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Influence of food availability and nutritional state of macroalgae on development of fouling bryozoans on cultivated *Saccharina latissima*

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Marine Coastal Development
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Abstract

Fouling of bryozoans is a major challenge in the Norwegian macroalgae industry. Bryozoans are one of the prominent epiphytes colonizing macroalgae, causing mechanical damage, competing for nutrient, shading and decreasing reproductive output. Farming of macroalgae has mainly been undertaken in relatively exposed waters, where nutritional conditions are most optimal and potentially harmful biological interactions less severe. Resource availability, physical conditions and the interactions among organism are different in sheltered and exposed waters and may affect the nutritional state of the macroalgae accordingly. Bryozoans feed on phytoplankton and their abundance might be different in sheltered and exposed locations.

This thesis examines how selected external conditions affected the bryozoan fouling on *S. latissima* cultured at different physical exposures, with a special focus on how nutritional state of the macroalgae affected the development and intensity of fouling, and how phytoplankton food concentrations affected bryozoan coverage. A field experiment was conducted from February to July 2017, where *S. latissima* were cultivated at 3m and 8m depths at three locations situated along an exposure gradient off the coast of Norway. Lamina were collected throughout the cultivation period for estimates of bryozoan coverage and intracellularly nitrate concentrations. Water samples were taken with regular intervals for inorganic nutrient and chlorophyll *a* analysis.

Physical exposure was found to have no significant ($P > 0.05$) impact on seaweed cultivated at 3m depth. With regards to cultivation depth, the results revealed a depth dependent growth with higher growth at 3m than at 8m depth, at Sheltered location. Intracellular dissolved nitrate content decreased towards the summer, with significantly higher concentrations at the Exposed location ($p<0.05$). The results revealed a weak positive correlation between external nitrate concentrations and intracellular dissolved nitrate concentrations. Bryozoan colonies settled in mid-May. Although abundant at all cultivation depths, the results revealed a decrease in bryozoan coverage with an increase in exposure at 3m depth, and an increase in coverage with an increase in exposure, at 8m depth. There was a weak relationship between bryozoan coverage and intracellular nitrate concentrations in the macoalgae, with an increase in coverage with a decrease in intracellular nitrate concentrations over the cultivation period. All locations showed good bryozoan food availabilities with no statistical differences. The less exposed locations showed a strong relationship were the variation in bryozoan coverage can be explained by the variation in chlorophyll a food fraction.

Samandrag

Påvekst av epifytt er ein stor utfordring i norsk tareindustri. Mosdyr er ein av dei dominante epifyttane som koloniserer tare. Mosdyr kan forårsake mekanisk skade, konkurrere om næringsstoff, skygge for lys eller redusera utsikta til reproduksjon. Oppdrett av tare vert vanlegvis gjort i eksponerte farvatn der næringstilhøva er mest optimale og skadepotensialet for biologiske interaksjonar er lågare. Taren sin ernæringsmessige tilstand kan være ulik mellom skjerma og eksponerte farvatn, då både tilgang på ressursar og dei fysiske tilhøva er ulike. Samspelet mellom ulike organismar kan også være forskjellig langs kvar eksponeringsgradient. Mosdyr beitar på planteplankton, og deira mattilgang kan variere mellom skjerma og utsette lokalitetar.

Denne studien tar sikte på å undersøka korleis taren sin næringsstatus påverkar utviklinga og intensiteten av påvekst av mosdyr på *Saccharina latissima* dyrka i ulike fysiske forhold, og om konsentrasjonen av planteplankton påverkar påvekst av mosdyr. Felteksperiment blei gjennomført frå februar til juli 2017 på kysten av Trøndelag. Tare blei dyrka på 3 og 8 meters djup på tre ulike stader langs ein eksponeringsgradient. Tarelamina blei regelmessig samla inn gjennom heile forsøksperioden for å kunne estimere påvekst av mosdyr og analysere nitratinnhald. Prøver av vatnet blei også tatt regelmessig for å kunne estimere konsentrasjonen av klorofyll-*a* og uorganisk næringsstoff.

Ulik fysisk eksponering hadde ingen signifikant ($P<0.05$) påverknad på tarevekst på 3 m djupn. Resultata viste ein djupnavhengig vekst med redusert eksponering, der tare dyrka skjerma på 3 m djupn hadde betre vekst enn på 8 m djupn. Intracellulært oppløyst nitratinnhald sank med sesongen. Eksponert lokalitet hadde betrakteleg høgare konsentrasjonar enn skjerma lokalitet. Resultata viste ein svak korrelasjon mellom det eksterne nitratinnhaldet og det intracellulære oppløyste nitratinnhaldet. Koloniar av mosdyr blei observert i midten av mai. Mosdyr var til stades på alle lokalitetar, men viste ein reduksjon i påvekst ved aukande eksponering på 3 m djupn, og ein reduksjon i påvekst ved mindre eksponering på 8 meters djupn. Det var inga klar samanheng mellom påvekst av mosdyr og intracellulære nitratkonsentrasjonar i løpet av forsøksperioden. Alle lokalitetane viste god mattilgang for mosdyr utan statistiske forskjellar. Skjerma lokasjon viste eit klart forhold der variasjonen i mosdyr på vekst kan forklarst av variasjon i klorofyll-*a* konsentrasjonar.

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Acronyms

	Explanation	**Unit**
N	Nitrogen	
PON	Particulate organic nitrogen	
DON	Dissolved organic nitrogen	
I-Nitrate	Intracellular dissolved nitrate	mg NO_3-/g DW
E-Nitrate	External nitrate + nitrite	µg/L
E-Phosphate	External phosphate	µg/L
E-Ammonim	External ammonium	µg/L
Chl *a*	Chlorophyll *a*	µg/L
DW	Dry weight (% of wet weight) of algae	
RGR	Relative growth rate	

1 Introduction

The world's population is growing, and it is estimated that by 2050 it will have reached 9 billion. However, with a growing population, it comes a growing demand for food sources. The demand for human food, animal feed, and biofuels are growing and thus is the increased need for new cultivation areas. However, these aspects are challenging in the context of climate change, economic and financial uncertainty, and growing competition for natural resources. The need for renewable resources have never been bigger, and the farming of seaweed may be one step in the right direction to meet these needs.

1.1 Seaweeds

Seaweed, also referred to as marine macroalgae, are among the most ecologically and economically important living resources in the world oceans, offering a wide range of applications without the need for fresh water, land area and fertilizers. Even though seaweeds only occupy a fraction of the world's oceans, they are responsible for 5-10 % of the marine primary production (Hurd, Harrison, Bischof, & Lobban, 2014a). Seaweed are classified into three different main groups based on pigmentation; brown (Phaeophyta), red (Rhodophyta) and green (Chlorophyta). Brown seaweed usually range in size from giant kelp that can grow up to 20 m long, to smaller species approximately 30 – 60 cm long. Red and green seaweeds are smaller, ranging from a few centimeters to meters in size.

Over the last decades' aquaculture has become one of the biggest and fastest growing food industries. In 2014 the world aquaculture production, including fish and aquatic plants, reached 101 million tonnes in live weight (FAO, 2014). Furthermore, the global production of aquatic algae (marine macroalgae, and marine and freshwater microalgae) has been growing rapidly and reached around 31 million tonnes in 2016 (FAO, 2018). Most of the produced biomass is the macro algae species *Eucheuma spp, Kappaphycus Alvaerzii* and *Saccharina japonica* (FAO, 2014). Around 80 % of the total produced biomass is used for food, while the rest are used in feed, fertilizers, biotechnology and for medical use (Bartsch et al., 2008; McHugh, 2003). The production take place in 50 different countries, with around 85 % of the production situated in Asia while only a small percentage is produced in Europe (FAO, 2014).

In Europe, the history of using macroalage as food and feed goes all the way back to the Vikings. They brought dried, nutrient rich macroalgae with them on their expeditions to protect themselves from diseases (Mouritsen et al., 2013). Today there is increasing interest in macroalgae cultivation in Europe due to the high nutritional value of natural vitamins, minerals,

and proteins, with several countries developing a macroalgae industry, such as; France, Spain, Portugal, Ireland and Norway (Taelman, Champenois, Edwards, De Meester, & Dewulf, 2015).

Norway has one of Europe's lagers economic zones with a coastline reaching 2.5 times around equator, and large areas suitable for macroalgae cultivation (Skjermo et al., 2014). In Norway, the species *Saccharina latissima*, *Alaria esculenta*, *Laminaria digitata* and *Laminaria hyperborea* is seen as the macroalgae with the greatest potential for large scale cultivation (Handå et al., 2009; Rueness & Steen, 2008). The area allocated to seaweed cultivation reached 277 hectare (ha) in 2017 and corresponds to a production potential of approximately 16 000 tonnes. Furthermore, there is a rapid development of efficient farming strategies, mechanisation of seedling deployment, biomass and crop handling logistics (Stévant, Rebours, & Chapman, 2017).

1.2 Saccharina latissima (Experimental species)

Saccharina latissima (Linnaeus, Lanes, Mayes, Druehl, and Saunders) [synonym: *Laminaria saccharina]* is a brown alga belonging to the phylum Ochrophyta, the class of Phaeophyceae (brown algae), and the Laminariales order (commonly referred to as kelp), with the common name sugar kelp. *S. latissima* is an attractive candidate for culturing, being one of the fastest growing kelp species in European waters and containing high amount of carbohydrates (Lane, Mayes, Druehl, & Saunders, 2006). The species has a circumpolar distribution on the northern hemisphere, from Spitsbergen in the north to Portugal in the south, at temperatures below 19 °C. It inhabits sheltered locations from the sublittoral zone to the lower photic zone (Handå et al., 2009).

S. latissima is a short-lived species, well adapted to low temperatures and seasonality in the northern hemisphere. This perennial species has a seasonal development with maximum growth during winter and spring followed by period of reduced growth during summer, and sorus formation during autumn and early winter (Bartsch et al., 2008; Kain, 1979; Lüning, 1979). Forbord et al. (2012) registered the highest growth rate from February to June when *S. latissima* was cultivated in Trøndelag, Norway.

Kelp sporophytes can be divided into holdfast, stipes, and lamina (Figure 1). *S. latissima* has a branched holdfast and a smooth stipes. New tissue is produced in the meristem, located by the stipes (Lüning, 1990). *S. latissima* has a diploid macroscopic sporophyte phase and microscopic haploid gametophyte phase. Sporangia is a spore holding cell, produced at the distal end, where the production of zoospores occurs by meiosis (Kain, 1979). A dense area of sporangia is called

sori. In Norway, sori occurs on the sporophyte from October to December, and by meristem removal and exposure to a short-day regime the production of sori can be artificially triggered (Buchholz & Lüning, 1999; Forbord et al., 2012).

Figure 1 Illustration of the subdivision of the sporophyte thallus, with holdfast, stipes and lamina in S. latissima (Førde et al., 2016).

1.3 Macroalage growth conditions

Worldwide, macroalgae are normally cultivated in shallow water and sandy sea bottoms (Taelman et al., 2015). The most important factors for macroalgae growth are light, temperature, nutrients, and current conditions, which are different in sheltered and exposed conditions. Photosynthesis and nutrient uptake is shown to increase with increasing velocity, as the supply of nutrient across the lamina increases and metabolic waste products are removed (Hurd, Harrison, Bischof, & Lobban, 2014b). High current conditions assures nutrient supply and prevents potentially harmful biological interactions, as well as, preventing shading of sediment particles (Handå et al., 2009).

Grazing animals and diseases are a common problem in cultivation. One example is the ice-ice disease, a common disease affecting macroalgae cultivated under low light conditions, slow water movement or low nutrients concentrations (Titlyanov & Titlyanova, 2010).

Macroalgae cultivation in the western world have normally been undertaken in relatively exposed waters were nutritional conditions believed to be more optimal and other potentially harmful interactions are less severe. In Norwegian cultivation of macroalgae there are no documented experiences with diseases, however biofouling has been and is a major problem (Skjermo et al., 2014). The cultivated crops are normally harvested before onset of fouling to ensure the best product.

1.4 Growth and nitrogen utilization

Nutrients can be found in different forms in seawater. Nitrogen are found in inorganic forms such as nitrate (NO_3^-), nitrite (NO_2) or ammonium (NH_{4+}), or in organic forms as particulate organic nitrogen (PON) or dissolved organic nitrogen (DON) (Hanisak, 1990). Inorganic nutrients are required for growth and photosynthesis in macroalgae. Due to stratification in the water column, macroalgae cultivated at different depth will experience different nutrient concentrations, because surface water normally contains less nutrients than deeper water, since there is higher primary production in the upper water column (Butler, Knox, & Liddicoat, 1979).

The availability of light and nitrogen is considered to be some of the main resource limiting macroalgae growth. The growth rate of *S. latissima* follows the seasonal abundance of nitrate in the water from high concentrations in late winter and early spring, to low in late spring, summer and autumn (Gagné, Mann, & Chapman, 1982). However, when light levels are low during autumn and winter there is little macroalgal growth, despite high nutrient concentrations in the water. Growth is initiated in spring, when light levels are higher (Nielsen et al., 2014).

Nitrogen is passively absorbed in the macroalgae as ammonium or actively as nitrate (Raven, Wollenweber, & Handley, 1992). Ammonium requires less energy for transport and metabolism and can be used for amino acid synthesis immediately, while nitrate must be reduced to nitrite, and further to ammonium in cells before utilization (Raven, 1984). In some Laminaria species, nitrate can be accumulated intracellularly when external nitrate concentrations are greater than 10 µmol/L (Chapman, Markham, & Lüning, 1978; Wheeler & Weidner, 1983), These nitrogen reserves, normally accumulated in the winter, can be utilized for growth, in early summer, when nitrogen is limited in the environment (Chapman & Craigie, 1977; Gordillo, Dring, & Savidge, 2002; Wiencke & Bischof, 2012).

The internal concentrations of a nutrient is dependent on the external concentrations in the environment and is a result of macroalgal uptake (Hurd et al., 2014a). Wheeler and Weidner (1983) showed that the intracellular nitrate concentrations in *S. latissima* increases linearly with the external nitrate concentrations. Chapman et al. (1978) found that internal nitrate concentrations affect photosynthetic activity. *S. latissima* cultivated in low nitrate concentrations, 0-3 µmol/L, have a lighter pigmentation than those cultivated in higher nitrate concentrations up to 20 µmol/L, and that chlorophyll concentrations in the algae increases with increasing nitrate concentrations.

1.5 Bryozoans

Bryozoan colonies are compiled by small box-like shaped individuals (zooids) that arises by asexual budding from a cestrula, originating from a sexually produced larva. They feed on phytoplankton by filtering particles from the water with their lophophore, bearing ciliated tentacles. The phylum consists of two living classes of marine bryozoa; Stenolaemata and Gymnolaemata, and three orders; Cyclostomatida, Ctenostomatida and Cheilostomatida (Hayward & Ryland, 2017).

1.5.1 Membranipora membranacea and *Electra pilosa*

The most common epiphytic bryozoan species colonizing kelp in North-Atlantic waters are the calcified and highly polymorphic *Membranipora membranacea* (Linnaeus) and *Electra Pilosa* (Linnaeus) (Figure 2). The individual zooids of *M. membranacea* (size approximately 0, 4 x 0, 15 mm) and *E. pilosa* (size approximately 0, 45 x 0, 3 mm) are lightly calcified forming mat, sheet-like colonies on the fronds of the seaweed (Hayward & Ryland, 2017). These species are commonly found in shallow and coastal waters, *M. membranacea* is normally colonizing Laminaria species, while *E. pilosa* have a broader habitat distribution (Ryland, 1962). Both species have a high fecundity, producing planktonic cyphonautes larvae that settle on the macroalgae (Seed & O'Connor, 1981). After settlement, they metamorphose into a feeding stage and form colonies by asexually bud new zooids (Saunders & Metaxas, 2007).

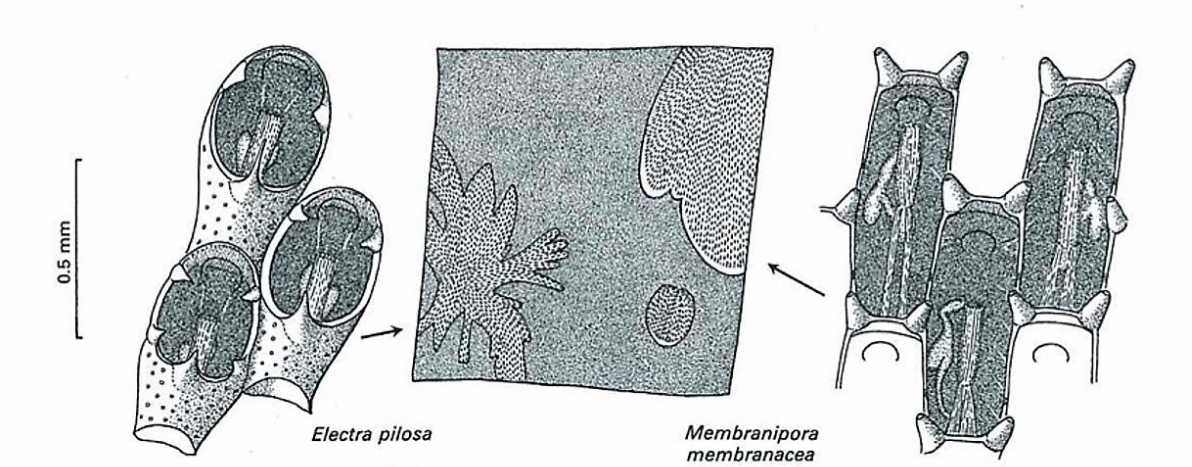


Figure 2 Illustration of zooids and colonies of Electra pilosa and Membranipora membranacea (Hayward & Ryland, 2017).

1.5.2 Environmental adaptations

In ectothermic animals the growth rate is normally positively related to temperature (Trudgill, Honek, Li, & Straalen, 2005). Studies have found that higher temperatures during macroalgae growth period could result in higher growth rates of *M. membranacea* colonies. Higher growth rates was observed with temperatures between 5.7°C and 16.2°C (Saunders & Metaxas, 2007, 2009). In a Norwegian fjord the maximum growth was measured during late spring and summer when the temperature increased from 8°C in May to 16.5°C in July (Nair, 1962). A study conducted by Menon (1972) showed that increasing temperatures during spring and summer initiated zooid growth in *M. membranacea* and *E. pilosa*, and that elevation in temperature triggered faster growth in these species.

Level of exposure is one of the abiotic factors affecting abundance of bryozoans settling on Laminaria species. Continuous exposure to moderate wave activity control epiphytic growth by washing away new settlers (Sogn Andersen, Steen, Christie, Fredriksen, & Moy, 2011; Strand & Weisner, 1996). *M. membranacea* is known for coping well with high water flows around the lamina it is covering, as the species can reduce its zooid size in high water velocities. These adaptable zooids, enable *M. membranacea* to position the polypide into the boundary layer around the lamina with lower flow regimes where food can be collected (Okamura & Partridge, 1999). The fronds of the Laminarians are highly flexible and the bryozoan colonies will experience unidirectional flow regimes with ingoing and outgoing tidal currents (Bartsch et al., 2008). *E. pilosa* is known for being tolerant for a wide range of environmental conditions like high turbulent water conditions and slit loading. The light calcification, in both species, is an adaptation needed to withstand the algae flexure caused by wave action and protection against sediment particles (Seed & O'Connor, 1981).

1.5.3 Polypide morphology and feeding behaviour

Bryozoan abundance may also be regulated by food supply acting independently or in interaction with temperature (O'Dea & Okamura, 1999; Saunders & Metaxas, 2009). Bryozoans are distributed over a wide range of environmental conditions, and like other suspension feeders they most likely have a broad diet of different phytoplankton species. The phytoplankton diversity can differ greatly in the different seasons and at the different localities the bryozoans inhabit (Winston, Woollacott, & Zimmer, 1977).

In *Membranipora sp*., polypides are organized into fixed clusters with chimneys between them (Cook, 1977). When feeding, polypides actively pops out of the zooid and move the tentacles

in a constant flickering motion, to steer selected particles down towards the mouth (Winston, 1978). The feeding apparatus in bryozoans (Figure 3) is a part of the polypide and consist of a ring of ciliated tentacles forming a lophophore. The lophophore is shaped like a cone with its tentacles bent outward with the mouth at the centre. The tentacles are covered with cilia organized in three different rows; lateral, frontal and laterofrontal. The lateral cilia produce water current which the frontal cilia steer towards the mouth (Riisgård & Manríquez, 1997). The laterofrontal cilia might be important for particle capturing acting as a sensor, detecting the presence of particles, or as a sieve which retains the suspended food particles (Gordon, 1974; Riisgård & Manríquez, 1997; Winston, 1978).

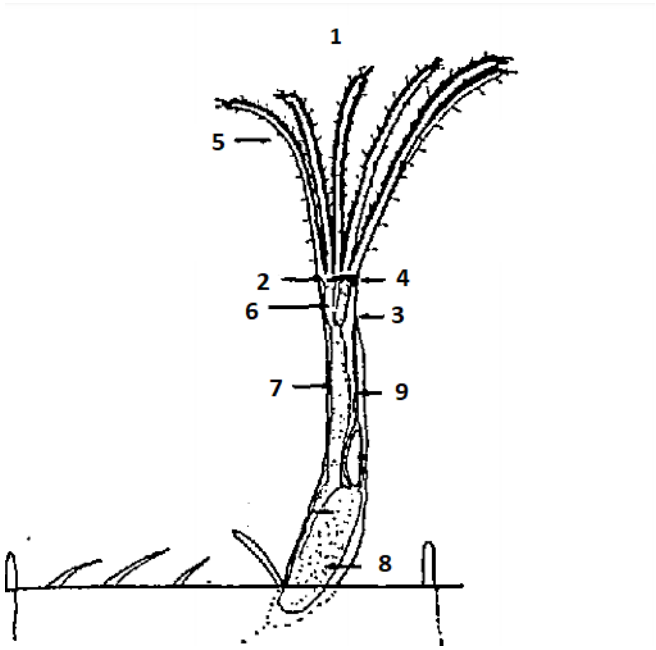


Figure 3 Bryozoan polypide showing orientation of main features. 1: Lophophore, 2: Mouth, 3: Anus, 4: Nerve ganglion, 5: Tentacles with cilia, 6: Ciliated pharynx, 7: Cardial region, 8: Stomach, 9: Rectal region. (Winston, 1978).

Studies of bryozoan growth shows that small bryozoan colonies have an exponential growth rate when high food concentrations are available. Growth rate decreases when the colonies become bigger and less substrate is available for colonization. *M. membranacea* and *E. pilosa* showed a doubling of colony area in 5 to 6 days when food concentrations were adequate (Hermansen, Larsen, & Riisgård, 2001). Riisgård and Goldson (1997) found that 100 % of the zooids in *E. pilosa* were feeding when the phytoplankton concentrations was between 0.5 to 5 µg chl *a*/L . Maximum growth of *E. pilosa* has been measured when *Rhodomonas sp.* concentrations was between 1000 and 1500 cells/mL, which is equivalent to 1.3-1.9 µg chl *a*/L (Hermansen et al., 2001).

The different morphological characteristics like mouth size, lophophore shape and length of tentacles may set a physical limit on the size of particles that can be collected. The most limiting factor is imposed by mouth size, ranging from 15 to 91 µm in the species measured by Winston (1978). *Electra sp*. have small round mouths with a mean diameter around 17 µm, while *Membranipora sp*. have a bigger oblong to round mouths with a mean diameter around 28 µm (Winston, 1978), making *Membranipora sp*. able to ingest larger particles.

Riisgård and Manriques (1997) found that that the particle retention efficiency drops when particles diameter is below 6µm, in Electra sp. The row of stiff laterofrontal cilia on the tentacles, each about 20 µm long and spaced approximately 5µm apart, act as a filter. These observations indicate that Electra sp. can only efficiently filter particles >6 µm, while particles <5 µm are passing through the filter apparatus, e.g. *Isochrysis* (4µm).

Growth and feeding behaviour of bryozoans have mainly been studied under controlled conditions in the laboratory, were they have successfully been fed the phytoplankton species *Rhodomonas sp*., *Rhinomonas sp*. (6µm) and *Tertraselmis sp*. (14µm) (Bayer, Cormack, & Todd, 1994; Riisgård & Goldson, 1997; Riisgård & Manríquez, 1997). Hunt (1925) analysed the gut of *M. tuberculata* and found diatoms, coccolithophores, dinoflagellates and nematocysts.

1.6 Biofouling by bryozoans

Biofouling of epiphytes is a major challenge in global commercial macroalgae cultivation (Fletcher, 1995; Forbord et al., 2012; Peteiro & Freire, 2013), and the biofouling is dominated by colonies of the sessile filter feeding bryozoan (Ryland, 1962). Bryozoan epiphytes constitute a mechanical barrier affecting nutrient uptake, defoliation, photosynthesis, and reproductive output (Bartsch et al., 2008; Hepburn, Hurd, & Frew, 2006; C. Hurd, Durante, Chia, & Harrison, 1994; Kain, 1975; Seed & O'Connor, 1981). Additionally, heavy coverage of bryozoans' causes the macroalgae to become brittle and more susceptible to breakage (Dixon, Schroeter, & Kastendiek, 1981; Hepburn & Hurd, 2005; Hepburn et al., 2006). The flexible and wide lamina of kelp serves as an excellent substrate and habitat for these sessile organisms (Bartsch et al., 2008).

Hurd et al. (1994) found that nitrate and ammonium uptake rate decreased with 30-50% when the seaweed *Agarum fimbriatum* and *Macrocystis integrifolia* were colonized with the bryozoan *Membranipora membranacea*. Additionally, a study on the kelp *Macrocystis pyrifera* showed that pigment concentrations was reduced and photosynthetic activity was lowered when it was

colonized with bryozoans (Hepburn et al., 2006). Gómez et al. (2011) found that physiological parameters such as maximum quantum yield and overall photosynthetic activity declined with increasing bryozoan colonization on the kelp *M. pyrifera*. Finally, the bryozoan species *M. membranacea* has been found to inhibit sporulation in *Laminaria sp.* and affecting sori size in *Laminaria hyperborea* (Kain, 1975; Seed & O'Connor, 1981).

The relationship between the bryozoan and their macroalgae host can be thought to be mutual beneficial, but this physical relationship depends on the degree of cover and the physiological state of the macroalgae (De Burgh & Fankboner, 1978; C. Hurd et al., 1994). Bryozoan coverage might enhance nutrient uptake and growth in seaweed when ammonium is excreted directly onto lamina, while the macroalgae offers a continuous renewable and highly flexible substrate for the bryozoans (Hepburn & Hurd, 2005). Furthermore, macroalgae might provide organic carbon to the bryozoans, while the bryozoans provide carbon dioxide to the colonized host (De Burgh & Fankboner, 1978; Muñoz, Cancino, & Molina, 1991).

Whether the relationship causes cost or is beneficial depends on the macroalgae species, bryozoan species, intensity of colonization, physiological status of the macroalgae and nutrient availability in the seawater (De Burgh & Fankboner, 1978; C. Hurd et al., 1994).

1.7 Aim of study

This study examines how selected external conditions affect the bryozoan fouling on *Saccharina latissima* cultured at different physical exposures, with a special focus on how nutritional state of the macroalgae affected the development and intensity of biofouling, and how phytoplankton food concentrations affected bryozoan abundance on *S. latissima*.

The study aims to:

- Investigate growth and nutritional state at the different physical exposures at 3m and 8m depths, by taking regular samplings of cultivated *S. latissima* during the cultivation period.
- Investigate exposure and depth dependencies in bryozoan colonization, by taking measurements of bryozoan coverage during cultivation period.
- Investigate environmental dependencies of bryozoan growth, by looking at environmental measurements such as temperature and inorganic nutrient in the water.
- Investigate if bryozoan food concentrations affect bryozoan growth and colonization, by taking regular samplings of chlorophyll *a* particles between <70µm and <5µm of size.

The following hypotheses were formulated:

H1: Growth and nutritional state of *S. latissima* will vary across categories of exposure and depths

H2: Bryozoan coverage on *S. latissima* will vary across categories of exposure and depths

H3: Bryozoan coverage on *S. latissima* will increase as intracellular nitrate concentrations in macroalgae decrease

H4: Higher phytoplankton concentrations will affect bryozoan growth and colonization

2 Methods

2.1 Study area and experimental design

The field experiment was carried out in Skjørafjord (64°09' N, 10°17' E), which is a potential new macroalgae cultivation site, off the coast of Norway (Figure 4). Skjørafjord is a 12 km long southeast facing fjord, separating Roan and Åfjord municipality northwest of Trondheim. *S. latissimia* was cultivated at three different locations situated along an exposure gradient. Location 1 – Sheltered in the inner part of fjord, location 2 – Intermediate, in the mid-fjord, and location 3 – Exposed at the mouth of the fjord (Figure 4).

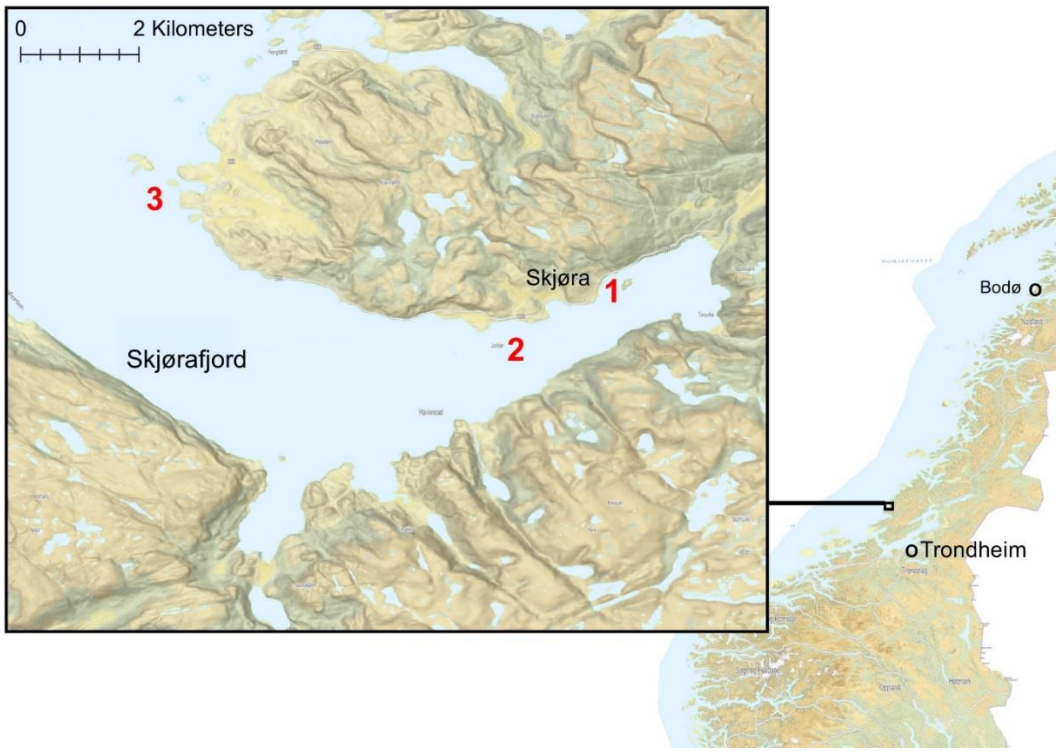


Figure 4 Geographical location of the three sampling stations in Skjørafjord, Norway (64°09' N, 10°17' E). 1: Sheltered location, 2: Intermediate location and 3: Exposed location. (Statens Kartverk, 2018).

Algae were cultivated at 1 – 3 m and 6 – 8 m below the surface attached on ropes hanging vertically from a longline system (Figure 5**)**. Six 12 mm polyester silk ropes were attached to each locations' longline system, approximately 2.5 meters apart. Three ropes were used for sampling and three ropes were used for weight measurements of the seaweed. The algae were seeded on a string in the laboratory and grown to a size of approximately 1 – 2 cm and winded around the ropes before deployment.

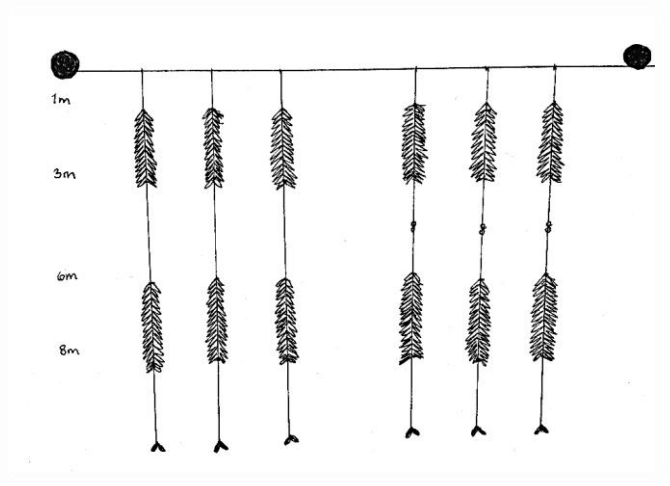


Figure 5 Illustration of the ropes hanging from a longline system. S. latissima was cultivated from 1 – 3m and 6 – 8m. Three of the ropes was used for sampling and three of the ropes was used for weight measurements.

2.2 Algae cultivation

The sporelings used for cultivation was produced in November 2016 by Seaweed Energy Solutions AS (Trondheim, Norway) by inducing spores from mother algae. *S. latissima* with natural sori was collected from a wild population in Skjørafjord in October 2016. The plants were kept in tanks with aeration and 16:8 light: dark regime until disinfection of sori and spore release.

Mature sori was cut and disinfected with 2 mL NaOCl/l sterile seawater. Disinfected sori was dried and stored in plastic zip-lock bags in a fridge, overnight. For spore release, the dehydrated sori was placed in sterile seawater in 60 min. Cultures were inoculated with 250 000 cells/mL and cultivated for 6 weeks in red light conditions at 12°C in a 16:8 h light: dark regime with a light intensity of 20 µmol/m^{-2}/s^{-1}. The gametophyte culture was placed in white light conditions with aeration for 14 days, at 10°C in a 17:7 h light: dark light regime with an intensity of 40 µmol/ m^{-2}/s^{-1}. Sporophytes were seeded onto polyester string and cultivated for 8 weeks in 35L tanks with constant flow through sterile seawater and gentle aeration.

Seedlings were deployed in Skjørafjord February 15th, 2017.

2.3 Sampling and measurements

The sampling period lasted from March 30th to July 20th, 2017. Samples were collected at the three different locations in Skjørafjord approximately every fortnight when the weather made it possible.

Sampling dates are summarized in Table 1 together with total number of lamina sampled at each location, CTD and water samples.

*Table 1 Overview of sampling dates and number of seaweed samples for both depth combined, CTD and water samples. CTD samples were only depth and temperature were measured is marked with *.*

Sampling number	Date	Total number or individuals sampled			CTD	Water samples
		Sheltered	*Intermediate*	*Exposed*		
Deployment	15.02.2017	N/A	N/A	N/A	X	X
1.	30.04.2017	12	12	12	X	X
2.	03.05.2017	12	12	12	X*	X
3.	15.05.2017	12	12	12	X*	X
4.	26.05.2017	12	12	12	X*	X
5.	06.06.2017	12	12	12	N/A	X
6.	26.06.2017	12	12	12	X	X
7.	20.07.2017	12	12	12	X	X

2.3.1 Biomass samples

On each sampling day, four individuals from each replicate rope were randomly collected, two individuals from each depth, and placed in plastic zip-lock bags. When returning from the field (approximately 10h after sampling), the algae were deep-frozen at -20°C and stored for later picture and nutritional analysis.

2.3.2 Environmental and nutrition conditions

Temperature, salinity, and depth were measured using a CTD (RBR Concerto and SAIV A/S SD204 Model). A CTD profile was taken from 0 to approximately 10 m at each location, when the weather made it possible.

Water samples of 5 L were taken from three meters and eight meters at each station using a Nansen water collector. The sample were kept cool and shaded until filtration and fixation was

done. Water was filtered into three different water fractions, <200 µm, <70 µm and <5 µm, and 200 mL x 3 of each fraction were filtered onto a 25 mm GF/F filters for later chlorophyll *a* analysis. The filters were packed in aluminium foil and 2 x 100 mL GF/F filtrated sea water were transferred to 100 mL bottles, transported back to the laboratory, where they were frozen at -20°C for further analysis.

The filters were extracted in 100 % methanol for two hours at 4°C. After extraction, the extractant with filters were filtered through a 0.2 µm syringe filter before chlorophyll *a* concentrations were measured on a Turner Design Trilogy fluorometer with the fluorescence module chlorophyll *a* (Non-Acid) (485 nm excitation filter, 685/10 nm emission filter). Chlorophyll *a* concentrations µg/L was calculated using Equation 1.

Equation 1: $$\mu g\ Chl\ a/L = \frac{((FL-BL)\times F\times R\times 1000)}{V\times 1000}$$

FL is the fluorescence of sample, BL is the fluorescence of blank, F is the calibration factor, E is the extraction volume, and V is the filtered volume.

The concentrations of phosphate (PO_4^{3-}), nitrate ($NO_3 + NO_2$) and ammonium (NH_4) in the GF-F filtered water samples was determined with a Flow solution IV system O.I. Analytical Auto analyser. The concentrations were estimated according to standard procedure in the laboratory.

2.4 Macroalgae Analysis

2.4.1 Intracellular dissolved nitrate (NO_3-) analysis

0. 06 g half frozen *S. latissima* was transferred to 15 mL glass tubes and mixed with 6 mL distilled water. The glass tubes were boiled for 30 min, with a marble on top. The samples were cooled down to room temperature and filtered with a 0, 45 µm polysulfone syringe filter to remove algae particles. Intracellular nitrate concentrations were determined with a Flow Solution IV system O.I. Analytical Auto analyzer, after the NS 4745 standardization. Intracellular dissolved nitrate concentrations were calculated using Equation 2.

Equation 2: $$mg\ NO_3\text{-}N/g\ DW = \frac{V\times N\times DM}{1000}$$

V is the total volume in liter, N is the NO_3-N measured (µg/*L*) and DM is dry matter of the algae. DM was set to 0.1. This number is based on the assumptions that the dry matter of the algae is 10 % of wet weight.

2.4.2 Image analysis

The individual lamina of *S. latissima* were stretched out on an 8400 cm^2 light table with an opaque Perspex screen and a ruler. An image of the whole lamina was taken with a SONY α 58 camera mounted above the light table. For bryozoan coverage measurements, photos were taken in a dark room while the frond was lighted up with ultra violet light (UV, 365 nm, 230 V). Ultra violet light was used to separate the bryozoan colonies from the frond. Pictures were taken with a shutter speed between 15 and 10 seconds, and ISO 6400. When the bryozoan colonies were not separated enough from the seaweed, camera setting were slightly modified until a satisfactory result was obtained. The procedure was repeated for both sides of the lamina.

Area analysis

The pictures taken with natural light were analysed using the image processing program ImageJ (win64) for area measurements. For the total area measurements, the digital scale was set by measuring 1 cm on the ruler on the image and using the function "Set Scale" in ImageJ. The image was then converted to an 8-bit type and threshold applied. The "Wand tool" was used to select the outline of the frond, and the area was measured by using the "Analyse particles" function. An average of the area of both lamina sides, relative growth rate (RGR) and standard error was calculated in Excel.

Growth rate is defined as the rate of growth in area over time (Yong, Yong, & Anton, 2013). The relative growth rate was calculated using Equation 3.

Equation 3:
$$\frac{(\frac{X_t - X_0}{t})}{X_0}$$

X_t is the area at the end of the growth period t and X_0 is the length at start.

Bryozoan coverage calculations

The pictures taken with ultra violet light (Figure 6 A) was processed in GIMP, a GNU Image manipulation program, by the use of "Analyze and select by colour range" plug-in. An area displaying the pixel range of the bryozoans were chosen, and thus used to define the range of pixels for the bryozoan colonies. All pixles that fell within this given pixel range was selected. The area containing these pixel values was filled with a green colour value, the backgound of the picure was converted to a blue colour value, and lamina converted to a red colour value (Figure 6 B). The percentage coverage was recorded in the historgam function in GIMP. The pixel count for each of the three different colours was recorded. Percent covergae was calculated the use of Equation 4.

Equation 4: $\frac{GPC}{(GPC+RPC)} \times 100$

GPC are the green pixel count, the sum of green and red pixel count (RPC) is eqivalent to total lamina area. Pixel count of both sides of the lamina was added together when calculating total bryozoan coverage on each specimen.

The method used for estimating bryozoan coverage was developed during this study. A more detailed metodology will be found in the master thisis of David Cohen, which is under preparation.

A)

B)

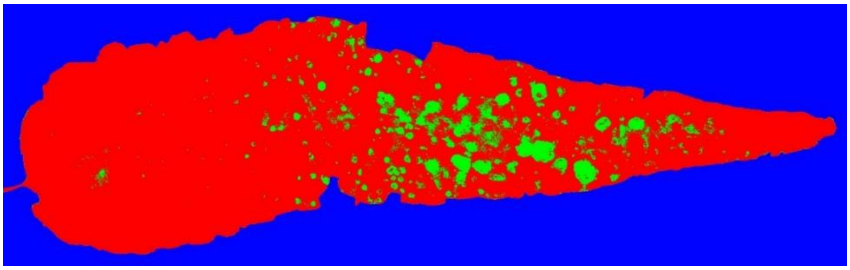


Figure 6 Example of images used for bryozoan coverage calculations. A) Picture of frond lighten up with UV light (upper panel) B) Picture of frond converted to red, green, and blue colours (lower panel).

Carbon:Nitrogen ratio analysis

This study aimed to analyse the carbon: nitrogen ratio in the cultivated *S. latissima*. However, this analysis were not performed due to sick leave.

2.5 Statistical analysis

Microsoft Excel 2016 was used for average and standard error (SE) calculations. All statistical analysis was performed by use of IBM SPSS statistics 25. Graphs was made using the software programming language for statistical computing and graphics R, version 3.0.4 (R Core Team, 2017) through RStudio ™, version 1.1.423, and Microsoft Excel 2016.

The data for area measurements, bryozoan coverage, chl *a* concentrations and I-Nitrate concentrations were tested for normality using a Shapiro-Wilk test. Non-parametric tests were chosen due to non-normal distribution and low sample size (Siegal, 1956).

The Freidman test followed by a post hock Wilcoxon signed rank test were used to test for differences between the different locations and depths.

Spearman's rank correlation coefficient was used to calculate the correlation between E-Nitrate concentrations and I-Nitrate concentrations, between bryozoan coverage and I-Nitrate concentrations, and between bryozoan coverage and chl *a* concentrations.

In the result chapter, the significant differences found refers to this value, unless stated otherwise. Values are given with ± SE.

3 Results

3.1 Environmental conditions, E-nitrate, E-phosphate and E-ammonium

Figure 7 shows water temperature at the different locations at 3 m and 8 m depth throughout the experimental period (March – July 2017). The temperature increased slowly at each location and was generally similar at all locations and both depths.

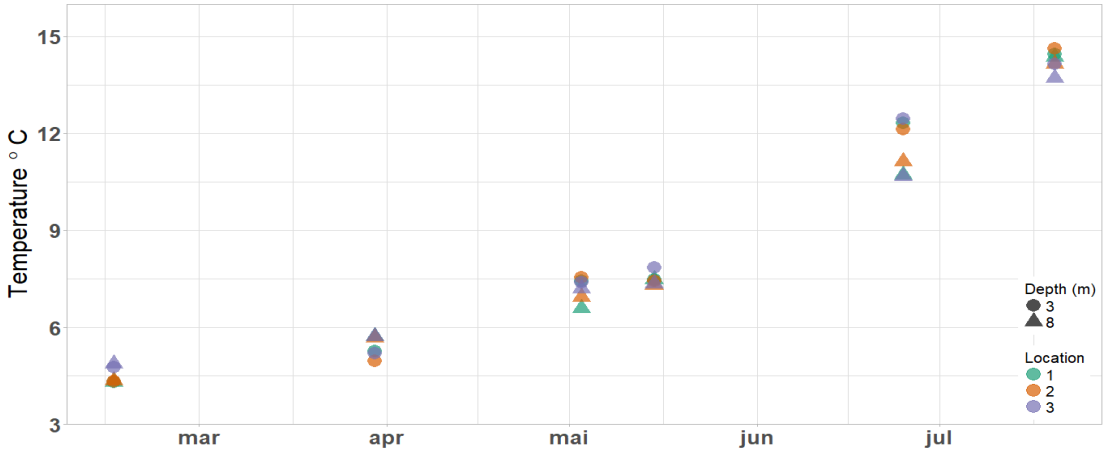


Figure 7 Water temperature (C°) at 3 m and 8m depth at Sheltered-(1), Intermediate-(2) and Exposed location (3) as a function of time (February to July 2017).

Figure 8 shows water temperature (C°) from 1m to 9 m depth at Sheltered and Exposed location from four samples in the time interval between May and July 2017. The temperature transects show the same pattern at both locations.

A) B)

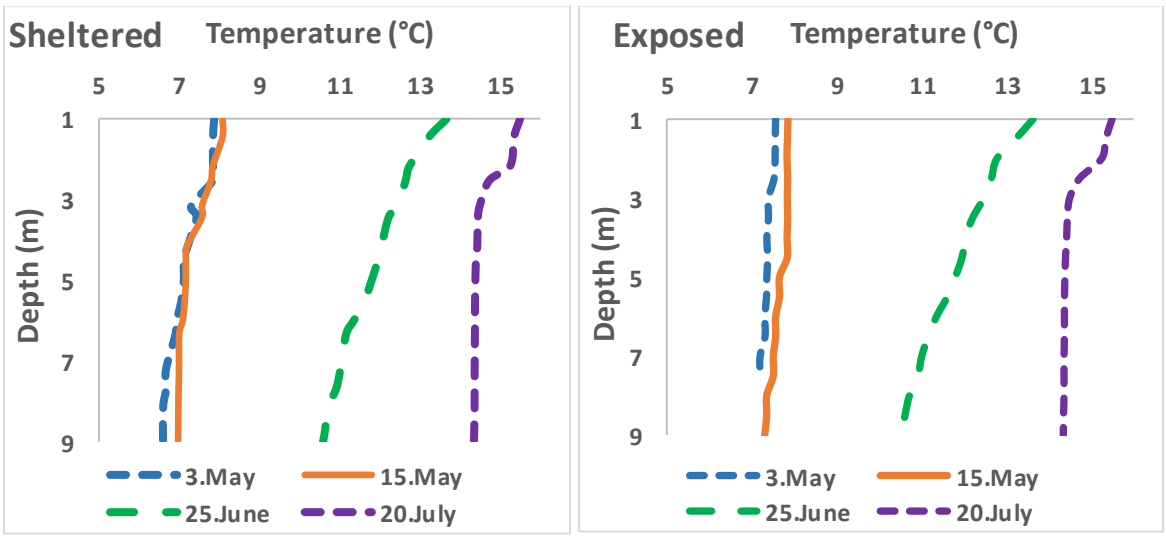


Figure 8 Water temperature from 1 m to 9 m depth at A) Sheltered and B) Exposed location from May to July 2017.

Figure 9 shows salinity at Sheltered-, Intermediate-, and Exposed location at 3 m and 8 m depth as a function of time. The two first measurements at each location show values of normal winter water with a salinity above 32 and the two-last show summer water with lower salinity. The data supports the temperature data, showing throughout efficient mixing. Measurements for May – June 2017 is lacking due to equipment failure.

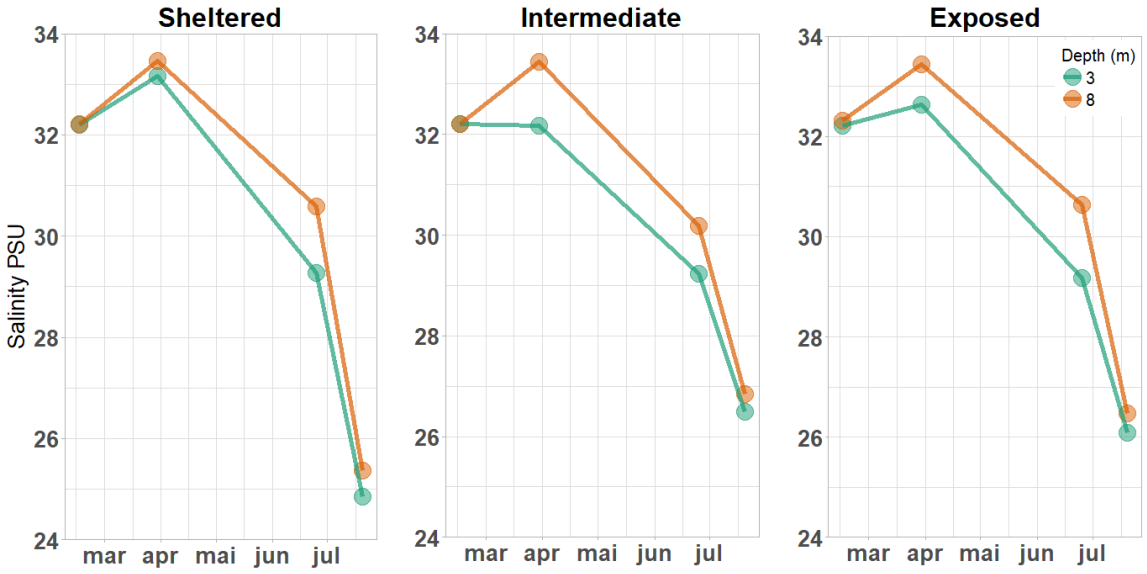
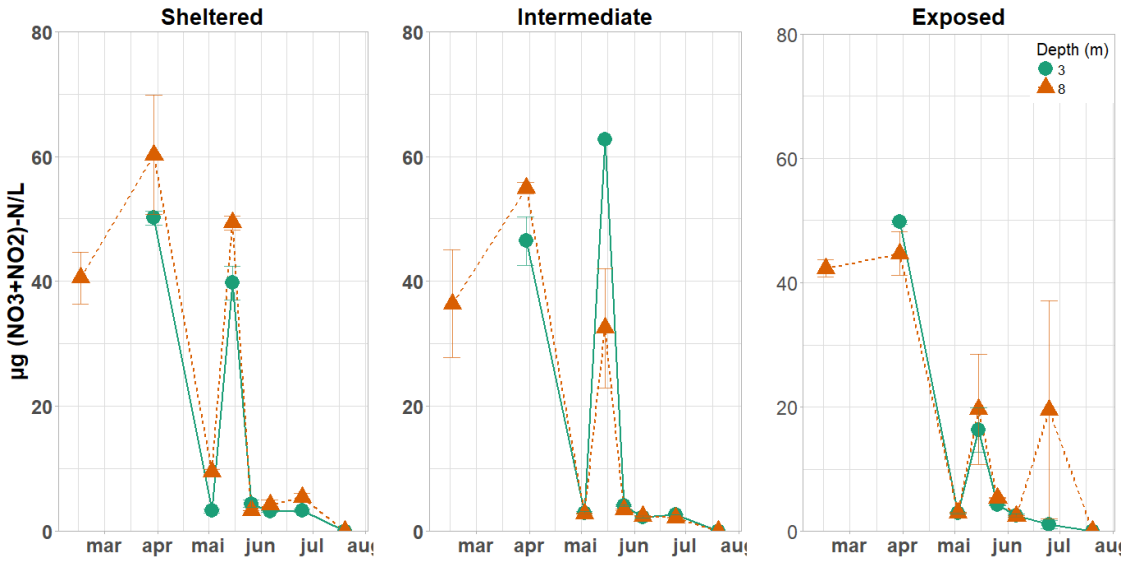


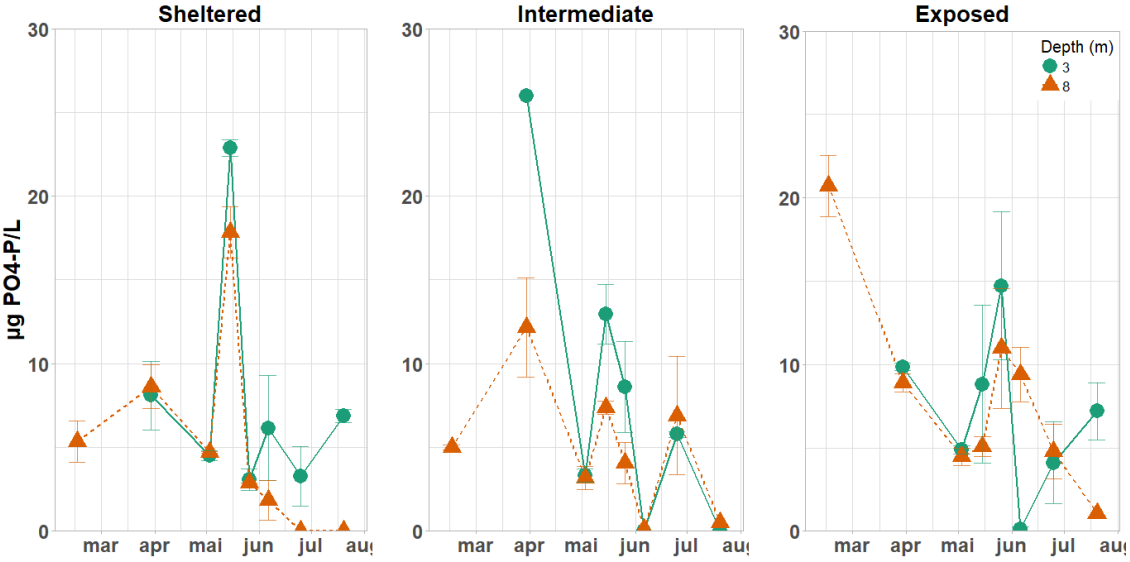
Figure 9 Water salinity (PSU) at 3 m and 8 m depth at Sheltered-(1), Intermediate- (2) and Exposed location (3), from February to July 2017.

Figure 10 shows nitrate (A) (E-nitrate), phosphate (B) (E-phosphate) and ammonium (C) (E-ammonium) concentrations at Sheltered-, Intermediate-, and Exposed location at 3 m and 8 m depths throughout the experimental period. Both E-Nitrate and E-Phosphate concentrations were generally low during summer and there were no clear differences between depths. Late winter concentrations were measured in March and showed typical higher winter values. The concentration of E-Nitrate and E-Phosphate showed a peak in mid-May which can indicate influence of deep-water. E-Ammonium concentrations shows a generally similar fluctuating behaviour at all locations and depth. Sheltered locations showed the highest ammonium concentrations.

A)


Sheltered
Intermediate
Exposed
Depth (m)
3
8
µg (NO3+NO2)-N/L
0
20
40
60
80
mar
apr
mai
jun
jul

B)


Sheltered
Intermediate
Exposed
Depth (m)
3
8
µg PO4-P/L
0
10
20
30
mar
apr
mai
jun
jul

C)

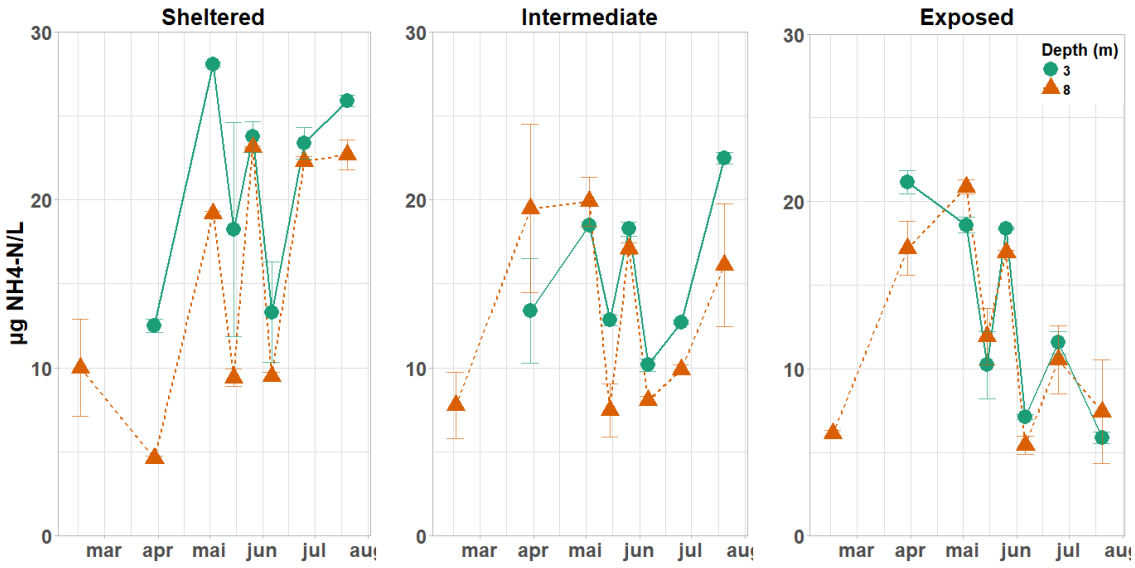


Figure 10 Nitrate (upper panels), phosphate (mid panels) and ammonium (lower panels) concentrations at 3 m and 8 m depth at Sheltered- (1), Intermediate- (2) and Exposed location (3) as a function of time (February to July 2017). A: Nitrate + nitrite, B: Phosphate, C: Ammonium. Whiskers shows standard error and dots shows mean values.

3.2 Macroalgae growth and I-Nitrate concentrations

Figure 11 shows average of the total lamina area throughout the experimental period. Overall there was a steady increase in area at all stations and at both depths. The algae cultivated at sheltered 8 m showed the lowest growth, with a maximum area of around 1000 cm^2 in June. The algae cultivated at Exposed 3 m showed the highest area with a maximum above 2000 cm^2, in June. There was a significant difference in area between the different locations and between depths (P = 0.05). There was no significant difference in area between the different locations at 3 m depth (P = 0.65), but there was a significant difference between the locations at 8 m depth (P = 0.01), were Sheltered 8 m was significantly different from Exposed 8 m (P = 0.02) and Intermediate 8 m (P =0.03). Most locations showed a decrease in biomass the 20th of July. When date from this date was excluded, there was a significant difference in area between Sheltered 3 m and Sheltered 8m (P=0.05).

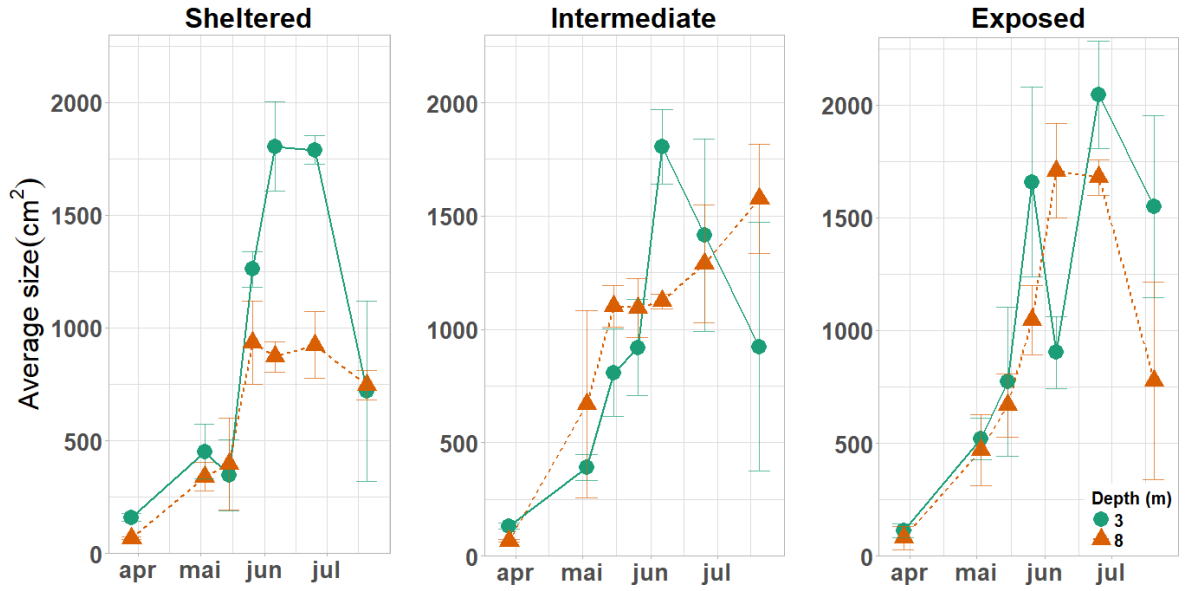


Figure 11 Average total lamina area (cm²) of S. latissima *at the different locations at 3 m and 8 m depth at Sheltered- (1), Intermediate-(2) and Exposed location (3) as a function of time (March to July 2017). Whiskers shows standard error and dot shows mean of n=3. Growth was determined by measuring total lamina area.*

Figure 12 shows relative growth rate (RGR) of the lamina area throughout the experimental period. RGR shows the growth each day relative to the laminas size. Calculations are based on means of 3 samples and the use of Equation 3.

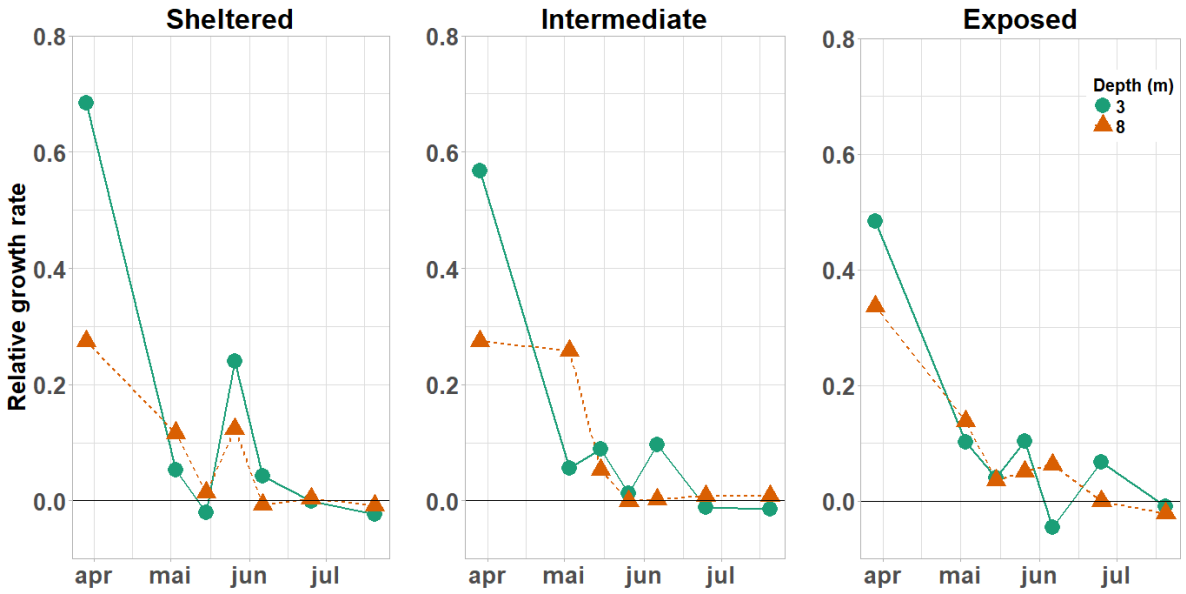


Figure 12 Relative growth rate of S. latissima between samplings at 3 m and 8 m depth at Sheltered- (1), Intermediate- (2) and Exposed location (3) as a function of time (March to July, 2017). Relative growth rate was calculated using Equation 3.

Figure 13 shows intracellular dissolved nitrate concentrations (I-Nitrate) at the different locations at 3 m and 8 m depths throughout the experimental period. In general, the highest I-Nitrate were measured in early spring. Exposed 8 m showed the highest I-Nitrate in mid-May with 0, 11 mg NO_3-N/g DW alga. There was a significant difference in I-Nitrate concentrations between the different locations and between depths (P =0.02). Sheltered 3 m was significantly different from Exposed 3 m (P=0.03), and Sheltered 8 m was significantly different from Exposed 8 m (P=0.02). There was no significant difference between the intermediate and the exposed locations, and between the intermediate and the sheltered location.

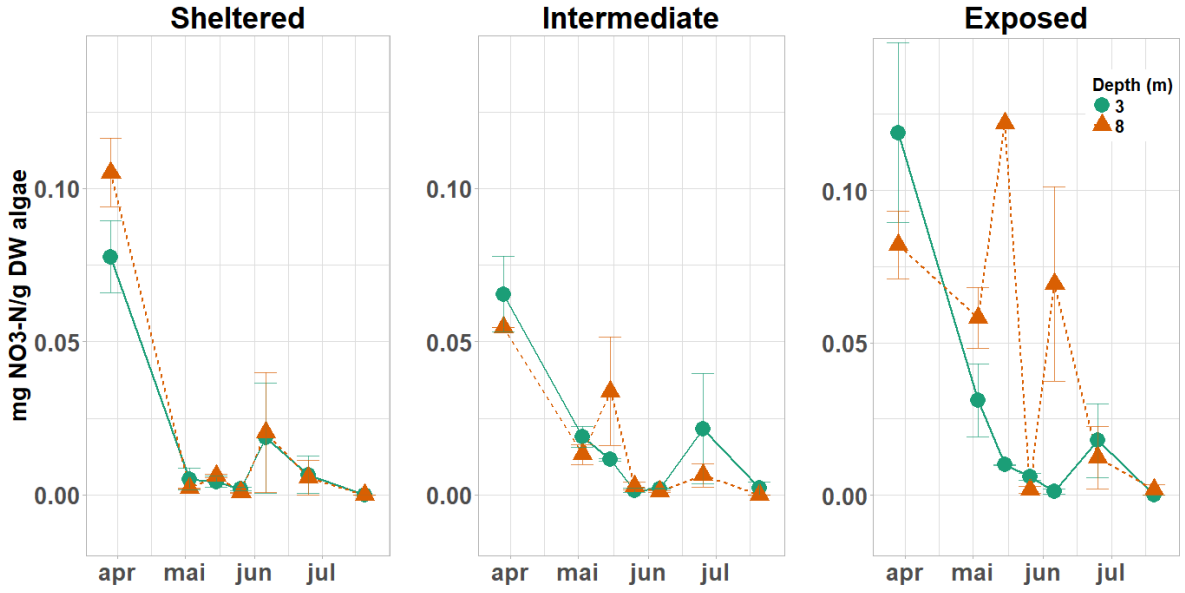


Figure 13 Concentrations of intracellular dissolved nitrate (mg NO_3-N/g dry weight algae) at 3 m and 8 m depth at Sheltered- (1), Intermediate- (2) and Exposed location (3) as a function of time (March to July 2017). Points are mean I-Nitrate concentrations of three or two plants, whiskers show standard error.

Figure 14 shows external nitrate concentrations (E-Nitrate) in the water column and I-Nitrate at the different locations at 3 m and 8 m depth, as a function of time (correlation coefficients are given in (Table 2). Values are given as means of two water samples and three seaweed individuals, sampled from 30th of March to 20th of July. All values of I-Nitrate followed, to some extent, the E-Nitrate variable. All locations showed both high E-Nitrate and I-Nitrate concentrations in end of March, before they became reduced in April. The E-Nitrate variable shows a peak in mid-May, at all locations. From May and onward I-Nitrate was low, but most of the locations shows a small peak in early June, except for Exposed 8 m and Intermediate 8m which showed a peak in mid-May. Both variables fluctuated in Exposed 8 m over experimental period. Most of the locations showed a strong to moderate relationship between E-Nitrate and

I-Nitrate concentrations, but only Intermediate 8m had a significant relationship (P=0.02). Sheltered 3 m and Intermediate 3 m showed the weakest relationship between E-Nitrate and NO_3-I.

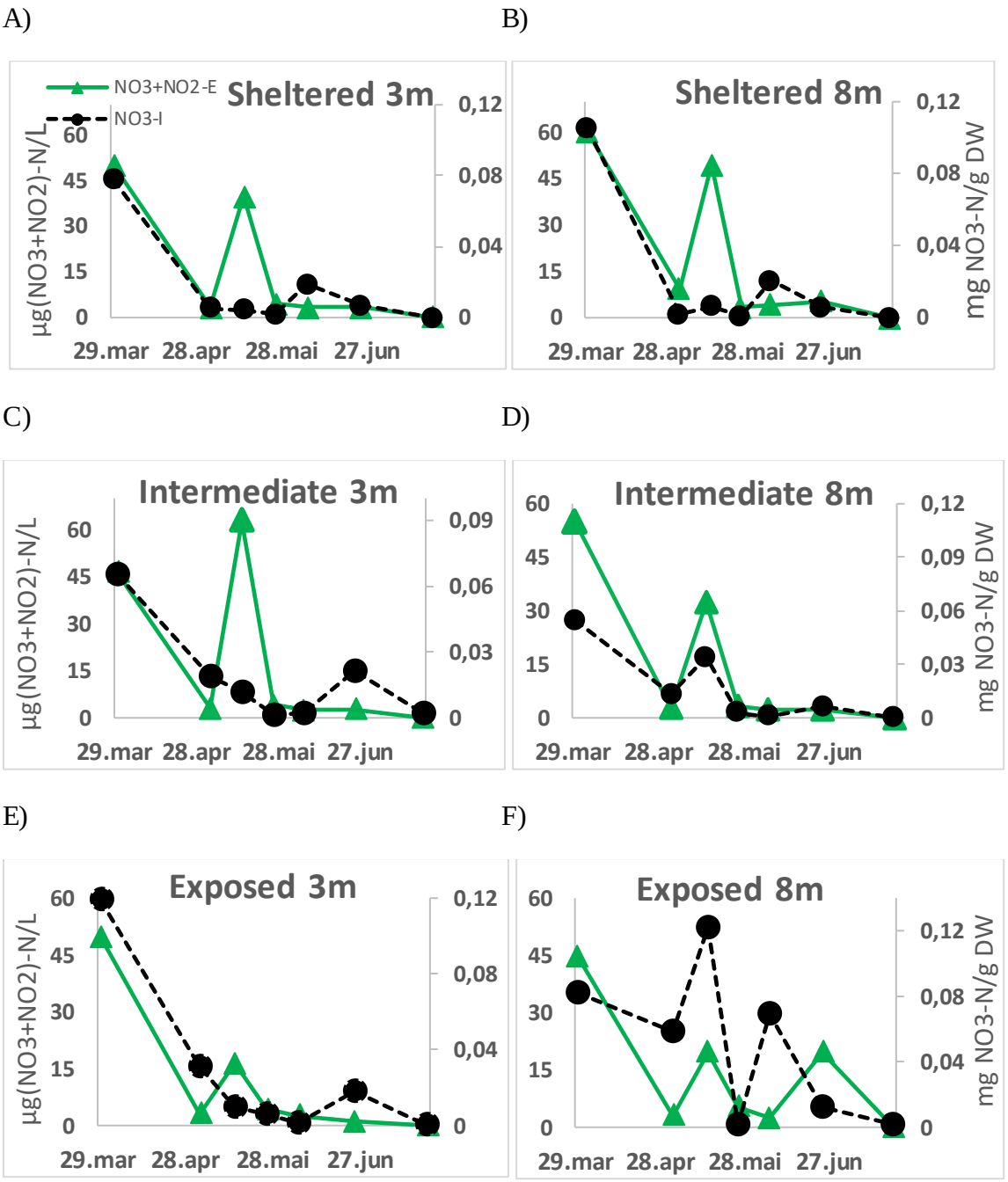


Figure 14 E-Nitrate concentrations (µg (NO_3+NO_2)-N/L on the y-axis and I-Nitrate concentrations (mg NO_3-N/g dry weight algae) on z-axis as a function of time (March to July, 2017). A) Sheltered 3m, B) Sheltered 8m, C) Intermediate 3m, D) Intermediate 8m, E) Exposed 3m and F) Exposed 8m.

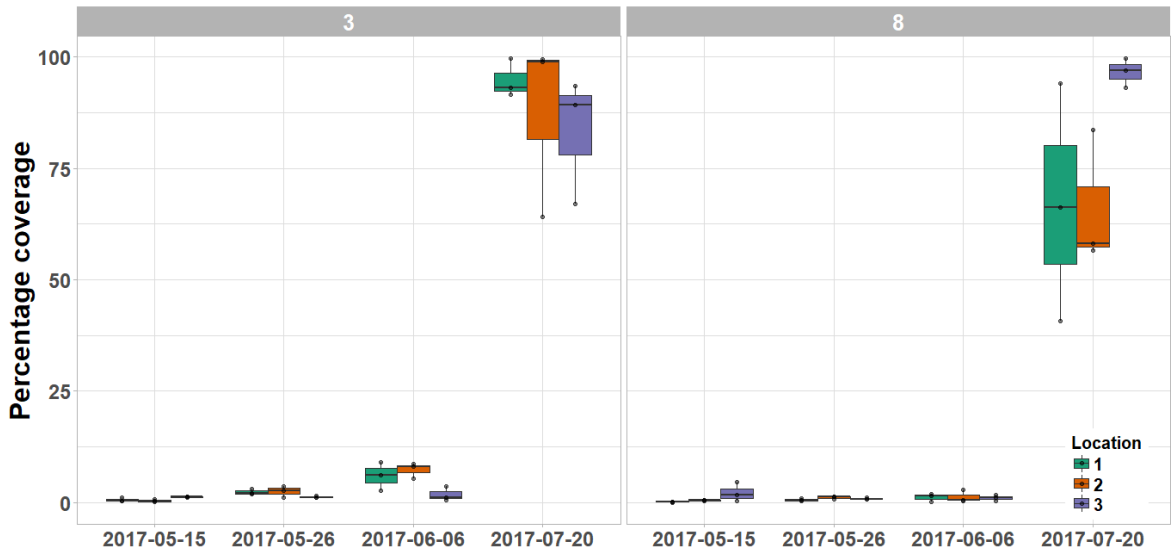
*Table 2 Correlation between external NO_3-N concentrations and intracellularly NO_3-N concentrations at 3m and 8m depth at Sheltered- (1), Intermediate- (2) and Exposed location (3). Spearman correlation r_s, a significant relationship $p < 0.05$ is marked with *.*

Location	Correlation coefficient (r_s)	P-value
Sheltered 3m	0.357	0.432
Sheltered 8m	0.750	0.052
Intermediate 3m	0.286	0.535
Intermediate 8m	0.821	0.023 *****
Exposed 3m	0.607	0.148
Exposed 8m	0.643	0.119

3.3 Bryozoan coverage

Figure 15 shows percentage bryozoan coverage on lamina throughout the sampling period (A) and in the settling phase (B) at the at Sheltered-, Intermediate-, and Exposed location at 3 m and 8 m depths. From May the algae were covered with bryozoans. *M. membranacea* was the dominant bryozoan species, while *E. pilosa* was only present to a small extent. The first newly settled bryozoan colonies were observed at the 15th of May, but they had most likely settled between 3rd and 15th of May, as some laminas had reached 2 % coverage at this time. In general, the bryozoan coverage increased from low values in May to approximately 90 % coverage at 3 m depth in end of July. In late July, the fronds were heavily fouled with bryozoans (shown in Figure 16) and thallus started to degrade. Most of the macroalgae had started to disintegrate because the bryozoan colonies covering the lamina made it heavy, brittle, and easily breakable. There was a significant difference in bryozoan coverage between the different depths and locations (P=0.003), and between the different depths at the sheltered location (P=0.006), and the intermediate location (P=0.01), but no significant difference was found between depths at the exposed location (P=0.58).

A)



B)

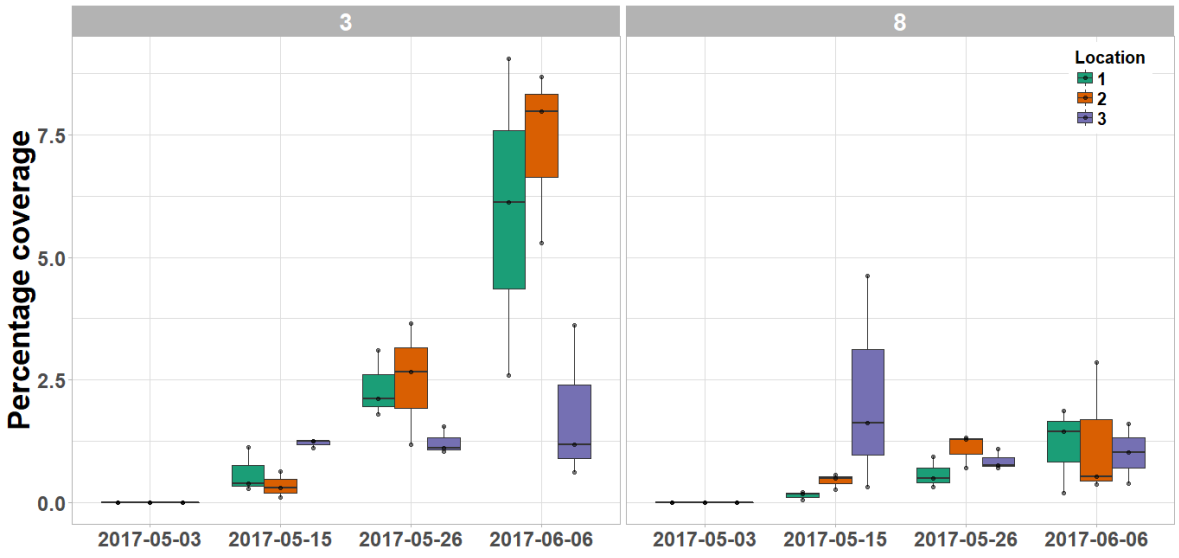


Figure 15 Boxplot of the percentage coverage of bryozoans on S. latissima *during the sampling period. A: Total sampling period (upper panel), B: Early settling phase (lower panel). Sheltered-(1), intermediate-(2) and Exposed location (3). The boxplot shows maximum and minimum values (whiskers), the lower and upper quartile (box), the median (horizontal line) and the individual samplings (dots).*

Figure 16 shows a photograph series of the algae from each sampling date to illustrate the development of bryozoan coverage.

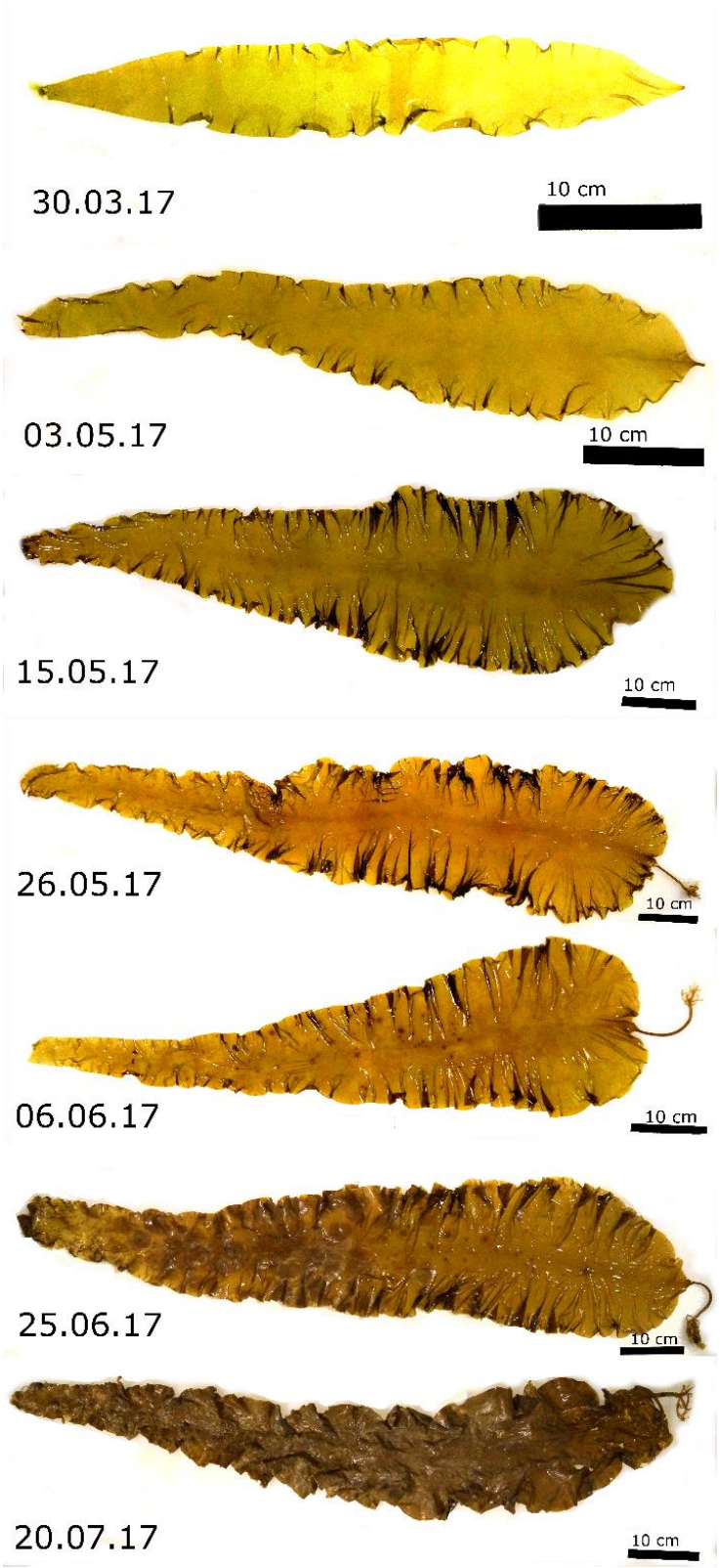
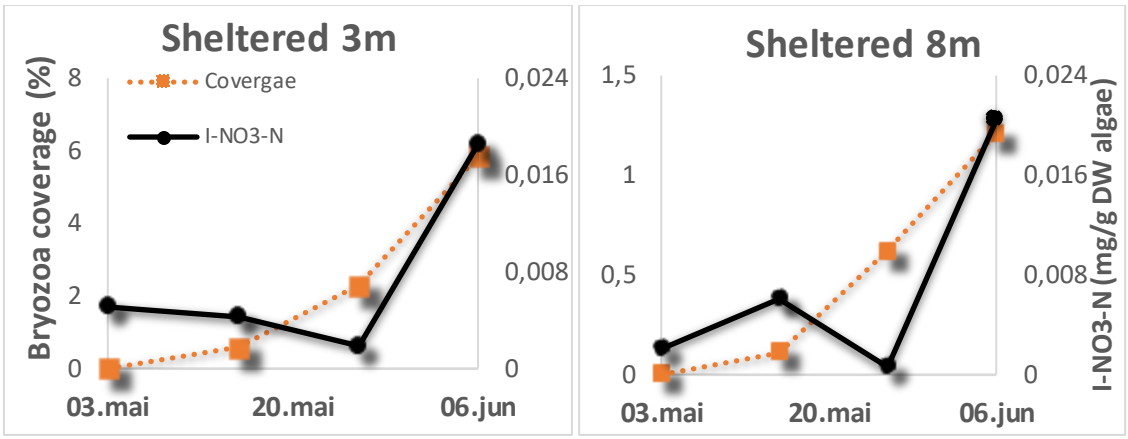


Figure 16 Photograph series of the experimental algae from every sampling date (March to July 2017). Pictures shows selected samples collected at Exposed 3 m.

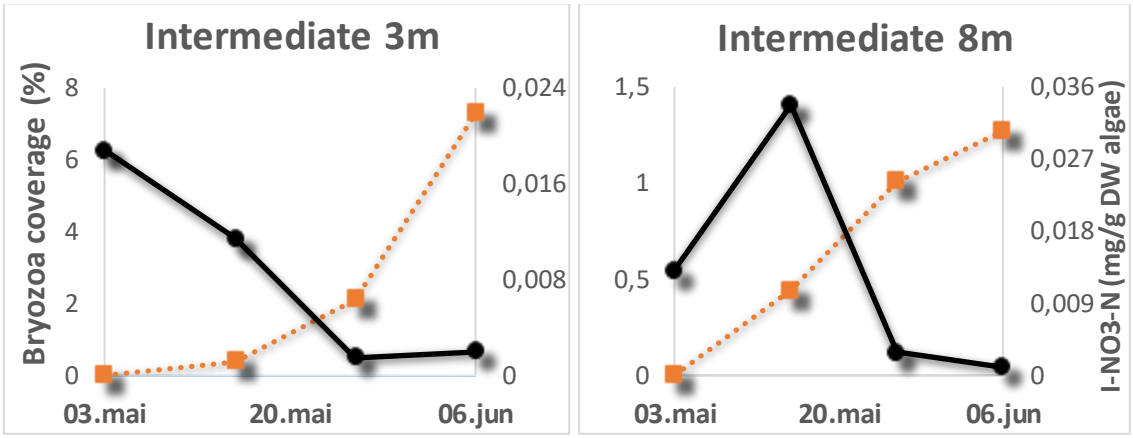
3.3.1 Bryozoan coverage and intracellular dissolved nitrate concentrations in algae

Figure 17 shows average bryozoan coverage and average I-Nitrate at the different locations at 3m and 8m depth as a function of time. Values are given as a mean of three samples sampled from 3rd of May to 6th of June 2017 (values are given in Appendix 1). There was no clear relationship between bryozoan coverage and I-Nitrate concentrations at the different locations, but five out of six cases showed a negative relationship (correlation coefficients are given in Table 3). Intermediate 3 m showed a significant relationship ($P<0.01$) were I-Nitrate concentrations decreased as bryozoan coverage increased. This pattern can also be observed in Exposed 3 m and Intermediate 8 m, but there was no significant relationship. Sheltered 3 m and Sheltered 8 m showed an increase in bryozoan coverage and an increase in I-Nitrate concentrations. Exposed 8 m showed the weakest relationship were both variables are fluctuating.

A) B)



C) D)



E) F)

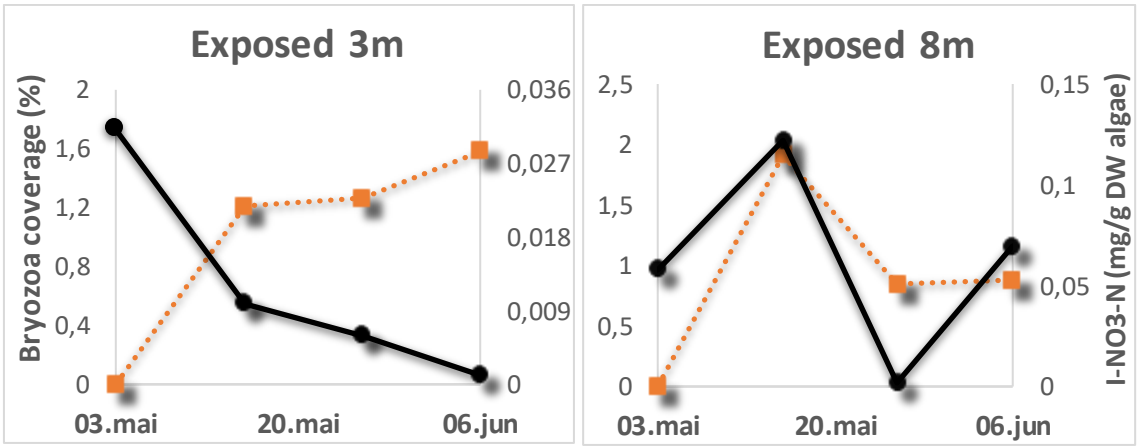


Figure 17 Bryozoan coverage (%) on y-axis and I-Nitrate (mg NO_3-N/g dry weight algae) in algae on z-axis as a function of time (May to June, 2017). A) Sheltered 3m, B) Sheltered 8m, C) Intermediate 3m, D) Intermediate 8m, E) Exposed 3m and F) Exposed 8m.

*Table 3 Correlation between bryozoan coverage and I-Nitrate concentrations at 3 m and 8 m depth at Sheltered- (1), Intermediate- (2) and Exposed location (3). Spearman correlation R_S, significant relationship p< 0.05 is marked with *.*

Location	Correlation coefficient (r_s)	P-value
Sheltered 3m	-0.437	0.156
Sheltered 8m	-0.311	0.353
Intermediate 3m	-0.845	0.001*
Intermediate 8m	-0.563	0.056
Exposed 3m	-0.532	0.092
Exposed 8m	0.132	0.699

3.3.2 Bryozoan coverage and chlorophyll *a* concentrations

Figure 18 shows chlorophyll *a* (chl *a)* concentrations in phytoplankton between 70 µm and 5 µm of size at Sheltered-, Intermediate-, and Exposed location at 3m and 8m depths throughout the experimental period. There was a small difference in chl *a* between the Sheltered and the Intermediate locations. Exposed location showed the highest concentrations in end of May with around 1. 9 µg/L. There was no significant difference in chl *a* between the different locations and between depths (p=0.54).

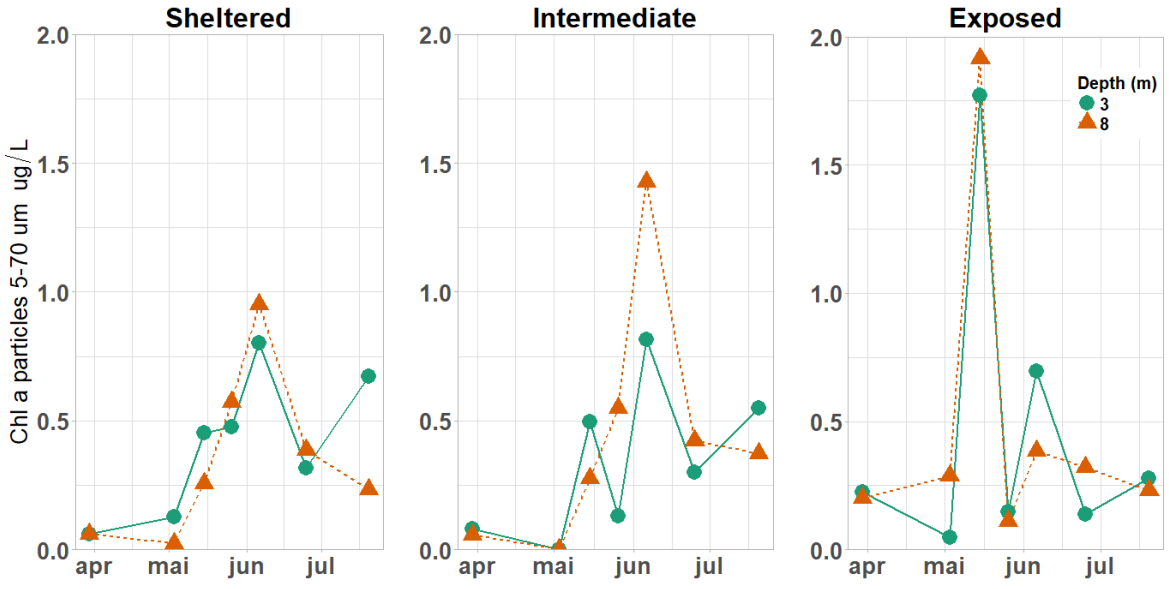


Figure 18 Chlorophyll a concentrations (µg/L) in phytoplankton between 70 µm and 5 µm of size at 3m and 8m depth at Sheltered- (1), Intermediate- (2) and Exposed location (3), as a function of time (March to July 2017).

Figure 19 shows mean bryozoan coverage on lamina as a function of the mean chl *a* concentrations at Sheltered-, Intermediate-, and Exposed location at 3m and 8m depth (see Table 4 or regression coefficients). Values are given as bryozoan coverage as a function of the mean of 3 chlorophyll *a* replicates, sampled from 3rd of May to 6th of June 2017 (values are given in Appendix 1). All locations showed a positive correlation between bryozoan coverage and chl *a* food concentrations and the correlation was significant at all locations, except for Intermediate 3 m and Exposed 3 m (P-values are given in Table 4). Sheltered 8 m showed the highest significant linear relationship were 93 % of the variation in the bryozoan coverage values can be explained by the variation of chl *a* food concentrations ($P<0.001$). Exposed 3 m showed the weakest linear relationship were 14 % of the variation in the bryozoan coverage values can be explained by the fitted line together with the chl *a* values, and no significant relationship ($P=0.14$).

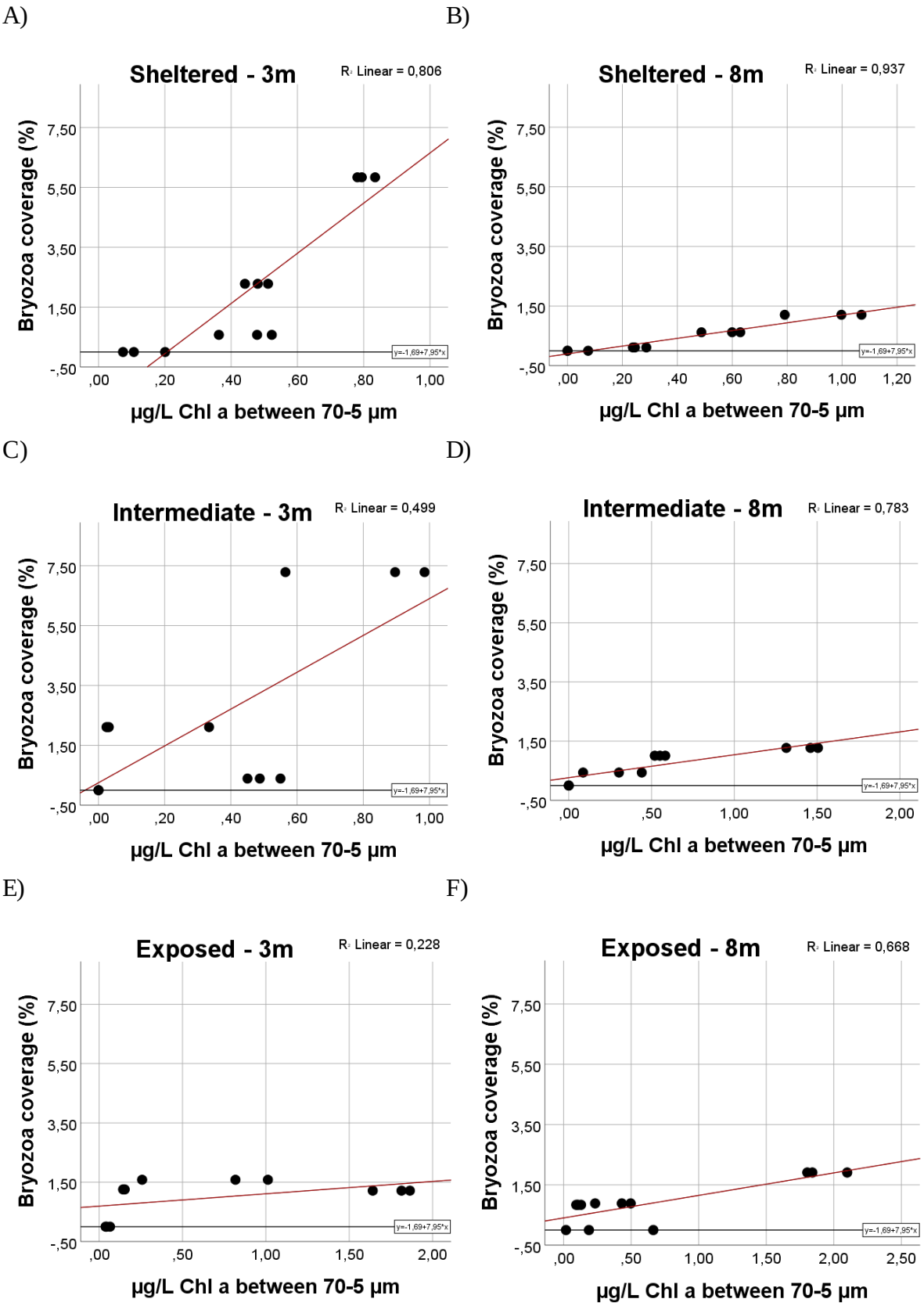


Figure 19 Bryozoan coverage (%) as a function of chlorophyll a particles (µg/L) between 70 µm and 5 µm of size. A) Sheltered 3m, B) Sheltered 8m, C) Intermediate 3m, D) Intermediate 8m, E) Exposed 3m and F) Exposed 8m.

*Table 4 Regression coefficients (intercept, slope, r^2 adjusted, R and P) to the curves shown in figure 17. P values < 0.05 marked with *.*

Location	Intercept $\beta_0 \pm$ SE	Slope $\beta_1 \pm$ SE	r^2 adjusted	R	P
Sheltered 3m	-1.73 ± 0.67	8.37 ± 1.30	0.786	0.898	<0.001*
Sheltered 8m	-0.10 ± 0.06	1.30 ± 0.10	0.931	0.968	<0.001*
Intermediate 3m	0.25 ± 1.06	6.15 ± 2.05	0.444	0.707	0.015*
Intermediate 8m	0.26 ± 0.11	0.77 ± 0.13	0.759	0.885	<0.001*
Exposed 3m	0.69 ± 0.25	0.41 ± 0.25	0.142	0.478	0.137
Exposed 8m	0.40 ± 0.16	0.74 ± 0.16	0.634	0.817	0.001*

3.3.3 Bryozoan coverage, chl *a* – *and* I-Nitrate *concentrations*

Table 5 shows mean of bryozoan coverage, chl *a* concentrations and I-Nitrate concentrations from samples collected between 3rd of May to 6th of June 2017, reflecting the conditions in late spring and early summer in the Northern Hemisphere. The highest bryozoan coverage mean value was measured at Sheltered 3 m and Intermediate 3 m with a mean coverage above 2 %, while the lowest mean was measured at Sheltered 8 m and Intermediate 8 m. Exposed location showed a similar bryozoan coverage at both depths, with a mean value around 1 % coverage. The highest chl *a* concentrations were measured at Exposed location and Intermediate 8 m, with a mean above 0.6 µg chl *a* /l^{-1}. Sheltered location and Intermediate 3 m showed lower mean value with around 0.4 µg chl *a* /l^{-1}. The highest mean of intracellular nitrate concentrations was measured at Exposed 8 m, with 0.063 mg NO_3-N/g DW algae. Sheltered location and Intermediate 3 m shows the lowest mean in I-Nitrate concentrations.

Table 5 Mean values for bryozoan coverage, chl a concentrations and I-Nitrate concentrations at 3m and 8m depth at Sheltered-, Intermediate- and Exposed location, sampled from 3rd of May to 6th of June 2017, ± SE.

Location	% coverage	µg chlorophyll *a*/L	mg NO_3-N/g DW algae
Sheltered 3m	2.215 ± 0.808	0.466 ± 0.07	0.007 ± 0.004
Sheltered 8m	0.471 ± 0.178	0.452 ± 0.11	0.008 ± 0.005
Intermediate 3m	2.539 ± 0.921	0.393 ± 0.11	0.008 ± 0.005
Intermediate 8m	0.696 ± 0.234	0.615 ± 0.17	0.013 ± 0.005
Exposed 3m	1.057 ± 0.281	0.714 ± 0.23	0.012 ± 0.004
Exposed 8m	1.008 ± 0.368	0.675 ± 0.22	0.063 ± 0.021

Figure 20 shows mean bryozoan coverage as a function of A) mean I-Nitrate concentrations and B) mean chl *a* concentrations, from all locations at 3 m and 8 m depth. Values are found in Table 5 and are means of samples collected between 3rd of May to 6th of June 2017. In figure A) there are no clear pattern. The 3 m depth bryozoan coverage decrease as I-Nitrate concentrations slightly increase, while the 8 m depth bryozoan coverage increase as I-Nitrate concentrations increases. In figure B), the different depth shows a conflicting pattern, where 3m depth bryozoan coverage decrease as chl *a* food concentrations increases. The 8 m depth shows the opposite were bryozoan coverage increase as chl *a* food concentrations increases.

A) B)

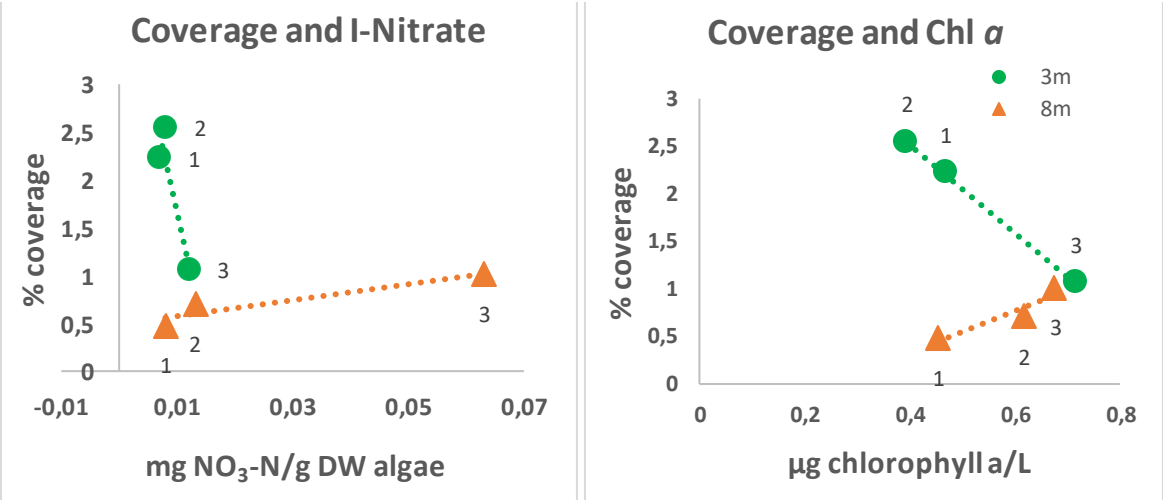


Figure 20 Bryozoan coverage mean as a function of A) intracellular dissolved nitrate mean and B) chlorophyll a mean concentration with a trend line, all locations at 3 m and 8 m depth sampled from 3rd of May to 6th of June 2017. 1) Sheltered-, 2) Intermediate-, 3) Exposed location.

Table 6 shows the correlation between bryozoan coverage and chl *a* concentrations, and the correlation between bryozoan coverage and I-Nitrate concentrations. Values are based on samples from all locations and depths, sampled from 3rd of May to 6th of June (settling phase), and from 3rd of May to 20th of July (total sampling period). There was a moderate, although significant ($P<0.01$) positive relationship between bryozoan coverage and chl *a* concentrations, were bryozoan coverage increased as chl *a* increased, with a higher correlation in the settling phase. There was moreover a weak significant ($P<0.05$) negative relationship between bryozoan coverage and I-Nitrate concentrations, were bryozoan coverage increased as I-Nitrate concentrations decreased in algae. There was a lower correlation between bryozoan coverage and I-Nitrate concentrations in the settling phase compared with total sampling period.

*Table 6 Correlation between bryozoan coverage and I-Nitrate concentrations, and bryozoan coverage and chl a concentration at all locations, May to June and May to July 2017. Spearman correlation, R_S significant relationship p< 0.05 is marked with *.*

	Bryozoan coverage (%)		
	Correlation coefficient, r_s	*P*	*n*
Chl *a* - May to June	0.572	<0.01*	69
I-Nitrate - May to June	-0.337	0.05*	69
Chl *a* - May to July	0.334	<0.01*	87
I-Nitrate - May to July	-0.605	<0.01*	87

4 Discussion

This study aimed to investigate how selected external conditions affected the bryozoans fouling on *S. latissima* cultured at different physical exposures, with a special focus on how nutritional state of the macroalgae affect the development and intensity of biofouling, and how phytoplankton food concentrations affected bryozoan abundance on *S. latissima*.

The study found that there was a steady increase in *S. latissima* area growth over the experimental period, were Exposed location showed the highest growth and Sheltered 8m showed the lowest (Figure 11). Intracellular nitrate (I-Nitrate) concentrations, decreased towards the summer, with significantly higher concentrations at Exposed location ($P<0.05$). Bryozoan coverage became a problem from May onwards, with loss of biomass as a direct result of epiphytes in the end of July (Figure 15 and Figure 16). Although abundant at all cultivations depths, results revealed significantly less bryozoan coverage at 8 m depth ($P=0.003$) at the Sheltered and the Intermediate locations. Exposed location showed no depth dependent bryozoan coverage pattern. There was no clear relationship between bryozoan coverage and I-Nitrate concentrations. The general trend suggests, however, that I-Nitrate concentrations decreased as bryozoan coverage increase (Figure 17 and Table 3). There was in most cases a clear relationship between food concentrations and bryozoan coverage at all stations (Figure 19 and Table 4). All locations showed good bryozoan food availabilities with no statistical differences. The less exposed locations showed a strong relationship were the variation in bryozoan coverage can be explained by the variation in chlorophyll *a* (chl *a*) concentrations.

4.1 Environmental variables

The uniformity in temperature between 3 m and 8 m depths suggest an efficient mixing of the water column at all locations throughout the sampling period (Figure 7 and Figure 8). The salinity data supported this finding, showing relatively similar PSU levels for stations and depths (Figure 9). Both variables were relatively similar at all locations, which suggested a high-water exchange rate of the fjord system. A lower salinity was measured at sheltered location in end of July, reflecting and increased influx of fresh water.

The measured nitrate (E-Nitrate) and phosphate (E-Phosphate) concentrations show throughout mixing, with no clear differences in concentrations between depths at all locations, except for Exposed 8 m which showed higher concentrations at 8 m depth (Figure 10). The external concentrations of E-Nitrate and E-Phosphate show an inverse correlation with temperature. In

end of March, the temperature was around 4°C while the ambient concentrations of nitrate were above 40 µg/L. In June, the temperature showed an increase to around 7°C, while the ambient concentrations of nitrate were depleted. The extreme transition from high to low concentrations of inorganic nitrogen is normally related to phytoplankton spring bloom. The concentrations of E-Nitrate and E-Phosphate showed a peak in mid-May, indicating influence of deep water, likely caused by an upwelling event caused by strong winds in the region. The first E-Phosphate measurement from Intermediate 3m is higher than the concentrations in normal deep water, suggesting that there may have been some contamination of the sample.

Ammonium (E-Ammonium) showed high concentrations throughout the cultivation period, whit the highest measurements found at Sheltered locations. The fish farm Seiskjæra is situated in Skjørafjord between Intermediate and Exposed location. This location has a capacity of 3120 tonnes fish, and is most likely contributing to the high E-Ammonium concentrations in the fjord (Barentswatch, 2018).

Both Sheltered and Intermediate locations showed a relatively stable nutrient concentrations, with small differences between depths (Figure 10). Exposed location on the other hand, showed a more fluctuating behaviour with higher differences between depths as expected due to higher water velocities and mixing.

4.2 Macroalgae growth and I-Nitrate

As found in previous macroalgae cultivation project in Trøndelag (Forbord et al., 2012), the best growth of *S. latissima* was recorded between February and June (Figure 11). The macroalgae cultivated at 3m depth showed a similar growth at all location, whereas those cultivated at 8 m depths showed a slower growth the reduced physical exposure, were Sheltered 8 m showed the lowest growth.

Handå et al. (2013) found a depth- dependent growth pattern with faster growth in length at 2 m and 5 m than at 8m depth. This growth pattern was also revealed at the Sheltered location, were the cultivated macroalgae showed a significantly slower growth at 8m depth (P=0.05). Light limitations might cause the slower growth, due to high turbidity in the water or shading from the macroalgae cultivated at 3 m depth. The locations with less physical exposure was expected to have less nutrient supply and higher turbidity, and hence lower growth. According to the E-Nitrate concentrations, Sheltered 3 m and Sheltered 8 m was exposed to similar concentrations throughout cultivation period (Figure 10). This may suggest that the low growth

was caused by light limitations presumable caused by particulate organic matter in the water column.

S. latissima is well adapted to seasonality and accumulates nitrate when light is limited (Gagné et al., 1982). There was no significant difference between Sheltered 3 m and Sheltered 8 m in I-Nitrate concentrations, indicating that nitrate was not accumulated intracellularly even though light was limited (Figure 13). Previous studies have shown that photosynthesis and nutrient uptake increase with increasing water velocities as the supply of nutrients increases and metabolic waste products in the macroalgae are removed (Hurd et al., 2014b). This suggest that even though there are nutrients available in the water masses, *S. latissima* is not able to utilize it when the water flow over the macroalgae is low. Macroalgae cultivated at 3 m depth are prone to higher wind mixing and higher water flow over the macroalgae and therefor able to utilize E-Nitrate. There was no statistical difference in growth between depths at Exposed and Intermediate location, confirming the presumed difference in exposure between the locations.

The growth rate of *S. latissima* follows the fluctuations of E-Nitrate from high concentrations in late winter and early spring, to low in the summer (Gagné et al., 1982). The results revealed high E-Nitrate and I-Nitrate concentrations in early-spring, and as the macroalgae grew, the concentrations decreased (Figure 10 and Figure 13). Inorganic nitrogen is believed to be one of the main resources limiting macroalgae growth (Hurd et al., 2014a). The intracellular nutrient concentrations is dependent on the external concentrations and macroalgae uptake (Hurd et al., 2014a). Wheeler & Weidner (1983) showed in a controlled experiment with *S. latissima* that intracellular nitrate concentrations increased linearly with external nitrate concentrations. When cultivated in its natural environment, this linearly relationship could not be seen. The results of the study revealed a weak correlation between I-Nitrate and E-Nitrate (Figure 14 and Table 2). E-Nitrate concentrations might give you a snapshot of the environment, while I-Nitrate concentrations give you a continuous picture of the actual nitrate conditions in the environment. The mean I-Nitrate concentrations and growth increased with increased physical exposure. Sheltered 8 m showed low growth rate and a mean I-Nitrate concentrations of 0.008 ± 0.004 mg NO_3-N/g DW, while Exposed 8 m showed high growth and higher mean I-Nitrate, 0.063 ± 0.021 mg NO_3-N/g DW (Table 5). Resultingly the study did not find a linear relationship between I-Nitrate concentrations and E-Nitrate concentrations.

From May and onward both E-Nitrate and I-Nitrate concentrations were low, suggesting that the reservoir accumulated in the winter is accumulated by macroalgae which then becomes

potentially Nitrogen-limited. E-Nitrate concentrations showed a peak in mid-May. This peak could immediately be seen in I-Nitrate concentrations at Intermediate 8 m and Exposed 8 m. Inorganic nitrate is stored intracellularly when the external concentrations are high, and utilized for growth directly when the macroalgae is N-limited (Chapman & Craigie, 1977; Wheeler & Weidner, 1983). Sheltered location and Intermediate 3 m shows no immediate response to the E-Nitrate input. Thomas and Harrison (1987) found that there may be a lag in nitrate uptake when the algae is N-limited, and uptake may increase first after 1-2 hours after nutrient input (Thomas, Harrison, & Turpin, 1987). This may be revealed in the results were *S. latissima* cultivated at Sheltered location and Intermediate 3 m were N-limited before nutrient input, and hence showed a lag phase that was not detected in the sampling interval. Intermediate 8 m and Exposed 8 m might not be N-limited and were able to respond faster. The response seen in Intermediate 8 m and Exposed 8 m supports the assumed difference between the locations where Intermediate 8 m and Exposed 8 m had greater nutrient availabilities and were not N-limited. The peak in I-Nitrate and RGR seen at 3 m depth in early June indicates that there have been high E-Nitrate concentrations earlier that enabled the algae to accumulate nitrate intracellularly (Figure 12).

In microalgae ammonium generally inhibit uptake of nitrate (Hurd et al., 2014a). Whether this is happening in *S. latissima* is unclear. The relatively high concentrations of ammonium appear to be poorly utilized by the macroalgae (Figure 10). However, high ammonia may explain the lacking response in I-Nitrate upon an increased external concentration. Improved knowledge of a potential selectivity of nitrate *versus* ammonia in *S. latissima* is needed to further elaborate on the observed patterns.

The Droop equation, established for microalgae, relates growth rate to the intracellular nitrogen concentrations, and can be used to estimate the length of time that an algae would be capable of maintaining growth in the absence of the external nutrient source (Droop, 1974; Hurd et al., 2014a). This equation was not used in this study, but it could have been applied to reveal maximum growth rate and determine the growth limiting resource *S.latissima*. Wheeler and Weidner (1983) showed that the growth rate of *S. latissima* increased until the external nitrate concentrations exceeded 5-10 µmol/L (equivalent to 70-140 µg NO_3-N/L), beyond which is was saturated, or constant. According to this, it could be suggested that the maximum growth rate was not achieved at the different locations of the this study because the highest E-Nitrate was between 40-60 µg NO_3-N/L, below saturation for *S. latissima* according to Wheeler and Weidner (1983).

In its natural environment, macroalgae growth rate cannot easily be expressed directly as a function of external nutrient concentrations. The rapid changes in the external nutrient concentration in the environment and the ability for macroalgae to accumulate nitrate leads to an uncoupling of nutrient uptake and the further use for growth (Rosenberg & Ramus, 1982). However, RGR showed clear relationship E-Nitrate concentrations (Figure 12 and Figure 10). All locations showed high growth rate in late spring, before RGR decrease, and later responds to the mid-May peak in E-Nitrate. Sheltered 3 m showed the highest RGR at the start of the cultivation period when E-Nitrate were high. Exposed 8 m showed a lower but more stable growth rate suggesting better nutrient availabilities over the cultivation period.

Sampling revealed clearly, that the macroalgae cultivated at the different sites adapted quickly to their external environment. *S. latissima* cultivated at sheltered location was in general more fragile and the holdfast was not attached well to the ropes, hence a lot of biomass was lost when handling. For growth measurements random individuals were collected at each sampling. For a more accurate growth measurement the hole punching method for length increase could have been used.

According to hypothesis 1 the growth and nutritional state of *S. latissima* will vary across categories of exposure and depths. The results partly support this hypothesis. The results showed no significant difference in growth at 3 m depth at the different exposures. There was however a significant different at 8 m depth, were Exposed location showed the best growth. The I-Nitrate concentrations at Sheltered location were significantly lower than at Exposed location. There was however no difference in I-Nitrate concentrations between 3 m and 8 m depths.

4.3 Bryozoan coverage

The first bryozoan colonies was observed in May which is earlier than expected from previous cultivation projects in Norway, where the first settlement occurred in June (Forbord et al., 2012; Handå et al., 2009). A rapid increase in bryozoan coverage followed in June and July (Figure 15 and Figure 16). This may be related to higher water temperatures in the fjord system, than in previous studies.

The temperature at all locations increased gradually from 7°C in May to around 14-15°C in July (Figure 7). The first colonies was observed in the beginning of May when the temperature exceeded 6°C. Increasing temperatures during spring and summer initiated zooid growth of *M. membranacea* and *E. pilosa*, and elevation in temperature can trigger faster growth (Menon,

1972). In July, the temperature exceeded 14°C and most of the locations had more than 75 % bryozoan coverage. Previous studies have shown that the growth rate and size of the colonies can be positively correlated with increasing temperatures, within the range of 6-16°C in the Norwegian waters (Nair, 1962; Saunders & Metaxas, 2007, 2009). Therefore, with global warming and climate change, the ocean temperature are expected to increase between 3 – 4 °C degrees – and may trigger bryozoan appearance earlier (IPCC, 2001).

Saunders & Metaxas (2007) demonstrated that "growing degree-day", a measure of thermal history, explained up to 81 % of the variability in the abundance of *M. membranacea*. However, growing degree-day was not measured in this study, but could have been used to explain the high variability seen in bryozoan abundance. Saunders and Metaxas (2007) calculated growing degree-day by sequentially adding daily average temperatures that are above a threshold temperature for growth and reproduction in *M. membranacea*.

The level of exposure is considered to be one of the abiotic factors affecting settlement of epiphytes on the Laminaria species (O'Dea & Okamura, 1999). This study found that the Sheltered and the Intermediate locations showed a higher bryozoan coverage, most significant at 3m depths. *M. membranacea* is known for coping well with high water flows around the lamina it is covering, adapting its morphology to the different environmental conditions (Okamura & Partridge, 1999). The bryozoan colonization at Exposed location showed a more random coverage, especially in settling phase, with no statistical difference between depths (Figure 15).

Andersen et al. (2011) suggested that continuous exposure to wave activity control epiphytes by washing away the new settlers. This mechanism may explain the difference in coverage at the different exposures. Higher water movement might make it more difficult for the bryozoans to settle on the macroalgae, since they might be transported away before they are able to settle. In July, Exposed 8 m showed the highest bryozoan coverage with around 90 %, suggesting optimal growth conditions for the epiphytes at this location when settled. Thus, higher level of exposure may contribute to harder settling conditions for the bryozoans, but when settled the epiphytes may grow more rapidly as the conditions are ideal.

Intermediate and Sheltered locations showed a significant difference in bryozoan coverage with increasing depths ($P=0.006$ and $P=0.012$, respectively). When linking this to other results, this colonisation behaviour might be a result of better food availabilities in the upper layer. Light availabilities might also be one of the factors determining bryozoan settlement, as the bryozoans

may use light as a cue for food and colonization substrate availabilities and therefore seek towards the light.

The results revealed higher macroalgae growth rate at 3 m depth, were the cultivated macroalgae have greater access to sunlight. Sheltered 8 m and Intermediate 8 m showed lower growth and lower bryozoan coverage, with a total mean of 0.471 ± 0.178 % and 0.696 ± 0.234 % coverage, respectively (Table 5). Light penetration goes deeper in water masses prone to less turbidity. The higher macroalgae growth measured at Exposed 8 m indicates good light penetration and higher nutrient availabilities. The less exposed the locations shows less macroalgae growth and bryozoan coverage, suggesting less light penetration due to higher turbidity and organic deposition.

The method used for bryozoan coverage estimations was developed during this study. Due to photos of low quality, it was not possible to analyse the pictures taken of the 25th of June sampling. This date was the link between the settling phase and total overgrowth. A different colonization behaviour was then observed were small colonies appeared in large numbers. Førde et al. (2016) found that bryozoan larvae abundance was low until June, before it increased in numbers presumable caused by an increase in temperature or spawning from the already settled colonies. The 25th of June 2017 sampling showed small colonies in great abundance, confirming what Førde et al.(2016) observed. Bryozoan coverage was estimated by calculating percentage of the total lamina covered with bryozoans. Exposed 8 m showed a significantly better lamina growth than Sheltered 8 m and the macroalage had more surface area available for bryozoans to cover. This has not been accounted for in the study but could have been done by taking regular sampling of the bryozoan larvae abundance at the different locations

Hypothesis 2 said that the bryozoan coverage on *S. latissima* will vary across exposure and depths. The results partly support this hypothesis. The results showed there was a depth dependent coverage pattern at Sheltered and Intermediate location, were 3 m depth showed the highest bryozoan coverage. At Exposed location there was no significant difference in coverage between 3 m and 8m depth. There was a significant different bryozoan coverage at Sheltered and Exposed location at 3 m depth, were Sheltered showed the highest coverage. However, there was no significant difference in bryozoan coverage at Sheltered-, Intermediate- and Exposed location at 8m depth.

4.3.1 Bryozoan coverage and I-Nitrate

The results revealed a general pattern were I-Nitrate decreased as bryozoan coverage increased (Figure 17 and Table 3). *S. latissima* is naturally adapted to the seasonal abundance of nitrate, from high concentrations in late winter and early spring, to low in late spring, summer and autumn (Gagné et al., 1982). Due to the high growth in early spring the I-Nitrate reserves normally becomes exhausted when the bryozoan settles in end of May.

Saunders and Metaxas (2007) observed a lower settlement of *M. membranacea* at 4 m depth than at 8 m and 12 m depth throughout the season, both in the presence and in the absence of stratification in the water coloum. In this study, a reversed depth dependent coverage was revealed, with a greater bryozoan coverage at 3 m depth than on 8 m depths at the Sheltered and the Intermediate location (Figure 15). Exposed location, however, showed no significant difference in bryozoan coverage between depths, suggesting that other biotic or abiotic factors are important when bryozoans settle on macroalgae.

Five out of six locations showed a negative relationship were bryozoan coverage increased as the I-Nitrate concentrations decreased (Figure 17). The E-Nitrate concentrations, which was linked to the I-Nitrate concentrations, showed an inverse correlation with temperature, while bryozoan coverage shows a positive correlation with temperature. Both the decreasing E-Nitrate concentrations and increase in bryozoan coverage can be related to phytoplankton abundance.

The results revealed a correlation between mean bryozoan coverage and mean I-Nitrate concentrations at 8 m depth, were the bryozoan coverage and I-Nitrate concentrations increased with increasing physical exposure (Figure 20). Hill et al. (2007) found that the abundance of freshwater bryozoans correlates positively with external nitrate and phosphate levels in the water. The results of the this study suggested that can this apply for marine bryozoans as well. More exposed waters are thought to have higher nutrient supply, and the intracellular nutrient concentrations in the macroalgae can be used as an indicator for the external nutrient concentrations in the water (Hurd et al., 2014a, 2014b). Surface water are normally prone to higher wind mixing and higher water flows, with similar conditions at Sheltered and Exposed locations. At 3 m depth there was a weaker correlation, confirming that external nutrient supply might affect bryozoan abundance.

Hurd et al. (1994) found that *M. membranacea* reduces the nitrate uptake rate in the macroalgae with 50 %, creating a physical barrier that prevented nutrient uptake. At the 6th of June, Sheltered 3 m showed the highest bryozoan coverage with 6 %, while Sheltered 8m only had

around 1 % coverage (Figure 15). I-Nitrate concentrations showed a peak of around 0.0016 mg NO_3-N/g DW macroalgae, in both locations, suggesting that 6% coverage might not affect the nutrient uptake in the macroalgae (Figure 20).

By end of July, almost all locations showed a coverage of around 90 % at 3 m and around 60 % at 8 m, while I-Nitrate concentrations were around 0. Intermediate 8 m was the only location that showed a positive net growth at this time. Hepburn (2006) presented a theory stating that ammonium excreted by the bryozoans is the main nitrogen source for the colonized macroalgae during summer. This theory might also have affected the result, were *S. latissima* cultivated at Intermediate 8 m was able to utilize ammonium excreted by the bryozoans for growth. Intermediate location was located near Seiskjæra fish farm and showed relatively high E-Ammonium concentrations throughout the cultivation period. The increase in growth seen at Intermediate 8 m is difficult to directly track back to the bryozoan coverage, since the E-Ammonium concentrations also was high.

M. membranacea is thought to prefer laminaria species due to their high flexibility and high water flow around them (Bartsch et al., 2008; Okamura & Partridge, 1999). Exposed 8 m showed the highest mean NO_3-I, 0.063 ± 0.021 mg NO_3-N/ g DW macroalgae, and a low bryozoan coverage. The nutritional state of the macroalgae might have affected bryozoan settlement. If the *M. membranacea* larvae have a preference when it comes to the nutritional state of the macroalgae they settle on, remains unclear. The bryozoans might be able to detect the nutritional state of the macroalgae, and thereafter get an indication of the nutrient availability in the environment. To study this, bryozoan coverage and I-Nitrate concentrations should have been estimated from the same individual.

Sheltered location showed a pattern were I-Nitrate concentrations increased as bryozoan coverage increased (Figure 17). The relationship between I-Nitrate and bryozoan coverage was estimated for the bryozoan settling phase, from 3rd of May to 6th of June. A peak in I-Nitrate was observed the 6th of June, with values measured to 0 before and after. If the total sampling period was considered instead of only the settling phase, the results might have looked different. In the settling phase, when all locations and both depth were combined, there was a weak negative relationship between bryozoan coverage and I-Nitrate concentrations (Table 6). There was no significant difference in I-Nitrate concentrations with increasing depth, but a clear difference in bryozoan coverage with increasing depth. This suggest that different factors may affect bryozoan abundance at 3 m and 8 m depths. Bryozoans at 8 m depth might be more able

to respond to a gradient in productivity. The total sampling period, 3rd of May to 20th of July, when all locations and depths were combined, showed a stronger relationship between bryozoan coverage and I-Nitrate concentrations (r_s= -0.605). These findings may suggest that there may be a relationship at both depths, but bryozoans at 3 m might be stronger influenced by other environmental factors in the settling phase.

According to hypothesis 3 bryozoan coverage on *S. latissima* will increase as intracellularly nitrate concentrations in macroalage decrease. The results partly support this hypothesis. Five out of six locations showed a negative correlation were I-Nitrate concentrations decreased as bryozoan coverage increased. Whether those variables influence each other remains unclear. Both the decrease in I-Nitrate concentrations and increase in bryozoan coverage can be related to other variables such as higher temperatures, salinity, water velocity, light abundance, and phytoplankton concentrations.

4.3.2 Bryozoan coverage and food concentrations in water

Bryozoan abundance could be regulated by food availabilities (O'Dea & Okamura, 1999). To investigate this, phytoplankton concentration between <70 µm and <5 µm of size expressed in terms of chl *a* was estimated, since this is the food size the bryozoans species *M. membranacea* and *E. pilosa* are believed to feed on (Riisgård & Manríquez, 1997; Winston et al., 1977). The results revealed a clear relationship between bryozoan coverage and food chl *a* concentrations, were most locations showed a significant relationship with an increase in bryozoan coverage following an increase in chl *a* (Figure 19 and Table 4).

From May and onward all locations showed good chl *a* food availabilities (Figure 18). There was no statistical difference between the different locations and depths. Riisgård and Goldson (1997) found that all of the individual zooids of *E.pilosa* were feeding when phytoplankton concentrations was between 0.5 to 5 µg chl *a*/L. In this study in mid-May, all locations showed chl *a* food concentrations greater than 0.5 µg chl *a*/L, indicating perfect feeding conditions for *E. pilosa*. Furthermore, Hermansen et al.(2001) suggested that *M. membranacea* and *E. pilosa* double their colony area in 5-6 days when food concentrations are adequate. Between the samplings 26th of May and 6th of June (11 days, respectively), the Sheltered 3 m mean bryozoan coverage was calculated to 2.3 % and 5.9 %, and Intermediate 3 m mean was calculated to 2.5 % and 7.3 %. These results cohere with Hermansen et al. (2001) with almost a three time increase in coverage, suggesting adequate food concentrations in this period.

The Exposed location showed the highest food availability (Figure 18). The weak linear relationship at Exposed 3 m can be explained by the mismatch between high chl *a* food concentrations and low bryozoan coverage. As previously mentioned, the bryozoans may have problems settling in exposed conditions and therefore not able to utilize the food available. Exposed 8 m showed a stronger linear relationship with chl *a* food concentrations. The bryozoans at 8 m depth might be more protected from wave action and turbulence than those closer to the surface, and hence better able to settle and utilize the food available. The Sheltered and the Intermediate location showed a more stable chl *a* abundance, while the Exposed location showed a pulsing behaviour fluctuating between high and low chl *a* food concentrations (Figure 18). The chl *a* measurement represents snapshots of the environment with no indication of what is happening in-between the samplings. The Exposed location shows high chl *a* food concentrations of 1.9 µg chl *a*/L in mid-May and close to 0 in end of May, suggesting unstable food concentrations, which might not be favourable for the bryozoans.

There was a closer relationship between bryozoan coverage and chl *a* concentrations at all locations, at 8 m depth. As mentioned earlier, 8 m depth showed generally lower bryozoan coverage, while chl *a* concentrations were relatively similar to those at 3 m depth, at all locations. This suggest that the bryozoans colonizing at 3 m depth may be potentially food limited and prone to intraspecific competition.

When combining all locations at 3 m depth, the bryozoan coverage decreased and chl *a* food concentrations increased with increasing physical exposure (Figure 20). When 8 m depths were combined, on the other hand, a different pattern was revealed were both bryozoan coverage and chl *a* food concentrations increased with increasing physical exposure. This may suggest that chl *a* food concentrations is an important factor explaining bryozoan abundance at 8 m depth, while bryozoans at 3 m might be responding more to other environmental variables, such as temperature, salinity, light availability, or water velocity. When all locations and both depths were combined, there was a weak correlation between bryozoan coverage and chl *a* food concentrations, suggesting that there are different factors limiting bryozoan coverage at the different depths.

In the bryozoan settling phase when all locations and depths were combined, there was a moderate relationship between bryozoan coverage and chl *a* food concentrations. However, when the total sampling period was combined, the relationship was weaker (r_s= 0.334). These

findings may suggest that chl *a* food concentrations may have a stronger influence on bryozoans settling than on older well-established colonies.

Hypothesis 4 said that higher phytoplankton concentration will affect bryozoan growth and colonization. The results partly support this hypothesis. Five out of six locations showed a high positive correlation between bryozoan coverage and chl *a* concentrations in the water. However, both bryozoan and phytoplankton abundance follow the seasons in temperate waters. The relationship between bryozoan coverage and phytoplankton concentrations might be two independent events happening at the same time. Even though there is a correlation between the events, they might be triggered by some other factors, and bryozoan coverage might not be a result of higher food concentrations.

4.4 Summary

The different environmental variables, temperature, salinity, and the external inorganic nutrient all suggest a uniformity between 3 m and 8 m depth at all locations, suggesting an efficient mixing of the water column.

The results showed no significant difference in macroalgae growth at 3 m depth at the different exposures, suggesting adequate light availabilities, nutrient concentrations and water velocities at this depth, at all locations. There was however a significant different at 8 m depth, were Exposed location showed the best growth. The result revealed a depth dependent growth pattern at the Sheltered location were macroalgae cultivated at 3 m depth showed the highest growth. Both E-Nitrate and I-Nitrate showed similar concentration at both depths, suggesting that macroalgae is not able to utilize the available nutrients when water velocities are low. At the Exposed and the Intermediate location, it was neither not found a difference in growth or I-Nitrate concentrations between the different depths, confirming the presumed difference in exposure between the locations. It was found a correlation between E-Nitrate and I-Nitrate concentrations, however, not a linear relationship, suggesting that E-Nitrate concentrations might give you a snapshot of the environment while I-Nitrate concentrations gives you a continuous picture of the actual nitrate concentrations in the environment.

There was a significantly different bryozoan coverage at Sheltered and Exposed location at 3 m depth, were Sheltered showed the highest coverage. However, there was no significant difference in bryozoan coverage at Sheltered-, Intermediate- and Exposed location at 8 m depth. Bryozoan abundance and development was found to be positively correlated with increasing temperatures. Sheltered and Intermediate location showed a depth dependent bryozoan coverage, with highest coverage at 3 m depth. The Exposed location, which is prone to higher mixing, showed no statistical difference in coverage, confirming that physical exposure are one of the abiotic factors affecting bryozoan settlement. The first colonies were observed in mid-May when the temperature exceeded 7°C. There was in general a high variability in bryozoan coverage at all depths and location, especially in the settling phase.

The results revealed a pattern were I-Nitrate concentrations decrease as bryozoan coverage increase. Due to the high growth in early spring the I-Nitrate reserves in *S. latissima* normally becomes exhausted when the bryozoan settles in end of May, and it might not be correct to link those two events together. However, when combining all location, 8 m depth showed a positive correlation between bryozoan coverage and I-Nitrate concentrations, suggesting that bryozoan

abundance might be responding to a gradient in productivity which is aligned with E-Nitrate concentrations. The E-Nitrate concentrations, which is linked with the I-Nitrate concentrations, can be related to phytoplankton abundance which the bryozoans feed on.

The first bryozoans were observed in mid-May, when all locations showed adequate chl *a* food concentrations. Five out of six locations showed a strong positive correlation between bryozoan coverage and chl *a* concentrations in the water, confirming that bryozoan abundance was correlated to chl *a* food concentrations in the water column. When all location were combined, 8 m depth showed a correlation were bryozoan coverage increase as chl *a* food concentrations increase, with increasing exposure. The same pattern was also revealed for macroalgae growth at 8 m, confirming the influence chl *a* food concentrations and E-Nitrate concentrations might have on bryozoan abundance. When combining all location at 3 m and 8 m depth, there was a weaker correlation between bryozoan coverage and I-Nitrate concentrations, and between bryozoan coverage and chl *a* food concentrations, suggesting that different factors influences bryozoan coverage at the different depths.

Both bryozoan and phytoplankton abundance follow the seasons in temperate waters and the correlations seen between I-nitrate concentrations and bryozoan coverage, and chl *a* concentrations and bryozoan coverage might be related to other variables such as elevation in temperatures, salinity, water velocity, light abundance, or nutrient availabilities. It is therefore difficult to settle on a specific factor explaining the correlation. Even though there is a correlation between the events, they might be triggered by different factors.

The correlations revealed in the results are based on small samples sizes which should be taken into consideration when interpreting the results. In future bryozoan abundance investigations related to bryozoans fouling on macroalgae a higher number of macroalgae individuals should be collected within a shorter sampling interval, in order to get a higher statistical significance. To be able to reveal the importance of the macroalgaes nutritional state when bryozoans are colonizing, intracellular nitrate concentrations and bryozoan coverage should be estimated on the same individual.

The Intermediate location shows the most optimal conditions for bryozoan growth, with high productivity, good nutrient availabilities and intermediate water velocities. The Exposed location showed the best growth with low bryozoan coverage, suggesting that future macroalage cultivation should be located in exposed waters with high water velocities and good nutrient availabilities.

5 Conclusion

Hypothesis 1 state that the growth and nutritional state of S. latissima will vary across categories of exposure and depths. The results showed no significant difference in growth at 3 m depth at different exposures, however there was a significant difference at 8 m depth, where Exposed location showed most growth. The I-Nitrate concentrations at Sheltered location were significantly lower than at Exposed location. There was no difference in I-Nitrate concentrations between 3 m and 8 m depths. The results of this study partly support Hypothesis 1.

Hypothesis 2 state that the bryozoan coverage on *S. latissima* will vary across exposure and depths. The results showed a depth dependent coverage pattern at Sheltered and Intermediate location, where 3 m depth showed the highest bryozoan coverage. At Exposed location there was no significant difference in coverage between 3 m and 8 m depth. There was a significant different bryozoan coverage at Sheltered and Exposed location at 3 m depth, where Sheltered location showed the highest coverage. However, no significant difference was found in bryozoan coverage at Sheltered-, Intermediate- and Exposed location at 8 m depth. The results partly support hypothesis 2.

According to hypothesis 3 bryozoan coverage on *S. latissima* will increase as intracellular nitrate concentrations (I-Nitrate) in macroalgae decrease. Five out of six locations showed a negative correlation were I-Nitrate concentrations decreased as bryozoan coverage increased. Further study is needed to identify if these variables influence each other. At 8 m depth there was a positive correlation between bryozoan coverage and I-Nitrate concentrations, suggesting that bryozoan abundance might be responding to a gradient in productivity which is aligned with E-Nitrate concentrations. However, both the decrease in I-Nitrate concentrations and increase in bryozoan coverage can be related to other variables such as higher temperatures, salinity, water velocity, light abundance, and phytoplankton concentrations. The results partly support hypothesis 3.

Hypothesis 4 said that higher phytoplankton concentration will affect bryozoan growth and colonization. Five out of six locations showed a strong positive correlation between bryozoan coverage and chl *a* concentrations. However, both bryozoan and phytoplankton abundance follow the seasons in temperate waters. The relationship between bryozoan coverage and phytoplankton concentrations may be two independent events occuring at the same time. Even

though there is a correlation between these events, they may be triggered by different factors. The results partly support this hypothesis.

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Appendix 1

Raw data

Table 7 Raw data from bryozoan coverage (%), chlorophyll a concentrations (µg/l) and intracellularly nitrate concentrations in macroalgae from 3rd of May to 20th of July 2017. At Sheltered- (1), Intermediate- (2) and Exposed location (3), at 3 and 8m depth.

Date	Depth	Location	Coverage %	Chl a (µg/l)	I-Nitrate NO_3-N (mg/g dw)
3. mai	3	1	0	0,106	0,012
3. mai	3	1	0	0,073	0,002
3. mai	3	1	0	0,200	0,001
3. mai	3	2	0	0,075	0,016
3. mai	3	2	0	0	0,025
3. mai	3	2	0	0	0,014
3. mai	3	3	0	0	0,019
3. mai	3	3	0	0	0,055
3. mai	3	3	0	NA	0,018
3. mai	8	1	0	0	0,002
3. mai	8	1	0	0	0,001
3. mai	8	1	0	NA	NA
3. mai	8	2	0	0,065	0,019
3. mai	8	2	0	0,043	0,007
3. mai	8	2	0	0,038	0,012
3. mai	8	3	0	0,187	0,068
3. mai	8	3	0	0,663	0,048
3. mai	8	3	0	0,017	NA
15. mai	3	1	0,382	0,522	0,007
15. mai	3	1	0,284	0,478	0,003
15. mai	3	1	1,120	0,369	0,001
15. mai	3	2	0,635	0,286	0,011
15. mai	3	2	0,096	0,245	0,011
15. mai	3	2	0,289	0,238	0,010
15. mai	3	3	1,250	0,487	0,009
15. mai	3	3	1,240	0,450	0,009
15. mai	3	3	1,102	0,549	NA
15. mai	8	1	0,201	0,303	0,007
15. mai	8	1	0,174	0,441	0,005
15. mai	8	1	0,041	0,086	0,005
15. mai	8	2	0,264	1,864	0,062
15. mai	8	2	0,494	1,641	0,037
15. mai	8	2	0,561	1,813	0,001
15. mai	8	3	0,305	1,841	0,062
15. mai	8	3	1,615	1,803	0,059
15. mai	8	3	4,616	2,099	0,244

26. mai	3	1	1,800	0,441	0,002
26. mai	3	1	2,111	0,487	0,002
26. mai	3	1	3,108	0,511	0,001
26. mai	3	2	2,665	0,487	0,002
26. mai	3	2	1,177	0,599	0,001
26. mai	3	2	3,648	0,629	0,001
26. mai	3	3	1,106	0,024	0,005
26. mai	3	3	1,031	0,031	0,008
26. mai	3	3	1,541	0,334	0,001
26. mai	8	1	0,928	0,5193	0,001
26. mai	8	1	0,489	0,550	0,001
26. mai	8	1	0,315	0,582	0,001
26. mai	8	2	1,278	0,143	0,002
26. mai	8	2	1,310	NA	0,001
26. mai	8	2	0,691	0,153	0,001
26. mai	8	3	1,081	0,106	0,001
26. mai	8	3	0,753	0,093	0,001
26. mai	8	3	0,704	0,128	0,004
6. juni	3	1	2,592	0,834	0,054
6. juni	3	1	9,056	0,780	0,001
6. juni	3	1	6,128	0,794	0,001
6. juni	3	2	8,690	1,070	0,039
6. juni	3	2	5,299	0,997	0,003
6. juni	3	2	7,977	0,790	NA
6. juni	3	3	0,618	0,896	0,007
6. juni	3	3	1,183	0,564	0,042
6. juni	3	3	3,612	0,985	0,003
6. juni	8	1	0,195	1,313	0,001
6. juni	8	1	1,861	1,460	0,001
6. juni	8	1	1,438	1,505	0,059
6. juni	8	2	2,862	1,012	0,001
6. juni	8	2	0,518	0,257	0,001
6. juni	8	2	0,367	0,817	0,001
6. juni	8	3	1,608	0,496	0,111
6. juni	8	3	1,024	0,430	0,006
6. juni	8	3	0,384	0,233	0,089
20. juli	3	1	91,557	0,667	0
20. juli	3	1	93,092	0,598	0
20. juli	3	1	99,663	0,750	0
20. juli	3	2	99,546	0,702	0
20. juli	3	2	64,027	0	0
20. juli	3	2	98,925	0	0,006
20. juli	3	3	89,186	0,490	0
20. juli	3	3	93,408	0,549	0
20. juli	3	3	66,924	0,608	0

20. juli	8	1	40,737	0,309	0
20. juli	8	1	94,081	0,395	0
20. juli	8	1	66,277	0,414	0
20. juli	8	2	58,152	0,271	0
20. juli	8	2	56,487	0,257	0
20. juli	8	2	83,671	0,306	0
20. juli	8	3	96,951	0,086	0,005
20. juli	8	3	93,140	0,178	0
20. juli	8	3	99,577	0,429	0

Appendix 2

Calculations

Relative growth rate

Table 8 Relative growth rate of S. latissima sampled from March to July 2017. At Sheltered-, Intermediate-, and Exposed location at 3 and 8m depth. Calculations are based on three samples were lamina area was calculated for both sides. Calculations was done by the use of Equation 3.

Location	*Shel. 3m*	*Shel. 8m*	*Interm. 3m*	*Interm. 8m*	*Expo. 3m*	*Expo. 8m*
30.03	0,684	0,275	0,567	0,275	0,483	0,335
03.05	0,052	0,116	0,055	0,257	0,102	0,138
15.05	-0,019	0,013	0,088	0,053	0,040	0,035
26.05	0,243	0,123	0,012	-0,001	0,103	0,051
06.06	0,043	-0,006	0,096	0,002	-0,045	0,063
25.06	-0,001	0,003	-0,011	0,007	0,066	-0,001
20.07	-0,023	-0,007	-0,013	0,008	-0,009	-0,021
$\bar{X}$ RGR	0,139	0,073	0,113	0,086	0,105	0,085

Area

Table 9 Mean area of S. latissimi sampled from March to July 2017. At Sheltered-, Intermediate-, and Exposed location at 3 and 8m depth.

Location	*Shel. 3m*	*Shel. 8m*	*Interm. 3m*	*Interm. 8m*	*Expo. 3m*	*Expo. 8m*
30.03	0,684	0,275	0,567	0,275	0,483	0,335
03.05	0,052	0,116	0,055	0,257	0,102	0,138
15.05	-0,019	0,013	0,088	0,053	0,040	0,035
26.05	0,240	0,123	0,012	-0,001	0,103	0,051
06.06	0,043	-0,006	0,096	0,002	-0,045	0,063
25.06	-0,000	0,003	-0,011	0,007	0,066	-0,001
20.07	-0,023	-0,007	-0,013	0,008	-0,009	-0,021
$\bar{X}$ RGR	0,13951	0,07398	0,11365	0,08646	0,10592	0,08591

Freidmann test output

Freidmann test output based on values in table 9, sampled from March to July 2017. N=7, P<0.05.

Ranks	
	Mean Rank
L1D3	3,71
L1D8	1,50
L2D3	3,71
L2D8	4,07
L3D3	4,57
L3D8	3,43

Test Statistics[a]	
N	7
Chi-Square	11,189
df	5
Asymp. Sig.	0,048*
a. Friedman Test	

*Table 10 Post Hock, Wilcoxon signed rank test output. P<0.05 marked with *.*

Location	L1D3	L1D8	L2D3	L2D8	L3D3	L3D8
L1D3		0.063	0.735	0.735	0.310	0.499
L1D8	0.063		0.028*	0.028*	0.018*	0.018*
L2D3	0.735	0.028*		0.499	0.499	0.866
L2D8	0.735	0.028*	0.499		0.866	0.612
L3D3	0.310	0.018*	0.499	0.866		0.237
L3D8	0.499	0.018*	0.866	0.612	0.237	

Bryozoan coverage

Table 11 Bryozoan coverage from May to July 2017. At Sheltered- (1), Intermediate- (2), and Exposed location (3) at 3 and 8m depth.

Date	L1D3	L1D8	L2D3	L2D8	L3D3	L3D8
03.05.17	0,00	0,00	0,00	0,00	0,00	0,00
03.05.17	0,00	0,00	0,00	0,00	0,00	0,00
03.05.17	0,00	0,00	0,00	0,00	0,00	0,00
15.05.17	0,38	0,20	0,64	0,26	1,25	0,31
15.05.17	0,28	0,17	0,10	0,49	1,24	1,62
15.05.17	1,12	0,04	0,29	0,56	1,10	4,62
26.05.17	1,80	0,93	2,67	1,28	1,11	1,08
26.05.17	2,11	0,49	1,18	1,31	1,03	0,75
26.05.17	3,11	0,32	3,65	0,69	1,54	0,70
06.06.17	2,59	0,20	8,69	2,86	0,62	1,61
06.06.17	9,06	1,86	5,30	0,52	1,18	1,02

06.06.17	6,13	1,44	7,98	0,37	3,61	0,38
20.07.17	91,56	40,74	99,55	58,15	89,19	96,95
20.07.17	93,09	94,08	64,03	56,49	93,41	93,14
20.07.17	99,66	66,28	98,93	83,67	66,92	99,58
Mean	20,72	13,78	19,53	13,77	17,48	20,11

Freidmann test output

Freidmann test output based on values in table 11, sampled from May to July 2017. N=15, P<0.05.

Ranks	
	Mean Rank
L1D3	4,43
L1D8	2,23
L2D3	4,23
L2D8	2,90
L3D3	3,63
L3D8	3,57

Test Statistics[a]	
N	15
Chi-Square	18,190
df	5
Asymp. Sig.	0,003*
a. Friedman Test	

*Table 12 Post Hock, Wilcoxon signed rank test p<0.05 marked with *.*

Location	L1D3	L1D8	L2D3	L2D8	L3D3	L3D8
L1D3		0.006*	0.937	0.008*	0.028*	0.308
L1D8	0.006*		0.028*	0.347	0.028*	0.099
L2D3	0.937	0.028*		0.012*	0.136	0.388
L2D8	0.008*	0.347	0.012*		0.158	0.099
L3D3	0.028*	0.028*	0.136	0.158		0.583
L3D8	0.308	0.099	0.388	0.099	0.583	

Chlorophyll *a*

Table 13 chlorophyll a concentrations *from May to July 2017. At Sheltered- (1), Intermediate- (2), and Exposed location (3) at 3 and 8m depth.*

Date	L1D3	L1D8	L2D3	L2D8	L3D3	L3D8
03.05.17	0,11	0,08	0,00	NA	0,07	0,19
03.05.17	0,07	0,00	NA	0,00	0,04	0,66
03.05.17	0,20	0,00	0,00	0,00	0,04	0,02
15.05.17	0,52	0,29	0,49	0,30	1,86	1,84
15.05.17	0,48	0,25	0,45	0,44	1,64	1,80

15.05.17	0,36	0,24	0,55	0,09	1,81	2,10
26.05.17	0,44	0,49	0,02	0,52	0,14	0,11
26.05.17	0,48	0,60	0,03	0,55	0,15	0,09
26.05.17	0,51	0,63	0,33	0,58	NA	0,13
06.06.17	0,83	1,07	0,90	1,31	1,01	0,50
06.06.17	0,78	1,00	0,56	1,46	0,26	0,43
06.06.17	0,79	0,79	0,99	1,51	0,82	0,23
20.07.17	0,67	0,70	0,49	0,31	0,27	0,09
20.07.17	0,60	0,00	0,55	0,40	0,26	0,18
20.07.17	0,75	0,00	0,61	0,41	0,31	0,43
Mean	0,5760	0,4516	0,4698	0,6083	0,7147	0,6514

Freidmann test output

Freidmann test output based on values in table 13, sampled from May to July 2017. N=15, P<0.05.

Ranks	
	Mean Rank
L1D3	4,25
L1D8	3,08
L2D3	3,25
L2D8	3,83
L3D3	3,58
L3D8	3,00

Test Statistics[a]	
N	12
Chi-Square	4,038
df	5
Asymp. Sig.	0,544
a. Friedman Test	

Intracellular dissolved nitrate

Table 14 intracellular nitrate concentrations *from March to July 2017. At Sheltered- (1), Intermediate- (2), and Exposed location (3) at 3 and 8m depth.*

Date	L1D3	L1D8	L2D3	L2D8	L3D3	L3D8
30.03.17	0,093	0,086	0,077	0,05467938	0,14834678	0,07104946
30.03.17	0,054	0,125	0,053	NA	0,08957488	0,09307756
30.03.17	0,085	0,103	NA	NA	NA	NA
03.05.17	0,012	0,002	0,016	0,019	0,019	0,068
03.05.17	0,002	0,001	0,025	0,007	0,055	0,048
03.05.17	0,001	NA	0,014	0,012	0,018	NA
15.05.17	0,007	0,007	0,011	0,062	0,009	0,062
15.05.17	0,003	0,005	0,011	0,037	0,009	0,059
15.05.17	0,001	0,005	0,010	0,001	NA	0,244
26.05.17	0,002	0,001	0,002	0,002	0,005	0,001
26.05.17	0,002	0,001	0,001	0,001	0,008	0,001
26.05.17	0,001	0,001	0,001	0,005	0,004	0,004
06.06.17	0,054	0,001	0,001	0,001	0,001	0,111
06.06.17	0,001	0,001	0,002	0,001	0,001	0,006
06.06.17	0,001	0,059	0,002	0,001	0,002	0,089

25.06.17	0,001	0,017	0,039	0,005	0,007	0
25.06.17	0	0	0,003	0	0,042	0,032
25.06.17	0,01	0	0	0,013	0,003	0,004
20.07.17	0	0	0	0	0	0,005
20.07.17	0	0	0,006	0	0	0
20.07.17	0	0		0	0	0
Mean	0,011	0,0115	0,012	0,0124	0,019	0,035

Freidmann test output

Freidmann test output based on values in table 14, sampled from March to July 2017. N=16, P<0.05.

Ranks	
	Mean Rank
L1D3	2,97
L1D8	2,47
L2D3	3,97
L2D8	3,28
L3D3	4,09
L3D8	4,22

Test Statistics[a]	
N	16
Chi-Square	13,212
df	5
Asymp. Sig.	0,021*
a. Friedman Test	

*Table 15 Post Hock, Wilcoxon signed rank test p<0.05 marked with *.*

Location	L1D3	L1D8	L2D3	L2D8	L3D3	L3D8
L1D3		0.959	0.093	0.460	0.030	0.009*
L1D8	0.959		0.179	0.363	0.191	0.022*
L2D3	0.093	0.179		0.255	0.109	0.017*
L2D8	0.460	0.363	0.255		0.334	0.017*
L3D3	0.030*	0.191	0.109	0.334		0.469
L3D8	0.009*	0.022*	0.017*	0.017*	0.469	