Master's thesis

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

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Bryozoan larvae (cyphonauts) growth and encrustation in *Saccharina latissima:* analysis of biotic and abiotic interactions.

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Supervisor: Glaucia Fragoso
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Abstract

Seaweed aquaculture is an essential tool for feeding the world's population in the 21st century, as well as assisting to achieve several United Nations sustainable development goals such as climate action and life below water. Biofouling, the growth of organisms on the seaweed, reduces the quality of biomass and poses a huge challenge to the profitability of the industry. The main fouling organisms that the seaweed industry primarily in Norway is facing are the bryozoans *Membranipora membranacea* and *Electra pilosa*. Prior to fouling, the bryozoans occur as a larval stage (cyphonaut) in the plankton for several weeks. After settling on the lamina of kelp, they undergo metamorphosis and develop a sessile stage where colonies develop along the lamina's surface. Consequently, the seaweed becomes brittle and the quality of the biomass for industry purposes (particularly food consumption) is reduced. The present study aims to look at which biotic and abiotic factors affect towards bryozoans' larval growth and subsequent settlement on the seaweed.

The abundance of bryozoans was documented during its planktonic stage from February to June 2022, along with several abiotic and biotic factors, such as temperature, salinity, nutrient and chlorophyll a concentration, sporophyte length, and photo physiological health state of the *Saccharina latissima*. Sampling was performed at a cultivation site belonging to Seaweed Solutions and was undertaken at two locations one inside the seaweed farm and one outside. Water samples were taken from a depth of 3m, the same depth as where the seaweed is cultivated, for analysis of nutrient and chlorophyll a concentration. Plankton samples for quantification of cyphonaut abundance as well as phytoplankton community analysis were taken from a depth of 5m using a plankton net.

Abundance of cyphonauts showed an overall increase throughout the sampling period, reaching the first peak in abundance on the 5th of April, slightly decreasing over subsequent sampling days and reaching the highest abundance on the 3rd of June. The appearance of the first colony was noted on the same day as the first peak of bryozoan larval abundance and colonies increased in number and size over the rest of the sampling period. Temperature and food availability (phytoplankton) were found to be key facilitators in bryozoan larval growth and settlement. The results of the study suggest that cultivated S. latissima in the Norwegian Sea and the North Atlantic, should be harvested from early to mid-May to avoid effects of biofouling.

Sammendrag

Havbruk av tang er et essensielt verktøy for å fø verdens befolkning i det 21. århundre, i tillegg til å bidra til å nå flere FNs bærekraftige utviklingsmål som klimahandling og liv under vann. Biobegroing, vekst av organismer på tangen, reduserer kvaliteten på biomassen og utgjør en stor utfordring for lønnsomheten i næringen. De viktigste begroingsorganismene som tangindustrien først og fremst i Norge står overfor er mosdyrene Membranipora membranacea og Electra pilosa. Før begroing opptrer mosene som larvestadium (cyphonaut) i planktonet i flere uker. Etter å ha satt seg på lamina av tare, gjennomgår de metamorfose og utvikler et fastsittende stadium hvor kolonier utvikler seg langs laminas overflate. Følgelig blir tangen sprø og kvaliteten på biomassen til industriformål (særlig matforbruk) reduseres. Denne studien tar sikte på å se på hvilke biotiske og abiotiske faktorer som påvirker mosdyrenes larvevekst og påfølgende bosetting på tangen.

Overfloden av mosdyr ble dokumentert i planktonfasen fra februar til juni 2022, sammen med flere abiotiske og biotiske faktorer, som temperatur, saltholdighet, næringsstoff og klorofyll a-konsentrasjon, sporofyttlengde og fotofysiologisk helsetilstand til Saccharina latissima. Prøvetakingen ble utført på et dyrkingssted tilhørende Seaweed Solutions og ble foretatt på to steder, en innenfor tangfarmen og en utenfor. Det ble tatt vannprøver fra 3m dyp, samme dybde som der tangen dyrkes, for analyse av næringsstoffer og klorofyll a-konsentrasjon. Planktonprøver for kvantifisering av cyphonautoverflod samt planteplanktonsamfunnsanalyse ble tatt fra en dybde på 5m ved bruk av et planktonnett.

Overflod av cyfonauter viste en generell økning gjennom prøvetakingsperioden, og nådde den første toppen i overflod den 5. april, noe avtagende over påfølgende prøvetakingsdager og nådde den høyeste forekomsten den 3. juni. Utseendet til den første kolonien ble notert samme dag som den første toppen av larvemengden og koloniene økte i antall og størrelse i løpet av resten av prøveperioden. Temperatur og mattilgjengelighet (fytoplankton) ble funnet å være viktige tilretteleggere for larvevekst og bosetting av bryozoer. Resultatene av studien tyder på at kultivert S. latissima i Norskehavet og Nord-Atlanteren bør høstes fra tidlig til midten av mai for å unngå virkningene av biobegroing.

Preface

The present study was undertaken to analyse the links between biofouling and other biotic/abiotic factors and use the acquired knowledge as a tool to predict biofouling settlement on cultivated *Saccharina latissima*. The research is part of the MoniTARE project (Autonomous underwater monitoring of kelp-farm biomass, growth, health and biofouling using optical sensors), funded by the Norwegian research council (315514). Research was carried out at a seaweed farm belonging to Seaweed Solutions in Frøya during early February to late June 2022 and samples were analysed at Trondheim biological station (TBS, NTNU).

During the research and the writing process I have gained valuable knowledge and experiences. I would like to thank all my supervisors; Glaucia Fragoso, Ana Borrero, Geir Johnsen, and Nicole-Aberle Malzahn for guiding me throughout the master's thesis. A warm thank you goes out to Glaucia Fragoso and my classmate Martin Overrien whom provided valuable support during the fieldwork at the SES seaweed farm in Frøya and made the trips fun and memorable. I would also like to give special thanks to Ana Borrero, from SES, for helping organise the fieldwork at SES and providing valuable advice and input during the writing process.

I would also like to thank in general the staff at SES, for allowing me to take part in the research at their seaweed farm as well as using their facilities for field and lab work. And lastly, I would like to thank my family for their support during the writing process. This master thesis has been a challenging but memorable experience thanks to the students and staff at NTNU and SES.

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1 Introduction

1.1 Seaweed aquaculture

Seaweed or macroalgal aquaculture is a multibillion-dollar industry, comprising half of the global mariculture production and the fastest growing aquaculture sector, at an estimated expansion rate of 8% per year. To scale this, production of seaweed has more than doubled; from 13.5 million tonnes in 2005 to 29.4 million tonnes in 2015 (FAO, 2015). Seaweed farming can greatly contribute to the sustainability of aquaculture due to the numerous environmental benefits it provides. Seaweed farming offers nature-based and local solutions for climate change and ocean acidification mitigation, through its ability to fixate inorganic carbon as a primary producer. By sequestering carbon, seaweed can help lower atmospheric levels of carbon dioxide (Krause-Jensen and Duarte, 2016; Duarte, Middelburg and Caraco, 2005). Cultivation of seaweed can also improve water quality by supplying oxygen and taking up excess nutrients in eutrophic waters (Duarte *et al.*, 2017). Seaweed cultivation can contribute to a more sustainable aquaculture, achieving several of the United Nations sustainable development goals including, zero hunger, responsible consumption and production, life below water, and climate action (Duarte, Bruhn and Krause-Jensen, 2021).

Seaweed is a sustainable food alternative for a world approaching 10 billion by 2050 (Banach *et al.*, 2022). It can provide food security and it has recently been often hailed as the "new superfood" (Blikra *et al.*, 2021). Seaweed is rich in several important nutrients, vitamins, minerals, dietary fibre, peptides, and polyunsaturated fatty acids (PUFA) (FAO, 2015). In addition, it has been discovered that seaweed contains many substances for nutraceuticals purposes and having a therapeutic role in the treatment of several metabolic diseases, such as cancer, hypertension, and diabetes (Peñalver *et al.*, 2020). Due to the high composition of PUFA in seaweed it is an excellent alternative for fish feed, being able to replace terrestrial vegetable ingredients such as soybeans, which are much less sustainable (Lundeberg and Grønlund, 2017; Mwendwa, Wawire and Kahenya, 2023). Besides this, seaweed can be used as fertiliser, in cosmetics, animal feed (specifically for pigs) and as biofuel (Costa *et al.*, 2021). As a multi-functional product seaweed has an excellent standing in helping to achieve the sustainable development goals (Duarte, Bruhn and Krause-Jensen, 2021).

Seaweed aquaculture has a long history in Asia, where the well-established industry uses its products for natural extracts such as agar and carrageen, as well as in Asian cuisine (FAO, 2015). China is the biggest aquaculture producer, contributing to 60% of the global production (FAO, 2015). Despite its successful establishment in Asia, seaweed cultivation is still in its infancy in the rest of the world and in key aquaculture producers in Europe such as Norway. Norway has had a late start, with the first commercial license only being granted in 2014 (Stévant, Rebours and Chapman, 2017). However, it has taken off since then: between 2014 and 2016 the surface area allocated to seaweed farming has more than tripled. In other words, approximately 277 hectares km^2 have been allocated to seaweed farming along the Norwegian coast (Stévant, Rebours and Chapman, 2017).

1.2 Seaweed aquaculture in Norway

Although seaweed farming has a long history in Asia, amounting to 97% of worldwide production, it has only become established in Europe in the last 5-10 years (Zhang *et al.*, 2022). Seaweed aquaculture in Norway has started to take root, and it has huge potential for growth. Firstly, Norway has a vast coastline which extends over 100,000 km meaning there are plenty of sites available for seaweed cultivation (Stévant, Rebours and Chapman, 2017).

Seaweed cultivation in Norway has mainly been directed to cultivation of sugar kelp (*Saccharina latissima*) due to its high content of polysaccharides and other important nutrients, as well as its excellent growth potential (Marinho *et al.*, 2015 ; Bojorges *et al.*, 2022). Winged kelp (*Alaria esculenta*) is also another commonly cultivated species, and popularity has been increasing over the last few years. In 2021, 180 metric tonnes of *S. latissima* and 66 tonnes of *A. esculenta* were produced from aquaculture (Directorate of Fisheries, 2022).

1.3 Biology of Sugar Kelp

Saccharina latissima, commonly known as sugar kelp, is a perennial species of brown algae that can be found between in the intertidal and photic zones of the northern hemisphere (Handå *et al.*, 2013). Its optimal growth is found in temperate to polar water waters between 10 and 17 °C and salinities of 33-35 (Kerrison *et al.*, 2015). Approximately 50% of the world's wild *S. latissima* populations are confined along the coast of Norway (Moy, 2009).

Saccharina latissima has a diplohaplontic lifestyle, where the sporophyte (2n) in the winter becomes sexually mature, releasing haploid spores (n) into the water column (Schiel and Foster, 2006). The spores germinate into male and female gametophytes and subsequently settle onto a suitable substrate. The mature male gametophyte then releases spermatozoids that fertilize the eggs on the female gametophyte, thereby entering the diploid stage in their lifecycle. The resulting sporophyte disperses into the water column through ocean currents and attaches to a suitable substrate (Schiel and Foster, 2006 ; Torp, 2018). Fully grown the sporophyte can reach up to 3m in length (Parke, 1948) (Fig. 1).

Life cycle processes of sugar kelp, including sorus induction, spore release and gametophyte growth can be controlled under laboratory conditions. Once the resulting sporophyte is suitably developed it can be easily seeded onto long lines or other structures used for cultivation, which typically occurs in the winter season, followed by harvesting in the summer months (Forbord *et al.*, 2012; Matsson, Christie and Fieler, 2019).

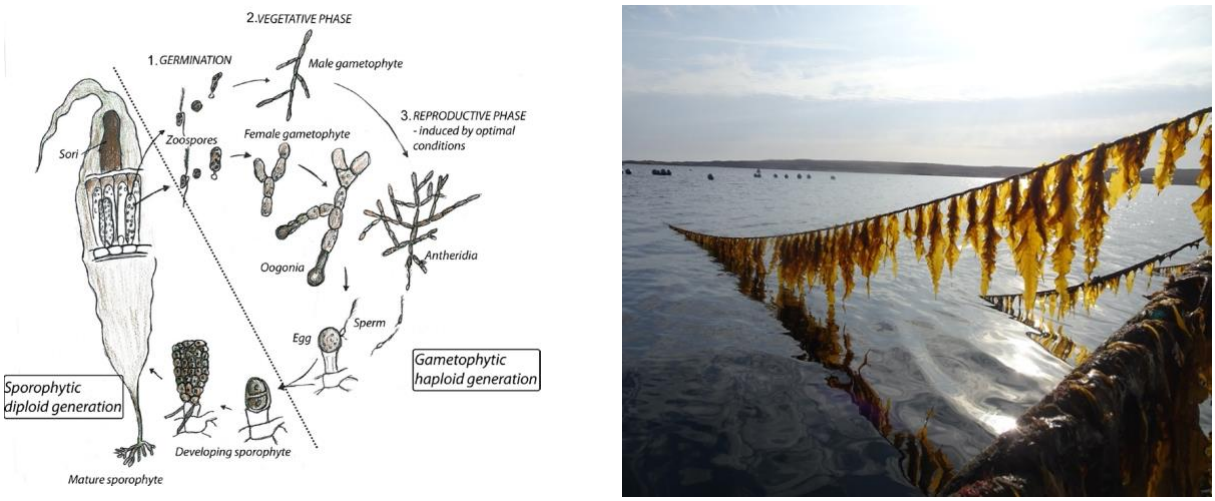

Figure 1- Schematic diagram of the life cycle of S. latissima (Forbord, 2020) and a picture of a long line of S. latissima cultivation at seaweed solutions AS.

1.4 Biofouling by bryozoans

In the Norwegian Sea and on a larger scale in the North-East Atlantic Ocean, the bryozoans *Membranipora membranacea* and *Electra pilosa* are the dominant biofouling community for

seaweed aquaculture (Førde *et al.*, 2016). In their larval, or cyphonaut, stage they feed on phytoplankton and other flowing particles in the water column, using a specialised organ known as a lophophore. This organ is made up of an array of ciliated tentacles and is used to generate their own feeding current, which in association with natural currents directs the food particles towards the mouth (Winston, Woollacott, and Zimmer, 1977).

Larvae of both species can be found within the plankton community year-round, although *M. membranacea* cyphonauts are especially abundant from May to September (Ryland, 1965). Once settled, the larva undergoes metamorphosis and forms an ancestrula, the primary zooid of a bryozoan colony. Each ancestral zooid develops a colony through producing a series of identical zooids by asexually budding. Both the ancestrula zooid and the asexually produced zooids grow calcified cell walls, creating a lattice-like structure that is clearly visible on the lamina (Seed and O'Conner, 1981; Førde, 2014). The colonies of the two species can be clearly distinguished by the pattern of the lattice on the lamina: *M. membranacea* colonies have a uniform rectangular/oval shape, whereas *E. pilosa* colonies are typically star-shaped (Hayward and Ryland, 1995; Hayward and Ryland, 1998) (Fig 2).

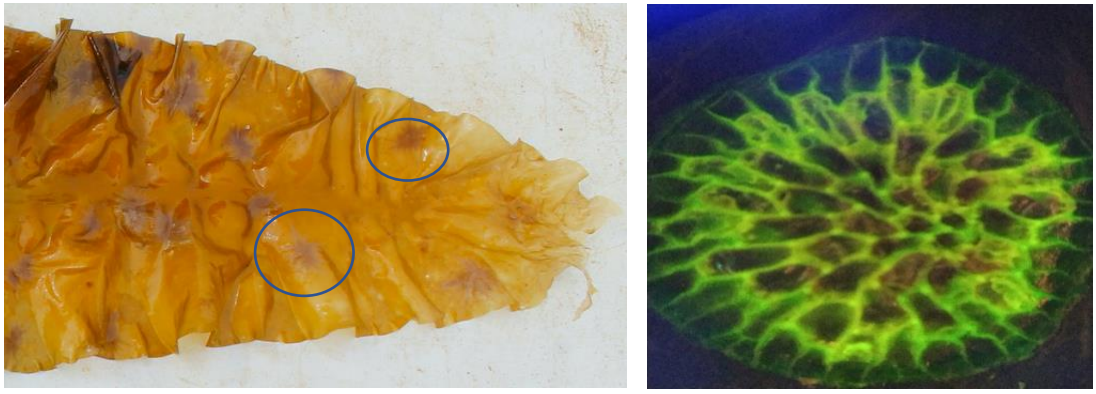

Figure 2- Heavy biofouling by E. pilosa on the tip of the lamina during late spring (shown left). Microscopic photo of a M. membranacea colony, marked with calcine florescent dye (right).

Due to biofouling by epiphytes, particularly by bryozoans, kelp harvesting must be carefully timed. Harvesting must occur before the onset and encrusting of bryozoans, which typically occurs in early May to early June in Trøndelag (Førde, 2014). However, the optimal macroalgal growth phase can extend until autumn. It is, therefore, suggested that *S. latissima* deployed in Norwegian temperate coastal waters in winter should be harvested from late

April to early June at the latest, to avoid the negative effects of bryozoan fouling (Førde *et al*., 2016).

Larval recruitment and the extent of bryozoan biofouling vary among cultivation sites but seem to be linked to the timing of the phytoplankton bloom timing which itself is controlled by light regimes including intensity and day length, and water column stratification (triggered by temperature) as well as nutrients (Matsson, Christie and Fieler, 2019; Saunders and Metaxas, 2008). In agreement, it has also been observed that epifauna species richness, including bryozoan, on *L. digitale and S. latissima* lamina is strongly correlated with water column processes (Carlsen *et al.*, 2007).

However, bryozoan encrustation may also be related to the weakening of defence mechanisms of the algae because of nutrient stress in the algae. Accordingly, encrustation seems to be more severe in the winter despite greater macroalgal growth (Matsson *et al.*, 2021). It is unclear how these factors interact and trigger the settling and the growth of bryozoan on the kelp lamina. Understanding the ecology of fouling organisms, and how biotic and abiotic factors affect growth is required to optimize productivity.

1.5 Phytoplankton succession

Since bryozoan larvae are filter feeders, their abundance is likely influenced by the abundance and community composition of their phytoplankton diet their food source present in the water column (O'Dea and Okamura, 1999; Saunders and Metaxas, 2009). Abundance and growth of phytoplankton in temperate areas typically follows a natural seasonal cycle controlled by light, nutrient concentrations, and grazing (Blauw *et al.*, 2012). Nutrient concentrations are controlled by physical processes, such as strong mixing in winter and thermal and/or haline stratification towards summer (Lindemann and St. John, 2014). Blooms, or exponential growth of phytoplankton, have been documented to occur in the temperate regions such as the North-Atlantic in spring followed by low abundance in summer and a subsequent short bloom in the late summer/autumn months (Silva *et al.*, 2021).

While it is known that light is the major driver of the spring bloom, Sverdrup's critical depth hypothesis describes changes in the hydrography as being key drivers for the onset of the bloom. The hypothesis states that shoaling of the mixed layer above the critical depth, which

is the depth of where the integrated phytoplankton growth through photosynthesis is equal to the integrated losses due to respiration, initiates the spring bloom (Smetacek and Passow, 1990). High nutrient concentrations and increased irradiance have been documented to be major factors contributing to the development of the spring bloom in areas around the mid coast of Norway (Sakshaug and Myklestad, 1973; Magnesen and Christophersen, 2008).

Throughout the duration of the spring bloom, diatoms are typically the most dominant phytoplankton group in the initial phase of the bloom as they naturally use up silicate in the water column, followed by large dinoflagellates as the silicate levels become depleted (Sakshaug and Myklestad, 1973; Hostyeva, 2011). Accordingly, diatoms including *Thalassiosira* sp., *Chaetoceros* sp. and *Skeletonema* sp. have been observed during the spring in the water column in waters off the Froan archipelago (north-east Norway), with the species *Skeletonema costatum* being highly prevalent within the community (Fragoso *et al.*, 2021). In addition, a high prevalence of flagellates and rhizosolenid diatoms during the summer when the water column becomes stratified, and a prevalence of the diatoms *Pseudo-nitszchia* spp. during the fall, through mixing by stormy conditions, has been observed (Fragoso *et al.*, 2021).

1.6 Photophysiology

It has also been suggested that the health state of kelp directly correlates with extent of biofouling (James *et al.*, 2020). Meaning that biofouling can negatively impact the photo physiology or photosynthetic efficiency of a primary producer and induce stress responses (James *et al.*, 2020 ; Dethier, Williams and Freeman, 2005). However, it is uncertain whether a stressed kelp with poor health state is more vulnerable to biofouling.

Light energy is first harvested by pigment molecules in the antenna pigment complex. As each pigment molecule in the antenna pigment complex is elevated to a higher energy state after absorption and then are subsequently reverted to its initial state, energy is released (Johnson, 2016). This energy can either be 1) dissipated by heat, a process called non-photochemical quenching 2) redirected to the photochemical process (photosynthesis) or 3) re-emitted as fluorescence (Fig 3) (Giannini, 2016; Johnson, 2016). These pathways are a function of the maximum quantum yield of photosystem II (PSII) or here stated as the Fv/Fm

which reflects photochemical efficiency, calculated from number of redirected photons to photosynthesis divided by number of photons absorbed (Gorbunov and Falkowski, 2022).

In dark conditions, the PSII reaction centres are open, meaning that they are ready to receive the photons absorbed. Naturally, as the electron carrier within the photosynthesis apparatus accepts an electron, the PSII reaction centre "closes" until the electron is passed onto the next carrier (Murchie and Lawson, 2013). When the reaction centres are closed, the photons are re-emitted as fluorescence. In situations where the photosynthetic organism becomes oversaturated with light, a process known as non-photochemical quenching occurs, which is a photo-protective mechanism employed when the excess of light is converted to heat, relieving the PSII reaction centres from the excess of energy (Gorbunov and Falkowski, 2022). (Fig 3c). The relationship between photosynthetic quenching (photosynthesis) and re-emission to fluorescence can be used to determine the photosynthetic efficiency of an organism (Gorbunov and Falkowski, 2022).

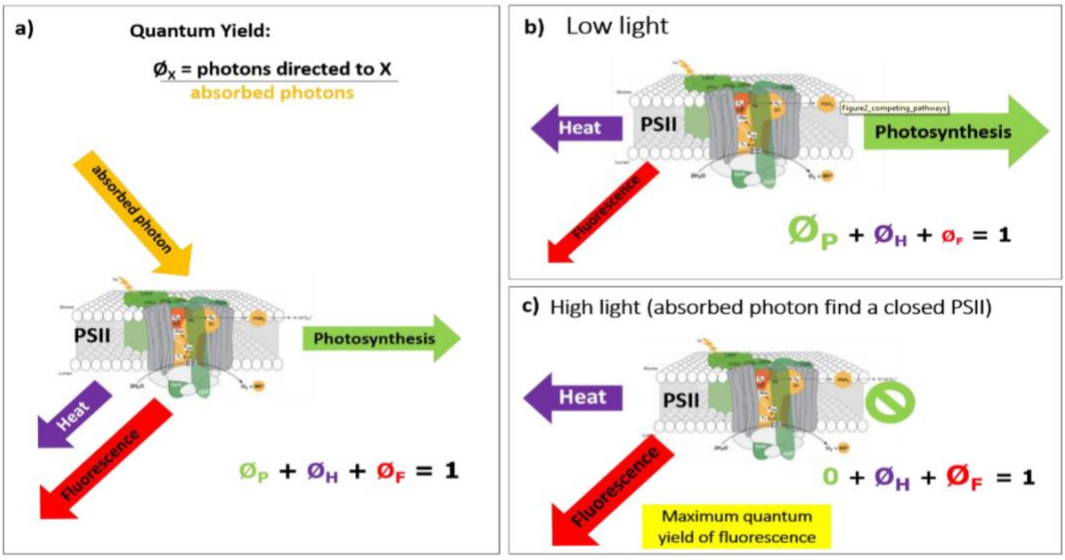

Figure 3- Summary of the potential pathways of absorbed photons by the PSII. Showing a) in normal conditions the probability of a photon to be directed to one of the three paths are equal, b) Reaction centres are open, and the amount of energy being directed to photosynthesis is higher due to low light conditions and c) the probability of fluorescence increases as the PSII reaction centres are closed and fluorescence is thus maximal. light increases until the PSII reaction centres are closed, and fluorescence is thus maximal. Figures taken from (Giannini, 2016).

1.7 Aims and objectives

This MSc project was a part of the MoniTARE project (2021-2025), funded by the Norwegian Research council (NRC). The main objective was to look at the interactions

between the abiotic and biotic factors that affect bryozoan larvae growth in the water column and settlement on the cultivated kelp species *S. latissima* (Fig.4). Research was performed in Frøya, in one of the Seaweed Solutions AS (SES) farms, Måsskjaeret. Sampling occurred throughout the growing season (February-June 2022). The factors affecting degree of biofouling and the ecology of bryozoans, including their development will be useful to predict when biofouling will occur and hence it is essential to ensure the quality of the lamina.

To achieve this, three subobjectives were proposed: (1) Studying the relationship between abiotic factors; light regime, temperature, salinity, wind speed, nutrient concentrations, water column stratification, and the timing and abundance of bryozoan larvae. (2) Evaluating the phytoplankton community and the bryozoan larvae concentration (3) Analysing the health state of the kelp and settlement of cyphonauts in relation to the phytoplankton bloom.

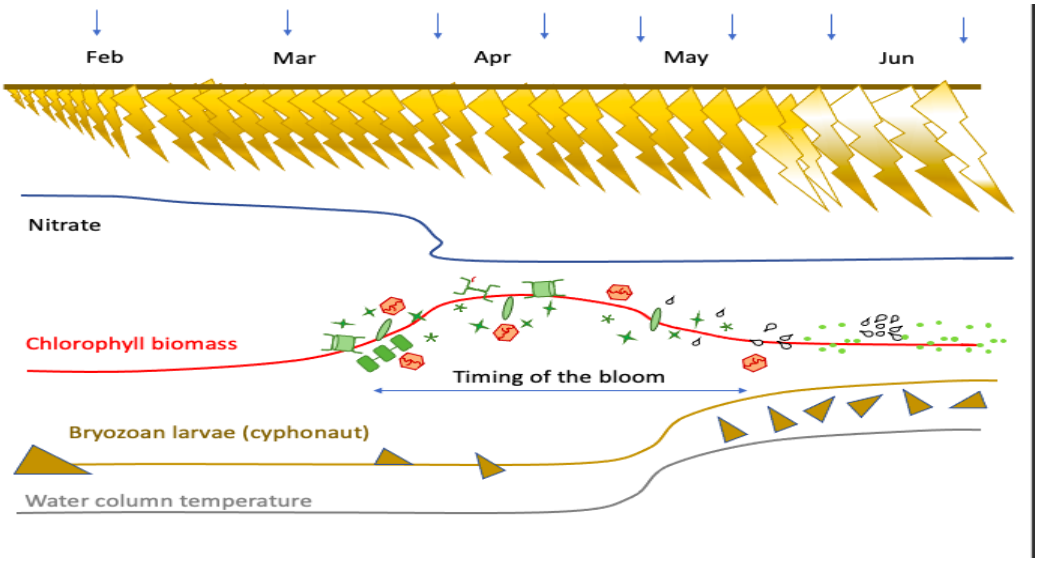

Figure 4- Conceptual diagram of how the different abiotic factors are expected to interact, with nitrate, chlorophyll a biomass, and water temperature as key factors. Figure taken from (Fragoso, n.d.)

2 Methods

2.1 Study area

Fieldwork was carried out at Seaweed Solutions kelp farm in Frøya, an island located off the coast mid-Norway (Trøndelag) (Fig 5). The Norwegian Sea is categorized by high primary productivity, with an annual primary production of 80-120 gC m^{-2} y^{-2} (Planque *et al.*, 2022). One reason for this is strong mixing, which occurs throughout the year, with down-welling favourable conditions in the winter and upwelling favourable winds during the spring and summer, supplying nutrient-rich deep water to the surface, and boosting primary productivity (Skagseth, Drinkwater and Terrile, 2011; Terrile; Jolivet *et al.*, 2015). Thus, the seafood and fishing industry in the Froan archipelago is highly productive and can sustain large populations of Atlantic cod and edible crabs, for example (Fragoso *et al.*, 2019). Due to the high productivity of the area, it is also an excellent location for seaweed farming.

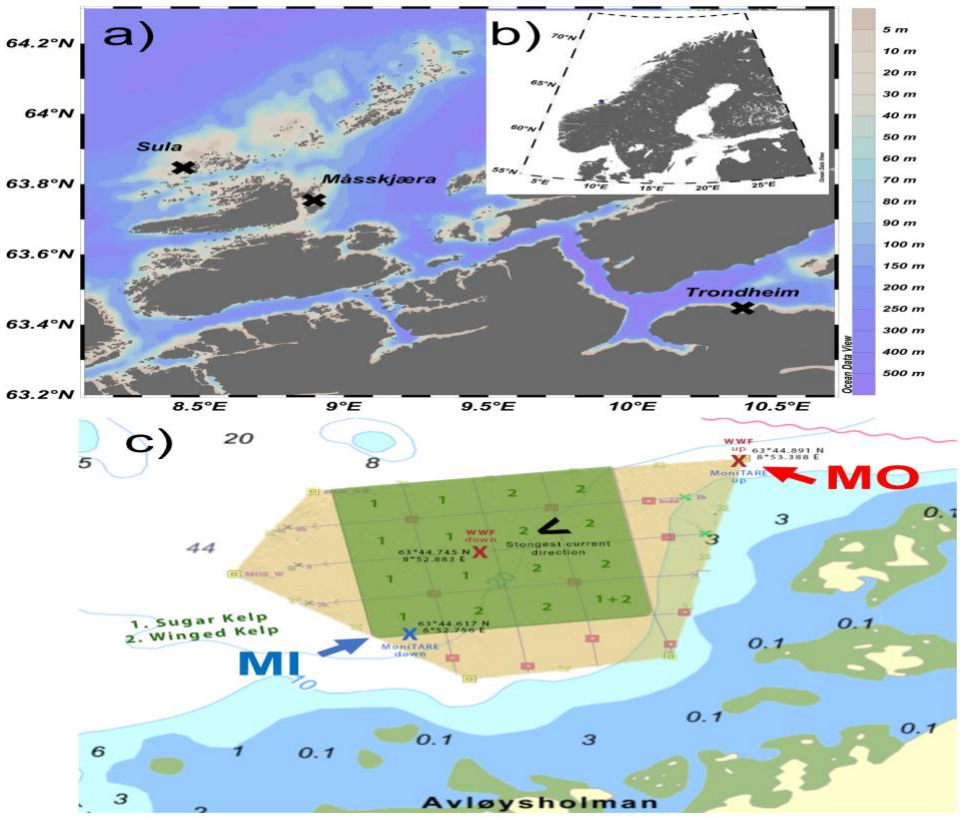

Figure 5- Geographical location of a) the study site, the Måsskjaere kelp-farm and the Sula meteorological station for wind speed and b) location of the farm off the coast of mid-Norway. C) Måsskjaere farm where the two sites (MI- inside the farm and MO- outside the farm) was sampled.

The SES farm is approximately a 15-minutes boat ride from the shore. Sampling was undertaken at two sites, one inside (station MI) and the other one outside the seaweed farm (station MO) (Fig 5c). Sampling took place every two weeks or month, mostly during low tides, starting from the 18th of February to the 15th of June 2022, amounting to a total of eight sampling days.

Abiotic parameters such as temperature, wind speed, salinity, wind speed, turbidity, and nutrient concentrations (nitrate, phosphate, silicate, and ammonium) were collected. In parallel, biotic parameters such as phytoplankton community structure, abundance of bryozoan larvae and chlorophyll a concentration were measured at both sampling stations. Photophysiological health state of *S. latissima* was collected from MI only. A C3 submersible fluorometer sensor (Turner Designs, USA) was located at 3m depth and was attached in a frame at the edge of the farm (station MI). This sensor collected time-series data of chlorophyll fluorescence (calibrated later to concentration in mg m), turbidity (FTU) and temperature (°C) every 10 minutes from mid-February to mid-June.

2.2 Water and net sampling

The physical properties of the water column were recorded at each station using conductivity-temperature-density (CTD) device, coupled with sensors to measure chlorophyll and turbidity (model SD204 SAIV A/S™). The device was attached to a rope then lowered off the side of the boat into the water column to achieve a vertical depth profile of 15m which was approximately the depth of the fjord at the sites sampled. Seawater samples were obtained using a water sampler from a depth of 3 metres, equivalent to the cultivation depth of the kelp, and collected into two 8L acid-washed brown bottles for later analysis of: nutrients and *in vitro* chlorophyll a.

After water collection, samples were taken back to the SES laboratory onshore, located a few km from the farm. The samples were filtered (0.25-0.5L, depending on the biomass) for *in vitro* chlorophyll a concentration using Whatman GF/F glass fibre filters. Filters were carefully folded in half and wrapped in aluminium foil, and temporarily kept in dry ice for transportation and stored in an 80°C freezer for posterior analyses in the lab at Trondheim Biological Station (TBS).

For nutrient concentrations, triplicate water samples for each station were filtered with a 0.8 µm polycarbonate filter after flushing with seawater for ammonium removal. Approximately 45ml of water was filtered through the filter for each sample into a centrifuge tube, kept temporarily in dry ice for transportation, for several hours, and then stored in a -20°C freezer for later analyses in the lab.

Plankton net samples for bryozoan larvae abundance estimations were taken using plankton net (Ø= 40cm) (Hydro-Bios, Germany) which was deployed to a depth of 5 metres and then subsequently hauled up (Fig. 6). The plankton net was rinsed with 96% ethanol and kept at an approximate concentration (>70%). The samples were then stored in dark conditions and at room temperature, for later microscopic analysis in the laboratory.

For phytoplankton identification and quantification, seawater samples were directly transferred into dark glass amber bottles and immediately fixed with neutral Lugols iodine solution to a final concentration of ~1%. Lugols samples were stored in dark conditions and at room temperature before being sent to a laboratory in Poland for microscopic analysis.

Figure 6- deployment of the plankton net from the side of the research vessel (shown left) and shown right, a (very brown) plankton net sample, indicating a zooplankton bloom.

2.3 Fv/Fm measurements

Five *S. latissima* specimens with varying lengths were collected from the cultivation site at the SES farm in each sampling period and stored temporarily in the dark using closed styrofoam boxes filled with local seawater to keep the local water temperature. Once back to the pier in the SES facilities (approx. 1 hour after seaweed collection) the maximum quantum yield of the kelp was analysed using a diving PAM under dark conditions. For this, a kelp specimen was placed in a tray, partially filled with local seawater of the same temperature to

prevent stress due to temperature change. Each specimen was first measured with a standard measuring tape, from the base of the holdfast to the tip of the lamina. Triplicate measurements of the maximum quantum yield (Fv/Fm) were taken at three different parts of the kelp lamina, meristem (new tissue) at the base, the middle, and the old tissue at the tip.

2.4 Chlorophyll analysis

Chlorophyll *a* concentration (μgL^{-1}) was analysed at the laboratory at TBS, a few months after collection. For chlorophyll a extraction., frozen filters were individually transferred to a 15ml glass tube, which was then filled up with chilled 100% methanol. The tubes were then lightly mixed using a vortex and immediately placed in the freezer and left for 24 hours prior to analysis.

After incubation, the filters were removed from the tubes and a 0.22 µm syringe filter was used to take up the filtrate. The extracted solvent was then transferred into clean test glass tubes. An aliquot of 1.6 µL of the filtrate solvent was then pipetted into a small glass vial, which was placed into a Turner Design Fluorometer for analysis of fluorescence. Before the first reading and after every 10 readings, a blank sample containing 1.6 µL 100% methanol was taken to calibrate the machine. The *in-vitro* concentration of chlorophyll (µg chl a L^{-1}) in the water was calculated using the reading of fluorescence and the blank values obtained:

$$\mu g \text{ chl } \underline{a} \text{ / liter} = (FL\text{-}BL) \times f \times E \times 1000) / (V*1000)$$

2.5 Nutrient analyses

Filtered seawater samples for nitrate and nitrite concentrations, phosphate and ammonium were sent to a laboratory in Poland (Eurofins) for analyses. Silicate concentration was analysed at TBS, using the instrument: OI Analytical flow solution IV and analysis was performed according to (Strickland and Parsons, 1972).

2.6 Bryozoan larvae counts

Taxonomic identification and quantification of (*M. membranacea* and *E. pilosa*) was performed at TBS using a Leica (model M205C) dissecting microscope. In the lab, the ethanol preserved samples were first rinsed with filtered seawater by flushing out the samples onto a counting mesh (106 µm), which was placed into a shallow tub filled with filtered seawater level to the mesh and left for approximately 1 hour. After rinsing, the samples were flushed into a plastic beaker for counting and diluted to a known concentration.

Subsamples of 8ml were pipetted into gridded petri dishes and number of *M. membranacea* and *E. pilosa* larvae were counted, with the help of a handheld counter to keep track of the counts. Subsamples were taken from each sample until a total of 100 bryozoans (*M. membranacea* and *E. pilosa*) had been counted. Larvae species were identified according to (Newell and Newell, 1970) and calculations were made to provide the taxa individual counts per m^3.

2.7 Statistical analysis

Graphic visualisations of the results and statistical analysis were performed using the programming language R, version 4.1.2 (2022) and its interface R studio, version 4.1.2 (2021). The downloadable package ggplot2, was used to make most of the graphs, and CTD graphs were made in excel (2016).

3 Results

3.1 Hydrography

Temperature and salinity profiles for the inside (MI) and outside (MO) stations showed similar patterns (Fig.7), however there was more variability in salinity with high values (approximately 33.5) at the start of the sampling period around, followed by a decrease and then a subsequent rise during the spring and summer months. Furthermore, profiles on the 22nd of March and the 20th of May were much more variable in MI than MO, possibly because of the greater influence of tides in MI. A clear trend for temperature in both stations can be observed, with temperatures increasing from approximately 5.5 °C in mid-February to around 9.5 °C in mid-June. Stratification of the water column starts on the 20th of April, breaks down on the 4th of May, and becomes stratified again from 20th May to 15th June.

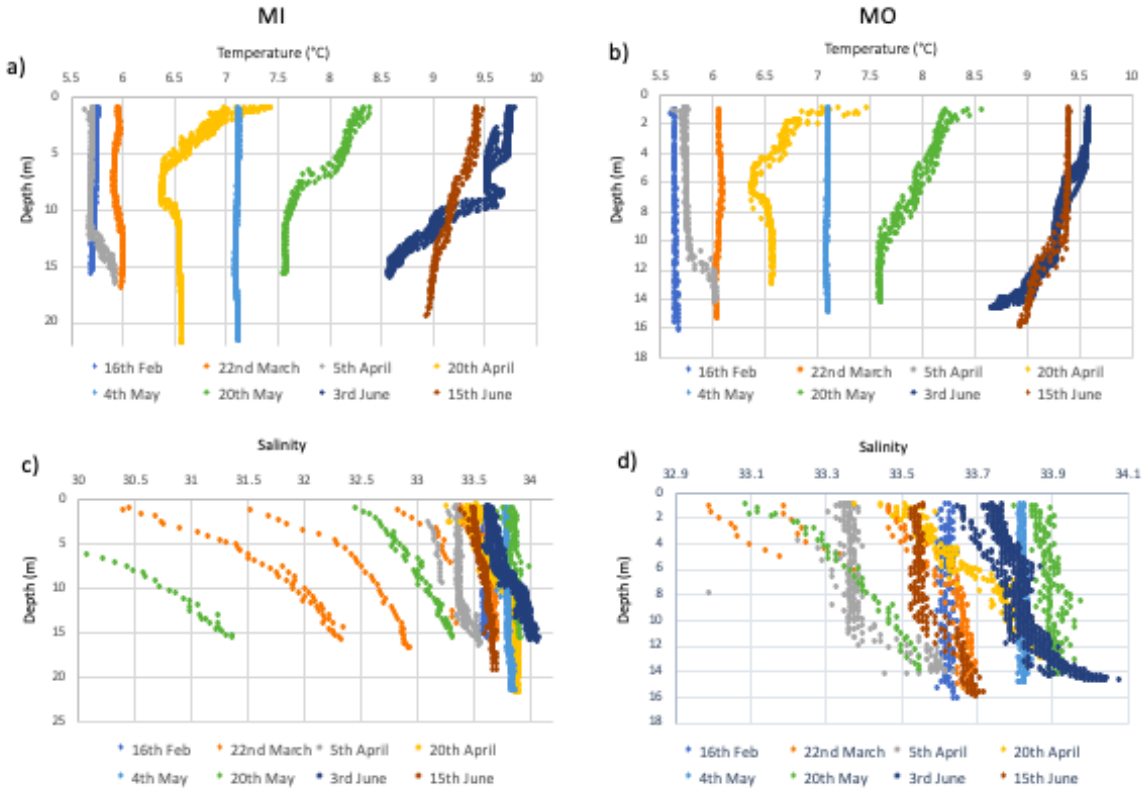

Figure 7- (a-b) Temperature and (c-d) salinity profiles of the two stations, MI (inside the farm-left) and MO (outside the farm-right)

Chlorophyll and turbidity were highest in the upper 10m on the more stratified waters, such as the 22nd of March, 20th of May, 3rd of June and 15th of June, and lowest when the water column was more mixed (Fig.8). The water column was the most turbid (up to 1.35 FTU) and had the highest chlorophyll a concentration (up to 7 µg L^{-1}) on the 20th of May and the 20th of April.

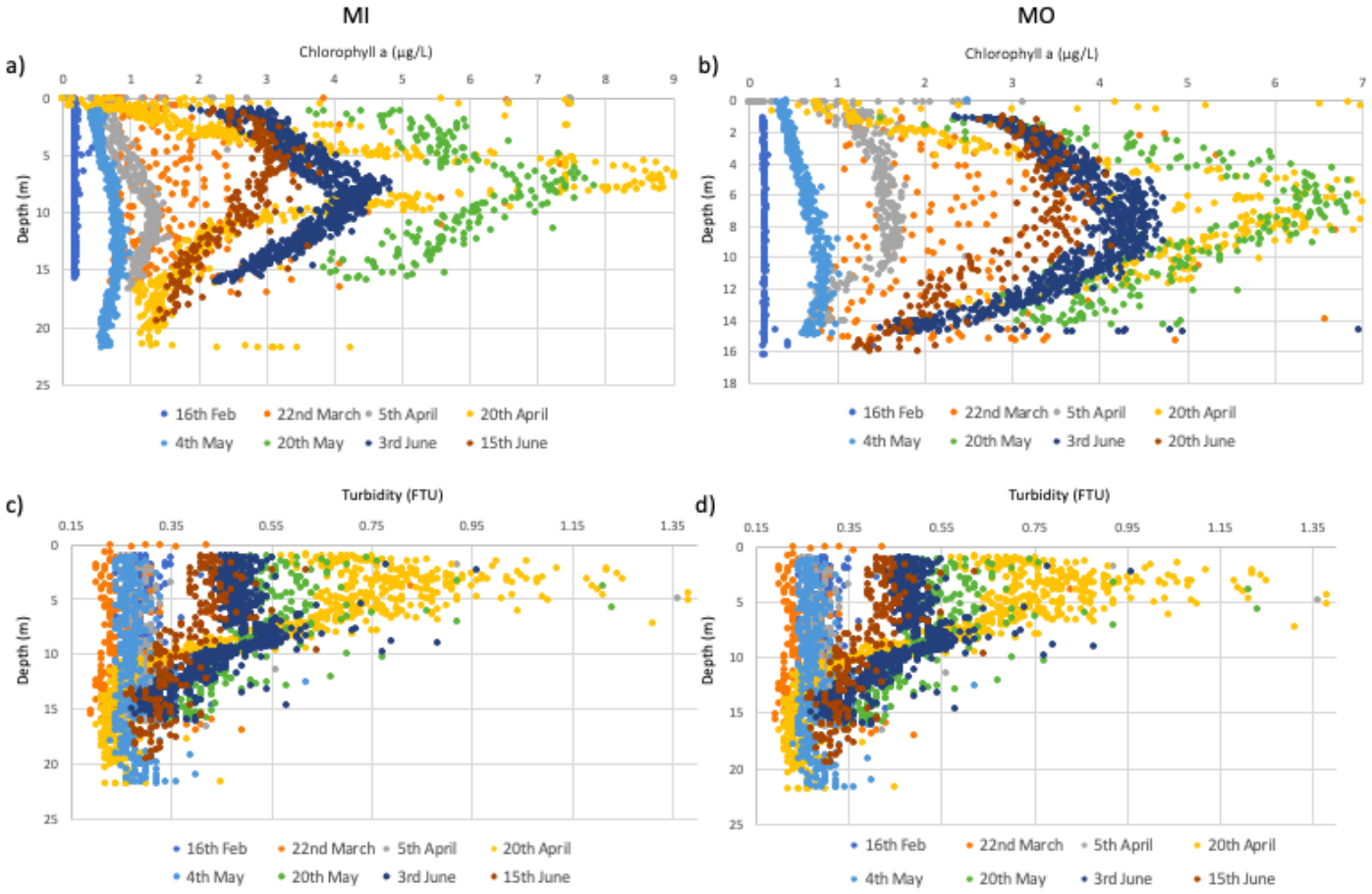

Figure 8- Vertical profiles of (a-b) chlorophyll (µg L^{-1}) and *(c-d) turbidity (FTU) at the two stations: MI (inside the farm- left) MO (outside the farm-right).*

Average wind speed was strongest and most variable in March, reaching up 20 ms^{-1}, with some variability in April, and less variability towards the summer months (Fig. 9a). Overall, seawater temperature increased over the sampling period from approximately 5°C in late February to 11°C in mid-June (Fig.9b). Seawater temperatures remained constant in March and April, averaging at approximately 5°C, and slowly increased reaching the first peak in mid-April (ca. 8°C). Temperatures then dipped slightly before rising at a consistent rate towards the summer months. Chlorophyll a concentration (Fig.9c) exhibited a similar pattern,

with low values (<1.5 mg m^{-3}) in March and April, except for a small peak ~2 mg m^{-3} in late March. Chlorophyll a values began to steadily increase in early to mid-April before plateauing in late April (up to 4 mg m^{-3}) and then subsequently steadily decreasing to the beginning of May and bouncing up and down from mid-May to mid-June.

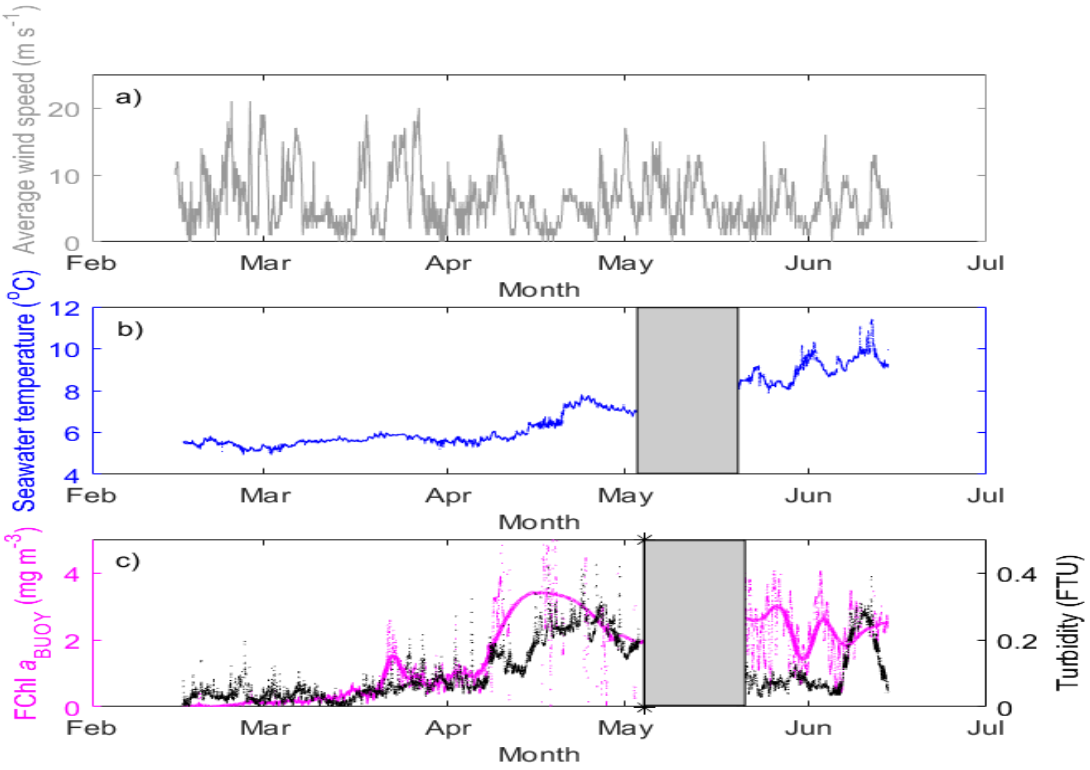

Figure 9- Time series data showing a) average wind speed (m s^{-1}) collected from Sula weather station (Fig. 1a), b) seawater temperatures (°C) and c) chlorophyll a (mg m^{3}) and turbidity data collected every 10-min using the C3 submersible fluorometer. Data from early to mid-May was not recorded due to the malfunctioning of the C3 instrument (as shown by grey boxes).

3.2 Nutrients

Nutrient concentrations (Phosphate, Nitrate, Silicate, Ammonium) generally decreased over the sampling period, except during 4th of May, when they increased, followed by a sharp decrease on the 20th of April (Fig.10). In general, the station MO had higher nitrate concentrations towards the beginning of the sampling period than MO. Noticeably ammonium concentrations (graphs g and h) were the highest on the 22nd of March, at 1.48 and 1.88 µm (average, mean) for the MO and MI station, respectively.

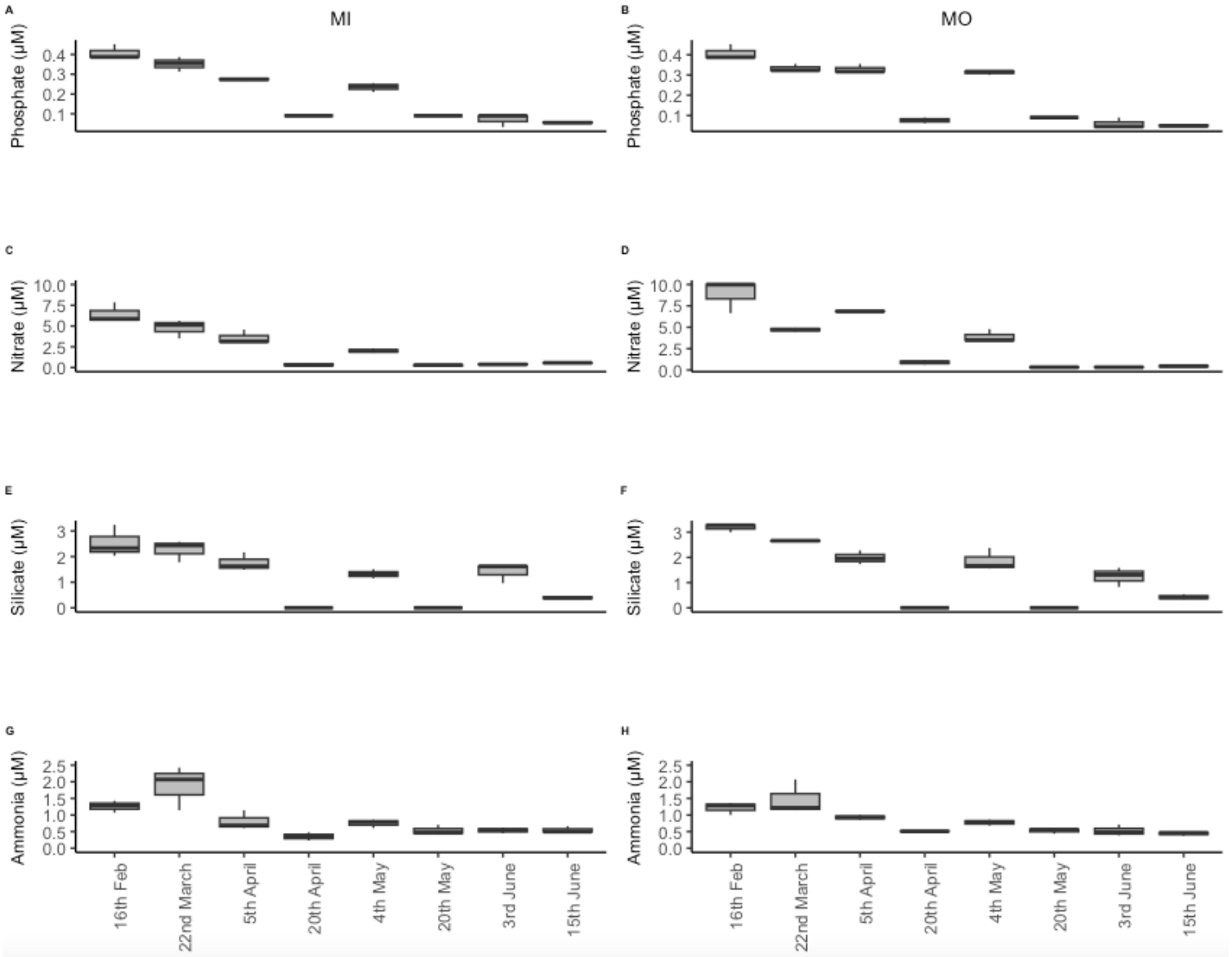

Figure 10- Nutrient concentrations (µM) over time: (a-b) Phosphate, (c-d) nitrate, (e-f) silicate and (g-h) ammonium. Results for MI are displayed on the left and MO are shown on the right.

3.3 Phytoplankton community

A similar pattern of phytoplankton abundance was found at each station, with a slightly higher abundance found at the MI station that MO (Fig.11a and 11b.) Relatively high abundance on the 16th of February was observed, followed by a dip, and then reaching the maximum abundance on the 20th of April (10883354 cells/L) and (816040 cells/L) for the MI and MO station respectively. There was then a sharp drop in abundance, on the 4th of May before a sharp increase on the 20th of May, followed by a fall in the June months.

The phytoplankton samples also showed similar patterns in community composition, with diatoms, dinoflagellates, and other flagellates (a group made up of chrysophyceae, cryptophycea, raphidophycecea and pyramimonas spp.) being the most dominant groups (Fig. 11c and 5d). Diatoms were most dominant on the 22nd of March, 20th of April and the 20th of May in the inside station. However, prymnesiophytes were the most dominant instead of diatoms on the 22nd of March at the MO station. The composition of dinoflagellates showed similar patterns, taking up a significant percent of the composition on the 16th of February, 5th of April, 4th of May and to a slightly lesser extent the 3rd and 15th of June. Other flagellates were most dominant out of other classes on the 5th of April (with >50% composition, for MI and MO), and their abundance peaked on the 3rd of June with a relative abundance of around 75% for the MI and MO stations.

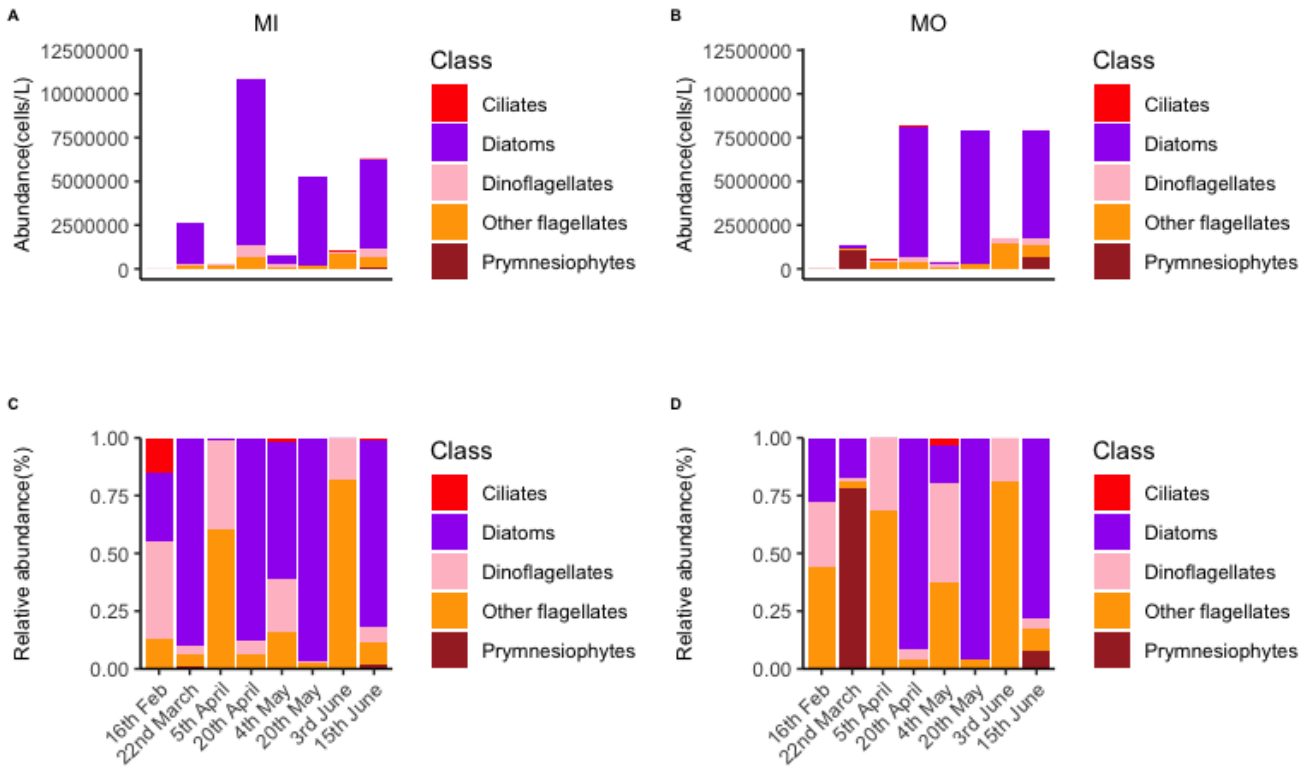

Figure 11- Phytoplankton community data, with (A-B) showing the total abundance (cells/L) and (C-D) showing the proportion of each class (%). Results for MI and shown on the left and for MO on the right. The "other flagellates" category includes the classes: chrysophyceae, raphidophycecea, cryptophycea and pyramimonas spp.

3.4- Bryozoan larvae abundance

There was a general increase in *M. membranacea* concentrations over the sampling period and higher concentration in MI than MO (Fig. 12). The lowest concentration of *M. membranacea* was 3 ind.m^{-3} for the MO and MI station, respectively on the 16th of February and the highest concentration on the 3rd of June with (MO/MI) 219 ind.m^{-3} and 266 ind.m^{-3}. *E. pilosa*, however, was a lot less abundant in the water column, comparatively, and showed a very different trend. The highest concentrations of *E. pilosa* were found on the 5th of April, at concentrations of 83 and 96 ind.m^{-3} for MO and MI stations, respectively, which noticeably coincided with the first peak of *M. membranacea* abundance (Fig.12).

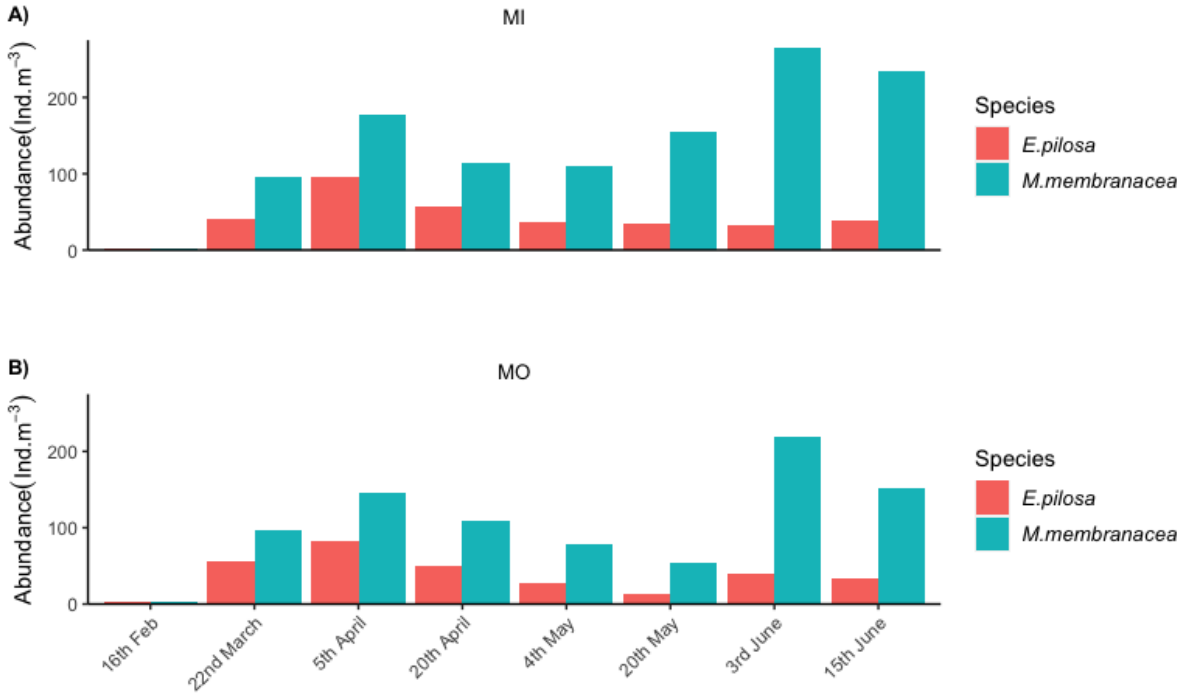

Figure 12- Bryozoan larva abundance for each species over the sampling period, with MO (outside station) and MI (inside station).

The two bryozoan species grew at a steady rate, with characteristics such as the apex or the "mouth" shape and the general body shape becoming more distinguished around the 5th of April and becoming most pronounced during the summer months. *M.membranacea* in its final stages is overall larger, and has a more triangular shape, with a more beak-like mouth whereas *E. pilosa* is smaller, has a bell-shaped body, and a blunter apex/mouth (Fig.13). The first settlement of bryozoans was recorded on the 20th of May, with a colony of just a few cm, by the 3rd of June the colonies had grown to several cm and on the 15th multiple colonies were observed reaching up to 10cm (Fig.14). Noticeably a much higher proportion of *M. membranacea* colonies compared to *E. pilosa* colonies were observed.

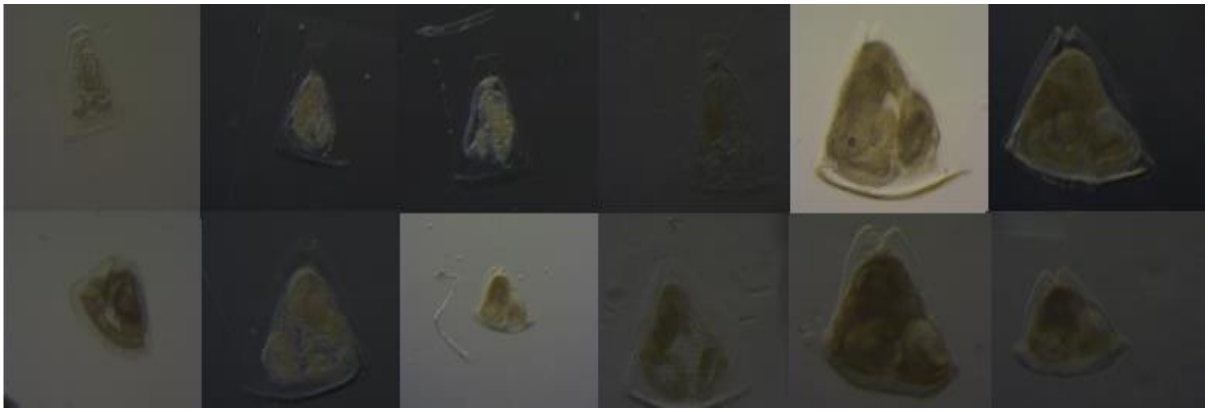

Figure 13- Bryozoan growth and development through the sampling period, with M. membranacea development showed on the top palette and E. pilosa development shown on the bottom palette.

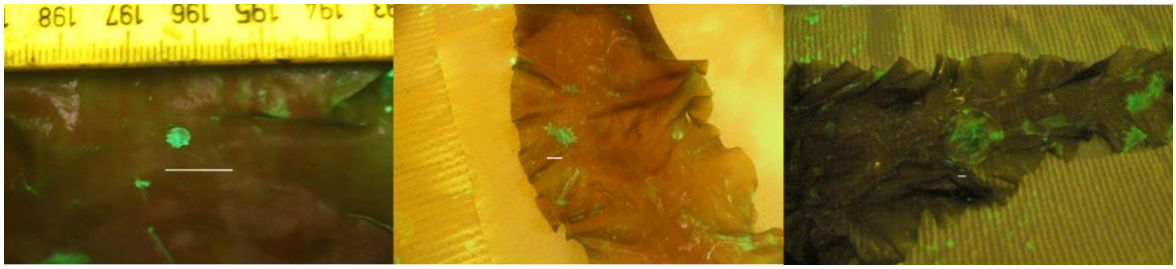

Figure 14 - Development of bryozoan colonies throughout the sampling period, with a) a single colony of a few mm on the 20th of May, b) larger colony of few cm on the 3rd of June and c) multiple large colonies (up to 10cm) on the 15th of June. White line under the colony refers to a scale-bar of 1cm.

3.5 Statistical analysis

A positive correlation was found between the abundance of *M. membranacea*, and chlorophyll a concentration, while no significant relationship was found for *E. pilosa* (Fig.15) in both stations. However, the correlation was found to be much stronger at MI compared to MO. The correlation between *M. membranacea* and chlorophyll a inside the seaweed farm was 0.34 (r^2 value) and 0.0025 for *E. pilosa* (Fig.15a). Whereas the correlation between *M. membranacea* and chlorophyll a concentration outside the seaweed farm was 0.58 and 0.0038 for *E. pilosa.*

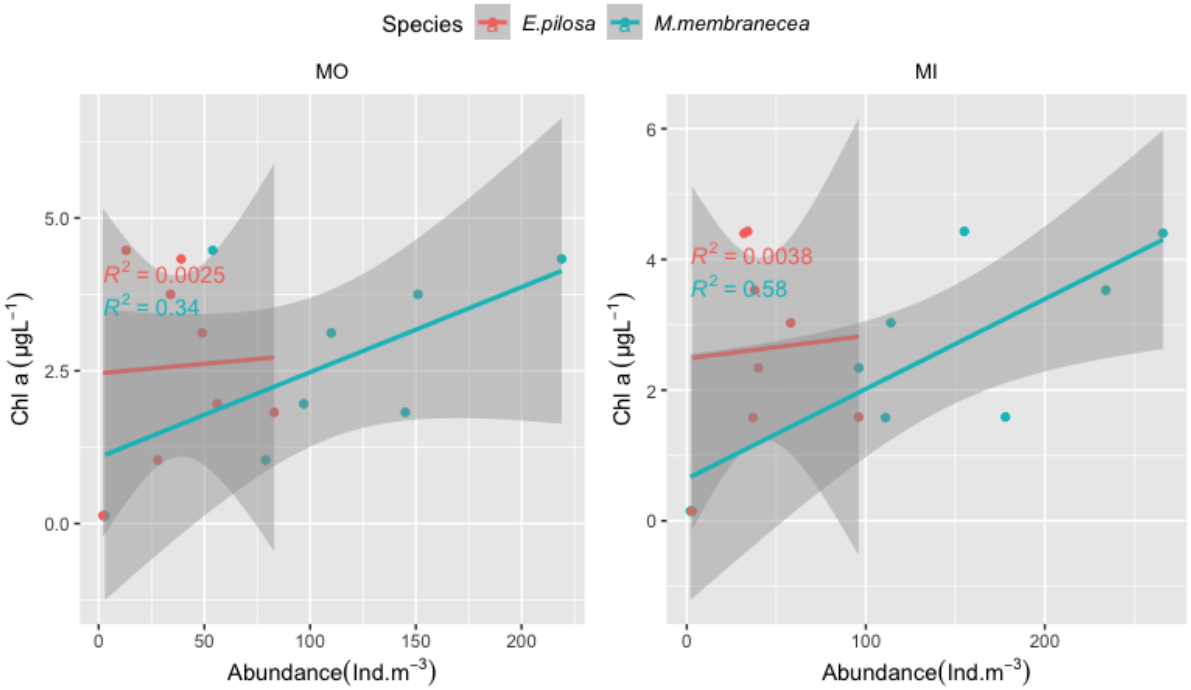

Figure 15- The correlation between abundance and chlorophyll a concentration, with trends and r values shown.

A positive correlation was also found between *M. membranacea* abundance and flagellates, including dinoflagellates, chrysophyceae, raphidophycecea, cryptophycea, and pyramimonas spp. No significant relationship was found for *E. pilosa*, for both stations (Fig.16). Conversely, the correlation was found to be stronger at MO than MI. The correlation between *M. membranacea* and the concentration of other flagellates inside the seaweed farm was 0.32 (r^2 value) and 0.0035 for *E. pilosa* (Fig 16a). Whereas the correlation between *M. membranacea* and chlorophyll a concentration outside the seaweed farm was 0.44 and 0.0055 for *E. pilosa.* And combined the correlation between bryozoan abundance and other flagellates, for both stations was 0.57 (r^2).

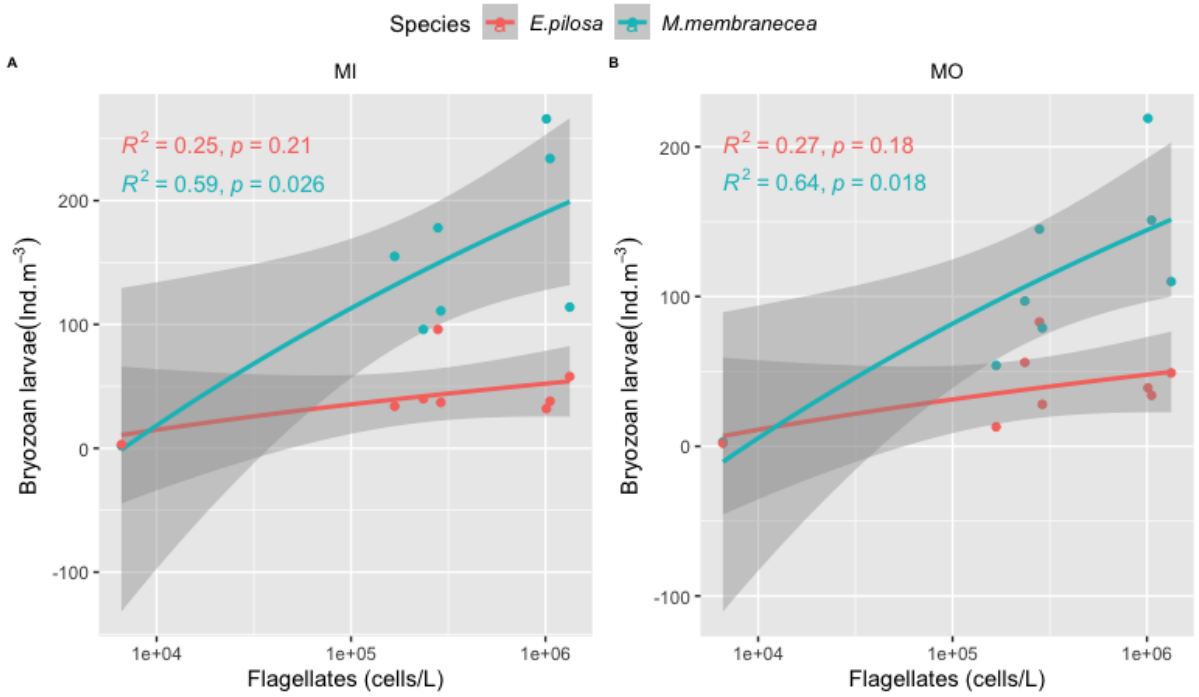

Figure 16- Correlations between Bryozoan abundance (ind.m^{-3}) and abundance of flagellates (log transformed) comprising of the groups: dinoflagellates, chrysophyceae, raphidophycecea, cryptophycea, and pyramimonas (cells/L), with MI and MO shown left and right, respectively.

3.6 Fv/Fm Data

The length of the kelp lamina steadily increased over time, as expected, with the fastest growth from 4th of May until 3rd June (Fig.17). The average kelp length on the 3rd of June was 111.2cm and 104cm on the 15th of June. The health state (Fv/Fm) varied a lot among the specimens, however, in general, a slight decrease over time (from 20th of April), particularly in older tissues was observed (Fig.18a). The Fv/Fm of older tissues was found to have a negative relationship with length (r^2= 0.21), while not significant for new (r^2 = 0.024) and mid-tissue (r^2 = 0.077) (Fig.17b).

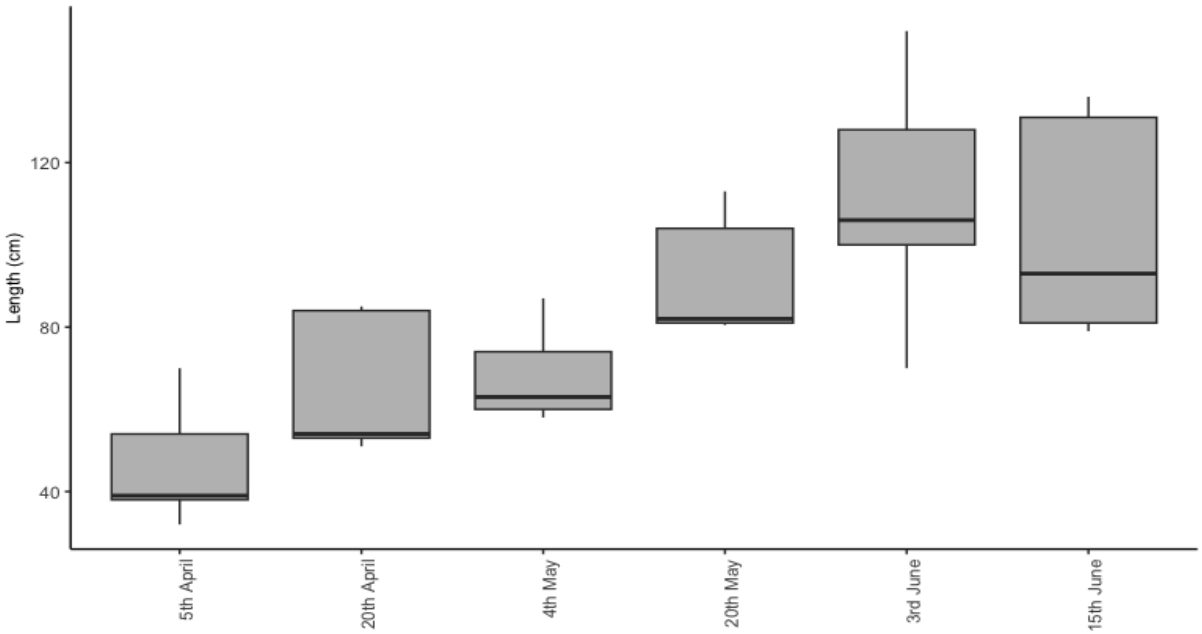

Figure 17- Length of S. latissima lamina from the 5th of April to the 15th of June

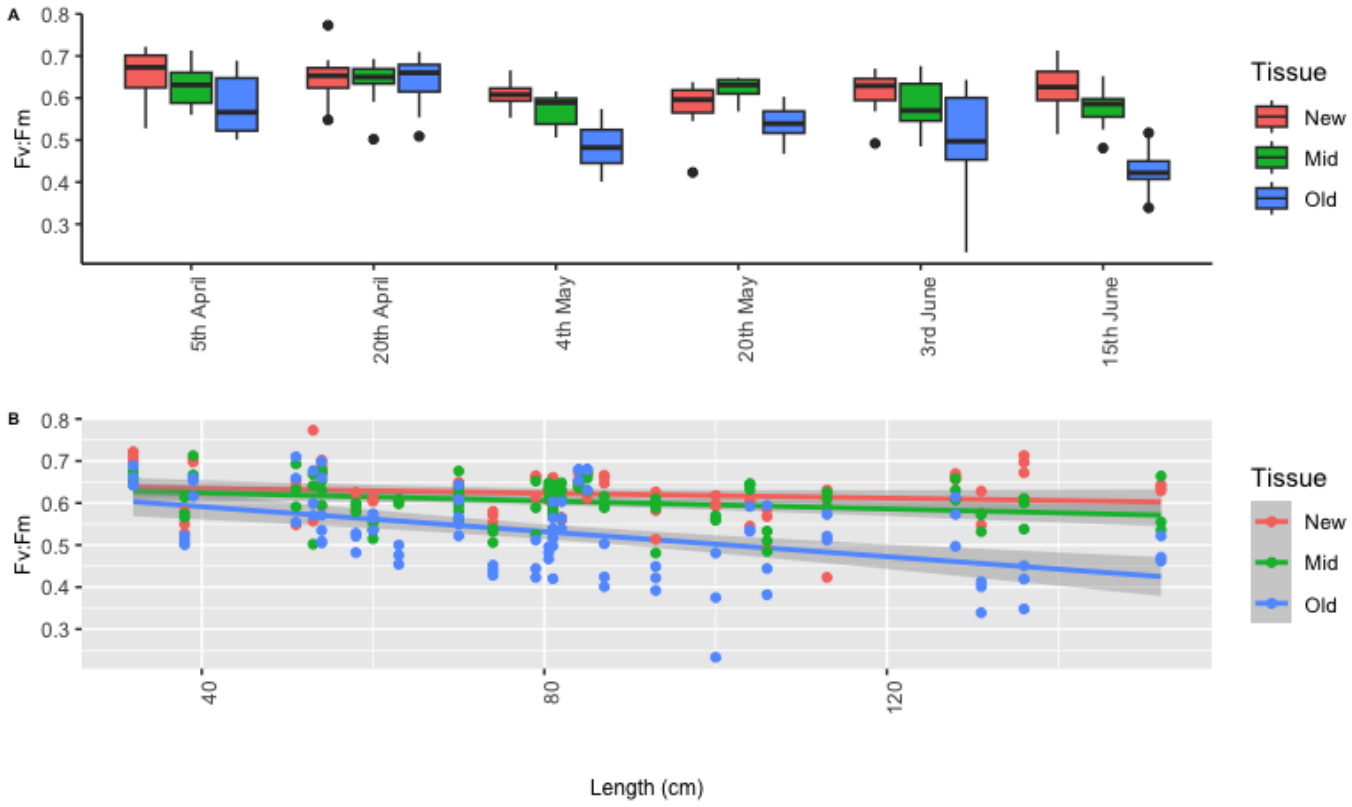

Figure 18- Health state of S. latissima, with A) changes throughout the sampling period and B) correlation of fv/fm with length

4. Discussion

4.1 Biofouling

Membranipora membranacea larvae were much more abundant in its larval stage throughout the entire sampling period and had a greater presence of colonies on the *S. latissima* compared to *E. pilosa*. In agreement, in a similar study undertaken at Frøya, *M. membranacea* larvae were found to be the most abundant Bryozoa species, with an average of 49 ind.m^{-3} (SD±19.68) compared to 29 ind.m^{-3} (SD±14.38) of *E. pilosa* larvae (Førde, 2014). Similarly, to the results from (Førde, 2014) who found the highest abundance of both species in late June, the highest abundance of both species in the present study was found in early June with the second largest abundance in late June.

In addition, Førde (2014), observed that *M. membranacea* accounted for 97.1% of the coverage on *S. latissima* samples (combined) whereas *E. pilosa* only accounted for 2.9% of the coverage. In agreement, *M. membranacea* is more selective when it comes to choosing a suitable substrate, and *S. latissima* has been demonstrated to be the preferred material to settle on, whereas *E. pilosa* is less selective and has been known to settle on rocks and shells as well as algae (Ryland, 1962; Førde, 2014). This could explain why less *E. pilosa* larvae and a lesser extent of biofouling by *E. pilosa*, compared to *M. membranacea*, was observed throughout the study. In addition, it has been noted that *E. pilosa* is typically outcompeted by *M. membranacea* (Yorke and Metaxas, 2011; Førde, 2014) when it comes to the colony cover and size (Yorke and Metaxas, 2011).

4.2 Larvae abundance and settlement

Generally, bryozoan larvae abundance was found to be higher in the inside station (MI) than the outside station (MO), even early during the sampling season, indicating that there are possible factors that attract them to the kelp species. One possible explanation could be that the kelp emits a chemical cue (Seed and O'Connor, 1981). However, *M. membranacea* colonies have been observed to "overwinter" meaning that a few colonies can survive on kelp

substrates over the winter period, being a potential source for recruitment during the consecutive spring and summer period. Moreover, with warmer winters there is an increased survival rate of the colonies. Wild kelp species which may occur on the seabed of the seaweed farm or on the frame or supports of the farm, could be an additional possible source of bryozoan larvae which may account for the slightly higher larval abundance in the seaweed farm overall as well as earlier in the season.

A positive correlation between larvae abundance and biofouling was observed, most notably in June, where the two highest abundances (3rd and 15th of June) of both bryozoan species and largest coverage on kelp was noted. The rapid growth of the larvae and development of colonies has been found to be strongly linked with temperature, and rapid growth in the colonies or zooids, has been documented at 12 °C and 18 °C compared to lower temperatures such as 6 °C which showed low growth (Menon, 1972; Førde, 2014). This is in line with the present study, which shows that higher temperatures were observed in June (>8°C) suggesting that it is a key factor in larval development, growth, settlement, and colony development.

However, in the beginning of April larval abundance reached its first peak, but no fouling was observed. This can be attributed to low temperatures, which were still relatively low (~6°C) before the longest peak of the spring bloom which arrived on the 20th of April. Furthermore, microscopic observations of the two bryozoan species showed that they were undeveloped in April. Moreover, it has been found that *M. membranacea* larvae may remain in the plankton community for weeks or months prior to settling (Ryland and Stebbing, 1971). The first colony was observed in May, suggesting that perhaps the larvae were still too immature to settle and develop colonies. Likewise, other studies on the settlement on *S. latissima* from Frøya have noted the first settlement occurring in May or June (Njåstad, 2018).

4.3 Hydrography and the phytoplankton community

Density gradients caused by temperature and salinity are known to contribute towards the initiation of the spring bloom as well as the distribution of larvae such as bryozoans (Saunders and Metaxas, 2010). The peak of the spring bloom in the present study was found

to occur on the 20th of April, indicated by a prominent increase in chlorophyll a in the surface.

A notable increase in temperature as well as the development of thermohaline stratification was observed in April, which likely played a key role in the initiation of the spring bloom. Enhanced stratification and food availability during the spring bloom possibly created ideal conditions for the hatching and development of bryozoan larvae observed in this and other studies (Saunders and Metaxas, 2010). A shallow thermocline, means that phytoplankton is confined within a shallow mixing layer depth, with more access to light (Huisman and Sommeijer, 2002). Additionally, increased irridance in the spring gives optimum conditions for rapid phytoplankton growth (Rumyantseva *et al.,* 2019). A drop in wind speeds were also noted, confirming that a breakdown of winds is an important factor in the development and stabilisation of thermoclines (Rumyantseva *et al.,* 2019).

Bryozoan abundances correlated positively with chlorophyll a and phytoplankton flagellate concentrations, particularly for *M. membranacea* in areas within the seaweed farm. Chlorophyll a concentrations or food availability has been found to be an important factor in the growth of bryozoan larvae populations and colonies. For instance, studies have found that 100% of zooids in *E. pilosa* were feeding when phytoplankton concentrations were between 0.5 to 5 µg chl a/L (Riisgård and Goldson, 1997) and that *M. membranacea* and *E. pilosa* colonies doubled in area within 5-6 days when food concentrations were adequate (Hermansen, Larsen and Riisg, 2001). In the present study, a high percentage of colony coverage was found in June where chlorophyll concentrations were typically >2 ug chl a/L.

In addition, the species composition and size distribution of phytoplankton was found to be important in the growth and settlement of bryozoan larvae. It has been suggested that mouth size of the bryozoans is a limiting factor in their growth as it determines what food they are able to eat (Winston, 1978) and hence community composition and size distributions of phytoplankton are limiting factors.

Membranipora sp. have a relatively wide mouth with a mean diameter of ~28 µm whereas *Electra* sp. have a much smaller mouth around 17 µm (Winston, 1978). This means that *Membranipora sp*. can ingest much larger particles and can account for why it had a stronger correlation with chlorophyll a concentration compared to *E. pilosa*. The first peak of

bryozoan abundance unexpectedly did not overlap with the spring bloom, however, a high proportion of diatoms were found at both stations. Diatoms are relatively large phytoplankton particles, and some species may have been too big to eat. In addition, a high proportion of "other flagellates" typically consisting of smaller-sized phytoplankton (<20 µm) could be observed on the 5th of April, which overlapped with the first peak in bryozoan abundance.

A high proportion of other flagellates could also be observed in June, which is when the highest bryozoan larvae abundances were recorded. In agreement, it was found that bryozoan larvae abundance had a (combined) positive relationship with the abundance of flagellates (r^2 = 0.57). This suggests that although food availability is an important factor in larvae growth and settlement, size distribution of the phytoplankton or food, is an important consideration.

4.4 Nutrients

Nutrient concentrations (ammonium, nitrate, silicate, and phosphate) were high earlier in the sampling period and before the spring bloom and remained low during June. In the MI station, fast growth of *S. latissima* was recorded from the 4th of May to 3rd of June. This corresponded with low ambient nutrient concentrations, showing that concentrations naturally decreased as the macroalgae started growing. A sharp drop in nutrient concentrations was observed on the 20th of April, which corresponded with the peak of the spring bloom, following high uptake by phytoplankton (Jevne, Forbord and Olsen, 2020); (Ibrahim *et al.*, 2014). An increase in colony coverage on the lamina of kelp a month after the spring bloom was noted, which could suggest that nutrient stress on the kelp may play a role in the susceptibility for bryozoan colony development.

However, it has been shown that *S.latissima* can store nutrients internally when concentrations are high during the winter and utilize them later in the year, when external nutrient concentrations are low (Matsson *et al.*, 2021). Meaning that ambient nutrient concentrations may not strongly affect biofouling due to internal stores of nutrients such as nitrogen. Concentrations of environmental dissolved inorganic nitrogen (E-DIN) have been found to be important in shedding rates of *S. latissima*. Through shedding, *S. latissima*, can actively remove epibionts, by discarding surface tissue on the lamina (Matsson *et al.*, 2021). When E-Din concentrations increase, even more shedding activity can be employed removing a greater number of epiphytes.

4.5 Kelp physiology

The health state of kelp remained consistent in young tissues, with only a slight decrease when nutrients became depleted after the spring bloom, while the largest differences occurred in old tissues of the lamina (tip). This was found to correlate with length, meaning that the longer the lamina, the older the tissue from the tip is, and the more stressed the tissue is. These large variations in the fv/fm values for the different tissue types may be attributed towards the productions of compounds such as phlorotannins, which are compounds in kelp that can help protect against UV damage and pathogens including epiphytes (Van Alstyne *et al.*, 1999)

Concentrations of these compounds have been documented to not only differ among species but also among tissue types. According to the Optimal defence theory, meristematic and reproductive tissue will have a higher allocation of these compounds due to higher fitness of the tissue types (Van Alstyne *et al.*, 1999). Higher abundance of these compounds in the newer tissue types means these tissues are more protected against UV radiation and may account for the higher fv/fm values in these tissue types.

Algal photosynthetic performance has been documented to be negatively affected by presence of epiphytes or biofouling (Muñoz, Cancino and Molina, 1991). This means that the fv/fm value may decrease due to settlement of bryozoan colonies. However, there may be a link between low fv/fm values or health state of the kelp due to suboptimal abiotic factors such as salinity, irradiance, temperature and nutrient stress and biofouling. Nutrient stress, specifically low concentrations of E-DIN have been known to reduce shedding rates in *S.latissima* (Matsson *et al.*, 2021) meaning that a low fv/fm value caused by nutrient stress could increase biofouling load by impairing its ability to actively remove epiphytes by shedding.

5. Conclusion

Biofouling in seaweed aquaculture is a huge problem and encrustation of bryozoans can lead to a partial loss or whole loss of the product under stormy conditions. Additionally, the colonies serve as natural competitors for nutrients and sunlight, reducing growth and thus profitability of the product. Temperature, food availability, hydrography and health state of the kelp were found to be important factors in promoting larval growth and subsequent encrustation, with many of the factors having a cascading effect on each other. The results of the study suggest that *S. latissima* should be harvested in early to mid-May, to avoid large amounts of biofouling.

However, since temperature is a key factor in the growth and settlement of bryozoans, the impacts of climate change/global warming and subsequent increases in temperature are important to take into consideration. Climate change not only means a change in temperature but the physio-chemical properties of our oceans, with changes in ocean currents, frequency and intensity of storms, salinity, and thus overall distribution of plankton species (Saunders, Metaxas and Filgueira, 2010). Higher temperatures may mean that harvesting should occur earlier in the season since biofouling by bryozoans is higher earlier in the season due to higher temperatures throughout the whole growing season.

In the future a more robust study with more focus on the development of colonies, during the whole growth season of *S. latissima* would be beneficial. A larger focus on intracellular nutrient concentrations rather than environmental nutrient concentrations would be more beneficial in determining the role of nutrients in both the growth of *S. latissima* and onset of biofouling, due to the ability of this species to store nutrients and thereby becoming less reliant on ambient nutrient concentrations.

6. References

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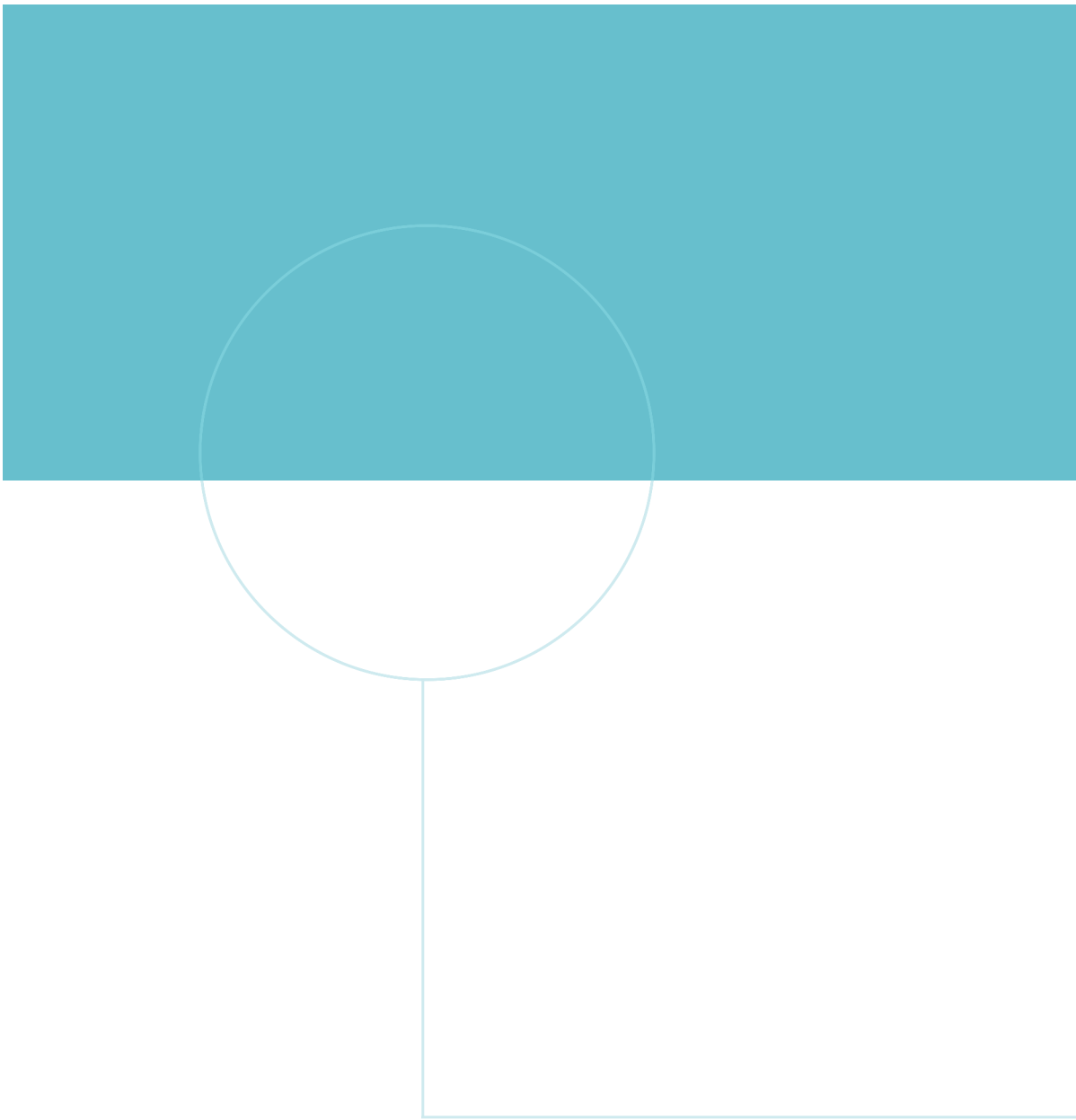

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