Master's thesis

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

June Valla and Ylva E. H. Rydningen

An assessment of critical steps in cultivating *Palmaria palmata* in Norwegian waters.

Master's thesis in MSc Ocean Resources
Supervisor: Kjell Inge Reitan
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Ylva E. H. Rydningen and June Valla

Sammendrag

Makroalger som et bærekraftig alternativ har de siste tiårene fått økt oppmerksomhet da de har vist stort potensiale med mange bruksområder. Rødalgen *Palmaria palmata* er en av artene som har fått økt fokus grunnet dens mange gode egenskaper. Sammenlignet med andre makroalger som har vært kultivert i en årrekke, er kultiveringen av *P. palmata* i et tidlig stadium. Metoden for å kultivere arten er ikke tilstrekkelig god nok for en effektiv produksjon i storskala. Noen punkter i metoden som trenger oppdatert forskning for en mer suksessfull kultivering er 1) produksjonen av fertilt vev for å øke tilgangen til sporebærende sori utenom den fertile sesongen, 2) utførelsen av sporeslipp, 3) metode for å spire levedyktige kimplanter for videre vekst i sjø, og 4) egnede substrater for videre vekst i sjø. Disse fire punktene var hovedfokuset i denne masteroppgaven med et overordnet mål om å forbedre kritiske steg i kultiveringsmetoden for *P. palmata*.

Eksperimentene som ble utført viste at fertilitet kan induseres i *P. palmata* utenom det som er den fertile sesongen her i Norge. Et lysregime som simulerte korte dager, resulterte i et høyere antall fertile vev sammenlignet med tilsvarende forsøk med lange dager. Videre forsøk viste *P. palmata* sin evne til å utføre sporeslipp fra samme sori flere ganger etter hverandre. I forsøket ble en etablert protokoll for å utføre sporeslipp benyttet. For å undersøke hvordan hviletid mellom sporeslipprundene påvirket sori, ble to ulike hviletider testet; to og syv dager. I forsøket med to dagers hviletid ble protokollen for sporeslipp repetert fem ganger, og for syv dager ble den repetert tre ganger. Alle rundene resulterte i sporeslipp. I samme forsøk ble forskjellige lysregimer undersøkt i hviletiden. Et lysregime som simulerte korte dager i hviletiden på syv dager resulterte i høyest sporetetthet i sporeslipp, samtidig som det resulterte i nesten like høy sporetetthet i hviletiden. Med et lysregime som simulerte lange dager i hviletiden på syv dager, ble flere sporer sluppet i hviletiden sammenlignet med sporeslipprundene. Resultatene viste at en hviletid på to dager, med et lysregime som simulerte korte dager i hviletiden, virket å være en anbefalt metode. Fremgangsmåten bidro til et høyt uttak av sporer fra samme blad, samtidig som sporeslipp i hviletiden var minimalt.

To ulike metoder for å utvikle kimplanter på laboratoriet ble testet for å sammenligne veksten frem til utsett i sjø; fritt flytende i boblekultur og direkte sådde substrater. Metoden med å utvikle kimplanter i boblekulturer virket å stimulere til høyre vekst sammenlignet med kimplantene som var blitt direkte sådd på ulike substrater. I metoden hvor kimplantene vokste i boblekulturer ble tre ulike behandlinger testet for å optimalisere metoden. Resultatet fra dette tydet på at en behandling med lavt lys og lavt næringsinnhold var bedre for vekst sammenlignet med en behandling med økt lys eller økt næringsinnhold.

For å undersøke hvilke typer substrater som egnet seg best for å kultivere *P. palmata* videre i sjø, ble kimplantene fra boblekulturene og de direkte sådde substratene satt ut i et sjøanlegg. De direkte sådde substratene besto av to ulike nett i tillegg til tråder surret på tau, og boblekulturene var limt på algetau før utsett. Basert på veksten i sjøanlegget, antydet resultatene til at nett var det foretrukne substratet når *P. palmata* ble direkte sådd. Veksten på algetauene og trådene ble ikke vellykket. Generelt var det mye vekst av andre organismer på alle substratene, og kultiveringen ble ikke regnet som suksessfullt totalt sett. Resultatene underbygger hvor viktig det er med høy sporetetthet og kimplante-tetthet på substratene i vekstfasen på laboratoriet før de settes ut for videre vekst i sjø.

Abstract

The use of macroalgae as a sustainable biomass for multiple applications has gained an increased interest the last decades. The red algae *Palmaria palmata* is one of the species in focus due to its vast characteristics. Compared to other macroalgae that have been cultivated for decades, cultivation of *P. palmata* is in an infant stage with underdeveloped cultivation techniques, inefficient for large-scale production. For successful cultivation, further knowledge regarding the following steps is essential 1) production of fertile tissue to provide spore-containing sori out of its main fertility season, 2) conditions and execution of spore release, 3) the conditions and method for the nursing stage to grow robust seedlings for deployment, and 4) the best suited substrates for cultivation at an open-sea farm. These four steps were the focus of this master's thesis, with the overall aim to improve critical steps in the cultivation process of *P. palmata.*

An important finding in the present study was a demonstration of induction of fertility outside the fertility season of *P. palmata* in Norway. Results revealed that a manipulation of light regime to resemble short day conditions secured the highest number of fronds induced with sori, compared to long day conditions. The same study revealed a possibility of repeated spore release from the same fronds. For maturing, two different recovery times between the repeated spore release rounds was tested: two and seven days. For two days recovery time, sori could release spores up to five times when repeating the spore release protocol. With seven days recovery time, the same fronds could release spores up to three times. In the same experiment, different light regimes were tested during recovery time. The light regime simulating short day conditions during seven days recovery time seemed to have superior effect on spore density in spore release. However, a considerable number of spores were released during recovery time as well. During seven days recovery time and a light regime simulating long days, higher number of spores were released during recovery time compared to the three days of spore release. Results from this experiment indicate that two days recovery with short day conditions for maturing of the fronds seems to be a recommended method. This approach enhanced the spore density retrieved from the same sori and kept the spore release during recovery time to a minimum.

Two different methods for the nursing stage were tested to compare growth of seedlings ready for deployment at an open-sea farm: free floating in bubble cultures and directly seeded substrates. By comparing the two different methods, results revealed that the method of nursing seedlings of *P. palmata* in bubble cultures seemed to give a higher growth rate compared to seedlings directly seeded on substrates. In the method for nursing seedlings in bubble cultures, three different treatments were tested to optimise the conditions for growth. Nursing seedlings in bubble cultures treated with low light and low nutrient content, resulted in a higher growth rate compared to seedlings nursed with high light intensity or high nutrient treatments.

To test which substrates are best suited for *P. palmata* at an open-sea farm, the substrates from nursing stage were deployed together with seedlings from bubble cultures glued to algae ropes. Based on growth at the open-sea farm, the present study suggests using nets as substrates when seeding *P. palmata* directly on substrates. Growth on algae ropes and entwined ropes were not successful. Overall, the substrates were densely covered by fouling and the cultivation trial was regarded as unsuccessful. The results amplify the importance of high spore density in the nursing stage before deployment at an open-sea farm.

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Definitions

Diploid	Twice the basic (i.e., haploid) numbers of chromosomes (2n)
Haploid	Single set of chromosomes (n)
Sporophyte	Diploid phase in the life cycle of macroalgae and plants, spores for reproduction (2n)
Gametophyte	Haploid phase in the life cycle of macroalgae and plants (n)
Tetrasporophyte	Diploid phase of the life history of many red algae. Produces tetraspores for reproduction
Sorus, sori	Cluster of spore-containing structures (sporangia) found in algae, fungi, and lichens. The spores that are produced are haploid meiospores

Abbreviations

N40	40 mm mesh thread nets
N150	150 mm mesh thread nets
SD	Short day (8h light and 16h darkness)
LD	Long day (14h light and 16h darkness)
SR	Repeated spore release round
R	Recovery time
SE	Standard error

1 Introduction

1.1 Brief introduction to macroalgae

Macroalgae are multicellular plant-like organisms that usually live attached on hard substrata in coastal areas. They are essential components of temperate to polar coastal ecosystems, contributing to production, biodiversity, and functioning (Barbier et al., 2019). In coastal zones many functions as structuring species which modify the environment by changing light, hydrodynamics, and sedimentation rates. Macroalgae supports complex food webs, and provide ecosystems services such as habitats, food, and reproductive refugia that offers shelter to a variety of organisms (Barbier et al., 2019).

Worldwide there is recorded about 10,000 different species of macroalgae, of which 6,500 are red algae (Rhodophyta), 2,000 are brown algae (Phaeophyceae), and 1,500 are green algae (Clorophyta and Charophytes) (Barbier et al., 2019). These three divisions are phylogenetically diverse reflecting distinctive evolutionary histories, different life cycles and they display specific ultrastructural and biochemical features. Their global distribution and adaption to various environments is also distinctive.

1.2 Global production and applications of macroalgae

Macroalgae cultivation takes place in about 50 countries. China, Philippines, Indonesia, and South Korea are the leading producing countries, where species such as *Eucheuma*, *Laminaria japonica*, *Gracilaria* and *Undaria pinnatifida* are mainly cultivated (Smith et al., 2018). Around the world, it is estimated that around 221 different species of macroalgae have a commercial value (FAO, 2018; Hudek et al., 2014). Macroalgae cultivation provides low trophic and nutrient extractive source of biomass and is globally the fastest growing aquaculture sector, where the production of macroalgae has more than tripled, up from 10.6 million tonnes in 2000 to 32.4 million tonnes in 2018 (FAO, 2020). Globally, macroalgae production has grown yearly at 8% since 2014, and is undergoing an expansion.

The use of macroalgae dates way back, where throughout history, coastal people have collected macroalgae and used it as fertilizer in horticulture and agriculture. Where macroalgae in Asia traditionally has been used for thousands of years, being of great importance of the total food intake (Tiwari & Troy, 2015). There is also a long tradition for macroalgae consumption in European countries like Ireland, Iceland, and Norway (Araújo et al., 2021). Iceland is the country where the use of macroalgae has been documented the most showing it has been used since the 700's, then as an exchange commodity (Kristjánsson, 1980). Ireland used macroalgae in various ways, such as medicine, food, and chewing tobacco (Mouritsen et al., 2013). Historically, the use of macroalgae in Norway is known for likely serving as provisions on long expeditions conducted by Norwegian Vikings (Coyer et al., 2006).

Today, the global macroalgae industry is worth more than USD 6 billion per annum, and of this, 85% comprises food products for human consumption (Bourgougnon et al., 2021). Almost 40% of the world's hydrocolloid market in term of foods are made up by macroalgae-derived extracts such as carrageenan, agar, and alginates (Liao et al., 2021).

Macroalgae is widely used in many industries and contributes considerably to nutritional status of communities due to its rich composition of both macronutrients (e.g., sodium, calcium, and phosphorus), micronutrients (e.g., iodine, iron, and zinc) and vitamins (B12, A, K) (Rajapakse & Kim, 2011; Shannon & Abu-Ghannam, 2019). World Health Organization has enlightened that iodine deficiency is the most prevalent and easily preventable cause in the world of impaired cognitive development in children (FAO, 2020). Even small quantities of macroalgae will meet the daily adult requirement of 150 µg/day iodine. In addition to well-known applications, macroalgae farming is gaining increasing attention to be promoted and monitored for climate and environmentally friendly bioeconomy development.

The red macroalgae *Palmaria palmata* (*P. palmata*), is one if the species that historically has been important in the Nordic countries. The nutritious composition, appearance, colour, taste, and characteristics has attracted interest among different researchers, companies and consumers based in the Northern Europe (Werner & Dring, 2011a). Although the primary uses of *P. palmata* has been as a supplement of food source, it is known for other uses such as animal feed, skin care products and as a resource for biofuel production (Jard et al., 2013; Mouritsen et al., 2013). *P. palmata* is a promising candidate for cultivation due to its promising characteristics, but as of today there is no commercial culture of *P. palmata* in Europe (Grote, 2019). Due to increased demands, a standard and improved cultivation technique is needed to offer dependable harvests in terms of biomass, quality, and food safety.

1.3 Characteristics of *P. palmata*

P. palmata is used for human consumption due to its richness in vitamins and high protein content compared to other macroalgae (Table 1). Further, it consists of essential amino acids and has a great benefit by being rich in omega-3 fatty acids - eicosapentaenoic acid (EPA) (Fleurence et al., 2018; Harnedy & FitzGerald, 2011; Peñalver et al., 2020). It also consists of important dietary fibres, in addition to trace elements and minerals, such as potassium, phosphorus, calcium, and sodium (Fleurence et al., 2018). Phycobiliproteins, is a protein-pigment complex and is one of the most important groups of proteins in *P. palmata* as it has interesting antioxidant properties and contains pigments that can be extracted. By this, it may be useful in the prevention of various diseases (Cian et al., 2015; Hamed et al., 2015). All of this contributes to *P. palmata* being characterized as a promising dietary supplement for both humans and salmon feed (Schmedes et al., 2019).

With all promising areas of use, *P. palmata* also has some natural substances that can be isolated to provide a great variety of biotechnological applications. Of these, mycosporine-like amino acids (MAAs) are becoming promising due to their UV screen properties and potential antioxidant activities. MAAs can serve as passive sunscreen that absorb UV wavelength protecting the cells against DNA damage, photoinhibition of photosynthesis and inhibition of growth (Lalegerie et al., 2020). This is unique to red macroalgae making *P. palmata* of particular interest if MAAs could be used in commercial applications (Yuan et al., 2009). With all its characteristics, there is an increased demand of *P. palmata* which raises the interest of cultivating the species (Werner & Dring, 2011a).

Table 1: Chemical composition per weight of *P. palmata* (Morrissey et al., 2001).

Protein	12-21%
Fat	0.7 – 3%
Carbohydrates	46 – 50%
Vitamin C	150 – 280 ppm
Beta-Carotene	663 i.u.
Vitamin B1	7 ppm
Vitamin B2	2 – 5 ppm
Vitamin B3	2 – 19 ppm
Vitamin B6	9 ppm
Vitamin B12	6.6 ppb
Vitamin E	1.71 ppm
Calcium	2000 – 8000 ppm
Iodine	150 – 550 ppm
Iron	56- 350 ppm
Magnesium	0.2 – 0.5%
Manganese	10 – 155%
Sodium	0.8 – 3%
Zinc	3 ppm

1.4 Biology and life cycle of *P. palmata*

P. palmata is a relatively small, intertidal, or shallow subtidal, red macroalgae in the division Rhodophyta. The size of the fronds can grow to a length of 50 cm and a width of 3-8 cm, with a flat surface and a leathery consistency, in a purple or brownish-red colour. In the wild, its normally found in cold and turbulent areas with rocky shores. From a round stable disc, it grows on rocks or as an epiphyte of other large macroalgae (Mouritsen et al., 2013). In Norway, a study conducted on their reproductive season revealed that the species had a peak in fertility from December to April (Bøe, 2019). Although, fertile tissue can be found outside the peak season in October/November and late April/May.

The life cycle of *P. palmata* is diplohaplontic, consisting of two phases: a diploid tetrasporophyte phase (2n) and a haploid gametophyte phase (n) (Fig.1). These two phases are isomorphic and can only be distinguished at ripe fertility. The diploid phase release tetraspores that are both male and female haploid gametophytes (Le Gall et al., 2004). The gametophyte part of the life cycle differs from other algae because the male and female phase have different morphology and longevity.

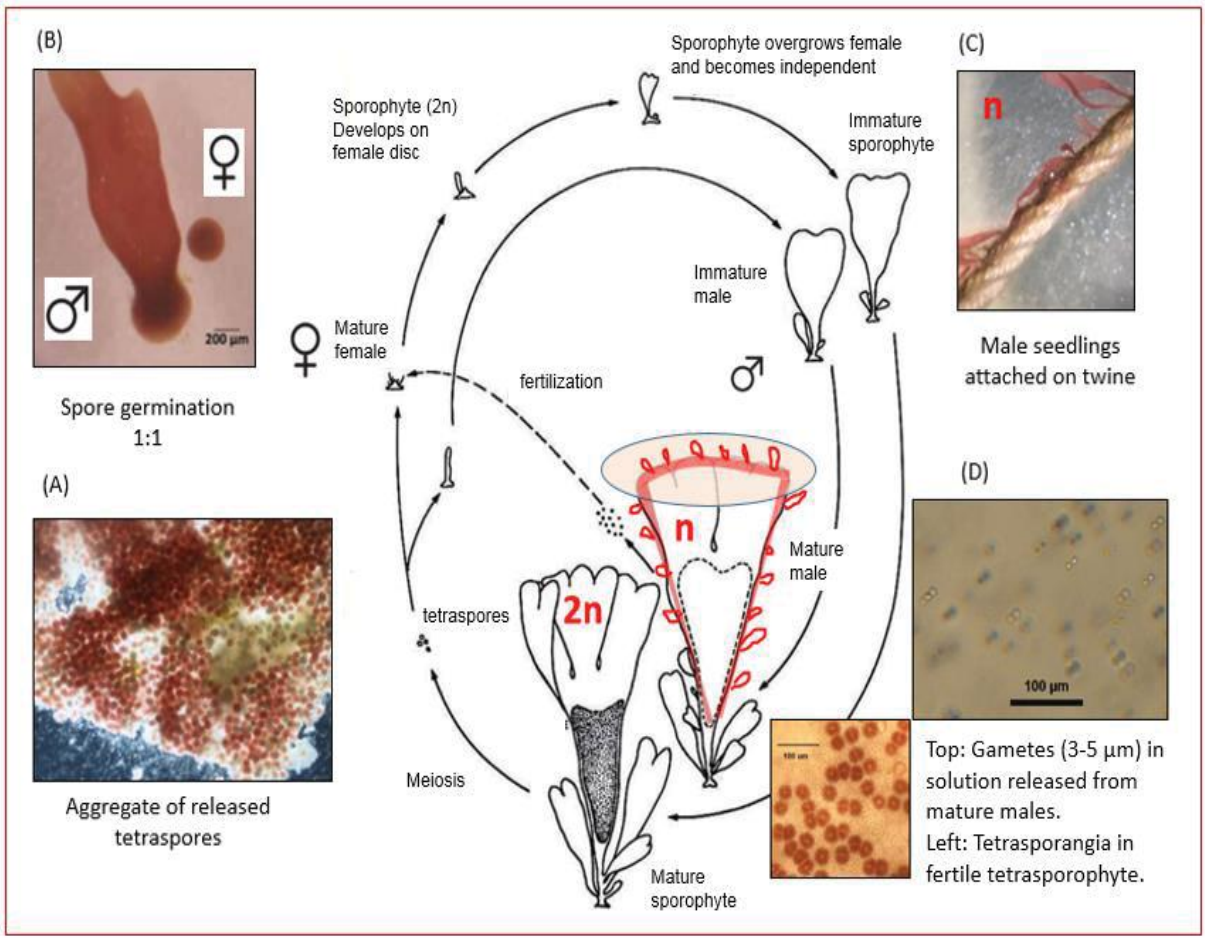

Figure 1: Presentation of the diplohaplontic life cycle of *P. palmata* with its isomorphic phases, diploid tetrasporophyte (2n) and the haploid male gametophyte (n). Fertile tissue is indicated as dark-shaded tetrasporangial basal sori (2n) and as a dashed line (n). **A**) Aggregate of released tetraspores. **B**) After release, spores germinate into male and female gametophytes. **C**) Male seedlings attached to a twine. **D**) Top: solution containing male gametes. Left: fertile tetrasporophyte. Figure made by Schmedes (2020).

The male gametophyte phase (n) is indistinguishable from the diploid tetrasporophyte phase (2n) at an immature stage, before reproduction starts after about one year. The female gametophyte develops into microscopic crustose thalli, 0.1 mm in diameter. Consequently, female gametophytes can only be observed in the laboratory (Werner & Dring, 2011a). Since it takes about a year before the male gametophyte becomes reproductive, the female gametophyte will be fertilized by gametes released by the male gametophyte the previous year (Le Gall et al., 2004). If the eggs from the female gametophyte are not fertilised within a few days, they die (Werner & Dring, 2011a). If they are fertilised, a tetrasporophyte grows directly on the female gametophyte from a zygote, which is retained in the gametophyte. The tetrasporophyte will shortly overgrow the female gametophyte. After a year of growth, the tetrasporophytes become reproductive and develop tetrasporangial sori on the fronds. This is observed as dark red, irregularly, and slightly elevated tissue areas, and contains spores clustered together as packs of four (Fig.2) (Werner & Dring, 2011a).

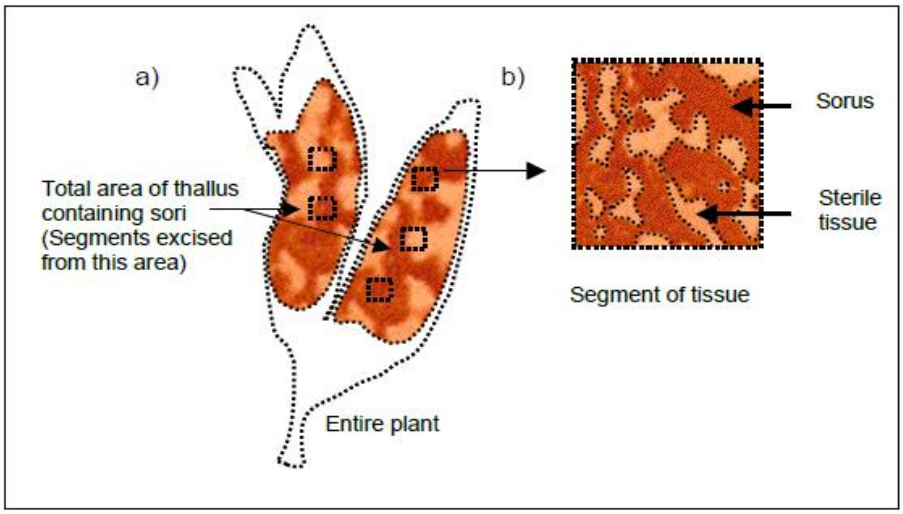

Figure 2: Tetrasporangial sori on the fronds, shown as dark red, irregularly shaped, and slightly elevated tissue area. **a**) Shows thallus containing sori, **b**) shows segment of tissue. Figure made by Schmedes (2020).

After the release of spores, the non-motile spores settle onto a substrate and develop into male and female gametophytes in a 1:1 ratio (Le Gall et al., 2004; Werner & Dring, 2011a). Released spores are covered in mucilage made of proteins and carbohydrates, which remains as a surrounding layer after spore release (Boney, 1975, 1978; Oza, 1975). Due to its sticky composition the mucilage is involved in the initial substrate attachment (Schmedes, 2020).

1.5 Cultivation of *P. palmata*

Around 99% of the exploitation of macroalgae in Europe is based on harvested wild stocks (Pang & Lüning, 2006; Schmedes, 2020). Cultivation trials of *P. palmata* began when pressure on the wild populations increased. For a sustainable production and to avoid diminishment of wild populations, it is important to focus on cultivation rather than wild harvest (Schmedes, 2020). Cultivation of macroalgae can be divided into two major stages (Forbord, 2020). First, a nursing stage providing necessary conditions for development of microscopic gametophytes through their sexual phase, and further, subsequent development of small sporophytes suitable for deployment at sea. Second, a sea-farming stage that involves cultivation of deployed juvenile sporophytes at sea, until they reach a size and chemical composition suitable for the end-products. The research activities aiming the use of and cultivation of *P. palmata* has been in focus the last two decades as its nutritional content and potential is valued, but no cost-effective and optimal cultivation method has yet been established (Schmedes, 2020).

1.5.1 Cultivation technology

The current cultivation method for *P. palmata* is a spore-based method with a land-based hatchery phase prior to cultivation at sea (Schmedes, 2020). From the released spores, the male gametophytes are the desired form to cultivate as they will grow into seedlings, while female gametophytes die if fertilization is not a part of the protocol (Browne, 2003). Mainly, the hatchery phase involves three steps (Schmedes, 2020): 1) collection of mature gametophytes or induction of sori in immature *P. palmata*, 2) release and dispersal of spores, and 3) spore attachment and growth. As these three steps are interlinked, the success and efficiency of all steps determine the value of a given hatchery protocol (Schmedes & Nielsen, 2020b). Together, all steps depend on the access to *P. palmata* in its tetrasporophytic phase, the mature male gametophyte.

Currently, the most common hatchery protocol for producing seeded substrates, is by using flat horizontal tanks with a 1:1 areal coverage of seeding-substrates, together with sori for a three-day spore release duration (Schmedes & Nielsen, 2020b). As ripeness of the sori varies even within the tissue sections, the technique represents a highly unpredictable method and one of the main challenges: the protocol requires a high input of fertile fronds, gives poor dispersal and relative low yield as there is a high spore mortality (60-90%), with reasons unknow (Schmedes, 2020; Werner & Dring, 2011a). As described in section regarding biology and life cycle, half of the tetraspores germinate into male gametophytes, whereas the other half (female gametophytes) remain microscopic or die off as fertilization is not a step in the current method. Altogether, this plays a role in the hatchery protocol having low seeding efficiency and contributes to the high mortality of spores.

Back in 2006, Pang and Lüning (2006) conducted a study that included spore release in darkness. The study indicated that darkness throughout spore release triggered spore release from *P. palmata* to be more efficient. Released spores are non-motile and have in addition a sticky surface due to mucilage, making them clump together when they settle. Schmedes and Nielsen (2020b) stated in their research that the current method for spore release often leads to a considerable number of spores settling on the tank surfaces instead of the seeding-substrates. This results in seeding-substrates with a highly variable spore density and can cause low quality seedlings for deployment for growth out at sea. In the work of optimizing the hatchery phase, Schmedes and Nielsen (2020b) demonstrated that agitation during the seeding phase and spore release had some positive effects on the density of released spores. This effect is likely due to agitation mediates a physical disturbance at the interface between water and sori, enhancing sori wall rupture and hence increase the spore release rate (Pueschel, 1979). Thus, agitation might be important for a faster spore release and even survival (Schmedes & Nielsen, 2020b). Agitation might also increase evenness of settled spores as it simulates the conditions in the lower intertidal and sublittoral habitat where *P. palmata* lives, with moderate exposure and strong currents.

The last part of the land-based hatchery phase is growth of seedlings on seeded substrates. This period can be referred to as the nursing stage and is where the seeded substrates are stored in larger holding tanks for about 1-5 months to grow into seedlings (Werner & Dring, 2011a). Earlier transfer to larger holding tanks with frequent water renewal maintains a higher spore survival. In the work of reducing negative effects of fouling organisms in the sea phase, research has shown that the recommended size of the deployed seedling of *P. palmata* should preferably be 0.5-1 cm (Werner & Dring, 2011b). Throughout the whole

hatchery phase, from collection to nursing stage, factors such as different light qualities, different daylength, agitation, high and low nutrient conditions, and temperatures can be manipulated in order to optimize the protocol (Grote, 2019).

An overall problem regarding cultivation of macroalgae is general difficulties associated to diseases and pests, a problem also highly relevant for *P. palmata* (Kim et al., 2017). There are three main challenges: 1) competition of space, often by fouling organisms; 2) epiphytes and endophytes growth; 3) pathogen attacks, such as viruses and bacteria (Fletcher, 1995). For laboratory studies, macroalgae is collected in nature, bringing natural epiphytic flora and fauna along with them (Borowitzka et al., 2007). These organisms have the potential to multiply and compete with the desired species stock, and potentially lead to a collapse. In order to reduce the contaminants, treatments with chemical disinfectant can be helpful (Kerrison et al., 2016). Early life stages of macroalgae are exposed to contaminants such as microalgae and diatoms (de Almeida, 2017). In this life stage, germanium dioxide (GeO_2) inhibits diatom growth by interfering with the frustule of the diatom formation (Shea & Chopin, 2007). This makes GeO_2 suitable for use in algae laboratory cultures, in order to inhibit growth of contaminating diatoms.

Cultivation at sea is linked to the hatchery phase, as it is based on the seedlings grown in the nursing stage. Due to low hatchery efficiency, the production at sea is currently insufficient for feasible production (Werner & Dring, 2011a). Studies conducted by Kim et al. (2017) and later Schmedes (2020), emphasized the importance of deployment timing, seedling size and density, cultivation depth, cultivation site, optimal deployment, and configuration for maximizing the production. All this together emphasises the importance of local farming experience to maximise the production. Unwanted growth of epiphytes on the cultivated biomass at open-sea facilities is one of the main challenges (Pang & Lüning, 2004). Presence of epibionts affects the productivity of the algae and decreases the commercial value of the end-product. Both abiotic and biotic factors influence the presence of epibionts (Walls et al., 2017). Control of the epiphytes are difficult to maintain when grown in non-uni-algae environment. Biofouling organisms, like other algae, has free access to the surface of the macroalgae when cultivated in open-sea farm. To promote growth of the macroalgae, the cultivation takes place at depths with large amounts of natural light which also promotes growth of algae epiphytes. However, by growing the algae in high densities on substrates, control over the epiphytes can be achieved (Pang & Lüning, 2004).

Hight growth rate after deployment is paramount for feasible cultivation to obtain successful yield (Schmedes, 2020). Cultivation at sea could be done in several dimensions using a suspended longline as the supporting construction (Fig.3) with different substrate types and deployment configurations. Depending on the right choice of substrate types and deployment configurations that functions well with the local water body type and light transmissions, open-sea farming holds the potential for high-area specific productivity (Schmedes, 2020).

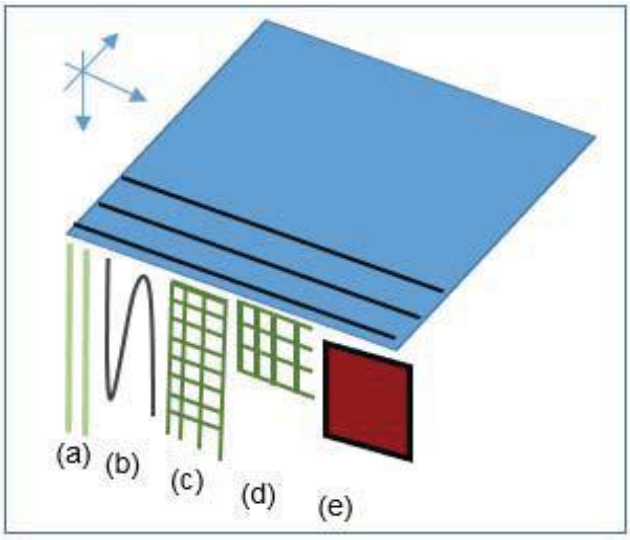

Figure 3: Deployment configuration of substrates using a longline (black line), here shown as a three-dimensional farming. **a**) Single vertical dropper line, **b**) continuous loops, **c**) vertical net, **d**) horizontal net, **e**) solid textile sheet. Picture made by Schmedes (2020)

Studies has shown that depth-dependent biomass growth influences the biomass yield on the deployed substrates and varies within the single deployed substrates (Schmedes, 2020). In the work of optimizing cultivation strategies at sea, research has shown that the use of substrates with more surface area such as thick ropes, nets, textile ribbons and textile sheets provide an increased cost-effective production at sea.

1.5.2 Site selection and environmental factors

Growth, survival, and reproduction is all controlled by a complex set of abiotic and biotic factors (Lobban & Harrison, 1994; Lüning, 1991). Of these factors, particularly light, temperature and salinity are of major importance (Werner & Dring, 2011a). The selection of a site optimal for growth of *P. palmata* is important for successful aquaculture.

In temperate regions, such as Norway, the growth of wild and cultured macroalgae follows a seasonal pattern: a rapid growth rate during spring and reduced or negative growth during autumn and winter. As autotrophic organisms, their life history is controlled by environmental factors such as daylength and temperature (Schmedes, 2020; Yuan et al., 2009). Light availability at sea is determinative for growth of macroalgae and is likely the most important abiotic factor as it is used to drive photosynthesis and generate energy for the alga (Dring, 1992; Gerard, 1988; Lobban & Harrison, 1994; Lüning, 1991). Light also impacts pigments and possibly the UV-absorbing MAAs found in *P. palmata* (Yuan et al., 2009).

Temperature influences the whole life-cycle due to its fundamental effect on chemical reaction rates (Lobban & Harrison, 1994; Yokoya et al., 1999). The optimal temperature for growth is species-dependent, but is often found to correlate with the temperature from the species habitat (Lobban & Harrison, 1994). As a cold-water species, it is found that the

optimal growth temperature is between 6-15℃ for *P. palmata* (Edwards & Dring, 2011). High water temperatures in summer and autumn are a limiting factor for growth and development. Minimum winter temperatures are not considered to be damaging, but sites with large fluctuations in temperature should be avoided for *P. palmata* (Werner & Dring, 2011a). The range of salinity *P. palmata* can be subjected to when cultivated is unknown, but research indicates that it may be adaptive based on the environment it originates from naturally (Schmedes & Nielsen, 2020c). The potential adaptation is supported by findings of other intertidal macroalgae species, that are found to be able to grow in salinities of 1-12‰ if cultivated at higher salinities, indicating a high salinity tolerance of intertidal species (Khfaji & Norton, 1979).

Another consideration regarding site location for *P. palmata* is a location where it is moderately exposed to wave action, but with a high-water current, as it is crucial for good and healthy growth (Werner & Dring, 2011a). High-water current facilitates good nutrient and CO_2 exchange across the fronds and is essential for keeping the fronds relatively clear from settlement of fouling organisms and sediments. Research has shown that currents with flow rates of 5-10 cm s^{-1} are considered useful for open-sea cultivation of *P. palmata*. Where the longlines preferably is placed in line with the main current flow (Werner & Dring, 2011a).

Research provides consensus that algae can be manipulated by environmental parameters such as: light exposure, salinity, temperature, and nutrient availability throughout its life cycle (Schmedes, 2020; Yuan et al., 2009). Thus, the optimum season for deployment of *P. palmata* seedlings at sea and the length of the cultivation period is so far not determined for the Norwegian coast.

1.6 Study objectives and approach

The overall objective of this master's thesis was to improve critical steps in the cultivation process of *P. palmata*. To achieve this, five strategies for improvement of the cultivation protocol were evaluated. These were grouped into A) hatchery and seedling production phase and B) cultivation in an open-sea farm phase.

The experimental work was structured into these two phases with following objectives:

A. Hatchery and seedling production phase:

1. Evaluate effects of manipulation of the light regime on induction of fertility outside the fertility season of *P. palmata.*

2. Evaluate if repeated spore release from the same fronds is possible, as well as how different recovery times between spore release impacts the spore release.

3. Evaluate how two different methods, pre-incubation method of directly seeded nets and ropes, and method with seedlings in bubble cultures, effects the development of seedlings and fouling organisms during the nursing stage.

B. Cultivation at an open-sea farm:

4. Compare the two different methods used in nursing stage, pre-incubation method of directly seeded nets and ropes and method with seedlings in bubble cultures, on the effect on cultivation success in an open-sea farm.

5. Evaluate how the different substrates, big-masked nets, small-masked nets, and ropes used in the pre-incubation method in the nursing stage, affects the growth of *P. palmata* at an open-sea farm.

1.7 Hypotheses

The research hypotheses of the work were:

H1.1	Fertility can be induced with manipulation of the light regime.
H2.1	Repeated spore release from the same individuals is possible after a period of maturation in the recovery tanks.
H2.2	The light regime 08:16 will lead to a higher spore density in the repeated spore release, compared to light regime 14:10.
H2.3	The light regime 08:16 will make fronds release spores during recovery time.
H3.1	Bubble cultures with enhanced nutrition will lead to fast growth of the seedlings.
H3.2	Bubble cultures with high light intensity will lead to fast growth of the seedlings.
H3.3	The seedlings in bubble cultures have a higher growth rate in the nursing stage compared to the pre-incubation method of directly seeded substrates.
H4.1	Algae ropes seeded with seedlings from bubble culture has a higher growth rate compared to pre-incubation method of directly seeded substrates.
H5.1	From the pre-incubation method, the directly seeded nets have a higher growth rate compared to directly seeded twines.

2 Material and Methods

2.1 Overview of the experimental work

Figure 4 shows an overview of the experimental work. The diagram is structured into two main phases of cultivating *P. palmata* with following five strategies to improve the protocol for cultivation. The light grey box A) indicates the spore-based hatchery phase for seedling production, and the dark grey coloured box B) indicates the open-sea phase.

In phase A) three different strategies were set up to improve the hatchery protocol. The first one was to induce fertility in immature *P. palmata* using two different light regimes that followed the frond throughout the whole experimental period. One of the light regimes was set to simulate short days (SD), and the other was set to simulate long days (LD). Fronds with induced fertility was used further in the second strategy: to test if it was possible to perform spore release from the same fronds repeatedly until the spore-containing sori was emptied. Between the rounds of repeated spore release, two different recovery times were tested in the two different light regimes that followed the fronds from the experiment of inducing fertility. This experiment was to study how recovery time for maturing the fronds would affect the protocol for spore release. The third strategy in phase A) was to improve the nursing of the seedlings, where two different methods for the nursing stage were tested to improve the development of seedlings: pre-incubation of directly seeded spores on big-masked nets, small-masked nets and entwined spools, and nursing of free-floating seedlings in bubble cultures holding three different conditions, "low light-low nutrient", "high light intensity" and "high nutrient enrichment".

In phase B) two different strategies were performed to improve the open-sea cultivation. The first one was to compare the effect of the two different nursing methods chosen in phase A), pre-incubation method and bubble cultures. The second strategy was to compare the three different substrates from the pre-incubated method, and how they performed at the open-sea farm.

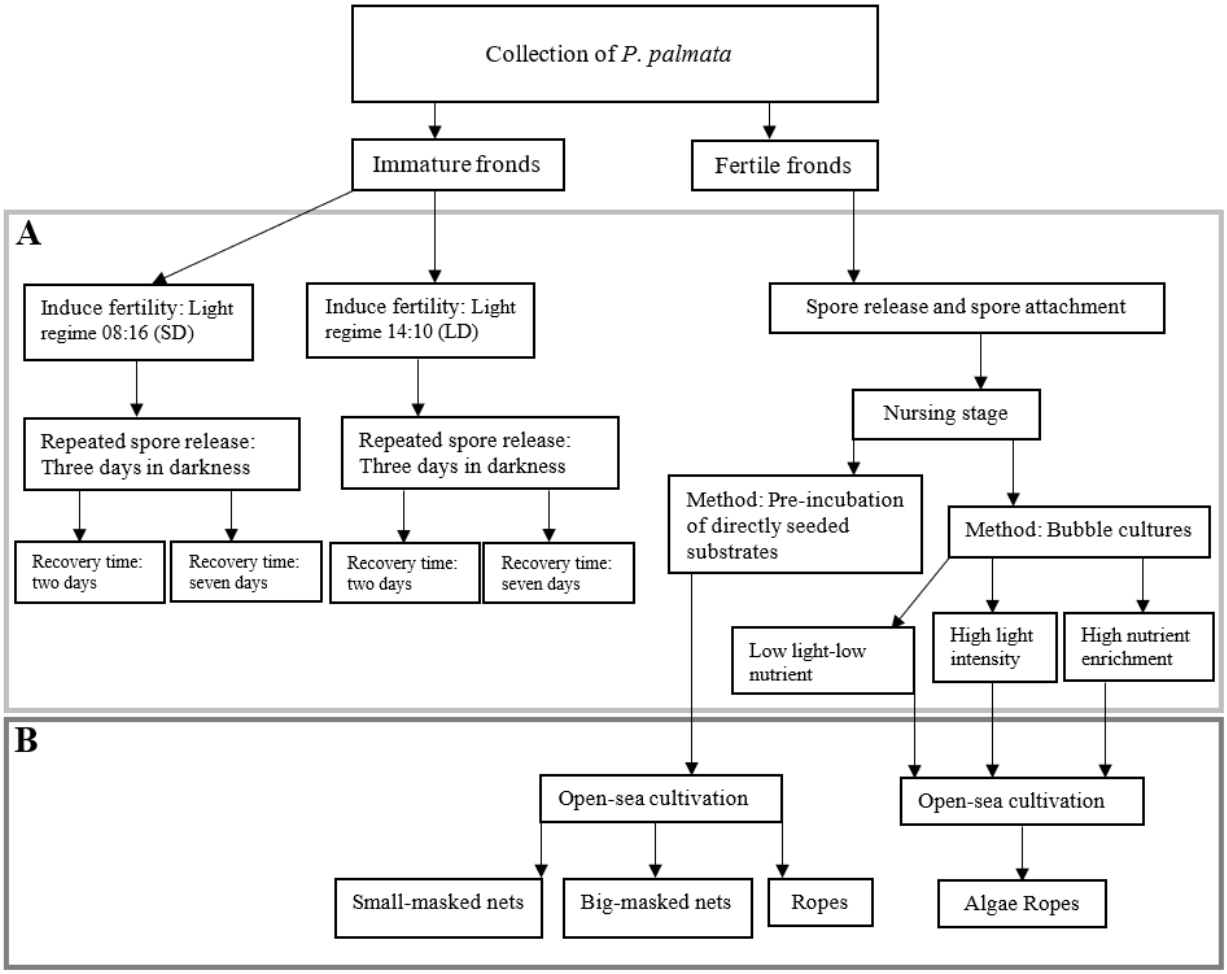

Figure 4: Diagram showing an overview of the experimental work. Phase **A**) shows the steps in the hatchery and seedling production phase. The experiments consist of three different strategies to improve the phase. From immature fronds two different light regimes were tested to induce fertility. Further, the induced ones were subjected to two different recovery times. From fertile fronds spore release was conducted to produce seedlings. These were grown by two different nursing strategies: pre-incubation of directly seeded substrates and in bubble cultures holding different condition throughout the experimental period. Phase **B**) takes the seedlings produced in phase A) out to an open-sea farm where the growth success was tested.

2.2 Collection of *P. palmata*

Fresh material of *P. palmata* was collected by hand on low tides (~60 cm) from February 2021 to November 2021 (Table 2). It was transported to the laboratory in plastic containers, where it was kept hydrated and cool by sea water. All collections were conducted at Korsvika in the Trondheim area (Fig.5).

Table 2: Date and location for collection of fresh material of *P. palmata*. Dates for dehydration, seeded on substrates, and removed are also listed.

Date	Location	Fertile status	Dehydration (24h)	Spore release	Removed
03.03.21	Korsvika	Mature	12. April	13. April	16. April
19.03.21	Korsvika	Mature	19. April	20. April	23. April
28.04.21	Korsvika	Mature	28. April	29. April	4. May
02.11.21	Korsvika	Immature	Repeatedly	Repeatedly	Repeatedly

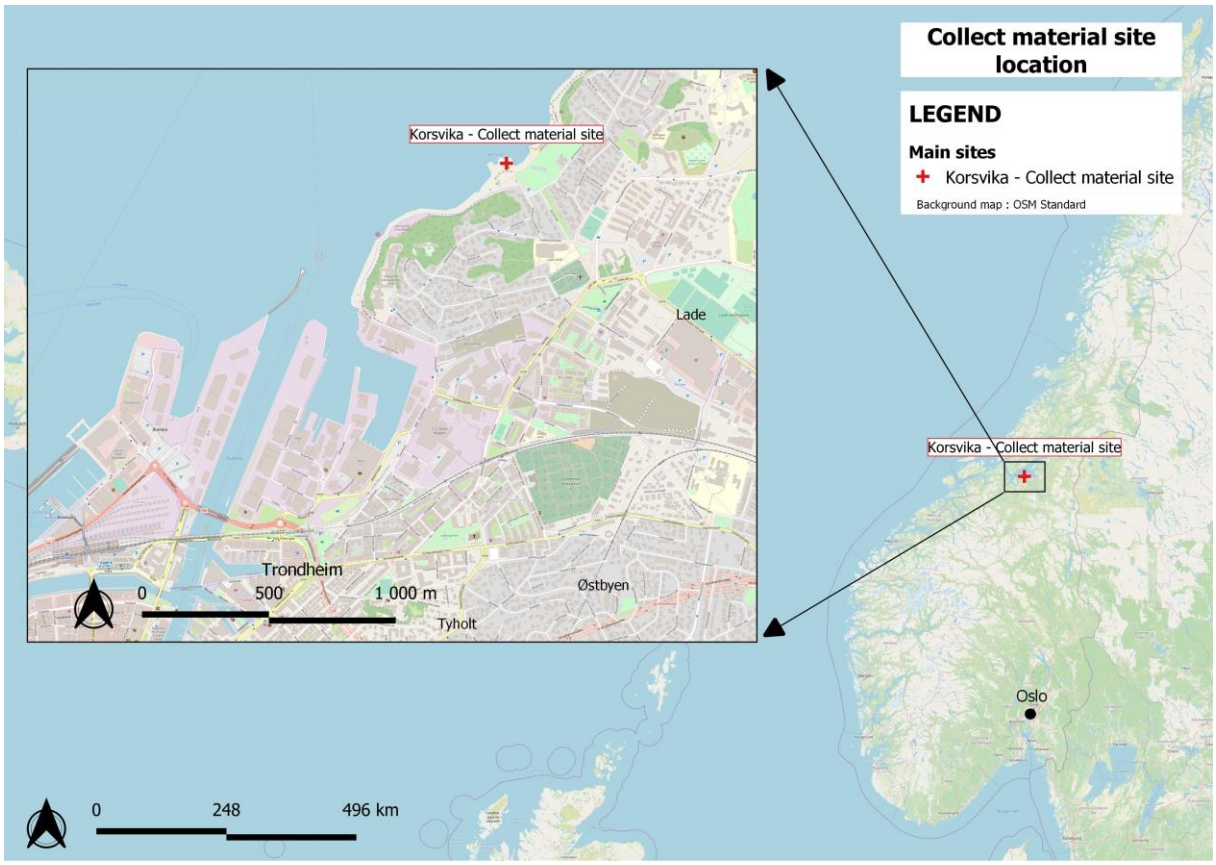

Figure 5: Material collection site in Korsvika, Trondheim (63.4305° N, 10.3951° E). Produced from QGIS application.

2.2.1 Processing of collected P. palmata

Fresh material was processed immediately after collection in a laboratory at 10℃ (± 1). Material was divided into four batches: fertile tetrasporophyte (2n), immature, fertile male gametophyte (n) and unfit. Maturity of the fronds was assessed by eye under bright light. Fronds were assessed by looking for dark red, irregularly, and slightly elevated tissue areas containing spores indicating fertile tetrasporophytes (Fig.6a,c). Fronds without irregularly and structured shaped areas were considered as not fertile. Fronds with lighter coloured, irregularly shaped areas were considered as fertile male gametophytes (n) containing gametes (Fig.6a, b). Fronds with rotten parts or growth of epibionts were categorized as unfit. The batches of fertile male gametophyte (n) and unfit fronds were thrown away.

The batches of fertile tetrasporophytes and immature *P. palmata* were stored in two separate plastic tanks (80 L) with running seawater (1.5 L min^{-1}) pumped up from 70 m depth in the Trondheimsfjord, at 10℃ (± 1). The seawater was both sand and particle filtrated, and UV light treated. The climate room (10℃) where the tanks with *P. palmata* was stored held a light regime of 10:14 (light:darkness). Material from the first collection of fresh material was kept in these tanks for 40 days. Before proceeding with the experiment, the immature *P. palmata* was assessed again to evaluate if some had become fertile during the 40 days of storage. Only the mature fronds (fertile tetrasporophytes) were transferred to the next step in the experiment.

Based on experience made from the first collection, the rest of the collections were sorted in a way which only the mature fronds were used further and directly transferred to the next step in the experimental setup.

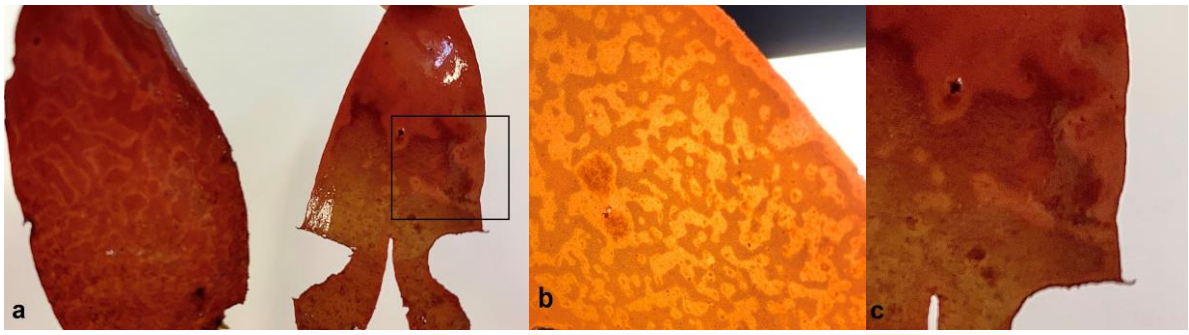

Figure 6: a) To the left a fertile male gametophytic frond and to the right a fertile tetrasporophytic frond where the black box focuses on a fertile area. **b**) zoomed in on the fertile male gametophyte. **c**) zoomed in on the fertile tetrasporophyte shown as a black box in picture a. Fronds collected at Korsvika, Trondheim 2021.

2.3 Induction of fertility and repeated spore release

Material for this experimental work was collected at low tide in Korsvika, Trondheim 02.11.2021. The material was transported and kept in the laboratory as described in section 2.2.1, except the desired fronds for this experiment were the immature ones.

2.3.1 Sori induction

The purpose of the first experiment in phase A) (Fig.4) was to induce fertility in immature fronds by manipulation of the light regime. Two different light regimes were used to characterize the impact on the inducement of fertility in *P. palmata* outside of the main fertile season (Table 3). One of the light regimes was set to 08:16 (light:darkness) to simulate short day (SD) and winter-like conditions, based on work conducted by Pang and Lüning (2006). The second light regime was set to 14:10 to simulate long days (LD), and spring conditions. The two different light regimes were both set up with three horizontal plastic tanks (25 L), measuring 60 cm (l) x 35 cm (w) x 17 cm (d), with white light (cold fluorescent lamp) as light source above the tanks.

During the early stages, macroalgae are exposed to contaminants such as microalgae and especially benthic diatoms. Studies on the effect of germanium dioxide (GeO_2) as a disinfection on various macroalgae has been conducted for several years. Based on work done by Shea and Chopin (2007), and later followed up by Bøe (2019), a treatment of GeO_2 was applied to inhibit the growth of diatoms and showed promising results as a disinfectant for *P. palmata*. The used method for disinfection and recipe of disinfectant was based on the findings conducted by Bøe (2019). Disinfection of *P. palmata* was conducted in a climate room at 10℃ (± 1) to secure minimal temperature fluctuations. Sterile seawater (SSW) was used in the following steps. To sterilise, seawater as described in section 2.2.1, was autoclaved in bottles (Schott Duran 2 L) in a high-pressure stream sterilizer (TOMY SX-700E) at 120℃ for 20 minutes. All equipment was autoclaved in the same steam sterilizer. Plastic equipment that could not be autoclaved was sterilized using 70% ethanol solution.

Three autoclaved glass bowls were used, two were filled with 500 mL SSW (10℃) and one was filled with 500 mL solution of disinfectant GeO_2 (0.5 mL L^{-1}) (10℃). Batches of 15-20 g sori were transferred to a sieve and immersed into the first glass bowl containing GeO_2 solution. It was immersed for two minutes while stirred using a magnetic stirrer. Two rounds of rinsing were performed in the following two glass bowls with SSW, each round for 30 seconds while carefully shaking the sieve so all parts of the sori were properly rinsed. A stopwatch was used to time the three different steps in the disinfection.

Disinfected material was equally split (30 fronds) into six horizontal tanks, three tanks in each light regime experiment with a continuous water flow (Table 3). The temperature was set to 10℃ (± 1) throughout the entire experiment. There was a weekly check for fertile fronds in the horizontal tanks.

Table 3: Light regime parameters for induction of sori in the fronds.

Light regime (Light: Dark) (h)	08:16 (SD)	14:10 (LD)
Light intensity (µmol photons m^{-2} s^{-1})	20	20
Water flow (L min^{-1})	0.5 – 1.5	0.5 – 1.5
Temperature	10℃	10℃

2.3.2 Repeated spore release and recovery time

Repeated spore release was a subsequent experiment based on the fronds induced with fertility in the first experiment in phase A) (Fig.4A). The two different light regimes, short day (SD) and long day (LD), that were used during induction of fertility followed the same fronds throughout this experiment. The same six horizontal tanks placed under the light source that were used for the sori induction were used in this experiment for recovery time. The aim of this part of the experiment, was to use the fertility-induced fronds to repeatedly subject them to three days of spore release until there were no more spores in the sori.

A protocol was made to conduct repeated spore release on the same fronds. The steps in the protocol consisted of 24h in a refrigerator, three days of spore release in darkness and a period of recovery time conducted in either SD or LD conditions (Fig.7).

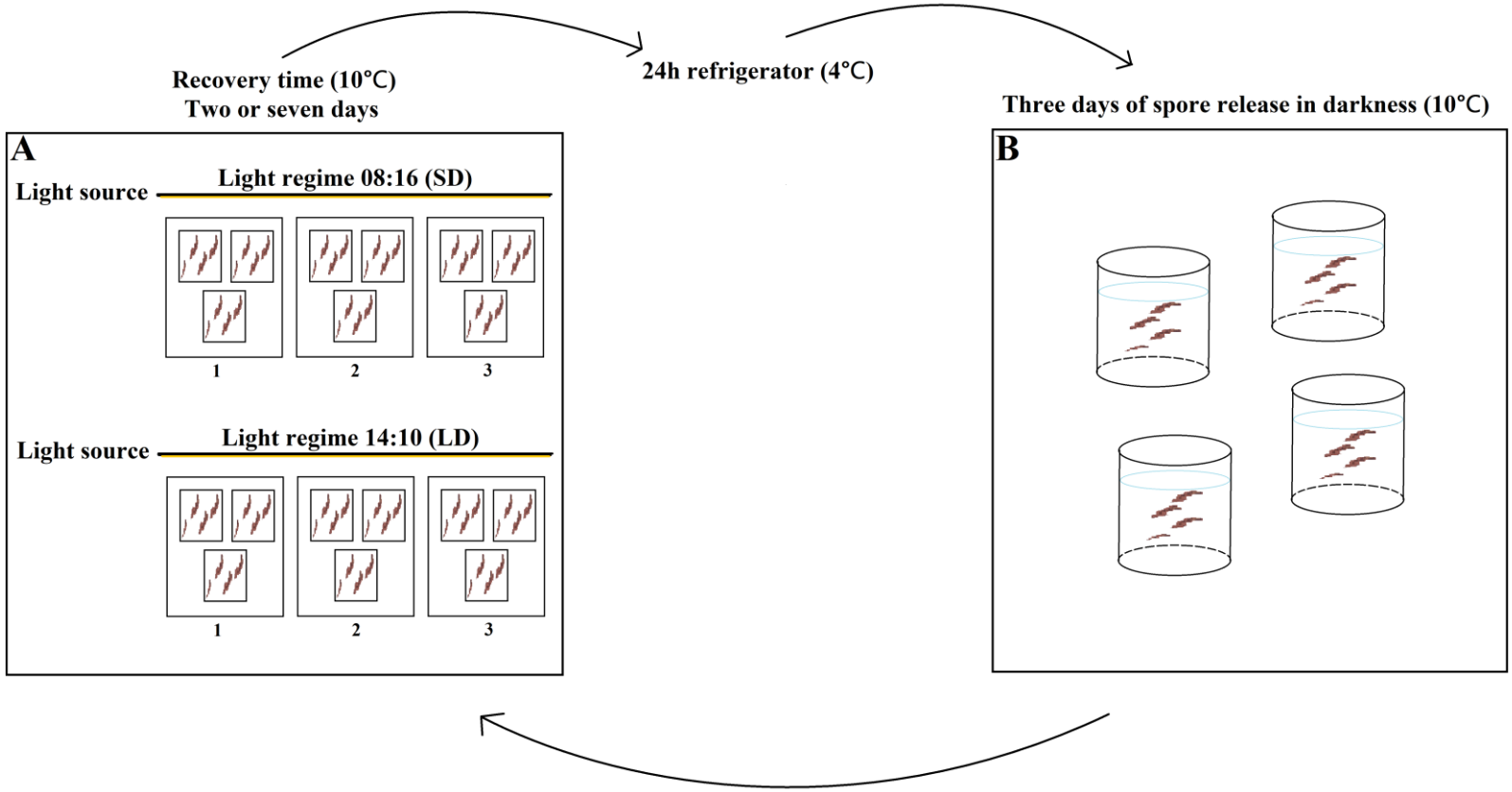

Figure 7: Diagram showing an overview of the experimental work for repeated spore release. Box **A** shows the set up for the recovery time with two light regime conditions (SD and LD) with the same irradiance (20 µmol photons m^{-2} s^{-1}) and where the fertile fronds were left for either two or seven days. After a recovery time all the fronds were put 24h in a refrigerator before entering box B. Box **B** indicates the period of three days spore release in total darkness. Diagram not to scale.

The protocol starts with the fronds from the refrigerator, where they after 24h became dehydrated. This created a stress-response in the fronds, making them release spores when they were rehydrated again (Forbord et al., 2018). Rehydration was conducted by placing the fronds in 1 L sterilized glass beakers containing 450 ml of SSW, placed in a dark climate room at 10℃ (±1). Spore release was conducted over a three-day period in total darkness and with agitation (Fig.7B). Each beaker was covered with aluminium foil to secure dark conditions and to protect against contamination. For agitation, they were placed on an orbital shaker with a relatively high speed (90 RPM) throughout spore release. After spore release, the fronds were put for a period of "recovery time" for maturation (Fig.7A).

In both the different light regimes, one short and one long recovery time were tested to mature the fronds, to examine if one of the recovery times influenced sori to release more spores in repeated spore release. The fronds were subjected to either two- or seven-days recovery time in both light regimes. The plan of which recovery time the fronds were going to be subjected to were determined before starting the protocol. The fronds picked out for either two- or seven-days recovery in SD and LD conditions, followed the same light regime throughout the entire experimental period.

Throughout this thesis, the different rounds of repeated spore release will be referred to as "SR", followed by a number indicating the number of rounds. The period the fronds spent in recovery time will be referred to as "R", followed by a number indicating the number of recovery time. E.g., SR1 indicates the first round of repeated spore release and is followed by R1 that indicates the first round of recovery time.

Figure 8 shows a schematic overview of the protocol for repeated spore release when it consisted of a recovery time of two days. The setup was the same regardless of which light regime the fronds were subjected to in the recovery time. The fertile fronds picked out for two days recovery time started in SR1, where they were placed in a refrigerator for 24h before three days of spore release, hereby referred to as 24, 48 and 72h. The next step is shown as R1, which consisted of two days for the two-day recovery time protocol. The experimental period for two-day recovery time lasted until the end of SR5. Meaning the same fronds were subjected to five rounds of repeated spore release and four rounds of recovery time, and a total experimental period of 28 days before sori were emptied.

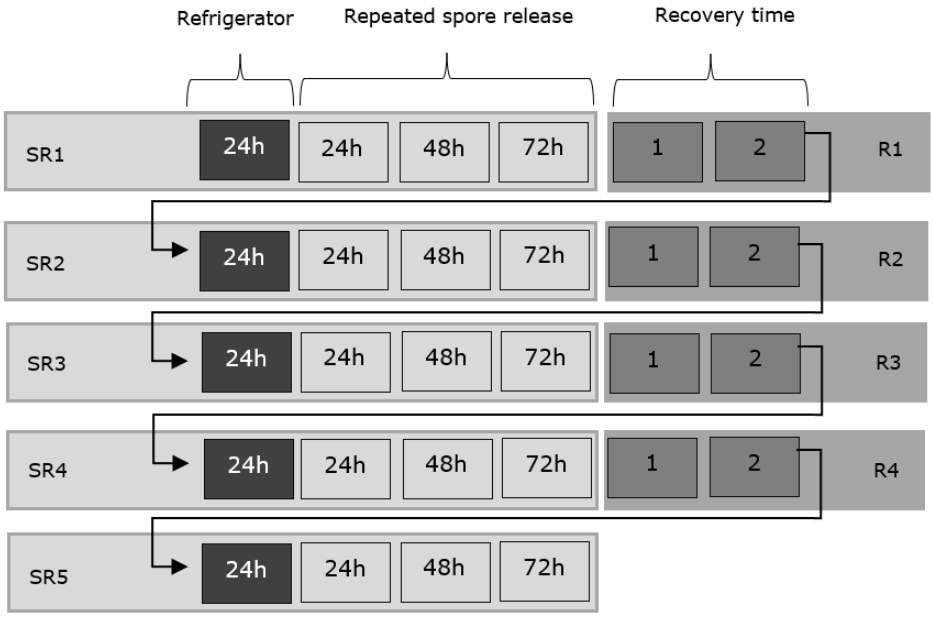

Figure 8: Overview over the experimental work, where the same fronds were subjected to 24h in refrigerator, three days in repeated spore release in darkness and with agitation, and two days of recovery time in either SD or LD condition. The different rounds were conducted repeatedly until the fronds had emptied their sori after SR5. After the last round the fronds were discharged.

Figure 9 shows a schematic overview of the protocol for repeated spore release when it consisted of a recovery time of seven days. The setup was the same regardless of which light regime the fronds were subjected to in the recovery time. The fronds picked out for seven-day recovery time followed the same procedure as for the fronds with two-day recovery time, only different recovery time duration. The longer time in recovery time led to fewer repeated rounds of the protocol, in comparison to two-day recovery time. The experimental period for seven-day recovery time lasted until the end of SR3. Meaning the same fronds were subjected to three rounds of repeated spore release, and a total experimental period of 26 days before sori were emptied and the fronds were discharged.

Figure 9: Overview over the experimental work, where the same fronds were subjected to 24h in a refrigerator, three days in repeated spore release in darkness and with agitation, and seven days of recovery time in either SD or LD condition. The different rounds were conducted repeatedly until the fronds had emptied their sori after SR3. After the last round the fronds were discharged.

To keep control of which recovery time the fronds belonged to, the fronds were put in open plastic boxes dedicated to its recovery time, placed in their respective horizontal tanks and light regime, to separate between different rounds of spore release. Colour codes were used to identify the fronds, indicating duration of recovery time and how many rounds of spore release they have had.

Before repeating the protocol for spore release, the fronds were examined to check for spore-containing sori. All the fronds in each horizontal tank were inspected using a light microscope (NIKON E200) (Fig.10A, D-F) before a new round of the protocol was initiated. The analysed fronds containing sori with spores were patted dry with clean paper towels, placed in marked plastic bags and kept for 24h in a dark refrigerator at 4℃ (± 1).

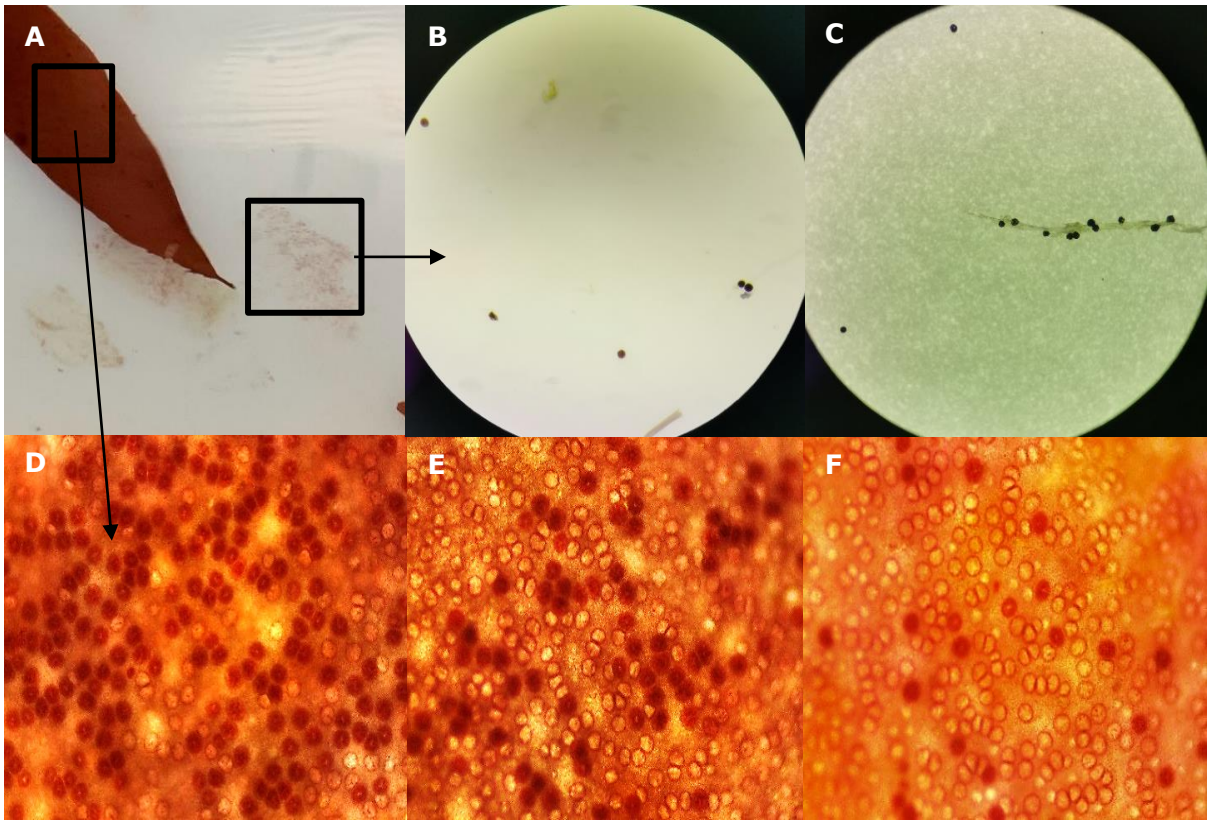

Figure 10: A photo guide of early sporophyte identification. **A)** To the left a zoomed in black box focused on released spores of *P. palmata* at the bottom of a tank and observed by eye as red patterns. **B, C)** Released spores from sori of different fronds. **D-F)** Stereomicroscopy views of sori are visible as marbled dark-red areas containing tetraspores visible in different stages of spore release. One tetrasporangium (spore compartment) contains four tetraspores (magnification x10 in all pictures).

For analyses of spore density from repeated spore release, samples were drawn every 24, 48 and 72 hours from the bottom of the beakers, after stirring it to homogenise the solution of SSW and spores. Between the SR-rounds the beakers were washed and sterilized. To study if spore release was present during recovery time, samples were taken from the recovery boxes, before placing them for dehydration to prepare them for another round of spore release. The samples were added a fixative to preserve them until analysis. The chosen fixative was Lugol's iodine solution, as it is recognised to be a suitable preservative to use when studying phytoplankton taxa (Hallegraeff, 2003). Samples fixed with Lugol's

show reduced range of microbes, which is good for long term storage, and it stains the density of individual algal cells whilst reducing the settling time (Choi & Stoecker, 1989; Lund et al., 1958). Three to four drops of Lugol's solution were added to all samples taken throughout the experiment.

2.3.3 Method for counting tetraspores

The method for counting tetraspores was based on Bøe (2019). Samples taken as described in section 2.3.2 were filtrated through a 50 µm filter, on a 0,8 µm syringe filter (cellulose nitrate) to eliminate objects lager than *P. palmata* spores. By using a tweezer, the filter was placed on a microscope slide and dried for one minute, before spores were counted manually by using a light microscope (Nikon E200) with objective 10x (Fig.10B, C). The counted spores were calculated to tetraspores mL^{-1}.

2.4 Nursing stage

The purpose of the second experiment in phase A) was to use mature fronds (fertile tetrasporophytes) collected in the fertility season of *P. palmata* and subject released spores to two different methods in the nursing stage (Fig.4A). Fronds were field collected as described in section 2.2 and processed as described in section 2.2.1.

Disinfection of the mature fronds was conducted as described in section 2.3.1. Disinfected and rinsed fronds were carefully patted dry using clean paper towels. Dried fertile fronds were equally split into four batches. For dehydration, the batches were placed on a tray packed in plastic bags, marked, and kept in a dark refrigerator at 4℃ for 24h.

For the spore release and seedling growth experiment, three different types of substrates were tested: 40 mm mesh thread nets (N40), 150 mm mesh thread nets (N150), and five entwined spools (size: 1.2 mm twines). Before use the nets were washed at 60℃ in a washing machine, and the entwined spools were flushed with high water pressure. The three different types of substrates were placed in separate horizontal tanks with a 1:1 areal coverage of substrates. Each tanks had a plate placed at the bottom, for easy access to the released spores that did not attach to the substrates. The fourth tank was kept empty of substrates, with only a plate at the bottom, where the released spores were intended to use in the bubble cultures.

Spore release was conducted by rehydration of the fertile fronds. This was done directly in the four horizontal tanks containing substrates and plates, filled with SSW. Conditions throughout the experiment was based on methods from Werner and Dring (2011): the tanks were placed in a climate room at 10℃ (± 1), under a low light source (10 µm m^{-2} s^{-1}), with a light regime of 12:12 (light:darkness). Spore release directly on the substrates was conducted over a three-day period, where gentle aeration by bubbles was used the last two days. The fronds were gently stirred by hand once a day to promote mixing and settlement of spores. The fronds were thrown away after three days, while the substrates were kept in the tanks waiting for a new round of spore release from new dehydrated fronds.

This method for spore release directly on substrates was conducted two more times on the same substrates.

2.4.1 Pre-incubation method

The three types of directly seeded substrates from section 2.4 were transferred to bigger holding tanks for the last step in the hatchery step, the nursing stage. The following experiment was one of two methods used in the nursing stage and is referred to as the pre-incubation method (Fig.4A).

This method was for growing *P. palmata* attached to substrates, into suitable sizes for deployment at an open-sea farm. Except from a few modifications, the method for nursing the seeded substrates were based on a manual created for cultivation of *P. palmata* (Schmedes & Nielsen, 2020a).

The three different types of substrates were each hung in three cylinder-tanks (300 L) (Fig. 11E). Before hanging the nets, they were cut into smaller parts. The N40 net was cut into three parts measuring 95x36 cm, and the N150 net was cut into three nets measuring 95x80 cm. All cylinders-tanks were treated with the same conditions throughout the nursing stage (Table 4), and all substrates were placed in approximately equal distance to the light source. The cylinder-tanks were checked once or twice every week for fouling and cleaned if necessary. In total they were nursed for 122 days.

Table 4: Key parameters for pre-incubation in cylinder-tanks based on method as described by Schmedes et al., (2020).

	Key parameters Day 0-3	**Key parameters Day 3-30**	**Key parameters Day 30-122**
Light intensity (µmol m^{-1} s^{-1})	35	35	15
Light regime (Light:Darkness)	10:14	10:14	10:14
Temperature (°C)	10	10	10
Water treatment	Sand-filtrated, particle-filtrated, UV	Sand-filtrated, particle-filtrated, UV	Sand-filtrated, particle-filtrated, UV
Aeration	No	Yes	Yes
Water flow (L min^{-1})	No	1.5-2.5	1.5-2.5

2.4.2 Bubble cultures

The use of bubble cultures to grow adequate *P. palmata* was the second method used in the nursing stage (Fig.4A). Within this experiment the aim was to optimize the conditions during the nursing stage when seedlings are grown free-floating in bubble cultures.

The spores for this experiment came from the same four horizontal tanks used in section 2.4 (Fig.11). Spores that did not attach to the substrates were attached to the tank wall, the plates placed at the bottom of the tank and underneath the plates at the tank floor. Spores attached to the plates were directly scraped into a beaker. While spores attached to the walls and floor of the tanks were scraped, washed with a small amount of sea water, and collected using a pipette to transfer them into the same beaker as the spores collected from the plates.

Figure 11: Overview of the experimental set up in the nursing stage from collection of mature fronds. **A)** Collection spot in Korsvika covered by *P. palmata*. **B)** Fronds with tetrasporangial sori. **C)** Mature tetrasporophyte with spores (magnification x10). **D)** Fertile fronds placed in a horizontal tank above net substrate for directly seeding. **E)** Pre-incubation of three types of substrates directly seeded with *P. palmata* placed in cylinder-tanks for nursing stage, **F)** Nursing stage method with bubble cultures for growth of free-floating seedlings.

The collected spores constituted a 200 ml culture consisting of SSW and *P. palmata* spores. This culture was transferred into a blender to homogenize it, using the slowest tempo for a few seconds. The homogenized solution was split into nine bottles. In addition, a small sample was taken into a centrifuge vial and fixed with Lugol's solution.

The setup for nursing free-floating seedlings in bubble cultures were based on Schmedes et. al (2020). Each of the nine bottles were filled up to 400 ml with autoclaved sea water and a nutrient enriched medium, Conway, together with the culture of *P. palmata* (Walne, 1966). The cultures were placed in approximately equal distance to the light sources. Then they were covered by a silicone top with a hole for aeration, where a glass pipette was placed to connect the culture to a silicone tube adding filtrated air to the cultures. The nine batches were split into three different batches for different treatments, with distinct incubation parameters throughout the experiment for each of the three batches (Table 5). Within the three different treatments there were three parallels. In total the bubble cultures were nursed for 122 days.

Table 5: The incubation parameters for the three different batches of bubble cultures of free-floating seedlings.

Batch number and treatment	Days	Light intensity (µmol)	Conway (%)	Light regime (Light:Dark)
A – Low light-low nutrient	0-14	10	10	10:14
	15-30	10	10	10:14
	31-122	10	10	10:14
B – Higher light intensity	0-14	10	10	10:14
	15-30	40	10	10:14
	31-122	40	10	10:14
C – Higher nutrient enrichment	0-14	10	10	10:14
	15-30	10	100	10:14
	31-122	10	100	10:14

The nine cultures were checked once a day to refill distilled water after evaporation, if necessary. The salinity in the cultures was also tested and adjusted if required (35 ppt). Throughout the experimental period, parameters were adjusted according to plan to get the correct light intensity and nutrition content. The cultures were changed twice, with new SSW to prevent contamination and correct amount of Conway was added. This was done by letting the cultures of gametophytes accumulate, before slowly pouring sea water and Conway out and replacing it.

For analyses of seedling growth there was taken samples once a month from each of the cultures. The samples were analysed under an inverted microscope (Nikon TE2000-E PFS Inverted Fluorescence Motorized Phase Contrast DIC Microscope), where 20 seedlings from each bottle were taken photos of and measured in imageJ (Fig.12).

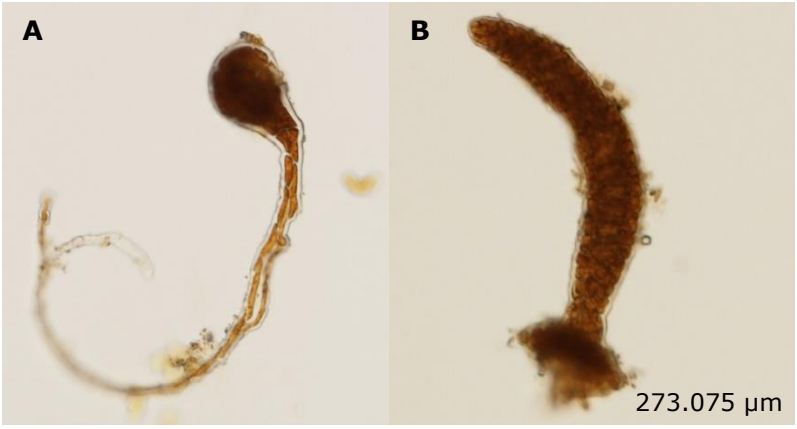

Figure 12: Seedling development. **A)** A female gametophyte with two trichogynes, not measured. (**B)** Developing male gametophyte (seedlings) (13.07.21).

2.5 Cultivation of *P. palmata* at an open-sea farm

The aim of this experiment was to evaluate the growth success of *P. palmata* at an open-sea farm. The main intention was to examine if there were any differences in growth success between the two different nursing methods tested in the laboratory, as described in section 2.4.1 and 2.4.2.

2.5.1 Study site

The experimental work was conducted at Skarvøya in Central Norway (63° 39’ N, 8° 39’ E) (Fig.13). The open-sea farm is situated at a sheltered location.

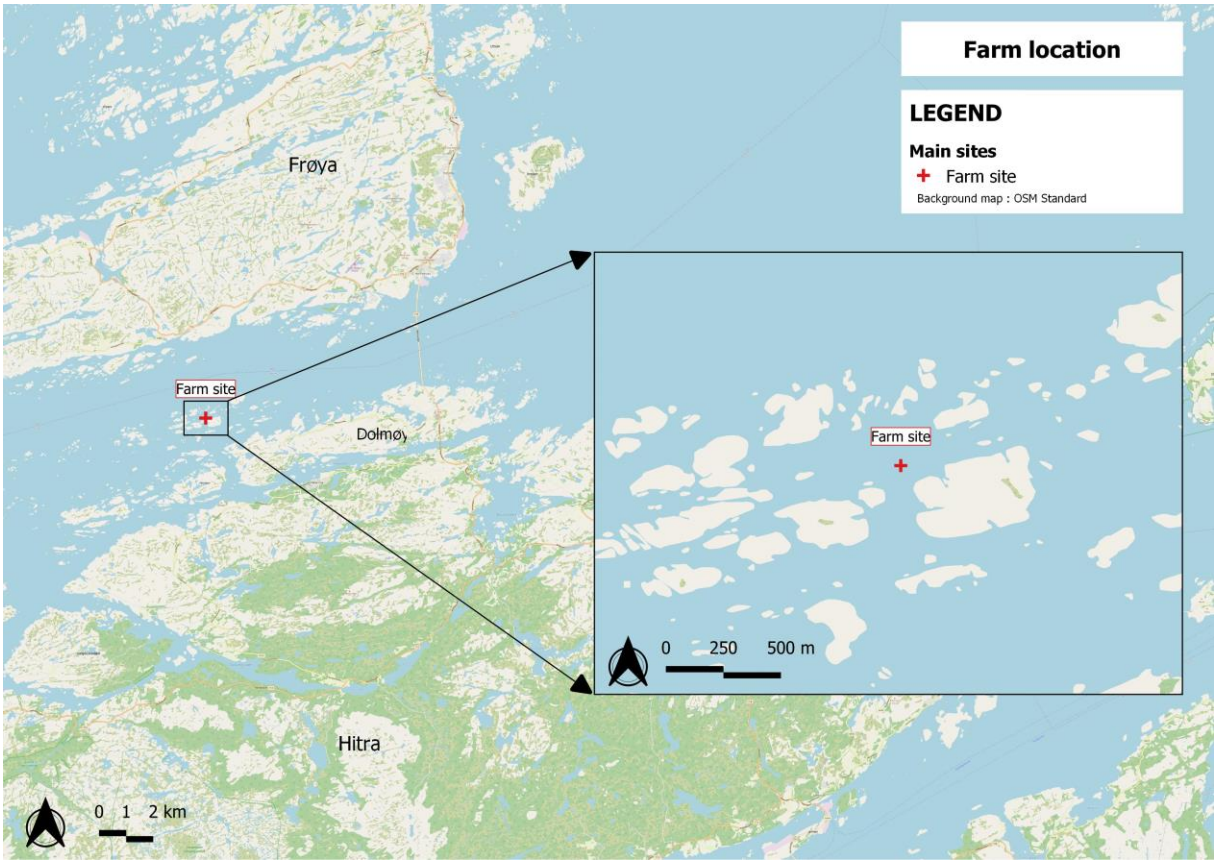

Figure 13: Geographical location of the experimental macroalgae farm Skarvøya, Frøya. Directly seeded nets, strings and seeded algae ropes from the bubble cultures were deployed at this location. Produced from QGIS application.

2.5.2 Method for preparation of pre-incubated substrates

The pre-incubated substrates from section 2.4.1 were in this part of the experimental work prepared for deployment at an open-sea farm.

The day before deployment, the entwined spools were prepared by twinning them on to thicker polypropylene ropes (14 mm), using a twisting machine from SINTEF Ocean. In total there were made nine 3 m and three 5 m entwined ropes containing seedlings. The ropes were stored overnight in tanks (80 L) filled with seawater (10℃) in a climate room at 10℃ (± 1).

The day of deployment, the entwined ropes, and nets (N40 and N150) were packed into black plastic bags, with bubble plastic between layers of substrates and 10℃ (± 1) seawater to secure stable conditions during transportation to the open-sea farm.

2.5.3 Bubble cultures for binder seeding

The bubble cultures nursed as described in section 2.4.2 were in this part of the experimental work prepared for deployment at an open-sea farm.

Within the three different conditions, the three parallels were mixed into one for further use. This was done by scraping the wall and bottom of the bottles, since *P. palmata* had attached to it, leaving it to sediment for 30 minutes before excess SSW was drained. The only desired product was the sedimented *P. palmata*. The seeding substrates for the bubble cultures were algae ropes together with a special binder to attach the culture of *P. palmata*, which were products recommended and delivered by a company named Hortimare. A standard procedure regarding the ratio between substrates, binder and substrates composed by Hortimare were followed to prepare the algae ropes for open-sea cultivation.

Before use, a water-test was conducted on the algae ropes to check how much water they absorbed. This was done by weighting a small part of the rope before and after one hour soaking in sea water. The wet weight was noted after 30 minutes of dripping. The difference in weight was used to calculate the amount of water absorbed by the algae rope, and further used to calculate the amount of binder mixture. To minimize contamination, the algae ropes were washed and properly dried before use.

The mixture of the binder was made after methodology according to the Hortimare procedure. The used algae rope had 0.5% (5 g binder/L seawater) as desired concentration of the binder. The mixture, consisting of SSW, binder, and *P. palmata*, was made the day before it was attached to the algae ropes, and the amount of mixture was based on the performed water-test. The mixture of SSW and binder was first made as double concentration (1%), before diluting it with *P. palmata* and adding SSW, so that the desired concentration of 0.5% was reached. This was done for each of the three different batches, making it three different mixtures containing *P. palmata*.

The day of the deployment, the binder mixture was attached to the algae ropes. For each of the three batches nine meters of rope (3x3 m) were placed in a bucket and the binder mixture was poured over the ropes and properly mixed until all the mixture was soaked up. After soaking up, the mixture was pressed deeper into the algae ropes by using a rolling pin. The algae ropes were packed in black plastic bags, marked, and stored in a

plastic container filled with seawater (10℃), to secure stable conditions during transportation to the open-sea farm. This preparation of the ropes was conducted in time to secure at least two hours soaking before deploying them at the open-sea farm.

2.5.4 Deployment at an open-sea farm

P. palmata from section 2.5.2 and 2.5.3 were deployed at the study site the 25th of October, at the site presented in section 2.5.1. The open-sea farm consisted of horizontal lines at 2 m depth attached between buoys and anchored to the sea bottom with mooring blocks (Fig.14).

Deployment of the different substrates with *P. palmata* were based on a randomized set up. All substrates with *P. palmata* were attached with a 10 m distance from each other and at 2 m depths. The algae ropes, nets (N40 and N150), and nine entwined ropes were deployed as vertical droppers from the horizontal lines. The three 5 m entwined ropes were deployed as horizontal lines. Each rope and net were colour-coded to identify which treatment they had gone through in earlier stages of the experimental work. All the different substrates were checked for seedling growth two times before harvesting.

2.5.5 Growth status from open-sea farming

The status on growth success at the open-sea farm was evaluated by checking the different substrates deployed as described in section 2.5.4. The first evaluation was performed March 21st, where pictures of all types of substrates was taken to document growth. The second, and last evaluation was performed April 27th, where growth success was evaluated by measurements and pictures.

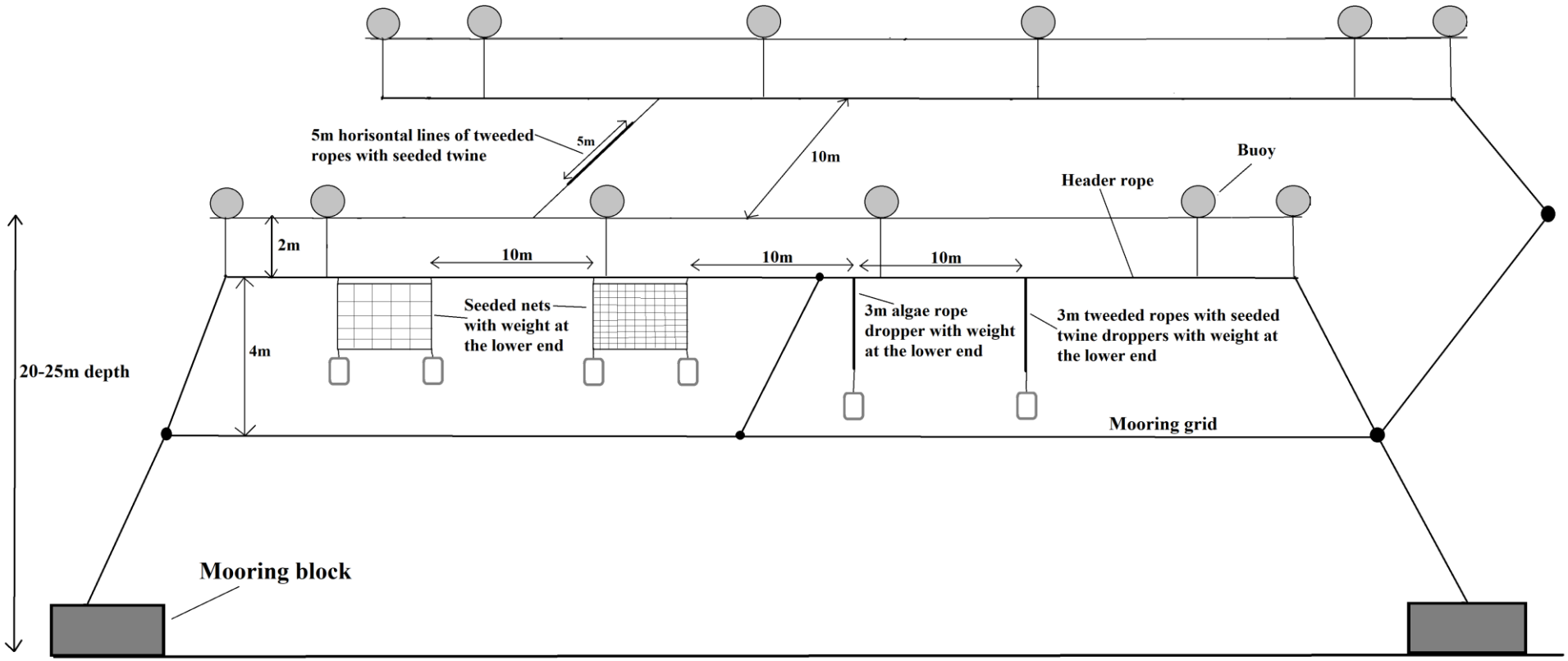

Figure 14: Set up at the open-sea farm and deployment of *P. palmata*. Longlines with two different vertical droppers and two different nets, all with weight at the lower end. Horizontal lines were attached between the different header ropes. Diagram not to scale. The macroalgae farm site, Skarvøya, once it was installed by Frøya Akvaservice in October 2021.

2.6 Statistical analysis of data

All data pertaining to the ecological variables of interest were non-normally distributed, based on Shapiro-Wilks normality test results, QQ-plots and histograms. Therefore, non-parametric tests were chosen for all statistical analyses. All data are presented as mean ± standard error (SE). Statistical analyses were performed using IBM SPSS Statistics V28, except for the analysis in section 2.6.4. Figures were made in Excel, Microsoft Office 365, except for figures related to section 2.6.4.

2.6.1 Effect of induced fertility

Mann-Whitney U tests were conducted to test the differences between sum of induced fertility and the two light regimes.

2.6.2 Rounds of repeated spore release

A series of non-parametric Kruskal-Wallis tests were conducted to investigate number of spores released within spore release rounds with either two- or seven-days recovery time. Further, Non-parametric Kruskal-Wallis tests were conducted to investigate the difference between spore release rounds for SD and LD, and which time of 24, 48 and 72h had most spore release.

2.6.3 Spores released during recovery time

Mann-Whitney U tests were conducted to test the differences of all factors between number of spores and light regimes. A series of non-parametric Kruskal-Wallis tests were conducted to investigate number of spores released within the different recovery rounds. Further, a series of Mann-Whitney test were conducted to investigate the comparison between spore release and connected recovery time in given light regime.

2.6.4 Size of seedlings in bubble culture

Non-parametric Kruskal-Wallis tests were conducted to investigate the differences in all factors between size of seedlings and days for the treatment with low light and low nutrient content. Pairwise comparisons using Wilcoxon rank sum test with continuity correction was tested to further investigate each of the days (0, 18, 122) and growth of length of *P. palmata.* Dots in the boxplot figures represents outliers, which are numerically distant from the rest of the data. All analyses were performed using R Statistical Software (v4.1.2; R Core Team 2021). Figures were produced using package ggplot2. Experimental results were analysed using mixed models.

2.7 Cooperation between students

The master's thesis was a cooperation between Ylva E. H. Rydningen and June Valla. The collections of *P. palmata* out in the field, planning all the experiments and the execution was conducted together. Exception from this is experiment 2.3.2 and 2.4.2. Ylva set up the station for repeated spore release and had the main responsibility of taking samples from the different repeated spore release rounds. June started up the nursing stage for bubble cultures and checked them every week throughout the summer. Ylva conducted the harvesting of *P. palmata* from the open-sea farm. After this, time spent on the experiments was set to be even between the students.

A lot of time was spent planning the writing process and the decision on what to include in the thesis was based on teamwork. The writing, statistical analyses and alterations were conducted in collaboration, where both the students feel attachment to each part of the thesis.

3 Results

3.1 Induction of fertility

The mean percentages of immature fronds that developed fertility by manipulation of light regime under laboratory conditions, are shown in Figure 15. Short day (SD) with 8h light and 16h darkness (08:16), and long day (LD) with 14h light and 10h darkness (14:10), were used as light regimes. There was no significant difference between induced fertility and the light regimes (Mann-Whitney U test, $p>0.05$).

In the field-collected material (late autumn, Nov. 2nd) no signs of sori was observed in any of the two light regimes, until the third assessment of the fronds (Nov. 16th). At this date, the result revealed that fronds with LD condition had induced most sori (25% ± 3.9) compared to fronds with SD condition (21% ± 5.1). The number of fronds induced with sori reduced with LD condition after this date, whereas with SD condition number of induced fronds with sori inclined. The last induction date shows that the SD had in total induced the most fronds with sori compared to fronds with LD condition (48% ± 6.4 and 41% ± 9.9, respectively).

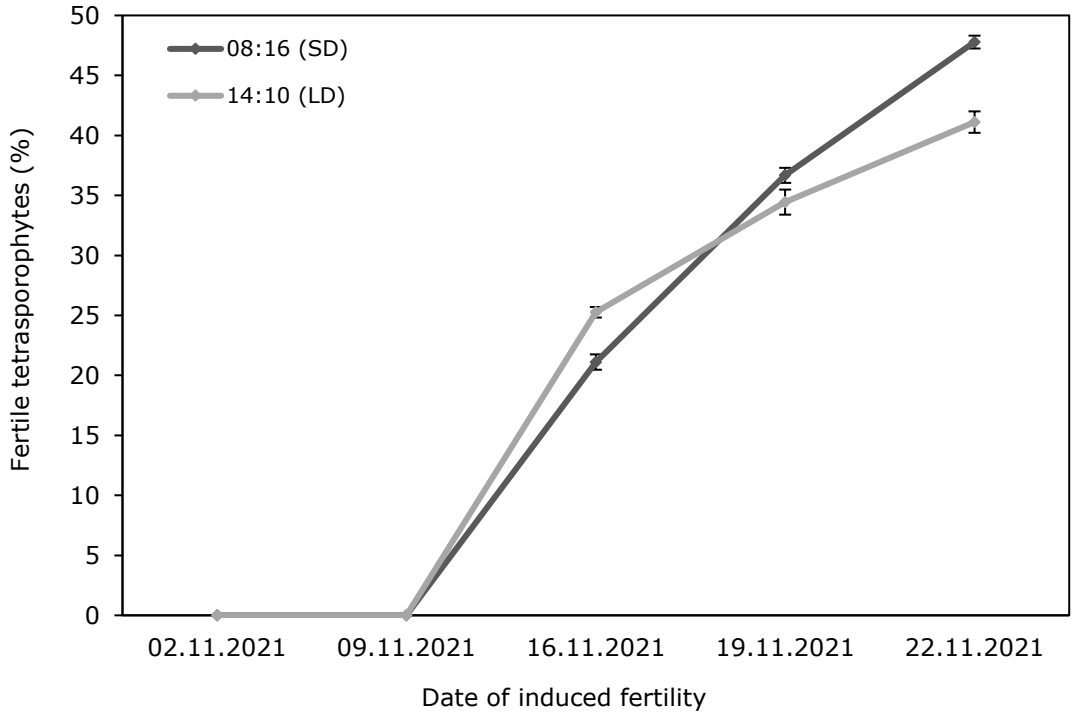

Figure 15: The percentages of field-collected immature fronds that induced fertility after manipulation of light regime are shown at different dates at the x-axis. Lines indicate the two different light regimes, 08:16 (Light:Dark) and 14:10 (Light:Dark), that were tested. The last date, 22.11.2021, indicates the total induced fronds in each light regime. Data points given in Sum ± SE (n=3).

3.2 Repeated spore release

The fronds induced with sori as shown in section 3.1, were the groundwork for the results presented in this section. The protocol for the experiment regarding repeated spore release consisted of three steps; 24h in a refrigerator, three days of spore release (SR) in darkness and a period of recovery time (R) conducted in either SD or LD conditions. The spore density in the results presented in this section were based on samples taken throughout the three days of spore release at given times 24, 48 and 72h.

With a repeated spore release protocol with two days recovery time, the number of repeated spore release rounds was in total five rounds (SR1-SR5) for both light regimes (Fig.8).

Figure 16 shows a comparison between spore density in SD and LD in the different spore release rounds. Looking at all repeated spore release rounds, there was no significant difference in spore release (Kruskal-Wallis H test, $p>0.05$) between SR1, SR2, SR3 and SR5 at given times in SD and LD. However, there was a significant difference in spore release in SR4, where spore density in LD were significantly higher compared to SD at 24 and 48h (Kruskal-Wallis H test, $p<0.05$).

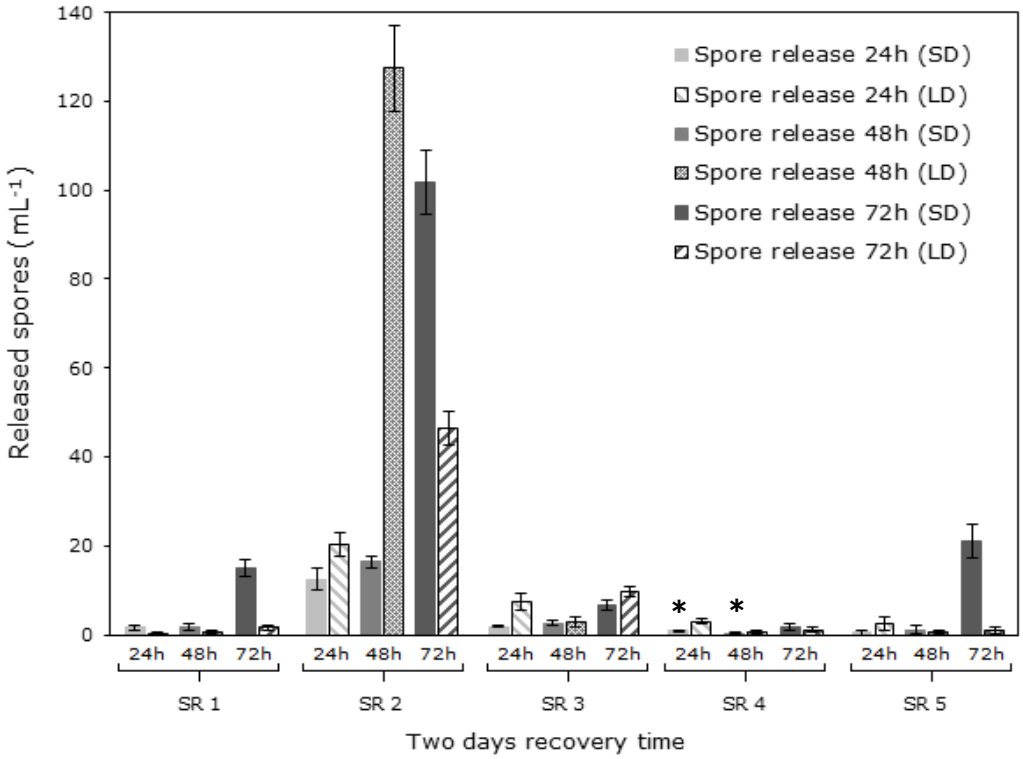

Figure 16: Spore density from repeated spore release with short day (SD) and long day (LD) conditions and two days recovery time shown at the x-axis. Bars indicate spore density at given times from SR1 to SR5. Data points given in mean ± SE (n=4 for SR1-SR4, n=2 for SR5). Significant differences between mean spore density in LD and SD between the same given times expressed as "*".

The number of repeated spore release rounds when subjected to seven days recovery time, was in total three times for both light regimes (SR1-SR3) (Fig.9).

Figure 17 shows a comparison between spore density in SD and LD in the different spore release rounds at given times. Looking at all repeated spore release rounds together (SR1-SR3) in each light regime, there was no significant difference in spore density (Kruskal-Wallis H test, $p>0.05$) between SD and LD. Looking within SR1, there was a significantly higher spore density (Kruskal-Wallis H test, $p<0.05$) after 72h in SD compared to 72h in LD.

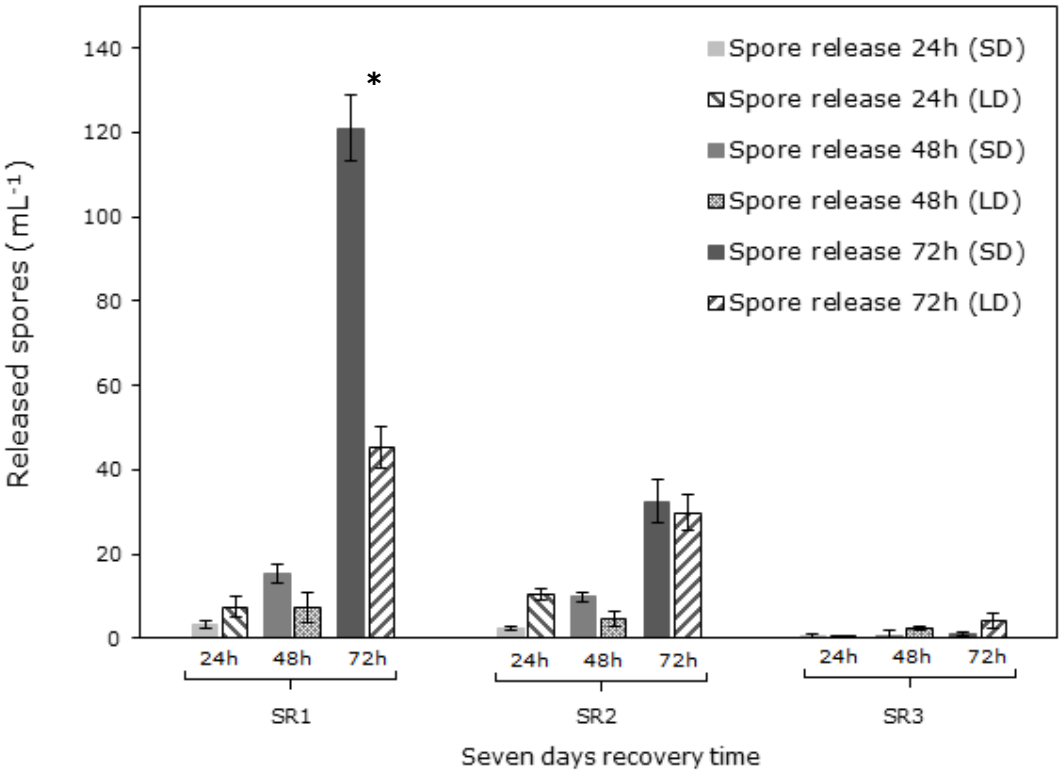

Figure 17: Spore density from repeated spore release with SD and LD and seven days recovery time shown at the x-axis. Bars indicate spore density at given times from SR1 to SR3. Data points given in mean ± SE (n=3 for SR1-SR3). Significant difference between mean spore density in LD and SD in samples from 72h expressed as "*".

To evaluate which recovery time and light regime that resulted in highest spore density in repeated spore release, the mean value of released spores in each given time 24, 48 and 72h in all repeated spore release rounds were calculated. Data based on spore release throughout SR1-SR5 with two days recovery time, and SR1-SR3 with seven days recovery time.

Figure 18 shows a comparison between the mean value of spore density from all spore release rounds given in 24, 48 and 72h in both SD and LD, and the differences between the two- and seven-days recovery time. For both recovery times, there was no significant difference in the spore density (Mann-Whitney test, $p>0.05$) at 24, 48, 72h between SD and LD.

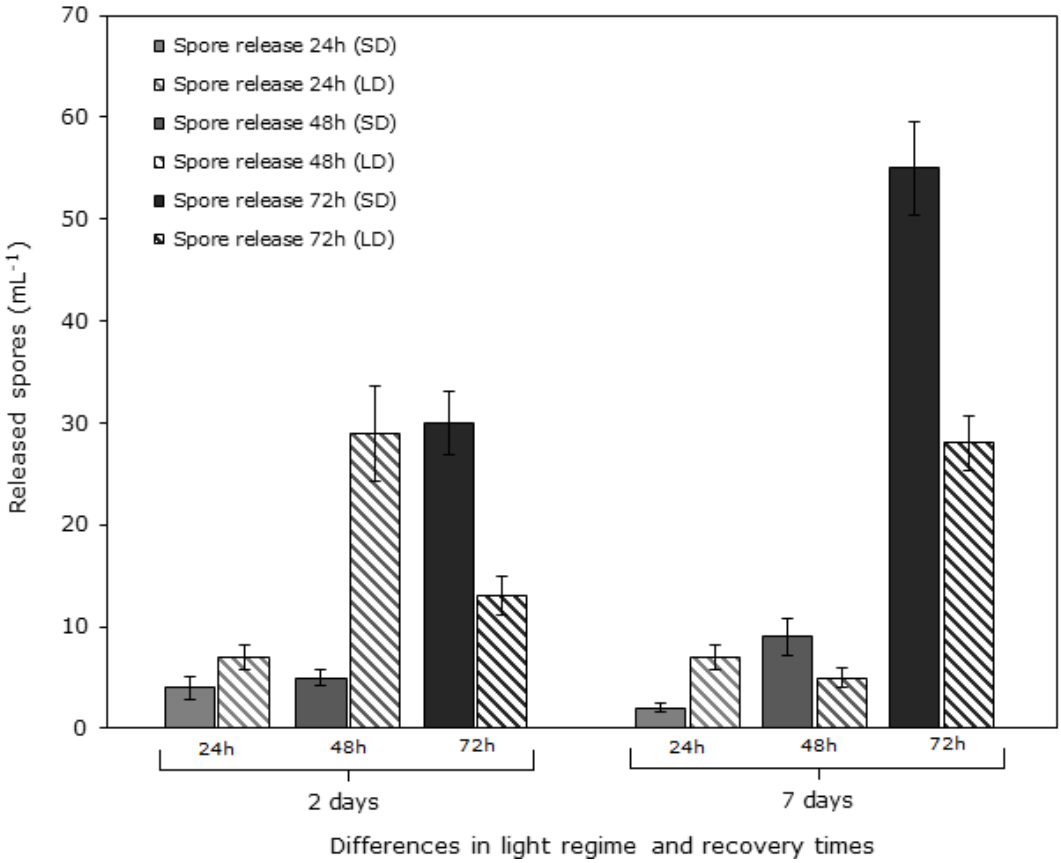

Figure 18: Spore density at 24, 48 and 72h during the three days of spore release. X-axis shows released spores in given times with a comparison between SD and LD in both two- and seven-days recovery time. Data points given in mean ± SE (n=18 for two days recovery for SD and LD, n=14 for seven days recovery in SD and n=11 for seven days recovery in LD).

3.2.1 Spores released during recovery time

To analyse if spores were released during recovery time, a time where spore release was not wanted, samples were taken at the end of each recovery time.

Figure 19 shows a comparison between mean value of spore density in SD and LD with two days recovery time. With two-day recovery time there were four rounds of recovery time, R-1 to R-4 in both light regimes.

Looking at all the rounds together (R-1 to R-4), spore density in LD was significantly higher (Mann-Whitney test, $p<0.05$) compared to SD. Looking within the rounds, there was a significant difference in spore density (Kruskal-Wallis H test, $p<0.05$) between SD and LD in R-1.

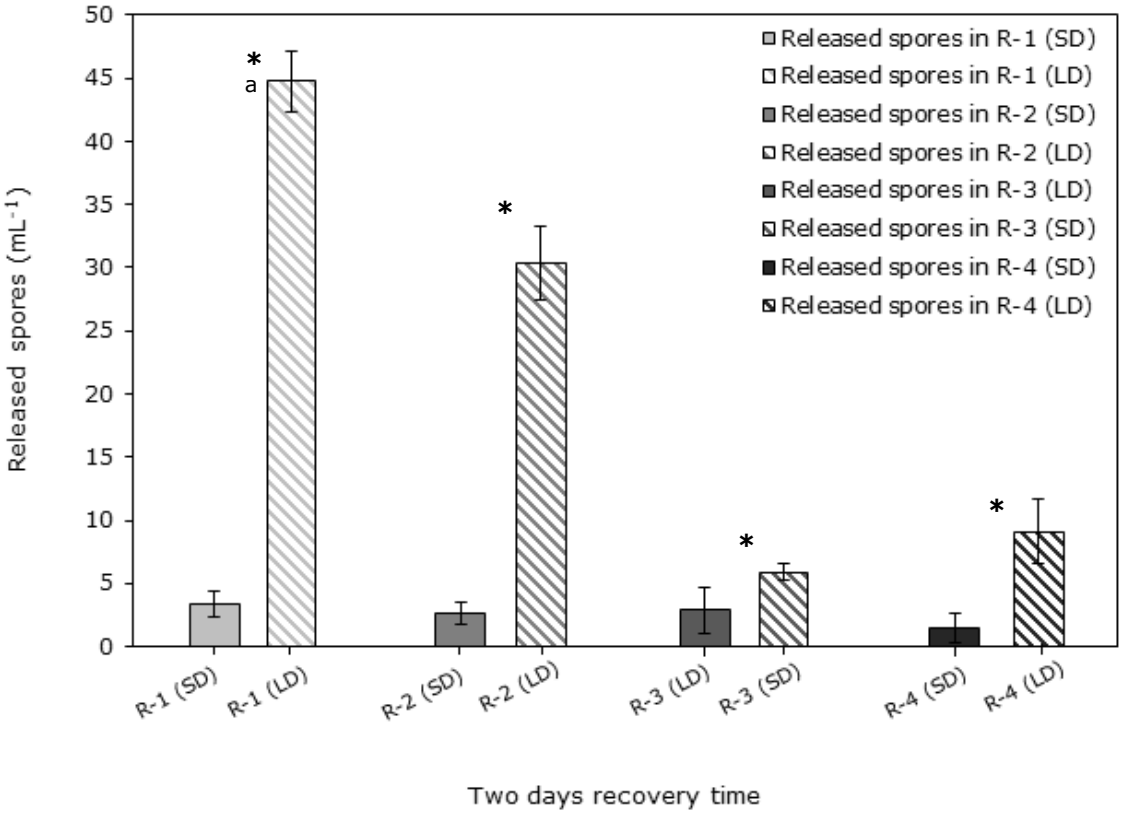

Figure 19: Spore density in the different rounds of recovery time (R) with two days recovery time. The x-axis shows the number of rounds of recovery time for both short day (SD) and long day (LD) conditions. Bars indicate the mean spore density in each round. Data points given in mean ± SE (n=4 for R-1 to R-3, and n=2 for R-4). Significant difference when looking at all spore density off all rounds (R1-R4) in LD and SD are expressed as "*". Significant difference within the rounds and between SD and LD are expressed as "a".

Figure 20 shows a comparison between the different light regimes and spores released during seven days recovery time given in mean value. With a seven-day recovery time there were two rounds of recovery time, R-1 to R-2 in both light regimes.

Looking at all the rounds together (R-1 to R-2), there was no significant difference in spore density (Mann-Whitney test, $p>0.05$) between SD and LD. Looking within the rounds, spore density in LD was significantly higher (Mann-Whitney test, $p<0.05$) compared to SD in R-2. Conversely, there was no significant difference in spore density in R-1 (Mann-Whitney test, $p>0.05$) between SD and LD.

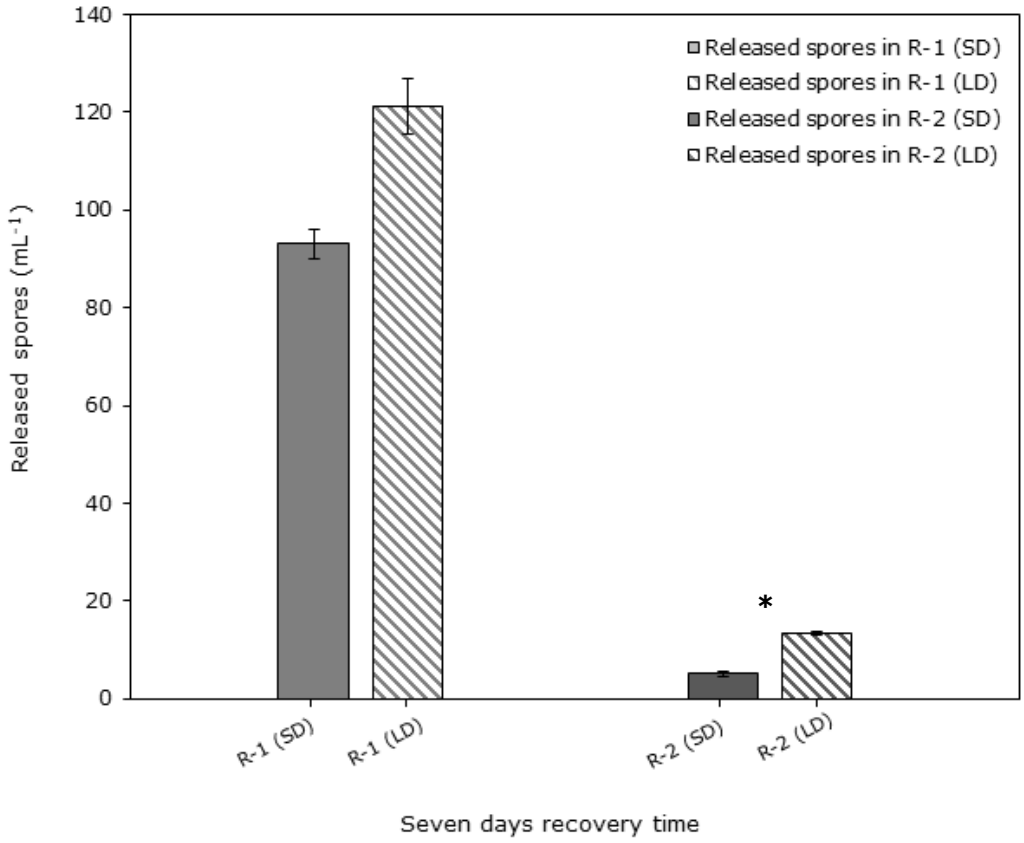

Figure 20: Spore density in the different rounds of recovery time (R) with seven days recovery time. The x-axis shows the number of rounds of recovery time for both short day (SD) and long day (LD) conditions. Bars indicate the mean spore density in each round. Data points given in mean ± SE (n=5 for R-1 in SD, n=4 for R-2 in SD, n=4 for R-1 in LD and n=3 for R-2 in LD). Significant difference within the rounds and between SD and LD are expressed as "*".

Since samples were taken at given times 24, 48 and 72h during spore release, and only one time from recovery time, some calculations were performed before using the data further. This was done to make spore density in spore release and recovery time comparable. By summarising the total number of spores that were released at 24, 48 and 72h for each spore release round (SR), the average number of released spores was calculated to include every round of spore release (SR1 to SR5 for two-day and SR1 to SR3 for seven-day recovery time). The average number of released spores in recovery time (R) was calculated to include every round of recovery time (R1 to R4 for two days and R1 to R2 for seven days).

Figure 21 shows a comparison between spore release and connected recovery time in given light regime. In SD and with two days recovery time, mean spore density was significantly higher in SR1-SR5 (Mann-Whitney test, $p<0.05$) compared to connected R1-R4. With seven days recovery time there was no significant difference in spore density (Mann-Whitney test, $p>0.05$) between SR1-SR5 and R1-R2 in SD. In LD and with two days recovery time, there was no significant difference in spore density (Mann-Whitney test, $p>0.05$) between SR1-SR5 and R1-R4. Further, there was no significant difference in spore density (Mann-Whitney test, $p>0.05$) between SR1-SR3 and R1-R2 with seven-day recovery. Comparison between spore density in each round of repeated spore release and connected recovery time is shown in Appendix 1.

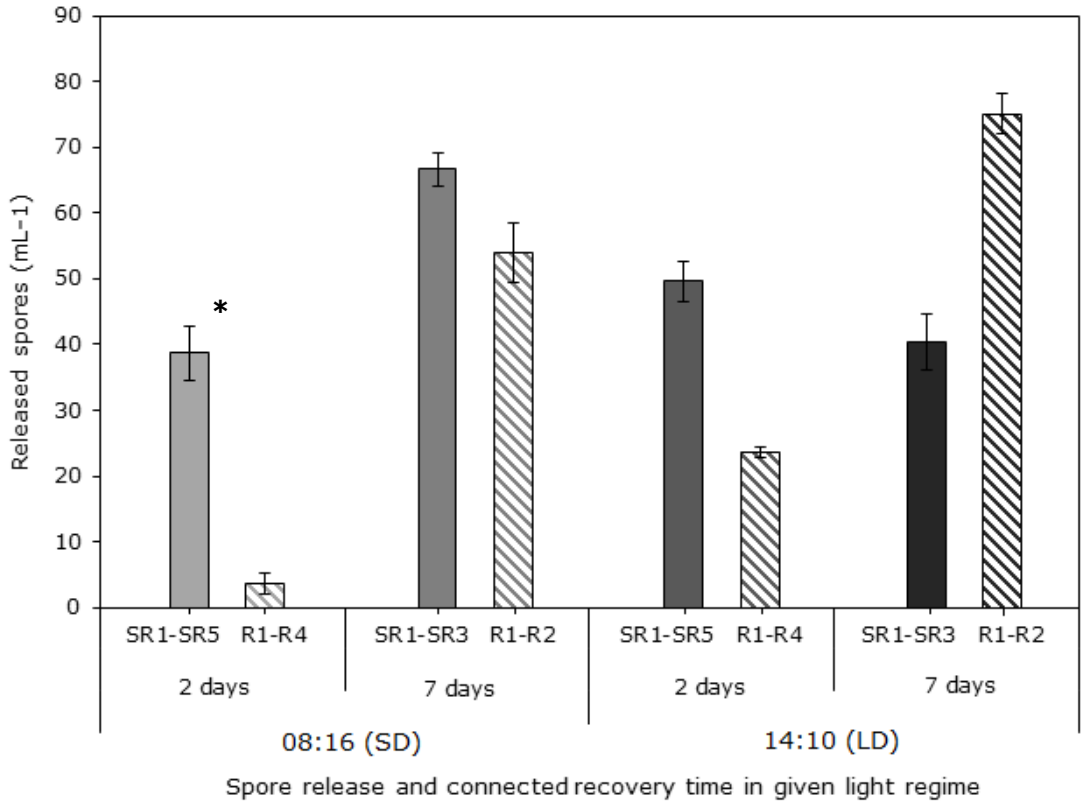

Figure 21: Spore density in all rounds of spore release (SR) and recovery time (R) are shown at the x-axis, with a comparison between short days (SD) and long days (LD) conditions and recovery time of either two or seven days. Bars indicate the mean spore density. Data points given in mean ± SE (n=18 for SR1-SR5, n=14 for SR1-SR3, n=14 for R1-R4 and, n=9 for R1-R2 in SD, n=7 for R1-R2 in LD). Significant difference between mean spore density in SR-rounds and R-rounds in each recovery time and light regime expressed as "*".

3.3 Nursing stage

The following results is from the two different nursing methods used in the nursing stage, as presented in section 2.4, on their development during the nursing stage in the hatchery phase (Fig.4A).

3.3.1 Pre-incubation method

The pre-incubated substrates were transferred to bigger holding tanks for the last step in the hatchery phase, the nursing stage, as described in section 2.4.1.

Figure 22 shows small-masked net (N40) seeded with *P. palmata* hanging in the cylinder-tanks used during the nursing stage. During the inspections, pictures were taken to document the growth of *P. palmata*. Picture A-D documents the growth during the nursing period at different given times, where only fouling organisms are visible. Samples were taken of the net to analyse together with samples from the water after cleaning of the substrates. Figure 23 shows analysed samples from the water and the nets where no seedlings of *P. palmata* were detected, only fouling organisms.

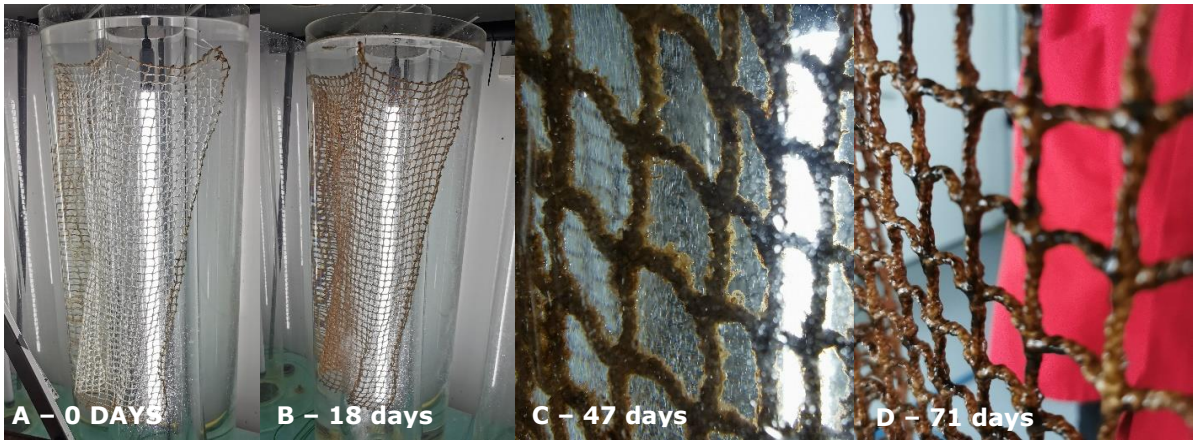

Figure 22: A timeline showing the net (N40) hanging in the cylinder-tanks during the nursing stage at different dates; **A**) The starting date 25.06.2021, **B**) 13.07.2021, **C**) 11.08.2021. and **D**) 03.09.2021.

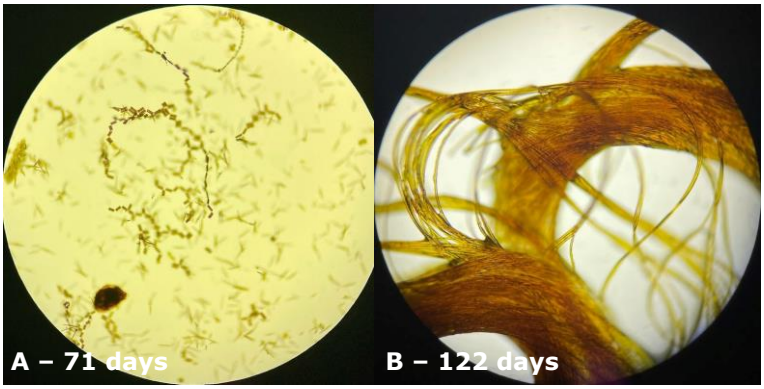

Figure 23: Pictures taken in microscope showing samples from the net (N40) during the nursing stage. **A**) Water samples 03.09.2021, 10x. **B**) Samples from the nets 22.10.2021, 4x.

The cylinder-tanks where the big-masked net (N150) seeded with *P. palmata* hung during the nursing stage are shown in figure 24. To document the growth of *P. palmata*, pictures were taken during the nursing period. Picture A-D documents the growth during the nursing period at different given times, where only fouling organism are visible. Samples were taken of the net to analyse together with samples from the water after cleaning of the substrates. Figure 25 shows analysed samples from the water and the nets, where no seedlings of *P. palmata* were detected, only fouling organisms.

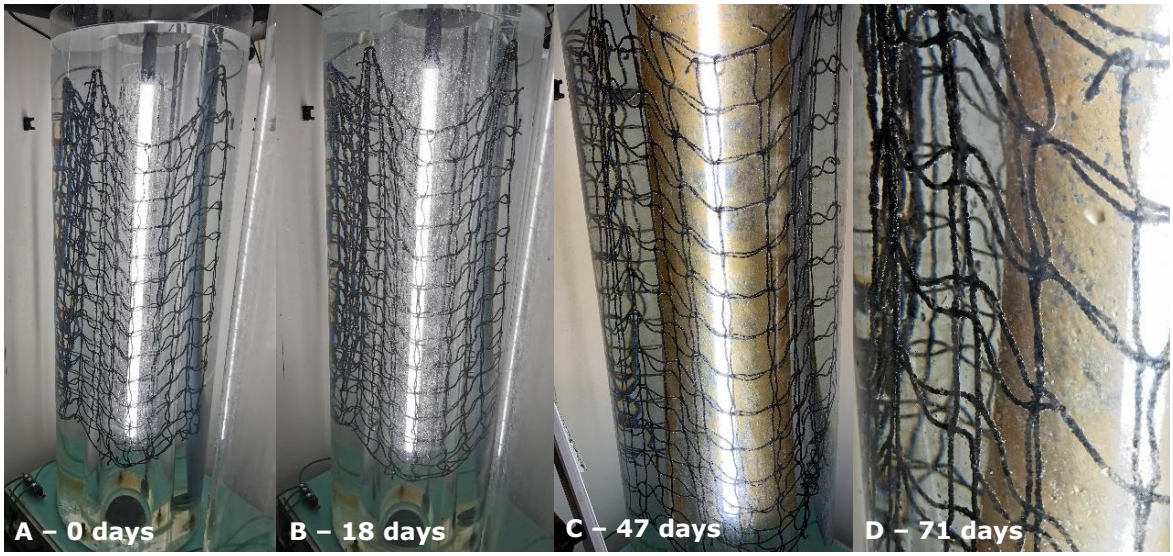

Figure 24: A timeline showing the net (N150) hanging in the cylinder-tanks during the nursing stage at different dates; **A**) The starting date 25.06.2021, **B**) 13.07.2021, **C**) 20.08.2021 and **D**) 03.09.2021.

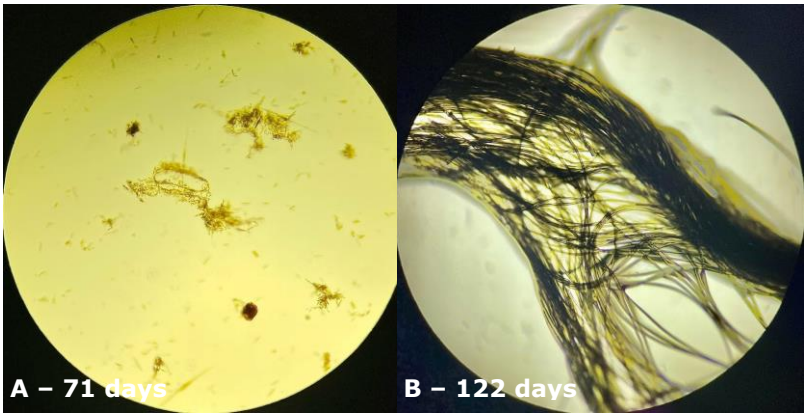

Figure 25: Pictures taken in a microscope showing samples from the net (N150) during the nursing stage; **A**) Water samples 03.09.2021, 10x. **B**) Samples from the nets 22.10.2021, 4x.

Entwined spools seeded with *P. palmata* hung in a cylinder-tank during the nursing stage (Fig.26). To document the growth of *P. palmata*, pictures were taken during the nursing period. Picture A-D documents the growth during the nursing period at different given times, where only fouling organism are visible. Substrate samples were taken of the spools to analyse together with samples from the water after cleaning the substrates. Figure 27 shows analysed samples from the water and the spools, where no seedlings of *P. palmata* were detected, only fouling organisms.

Figure 26: A timeline showing the entwined spools in the cylinder-tanks during the nursing stage at different dates; **A**) The starting date 25.06.2021, **B**) 13.07.2021, **C**) 28.07.2021 and **D**) 03.09.2021.

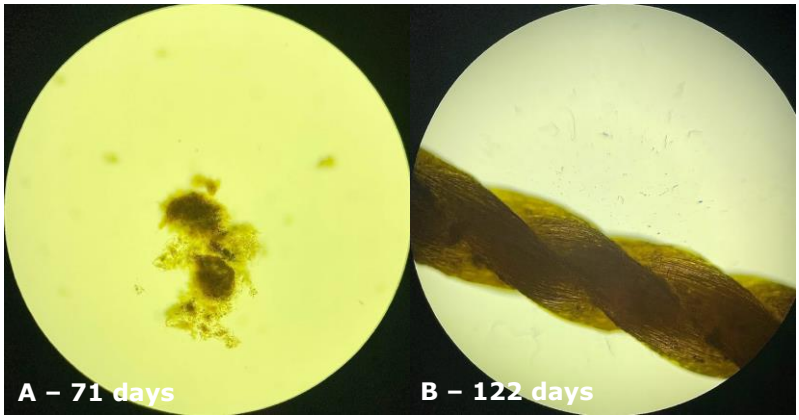

Figure 27: Pictures taken in a microscope showing a piece of the twine from the entwined spools during the nursing stage; **A**) Water samples 03.09.2021, 10x. **B**) Samples from the nets 22.10.2021, 4x.

3.3.2 Bubble cultures

The use of bubble cultures was the second method used in the nursing stage, as described in 2.4.2. Within the experiment the aim was to optimize the conditions during the nursing stage for growth of adequate quality of free-floating male gametophytes (seedlings). The different parameters for the three different treatments; low light-low nutrient, higher light intensity and nutrient enriched cultures are shown in table 5 in section 2.4.2. No seedlings of *P. palmata* were observed in the treatments with higher light intensity and nutrient enriched at day 122 before deployment, and was for that reason not included in the figures.

The mean growth (µm) of seedlings in the different treatments; low light-low nutrient, higher light intensity, and higher nutrient enriched are shown in Figure 28. There was a significant difference (Kruskal-Wallis test, $p<0.05$) in growth at 18 days between the different treatments.

In the low light-low nutrient treatment (A) there was a significant difference (Kruskal-Wallis test, $p<0.05$) between growth of the seedlings throughout the entire nursing period at given days 0, 18 and 122. The mean seedling length at day 122 was significantly higher (Pairwise Dunn's, $p<0.05$) compared to both days 0 and 18. However, mean seedling length on day 18 was not significantly different (Pairwise Dunn's, $p>0.05$) compared to day 0. Looking at the higher light intensity treatment (B), the mean seedling length at day 18 was significantly lower (Pairwise Dunn's, $p<0.05$) compared to day 0. Similarly, mean seedling length at day 18 was significantly lower (Pairwise Dunn's, $p<0.05$) compared to day 0 with the nutrient enriched treatment (C).

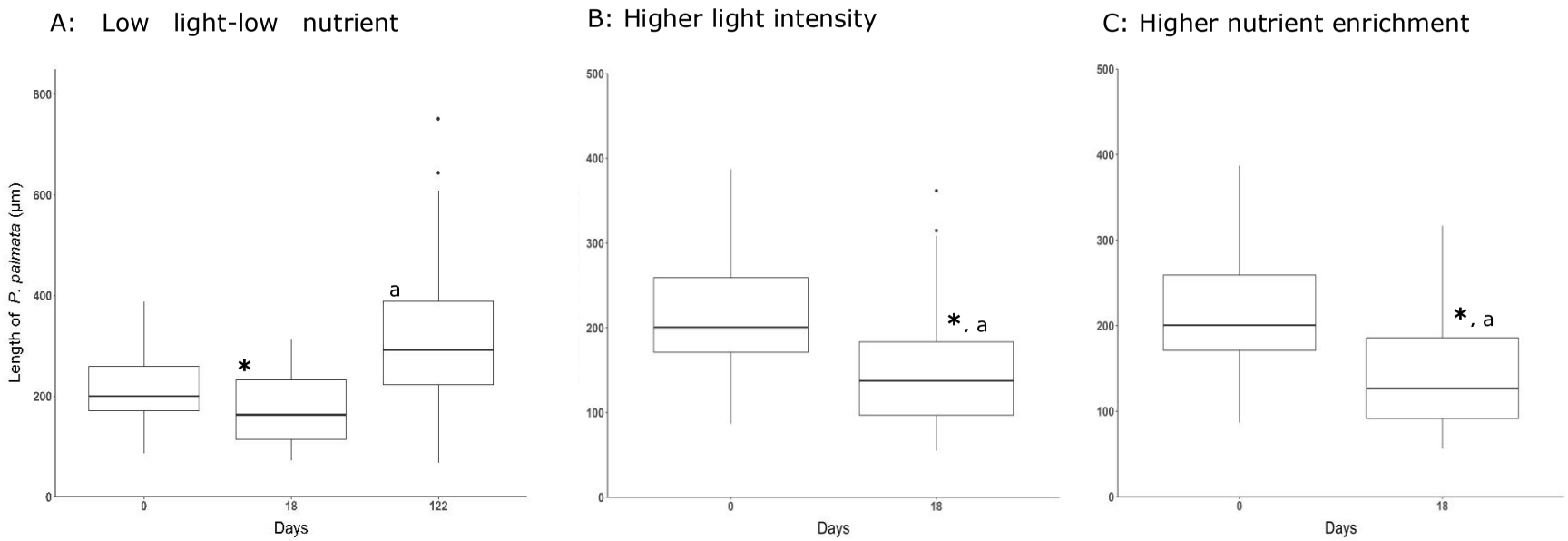

Figure 28: Mean growth (µm) during nursing stage at different conditions **A**) Low light-low nutrient treatment, **B**) Higher light intensity and **C**) Higher nutrient enrichment, at given days 0, 18 and 122. Data points represents mean ± SE (n=20 day 0, n=60 day 18 and 122). Dots in the boxplot represents outliners which are numerically distant from the rest of the data. Y-axis given in 800 µm in A, and 500 µm in B and C. Significant difference in growth at 18 days between the different treatments are expressed as "*". Significant difference between the days in the treatment are expressed as "a".

Samples were taken once a month from each of the treatments in the bubble cultures to estimate growth during the nursing stage. The samples were measured and analysed in ImageJ.

The length of *P. palmata* seedlings varied between the parallels (n=3) within the same treatment. Two samples from each treatment are shown to illustrate the differences in seedling length (µm) (Fig. 29). Seedlings from the treatment with higher light intensity and higher nutrient enrichment showed more growth of other fouling organisms, compared to low light-low nutrient treatment.

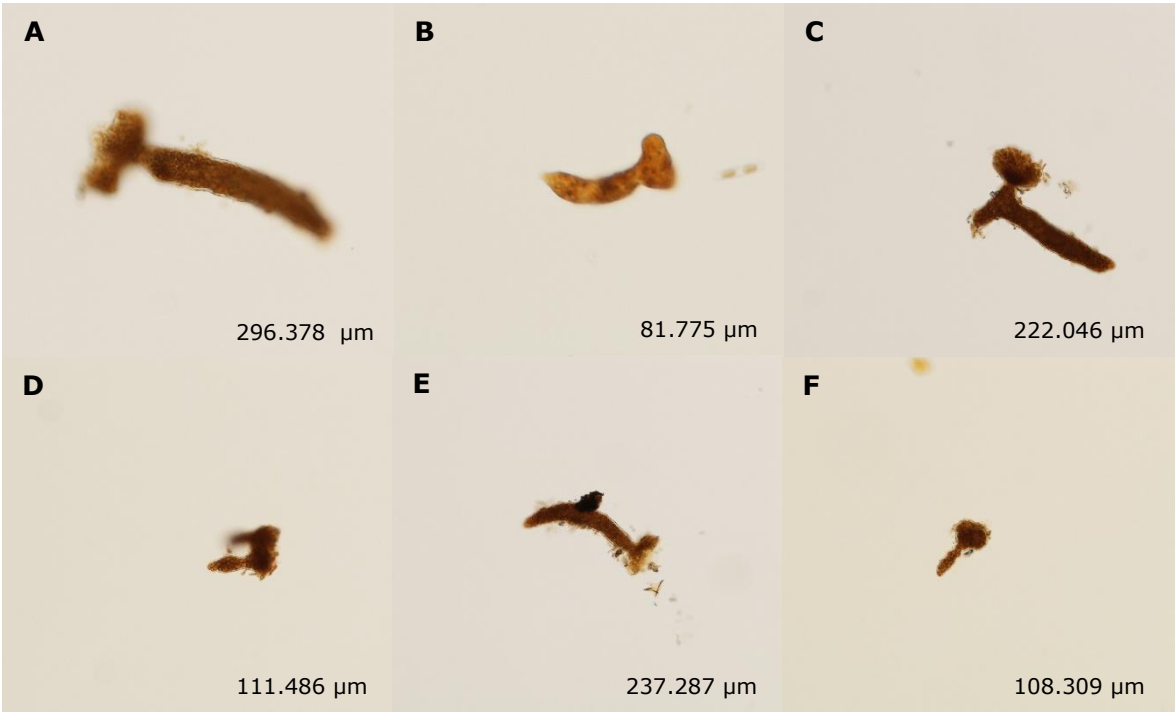

Figure 29: Differences in seedling length within the different treatments after 18 days of growth. **A-B**: Low light-low nutrient treatment. **C-D**: Higher light intensity treatment. **E-F**: Higher nutrient enrichment. Pictures with objective 10x.

3.4 Cultivation of *P. palmata* at an open-sea farm

The results from cultivation at an open-sea farm for both methods used in the nursing stage, pre-incubation method and bubble cultures, are presented in this section. The prepared substrates as described in section 2.5.4 and 2.5.5 were all deployed on the same date, 25th of October, and were grown in a total of six months. During the cultivation at sea, the growth was checked two times, once in March and once in April.

3.4.1 Cultivation on algae ropes and entwined ropes

After six months of growth (April 27th) a small *P. palmata* had developed on one of the binder-seeded algae ropes from the bubble culture with low light and low nutrient (Fig.30). The remaining eight algae ropes did not have any growth. The entwined ropes that were hung as droppers did not grow successfully. Both substrates were covered with large growth of *Saccharina latissima* and *Alaria esculenta*, making it hard to detect if there was any growth of *P. palmata*. Further, of the three entwined ropes deployed as horizontal lines, only one had growth of a single *P. palmata*. All ropes were densely covered by epiphytic micro- and macroalgae.

Figure 30: *P. palmata* growth at the different ropes after six months in the sea; **A**) One small *P. palmata* on an algae rope from the bubble culture with low light and low nutrient content. **B**) One small *P. palmata* on a horizontal entwined rope.

3.4.2 Cultivation on 40- and 150-mm mesh nets

The growth of *P. palmata* on both nets (N40 and N150) were more successful than on the ropes (Fig.31). However, the outcome was unsuccessful in a commercial harvest point of view, where the size of *P. palmata* should have been bigger and the density higher. The overall highest growth density among all substrates, was found on N150, with a total of 21 *P. palmata*. The largest *P. palmata* was measured to be 6 cm in length, whereas the others were between 0.5-3 cm in length. Conversely, there were 14 growing *P. palmata* on N40. On that net, the largest *P. palmata* was measured to be 4 cm in length, whereas the others were between 0.4-3 cm.

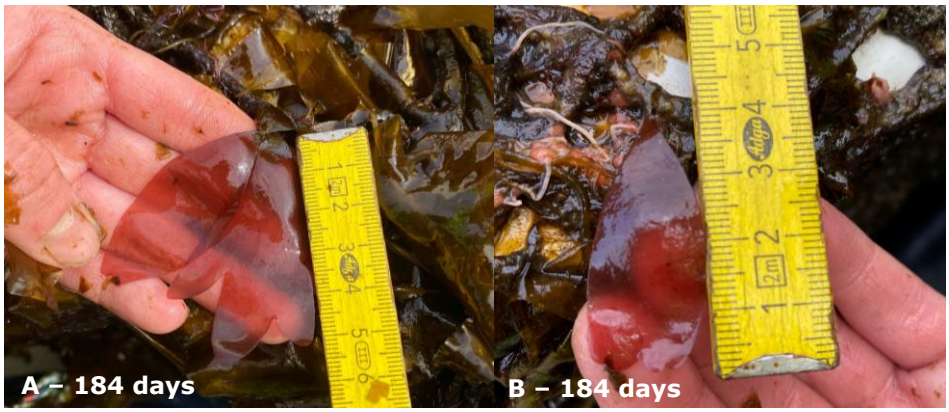

Figure 31: *P. palmata* growth on different mesh net types after six months in the sea; **A**) One small *P. palmata* on the big mesh width net. **B**) One small *P. palmata* on the small mesh width net.

As clearly displayed in pictures below, the nets were densely covered by fouling organisms, as well as large growth of *S. latissima* and *A. esculenta* (Fig.32). The largest number of *P. palmata* were found on the top part of the nets compared to the lower parts.

Figure 32: *P. palmata* growth on different mesh net types; **A**) Big mesh width net covered with micro- and macro algae. **B**) Small mesh width net covered with various fouling organisms.

4 Discussion

4.1 Induction of fertility

The present experiment demonstrated induction of sori in *P. palmata* collected late autumn, just before the natural occurrence of spore-containing fronds are present. A study conducted by Bøe (2019) indicated that the natural peak in fertility-season was between December and April in Norway. Thus, the optimal season for deployment of *P. palmata* at sea and the length of the cultivation period has not determined for the Norwegian coast so far (Schmedes, 2020; Yuan et al., 2009). Whereas the time from spring and well into summer is known as a season to elicit high growth rate, as solar light fuels autotrophic photosynthesis and promotes the build-up of nutrient reserves in macroalgae (Faes & Viejo, 2003; Martínez et al., 2006; Sanderson, 2006). As the natural Norwegian season for fertility in *P. palmata* is short and occurs just before and during the best season for macroalgae grow out, it constitutes a mismatch: between growing *P. palmata* in the laboratory of sufficient size for grow out at sea, and deploying them in the most suitable season for macroalgae (Pang & Lüning, 2006). This makes the need for a successful induction method crucial to exploit the season with the highest growth rate in the open sea.

Looking at the total sori-induced fronds (Nov. 22nd) and the difference in percentage induced fronds between short day (SD) and long day (LD) conditions (Fig.15), the difference in induced fronds is small. Of the vegetative fronds collected in November, 21-48% of the fronds exposed to SD and 25-41% of the fronds exposed to LD, developed sori within 14-20 days. The small differences in development of sori in fronds suggests that long daylength does not inhibit fertility induction in fronds collected in the season just before the peak in fertility, supportive of earlier studies (Edwards, 2008). The findings support previous studies regarding the fact that the change in daylength and spectral light in autumn have been perceived by the fronds, and that the cellular mechanism to onset sporangial initials has already been triggered. Hence, abrupt changes in light regime does not reverse their maturation when first initiated (Breeman & Hoopen, 1987; Dring & West, 1983; Schmedes, 2020).

The fact that fronds with SD conditions induced the most fronds with sori, correlates with studies showing that algae that are naturally reproductive in the winter season, reproduce during SD conditions in the laboratory (Agrawal, 2012). With this, SD with low temperatures function as an "early warning system" for inducing fertility (Pang & Lüning, 2006). The reason why it functions as a trigger may be seen in a biographical view, where it reflects the environmental conditions of the species (Kain, 1986). In this experiment when the fronds were field-collected late autumn and placed in tanks to induce fertility in SD and 10℃, they were taken from an environment that was similar to the laboratory environment. This emphasises the fact that the fronds were collected in a time near the start of the peak season of fertility, where similar conditions at the laboratory as in nature functioned as a simulation of the biographical environment in the reproductive season. This caused a higher number of fronds induced in SD condition to become fertile compared to fronds induced in LD condition. To our knowledge there are no other studies on induction of fertility in *P. palmata* in LD conditions, resulting in no literature to compare the result from the induction in this experiment. There were registered 25% sori-induced fronds in LD conditions on the first date of notable fertility (Nov. 16th), compared to 21% in SD

conditions at the same date. This is in contrast with the remaining dates of registered induced fertility, where SD had a higher number compared to LD. This may be due to individual differences between the fronds, as there may be individual perceptions of the environment which controls the timing of fertility in each frond (Edwards, 2008). A large-scale laboratory study on the differences in induction in different light regimes would provide a better answer to whether the differences are due to individual differences in fronds, or as an effect of light regime.

The results show that fertility can be induced in the laboratory, preferably under SD conditions, when induced in late autumn approaching the peak season of fertility. The possibility to manipulate the induction of sori during a season where it is naturally absent, gives huge opportunities to extend the seedling production season, and thus time it with a suited season for growth at sea (Schmedes, 2020). Controlling and inducing fertility in the laboratory can lead to a better exploitation of the fertile fronds, by initiating the process of spore release when the first signs of sori appears.

4.2 Repeated spore release

Compared to other macroalgae species such as *S. latissima*, where short-term spore release results in sufficient spore release (Kerrison et al., 2018), the most used method for a sufficient spore release of *P. palmata* is a three-day spore release method (Pang & Lüning, 2006). This method was the basis of how spore release was conducted in the present study. For the fronds subjected to two days recovery time, five rounds of repeated spore release in both SD and LD conditions were performed, whereas for the fronds with seven days recovery time, it was repeated three times. All rounds resulted in released spores, revealing that the same fronds were able to release spores repeatedly after a period of recovery. The present study suggests an opportunity to enhance a higher spore density from *P. palmata* with a long-term spore release, prolonged from the three-day spore release method. This would also reduce a problem in cultivation of *P. palmata,* regarding the requirement of large amounts of fertile tissue to obtain sufficient amounts of spores (Le Gall et al., 2004).

To examine when sori released its spores during the three days of spore release method, samples were taken every 24, 48 and 72h. Looking at the results from both recovery times and light regimes, the highest spore density was mainly measured after 72h spore release inn all repeated rounds. The exception is the fronds with two days recovery time subjected to LD conditions, where the highest density was measured after 48h in SR2. These results are a bit conflicting compared to a similar study conducted by Bøe (2019), where the highest spore density was found after 48h in a three-day dark phase spore release. In the present study, significant difference in spore density in SR4 for two days recovery were measured, where spore density in LD were significantly higher than SD after 24 and 48h. The opposite was measured for seven days recovery, where in SR1 spore density from SD conditions was significantly higher than spore density in LD conditions after 72h. It should be kept in mind that the results are based on small sample sizes, which affects the reliability and gives varying results when testing the relationship in the experiment for repeated spore release. From the repeated spore release rounds, there were no significant differences in spore release between the rounds with either two days or seven days recovery. Although, when looking at spore density between the repeated spore release rounds in both recovery times (Fig.16;17), big differences between the rounds are visible

in the figures. As a small-scaled experiment, extreme outliers in the data become more prominent and influence the results when visually exploring them in the figures, making the differences looks more extreme than the reality of the experiment. Besides this, spores from *P. palmata* are naturally sticky as it releases mucilage with spore release, resulting in settlement of nested spore aggregates in the bottom of the beakers (Hoffmann, 1987). The sticky nature may have affected the sampling from the beakers in each given times, giving varying results in the experiment as well as the conflicting results compared to the study conducted by Bøe (2019).

The photoperiod in the present study were unique during recovery time, by the timing of light and darkness in a 24-hour cycle. Several experimental studies have demonstrated photoperiodic control of reproduction status in species with isomorphic phases, where species of red algae have shown true photoperiodic control of spore production under laboratory conditions (Guiry, 1984; Guiry & Cunningham, 1984; Hoffmann, 1987; Lobban & Wynne, 1981; Rietema & Breeman, 1982). With a genuine photoperiodic response (Lobban & Wynne, 1981; Santelices, 1990) it was thought that *P. palmata* would have a response to the different recovery times and light regimes that would influence spore density in the three day spore release method. An old study found that *P. palmata,* with its distinct reproductive period, was most likely to respond to short days (Kain, 1986). Because short day condition simulates winter-like conditions, and is thought to function as a warning system, making sori release spores as it simulated the natural environment during fertile season for *P. palmata* (Dring, 1984; Lüning, 1991). Contradictory, LD conditions mimic spring season (Martínez et al., 2006; Schmedes, 2020), and studies have implied that long day condition inhibits reproduction (Kain, 1987; Lüning, 1988). No significant differences in spore density between the different light regimes and recovery times were detected. Yet, in accordance with the literature the mean highest spore density from all rounds of repeated spore release was found for the fronds subjected to SD in both recovery times (Fig.18).

Looking simultaneously at spore density from spore release and spore density in connecting recovery time, it varies when the peak in spore density is observed. For two days recovery time, the highest spore density was observed in SR2, for both light regimes. For seven days recovery time, the highest spore density was observed in SR1 for both light regimes, but for LD the spore density in R1 was higher than SR1. An earlier experiment on consecutive use of the same sori for seeding purposes reported promising results after using the same sori for nine days (Schmedes & Nielsen, 2020b). The study showed promising results compared to the conventional three-days method, where the highest seeding densities were found after periods of 3-6 and 6-9 days. The finding of highest spore density after a prolonged period, and not in the first three days of spore release, seems to agree somewhat with the present study. The fact that the highest density was observed in SR2 with two days recovery time, means it had already been subjected to SR1 and R1 (total of five days) before peaking (Appendix 1, Fig.33;35). As the highest density was observed in R1 for fronds with seven days of recovery and LD condition, the fronds had been subjected to SR1 and seven days of recovery time (total of ten days) before peaking (Appendix 1, Fig.36). The late peak of spore density can be explained by several studies, showing large differences in area of sori and sori ripeness in fertile tissue, causing high variations in timing and yield of spore release (Fleurence et al., 2018; Werner & Dring, 2011a; Wood, 2018). These studies seem to agree with the current experiment, where extra time to develop sori seemed to be necessary due to delayed peak in spore density. The individual differences in ripeness can also explain why the fronds with seven-day

recovery time and SD condition peaked in SR1, in contrast to the other fronds in the experiment these fronds might have been riper. In addition, studies shows that fronds collected in early stages of natural fertility only have sori covering 8-10% of the total frond surface (Werner & Dring, 2011a). Which end of the scale fronds in spore release are, influences the result in a small-scaled laboratory experiment as this. Overall, the results shows that a prolonged period of spore release seems to reduce the problem with unpredictable ripeness and small area of sori.

When subjected to recovery time, the fronds were simultaneously exposed to SD or LD, with dark phases of 16h and 10h, respectively. Pang & Lüning (2006) found in their study that around 80% of the spores were released during the dark phase, and the remaining 20% were released during the light phase. This means, when exposing fronds to recovery time between three days of spore release with a light regime consisting of light and darkness, the fronds will continue to release spores, as the results from this study have confirmed. The sample sizes were small, and there was a large variability in spore releases between the different rounds of spore release. Due to this, the probability of a type II error in the statistical analysis is high (failing to find a statistic difference when there really is one). This makes it difficult to conclude whether the variation in spore release was because the fronds were subjected to different light regimes and recovery times, or mainly because of casualties. Even without significant difference, comparing the spore density in repeated spore release and recovery time shows a spore density that is almost just as high in spore release and recovery time (Fig.21). From this, the result from fronds with SD and two days recovery time differs from the other fronds. The spore density in SR1-SR5 was significantly higher compared to spore density in R1-R4. Based on that finding, a large-scaled laboratory study should be performed to control the findings to remove uncertainties regarding individual differences in the fronds that become prominent in a small-scaled experiment as this present study.

In conclusion, by ensuring a recovery time of two days with SD conditions consisting of 8h light and 16h darkness, repeated spore release from *P. palmata* can be performed. This represents an opportunity for a more sustainable utilisation of the sori as it reduces the requirement of large amount of fertile tissue.

4.3 Nursing stage

The present study was conducted with two different approaches regarding how the seedlings were nursed and it was investigated how spores released from fertile fronds of *P. palmata* developed using the two different methods. The nursing stage is important for spore attachment and further growth of seedlings to be ready for deployment, where young male seedlings (gametophytes) are nursed for approximately 1-5 months before deployed at sea (Morgan & Simpson, 1981; Werner & Dring, 2011a).

In the pre-incubation method, where directly seeded substrates were placed in cylinder-tanks, samples from the substrates revealed an insufficient seedling density and growth. This in turn created a lot of room for other fouling organisms to attach and grow. Weekly inspections revealed that opportunistic organisms started to grow on all types of substrates a short period after placing them in the cylinder-tanks. The light source was placed in the middle of the tank, and inspection of the substrates revealed that all the substrates had high growth of fouling organisms, where the effect of light was especially noticeable on the

entwined spools. On the side of the spool facing the light source, there was markedly more fouling present (Fig.26C). This indicates that the light was favourable for the contaminating organisms, together with the fact that the substrates hung quite near the light source. To prevent a higher fouling rate on one side of the spools, they should have been turned and shifted position more frequently. The same could have been done for the nets for a more even distribution of light. The entwined spools should also have been prepared before it was put to be directly seeded. The number of seedlings compared to the surface of the entwined spools were probably a mismatch, with too small a number of spores per substrates. To reduce the amount of seeding-substrate, the twines could have been shortened and attached to a frame, making it a flat substrate with a big surface for spores to settle on (Schmedes & Nielsen, 2020a), instead of using whole spools with twine, which is the common method for cultivating *S. latissima* (Forbord et al., 2018).

According to literature, an irradiance of about 20 µmol m^{-2} s^{-1} will maintain a slow growth rate and reduce fouling of more light-hungry competitors (Werner & Dring, 2011a). The light irradiance in the present study was set to automatically reduce to 15 µmol m^{-2} s^{-1} after 30 days, and hence below the recommended irradiance according to literature. All three of the cylinder-tanks were connected to the same control system regulating the environmental conditions during nursing stage. The automatic adjustments were not working properly the entire time during the nursing stage, making light irradiance fluctuate, which was revealed by light measurements conducted manually during the period. When light fluctuated it was manually fixed in the program, but fluctuations still occurred, contributing to less control over the conditions. The light irradiance was measured to be higher than programmed. Whereas higher light irradiance was in favour of the light-hungry competitors (Werner & Dring, 2011a), literature shows that newly released macroalgae spores settles in shaded habitats to prevent high irradiance (Santelices, 1990), which makes unwanted high irradiance a contribution to poor seedling growth. This can be one explanation to why this present experiment was unsuccessful compared to the study it was based on. The method for setting up the experiment on nursing directly seeded substrates and the conditions during the period was based on earlier studies (Schmedes, 2020), where the growth success of seedlings was effective. However, other factors such as human errors can affect growth success rate. In addition, *P. palmata* is a quite new species for cultivation and it is challenging to achieve a successful cultivation process, even by following a detailed protocol that is often experience-based.

In the method for the nursing stage where seedlings were grown free-floating in bubble cultures, the ones nursed with the "low light-low nutrient"-treatment had the highest growth, compared to the "higher light intensity" and "higher nutrient enrichment" treatments. In all the treatments there was observed less growth after 18 days, but further at day 122 the mean seedling growth was significantly higher compared to day 0 in the "low light-low nutrient"-treatment. The other two treatments had too high amounts of fouling present at day 122, making the samples impossible to analyse. High level of nutrients stimulates growth of other algae and not just for *P. palmata*. It is nearly impossible to keep other algae out of the culture as they can find their way in multiple ways; either through the seawater supply or by being attached to the fronds of *P. palmata,* if the disinfection step was insufficient (Werner & Dring, 2011a). This result coincide with the work conducted by Schmedes and Nielsen (2020a), where they nursed bubble cultures with low light, low temperature, and low nutrient enrichment. The setup of the "low light-low nutrient"-treatment in the current experiment was based on that study, with the only difference being temperature settings. Their study was conducted in temperatures at 5℃,

whereas this experiment was conducted at 10℃, due to limitations related to laboratory facilities. The result from growing the cultures at 10℃ shows that the temperatures can be set to 5-10℃ and still give satisfying growth. The result from both "higher light intensity" and "higher nutrient enrichment" treatments, supports the use of the "low light-low nutrient"-treatment protocol, showing that both higher light and higher nutrient seem to give opportunistic organisms the perfect conditions to grow and dominate the cultures, and by this preventing successful growth of *P. palmata* seedlings.

As a comparison between the two different nursing stage methods, it was hypothesised that the directly seeded, pre-incubated substrates would have more fouling compared to seedlings nursed in bubble cultures. Based on manual inspections, the directly seeded nets showed a higher occurrence of fouling organisms compared to the bubble cultures. However, higher light intensity and higher nutrient enrichment in the bubble cultures appeared to have almost just as high growth of fouling as the seeded substrates. Both methods demonstrate that the main problem in the nursing stage is fouling of other organisms. It is nearly impossible to cultivate without interruptions of other algae, the challenge is to optimise the condition to be only in favour of *P. palmata* (Schmedes, 2020; Werner & Dring, 2011a). After the nursing stage, the seedlings should preferably have been around 0.5-1 cm in length, a recommended size for deployment at sea to reduce negative effects of fouling and to enhance further growth (Schmedes, 2020). Neither of the two methods showed seedlings in that size order and was hence a deficient starting point for deployment for further sea cultivation.

Combining two methods of producing *P. palmata* seedlings, may lead to a better exploitation of the spores. As Schmedes and Nielsen (2020a) stated, the method using 1:1 areal coverage of seeding-substrates and sori leads to a considerable number of spores settling on the tank surfaces, instead of the seeding-substrate. By scraping the settled spores on the tank surface, they could come to use instead of being a waste of the directly seeding of substrates for pre-incubation method. By cultivating the scraped spores further in bubble cultures, the method of directly seeded substrates could have two outcomes in seedling production. Additionally, by having a long nursing stage in the hatchery phase it constitutes a high cost (Morgan & Simpson, 1981). Except, the cost might be worth it if the nursing phase could be used to time the cultivation at sea to a deployment timing suitable for *P. palmata* and providing sufficient biomass for harvest.

4.4 *P. palmata* seedlings deployed at an open-sea farm

The substrates deployed at the open-sea farm did not have enough *P. Palmata* and were covered with a lot of fouling organisms. Literature shows that epiphytes can be controlled for cultures on rope in sea by growing in high densities (Lüning & Pang, 2003). Previous cultivation trials show that a spore density around 100 spores cm^{-1} on seeding-substrates is ideal for a successful growth (Demetropoulos & Langdon, 2004). In this present study, the seedling density was not taken in consideration during seeding in either of the nursing methods. Samples taken from the pre-incubated substrates did not show any signs of seedlings, and based on this, the seedling density was thought to be low and sporadic. For the algae ropes, calculations were performed to estimate the relationship between rope, algae binder, and bubble culture, but due to varying success in the seedling growth in the bubble cultures together with fouling organisms, the density of seedlings is thought to have not been ideal.

In January, the whole open-sea farm fell two meters down and hung at 4 m depth instead of the set-up of 2 m, until it was discovered and adjusted the same month. The extra depth of two meters resulted in reduced light exposure for a period, and a reduction of light absorption is a limiting factor for growth of macroalgae (Burrows, 2012; Cronin & Hay, 1996). Consequently, the environment for the seeded substrates was not ideal for a short period of time and is thought to have played a negative impact on the growth of *P. palmata*, as light availability in sea is determinative for growth (Gerard, 1988; Lobban & Harrison, 1994).

The first inspection on the growth success for *P. palmata* in March revealed that both the nets, lines, and algae ropes were densely covered by contaminating epiphytic micro- and macroalgae, which seemed to have outcompeted seedlings of *P. palmata*. Only small fronds of *P. palmata* were discovered, mainly on the two different nets (N40 and N150). The growth conditions during autumn includes high nutrient availability, temperature, currents, and light conditions which affected the development of the seedlings and the biomass yield in the sea (Kerrison et al., 2015). According to some literature, the best season for deployment is winter, when there is low temperature and little light for growth of fouling algae (werner & Dring, 2011a), allowing seedlings of *P. palmata* to cover the substrates before fouling organisms get the right environment for growth. The present study disagrees with the literature regarding suitable conditions for *P. palmata*, but it is thought to be a result of substrates that were already covered with fouling organisms before deployment, thus the results cannot be used to reject if winter is a suitable season to deploy *P. palmata*.

The second inspection at the open-sea farm was in late April and revealed further growth of *P. palmata* on the nets that was discovered during the first inspection. Just a few *P. palmata* were discovered on the ropes, whereas only one was discovered on the algae ropes. The net with the biggest mesh width had the densest growth of seedlings. Yet, a large amount of fouling of other organisms was still observed. A similar study on the use of different mesh width, discovered that the smallest mesh width had the densest growth of seedlings (Bøe, 2019). However, in this study the density of seedlings on both mesh nets were close to similar; with 21 seedlings for the big mesh net and 14 seedlings for the small mesh net. As mentioned in section 2.5.2, the entwined spools were twisted on thicker polypropylene ropes by using an electrical twisting machine before deployment in sea. Consequently, during the twisting machine process seedlings were prone to fall off, affecting the growth out in the sea. Even with small amounts of growth, the present study suggest that nets might be suitable for cultivation of *P. palmata* in comparison to ropes and algae ropes.

With deployment at an open-sea farm with monoculture conditions in the present study, the results can be compared to studies conducted by Schmedes (2020) and Bøe (2019). In their studies, seedlings were deployed in an integrated multi-trophic aquaculture (IMTA), inside a salmon farm with exposed waters. Their results showed that the growth of seedlings on nets were successful. Conversely, *P. palmata* seedlings on strings were unsuccessful. An earlier experiment investigated growth of seaweed close to fish farms cages in the Northwest Scotland (Sanderson et al., 2012). Yields and enhanced growth rate of *P. palmata* cultures were found grown adjacent to fish farm cages. The nitrogen content of the macroalgae grown adjacent to the fish farm cages was greater, compared to those grown at a reference site and away from the cages (Sanderson et al., 2012). Comparing it with the success from the present study, it shows that deploying *P. palmata*

in an IMTA situation could potentially increase growth compared to a monoculture farm system.

In the present study, no fronds of *P. palmata* had an optimal size for harvesting, where optimal harvesting size of *P. palmata* should be around 30-40 cm (Sanderson et al., 2012). Hence the grow out at sea was not promising, due to low density and sizes of *P. palmata*, and that high fouling of other micro- and macroalgal species were observed. Seedlings of *P. palmata* were deployed at a new system and in a new location during autumn. Consequently, there was not enough knowledge about the conditions in the sea where the farm was placed for an optimal cultivation process of the species *P. palmata*. Literature emphasize the importance of deployment timing, seedling size and density, cultivation depth, cultivation site, optimal deployment, and configuration for maximizing the production (Kim et al., 2017; Schmedes, 2020). With all the contributing factors, it could potentially take up to several years to cultivate *P. palmata* in an efficient way. It must also be taken in consideration that the cultivation of *P. palmata* was conducted together with other macroalgae species. This might have influenced the growth success, and further studies should be performed to discover the impact of co-cultivation of *P. palmata*.

4.5 Implications for cultivation of *P. palmata* and further studies

In retrospect, the experimental set up of the repeated spore release could have been performed in a more controlled manner: as it was performed in the experiment, it was based on the number of fronds that induced sori at the laboratory, and how many spores that were released at given times from these fronds. The number of released spores was counted manually and divided by the number of fronds to get a number on released spores from one frond. This was done because the number of fronds during spore release differed between the parallels. To be able to say more about the ripeness and number of released spores, the area of sori should have been measured so the number of released spores could have been counted as number of spores released per mm^2 sori. Along with washing each beaker to get more accurate number of released spores at given times, the results would have been more accurate. However, the spore release revealed that the same fronds could release spores repeatedly from the same sori. This could provide valuable information in the work of improving the hatchery protocol in the future. The present experiment did not include any further use of the released spores. As Schmedes (2020) demonstrated in a study, seedlings were found after 17 days of spore release. A prolonged spore release protocol requires a use of the released spores before the end of the prolonged spore release period is over, as spores starts to grow into seedlings before the period is over. A possible way of using the spores further is to directly seed spores on substrates, or to store them in bubble cultures, both ways were demonstrated in another part of this study. Further investigation on how recovery time between repeated spore release rounds affects the spore release, compared to continuous spore release without recovery time needs to be studied.

P. palmata is known to be epiphytic on several algae and in particular near shallow water (Whittick, 1983). It occurs in abundance as an epiphyte on the smooth stipe of *Laminaria digitata* and on the upper part of *Laminaria hyperborean* (Whittick, 1983). This indicates either a restricted distribution of spores by specific requirements, or spore dispersal for survival and settlement (Irvine & Guiry, 1983; Santelices, 1990). However, it would be interesting to include these species in the cultivation of *P. palmata* in further studies, to

investigate if it might increase yield and biomass due to co-cultivation with familiar species, by simulating a natural environment. The use of disinfectant in macroalgae cultivation is a costly part of the process, and if co-cultivation with other species would result in promising outcomes, the disinfection would play an even bigger part. During the whole experimental period, GeO_2 was used due to fouling of benthic diatoms. The problem with this disinfection is the cost, GeO_2 is expensive and cannot be used on an industrial scale, but since it is a good disinfectant it was used in this small-scaled experiment (Shea & Chopin, 2007). In further research, something new should be tested to minimize the disinfection-process expenses.

As the treatments for bubble cultures in this present study were not as successful as expected, a new attempt to optimize the nursing stage should be performed. Since the most prominent bubble culture was the one with a "low light-low nutrient" treatment, small adjustments based on this could result in a better outcome: by small adjustments on the light regime and contents of nutrients. A better treatment during the nursing stage to grow adequate seedlings for deployment is crucial for the development of the seedlings in an open sea system (Schmedes, 2020).

Deployment of *P. palmata* based on fronds induced prior to the fertility season should be timed to be conducted in February. This would give the seedlings time to develop for four months in the nursing stage if they are induced late October or early November, before being deployed in a time where there is a rising level of nutrients and adequate growth opportunities for the algae (Faes & Viejo, 2003; Martínez et al., 2006; Sanderson, 2006). However, the effect of substrate type for outgrowth at sea is a key importance regarding density of *P. palmata* biomass and to lower the production cost of macroalgae grow out (Kerrison et al., 2017). Future research should investigate optimal net dimensions for the maintenance and handling of seeded substrates in the nursery, when kept for long periods, in order to achieve a more successful cultivation at sea (Kerrison et al., 2017). An important task in *P. palmata* cultivation at open-sea farm is to identify the best-suited grow out strategy according to environment, harvest technology, operational costs, and production volume (Kerrison et al., 2017). In future studies it would be interesting to determine *P. palmata* growth by measuring the plant every month to specify growth rate in the Norwegian open waters.

5 Conclusion

Fertility can be induced in fronds collected prior to the reproductive season in Norway, by manipulating the light regime under laboratory conditions. The study suggests that sori may have a higher chance of getting induced in fronds subjected to short day conditions (8h light, 16h darkness), compared to long day conditions (14h light, 10h darkness).

A way to enhance higher spore density from *P. palmata* by exposing the same fronds to repeated spore release was demonstrated. Fronds subjected to SD in recovery time showed the highest spore density in the repeated spore release rounds. The highest spore density during the recovery time were in the light regime with LD condition and seven days of recovery time. With a hatchery protocol with recovery time between repeated rounds of spore release, a recovery time of two days and a light regime simulating short days is recommended. This as the approach provided a significantly higher spore density in spore release compared to recovery time.

Nursing seedlings in bubble cultures with "low light-low nutrient"-treatment resulted in a higher growth rate, compared to seedling nursed in the treatments "higher light intensity" and "higher nutrient enrichment". In bubble cultures with treatments "high light intensity" and "nutrient enrichment", the growth of *P. palmata* seedlings was supressed because of competition with opportunistic microalgae, which were unintentionally given a favourable environment to overtake the cultures. The growth success of seedlings was higher in bubble cultures compared to seedlings grown directly on substrates.

Special algae ropes, binder-seeded with seedlings from bubble cultures before deployment in the sea farm, had no successful growth. The present study suggests using nets as substrates when it's directly seeded for pre-incubation until deployment at an open-sea farm for further cultivation. In accordance with previous literature, the use of twines, i.e., thin, seeded lines that are spooled onto thicker carrier ropes in the sea farm, was unsuccessful, contributing to a recommendation to not use twines as seeding substrate for future cultivation trial of *P. palmata*. The unsuccessful cultivation trial at an open-sea farm is thought to be a result of too low seeding density prior to sea deployment and shows how sensitive *P. palmata* is regarding seeding density on substrates. Low seeding densities at the substrates may in turn enable fast growing of benthic diatoms to take over.

In line with the overall objective of this master's thesis, conducted experiments have provided results that might be implemented to improve critical steps in the cultivation process of *P. palmata* in the future, during both A) hatchery and seedling production phase and B) cultivation at an open-sea farm phase.

6 References

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Appendix 1

Figure 33 shows mean value of released spores over a three-day period in each spore release round (SR1-SR5) and released spores in two days recovery time for each round of recovery time (R-1 to R-4) in SD.

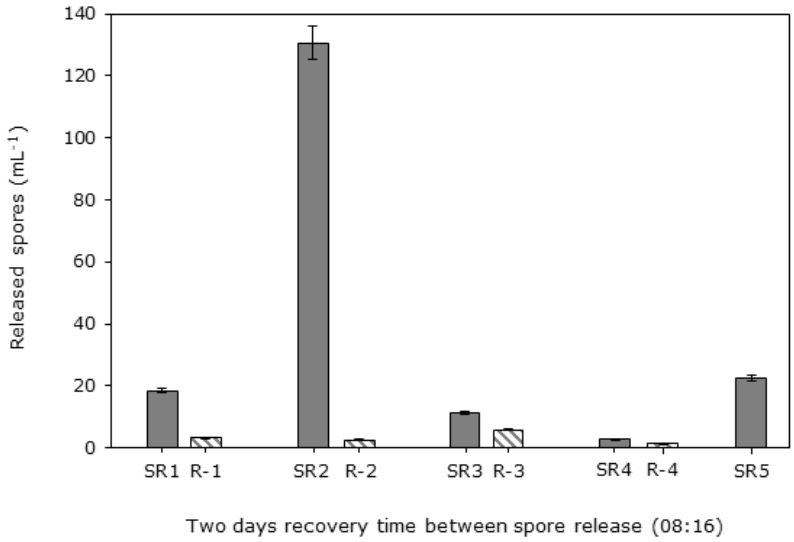


Figure 33: A comparison between spore density in repeated spore release with a three-day duration, and the released spores in the two days recovery time between the repeated spore release rounds. The bars indicate spore density. Data points given as mean ± SE (n=12 for SR1-SR4 and n=6 for SR5, n=4 for R-1 to R-3, and n=2 for R-4).

Figure 34 shows mean value of released spores over a three-day period in each spore release round (SR1-SR3) and released spores in seven days recovery time for each round of recovery time (R-1 to R-2) in SD.

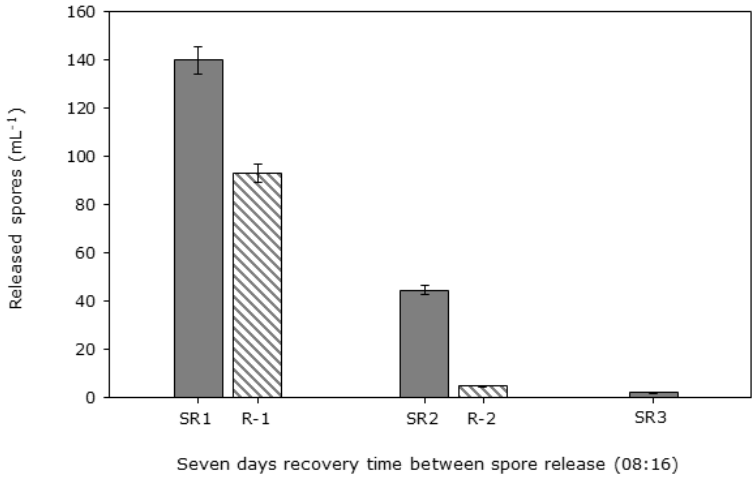


Figure 34: A comparison between the released spores in repeated spore release with a three-day duration, and the released spores in the seven days recovery time between the repeated spore release rounds. The bars indicate spore density in. Data points given as mean ± SE (n=15 for SR1-SR2 and n=12 for SR3, n=4 for R-1 and R-2).

Figure 35 shows mean value of released spores over a three-day period in each spore release round (SR1-SR5) and released spores in two days recovery time for each round of recovery time (R-1 to R-4) in LD.

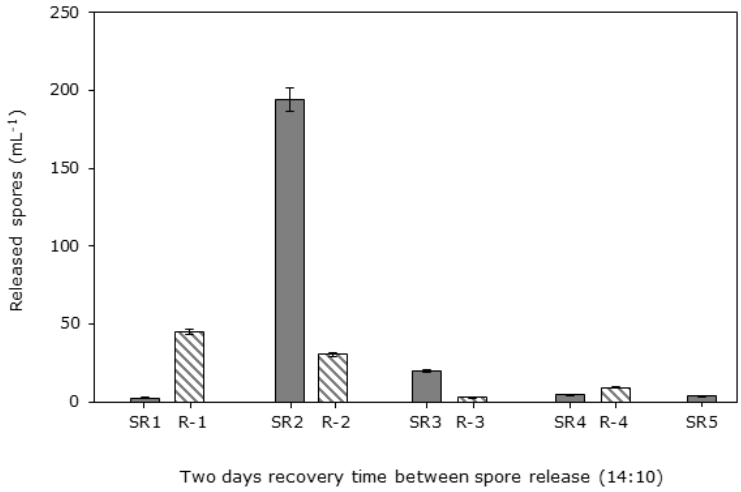


Figure 35: A comparison between the released spores in repeated spore release with a three-day duration, and the released spores in the two days recovery time between the repeated spore release rounds. The bars indicate spore density. Data points given as mean ± SE (n=12 for SR1-SR4 and n=6 for SR5, n=4 for R-1 to R-3, and n=2 for R-4).

Figure 36 shows mean value of released spores over a three-day period in each spore release round (SR1-SR3) and released spores in seven days recovery time for each round of recovery time (R-1 to R-2) in LD.

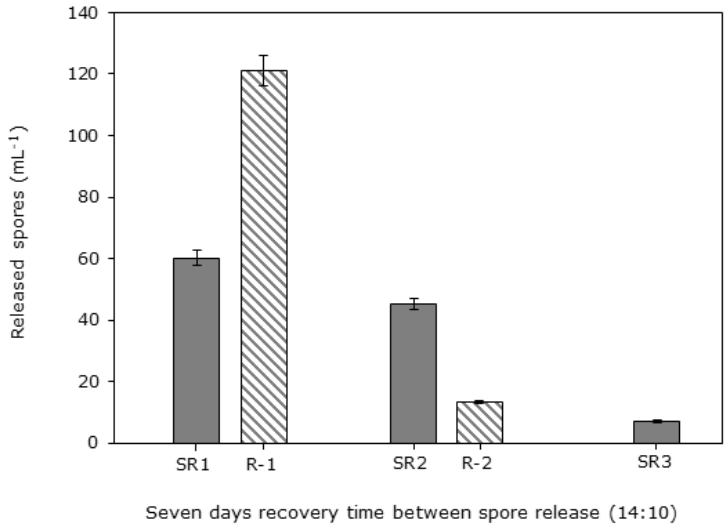
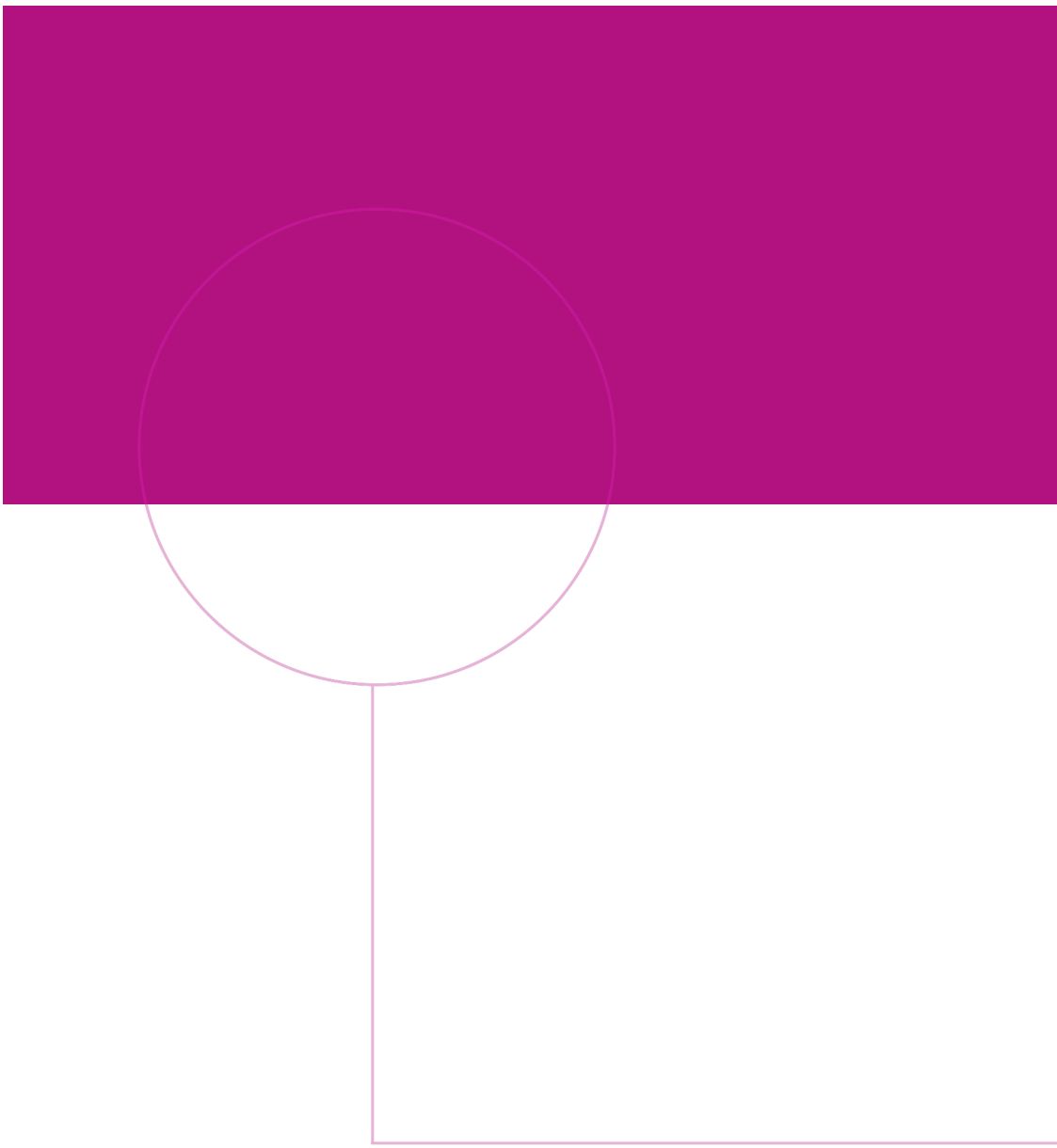


Figure 36: A comparison between the released spores in repeated spore release with a three-day duration, and the released spores in the seven days recovery time between the repeated spore release rounds. The bars indicate spore density. Data points given as mean ± SE (n=15 for SR1-SR2 and n=12 for SR3, n=4 for R-1 and R-2).



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