Renate Rimstad Bøe

Investigation of important steps in *Palmaria palmata* cultivation

Master's thesis in Ocean Resources
Supervisor: Kjell Inge Reitan
August 2019

Master's thesis

NTNU
Norwegian University of Science and Technology
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Kunnskap for en bedre verden

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Trondheim, August 2019

Renate Rimstad Bøe

Abstract

Increased demand of the red macroalgae *Palmaria palmata* raises the interest of cultivating this specie in Norway. More research within important steps in *P. palmata* cultivation is necessary in order for successful large-scale cultivation. In this present thesis, it was desirable to investigate life stages of *P. palmata* to solve some of the bottleneck in *P. palmata* seedling cultivation.

A survey on the year-round health and reproductive status of *P. palmata* in Trondheim revealed that collecting sporophytes during winter may secure high amount of fertile and healthy sporophytes suitable for spore release (highest in January). The survey also investigated the distribution of fertile tetraporophytes and fertile male gametophytes, suggesting that high availability (20- 40 %) of fertile male gametophytes during the reproduction season can be utilize.

Two spore release trials over three days, one in January and one in November, examined when spore release had its highest density. Spore release rate seemed to be seasonal dependent, and spore release with sori from sporophytes collected within the reproductive season demonstrated highest spore density after 28 hours of spore release.

The effect of four chemical disinfectants at different concentrations was investigated in order to reduce diatom contamination. The most promising disinfection treatments was immerging sori from sporophytes collected within the reproductive season in 0.5 mL L^{-1} germanium dioxide solution (GeO_2) for 2 minutes in an exposure temperature of 10 °C followed by two rinsing baths of sterile seawater for 30 seconds. This treatment significantly reduced diatom contamination without affecting seedling development and survival and gave the longest seedlings after 21 days of incubation. Disinfection of sori from sporophytes collected outside the reproductive season in GeO_2 showed a delaying effect on spore to seedling development. 0.1 mL L^{-1} GeO_2 added in growth medium in *P. palmata* cultures was also investigated as a way of controlling diatom contamination. The addition of GeO_2 showed no significant effect on seedling survival. Sori disinfection with 0.02 % and 0.2 % Lugol, 1 % and 7 % acidic acid and 300 ppm and 600 ppm of sodium hypochlorite reduced tetraspore survival and should not be recommended in *P. palmata* cultivation.

The present study also includes the first deployment of cultivated *P. palmata* seedling in Norwegian waters. Seedlings were deployed on nets and strings in an integrated multitrophic

aquaculture (IMTA) farm, and seedling on nets showed successful growth after 4 months in sea.

Sammendrag

Økt etterspørsel av rødalgen *Palmaria palmata* øker interessen for kultivering av denne arten i Norge. Mer forskning innen viktige trinn i *P. palmata* kultivering er nødvendig for at stor skala kultivering skal bli mulig. I denne masteroppgaven var det ønskelig å undersøke livsstadier av *P. palmata* for å kunne løse noen av flaskehalsene i *P. palmata* kultivering.

En undersøkelse av den helårlige helse og reproduktive statusen av P. palmata i Trondheim viste at innsamling av sporofytter gjennom vinteren kan sikre høyt antall fertile of friske sporofytter passende for sporeslipp (høyest i januar). Undersøkelsen så også på distribusjonen av fertile tetrasporofytter og fertile hann gametofytter, og forslår at høy tilgjengelighet (20-40 %) av fertile hann gametofytter gjennom den reproduktive sesongen kan bli utnyttet.

To sporeslipp forsøk over tre dager, et i januar og et i november, undersøkte når sporeslipp har den høyeste tettheten. Sporeslipp raten syntes å være sesongavhengig, og sporeslipp med sori fra sporofytter innsamlet innenfor den reproduktive sesongen demonstrerte høyest spore tetthet etter 28 timer sporeslipp.

Effekten av fire desinfeksjonsmidler ved ulike konsentrasjoner var undersøkt for å redusere diatom kontaminering. Den mest lovende desinfiserings behandlingen var å senke sori fra sporofytter innsamlet i den reproduktive sesongen i 0,5 mL L^{-1} germanium dioxide løsning (GeO_2) i 2 minutter i en eksponeringstemperatur på 10°C etterfulgt av to rensende bad med sterilt sjøvann i 30 sekunder. Denne behandlingen redusere diatom kontamineringen signifikant uten å påvirke utviklingen og overlevelsen til kimplantene og gav de lengste kimplantene etter 21 dages inkubering. Desinfisering av sori fra sporofytter innsamlet utenom den reproduktive sesongen i GeO_2 viste en forsinkende effekt på spore til kimplante utviklingen. 0,1 mL L^{-1} GeO_2 tilsatt i vekstmediet i *P. palmata* kulturer var også undersøkt som en måte å kontrollere diatom kontaminering. Tilsetningen av GeO_2 viste ingen signifikant effekt på kimplante overlevelse. Sori desinfisert med 0,02 % og 0,2 % Lugol, 1 % og 7 % eddiksyre of 300 ppm og 600 ppm natriumhyperkloritt reduserte teraspore overlevelsen og bør ikke anbefales i *P. palmata* kultivering.

Denne masteroppgaven inkluderer også den første utplasseringen av kultiverte *P. palmata* kimplanter i norsk farvann. Kimplanter ble utplassert på nett og tau i en integrert multitrofisk akvakultur (IMTA) lokalitet, og kimplanter på nett viste vellykket vekst etter 4 måneder i sjø.

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1 Introduction

1.1 Global aquaculture of macroalgae

The global seaweed production more than doubled from 2005 to 2016 and is today worth more than USD 6 billion per annum (FAO, 2018). Macroalgae cultivation has been a fast-growing industry. The variety of possible uses have created a demand of macroalgae biomass that exceeded what the supply of natural resources can offer (McHugh, 2003). In 2016, around 96 % of the volume of total 31.2 million tonnes aquatic plants were cultivated. The majority of these aquatic plants were macroalgae, often referred to as seaweed (FAO, 2018).

The biggest aquaculture seaweed producing countries are China, Indonesia, the Philippines and the Republic of Korea. In 2016, China produced 14.4 million tonnes, which stands for about 48 % of the total global production. These countries are mainly cultivating *Eucheumatoids* spp, *Shaccarina japonica, Gracilaria* spp, *Undaria pinnatifida, Kappaphycus alverezii* and *Porphyra tenera*. Of the total global production, *Eucheuma* sp. stands for around 10.5 million tonnes and *S. japonica*, for around 8.2 million tonnes. However, there are in total 221 different species of seaweed with commercial value around the world (FAO, 2018).

Seaweed production in Europe is quite low compared to Asian countries. More than 90 % of the volume are harvested from the wild in Norway, France and Ireland, with *Laminaria digitata, Laminaria hyperborea* and *Ascophyllum nodosum* being the most important species. Norway are harvesting around 90 % of biomass almost exclusively on wild stocks and has the largest seaweed industry in Europe (Friis Pedersen et al., 2013; Meland & Rebours, 2012).

The interest in seaweed cultivation has rapidly increased in the European countries. Several experimental sites are being established in order to industrialize the cultivation and advance the cultivation of native species. Several different implementation studies are also being carried out. These factors are all supporting the rising interest of seaweed cultivation (Walls et al., 2017). Norway has high competence within aquaculture and off-shore construction, as well as a long coast with high-productive areas, which are major advantages for seaweed cultivation (Skjermo et al., 2014). Number of licences for seaweed farming in Norway have reached 400 and have tripled since 2014 (Fiskeridirektoratet, 2019). Most of these companies farming sites are not commercial yet and are in the early phase of development (Stévant et al., 2017). Around 97 % of total biomass produced are *Saccarina latissima* while the remaining 3 % consists of *Alaria esculeta* and other species (Fiskeridirektoratet, 2019).

1.2 Applications of macroalgae

Seaweed products are important food resources (Mouritsen et al., 2013). In Asia, they have traditionally been used for more than 2000 years, and constitutes a great part of the total food intake (Tiwari & Troy, 2015). European countries like Ireland, Norway and Iceland, also have a long tradition of seaweed consumption. The most documented use has been on Iceland, where the consumption of "dulse" (*Palmaria palmata*) was widespread in the coastal areas. Since the 700's, this species has been used as an exchange commodity between coastal and inland residents. Irish people used seaweed in several different ways; as food, for medicine and as a sort of chewing tobacco (Mouritsen et al., 2013). Norway also have a rich cultural history associated with seaweed and it was likely used as provisions for the Norwegian Vikings long expeditions (Mouritsen & Mouritsen, 2013). Nowadays, Ireland may be the most remarkable European country within seaweed consumption. Some of the species consumed are "dulse" (*P. palmata*), "Irish Moss" (*Chondrius crispus*), and "sloke" (*Porphyra* sp).

Today, a wide range of products are provided by the seaweed industry. Products for human food consumptions contributes for about US$ 5 billion of total US$5.5 -6 billion. Hydrocolloids, substances extracted from seaweeds, are the second largest part of the total value. The remaining consists mainly of fertilizers and animal feed additives (McHugh, 2003).

A holistic health- food movement carried by the demand of new, healthy and clean food sources produced in a sustainable way are making consumption of macroalgae a trend (Mouritsen et al., 2013). The demand for macroalgae foods is growing and it is increasingly being consumed as functional food (Wells et al., 2017). Generally, macroalgae have a high content of essential amino acids, proteins and vitamins. It also consists of several trace elements and minerals, such as Ca, K, P and Na (Kim, 2011) in addition to vitally important fatty acids and dietary fibres (Mouritsen et al., 2013). Several biologically active phytochemicals such as polysaccharides, carotenoids and phycobilins are also present in seaweed (Tiwari & Troy, 2015). However, considerable challenges associated to possible adverse effects and quantifying the health benefits remains (Wells et al., 2017).

P. palmata is one of the most popular species of macroalgae for human consumption (Mouritsen et al., 2013). Today, several countries in the western world uses *P. palmata* as a dietary supplement (Mouritsen & Mouritsen, 2013). The specie constitutes high levels of

protein, normally in a range between 8-35 % of dry weight (Galland-Irmouli et al., 1999; Le Gall et al., 2004; Morgan et al., 1980). *P. palmata* is also a rich source of a variety of vitamins and the polyunsaturated fatty acid, EPA (Mishra et al., 1993). Additionally, the specie is reported to be a rich source of antioxidants (Yuan et al., 2005), and derived extract are alleged to be able to inhibit cancer cell lines with up to 78 % (Yuan & Walsh, 2006). In the cuisine and gastronomy, *P palmata* has a greatly unexplored potential (Mouritsen, 2012).

1.3 Biology of *P. palmata*

The distribution of *P. palmata* is spread from the North Arctic to Portugal in the eastern Atlantic and mid-Connecticut in the western Atlantic. It grows down to maximum 20 meters depth in the lower intertidal and shallow subtidal zone. It is found on hard substrates or as an epiphyte of *L. hyperborea, L. digitata* and on *Fucus* sp (Lüning, 1990). Normally it grows in moderately wave or current -exposed rocky shore areas. *P. palmata* is a small species with fonds being maximum 50 cm in length and 2-8 cm in width. The texture is leathery and soft, and it has a small disc resemble attachment (Mouritsen et al., 2013).

van derMeer and Todd (1980) completed the life history of *P. palmata* in cultures (Figure 1). They demonstrated the diphasic life cycle, with gametophyte generation and one diploid life stage. Tetraspores from diploid plants give male and female haploid gametophytes with different morphology. Male gametophytes require 9-12 months of vegetative growth before forming spermatangial sori. Male gametophytic fronds and tetrasporophytic fronds have similar morphology.

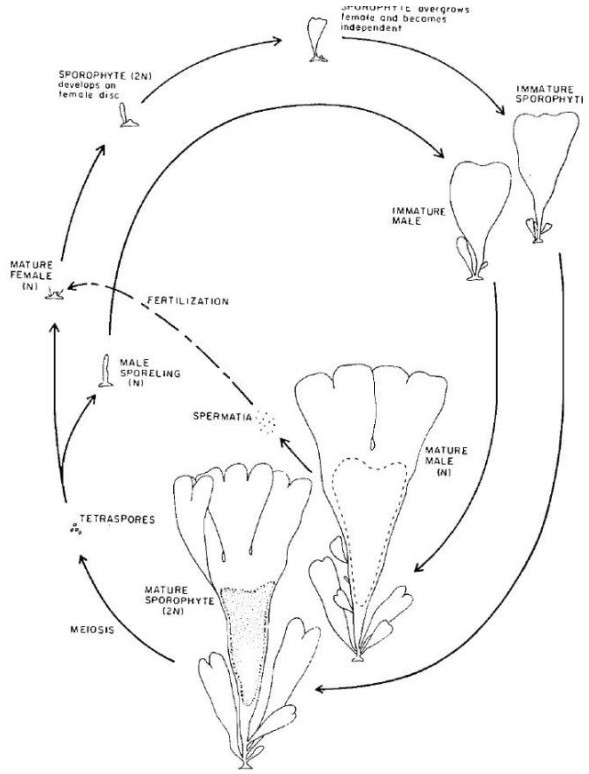

Figure 1: The life cycle of *P. palmata* (van derMeer & Todd, 1980).

Female gametophytes are microscopic and are sexual mature only days after tetraspore release. Trichogynes, the receptive organ for spermatium, can be observed on the female gametophytes only 4 days post tetraspore release. Because of this inequality between male's and female's, female gametophytes must be fertilized by older males. Tetrasporophytes develops directly on the fertilized females after a zygote is formed and overgrows the female gametophytes (van derMeer & Todd, 1980). After a year of growth, tetrasporophytes reaches maturity and develops tetraspongial sori on the fronds. Dark red, irregularly and elevated areas on the fronds characterizes the tetrasporangil sori which contains spores clustered together in packs of four. After tetraspore release, each spore measures approximately 30 µm (Le Gall et al., 2004).

1.4 Cultivation of *P. palmata*

The demand of *P. palmata* has lately increased in Europe due to the raising interest for this species in human consumption. The increasing demand, leading to high pressure on the wild resources have led to studies on large-scale cultivation of *P. palmata* in open sea culture and

in tanks (Le Gall et al., 2004; Pang & Lüning, 2006; Werner & Dring, 2011). Researchers in several countries have focused on *P. palmata* cultivation with different cultivation techniques for several years. As example, Le Gall et al. (2004) and Pang and Lüning (2004) have focused on vegetative growth in tank cultivation, while Martínez et al. (2006) have focused on growth trials in sea.

Improvements in the current cultivation techniques are needed in order to support large scale cultivation of *P. palmata*. The current techniques involve using high amounts of sori from fertile tetrasporophytes to cover substrates during spore release. After a few days, male gametophytes (seedlings) develops while female gametophytes die off. Seedlings are visible on growth substrate after 1-3 months of nursery. One of the main challenges is low spore-to seedling efficiency (Schmedes et al., 2019). General difficulties associated to seaweed cultivation such as reduction of fouling organism (Kim et al., 2017), are also highly relevant for *P. palmata* cultivation. A recent study by Schmedes et al. (2019) showed improved hatchery strategies for tetraspore release and more efficient spore use and seedling distribution. Spore release in high- agitated tanks showed increased spore yield compared to calm waters. The settlement and disposals of spores and seedlings on twine substrates were also highest in high-agitated cultures compared to cultures with non-agitated water. A study by Pang and Lüning (2006) including tetraspore release in darkness, indicated that darkness triggered spore release for *P. palmata*. In Norway, 60 allocated areas have permission to produce *P. palmata* (Fiskeridirektoratet, 2019), but the specie is still not currently grown in sea (Stévant et al., 2017).

1.5 Contamination problems in macroalgae cultivation

A large challenge in the seaweed cultivation industry is diseases and pests. Fletcher (1995) stated three main challenges; 1) competition of space, normally by fouling organisms; 2) growth of epiphytes and endophytes; 3) pathogen attacks such as viruses and bacteria.

Growth of unwanted epiphytes on the cultivated biomass are an especially great issue for the industry (Lüning & Pang, 2003). Both the commercial value of the products and the productivity of the plants are affected by the presence of epibionts. A variety of abiotic and biotic factors influences the existence of epibionts (Walls et al., 2017). When grown in non uni-algae circumstances, controlling the epiphytes are difficult. The surface of the seaweed is

exposed and gives free access to a variety of biofouling organisms like other algae when grown in sea. In addition, seaweeds are normally cultivated on a depth with large amounts of natural light, which promote the growth of algae epiphytes. However, according to Lüning and Pang (2003), epiphytes can be controlled on rope cultures in sea or in land-based tank cultures by growing the plants in high densities.

Seaweed is collected in the nature in order to stock cultivation tanks and to gather materiel for laboratory studies. These wild-harvested seaweed carries different natural epiphytic flora and fauna. These organisms can multiple and compete with the desire specie stock, which possibly can lead to a collapse. (Borowitzka et al., 2007). Different treatments have been proposed as a way of controlling and reduce the contaminants.

1.5.1 Chemical disinfection of sori

Treatments with chemical disinfectants can be helpful in order to reduce or remove contaminants (Kerrison et al., 2016c). The type of agent, the duration of exposure, the intensity, and the type of organism, are all factors affecting the response of microorganisms to disinfectants (Block, 2001).

Different chemical treatments are introduced by earlier studies. Substances successfully used for disinfection of macroalgae by forming monocultures are sodium hypochlorite (NaOCl), organic solvents, detergent, reactive iodine and formaldehyde (Kerrison et al., 2016c). For the brown algae, *S. latissima*, Forbord et al. (2018) suggests disinfection by NaOCl, but also iodine-based Betadine or Lugol's solution. Pieces of mature sori are immersed in a bath consisting of 200 ppm NaOCl, 10°C for 2 min. A study by Kientz et al. (2011) tested various disinfection solutions on five different macroalgae. A mix of ethanol (40-50 %) and NaOCl (1 %) had the greatest removal rate of epibionts without damaging the macroalgae tissue. 0.1-1 % NaOCl for 10 minutes, 0.5 % potassium iodine for up to 10 minutes, or 0.25 % Kickstart for 1 to 5 minutes are suggested for *P. palmata* sori disinfection (Kerrison et al., 2016b).

1.5.2 Use of Germanium dioxide (GeO_2)

During cultivation, the early life stages of macroalgae are exposed to contaminants like e.g. diatoms and microalgae. Germanium dioxide (GeO_2) inhibits diatom growth by interfering

with the formation of the diatom's frustule (de Almeida, 2017). Treatments with GeO_2 can be used in algae laboratory cultures in order inhibit growth of contaminating diatoms (Shea & Chopin, 2007).

Species of brown algae have demonstrated negative respond to GeO_2 (Markham & Hagmeier, 1982). Normally, concentrations between 0.1 and 0.5 mL L^{-1} of GeO_2 are used to control contamination in algae cultures (Kerrison et al., 2016b; Shea & Chopin, 2007). Shea and Chopin (2007) concluded that the effect of GeO_2 is positive until a critical point (0.5 -1.0 mL L^{-1}) is reached, where it inhibits the growth of the macroalgae seedlings. Only very high concentrations of GeO_2 have shown growth inhibitory effect on red algae (Tatewaki, 1979).

1.5.3 Acetic acid

Acetic acid has been used as a disinfectant for several centuries (Fraise et al., 2013). It has a wide microbiological range, and are useful even at low concentrations (3 %) (Ryssel et al., 2009). A major component in vinegar is acetic acid, and some of vinegars useful functions are control of microbial growth and pH reduction. Vinegar is a cheap alternative to many other disinfectants and can easily been bought in grocery shops in Norway.

1.5.4 Lugol's solution

Lugol's solution is used to preserve microorganisms and are normally consisting of around 10% potassium iodine (KI) and 5% iodine (I_2). Iodine was in 1874 found to be the most efficacious antiseptics and has been used widely in medical practices for more than a century. It is excellent in reducing the infectivity of a wide range of microbes (Guillard & Sieracki, 2005). Reactive iodine is also one of the most common disinfectants utilized for macroalgae culturing (Kerrison et al., 2016b). Different iodine disinfectant species are in a pH range between 6 and 8 very stable in water, and it is therefore also often used in water disinfection (Brion & Silverstein, 1999).

1.5.5 Sodium hypochlorite (NaOCl)

Sodium hypochlorite is a common sterilization medium in aquaculture (Qin et al., 2014) and is one of the most common disinfectant in macroalgal culturing (Kerrison et al., 2016a). The extensively use of chorine as a biocide has among other things also been used in water sterilization, sanitation of agriculture farms and in control of biofouling. Sodium hypochlorite damages the cell walls of bacteria leading to autolysis, meaning cell destruction. Normally, hypochlorite solution contains a mix of hypochlorite ion (OCl^-), hyperclonious acid (HOCl) and chloride (Cl_2) (Añasco et al., 2008).

1.5.6 Mechanical disinfection of sori

The existence of epibionts is significantly reduced by mechanical removal such as washing and sorting by hand. Nevertheless, such methods are not able to completely remove the epibionts, and the methods are considerable time-consuming. Epibionts could also be introduced later by cross-contamination or seawater refreshment (Kerrison et al., 2016b).

Protocols for reducing contamination of macroalgae are an essential tool for laboratory culture studies. These protocols must be adjusted to the specific specie and contains procedures of both physical and chemical treatments. A study found that a combination of both mechanical and chemical treatment was effective in obtaining uni-algal cultures (Fernandes et al., 2011). If fouling or sediments are present on the algae, mechanical cleaning with paper towels can be conducted before further chemical disinfection (Forbord et al., 2018). In some cases, minimizing or prevention of contaminants can be conducted through manipulation of culture conditions. Such manipulations may be high stocking densities, low nutrient levels and low light (Lüning & Pang, 2003).

1.6 Study aims and approach

The aim of this study was to investigate steps in *P. palmata* life cycle in order to solve some of the bottlenecks of the hatchery protocol. This was done by focusing on the following sub-objectives;

1) Evaluate the health and reproductive status of *P. palmata*

2) Evaluate spore release of *P. palmata*
3) Evaluate the effect on spore release, sporophyte density and sporophyte growth after exposure to different disinfecting chemicals
4) Evaluate the effect on contamination after exposure to different disinfecting chemicals
5) Evaluate methods that can be used to measure effect of treatments tested in cultivation of initial life stages of *P. palmata*

A final test was conducted to evaluate the growth success of *P. palmata* seedlings in sea.

2 Material and Methods

To prepare for a more optimised hatchery protocol for *P. palmata*, the following experiments were conducted from January 2018 to March 2019;

1. A year-round field survey on the reproductive and health status of *P. palmata*
2. Spore density from spore release over three days
    a) Spore release conducted in January 2018
    b) Spore release conducted in November 2018
3. Chemical disinfection of *P. palmata* sori
    a) Chemical sori disinfection with four disinfectants
    b) Chemical sori disinfection with GeO_2
4. Cultivation of *P. palmata* gametophytes in growth medium
5. Deployment of *P. palmata* seedlings in sea for cultivation of biomass

2.1 A year-round field survey on the reproductive and health status of *P. palmata*

The aim of this experiment was to investigate the year-round reproductive status of *P. palmata* in the Trondheim area. The main intention was to examine when it is possible to collect fertile tetrasporophytes for the purpose of spore release for laboratory cultures. It was also interesting to examine the amount of fertile male gametophytes that potentially can be used to fertilize female gametophytes.

2.1.1 Sampling

Fresh material was collected at Storsteinan (63°27'N 10°15'E), in the Trondheimsfjord, once a month from October 2017 to September 2018. The material was collected by hand at 40-60 cm on low tides and kept wet in plastic containers during collection and transport to the laboratory.

2.1.2 Experimental setup for examination of fertility and health status of *P. palmata*

A number between 150 and 250 random sporophytes of *P. palmata* were collected at each sampling. In the laboratory (10°C, ± 1), material was divided into four batches; fertile (2n), apparently fertile (n), sterile and unfit. Sporophytes with dark red, irregularly and structure shaped areas on thallus was assessed at fertile tetrasporophytes (fertile, 2n). Sporophytes with lighter coloured irregularly shaped areas without structure were considered as male gametophytes (apparently fertile, n). Maturity of tetrasporophytes was assessed by eye under bright light. Sporophytes with no visible pattern or colour variations were assessed as sterile. Unfit sporophytes were characterized with distinct on growth of epibionts or rotten parts. Number of unfit sporophytes out of total collected sporophytes indicated the monthly health status. Each of the apparently fertile plants (n) were further evaluated in a stereo-loupe in order to determine whether it was fertile or sterile. If spermatangia was detected on sori, plants were assessed as fertile.

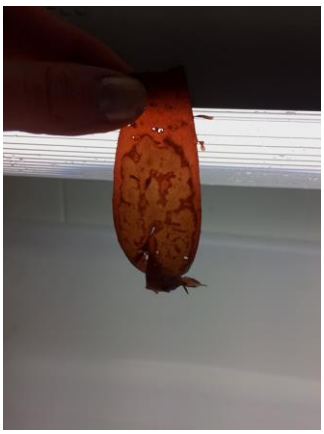

Figure 2: Fertile (2n) *P. palmata* tetrasporophyte collected at Storsteinan, outside of Trondheim in February 2018.

2.2 Spore density from spore release over three days

Two different trials were preformed, one in January 2018 and one in November 2018. The aim of these trials was to investigate how spore density changed in spore release over three days. Some modifications were conducted in trial b), for optimization based on results and experiences from trial a).

2.2.1 Orgin of sorus

Fertile tetrasporophytes were collected in January 2018 and in November 2018 (Table 1) by hand at low tide at Lade, Trondheim (63°27'N 10°26'E). The material was transported to the laboratory in a plastic container with seawater. At the laboratory (10°C ± 1), healthy tetrasporophytes were placed in plastic tanks measuring 60 cm (l) x 35 cm (w) x 17 cm (d) with running seawater holding 10°C (1,5 L min^{-1}) pumped from 70m depth in the Trondheimsfjord. The seawater was both sand and particle filtrated and treated with UV light. A 16:8 h (light: dark) light regime was used. The tetrasporophytes were kept in the laboratory until the experimental start (Table 1).

Table 1: Dates for collection of material and experimental start.

Trials	Date for collection	Start of experiment
a	23.01.2018	30.01.2018
b	08.11.2018	12.11.2018

2.2.2 Method for survey of spore density used in experiment a) and b)

All vegetative areas of the fertile tetrasporophytes were removed before it was washed with sterile seawater (SSW) and patted dry with clean paper towels. Dried sori were placed in marked plastic bags and kept for 24 hours in a dark refrigerator holding 4°C for dehydration.

Sori was equally split by weight in three replicates (n=3) after dehydration. Spore release was conducted by rehydration (5 g sorus/10 mL SSW) of sori in sterilized beakers in a climate room holding 10°C (±1). The beakers were wrapped in aluminium foil to secure dark conditions and placed on an orbital shaker with relatively high speed for agitation throughout spore release. In trial a), samples for counting of spore density were collected from the centre of the beakers after 1, 2, 3, 6, 24, 28, 48 and 72 hours and Lugol's solution was added as a fixative.

The fixed samples were counted both manually and automatically to registrate spore density (number of tetraspores mL^{-1}). A subsample of 125 µL was taken out of each sample, placed on an object glass with a grid, and counted manually in a stereo loupe. The samples were counted automatically by Coulter Counter (Multiziser 3)

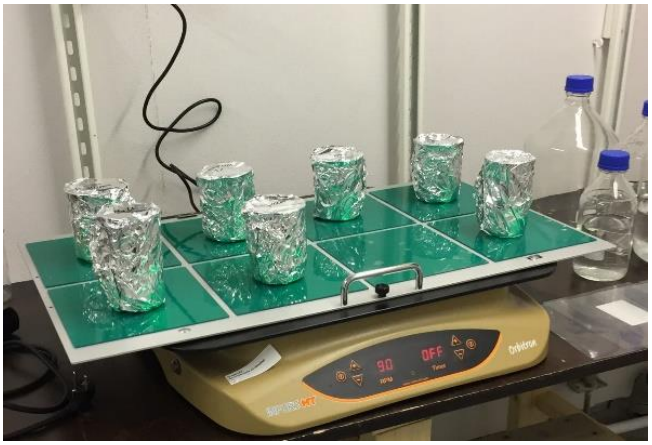

Figure 3: Spore releases in wrapped beakers placed on an orbital shaker in a climate room holding 10°C.

Based on results from trial a), some modifications were conducted in trial b). An extra sampling was included after 32 hours of spore release. Some of the samples contained high amounts of dissolved debris of *P. palmata* tissue. At times, this made it difficult to count the spores manually. It was therefore decided to use a different method for estimating spore densities manually in trial b. A Lugol fixated sample was filtrated through a 50 µm filter to eliminate larger objects than *P. palmata* spores. The filtrated spore solution was re-filtered using a 0.8 µm syringe filter (Cellulose Nitrate). By using a tweezer, the syringe filter was placed on a microscope slide and left for drying for 30 seconds. Spores were easily detected on the dried filter and were counted in a microscope (magnification 10x) and calculated (tetraspores mL^{-1}).

Results from the manual and automatic counting in trial a did not match, and the automatic counting showed higher densities than the manual counting. There was reason to believe that the Coulter Counter (limit of detection set to 24-40 µm) results included small pieces of dissolved *P. palmata* tissue, which were relatively similar sized as *P. palmata* spores. Based on this, automatic counting was eliminated in spore release trial b) and automatic counting from trial a) are not included in the result section.

2.3 Chemical disinfection of *P. palmata* sori

Two disinfection trials were conducted, one in April 2018 and one in November 2018. The aim of the trials was to investigate the tolerance of *P. palmata* to chemical disinfectants. The disinfection treatment should limit diatom growth while not inhibit spore survival and growth.

The results from disinfection trial a) was the basis for the treatments chosen in the second experiment (trial b). A few modifications were conducted in trial b) based on the results and experiences from trial a).

2.3.1 Origin of sorus

Fertile tetrasporophytes for both disinfection experiments were collected at Storsteinan (63° 27'N 10°15'E), in the Trondheimsfjord. The material for trial a was collected 14.04.2018, and for trial b, 22.11.2018. The material was collected, transported and kept in the laboratory as previously described in section 2.1.1.

2.3.2 Growth medium

SSW was used in the cultivation experiments of *P. palmata*. Seawater pumped from 70 m depth as described in section 2.1.2 was autoclaved in bottles (NALGEL 5GAL, 5L) in a high-pressure steam sterilizer (TOMY SX-700E) at 120 degrees for 20 min.
Natural seawater is normally not nutrient sufficient in algal cultures. In order to sustain an algal yield sufficient for laboratory experiments, natural seawater enrichment is required. A medium often used in macroalgae cultures is Provasoli's Enriched Seawater medium (PES). PES medium made by SINTEF Ocean was applied in the following experiments (20 mL PES L^{-1} SSW) (See recipe in Harrison and Berges 2005).

2.3.3 Method for sori disinfection and spore release used in trial a) and b)

The two trials were conducted a climate room holding 10°C (±1). This secured minimal temperature fluctuations between the different steps in the experiments and a stabile reaction temperature.

Tetrasporophytes were prepared as described in section 2.2.2 and was divided equally by weight into seven batches. Three autoclaved glass bowls were used under the disinfection for each treatment. The first glass bowl was filled with 400 mL of the chosen disinfectant and SSW (10°C) while the second and the third was filled with 400 mL SSW (10°C). Sori were transferred to a sieve and immersed into the first glass bowl containing the chosen

disinfectant. After 2 min, sori were rinsed twice in the following two glass bowls by immersing the sieve for 30 sec in each bowl. The sieve was carefully shaken during disinfection and rinsing so all parts of sori were disinfected and rinsed properly. The bowls were placed on an orbital shaker during disinfection, which created movements in the solution and prevented a possible merging of material. A stopwatch was used to keep the reaction time. The disinfected material was placed on paper towels and dried as much as possible with clean paper cloth with even pressure. Dehydration and spore release by rehydration are described in section 2.2.2. In trial a), the spore release was terminated after 28 hours based on results from spore release trial a). Due to alterations between the results in spore release trial a) and b), the spore release in disinfection trial b) was not terminated before 48 hours.

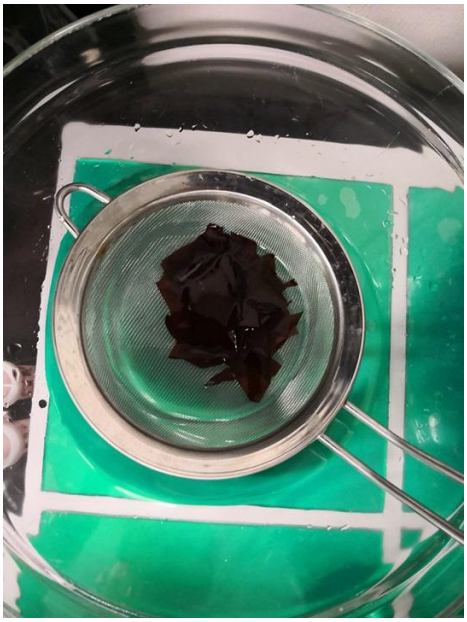

Figure 4: Pieces of sori in an immersed sieve under disinfection on an orbital shaker in a climate room holding 10°C.

Marked petri dishes (60*15 mm, 23 mL) (Nuclon TM) were filled with 12 mL of precooled (10°C) SSW with PES enrichment. 8 mL of spore solution was added after stirring around in the beakers for 10 seconds in order to make the spore solution more homogenised. A sample was taken of the spore solutions (n=3), fixated (Lugol´s solution) and counted in order to determine spore density.

2.3.4 Incubation of *P. palmata* spores in trial a) and b)

In disinfection trial a), all trials were incubated for 25 days in a climate room holding 10°C (± 1), light intensity 40 µmol m^{-2} s^{-1} and light regime 16:8 h (day: night). All trials were placed in approximately equal distance to the light source. PES enrichment was changed after 2 weeks. Dishes were inspected in a stereo-loupe to see if there were any spores left to germinate after 21 days. The incubation was terminated after 25 days. All male gametophytes in each trial were counted manually in a stereo-lupe. Pictures were taken of the 10 first detected seedlings and measured in imageJ. Trials without developed seedlings, were also photographed.

Number of benthic diatom cells were counted in imageJ from the pictures taken after incubation. Three pictures of each dish were counted (n=3). The pictures were divided into 9 equal squares, and 3 squares were counted. An average number of benthic diatom cells per mm^2 were calculated.

Some modifications were conducted between trial a) and b) and these differences are described in Table 2.

Table 2: Differences in experimental setup between trial a and b.

Experimental setup	**Trial a**	**Trial b**
Incubation period	25 days	21 days
Pictures of trials	Post incubation	Every second/third day and post incubation
Number of replicates	n=3	n=5

2.3.5 Trial a)- Chemical sori disinfection with four disinfectants

Four chemicals were tested in order to identify a proper disinfection treatment for *P. palmata* sori. A total of 13 different conditions were tested. The trials were a combination of four chemicals in two concentrations, addition or no addition of germanium dioxide (GeO_2), and one given reaction temperature and time (n=3) (Table 3). One positive (untreated sori) and one negative control (untreated sori grown in growth medium with addition of GeO_2) was additionally tested. The GeO_2 solution was prepared by adding 0.894 g GeO_2 in 200 mL

distilled water in an autoclaved bottle (NALGEL 5GAL, 250 mL). A magnetic stirrer was used to secure a hegemonized GeO_2 solution.

Table 3: Disinfection chemicals, abbreviations, concentrations, exposure time and exposure temperature.

Chemical	**Abbreviation**	**Concentration**	**GeO_2***	**Exposure time**	**Exposure temperature**
Lugol´s solution	L	0.02 %, 0.2 %	+/-	2 min	10°C
Acidic Acid	AA	1 %, 7 %	+/-	2 min	10°C
Sodium hypochlorite	H	300 ppm, 600 ppm	+/-	2 min	10°C
Control	C		+/-	2 min	10°C

*Added a final concentration of 0.1 mL^{-1}

Parameters that were registered:

1. Spore density after spore release
2. Seedling density after the incubation period
3. Length of seedlings after the incubation period

2.3.6 Trial b)- Chemical sori disinfection with GeO_2

Based on the results from disinfection trial a GeO_2 was tested as a disinfection treatment of fertile *P. palmata* sori. A total of 4 conditions based on three different concentrations of GeO_2, one reaction time and one reaction temperature (n=5) (Table 4) were tested. GeO_2 was prepared as described in section 2.3.5.

Table 4: Concentrations of GeO_2, exposure time and exposure temperature.

Concentration	**Exposure time**	**Exposure temperature**
0, Control	2 min	10°C
0.05 mL L^{-1}	2 min	10°C
0.1 mL L^{-1}	2 min	10°C
0.5 mL L^{-1}	2 min	10°C

The same parameters as described in section 2.3.5 was registered. Pictures of seedlings were taken every second/third day (Table 1).

2.4 Cultivation of *P. palmata* gametophytes in growth medium

The aim of this experiment was to examine if GeO_2 could be utilized in the development of gametophyte cultures of *P. palmata* by increasing survival and growth. A second aim was to examine whether 28 hours or a 48 hours spore release resulted in best survival and growth of *P. palmata* spores and seedlings.

2.4.1 Orgin of sorus

Fertile tetrasporophytes were collected at low tide at Lade (63°27'N 10°26'E) in the Trondheimfjord in January 2019. The material was transported and kept in the laboratory as described in section 2.2.1

2.4.2 Method for sori disinfection and spore release

The disinfection was conducted as described in section 2.3.3 where, disinfection trial b); Chemical sori disinfection with GeO_2, indicated that 0.5 mL L^{-1} GeO_2 could be helpful to a certain extent to eliminate unwanted contamination. Table 5 gives the disinfection concentration, exposure time, exposure temperature and timing of spore release.

Table 5: Concentration of GeO_2, exposure time, exposure temperature and spore release durations.

Concentration GeO_2	**Exposure time**	**Exposure temperature**	**Spore release**
0, Control	2 min	10°C	28 and 48 h
0.5 mL L^{-1}	2 min	10°C	28 and 48 h

The spore releases were conducted as described in section 2.2.2. Spore release trial a) and b) showed conflicting results, and it was therefore decided to run two different spore releases, one terminated after 28 and one terminated after 48 hours.

2.4.3 Method for starting up gametophyte cultures of *P. palmata*

Except from a few modifications, the method for starting up gametophyte cultures of *P. palmata* was similar to the method used for *S. latissima*, which are described by Forbord et al (2018). After 28 hours of spore release, spore solutions were filtrated though 40 µm filter and divided equally in 3 marked culture flasks (n=3). Spore density was counted in each culture. Cultures were diluted until all of them had approximately equal spore densities (600 spores mL^{-1}) and refilled with culture medium (PES). Incubation parameters were 10°C, light intensity 40 µmol m^{-1} s^{-1} and light regime 16:8 h (day: night). All cultures were placed in approximately equal distance to the light sources. The cultures were covered by silicon tops with a hole for a glass pipette. A silicon tube was attached to the glass pipette and provided filtered air into the cultures. Culture medium was changed after 2 weeks (equal concentration, 20 mL PES L^{-1} SSW). The procedure was repeated for the spore solutions from the 48 hours spore releases. In total 12 gametophyte cultures were started (n=3).

The cultures were supposed to be incubated for 6 weeks, but due to personal matters, they were terminated after 28 days. Gametophyte development was checked once a week in a microscope. The cultures were supposed to be weekly analysed for gametophyte density, but this was not conducted since major part of the gametophytes attached to the glass surface in the culture flasks.

2.5 Deployment of *P. palmata* seedlings in sea for cultivation of biomass

Seeded nets and strings were deployed in sea at Frøya 14.02.2018. The aim of the experiment was to evaluate growth success of deployed *P. palmata* seedlings in an open sea-farm.

2.5.1 Orgin of sorus

Fertile material (sori) were collected at Storsteinan, outside of Trondheim, and transported to the laboratory as described in section 2.1.1. The sori used to seed the nets were collected 05.11.2017 while the material for the strings was collected 04.12.2017.

2.5.2 Method for seeding *P. palmata* spores and cultivation of seedlings

Panels twisted with string, cylindrical Polyvinyl chloride (PVC) spools twisted with string and 2 different nets were seeded, one with small mesh width and one with larger mesh width. Panels, spools and nets were seeded directly from spore release from 15 fertile plants for 4 days in 8:16 h light regime (day: night) with light intensity 30 µmol s^{-1} m^{-1}. Thin strings (1-2 mm) twisted around horizontal glass panels and spools were placed on the bottom of the plastic tanks (25 L) described in section 2.2.5 with running seawater (2,2 L^{-1}). In a larger tank wit aeration (80 L), nets were hung from the edge of the tank while 15 fertile plants were moved around from the turbulence of air bubbles from the bottom of the tank.

The incubation method for *P. palmata* sporophytes were similar to the method used in *S. latissima* cultivation, described by Forbord et al. (2018). Nets, panels and spools were transferred to an incubator, a large cylindric plastic tank (300 L) filled with sand-filtrated, particle-filtrated and UV treated seawater holding 10°C. Four evenly distributed fluorescent tubes on the outside wall of the cylinder illuminated the tank. The light intensity outside of the incubators were set to around 70 µmol m^{-1} s^{-1}. The incubator was left without aeration and water flow for the first three days to allow the spores to settle. After the initial three-day period, water flow started at 1.5 L min^{-1}, but were increased gradually to 2- 2.5 L min^{-1} after 3-4 weeks.

Table 6: Key parameters for seedling cultivation (Forbord et al., 2018)

	Key parameters
Light intensity (µmol m^{-1} s^{-1})	20-70
Light regimes (Day: night) (h)	16:8
Light source	Fluorescent lamps (day light)
Temperature °C	10
Water treatment	Sand-filtrated, particle-filtrated, UV
Aeration	Unfiltered, cooled
Water flow (L min^{-1})	1.5-2.5

Seedlings were incubated for 9 weeks before deployment in sea. The nets and strings were gently washed with UV-treated seawater to reduce the on-growth of contaminants after 7 weeks. The seedlings were deployed in a sea-farm in exposed waters at Rataren (63° 46'N 8°31'E) outside of Frøya in Central-Norway, around 3 hours' drive from the laboratory. The

panels, nets and spools were kept wet and cooled during transport. The seeded 1.2 mm strings on panels and spools were further twisted around 21 ticker nylon ropes with a diameter of 14 mm by an electrical twisting machine before it was attached to horizontal carrier lines in the sea (from 1-6 meters depth from the surface). The strings had a density of around 1-2 seedling cm^{-1}. The nets and ropes were checked for seedling growth once a month.

2.6 Statistics

2.6.1 Spore density in spore release trial 1 and 2

Non-parametric tests were chosen due to due to low replicate numbers (n=3) and non-normal distribution. Kruskal Wallis significant test was run on the different sampling groups. A significant level of $p \leq 0,05$ was used. The Kruskal-Wallis test was followed up by an inspection of the graph and a Mann-Whitney test on groups with major different densities.

2.6.2 Effect of sori disinfection

Non- parametric tests were also chosen for statistical analyses of the disinfection results due to the same reasons described in section 2.5.1. Kruskal- Wallis test was run on control and groups with the same disinfection chemical. A significant level of $p \leq 0,05$ was used. When Kruskal- Wallis test revealed a significant difference, a Mann- Whitney test was run on control and each group. A Mann-Whitney test were also run on the different concentrations of the same disinfection chemical and on groups with and without GeO_2 addition. Statistical analyses were performed in IMP SPSS Statistics, version 25. Graphs were made in Excel, Microsoft Office 365.

3 Results

3.1 A year-round field survey on the reproductive and health status of *P. palmata*

3.1.1 Reproductive season

Figure 5 shows the year-round reproductive status of *P. palmata* collected at Storsteinan outside of Trondheim. The result reveals a peak of reproductive tissue from December to April, with highest percentage of fertility in January. None of the sporophytes collected between June and September were reproductive.

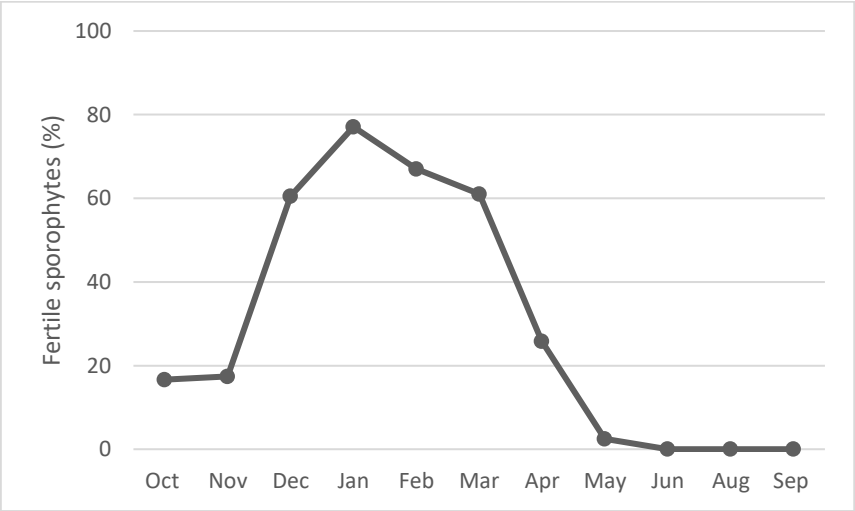

Figure 5: The year-round reproductive status (%) for *P. palmata* sporophytes collected at Storsteinan, outside of Trondheim, from October 2017 to September 2018. The line represents percentage fertile sporophytes of total collected sporophytes. The different months are given on the x-axe.

The percentage of fertile tetrasporophytes (2n) and fertile male gametophytes (n) are given in Figure 6. Number of both fertile tetrasporophytes and fertile male gametophytes were highest between December and March. However, a small decrease of fertile tetrasporophytes was registered in January, when the amount of fertile male gametophytes was at its highest. In January, around 45 % of total collected sporophytes were fertile male gametophytes, which possibly can be used to fertilize female gametophytes.

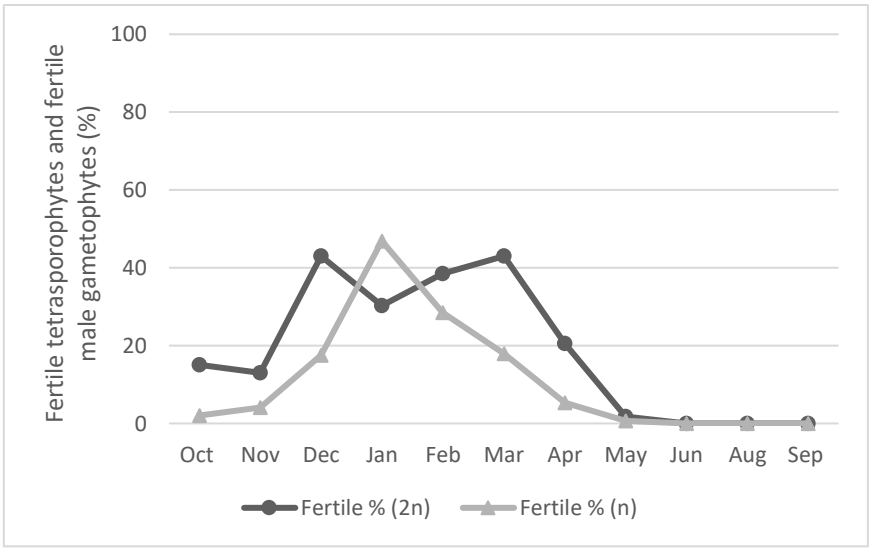

Figure 6: The percentage of fertile tetrasporophytes (2n) and fertile male gametophytes (n) of total collected sporophytes at Storsteinan outside of Trondheim, from October 2017 to September 2018. The line with circles represents fertile tetrasporophytes (2n) and the line with triangles represents fertile male gametophytes (n). The different months are given on the x-axe (except from July).

3.1.2 Health status

The year-round health status of *P. palmata*, based on the share of unfit sporophytes, at Storsteinan, Trondheim is given in Figure 7. The highest percentage of unfit sporophytes are found in the summer months (June and August). Number of unfit sporophytes was low when number of fertile sporophytes was high (Figure 5). Collecting *P. palmata* sporophytes during winter will secure high percentage of fertile and healthy sporophytes suitable for spore release.

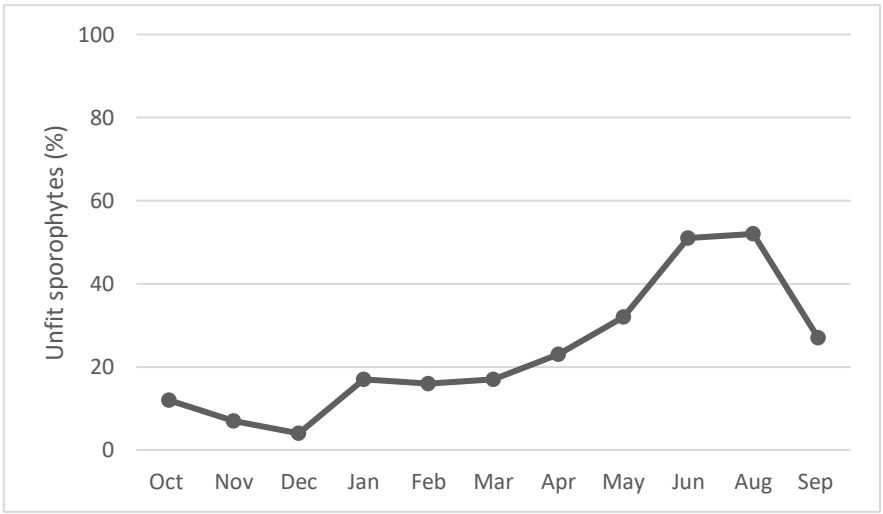

Figure 7: The year-round health status (%) for *P. palmata* sporophytes collected at Storsteinan outside of Trondheim from October 2017 to September 2018. The line represents percentage of unfit sporophytes of total collected sporophytes. The x-axis represents the different months.

3.2 Spore density from spore release over three days

3.2.1 Trial a)- Spore release conducted in January 2018

Figure 8 shows spore density from spore release over 3 days. Samples taken 28 hours into spore release revealed a spore density of 1304 ± 294 spores mL^{-1}, which was significantly higher than spore density after both 24 hours and 48 hours (Mann Whitney, $p<0.05$). Few spores were released until 24 hours, and a rapid decrease was registered after 28 hours. The result indicates that spore release with 28 hours duration will be the most suitable for *P. palmata* cultivation.

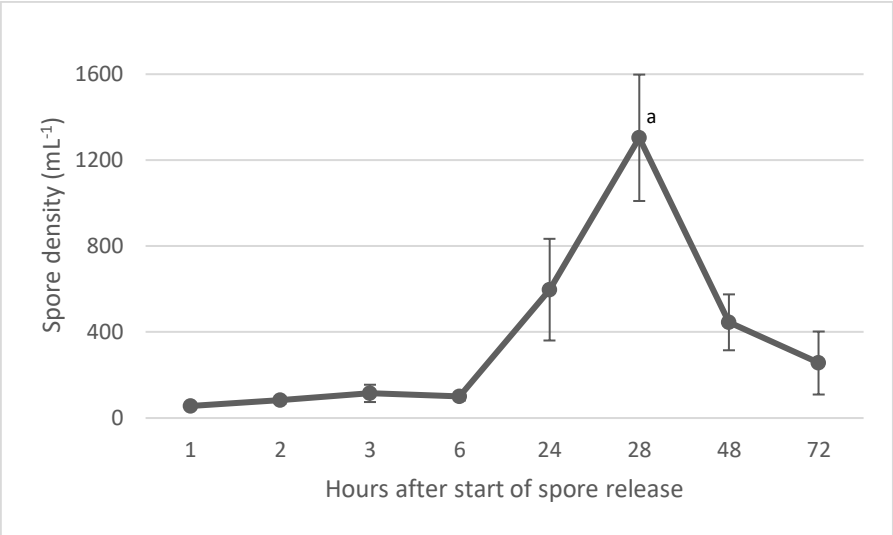

Figure 8: Spore release from *P. palmata* sorus over 72 hours incubation with agitation. The line shows spore density during spore release (mL^{-1}) (mean value ± SD, n=3). The x-axe indicates hours into the spore release. a= Significant higher spore density than after 24 and 48 hours into spore release.

3.2.2 Trial b)- Spore release conducted in November 2018

Spore density in spore release over 3 days is given in Figure 9. The highest density was observed after 48 hours with 665 ± 333 spores mL^{-1}. The density had decreased slightly after 72 hours (558 ± 203 spores mL^{-1}) and a significant difference was registered (Mann Whitney,

p<0.05). Compared to the trial run in January few spores were released at a later stage. The highest spore density in trial b) (666 ± 333 mL^{-1}) was only around half of the density in trial a) (1300 ± 294 mL^{-1}).

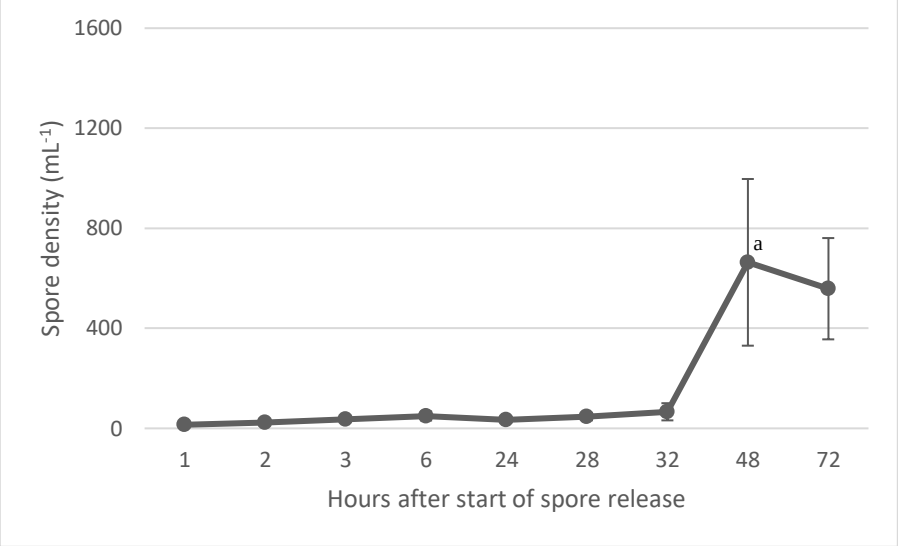

Figure 9: Spore release from *P, palmata* sorus over 72 hours incubation with agitation. The line indicates the spore density during spore release (mL^{-1}) (mean value ± SD, n=3). The x-axis shows sampling times, hours after start of spore release. a= Significant higher spore density compared to 32 hours into spore release.

3.3 Chemical sori disinfection with four disinfectants

3.3.1 Control treatments

Spore density after spore release and seedling density after incubation of control treatments are showed in Figure 10. Control showed an average spore density of 515 ± 198.07 spores mL^{-1}. Spores from untreated sori grown with GeO_2 addition (negative control for seedling density) showed a lower seedling density compared to those not grown with GeO_2 addition (positive control of seedling density) after incubation (4.12 and 8.54 seedlings, respectively). However, no significant difference was observed (Mann Whitney test, p>0.1).

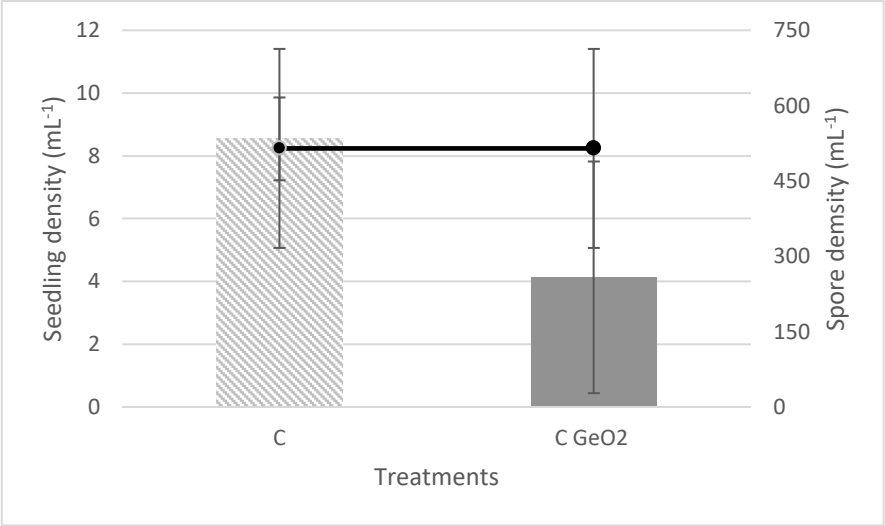

Figure 10: Effect of GeO_2 on the *P. palmata* spore release and seedling density after 25 days of incubation. Bars represent seedling density (mL^{-1}) for control treatments (mean values ± SD, n=3). Spore density (mL^{-1}) is given on the secondary y-axis and as a line (mean value ± SD, n=3). The different control treatments are given on the x-axis.

Diatom contamination in control treatments after 25 days of incubation is given in Figure 11. Surprisingly, diatom density increased when untreated spores were grown with addition of GeO_2, but no significant difference was registered (Mann Whitney, $p>0.05$). Results indicates that addition of GeO_2 did not show the desired effect and both increased the contamination density and decreased the survival of *P. palmata* spores.

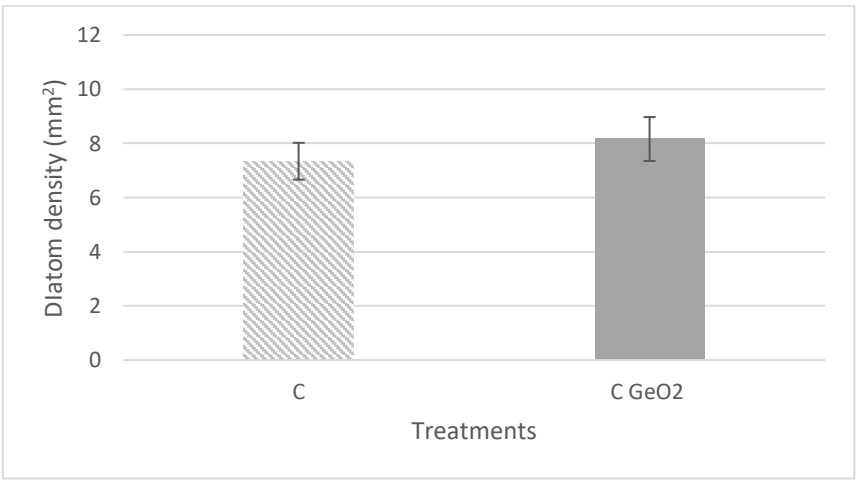

Figure 11: Effect of GeO_2 on the diatom density in *P. palmata* cultures after 25 days of incubation. Bars indicate diatom density (mm^2) in positive (untreated spores) and negative control (untreated spores grown with addition of GeO_2) treatments (mean value ± SD, n=9). The different treatments are given on the x-axis.

3.3.2 Lugol treatments

Spore density after spore release and seedling density after incubation from disinfected treatments with Lugol are showed in Figure 12. All disinfected treatments showed low densities of both spores and seedlings, which indicates that the chosen treatments were harmful for *P. palmata* spores. The spore density was significantly lower in both concentrations compared to control (Mann Whitney, $p<0.05$). Treatments with the highest disinfection concentration (0.2 %) did not developed into seedlings, and all chosen treatments revealed a significant lower seedling density than the control (Mann Whitney, $p<0.05$). For treatments disinfected with the weakest concentration, addition of GeO_2 resulted in decreased seedling density, but no significant difference was observed (Mann Whitney, $p>0.05$).

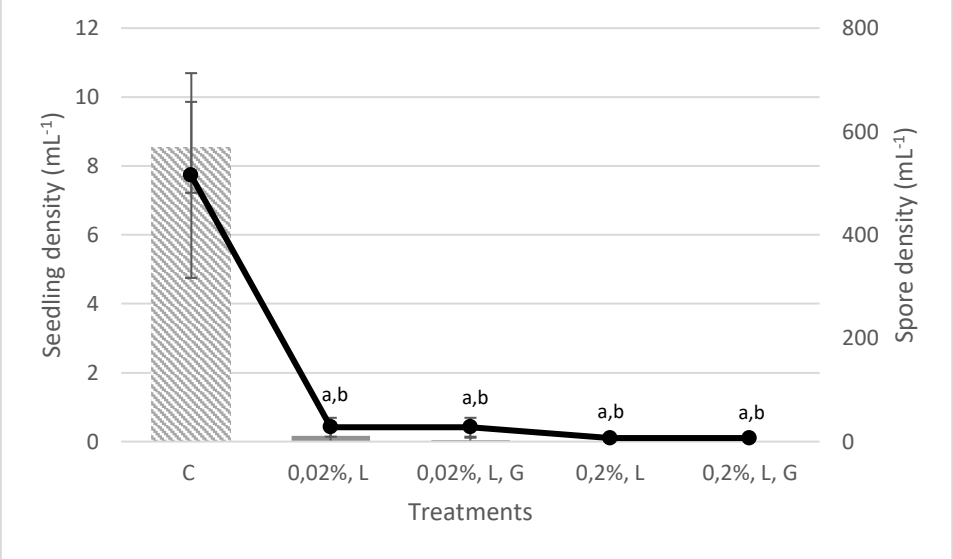

Figure 12: Effect of disinfection of sorus with Lugol on the *P. palmata* spore release and seedling density after 25 days of incubation. Bars indicate seedling density (mL^{-1}) (mean values ± SD, n=3). The line and the secondary y-axis represent spore density (mL^{-1}) after spore release (mean value ± SD, n=3). The x-axis shows the different treatments with Lugol and control (concentration, first letter of chemical, addition of GeO_2). a= significantly lower spore density then control treatment, b= significantly lower seedling density than control.

Registrations of diatom contamination in treatments disinfected with Lugol are given in Figure 13. All chosen treatments revealed significantly lower diatom contamination than control (Mann Whitney, $p<0.05$). The highest concentration (0.2 %) gave significantly lower density of contamination compared to the weakest concentration (0.02 %) (Mann Whitney, $p<0.05$). Treatments with addition of GeO_2 showed lower contamination density than treatments without GeO_2, and a significant difference was observed for both disinfection concentrations (Mann Whitney $p>0.05$).

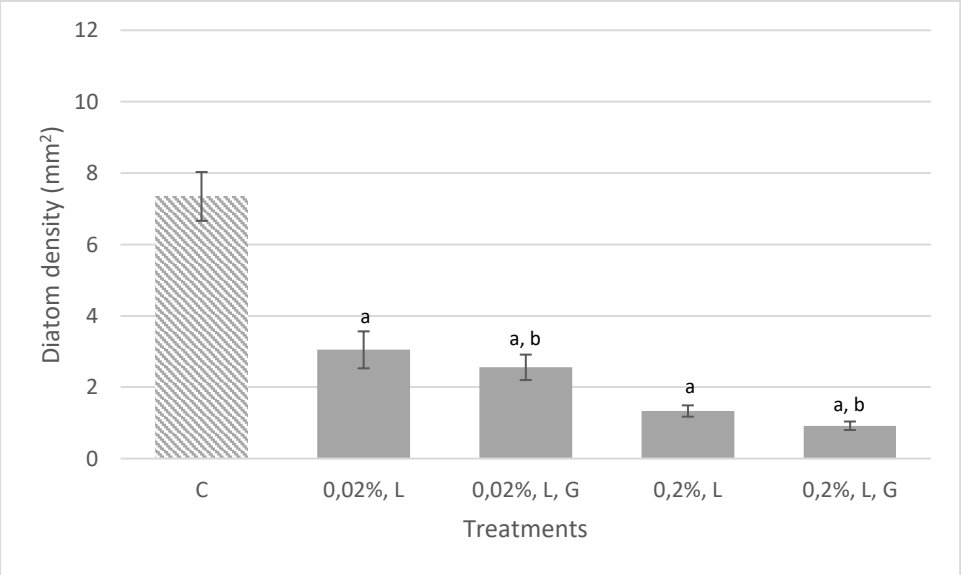

Figure 13: Effect of disinfection of sorus with Lugol on the diatom density in *P. palmata* cultures after 25 days of incubation. Bars indicate number of benthic diatom cells (mm^2) (mean value ± SD, n=9). The x-axis represents the different treatments with Lugol and control (concentration, first letter of chemical, addition of GeO_2). a= significantly lower diatom contamination than control, b= significantly lower diatom contamination than without addition of GeO_2.

The chosen disinfection treatments with Lugol did not give the wanted effect, and both diatom contamination the survival of *P. palmata* spores were significantly reduced. It is clear from the results that the contamination was further reduced by addition of GeO_2.

3.3.3 Acetic acid treatments

Figure 14 gives spore density (after spore release) and seedling density (after incubation) from treatments disinfected with acetic acid. The disinfected treatments showed low spore density after spore release (14.6 and 12.2 spores mL^{-1}, respectively). A significant difference was observed from both concentrations (1 % and 7 %) compared to control (Mann Whitney, $p<0.05$). Registrations from all acetic acid exposed treatments showed non seedlings after 25 days of incubation.

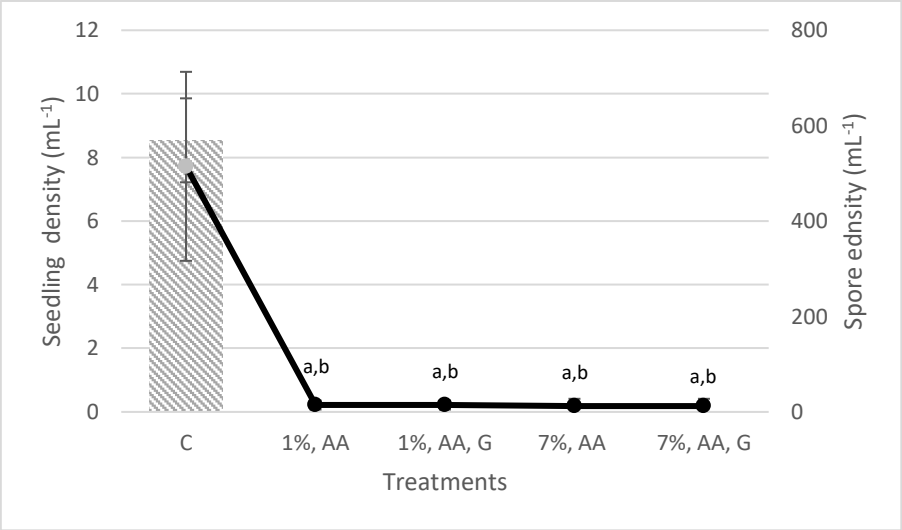

Figure 14: Effect of disinfection of sorus with acidic acid on spore release and seedling density after 25 days of incubation. Bars indicate seedling density (mL^{-1}) (mean value ± SD, n=3). The line and the secondary y-axis represent spore density (mL^{-1}) after spore release (mean value ± SD, n=3). The different treatments and control are given on the x-axis, named as in Figure 12. a= Significant lower seedling density than control, b= Significant lower spore density than control.

Diatom contamination was only found in the weakest concentration of acetic acid (1 %), but in limited amounts. Addition of GeO_2 revealed lower contamination than without addition of GeO_2, but no significant difference was registered (Mann Witney $p>0.05$).

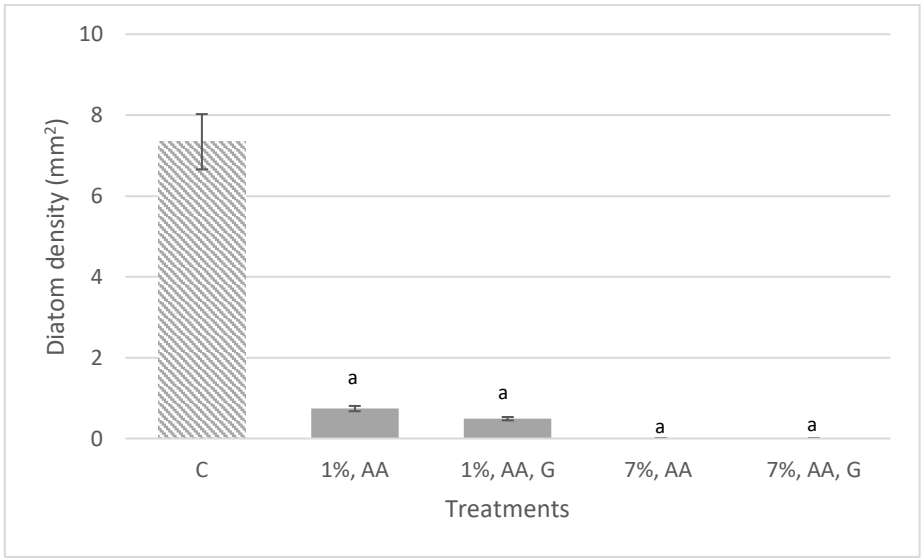

Figure 15: Effect on disinfection of sorus with acetic acid on diatom density after 25 days of incubation. Bars show number of benthic diatom cells (mm^2) (mean value ± SD, n=9). X-axis gives the different treatments and control, named as in Figure 3. a= Significant lower diatom contamination than control.

Results indicates that the chosen disinfection treatments of acetic acid were lethal for *P. palmata* spores at the same time as they decreased the density of contamination. The results also show that the contamination was further reduced by addition of GeO_2.

3.3.4 Sodium hypochlorite treatments (NaOCl)

Both concentrations of NaOCl (300 ppm and 600 ppm) revealed significantly lower spore density after spore release (35.7 and 28.1 spores mL^{-1}, respectively), compared to control (Mann Whitney, $p<0.05$). Spore density was reduced with increasing concentration of NaOCl, but no significant difference was observed between the two concentrations (Mann Whitney, $p>0.05$). Figure 16 also shows a significantly lower seedling density for all treatments compare to control (Mann Whitney, $p<0.05$). Seedling density was highest in the weakest concentration, and no seedlings were observed in the highest concentration without addition of GeO_2.

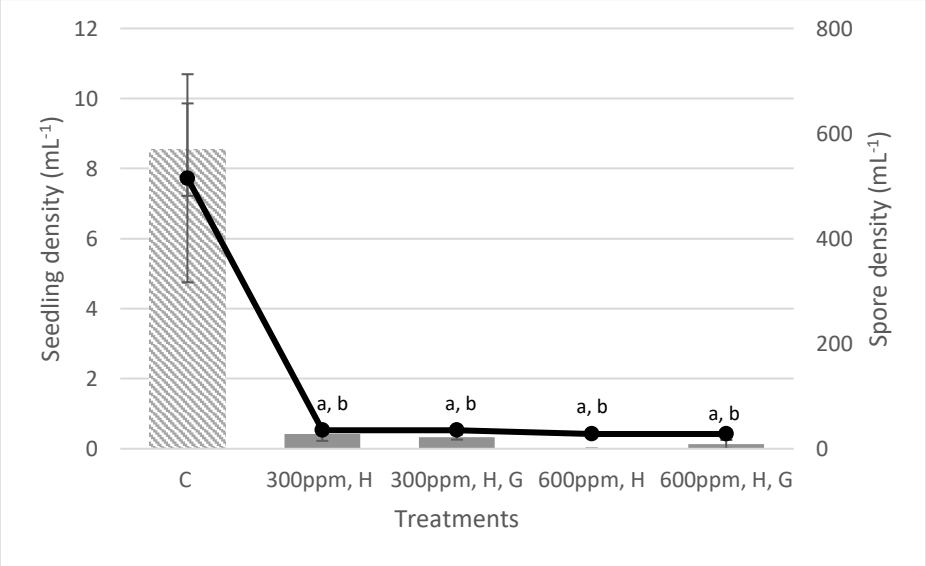

Figure 16: Effect of disinfection of sorus with hypochlorite on spore release and seedling density after 25 days of incubation. Bars indicate seedling density (mL^{-1}) (mean value ± SD, n=3). The secondary y-axis and the line show spore density (mL^{-1}) after spore release (mean value ± SD, n=3). The different treatments with hypochlorite and control is given on the x-axis, named as in Figure 12. a= Significant lower sporophyte density than control, b= Significant lower spore density than control.

Diatom density in treatments disinfected with NaOCl is given in Figure 17. The weakest concentration (300 ppm) contained the highest amounts of contamination, with densities close to control. The highest concentration (600 ppm) also contained noteworthy diatom density. However, all disinfected treatments show significantly lower diatom density compared to

control (Mann Whitney, $p<0.05$). For both concentrations, samples with addition of GeO_2 had significantly fewer diatoms than samples without addition GeO_2 (Mann Whitney, $p<0.05$).

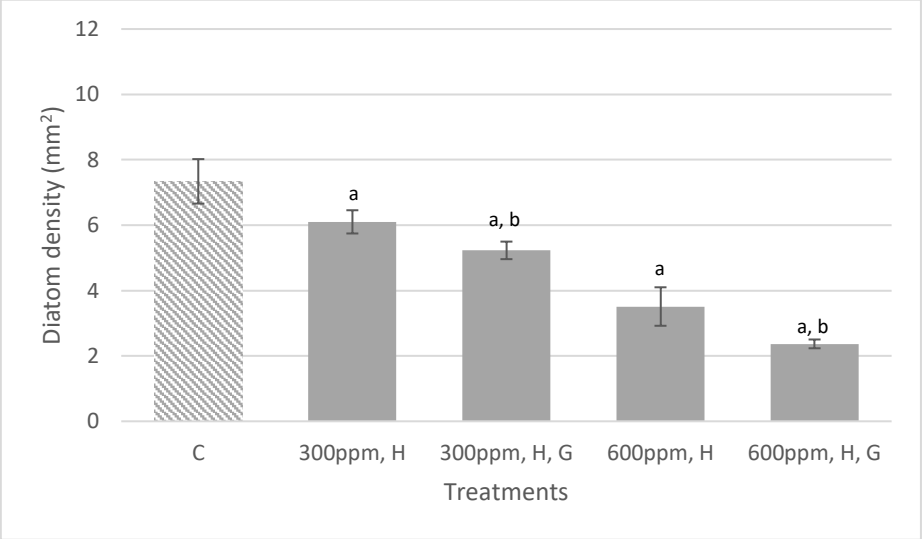

Figure 17: Effect of disinfection of sorus with NaOCl on diatom density after 25 days of incubation. Bars indicate number of benthic diatom cells (mm^2) (mean value ± SD, n=9). The x-axis represents control and the different treatments of NaOCl. a= Significant lower diatom density than control, b= Significantly lower diatom density than samples with the same concentration, but without addition of GeO_2.

Disinfecting *P. palmata* sori with the chosen treatments of NaOCl was harmful for *P. palmata* spores while the diatom contamination was significantly reduced. Nevertheless, the weakest concentration (300 ppm) contained high densities of diatoms and contamination was further reduced by addition of GeO_2.

3.3.5 Variations in seedling length

As described in material and method section 2.3.4, 10 seedlings from each treatment was supposed to be measured in image J after incubation. Only positive (untreated sori) and negative control (untreated sori grown in addition of GeO_2) treatments developed more than 10 seedlings in each sample. Control treatments showed similar mean length on seedlings after incubation (253 and 259 µm, respectively).

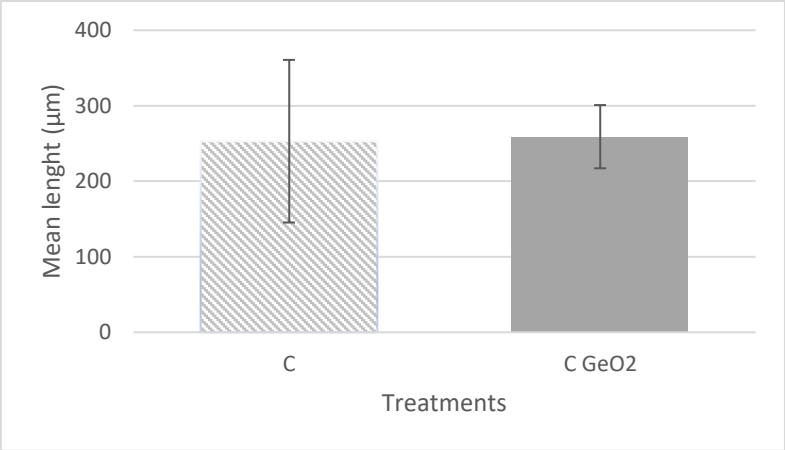

Figure 18: Effect of growing untreated spores in growth medium with addition GeO_2 on seedling length after 25 days of incubation. Bars indicate length of seedlings (µm) in positive and negative control treatments (mean value ± SD, n=30). The x-axis represents the different control treatments.

Seedling length varied between replicates within the same treatments. Figure 19 and 20 is an example on length differences between replicates. Two replicates from positive control (untreated spores) and NaOCl (300 ppm) after 25 days of growth are given in Figure 19. Three replicates of untreated spores grown in addition of GeO_2 (negative control) is represented in Figure 20. Figure 19 B and D shows a relatively small seedling which consists of more pale cells than the larger seedlings (19 A and C).

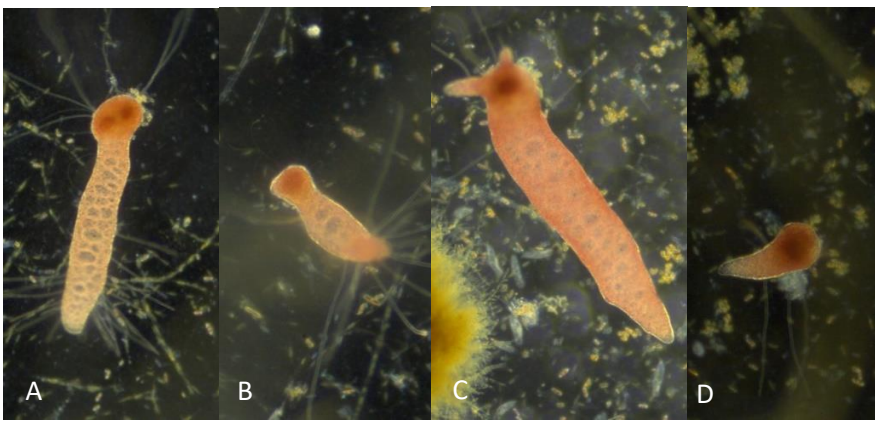

Figure 19: Difference in seedling length within the same treatments after 25 days of growth. **A-B**: Replicates of positive control (20x). **C-D**: Replicates of NaOCl 300 ppm (20x).

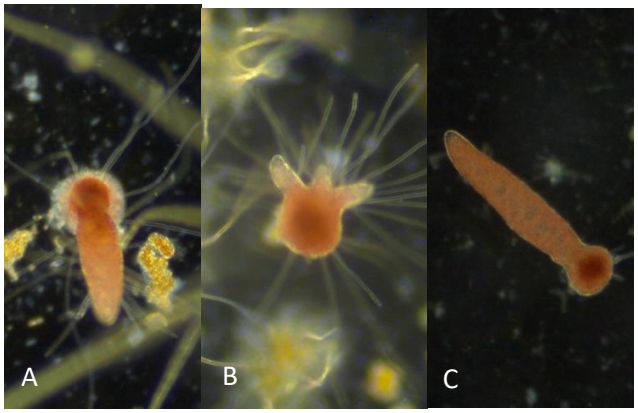

Figure 20: Difference in seedling length of *P. palmata* within the same treatment after 25 days of growth. A-C: Replicates of C, GeO_2 (negative control) A-C: 20x.

3.4 Chemical sori disinfection with GeO_2

Based on results from trial a GeO_2 was chosen as the disinfection chemical in trial b. Spore density was registered after 48 hours of spore release (see material and method, section 2.3.3). Seedling and contamination density were registered after 21 days of incubation.

3.4.1 GeO_2 treatments

Figure 21 shows spore density after spore release and seedling density after incubation after disinfection with GeO_2. Spore density increased with increasing concentrations of GeO_2. However, no significant difference was observed compared to control (Kruskal Wallis, $p>0.05$). Sori disinfected with the highest concentration (0.5 mL L^{-1}) gave a spore density close to control. Figure 21 reveals significantly lower seedling density in treatments disinfected with the lowest and the intermediate concentrations compared to control (Mann Whitney, $p<0.05$). The highest concentration (0.5 mL L^{-1}) gave the highest seedling density of all treatments, including control. The seedling density increased with increasing concentrations of GeO_2.

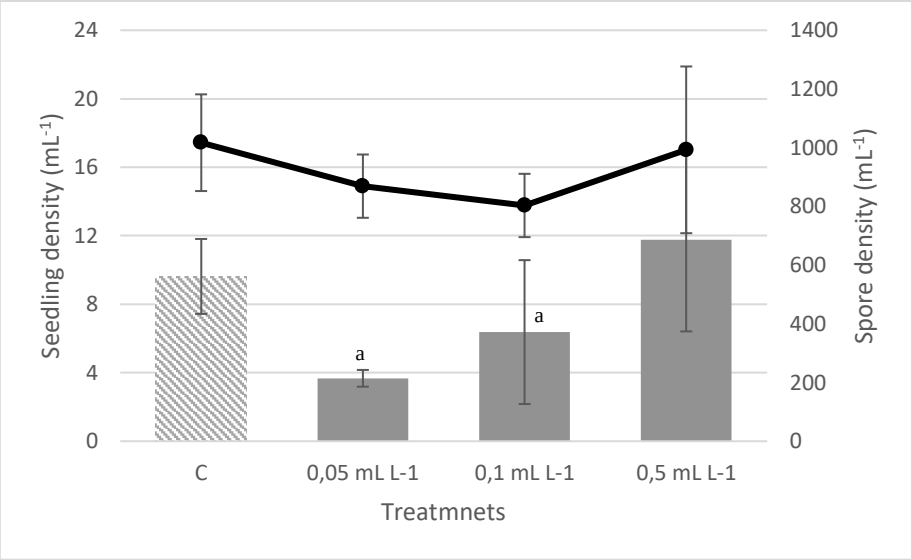

Figure 21: Effect of disinfection of sorus with GeO_2 on spore release and seedling density after 21 days of incubation. Bars indicate seedling density (mL^{-1}) (mean value ± SD, n=5). The line and the secondary y-axis represent spore density ($mL^{-}1$) after spore release (mean value ± SD, n=5). The x-axis shows control the different treatments of GeO_2. a= Significant lower sporophyte density than control.

Figure 22 gives the diatom density of treatments disinfected with GeO_2. Contamination decreased with increasing concentrations of GeO_2. The weakest disinfection treatment (0.05 mL L^{-1}) gave the absolute highest diatom density, but not significantly higher than control (Mann Whitney, $p>0.05$). Mann Whitney tests revealed significantly lower diatom density in treatments disinfected with the intermediate and lowest concentration of GeO_2 compared to control ($p<0.05$).

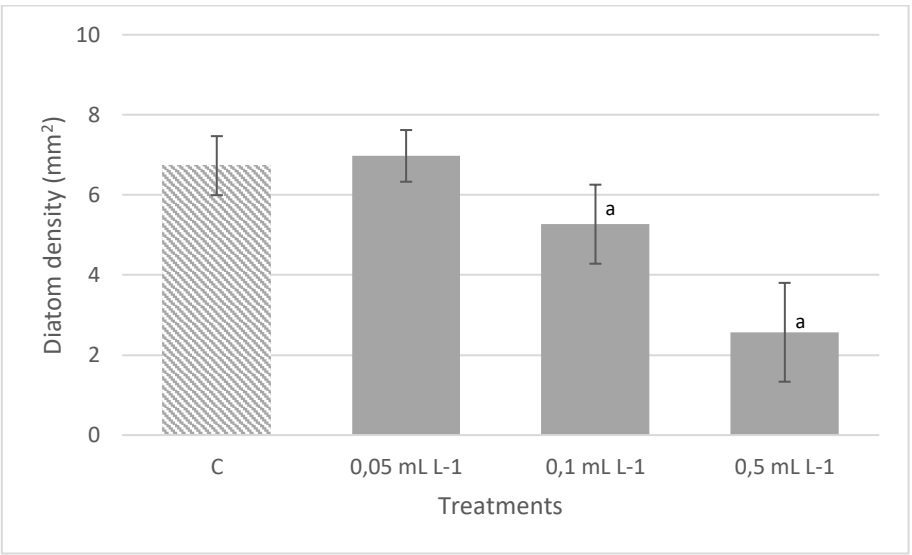

Figure 22: Effect of disinfection of sorus with GeO_2 on diatom density after 21 days of incubation. Bars indicate diatom density (mm^2) (mean value ± SD, n=15). The different treatments are given on the x-axis. a= Significantly lower diatom density than control

The results indicate that treatments disinfected with the highest concentration of GeO_2 gave similar spore density as control, the highest density of seedlings, and the lowest density of contamination.

3.4.2 Variation in seedling length

Ten seedlings from each trial were measured in imageJ after incubation (n=50). Figure 23 gives the length on seedlings disinfected with GeO_2 after 21 days of incubation. Treatments disinfected with the weakest and highest concentration gave the longest seedlings while the control treatment gave the shortest seedlings. However, no significant difference was registered between disinfected treatments and control (Kruskal Wallis, $p>0.05$).

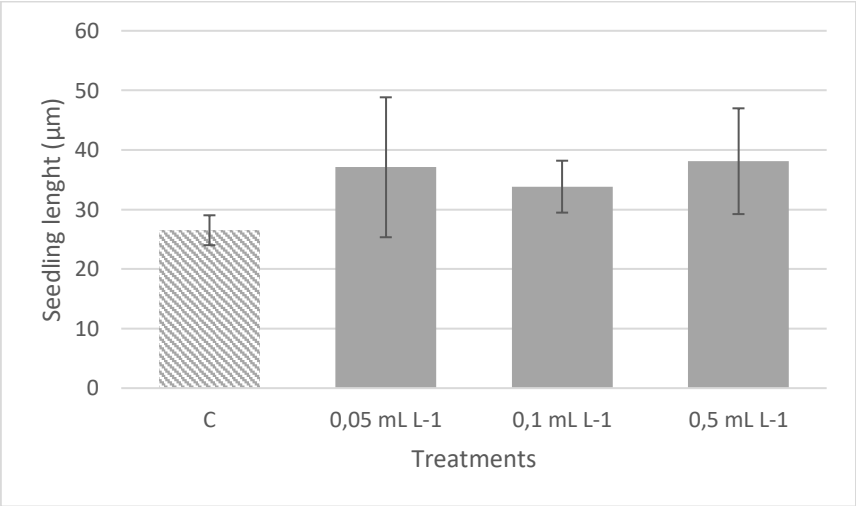

Figure 23: Effect of disinfection of sorus with GeO_2 on seedling length after 21 days of incubation. Bars indicate seedling length (µm) (mean value ± SD, n=50). The different treatments are given on the x-axis.

Seedling length varied between replicates within the same treatment. Figure 24 is an example on length differences between replicates. The smaller seedling in Figure 24 B looks paler than the larger seedlings (24 A and C).

Figure 24: Differences in seedling length between replicates after disinfection of sorus with GeO_2 after 21 days of incubation. A-B: Replicates of 0.05 mL L^{-1} (10x). B-C: Replicates of 0.1 mL L^{-1} (10x).

Figure 25 and 26 shows the seedling development in all GeO_2- treatments after 2, 4, 11, 15, 18 and 21 days of incubation. Despite length differences after 21 days of incubation (Figure 24), no variations in seedling development were observed between treatments. All treatments had ongoing cell-divisions at day 4. Seedlings were observed after 15 days of incubation in all treatments.

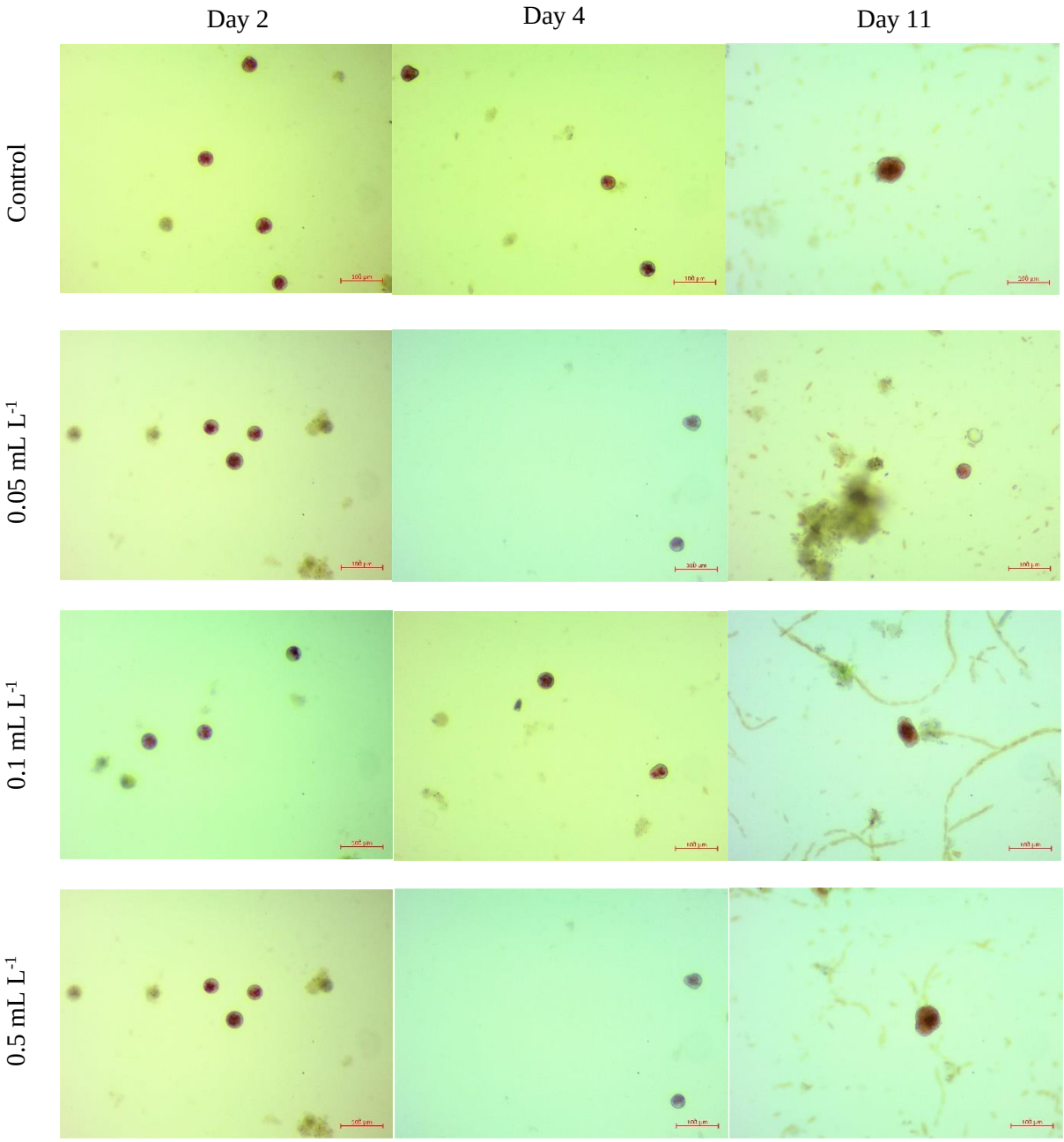

Figure 25: Spore development at day 2, 4 and 11 for disinfected treatments with GeO_2. The different treatments are represented along the left side and the different days are given on the top of the figure.

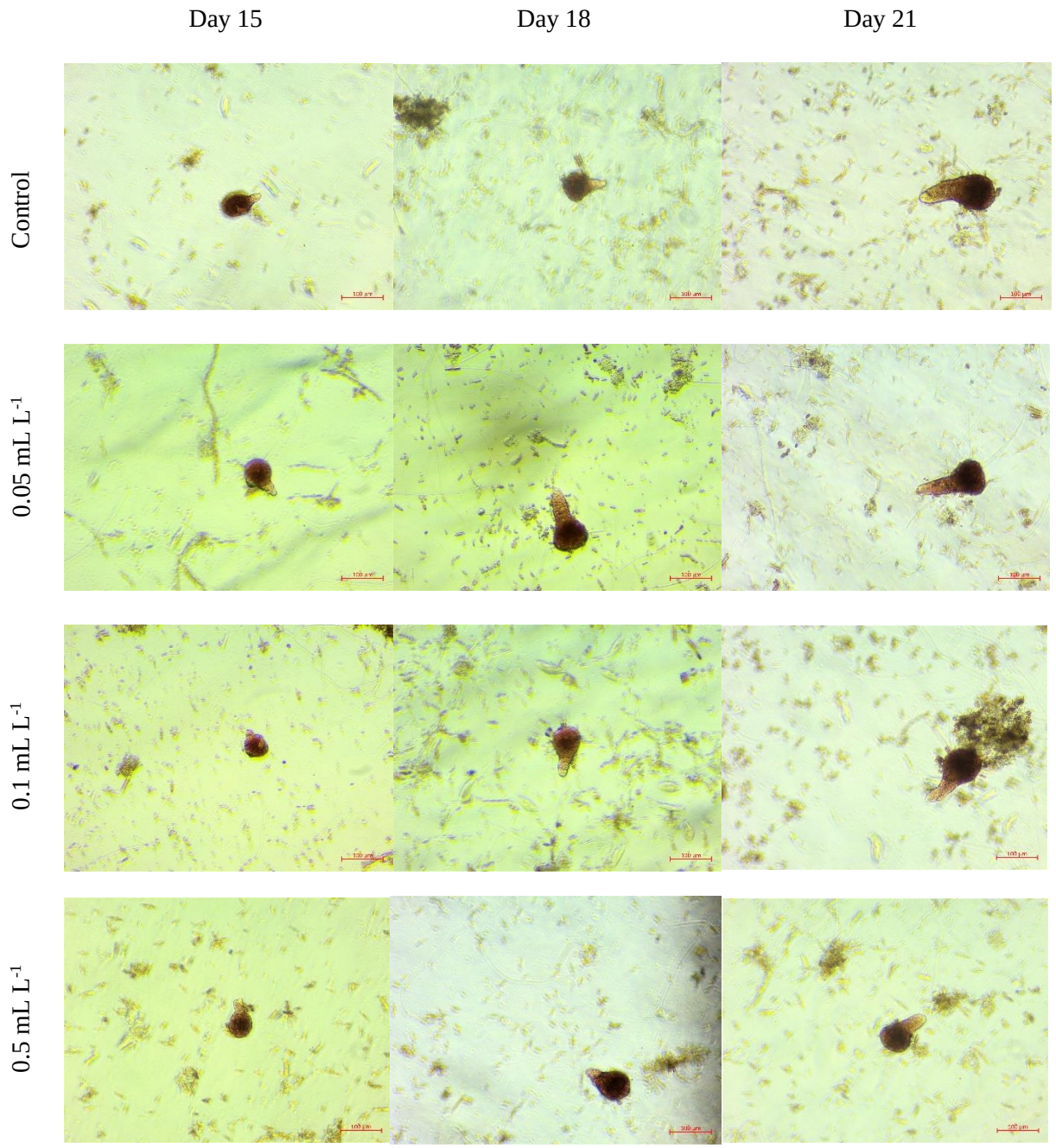

Figure 26: Seedling development at day 15, 18 and 21 of incubation for treatments disinfected with GeO_2. The different treatments are given along the left side while the days of incubation are represented on top of the figure.

3.5 Cultivation of *P. palmata* gametophytes in growth medium

The most promising disinfectant from the previous disinfection trial (0.5 mL L^{-1} GeO_2) was tested in the development of *P. palmata* gametophyte cultures. Spores from both 28- and 48-hours spore releases were applied. All treatments were weekly checked for gametophyte development at day 8, 14, 21 and 29.

All treatments contained spores undergoing cell-division at day 8. At this point, a few small gametophytes were observed in treatments disinfected with GeO_2, but no differences in

treatments from 28 hours and 48 hours spore release were registered. At day 14, all treatments had developed gametophytes. Figure 27 shows length of gametophytes in all treatments after 14, 21 and 29 days of incubation. The treatment disinfected with GeO_2 from 28 hours spore release had the longest gametophytes in all registrations. The treatment not disinfected with GeO_2 from 48 hours spore release (C, 48 h) gave the shortest gametophyte length in all registrations. No significant difference was observed between treatments after 21 days of incubation (Kruskal Wallis, $p>0.5$). A significant difference (Mann Whitney, $p< 0.05$) was observed between treatments with longest and shortest gametophytes length (GeO_2, 28 h and C, 48 h, 1143 ± 8.77 µm and 736 ± 71 µm, respectively) after 29 days of incubation. During the incubation period, treatments from 28 hours spore release showed longer gametophytes length than treatments from 48 hours spore release. However, no significant difference was observed (Mann Whitney, $p>0.05$). Figure 28 shows an example on differences in gametophyte development after 14 days of incubation between treatments with most developed and least developed gametophytes (GeO_2, 48 h and C, 28 h, respectively).

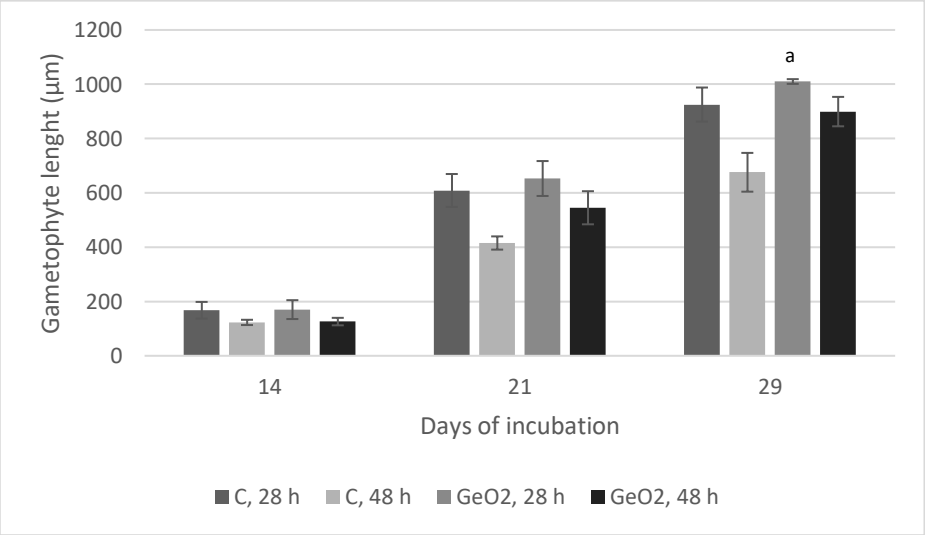

Figure 27: Gametophyte length (µm) in treatments from 28- and 48-hours spore release disinfected with GeO_2 after 14, 21 and 29 days of incubation (mean value ± SD, n=30). Days of incubation is given on the x-axis. a= Significant longer gametophyte length than C, 48 hours.

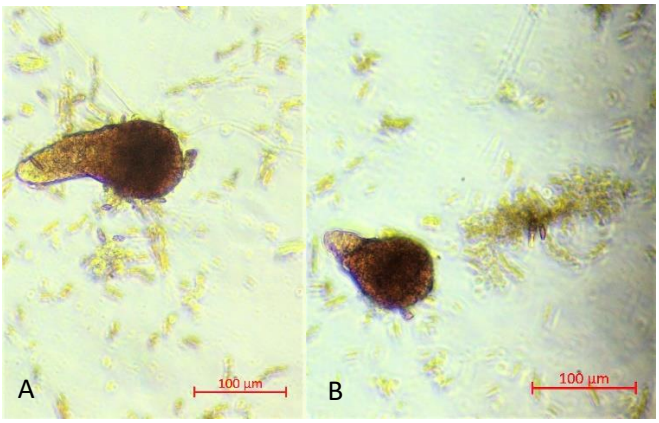

Figure 28: Differences in gametophyte development after 14 days of incubation after disinfection of sorus with GeO_2 followed by 28 hours spore release and untreated sorus followed by 48 hours spore release. A: Gametophyte in GeO_2, 28 h treatment (10x). B: Gametophyte in C, 48 h treatment (10x).

3.6 Deployment of *P. palmata* seedlings in sea for cultivation of biomass

21 strings and 2 nets of *P. palmata* seedlings were deployed in sea 13.02.2018. Growth success were checked once a month until harvest 13.06.2018.

The growth of *P. palmata* seedlings on strings twisted around vertical ropes, reaching from 1-5 meters depth, were unsuccessful. Of the 21 strings deployed only 3 had developed between 1 and 3 small seedlings after 4 months of growth (Figure 29). Ropes were densely covered by contaminating on-growth by epiphytic macro- and microalgae.

Figure 29: A small seedling on one of the strings after 4 months of growth in sea.

The growth of seedlings at both net types were successful. Figure 30 shows seedling growth at both nets after around 3.5 months of growth (29.05.2018) and Figure 31 the seedling

growth after 4 months in sea (13.06.2018). Both nets were densely coved by *P. palmata* seedlings after 4 months of growth. The net with smallest mech width had the densest growth after both 3,5 months and 4 months. Three long seedlings from the net with the smallest mesh width was measured to be 21.8 cm, 17.0 cm and 15.8 cm after 4 months in sea.

Both nets and strings were deployed in an integrated- multitrophic aquaculture (IMTA) situation. Strings were dispersed on a 300 meters gradient from the salmon cages while nets were placed between cages at the start of the gradient.

Figure 30: Seedling growth the 29.05.2018 on both nets deployed 14.02.2018. A: Seedling growth on the net with widest mech width. B: Seedling growth on the net with smallest mesh width. Size measurements would have required destructive harvesting of samples and where therefore not conducted.

Figure 31: Seedling growth at both nets deployed 14.02.2018 after 4 months in sea, 13.06.2018. A: Seedling growth at the net with widest mesh width. B: Seedling growth at the net with smallest mesh. Size measurements would have required destructive harvesting of samples and where therefore not conducted.

4 Discussion

4.1 The year-round reproductive and health status of *P. palmata*

The results revealed a peak of reproductive season from December to April. This coincides with studies of *P. palmata* in both North- France and Ireland, which indicated that tetrasporophytic fronds were fertile during winter (Le Gall et al., 2004; Werner & Dring, 2011). The present study also reveals the year-round distribution of fertile tetrasporophytes and fertile male gametophytes. A low spore to seedling efficiency for *P. palmata* (Schmedes et al., 2019) requires large quantities of fertile tetrasporophytes for successful cultivation. According to Edwards (unpublished work), this may be unnecessary since microscopic mature female gametophytes possibly can be fertilized by fertile male gametophytes. During the reproductive season, around 30- 40 % of total collected sporophytes were fertile tetrasporophytes (2n). Fertile male gametophytes accounted for 20- 30 %, except from January, when it exceeded the amount of fertile tetrasporophytes (47 %). Exploiting the relatively high amount of fertile male gametophytes (Figure 6) can eventually supply *P. palmata* cultivators with sufficient culture stings while reduce the utilization of local wild populations. The present study also suggests that fertile male gametophytes can be utilized in months with low availability of tetrasporophytes in order to obtain the quantity of culture strings.

The result indicates high frequency of unfit *P. palmata* sporophytes in the summer months (June and August) at Storsteinan. Unfit sporophytes were characterized by distinct on growth of epibionts or rotten parts. According to Sanderson et al. (2012) high amounts of epiphytic growth during summer are due to high temperatures and sun radiation. The results suggest that collecting *P. palmata* sporophytes during winter secures both high amounts of reproductive and healthy sporophytes suitable for spore release.

4.2 Spore density in spore release of *P. palmata*

The results from the two spore release trials were somewhat conflicting. Trial a) revealed highest spore density after 28 hours of spore release, while trial b) showed highest spore density after 48 hours. Regardless, the density after 28 hours in trial a) were around two times higher than after 48 hours in trial b). The method for spore release were similar in spore

release trial a) and b). Sporophytes were kept moist under collection and transport to the laboratory and all processing of material was conducted in climate rooms holding 10 °C. This was done in order to avoid early stress-induced spore release due to desiccation and high temperatures (Werner & Dring, 2011). According to Kraft (1984), spore release of *P. palmata* is unpredictable due to high variations in ripeness, also within the same tetrasporophytic fronds. The maturity varies according to several factors such as geographical location and season (Werner & Dring, 2011). In order to minimize individual variations of ripeness, all vegetative tissues were removed, and only ripe sori were used. In both trials, sporophytes were collected at the same geographical position and spore release were conducted in high-agitated waters, which, according to Schmedes et al. (2019), increases the spore yield in spore release. Sporophytes used in spore release trial a) was collected in January while sporophytes for trial b) was collected in November. As mentioned, the year-round field survey on reproductive status of *P. palmata* indicated a reproductive season from December to April. This suggests that sporophytes collected in January, within the reproductive season, gave a higher yield of spores than sporophytes collected in November. Additionally, sporophytes collected within the reproductive season appeared to release tetraspores faster than sporophytes collected at other times.

Spore density after 48 hours spore release in the second disinfection trial (control), with sporophytes collected in November, was surprisingly high, 1017 ± 165 spores mL^{-1} (Figure 21). Highest spore density after 48 hours spore release reinforces the suspicion of spore release rate in context to reproductive season. However, the high spore yield does not agree with previous assumption associated with higher yield of spores from sporophytes collected during the reproductive season. It should however be kept in mind that different methods were used to count spores in spore release trial a) and the first disinfection trial and in spore release trial b).

4.3 Methods for counting of tetraspores

In the first method, a subsample of 125 µL of spore solutions was placed on an object glass with grid. All tetraspores within the grind were counted in a stereo-loupe. Due to dissolved debris of *P. palmata* tissue in samples, especially after sori disinfection, spores were at times hard to detect. Based on this, a different method was tested. In this second method, samples were first filtrated in order to eliminate larger objects than *P. palmata* tetraspores. The spore

solution was then re-filtered using a syringe filter. The syringe filter was placed on a microscope slide and after 30 seconds of drying, tetraspores were counted in a microscope (Material and method, section 2.2.2) Tetraspores were easily detected on the dried filter and somehow looked like they popped out of the filter. This method made the counting results more reliable and based on this it is recommended to use the second method for further laboratory studies of *P. palmata*.

Counting tetraspores automatically would be considerable time saving in laboratory studies of *P. palmata*. Coulter Counter was therefore tested as a method for automatic counting. Unfortunately, results from Coulter Counter and manual counting did not match, and number of spores counted in the Coulter Counter were disturbingly high. This suggests that more than *P. palmata* tetraspores were included in the Coulter Counter results. As earlier mentioned, spore solutions often contained dissolved debris of *P. palmata* tissue. The debris were small and often similar sized as *P. palmata* tetraspores. It is therefore reasons to believe that the Coulter Counter counted small dissolved debris as tetraspores, suggesting that the method cannot be recommended in *P. palmata* laboratory studies.

4.4 Evaluation of chemical sori disinfection

Sori disinfection with use of Lugol, acidic acid and sodium hypochlorite significantly reduced tetraspore survival. Disinfection with 0.2 % Lugol, 1 % and 7 % acidic acid and 600 ppm hypochlorite were lethal for *P. palmata* tetraspores. 0.02 % Lugol and 300 ppm hypochlorite gave significantly lower seedling density than the control treatment. However, diatom contamination was present in al trials disinfected with Lugol and hypochlorite despite a significant reduction compared to control and was only eliminated in acidic acid treatments. Diatom density in the weakest concentration of hypochlorite (300 ppm) were surprisingly high. Noteworthy diatom contamination was also registered in the highest disinfection concentration (600 ppm). According to Kerrison et al. (2016a) disinfection treatments should ideally remove all contaminants while not be harmful for the host or at least allowing quick recovery. Based on this, none of the chosen Lugol, hypochlorite and acidic acid treatments for sori disinfection can be recommended in *P. palmata* cultivation.

According to Druehl and Hsiao (1969); Rød (2012) a mucilage layer covers macroalgae sori and may weaken the capacity of a disinfectant. Knowing that iodine interacts with

polysaccharides (Gaillard et al., 1969), Brown et al. (1995) suggested that the glycocalyx creates an iodine resistance which increases the iodine time requirement to interact with the deepest cell layer. Sporophytes were kept in tanks with running seawater until experimental starts, and prior to disinfection, sori was physically cleaned by paper towels with sterile seawater. According to Characklis and Dydek (1976), available chlorine can be consumed by organic matter in a solution. The study reported that extracellular polysaccharides from microbial aggregates consumes hypochlorite. It is therefore possible that some organic matter still was attached to the sori after physical cleaning and prevented the full potential of hypochlorite disinfection. Acidic acid is interesting to test as a sori disinfectant due to its limiting environmental threat and low cost. Locke et al. (2009) reported that acidic acid at 4 to 5 % is lethal to macroalgae. Forrest et al. (2007) suggested that far weaker concentrations (<1 %) were lethal for early life stages of some macroalgae. Based on this, in order for Lugol to interact with a greater share of diatoms while the effect on *P. palmata* reduces, weaker Lugol concentrations and longer exposure times should be tested. A possible hypochlorite treatment to reduce contaminants without negatively affect *P. palmata* development could be disinfection with weaker concentrations after total elimination of organic matter from sori. This will however be labour intensive and most likely not suitable for large-scale cultivation of *P. palmata.* It would additionally be interesting to test the tolerance limit for *P. palmata* to acidic acid in further studies. Testing the tolerance limit to acidic acid has also been suggested in *S. latissima* disinfection (Rød, 2012).

No significant variation in seedling density between treatments with GeO_2 in growth medium (0.1 mL L^{-1}) (negative control) and treatments without (positive control) was detected (Figure 7). Markham and Hagmeier (1982) tested growth inhibiting effect of GeO_2 on the Rhodophyta species *Porphyra umbilicas*, *Polysiphona urceolata* and *Chondrus crispus*. The study suggested that the majority of diatoms could be controlled with no negative effect on macroalgae in these Rhodophyta cultures. A different study by Tatewaki (1979) reported that only very high concentrations of GeO_2 (20-30 mg L^{-1}) showed growth inhibitory effects on red algae. It should however be kept in mind that small sample size was used (n=3) in the present study, which decreasingly represent the population and affects the reliability. Additionally, seedling density in negative control (Figure 6) had a high SD, indicating high variability of the populations (Altman & Bland, 2005).

In agreement with Markham and Hagmeier (1982) contamination was significant positively affected by addition of GeO_2 in Lugol and hypochlorite treatments. Surprisingly, addition of

GeO_2 in control treatment (negative control) negatively affected diatom density. However, the difference in diatom density between negative and positive control was very small, and not significant (7.3 ± 0.7 and 8.2 ± 0.8, respectively). Based on this, in addition to the SD and small sample size (n=9), it is suggested that diatom density did not represent the entire populations. This can indicate that negative control might not have had higher diatom density than positive control.

Control treatments in both disinfection trials showed poor spore survival rate. In disinfection trial a), 514 spores mL^{-1} after spore release developed into 8.5 seedlings mL^{-1} after incubation. 9.6 spores mL^{-1} out of 1017 spores mL^{-1} developed into seedlings in disinfection trial b). Small seedings with more pale cells than larger seedlings were also observed in both disinfection trials (Figure 19 and 24). Santelices (2014) reported that photosynthesis can be inhibited by high irradiances in newly released macroalgae spores, which is probably one of the reasons why spores in nature settles in shaded habitats. Irradiance of 50 µmol m^{-1} s^{-1} is suggested to be destructive to *P. palmata* seedlings (Edwards) (unpublished work). In the present study, both disinfection trials were incubated in 40 µmol m^{-1} s^{-1}. Pigments responsible for the coloration in red algae, such as phycocyanin and phycoerythrin are necessary for their photosynthesis (Dring et al., 1991). An important component in these pigments and their associated proteins is nitrogen (Morgan et al., 1980). As described in Edwards and Dring (2011), decreased pigmentation in red algae can be an indicator of nitrogen limitations (Rico & Fernández, 1996). Additionally, cultivation under high irradiances have shown reduced nitrogen content in adult *P. palmata* sporophytes (Morgan & Simpson, 1981). Growth medium were changed after two weeks in both disinfection trials. According to Sanderson et al. (2012) growth medium should be changed at least once a week to secure high macroalgae survival and growth in small volumes. Pale seedlings can therefore suggest reduced photosynthesise rate due to limiting nitrogen. Based on this, it is suggested that chosen culture parameters were not optimal for *P. palmata* growth. Further laboratory studies on *P. palmata* growth should test incubation with lower irradiances than 40 µmol m^{-1} s^{-1} and more frequent changing of growth medium.

4.6 Effect of GeO_2 as a sori disinfection treatment for *P. palmata*

The result showed no significant difference in spore density between control and treatments disinfected with GeO_2 (Figure 21). Surprisingly, spore density increased with increasing

disinfection concentrations, and the highest concentrations (0.5 mL L^{-1}) of GeO_2 showed spore density close to the control. Seedling density was significantly lower than the control after disinfection with the weakest and the intermediate concentrations of GeO_2 (0.05 mL L^{-1} and 0.1 mL L^{-1}) However, treatments disinfection with the highest concentration (0.5 mL L^{-1}) developed a higher seedling density than the control after 21 days of incubation. Diatom contamination were significantly lower than the control in treatments disinfected with 0.1 mL L^{-1} and 0.5 mL L^{-1} GeO_2 (Figure 18). Results indicates that sori disinfection with 0.5 mL L^{-1} GeO_2 for 2 minutes in an exposure temperature of 10 °C followed by two rinsing baths of sterile seawater for 30 seconds prior a 48 hours spore release reduced contamination without reducing the survival of *P. palmata* spores.

No seedlings were observed until 15 days of incubation after disinfection of GeO_2 (Figure 26). However, seedlings in disinfected treatments were longer than seedlings in control after 21 days of incubation. This agrees to Shea and Chopin (2007) who reported that seedlings treated with 0.1 mL L^{-1} and 0.5 mL L^{-1} GeO_2 were longer than seedlings in other treatments. In a *P. palmata* cultivation study by Werner and Dring (2011), seedlings were observed after 10 days. This suggests that GeO_2 showed an inhibitory effect and delayed spore to seedlings development but increased the seedling growth after this transition. Despite length differences, no differences were detected between disinfection in 0.05 mL L^{-1} and 0.5 mL L^{-1} GeO_2, which proposes that the delaying effect did not deteriorate between these concentrations of GeO_2.

However, seedlings were observed at day 8 in gametophyte cultures disinfected with 0.5 ml L^{-1} GeO_2 prior 28 hours spore release. Seedlings were not developed until 14 days of incubation in cultures with untreated sori prior 28- and 48-hours spore releases and GeO_2 disinfected sori prior 48 hours spore release. Seedlings disinfected with 0.5 mL L^{-1} GeO_2 prior a 28 hours spore release were longest in all four registrations and were significantly longer than disinfected seedlings from 48 hours spore release after 29 days of incubation. This might this be interpreted as if GeO_2 did not have the delaying effect on spore to seedling development on spores from 28 hours spore release. Additionally, since sori disinfection with 0.5 mL L^{-1} GeO_2 positively affected seedling survival and significantly reduce diatom contamination, should this treatment prior 28 hours spore release be further assessed.

In the sori disinfection trial with four disinfectants, seedlings from untreated sori grown in addition of GeO_2 showed a slightly longer length than seedlings in positive control, despite, as earlier mentioned, a lower seedling density. In the sori disinfection trial with GeO_2, the

treatment with lowest seedling density (0.05 mL L^{-1}) were one of the treatments with longest seedlings after incubation. Apparently, this suggests that low seedling density in cultures improves individual seedling growth. This coincides with a study within experimental density and plant size in brown macroalgae (Creed et al., 1998), which demonstrated that high population densities reduced seedling survival and growth. On the other hand, the present study's results are ambiguously, and disinfection with 0.5 mL L^{-1} GeO_2 gave the trial's highest seedling density in addition to long seedlings.

4.8 Growth success for *P. palmata* seedlings deployed in sea

P. palmata seedlings were deployed in an IMTA situation with intensive cultivation of salmon in exposed waters and the result showed that the growth of seedlings on nets were successful. However, the deployment of *P. palmata* seedlings on stings were unsuccessful. This was the first attempt to grow *P. palmata* seedlings in Norwegian open waters.

Strings were dispersed on a 300-meter horizontal gradient from the salmon cages, while nets were placed in the start of the gradient, in the middle of the salmon cages. It is known that the most important elements for algae metabolism in coastal waters are nitrogen and phosphorus. According to Dring et al. (1991) nitrogen is the limiting factor for algal productivity since phosphorus in open sea is in relative excess. Sanderson et al. (2012) demonstrated that *P. palmata* utilizes up to 12 % of waste nitrogen from salmon cages. However, strings close to salmon cages also showed poor seedling growth. Based on this, less additional nitrogen available for seedlings on strings than on nets is most likely not the reason for unsuccessful growth on strings. The strings had low density of seedlings before deployment (1-2 seedlings cm^{-1}) in February. After 4 months in sea, ropes were densely covered by contaminating epiphytic macro and microalgae, that seemed to have outcompeted the *P. palmata* seedlings. According to Werner and Dring (2011), the best season for deployment is late autumn/winter when the growth of fouling algae is inhibited by low light and temperatures. This will allow *P. palmata* to cover the culture stings before fouling organism's growth conditions improve. Regardless, growth of cultivated *P. palmata* seedlings in Norwegian open waters should be tested in a monoculture. Deployment of denser cultures strings in late autumn/winter should additionally be tested. As mentioned in material and method, section 2.5.2, seeded strings were twisted around ticker nylon ropes by an electrical twisting machine before deployment

in sea. This can as well have affected the unsuccessful growth on the stings since seedlings may have fallen of during the twisting process.

The results demonstrate that the net with smallest mesh width had the densest growth of seedlings (Figure 27) and few epiphytic macro and microalgae were observed. Seedling density on nets were unfortunately not checked prior to deployment. Assuming that the density of seedlings was close to similar on both nets, this agrees with Lüning and Pang (2003), which stated that epiphytes can be controlled on rope cultures in sea by growing plants in high densities. According to Sanderson et al. (2012), the optimal harvesting size of *P. palmata* sporophytes is between 30 to 40 cm, but depends on geographical criteria, growth rates and intended use. Size measurements were not conducted in the present study since it would have required destructive harvesting of samples. Nevertheless, three long seedlings from the net with the smallest mesh width were measured to 21.8 cm, 17.0 cm and 15.8 cm after 4 months in sea, interpreting a successful deployment. However, it would be interesting to measure seedling size once a month to determine the specific growth rate of cultivated *P. palmata* in Norwegian open waters.

5 Conclusion

The present study demonstrated a reproductive season from December to April for *P. palmata* sporophytes in the area of Trondheim. Collecting sporophytes during winter may secure high amounts of both mature and fit sporophytes suitable for spore release. This study also revealed the year-round distribution of fertile tetrasporophytes and fertile male gametophytes and demonstrated high availability of fertile male gametophytes especially in January.

The study suggests that spore release rate is seasonal dependent. Sporophytes collected during the reproductive season released spores earlier than sporophytes collected at other times in 10 °C agitated spore releases in darkness. During the reproductive season (January) spore density was highest after 28 hours of spore release, while after 48 hours prior the reproductive season (November).

None of the chosen treatments of Lugol, hypochlorite and acidic acid for sori disinfection can be recommended in *P. palmata* cultivation. Despite significant reduction of diatom contamination, all treatments gave significantly lower spore density after spore release and seedling density after 25 days of incubation than control. Sori disinfection with 0.2 % lugol, 600 ppm hypochlorite and 1 % and 7 % acidic acid was lethal for *P. palmata* spores.

Sori disinfection with 0.5 mL L^{-1} GeO_2 for 2 minutes at an exposure temperature of 10 °C, followed by two rinsing baths of sterile seawater for 30 seconds can be a promising treatment in *P. palmata* cultivation. The treatment significantly reduced contamination without any observed effect on seedling survival and developed the longest sporophytes after 21 days of incubation. GeO_2 (0.1 mL L^{-1}) added in growth medium in *P. palmata* cultures showed no significant effect on seedling survival.

The study suggests that spores from a 28 hours spore release was more resilient than spores from a 48 hours spore releases. Sori disinfection with GeO_2 (0.05 – 0.5 mL L^{-1}) showed a delaying effect on spore to seedling development in cultures with spores from a 48 hours spore releases. This effect was not observed in cultures with spores from a 28 hours spore release.

The first deployment of *P. palmata* seedlings in Norwegian open waters in February 2018 showed successful growth on nets in an IMTA situation. The growth success for seedlings on

strings were unsuccessful. The net with smallest mesh width appeared to have the densest growth of sporophytes after 4 months in sea.

Finally, due to dissolve debris of *P. palmata* tissue, an automatic counting of tetraspores using Coulter Counter cannot be recommended in laboratory studies of *P. palmata*. Manual counting of tetraspores from a 0.8 µm Cellulose Nitrate filter after syringe filtration can on the other hand be a good tool for spore counting.

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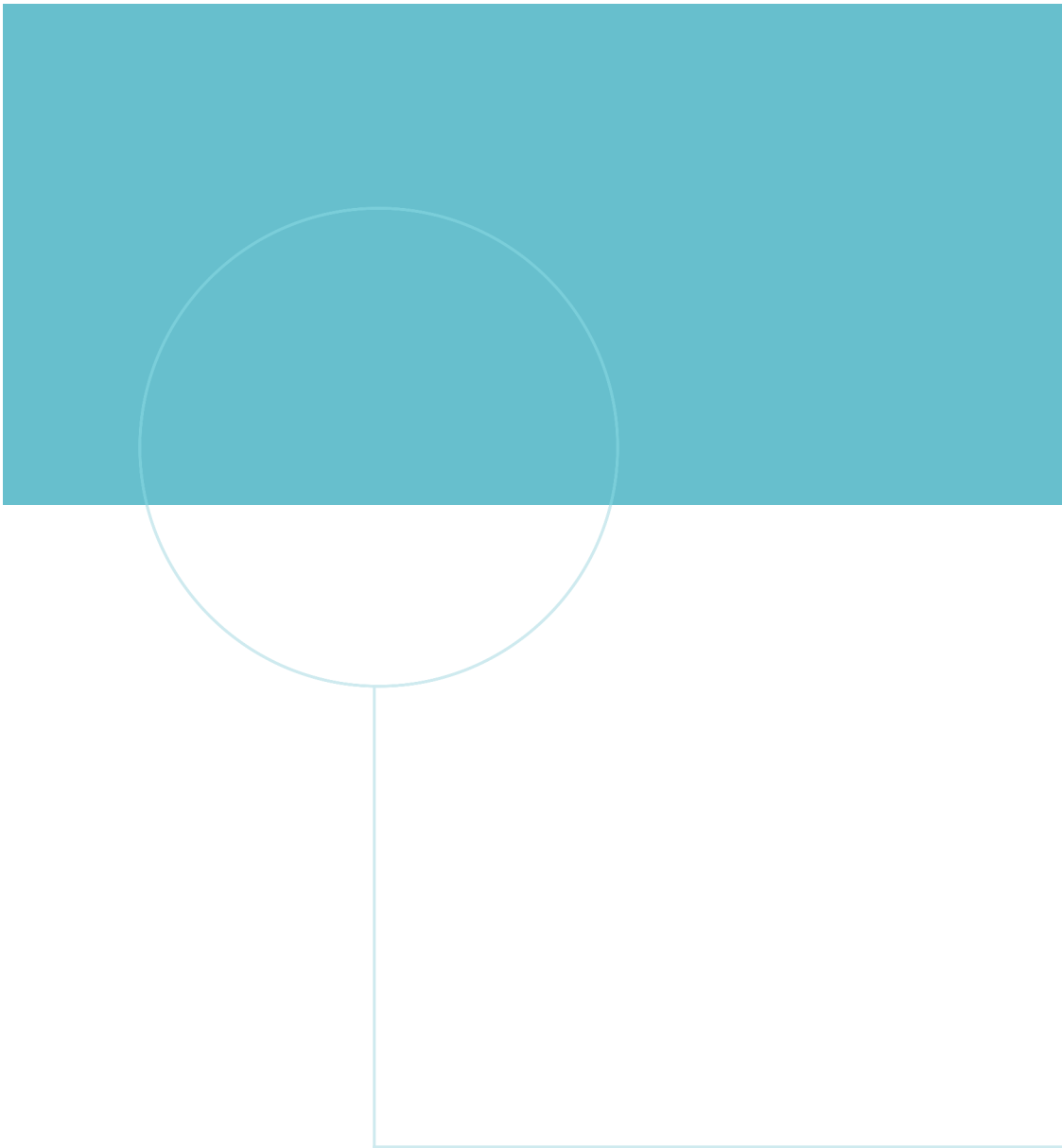

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