



Faculty of Biosciences, Fisheries and Economics

**Fouling of macro epibionts on cultivated *Saccharina latissima*
(Phaeophyceae)**

In situ temporal and spatial variation

Böris Sanna Christina Angelica Matsson

A dissertation for the degree of Philosophiae Doctor, November 2020

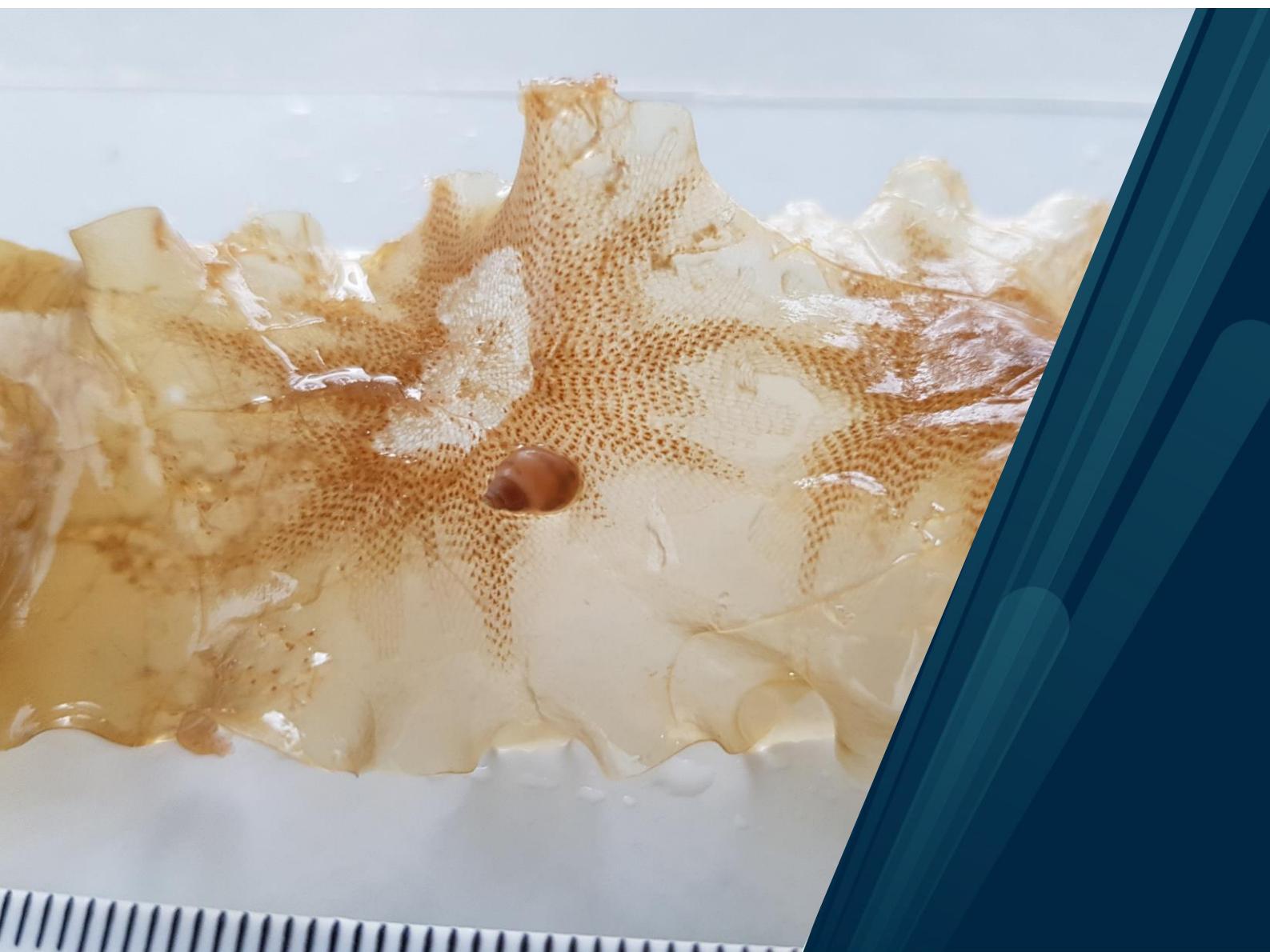


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Acknowledgements

First and foremost I would like to thank my main supervisor, Bodil Bluhm (UiT – The Arctic University of Norway). She is a unique supervisor and has patiently taught me structure, procedures and ensured constant progression. She genuinely cares about her PhD students, which makes this journey feeling much less lonely. Many thanks to my co-supervisors Anna Metaxas (Dalhousie University) and Hartvig Christie (NIVA). Anna has been a true asset for my thesis and she has ensured high scientific quality, and her solid statistical knowledge has come in handy. Few people have as much experience and as many hours under the sea surface, studying and recording the seaweed and its associated community, as Hartvig has. This knowledge, along with his nice nature has been very valuable for my thesis. Together my three supervisors have made the perfect supervisor team, guiding and caring the entire way.

I would like to express my biggest gratitude to the MACROSEA team. Project leader, Aleksander Handå, for letting me join the project and leading it positively and rigidly. Silje Forbord for keeping me company along this road, for answering all my question (instantly!), and also being responsible for me being a part of this field in the first place (by giving such an inspirational lecture at NTNU when I took my bachelor degree). Ole Jacob Broch for helping me with mathematical questions and discussions. Also, great thanks to Jorunn Skjermo, Yngvar Olsen, and the rest of the team for being knowledgeable and making me feel as a part of the MACROSEA-family.

I have spent my PhD period at Akvaplan-Niva, and I would like to thank my co-workers here. Especially, I would like to thank Reinhold Fieler for believing in me and employing me in the first place, for being passionate about seaweed cultivation, and for organising a PhD-position for me whilst I was on maternity leave. I would also like to thank Paul Renaud for being helpful, Magnus Aune and Kjetil Sagerup for guidance in R, Michael Greenacre for statistical help, and last, but not least my fellow PhD-student at Akvaplan-Niva, Eli Børve, for keeping me company.

I would like to thank all industrial partners in the MACROSEA project, especially Seaweed Solutions AS (former Seaweed Energy Solutions), with Jon Funderud and Luiza Neves and the rest of the team, for providing valuable knowledge based on years of experience.

Thanks to my friends for activating me and making my time enjoyable. Thanks to my wonderful parents and brothers for believing in me and always being proud of me (at least for giving an impression of it). Last, but certainly not least, thanks to my partner, Yngve, for supporting, cherishing, and loving me and for being the balanced part. Thanks to Astrid and Sigve for being absolutely amazing and providing perspectives in life (and I forgive you for ruining my sleep for the last five years).



Sanna Matsson,
Tromsø November 2020

Abstract

The motivation for macroalgal cultivation is to meet the global demand for food, energy and biomaterials for a rapidly growing human population in the context of the challenges of limited terrestrial food resources. Over the last 20 years the interest in seaweed cultivation has increased in European countries, and the kelp *Saccharina latissima* is one of the best-suited species for cultivation in North Atlantic waters. Norway, with its extensive coastline and marine knowledge and history, has a great potential to develop this nascent industry in Europe. Seaweeds also provide a substratum for a wide array of benthic organisms for colonization, as well as food supply and permanent or temporary shelter. These organisms are called epibionts, i.e. organisms living on the surface of another organism. From an industrial perspective, epibiosis is negative, as the goal of seaweed farming is to obtain high yield of high quality biomass. As such, epibiosis in this sense is also called biofouling. Epibiosis results in seaweed biomass being less attractive for human consumption, affecting the commercial value of the yield. Epibiosis on cultivated seaweed in mid and high latitudes usually occurs from spring to summer, forcing the farmers to harvest the seaweed biomass before it reaches its potential maximum and higher carbohydrate content. Therefore, epibiosis is considered one of the main challenges in industrial seaweed farming.

There has been a lack of knowledge about timing and species fouling cultivated *S. latissima* under different environmental conditions, including different latitudes, seasons and depths, as well as sporophyte age and nutritional history. This topic was investigated through three *in situ* studies, one with a large latitudinal range covering eleven degrees in latitude, one smaller-scale study within a geographical region characterized by differing environmental characteristics, and one with different outplanting dates resulting in various sporophyte ages within the same location at a given calendar date. The papers that this thesis is built upon show that there is spatial variability in phenology, degree and density of epibiosis on multiple scales on cultivated *S. latissima*.

The large-scale latitudinal study revealed a south to north gradient in the onset of epibiosis, with visible epibionts appearing ~2 months later at the northernmost location, with associated implications for the harvesting period. The study within one geographical region revealed strong differences in the amount and type of epibionts among sites within a relatively short distance from one another. Temperature had the highest impact on the amount of epibiosis of the environmental parameters observed. Further, weaker currents, increased light, and most likely lower salinity were associated with lower amount of epibiosis. Combined, these results show the possibilities for a temporally shifting harvesting approach with later harvesting towards the northern Norwegian coast. Due to the large local variations shown, however, pilot investigations should be undertaken when considering a new farm location in order to acquire knowledge about the species fouling a particular location and their temporal variation.

The epibiont community had an overarching seasonal pattern in density or cover and composition in all studies. An initial onset of a few organisms was followed by a period of slowly increasing cover and density with time, and a sharp increase later in the season. The succession of fouling species began with filamentous algae and diatoms fouling the tips of the fronds. The bryozoan *Membranipora membranacea* was the most prevalent fouling species in all three studies of this thesis, with an increasing relative contribution over time. There was a trend for larval settlement on the meristematic regions, which eventually resulted in larger colonies and more area fouled at the seaweed tips.

Besides choice of location, environmental history and/or age of the seaweed host may affect the epibiosis. *S. latissima* outplanted later in the season had no difference in concentration of nitrogen compounds, but had a higher content of carbon and a lower density of fouling organisms. One of the reasons for this result was both a higher growth and shedding of seaweed fronds in this treatment.

The present study has increased our knowledge about one of the bottlenecks for seaweed cultivation; epibiosis. Furthermore, this new fundamental understanding of timing and species diversity of epibiosis on cultivated *S. latissima* contributes to an overall understanding of the fouling issue along the Norwegian coast, enabling a broader view with important implications for the seaweed industry.

List of papers

The following papers are included in my PhD thesis:

- I. Sanna Matsson, Reinhold Fiebler, Hartvig Christie. Variation in biomass and biofouling of kelp, *Saccharina latissima*, cultivated in the Arctic, Norway. *Journal of Aquaculture* 506, 445-452. Published 15 May 2019. <https://doi.org/10.1016/j.aquaculture.2019.03.068>
- II. Silje Forbord, Sanna Matsson (shared first-authorship), Guri E. Brodahl, Bodil A. Bluhm, Ole Jacob Broch, Aleksander Handå, Anna Metaxas, Jorunn Skjermo, Kristine Braaten Steinhovden, Yngvar Olsen. Latitudinal, seasonal and depth-dependent variation in growth, chemical composition and biofouling of cultivated *Saccharina latissima* (Phaeophyceae) along the Norwegian coast. *Journal of Applied Phycology*, 32, 2215-2232. Published 23 January 2020. <https://doi.org/10.1007/s10811-020-02038-y>
- III. Sanna Matsson, Anna Metaxas, Silje Forbord, Svein Kristiansen, Aleksander Handå, Bodil A. Bluhm. Effects of outplanting time on growth, shedding and quality of *Saccharina latissima* (Phaeophyceae). Submitted to *Journal of Applied Phycology* 22 October 2020.

Author contributions

Table 1:

	Paper I	Paper II	Paper III
Concept and idea	SM, HC, RF	SM, SF, OJB, AH, JS, YO	SM
Study design and methods	SM, HC, RF	SM, SF, OJB, AH, JS, YO, HC, AM, BB	SM, BB, AM, SK
Data gathering, analysis and interpretation	SM	SM, SF, GB, OJB	SM, SF
Manuscript preparation	SM, HC, RF	SM, BB, AM, GB, OJB, AH, JS, KBS, YO	SM, BB, AM, SF, AH, SK

SM = Sanna Matsson

HC = Hartvig Christie

RF = Reinhold Fieler

BB = Bodil Bluhm

AM = Anna Metaxas

SF = Silje Forbord

GB = Guri E. Brodahl

OJB = Ole Jacob Broch

AH = Alexander Handå

JS = Jorunn Skjermo

KBS = Kristine Braaten Steinhovden

YO = Yngvar Olsen

SK = Svein Kristiansen

1. Preface

With respect to the observed climate change, biodiversity crisis, increased human population and pollution of the environment, seaweed cultivation has the potential to be a part of the solution by providing biomass for consumption, binding CO₂, and removing some pollutants. Seaweed cultivation has been my major work interest since the time I first heard about it. The aim of my thesis is to provide knowledge to the growing seaweed industry, especially in the Western world, and to provide important information to one of the bottlenecks in seaweed cultivation today: the settlement of fouling organisms on the seaweed fronds known as biofouling/epibiosis. Biofouling is a bottleneck because organisms settling on the seaweed fronds degrade and deteriorate the quality of the seaweed product, and as a consequence the growth season and production potential is reduced. Therefore, questions such as these arise: where and when does the epibiosis occur? Can the onset of epibiosis be delayed? Are there environmental parameters that make certain sites better-suited for cultivation, i.e. with later onset of or less epibiosis?

The work presented in this thesis is a result of a three-year PhD project funded by the Research Council of Norway (project number 254883, MACROSEA, led by Aleksander Handå SINTEF Ocean Trondheim), and conducted within Akvaplan-Niva and as an external student of UiT The Arctic University of Norway. Additionally, financing for field work in **Paper I** came from Troms County (RDA 12/234 "Pilotstudie på bioenergi fra tare", led by Reinhold Fieler, Akvaplan-Niva). The primary objective of the MacroSea project was "to establish an *interdisciplinary knowledge platform* on fundamental production biology and technology for macroalgae cultivation over a wide range of climatic, ecological and physical regimes".

Supervisors

Professor Bodil Bluhm, Department of Arctic and Marine Biology, Faculty of Bioscience, Fisheries and Economics, UiT – The Arctic University of Norway

Professor Anna Metaxas, Dalhousie University, Halifax, Nova Scotia, Canada

Senior Research Scientist Hartvig Christie, NIVA

Definitions

Epibiont: Organism growing attached to a living surface

Epizoan: Sessile epibiotic animal

Epiphyte: Epibiotic plants/algae

Basibiont: Substrate organisms/host

2. Introduction

2.1 Seaweed aquaculture

The motivation for macroalgal cultivation is to meet the global demand for food, energy and biomaterials for a rapidly growing human population in the context of the challenges of limited terrestrial food resources (Olafsen et al. 2012). To meet these challenges, a larger component of human food consumption has to originate from lower trophic levels and from marine production than it currently does (Olafsen et al. 2012). Macroalgae, and particularly the algal order Laminariales (kelp), are among the fastest growing photosynthesizing organisms in the world (Broch et al. 2013) and thus have the potential to contribute substantially to the resource demands. Additionally, seaweed cultivation is considered to be *sustainable* by simultaneously providing the listed ecosystem services, while at the same time mitigating ocean acidification, and carbon sequestration (Visch et al. 2020). As autotrophic organisms, macroalgae require dissolved organic and inorganic compounds and light for growth, and do not require addition of feed or fertilizer. Seaweed biomass can be cultivated on a large scale in coastal areas without competing for freshwater or land area. In 2018, a total of approximately 114.5 million tons of freshwater and marine aquaculture products were produced, with an estimated value of 263.6 billion USD, of which seaweeds (red, green and brown algae) accounted for 32.4 million tons and 13.3 billion USD, respectively (FAO 2018, 2020). Most of these seaweeds (>99.5 %) were produced in Asian countries (Chopin 2014), as Asia has a strong cultural and historical link of seaweed use in food (Mouritzen and Mouritzen 2013; Rioux et al. 2017). Europe has a long tradition of wild kelp harvesting, but algal cultivation is in its early stages. Over the last 20 years, however, the interest in seaweed cultivation has increased in European countries, such as Norway, Sweden, Spain, Scotland, and Denmark (Peteiro and Freire 2009; Kraan 2013; Marinho et al. 2015a; Walls et al. 2017; Broch et al. 2019; Visch et al. 2020). Still, in 2018 the European seaweed production accounted for less than 0.017 % of the world seaweed production. In Norway in particular, the production has risen from 0 tons in 2014 to 175 tons in 2018 (FAO 2018). This new interest has resulted in several pilot-scale seaweed farms with native kelp species to facilitate and develop the cultivation techniques and advance the seaweed aquaculture industry in these areas (Edwards and Watson 2011; Marinho et al. 2015a).

The seaweed biomass produced is utilised for both low-tech low value and high-tech high value products. Low-tech low value products include highly nutritional human food and animal feed (Stévant et al. 2017; Délérès et al. 2016). The seaweed properties that can enhance the physico-chemical characteristics of foods such as water- and oil-binding, and swelling capacities (Rioux et al. 2017) can potentially be used in high-tech high value products. Specifically, seaweed additions provide thickening, stabilising and emulsifying properties to gelatine substitutes, processed meat and dairy.

Macroalgae also have the potential for various other applications in need for further development, such as biofuels, bioplastics, cosmetic and pharmaceutical products. A bio-refinery concept is being developed that aims to enable processing of seaweed biomass for complete utilization of feedstock without compromising yield or quality of products (Baghel et al. 2016).

2.2 *Saccharina latissima* – study focal species

Saccharina latissima (Linnaeus) Lane, Mayes, Druehl and Saunders, the focal study species, is extensively distributed circumpolar in the northern hemisphere (Bolton et al. 1983). The species is present on both sides of the Atlantic Ocean, in the Gulf of Maine, eastern Canada and the European coasts, along the North American Pacific coast, and in some regions in Japan and Arctic Russia (Druehl 1970; Druehl and Kaneko 1973; Lüning 1990; Bartsch et al. 2008). It has an average life span of 2-4 years (Forbord et al. 2012). Optimal conditions for *S. latissima* growth are met along most parts of the Norwegian coast with optimal water temperatures between 10 and 17 °C (Druehl 1967; Fortes and Lüning 1980a) and salinities of 30–35 psu (Kerrison et al. 2015). Roughly half of the world's natural kelp beds of *S. latissima* are in fact found along the Norwegian coast (Moy et al. 2006), suggesting suitable conditions for farming along the entire latitudinal gradient from 58 to 71 °N (Broch et al. 2019). Consequently, along with its high growth rate (Handå et al. 2013; Peteiro and Freire 2013b; Bak et al. 2018), high content of valuable components (Holdt and Kraan 2011; Sharma et al. 2018; Bak et al. 2019), and a well-described life cycle (Forbord et al. 2018), *S. latissima* is one of the best suited species for cultivation in North Atlantic waters. Accordingly, commercial actors have prioritized cultivation of *S. latissima*. Maximum potential annual production capacity of *S. latissima* along the Norwegian coast has been estimated at 150-200 tons wet weight ha⁻¹ (Broch et al. 2019). Additionally, in Norway there exists much knowledge from other marine industries (e.g. the salmon farming-, fish - and oil industries) on processing of marine raw materials and related infrastructures, and the future perspectives for industrial developments of seaweed farming in Norway are positive (Stévant et al. 2017).

The cultivation process of *S. latissima* includes a microscopic and macroscopic phase of the heteromorphic life cycle (figure 1), typical for the order Laminariales (Kain 1979). **1.** The process starts with specialized cells in the adult sporophyte producing sporangia which are spore producing cells. During this process, darker areas are formed on the seaweed frond, called sori. These occur naturally in Norwegian waters around October to December or can be induced in lab cultures by an artificially controlled day-night rhythm, thus enabling year-around access to spores (Forbord et al. 2012). **2.** In the laboratory, sori can be stressed to release free-swimming spores produced by meiosis (Rød 2012; Forbord et al. 2018), and these spores settle on a growth substrate where they germinate into female and male multicellular gametophytes. **3.** In the reproductive phase, either female egg-producing

structures (oogonia) or male sperm-producing structures (antheridia) develop (Kain 1979). **4.** When fertilization has taken place a zygote is developed and grows into a new sporophyte (Kain 1979; Edwards and Watson 2011). Presently, there are four ways to produce small sporophytes for seaweed cultivation. (1) 'Direct seeding' where the spores are sprayed on growth substrate with a 'binder'. (2) The growth substrate is submerged in a spore solution and thereafter outplanted directly in the sea (Forbord et al. 2019; Kerrison et al. 2019). (3) The zoospores are sprayed on growth substrate (or the strings are submerged in a zoospore solution) for rearing of young sporelings in a greenhouse until the desired sporeling size is reached (Forbord et al. 2012). (4) The gametophytes are kept in non-optimal conditions, such as red light, to enable vegetative growth and prevent sexual reproduction with the goal of keeping a stock-solution of gametophytes for future cultivation (Matsson 2013). By changing the conditions to optimal (i.e. changing from red to white light), the gametophytes turn fertile (Lüning 1980; Cuijuan et al. 2005). This method provides continuous cultures for year-round seeding of gametophytes or production of juvenile sporophytes for direct seeding. **5.** The growth substrates with young seedlings/gametophytes/spores are outplanted in the sea. **6.** In the grow-out phase, the sporophytes increase in surface area, weight and content. The length of this stage is dependent on when sporophytes are outplanted and when they are harvested. **7.** The timing for harvesting depends on the end-product but commonly maximum biomass of clean seaweed is desired, i.e. before larger organisms settle on the seaweed frond in substantial numbers. **8.** During a process called epibiosis or biofouling, the seaweed frond provides a substrate for other algae (epiphyte) or planktonic larvae (epizoan) of invertebrates to settle on and metamorphose into the adult forms.

This thesis focuses on stages 6-8, with the main emphasis on stage 8. The growth rates of perennial Laminariales such as *S. latissima* at mid- and high latitudes are reduced in summer, when ambient nutrient levels are depleted after the spring bloom, in favor of internal carbohydrate storage. This storage ability enables the seaweeds to utilize the higher nutrient levels in the seawater in winter, when growth rates are consequently increased. This growth strategy has important implications for the cultivator, as reduced seaweed growth in summer facilitates the growth of epibionts on the seaweed fronds (Lüning and Pang 2003). Seasonal cycles of seaweed growth and shedding and their response to environmental variables differ among seaweed species and vary both temporally and spatially within species (Gerard and Mann 1979; Schaffelke and Lüning 1994).

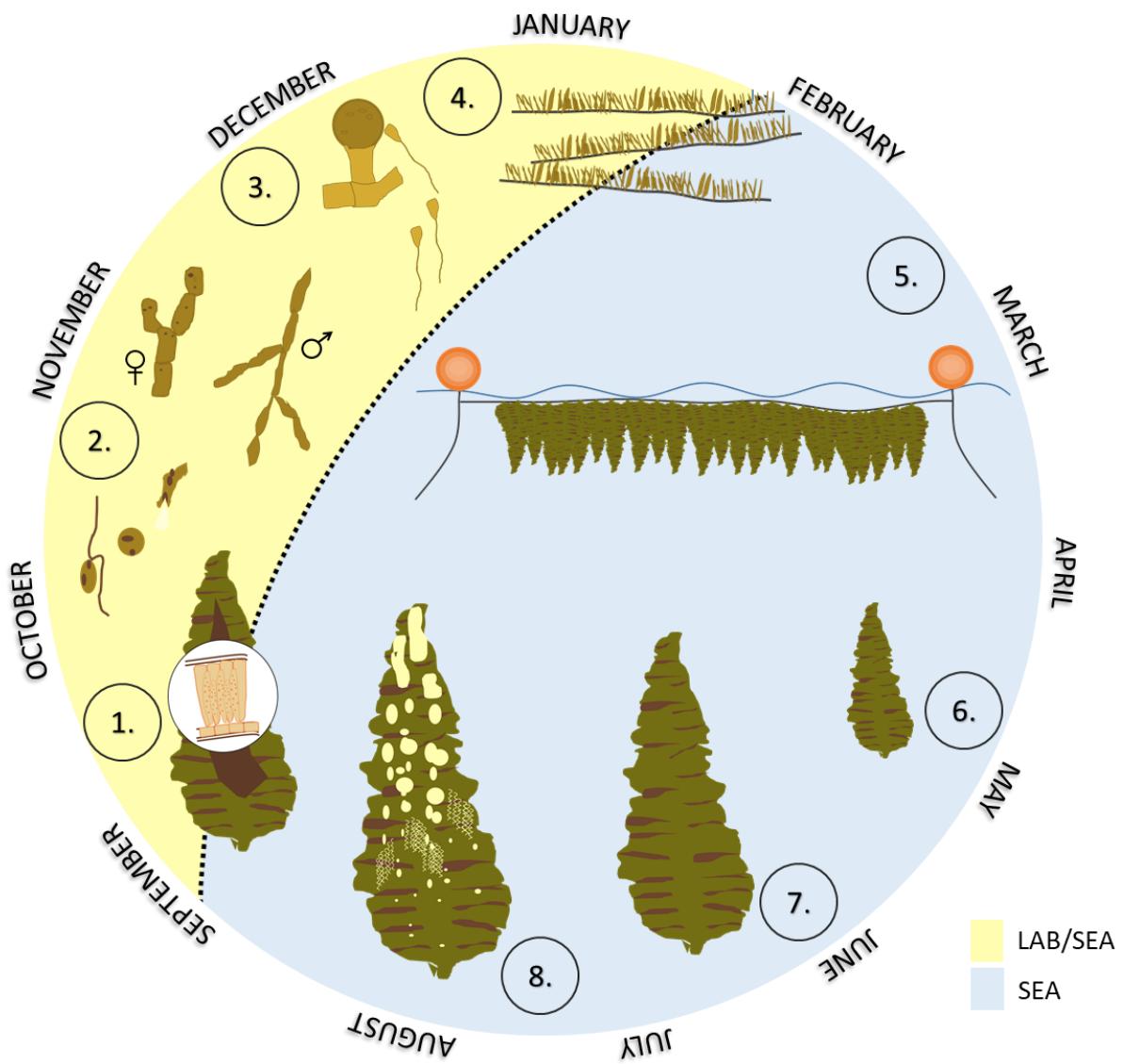


Figure 1 Graphic illustration of the heteromorphic life cycle of *Saccharina latissima* in cultivation, consisting of the microscopic gametophyte stages, and the macroscopic sporophyte stage. Months are for approximate stages at 69°N. **1.** Dark brown sori (spore-producing bodies) are either produced naturally (in the sea) or induced in the lab. **2.** Haploid spores are released naturally or through stress treatment. After finding a substrate to settle on spores lose their flagellae, and develop either into female or male gametophytes. In cultivation, the spores can either be used for direct seeding on a growth substrate deployed straight into the sea, vegetative growth of gametophytes for gametophyte rearing in the lab, or sprayed on growth substrate for indoor seedling production. **3.** Reproductive female gametophytes produce egg-producing oogonia and male gametophytes develop antheridia producing sperm released into the water. **4.** Fertilized zygotes develop into diploid sporophytes. **5.** The young seedlings/gametophytes/spores are outplanted in the sea. **6.** Grow-out phase. **7.** Harvesting of clean biomass. **8.** Onset of biofouling organisms.

2.3 Epibiosis

Seaweeds provide a substratum for a wide array of benthic organisms for colonization, as well as for food supply and permanent or temporary shelter (figure 2a). The attached organisms are called epibionts, i.e. organisms living on the surface of another organism, consist of animals (epizoans) and other algae (epiphytes), and may be macroscopic or microscopic (Wahl 1989). Due to fast regeneration of frond tissue, the life-span of epibionts living on these fronds also has to be short, and therefore epiphytes of Laminariales are restricted to a few species that can grow and reproduce within this limited amount of time (Russell 1983). Consequently, variations in epibiont abundance could be caused by differential longevity on different hosts. Macroscopic epibionts include calcareous hard bodied organisms such as moss animals (figure 2c), acorn barnacles, hydroids, mussels and tubeworms and soft bodied organisms such as non-calcareous algae, sponges, anemones and tunicates. These sessile epibionts along with mobile invertebrates such as polychaetes, isopods, amphipods and gastropods are commonly observed on the surface of seaweeds and may in turn form an important food source for juvenile fishes. Epibionts may affect cultivated and wild kelp forests differently, because wild kelp habitats have a heterogenic composition of seaweed species, are genetically more diverse, and community phenology is temporally asynchronous. Additionally, wild seaweed habitats grow on a bottom substrate (as compared to a rope when cultivating), possibly enabling some larval removal with the joint efforts of water motion and a harder bottom substrate whipping off the larva (Wiencke and Bischof 2012).

Epibiosis has a range of effects on the surface of the host (basiphyte) that depend on the nature of both the epibiont and the basiphyte. This relationship between the basiphyte and epibiont ranges from mutualistic to parasitic (Potin 2012), even though most studies found negative effects of epibionts on their host. Negative effects of macroscopic epibionts on the seaweed host include shading (Rohde et al. 2008; Andersen 2013), hindering nutrient and gas exchange (Hurd et al. 1994; Hurd et al. 2000), reducing frond flexibility (Krumhansl et al. 2011; figure 2c), causing reduced growth rate (Honkanen and Jormalainen 2005) and reducing spore release from fertile fronds (Saier and Chapman 2004). As a consequence of these effects, epibiosis causes considerable quality deterioration and biomass loss of the host (Kuschel and Buschmann 1991; Lüning and Pang 2003; Titlyanov and Titlyanova 2010; Krumhansl et al. 2011; figure 2d). Epiphytes can also influence trophic interactions, affecting their host negatively or positively (Karez et al. 2000). For example, one of the main epibionts on cultivated *S. latissima* along the coast of Norway is the encrusting bryozoan *Membranipora membranacea* (Førde et al. 2015; Matsson S. 2015). *M. membranacea* has an inflexible CaCO₃ exoskeleton and, therefore, its presence on seaweed fronds increases the brittleness which can result in heavy defoliation of natural and cultivated kelp (Scheibling and Gagnon 2009; Krumhansl et al. 2011; figure 3d).

Overall, the effects of epibionts are not always straight forward to disentangle, but from an industrial perspective, epibiosis is negative, as the goal of seaweed farming is to obtain high yield of high quality biomass. As such, epibiosis in this sense is also called biofouling. Epibiosis results in seaweed biomass being less attractive for human consumption, affecting the commercial value of the yield (Park and Hwang 2012). Seaweed with low value for human consumption may, however, still be used in other industries, for example in the production of animal feed (Bruton et al. 2009). To avoid biomass loss and reduced monetary value, the current practice is to harvest the seaweed biomass before substantial biofouling occurs (Fletcher 1995; Park and Hwang 2012). Epibiosis on cultivated seaweed in mid and high latitudes usually occurs from spring to summer (Peteiro and Freire 2013a; Skjermo et al. 2014; Førde et al. 2015), forcing the farmers to harvest the seaweed biomass before it reaches its potential maximum and highest carbohydrate content. Therefore, Skjermo et al. (2014) lists biofouling as one of the main challenges in industrial seaweed farming. To date, there are no established standards in Norway for an acceptable amount of biofouling on the seaweed biomass yield, but when the primary end-use is human consumption or the biochemical industry, the seaweed biomass should be as clean as possible (personal communication with seaweed farmers, Seaweed Solutions AS).

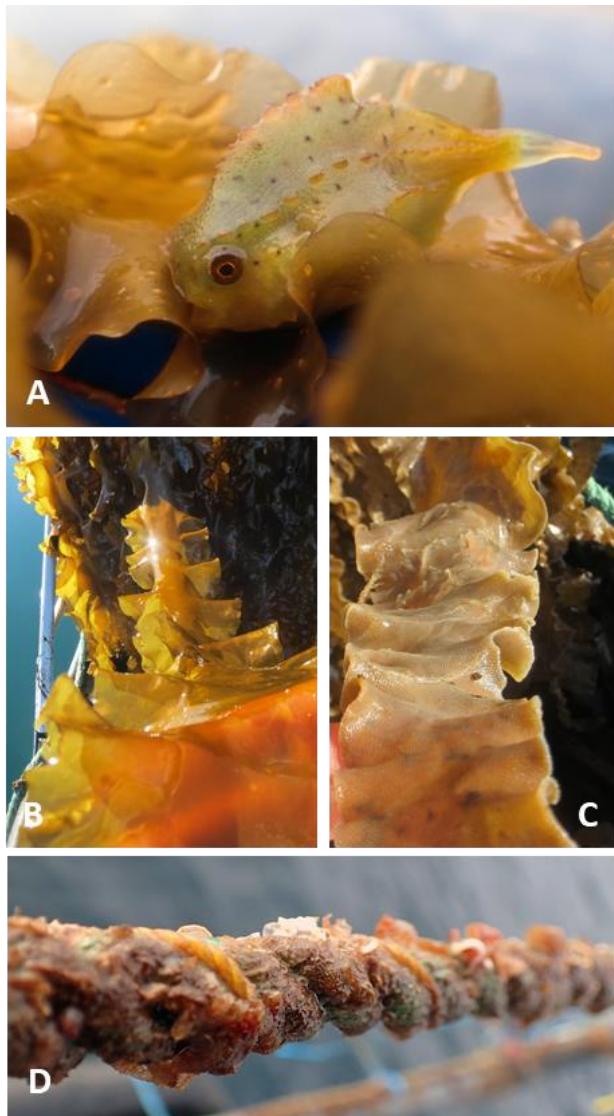


Figure 2 Cultivated seaweed interacting with the surrounding environment. A) Ecosystem services provided by seaweed aquaculture, here in the form of shelter for a juvenile lump fish. B) Clean seaweed frond. C) Seaweed frond covered with the bryozoan *Membranipora membranacea*. Pictures B and C are from the same date, but from different locations within the same region. D) Seaweed-cultivation ropes after all seaweed was lost due to the joint actions of epibionts, grazers and waves.

The colonisation of the seaweed frond is a complex process involving both micro-foulers, such as viruses, bacteria, cyanobacteria, fungi, protozoa and microalgae, and macro-foulers (Wahl 1989). The process of colonisation has been described as a succession of four main stages (figure 3) in a 'fouling sequence model', and is mostly based on studies from inert surfaces; however, similar results have been achieved from living surfaces, such as seaweed (Wahl 1989). (1) After immersion into seawater, an instant adsorption of dissolved chemical compounds (mostly macromolecules) creates a biofilm on the algal surface. This process is purely physical, and reaches a steady state within a few hours. (2) Within hours, bacteria adhere to the substratum, facilitating the settlement of macro-foulers. This

state is essentially physically driven, evolves continuously, and never reaches steady state. (3) After several days, unicellular eukaryotes such as yeasts, protozoa and mainly diatoms arrive. (4) Within weeks to months, depending on the biological activity in seawater, the seaweed fronds hold a three-dimensionally structured microbial community. At this point, the last and longest colonisation stage starts, with settlement of meroplanktonic larvae (epizoans) and algal spores (epiphytes). As they grow and age, macro-epibionts in turn can attract and repel further settlers, and the fouling community continues to evolve (Wahl 1989). This classic model may represent important major patterns, but it oversimplifies the process, and in reality the colonisation process is more dynamic (Vinagre et al. 2020). The biofouling community, consisting of both sessile and mobile species, reaches maturity within a few years, increasing in species diversity and richness (Wahl 1989). Wahl (1989) suggested that the initial phases are purely physically driven, whereas the later phases are driven increasingly by biological processes and their interactions.

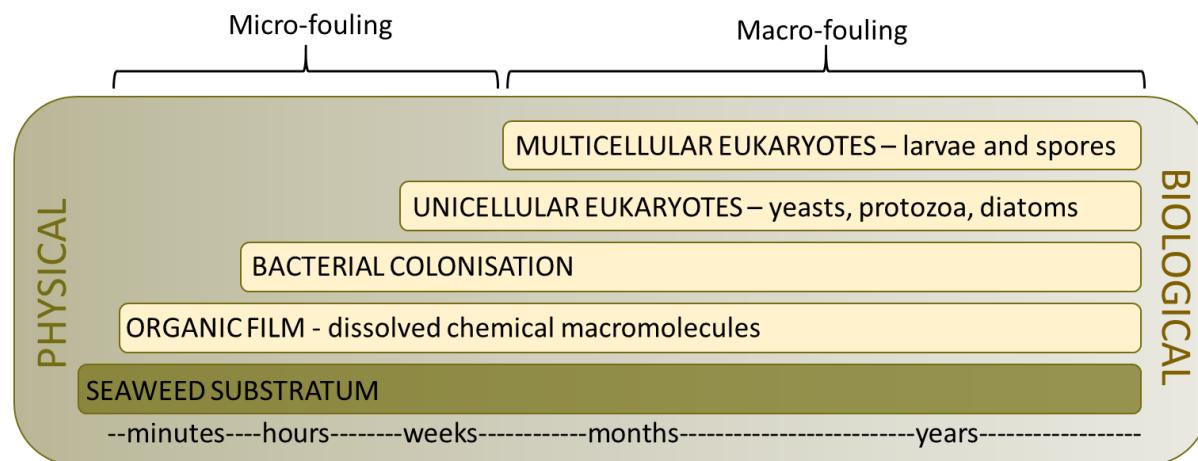


Figure 3 Highly schematized colonizing sequence leading to the establishment of a fouling community on seaweed, driven by physical and biological factors. The nearly instantaneous adsorption of macromolecules is followed several hours later by prokaryotic fouling. Diatoms and other protists settle from the second day onward. Larvae of invertebrates and algal spores may settle after one to several weeks (depending on latitude, season, etc.). Figure modified from Wahl (1989).

2.4 Physical and biological factors influencing epibiosis

The most important environmental (abiotic) factors affecting epibionts, directly or indirectly, are light, nutrient availability, temperature, salinity, and water motion. In addition, biological (biotic) factors include interactions among epiphytic bacteria, fungi, other macro-fouling epibionts, and grazers (Vinagre et al. 2020). The biotic factors depend on many species-specific interactions between host and epibionts and can differ greatly from species to species and among geographic locations (Vinagre et al. 2020). Temporal and spatial patterns of the epibiont community vary greatly on small and large-scales and across depths, again influenced by numerous abiotic and biotic factors. Important biotic

factors are related to the biology of the different organisms which in turn determine settlement on the seaweed frond. The epibionts may also produce chemical cues released in response to competition, reproduction, grazing and predation affecting further settlement and surface recruitment of different organisms (Dayton 1971).

Among the abiotic factors, seawater temperature is a major one relevant to epibiosis and is clearly related to latitude and season. Composition and phenology of marine communities in general are highly dependent on temperature, and so are the life cycles of epibionts, including spawning period, timing of settlement, growth rates and reproduction (Newell and Branch 1980). Increased temperature usually results in shorter developmental times and higher growth rates of ectotherms (Atkinson 1994) since growth, development and reproduction are all regulated by thermal history (Trudgill et al. 2005). There have been many studies linking temperature with *M. membranacea* outbreaks in Nova Scotia, Canada (Saunders and Metaxas 2007, 2009; Scheibling and Gagnon 2009; Saunders et al. 2010). Concluding from these studies, less epibiosis is expected at higher than lower latitudes due to generally lower temperatures in the north. In temperate and boreal areas, epibiosis tends to show strong seasonality, with most spawning and growth occurring between spring and summer depending on location.

Water depth and light availability also affect the composition and growth of biofouling organisms. Epiphytes, i.e. photosynthesizing algae, are usually more abundant in shallow water where more light is available. Shallower waters are generally warmer in summer than deeper waters, have higher light levels and therefore also higher phytoplankton concentration. Phytoplankton may serve as a food source for certain fouling animals (epizoans) and compete for nutrients and light with epiphytes and farmed seaweed; as a result, epibiosis and seaweed growth generally decrease with increasing depth (Vinagre et al. 2020).

Water currents can also affect the species composition and amount of epibionts on the seaweed fronds. Many epizoans, such as mussels, hydroids, and bryozoans benefit from currents as they feed on phytoplankton and other suspended particles (Railkin 2003). Feeding success depends on the resupply of food particles and, thus the velocity of the currents and varies with fouling species. For example, the bryozoan *Membranipora serrilamella* (Arkema 2009) showed the highest feeding success at sites with intermediate ambient flow speed ($10\text{--}12 \text{ cm s}^{-1}$). Very strong currents may dislodge organisms from the seaweed frond, and facilitate or complicate the settlement of larvae or spores from epibionts (Vinagre et al. 2020).

2.5 Defence mechanisms

Seaweeds have several strategies to defend themselves against epibionts, either by preventing settlement, removing epibionts or killing them (Hurd et al. 2014). In general, however, the defence mechanisms in algae are largely undocumented in marine systems (Amsler 2008). Environmental conditions affect the defence strategies, growth and amount of chemical content of seaweeds. The dynamic nature of seaweed fronds allows the removal of epibionts by continuously producing new, clean frond area and shedding old, fouled tips. This process clearly affects overall growth potential and is considered in this thesis. Other defence mechanisms include peeling off the outer epibiont-infected layer (Bartsch et al. 2008), and production and release of toxic defence compounds or antifouling metabolites inhibiting epibionts, a process called allelopathy (Harlin and Rice 1987). Most often, these compounds have been found to be affected by the surrounding environment (Amsler 2008). Phlorotannins (polyphenolics) have been identified as one group of these defence compounds of seaweeds, although the evidence is still equivocal (Hurd et al. 2014). Oxidative bursts, producing huge amounts of reactive oxygen species, a common defence mechanisms in plants (Wojtaszek 1997) have also been interpreted as a mechanism to deter epibionts, and in particular pathogens, and this response has been found consistently in all Laminariales studied (Küpper et al. 2002).

3. Scope of thesis

In this thesis, I examined the spatial and temporal patterns of epibiosis on the fronds of cultivated seaweed, *Saccharina latissima*, and their effects on seaweed growth (figure 4). Additionally, I examined whether outplanting time of the seaweed can affect seaweed quality, quantity and epibiont occurrence and composition. The main objectives and hypotheses of the individual papers that this thesis is composed of were:

Paper I: To study the variation of epibiosis on a regional scale in northern Norway and determine key environmental parameters affecting epibiosis.

The primary aims were to document seaweed biomass production, along with variation in abundance, taxonomic composition and the distribution of epibionts along seaweed fronds on cultivated *S. latissima* at three close-by sites with different water mass characteristics.

Paper II: To describe the variation of epibiosis cover and species composition along a latitudinal gradient spanning 11 degrees across coastal Norway, and identify environmental parameters affecting this variation.

Embedded in a comprehensive study, the overall objective in relation to epibiosis was to examine the effects of latitude, season and cultivation depth on epibiosis of *S. latissima*. Specifically, I hypothesized that a latitudinal pattern of abiotic factors would provide the potential of a northward delay in development of epibiosis during the growing season, with associated implications for the harvesting period. It was also hypothesised that seaweed cultivated at deeper waters would exhibit a lower amount of fouling organisms than seaweed cultivated on shallower water.

Paper III: To study the effect of outplanting time of seaweed on seaweed frond area, growth and shedding rates and on epibiont abundance.

I hypothesized that the environmental conditions at outplanting would affect the growth and composition of the seaweed differently, along with creating different phenology of the seaweed when the bulk of epizoan larvae arrived.

Epibiosis varies in space (addressed in **papers I and II**) and time (addressed in **papers II and III**). Together these papers give an overall picture of the epibiosis of cultivated *S. latissima in situ*, including identification and description of the fouling species, succession of the fouling community, onset and rate of fouling, and environmental parameters affecting epibiosis. The key results are briefly presented and discussed in **chapter 5** (and in detail in the articles).

Fouling of macro epibionts on cultivated *Saccharina latissima* (Phaeophyceae): *in situ* temporal and spatial variation

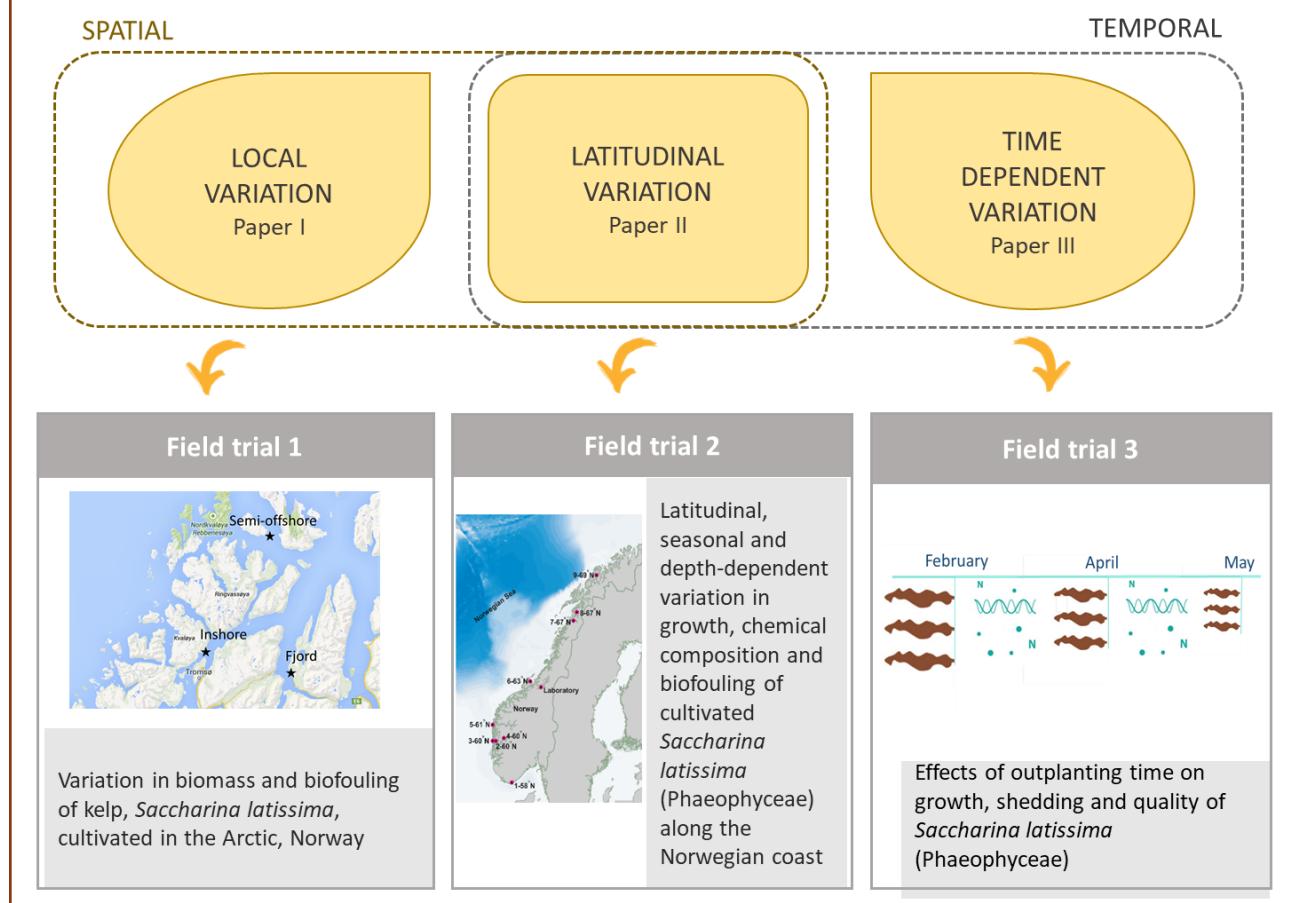


Figure 4 Graphical presentation of the different topics studied and field trials conducted in this thesis. Key results of spatial variation are given and discussed in section 5.1, and key results from natural temporal variation are showed and discussed in section 5.2, and the effect of outplanting time on seaweed response in section 5.3.

4. Methods

4.1 Epibiosis measurements

The methods applied are similar across articles but modified to suit the specific study questions and are more thoroughly described in the respective papers. Briefly, in **paper I**, a modified version of the point-sampling-method described in Christie (1980) was used to estimate frond area covered by epibionts. Here, sporophytes collected on the last sampling day were transported to land and laid flat on a white background. A grid system with vertical and horizontal grid lines was placed on top of the kelp fronds at each of three parts: distal, middle, and proximal, and each grid was photographed. Percentage cover of epibionts per frond area was estimated based on the overlaid proportion of 30 points (at the intersection of the grid lines). In **paper II**, a similar method was used, but further modified and upgraded to fit the large spatial and seasonal extent of the study. Here, sporophytes were collected on every sampling date, transported to land and again laid flat on a white background. Epibiosis was quantified as percentage cover on each frond, using image analysis. To image the entire frond, 1–3 images were taken depending on frond size, with a digital camera mounted on a tripod 25 cm above the frond. Percent cover 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 fouling organisms underneath the points were identified and recorded for each point. The advantages of these two methods used in **paper I** and **II** are that they are relatively easy to perform with little need for expensive equipment, they can be used in the field, and/or close to the sampling site, and include analysis of all species identified. Alternative methods include a fiber optic light table as in Førde et al. (2015), which may give an even more precise estimate of total area fouled, but is more equipment and time demanding. In **paper III**, the differences between the treatments were assumed to be smaller than in **papers I** and **II**, and a method with higher resolution was, therefore, used where epibiosis was quantified by the absolute number of fouling individuals and colonies instead. Also here, the seaweed frond was divided into three equally long sections representing meristematic (proximal), middle, and distal (tip) regions to test for effects of blade age on epizoans fouling the seaweed frond, and all epizoan individuals/colonies were identified and counted. Additionally, colonies of the abundant bryozoan *M. membranacea* were subdivided into two size classes: < 2 zooid rows were categorized as (early) settlers and ≥ 2 zooid rows as colonies as in Saunders and Metaxas (2007), using magnifying eyewear (Watch Repair Magnifyer) (25x). The reason for this separation was due to known preferential settlement by this species on the meristem, and therefore any potential difference in amount of chemical cues among the outplanting treatments would affect the preferences of initial settlement of this species, and the separation into early settlers and larger colonies allowed me to identify this preferential settlement.

4.2 Growth measurements

Seedlings of *S. latissima* were produced according to the cultivation protocol in Forbord et al. (2018) using hatchery at FISK Tromsø for **paper I**, and SINTEF Sealab in Trondheim for **papers II and III**. Seaweed growth can be measured in several ways, including measuring the increase in seaweed biomass produced, and seaweed frond. In **papers I and II**, seaweed biomass was weighed with a scale. In **paper II**, total frond length was measured to obtain net growth of the seaweed frond including both frond elongation and lost material as shedding of the tips. The advantage of this method is that it is simple, and sources of errors are minimal. In **paper III**, the hole-punching method (Parke 1948) was used to measure gross growth in frond length and loss through shedding of the seaweed frond. Here, a hole was punched 5 cm from the transition between the stipe and the frond. A new hole was punched on a certain time-interval and the distance between the new and the old holes and between the old holes was measured. From the distance measurements between holes, the relative Daily Growth Rate (DGR), and relative Daily Shedding Rate (DSR) were calculated as:

$$DGR \text{ (day}^{-1}\text{)} = \left[\left(\frac{L_0 + G}{L_0} \right)^{\frac{1}{t}} \right] - 1$$

$$DSR \text{ (day}^{-1}\text{)} = \left[\left(1 + \left(\frac{L_0 + G - L_t}{L_0} \right)^{\frac{1}{t}} \right) - 1 \right]$$

where L_0 is the total frond length on the previous sampling date, L_t is the total frond length on the following sampling date, G is gross frond growth since previous sampling, calculated by adding the length increase between the punched holes, and t is days since last sampling date.

Additionally, seaweed frond area was estimated in **paper III**. Measuring growth of the seaweed frond area is not straight forward as frond area includes frills and in paper III, the area of the frond was estimated from length and width measurements, corrected for frills. The correction factor was estimated based on the relationship of frond length and width to actual area as in Yorke and Metaxas (2012). To establish a correction factor for frills, the seaweed frond was cut into small pieces and laid flat on a white background, and each section was photographed with an Olympus Tough F2.0 digital camera. The pictures were analysed in ImageJ (Schneider et al. 2012) and total area and frond areas were calculated from this as:

$$\text{Frond area} = 0.289 \cdot (L \cdot W)^{1.15}, R^2 = 0.98$$

where L is the total frond length and W is the width of the widest part of the frond.

4.3 Analyses of chemical contents

In **papers II and III**, dried seaweed tissue material was used to analyse seaweed tissue carbon (C) and nitrogen (N). This was analysed with a CHN elemental analyser (Leeman Lab CEC 440 CHN analyzer)

with acetanilide as standard. For internal dissolved inorganic nitrogen (I-DIN) measurements, seaweed tissue material were boiled for 30 minutes in distilled water to cause rupture to the seaweed cells and thereby the NO_3^- content leaked into the surrounding water (Fujita et al. 1988; Hurd et al. 1996). This water was analysed for its nitrate content. Both I-DIN as well as the nitrate concentration in ambient seawater (external, E-DIN) were analysed by standard seawater methods (Randelhoff et al. 2018) using a Flow Solution IV analyzer from O.I. Analytical, USA. The nutrient analyser was calibrated using reference seawater from Ocean Scientific International Ltd. UK.

5. Key findings and discussion

The papers that this thesis is built upon show that there is spatial variability in phenology, degree and density of epibiosis on multiple scales on cultivated *Saccharina latissima*. **Papers I and II** showed that temperature explained most of this variation, a finding consistent with earlier literature.

5.1 Spatial variation of epibiosis

5.1.1 Large-scale spatial variation of epibiosis

In **papers I and II**, I ask how epibiosis varies on different spatial scales, and I focus on larger, latitudinal scales in **paper II**. In **paper II** the hypothesised latitudinal pattern in abiotic factors of light intensity, day length and temperature from 58 °N-69 °N was confirmed along with associated patterns in seaweed production of biomass, chemical composition and epibiosis. Despite local variation within a region (shown in section 5.1.2), visible epibionts appeared ~2 months later at northern compared to southern locations (figure 5), with associated implications for the harvesting period. Freshwater-influenced locations deviated from this latitudinal gradient pattern. The latitudinal pattern in phenology of epibiosis was partly explained by the variation of environmental factors, with a positive effect of increased temperature and negative effect of increased light. Temperature has a direct effect on ectothermic organisms, such as the epibionts in this study including *M. membranacea*, as increased temperatures usually results in shorter development times and higher growth rates in these species (Atkinson 1994). Also the experienced thermal history regulates many life-history characteristics in ectotherms, such as growth, development and reproduction (Trudgill et al. 2005). In temperate regions, increasing temperature at the sea surface during spring causes stratification of the water column, the timing and strength of which vary along a latitudinal gradient, resulting in substantial seasonal differences in nutrient availability available for epiphytes (fouling plants/algae) along the coast (Rey et al. 2007; Ibrahim et al. 2014; Broch et al. 2019). *S. latissima* is a cold-water species, showing reduced tissue strength after exposure to 14 °C for three weeks (Simonson et al. 2015), a common summer temperature in latitudes of mid and southern Norway. This weakening of tissue can act synergistically with fouling by epibionts and further impact seaweed crops.

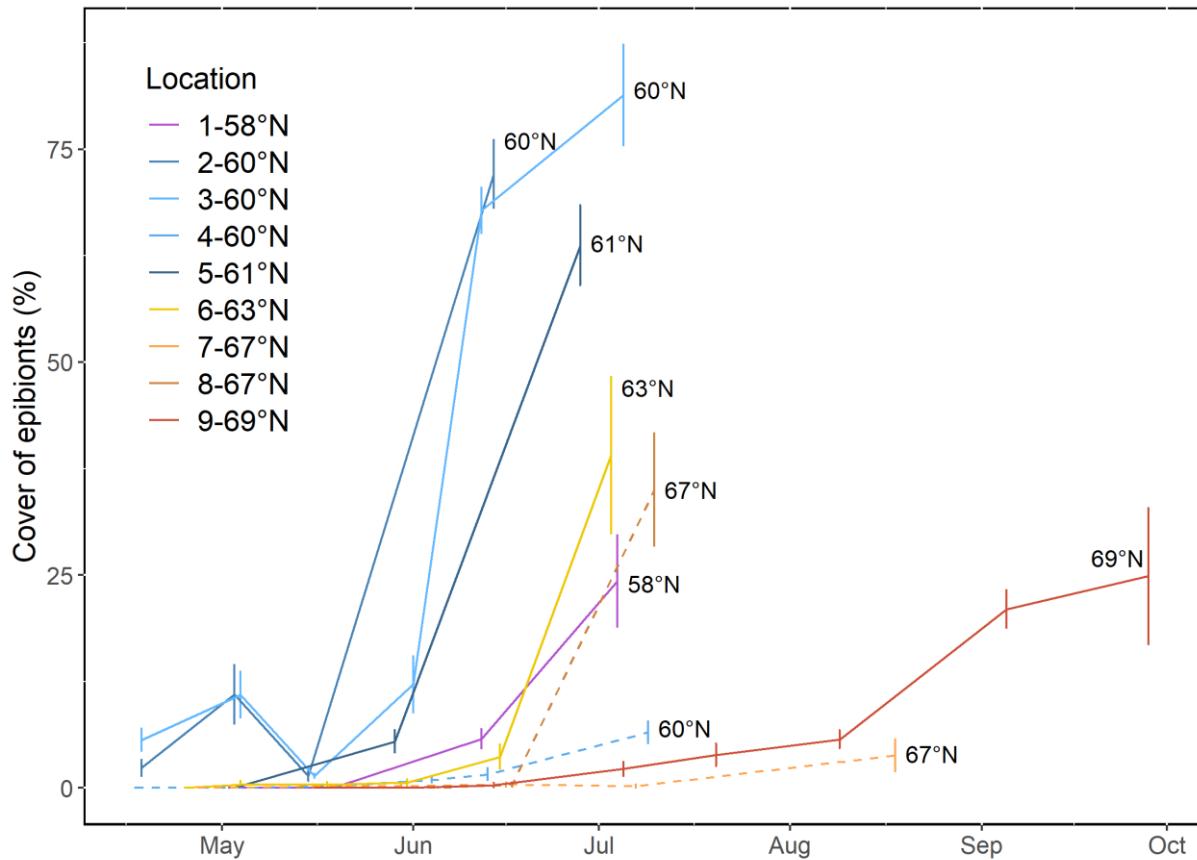


Figure 5 The effect of latitude and season on epibiosis as percent cover of the *S. latissima* frond in 2017 from 58 °N -69 °N at 1-2 m depth. Stippled lines indicates freshwater impact. Figure modified from [paper II](#).

An extended grow-out phase before harvesting of the seaweed cultivated in the north may have implications beyond an extended seaweed growth period. Specifically, my finding may also be beneficial for developing Integrated Multi-Trophic Aquaculture (IMTA). In IMTA, organisms produced at a higher trophic level (i.e. in finfish aquaculture) release dissolved inorganic nutrients (DIN) which can be utilised by lower trophic levels, such as seaweed. When water temperature rises in summer and fall, faunal metabolism increases and more DIN is released from the high-trophic species. Usually, there is a mismatch with seaweed aquaculture, as the seaweeds are harvested before finfish aquaculture peaks in nutrient release (Broch et al. 2013). However, this study shows that there may be possibilities for a better match of the algal growth phase with nutrient peaks in more northern than mid-latitude and southern Norwegian locations.

5.1.2 Small-scale spatial variation of epibiosis with location

One interesting result from northern Norway near Tromsø was the strong difference in the amount and type of epibionts among sites within a relatively short distance from one another (<50 km) but with slightly different environmental characteristics (figure 6, **paper I**). The differences in epibiont cover were partly explained by the variation in abiotic factors among the sites, representing semi-offshore, inshore and fjord conditions, respectively, with temperature having the highest impact. Also, increased currents had a significantly positive relationship with more epibiosis. Other studies have found contrasting results with more epibiosis on less current-exposed sites on cultivated kelp (Peteiro and Freire 2013a; Mols-Mortensen et al. 2017), and on wild *S. latissima* (Moy and Christie 2012). Currents affect a range of other abiotic and biotic factors, and for sessile filter-feeders, such as bryozoans, feeding success is highly affected by flow speeds. Based on my results, pilot studies before establishing a seaweed farm should compile (or even measure) environmental conditions of prospective sites. In addition, industry would benefit from a summary of experimental studies that establish effect sizes of relevant environmental factors on epibiosis levels.

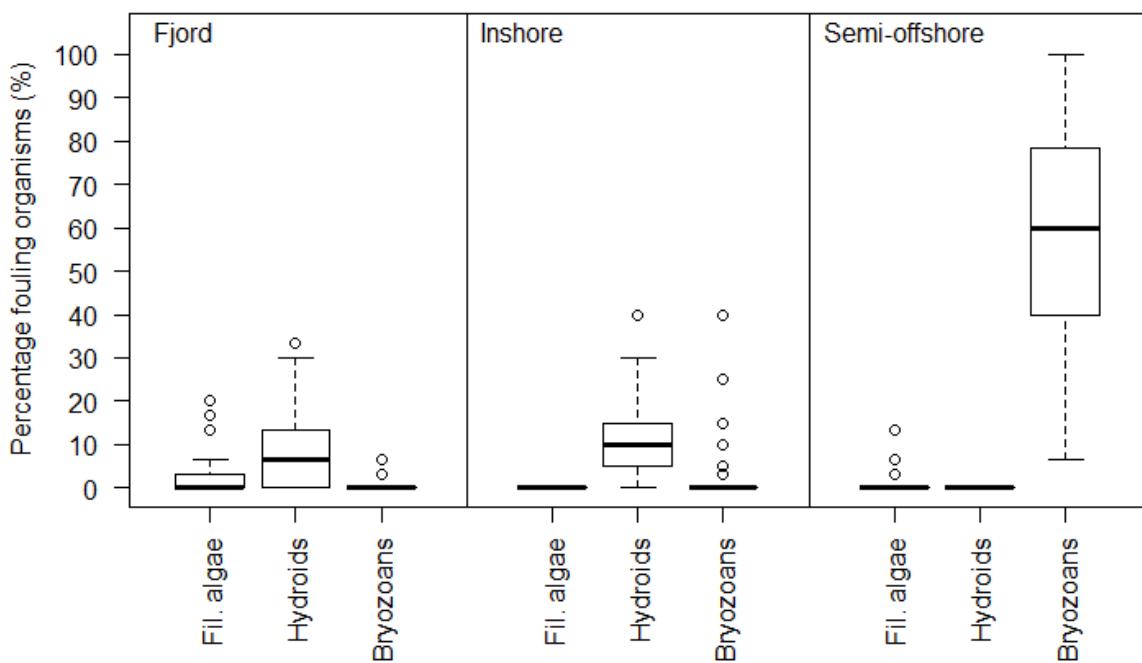


Figure 6 Proportion of *S. latissima* frond area covered by epibiont taxa; filamentous algae (Fil. algae), hydroid cnidarians *Obelia geniculata* and the bryozoan *Membranipora membranacea* at three locations in close vicinity of each other between 69-70°N, sampled 18-22 August 2014. Figure from **paper I**.

5.1.3 Small-scale spatial variation of epibiosis with depth

Lowering the seaweed from the sea surface to greater depths has been suggested as a method to minimize epibiosis by suspension-feeders on cultivated seaweed (Førde et al. 2015) as a result of lower photoautotrophic food particles available where the light is limiting. Testing this earlier finding in **papers I and II**, I did not find consistent results. In **paper I**, I did not detect a statistically significant difference in epifouling cover between 3 and 8 m depth, while in **paper II**, the effect of depth was location-specific, with some locations experiencing higher epibiont cover in shallower waters (1-2 m), and some in deeper waters (8-9 m, figure 7). I found, however, a significantly negative effect of light on epibiosis in **paper II**. In conclusion, the results in **paper I and II** did not reveal any clear benefit of cultivation at deeper than shallower depths, but could not definitely exclude this benefit either. My result suggests that lowering the seaweed to greater depths later in the cultivation season – i.e. at the onset of epibiosis - may be beneficial in some areas, but the effects appear to be location specific. Experimentally determining ideal cultivation depths should in the future not just consider light for the benefit of algal growth as is commonly done (Azevedo et al. 2019) but also the effects on epibiosis.

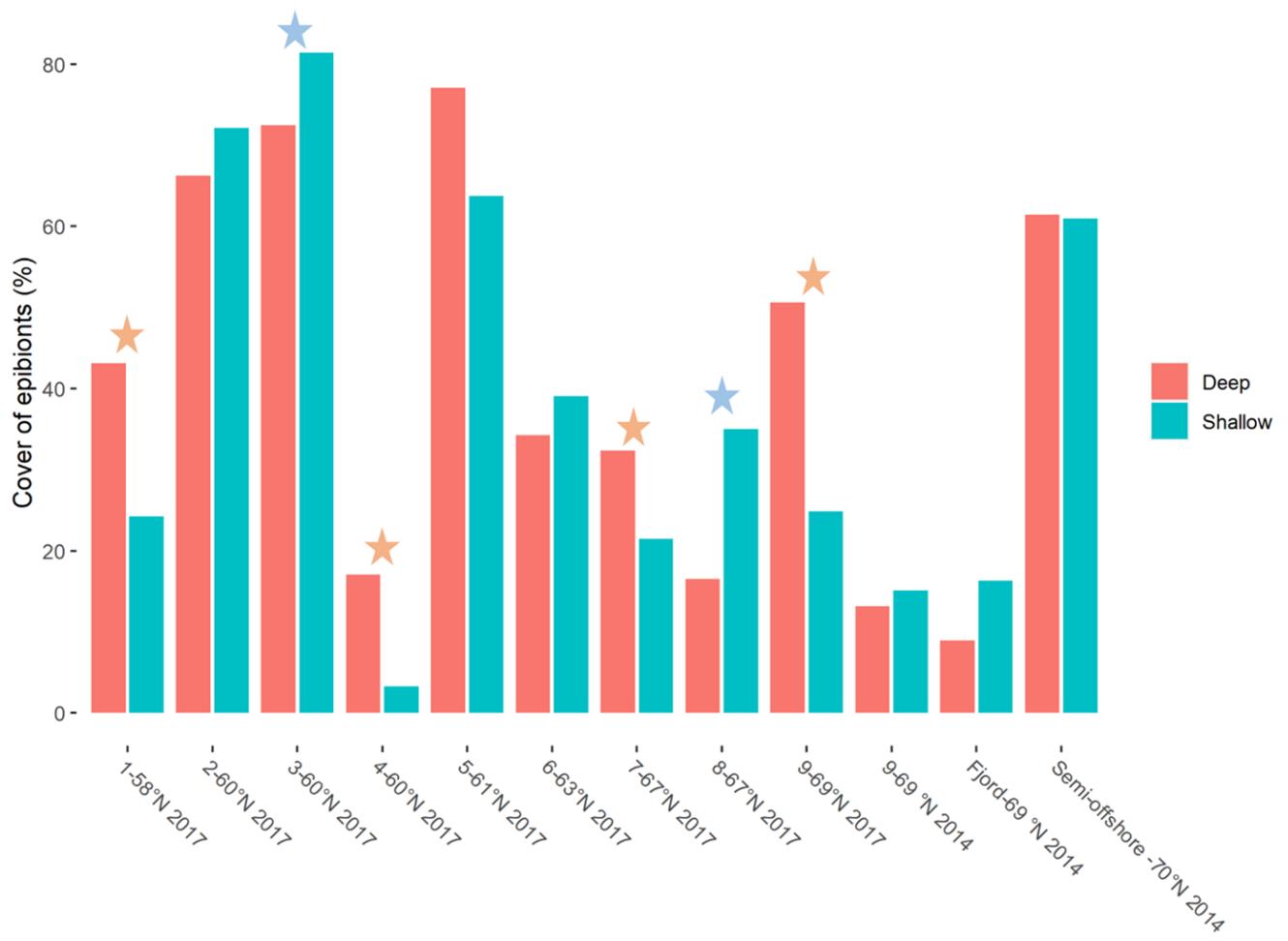


Figure 7 The effect of depth on mean percent cover of epibionts on fronds of *S. latissima* from 58 °N - 70 °N. Data are from the last sampling date at each location (July-September) and from two years, 2014 and 2017. Orange stars show significantly higher epibiont cover on kelp growing on ropes at 8 m depth and blue stars indicate a significantly higher cover on kelp at 1 (in 2017) and 3 (in 2014) m depth. Figure modified from **papers I and II**.

5.1.4 Small-scale spatial variation of epibiosis along the seaweed frond

The spatial variation of total epibiosis along each seaweed frond was studied in **paper I**, with a clear trend of greater cover on the older, distal parts of the fronds. In **paper III**, however, microscopic settlers of the bryozoan *M. membranacea* showed a preference for the younger meristematic regions of the frond, while in **paper I** the older tips were more severely covered by older (hence larger) *M. membranacea* colonies (figure 8). This finding corroborates earlier studies, where the younger meristematic regions were also preferred by *M. membranacea* settlers (Matson et al. 2010; Denley et al. 2014). The meristematic regions are the growth zones of kelps, while the tips constitute the oldest tissue. Consequently, the heavier epibiosis of the seaweed tips are most likely an effect of initial accumulation of settlers on the meristematic region and subsequent growth of both seaweed and

epibionts over time (Jennings and Steinberg 1997), resulting in an ‘assembly line’ that moves settlers towards the tips while they undergo growth. Therefore, the results from **paper I** and **III** agree with the above cited studies, where cyphonautes larvae settle on the younger, newly produced, tissue, to achieve a maximal amount of time to grow and reproduce, before the seaweed shed its heavily fouled tips.

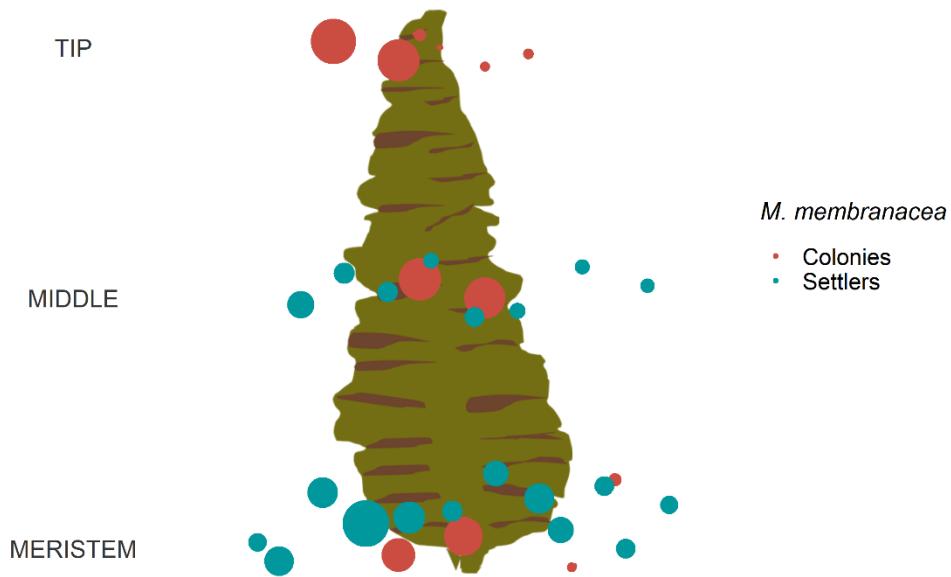


Figure 8 Fouling by *Membranipora membranacea* on the *S. latissima* frond as an effect of frond area (meristem, middle and tip) cultivated at 69 °N -70 °N. Each circle represents the mean for each treatment (location and depth in **paper I** and outplanting time in **paper III**). Amount of fouling is represented by the size of the circles (where the data for colony abundance in **paper I** come from percent cover, and that of settlers in **paper III** from number per area). The data presented are from August and from two years, 2014 and 2018. Figure modified from data in **paper I** and **III**.

5.2 Natural temporal variation of epibiosis – amount and species composition

The epibiont community had an overarching seasonal pattern in density/percent cover, and composition (**papers II** and **III**) at all 11 locations, across 3 years and 3 depths. An initial onset of a few organisms was followed by a period of slowly increasing cover and density with time, and a sharp increase later in the season. The succession of fouling species began consistently with filamentous algae and diatoms, when present, fouling the tips of the fronds (figure 9 & 10, **paper II**). *M. membranacea* was the most prevalent fouling species in all three studies of this thesis. **Papers II** and **III** showed that there was a temporal developmental pattern in the density and cover of this species. Overall, an increasing relative contribution of *M. membranacea* over time was observed at most locations (**papers II** and **III**), except at those with freshwater input, which had a lower amount and

proportion of *M. membranacea* (figure 6 & 10, **paper I and II**). Temperature had a significant, positive relationship with total epibiosis in **papers I and II**. Thermal history also explained 81% of the variability in the abundance of settlers of *M. membranacea* in Nova Scotia, Canada (Saunders and Metaxas 2007). At the same locations, changes in winter and spring temperatures had the most pronounced effects on the timing of settlement and abundance of *M. membranacea* colonies while changes in summer temperature had the most pronounced effect on colony size and coverage on kelp blades (Saunders et al. 2010). While determining the abundance of larvae in the water column was not within the scope of this thesis, larval supply and settlement rates have been shown to coincide (Førde et al. 2015), and the sudden rise in settlement may, therefore, be explained by an increase in larval supply. Consequently, the sudden rise in epibiosis by *M. membranacea* in the present study may be a consequence of the thermal history experienced in the area (triggering larval recruitment), consistent with temperature being the primary explanatory variable for epibiont cover in **papers I and II**.

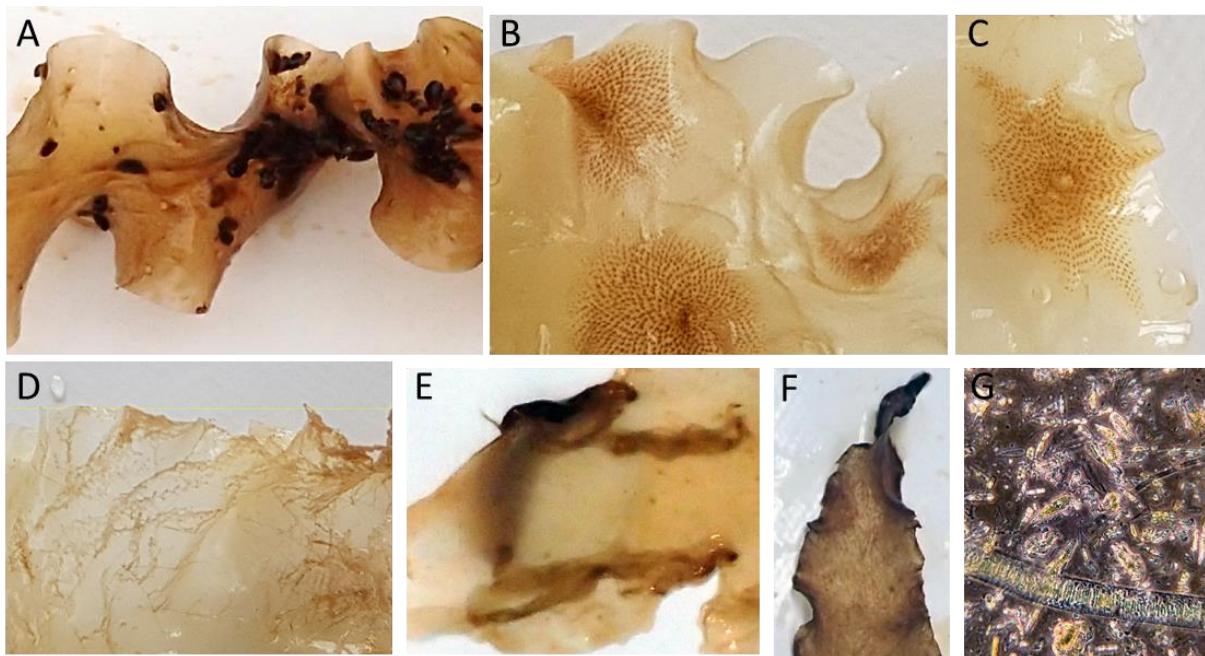


Figure 9 Images of the epibionts found in this study. A) Bivalvia. B) *Membranipora membranacea*. C) *Electra pilosa*. D) Hydroids. E) Filamentous algae. F) Diatoms. G) Diatoms at $\times 40$ magnification. Figure from **paper II**

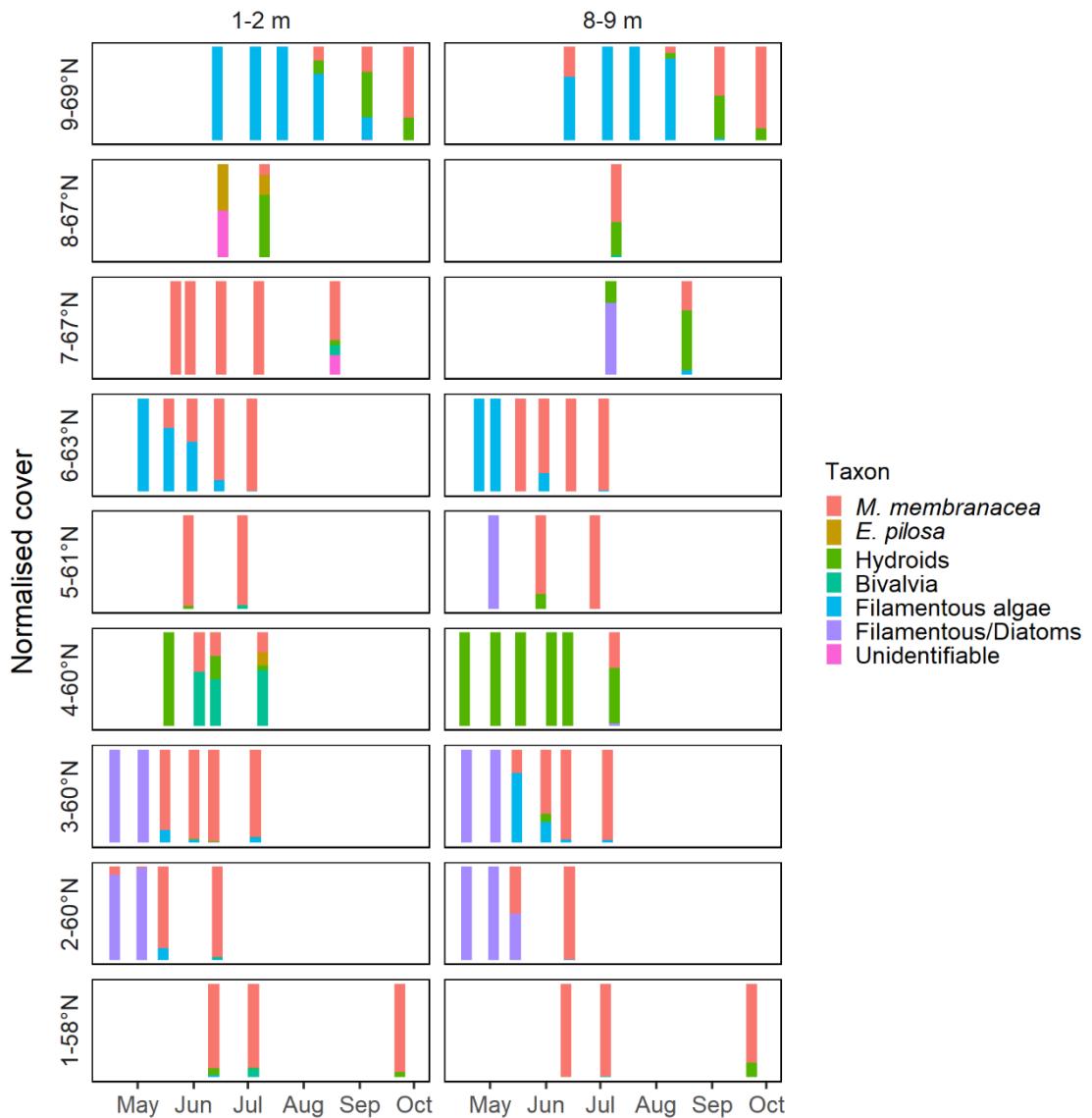


Figure 10 Epibionts fouling *S. latissima* in 2017 from 58 °N-69 °N at 1-2 m depth and 8-9 m depth. Data are showed as normalised (relative) cover with the proportion each taxon contributed to the total cover of all epibionts. Sites 4-60 °N, 7-67 °N and 8-67 °N were influenced by freshwater run-off. Figure from **paper II**.

5.3 Temporal variation of epibiosis controlled by outplanting time

In **paper III**, I examined whether the amount of epibiosis on cultivated seaweeds at a particular location can be regulated by outplanting time. *S. latissima* were outplanted at three different times in late winter to late spring with varying physico-chemical environmental conditions (particularly light, nutrients and temperature). I hypothesized that the environmental conditions at outplanting would affect the growth and composition of the seaweed differently, along with creating different phenology of the seaweed when the bulk of epizoan larvae arrived. Earlier outplanting resulted in a higher density

of epizoans at any given time (figure 11), but with a similar phenology of settlement of *M. membranacea* for both the February and the April outplanting treatments. There was specifically a significantly higher amount of settlers on the earlier outplanting date, as well as on the younger meristematic regions on the seaweed frond on all outplanting dates, corroborating earlier studies (Denley et al. 2014). Epizoan larvae can exhibit several behaviors before attaching to a substrate. After larvae contact a substrate (i.e. seaweed frond), they may crawl, tumble or swim away, to more suitable locations. In the absence of such post-contact behaviour, the larvae will attach to the surface which they first encounter (Walters 1992). Larvae of *M. membranacea* show different behaviours on different seaweed species and frond areas (Matson et al. 2010). This ability suggests that larvae of *M. membranacea* can detect small-scale differences in substrate quality such as chemical composition between meristem and tips. Further, it has been suggested that the larvae use physical or chemical cues or deterrents for settlement (Brumbaugh et al. 1994), possibly including defense compounds produced by the seaweed. In **paper III**, I recorded a preference of *M. membranacea* settlers for young, meristematic tissue in all outplanting experiments, as well as a higher density of these settlers in the earlier outplanting, perhaps indicating a choice for these sporophytes over the later outplanting dates. While results from **paper III** did not reveal any difference in the seaweed tissue nitrogen contents measured among outplanting experiments preventing conclusions about possible cues related to nitrogen compounds, sporophytes outplanted in April had a higher carbon content and a higher C:N ratio than those outplanted in February, suggesting a higher accumulation of carbohydrates for the former. Polyphenolic C-based defense products known as phlorotannins, may provide a chemical cue preventing the settlement of fouling organisms in brown seaweed, as documented in *Fucus evanescens* (Wikström and Pavia 2004). Phlorotannins show a high degree of spatial and temporal variation among seaweed species (Van Alstyne et al. 1999). This might be explained by the Carbon Nutrient Balance Model (CNBM) in algae (Pavia and Toth 2000), stating that when nutrients are limiting growth (indicated by a high carbon:nitrogen ratio), photosynthetically fixed carbon will be allocated to the production of defense compounds such as phlorotannins instead of growth. Thus, seaweed cultivated in shallow waters, where attenuation of light is high and nutrient concentration after the phytoplankton bloom is low, may contain a higher concentration of phlorotannins than seaweeds cultivated in deeper waters where light is limiting and the carbon:nitrogen ratio is low. The amount of chemical components on the seaweed frond may also vary with age (Sjøtun et al. 1996). The results also showed a significant variation in relative daily growth rate (DGR) and higher relative Daily shedding rate (DSR) among the three outplanting dates. Continuous growth and shedding in summer, may help reduce the density of fouling organisms and can, therefore, partly or fully explain the difference between the densities of epibionts. As there was a difference in density of epizoans, but not in the phenology of epizoan settlement, an earlier outplanting gave a prolonged time for grow-out at sea

prior to the main recruitment event in fall and therefore resulted in significantly larger frond area. In conclusion, fine-tuning outplanting date in relation to local phenology of light, ambient nutrient conditions and epibiosis may pay off for seaweed farmers.

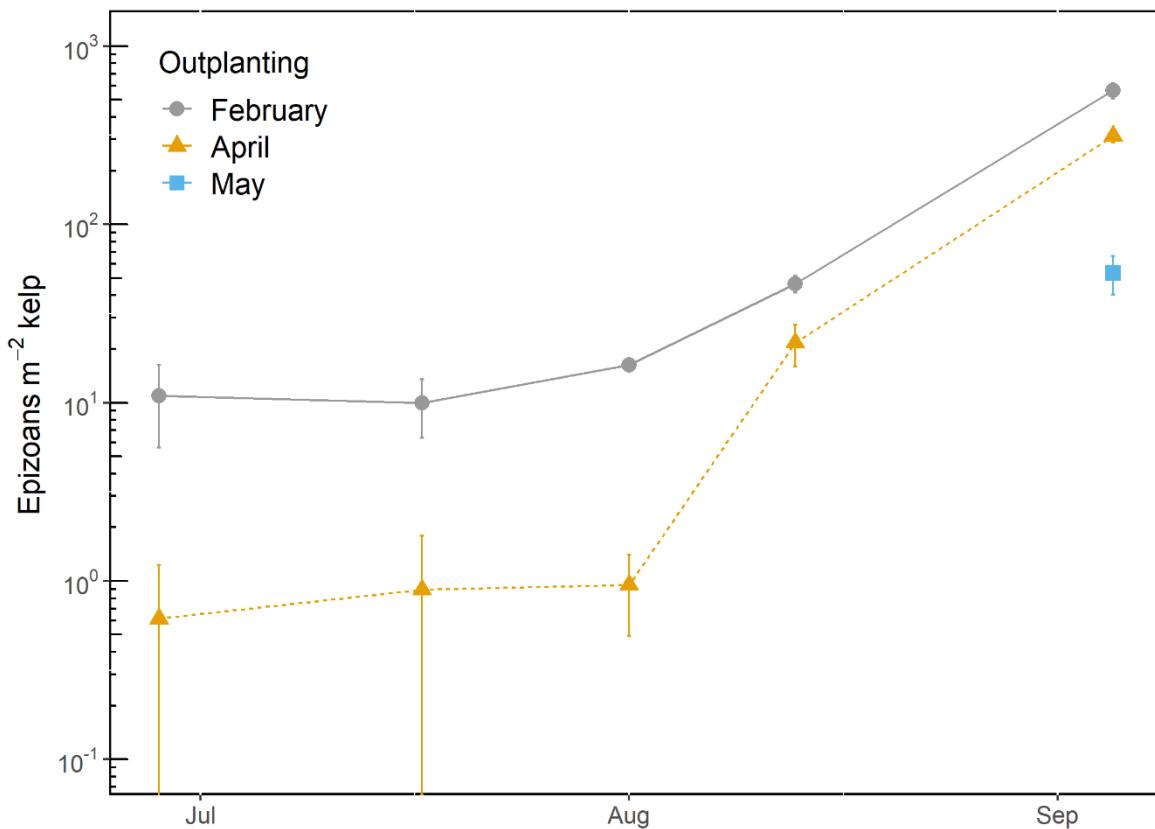


Figure 11 Organisms fouling *Saccharina latissima* outplanted at three different times (February, April and May). Density (number of epizoans m^{-2} kelp) over time for the three outplanting dates from 28 June until 5 September. Mean \pm SE, n=7. Kelp outplanted in May was only sampled 5 September.

5.4 Seaweed yield in relation to epibiosis

Seaweed yield, one measure of seaweed farming success, is generally negatively affected by epibiosis. Mean frond length and biomass yields showed a latitudinal related pattern, with locations in the south reaching their maximum length and biomass earlier in the cultivation period than locations further north (**paper II**). However, latitude did not affect the total yield produced, but rather the timing of when maximum yield was reached. This timing was affected by the onset of epibiosis (figure 5), as a particular level of epibiont cover leads farmers to harvest the seaweed biomass. There was a clear reduction in area fouled at locations influenced by freshwater, this was accompanied by a lower growth in seaweed frond length and biomass. This finding is consistent with previous trials in Denmark during periods of low salinity (Marinho et al. 2015b; Bruhn et al. 2016). On a regional scale, in **paper I**, the location with highest water temperature and fully marine salinity had the highest seaweed biomass

production, both before and after the arrival of epibionts, which however was the same location with the highest epibiont cover. The shallower cultivation depth, with more light, gave significantly larger seaweed fronds and higher biomass (**papers I and II**). This is similar to findings from earlier studies in Central Norway (Forbord et al. 2012; Handå et al. 2013; Sharma et al. 2018). The effect of depth is not constant but depends on local environmental variations, and in **paper II**, in summer, shorter frond lengths and lower biomass yields were found in shallower than deeper water at several locations. This was presumably an effect of high freshwater runoff in the surface layer or of high irradiance that may suppress algal growth (Fortes and Lüning 1980b; Spurkland and Iken 2011). And hence, several depths should be tested for new farm locations if uniformly seeded drop lines are not used.

In addition, seaweed yield varied among outplanting dates (**paper III**). Outplanting date had the highest impact on seaweed yield as the recruitment of epizoan larvae increased rapidly in fall – when seaweeds are largest - in all outplanting treatments. Given the duration in culture, seaweed yield was greater for earlier than later outplanting dates. This result is consistent with earlier literature (Peteiro and Freire 2009; Edwards and Watson 2011; Handå et al. 2013). A sharp increase in epizoan abundance occurred approximately at the same calendar time for all outplanting dates, and thereby, the seaweed outplanted earliest also had the longest time to grow before this event (figure 12). A prolonged time for grow-out at sea prior to the main recruitment event in September resulted in double the frond area for the February outplanting than in the April outplanting. Based on my research, early outplanting should be considered to increase overall seaweed yield before the epibionts settle.

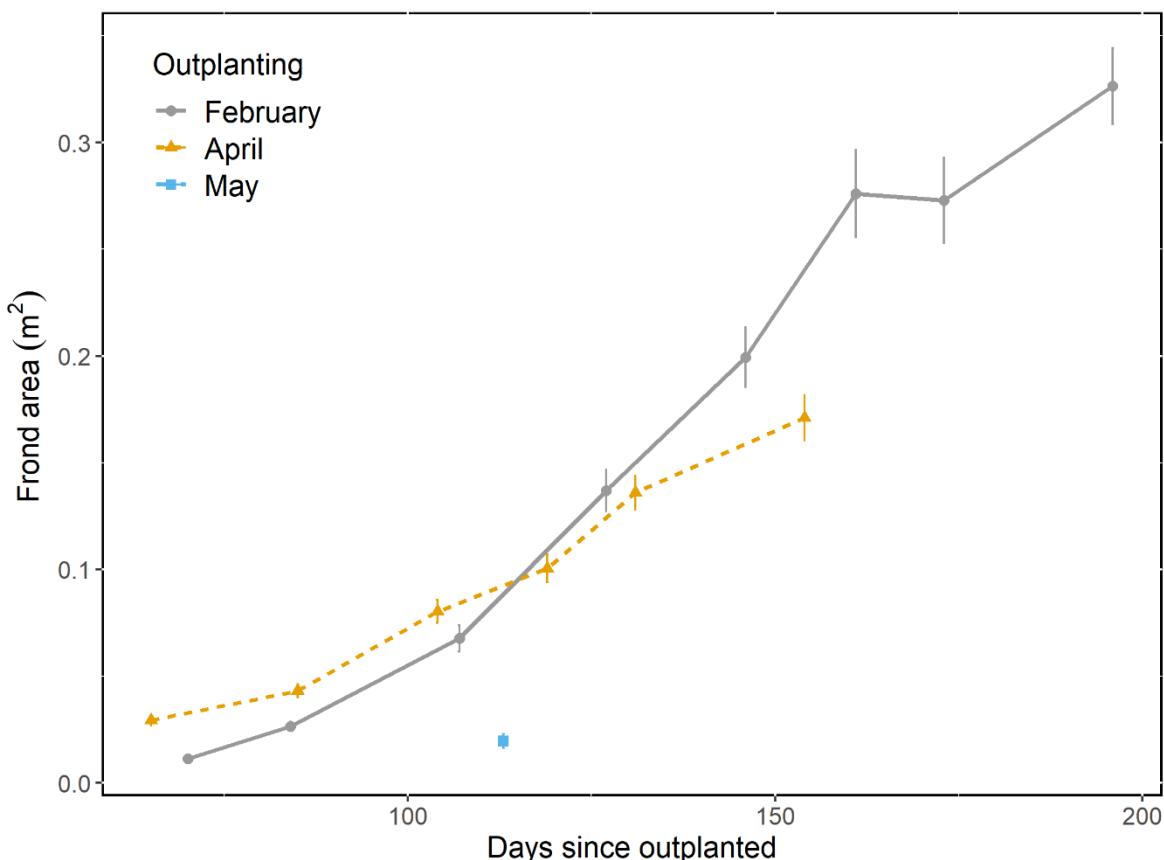


Figure 12 Variation in seaweed fond size linked to days of grow-out in sea restricted by the settlement of epibionts at 69°N in 2018. Mean ± SE, n=7.

6. Conclusions and future perspectives

My thesis shows that epibiosis on cultivated *Saccharina latissima* varies on small and large spatial scales, temporally and with the growth history of the seaweed host. A delayed onset of epibiosis with increased latitude follows a seasonal progression, and epibiosis is lower when seaweeds are outplanted later in the season. The variation on regional scales is a result of complex interactions of both biotic and abiotic factors. Table 1 shows a summary of the abiotic and biotic factors that affected the timing and extent of epibiosis in this study. Given there was a tendency of lower epibiont cover and occurrence of *M. membranacea* at freshwater influenced sites, I 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, and this should be further tested. The underlying mechanisms are not fully understood, for which more environmental data and experiments aiming at establishing response curves to environmental parameters, such as temperature, currents and light and combinations of these are required. This was not within the scope of this thesis, and further studies

on environmental impacts are needed in order to make more accurate predictions about where and when epibiosis occurs, and to find so-called "hot-spots" with reduced fouling pressure.

Table 1 Summary of factors that affect epibiosis and reference to papers in this thesis. Upward facing arrow indicates a positive relationship, and downward facing arrow indicates a negative relationship. Hyphens indicate absence of a significant effect and brackets means that it most likely is an effect, but that this was not measured.

	Factor	Epibiosis	Paper
Abiotic	Temperature	↑	I, II
	Nutrients	-	II, III
	Salinity	- (↑)	I (II)
	Light	↓	II
	Currents	↑	I
Biotic	Host length/biomass	-	II, III
	Host age	↓	III

The ultimate goal is to reduce epibiosis on cultivated kelp to improve the quality and quantity of the seaweed biomass. The seaweed quality is improved by a reduced (preferably zero) amount of epibionts as well as a prolonged growth season, accompanied with a higher carbohydrate storage. A reduced amount of epibionts can prolong the growth season of seaweeds possibly enabling a higher quantity of seaweed biomass.

Paper I showed that on a smaller scale the variation of epibionts, both species and cover, was highly variable, as was seaweed biomass. As such, an improved seaweed quality (i.e. less epibiosis), also gave a reduced seaweed biomass yield. On a larger scale, **Paper II**, revealed a delayed onset of epibionts of ~2 months at northern compared to southern locations along the Norwegian coast, resulting in a temporal shift of the seaweed quality and quantity related to latitude (figure 12). This gradient allows for delayed kelp harvest with increasing latitude. **Paper III** showed that a later outplanting time could reduce the amount of epibionts, but that an associated shorter grow-out phase in sea results in reduced seaweed yield.

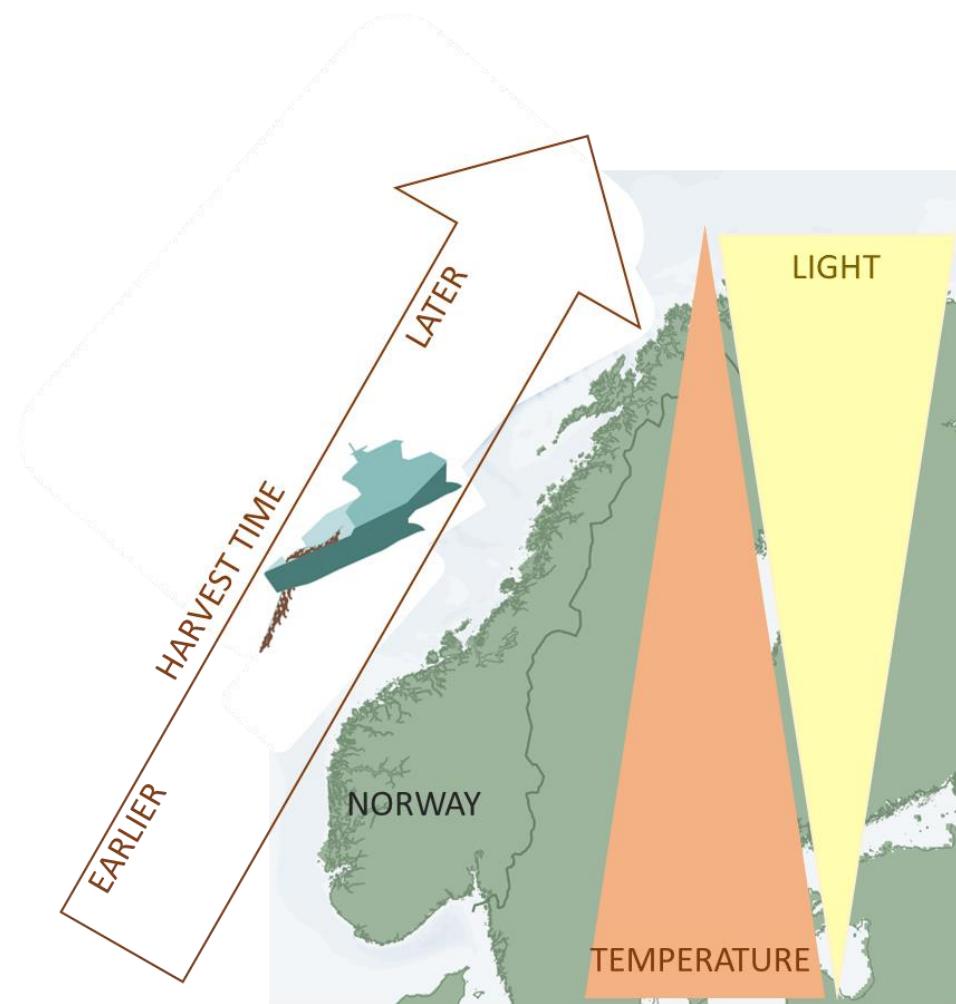


Figure 12 Graphical representation of the main environmental parameters in summer causing a northward shift in timing of onset of epibionts along the Norwegian coastline. Temporal variation of epibiosis is studied in **paper II** and **III**, spatial variation are studied in **paper I** and **II**. As the harvesting of seaweed biomass is recommended to occur before the onset of epibiosis to achieve high quality yield, these results suggest an advance in harvest of *S. latissima* biomass by 2 months in southern latitudes (58°N) compared to northern latitudes ($69\text{-}70^{\circ}\text{N}$), enabling a joint harvest from south to north.

7. References

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Paper I



Variation in biomass and biofouling of kelp, *Saccharina latissima*, cultivated in the Arctic, Norway

Sanna Matsson^{a,b,*}, Hartvig Christie^c, Reinhold Fieler^a

^a Akvaplan-niva AS, Fram Centre, 9296 Tromsø, Norway

^b Department of Arctic and Marine Biology, UiT The Arctic University of Norway, Breivika, 9037 Tromsø, Norway

^c Norwegian Institute for Water Research (NIVA), Gaustadalléen 21, 0349 Oslo, Norway

ARTICLE INFO

Keywords:

Seaweed farming
Epibionts
Bryozoans
Biomass yield
Low trophic level aquaculture

ABSTRACT

In the present study, the kelp *Saccharina latissima* was cultivated at three sites in Troms, northern Norway (at 69–70°N). These sites, while close to each other geographically, were characterized by differences in exposure to waves and influence from oceanic water, and arranged in a gradient from semi-offshore through intermediate exposure to a more sheltered fjord with a stronger influence from freshwater run-off. The effect on kelp biomass and biofouling was studied from February to August in 2014. Large variation in biofouling cover was observed between sites. The site with highest exposure had the maximum kelp biomass, but also the highest cover of fouling organisms. The frond area covered by epibionts varied by a factor of 8 with lowest cover at the fjord site, intermediate cover at the inshore site and highest cover at the semi-offshore site. Species composition of the biofouling community also varied between sites, with the dominant taxa being hydroids at the most protected, and bryozoans at the most exposed site. The present study shows that both biomass yield and biofouling can vary profoundly within short geographical ranges and it thereby underlines the importance of thorough site selection for kelp cultivation in order to achieve maximum kelp biomass and minimum biofouling. It also reveals promising opportunities for kelp cultivation at higher latitudes.

1. Introduction

1.1. Macroalgae cultivation

Macroalgae cultivation, including brown, red and green algae, is gaining interest in Europe. This is due to a general need for ingredients in a number of products such as food, fish diets, cosmetics, pharmaceuticals, biofuel etc. (Skjermo et al., 2014). In Norway, as in the rest of Europe, macroalgal cultivation is at an early stage of development. The Norwegian coastline has a potential for macroalgae cultivation along the entire latitudinal gradient from 58 to 71°N, but cultivation activities in Norway have so far been located in southern and in mid Norway (Stévant et al., 2017). These activities have been classified as research and pilot-scale production, with some of the first commercial permits granted in 2014.

Sugar kelp, *Saccharina latissima* (L.) C.E. Lane, C. Mayes, Druehl, and G.W. Saunders, is distributed from Portugal to Spitsbergen (Araújo et al., 2016) and in the NW Atlantic. It is the favoured species for cultivation in both the North Atlantic region and Norway. This is due to its high growth rate and comparatively simple life cycle where sorus

induction, spore release and gametophyte growth can be controlled in the lab (Forbord et al., 2012; Marinho et al., 2015b; Marinho et al., 2015a), and it can easily be seeded and grown on rope or other convenient structures.

1.2. Biofouling

The kelp fronds provide an attractive substrate for a variety of epibionts, such as filamentous algae, bryozoans, hydroids, tunicates and herbivorous invertebrates (Andersen, 2013; Moy and Christie, 2012; Scheibling and Gagnon, 2009). The epibionts usually settle during late spring and early summer, coinciding with the time at which biomass and nutritional values of kelp are high (Marinho et al., 2015b; Marinho et al., 2015a). Epibionts may cause loss of kelp biomass (Scheibling and Gagnon, 2009; Skjermo et al., 2014) through increased drag and friction, decreased flexibility, mechanical damage (Krumhansl et al., 2011) and light retention (Andersen, 2013). Epibiont settlement can occur at different parts of the kelp and may vary by taxonomic group of biofouling organism (Wahl, 1989). Due to deterioration of kelp tissue, epibionts can make the biomass unsuitable for human consumption

* Corresponding author at: Akvaplan-niva AS, Fram Centre, 9296 Tromsø, Norway
E-mail address: sanna.matsson@akvaplan.niva.no (S. Matsson).

(Marinho et al., 2015a). The colonial encrusting bryozoan, *Membranipora membranacea* (L.) is one of the most common epibionts with detrimental effects on kelp fronds on both sides of the North Atlantic, on both wild kelps (Dixon et al., 1981; Saunders and Metaxas, 2009b; Scheibling and Gagnon, 2009) and cultivated kelps (Førde et al., 2015; Gendron et al., 2007; Marinho et al., 2015b). To ensure high quality of kelp biomass and to avoid biomass loss from the farm, the kelp is often harvested before the onset of colonisation by epibionts, reducing the growth season. Thus epibionts may cause a problem for the fledgling Norwegian kelp industry (Skjermo et al., 2014).

Mortality, possibly due to fouling, of wild stands of sugar kelp has been found mainly in southern and in mid Norway (Andersen et al., 2019). However, due to heavy grazing by sea urchins, sugar kelp beds are hardly found north of the Arctic Circle (Norderhaug and Christie, 2009). In recent years, monitoring programs have observed natural kelps without fouled laminas in Northern Norway (NIVA personal observations). Moy and Christie (2012) indicated that fouling showed a discontinuous pattern which seemed to be enhanced with decreasing wave exposure in natural sugar kelp beds. So far, the Norwegian experience with kelp farming, including damage caused by epibionts (Førde et al., 2015), has only been observed in areas south of the Arctic circle.

The objectives of this field study were to study the effect of site and cultivation depth on the (1) temporal variation of *S. latissima* biomass yield, (2) abundance and taxonomic composition of biofouling organisms, by examining the environmental parameters such as current strength, temperature, salinity and nitrate concentration. Lastly, (3) we wanted to quantify the magnitude of difference in biofouling levels between different areas on the kelp lamina.

2. Methods

2.1. Description of study sites

The experiment was carried out between February and August 2014 at three sites in Troms, Northern Norway (Fig. 1). The three sites were chosen to represent semi-offshore, inshore and fjord habitats. Currents and flow direction for the sites were collected prior to this study, following procedures in Norwegian standard NS 9415:2009 for analyses of aquaculture sites.

The fjord site was located in Ullsfjorden ($69^{\circ}40.452' N / 019^{\circ}46.043' E$), with a water depth of 90–95 m. This site is sheltered. Currents were measured at 6 m depth through 31 days from June to July 2010. The measurements show a moderate to high current velocity, averaging 11 cm s^{-1} , and a maximum of 42 cm s^{-1} . The flow direction was uniform throughout the water column from 5 m to 21 m with the main direction towards the northwest.

The inshore site was located on the island of Kvaløya ($69^{\circ}45.259' N / 019^{\circ}02.176' E$) near Tromsø, with a water depth of 15–20 m. This is a sheltered site, yet it has well-mixed water masses through tidal forcing. Current velocity was measured during 120 days at 12 m depth from March to July 2011, and is moderate, with an average of 3.4 cm s^{-1} , and a maximum of 22 cm s^{-1} . The main current direction was towards the northwest.

The semi-offshore site was located southeast of Helgøya ($70^{\circ}06.311' N / 019^{\circ}34.871' E$), with a water depth of 40–60 m. This site is relatively close to the open ocean (20 km) and is somewhat exposed to ocean swell but sheltered from wind-driven waves (Fig. 1a). Currents were measured in January and February 2012 at 5 m depth through 31 days. Current velocities were moderate to strong, with an average of 17 cm s^{-1} , and a maximum of 55 cm s^{-1} . Flow direction was uniform throughout the water column from 5 m to 21 m with the main direction towards the north and northwest. From examining ocean models, median temperature throughout the year was expected to be slightly higher than at the other two sites (Fig. 1b).

2.2. Seaweed material and experimental set-up

Fronds of adult sporophytes of *S. latissima* were collected in Grøtsund ($69^{\circ}45.259' N / 019^{\circ}02.176' E$) during the summer 2013. Sori were induced in culture as described by Forbord et al. (2012) and spore release was induced as described by Rød (2012). *S. latissima* were cultured using methods modified from those described in Edwards and Watson (2011). In the laboratory, the spores were sprayed onto nylon strings where young *S. latissima* sporophytes emerged within three to four weeks. The strings with densely grown sporophytes were spun around two horizontal polypropylene led ropes in $100 \times 50 \text{ cm}$ PVC-frames (2 m seeded ropes per frame). The sporophytes were approximately 10 mm in length when placed at the cultivation sites on 26–27 February, 2014. Three frames were employed at 3 m and at 8 m depth at each site, giving 18 frames and 36 m of seeded ropes in total (Fig. 2).

Once a month throughout the year 2014, salinity and temperature transects were collected at each of the three cultivation sites with a CTD, model SD 204. Water samples for nitrate concentration measurements were collected at 3 and 8 m depth with a Ruttner water sampler and analysed with an autoanalyzer (Flow Solution IV System, I.O. Analytical) according to the Norwegian Standard 4745.

2.3. Biomass and biofouling analyses

Approximately once a month from deployment until final harvest, the kelp was monitored for biomass growth, weighted with a Rapala scale, and for epibiont cover. After 6 months in the sea, the frames were collected between 18 and 22 August 2014, and a minimum of three randomly chosen fronds per frame were analysed for epibionts, giving a minimum of 9 kelp fronds per depth and site. A modified version of the point-sampling-method described in Christie (1980) was used to estimate frond area covered by epibionts. A $15 \times 15 \text{ cm}$ grid system with 11 grid lines each vertically and horizontally was placed on top of the kelp fronds at each of three parts: distal, middle, and proximal (Fig. 3a), and each grid was photographed with a digital camera without magnification. The points below the intersection of the grid lines were analysed for presence/absence of epibionts. Where epibionts were present, the species were identified using the World Register of Marine Species (WoRMS, 2017). Ten specific points per grid were analysed in this fashion in a standardised pattern (Fig. 3b) starting in the uppermost left corner, moving diagonally down towards the right edge until the end of the grid or frond area, and then diagonally down the left and so on, thereby ensuring analyses of both edges and middle parts of each kelp frond. This was done on one side of the frond. The different epibiont species on the fronds differed in morphology and were easy to distinguish on the photographs.

The percentage biofouling cover was calculated by dividing 100% with number of points analysed, thereafter multiplying by the amount of points that covered any fouling. The percentage biofouling cover was visualised with boxplots separated into depths, species and blade regions as a function of site, with the remainder pooled.

2.4. Statistical analysis

The environmental parameters were presented through graphs made in Microsoft Excel. All other graphs and statistical analyses were conducted using R, version 3.5.1 (R Core Team, 2018) through RStudio version 1.1.456 (RStudio Team, 2016), using the package lme4 (Bates et al., 2015).

The kelp biomass was modelled by a linear mixed effects analysis of the relationship between biomass, depth and environmental parameters. As fixed effects, we used depth, and the environmental variables with significant impact on the biomass. For random effects, we had intercepts for site and date. Residual plots did not reveal any obvious deviations from homoscedasticity or normality.

The biofouling cover was modelled by a binomial generalized linear

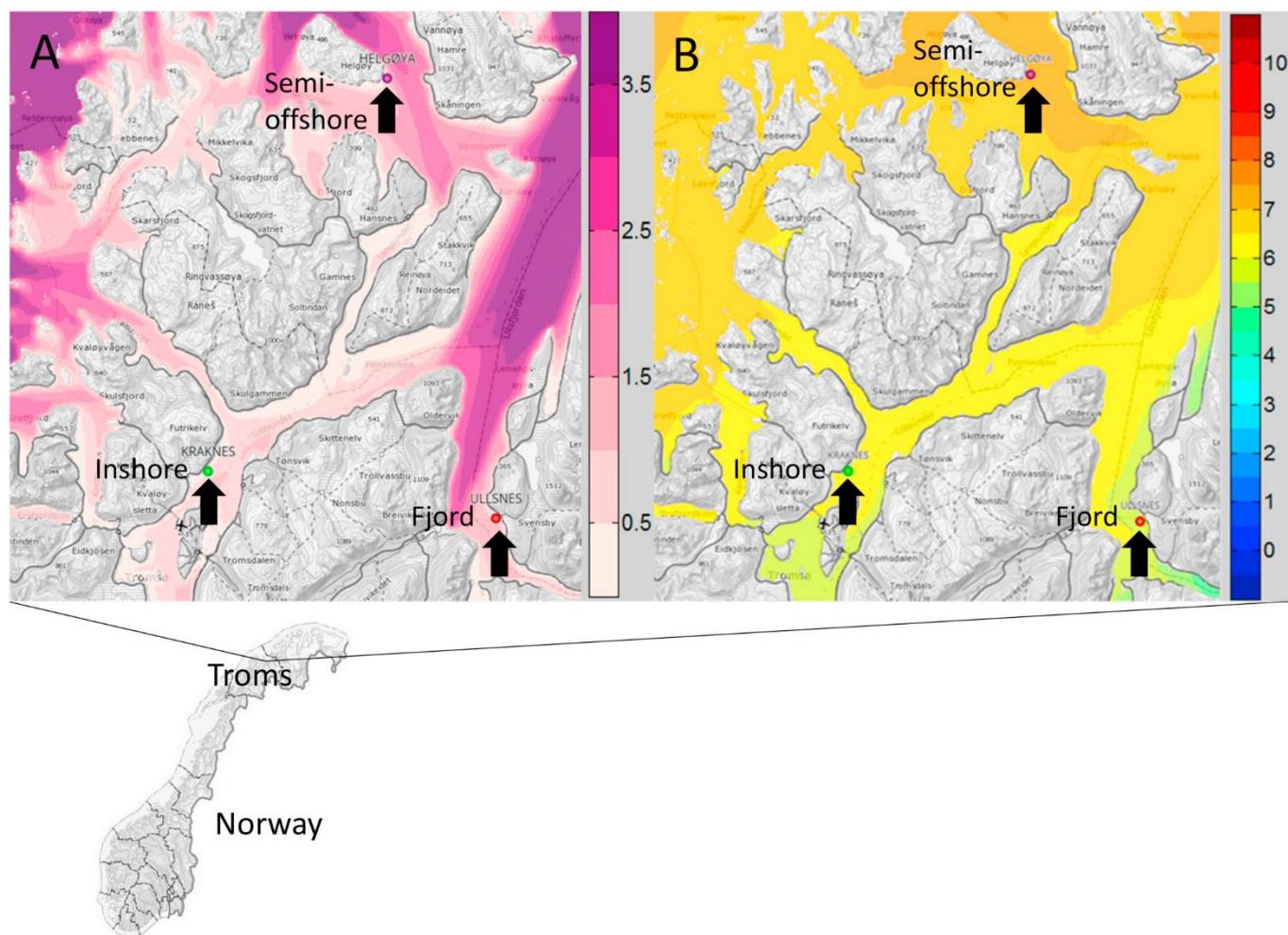


Fig. 1. Map of the three cultivation sites for *Saccharina latissima* in northern Norway with black arrows indicating the site and illustrating, A) modelled extreme wave heights in metres, B) the 50 percentile of modelled water temperature throughout the year in degrees Celsius.

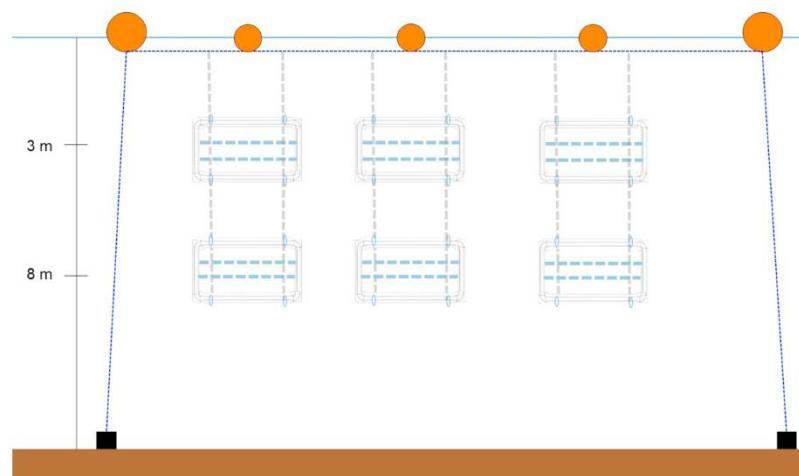


Fig. 2. Experimental set-up with PVC-frames at 3 m and 8 m and three replicates at each depth. Each frame (grey rectangles) consisted of two sets of ropes (stippled lines) with *S. latissima* sporophytes, in total 2 m of rope per frame. Circles denote buoys, black squares are anchors attached to the bottom substrate, and the blue solid lines show the anchoring and horizontal carrying ropes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mixed model (GLMM) (Bates et al., 2015), with logit link function, analysing the relationship between biofouling cover, depth, blade age and environmental parameters. As fixed effects, blade age, and the environmental variables with significant impact on the biofouling cover were selected. Fixed factors without significant impact on the dependent variable were omitted from the model. For random effects, we had intercepts for frames nested in site. Interpretation of residual plots

where conducted with the DHARMA package (Hartig, 2018), as residual interpretation for GLMMs often are problematic. These did not reveal any apparent deviations from homoscedasticity or normality. *P*-values were achieved by likelihood ratio tests of the full model with the effect in question against the models without the effect in question. R2 values for the GLMMs was calculated using the package r2glmm (Jaeger, 2017) with the Nakagawa and Schielzeth approach applied.

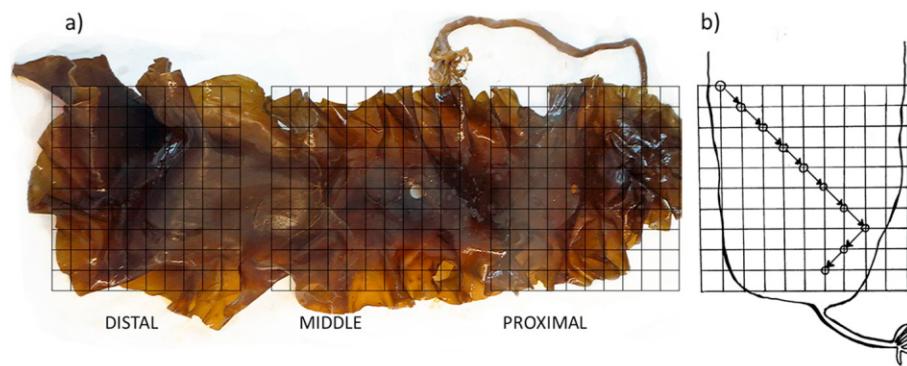


Fig. 3. a) The system of grids used for biofouling analysis of *Saccharina latissima*. The frond was divided into three parts: the distal, middle and proximal region. b) Standardised pattern for point-sampling analyses of biofouling.

3. Results

3.1. Environmental conditions

The temperature slowly increased at all sites and both depths until June, and then increased more rapidly until August (Fig. 4). The semi-offshore site had a slightly higher temperature compared to the inshore site (averaging $+0.4 \pm 0.2^\circ\text{C}$ at 3 m depth and $+0.25 \pm 0.2^\circ\text{C}$ at 8 m) and the fjord site (averaging $+0.8 \pm 0.2^\circ\text{C}$ at 3 depth and $+0.7 \pm 0.2^\circ\text{C}$ at 8 m). The salinity was quite constant at both depths throughout the five first months of the study (Fig. 4), but after July the readings at the semi-offshore site increased, while they dropped at the fjord site. The ambient nitrate levels were overall slightly higher at the semi-offshore site, and lowest at the fjord site (Fig. 5). The nitrate levels dropped rapidly at the end of April, coinciding with the spring bloom. The drop occurred faster, and earlier at the fjord site compared to the two other sites, as was expected due to less mixing of the water masses. The nitrate values were similar between the two depths studied.

3.2. Kelp biomass

The kelp biomass initially increased slowly from February to May (Fig. 6), and thereafter increased rapidly until August. In August, biofouling added to the total biomass, especially on the semi-offshore site with the highest amount of biofouling cover. The highest weight pre-fouling was $15.0 \pm 0.3 \text{ kg per meter of rope}$ at 3 m at the semi-offshore site on 15 July. The inshore site had the lowest biomass growth from June onwards. The inshore site experienced impact from a storm in May that caused some loss of biomass. This is visualised by the flat portions of the curves for the inshore site.

In the Linear Mixed-Effects Model of kelp biomass as a function of the fixed factors depth and environmental factors, currents and nitrate

were omitted due to non-significant contributions shown in the analysis. The AIC comparison of the models including and excluding the fixed factors in question, significantly improved the model (Table 1). Model comparisons showed that depth had the highest impact on kelp biomass (likelihood ratio test: $\chi^2 = 57.924$, df = 1, p value < .001), thereafter temperature (likelihood ratio test: $\chi^2 = 25.813$, df = 1, p value < .001), and salinity (likelihood ratio test: $\chi^2 = 6.611$, df = 1, p value < .05) impacted the seaweed biomass in declining degree.

Model comparisons with currents did not show any impact (likelihood ratio test: $\chi^2 = 0.450$, df = 1, p value = .502), neither did nitrate (likelihood ratio test: $\chi^2 = 0.257$, df = 1, p value = .613).

3.3. Biofouling

No epibionts were observed at any of the sites between February and late June. The first colonies of epibionts were observed in field, but not analysed, at all sites in mid-July. The semi-offshore site had the highest fouling with a coverage of $61.0 \pm 6.5\%$ (mean of 3 frames \pm SE) at 3 m depth and $59.0 \pm 9.5\%$ at 8 m depth (Fig. 7a). The fjord site had $15.7 \pm 2.7\%$ at 3 m depth and $7.0 \pm 4.7\%$ at 8 m. The inshore site had $14.8 \pm 4.2\%$ at 3 m depth and $13.3 \pm 0.87\%$ at 8 m.

Three major biofouling taxa (Fig. 8) were identified on the fronds of *S. latissima*: the bryozoan, *Membranipora membranacea* (Linnaeus, 1767); the hydroid, *Obelia geniculata* (Linnaeus, 1758); and a filamentous brown alga, *Ectocarpus* sp. In addition, the blue mussel, *Mytilus edulis*, and the gastropod *Lacuna vincta* were occasionally observed on the kelp fronds, but as these organisms did not cover any of the points sampled in the image processing they were omitted from the analysis. The composition of species covering the fronds varied between the three sites (Fig. 7b). The frond community at the fjord site was dominated by the hydroid *O. geniculata*, followed by *Ectocarpus* sp. and only $0.67 \pm 0.16\%$ of the fronds were covered by *M. membranacea*. At the

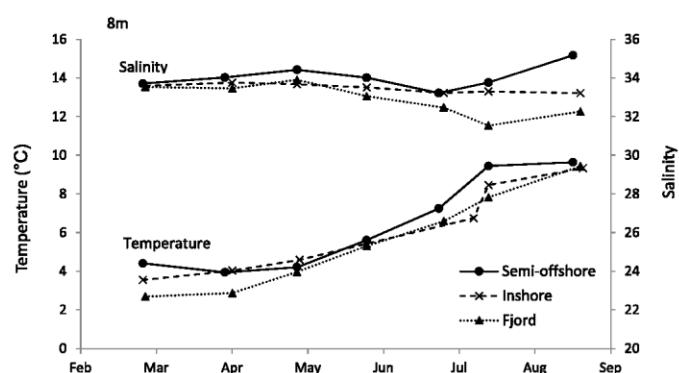
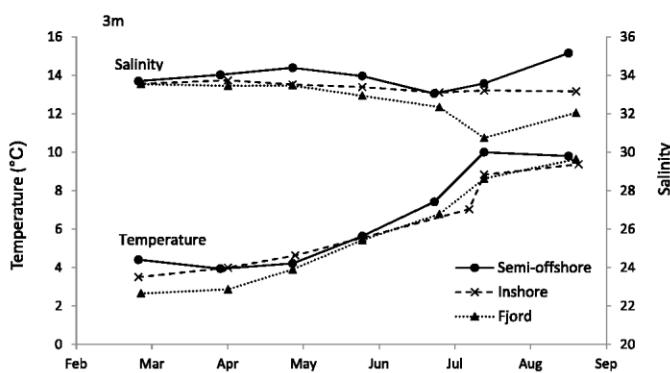


Fig. 4. Temperature and salinity measured at 3 and 8 m depth at the semi-offshore site (marked with ●), the inshore site (marked with X), and the fjord site (marked with ▲).

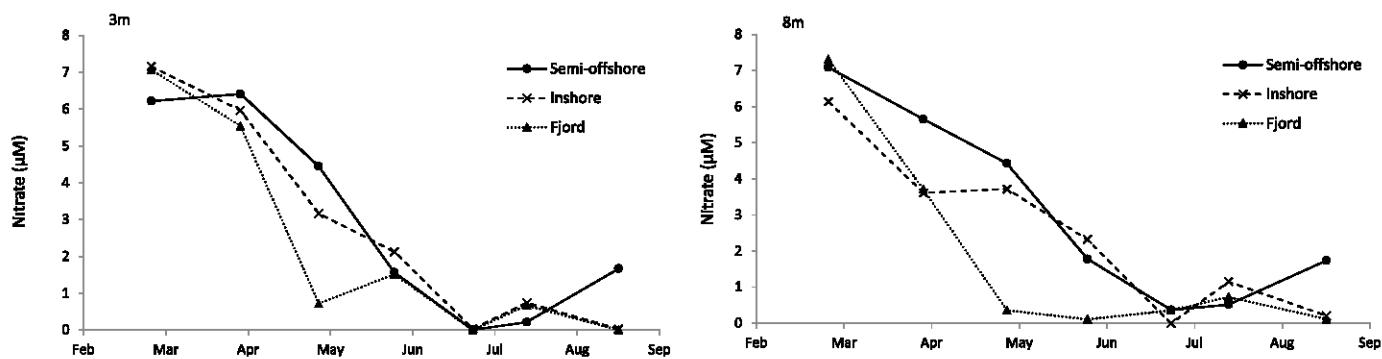


Fig. 5. Nitrate concentration measured at 3 and 8 m depth at the semi-offshore site (marked with ●), the inshore site (marked with X), and the fjord site (marked with ▲).

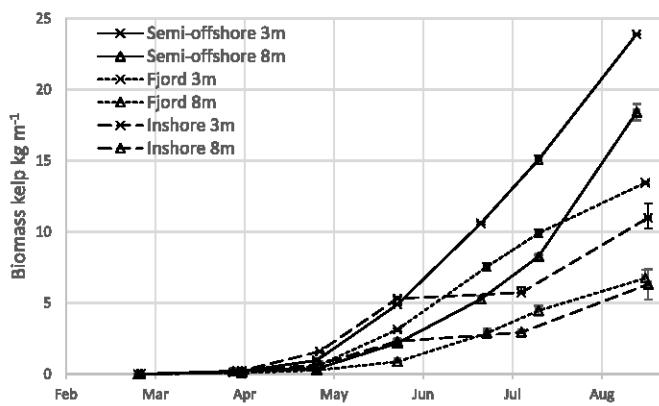


Fig. 6. Kelp biomass in kg per meter of rope on each sampling date. All three sites are shown at 3 m (marked with X) and 8 m depth (marked with Δ). The error bars show the standard error.

inshore site the frond community consisted of *O. geniculata* and some *M. membranacea*. The frond community at the semi-offshore site was dominated by *M. membranacea* and had no registered *O. geniculata* and very little *Ectocarpus* sp.

There was a clear trend for increased blade area fouled towards the older, distal parts of the kelp fronds (Fig. 7c). The largest differences of biofouling cover on proximal and distal regions were seen at the inshore and fjord sites, where the inshore site had $27.0 \pm 3.2\%$ at the distal ends and $6.3 \pm 2.6\%$ at the proximal parts, and the fjord site had $21.1 \pm 4.5\%$ at the distal and $4.0 \pm 2.1\%$ at the proximal parts. Total biofouling was modelled with GLMM as a function of the fixed factors temperature, blade age and temperature. Salinity and depth were omitted due to non-significant contributions. An AIC comparison of the full model, with reduced models excluding factors in question, showed a significant difference between models (Table 2). Model comparisons showed that temperature (likelihood ratio test: $\chi^2 = 13.292$, df = 1, p value < .001), blade age (likelihood ratio test: $\chi^2 = 124.83$, df = 2, p value < .001), and currents (likelihood ratio test: $\chi^2 = 8.89$, df = 1, p value < .01), had a significant impact on total biofouling. Whereas, depth (likelihood ratio test: $\chi^2 = 0.0069$, df = 1, p value = .934), and salinity (likelihood ratio test: $\chi^2 = 0.45$, df = 1, p value = .5039), did

not. R^2 showed that temperature had the highest influence on total biofouling of the fixed factors.

4. Discussion

4.1. Kelp biomass

Saccharina latissima was successfully cultivated at all three sites. Before the onset of heavy fouling we registered at 3 m depth an average biomass yield per meter rope of 5.7 kg at the inshore site, 11.0 kg at the fjord site, and 15.1 kg at the semi-offshore site. In comparison, Druhl et al. (1988) registered 8 kg m^{-1} cultivation rope, Bruhn et al. (2016) 0.51 kg m^{-1} , and Peteiro and Freire (2013b) registered 16 kg m^{-1} .

Our results show that elevated sea temperatures, cultivation at shallower waters (3 versus 8 m), and increased salinity had a positive correlation with higher kelp biomass in Arctic waters, while currents and nitrate did not have any significant impact on the kelp biomass in our field study. Mols-Mortensen et al. (2017) conducted a similar field study at the Faroe Islands. They did not find a relationship between current exposure and *S. latissima* biomass yield either. Gerard (1982) found that current speeds of 2.5 cm s^{-1} were sufficient to saturate the nutrient uptake of the macroalgae *Macrocystis pyrifera*. Thus current speeds are within the measured values at all our sites.

4.2. Effects of environmental variables on biofouling

The biofouling model showed that temperature had the highest influence on total cover of biofouling. There are many other studies (Saunders and Metaxas, 2007, 2009a; Saunders et al., 2010; Scheibling and Gagnon, 2009) showing the correlation between elevated sea temperatures with a higher degree of fouling by *M. membranacea*. A model developed by Saunders et al. (2010) predicted that a temperature difference of 1°C and 2°C will increase the coverage by *M. membranacea* on wild kelp beds by a factor of 9 and 62, respectively. At the semi-offshore site in this study, the average daily temperature was 0.8°C higher than at the fjord site, but the coverage by *M. membranacea* at the semi-offshore site was 64 times as high as at the fjord site. Hence the temperature could have had some impact, but is most likely not the only causative factor involved in increased epibionts at the semi-offshore site. At the same time, this study only measured ambient

Table 1

AIC model comparison and associated R^2 values for the effect on kelp biomass with and without depth, salinity and temperature as fixed factors.

Rank	Formula	K (parameters)	AIC	ΔAIC	R2
1	log(Biomass+1)~Depth + Salinity + Temp + (1 Date) + (1 Site)	7	44.39	0.0	0.853
2	log(Biomass+1)~Depth + Temp + (1 Date) + (1 Site)	6	48.69	4.3	0.874
3	log(Biomass+1)~Depth + Salinity + (1 Date) + (1 Site)	6	67.89	23.5	0.058
4	log(Biomass+1)~Salinity + Temp + (1 Date) + (1 Site)	6	100.00	55.6	0.859

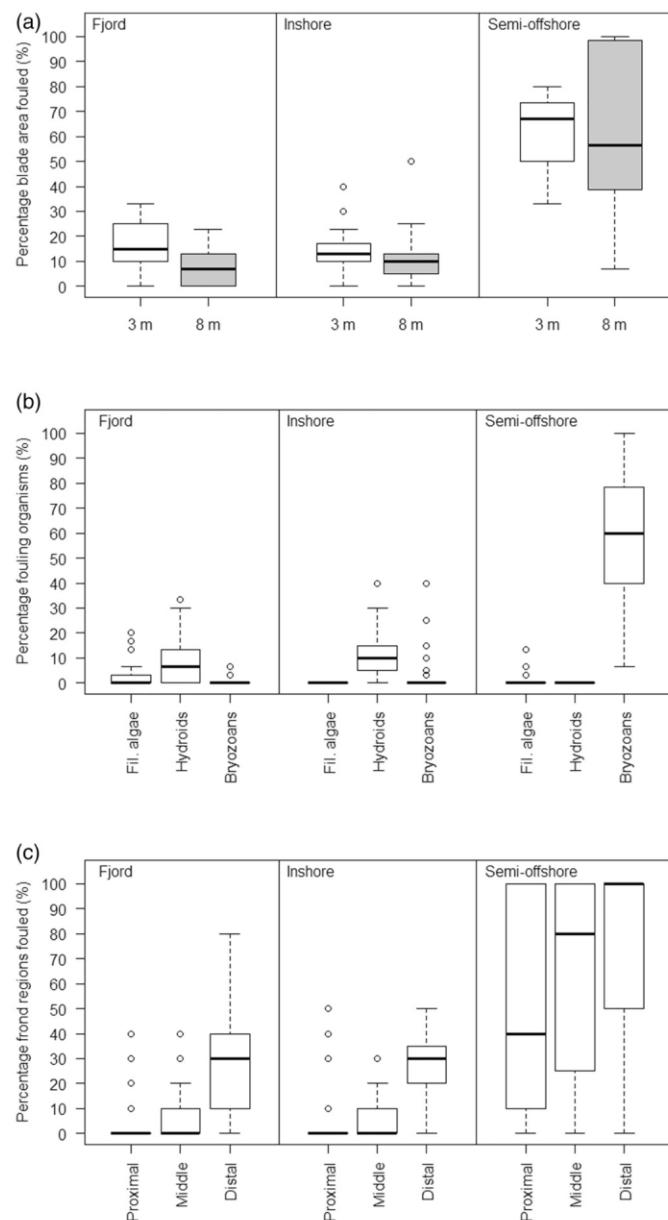


Fig. 7. a–c) Epibionts on *S. latissima* at three sites (fjord, inshore and semi-offshore) studied in late August 2014; a) Percentage of whole kelp fronds covered by epibionts at two depths, 3 and 8 m. b) Percentage of whole kelp fronds covered by epibionts, separated by taxon. c) Percentage of epibionts on the kelp fronds divided into three parts: proximal (younger tissue)-, middle- and distal (older tissue) regions. The box plots show the median percentages (the horizontal lines within the boxes), the 25th and the 75th percentiles (top and bottom of the boxes) and the interquartile range of 3/2 (whiskers) separating the main body of the data from the outlying values (points). In b–c) the two depths are pooled.

temperatures between February and August, whereas the life cycles of the epibionts are affected by the physical environment throughout the year.

Salinity did not have any significant impact on the cover by biofouling. The impact of varying salinity on *M. membranacea* is not well studied, but it is a eurohaline species that can be found at salinity levels as low as 20 psu in fjords (personal observation Christie).

The semi-offshore site, which was most exposed to currents, had significantly higher biofouling cover in comparison with the two more sheltered sites. The same site also had the highest kelp biomass. This difference was significant despite all sites being located within a

distance of 50 km from each other.

The biofouling model showed that increased currents had a significantly positive relationship with higher biofouling cover. In contrast, Peteiro and Freire (2013a) found more biofouling on cultivated *S. latissima* and *Undaria pinnatifida* on a less current-exposed site compared to a higher current-exposed site in Spain. Also, Mols-Mortensen et al. (2017) observed more heavily fouled individuals on *S. latissima* cultivated at wave exposed and sheltered sites than at a site exposed to high current velocities at the Faroe Islands in 2015. Among wild sugar kelp in Norway, Moy and Christie (2012) found more fouling at the sheltered sites. In natural kelp beds, the interaction between kelps, waves and bottom-substrate may act differently than in kelps cultivated in the water column without the possibilities to interact with a bottom-substrate. A field study on the bryozoan *Membranipora verrucosa* (Arkema, 2009), a closely related species of *M. membranacea* (Schwaninger, 2008), showed that sites with intermediate ambient flow speed ($10\text{--}12 \text{ cm s}^{-1}$) gave the highest feeding success, and that both flow speeds $< 5 \text{ cm s}^{-1}$ and $> 20 \text{ cm s}^{-1}$ had the lowest feeding success. The feeding success was positively correlated with percent cover. In this perspective, the conditions in the fjord site would offer the best feeding base to *M. membranacea*, but only a few colonies of this species were registered at this site.

In this study we did not find a correlation between biofouling cover and depth, but we saw a non-significant trend of less biofouling at 8 m compared to 3 m. Earlier studies on depth dependencies indicated decreased fouling on cultivated kelp with increasing water depth from 1 to 15 m (Førde et al., 2015) while on wild kelp fouling increased with depth (Saunders and Metaxas, 2007).

4.3. Species composition of frond community

S. latissima was colonized by the bryozoan *M. membranacea*, the hydroid *O. geniculata* and filamentous brown algae as the major fouling organisms, with prevalence and abundances varying between the three study sites. The method used in this study registered only sessile organisms. The sessile species fouling the kelp fronds may have different impacts on the kelp (Hepburn et al., 2006). *M. membranacea*, which has a sheet-like growth, forms a barrier for nutrients and light between the kelp and the surrounding water (Andersen, 2013). The hard calcium carbonate skeleton reduces flexibility and increases brittleness on the kelp fronds, potentially causing substantial loss of algal biomass up to 100% (Krumhansl et al., 2011; Skjermo et al., 2014). Epibionts with an erect growth form, such as *O. geniculata*, are less likely to have such severe effects (Hepburn et al., 2006). *O. geniculata* is also protected by a calcium carbonate shell, but the stoloniferous growth form usually provides no barrier for nutrient uptake on the host (Hepburn et al., 2006). The filamentous brown algae were mostly found on the edge of the kelp fronds, associated with low coverage. Therefore, it is expected that filamentous brown algae here only had a slight impact on the availability of light and nutrients for the kelp. A high occurrence of bryozoans at the semi-offshore site may have had a high impact on the kelp biomass, whereas the relatively high occurrence of hydroids in the fjord site may not have the same detrimental effect, possibly allowing for an extended cultivation season in aquaculture systems. The fragile state of the bryozoan covered kelp individuals at the semi-offshore site, as well as the storm event at the inshore site, resulted in some loss of the distal ends from several individuals. This may have affected the amount of filamentous algae at these sites.

The amount of species found on the kelp blades in this study were lower than in some other studies. Walls et al. (2016) found 32 species (both sessile and mobile species) inhabiting cultivated *Alaria esculenta* in Ireland. Sogn Andersen et al. (2011) observed blue mussels, sponges, filamentous algae, bryozoans (both *M. membranacea* and *Electra pilosa*) and a high number of the vase tunicate *Ciona intestinalis* on wild *S. latissima* in Skagerak, Norway. Rolin et al. (2017) found three main epibionts on cultivated *S. latissima* in the Shetland Islands, and in their



Fig. 8. The three main taxa of epibionts found at the three locations studied in northern Norway. Yellow arrows (left picture) shows examples of the bryozoan *Membranipora membranacea*, green arrow (middle) show filamentous algae, *Ectocarpus* sp., and red arrow (right picture) point at colonies of the hydroid, *Obelia geniculata*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

study the kelp was highly fouled by bryozoans, but they also had a high occurrence of tunicates.

4.4. Total biofouling on frond area

The highest abundances of biofouling organisms was found on the distal ends of the blades. As the proximal regions are the growth zone of kelps, and the distal ends have the oldest tissue, the variation in biofouling cover are most likely an effect of accumulation and growth of epibionts over time (Jennings and Steinberg, 1997). The place of fouling may be relevant for regrowth potential. If the main occurrence of fouling occurs at the distal tips these can be removed, either naturally or through cutting, enabling new, clean kelp tissue to grow (Rolin et al., 2017). In this study, two of the locations, the fjord and the inshore site, had very little occurrence of biofouling on the meristematic region. Further studies may reveal whether cutting of the older blade parts would result in regrowth of clean biomass.

4.5. Latitudinal differences

In *S. latissima* cultivated at the southwest coast of Norway in Lysefjord ($59^{\circ} 0' N$, $6^{\circ} 16' E$) in 2012 (Lüning and Mortensen, 2015) epibionts were first recorded in early May. By 31 May approximately 50% of the blade area was fouled, and by 22 August the majority of the blades had disappeared. On *S. latissima* cultivated in central Norway at Frøya ($63^{\circ} 42' N$, $8^{\circ} 51' E$) and Reksta ($61^{\circ} 34' N$, $4^{\circ} 48' E$) in 2013 the first epibionts were observed in mid-June, followed by a rapid increase of biofouling (Førde et al., 2015). At the end of July the biofouling covered around 75%, and in the end of August many of the blades were degraded and lost. Based on this observation, the authors suggest that cultivation of *S. latissima* is impracticable during July and August in that part of Norway. In Galicia ($43^{\circ} 22' N$, $8^{\circ} 15' W$ and $43^{\circ} 25' N$, $8^{\circ} 16' W$), Spain, Peteiro and Freire (2013a) estimated the kelp blades of *U. pinnatifida* and *S. latissima* to be 33–53% covered by fouling, depending on site and kelp species, as early as on the 26 April. At present, common

practice is to harvest farmed sugar kelp in central Norway before the end of May for the food market (Seaweed farming industry, personal communication), to avoid any biofouling. The consequence of this is that farmers cannot utilize the favourable light conditions with related biomass gain that would be possible during June, July and August.

However, we hardly observed any fouling before August at either of the sites, suggesting that harvesting of kelp biomass with little or no epibionts can be successful during the late summer growth period in the High North, particularly at the fjord and inshore sites in this study. North of the Arctic Circle, farmers can therefore take advantage of the summer season with 24 h daylight at these high latitudes. Yet the current study also shows a large variation in fouling on kelp at sites which are located < 50 km apart from each other. Both when it comes to biomass yield and biofouling, our results emphasize the importance of thorough site selection and pre-testing using small scale farms, before establishing large scale kelp farming operations.

5. Conclusions

All sites in this study had promising biomass yields, showing that cultivation of *S. latissima* is favourable in Northern Norway. Biomass was positively correlated with elevated sea temperatures, higher salinity and rather shallow depths. The variation of spatial patterns in abundance and in taxonomic groups of epibionts, fouling cultivated *S. latissima*, was surprisingly large between sites within the same geographical region. Such variation in fouling can affect the period for harvesting of cultivated kelp by several month, depending on end-use. The highest coverage of fouling organisms was found on the oldest parts of the kelp blades, and there was no significant difference in biofouling with regard to coverage between the two cultivation depths. It is of large interest to further investigate factors influencing the life cycle of epibionts, in particular the highly destructive *M. membranacea*. With regard to differences in productivity caused by extended growth periods and destructive effects from fouling, our study gives evidence for the importance of thorough site selection for kelp farms, even for sites in

Table 2

AIC model comparison and associated R² values for total biofouling with and without the fixed factors; temperature, currents and blade age.

Rank	Formula	K (parameters)	AIC	ΔAIC	R2
1	Total biofouling~Temperature + Currents + Bladeage + (1 Site/Frame)	7	1692.47	0.00	0.154
2	Total biofouling~Temperature + Bladeage + (1 Site/Frame)	6	1699.35	6.88	0.128
3	Total biofouling~Currents + Bladeage + (1 Site/Frame)	6	1703.75	11.28	0.101
4	Total biofouling~Temperature + Currents + (1 Site/Frame)	5	1813.27	120.80	0.129

the same geographical region.

Ongoing studies indicate that our results also can be applied to other regions, showing that fouling, both in abundance and species composition, has large spatial variation and fundamentally effects the productivity of kelp farms.

Acknowledgements

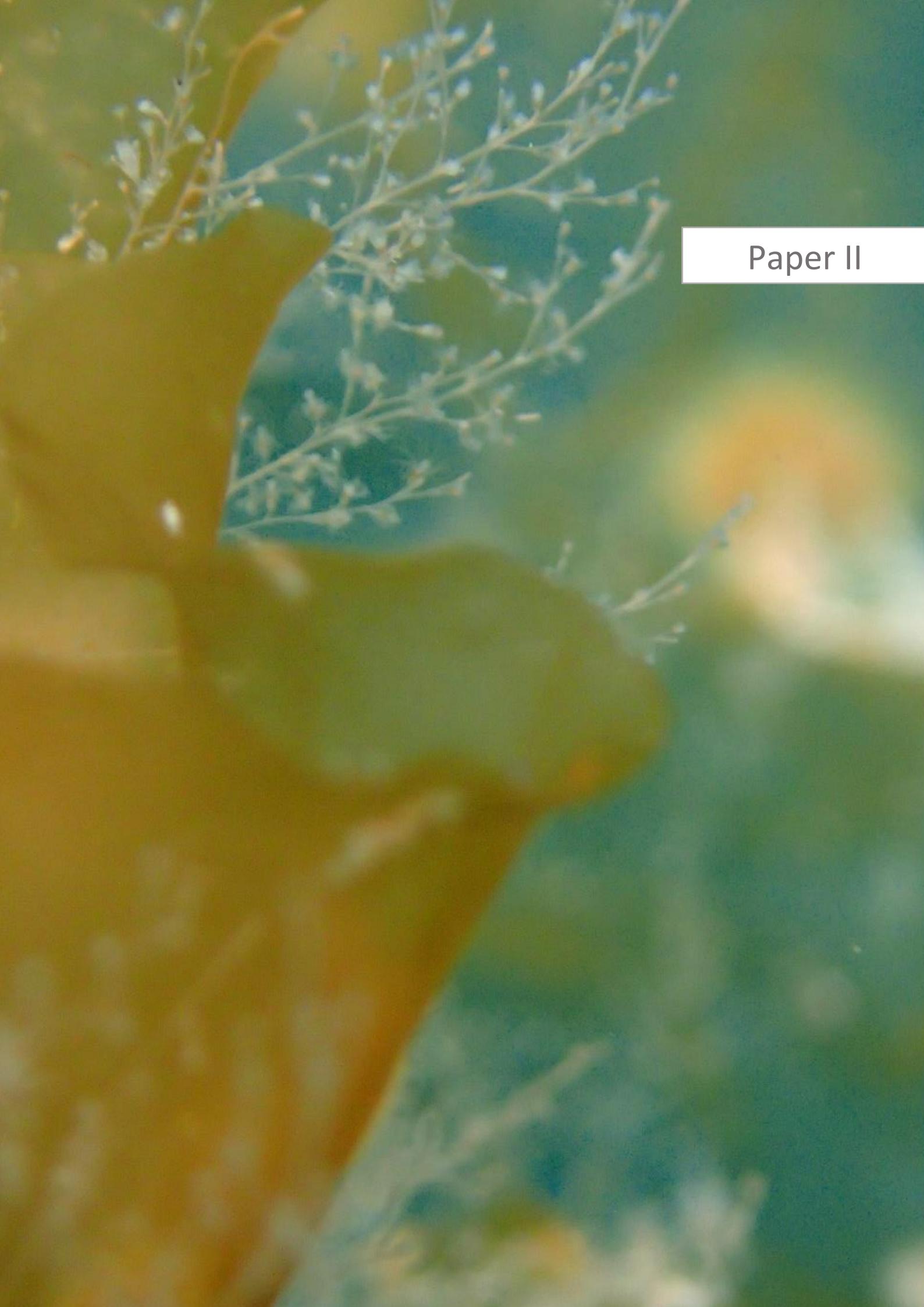
The authors would like to thank Professor Bodil Bluhm and Professor Malcolm Jobling for constructive comments to the manuscript, Professor Nigel Gilles Yoccoz and Dr. Martin Biuw for valuable input on the statistical work. Lerøy Aurora is thanked for deploying the rigs. We would also like to thank the anonymous reviewer who contributed with valuable and constructive input, improving this manuscript substantially. This research was supported by projects from Troms County (RDA12/234 "Pilotstudie på bioenergy fra tare") and from the Research Council of Norway (MACROSEA, No. 254883/E40).

Conflicts of interest

None.

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Paper II



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

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

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

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Abstract

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

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

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

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

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

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

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

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

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

Materials and methods

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

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

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

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

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

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

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

Growing degree-day (GDD, $^{\circ}\text{C day}^{-1}$) 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 ($N58^{\circ}13.3' E08^{\circ}28.2'$) 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^{-2}) 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 (Hetrosicc 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 $\sim 2\text{--}3$ mg freeze-dried samples on an elemental analyser (Elementar vario EL cube, with acetanilide as standard). For analysis of amino acids, freeze-dried samples (50–100 mg) were hydrolysed in 6 M HCl containing 4% mercaptoethanol for 24 h at 110°C and neutralized to pH 1.5–3.0 by 5 M NaOH. The samples were filtered through a GF/C Whatman filter and diluted either 1:1 or 2:1 with a citrate buffer (Sodium Diluent Na220, pH 2.2). The analysis was performed by High-Performance Liquid Chromatography, HPLC (Agilent Infinity 1260, Agilent Technologies) coupled to an online post-column

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



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

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

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

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

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

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

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

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

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

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

Results

Environmental conditions

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

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

Growth measurements

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

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

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

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

Chemical composition

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

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

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

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

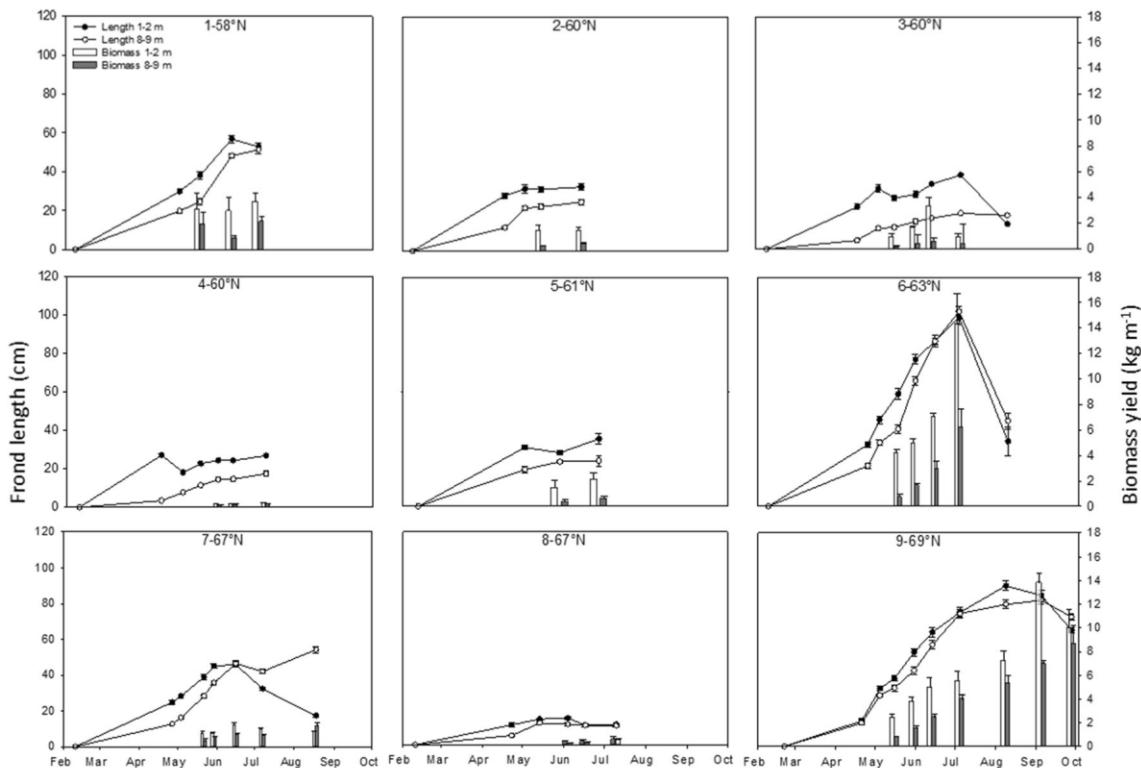


Fig. 2 Length (solid line, left y-axis) and biomass (bars, right y-axis) for both cultivation depths for all nine locations during the experimental period (February–September). Mean \pm SE, $n = 50$ for length and $n = 4$ for biomass

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 NO₃⁻ 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	Δ AIC	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 $101 \pm 4.0 \text{ mg g}^{-1} \text{ DW}$ at 1–2 m depth and from 22.0 ± 0.1 to $110 \pm 0.6 \text{ mg g}^{-1} \text{ DW}$ 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

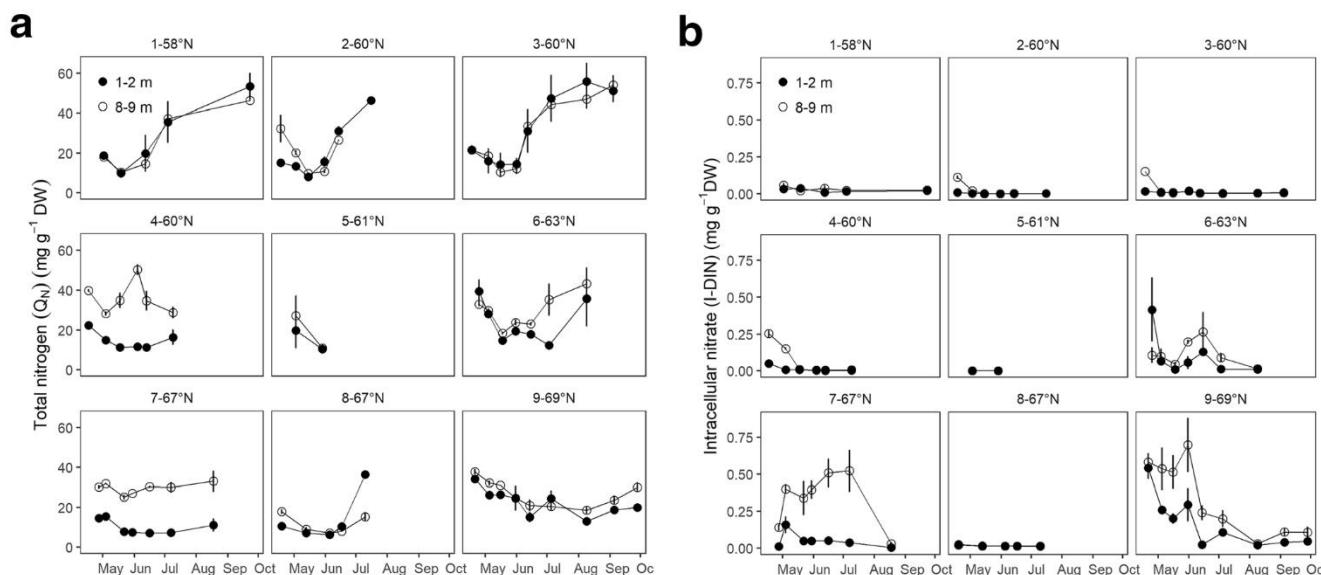


Fig. 4 Latitudinal and seasonal pattern in **a** total nitrogen content (Q_N) ($\text{mg N g}^{-1} \text{ DW}$) and **b** intracellular nitrate content ($I\text{-DIN}$) ($\text{mg NO}_3^- \text{ g}^{-1} \text{ DW}$) of *S. latissima* from all nine experimental sites at 1–2 m and 8–9 m depth across the sampling period. Mean \pm SE, $n = 3$

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

K_p	1–58°N	2–60°N	3–60°N	4–60°N*	5–61°N	6–63°N	7–67°N*	8–67°N	9–69°N
1–2 m	3.9 ± 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 ($81.4 \pm 5.9\%$) at 1–2 m depth at 3–60°N and lowest ($6.5 \pm 1.3\%$) at 4–60°N at the same depth in early July (Fig. 7). The biofouling community varied between these two locations, with *M. membranacea* dominating at 3–60°N and Bivalvia at 1–2 m depth at 4–60°N (Fig. 8). On the following sampling event in early July, most seaweed biomass was lost at both locations.

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

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

Discussion

Growth performance The frond length and biomass yield peaked 5 and 8 weeks later in the northern (9–69°N) than in the central (6–63°N) and southern (1–58°N) locations, respectively, likely because of seasonal differences in temperature, daylight and an earlier depletion of ambient inorganic nutrients by phytoplankton blooms in the low than high latitudes (Rey et al. 2007; Ibrahim et al. 2014). Maximum frond length and biomass yield were greatest at central (6–63°N, in summer) and northern (9–69°N, in autumn) locations, with levels comparable to *S. latissima* previously cultivated in Norway (Handå et al. 2013; Fossberg et al. 2018; Forbord et al. 2019; Matsson et al. 2019) and as high as or higher than several cultivation trials across Europe under variable conditions (Peteiro et al. 2014; Mols-Mortensen et al. 2017; Bak et al. 2018). The maximum yield of 14 kg m^{-2} found in our study is far lower than registered for other cultivated kelp species like *Macrocystis pyrifera* in Chile (up to 22 kg m^{-2}) (Macchiavello et al. 2010) and hybrids of *Undaria pinnatifida* and *Undariopsis peterseniana* in Korea (37.5 kg m^{-2}) (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

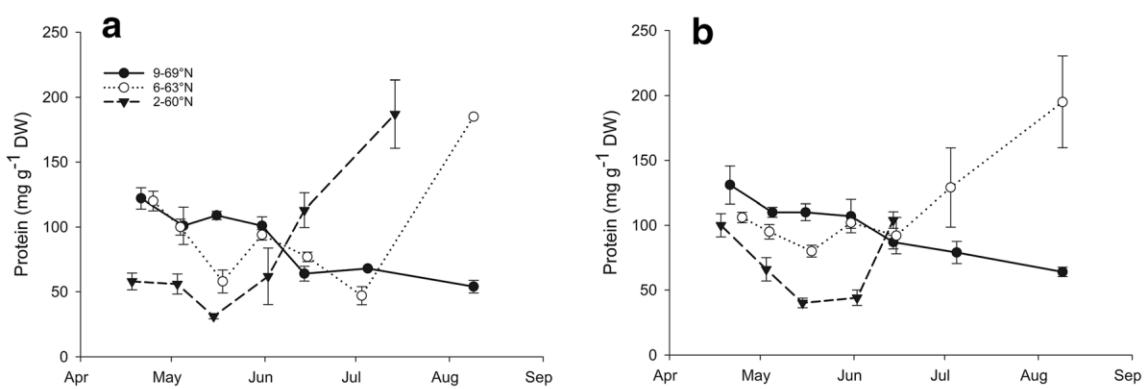


Fig. 5 Development in protein content ($\text{mg protein g}^{-1} \text{ DW}$) of cultivated *S. latissima* over the entire sampling period at locations 2–60°N, 6–63°N, and 9–69°N at **a** 1–2 m depth and **b** 8–9 m depth. Mean \pm SE, $n = 3$

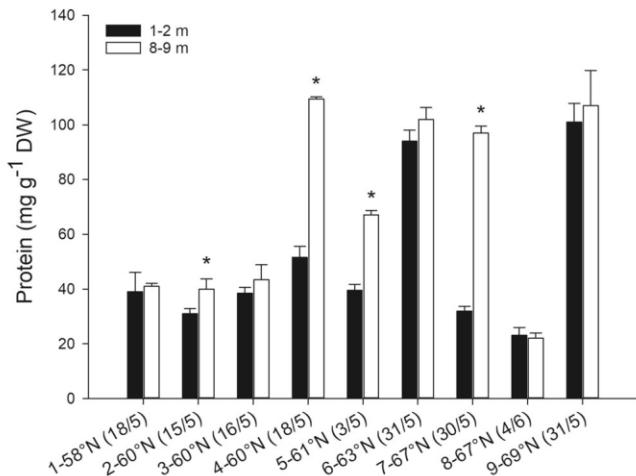
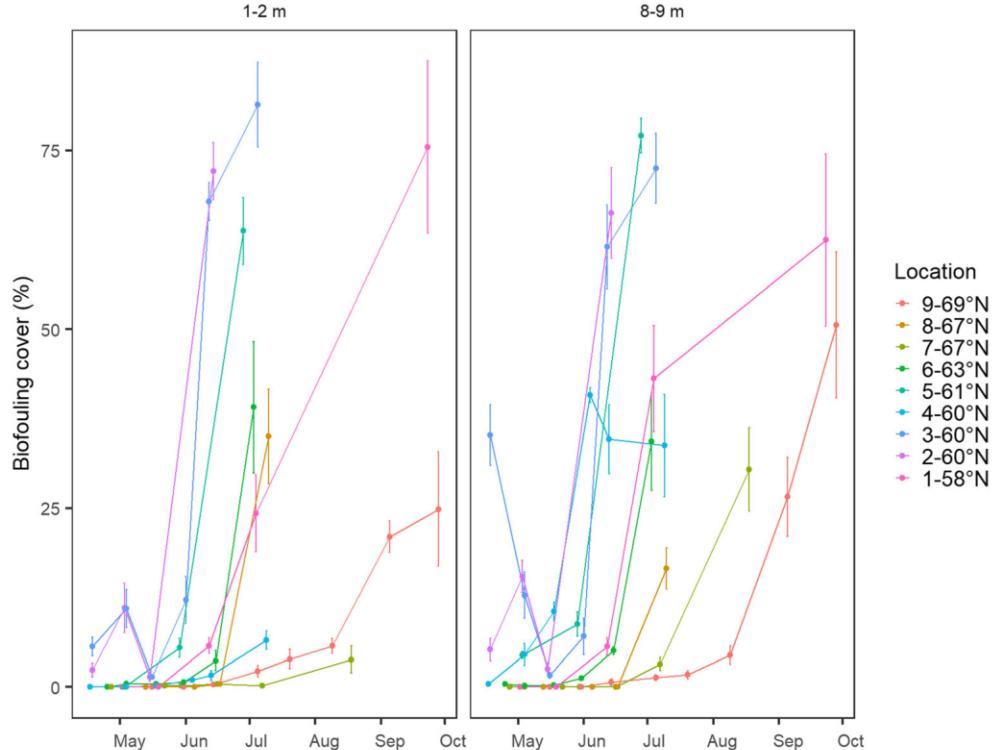


Fig. 6 Protein content ($\text{mg protein g}^{-1} \text{DW}$) for all experimental sites and both depths measured before clearly visible biofouling occurred (sampling date indicated in parentheses after each location name). Asterisk on top of the bars indicates significant differences ($p < 0.001$) between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

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

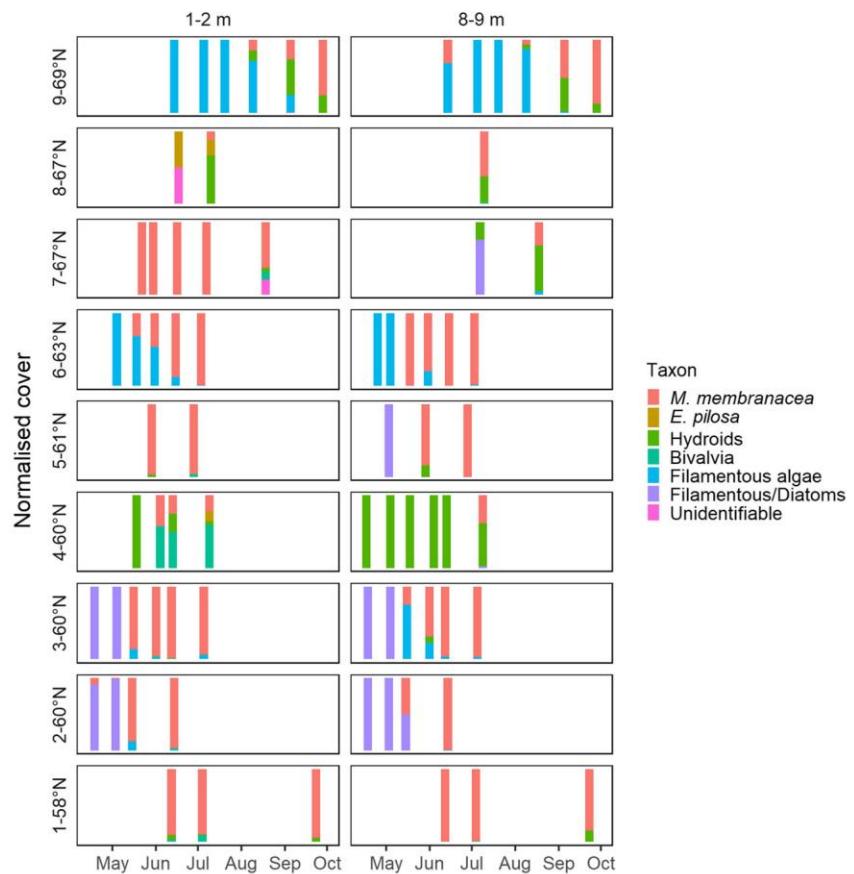
Fig. 7 Cover of biofouling (% of fouled frond area) as a function of time for all study locations at 1–2 m and 8–9 m depth. Mean \pm SE, $n = 4–5$



suppress kelp growth (Spurkland and Iken 2011; Kerrison et al. 2015). Frond length and biomass measured at the southern locations (1–5°N to 5–6°N) did not reach those at central and northern locations (6–6°N, 9–6°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. 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 $\text{m}^{-2} \text{s}^{-1}$ can lead to significant photoinhibition and photodamage in *S. latissima*, in turn causing

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

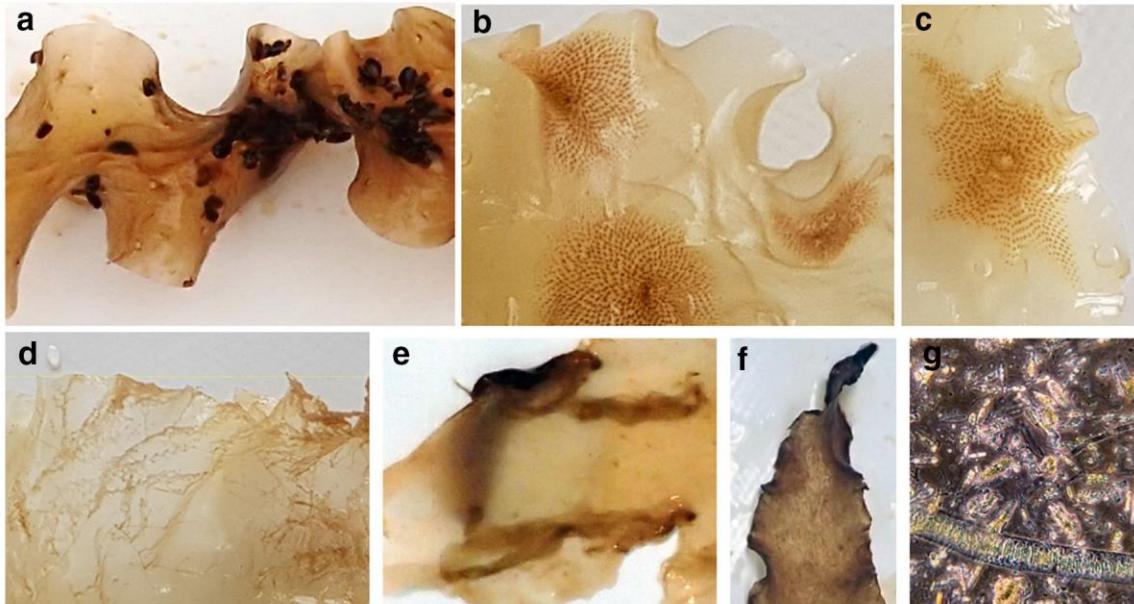


Fig. 9 Images of the epibionts found and registered in this study. **a** Bivalvia. **b** *Membranipora membranacea*. **c** *Electra pilosa*. **d** Hydroids. **e** Filamentous algae. **f** Diatoms. **g** Diatoms at $\times 40$ magnification

Table 5 Linear mixed effects models for the log-transformed dependent variable biofouling cover, with and without temperature (GDD) and light as fixed factors. Location and sample date are random intercepts 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 NO₃⁻ g⁻¹ DW) is suggested to express the nutritional state of the alga, but unlike Q_N and protein content, it is not affected by onset of biofouling during summer. I-DIN is easily measurable and studies have revealed that there is a close and significant relationship between I-DIN and both growth rate and ambient nitrate concentrations (Wheeler and Weidner 1983; Young et al. 2007;

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

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

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

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

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

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

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

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

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

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

Conclusions

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

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

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

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).

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Online Resources 1 -Table 1: Results of three-way ANOVA analyzing the effect of depth, location and month on temperature ($^{\circ}\text{C}$), GDD ($^{\circ}\text{C day}^{-1}$) 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

Location	Effect	Frond length (cm)			Biomass yield (kg m^{-1})		
		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

Location	Effect	DW (% of WW)			Ash (mg g ⁻¹ DW)		
		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

Location	Effect	I-DIN (mg NO ₃ ⁻ g ⁻¹ DW)			Q _N (mg N g ⁻¹ DW)		
		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	0.022	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

Location	Effect	Biofouling coverage (%)		
		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

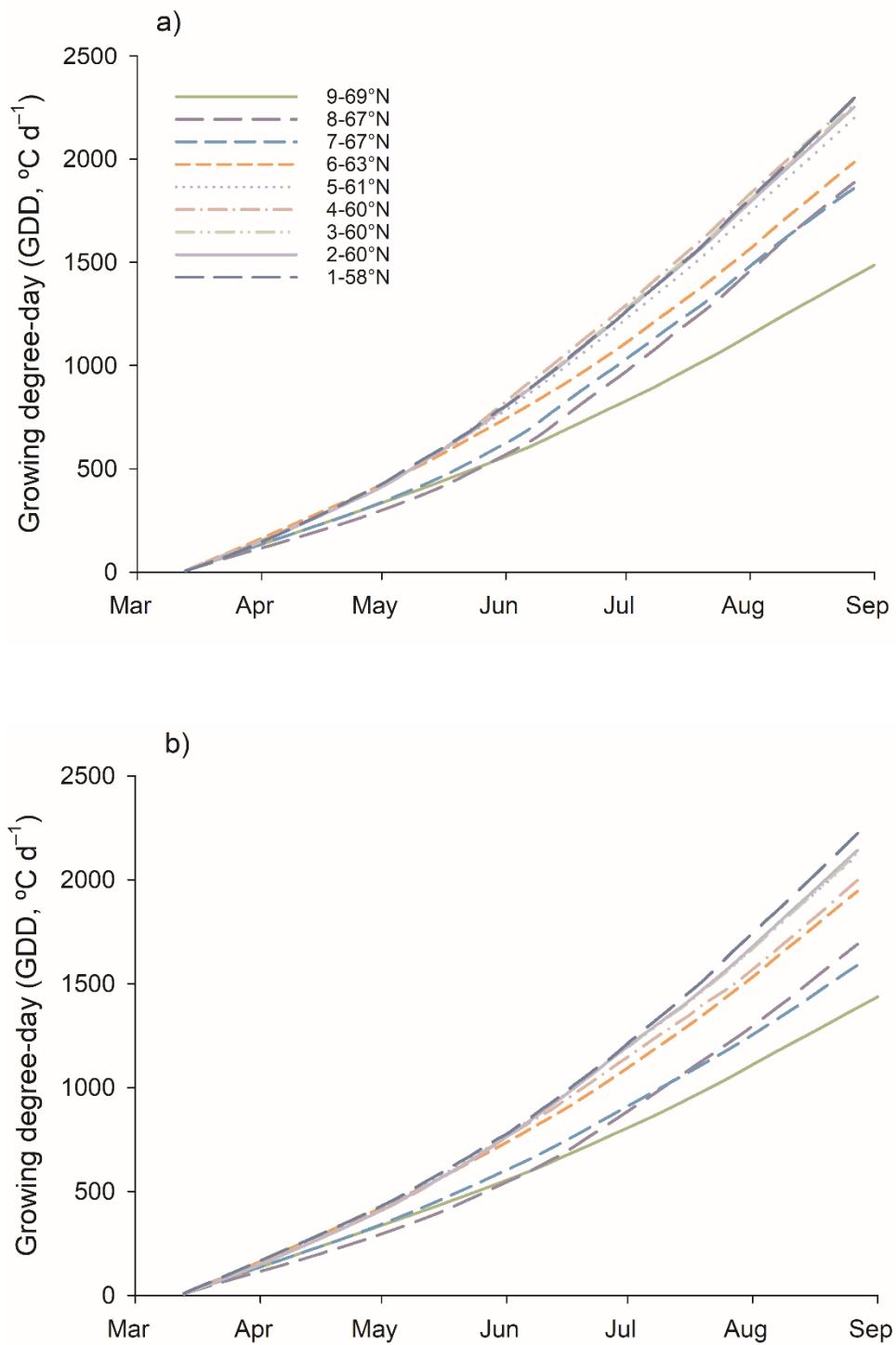


Figure 1 (Online Resources 1) Growing degree-day (GDD,) for all locations for a) 2 m depth and b) 8 m depth.

Online Resources 2- Figure 2

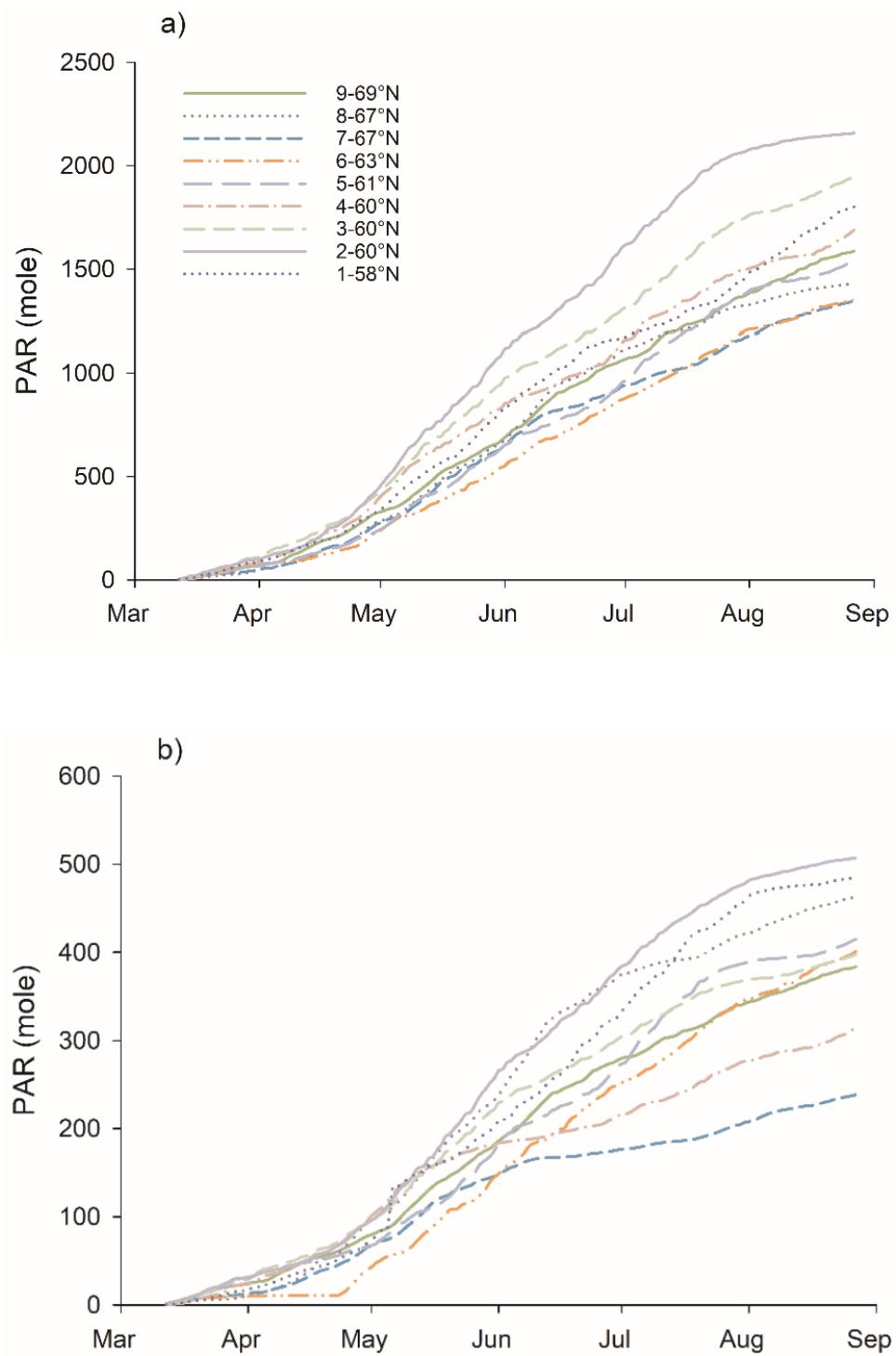


Figure 2 (Online Resources 1) Accumulated PAR (mole) for all locations for a) 2 m depth and b) 8 m depth. Notice the different scale on the y-axis.

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

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

Location		DM (% of WW)		Ash (mg g ⁻¹ DW)	
		1-2 m	8-9 m	1-2 m	8-9 m
8-67°N**	<i>23-Apr</i>	9.9 ± 0.3	10.8 ± 2.5	390.3 ± 11.7	364.3 ± 59.0
	<i>14-May</i>	10.4 ± 1.1	9.4 ± 0.1	366.4 ± 34.5	436.6 ± 18.0
	<i>04-Jun</i>	9.6 ± 1.2	10.8 ± 1.8	335.0 ± 41.4	313.2 ± 28.3
	<i>17-Jun</i>	9.6 ± 1.0	15.9 ± 5.2	332.7 ± 7.5	267.4 ± 8.9
	<i>10-Jul</i>	12.0 ± 0.3	23.7 ± 4.7	300.6 ± 7.1	286.2 ± 1.7
9-69°N	<i>**21-Apr</i>	11.8 ± 1.0	8.8 ± 0.4	350.8 ± 35.3	434.5 ± 36.1
	<i>**5-May</i>	14.5 ± 2.0	8.1 ± 1.6	263.4 ± 69.3	473.3 ± 19.6
	<i>16-May</i>	12.5 ± 0.6	10.0 ± 0.7	427.6 ± 40.7	474.3 ± 27.1
	<i>31-May</i>	12.4 ± 2.6	11.6 ± 1.3	420.4 ± 58.6	442.5 ± 39.2
	<i>14-Jun</i>	22.6 ± 2.3	13.3 ± 0.7	228.3 ± 1.5	398.0 ± 13.2
	<i>05-Jul</i>	13.6 ± 0.8	12.6 ± 0.9	401.0 ± 17.6	474.3 ± 6.9
	<i>09-Aug</i>	14.1 ± 1.8	15.5 ± 3.7	407.0 ± 45.8	375.9 ± 31.2
	<i>05-Sep</i>	22.6 ± 3.5	10.8 ± 1.5	324.1 ± 42.1	454.7 ± 24.1
	<i>28-Sep</i>	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 \pm SE, n=5, *n=4

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

	29-May	<i>M. membranacea</i>	5.3 ± 1.3	7.4 ± 1.0		
		Hydrozoa	0.2 ± 0.2	1.4 ± 1.0		
	28-Jun	<i>M. membranacea</i>	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	<i>M. membranacea</i>	0.1 ± 0.1	0.2 ± 0.2		
		Filamentous algae	0.3 ± 0.3			
	31-May	<i>M. membranacea</i>	0.3 ± 0.3	1.0 ± 0.3		
		Filamentous algae	0.3 ± 0.3	0.2 ± 0.1		
	15-Jun	<i>M. membranacea</i>	3.0 ± 1.2	5.0 ± 0.6		
		Filamentous algae	0.4 ± 0.4			
	3-Jul	<i>M. membranacea</i>	38.9 ± 9.2	33.7 ± 7		
		Filamentous algae	0.2 ± 0.2	0.5 ± 0.4		
7-67°N	22-May	<i>M. membranacea</i>	0.2 ± 0.2			
	30-May	<i>M. membranacea</i>	0.2 ± 0.2			
	16-Jun	<i>M. membranacea</i>	0.4 ± 0.2			
	7-Jul	<i>M. membranacea</i>	0.3 ± 0.3	*		
		Hydrozoa		0.7 ± 0.4		
		Filamentous algae/Diatoms		2.4 ± 0.9		
	18-Aug	<i>M. membranacea</i>	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	<i>E. pilosa</i>	0.2 ± 0.2			
		Unidentifiable	0.2 ± 0.2			
	10-Jul	<i>M. membranacea</i>	3.9 ± 2.4	10.3 ± 3.3		
		<i>E. pilosa</i>	4.0 ± 2.3			
		Hydrozoa	22.4 ± 5.9	5.8 ± 2.1		
		Bivalvia		0.4 ± 0.2		
9-69°N	14-Jun	<i>M. membranacea</i>		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	<i>M. membranacea</i>	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	<i>M. membranacea</i>	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	<i>M. membranacea</i>	18.8 ± 8.1	44.0 ± 9.9		
		Hydrozoa	6.1 ± 1.8	6.6 ± 1.9		

Paper III



Effects of outplanting time on growth, shedding and quality of *Saccharina latissima* (Phaeophyceae)

Sanna Matsson^{1,2*}, Anna Metaxas³, Silje Forbord^{4,5}, Svein Kristiansen², Aleksander Handå⁴, Bodil A. Bluhm²

¹Akvaplan-niva, N-9296 Tromsø, Norway

²UiT - The Arctic University of Norway, Institute of Arctic and Marine Biology, N-9037 Tromsø, Norway

³Dalhousie University, Department of Oceanography, B3H 4R2 Halifax, Nova Scotia, Canada

⁴SINTEF Ocean, Department of Environment and New Resources, N- 7465 Trondheim, Norway

⁵Norwegian University of Science and Technology, Department of Biology, Centre of Fisheries and Aquaculture, N-7491 Trondheim, Norway

*Corresponding author:

Sanna Matsson, Akvaplan-niva, N-9296 Tromsø, Norway, Phone 0047 95070503,
sma@akvaplan.niva.no

Key words: Biofouling, Deployment timing, Epibionts, Kelp cultivation, Seaweed aquaculture

Submitted to Journal of Applied Phycology 22 October 2020

Abstract

To reach the goal of large-scale seaweed cultivation in Norway and the rest of Europe, new knowledge about the commercially important species *Saccharina latissima* is central. Efforts to maximise seaweed biomass by outplanting the seaweed at different seasons may affect the seaweed quality. Here, we investigate the effects of outplanting time (February, April and May 2018) when cultivating *S. latissima* in the northern range of the species' distribution. We studied the quantity and quality of the seaweed biomass produced in the autumn following outplanting: effects on quantity were evaluated as seaweed frond area, relative Daily Growth Rate (DGR) and relative Daily Shedding Rate (DSR); quality was evaluated by tissue content of carbon and nitrogen compounds and number of fouling epizoans. Cultivation was successful when seedlings were outplanted in both February and April, but not in May. An earlier outplanting, in February, gave a prolonged time for grow-out at sea prior to the main recruitment event of epizoans that occurred in September, thereby earlier outplanting resulted in larger frond areas. The frond area reached in September was doubled when seedlings were outplanted in February compared to April, whereas a later outplanting in April gave a higher DGR and DSR, higher carbon content, and lower amount of fouling epizoans. The outplanting season did not affect tissue nitrate concentration or internally stored nitrate. These results show that outplanting time is an important factor to consider especially for biomass yield, but also for seaweed quality, including epibiosis of the seaweed biomass.

Introduction

Due to a steadily increasing food and energy demand, the UN have declared 17 Sustainable Development Goals (SDG) for 2030. Seaweed aquaculture can contribute to several of these (SDG 2 – zero hunger; SDG 3 – good health and well-being, SDG 12- Responsible consumption and production; SDG 13 – Climate action; SDG 14 - life below water) (Custódio et al. 2020; FAO 2020), by producing nutritional and healthy biomass (García-Poza et al. 2020), and supporting ecosystem services such as removal of dissolved inorganic nutrients and carbon dioxide, decreasing eutrophication and acidification of coastal waters (Jiang et al. 2020), and habitat provision (Visch et al. 2020). In 2018, seaweed aquaculture (red, green and brown algae) accounted for 32.4 million of 114.5 million tons of biomass from aquaculture and 13.3 of 263.6 billion USD (FAO 2018, 2020). Presently, the bulk of this seaweed production occurs in six Asian countries (Chopin 2014), but is also one of the fastest growing industries in countries with developed economies (Buck et al. 2017).

In the northwest Atlantic Ocean, sugar kelp, *Saccharina latissima* (L.) C.E. Lane, C. Mayes, Druehl, and G.W. Saunders 2006, is the preferred cultivated seaweed because of its high growth rate (Handå et al. 2013; Bak et al. 2018; Sharma et al. 2018), tissue content (Marinho et al. 2015b; Stévant et al. 2017; Sharma et al. 2018), and a life cycle that can be regulated (Forbord et al. 2012). Since seaweed farming is in an early phase in Europe, the conditions under which to maximize quantity and quality of the yield are not well known. Yet, optimisation for these conditions is essential for establishment and further development of seaweed aquaculture. Depending on location and latitude, the cultivation period for this species is determined by seasonal changes of environmental parameters (i.e. light, temperature and nutrients) that affect growth and build-up of desirable chemical compounds (Broch et al. 2019; Forbord et al. 2020). Following seasonal environmental changes, epizoans (i.e. sessile epibiotic animals (Wahl 1989)) begin to attach to the seaweed surface, altering seaweed biomass quantity and quality (Matsson et al. 2019; Forbord et al. 2020), and these epizoans limit the cultivation period.

The quantity and quality of produced biomass is affected by the chemical composition and growth and shedding rates of *S. latissima*. Both quality and quantity are regulated by a combination of abiotic factors and their seasonal interactions, along with biotic factors such as life stage and age of the seaweed sporophyte (Bartsch et al. 2008; Roleda and Hurd 2019; Forbord et al. 2020). Nitrogen (N) most commonly limits seaweed growth (Roleda and Hurd 2019), and variations in seaweed growth rates correspond to variations in ambient nitrogen supply and internally stored nitrate (Bartsch et al. 2008). Seasonal N fluctuations are high in the Arctic, and N is usually limited in summer (Hurd et al. 2014). The C:N ratio can vary from 5 to 40 for different macroalgae, where values above 10-15 indicate possible nitrate-limited growth, and values below that ratio indicate storage of nitrogen (Hanisak

1983). When environmental nutrient concentrations are high (i.e. in winter in temperate regions) Laminariales, including *S. latissima*, can store nutrients that can be used for growth later when ambient nutrient levels decrease. Additionally, sporophytes with higher tissue N can exhibit higher protein content (Mortensen 2017; Forbord et al. 2020); in turn an indicator of the seaweed quality. Later in summer when water temperature increases, light availability is high, and nutrients are depleted in surface layers, the seaweeds store energy in carbohydrates (Black 1950). Consequently, seaweed yield and quality vary with ambient environmental conditions particularly in the highly seasonal Arctic (Bartsch et al. 2008). The end-product will therefore be affected by the timing of outplanting and harvest (Peteiro and Freire 2012; Bruhn et al. 2016; Forbord et al. 2020). In Europe, much research is focused on maximising seaweed biomass yields by optimizing the timing for growth and quality for the intended end-products. It is, therefore, of high interest for seaweed farmers to be given guidelines on outplanting and harvest times that maximize quality and minimize biomass loss.

Epizoan species composition and peak abundance may vary with season and location (Forbord et al. 2020; Wahl 1989; Hepburn et al. 2006). The bryozoan *Membranipora membranacea* (L.) is one of the most common epizoans fouling seaweed fronds (Saunders and Metaxas 2009; Marinho et al. 2015b; Forbord et al. 2020). Its hard calcium carbonate skeleton deteriorates the seaweed quality and compromises the structural integrity of the frond, causing up to 100 % loss of biomass (Krumhansl et al. 2011; Skjermo et al. 2014). Seaweed frond elongation occurs at the base/meristem while the tips are shed continuously, and fronds of Laminariales can turnover 1 to 5 times a year (Mann 1973). Fouling organisms are thereby removed with the shed seaweed tissue, and growth and shedding rates can reduce amount of epizoans.

Here, we examined the effect of outplanting time (winter to spring) of *Saccharina latissima* in the northern range of the species' distribution by measuring the quantity (frond area, growth and shedding rates) and quality (tissue content of carbon and nitrogen compounds and density of epizoans) of seaweed biomass produced the following autumn. We hypothesised that earlier outplanting would: (1) result in higher content of nitrogen components in kelp tissue due to higher ambient nitrate concentrations at the time of outplanting; (2) produce larger frond areas, prolong the seaweed growth season, and increase rate of shedding; and thereby also (3) affect the occurrence of epizoans and bryozoan settlers. Considering the commercial importance of *S. latissima*, this trial has an industrial application in that it will provide important information on the cultivation of this species in its northern distribution range in the Norwegian Sea.

Methods

Material collection and site

Seedlings of *S. latissima* were prepared for three outplanting dates (February, April and May 2018). Parent plants with sori were collected for the February outplanting on 5 January 2018 at the harbour in Tromsø ($69^{\circ}39'07''\text{N}/18^{\circ}57'48''\text{E}$). Parent plants without sori were collected for the April and May outplantings from a seaweed cultivation site nearby Kvaløya ($69^{\circ}45'21''\text{N}\ 19^{\circ}02'17''\text{E}$) on 31 October 2017 and 21 February 2018, respectively. Fertile sorus tissue was induced, when not occurring naturally, by removal of the basal blade meristem, and kept in tanks indoors with running seawater from 30-m depth and a 16:8 h day:night regime as in Forbord et al. (2012). Contaminant-free spore release was achieved by disinfecting sori with 5% NaHCl (Rød (2012), blot drying them with paper towels and transferring them to zip-lock bags for 24 h. The disinfected and dehydrated sori were sent with freezing elements to SINTEF Sealab in Trondheim for spore release (number of fertile sporophytes: N=14, N=9 and N=13 for the first, second and third outplantings, respectively). A solution containing ~ 250.000 spores mL^{-1} (February and May outplantings) or ~ 150.000 spores mL^{-1} (April outplanting) was sprayed onto a 1.2-mm diameter twine coiled around PVC spools. The twines were then incubated for 6 weeks in nutrient-rich seawater ($148 \mu\text{g NO}_3^- \text{ L}^{-1}$, $20.6 \mu\text{g PO}_4^{2-} \text{ L}^{-1}$) in a flow-through (120 L h^{-1}), light- and temperature-controlled system at the seaweed hatchery ($70 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ at the surface and 10°C) as in Forbord et al. (2018). The twines were packed in polystyrene boxes and express-shipped to Tromsø, where they were spun around 14-mm diameter ropes and deployed (Figure 1) on the day of arrival (21 February, 4 April and 15 May).

Each outplanting consisted of seven vertical ropes attached to a horizontal carrying rope (Figure 2) with seaweed seedlings spread at 1-2 m depth, for a total of 21 ropes. Each rope had a 1-kg weight at 2 m depth. The farm was situated at ~ 100 m from the shore.

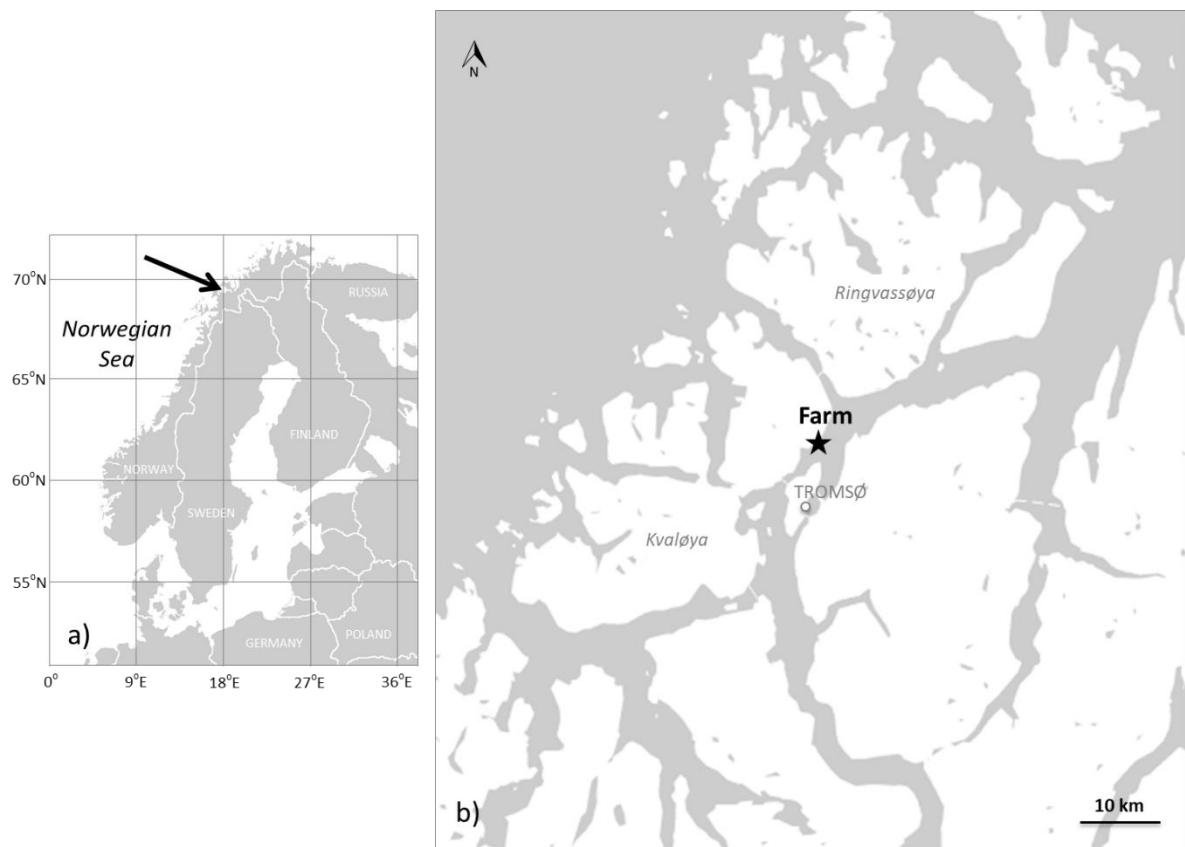


Figure 1 a) Location of Tromsø in northern Norway. b) Location of the experimental site marked with a star

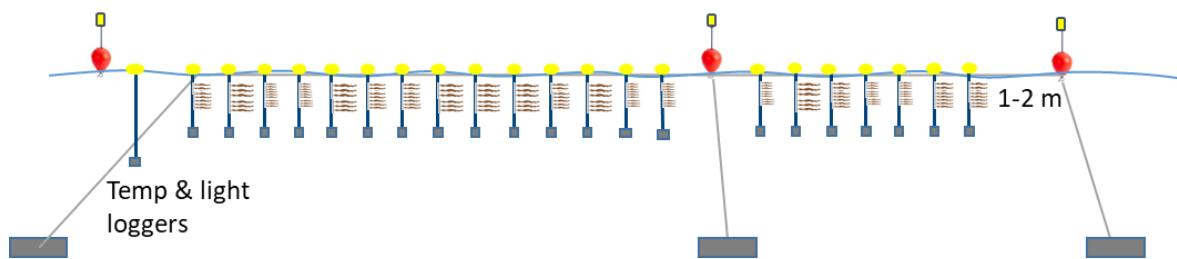


Figure 2 Experimental set-up with 7 vertical ropes per outplanting date, seeded with *S. latissima* at 1-2 m depth (not at scale). Each rope was attached to a buoy (yellow circles), placed approximately 6 m apart on a horizontal carrying rope. Marker buoys (orange dots), weights and mooring ropes (grey squares and grey lines) formed the cultivation rig

After three weeks, most sporelings from the May outplanting had disappeared, possibly because the spring bloom covered the ropes with other algae competing for light and nutrients. However, there were some surviving sporophytes for the last census of the experiment in September.

There was an observed difference in sporeling densities between the two other outplantings (February and April). To avoid confounding effects from different levels of intraspecific competition between outplantings, the ropes were thinned to 100 individuals per meter rope on 8 June by removing individuals, including the very smallest ones (≤ 10 cm in length), to achieve a more even distribution along the ropes.

Data Collection and analyses

Environmental variables

Temperature ($^{\circ}\text{C}$) and light intensity (LUX) were recorded at 2-m depth from 9 March to 5 September 2018 every 15 min using Onset HOBO pendant loggers (Bourne, MA; temperature accuracy $\pm 0.53\ ^{\circ}\text{C}$, resolution $0.14\ ^{\circ}\text{C}$) fixed to the rig (Figure 2). The LUX measurements were converted to PAR using the relationship $\text{PAR} = 0.0291 \text{ LUX}^{1.0049}$ (Broch et al. 2013; Long et al. 2012). Loggers were cleaned at every sampling date to minimize the effect of fouling.

Samples for ambient (extracellular) nitrate (E-DIN) concentration were collected using a Ruttner water sampler ($N=3$, per sampling period).

Tissue composition

Samples for total tissue nitrogen (Q_N), intracellular nitrate concentration (I-DIN), and carbon analyses were collected once to twice per month. Six seaweed fronds (without the stipes) were haphazardly collected from each of the seven replicate ropes on every sampling date from 2 May for the February outplanting, from 16 May for the April outplanting and until 5 September for both outplanting. The May outplanting only had enough biomass for one sampling date at the end of the experiment. The samples were shaken for 30 seconds to remove excess water and placed in pre-marked plastic zip-lock bags and plastic bottles. On shore, the samples were put into a $-18\ ^{\circ}\text{C}$ freezer and stored until analysis.

Fouling organisms were removed and the middle of the seaweed fronds was selected for all nutrient analyses modified from Forbord et al. (2020). Briefly, for analysis of intracellular nitrate content (I-DIN), 0.06 g semi-frozen *S. latissima* material from each sample was placed in test tubes with 6 mL distilled water, boiled for 30 minutes (with marbles at the surface to prevent evaporation), cooled, filtered into 15-mL plastic tubes using a $0.45\ \mu\text{m}$ polysulfone syringe filter and diluted by mixing 0.3 mL of the solution with 9.7 mL distilled water. The tubes with the diluted solution were placed in a $-20\ ^{\circ}\text{C}$ freezer until further analysis. Prior to analysis, the tubes were defrosted and shaken. E-DIN and I-DIN were analysed by standard seawater methods (Randelhoff et al. 2018) using a Flow Solution IV analyzer

from O.I. Analytical, USA. The nutrient analyser was calibrated using reference seawater from Ocean Scientific International Ltd. UK.

Total tissue C and N were analysed by drying samples at 60 °C for 24 h. The dried samples were homogenized and pulverized, and 0.55-0.75 mg weighed into 6x2.9 mm tin capsules using a Mettler Toledo MX5 ultra-microbalance and analysed with a CHN elemental analyser (Leeman Lab CEC 440 CHN analyzer) with acetanilide as standard.

The dry weight (DW) of the sporophytes used for I-DIN calculations was calculated by measuring the wet weight (WW) and DW of three individuals per rope from each outplanting harvested the 17 July (DW = 0.14 g g⁻¹ WW, SE 0.0047).

Seaweed growth (Frond area, DGR and DSR)

The area of the frond was estimated from length and width measurements, corrected for frills. The correction factor was estimated based on the relationship of frond length and width to actual area as in Yorke and Metaxas (2012). The seaweed frond was cut into small pieces and laid flat on a white background, and each section was photographed with an Olympus Tough F2.0 digital camera. The pictures were analysed in ImageJ (Schneider et al. 2012) and total area and frond areas were calculated as:

$$\text{Frond area} = 0.289 \cdot (L \cdot W)^{1.15}, R^2 = 0.98 \quad (1)$$

where L is the total frond length and W is the width of the widest part of the frond.

The hole-punching method (Parke 1948) was used to measure gross growth in frond length and loss through shedding of the seaweed frond. A hole was punched 5 cm from the transition between the stipe and the frond on 6-10 haphazardly chosen individuals from each of the seven replicate ropes in each treatment. A new hole was punched once to twice every month and the distance between the new and the old holes and between the old holes was measured. To minimise the impact from handling on the fragile fronds, hole punching was initiated when the sporophytes were considered robust enough (2 May and 8 June for the February and April outplantings respectively).

From the distance measurements between holes, the relative Daily Growth Rate (DGR), and relative Daily Shedding Rate (DSR) were calculated as:

$$DGR (\text{day}^{-1}) = \left[\left(\frac{L_0 + G}{L_0} \right)^{\frac{1}{t}} \right] - 1 \quad (2)$$

$$DSR (day^{-1}) = \left[(1 + \left(\frac{L_0 + G - L_t}{L_0} \right))^{\frac{1}{t}} \right] - 1 \quad (3)$$

where L_0 is the total frond length on the previous sampling date, L_t is the total frond length on the following sampling date, G is gross frond growth since previous sampling, calculated by adding the length increase between the punched holes, and t is days since last sampling date.

Epibiosis (total, species, and *M. membranacea* settlers)

At each sampling date, three sporophytes per rope were collected haphazardly and kept moist and cool until analysis. The seaweed frond was divided into three equally long sections representing meristematic, middle, and distal (tip) regions to test for effects of blade age on epizoans, and the number of epizoan individuals/colonies was identified and counted. Colonies of the abundant bryozoan *M. membranacea* were subdivided into two size classes: < 2 zooid rows were categorized as (early) settlers and ≥ 2 zooid rows as colonies as in Saunders and Metaxas (2007), using magnifying eyewear (Watch Repair Magnifyer) (25x). When a colony covered two frond areas, it was included in the blade area nearest the stipe.

Statistics/Data analysis

The effects of timing of outplanting (fixed factor, three levels) and date (random factor, seven levels) on Q_N , I-DIN, C:N and C were examined with a two-way analysis of variance (ANOVA). Outliers for Q_N and C were removed because the very low values were assumed to be the result of an analysis error. The data were normally distributed for most variables (except for I-DIN on 7 June, 17 July, 1 August and 13 August for the February outplanting and 7 June for April outplanting, C:N on 16 May for February outplanting and 7 July for April outplanting), as assessed by Shapiro-Wilk's test ($p > 0.05$). Variances were homogeneous ($p > 0.05$) for most variables (except for carbon $p=0.047$, and C:N $p=0.003$), as assessed by Levene's test. Significant differences between means were examined using *post hoc* tests with Bonferroni corrections. Relationships between E-DIN, I-DIN and Q_N were examined using linear regression, including a potential time-lag effect of external nitrogen tested using E-DIN data from succeeding sampling date ('delayed E-DIN'). ANOVA was used to examine the effects of the effects sampling date (repeated measures, random factor, five levels) and outplanting time (fixed factor, two levels) on the relative daily growth rate (DGR) and shedding rate (DSR). The data were normally distributed, as assessed by Shapiro-Wilk's test ($p > 0.05$) and variances were homogeneous ($p > 0.05$) for most data, as assessed by Levene's test. For the repeated-measures ANOVA, Mauchly's test was used to test the assumption of sphericity, which was met for DSR but not for DGR; therefore p-values for tests were adjusted using the Greenhouse-Geisser corrections (Queen et al. 2002).

Two-way ANOVA was used to test the effects of outplanting time (fixed factor, three levels) and date (random factor, 5 levels) on the amount of epizoans. Most data were normally distributed as assessed by Shapiro-Wilk's test ($p>0.05$), except data from the initial colonisation in the April outplanting (28 June; $p=0.012$, 17 July; $p=0.000$, 1 August; $p=0.000$) and February (28 June; $p=0.006$). The data were log-transformed without much improvement. Three-way ANOVA was used to examine the effects of outplanting time (fixed factor, two-three levels), sampling date (random factor, five levels), and frond section (fixed factor, three levels) on the dependent variable *M. membranacea* settlers. In cases where the variances were heterogeneous or deviated from normality, data were log-transformed which yielded little improvement. Since ANOVA is relatively robust to heterogeneity of variance when group sizes are approximately equal (Jaccard and Jaccard 1998) and to deviations from normality (see Maxwell et al. (2017)) the two-way ANOVA was done on the untransformed data. Statistical analyses were performed using IBM SPSS Statistical software (Version 25) and graphs produced by using R, version 3.5.1 (R Core Team 2018) through RStudio version 1.1.456 (RStudio Team 2016).

Results

Environmental data

Water temperature gradually increased from 2.9 °C on 9 March to 3.7 °C by 4 April (April outplanting) and 5.2 °C by 15 May (May outplanting) (Figure 3, left y-axis). By the end of July, seawater temperatures were rather stable at ~10 °C. The average daily irradiance in PAR increased rapidly from February onwards with increasing day length with an average of 32 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 81 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 116 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the February, April and May outplantings, respectively (Figure 3, right y-axis). From mid-May until the end of June, measured irradiance decreased, most likely because of shading caused by phytoplankton bloom and fouling on the loggers. E-DIN was highest in April (Figure 4) and steadily decreased to less than 0.1 μM in August, when it started to increase again.

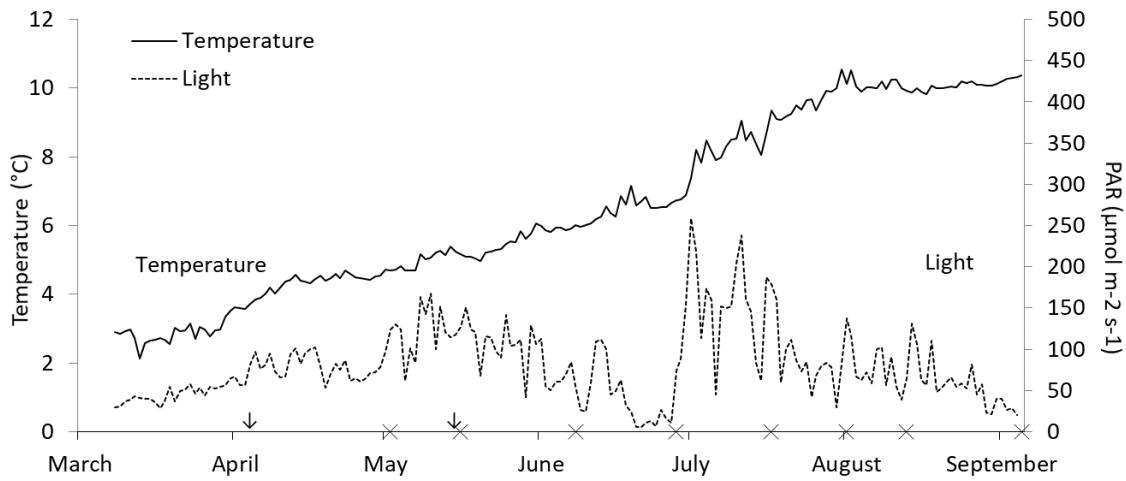


Figure 3 Seawater temperatures and light averaged over 24 h across the deployment period of *S. latissima* at 69°N at 2 m depth. × indicate sampling times, including cleaning of loggers. Arrows indicate April and May outplanting dates

Tissue composition

Q_N ranged from maximum mean values of 2.4 mg N g⁻¹ DW in May to a minimum of 0.78 mg N g⁻¹ DW in the beginning of August, and was significantly affected by date (Figure 4, Table 1). I-DIN peaked in June with maximum mean values of 0.96 mg NO₃⁻ g⁻¹ DW for the February outplanting and 0.51 mg NO₃⁻ g⁻¹ DW for the April outplanting, and minimum values for both outplantings in August. There was a significant interaction of outplanting time and sampling date on I-DIN (Figure 4, Table 1) and a significant effect of sampling date on I-DIN, and I-DIN storage was greater for the February than the April outplanting. After June, these elevated levels of I-DIN were reduced to similar levels between outplantings. Both I-DIN and Q_N were affected by the fluctuations of E-DIN (Table 2), where the variance of Q_N was better explained by E-DIN than by I-DIN. Both I-DIN and Q_N had a delayed response (2-3 weeks, i.e. subsequent sampling date) to changes in E-DIN (Table 2).

C:N ratios increased from low values around 10 in May to peak values of 30-40 in August (Figure 5a). There was a significant interaction between outplanting time and date on C:N ratio (Table 1). C:N ratio increased over sampling time, until the last sampling, where the E-DIN levels started to rise (Figure 4 & 5a). C:N ratio was higher for the April than the February outplanting from mid-July to mid-August (Table 1) and both were higher than the May outplanting in September (Table 1).

Carbon tissue content was significantly affected by both outplanting time and date (Figure 5b, Table 1), increasing with time and being higher for the seaweed outplanted in April compared to February.

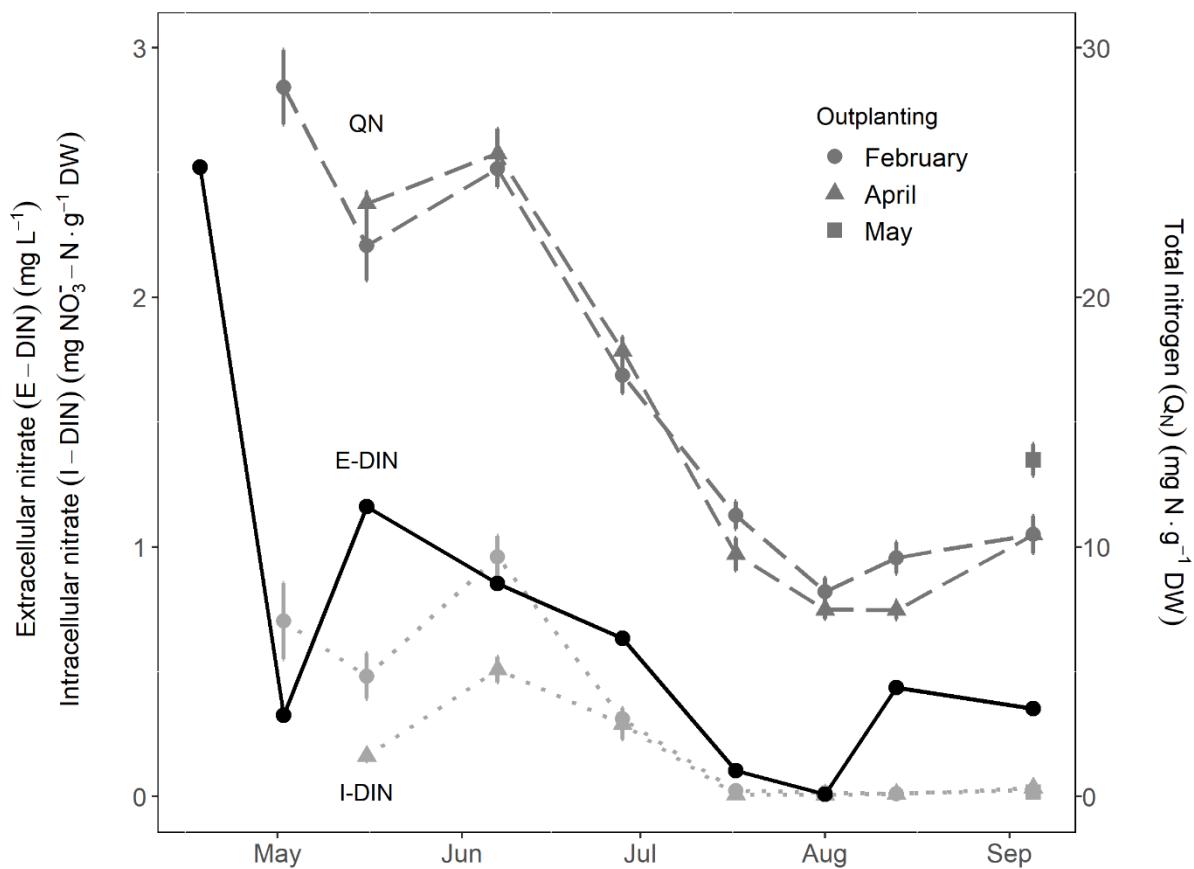
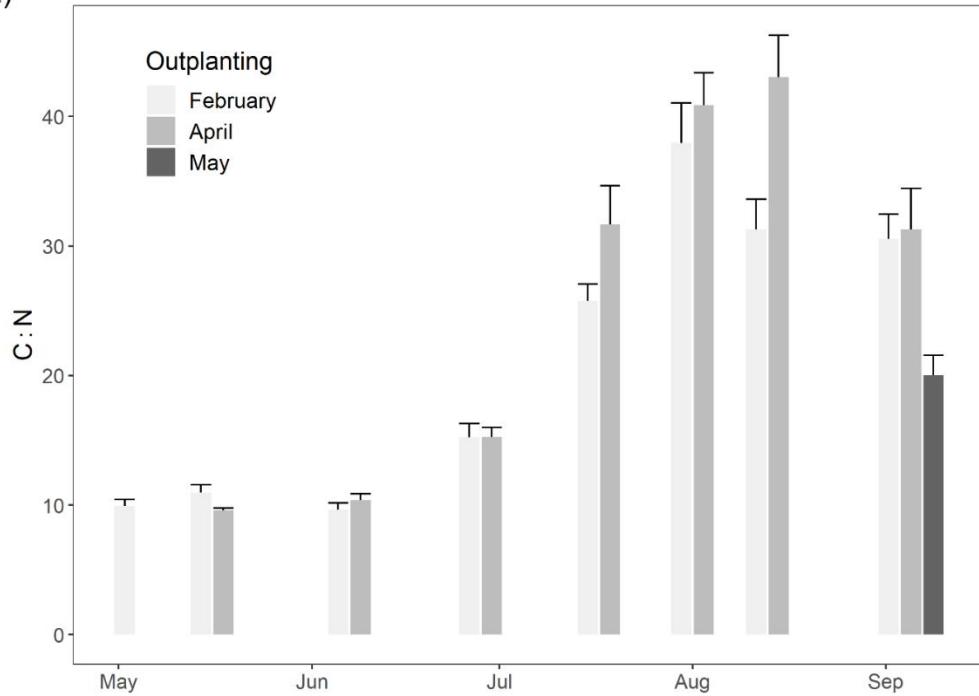


Figure 4 Extracellular nitrate (E-DIN) (μM) measured in the water column at 69°N at 1 m depth (black line), intracellular nitrate (I-DIN) ($\text{mg NO}_3^- \cdot \text{g}^{-1} \text{DW}$) (dotted line), and total nitrogen content (QN) ($\text{mg N} \cdot \text{g}^{-1} \text{DW}$) (stippled line) of *S. latissima* fronds. Mean \pm SE, n = 3

a)



b)

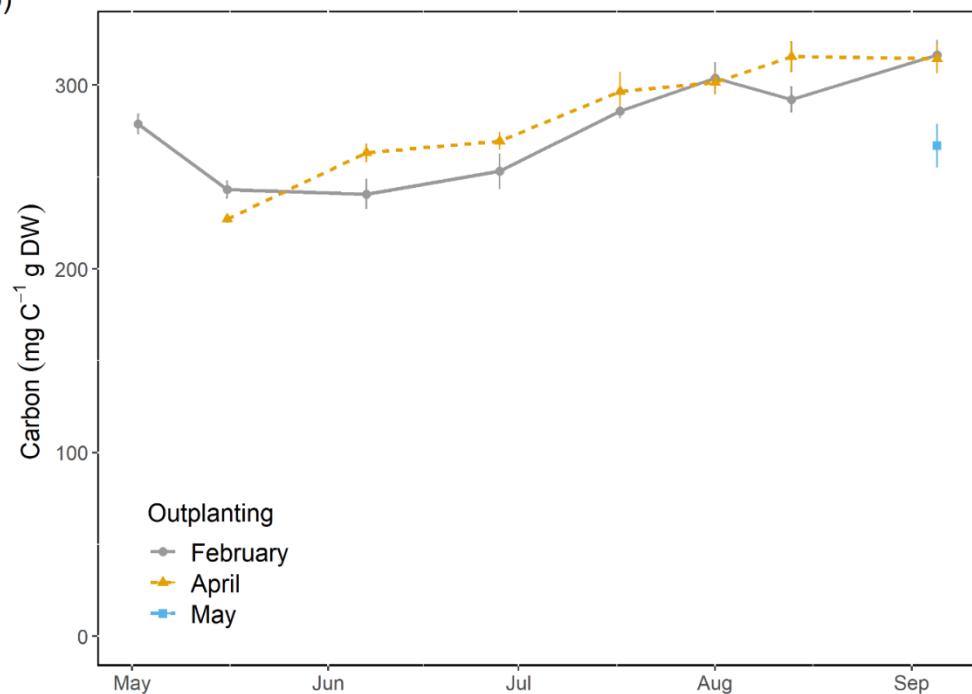


Figure 5 a) C:N ratios of tissue of *S. latissima* at 69° N outplanted at different times of the year. B) and carbon content ($\text{mg C g}^{-1} \text{ DW}$). Mean \pm SE, n=3

Table 1 Results of two-way ANOVA analysing the effects of outplanting time of *S. latissima* at 69° N (fixed factor, three levels) and sampling date (random factor, seven levels) on total nitrogen (Q_N), intracellular nitrogen (I-DIN), Carbon and C:N . P values are presented in bold for $\alpha_{crit}(0.05)$. F: February outplanting, A: April outplanting, M: May outplanting

Effect	df	MS	F	p	Post hoc (Bonferroni Correction)
Total nitrogen (Q_N)					
Outplanting time	2	0.17	2.94	0.120	
Date	6	6.85	116.51	0.001	
Outplanting time x Date	6	0.06	1.83	0.100	
Error	83	0.03			
Intracellular nitrogen (I-DIN)					
Outplanting time	2	0.15	1.35	0.330	
Date	6	1.04	8.74	0.009	
Outplanting time x Date	6	0.12	10.19	0.001	
Error	80	0.01			
C:N					
Outplanting time	2	313.39	5.16	0.046	
Date	6	1990.16	31.08	0.001	
Outplanting time x Date	6	0.06	2.49	0.029	17 July: F<A, 13 August: F<A, 5 September: F=A>M
Error	83	25.69			
Carbon					
Outplanting time	2	49.36	7.97	0.017	
Date	6	116.21	18.00	0.001	
Outplanting time x Date	6	6.46	1.92	0.087	
Error	83	3.37			

Table 2 Regression coefficients for the relationships between variables associated with nutrient status; extracellular nitrate (E-DIN), intracellular nitrate (I-DIN), and total nitrogen content (Q_N), and 'delayed E-DIN' as E-DIN data from succeeding sampling date . P values are presented in bold for $\alpha_{crit}(0.05)$

Relation y versus x	Intercept y-axis (b)	Slope (a)	R ²	Adjusted R ²	F (df1,df2)	P
I-DIN versus E-DIN	0.006	0.445	0.309	0.259	6.25 (1,14)	0.025
QN versus E-DIN	0.885	1.334	0.470	0.432	12.42 (1,14)	0.003
QN versus I-DIN	1.068	2.094	0.745	0.727	40.88 (1,14)	<0.0001
I-DIN versus delayed E-DIN	0.008	0.346	0.535	0.502	16.13 (1,14)	0.001
QN versus delayed E-DIN	0.995	0.869	0.573	0.542	18.76 (1,14)	0.001

Seaweed growth (Frond area, DGR and DSR)

Frond area increased with time (Figure 6a). The initial absolute growth was higher for the April than the February outplanting from outplanting date until the first sampling (at day 70 for the February outplanting and day 65 for the April outplanting) but the opposite was the case over the entire study period (196 and 154 days, respectively). Fronds were longest on the last sampling date (5 September) for both the February (147.8 ± 7.65 cm) and the April outplantings (87.5 ± 4.70 cm) (n=7), compared to 26.8 ± 5.43 cm (n=6) for the May outplanting. The length to width ratio (L:W) was consistently higher for the February than the April and May outplantings throughout the study period, with 6.1 ± 0.08 compared to 4.5 ± 0.07 and 4.5 ± 0.21 (Mean \pm SE, n=455; 288; 24) (Electronic Supplementary Material 1).

Relative Daily Growth Rate (DGR) was significantly greater for seaweed outplanted in April than February and was significantly affected by date (Figure 6b and Table 3), with higher rates in early than late summer. Relative daily shedding rate (DSR) was also significantly higher for the seaweed outplanted in April than February and decreased from June to early August for both outplantings (Figure 6b, Table 3), though there was no significant relationship between DSR and DGR (See Electronic Supplementary Material 2).

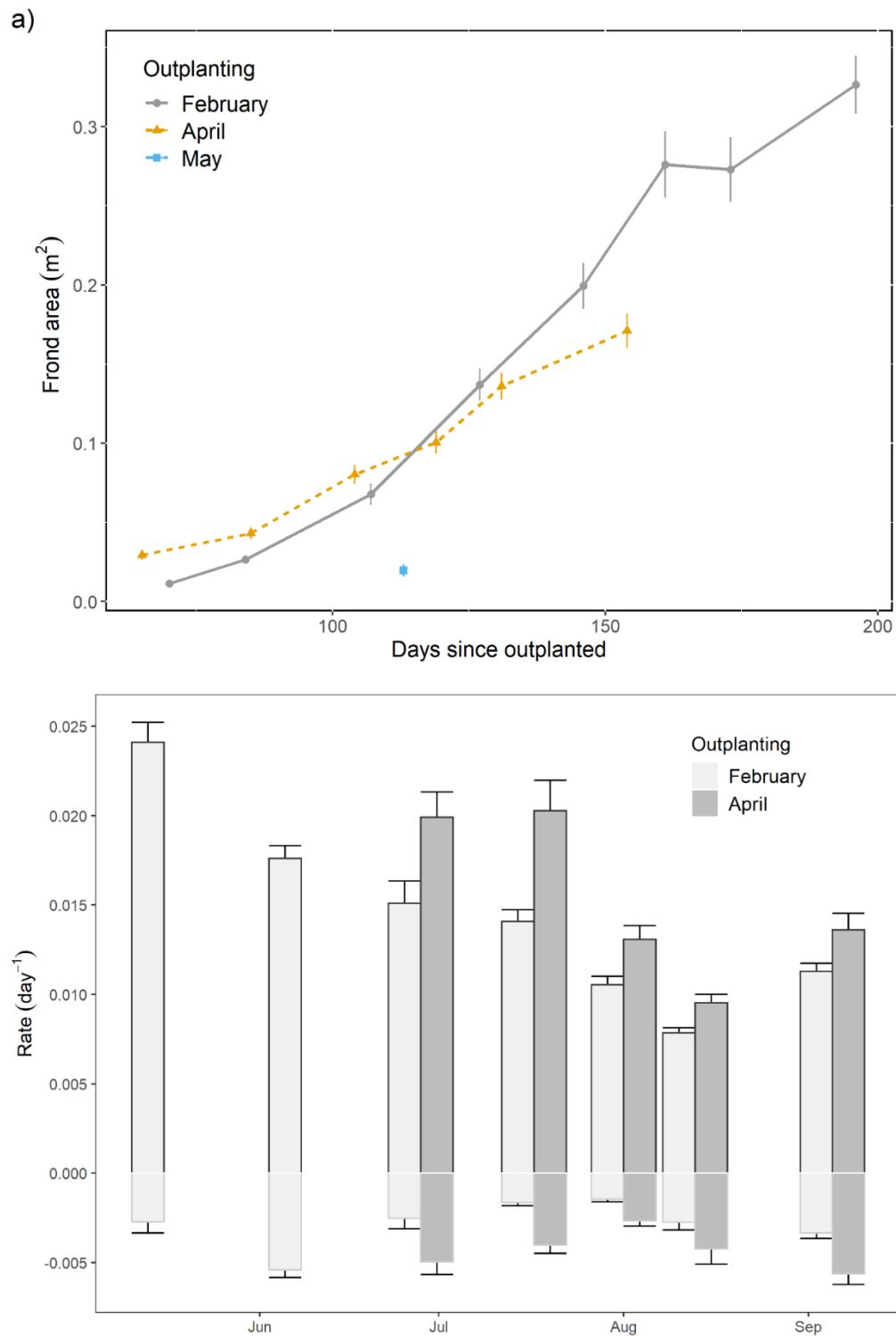


Figure 6 a) Area of the seaweed frond in m^2 as an effect of days in the sea, Mean \pm SE, n=7. B) Relative Daily Growth Rate (DGR) in length for *Saccharina latissima* outplanted in February and in April at 69°N (positive values) and relative Daily Shedding Rate (negative values) as lost algae material in length. Mean \pm SE, n=7

Table 3 Results of repeated measures ANOVA analysing the effects of outplanting time (fixed factor, two levels) and sampling date (random factor, five levels) on relative Daily Growth rates (DGR) and relative Daily Shedding Rate (DSR) of *Saccharina latissima*. P values are presented in bold for $\alpha_{\text{crit}}(0.05)$

Effect	Source	df	MS	F	p value	Partial η^2
DGR (day ⁻¹)	Within-subjects effects					
	Sampling date	4	0	48.04	<0.001	0.889
	Error (Date)	24	4.13E-06			
	Outplanting	1	0	15.21	0.008	0.717
	Error (Outplanting)	6	1.42E-05			
	Outplanting x Sampling date	1.6	1.24E-05	2.43	0.146	0.288
	Error (Outplanting x Date)	0	1.33E-05			
DSR (day ⁻¹)	Within-subjects effects					
	Sampling date	4	1.18E-05	5.23	0.004	0.466
	Error (Date)	24	1.25E-06			
	Outplanting	1	6.08E-05	120.59	<0.001	0.953
	Error (Outplanting)	6	5.04E-07			
	Outplanting x Date	4	1.61E-06	1.86	0.151	0.236
	Error (Outplanting x Date)	24	8.66E-07			

Epibiosis (total, species, and *M. membranacea* settlers)

Epizoans were first observed in late June, then their abundance increased slowly until a main fouling event occurred before the last sampling in September (Figure 7a). Epizoan density peaked 6.5 and 5 months after outplanting for the February and April outplantings, respectively (Figure 7a). There was a significant interaction between outplanting time and sampling date for the number of epizoans per area kelp frond (Figure 7a and b, and Table 4). Abundance of epizoans was significantly different among outplanting treatments in September only (Table 4). The number of fouling organisms per area at the last sampling in September was not affected by the total area of the seaweed (Figure 7b).

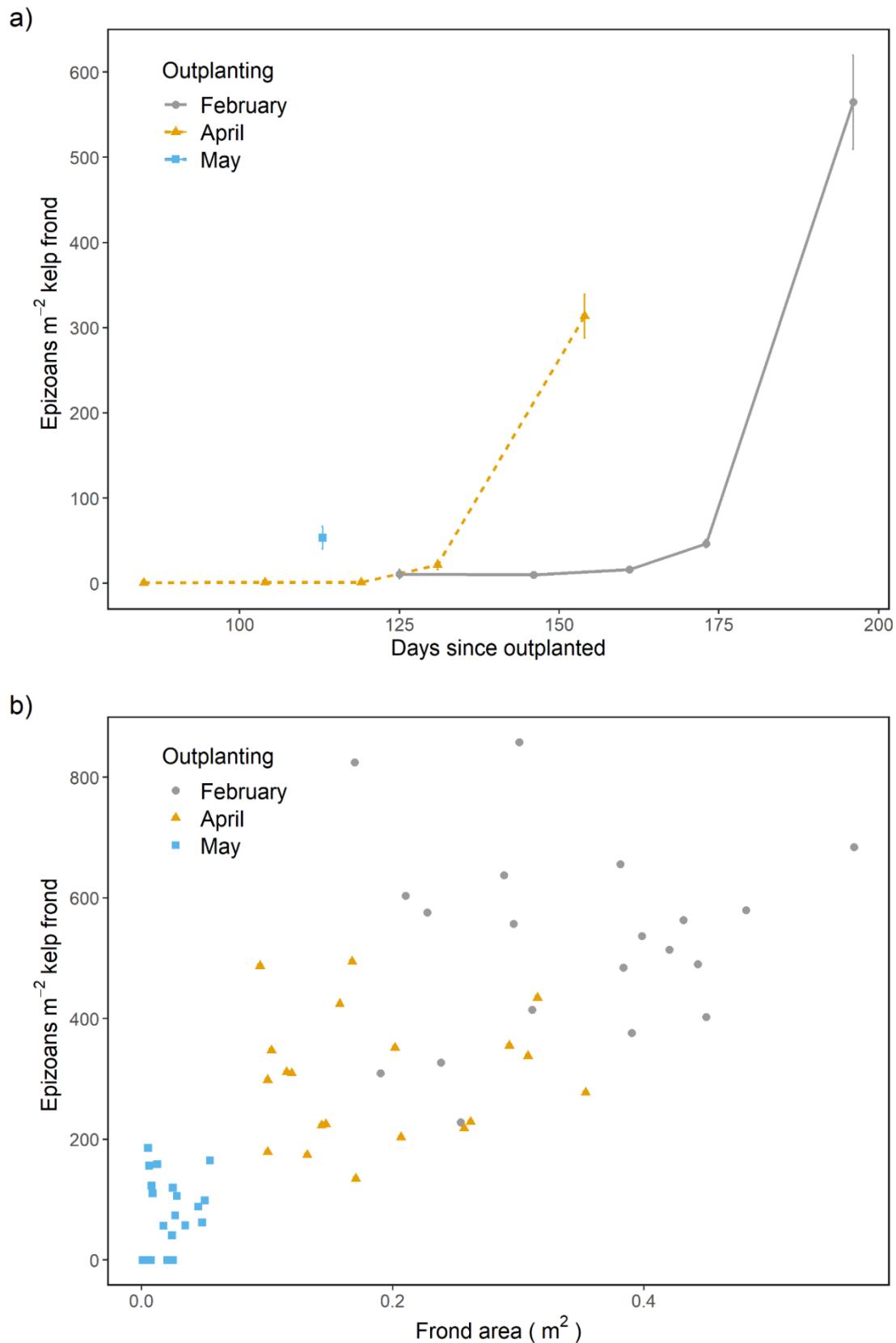


Figure 7 a) The number of epizoans per m^2 seaweed frond over the time period elapsed since outplanting for the three outplanting times. b) Number of epizoans per m^2 as an effect of the kelp frond area on the last sampling date (5 September 2018) for the three outplanting dates

Table 4 Results of two-way ANOVA analysing the effects of outplanting time (fixed factor, three levels) and sampling date (random factor, five levels) on total amount of fouling organisms. P values are presented in bold for $\alpha_{\text{crit}}(0.05)$. F: February outplanting, A: April outplanting, M: May outplanting

Effect	df	MS	F	p	Post hoc (Bonferroni Correction)
No. epizoans m⁻² kelp					
Outplanting time	2	320116.36	10.84	0.024	
Date	4	478762.43	15.44	0.011	
Outplanting time x Date	4	31017.36	24.56	<0.001	5.9.2018: F>A>M
Error	65	1262.81			

Five species were attached to the *S. latissima* fronds: the hydroid *Obelia geniculata*, the bivalve *Mytilus edulis*, the barnacle *Balanus* sp. and the bryozoans *Membranipora membranacea* and *Electra pilosa* (Figure 8b) but *M. membranacea* was the most abundant epizoan for all outplantings and dates. The relative contributions in total epibiosis abundance by *E. pilosa* were highest in early summer but only for the February outplanting, and were succeeded first by *M. edulis* and then *O. geniculata* which contributed substantially in August-September. Filamentous algae (not quantified) first occurred on kelp tips of the February outplanting in June and to a lesser extent on the April outplanting; by the last sampling in September they were similar for all three outplanting times. There were significant interactions between frond section and outplanting time, and between frond section and sampling date for the number of *M. membranacea* settlers (Table 5). More settlers were present on the young meristematic region than on the middle region and the tips for the February treatment. More settlers were present on the meristematic region than the tips for the April treatment, and more settlers were present on the mid-section than the meristem for the May outplanting (Figure 8c). Also, the number of settlers at each section was highest for the February outplanting and lowest for the May outplanting. Only in September was the number of *M. membranacea* settlers highest on the meristem and lowest on the tips for all outplantings.

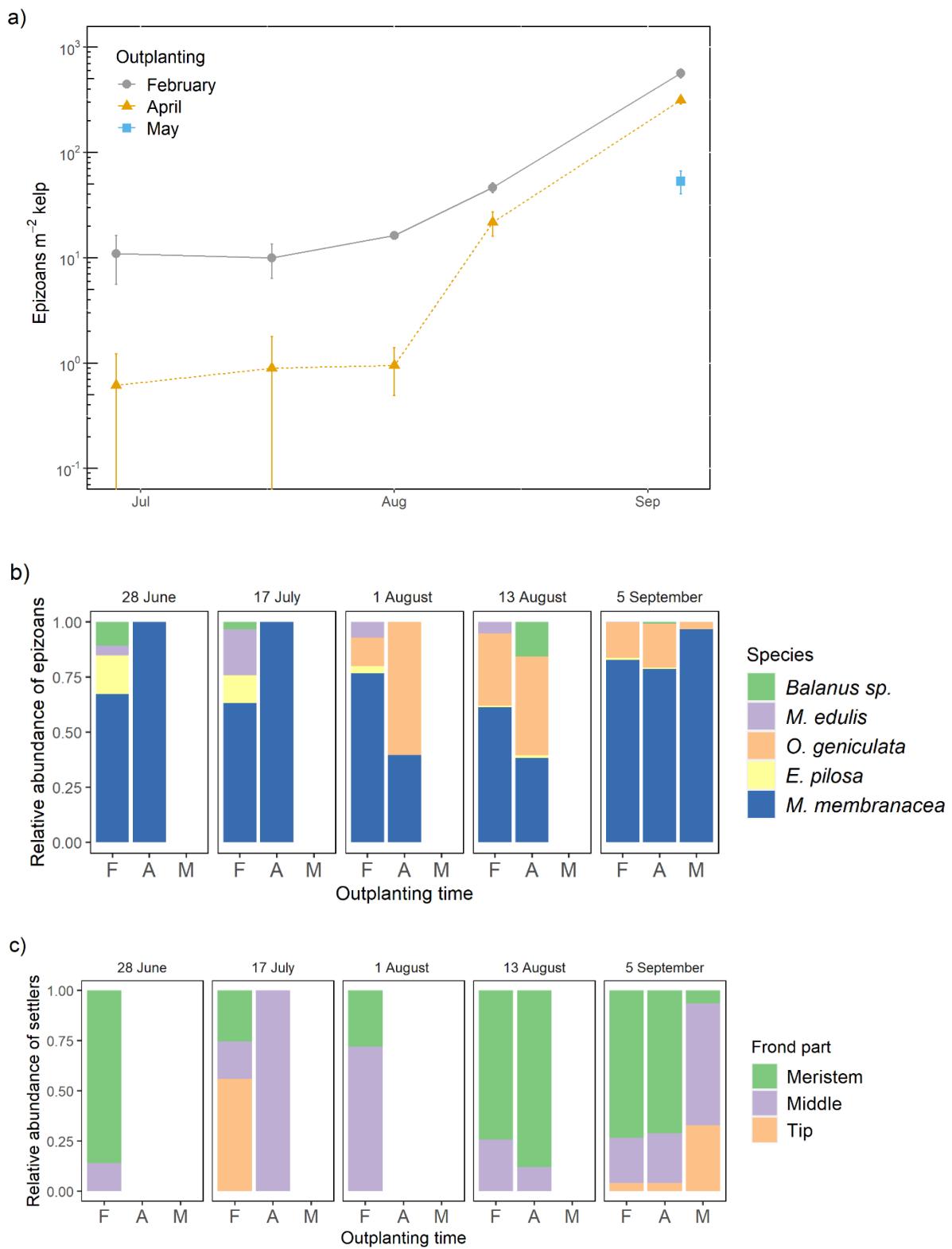


Figure 8 Organisms fouling *Saccharina latissima* outplanted at three different times (F:February, A:April and M:May) from 28 June until 5 September. a) Density (number of epizoans m^{-2} kelp) over time for the three outplanting dates. Mean \pm SE, n=7. b) Relative composition of epizoans for each sampling date: Balanus = *Balanus* sp., Mytilus= *Mytilus edulis*, Electra = *Electra pilosa*, Hydroids= *Obelia geniculata*, Membranipora = *Membranipora membranacea*. c) settlers of *M. membranacea* relative

abundance on three frond sections for the three different outplanting times. Kelp outplanted in May was only sampled 5 September

Table 5 Results of three-way ANOVA examining the effects of outplanting time, sampling date and frond section on *M. membranacea* settlers. P values are presented in bold for $\alpha_{\text{crit}}(0.05)$. Outplanting in F:February, A: April, and M: May at three regions of the frond; Mer: meristematic, Mid: middle, and T: tip

Effect	df	MS	F	p	Post hoc (Bonferroni Correction)
Section	2	10564.83	0.07	0.929	
Outplanting time	2	122421.16	4.49	0.092	
Date	4	443526.80	2.43	0.123	
Section x Outplanting time	4	211937.76	16.99	<0.0001	A: Mer > Tip, F: Mer > Mid, Mer > Tip, M: Mer < Mid, Mer/Mid/Tip: F>A>M
Section x Date	8	166793.10	13.20	0.001	5 September 2018: Mer>Mid>Tip
Outplanting time x Date	4	28212.33	2.23	0.155	
Section x Outplanting x Date	8	12635.21	1.33	0.230	
Error	195	9496.80			

Discussion

In this study, cultivation of *S. latissima* at 69° N was successful when outplanted in both February and April, but not in May. Our results generally supported our hypothesis that quantity and quality of harvestable seaweed are affected by outplanting time but this effect was not consistent across all examined variables or across the entire sampling period.

Tissue composition

There was no difference in total tissue nitrogen, Q_N, among outplanting times, and the initially elevated levels of intracellular nitrate, I-DIN, for the February outplanting were utilized fast when extracellular nitrate, E-DIN, dropped. These results were contrary to our hypothesis that earlier outplanting with accompanying elevated E-DIN would result in a higher content of intracellular nitrogen components (Q_N and I-DIN) in *S. latissima*. This result is also in contrast to a previous laboratory study with the same kelp species where nutrient depletion in the tissue did not occur until 9 weeks in nutrient replete water (Lubsch and Timmermans 2019). Similarly, depletion of the intracellular nitrate storage in *Laminaria longicruris* in Nova Scotia, Canada, followed the disappearance of the external nitrate with a lag period up to 2 months (Chapman and Craigie 1977). The reason for the fast depletion of I-DIN in the present

study may be that initial E-DIN concentrations were comparatively low, resulting in the internal-tissue nutrient pools and other N-compounds not being filled up before the external nutrient levels dropped to a minimum. The storage of I-DIN in *S. latissima* is a slow process (Forbord et al. 2021) and this species tends to store nitrate when the ambient nitrate concentrations are higher than 10 µM (Chapman et al. 1978), levels never recorded in our study (Figure 1).

I-DIN and Q_N concentrations followed a seasonal pattern, as also found by Forbord et al. (2020) across Norway, being highest in the beginning of the sampling period, when extracellular nitrate levels were also highest before summer stratification, and before the phytoplankton spring bloom reduces the E-DIN (Ibrahim et al. 2014). Both Q_N and I-DIN were, as hypothesized, significantly affected by the availability of extracellular nitrate (E-DIN) throughout the sampling period and as a result were also correlated with each other. I-DIN dropped to near zero in July, when Q_N dropped below 10 mg N g⁻¹ DW, which is likely because Q_N values exceeding 1% of DW (or 10 mg N g⁻¹ DW) allow internal storage of nitrate in *S. latissima* (Asare and Harlin 1983). Both the incorporation of nitrogen in the seaweed tissue (Q_N) and the intracellular storage of nitrate (I-DIN) responded with a 2-3 week delay relative to the altered levels of nitrate available in the water column. Q_N in members of the Laminariales order follow ambient nitrate level at various time lags (Chapman and Craigie 1977; Wheeler and North 1980; Wheeler and North 1981). Protein content is another indicator of kelp quality, and based on an average nitrogen-to-protein conversation factor (K_p) for the present location of 3.9 ± 0.3 (mean ± SE) (Forbord et al. 2020), protein concentration was estimated at 99 mg g⁻¹ DW proteins in June and declined to less than one third (30 mg g⁻¹) two months later for all outplanting treatments. Thereby, supplying a higher protein yield if the seaweed biomass is harvested earlier in the season.

While outplanting time did not affect nitrogen components, it did affect carbon content. Sporophytes outplanted in April had a higher carbon content and a higher C:N ratio than those outplanted in February, suggesting a higher accumulation of carbohydrates for the former. Photosynthetic rates are affected by biotic factors such as morphology, ontogeny, age, and circadian rhythms (Hurd et al. 2014), and the assumed higher surface area:volume ratio of the smaller April sporophytes may contribute to a higher photosynthetic rate, resulting in higher carbon content and also higher growth rates (Littler and Arnold 1982). In contrast, older individuals of a related species, *Laminaria hyperborea*, can have a higher C:N ratio than first-year sporophytes (Sjøtun et al. 1996). The critical nitrogen concentration (Q_N) to sustain maximum growth rate in *S. latissima* is ~ 1.9 % of DW (Chapman et al. 1978). When nitrogen content is above that value, carbon content is positively correlated to the nitrogen content for *Saccharina japonica* (Mizuta et al. 1997b). This is consistent with the stable C:N ratio of ~ 10 in our study which persisted until the beginning of July for both outplantings in the present study. Carbon content increased over time for all outplanting dates, when irradiances was higher and seaweed

growth rates slower. This pattern suggests an accumulation of carbohydrates in summer when reserve carbon storage compounds increase (Sjøtun 1993; Azevedo et al. 2019).

Seaweed growth (Frond area, DGR and DSR)

In support of our hypothesis, frond area was larger throughout the experiment in seaweed outplanted in February than April and May. Earlier studies of cultivated Laminariales, *S. latissima*, *Laminaria digitata* and *Undaria pinnatifida*, at 43 °N to 70 °N show a similar trend with increased production in yield when outplanted earlier (Peteiro and Freire 2009, 2012; Edwards and Watson 2011; Handå et al. 2013). In contrast, one study in the UK reported a lower biomass production in *S. latissima* when outplanted in November compared to December and February (Kain et al. 1990).

Contrary to our hypothesis, however, earlier outplanting did not result in an increased relative Daily Growth Rate (DGR) through the summer. In fact, DGR was significantly higher when kelp was outplanted later (April) than earlier (February). Given that the younger and smaller individuals from the April outplanting had a higher carbon content and, in summer (July to August), a higher C:N, indicating nitrogen limited growth; thus, the higher DGR in the April outplanting may be due to processes not restricted by nitrogen. One possible explanation is that younger individuals of several Laminariales species, including *S. latissima*, exhibit age-specific seasonal growth, with a prolonged duration of high vegetative growth in summer (Lüning 1979; Druehl et al. 1987), and the triggering mechanisms for seasonal growth for many kelp species is an underlying endogenous circannual rhythm (Lüning and Tom Dieck 1989; Lüning and Kadel 1993). Experiments have indicated that the development of the endogenous growth rhythm of juvenile *Laminaria* sporophytes occurred a few weeks after sporophyte ontogeny (Bartsch et al. 2008), possibly explaining the longer growth season for juvenile sporophytes, as well as the higher DGR of the younger April outplanting sporophytes in the present study. DGR declined significantly over the duration of both the February and April outplantings. Growth reduction for *S. japonica* has been shown to occur when Q_N falls below 21 mg g⁻¹ DW (Mizuta et al. 1997a). In our study, this value was reached around mid-June, approximately the same time when the C:N sharply increased. This growth pattern, with the main growth occurring during winter and carbon being stored during summer, is consistent with other studies in areas with nitrogen abundant in winter (Gagné et al. 1982).

Both later outplanting and season significantly increased the shedding of the tips (DSR). A higher amount of shedding of *S. latissima* has been positively correlated to E-DIN (Boderskov et al. 2016), whereas the opposite has been the case for *Undaria pinnatifida* (Yoshikawa et al. 2001). Our study does not support an effect of E-DIN on shedding rates. Frond age of several Laminariales has a positive correlation with shedding (Kurogi 1957; Nishikawa 1967; Zhang et al. 2012). However Sjøtun (1993) found that shedding per se is not related to age, but that longer fronds are more prone to shedding.

This is not consistent with our results of a relatively higher shedding of the smaller individuals in the April outplanting.

Epibiosis (total, species, and *M. membranacea* settlers)

Our results supported the hypothesis that outplanting time affects the amount of fouling organisms (epizoans) in general and *M. membranacea* settlers in particular. In September, when fouling was greatest, occurrence of epizoans was significantly higher in the seaweed outplanted earliest throughout the observation period than the later outplanting times. Epibiosis of perennial seaweeds at mid- and high latitudes typically peaks earlier than in our study, in summer, when seaweed growth rate is reduced (Lüning and Pang 2003) and recruitment rates of epizoic larvae increase (Lüning and Pang 2003; Saunders and Metaxas 2007; Forbord et al. 2020). Increasing temperature is the main driver for the timing of larval settlement (Saunders and Metaxas 2007). Continuous growth and shedding in summer, which was higher for the April outplanting, may help reduce the density of fouling organisms. The differences in epizoan densities among the outplanting times may, therefore, be a result of the relationship between larval supply timing and different turnover times of frond tissue caused by the varying growth and shedding rate. From an industrial point of view, however, it is more important to note that seaweed from both successful outplanting dates were in fact greatly fouled by September regardless of outplanting date (>550 epizoans m^{-2} frond for February experiment and >300 epizoans m^{-2} frond for April experiment). The seaweed outplanted in May was much less fouled (<60 epizoans m^{-2} frond), but the biomass produced was minimal.

The two most abundant epizoans, the bryozoan *M. membranacea* and the hydroid *Obelia geniculata*, were also reported as dominant taxa in earlier studies in this region (Matsson et al. 2019; Forbord et al. 2020). Both species are widespread and found on cultivated (Peteiro and Freire 2013; Førde et al. 2015; Walls et al. 2017) and wild seaweed in the boreal and sub-Arctic Atlantic (Lambert 1990; Fredriksen et al. 2005; Scheibling and Gagnon 2009). The other three less abundant species in this study, *Electra pilosa*, *Mytilus edulis* and *Balanus* sp., are not reported by the same studies, likely because of their low density on cultivated seaweed (Matsson et al. 2019; Forbord et al. 2020). Density of *M. membranacea* settlers was higher on seaweed outplanted in February compared to April and May, and on the younger regions of the frond, possibly as a result of preferential larval settlement (Denley et al. 2014). In addition, *M. membranacea* larvae may alter their behavior in response to habitat types and can detect small-scale differences in substrate quality (Matson et al. 2010), possibly through chemical cues (Brumbaugh et al. 1994). In the present study, there were no significant differences between the concentrations of N-compounds (I-DIN and Q_N) within the seaweed tissue of the different outplanting times, making a nitrogen cue unlikely. Production of defence compounds such as phlorotannins may provide an alternative cue. Pavia and Toth (2000) suggest a Carbon Nutrient

Balance Model for seaweeds, according to which photosynthetically fixed carbon will be allocated to production of defence compounds when nutrients are limiting growth (i.e. when C:N is high). While we did not measure defence compounds, the C:N ratio was significantly higher in seaweed outplanted in April than February, particularly in mid-August prior to the peak in epibiosis, implying a possible higher production of defence compounds in the April outplanting.

Conclusions

Our results indicated that, for the February outplanting, a prolonged time for grow-out at sea prior to the main recruitment event in September resulted in a doubled frond area than in the April outplanting. Therefore, we recommend outplanting in February over April in this area. Even earlier outplanting before the onset of the Polar night in late autumn may be advantageous and should be examined although it poses a higher risk of autumn and winter storms damaging the seaweed farm. Outplanting time affected the quantity of seaweed produced, and from an industrial perspective, outplanting time also affected the quality of the produced biomass. An earlier outplanting time resulted in lower carbon content and higher amount of fouling epizoans, but no difference in seaweed nitrogen compounds (I-DIN or Q_N). The most suitable harvesting time, therefore, depends on the type of desired end-product. When large biomass production is preferred, an extended grow-out phase with latest harvesting is recommended. For more delicate fronds with little epibiosis intended for direct human consumption, a delayed outplanting and earlier harvesting may be advantageous. Depending on the desired chemical composition when producing feed ingredients or to produce microbial growth media, protein-rich epizoans may be included in the end-product, allowing for a later harvesting. In conclusion, our findings improve the knowledge on optimal cultivation period as well as the effect of variation in cultivation timing, thereby improving the yield as well as the quality of cultivated seaweed.

Acknowledgements

This work was funded by the Research Council of Norway, project no. 254883 (MacroSea). The seeding production was carried out within the framework of the research infrastructure Norwegian Center for Plankton Technology (245937/F50). We would like to thank Paul Dubourg (UiT) for conducting the CN analysis, Zsolt Volent and Magnus Oshaug Pedersen (SINTEF Ocean) for operating the Hobo-loggers, Hartvig Christie (NIVA) for scientific discussion of the MS content, Ekaterina Storhaug (Akvaplan-Niva) for providing the map (figure 1), Magnus Aune for guidance with Rstudio, and Ole-Jacob Broch (SINTEF Ocean) for mathematical guidance with growth and shedding rates.

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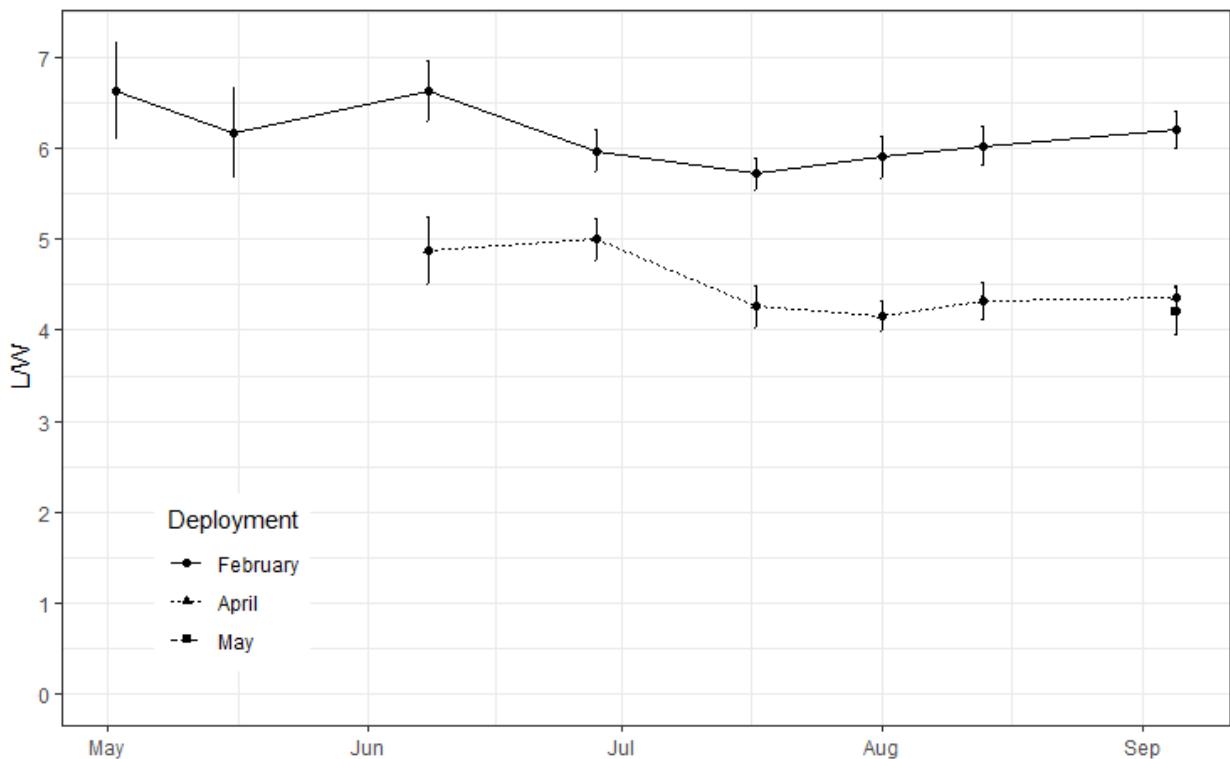
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Electronic Supplementary Material 1:



ESM 1 Length to width on *Saccharina latissima* fronds outplanted at three different times (February, April and May) from 5 May until 5 September.

Electronic Supplementary Material 2:

Regression coefficients for the relationships between variables associated with relative Daily Growth rates (DGR) and relative Daily Shedding Rate (DSR) of *Saccharina latissima* and nutrient status, extracellular nitrate (E-DIN), intracellular nitrate (I-DIN), and total nitrogen content (QN). P values are presented in bold for $\alpha_{\text{crit}}(0.05)$

Relation y versus x	Intercept y-axis (b)	Slope (a)	R ²	Adjusted R ²	F (df1,df2)	P
DGR versus E-DIN	0.008	0.012	0.297	0.226	4.22 (1,10)	0.067
DSR versus E-DIN	0.003	0.001	0.114	0.025	1.28 (1,10)	0.284
DGR versus I-DIN	0.013	0.009	0.296	0.226	4.21 (1,10)	0.067
DSR versus I-DIN	0.003	0.002	0.141	0.056	1.65 (1,10)	0.228
DGR versus QN	0.007	0.006	0.426	0.369	7.42 (1,10)	0.021
DSR versus QN	0.002	0.001	0.129	0.042	1.48 (1,10)	0.252
DSR versus DGR	0.002	0.073	0.065	-0.028	0.70 (1,10)	0.423
DGR versus delayed E-DIN	0.011	0.007	0.324	0.256	4.79 (1,10)	0.054
DSR versus delayed E-DIN	0.003	0.002	0.175	0.092	2.12 (1,10)	0.176

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