NTNU – Trondheim
Norwegian University of
Science and Technology

Development of bryozoan fouling on cultivated kelp (*Saccharina latissima*) in Norway

Henny Førde

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Supervisor: Geir Johnsen, IBI

Norwegian University of Science and Technology
Department of Biology

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Abstract

Biofouling of cultivated kelp is a major challenge for the seaweed industry, and hard to avoid during the cultivation process. Several species are involved in the fouling in temperate waters, and among them are the encrusting bryozoans *Membranipora membranacea* and *Electra pilosa*. The bryozoans planktotrophic larvae settles on kelp and give rise to widespread colonies that covers the surface of the kelp thalli. The colonies make the flexible kelp thalli brittle and susceptible to breakage, and thus loss of valuable biomass for the producers. The encrusting fouling also reduces the value of the product my making it indelicate and unsuitable for human food consumption.

The development of the bryozoan fouling on cultivated *Saccharina latissima* in temperate waters was documented during the cultivation period in the sea from April to September to establish the time of settling and development in area coverage of colonies of *M. membranacea* and *E. pilosa*. This was performed at two locations in Norway, one for frequent time registrations at a seaweed farm, and one for registrations of the development of bryozoan fouling in an integrated multi-trophic aquaculture (IMTA) system. The registrations were performed at three different cultivation depths at each location. Zooplankton samples were also taken regularly for registration of bryozoan larvae abundance.

The results showed that the bryozoan colonies settled on the cultivated kelp in mid June at both locations, followed by a rapid colony growth during late June and July. In August and September the kelp was highly degraded by the bryozoan coverage, and very subjective to breakage of the lamina. *M. membranacea* was the most prevailing of the two species, having the highest proportion of coverage during the whole sampling period, even though both species was present in the zooplankton samples in almost similar abundance. Although abundant at all cultivation depths, the statistical analysis of the data showed a decrease in bryozoan coverage with increasing depth. Cultivating kelp at lower depths may however reduce the production of kelp biomass, and may not be feasible for the industry. The zooplankton analysis showed presence of bryozoan larvae during the whole sampling season and a peak in abundance in late June, which coincided with the rapid increase in bryozoan coverage on the kelp. This study shows that, from a commercial point of view, harvest of cultivated *S. latissima* in temperate waters should occur in June to avoid the negative impact from bryozoan fouling.

Sammendrag

Begroing av dyrket tare er en stor utfordring for tareindustrien, og vanskelig å unngå i løpet av kultiveringsprosessen. Flere arter er involvert i begroingen i tempererte farvann, og blant dem er de skorpedannende mosdyrene *Membranipora membranacea* og *Electra pilosa*. Mosdyrenes planktotrofiske larver setter seg på taren og gir opphav til omfattende kolonier som dekker overflaten av tarebladet. Koloniene gjør at det fleksible tarebladet blir skjørt og brekker lettere av, noe som skaper tap av verdifull biomasse for produsentene. Den skorpedannende begroingen reduserer også verdien av produktet, og kan gjøre det udelikat og uegnet for salg som matvare.

Utviklingen av mosdyrbegroing på dyrket *Saccharina latissima* i tempererte farvann ble dokumentert gjennom dyrkingsperioden i sjøen fra april til september for å bestemme tidspunkt for nedslåing og utviklingen i kolonienes arealdekke av mosdyrene *M. membranacea* og *E. pilosa*. Dette ble utført på to steder i Norge, et for regelmessige tidsregistreringer på en tarefarm, og et for registreringer av utviklingen av mosdyrbegroing i et integrert multi-trofisk akvakultur (IMTA) system. Registreringene ble utført ved tre forskjellige dyrkingsdybder på hvert sted. Zooplanktonprøver ble også tatt regelmessig for registrering av mengde mosdyrlarver.

Resultatene viste at mosdyrkoloniene satte seg på den dyrkede taren i midten av juni på begge steder, etterfulgt av en rask kolonivekst i løpet av slutten av juni og i juli. I august og september var taren betydelig dekket av mosdyrkolonier, og svært subjektive til brekkasje av lamina. *M. membranacea* var den mest utbredte av de to artene og hadde den høyeste andelen av dekning under hele prøveperioden, selv om begge artene var til stede i zooplanktonprøvene i nesten lik mengde. Selv om mosdyrkoloniene var tilstede på alle dyrkningsdybder viste den statistiske analysen av dataene en reduksjon i mosdyrdekke ved økende dybde. Dyrking av tare på lavere dybder kan imidlertid redusere produksjonen av tarebiomasse, noe som ikke vil gagne bransjen. Zooplanktonanalysen viste tilstedeværelse av mosdyrlarver gjennom hele prøvetakingssesongen, og en mengdetopp i slutten av juni som falt sammen med den raske økningen i mosdyrdekket på taren. Denne studien viser at høstingen av dyrket *S. latissima* i tempererte farvann, fra et kommersielt synspunkt, bør skje i juni for å unngå de negative effektene fra mosdyrbegroing .

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1 Introduction

1.1 Seaweed cultivation

1.1.1 Global seaweed industry

On a global scale, about 23.8 million tonnes of aquatic algae (marine macroalgae, and marine and freshwater microalgae) was cultivated in aquaculture in 2012 according to the FAO Fisheries and Aquaculture Department's statistics (2014). Almost all of the production is situated in Asia, and mainly China that contributes with 12.8 million tonnes of the total. The industry is in rapid growth, showing a 10.4 % production growth just from 2010 to 2011 (FAO, 2013). The harvest of wild seaweed has however remained relatively stable for the last years, with a volume of 1.1 million tonnes in 2012 (FAO, 2014).

The seaweed is used both for human consumption and for industrial use (McHugh, 2003). Phycocolloids as alginate, agar and carrageenan extracted from brown and red seaweed are being used as thickening and gelling agents in various products (Jensen, 1993). The seaweed may also be used as soil fertilizer and in animal feed (Kain & Dawes, 1987). The potential for use of macroalgae in the production of biofuel is also being explored (Kraan, 2013).

1.1.2 Norwegian seaweed industry

In Norway the seaweed industry constitutes 100% of harvest of natural beds of brown algae. The most harvested species is the brown algae *Laminaria hyperborea* (150 thousand tonnes) that is used by FMC Biopolymer AS for production of alginate for pharma- and nutraceutical products, and less volumes of the fucoid brown algae *Ascophyllum nodosum* (10-20 thousand tonnes) that are used by Algea AS for production of seaweed meal for use in aminal feed, fertilizers and cosmetics (Meland & Rebours, 2012).

Certain ecological issues are believed to arise when harvesting natural beds of seaweed. The kelp forest provides habitat and nursery shelter for a vast number of marine species, and it is an uncertainty if the trawling may affect the stocks of some fish species. Such interference as the seaweed trawling may represent on the seafloor ecosystem will have a short-term effect, and it will take some years to regain the pre harvest ecosystem balance (Christie et al., 1998). The interest for cultivation of seaweed in Norway has increased the last years, but the production is still mainly on an R&D stage (Meland & Rebours, 2012).

1.1.3 Integrated multi-trophic aquaculture

Another form of seaweed production is to cultivate seaweed and other extractive species in vicinity to other fed aquaculture species (Chopin et al., 2001; Neori et al., 2004; Chopin et al., 2008; Barrington et al., 2009; Troell et al., 2009). This form of aquaculture has been named integrated multi-trophic aquaculture (IMTA), where one species will take advantage on the wastes produced by another species. IMTA has been tested and practiced in many Asian countries as well as other countries like Canada and Chile (Chopin et al., 2001; Chopin et al., 2008).

Large amounts of nutrients from faeces and excess feed, as well as excretory and respiration products in salmon aquaculture in Norway are released into the surrounding water masses around the fish cages (Olsen et al., 2008). Estimates calculated by Wang et al. (2013) show that as much as 62 % of the nitrogen and 76 % of the phosphorous in feed used in salmon aquaculture is released as excess nutrients in the marine environment. It is thus suggested to cultivate extractive species from lower trophic levels close to the salmon farm, like seaweed and mussels, which can extract the excess nutrients from the farm (Chopin et al., 2001; Troell et al., 2003; Neori et al., 2004; Chopin et al., 2008; Handå et al., 2013). The goal with IMTA is to maintain an increasing biomass production and at the same time utilize the feed investments in a better way, which in turn can give a more sustainable aquaculture production and environmental advantages (Barrington et al., 2009).

1.2 Saccharina latissima

An attractive candidate for Norwegian seaweed cultivation is the large brown kelp *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders, mainly because of its rapid growth and high content of polysaccharides, as well as being native and adapted to Norwegian coastal waters.

S. latissima is naturally common along the Norwegian coast, and is usually found in the sublittoral zone and down to the lower euphotic zone. It belongs to the phylum Ochrophyta, the class of Phaeophyceae (brown algae), and the Laminariales order (kelp). Until recent it was classified as the genus *Laminaria*, but the classification was changed in 2006 to the genus

Saccharina (Lane et al., 2006). The common name of *S. latissima* is sugar kelp, which may refer to the species' high content of polysaccharides.

Kelp sporophytes can usually be divided into three distinct parts: the holdfast/hapter, the stipe and the lamina (Figure 1-1). *S. latissima* has a highly branched holdfast and a smooth, flexible stipe that varies in thickness and length with respect to current strength (Lüning, 1990). The shape of the lamina also varies with grade of exposure. At sheltered locations it is usually wide and smooth compared to exposed locations where it can be more elongated and wrinkled (Lüning, 1990). Production of new tissue occurs at the meristem positioned by the stipe.

Figure 1-1 – Illustration of the subdivision of the sporophyte thallus, with holdfast, stipe and lamina

The life cycle of *S. latissima* alternates between a visible sporophyte phase and a microscopic gametophyte phase (Kain, 1979; Bartsch et al., 2008). The sporophyte produces spores by meiosis that is released into the sea. The spores develop into haploid female and male gametophytes, which in turn produce gametes by mitosis. A diplod zygote is formed when the gametes fuses to produce a new sporophyte. The spore releasing sporophyte can be perennial by shedding the lamina that can be regenerated from the meristem by the remaining stipe the following year. The *S. latissima* sporophyte has a high growth rate from late winter to spring, but the rate declines during the summer (Sjøtun, 1993).

The cultivation of *S. latissima* in temperate waters, as described in Forbord et al. (2012), starts by inducing spore release in motherplants by cutting off the meristem and giving the sporophyte a short-day light treatment. The released spores are then seeded onto strings of rope and sporelings incubated in tanks until they have reached a length of 5-8 mm. The ropes

with juveniles are then being deployed on longlines in the sea, where they stay for the rest of the cultivation period.

As other species of kelp, *S. latissima* provides substrate and habitat area for a variety of other species, both sessile and vagile (Bartsch et al., 2008; Christie et al., 2009). The smooth, flexible, wide lamina that flows with the current is excellent as habitat for small filter feeding organisms and small epiphytic algae (Ryland, 1962; Seed & O'Connor, 1981).

1.3 Challenge with fouling of seaweed

Fouling causes a major challenge for the seaweed industry (Fletcher, 1995; Forbord et al., 2012; Handå et al., 2013; Peteiro & Freire, 2013), and it is often advised to harvest the crops before the onset of fouling to extract the best product and to prevent loss of valuable biomass. When cultivating the kelp *Saccharina longicruris* in Canada, Gendron and Tamigneaux (2008) experienced bryozoa colonization on the kelp blade and stipe, reducing the blade by 68 %. In a study on macroalgae (*S. latissima*) cultivation in Trøndelag performed by Forbord et al. (2012) the best growth rate of the sporophytes was registered from February to June. In the following months the kelp was almost completely covered by epiphytes, which lead to necrosis in the distal end of the frown, and loss of biomass. The epiphytes included hydroids, mussels, other algae and particularly bryozoan colonies (Forbord, pers. comm.).

Both naturally growing and cultivated seaweed are subjected to fouling by epiphytes and epifauna. Encrusting fouling may hinder the kelps flexible nature by making the frond stiff and crispy (Dixon et al., 1981). This was also showed in a study by Krumhansl et al. (2011), where *S. longicruis* encrusted with the bryozoan *Membranipora membranacea* was more susceptible to breakage than non-encrusted specimens. The same study also showed increased lesions of the upper epidermal cells for the macroalgae exposed to encrustation. Other negative impacts of the encrusting fouling is inhibition of reproduction by preventing spore release (Saier & Chapman, 2004), creating a barrier to nutrient uptake (Hurd et al., 2000), and inhibition of photosynthesis by blocking of the surface area of the frond and reducing pigmentation (Hepburn et al., 2006). In a study on reduction of natural beds of *S. latissima* in Skagerrak, Norway, Andersen et al. (2011) concluded that the effect of heavy fouling, reducing access to light and disrupting the natural life cycle, was the main reason for reduction in the population.

1.4 Bryozoans

One of the conspicuous epifauna growing on seaweed is colonies of bryozoans. The phylum is well described by Hayward & Ryland (1998), and consists of three classes; Phylactolaemata, Stenolaemata and Gymnolaemata, and four orders; Plumatellida, Cyclostomata, Ctenostomata, and Cheilostomata. A bryozoan colony consists of small box-shaped individuals (zooids) that arises when a single ancestrula zooid, originating from a sexually produced larva, starts asexual budding and creates a series of identical zooids. They feed by filtrating phytoplankton with their lophophore, a ciliated tentacle.

Two common epiphytic, kelp encrusting bryozoan species in the North-East Atlantic Ocean are *Membranipora membranacea* (Linnaeus) and *Electra pilosa* (Linnaeus), both from the class of Gymnolaemata and Cheilostomata order (Hayward & Ryland, 1995). The walls that enclose each individual zooid (zoecia) are lightly calcified, and together the zooids create extensive, highly organized, mat-like colonies (Figure 1-2). The lightness of calcification makes the zoecia more flexible and more able to withstand bending at the flexible lamina of the macroalgae it inhabits (Seed & O'Connor, 1981).

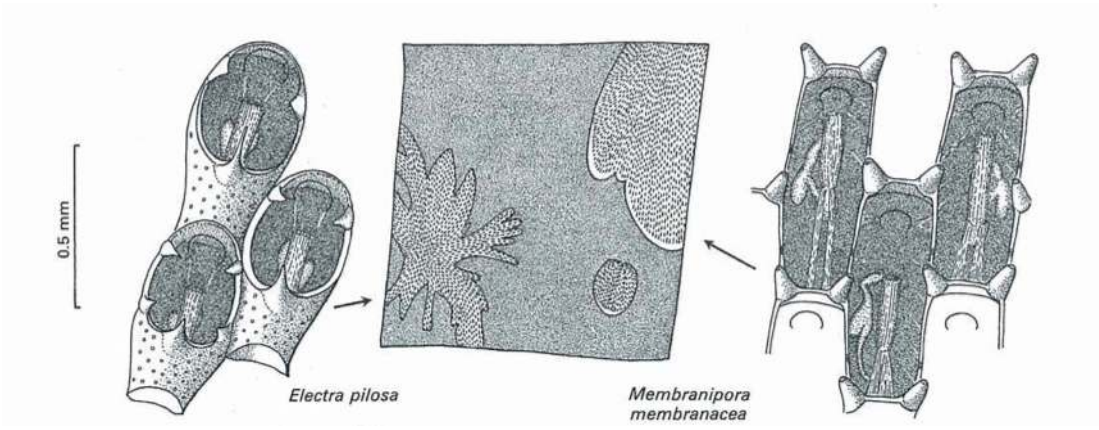

Figure 1-2 – Illustration of zooids and colony shape for *Electra pilosa* and *Membranipora membranacea* (Hayward & Ryland, 1995)

1.4.1 Membranipora membranacea

M. membranacea sheds fertilized eggs directly into the sea, which develops into feeding, planktotrophic cyphonaut larvae (size about 0,6 x 0,8 mm, Figure 1-3) that may remain in the plankton for weeks or months before settling (Ryland & Stebbing, 1971). Production of gametes occurs in the early spring, and follows continuously during the early summer. The triangular larva can be found in North Atlantic coastal plankton from February to November, especially between June and August (Ryland, 1965).

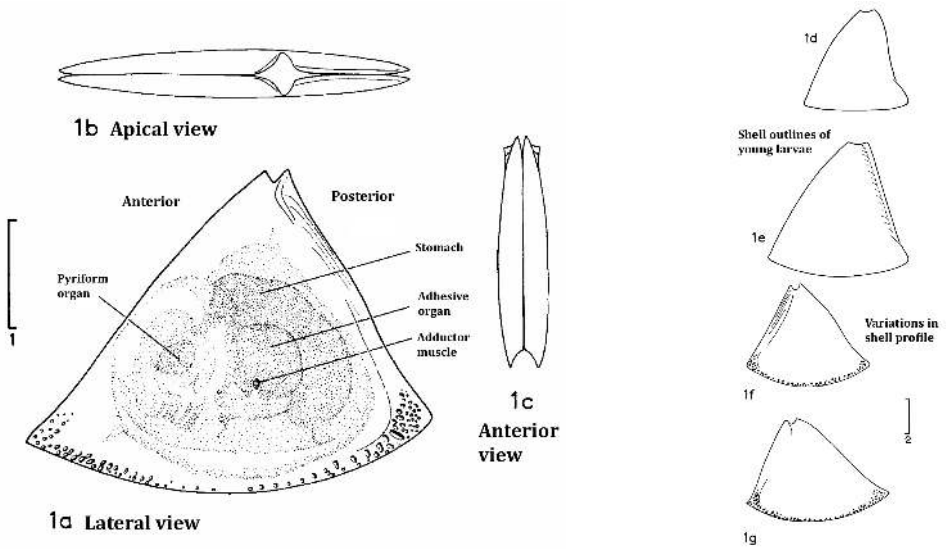

Figure 1-3 – Cyphonaut larvae of *Membranipora membranacea* (Ryland, 1965)

M. membranacea prefers fronds of kelp as substrate, especially species from the *Laminaria* genus (Hayward & Ryland, 1995). The cyphonaut larva of *M. membranacea* is shown to be highly locomotive when exploring suitable substrate and is able to move around in all directions, but usually possesses an upstream motion (Abelson, 1997). This ability may influence the positioning of settlement at the kelp frond, which is often at the base of the lamina (Ryland & Stebbing, 1971), and thus upstream when the kelp is flowing with the current in the sea. When settling, the cyphonaut larva give raise to twin ancestrula zooids (Atkins, 1955). By asexual budding from the twin ancestrula, *M. membranacea* produces roughly circular colonies (Figure 1-2)(Hayward & Ryland, 1998).

1.4.2 Electra pilosa

Gametes from *E. pilosa*, which also develops into planktotrophic cyphonaut larvae (Figure 1-4), are mainly produced in late summer and remains present in the plankton throughout the year (Ryland, 1965). The cyphonaut larva of *E. pilosa* is smaller (size about 0,4 x 0,5 mm) than of *M. membranacea,* and appears rather opaque without ornamentation along the basal edge (Atkins, 1955). When settling, the cyphonaut larva metamorphoses and give raise to a single ancestrula zooid (Atkins, 1955). The asexually produced colonies have a characteristic star-like shape (Figure 1-2), and occur on almost any substratum (Hayward & Ryland, 1995).

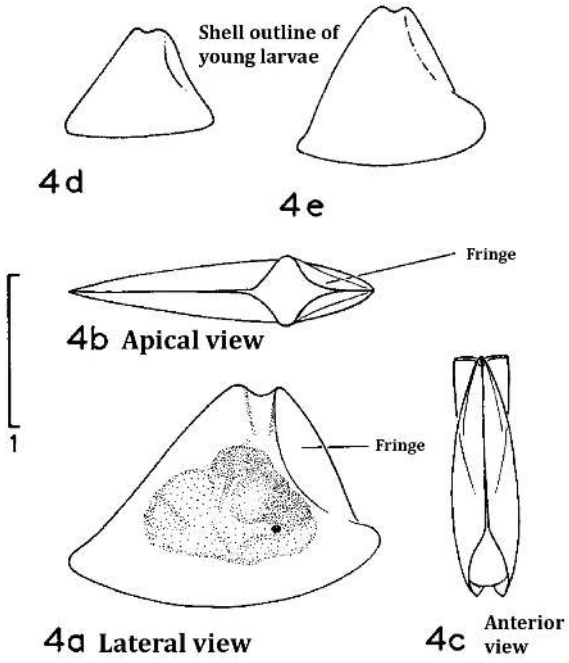

Figure 1-4 – Cyphonaut larvae of *Electra pilosa* (Ryland, 1965)

1.5 Study aims and approach

The main aim of this thesis is to describe the development of epifauna and the impact of bryozoan colonies on cultivated macroalgae *S. latissima* during the cultivation period. The reason for selecting this particular kelp fouling species was because of the encrusting and area covering nature of bryozoan colonies, which would have a greater impact on the production of seaweed than erect species with less direct area coverage.

This has been approached by:

- Taking regular sampling of cultivated *S. latissima* during the cultivation period in the sea, and by calculating the area coverage of bryozoan colonies at the different sampling dates
- Taking measurements of coverage at different cultivation depths to investigate depth dependencies of the bryozoan growth
- Comparing bryozoan growth at cultivation in a monoculture system and in an IMTA system
- Taking regular sampling of zooplankton and semi-quantitative analysis of cyphonaut larvae abundance during the cultivation period to investigate the effect of relative larvae abundance

2 Materials and method

2.1 Sampling areas

The sampling of cultivated *Saccharina latissima* was carried out at two different locations in Norway (Figure 2-1). One location was the study site for registration of bryozoan growth in an IMTA system (Florø), while the other was used for frequent time registrations of bryozoan growth during the growth season of *S. latissima* (Frøya).

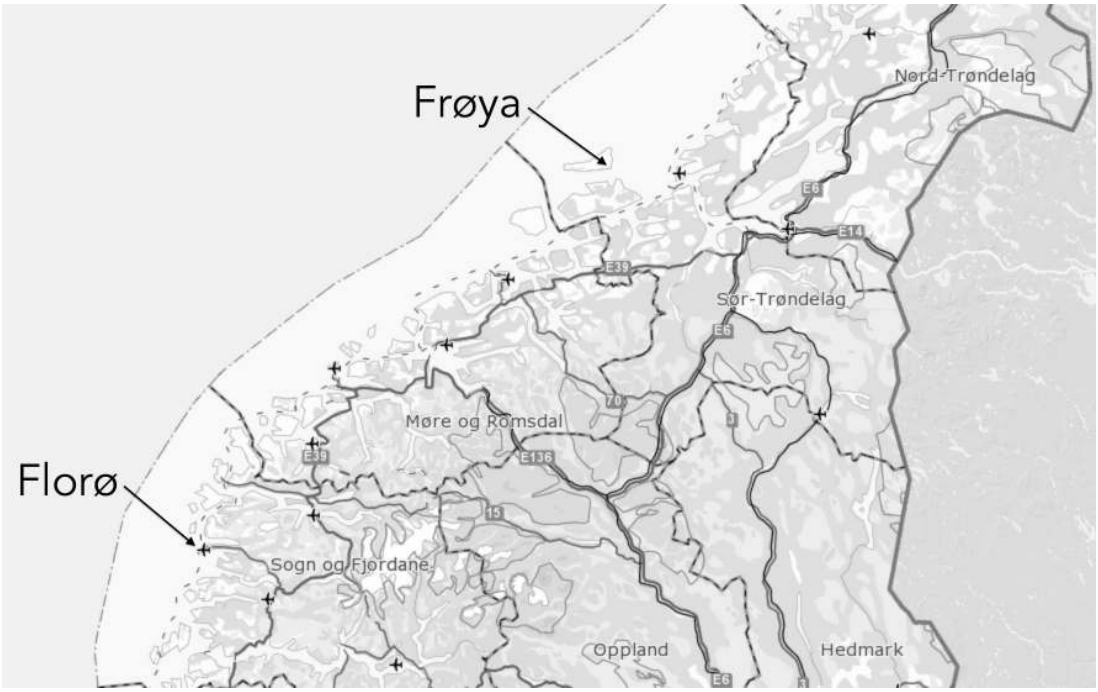

Figure 2-1 – Map of the two sampling locations in Norway (Statens kartverk)

2.1.1 Cultivation in IMTA system at Florø

The location for registration of bryozoan growth in an IMTA system was at one of Marine Harvest Norway AS fish farms by the island Reksta in Sogn og Fjordane county, close to Florø (Flåtegrunnen, 61° 34'N, 04° 48'E). These registrations were performed as a part of the IMTA-project EXPLOIT, funded by the Research Council of Norway, (project no. 216201/E40).

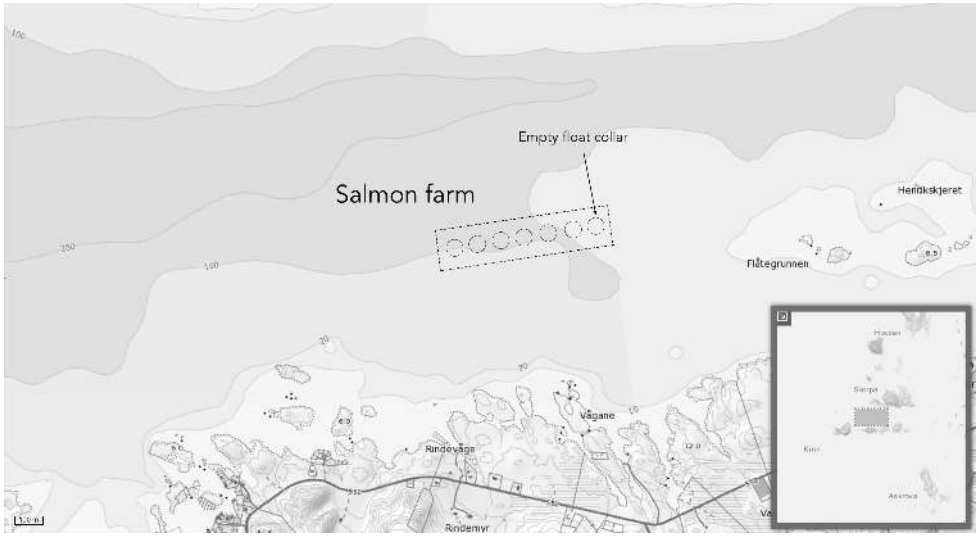

Figure 2-2 – Map of the Florø location with the position of the empty float collar where *S. latissima* was cultivated (Statens kartverk)

The fish farm consisted of seven circular net pens in a single row east-west direction, each with 80 m diameter. Total biomass of salmon in the fish farm ranged from 791 943 tonnes at the deployment of the sporophytes in February, to 3 045 552 tonnes in September when the registrations ended. Salmon was produced in six of the net pens, and the empty fish cage float collar where the seaweed was cultivated was situated at the east end of the farm (see Figure 2-2). Main current direction at 5 m was measured to be 90-110° (east) and 210-230° (southwest), depending on the tidal cycle. Current speed was measured to be 2,4 cm/sec in April 2013, and 8,6 cm/sec in September 2013. Average depth below the farm was 75-200 m.

2.1.2 Time registrations at Frøya

The location for time registrations was situated by the island Frøya in Sør-Trøndelag county (Taraskjæra, 63° 42'N, 08° 51'E). The position of the farm is shown in Figure 2-3. This is a seaweed farm owned by the company Seaweed Energy Solutions AS. This location was chosen for practical and economic reasons to get more frequent sampling during the season, as it is located closer to the university.

Figure 2-3 – Map of the Frøya location with the longlines at the seaweed farm (Statens kartverk)

The location has a semi-exposed position, sheltered from wind coming from south and west, but exposed from northeast. Average current speed (28 days) was measured to be 9,4 cm/sec at 6 m depth. Main current direction was 28° (northeast). Average depth below the farm was 30-50m.

2.2 Sampling period

The sampling period at both locations lasted from April to September 2013. Sampling dates are summarized in table Table 2-1 and Table 2-2, together with the total number individual samples of *S. latissima* and zooplankton samples for each sampling date.

Table 2-1 – Overview of sampling dates and number of seaweed and plankton samples at the Florø location

Sampling Florø

Sampling no.	Date	Total number of individual seaweed samples			Total number of zooplankton samples
		2 m	5 m	7 m	
1	11.04.13	12	12	12	0
2	10.06.13	12	12	12	6
3	07.08.13	12	12	12	6
4	12.09.13	6	7	5	6

Table 2-2 - Overview of sampling dates and number of seaweed and plankton samples at the Frøya location

Sampling Frøya

Sampling no.	Date	Total number of individual seaweed samples			Total number of plankton samples
		3 m	8 m	15 m	
1	30.04.13	6	6	6	2
2	14.05.13	6	6	3	6
3	29.05.13	12	12	12	9
4	18.06.13	11	12	9	4
5	27.06.13	12	12	9	6
6	12.07.13	12	8	9	6
7	24.07.13	12	12	9	6
8	29.08.13	11	12	6	6

2.3 Sampling from the seaweed cultures

2.3.1 Florø

The sporelings used for the cultivation at the Florø location was produced in November at the SINTEF Fisheries and Aquaculture laboratory in Trondheim by inducing zoospores from motherplants collected from a wild population near the deployment location according to the method used in Forbord et. al (2012). The seeded ropes with juvenile sporophytes were transported to and deployed at the fish farm in February.

At the Florø location *S. latissima* was cultivated on ropes hanging vertically from the floating collar of an empty fish cage situated in the fish farm (Figure 2-4). The sporophytes were cultivated from 2 to 7 meters depth, and samples were collected from 2, 5 and 7 meters depth on four different ropes by lifting the ropes to the surface. Three individual laminas from each depth were randomly chosen when it was possible. The samples from April and June were wrapped in aluminium foil, placed in marked zip-lock-bags, and stored at –20°C for 11 and 20 days respectively. The samples were defrosted before image analysis. This method caused the thalli to be very soft and intractable, but did not compromise the bryozoan colonies or the image analysis. The samples from August and September were therefore not wrapped in

aluminium foil or frozen but placed directly in the zip-lock-bags and kept cool in a portable cooler during transport to the laboratory by boat and car. The image analysis of these samples was performed within 24 hours after collecting the seaweed.

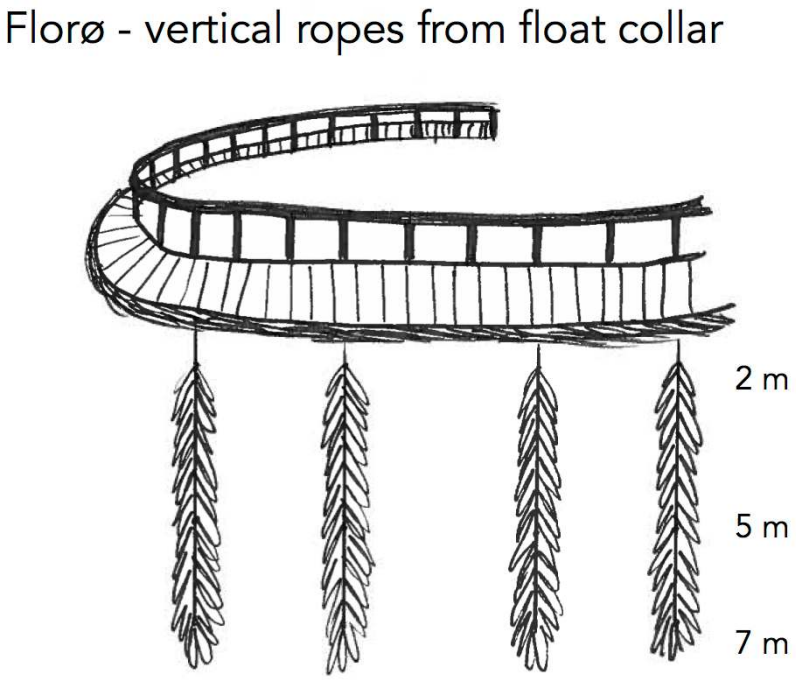

Figure 2-4 – Illustration of the cultivation of *S. latissima* on vertical ropes hanging from the floating collar of an empty fish cage

2.3.2 Frøya

The same method for production of sporelings as with the Florø cultivation was used for sporophytes deployed at Frøya. The sporophytes were deployed on frames hanging from longlines at 3, 8 and 15 meters (Figure 2-5), and samples were collected by lifting the frames to the surface. Three individuals were randomly chosen from each frame at 3, 8 and 15 meters depth. This was performed at four different frame stations whenever possible. These samples were also put in marked zip-lock-bags and kept cool in a portable cooler during transport to the laboratory by boat and car. Image analysis was performed within 24 hours after collecting the seaweed.

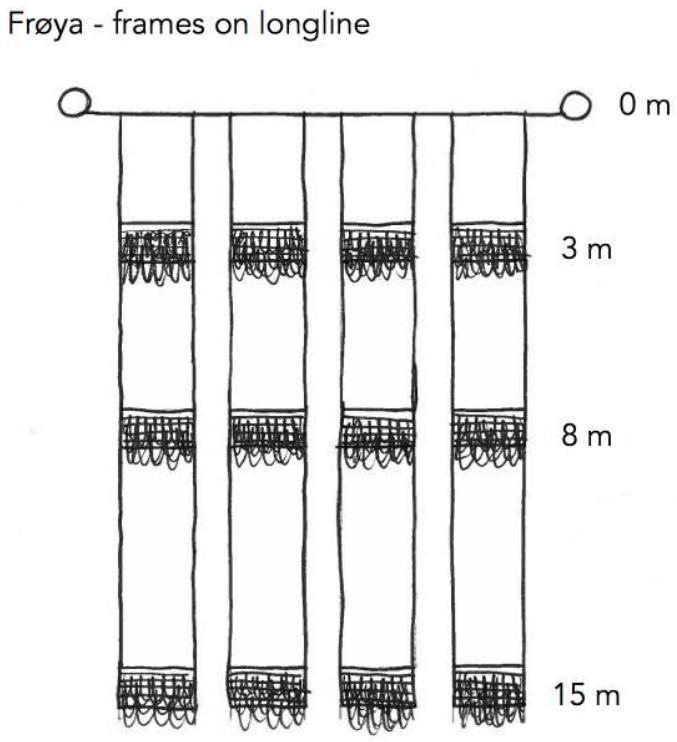

Figure 2-5 – Illustration of the frames hanging from longlines at the seaweed farm at Frøya

2.4 Image analysis

The individual fronds were stretched out as much as possible on a white background. An image of the whole lamina, including a ruler for measure, was taken on both sides of the frond to measure the total area of the lamina, using an Olympus E-500 digital camera (AF Olympus Zuiko digital 14-45 mm, 1:3,5-5,6).

Close up images of the bryozoan colonies were taken by placing the frond on to a fiber optic light table to more easily see the outline of the colonies. The images were taken using a Nikon D200 digital camera (AF Micro Nikkor 60 mm 1:2,8), which was placed on a rig to stabilize the camera (Figure 2-6). Whenever the fronds were too large for the light table, they were cut up in strips to match the width of the light table. Segmented images were then taken of the whole lamina. The use of the light table made it possible to measure colonies on both sides of the thalli on the same image (Figure 2-7). Whenever the bryozoan cover was so heavy that it covered both sides, the measured area was multiplied with two to correct for the layer on both sides.

Figure 2-6 – The rig used to stabilize the camera when taking close up images of the bryozoan colonies

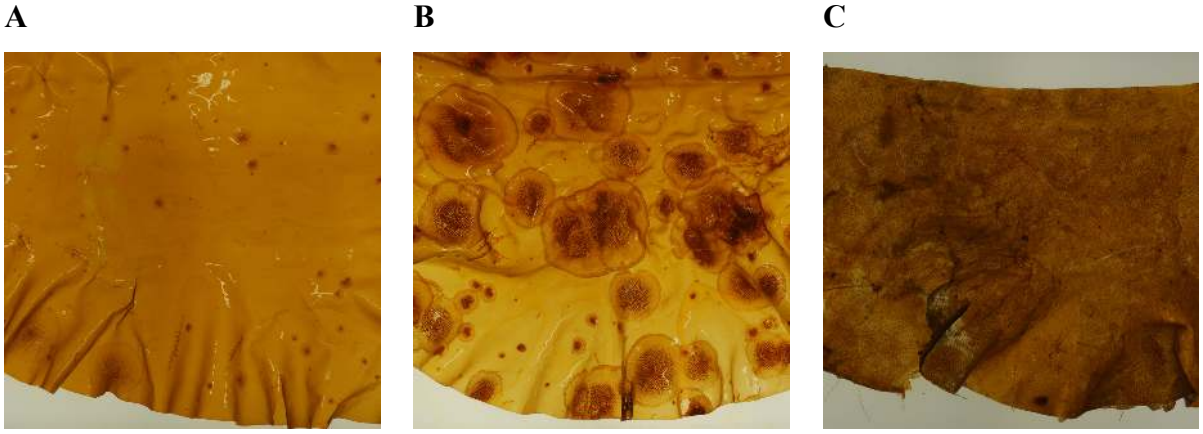

Figure 2-7 – Examples of close up images of the bryozoan colonies on light table. A: 18.06.13, small colonies of bryozoans on both side of the lamina. B: 27.06.13, larger colonies on both sides of the lamina. C: 24.07.13, the bryozoans are covering the entire lamina on both sides

The images were analyzed using the image processing program ImageJ 1.47v (Rasband, 1997-2014) for area measurements. For the total area images, the digital scale was set by measuring 1 cm on the ruler on the image and using the function “Set scale”. The image was then converted to 8-bit type and threshold applied. The Wand (tracing) tool was used to select the outline of the frond, and area was measured by using the “Analyze particles” function.

Because of the fronds corrugated nature, an average of the two sides multiplied with two was used for determine the total area.

To measure the area of the bryozoan colonies, a drawing tablet (Wacom Cintiq 12wx) was used to circle around every colony with the Freehand selections tool. The thallus was divided into three different areas (meristem, mid part and distal end, see Figure 2-8) by eye, and the size of the colonies measured within each area.

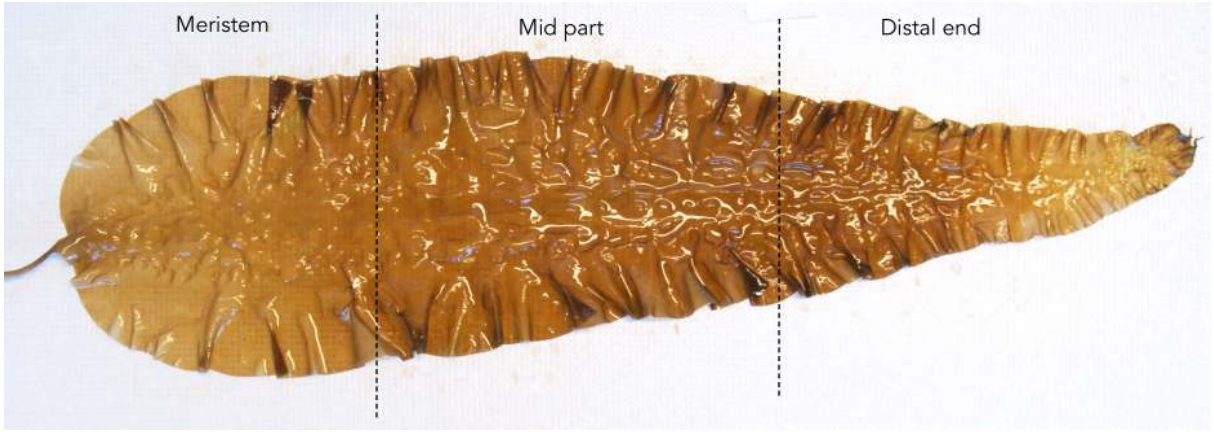

Figure 2-8 – Illustration of the approximate area division of the thalli in meristem, mid part and distal end

The percent coverage of bryozoa was calculated by dividing the surface area of bryozoa by the total surface area and multiplying it with 100.

2.5 Plankton samples

The plankton samples were taken at the same dates and locations as the collecting of seaweed. A standard plankton net with a 100 µm mesh and 30 cm diameter was lowered to 15 meters depth and vertically pulled up to the surface at a speed of approximately 1 m/sec. The method was performed consistently for every sample and sampling date to be able to compare the samples. Exact filtrated volume was not measured due to lack of required equipment, but assumptions that the volume would be approximately the same for each sampling by consistency in sampling method were made. Six replicates were taken on each sampling date when possible. The net sample was then transferred to a test tube and fixated with formalin. 1 drop (0.05 ml) of 20 % formalin was added per 25 ml of seawater, creating a 0.04 % end concentration.

Because formalin is known to be toxic and carcinogenic, the samples had to be rinsed to eliminate formalin fumes during microscope observations. The formalin containing plankton sample was therefore gently rinsed with tap water in a 100 µm mesh sieve to remove most of the formalin before analysis. The sample was then observed systematically in a petridish under a stereomicroscope (Leica MZ 12.5, 0.8-10.0x), and the number of cyphonaut larvae counted both for *M. membranacea* and *E. pilosa* in each sample. A key for identification of cyphonaut larvae (Ryland, 1965) was used to identify and differentiate between the two species. Other dominating plankton species in the samples was also noted.

2.6 Statistical analysis

All statistical analysis and graphs for the results was conducted using the software programming language for statistical computing and graphics R, version 3.0.2 (R Core Team, 2013) through RStudio™, version 0.98.501, a free and open source integrated development environment for R (RStudio, 2012).

Presence/absence of bryozoa was modeled in a binomial Generalized Linear Mixed Model (GLMM) with logit link function where nested dependencies between observations were fitted as random intercept structure. All models were fitted using the lme4-packages in R (Bates et al., 2014). Due to dependent variation among the observations such as replicates, ropes and frames, which is common in ecological studies, GLMM that handle nonnormal data and includes both fixed and random effects was used for the statistical modeling (Zuur et al., 2009). Binomial error distribution was used, as this is appropriate for proportional data.

As a consequence of lack of sufficient data from the sampling, an average of the replicates for each depth were used for statistical analysis. The random effect factor was chosen to be the ropes, as this would take variation dependencies within the stations into consideration.

Variance dependencies were tested for depth, date and site as fixed effect factors by comparing the alternative models using Akaike information criterion (AIC). AIC gives a relative measurement of quality for different models for a given data set compared to each other (Burnham & Anderson, 2002). The model given the minimum AIC value is considered to be the preferred model when choosing between the models that are compared against each other. The method differs from the likelihood-ratio test by giving a penalty for the number of

parameters used in the models. AIC can however not tell you how good the model fits or give you a p-value for the fit. Although likelihood ratio tests are not recommended for small to moderate sample sizes when using GLMMs (Bolker et al., 2009), as in this study, an ANOVA test was also performed.

2.6.1 Variables available for modeling:

Date – The date of sampling

Rope – Which rope or frameset the samples were taken from (1-4)

Depth – Which depth the sample was taken from (3, 8, and 15 m at Frøya, 2, 5 and 7 m from Florø)

Total – The total area of the lamina

Bryozoa – Area of bryozoan cover on the lamina

Site – Which site the sample was taken from (Frøya or Florø)

Neg – Integer of Total used for the binomial distribution

Pos – Integer of Bryozoa used for the binomial distribution

3 Results

3.1 Bryozoan coverage development

3.1.1 Time registrations of bryozoan growth on cultivated seaweed at Frøya

The samples collected at the seaweed farm owned by Seaweed Energy Solutions (SES) AS at Frøya were used for frequent time registrations of bryozoan growth on cultivated seaweed. Samples were taken on 8 different dates between 30.04.13 to 29.08.13 (Table 2-2) approximately every fortnight. Some of the sample sizes (Table 2-2) were smaller than planned, either due to bad weather or missing ropes/frames. The samples were taken randomly at the farm at the different depths, and selected dependent on availability. Thus the samples represent real replicates.

Early in the sampling period, from the end of April to the middle of June, no bryozoan colonies were observed on the cultivated seaweed at the Frøya location (Figure 3-2). Some of the fronds were however covered with pennate diatoms, especially at the distal end, during this period. This gave the seaweed a somewhat "hairy" coating, and seemed to cause small areas of necrosis of the tip of the distal end in some of the samples.

The first newly settled bryozoan colonies was observed at the 18th of June sampling (Figure 3-2), but were also observed the week before by the SES staff (Tine Solvoll Tønder, pers. comm). At this point it was difficult to differentiate between the two species from the pictures, *M. membranacea* and *E. pilosa*, because of the small size of the colonies (Figure 2-7, A). Unidentifiable settlements were noted as *M. membranacea*. The newly settled colonies were abundant, but due to the small size of the colonies the median coverage in June was only 0,7 % (Table 1 in Appendix I). The colonies then spread and grew rapidly during June and July (Figure 3-2 and Table 1 in Appendix I). In late July and in August the fronds were heavily fouled with bryozoans (Figure 3-2), and the thallus started to degrade (Figure 3-1). Most of the seaweed was by then not entirely intact (Figure 3-11). The bryozoan colonies covering the lamina made it heavy, brittle, and easily breakable.

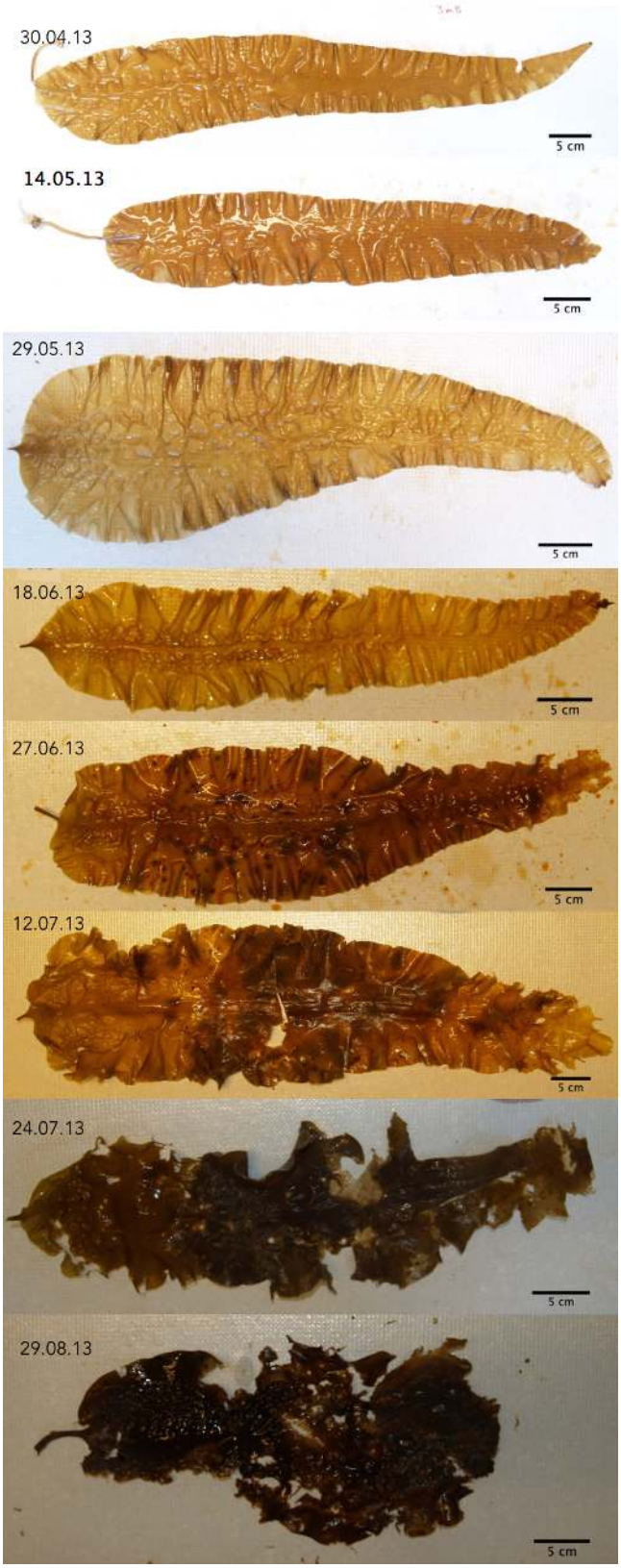

Figure 3-1 – Degrading of the sporophytes caused by bryozoans during the sampling period at Frøya. The pictures show selected examples of samples collected at 3 meters depth for every sampling date.

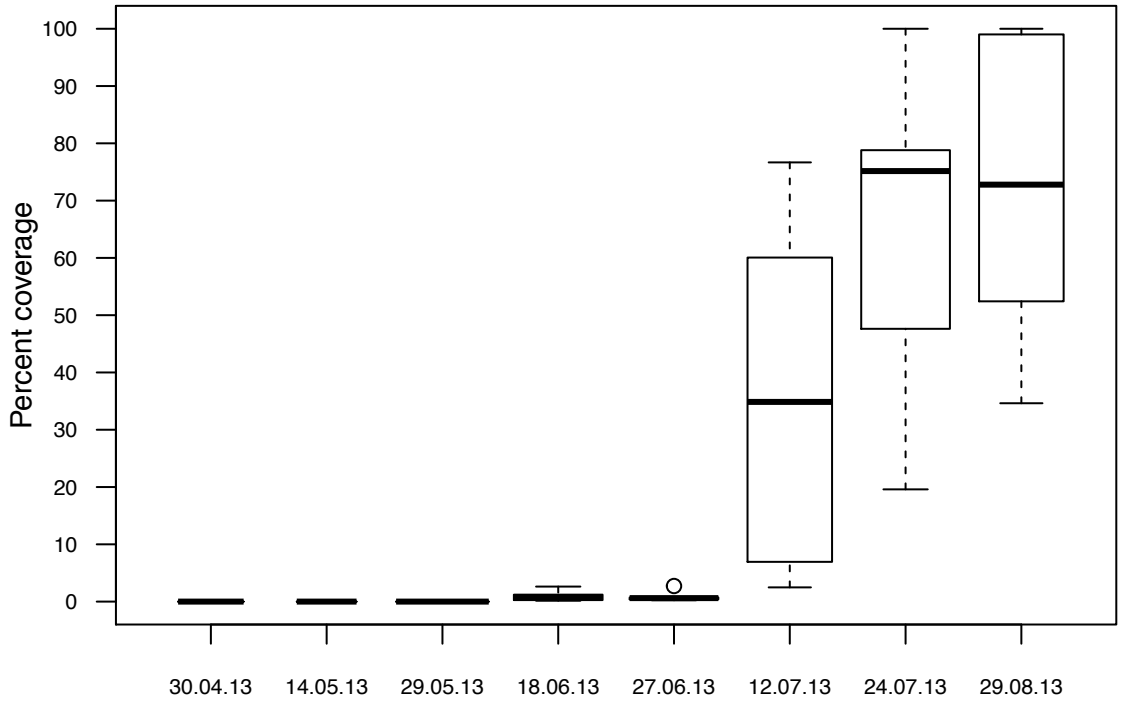

Figure 3-2 – Boxplot of the percentage coverage of bryozoans on the seaweed lamina during the sampling period at the Frøya location. The boxplot shows the maximum and minimum values (whiskers), the lower and upper quartiles (box), and the median (horizontal line). The width of the bars is proportional with sample size. Values are shown in **Table 1** in Appendix I.

M. membranacea was the most abundant of the two species during the whole sampling period (Figure 3-3). The proportion of *E. pilosa* was higher early in sampling season, but then decreased as the bryozoan coverage increased in July (Figure 3-3 and Figure 3-2). On average, 97,1 % of the coverage consisted of *M. membranacea*, and 2,9 % of *E. pilosa* for all samples combined during the sampling period. It did not appear to be any differences between the depths on the species composition.

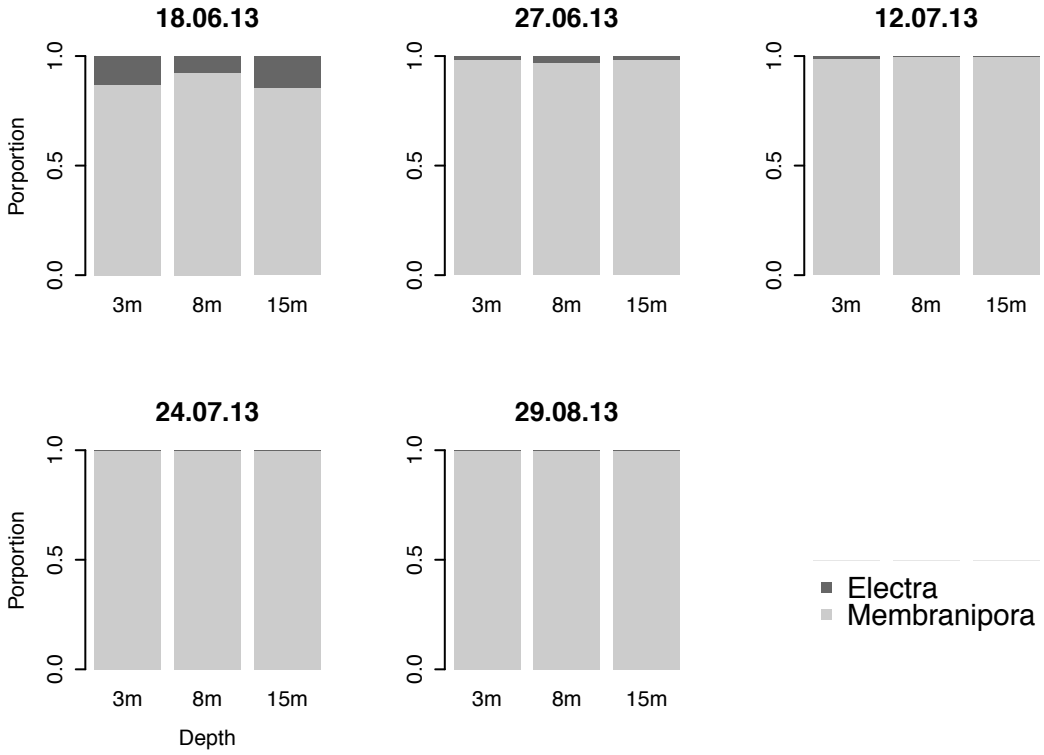

Figure 3-3 – Species composition shown as proportion of total bryozoan area at Frøya at different depths for each sampling date where bryozoans were observed

The median total area (cm^2) of the lamina (Figure 3-4 and Table 3 in Appendix I), and thus available substrate for bryozoan colonies, decreased during the late sampling period, and was in general smaller at increasing depths (Figure 3-5 and Table 5 in Appendix I).

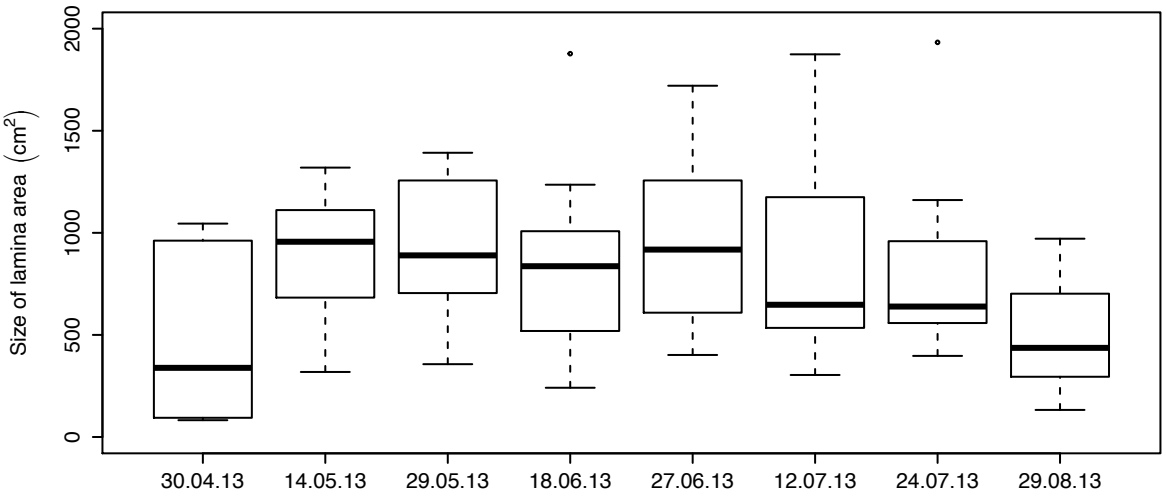

Figure 3-4 – Size of total area of sampled lamina at the different sampling dates at Frøya in cm^2. The boxplot shows the maximum and minimum values (whiskers), the lower and upper quartiles (box), and the median (horizontal line). Values are shown **Table 3** in Appendix I.

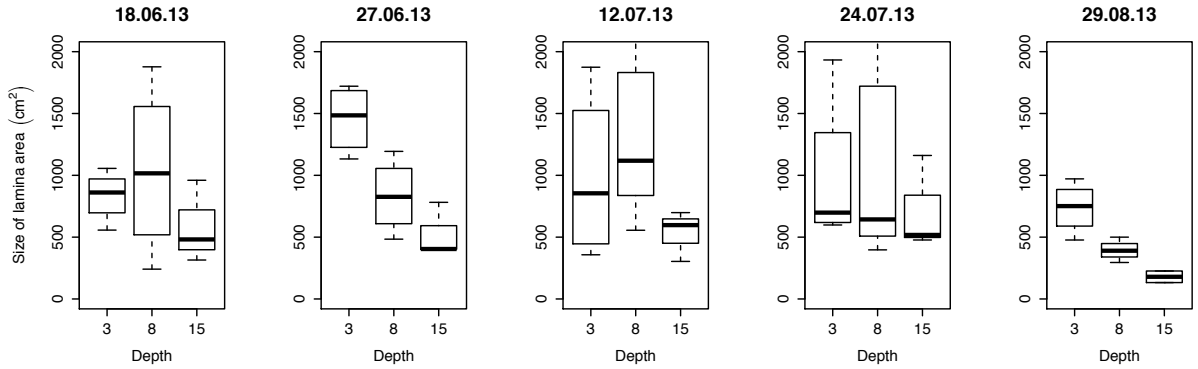

Figure 3-5 – Size of total area of sampled lamina at sampling dates with bryozoan coverage at Frøya in cm^2 at the different depths. The boxplot shows the maximum and minimum values (whiskers), the lower and upper quartiles (box), and the median (horizontal line). Median values are shown in **Table 5** in Appendix I.

3.1.2 Bryozoan growth on seaweed cultivated in an IMTA system at Florø

The samples collected from Florø were cultivated from an empty salmon cage next to a full size fish farm. Samples were collected once in April, June, August and September as a part of the IMTA-project EXPLOIT.

At the Florø location bryozoan colonies were not observed at the first sampling in April (Figure 3-7 and Table 2 in Appendix I), but pennate diatoms were observed, especially at the distal end. Early settled colonies were present in the June sampling, although covering only 0,05 % of the blade (median coverage, Table 2 in Appendix I) because of the small size of the colonies. While small in size, the colonies were abundant on most of the samples. In August many of the fronds were degraded and often only newly grown tissue at the meristem was left (Figure 3-13), which was not so heavily fouled by bryozoans. This was also observed in September, when most of the sporophytes were either damaged or entirely missing (Figure 3-6 and Figure 3-12). This resulted in lack of samples from every depth at some of the ropes.

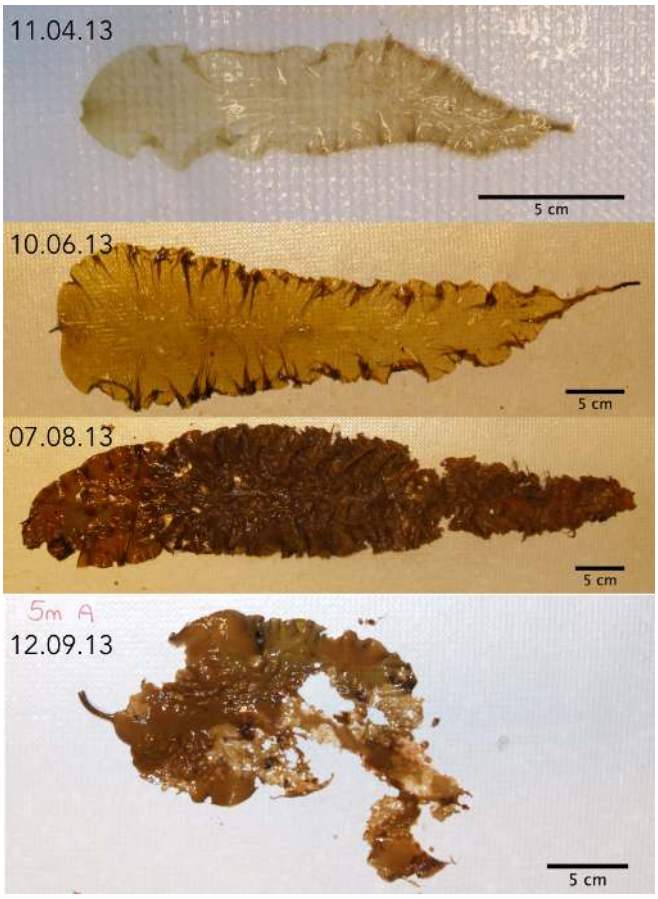

Figure 3-6 – Pictures of selected samples from 5 meters depth at Florø for each sampling date, showing the degradation of the sporophytes

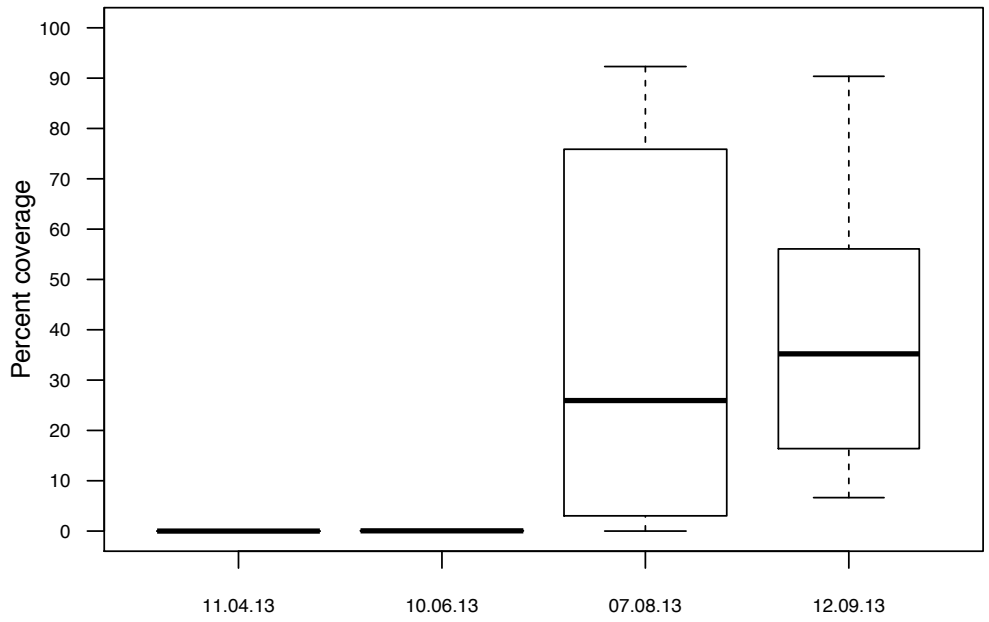

Figure 3-7 – Boxplot of the percentage coverage of bryozoans on the seaweed lamina during the sampling period at the Florø location. The boxplot shows the maximum and minimum values (whiskers), the lower and upper quartiles (box), and the median (horizontal line). The width of the bars is proportional with sample size. Values are shown in **Table 2** in Appendix I.

The species composition of the bryozoan cover on the seaweed where *M. membranacea* was the dominant species during the whole sampling period (Figure 3-8) showed in general little differences between the different cultivation depths. The average species composition of all samples from Florø showed that 99,7 % of the coverage consisted of *M. membranacea*, and only 0,3% of *E. pilosa*. Also here the proportion of *E. pilosa* was highest at the early sampling in June, but decreases as the total bryozoan coverage increases.

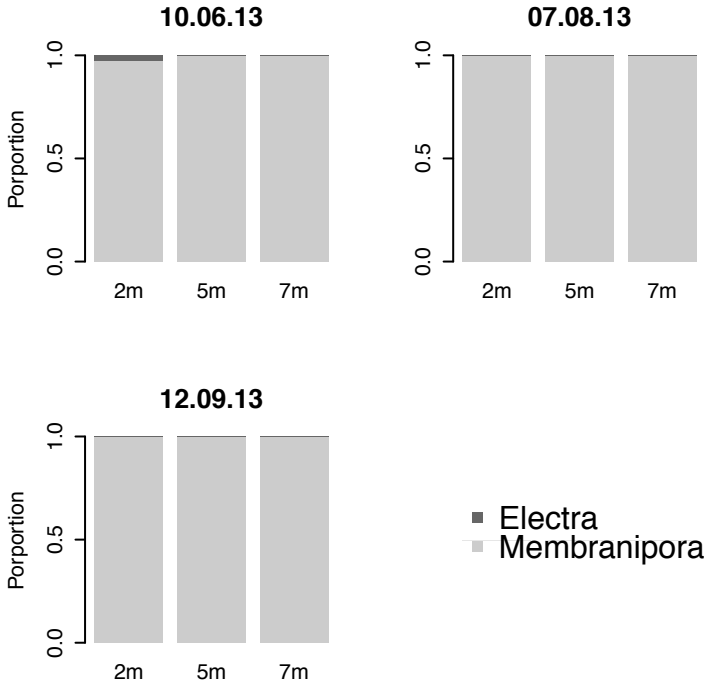

Figure 3-8 - Species composition shown as proportion of total bryozoan area at Florø for the different depths at the sampling dates where bryozoans were observed

The total median size (cm^2) of the lamina of the sporophytes sampled at Florø has a strong increase from April to June, but decreased in the late sampling season (Figure 3-9 and Table 4 in Appendix I). The difference in total area between the depths at Florø (Figure 3-10 and Table 6 in Appendix I) was not as apparent as the Frøya sampling.

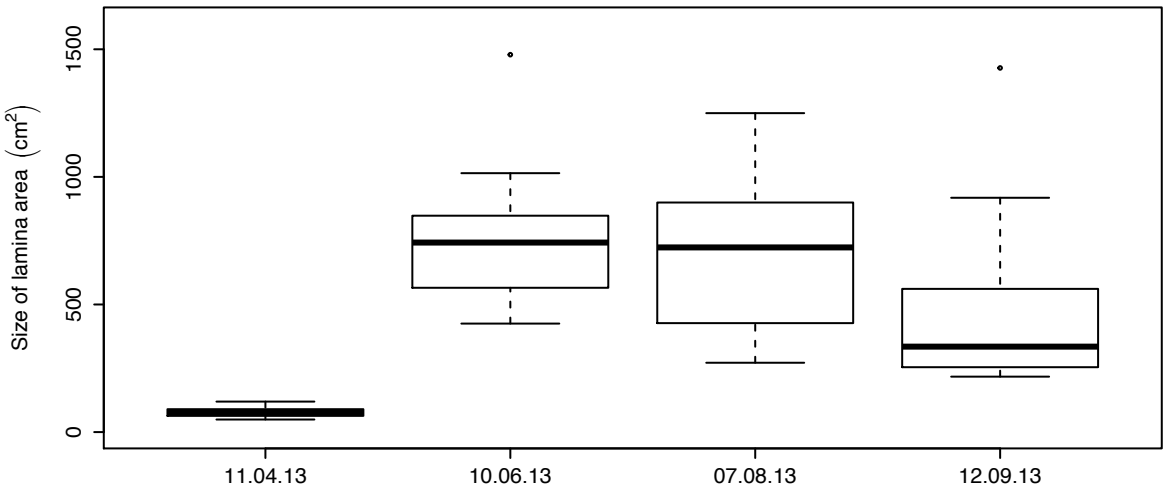

Figure 3-9 – Size of total area of all sampled lamina at Florø in cm^2 at the different sampling dates. The boxplot shows the maximum and minimum values (whiskers), the lower and upper quartiles (box), and the median (horizontal line). Values are shown in **Table 4** in Appendix I.

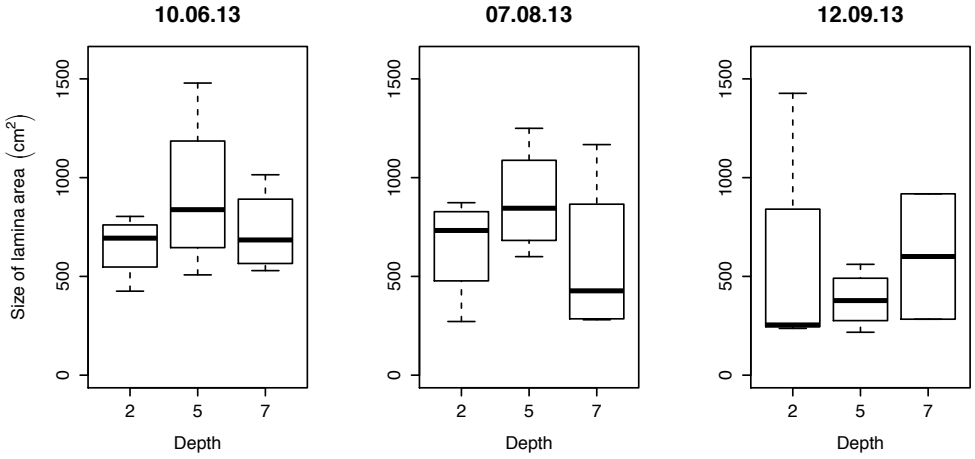

Figure 3-10– Size of total area of sampled lamina at sampling dates with bryozoan coverage at Florø in cm^2 at the different depths. The boxplot shows the maximum and minimum values (whiskers), the lower and upper quartiles (box), and the median (horizontal line). Median values are shown in **Table 6** in Appendix I.

3.2 Missing distal ends

As the bryozoan coverage increased, the *S. latissima* fronds became increasingly fragile and breakable. This resulted in loss of the distal end for several of the sampled individuals (Figure 3-11 and Figure 3-12) at both sampling locations. The samples taken in September at Florø were all broken off and the distal ends were missing (Figure 3-12). The new tissue at the meristem on the damaged frond was not as heavily fouled as the rest of the thalli (Figure 3-13).

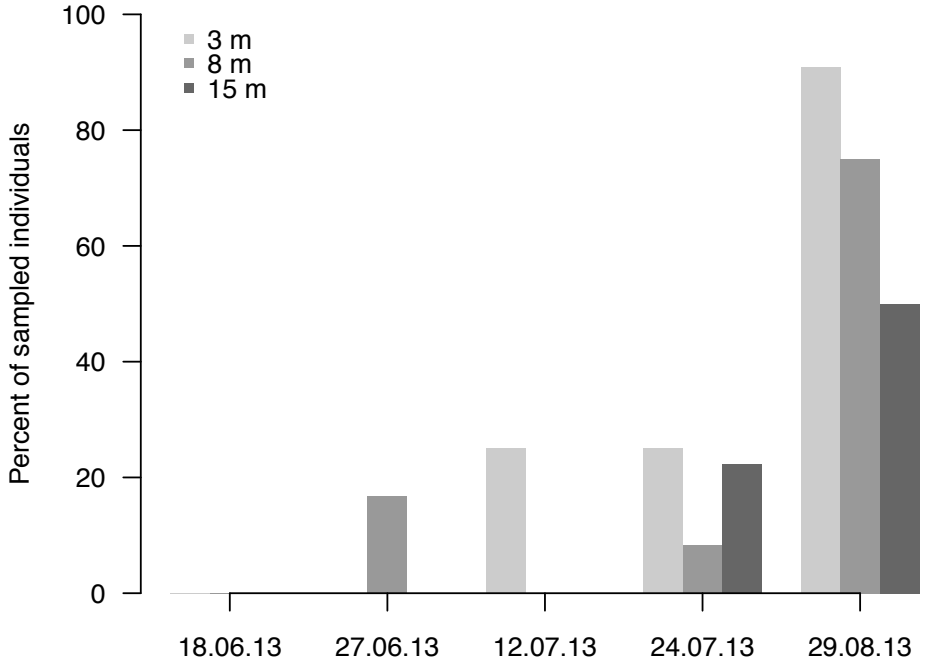

Figure 3-11 - Barplot showing percentage of the sampled individual seaweed that was missing the distal end at the Frøya location for each sampling date when bryozoans were observed.

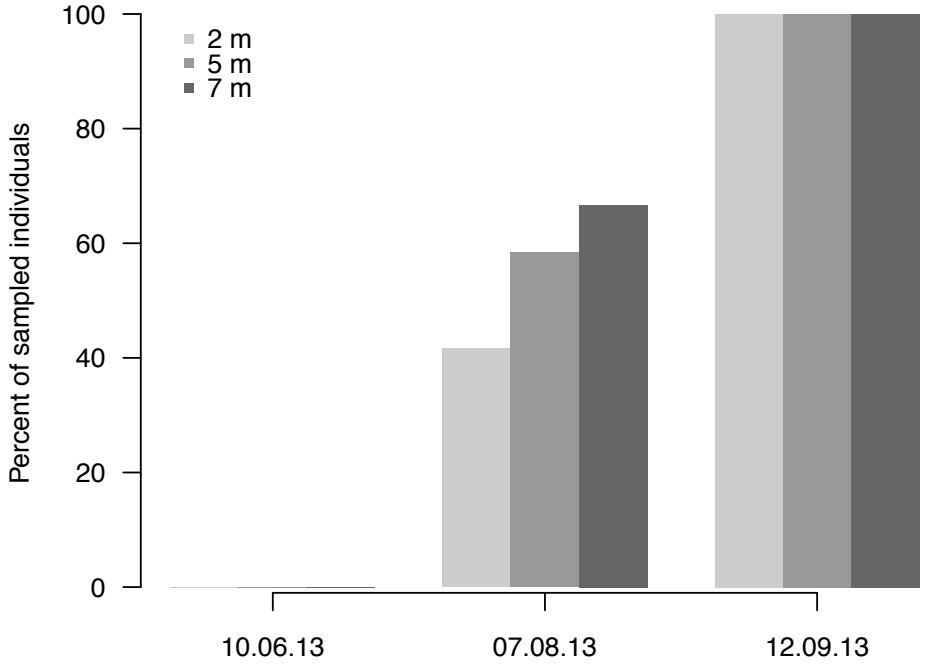

Figure 3-12 – Barplot showing percentage of the sampled individual seaweed that was missing the distal end at the Florø location for each sampling date when bryozoans were observed.

Figure 3-13 – Example of sporophyte missing distal end. The sample was collected from 3 meters depth at Frøya 29th of August.

3.3 Distribution of the bryozoan coverage on the lamina

The spatial distribution of the bryozoan coverage on different parts of the lamina from the Frøya sampling and the Florø sampling varied during the sampling period (Figure 3-14 and Figure 3-15). The results from Frøya show a decreasing trend in coverage at the meristem and increasing trend at the distal end, except for the August sample (Figure 3-14). At Florø most of the settlement occurs at the mid part of the frond for all sampling dates (Figure 3-15).

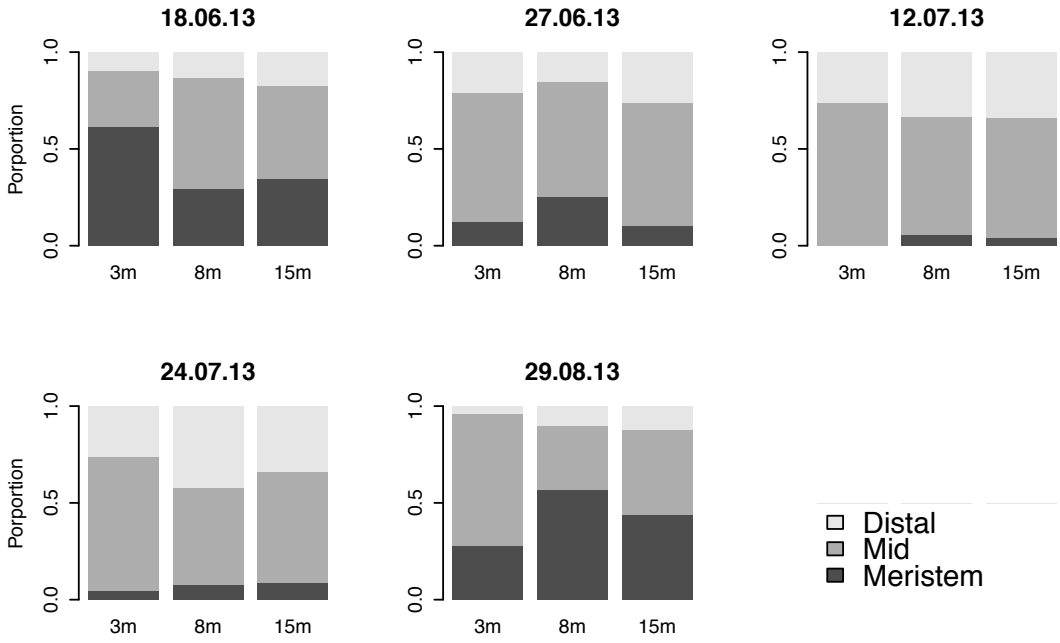

Figure 3-14 – Distribution of the bryozoan colonies on different parts of the fronds collected at Frøya sorted by depth at the different sampling dates when bryozoans were observed.

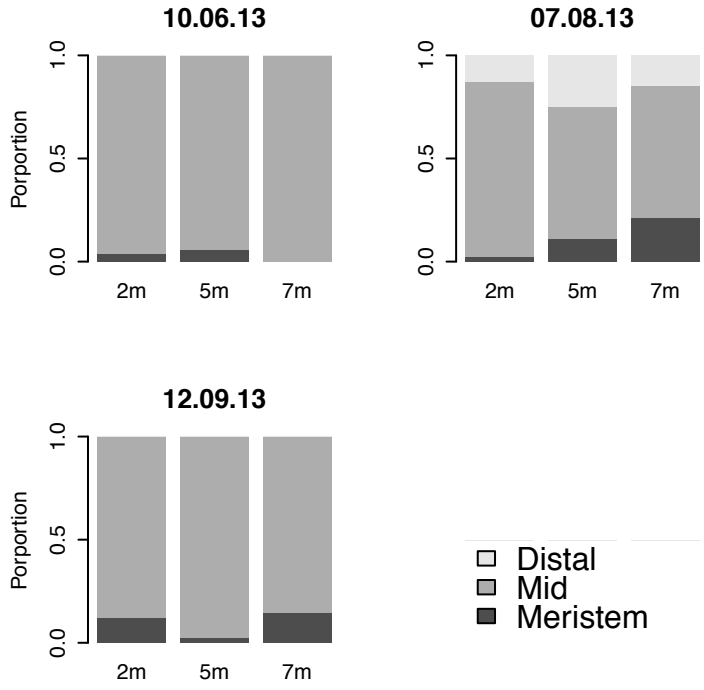

Figure 3-15 – Distribution of the bryozoan colonies on different parts of the fronds collected at Florø sorted by depth at the different sampling dates when bryozoans were observed.

3.4 Depth dependencies

The depth with most coverage differed for the different sampling dates at both locations (Figure 3-16 and Figure 3-17). A variance component analysis showed that 94% of the variance was between ropes rather than within depths for the Frøya location. This means that there was less variation within samples from a specific depth at a specific rope than the same depth at different ropes. The same applies for Florø where 67% of the variance was between ropes rather than within depths at different ropes.

3.4.1 Frøya

At Frøya, the samples from 3 meters were most heavily overgrown with bryozoa in the beginning, but ended up with having the least coverage at the last sampling (Figure 3-16). At 15 meters the coverage was the lowest of the different depth for all sampling dates besides the last sampling. The difference between the depths was greatest in the middle of July, where the 8 meters samples had the most coverage.

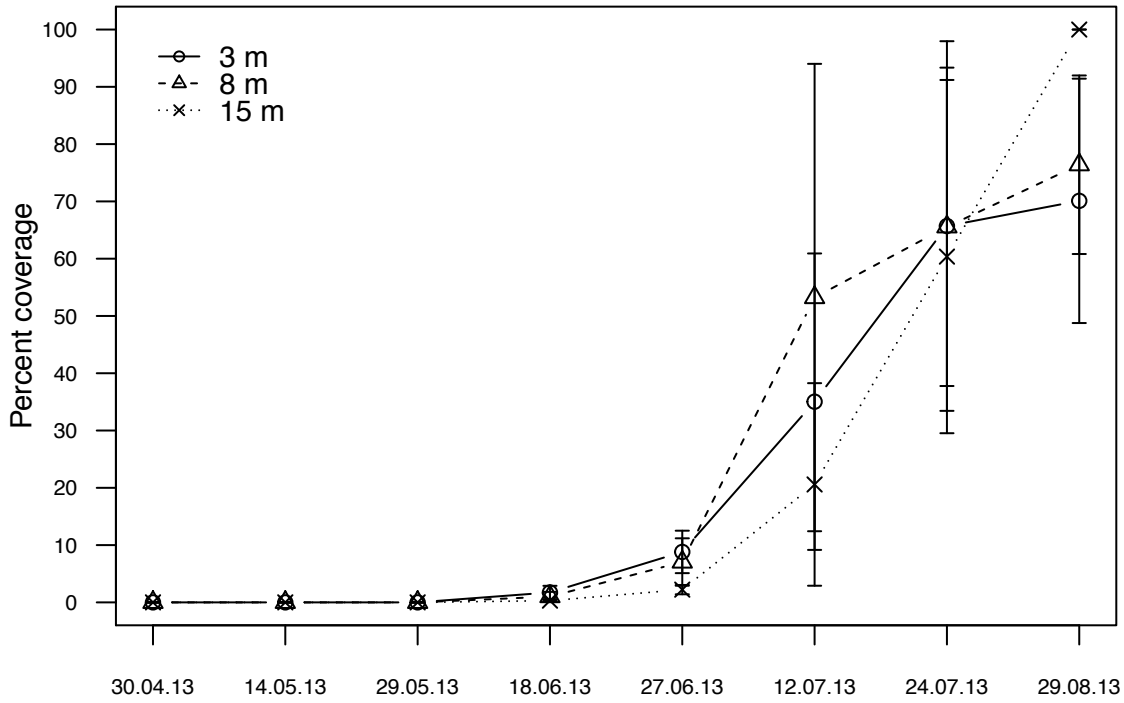

Figure 3-16 – Mean percentage coverage at 3, 8 and 15 meters depth at each sampling date at Frøya. Error bars show standard deviation.

From the statistical analysis based on AIC (Table 3-1), models significantly improved when including depth as fixed factor rather than having only a random intercept (ΔAIC = 408.95 (Burnham & Anderson, 2002)). In all, there was a significant decrease in coverage with increasing depth (likelihood ratio test: χ^2=412.95, df=2, p-value<0.001). Estimates from the model and their standard errors are shown in Appendix II.

Table 3-1 – AIC model comparison with and without depth as fixed factor for the Frøya location

Rank	Formula	K (parameters)	AIC	ΔAIC
1	Respons~Depth+(1|Rope)	3	43377.54	0
2	Respons~1+(1|Rope)	1	43786.49	408.95

3.4.2 Florø

The development of bryozoan coverage at Florø showed an increasing trend from June to August, except for the samples from 2 meters (Figure 3-17). In September the coverage however decreased for the samples from 5 and 7 meters depth, but increased for 2 meters.

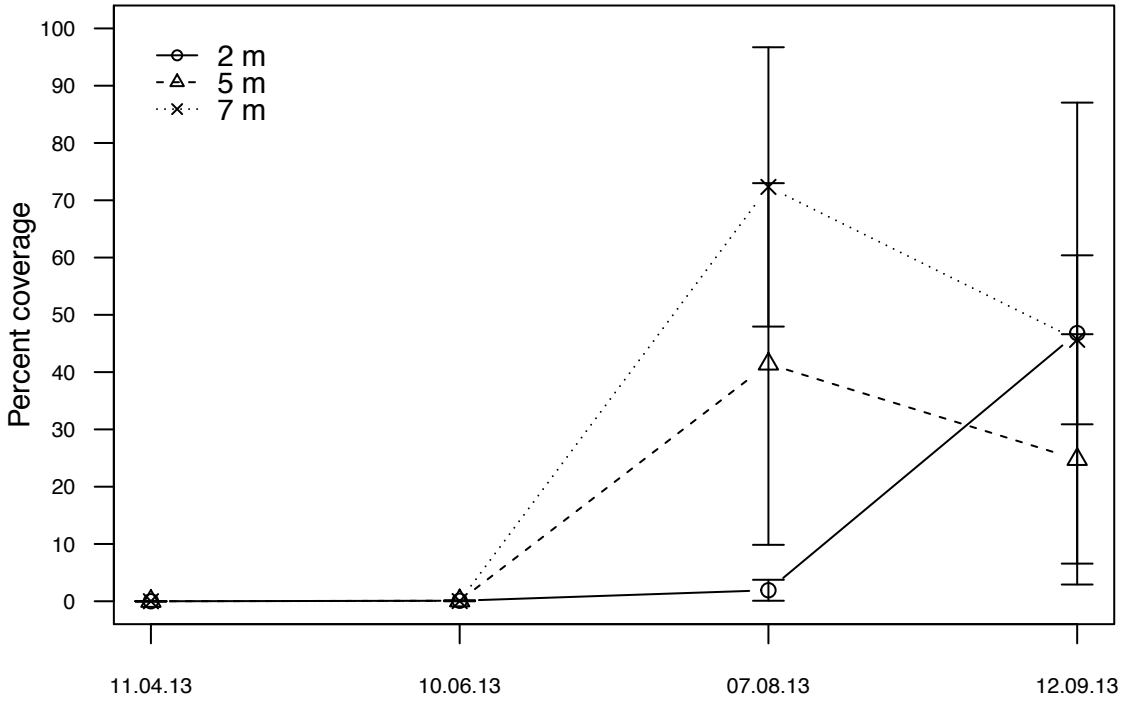

Figure 3-17 – Mean percentage coverage at 2, 5 and 7 meters depth at each sampling date at Florø. Error bars show standard deviation.

The AIC comparison on models including and excluding depth as fixed factor from the Florø location (Table 3-2) also significantly improved when depth was included (ΔAIC = 641.18 (Burnham & Anderson, 2002)). In all there was a significant decrease in coverage with increasing depth (likelihood ratio test: χ^2= 645.18, df=2, p-value<0.001). Estimates from the model and their standard errors are shown in Appendix II.

Table 3-2 – AIC model comparison with and without depth as fixed factor at the Florø location

Rank	Formula	K (parameters)	AIC	ΔAIC
1	Respons~Depth+(1|Rope)	3	10015.61	0
2	Respons~1+(1|Rope)	1	10656.79	641.18

3.5 Plankton samples

3.5.1 Plankton sampling at Frøya

The semi-quantitative abundance of cyphonaut larvae at the Frøya location for both *M. membranacea* and *E. pilosa* was registered during the sampling period. The cyphonaut larvae were observed at all sampling dates from April to September (Figure 3-18). The relative abundance between the samples was however highest in late June for both species. This sample had an average of 49 (SD±19.68) *M. membranacea* larvae and 29 (SD±14.38) *E. pilosa* larvae. The amount of *E. pilosa* larvae was also relatively high in late August.

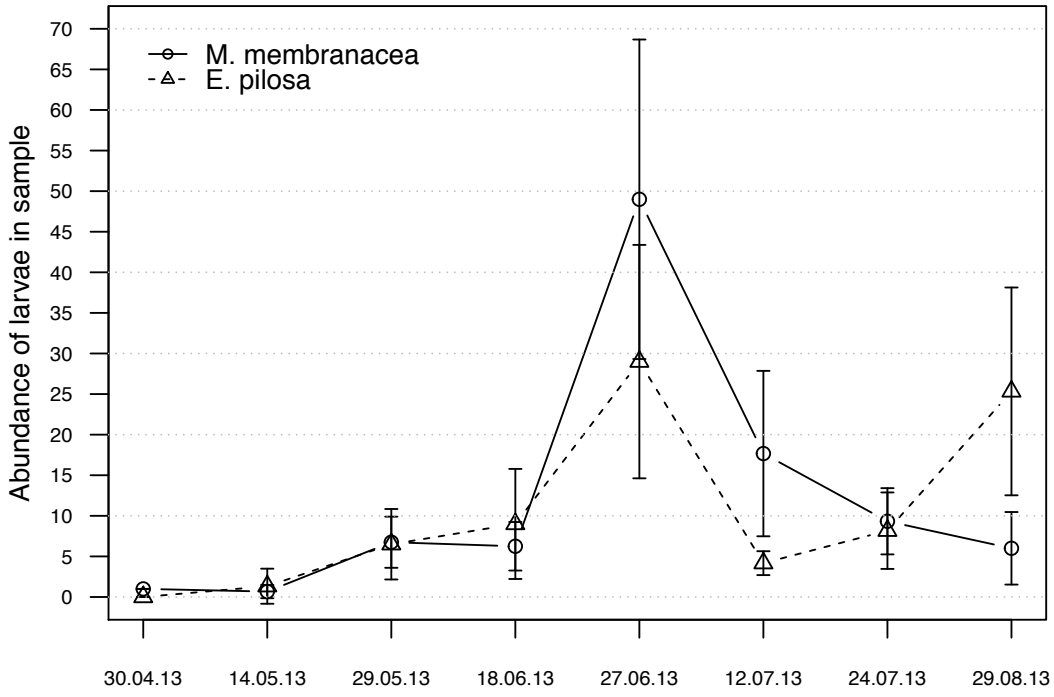

Figure 3-18 – Difference in average abundance of cyphonaut larvae found in plankton samples from Frøya for *M. membranacea* and *E. pilosa* during the sampling period. Error bars show standard deviation.

3.5.2 Plankton sampling at Florø

Bryozoan cyphonaut larvae were found at all sampling dates also at Florø (Figure 3-19), but the relative abundance was not as high as some of the samplings from Frøya. Note that the samples were not taken at Florø in late June and July when the peak in abundance at Frøya occurred. The number of *M. membranacea* larvae was relatively stable for all samplings, but *E. pilosa* showed an increase in relative abundance during August and September.

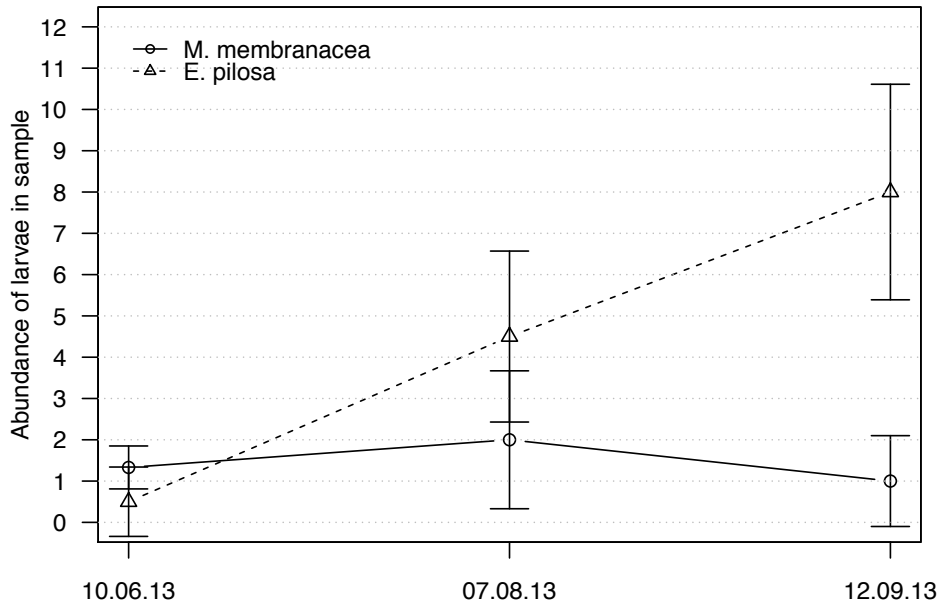

Figure 3-19 – Difference in average abundance of cyphonaut larvae found in plankton samples from Florø for *M. membranacea* and *E. pilosa* during the sampling period. Error bars show standard deviation.

3.6 Temperature measurements

Sea temperature was measured every week at 5 meters depth at both locations (Figure 3-20). At Florø the temperature was measured by the fish farm. The measurements from Frøya are taken from the near by fish farm Bukkholmen owned by Måsøval Fishfarm AS situated 3 km from the seaweed farm.

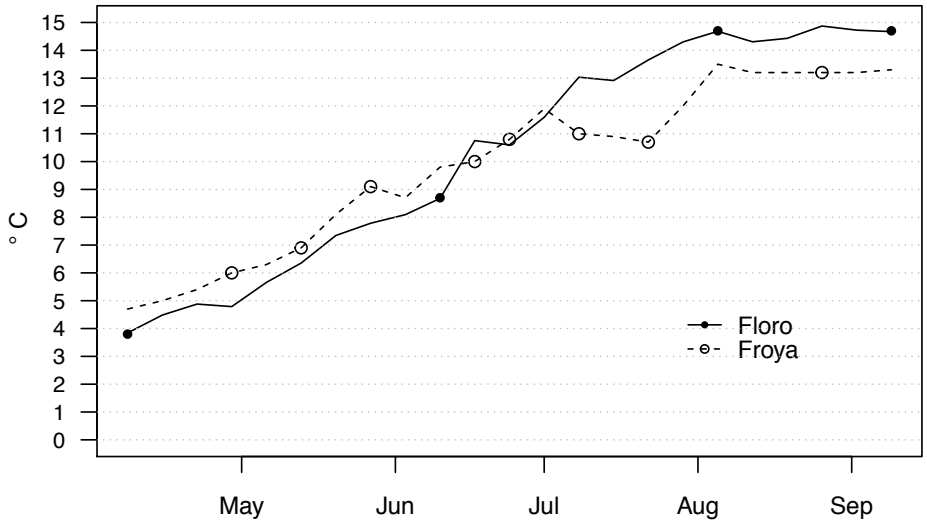

Figure 3-20 – Weekly measurements of sea temperature at 5 meters depth during the sampling period at the Florø and Frøya locations. Dots represent sampling dates for each location.

4 Discussion

4.1 Development of bryozoan coverage

As expected from previous seaweed cultivation projects in Norway (Forbord et al., 2012; Handå et al., 2013) the bryozoan settlement occurred during the month of June. The time of observation of the first settled colonies (week 24) was the same for both sampling locations. A rapid increase in coverage followed during late June and July. The increase in bryozoan coverage percentage tended to decrease during the late season. A reason for the reduced ratio may mainly be caused by the breakage of the distal ends, rather than decrease in coverage itself. The decrease was most probably due to the fact that the proportion of covered versus newly grown tissue by the meristem decreases as the covered distal end went missing.

The variance in coverage increased during the late season, where minimum and maximum values of coverage varied from 2.5 – 76.7 % for samples collected in mid July, 19.6 – 99.9 % in late July, and 34.6 – 100 % in late August at Frøya. At Florø the minimum and maximum values of coverage varied from 0 – 92.3 % in August and 6.6 – 90.4 % in September.

The total median bryozoan coverage was in general higher on Frøya than Florø. A reason for this may be the total biomass or density of kelp cultivated at the site. The amount of cultivated seaweed was higher at Frøya than the IMTA trial site at Florø, which could lead to higher densities of bryozoan colonies due to spawning and recruitment from the already settled colonies in close vicinity (Yoshioka, 1982).

Available space for settlement and growth is one of the limiting factors in algal epifauna (Seed & O'Connor, 1981). The spatial distribution on bryozoans on the different parts of the lamina showed a more even distribution between the different parts of the lamina when more space was available in June than during the rest of the sampling season. *M. membranacea* has in earlier experiments (Abelson, 1997; Matson et al., 2010) showed to be selective and locomotive when searching for suitable substrate, and may therefore be able to position itself on substrate free of other colonies. Colony growth of *M. membranacea* also tends to be directed towards the meristem (Ryland & Stebbing, 1971), which provides new tissue and free space for colonization. Combined with slow growth of the kelp during late summer, this

makes the bryozoa able to colonize the whole frond, as observed in some of the samples from the late sampling season.

As the sporophyte grows during July, the colonies have a larger distribution at the mid part and distal end rather than at the meristem. This may be because colonies settled in June continue to extend in size, while new settlers at the new tissue at the meristem have not had the time to reach the same size. Epibiont size and density usually correlates to the age of substrate, where older tissue is more densely covered (Ryland & Stebbing, 1971). In August and September the proportion of bryozoans at the distal end decrease, mostly due to the missing tissue, not a decline in bryozoans.

Another important, general factor for growth is temperature. A laboratory study on temperature affected zooid growth of *M. membranacea* and *E. pilosa* performed by Menon (1972) showed slow, exponential growth at 6 °C, but considerable faster growth at 12 °C and 18 °C. The temperature at both Frøya and Florø increased gradually from 4-5°C in April to around 9-10°C when the first settlement was observed in June. During the highest increase in coverage in July the temperature at Frøya ranged from 11-14 °C, which could have affected the bryozoan growth rate substantially according to Menons study.

4.2 Species composition

The species composition shows that the main bryozoan species growing on cultivated *S. latissima* is *M. membranacea*. This may be due to the species' preferences in selecting substrate. A collection of observations presented by Ryland (1962) showed that *M. membranacea* is more selective when it comes to substrate, and prefers macroalgae as substrate and especially the laminarian species. *E. pilosa* tends to be less selective when choosing substrate and occurs both on algae and hard substrates like rocks and shells. *E. pilosa* could thus more easily be outcompeted by *M. membranacea* by preferences. Much less *E. pilosa* was observed on the sporophytes at Florø (on average 0,3 % of total) than Frøya (on average 2,9 % of total). This may be due to the amount of nearby available substrate for the less selective *E. pilosa*. The Florø location was surrounded by fish farm installations, whereas this was not present at the Frøya location where the seaweed farm was far from other marine installations. According to the zooplankton samples (Figure 3-18 and Figure 3-19), cyphonaut

larvae of *E. pilosa* was definitively present, but according to the species composition (Figure 3-3 and Figure 3-8) not on the cultivated kelp.

Another reason for the dominance of *M. membranacea* on the kelp could be due to greater success for *M. membranacea* in the competition between the two species. Seed and O'Connor (1981) postulates that of the common bryozoans in Britain, *E. pilosa* tends to be overgrown by other species. Similar results were seen in interactions between *M. membranacea* and *E. pilosa* in a study from Canada (Yorke & Metaxas, 2011). This study also showed a slower growth rate for *E. pilosa* than *M. membranacea*. Overgrowing of colonies was also observed on Frøya and Florø, creating double layers of bryozoan cover. This was regarded as same as a one-layer coverage when this observation was recorded, and the area of the dominant/overlapping species calculated.

The higher abundance of *M. membranacea* of the two species may thus be a combination of its preferences in selecting substrate, higher growth rate, and the ability to grow over *E. pilosa*.

4.3 Depth dependencies

The statistical analysis of the data showed significantly less coverage with increasing depth. However, bryozoans were fouling sampled sporophytes at all depths in various amounts in this study.

As bryozoans are filter feeders it was expected to find more bryozoan coverage on the seaweed cultivated closer to the surface, where food availability might be higher. Although the statistical analysis showed a significant decrease in coverage with increasing depth, the depth with the most coverage differed during the sampling period. For instance, in August the seaweed samples from 15 meters depth at Frøya had the highest coverage (mean=100 %, SD±0.001). One way to explain these findings may be that the seaweed cultivated at greater depths are more sheltered from wave action and turbulence than the seaweed grown closer to the surface, thus experiencing less breakage of the lamina and loss of the distal end, and increasing coverage. The growth rate of the kelp also tends to be higher closer to the surface due to the increased access to sunlight, and new tissue has the chance to grow before new bryozoan colonies settles or extend. The total area of the lamina collected at 15 meters at

Frøya was in general less than the seaweed grown at 3 meters depth (Figure 3-5), and the bryozoans would have a greater chance to overgrow the smaller than the larger lamina during the same time span. The 15 meters samples also had less loss of distal ends than the other depths (Figure 3-11).

For the Florø location, bryozoan coverage on samples from 2 meters depth was relatively low in August (mean=1.92 %, SD±1.83) compared to 5 and 7 meters depth (mean=41.42 %, SD±31.57 and mean=72.33 %, SD±24.38, respectively). The reason for this difference may be the same as for Frøya, where kelp closer to the surface has a higher growth rate than the kelp at greater depths, thus lowering the coverage ratio. However, the depth difference (2-7 meters) was not as high as at the Frøya location (3-15 meters). Neither was the difference in median total size of the lamina between the depths.

4.4 Zooplankton sampling

The semi-quantitative method used for the zooplankton sampling allows for a relative comparison between the samples taken at the different sampling dates for this study.

The presence of bryozoan larvae at all the sampling dates supports the literature on cyphonaut larvae being present in coastal waters throughout the year in North Atlantic oceans (Ryland, 1965). The relative abundance in the samples was however low until the end of June. The highest abundance of cyphonaut larvae in this study was observed after the settlement of colonies on the seaweed. This may be due to an increase in plankton generally, a rise in temperature, or to spawning from the already settled colonies in the seaweed farm area.

A trend in the increase of bryozoan coverage was observed with the relative abundance of cyphonaut larvae (Figure 4-1). The percent point increase in mean bryozoan coverage was highest at the sampling date as the peak in relative abundance of cyphonaut larvae occurred at Frøya. The increase then declined, as well as the relative larvae abundance, during the late sampling period.

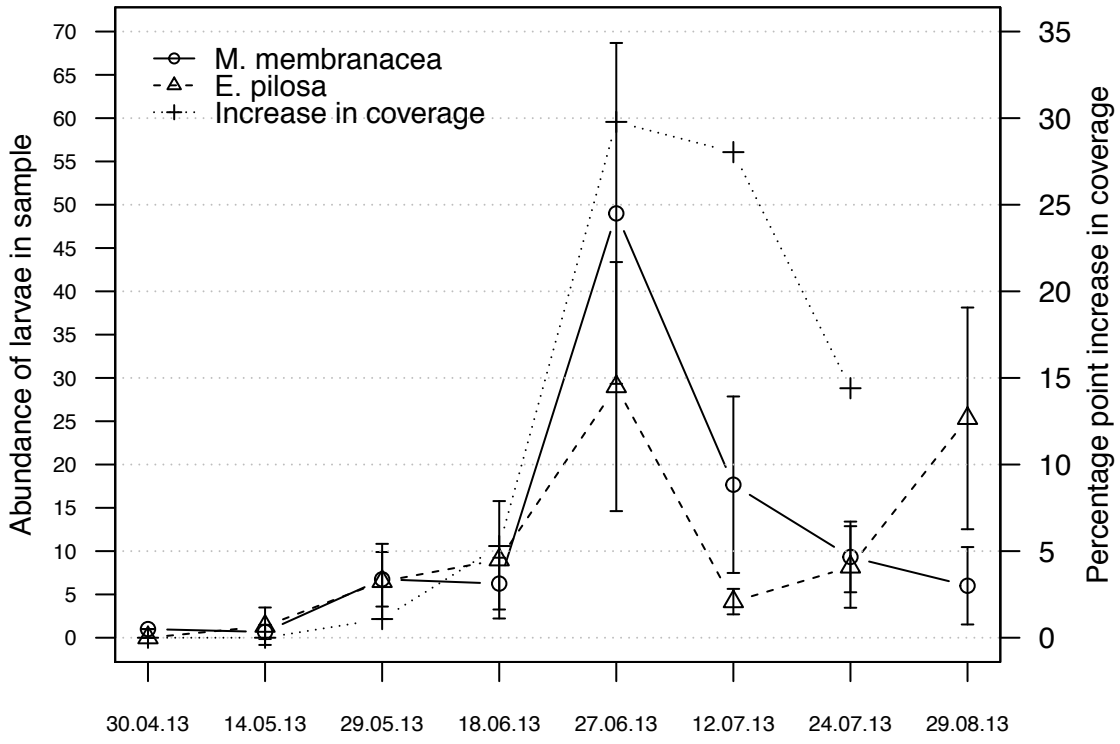

Figure 4-1 – Semi-quantitative abundance of cyphonaut larvae in the zooplankton samples and mean percentage point increase in bryozoan coverage relative to previous sampling date, for each sampling date

The presence of cyphonaut larvae without them settling on the seaweed in the early season indicates that some sort of cue is necessary for the settlement on the seaweed. Different theories have been proposed, as thermal history (growing degree-day) (Saunders & Metaxas, 2007), or that the kelp itself emits some sort of chemical cue (Seed & O'Connor, 1981).

A study of *M. membranacea* on the giant bladder kelp (*Macrocystis pyrifera*) in California by Yoshioka (1982) showed that temperature and larval abundance, which affects recruitment, played a major role in population fluctuations of *M. membranacea*. This was also shown in a study of the same species on the kelp *S. longicruris* in northern Canada (Caines & Gagnon, 2012). The rise in temperature may though not be causative, but indirectly affect other factors like phytoplankton increase, larval supply, and increased growth rate in general.

4.5 Comparing the two locations

At first it was desirable to compare the two locations used for sampling in this thesis. The difference in sample size and frequency made the statistical analysis by comparing models

with and without site as a variable difficult. At the two locations the seaweed was grown at different depths, which also made the comparison challenging. The two locations was therefore treated separately, Frøya for time registrations of bryozoan growth on cultivated seaweed, and Florø for observing bryozoan growth on seaweed cultivated in vicinity to a fish farm.

4.6 Biofouling and seaweed cultivation / concluding remarks

Biofouling is a major challenge in global commercial macroalgae mariculture (Fletcher, 1995; Forbord et al., 2012; Handå et al., 2013; Peteiro & Freire, 2013). Fouling organisms degrades the seaweed and decreases the value of the product. This study has documented the development of bryozoan growth and coverage on cultivated *S. latissima* in Norway, and the results show that cultivation during July and August is not feasible for the industry as the deterioration of the product is high during these months due to the heavy fouling of bryozoan colonies. The bryozoan coverage does not just make the product more indelicately, but causes a substantial loss of biomass due to breakage of the fronds.

From a commercial point of view the best solution at this point will be to harvest the crops of kelp in June, before the bryozoan colonies settles and spread extensionally. Other solutions like submerging the crops to greater depths during the late season will not have a large impact as the bryozoans settles and grow just as well at depths as 15 meters as measured in this study. This solution will also reduce the growth of the sporophyte due to decreased access to sunlight, and the chance of overgrowth increases, as the bryozoan growth rate may be greater than the sporophytes.

There are several environmental measurements that could be included in this study, as stratification, wave energy, current strength and phytoplankton abundance. Due to the limited scope of this thesis, this was however not conducted, but should be considered in further investigations on fouling organisms on cultivated seaweed.

5 Conclusion

This study has documented the bryozoan biofouling on the macroalgae *Saccharina latissima* cultivated in Norway. The results showed that the fouling starts in mid June, and continues to increase during late June and July. A reduction in the ratio of bryozoan covered lamina during August and September is most likely due to breakage of covered lamina and growth of new uncovered tissue at the meristem. The large amount of bryozoan colonies on the fronds made them heavy, brittle and easily breakable, and missing distal ends were prominent in the late sampling season.

The species composition of the bryozoan coverage was shown to constitute of mostly *Membranipora membranacea*, although cyphonaut larvae of both *M. membranacea* and *Electra pilosa* were observed in the zooplankton samples. The abundance of cyphonaut larvae in the zooplankton samples showed to coincide with the increase in coverage on the kelp, indicating that larvae abundance is an important factor in recruitment. The presence of cyphonaut larvae without them settling also indicates that some sort of cue is necessary for the actual settlement.

The statistical analysis of depth dependencies in bryozoan coverage showed a significantly decrease with increasing depth. It is however debatable, from a commercial point of view, if this decrease is sufficient to make up for the potential reduction in production of kelp biomass at lower depths. This study shows that, at the time being, harvest of the cultivated seaweed should be conducted in June before the bryozoan colonies spreads and degrades the product. Further investigations of this subject including more environmental variables should be explored.

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Appendix I

Boxplot values

Table 1 - Boxplot values of the percentage of bryozoan coverage at different sampling dates at Frøya

Date	30.04.13	14.05.13	29.05.13	18.06.13	27.06.13	12.07.13	24.07.13	29.08.13
Sample size	6	5	12	11	11	10	11	10
Min	0	0	0	0.154	0.234	2.481	19.594	34.626
Lower quartile	0	0	0	0.265	0.404	6.937	47.618	52.418
Median	0	0	0	0.714	0.611	34.860	75.160	72.785
Upper quartile	0	0	0	1.212	0.731	60.057	78.806	99.012
Max	0	0	0	2.623	0.781	76.670	99.999	100.000

Table 2 - Boxplot values of the percentage of bryozoan coverage at different sampling dates at Florø

Date	11.04.13	10.06.13	07.08.13	12.09.13
Sample size	12	12	12	9
Min	0	0.002	0.000	6.646
Lower quartile	0	0.029	3.028	16.382
Median	0	0.047	25.937	35.214
Upper quartile	0	0.102	75.871	56.069
Max	0	0.155	92.305	90.362

Table 3 - Boxplot values of lamina size in cm^2 at different sampling dates at Frøya

Date	30.04.13	14.05.13	29.05.13	18.06.13	27.06.13	12.07.13	24.07.13	29.08.13
Sample size	6	5	12	11	11	10	11	10
Min	82.332	318.611	356.6890	241.253	401.7330	303.758	397.1850	132.56
Lower quartile	94.045	682.691	704.9065	519.265	608.8695	534.384	558.1475	294.99
Median	338.676	956.381	889.5960	836.544	917.9850	647.768	638.9690	436.57
Upper quartile	961.747	1111.50	1256.565	1007.98	1256.689	1174.77	959.1095	701.92
Max	1045.24	1319.51	1392.199	1235.692	1720.5860	1874.261	1160.6290	971.28

Table 4 – Boxplot values of lamina size in cm^2 at different sampling dates at Florø

Date	11.04.13	10.06.13	07.08.13	12.09.13
Sample size	12	12	12	9
Min	49.2280	425.1560	271.6320	217.223
Lower quartile	63.5430	565.4245	426.8860	254.186
Median	76.8655	742.5790	723.6365	334.839
Upper quartile	89.6390	847.8805	899.4980	561.115
Max	119.4530	1014.5270	1249.6790	918.085

Table 5 – Median size of the lamina in cm^2 for each depth at sampling dates with bryozoan coverage at Frøya

Date	18.06.13			27.06.13			12.07.13			24.07.13			29.08.13		
Depth	3	8	15	3	8	15	3	8	15	3	8	15	3	8	15
Median	861	1016	481	1484	825	404	854	1118	597	698	643	517	751	390	179

Table 6 – Median size of the lamina in cm^2 for each depth at sampling dates with bryozoan coverage at Florø

Date	10.06.13			07.08.13			12.09.13		
Depth	2	5	7	2	5	7	2	5	7
Median	693	837	684	732	845	426	254	377	600

Appendix II

Summary with estimates and standard error for depth model

- **Frøya**

```
Generalized linear mixed model fit by maximum likelihood ['glmerMod']
 Family: binomial ( logit )
Formula: Respons ~ Depth + (1 | Rope)
   Data: froya

      AIC       BIC    logLik  deviance
 43377.54  43386.86 -21684.77  43369.54

Random effects:
 Groups Name        Variance Std.Dev.
 Rope   (Intercept) 0.003774 0.06144
Number of obs: 76, groups: Rope, 4

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  1.27437    0.03385   37.64   <2e-16 ***
Depth8      -0.35775    0.01992  -17.96   <2e-16 ***
Depth15      0.06903    0.02786    2.48   0.0132 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
        (Intr) Depth8
Depth8  -0.294
Depth15 -0.212  0.362
```

- **Florø**

```
Generalized linear mixed model fit by maximum likelihood ['glmerMod']
 Family: binomial ( logit )
Formula: Respons ~ Depth + (1 | Rope)
   Data: floro

      AIC       BIC    logLik  deviance
10015.610 10022.836 -5003.805 10007.610

Random effects:
 Groups Name        Variance Std.Dev.
 Rope   (Intercept) 0.02729  0.1652
Number of obs: 45, groups: Rope, 4

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  2.06236    0.09045   22.80   <2e-16 ***
Depth5      -0.55645    0.04590  -12.12   <2e-16 ***
Depth7      -1.12312    0.04591  -24.46   <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
       (Intr) Depth5
Depth5 -0.327
Depth7 -0.325  0.641
```

- **Florø and Frøya combined**

```
Generalized linear mixed model fit by maximum likelihood ['glmerMod']
 Family: binomial ( logit )
Formula: Respons ~ Depth + (1 | Rope)
   Data: data

      AIC       BIC    logLik  deviance
 53417.70  53437.27 -26701.85  53403.70

Random effects:
 Groups Name        Variance Std.Dev.
 Rope   (Intercept) 0.006034 0.07768
Number of obs: 121, groups: Rope, 4

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  2.06096    0.05335   38.63   <2e-16 ***
Depth3      -0.79013    0.03915  -20.18   <2e-16 ***
Depth5      -0.55064    0.04565  -12.06   <2e-16 ***
Depth7      -1.10010    0.04564  -24.11   <2e-16 ***
Depth8      -1.14920    0.03922  -29.30   <2e-16 ***
Depth15     -0.71860    0.04377  -16.42   <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
        (Intr) Depth3 Depth5 Depth7 Depth8
Depth3  -0.639
Depth5  -0.549  0.747
Depth7  -0.548  0.747  0.641
Depth8  -0.639  0.871  0.747  0.747
Depth15 -0.573  0.780  0.670  0.668  0.781
```

Summary from ANOVA test

- **Frøya**

Analysis of variance test for models including and excluding depth as a factor at the Frøya location

```
Models:
mod.Null: Respons ~ 1 + (1 | Rope)
mod.Depth: Respons ~ Depth + (1 | Rope)
          Df   AIC   BIC logLik deviance  Chisq Chi Df Pr(>Chisq)
mod.Null   2 43786 43791 -21891    43782
mod.Depth  4 43378 43387 -21685    43370 412.95      2  < 2.2e-16 ***
```

- **Florø**

Analysis of variance test for models including and excluding depth as a factor at the Florø location

```
Models:
mod.Null: Respons ~ 1 + (1 | Rope)
mod.Depth: Respons ~ Depth + (1 | Rope)
          Df   AIC   BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
mod.Null   2 10657 10660 -5326.4    10653
mod.Depth  4 10016 10023 -5003.8    10008 645.18      2  < 2.2e-16 ***
```