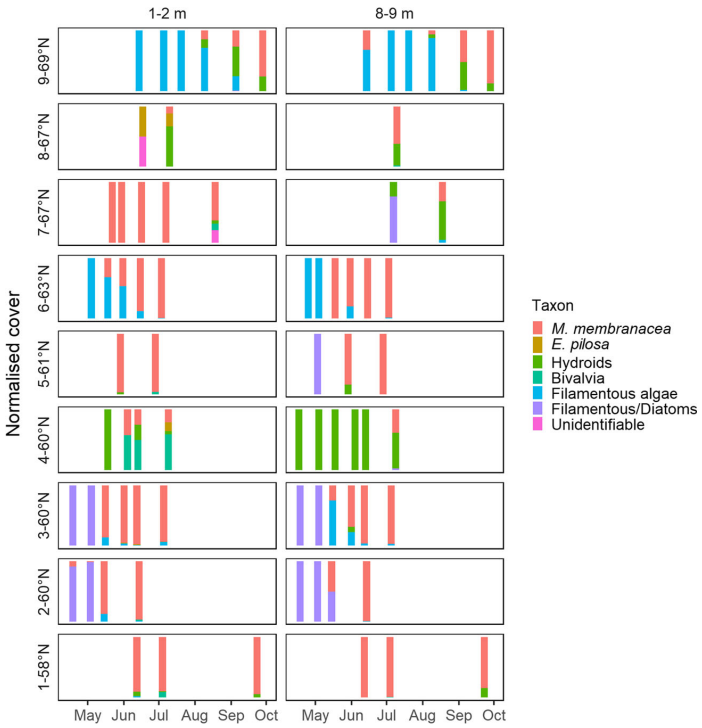

Fig. 8 Epibionts fouling *S. latissima* from all the locations at 1–2 m and 8–9 m depth. Data are shown as normalized cover, with the proportion each taxon constituted of the total cover of all epibionts. Mean, $n = 4–5$

irradiance that may suppress algal growth (Fortes and Lüning 1980; Spurkland and Iken 2011). Exposure of 1–2 h to light at 500–700 µmol photons m^{-2} 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 µmol photons m^{-2} s^{-1}) were only measured for less than 2 h at most of our sites (data not shown), low salinity was the

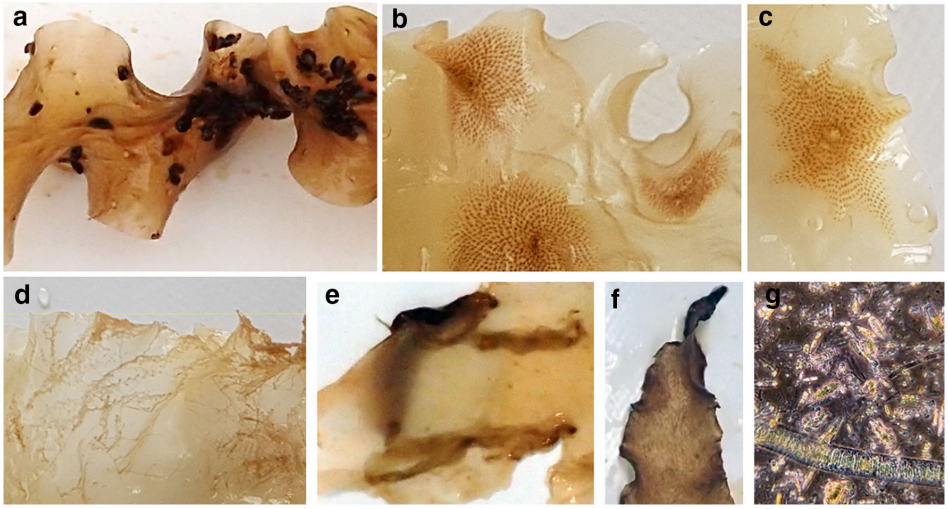

Fig. 9 Images of the epibionts found and registered in this study. **a** Bivalvia. **b** *Membranipora membranacea*. **c** *Electra pilosa*. **d** Hydroids. **e** Filamentous algae. **f** Diatoms. **g** Diatoms at × 40 magnification

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 and GDD 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	ΔAIC	R^2
1	log(Total biofouling + 1)~Light+GDD+(1 + GDD|Location) + (1|Sample date)	8	256.4	0.0	0.51
2	log(Total biofouling + 1)~GDD+(1 + GDD|Location) + (1|Sample date)	7	269.3	12.9	0.46
3	log(Total biofouling + 1)~Light+(1 + GDD|Location) + (1|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 Handå 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 NO_3^- 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 ecothermic 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, wheras 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.

Acknowledgements We are very grateful to Norway Seaweed, Austevoll Seaweed Farm, Hardangerfjord Seaweed Farm, Ocean Forest, Hortimare, Seaweed Energy Solutions, Salten Algae and Folla Alger for participating in this cultivation program and using time and resources on sampling and registration throughout large parts of 2017. Thanks to Saifullah (NTNU) and Solveig Foldal (NTNU) for helping with field measurements, Rasa Slizyte (SINTEF Ocean) for conducting the amino acid analysis, Synnøve Strand Jacobsen (NTNU) for helping prepare samples and Kjersti Andresen (NTNU) for conducting intracellular nitrate and CN analysis. Thanks to Zsolt Volent and Magnus Oshaug Pedersen (SINTEF Ocean) for operating the Hobo-loggers. Thanks to Hartvig Christie (NIVA) for scientific discussion of the MS content and Michael Greenacre (Universitat Pompeu Fabra and Barcelona Graduate School of Economics) for statistics support.

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.

Funding information Open access funding provided by SINTEF AS. This work was funded by the Research Council of Norway, project no. 254883 (MacroSea).

References

Ak İ, Yücesan M (2012) Effect of light intensity on the pigment composition of *Gracilaria verrucosa* (Rhodophyta). Fresenius Environ Bull 21:2126–2131

Andersen G (2013) Growth, survival and reproduction in the kelp *Saccharina latissima*–seasonal patterns and the impact of epibionts. PhD Thesis, University of Oslo, Oslo, Norway

Angell AR, Mata L, de Nys R, Paul NA (2016) The protein content of seaweeds: a universal nitrogen-to-protein conversion factor of five. J Appl Phycol 28:511–524

Atkinson D (1994) Temperature and organism size: a biological law for ectotherms? Adv Ecol Res 25:1–1

Bak UG, Mols-Mortensen A, Gregersen O (2018) Production method and cost of commercial-scale offshore cultivation of kelp in the Faroe Islands using multiple partial harvesting. Algal Res 33:36–47

Bak UG, Nielsen CW, Marinho GS, Gregersen Ó, Jónsdóttir R, Holdt SL (2019) The seasonal variation in nitrogen, amino acid, protein and nitrogen-to-protein conversion factors of commercially cultivated Faroese *Saccharina latissima*. Algal Res 42:101576

Barbier M, Charrier B, Araujo R, Holdt SL, Jacquemin B, Rebours C (2019) PEGASUS - PHYCOMORPH European guidelines for a sustainable aquaculture of seaweeds. COST action FA1406. Roscoff, France

Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R (2008) The genus *Laminaria* sensu lato: recent insights and developments. Eur J Phycol 43:1–86

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48

Biancarosa I, Espe M, Bruckner C, Heesch S, Liland N, Waagbø R, Torstensen B, Lock E (2017) Amino acid composition, protein content, and nitrogen-to-protein conversion factors of 21 seaweed species from Norwegian waters. J Appl Phycol 29:1001–1009

Bolton JJ, Germann I, Lüning K (1983) Hybridization between Atlantic and Pacific representatives of the Simplices section of *Laminaria* (Phaeophyta). Phycologia 22:133–140

Brenko MH, Calabrese A (1969) The combined effects of salinity and temperature on larvae of the mussel *Mytilus edulis*. Mar Biol 4:224–226

Broch OJ, Alver MO, Bekkby T, Gundersen H, Forbord S, Handå A, Skjermo J, Hancke K (2019) The kelp cultivation potential in coastal and offshore regions of Norway. Front Mar Sci 5:529

Broch OJ, Ellingsen IH, Forbord S, Wang X, Volent Z, Alver MO, Handa A, Andresen K, Slagstad D, Reitan KI, Olsen Y, Skjermo J (2013) Modelling the cultivation and bioremediation potential of the kelp *Saccharina latissima* in close proximity to an exposed salmon farm in Norway. Aquacult Env Interact 4:187–206

Bruhn A, Tørring DB, Thomsen M, Canal-Vergés P, Nielsen MM, Rasmussen MB, Eybye KL, Larsen MM, Balsby TJS, Petersen JK (2016) Impact of environmental conditions on biomass yield, quality, and bio-mitigation capacity of *Saccharina latissima*. Aquacult Env Interact 8:619–636

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

Bruton T, Lyons H, Lerat Y, Stanley M, Rasmussen M (2009) A review of the potential of marine algae as a source of biofuel in Ireland. Sustainable Energy Ireland

Busch K, Iversen K, Nashoug B, Kiele M, Gitmark J, Remen V, Krogstad M (2014) Miljøundersøkelse av Skjerstadfjorden (*in Norwegian*). SALT rapport

Buschmann AH, Camus C (2019) An introduction to farming and biomass utilisation of marine macroalgae. Phycologia 58:443–445

Cornelius PFS (1982) Hydroids and medusae of the family Campanulariidae recorded from eastern North Atlantic, with a world synopsis of genera. Bull Br Mus Nat Hist Zool 42:37–148

Cronin G, Hay ME (1996) Effects of light and nutrient availability on the growth, secondary chemistry, and resistance to herbivory of two brown seaweeds. Oikos 77:93–106

Directorate of Fisheries (2019) Akvakulturstatistikk (tidsserier)-Alger (***in Norwegian***). http://www.fiskeridir.no/Akvakultur/Statistikk-akvakultur/Akvakulturstatistikk-tidsserier/Alger . Accessed 08 Dec 2019

Druehl LD (1967) Distribution of two species of *Laminaria* as related to some environmental factors. J Phycol 3:103–108

Druehl LD (1970) The pattern of Laminariales distribution in the Northeast Pacific. Phycologia 9:237–247

Druehl LD, Kaneko T (1973) On *Laminaria saccharina* from Hokkaido. Bot Mag 86:323–327

FAO (2018) The global status of seaweed production, trade and utilization, vol 124. Globefish Research Programme, FAO, Rome

Flavin K, Flavin N, Flahive B (2013) Kelp farming manual: a guide to the processes, techniques, and equipment for farming kelp in New England waters. Ocean Approved LLC, Saco

Fletcher RL (1995) Epiphytism and fouling in *Gracilaria* cultivation: an overview. J Appl Phycol 7:325–333

Forbord S, Skjermo J, Arff J, Handå A, Reitan K, 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

Forbord S, Steinhovden KB, Rød KK, Handå A, Skjermo J (2018) Cultivation protocol for *Saccharina latissima*. In: Charrier B, Wichard T, Reddy CRK (eds) Protocols for macroalgae research. CRC Press, Boca Raton, pp 37–59

Forbord S, Steinhovden KB, Solvang T, Handå A, Skjermo J (2019) Effect of seeding methods and hatchery periods on sea cultivation of *Saccharina latissima* (Phaeophyceae): a Norwegian case-study. J Appl Phycol https://doi.org/10.1007/s10811-019-01936-0

Førde H, Forbord S, Handå A, Fossberg J, Arff J, Johnsen G, Reitan K (2016) Development of bryozoan fouling on cultivated kelp (*Saccharina latissima*) in Norway. J Appl Phycol 28:1225–1234

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

Fossberg J, Forbord S, Broch OJ, Malzahn AM, Jansen H, Handå A, Førde H, Bergvik M, Fleddum AL, Skjermo J, Olsen Y (2018) The potential for upscaling kelp (*Saccharina latissima*) cultivation in salmon-driven integrated multi-trophic aquaculture (IMTA). Front Mar Sci 5:418

Fredriksen S, Sjøtun I (2015) Risikovurdering ved utsetting av ikke-stedegen tare (in Norwegian), vol 2015. Miljødirektoratet, Oslo

Gendron L, Gauthier P, Savard G (2007) Expériences préliminaires de culture de l'algue brune *Laminaria longicruris* en laboratoire et en mer au large de Paspébiac (Québec) en 2006 (*in French*). Direction régionale des sciences, pêches et océans Canada, Institut Maurice-Lamontagne, Rapport technique canadien des sciences halieutiques aquatiques 2731: viii+53 pp

Gerard V, DuBois K, Greene R (1987) Growth responses of two *Laminaria saccharina* populations to environmental variation. Hydrobiologia 151:229–232

Getachew P, Kang J-Y, Choi J-S, Hong Y-K (2015) Does bryozoan colonization alter the biochemical composition of *Saccharina japonica* affecting food safety and quality? Bot Mar 58:267–274

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

Hanelt D, Wiencke C, Karsten U, Nultsch W (1997) Photoinhibition and recovery after high light stress in different developmental and life-history stages of *Laminaria saccharina* (Phaeophyta). J Phycol 33: 387–395

Harnedy PA, FitzGerald RJ (2011) Bioactive proteins, peptides and amino acids from macroalgae. J Phycol 47:218–232

Hasselström L, Visch W, Gröndahl F, Nylund GM, Pavia H (2018) The impact of seaweed cultivation on ecosystem services-a case study from the west coast of Sweden. Mar Pollut Bull 133:53–64

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

Hurd CL (2000) Water motion, marine macroalgal physiology, and production. J Phycol 36:453–472

Hurd CL, Durante KM, Harrison PJ (2000) Influence of bryozoan colonization on the physiology of the kelp *Macrocystis integrifolia* (Laminariales, Phaeophyta) from nitrogen-rich and-poor sites in Barkley sound, British Columbia, Canada. Phycologia 39:435–440

Hurd CL, Harrison PJ, Bischof K, Lobban CS (2014) Seaweed ecology and physiology. Cambridge University Press, Cambridge

Hwang EK, Gong YG, Park CS (2012) Cultivation of a hybrid of free-living gametophytes between *Undariopsis peterseniana* and *Undaria pinnatifida*: morphological aspects and cultivation period. J Appl Phycol 24:401–408

Ibrahim A, Olsen A, Lauvset S, Rey F (2014) Seasonal variations of the surface nutrients and hydrography in the Norwegian Sea. Int J Environ Sci De 5:496–505

Jaccard J, Jaccard J (1998) Interaction effects in factorial analysis of variance. Sage Publications, Thousand Oaks, CA

Jaeger B (2017) r2glmm: computes R squared for mixed (multilevel) models. R package version 0.1.2., the comprehensive R archive network (CRAN), Vienna, Austria

Kerrison PD, Stanley MS, Edwards MD, Black KD, Hughes AD (2015) The cultivation of European kelp for bioenergy: site and species selection. Biomass Bioenergy 80:229–242

Kohler KE, Gill SM (2006) Coral point count with excel extensions (CPCe): a visual basic program for the determination of coral and substrate coverage using random point count methodology. Comput Geosci 32:1259–1269

Krumhansl KA, Lee JM, Scheibling RE (2011) Grazing damage and encrustation by an invasive bryozoan reduce the ability of kelps to withstand breakage by waves. J Exp Mar Biol Ecol 407:12–18

Long MH, Rheuban JE, Berg P, Zieman JC (2012) A comparison and correction of light intensity loggers to photosynthetically active radiation sensors. Limnol Oceanogr Methods 10:416–424

Lourenço SO, Barbarino E, De-Paula JC, Pereira LOS, Marquez UML (2002) Amino acid composition, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical seaweeds. Phycol Res 50:233–241

Lüning K (1990) Seaweeds: their environment, biogeography, and ecophysiology. John Wiley & Sons, London

Lüning K, Mortensen L (2015) European aquaculture of sugar kelp (*Saccharina latissima*) for food industries: iodine content and epiphytic animals as major problems. Bot Mar 58:449–455

Macchiavello J, Araya E, Bulboa C (2010) Production of *Macrocystis pyrifera* (Laminariales; Phaeophyceae) in northern Chile on spore-based culture. J Appl Phycol 22:691–697

Mæhre HK, Dalheim L, Edvinsen GK, Elvevoll EO, Jensen I-J (2018) Protein determination—method matters. Foods 7:5

Manns D, Nielsen MM, Bruhn A, Saake B, Meyer AS (2017) Compositional variations of brown seaweeds *Laminaria digitata*

and *Saccharina latissima* in Danish waters. J Appl Phycol 29:1493–1526

Marinho GS, Holdt SL, Angelidaki I (2015a) Seasonal variations in the amino acid profile and protein nutritional value of *Saccharina latissima* cultivated in a commercial IMTA system. J Appl Phycol 27:1991–2000

Marinho GS, Holdt SL, Birkeland MJ, Angelidaki I (2015b) Commercial cultivation and bioremediation potential of sugar kelp, *Saccharina latissima*, in Danish waters. J Appl Phycol 27:1963–1973

Matsson S, Christie H, Fieler R (2019) Variation in biomass and biofouling of kelp, *Saccharina latissima*, cultivated in the Arctic, Norway. Aquaculture 506:445–452

Mols-Mortensen A, Ortind EG, Jacobsen C, Holdt SL (2017) Variation in growth, yield and protein concentration in *Saccharina latissima* (Laminariales, Phaeophyceae) cultivated with different wave and current exposures in the Faroe Islands. J Appl Phycol 29:2277–2286

Mosse J (1990) Nitrogen-to-protein conversion factor for ten cereals and six legumes or oilseeds. A reappraisal of its definition and determination. Variation according to species and to seed protein content. J Agric Food Chem 38:18–24

Moy F, Alve E, Bogen J, Christie H, Green N, Helland A, Steen H, Skarbøvik E, Stålnacke P (2006) Sugar kelp project: status report no 1–2006. Norsk institutt for vannforskning – NIVA

Myksvoll MS, Sundby S, Ådlandsvik B, Vikebø FB (2011) Retention of coastal cod eggs in a fjord caused by interactions between egg buoyancy and circulation pattern. Mar Coast Fish 3:279–294

Nakagawa S, Schielzeth H (2013) A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods Ecol Evol 4:133–142

Nielsen MM, Manns D, D'Este M, Krause-Jensen D, Rasmussen MB, Larsen MM, Alvarado-Morales M, Angelidaki I, Bruhn A (2016) Variation in biochemical composition of *Saccharina latissima* and *Laminaria digitata* along an estuarine salinity gradient in inner Danish waters. Algal Res 13:235–245

Norwegian Water Resources and Energy Directorate. (2019) Norwegian water resources and energy directorate. https://www.nve.no/english/ Accessed 25 Jun 2019

NSF (1975) NS 4746 Water analysis. Determination of the sum of nitrite-nitrogen and nitrate-nitrogen (*in Norwegian*). Norges Standardiseringsforbund, Oslo, Norway

Øie G, Olsen Y (1997) Protein and lipid content of the rotifer *Brachionus plicatilis* during variable growth and feeding condition. Hydrobiologia 358:251–258

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

Pavia H, Toth GB (2000) Influence of light and nitrogen on the phlorotannin content of the brown seaweeds *Ascophyllum nodosum* and *Fucus vesiculosus*. Hydrobiologia 440:299–305

Pedersen MF, Borum J (1997) Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake. Mar Ecol Prog Ser 161:155–163

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

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

Core Team R (2018) R: a language and environment for statistical computing. Foundation for Statistical Computing, Vienna

Redmond S, Green L, Yarish C, Kim J, Neefus C (2014) New England seaweed culture handbook-nursery systems. Connecticut Sea Grant CTSG-14-01, vol 7

Rey F, Aure J, Danielssen D (2007) Temporal and spatial distribution of nutrients. In: Sætre R (ed) The Norwegian coastal current- oceanography and climate. Tapir Academic Press, Trondheim, pp 73–88

Rickert E, Wahl M, Link H, Richter H, Pohnert G (2016) Seasonal variations in surface metabolite composition of *Fucus vesiculosus* and *Fucus serratus* from the Baltic Sea. PLoS One 11:e0168196

Rolin C, Inkster R, Laing J, McEvoy L (2017) Regrowth and biofouling in two species of cultivated kelp in the Shetland Islands, UK. J Appl Phycol 29:2351–2361

RStudio Team (2016) RStudio: integrated development for R. RStudio, Inc., Boston, MA

Sætre R (2007) The Norwegian coastal current: oceanography and climate. Tapir Academic Press, Trondheim

Saunders M, Metaxas A (2007) Temperature explains settlement patterns of the introduced bryozoan *Membranipora membranacea* in Nova Scotia, Canada. Mar Ecol Prog Ser 344:95–106

Saunders M, Metaxas A (2009) Effects of temperature, size, and food on the growth of *Membranipora membranacea* in laboratory and field studies. Mar Biol 156:2267–2276

Saunders M, Metaxas A, Filgueira R (2010) Implications of warming temperatures for population outbreaks of a nonindigenous species (*Membranipora membranacea*, Bryozoa) in rocky subtidal ecosystems. Limnol Oceanogr 55:1627–1642

Scheibling RE, Gagnon P (2009) Temperature-mediated outbreak dynamics of the invasive bryozoan *Membranipora membranacea* in Nova Scotian kelp beds. Mar Ecol Prog Ser 390:1–13

Schiener P, Black KD, Stanley MS, Green DH (2015) The seasonal variation in the chemical composition of the kelp species *Laminaria digitata, Laminaria hyperborea, Saccharina latissima* and *Alaria esculenta*. J Appl Phycol 27:363–373

Sharma S, Neves L, Funderud J, Mydland LT, Øverland M, Horn SJ (2018) Seasonal and depth variations in the chemical composition of cultivated *Saccharina latissima*. Algal Res 32:107–112

Sjøtun K, Gunnarsson K (1995) Seasonal growth pattern of an Icelandic *Laminaria* population (section Simplices, Laminariaceae, Phaeophyta) containing solid-and hollow-stiped plants. Eur J Phycol 30:281–287

Sjøtun K, Husa V, Asplin L, Sandvik AD (2015) Climatic and environmental factors influencing occurrence and distribution of macroalgae a fjord gradient revisited. Mar Ecol Prog Ser 532:73–88

Skjermo J, Aasen IM, Arff J, Broch OJ, Carvajal A, Christie H, Forbord S, Olsen Y, Reitan KI, Rustad T, Sandquist J, Solbakken R, Steinhovden KB, Wittgens B, Wolff R, Handå A (2014) A new Norwegian bioeconomy based on cultivation and processing of seaweeds: opportunities and R&D needs. SINTEF Fisheries and Aquaculture

Spurkland T, Iken K (2011) Salinity and irradiance effects on growth and maximum photosynthetic quantum yield in subarctic *Saccharina latissima* (Laminariales, Laminariaceae). Bot Mar 54:355–365

Stévant P, Rebours C, Chapman A (2017) Seaweed aquaculture in Norway: recent industrial developments and future perspectives. Aquac Int 25:1373–1390

Trudgill D, Honek A, Li D, Straalen N (2005) Thermal time–concepts and utility. Ann Appl Biol 146:1–14

Varela DA, Hernríquez LA, Fernández PA, Leal P, Hernández-González MC, Figueroa FL, Buschmann AH (2018) Photosynthesis and nitrogen uptake of the giant kelp *Macrocystis pyrifera* (Ochrophyta) grown close to salmon farms. Mar Environ Res 135:93–102

Wahl M (1989) Marine epibiosis. I. Fouling and antifouling: some basic aspects. Mar Ecol Prog Ser 58:175–189

Walls A, Edwards M, Firth L, Johnson M (2017) Successional changes of epibiont fouling communities of the cultivated kelp *Alaria esculenta*: predictability and influences. Aquacult Env Interact 9:55–69

Watanabe T, Kitajima C, Fujita S (1983) Nutritional values of live organisms used in Japan for mass propagation of fish: a review. Aquaculture 34:115–143

Westmeijer G, Everaert G, Pirlet H, De Clerck O, Vandegehuchte MB (2019) Mechanistic niche modelling to identify favorable growth sites of temperate macroalgae. Algal Res 41:101529

Wheeler W, Weidner M (1983) Effects of external inorganic nitrogen concentration on metabolism, growth and activities of key carbon and nitrogen assimilatory enzymes of *Laminaria saccharina* (Phaeophyceae) in culture. J Phycol 19:92–96

Xiao X, Agusti S, Lin F, Yu Y, Pan Y, Li K, Wu J, Duarte CM (2019) Resource (light and nitrogen) and density-dependence of seaweed growth. Front Mar Sci 6:618

Young EB, Dring MJ, Savidge G, Birkett DA, Berges JA (2007) Seasonal variations in nitrate reductase activity and internal N pools in intertidal brown algae are correlated with ambient nitrate concentrations. Plant Cell Environ 30:764–774

Online Resources 1 -Table 1: Results of three-way ANOVA analyzing the effect of depth, location and month on temperature (°C), GDD (°C day^{-1}) and accumulated PAR (mole)

	Effect	MS	$F_{(df)}$	P
Temperature	Depth	201.2	$1.916_{(1,3338)}$	0.201
	Location	767.5	$10.89_{(8,3338)}$	<0.001
	Month	6400	$47.66_{(6,3338)}$	<0.001
	Location x Month	51.88	$6.656_{(47,3338)}$	<0.001
	Location x Depth	28.57	$3.840_{(8,3338)}$	0.001
	Month x Depth	90.76	$11.79_{(6,3338)}$	<0.001
	Location x Month x Depth	7.795	$12.80_{(47,3338)}$	<0.001
	Error	0.609		
GDD	Depth	392353	$0.750_{(1,3338)}$	0.404
	Location	8311948	$11.61_{(8,3338)}$	<0.001
	Month	218530017	$257.2_{(6,3338)}$	<0.001
	Location x Month	532516	$28.17_{(47,3338)}$	<0.001
	Location x Depth	227999	$12.34_{(8,3338)}$	<0.001
	Month x Depth	342887	$18.25_{(6,3338)}$	<0.001
	Location x Month x Depth	18901	$1.807_{(47,3338)}$	0.001
	Error	10459		
Accumulated PAR	Depth	222495942	$8.510_{(1,3324)}$	0.024
	Location	2827422	$1.704_{(8,3324)}$	0.215
	Month	72998347	$2.720_{(6,3324)}$	0.123
	Location x Month	304419	$2.088_{(47,3324)}$	0.007
	Location x Depth	1508393	$10.870_{(8,3324)}$	<0.001
	Month x Depth	26685443	$185.5_{(6,3324)}$	<0.001
	Location x Month x Depth	145828	$35.11_{(47,3324)}$	<0.001
	Error	4154		

Online Resources 1- Table 2: Results of two-way ANOVA analyzing the effect of depth and sampling date on frond length and biomass yield for all nine locations

		Frond length (cm)			Biomass yield (kg m^{-1})		
Location	Effect	MS	$F_{(df)}$	P	MS	$F_{(df)}$	P
1-58°N	Depth	6843	$10.85_{(1,389)}$	0.046	11.76	$33.34_{(1,17)}$	0.027
	Sampling date	19978	$31.67_{(3,389)}$	0.009	1.020	$3.794_{(3,17)}$	0.209
	Depth x Sampling date	630.8	$4.529_{(3,389)}$	0.004	0.349	$0.195_{(2,17)}$	0.825
	Error	139.3			1.792		
2-60°N	Depth	10110	$70.28_{(1,392)}$	0.004	15.05	$4.860_{(1,12)}$	0.271
	Sampling date	968.7	$6.734_{(3,392)}$	0.076	4.494	$1.451_{(1,12)}$	0.441
	Depth x Sampling date	143.9	$1.391_{(3,392)}$	0.245	3.098	$19.18_{(1,12)}$	0.001
	Error	103.4			0.161		
3-60°N	Depth	37698	$25.84_{(1,686)}$	0.002	13.81	$6.872_{(1,24)}$	0.079
	Sampling date	3063	$2.100_{(6,686)}$	0.194	3.446	$1.715_{(3,24)}$	0.334
	Depth x Sampling date	1459	$12.91_{(6,686)}$	<0.001	2.009	$5.536_{(3,24)}$	0.005
	Error	113.0			0.363		
4-60°N	Depth	23163	$29.93_{(1,588)}$	0.003	0.036	$44.62_{(1,18)}$	0.022
	Sampling date	1114	$1.441_{(5,588)}$	0.349	0.005	$5.996_{(2,18)}$	0.143
	Depth x Sampling date	773.9	$21.63_{(5,588)}$	<0.001	0.001	$1.247_{(2,18)}$	0.311
	Error	35.79			0.001		
5-61°N	Depth	8832	$91.71_{(1,369)}$	0.002	5.382	$49.46_{(1,12)}$	0.090
	Sampling date	692.3	$7.224_{(3,369)}$	0.069	0.689	$6.326_{(1,12)}$	0.241
	Depth x Sampling date	95.83	$0.649_{(3,369)}$	0.584	0.109	$0.443_{(1,12)}$	0.518
	Error	147.8			0.246		
6-63°N	Depth	4139	$2.667_{(1,592)}$	0.151	165.2	$20.10_{(1,22)}$	0.020
	Sampling date	65058	$36.85_{(6,592)}$	<0.001	79.99	$9.629_{(3,22)}$	0.048
	Depth x Sampling date	1765	$6.775_{(6,592)}$	<0.001	8.307	$3.536_{(3,22)}$	0.031
	Error	260.6			2.349		
7-67°N	Depth	29.12	$0.004_{(1,676)}$	0.954	0.818	$2.786_{(1,28)}$	0.170
	Sampling date	9106	$1.138_{(6,676)}$	0.440	0.516	$1.745_{(4,28)}$	0.302
	Depth x Sampling date	8001	$111.6_{(6,676)}$	<0.001	0.296	$4.753_{(4,28)}$	0.005
	Error	71.69			0.062		
8-67°N	Depth	761.4	$5.431_{(1,490)}$	0.080	0.062	$13.73_{(1,18)}$	0.066
	Sampling date	441.4	$3.148_{(4,490)}$	0.146	0.099	$21.92_{(2,18)}$	0.044
	Depth x Sampling date	140.2	$5.111_{(4,490)}$	<0.001	0.005	$0.166_{(2,18)}$	0.848
	Error	27.43			0.027		
9-69°N	Depth	3228	$4.180_{(1,882)}$	0.075	77.24	$11.16_{(1,36)}$	0.015
	Sampling date	63442	$82.15_{(8,882)}$	<0.001	77.92	$10.82_{(6,36)}$	0.005
	Depth x Sampling date	772.3	$3.681_{(8,882)}$	<0.001	7.205	$3.899_{(6,36)}$	0.004
	Error	209.8			1.848		

Online Resources 1- Table 3: Results of two-way ANOVA analyzing the effect of depth and sampling date on dry weight (DW) and ash content for all nine locations

		DW (% of WW)			Ash (mg g^{-1} DW)		
Location	Effect	MS	$F_{(df)}$	P	MS	$F_{(df)}$	P
1-58°N	Depth	34.07	$8.764_{(1,18)}$	0.035	67627	$18.68_{(1,18)}$	0.010
	Sampling date	47.49	$12.23_{(4,18)}$	0.016	7219	$1.960_{(4,18)}$	0.265
	Depth x Sampling date	3.882	$0.980_{(4,18)}$	0.443	3683	$1.384_{(4,18)}$	0.279
	Error	3.963			2661		
2-60°N	Depth	36.03	$3.205_{(1,20)}$	0.147	19311	$1.415_{(1,20)}$	0.300
	Sampling date	18.28	$1.598_{(5,20)}$	0.337	7779	$0.556_{(5,20)}$	0.734
	Depth x Sampling date	11.31	$2.916_{(4,20)}$	0.047	13764	$10.99_{(4,20)}$	<0.001
	Error	3.879			1252		
3-60°N	Depth	24.54	$2.379_{(1,30)}$	0.165	36817	$6.580_{(1,30)}$	0.037
	Sampling date	31.06	$2.943_{(7,30)}$	0.089	3868	$0.662_{(7,30)}$	0.700
	Depth x Sampling date	10.55	$1.900_{(7,30)}$	0.105	5843	$8.958_{(7,30)}$	<0.001
	Error	5.555			652.2		
4-60°N	Depth	510.0	$12.80_{(1,24)}$	0.016	130441	$13.24_{(1,24)}$	0.015
	Sampling date	61.24	$1.537_{(5,24)}$	0.324	8980	$0.911_{(5,24)}$	0.539
	Depth x Sampling date	39.86	$7.058_{(5,24)}$	<0.001	9853	$13.69_{(5,24)}$	<0.001
	Error	5.647			719.6		
5-61°N	Depth	34.68	$650.3_{(1,8)}$	0.025	13940	$2.245_{(1,8)}$	0.375
	Sampling date	126.8	$2376_{(1,8)}$	0.013	57270	$9.221_{(1,8)}$	0.203
	Depth x Sampling date	0.053	$0.005_{(1,8)}$	0.944	6211	$2.735_{(1,8)}$	0.137
	Error	10.08			2271		
6-63°N	Depth	330.5	$11.69_{(1,28)}$	0.014	65491	$9.012_{(1,28)}$	0.024
	Sampling date	33.97	$1.201_{(6,28)}$	0.415	4401	$0.606_{(6,28)}$	0.721
	Depth x Sampling date	28.27	$2.782_{(6,28)}$	0.030	7267	$1.531_{(6,28)}$	0.205
	Error	10.16			4747		
7-67°N	Depth	408.0	$14.51_{(1,28)}$	0.009	156795	$12.92_{(1,28)}$	0.011
	Sampling date	51.65	$1.837_{(6,28)}$	0.239	21365	$1.761_{(6,28)}$	0.254
	Depth x Sampling date	28.12	$0.548_{(6,28)}$	0.767	12132	$2.541_{(6,28)}$	0.043
	Error	51.32			4775		
8-67°N	Depth	71.82	$2.654_{(1,10)}$	0.179	661.3	$0.266_{(1,10)}$	0.633
	Sampling date	44.78	$1.665_{(4,10)}$	0.319	9194	$3.703_{(4,10)}$	0.116
	Depth x Sampling date	27.06	$2.305_{(4,10)}$	0.130	2483	$1.597_{(4,10)}$	0.249
	Error	11.74			1555		
9-69°N	Depth	257.6	$8.821_{(1,32)}$	0.017	118606	$13.46_{(1,32)}$	0.006
	Sampling date	53.34	$1.800_{(8,32)}$	0.212	11229	$1.255_{(8,32)}$	0.378
	Depth x Sampling date	29.63	$2.298_{(8,32)}$	0.045	8947	$2.578_{(8,32)}$	0.027
	Error	12.90			3471		

Online Resources 1- Table 4: Results of two-way ANOVA analyzing the effect of depth and sampling date on intracellular nitrate (I-DIN) and tissue nitrogen (Q_N) for all nine locations

		I-DIN (mg NO_3^- g^{-1} DW)			Q_N (mg N g^{-1} DW)		
Location	Effect	MS	$F_{(df)}$	P	MS	$F_{(df)}$	P
1-58°N	Depth	0.001	$1.485_{(1,20)}$	0.290	29.77	$1.591_{(1,17)}$	0.265
	Sampling date	0.000	$0.594_{(4,20)}$	0.687	1282	$73.47_{(4,17)}$	0.001
	Depth x Sampling date	0.001	$1.223_{(4,20)}$	0.332	17.46	$0.225_{(4,17)}$	0.921
	Error	0.001			77.64		
2-60°N	Depth	0.005	$1.946_{(1,24)}$	0.222	166.7	$1.338_{(1,18)}$	0.331
	Sampling date	0.002	$0.647_{(5,24)}$	0.678	853.5	$6.848_{(4,18)}$	0.073
	Depth x Sampling date	0.003	$6.933_{(5,24)}$	<0.001	124.7	$6.23_{(3,18)}$	0.004
	Error	0.000			20.02		
3-60°N	Depth	0.003	$1.759_{(1,30)}$	0.226	17.97	$0.621_{(1,30)}$	0.448
	Sampling date	0.002	$1.343_{(7,30)}$	0.354	1714	$69.26_{(7,30)}$	<0.001
	Depth x Sampling date	0.002	$145.8_{(7,30)}$	<0.001	24.75	$0.219_{(7,30)}$	0.978
	Error	0.000			112.8		
4-60°N	Depth	0.033	$2.709_{(1,24)}$	0.161	4143	$29.55_{(1,36)}$	0.003
	Sampling date	0022	$1.842_{(5,24)}$	0.259	116.5	$0.831_{(5,36)}$	0.578
	Depth x Sampling date	0.012	$62.63_{(5,24)}$	<0.001	140.2	$8.097_{(5,36)}$	<0.001
	Error	0.000			17.31		
5-61°N	Depth	n.d	n.d	n.d	48.40	$1.385_{(1,8)}$	0.448
	Sampling date	n.d	n.d	n.d	496.4	$14.20_{1,8)}$	0.165
	Depth x Sampling date	n.d	n.d	n.d	34.95	$0.259_{(1,8)}$	0.625
	Error	n.d			135.1		
6-63°N	Depth	0.003	$0.086_{(1,28)}$	0.780	286.5	$3.469_{(1,24)}$	0.111
	Sampling date	0.051	$1.467_{(6,28)}$	0.327	378.5	$4.549_{(6,24)}$	0.044
	Depth x Sampling date	0.035	$2.182_{(6,28)}$	0.075	83.21	$1.647_{(6,24)}$	0.178
	Error	0.016			50.53		
7-67°N	Depth	0.836	$19.89_{(1,28)}$	0.004	3865	$276.1_{(1,27)}$	<0.001
	Sampling date	0.068	$1.618_{(6,28)}$	0.287	46.41	$3.312_{(6,27)}$	0.085
	Depth x Sampling date	0.042	$3.772_{(6,28)}$	0.007	14.01	$1.255_{(6,27)}$	0.310
	Error	0.011			11.16		
8-67°N	Depth	0.000	$1.592_{(1,19)}$	0.273	55.19	$0.310_{(1,20)}$	0.607
	Sampling date	0.000	$15.45_{(4,19)}$	0.011	369.2	$2.072(_{4,20)}$	0.249
	Depth x Sampling date	0.000	$0.335_{(4,19)}$	0.852	178.1	$47.81_{(4,20)}$	<0.001
	Error	0.000			3.726		
9-69°N	Depth	0.322	$11.34_{(1,33)}$	0.009	222.9	$9.371_{(1,36)}$	0.016
	Sampling date	0.236	$8.153_{(8,33)}$	0.004	229.5	$9.651_{(8,36)}$	0.002
	Depth x Sampling date	0.029	$1.604_{(8,33)}$	0.162	23.78	$1.501_{(8,36)}$	0.191
	Error	0.018			15.84		

Online Resources 1- Table 5: Results of two-way ANOVA analysing the effect of location and sampling date on the protein content (mg protein g^{-1} DW) of *Saccharina latissima* cultivated at 1-2 m and 8-9 m depth.

	Between-Subject effect	MS	$F_{(df)}$	P
1-2 m depth	Sampling date	1583	$16.32_{(6,35)}$	<0.001
	Location	402	$4.15_{(2,35)}$	0.024
	Sampling date x Location	5681	$58.57_{(11,35)}$	<0.001
	Error	97		
8-9 m depth	Sampling date	1654	$11.71_{(6,33)}$	<0.001
	Location	5431	$38.45_{(2,33)}$	<0.001
	Sampling date x Location	3475.9	$24.61_{(10,33)}$	<0.001
	Error	141		

Online Resources 1- Table 6: Results from the independent sample t-test for protein content between the cultivation depths (1-2 m and 8-9 m) for all experimental sites. Statistical significances ($p < 0.05$) are accentuated in **bold**.

Location	t-value	*df*	p-value
1-58°N	-0.42	3	0.705
2-60°N	-3.90	4	**0.017**
3-60°N	-1.49	3	0.231
4-60°N	-19.35	3	**< 0.001**
5-61°N	-15.00	3	**0.001**
6-63°N	-2.28	4	0.085
7-67°N	-36.12	4	**< 0.001**
8-67°N	0.55	4	0.613
9-69°N	-2.08	4	0.106

Online Resources 1- Table 7: Results of two-way ANOVA analyzing the effect of depth and sampling date on biofouling coverage (%) for all nine locations

		Biofouling coverage (%)		
Location	Effect	MS	$F_{(df)}$	P
1-58°N	Depth	19.85	$0.047_{(1,68)}$	0.838
	Sampling date	9178	$19.51_{(4,68)}$	0.007
	Depth x Sampling date	470.3	$4.042_{(4,68)}$	0.035
	Error	170.4		
2-60°N	Depth	3.740	$0.072_{(1,31)}$	0.805
	Sampling date	9970	$193.0_{(3,31)}$	0.001
	Depth x Sampling date	51.67	$1.036_{(3,31)}$	0.390
	Error	49.86		
3-60°N	Depth	50.25	$0.127_{(1,46)}$	0.736
	Sampling date	10042	$25.10_{(5,46)}$	0.001
	Depth x Sampling date	400.3	$6.434_{(5,46)}$	<0.001
	Error	62.20		
4-60°N	Depth	6132	$9.173_{(1,44)}$	0.039
	Sampling date	724.6	$1.084_{(5,44)}$	0.482
	Depth x Sampling date	668.5	$18.57_{(4,44)}$	<0.001
	Error	36.01		
5-61°N	Depth	338.0	$5.243_{(1,22)}$	0.149
	Sampling date	12314	$190.4_{(2,22)}$	0.005
	Depth x Sampling date	64.69	$3.336_{(2,22)}$	0.054
	Error	19.39		
6-63°N	Depth	3.295	$0.269_{(1,48)}$	0.626
	Sampling date	2120	$173.0_{(5,48)}$	<0.001
	Depth x Sampling date	12.25	$0.221_{(5,48)}$	0.952
	Error	55.42		
7-67°N	Depth	295.8	$1.187_{(1,56)}$	0.318
	Sampling date	404.3	$1.623_{(6,56)}$	0.286
	Depth x Sampling date	249.2	$18.11_{(6,56)}$	<0.001
	Error	13.76		
8-67°N	Depth	222.5	$1.059_{(1,32)}$	0.379
	Sampling date	1653	$7.861_{(3,32)}$	0.062
	Depth x Sampling date	210.2	$6.462_{(3,32)}$	0.002
	Error	32.53		
9-69°N	Depth	35.55	$0.085_{(1,79)}$	0.779
	Sampling date	2159	$4.972_{(7,79)}$	0.025
	Depth x Sampling date	434.2	$4.890_{(7.79)}$	<0.001
	Error	88.78		

Online Resources 2- Figure 1

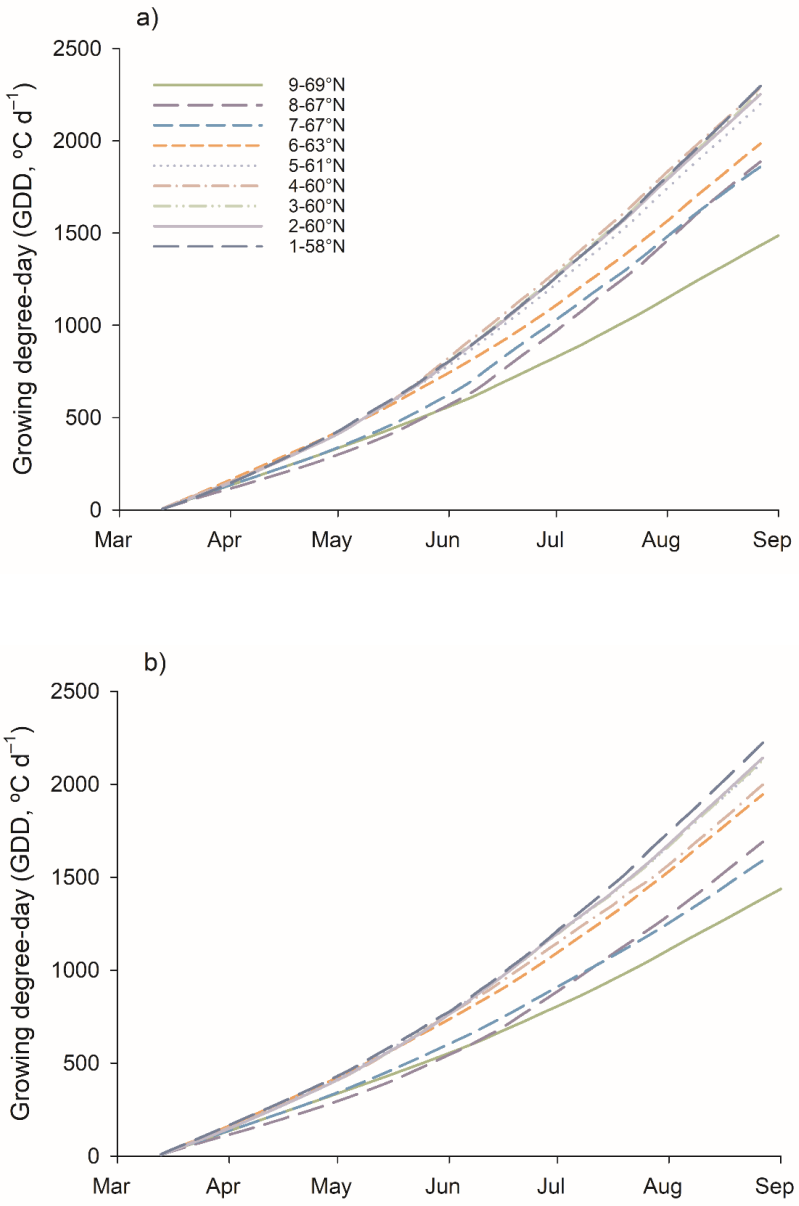

Figure 1 (Online Resources 1) Growing degree-day (GDD,) for all locations for a) 2 m depth and b) 8 m depth.

Online Resources 2- Figure 2

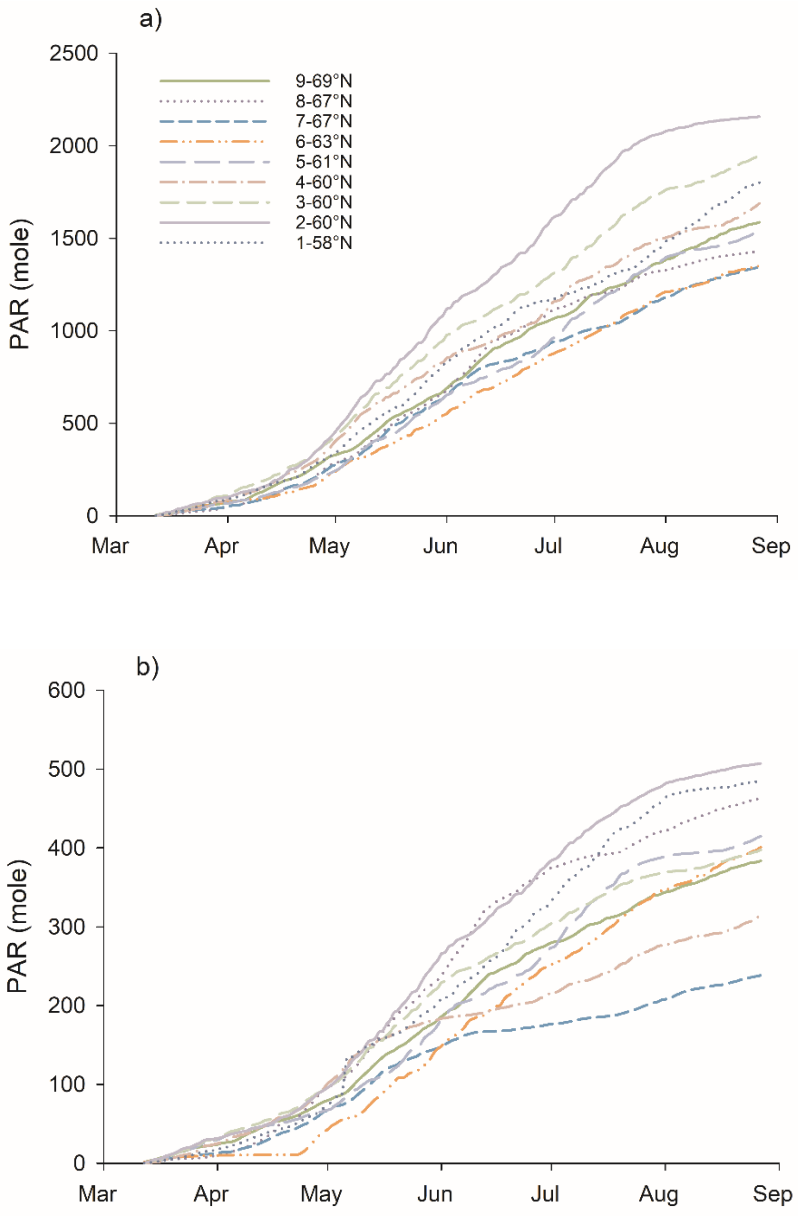

Figure 2 (Online Resources 1) Accumulated PAR (mole) for all locations for a) 2 m depth and b) 8 m depth. Notice the different scale on the y-axis.

Online Resources 3: Dry weight (% of wet weight) and Ash (mg g^{-1} DW) for all locations and all sampling dates. Mean ± SE, n=3, **n=2, *n=1

		DW (% of WW)		Ash (mg g^{-1} DW)	
Location		*1-2 m*	*8-9 m*	*1-2 m*	*8-9 m*
1-58°N	*2-May*	10.4 ± 0.4	7.4 ± 0.1	352.7 ± 17.8	518.9 ± 8.0
	18-May	11.6 ± 1.4	8.8 ± 0.9	347.0 ± 50.3	456.9 ± 35.4
	12-Jun	14.9 ± 0.6	11.7 ± 0.1	278.3 ± 29.9	405.2 ± 29.6
	03-Jul	16.5 ± 0.6	13.6 ± 0.4	367.4 ± 8.3	450.7 ± 15.6
	06-Sep	16.0 ± 2.5	16.6 ± 1.3	350.7 ± 41.1	374.2 ± 10.5
2-60°N	*18-Apr*	11.0 ± 0.9	8.4 ± 0.7	406.4 ± 7.7	505.9 ± 29.6
	03-May	14.4 ± 2.0	9.9 ± 1.4	318.9 ± 17.3	437.2 ± 35.5
	15-May	13.6 ± 0.1	9.5 ± 0.2	301.1 ± 18.7	416.5 ± 24.8
	02-Jun	11.5 ± 0.4	12.3 ± 0.8	403.3 ± 33.7	327.5 ± 3.9
	14-Jun	12.7 ± 1.1	12.9 ± 1.0	416.0 ± 12.6	384.8 ± 1.1
	14-Jul	17.8 ± 2.0		386.1 ± 14.3	
3-60°N	*18-Apr*	13.2 ± 1.8	10.4 *	367.8 ± 47.9	388.9*
	04-May	13.2 ± 0.7	11.6 ± 3.2	292.4 ± 8.6	477.7 ± 24.7
	16-May	13.7 ± 1.6	8.9 ± 0.6	323.1 ± 25.2	427.2 ± 28.6
	01-Jun	14.5 ± 2.3	15.2 ± 1.4	309.6 ± 30.7	350.1 ± 22.7
	13-Jun	13.9 ± 0.3	12.3 ± 0.7	411.4 ± 31.2	418.6 ± 21.2
	05-Jul	16.0 ± 0.5	17.0 ± 1.4	389.5 ± 4.2	402.8 ± 11.7
	09-Aug	20.0 ± 0.1	14.4 ± 1.5	348.2 ± 1.8	438.1 ± 42.8
	04-Sep	15.1 ± 1.0	17.2 ± 0.2	358.5 ± 21.3	366.7 ± 4.2
4-60°N	*17-Apr*	11.8 ± 1.8	11.3 ± 0.5	316.7 ± 21.7	292.5 ± 19.2
	04-May	14.6 ± 0.5	11.5 ± 0.2	232.8 ± 8.9	315.4 ± 6.9
	18-May	14.9 ± 1.3	7.9 ± 0.4	205.2 ± 4.0	344.1 ± 20.2
	01-Jul	18.4 ± 0.9	6.8 ± 0.3	118.2 ± 8.1	283.9 ± 8.4
	13-Jun	27.1 ± 2.6	12.7 ± 1.2	142.0 ± 11.1	298.0 ± 21.8
	09-Jul	19.1 ± 3.0	10.3 ± 0.4	140.2 ± 27.2	343.0 ± 3.6
5-61°N	*3-May*	11.7 ± 1.1	8.4 ± 0.5	367.1 ± 41.9	480.9 ± 25.4
	29-May	18.3 ± 2.2	14.8 ± 2.6	274.8 ± 11.5	297.4 ± 22.6
6-63°N	*25-Apr*	21.6 ± 0.8	12.4 ± 2.6	360.8 ± 29.9	441.2 ± 31.5
	04-May	14.1 ± 1.4	13.8 ± 2.3	339.1 ± 69.1	366.8 ± 79.8
	18-May	13.9 ± 1.9	13.5 ± 1.5	335.8 ± 155.0	369.1 ± 76.9
	31-May	15.3 ± 2.0	12.5 ± 1.6	418.8 ± 59.6	409.9 ± 90.1
	15-Jun	23.4 ± 0.4	12.9 ± 1.0	242.4 ± 54.8	440.9 ± 42.2
	03-Jul	21.9 ± 3.0	14.6 ± 1.1	309.4 ± 69.0	432.6 ± 34.0
	09-Aug	23.6 ± 2.9	14.8 ± 1.5	312.5 ± 19.2	411.3 ± 27.0
7-67°N	*27-Apr*	21.4 ± 4.8	18.9 ± 3.0	356.2 ± 21.0	406.3 ± 42.6
	04-May	18.1 ± 0.7	11.8 ± 1.8	400.9 ± 18.8	428.7 ± 7.9
	22-May	23.0 ± 10.8	11.9 ± 1.6	286.2 ± 78.0	455.3 ± 20.0
	30-May	16.0 ± 1.7	9.1 ± 0.7	317.0 ± 26.0	473.8 ± 20.6
	16-Jun	15.3 ± 1.8	10.6 ± 0.5	206.8 ± 22.8	405.7 ± 14.3
	07-Jul	22.8 ± 4.3	10.8 ± 2.7	146.1 ± 14.1	384.5 ± 16.3
	18-Aug	16.6 ± 6.2	21.8 ± 2.4	349.1 ± 75.5	212.3 ± 22.7

		DM (% of WW)		Ash (mg g^{-1} DW)	
Location		*1-2 m*	*8-9 m*	*1-2 m*	*8-9 m*
8-67°N**	*23-Apr*	9.9 ± 0.3	10.8 ± 2.5	390.3 ± 11.7	364.3 ± 59.0
	14-May	10.4 ± 1.1	9.4 ± 0.1	366.4 ± 34.5	436.6 ± 18.0
	04-Jun	9.6 ± 1.2	10.8 ± 1.8	335.0 ± 41.4	313.2 ± 28.3
	17-Jun	9.6 ± 1.0	15.9 ± 5.2	332.7 ± 7.5	267.4 ± 8.9
	10-Jul	12.0 ± 0.3	23.7 ± 4.7	300.6 ± 7.1	286.2 ± 1.7
9-69°N	***21-Apr*	11.8 ± 1.0	8.8 ± 0.4	350.8 ± 35.3	434.5 ± 36.1
	***5-May*	14.5 ± 2.0	8.1 ± 1.6	263.4 ± 69.3	473.3± 19.6
	16-May	12.5 ± 0.6	10.0 ± 0.7	427.6 ± 40.7	474.3 ± 27.1
	31-May	12.4 ± 2.6	11.6 ± 1.3	420.4 ± 58.6	442.5 ± 39.2
	14-Jun	22.6 ± 2.3	13.3 ± 0.7	228.3 ± 1.5	398.0 ± 13.2
	05-Jul	13.6 ± 0.8	12.6 ± 0.9	401.0 ± 17.6	474.3 ± 6.9
	09-Aug	14.1 ± 1.8	15.5 ± 3.7	407.0 ± 45.8	375.9 ± 31.2
	05-Sep	22.6 ± 3.5	10.8 ± 1.5	324.1 ± 42.1	454.7 ± 24.1
	28-Sep	22.7 ± 3.9	14.6 ± 1.7	264.1 ± 39.4	448.5 ± 32.7

Online Resources 4: Frond area covered (%) divided by taxon for all locations and all sampling dates when the taxon in question was registered. Mean ± SE, n=5, *n=4

			Frond area covered (%)			
Location		**Taxon**	*1-2 m*		*8-9 m*	
1-58°N	*12-Jun*	*M. membranacea*	5.2 ± 2.0		5.6 ± 2.1	
		Hydrozoa	0.2 ± 0.2			
		Filamentous algae	0.1 ± 0.2			
	4-Jul	*M. membranacea*	21.5 ± 8.0		42.8 ± 10.4	
		Hydrozoa	0.3 ± 0.3			
		Bivalvia	2.1 ± 0.9		0.3 ± 0.3	
	23-Sep	*M. membranacea*	71.3 ± 11.5	*	54.4 ± 19.1	*
		Hydrozoa	4.2 ± 1.3	*	10.3 ± 5.3	*
2-60°N	*18-Apr*	*M. membranacea*	0.2 ± 0.2			
		Filamentous algae/Diatoms	2.1 ± 0.9		5.2 ± 1.6	
	3-May	*M. membranacea*	0.1 ± 0.1			
		Filamentous algae/Diatoms	10.9 ± 3.4		15.4 ± 2.2	
	15-May	*M. membranacea*	1.0 ± 0.6		1.2 ± 0.4	
		Filamentous algae/Diatoms			1.2 ± 0.7	
		Filamentous algae	0.2 ± 0.2			
	14-Jun	*M. membranacea*	69.7 ± 4.2		65.7 ± 6.3	
		Bivalvia	1.3 ± 0.5		0.2 ± 0.2	
		Filamentous algae	1.1 ± 0.6		0.4 ± 0.2	
3-60°N	*18-Apr*	Filamentous algae/Diatoms	5.6 ± 1.3		31.6 ± 4.6	*
	4-May	Filamentous algae/Diatoms	10.9 ± 2.7		12.8 ± 3.3	
	16-May	*M. membranacea*	1.2 ± 0.4		0.4 ± 0.2	
		Filamentous algae	0.2 ± 0.2		1.2 ± 0.2	
	1-Jun	*M. membranacea*	11.6 ± 3.2		4.9 ± 1.6	
		E. pilosa	0.2 ± 0.2			
		Hydrozoa			0.6 ± 0.6	
		Filamentous algae	0.4 ± 0.4		1.6 ± 0.6	
	12-Jun	*M. membranacea*	76 ± 6.4		70.4 ± 5.6	
		Bivalvia	0.7 ± 0.4			
		Filamentous algae	4.8 ± 2.2		2.1 ± 1.2	
4-60°N	*4-May*	Hydrozoa	0.2 ± 0.2		10.5 ± 1.3	
	4-Jun	*M. membranacea*	0.4 ± 0.2			
		Hydrozoa			40.8 ± 1.1	
		Bivalvia	0.5 ± 0.2			
	13-Jun	*M. membranacea*	0.4 ± 0.2			
		Hydrozoa	0.4 ± 0.2		34.6 ± 4.8	
		Bivalvia	0.8 ± 0.4			
		M. membranacea	1.4 ± 1.2		12.8 ± 4.1	
		E. pilosa	0.9 ± 0.6			
		Hydrozoa	0.4 ± 0.2		20.0 ± 5.0	
		Bivalvia	3.9 ± 0.6			
		Filamentous algae/Diatoms			1.0 ± 0.7	
5-61°N	*3-May*	Filamentous algae/Diatoms			4.4 ± 0.5	*

	29-May	*M. membranacea*	5.3 ± 1.3		7.4 ± 1.0	
		Hydrozoa	0.2 ± 0.2		1.4 ± 1.0	
	28-Jun	*M. membranacea*	61.2 ± 4.7	*	75.9 ± 3.6	*
		Bivalvia	2.6 ± 1.0	*		
6-63°N	*25-Apr*	Filamentous algae			0.8 ± 0.3	
	4-May	Filamentous algae	0.2 ± 0.2		0.4 ± 0.4	
	18-May	*M. membranacea*	0.1 ± 0.1		0.2 ± 0.2	
		Filamentous algae	0.3 ± 0.3			
	31-May	*M. membranacea*	0.3 ± 0.3		1.0 ± 0.3	
		Filamentous algae	0.3 ± 0.3		0.2 ± 0.1	
	15-Jun	*M. membranacea*	3.0 ± 1.2		5.0 ± 0.6	
		Filamentous algae	0.4 ± 0.4			
	3-Jul	*M. membranacea*	38.9 ± 9.2		33.7 ± 7	
		Filamentous algae	0.2 ± 0.2		0.5 ± 0.4	
7-67°N	*22-May*	*M. membranacea*	0.2 ± 0.2			
	30-May	*M. membranacea*	0.2 ± 0.2			
	16-Jun	*M. membranacea*	0.4 ± 0.2			
	7-Jul	*M. membranacea*	0.3 ± 0.3	*		
		Hydrozoa			0.7 ± 0.4	
		Filamentous algae/Diatoms			2.4 ± 0.9	
	18-Aug	*M. membranacea*	2.4 ± 1.9		10.8 ± 6.8	
		Hydrozoa	0.2 ± 0.2		18.3 ± 4.5	
		Bivalvia	0.4 ± 0.4			
		Filamentous algae			1.3 ± 1.1	
		Unidentifiable	0.8 ± 0.8			
8-67°N	*17-Jun*	*E. pilosa*	0.2 ± 0.2			
		Unidentifiable	0.2 ± 0.2			
	10-Jul	*M. membranacea*	3.9 ± 2.4		10.3 ± 3.3	
		E. pilosa	4.0 ± 2.3			
		Hydrozoa	22.4 ± 5.9		5.8 ± 2.1	
		Bivalvia			0.4 ± 0.2	
9-69°N	*14-Jun*	*M. membranacea*			0.2 ± 0.2	
		Filamentous algae	0.3 ± 0.2		0.4 ± 0.4	
	5-Jul	Filamentous algae	2.2 ± 0.8		1.3 ± 0.4	
	20-Jul	Filamentous algae	3.9 ± 1.3		1.4 ± 0.6	
	9-Aug	*M. membranacea*	0.8 ± 0.8		0.3 ± 0.2	
		Hydrozoa	0.8 ± 0.5		0.3 ± 0.2	
		Filamentous algae	4.1 ± 0.9		4.0 ± 1.4	
	5-Sep	*M. membranacea*	6.2 ± 3.7		13.9 ± 7.1	
		Hydrozoa	11.1 ± 3.1		12.1 ± 6.1	
		Filamentous algae	5.4 ± 2.6		0.5 ± 0.3	
	28-Sep	*M. membranacea*	18.8 ± 8.1		44.0 ± 9.9	
		Hydrozoa	6.1 ± 1.8		6.6 ± 1.9	

Paper IV

Journal of Applied Phycology
https://doi.org/10.1007/s10811-019-01936-0

23RD INTERNATIONAL SEAWEED SYMPOSIUM, JEJU

Effect of seeding methods and hatchery periods on sea cultivation of *Saccharina latissima* (Phaeophyceae): a Norwegian case study

Silje Forbord[1,2] · Kristine B. Steinhovden[2] · Torfinn Solvang[2] · Aleksander Handå[2] · Jorunn Skjermo[2]

Received: 5 July 2019 / Revised and accepted: 16 September 2019

Abstract
To reach the goal of an industrialised macroalgae industry in Norway and other high-cost countries in the near future, a standardised seedling production method to improve quality control and predictability of cultivated biomass is essential. A total of 11 different treatments for seeding twine or rope with meiospores, gametophytes or juvenile sporophytes from the kelp *Saccharina latissima* were measured for growth (frond length, frond area, biomass yield and density) and protein content after 80 and 120 days at sea. Meiospore- and gametophyte-seeded twines were pre-cultivated in the hatchery for 14–42 days prior to deployment, while juvenile sporophytes of different ages were seeded on ropes directly on the day of deployment using a commercial binder to attach the seedlings. The results showed that seeding with meiospores pre-cultivated in the hatchery for 42 days (S42) before deployment gave significantly longer fronds (77.0 ± 6.7 cm) and a higher biomass yield (7.2 ± 0.1 kg m^{-1}) at sea compared to other treatments. The poorest growth was measured for the direct-seeded sporophytes pre-cultivated in free-floating cultures for 35 days prior to deployment (D35; 34.4 ± 2.4 cm frond length and 1.6 ± 0.4 kg m^{-1}). Image analysis was used to measure the coverage of the twine substrate before deployment, and a correlation was found between substrate coverage and frond length at sea, indicating that this can be used as a tool for quantity and quality control during the hatchery phase and before deployment. The protein content did not reveal any large differences between the treatments after 120 days of cultivation.

Keywords Cultivation strategies · Direct seeding · Kelp aquaculture · Image analysis · Optimising seaweed hatchery · Protein content

Introduction

The macroalga *Saccharina latissima* (Phaeophyceae) is one of the most attractive species for cultivation in the North Atlantic Ocean due to its fast growth and high content of valuable components (Holdt and Kraan 2011; Handå et al. 2013; Peteiro and Freire 2013; Sharma et al. 2018; Bak 2019). In 2017 Europe contributed less than 1000 t of *S. latissima* to the global production of cultivated macroalgae of about 30 million t (Ferdouse et al. 2018), with China and other Asian countries supplying the major part of the biomass using breeding as a strategy to improve yield and quality (Wu and Guangheng 1987; Zhang et al. 2007). Macroalgae for human consumption account for 83–90% of the value of the global market (Wei et al. 2013), and the Western market is expected to increase rapidly due to consumers' desire for new protein sources and healthy food supplements (Kim et al. 2017). This is a key driver for the ongoing development of an industrial macroalgae cultivation in Europe (Cottier-Cook et al. 2016), and high salary costs call for standardised solutions that are easy to scale up.

In aquaculture, seaweeds grow on artificial substrates or under free-floating conditions. Regardless of cultivation methods, land facilities are currently necessary to accommodate the hatchery units and facilities for the processing of harvested biomass. Although most cultivated macroalgae species can be grown through vegetative propagation, the production of seedlings is mandatory for several important commercial species. *Saccharina latissima* has a diplo-haplontic, heteromorphic life cycle

✉ Silje Forbord
Silje.Forbord@sintef.no

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

[2] SINTEF Ocean, Department of Environment and New Resources, N-7465 Trondheim, Norway

Published online: 23 October 2019

with alternation between a microscopic haploid (*n*) gametophyte generation and a macroscopic diploid (2*n*) sporophyte generation (Kain 1979). During the fertile season, a sorus with sporangia develops on the lamina and meiosis produces meiospores (spores) that are released into the surrounding water (Van Patten and Yarish 1993). The spores develop into female and male gametophytes, and fertilisation leads to the development of microscopic sporophytes that grow to adult size (Kain 1979). For any seaweed species grown through sexual reproduction, optimising hatchery production processes is crucial to the success of sea farming. Standardisation of cultivation procedures and strategies is essential to overcome low predictability of production quantity and quality, and to lower the production costs.

There are three main strategies for producing kelp seedlings, seeding the growth substrate with either (1) meiospores, (2) gametophytes or (3) juvenile sporophytes. Seedlings can be kept on a substrate in the hatchery for several weeks before deployment at sea or seeded directly before deployment using a binder.

Using spores for seeding requires fertile sporophytes, which is season-dependent if these are collected in natural habitats. Fertility can also be induced by artificial day rhythm and thus enable year-around access to spores (Pang and Lüning 2004; Forbord et al. 2012). *Saccharina latissima* development can be held in the gametophyte life stage (Zhang et al. 2008) by keeping the cultures in red light under controlled environmental conditions where fertilisation can be induced by changing from red to white light (Lüning and Dring 1972; Cuijuan et al. 2005). These continuous cultures are available for year-through seeding of gametophytes or production of juvenile sporophytes for direct seeding. This method can be advantageous as the use of incubation facilities might be shortened by several weeks or, in the case of direct seeding, omitted completely. The use of a binder to attach spores, gametophytes or sporophytes to the substrate is preferred by several commercial farmers and in research projects (Mols-Mortensen et al. 2017; Bak et al. 2018; Kerrison et al. 2018, 2019). Recent experiments have shown that a binder method of cultivation is not only as effective as traditional methods but also can be 100 times more space-efficient during the laboratory phase (Kerrison et al. 2018). When seeding spores or gametophytes on twine without using a binder, they need to be incubated in a hatchery for several weeks to be able to attach properly to the substrate before being deployed at sea (Xu et al. 2009; Forbord et al. 2018).

This study aimed to compare how three different seeding methods of *S. latissima*, using either meiospores, gametophytes or direct seeding with juvenile sporophytes, and time of hatchery periods affect growth in size (length and area), biomass yield, density and protein content during 80 and 120 days of sea cultivation.

Materials and methods

Preparation of gametophyte cultures

Sporophytes of *S. latissima* were collected by divers in August 2017 for induction of sori under low-light (70 μmol photons m^{-2} s^{-1} at the water surface)/short-day (8 h light:16 h dark) conditions for 6 weeks before maturation according to Forbord et al. (2012). Sorus pieces from around 20 sporophytes were disinfected and dehydrated for 24 h at 4 °C, and spore release was carried out the following day. The spore solution used for starting gametophyte cultures had a density of 400,000 spores mL^{-1}, which was added to culture flasks with Provasoli's enriched seawater (PES) (Provasoli 1968) kept at 10 °C under constant red light with a light intensity of 30 μmol photons m^{-2} s^{-1} and filtered air provided through silicon tubes for aeration. The cultures were up-scaled and maintained after 4 weeks and then every second week until used in the experiment in January 2018.

Preparation of cultures of juvenile sporophytes for direct seeding

Fertile *S. latissima* sporophytes were collected by divers in December 2017 and stored in tanks with running seawater and low-light/short-day conditions until used for different seeding trials in the current experiment. Sori tissue from around 20 sporophytes was used to obtain a spore solution with a density of 400,000–470,000 spores mL^{-1} used for starting cultures of free-floating seedlings in aerated flasks containing PES at 10 °C in white light (40 μmol photons m^{-2} s^{-1}) and a light regime of 16 h light:8 h dark to promote fertilisation and sporophyte development. Cultures were started 42 (D42), 35 (D35) and 28 (D28) days before they were seeded on ropes using a binder and deployed at sea the same day. The seedlings had an average length of 45–120 μm before seeding, and the fraction of sporophytes vs. gametophytes was in the range of 7.4–13.6% where one counted individual was equal to one sporophyte or one gametophyte filament (Table 1). The sporophyte cultures were diluted 50/50 with autoclaved seawater to aim for the same density as the spore solutions. One of the gametophyte culture was induced in white light for 14 days (GF0) before being seeded with the binder directly on ropes on the day of deployment and had an average length of 88 μm and a sporophyte vs. gametophyte fraction of 6.4%. A commercial binder (AtSeaNova, BE) was used to thicken the sporophyte/gametophyte cultures, which were applied to the ropes (⌀ 18 mm, braided AlgaeRope), preventing the suspended seedlings from being washed off before they could attach (Kerrison et al. 2018). The protocol for

Table 1 Detailed information for the different hatchery treatments before deployment in sea showing their abbreviation (Abb), the starting dates for cultures and for induction of fertility in white light, the culture density, the date of seeding on ropes, the seeding density (spores mL^{-1} for spores, mg mL^{-1} (DW) for gametophytes and individuals mL^{-1} for sporophytes) and the sporophyte lengths at seeding

Hatchery treatment	Abb	Date of culture start/date of fertility induction	Culture density (spores mL^{-1})	Date for seeding on ropes	Seeding density (spores mL^{-1}, *mg mL^{-1} (DW) or **individuals mL^{-1})	Length at seeding (μm)
Spores 42	S42	–	–	03.01.18	250,000	–
Spores 28	S28	–	–	17.01.18	250,000	–
Spores 21	S21	–	–	24.01.18	250,000	–
Gametophytes 28	G28	06.10.17	400,000	17.01.18	*0.35	–
Gametophytes 21	G21	06.10.17	400,000	24.01.18	*0.35	–
Gametophytes 14	G14	06.10.17	400,000	31.01.18	*0.35	–
Gametophytes fertile 14	GF14	06.10.17/17.01.18	400,000	31.01.18	*0.35	–
Gametophytes fertile direct 0	GF0	06.10.17/31.01.18	400,000	13.02.18	**Gametophytes: 2805 **Spores: 180	87.6
Sporophytes direct 42	D42	03.01.18	434,000	13.02.18	**Gametophytes: 1740 **Spores: 185	92.4
Sporophytes direct 35	D35	10.01.18	400,000	13.02.18	**Gametophytes: 5973 **Spores: 440	44.5
Sporophytes direct 28	D28	17.01.18	467,000	13.02.18	**Gametophytes: 1730 **Spores: 235	120.0

seeding with the binder is IPR of the AT~SEA project partners (http://www.atsea-project.eu/).

Seeding of meiospores and gametophytes on twine in the hatchery

The spore solution used for producing seedlings on twine had a density of 250,000 spores mL^{-1} (Table 1). Gametophytes used for seeding were either taken straight from the red-light conditions or induced for 14 days in white light to make the gametophytes fertile before seeding. The gametophyte densities were measured with optical density at 750 nm (OD_{750}) and diluted to 0.35 mg mL^{-1} (DW) before seeding (OD calculated to DW from standard curves), a density found to give adequate seedling growth on substrate in previous experiments with *Alaria esculenta* (Duarte 2017). Spores and gametophytes were seeded on ⌀ 1.2 mm twisted nylon string coiled around PVC spools. The spools were incubated in 300-L cylinders holding running, nutrient-rich deep water, a light intensity of 70 μmol photons m^{-2} s^{-1} outside the cylinders and a light regime of 16:8 (L:D). To let the propagules settle on the twine, the water was kept stagnant before a water flow of around 2 L min^{-1} and aeration was turned on 3 days after seeding. Seeding with spores started 42 (S42), 28 (S28) and 21 (S21) days before deployment, and gametophytes were seeded 28 (G28), 21 (G21) and 14 (G14) days before deployment (Table 1). One culture of gametophytes was induced in white light for 14 days before being seeded on twine and kept in the hatchery for 14 days before deployment (GF14). Details concerning all stages of seedling production are described in Forbord et al. (2018).

Substrate coverage measured by image analysis

To quantify the number of seedlings covering the twine substrate, images were collected 2 days prior to deployment at sea for later processing. The cylinders with the twine substrate were removed from the incubators into a small water-filled glass tank for depiction. A white LED ring light (Effilux 000 SD P2) was used for even illumination in a brightfield setup with the lens positioned inside the ring light. Each substrate cylinder was depicted at three distinct locations, resulting in three images per substrate (Alver et al. 2018). The working distance was kept identical between images, and the field of view was 30 × 40 mm. Images were collected using a Nikon D800E DSLR and a Sigma AF 105 mm f/2,8 macro lens. Software was developed in LabVIEW (National Instruments Co., USA), which extracted the saturation colour plane to identify seedlings on the white substrate. The International Commission on Illumination has defined six attributes describing a colour, saturation being one of them, defined as the colourfulness of an area relative to its brightness (CIE Standard S 017/E 2011). Using the saturation colour plane is a robust method of segmenting the growth from the cultivation substrate. The average pixel intensity from the saturation colour plane of the three images per cylinder is calculated to

represent the amount of growth using the developed software. The values presented here were normalised to a percentage of the output range, where 0% is a clean, white substrate and 100% is a substrate completely covered by sporophytes.

Deployment at sea, growth measurements and collection of samples for chemical analysis

The seed lines were entwined onto 6-m-long 18-mm carrier ropes using a spinning machine on the day of deployment (Alver et al. 2018; Forbord et al. 2018) and randomly distributed vertically from two longlines at the sea farm Skarvøya in Central Norway (63° 39′ N, 8° 39′ E) on 13 February 2018 (Fig. 1). This area has a mild maritime climate with the coldest season from January to March and the driest season in May to June (Sharma et al. 2018). The farm is sited at a sheltered location. The first registration was done on 4 May 2018 after 80 days at sea and the last one on 13 June 2018 after 120 days at sea. Length and width measurements of 20 sporophytes for all treatments on each of the two longlines were registered in May and June, and biomass and density were measured only in June, all from 1 to 2 m cultivation depth. Samples of 10 individuals, consisting of frond, stipe and holdfast, from all treatments at both lines, were collected for chemical analysis and kept cold in bags until frozen at − 20 °C immediately after arriving at the laboratory (Fig. 1).

The relative daily growth rate (RGR, day^{-1}) based on an increase of mean frond length was calculated as

$$\mathrm{RGR}\left(\mathrm{day}^{-1}\right) = \frac{\left(\frac{L_1 - L_0}{T}\right)}{L_0} \quad (1)$$

where L_1 represents length (cm) at a given sampling date, L_0 the length (cm) at the previous sampling date and T is the elapsed time (days) between these sampling days.

The area of the sporophytes was calculated using a factor of 0.75 × length × width (Broch et al. 2013).

Temperature

The temperature was recorded at 2 m depth every 15 mins using Onset HOBO pendant loggers (Bourne, USA; temperature accuracy ± 0.53 °C, resolution 0.14 °C) situated on a separate rope placed in the middle of the farm. The loggers were cleaned from fouling during the May registration.

Nitrogen analysis

Nitrogen content was analysed for the whole thalli. The samples were frozen at − 20 °C and later stored at − 80 °C until freeze-dried (Hetosicc CD 13-2) at − 40 °C for 48 h. The freeze-dried kelp was homogenised into a fine powder; samples of 0.4–1.0 mg were transferred to tin capsules, and nitrogen was analysed in parallels with a Carlo Erba element analyser (model 1106). The nitrogen content was used to calculate the protein content using season- and depth-specific

Fig. 1 Geographical location of the experimental seaweed farm Skarvøya and the laboratory producing the seed lines. The region to the right is indicated by a black rectangle in the large-scale map

nitrogen-to-protein conversion factors (K_p) of 3.6 for May and 4.3 for June (Forbord et al. submitted).

Statistics and data analyses

Independent-samples *t* tests were run to assess if there were differences between frond length and width between the two long lines after confirming the assumption of normality (Shapiro-Wilk's test) and homogeneity of variance (Levene's test). Significant differences were not found between any of the 11 treatments on the two lines, and the lines were pooled to get a sample size of $n = 40$. For frond length and sporophyte area, the assumption of homogeneity of variances was violated (Levene's test, $p < 0.001$). The Welsh ANOVA was used to look for significant differences and the Games-Howell post hoc test to compare all possible combinations of group differences. Linear regression analysis was performed to look for the relationship between density and individual sporophyte weight, and for mean frond length and the substrate coverage before deployment. Data are presented as mean ± standard error (SE) unless otherwise stated. Significance level was set to 0.05. Statistical analysis was performed using IBM SPSS Statistical Software (Version 25), and plots were made using Systat SigmaPlot software (version 14).

Results

Substrate coverage before deployment at sea

The treatment showing the highest substrate coverage was the spore treatment S42 with an average of 84% (Figs. 2 and 3). This treatment had the longest incubation time of 42 days in the hatchery before deployment at sea. S28 and S21 had a coverage of 58% and 25%, respectively. For the gametophyte seeding, the G28 treatment with 28 days of incubation had a substrate coverage of 43% on average compared to G21 and G14 with 25% and 9% coverage, respectively. The GF14 treatment that was induced in white light before seeding and incubated in the hatchery for the same number of days as the G14 treatment had a coverage of 10%.

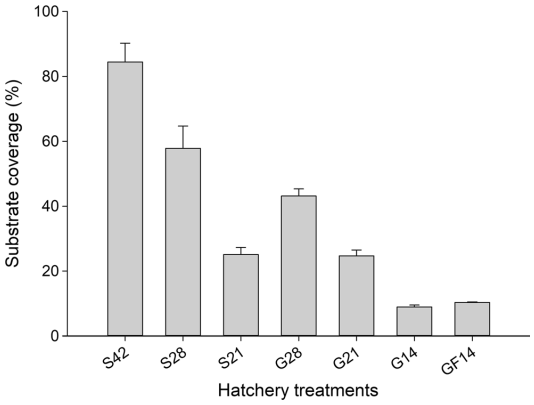

Fig. 2 Substrate coverage (%) before deployment of spore and gametophyte treatments on twine (see Table 1) incubated in the hatchery for 14–42 days. Mean ± SD, $n = 3$

Temperature

The sea temperature was at its lowest in mid-March at 4.5 °C and at its highest at the beginning of June at 10.2 °C (Fig. 4). Average monthly temperatures never reached more than 9.8 °C.

Growth performance at sea

Mean frond lengths of *S. latissima* varied pronouncedly between treatments, and a pattern was apparent where the treatments with a long hatchery period had longer fronds than the treatments with a shorter period in the hatchery (Fig. 5). The mean maximum frond length in June of 77.0 ± 6.7 cm was found for treatment S42, while the shortest fronds were found for treatment D35 with 34.4 ± 2.4 cm. The S42 sporophytes were significantly longer than the other treatments in both May (Welch's $F_{10,170.2} = 48.7$, $p < 0.001$) and June (Welch's $F_{10,171.2} = 20.4$, $p < 0.001$).

Generally, the direct-seeded treatments (GF0, D42, D35 and D28) showed higher relative growth rates (RGR, day^{-1}) between the two registrations than the spore- and gametophyte-seeded treatments. The RGR for all treatments fluctuated around 0.02–0.05 day^{-1} (Fig. 5).

The relationship between mean frond length (cm) and the substrate coverage (%) before deployment revealed a strong positive correlation for both May ($r = 0.84$) and June ($r = 0.90$). Linear regression was used to fit straight lines to the data (Fig. 6), and the linear association reached statistical significance for both May ($R^2 = 0.7$, $F_{1,5} = 11.8$, $p = 0.018$) and June ($R^2 = 0.8$, $F_{1,5} = 20.7$, $p = 0.006$), with a slightly better fit for June than for May.

The highest mean frond area in June was found for treatment S42 with 588 ± 52.4 cm^2, and the lowest was measured for D35 with 133 ± 11.8 cm^2 (Fig. 7). The S42, S28, G28 and G21 sporophytes had a significantly larger area than the other treatments in May (Welch's $F_{10,165.95} = 43.1$, $p < 0.001$), and in June, the S42 and G28 sporophytes showed a significantly larger area than the other treatments (Welch's $F_{10,170.3} = 19.5$, $p < 0.001$).

Biomass yield (kg m^{-1}) and sporophyte density (individuals m^{-1}) were measured in June, and as no fouling by diatoms and filamentous algae was visible, the weight represents only kelp biomass. The mean biomass yield across all treatments was 3.4 ± 0.5 kg m^{-1}, and the range was from 1.6 ± 0.4 for D35 to 7.2 ± 0.1 kg m^{-1} for the S24 treatment (Fig. 8a).

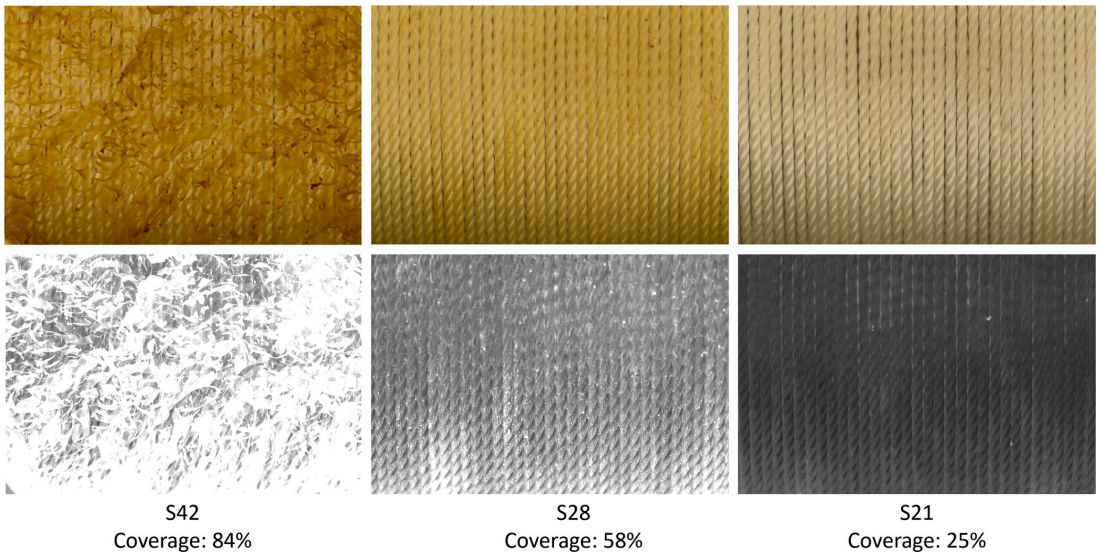

Fig. 3 Colour images of substrate (top row) and corresponding saturation image planes (bottom row). Treatment S42 (left) had an average substrate coverage of 84%, treatment S28 (mid) with an average substrate coverage of 58% and treatment S21 (right) with an average substrate coverage of 25%

The sporophyte density had a mean value across all treatments of 311 ± 87.9 individuals m^{-1} and was lowest for the G28 treatment with 175 ± 5.0 individuals m^{-1} compared to the highest density of 450 ± 35.4 individuals m^{-1} for the D35 treatment (Fig. 8b).

The relationship between sporophyte density and the individual sporophyte weight revealed a linear increase of individual weight with decreasing density with a positive correlation ($r = 0.73$) (Fig. 9). Linear regression was used to fit a straight line to the data, and the linear association reached statistical significance ($R^2 = 0.5$, $F_{1,9} = 10.0$, $p = 0.012$). The results from the S42 treatment (marked with a circle in Fig. 9) deviated strongly from the others, having both a high density (417 ± 31.8 individuals m^{-1}) and high individual weight (8.7 ± 0.6 g). With the removal of this treatment from the linear regression, the positive correlation was strong ($r = 0.95$), with a significant linear association ($R^2 = 0.9$, $F_{1,8} = 73.5$, $p < 0.001$).

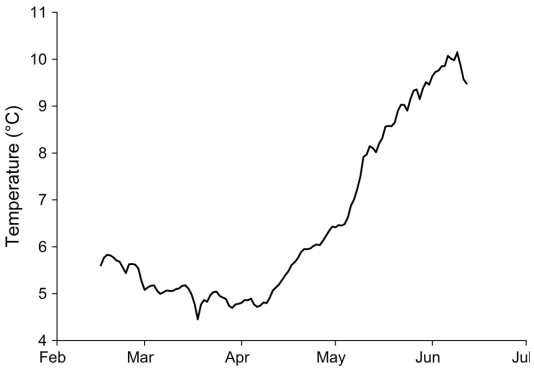

Fig. 4 Daily average sea temperatures (°C) at 2 m depth at the sea farm Skarvøya from deployment in mid-February until harvest in mid-June

Protein content

The protein content decreased from May to June for all treatments (Fig. 10), and the mean content across all treatments was 73.3 ± 2.4 mg g^{-1} DW in May and 57.2 ± 1.5 mg g^{-1} DW in June. In May, the highest protein content was found for the G21 sporophytes with 87.0 ± 5.3 mg g^{-1} DW, and in June, the highest content was measured in the G14 treatment with 67.3 ± 2.6 mg g^{-1} DW. Overall, the protein content did not vary a lot between the 11 different treatments over the cultivation period.

Discussion

Building up a full-scale seaweed hatchery can constitute a high investment cost for farmers and might not be manageable for newly established companies. A shortening of the incubation phase, thus enabling several production cycles or a total omittance, might therefore be worth looking into, especially when aiming for large-scale cultivation to reach the prospected production goals of 4 million t of macroalgae in Norway in 2030 and 20 million t in 2050 (Olafsen et al. 2012).

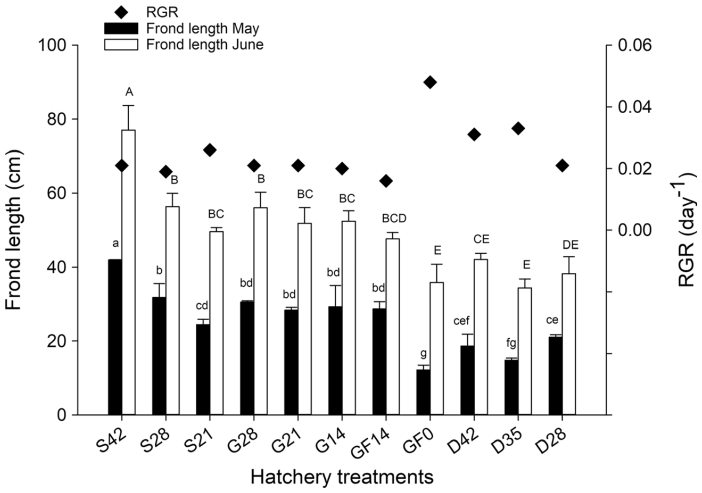

Fig. 5 Frond length for May and June sampling (left *y*-axis) and RGR (right *y*-axis) for the different hatchery treatments (see Table 1), mean ± SE, $n = 40$. Letters above bars denote significant differences in length between the treatments, lowercase letters for May and capital letters for June

Image analysis of substrate coverage before deployment at sea

Standardisation of production methods to improve quality control and predictability of produced quantity of seaweed biomass is needed for upscaling to industrial production volumes of macroalgae in high-cost countries, e.g. in Western Europe. Monitoring, automation and control techniques are necessary to replace manpower. In this study, we demonstrate a possible first step through the measurement of the substrate coverage as a form of early-stage control of the seedling quality and expected quantity of produced biomass. The method makes processing of a large number of images possible with little effort, compared to manual counting/analysis of the substrate itself or images of it. However, a weak point of this method is to separate the growth of target species from that of other contaminant species like diatoms or filamentous algae. If these species have a somewhat similar colour representation, contamination may be hard to distinguish from target species using image processing techniques. Although the measured growth may be correct using the saturation method presented here, it may not be accurately represented for the targeted species if unwanted species are increasingly present. The method may be refined, and one step in that direction would involve the calibration of reflectance from a standard target, as light quality, intensity and sensitivity in the source and camera are due to change between equipment and time. The light-reflecting properties of the substrate influence the definition of zero coverage, and hence, this should also be included in the calibration process. A comparison of the method against the manual counting of sporophytes on substrate was not within the scope of the experiment.

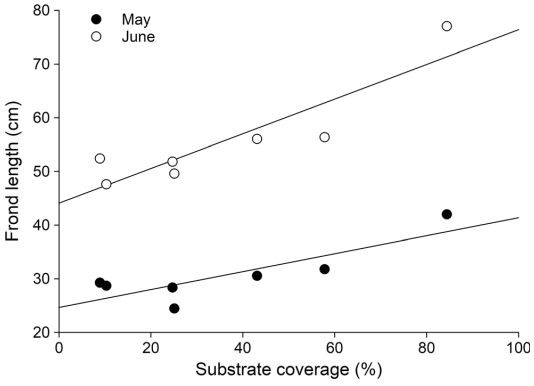

Fig. 6 The mean frond length (cm) for May and June as a function of the substrate coverage (%) at deployment in sea in February, with regression lines showing the linear trends

Growth performance at sea

All the 11 seeding treatments were cultivated successfully at sea but with significant differences in frond lengths. The overall best performance of all measured variables was obtained by seeding twine with spores and pre-cultivating them in the hatchery for 42 days (S42), a treatment used in previous experiments in Norway (Forbord et al. 2012; Handå et al. 2013; Fossberg et al. 2018; Sharma et al. 2018). Frond lengths in June for the S42 treatment were comparable to those of previous experiments in the Faroe Island and Norway (Handå et al. 2013; Mols-Mortensen et al. 2017; Bak et al. 2018), and the biomass was well within the range found by other trials in Europe (Peteiro and Freire 2009; Kraan 2013; Mols-Mortensen et al. 2017; Matsson et al. 2019). The D35 treatment, on the contrary, showed the shortest frond lengths and the lowest biomass yield, which was in the same range or higher as found in several Danish cultivation experiments

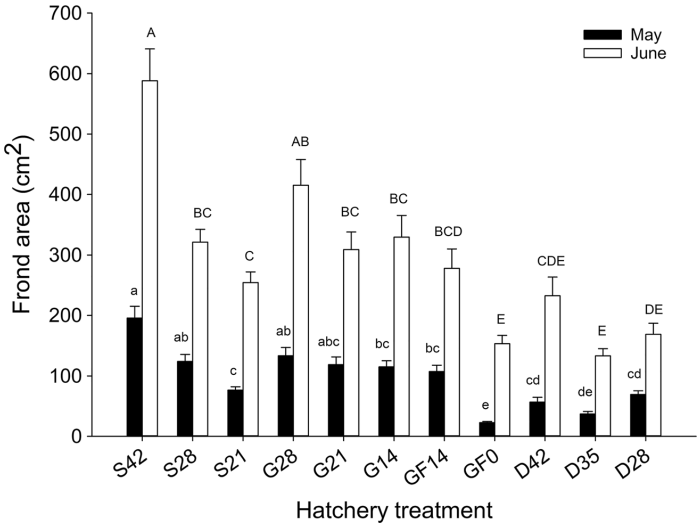

Fig. 7 Mean frond area (cm^2) for May and June sampling for the different hatchery treatments (see Table 1), mean ± SE, $n = 40$. Letters above bars denote significant differences between the treatments, lowercase letters for May and capital letters for June

(Marinho et al. 2015; Bruhn et al. 2016). All treatments in the current experiment resulted in shorter fronds than those found in a previous cultivation experiment in the same geographical area but at a more exposed location (Sharma et al. 2018). They found average lengths twice of the best growth in the present experiment after 134 days at sea, suggesting that local environmental conditions have a major impact on the growth at sea.

The frond area showed a similar trend as the length in June with the highest measured values for the S42 treatment and the lowest for D35, indicating that the width of the frond mainly followed the frond length at this sheltered location. Both the area and length of *S. latissima* have been found to give good estimates for the standing biomass (Stagnol et al. 2016; Foldal 2018).

The highest relative growth rate (RGR) for the cultivation period was found for the four direct-seeded treatments (GF0, D42, D35, D28), which had the shortest frond lengths compared to the others after 80 and 120 days at sea. This is explained by growth rates becoming reduced with increasing size of the sporophytes (Huges 1973; Kain 1991).

Sporophyte density on ropes can affect individual sporophyte growth and total yield (Reed et al. 1991; Creed et al. 1998; Steen and Scrosati 2004; Kerrison et al. 2015, 2016). The optimal density for the highest achievable biomass yield for *S. latissima* and other kelp species is, however, still unknown (Kerrison et al. 2015). The sporophyte density varied greatly between the treatments in the current experiment, with an almost three times higher density for D35 compared with G28, suggesting a better attachment to the substrate (Xu et al. 2009; Kerrison et al. 2018). The sporophyte density had a strong correlation with the individual sporophyte weight, showing that high densities led to low individual weights due to intraspecific competition and resource limitation such

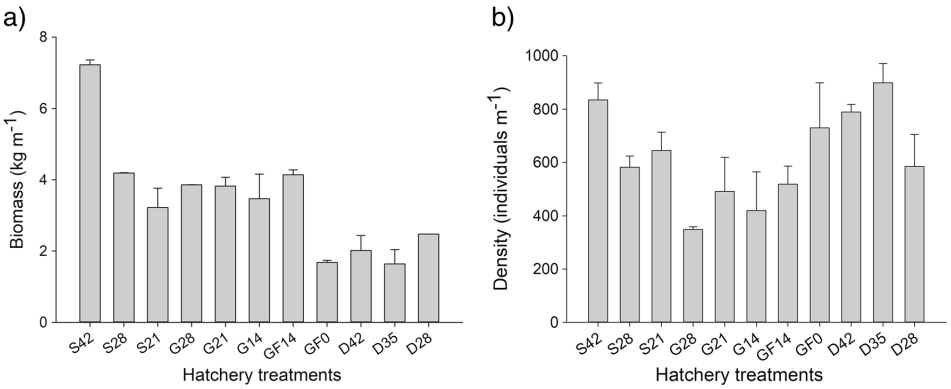

Fig. 8 **a** Biomass yield (kg m^{-1}) and **b** sporophyte density (individuals m^{-1}) for the different hatchery treatments (see Table 1) in June, mean ± SE, $n = 2$

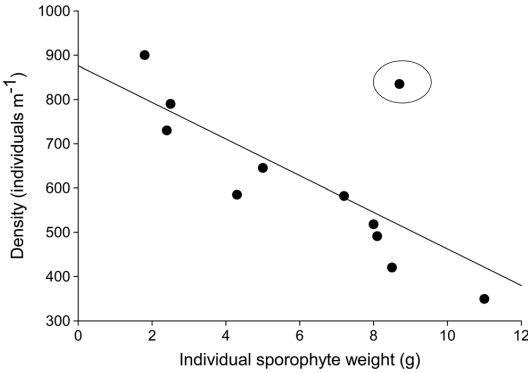

Fig. 9 The sporophyte density on the ropes (individuals m^{-1}) as a function of the individual sporophyte weight (g), with the regression line showing the linear trend. The S42 treatment deviated from the others with both high density and weight and is marked with a circle

as light and nutrients (Kain and Jones 1963). However, the only treatment deviated from this linear trend was the S42 with the highest yield but also one of the highest densities. One possible explanation for this can be that the long incubation period in the hatchery before deployment stimulated the development into sporophytes from a higher number of spores, but the high density obviously did not exceed the optimal density for growth at sea.

All 11 treatments were deployed the same day on the same farm and were exposed to the same environmental conditions during sea cultivation. Differences in growth performance were, therefore, most likely attributed to the size and density of the juvenile sporophytes at deployment. The range in temperature measured during the experiment showed that the treatments were cultured within the typical thermal range of 5–15 °C for *S. latissima* (Kerrison et al. 2015) and never encountered temperatures exceeding 17 °C that may cause loss of tissue (Gerard et al. 1987).

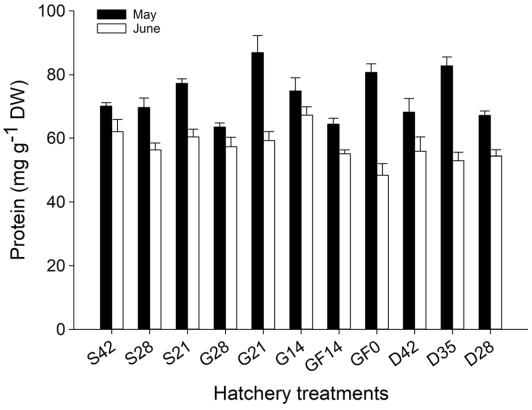

Fig. 10 Protein content (mg g^{-1} DW) as [N] × 3.6 in May and [N] × 4.3 in June for different hatchery treatments (see Table 1), mean ± SE, $n = 2$

Comparing similar seeding methods

By comparing growth performance at sea for treatments with the same seeding method (spores, gametophytes or juvenile sporophytes), a clear pattern was seen for spores (S42, S28 and S21), with a significantly increased growth at sea with number of days in the hatchery. This was also evident from the substrate coverage before deployment with a decrease from 84% for S42, 58% for S28 and 25% coverage for S21. For seeding with gametophytes (G28, G21, G14 and GF14), on the contrary, no significant differences were found at sea with days in the hatchery. However, a difference in the substrate coverage could be seen with the G28 having 43% coverage, the G21 25% and the G14 and the GF14 9 and 10% coverage, respectively. A clear correlation between the substrate coverage and frond length for all twine seeded treatments was found for both the May and June samplings, suggesting that image analysis can be used as a tool for easy quantification of frond lengths and quality assurance of the seed lines before deployment. The four direct-seeded treatments (GF0, D42, D35 and D28) showed the poorest growth at sea for all measured variables and no significant differences with seedling age. Seedling lengths and share of developed sporophytes compared to gametophytes in the free-floating cultures did not follow an age-specific pattern. This could be due to small and stochastic differences in spore development in the free-floating cultures caused by genetic variation, a different maturation degree of the selected sori, self-shading or minor different physical conditions like light and aeration. However, a clear coherence between the seedling's length at deployment and growth performance at sea was evident for the D35 treatment that had the poorest growth for all measurements in this study and that deviated most from the robust S42 treatment.

Comparing treatments of similar age

The gametophytes were at a more advanced stage in the development than spores when seeded on twine, but they usually need 8–10 days in white light before reaching fertility (Arbona and Molla 2006) and time to develop rhizoids to attach properly to the substrate in contrast to spores that actively attach (Xu et al. 2009). The substrate coverage before deployment at sea for treatments S28 vs. G28 and S21 vs. G21, which had the same number of days in the hatchery, was comparable. When comparing these treatments at sea after 120 days of cultivation, they had the same frond lengths and biomass yield, but the spore-seeded twine had a higher density than the gametophyte-seeded twine, most likely due to poorer adhesion properties of the gametophytes (Xu et al. 2009; Shan

et al. 2013). Loss of propagules after seeding and placement in the incubators could thus be a possible explanation for the gametophyte treatments resulting in the lowest density at sea. Techniques to avoid this can be disrupting the gametophyte fragments as small as possible for better attachment, increasing the period of stagnant water in the incubators (Xu et al. 2009) or using a binder for better attachment (Kerrison et al. 2018). The gametophytes transferred to white light for induction of gametogenesis (GF14) 14 days prior to seeding did not show a significantly better length growth at sea than the gametophytes seeded directly from red-light conditions (G14), indicating that the fertility induction in white light in reality can be omitted for *S. latissima* gametophytes.

Direct seeding

Gluing juvenile sporophytes or gametophytes directly on to the droplines before deployment using a binder saves both time and space by skipping the hatchery incubation and the time-consuming entwining process of the seed string onto the droplines. When cultivating juvenile sporophytes in free-floating cultures, the holdfast of the sporophytes will most likely not develop as rapidly as when pre-cultivated on substrates in a hatchery for several weeks prior to deployment at sea. Mols-Mortensen et al. (2017) explained low yields using a binder method by a possible detachment of the seedlings shortly after deployment. The sporophyte density of the direct-seeded ropes in the current experiment was among the highest in the experiment, suggesting that the direct-seeded sporophytes were not washed off the ropes after deployment but rather that extra time was needed to develop a holdfast and a tight attachment to the substrate before the frond elongation could start. The sheltered sea farm and the good light conditions at time of deployment could also have contributed to a successful attachment (Kerrison et al. 2018). A longer cultivation period, by deployment e.g. in autumn, or seeding with lower densities may have levelled out the growth between the different treatments when harvesting in June. No differences in growth measurements were found when comparing direct-seeded gametophytes induced in white light for 14 days to direct-seeded sporophytes cultured up to 42 days in white light. This is probably due to the low fraction of developed sporophytes in all treatments before seeding that might be the result of sub-optimal culture conditions for sporophyte development.

The rope structure and material used for seeding have proven to have a large impact on the harvesting yield. Twisted ropes have shown significantly better performance than braided ropes for both spore and juvenile sporophyte seeding (Kerrison et al. 2019). The spores and gametophytes were seeded on a twisted nylon string (⌀ 1.2 mm and wound around ⌀ 18 mm twisted rope when deployed), while the treatments using a binder were seeded directly on a braided rope (⌀ 18 mm, AlgaeRope). This dissimilarity could have impacted the final yield in this experiment and explained some of the differences.

Protein content

A decrease in protein content was measured from the first sampling in early May of 73.3 ± 2.4 mg g^{-1} DW to the second sampling in mid-June of 57.2 ± 1.5 mg g^{-1} DW on average. The protein content most likely followed the seasonal pattern of ambient nitrate fluctuations in seawater (Rey et al. 2007; Broch et al. 2019), where a higher ambient nitrate concentration is found to result in higher protein content (Harnedy and FitzGerald 2011). Brown seaweeds have been reported to have lower protein content than green and red seaweeds, but the single maximum value found in this study (111 mg g^{-1} DW) is within the range of some of the concentrations found in green (100–260 mg g^{-1} DW) and red (50–440 mg g^{-1} DW) seaweeds (Fleurence 1999; Holdt and Kraan 2011). The growth environment is likely to affect the biochemical composition of seaweeds, which may, in turn, affect the quality of the harvested biomass (Kerrison et al. 2015; Schiener et al. 2015). Because the sea cultivation conditions were similar for all treatments, no large differences were expected to be found for the protein content between them. It is worth noting that the protein concentration is calculated from the nitrogen content using season- and depth-specific nitrogen-to-protein conversion factors (K_p) found in a previous experiment from the same geographical area (Forbord et al. submitted). This may both have under- and overestimated the protein concentration at certain points in time (Manns et al. 2017; Mæhre et al. 2018).

Summary and conclusions

This study has demonstrated that different seeding methods and hatchery periods had high impact on the growth performance of *S. latissima* at sea. Twine seeded with spores pre-cultivated in the hatchery for 42 days gave significantly better growth measurements than any of the other treatments tested in this experiment. A clear coherence was found between days in the hatchery before deployment and growth performance at sea for the spore seeding method. In contrast, no differences were found between the hatchery period and growth at sea for the gametophytes, which indicated that the hatchery period can be reduced down to 14 days and that the induction of fertility in white light before seeding is not crucial. The gametophyte seeding showed the lowest density of all treatments at sea but had larger frond lengths, area and biomass compared to the direct-seeded treatments that used a commercial binder to attach juvenile sporophytes before deployment. All measured growth variables were poor for the direct-seeded

treatments during the relatively short cultivation period of 120 days at sea, but a longer cultivation period might have levelled out the differences between the seeding methods. The reduced costs by skipping the hatchery phase and entwining process may make up for this in a business prospective, but no attempts were made to compare the costs related to the different seeding techniques in this work. Image analysis of substrates before deployment seemed to be a useful tool when assessing frond lengths at sea, but the method needs to be further developed to include predictions about harvesting yields. One source of weakness in this study, which might have affected the comparison of growth measurements between the seeding techniques, is the different methods used to assess seeding density. An important area for further work should be to determine the optimal seeding density for the different life stages of kelp giving the maximum yield at sea and to standardise easy methodology for measurements before deployment.

Acknowledgements This work was conducted in the Research Council of Norway with project no. 254883 (MACROSEA). Thanks to Ole Jacob Broch (SINTEF Ocean) for preparing the temperature data and designing the maps used in this paper, Magnus Oshaug Pedersen (SINTEF Ocean) for calibrating the Hobo-loggers and downloading data, and Marte Schei (SINTEF Ocean) for analysing CN. The authors are also grateful to Åsmund Johansen (SINTEF Ocean) for helping with deployment and registrations, Synnøve Strand Jacobsen (NTNU) for registrations in field and lab, and Terje Bremvåg (SINTEF Ocean) and Bjørnar Johansen (Blått Kompetansesenter) for boat access during registrations. Thanks to the scuba diving group at NTNU for collection of sporophytes and Hortimare for providing AlgeRopes and binder for testing.

Author contributions JS conceived the idea of this study, and SF, KBS, AH and JS conceived and planned the article. SF, KBS and JS executed the seedling production, and SF and JS, the cultivation trials and samplings. TS was responsible for the image analysis. SF wrote the first draft of the manuscript, and all of the authors contributed to the writing/editing of the paper and approved the final manuscript.

References

Alver MO, Solvang T, Kvæstad B (2018) Proof of concept on seeding system. SINTEF Ocean report 2018:00785 A,. SINTEF Ocean, Trondheim

Arbona J, Molla M (2006) Cultivation of brown seaweed *Alaria esculenta*. Aquaculture explained, vol 21. Bord lascaigh Mhara, Dublin

Bak UG (2019) Seaweed cultivation in the Faroe Island - an investigation of the biochemical composition of selected macroalgal species, optimised seeding techniques, and open-ocean cultivation methods from a commercial perspective. Industrial PhD Thesis, Technical University of Denmark, Lyngby

Bak UG, Mols-Mortensen A, Gregersen O (2018) Production method and cost of commercial-scale offshore cultivation of kelp in the Faroe Islands using multiple partial harvesting. Algal Res 33:36–47

Broch OJ, Ellingsen IH, Forbord S, Wang X, Volent Z, Alver MO, Handa A, Andresen K, Slagstad D, Reitan KI, Olsen Y, Skjermo J (2013) Modelling the cultivation and bioremediation potential of the kelp *Saccharina latissima* in close proximity to an exposed salmon farm in Norway. Aquacult Env Interact 4:187–206

Broch OJ, Alver MO, Bekkby T, Gundersen H, Forbord S, Handå A, Skjermo J, Hancke K (2019) The kelp cultivation potential in coastal and offshore regions of Norway. Front Mar Sci 5:529

Bruhn A, Tørring DB, Thomsen M, Canal-Vergés P, Nielsen MM, Rasmussen MB, Eybye KL, Larsen MM, Balsby TJS, Petersen JK (2016) Impact of environmental conditions on biomass yield, quality, and bio-mitigation capacity of *Saccharina latissima*. Aquacult Env Interact 8:619–636

CIE (2011) CIE S 017/E:2011 ILV: international lighting vocabulary. Central Bureau of the Commission Internationale de L'Éclairage, Vienna

Cottier-Cook E, Nagabhatla N, Badis Y, Campbell M, Chopin T, Dai W, Fang J, He P, Hewitt C, Kim G et al (2016) Safeguarding the future of the global seaweed aquaculture industry. United Nations University and Scottish Association for Marine Science Policy Brief 12pp

Creed JC, Kain JM, Norton TA (1998) An experimental evaluation of density and plant size in two large brown seaweeds. J Phycol 34:39–52

Cuijuan S, Hironao K, Delin D (2005) Effects of blue light on gametophyte development of *Laminaria japonica* (Laminariales, Phaeophyta). Chin J Oceanol Limnol 23:323

Duarte A (2017) Optimization of seedling production using vegetative gametophytes of *Alaria esculenta*. Master thesis, University of Porto, Porto

Ferdouse F, Holdt SL, Smith R, Murua P, Yang Z (2018) The global status of seaweed production, trade and utilization, vol 124. FAO Globefish Research Programme. Food and Agriculture Organization of the United Nations, Rome

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

Foldal S (2018) Morphological relations of cultivated *Saccharina latissima* at three stations along the Norwegian coast (in Norwegian). Master's thesis, Norwegian University of Science and Technology, Trondheim

Forbord S, Skjermo J, Arff J, Handå A, Reitan K, 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

Forbord S, Steinhovden KB, Rød KK, Handå A, Skjermo J (2018) Cultivation protocol for *Saccharina latissima*. In: Charrier BTW, Reddy C (eds) Protocols for Macroalgae Research. CRC Press, Boca Raton, pp 37–59

Fossberg J, Forbord S, Broch OJ, Malzahn AM, Jansen H, Handå A, Førde H, Bergvik M, Fleddum AL, Skjermo J, Olsen Y (2018) The potential for upscaling kelp (*Saccharina latissima*) cultivation in salmon-driven integrated multi-trophic aquaculture (IMTA). Front Mar Sci 5:418

Gerard V, DuBois K, Greene R (1987) Growth responses of two *Laminaria saccharina* populations to environmental variation. Hydrobiologia 151:229–232

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

Harnedy PA, FitzGerald RJ (2011) Bioactive proteins, peptides and amino acids from macroalgae. J Phycol 47:218–232

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

Huges AP (1973) A comparison of the effects of light intensity and duration on *Chrysanthemum morifolium* cv. Bright Golden Anne in controlled environments: 1 Growth analysis. Ann Bot 37:267–274

Kain J (1979) A view of the genus *Laminaria*. Oceanogr Mar Biol Annu Rev 17:101–161

Kain J (1991) Why does *Delesseria sanguinea* stop growing in the summer. Oebalia 17:485–492

Kain JM, Jones N (1963) Aspects of the biology of *Laminaria hyperborea*: II. Age, weight and length. J Mar Biol Assoc UK 43: 129–151

Kerrison PD, Stanley MS, Edwards MD, Black KD, Hughes AD (2015) The cultivation of European kelp for bioenergy: site and species selection. Biomass Bioenergy 80:229–242

Kerrison P, Stanley M, Kelly M, MacLeod A, Black K, Hughes A (2016) Optimising the settlement and hatchery culture of *Saccharina latissima* (Phaeophyta) by manipulation of growth medium and substrate surface condition. J Appl Phycol 28:1181–1191

Kerrison PD, Stanley MS, Hughes AD (2018) Textile substrate seeding of *Saccharina latissima* sporophytes using a binder: an effective method for the aquaculture of kelp. Algal Res 33:352–357

Kerrison PD, Twigg G, Stanley M, De Smet D, Buyle G, Pina AM, Hughes AD (2019) Twine selection is essential for successful hatchery cultivation of *Saccharina latissima*, seeded with either meiospores or juvenile sporophytes. J Appl Phycol. https://doi.org/10.1007/s10811-019-01793-x

Kim JK, Yarish C, Hwang EK, Park M, Kim Y (2017) Seaweed aquaculture: cultivation technologies, challenges and its ecosystem services. Algae 32:1–13

Kraan S (2013) Mass-cultivation of carbohydrate rich macroalgae, a possible solution for sustainable biofuel production. Mitig Adapt Glob Change 18:27–46

Lüning K, Dring M (1972) Reproduction induced by blue light in female gametophytes of *Laminaria saccharina*. Planta 104:252–256

Mæhre HK, Dalheim L, Edvinsen GK, Elvevoll EO, Jensen I-J (2018) Protein determination—method matters. Foods 7:5

Manns D, Nielsen MM, Bruhn A, Saake B, Meyer AS (2017) Compositional variations of brown seaweeds *Laminaria digitata* and *Saccharina latissima* in Danish waters. J Appl Phycol 29: 1493–1506

Marinho GS, Holdt SL, Birkeland MJ, Angelidaki I (2015) Commercial cultivation and bioremediation potential of sugar kelp, *Saccharina latissima*, in Danish waters. J Appl Phycol 27:1963–1973

Matsson S, Christie H, Fieler R (2019) Variation in biomass and biofouling of kelp, *Saccharina latissima*, cultivated in the Arctic, Norway. Aquaculture 506:445–452

Mols-Mortensen A, Ortind EG, Jacobsen C, Holdt SL (2017) Variation in growth, yield and protein concentration in *Saccharina latissima* (Laminariales, Phaeophyceae) cultivated with different wave and current exposures in the Faroe Islands. J Appl Phycol 29:2277–2286

Olafsen T, Winther U, Olsen Y, Skjermo J (2012) Value created from productive oceans in 2050. DKNVS and NTVA, Trondheim

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

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

Peteiro C, Freire Ó (2013) 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

Provasoli L (1968) Media and prospects for the cultivation of marine algae. Proceedings of US-Japan Conference, Hakone, 12-15 September 1966: pp 63–75

Reed DC, Neushul M, Ebeling AW (1991) Role of settlement density on gemetophyte growth and reproduction in the kelps *Pterygophora californica* and *Macrocystis pyrifera* (Phaeophyceae). J Phycol 27: 361–366

Rey F, Aure J, Danielssen D (2007) Temporal and spatial distribution of nutrients. In: Sætre R (ed) The Norwegian coastal current- oceanography and climate. Tapir Academic Press, Trondheim, pp 73–88

Schiener P, Black KD, Stanley MS, Green DH (2015) The seasonal variation in the chemical composition of the kelp species *Laminaria digitata, Laminaria hyperborea, Saccharina latissima* and *Alaria esculenta*. J Appl Phycol 27:363–373

Shan TF, Pang SJ, Gao SQ (2013) Novel means for variety breeding and sporeling production in the brown seaweed *Undaria pinnatifida* (Phaeophyceae): crossing female gametophytes from parthenosporophytes with male gametophyte clones. Phycol Res 61:154–161

Sharma S, Neves L, Funderud J, Mydland LT, Øverland M, Horn SJ (2018) Seasonal and depth variations in the chemical composition of cultivated *Saccharina latissima*. Algal Res 32:107–112

Stagnol D, Macé M, Destombe C, Davoult D (2016) Allometric relationships for intertidal macroalgae species of commercial interest. J Appl Phycol 28:3407–3411

Steen H, Scrosati R (2004) Intraspecific competition in *Fucus serratus* and *F. evanescens* (Phaeophyceae: Fucales) germlings: effects of settlement density, nutrient concentration, and temperature. Mar Biol 144:61–70

Van Patten M, Yarish C (1993) Allocation of blade surface to reproduction in *Laminaria longicruris* of Long Island Sound (USA). Hydrobiologia 260:173–181

Wei N, Quarterman J, Jin Y-S (2013) Marine macroalgae: an untapped resource for producing fuels and chemicals. Trends Biotechnol 31: 70–77

Wu C, Guangheng L (1987) Progress in the genetics and breeding of economic seaweeds in China. Hydrobiologia 151:57–61

Xu B, Zhang QS, Qu SC, Cong YZ, Tang XX (2009) Introduction of a seedling production method using vegetative gametophytes to the commercial farming of *Laminaria* in China. J Appl Phycol 21:171–178

Zhang Q-S, Tang X-X, Cong Y-Z, Qu S-C, Luo S-J, Yang G-P (2007) Breeding of an elite *Laminaria* variety 90-1 through inter-specific gametophyte crossing. J Appl Phycol 19:303–311

Zhang QS, Qu SC, Cong YZ, Luo SJ, Tang XX (2008) High throughput culture and gametogenesis induction of *Laminaria japonica* gametophyte clones. J Appl Phycol 20:205–211