Temperature tolerance and distribution of the invasive non-indigenous red algae *Agarophyton vermiculophyllum* in seagrass meadows in Norway

Ingvild Kristine Sundal Joys

Master of Science in Biology – Marine Biology

September 2021

Supervised by

Vivian Husa - Institute of Marine Research

Inga Kjersti Sjøtun - Department of Biological Sciences, University of Bergen

Acknowledgements

First, I want to give a big thank you to my supervisors Vivian Husa and Kjersti Sjøtun for the guidance and feedback, and for all the help with fieldwork in sometimes challenging conditions. I am grateful for the opportunity to gain more knowledge from the world of algae and non-indigenous species.

A lot of people have made this thesis possible. Thank you to my co-supervisors Henning Steen and Barbro Haugland for excellent assistance in the field and invaluable help with species identification, equipment, and ideas. Thank you to my remaining co-supervisors Thijs Christiaan van Son and Jonas Thormar for ideas during the planning of the field investigations.

I want to thank Helen and Samuel Rastrick for help with set up, ideas and technical see through of the temperature experiment, and Erwann Legrand for help with equipment and ideas, the experiment could not have been done without you!

The Institute of Marine Research for analysing the nutrient samples and allowing me to use their facilities in Bergen and Austevoll.
A special thanks for great support from the bioCEED R- club, for keeping the Zoom-room open throughout the summer and helping us with all our data and code problems.

Last, I want to express my gratitude to my dear friends and family for the motivation and support. I honestly could not have asked for better people having my back though this challenging and uncertain last year during a global pandemic. Thank you!

Abstract

Invasive non-indigenous species are considered one of the main threats to the global biodiversity. The marine red alga *Agarophyton vermiculophyllum* originating from the northwest Pacific has been introduced to all the main coast in the northern hemisphere, Norway included, and is considered highly invasive. *A. vermiculophyllum* has been shown to negatively affect *Zostera*-species, which form the highly productive and biodiverse habitat of seagrass meadows. A field study was conducted to assess the abundance of *A. vermiculophyllum* in areas south-eastern Norway with important seagrass meadows, as well as environmental factors that could explain the difference in abundance of *A. vermiculophyllum* at the sites.

A large variability in abundance was detected between the four sites investigated. On the four sites combined *A. vermiculophyllum* was present on more stations outside the seagrass meadows than together with the seagrass *Zostera marina*. *A. vermiculophyllum* showed very low abundance in Viksfjorden where the nutrient concentrations are high, even though this alga is known for establishing in eutrophicated shallow waters. A model selection with the environmental variables returned depth as the most explaining factor of its presence. However, this does not provide the full representation of reality and wave exposure is suggested as a hypothetical factor affecting abundance.

In addition, a temperature experiment imitating Norwegian winter conditions was performed to investigate survival of *A. vermiculophyllum* by ability of regrowth and photosynthetic capacity once returned to favourable conditions. There was found no significant differences in the survival between the groups that had been exposed to water of 0°C, 2°C, 8.4°C or 11.7°C degrees.

Agarophyton vermiculophyllum does not seem to be a threat to the investigated seagrass meadows at this point, but areas where it is present together with the seagrass should be monitored to assess possible future negative impact on this ecosystem, and the data presented here provides a good basis of comparison in future studies. This study provides new insight in the ability of *A. vermiculophyllum* to tolerate the low temperatures of Norwegian winters, and its potential for further dispersal northwards.

Table of contents

1 Introduction

Nature and its vital ecosystem services it under elevated pressure from anthropogenic disturbances and biodiversity worldwide is rapidly declining (IPBES 2019). Invasive non-indigenous species (NIS) are considered to be one of the five largest direct drivers of these changes (IPBES 2019). The rising ocean temperatures and more rapid occurrence of extreme heat events (IPCC 2014) is likely to further facilitate invasions in the marine environment (Stachowicz et al. 2002). Invasive non- indigenous species are known to alter marine habitats and further knowledge of how is crucial for good management of the invaders (Katsanevakis et al. 2014).

1.1 The invasive red alga *Agarophyton vermiculophyllum*

Agarophyton vermiculophyllum (Ohmi) Gurgel, J.N.Norris & Fredericq, 2018 is a marine macro algae in the phylum Rhodophyta. It was previously known as *Gracilaria vermiculophylla*, but has recently been subject of a revision (Gurgel et al. 2018). It was first described by Ohmi (1956) in northern Japan as *Gracilariopsis vermiculophylla. Agarophyton vermiculophyllum* is native to the north-western Pacific and is common in Japan, China, Korea, Russia and Vietnam, where it is a valued species in agar production (Tseng and Xia 1999; Kim et al. 2010). However, this alga has now been introduced to all the main coasts in the northern hemisphere, including the Pacific east coast, and the eastern- and western Atlantic coasts (Bellorin et al. 2004; Rueness 2005; Freshwater et al. 2006; Kim et al. 2010). It is ranked in the top among the non-indigenous macroalgal species of Europe, with a high potential of invasion (Nyberg 2007).

This coarsely branched alga usually grows to a size between a few cm to 30 cm, but larger individuals are not uncommon. *A. vermiculophyllum* has a cartilaginous, somewhat flexible texture, with branches of cylindrical shape, which branches in an alternate or irregular pattern in three to four orders (Ohmi 1956; Tseng and Xia 1999; Rueness 2005). Its colour has been described as dark red or even black, to more brown (Ohmi 1956; Rueness 2005; Rinde et al. 2020). It is a perennial species and can be found in the intertidal attached to hard substratum with its basal disk (Ohmi 1956) or laying loosely on the seafloor sometimes forming large mats (Rueness 2005; Nyberg 2007; Davoult et al. 2017). It usually inhabits shallow estuarine habitats as soft- bottom bays and mudflats (Rueness 2005; Nyberg et al. 2009; Davoult et al. 2017).

Reproduction appears through a haplo-diplontic life cycle with almost identical tetrasporophytes and separate female and male gametophytes (Ohmi 1956; Krueger-Hadfield et al. 2016). Asexual reproduction appears trough fragmentation. The latter appears to be the main method of reproduction in the predominantly loose-laying introduced populations of *A. vermiculophyllum* (Krueger-Hadfield et al. 2016). It is known for its wide tolerance limits and has been shown to sustain considerable variability in temperature, salinity, moisture, and light (Raikar et al. 2001; Nyberg and Wallentinus 2009; Nejrup et al. 2013; Kim et al. 2016).

1.2 Dispersal of *A. vermiculophyllum*

The first records of *A. vermiculophyllum* in European waters were done in Brittany, France in the 1990s (Mollet et al. 1998; Rueness 2005). Since then, it has been discovered along the coasts of several European countries, including Portugal, Spain, the Netherlands (Rueness 2005), the British Isles (Krueger-Hadfield et al. 2017b), Italy (Sfriso et al. 2010), and Germany (Thomsen et al. 2007a). In 2003 the first Scandinavian observations were done when *A. vermiculophyllum* was discovered in Denmark and Sweden (Nyberg 2007; Thomsen et al. 2007b), with observation if subsequent rapid dispersal along the Swedish coastline (Nyberg 2007).

As the first observations of *A. vermiculophyllum* in Europe were done in close proximity to facilities with Japanese oysters, introduction together with Asian species imported for aquaculture has been a proposed as a plausible vector of introduction (Mollet et al. 1998; Rueness 2005). This theory has been strengthened by analysis showing large genetic similarities between the European *A. vermiculophyllum* populations and those in the Japanese main region of oyster export (Krueger-Hadfield et al. 2017a).

1.2.1 Introduction and dispersal in Norwegian waters

In 2012 the first registered observation of *A. vermiculophyllum* was done in Norway (Husa et al. 2013). During a mapping project of marine introduced species in inner- and outer Oslo fjord, 70 marinas were surveyed. Specimens of *A. vermiculophyllum* were found at three localities in outer Oslo fjord. Husa et al. (2013) discovered one specimen in Ødegårdskilen, Tjøme, while several larger specimens were detected in two sites at Nøtterøy (Figure 1, red). The species identification was confirmed by genetic analysis. It should be noted that the findings of *A. vermiculophyllum* happened towards the end of this survey, and hence it was looked more actively in suitable habitat for this species after this and it cannot be disregarded that it could have been overlooked earlier in the survey. The only station of this survey that

was situated in inner Oslo fjord was at the opera house ceiling on hard substrate. Due to this one cannot exclude the possibility that *A. vermiculophyllum* already was present in inner Oslo fjord at this point. In the years after the first detection in 2012 the surveying of this alga has been more systematic (V. Husa, personal communication, July 28, 2021).

In the following years *A. vermiculophyllum* has been observed along the coastline from Kristiansand to the Swedish border (Figure 1). The possibility that *A. vermiculophyllum* was present in Østfold near the Swedish border some time before the first registration in 2015 is quite probable due to the large amounts found when it was detected (V. Husa, personal communication, July 28, 2021). In 2018 findings of *A. vermiculophyllum* was again confirmed by genetic analysis. This time specimens from Slependrenna in inner Oslo fjord were tested to confirm its presence, as it can be hard to visually distinguish from the native *Gracilaria gracilis* (Rinde et al. 2020).

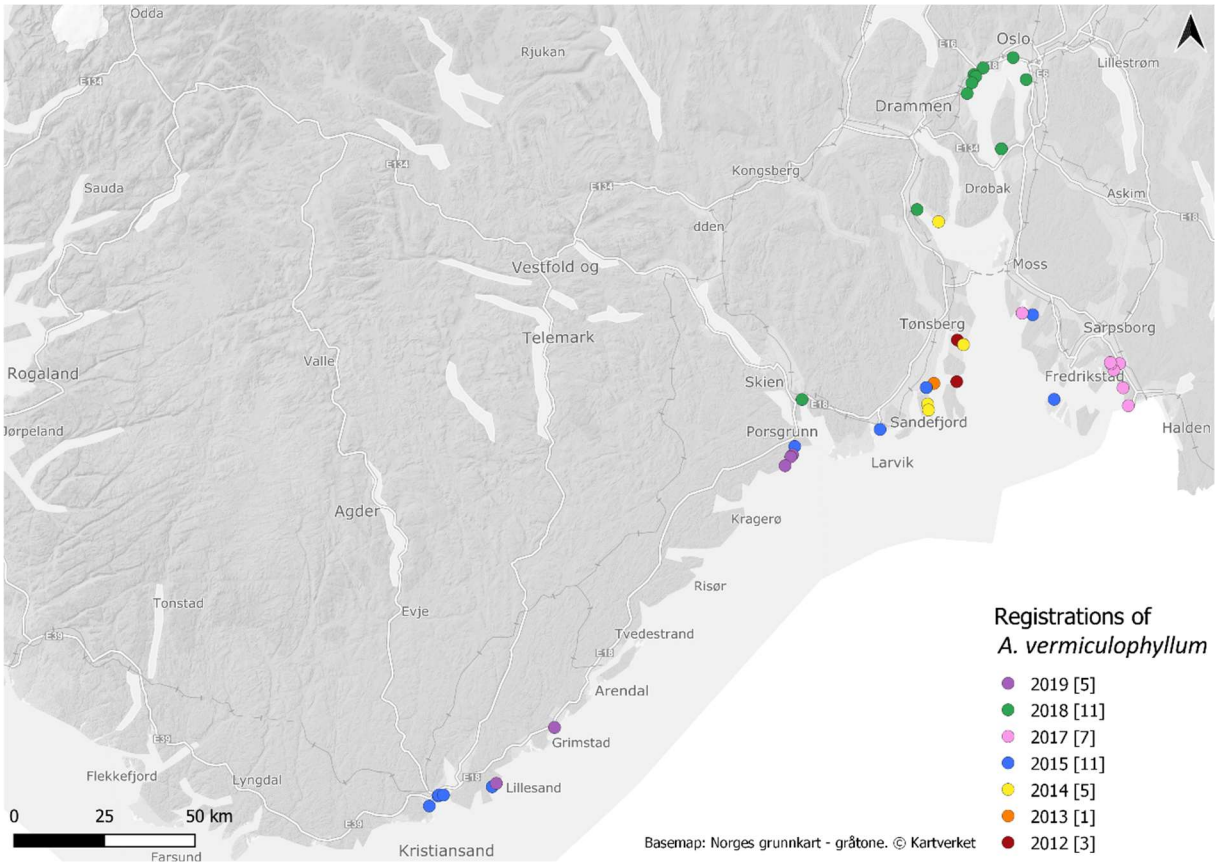

Figure 1 Map showing all registered observations of A. vermiculophyllum in Norway from 2012 to 2019. Coloured by year, number of observations in brackets. Based on data downloaded from the Norwegian Biodiversity Information Centre a, Artskart.artsdatabanken.no 03.06.2021. Observational data from: Norsk botanisk forening, BioFokus.

The species is suggested to have been introduced to Norway by secondary dispersal from Sweden or Denmark (Husa et al. 2013, 2018), possibly with boat traffic as the vector (Husa et al. 2013). Potential vectors for further dispersal in Norway includes entanglement in small boats and their propellers, fishing equipment, and other mobile marine equipment (Husa et al. 2018), the same as the believed vector of secondary dispersal in Denmark and Sweden (Nyberg 2007; Thomsen et al. 2007a). Fertile specimens of *A. vermiculophyllum* has been observed in Norwegian waters (V. Husa, personal communication, July 28, 2021) and it is therefore believed to have some degree of sexual reproduction in addition to vegetative reproduction.

1.3 Consequences from dispersal of *A. vermiculophyllum*

The combination of its ability to reproduce vegetatively through fragmentation (Krueger-Hadfield et al. 2016) and its capability to survive unfavourable conditions (Nyberg and Wallentinus 2009) has likely contributed to *A. vermiculophyllum*'s success in establishing worldwide (Nyberg 2007), as fragments can endure harsh periods of transport on the vector and continue dispersal by fragmentation in the new environment. This ability of fragments of red algae to survive long periods in ballast water-conditions has previously been demonstrated (Sjøtun et al. 2008). These two factors, as well as its wide tolerance limits (Raikar et al. 2001; Nyberg and Wallentinus 2009; Nejrup et al. 2013; Kim et al. 2016), and possible also by less grazing pressure from native herbivores (Nejrup et al. 2012) likely have facilitated the establishment of large mats of *A. vermiculophyllum* in Europe. The phenomenon of loose laying populations is reported especially from France where large estuarine habitats are completely dominated by *A. vermiculophyllum* (Rueness 2005; Surget et al. 2017; Davoult et al. 2017).

Agarophyton vermiculophyllum has been observed to form mats covering the seafloor also in Norway (Husa et al. 2018; Rinde et al. 2020), but findings to the extent detected in i.e. France are yet to be observed. Although *A. vermiculophyllum* is still believed to be in an early stage of establishment in Norway, it is categorised to "Severe impact" (SE) in the ecological risk assessment for alien species, based on its high invasion potential and potential negative ecological effect on native species and habitats (Husa et al. 2018).

The localities where *A. vermiculophyllum* has been observed in Norway are mostly sheltered, shallow bays with muddy substrate (Husa et al. 2013, 2018), and the species has been registered down to five meters depth (Rinde et al. 2020). This habitat is often overlapping

with that of seagrass meadows, and *A. vermiculophyllum* is hence considered a threat to the native seagrass species *Zostera marina* and the endangered *Zostera noltei* (Henriksen and Hilmo 2015; Husa et al. 2018). In addition to that their preferred habitats seem to be overlapping, the presence of *Zosters marina* has previously been shown to facilitate attachment of another drifting invasive algae (Tweedley et al. 2008).

1.3.1 Effect of *A. vermiculophyllum* on *Zostera* species

Zostera marina Linnaeus, 1753 and *Zostera noltei* Hornemann, 1832 are marine angiosperms native to Norwegian waters (Bekkby et al. 2008; Olsen et al. 2013; Enerstvedt et al. 2017). *Zostera marina* is the dominant species in Norwegian seagrass meadows (Enerstvedt et al. 2017), an important habitat that can be found in shallow, calm bays, often on sandy or muddy substrate (Bekkby et al. 2008; Olsen et al. 2013).

Seagrass ecosystems provide important habitats for juvenile fish (Lilley and Unsworth 2014) in addition to a high number of other species (Fredriksen et al. 2005; Christie et al. 2009), and subsequently maintains high biodiversity. Its capacity of carbon sequestering has been compared to that of the rain forest, as the roots trap carbon in the sediment acting as a carbon sink (Duarte et al. 2010). However, seagrass meadows represent an ecosystem under elevated pressure globally and is present on OSPARs list of Threatened and/or Declining Species & Habitats (OSPAR Commission, 2009).

Stressors as advancing anthropogenic ocean warming (Höffle et al. 2011), invasive species (Matheson et al. 2016), eutrophication (Duarte 1995), and loss of habitat due to coastal development (Waycott et al. 2009), are just a few of the factors that have caused great concern for the future of this highly productive system (Duarte 2002; Waycott et al. 2009).

Several studies have investigated the impact of *A. vermiculophyllum* on seagrass species, with the current invasive success and potential dispersal of this red algae in mind and have shown negative impacts on this habitat (Martínez-Lüscher and Holmer 2010; Höffle et al. 2011; Thomsen et al. 2013; Vieira et al. 2020).

High abundance of *A. vermiculophyllum* has been shown to have a significant negative impact on *Z. noltei* (Vieira et al. 2020), by causing reduced above- and below ground biomass of the seagrass. Thomsen et al. (2013) demonstrated that the presence of *A. vermiculophyllum* can significantly reduce the above ground biomass of *Z. marina*. Other studies have detected similar but weaker relationships where higher abundance of *A. vermiculophyllum* resulted in lower shoot survival in *Z. marina.* This was most apparent when temperatures reached 26°C

and above (Martínez-Lüscher and Holmer 2010). Höffle et al. (2011) detected similar trends, and found increased mortality of *Z. marina* with increasing *A. vermiculophyllum* algal cover at 27°C, the highest temperature tested.

1.4 The temperature tolerance of *A. vermiculophyllum*

Many experiments have investigated temperature tolerance of A. *vermiculophyllum*, focusing on temperature dependence of growth, and tolerance to high temperatures (Yokoya et al. 1999; Raikar et al. 2001; Rueness 2005; Abreu et al. 2011; Nejrup et al. 2013; Kim et al. 2016). *A. vermiculophyllum* has showed a wide temperature range for maximum growth rate, from 15-25°C (Yokoya et al. 1999; Raikar et al. 2001; Rueness 2005; Abreu et al. 2011; Nejrup et al. 2013; Kim et al. 2016), while a growth rate experiment performed on Scandinavian specimens gave maximum growth rate at 15-20°C (Nejrup et al. 2013).

Growth has been shown to decrease below this range (Yokoya et al. 1999; Nejrup et al. 2013; Kim et al. 2016). Previous research have focused on temperatures from 5°C and higher, and experiments including 5°C as a temperature treatment have showed significantly lower growth rates at this temperature (Yokoya et al. 1999; Nejrup et al. 2013). Abreu et al. (2011) tested spore germination success at various temperatures and found that although spores (carpospores and tetraspores) of *A. vermiculophyllum* mostly was able to germinate at 5°C , the spores did not survive long enough to be included in the following growth rate-experiment.

However, *A. vermiculophyllum* has also shown a tremendous ability to grow in and after long periods in low temperatures and darkness, what one might consider unfavourable conditions. Nyberg and Wallentinus (2009) illustrated this in a growth rate experiment where *A. vermiculophyllum* was kept in closed plastic bags within an 8°C, dark fridge for up to 141 days, and resumed growth when transferred to higher temperatures and light. In addition, *A. vermiculophyllum* has already appeared in colder areas with water temperatures below 5°C down to freezing point, and with occurrence of ice during winter (Weinberger et al. 2008; Nyberg and Wallentinus 2009; Kim et al. 2016). Despite of this no laboratory experiments have investigated how temperatures below 5°C effect survival and growth.

1.5 Objectives

Three aims are addressed in this thesis. The initial aim was to investigate abundance of *A. vermiculophyllum* in two locations in outer Oslo fjord, containing important seagrass meadows as well as nearby observations of the introduced algae within the recent years. This field survey was conducted to provide knowledge on abundance *A. vermiculophyllum* in seagrass meadows in Norway and its potential negative effects.

Agarophyton vermiculophyllum has often been found in *Zostera* meadows, and as *Zostera marina* previously has been shown to facilitate attachment of another large and branched invasive drifting algae (Tweedley et al. 2008) it is hypothesised that *A. vermiculophyllum* will be present on more stations with *Z. marina* than on stations without.

The second aim was to investigate the environmental factors determining abundance of *A. vermiculophyllum*. A collection of environmental variables together with presence/absence data on *A. vermiculophyllum* four sites provided the basis for a model selection to answer the research question of which environmental factors that are most important for abundance of *A. vermiculophyllum*.

The third aim of this thesis was to investigate *A. vermiculophyllum's* survival after exposure to temperatures down to 0°C through its ability to grow and photosynthesise when returned to favourable conditions. As *A. vermiculophyllum* is introduced in the northern part of Scandinavia, knowledge of the algae's response to low temperatures will be useful insight into its potential for future dispersal northwards. Based on the findings that a temperature of 5°C has resulted in significantly lower growth rate (Yokoya et al. 1999; Nejrup et al. 2013) it is hypothesised that *A. vermiculophyllum* will have lower survival after exposure to the below 5°C -temperature treatments. The hypothesis will be tested by measuring growth and photosynthetic activity after returned to favourable conditions.

With these three objectives in mind this thesis aims to provide more understanding on the current distribution of *A. vermiculophyllum* in Norway, its abundance in areas with seagrass meadows, environmental drivers for presence, and the physiology of *A. vermiculophyllum* to better understand and possibly mange further dispersal of this invasive non-indigenous alga.

2 Material and Methods

2.1 Study area

In order to obtain data on the distribution and abundance of *A. vermiculophyllum,* four sites on the east coast of Norway were investigated (Figure 2). All of them on the west side of the Oslo Fjord, with two sites in the outer part, and two in the inner part. The combination of previously detected *A. vermiculophyllum* and the presence of larger seagrass meadows in the area were the criteria that lead to the decision of these sites.

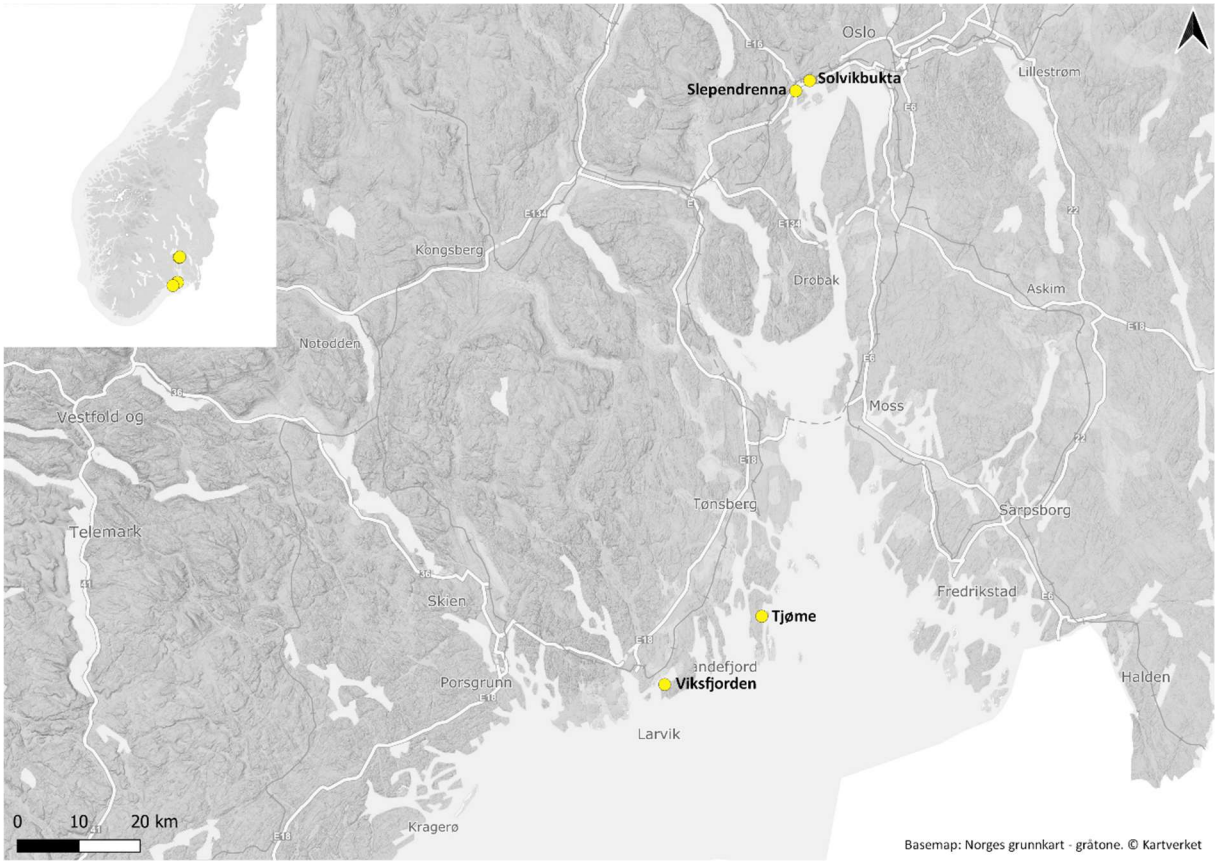

Figure 2 Overview map of the four sites investigated in the inner and outer Oslo fjord, southeast in Norway in August and September 2020.

Viksfjorden is situated in Larvik municipality (Figure 2) and is mainly surrounded by farmland. It has large seagrass meadows dominated by *Z. marina* (Figure 3, left panel), as well as presence of the endangered species *Z. noltei*, and previous investigations have looked at the accumulation of nutrients giving rise to large mats of green alga, a widespread problem in this area (Moy et al. 2014). Presence of *A. vermiculophyllum* was detected in Hølen marina in Larviksfjorden next to Viksfjorden in 2015 (Figure 1, blue dot in Larviksfjorden) (Norwegian Biodiversity Information Centre b, retrieved 31.08.2021).

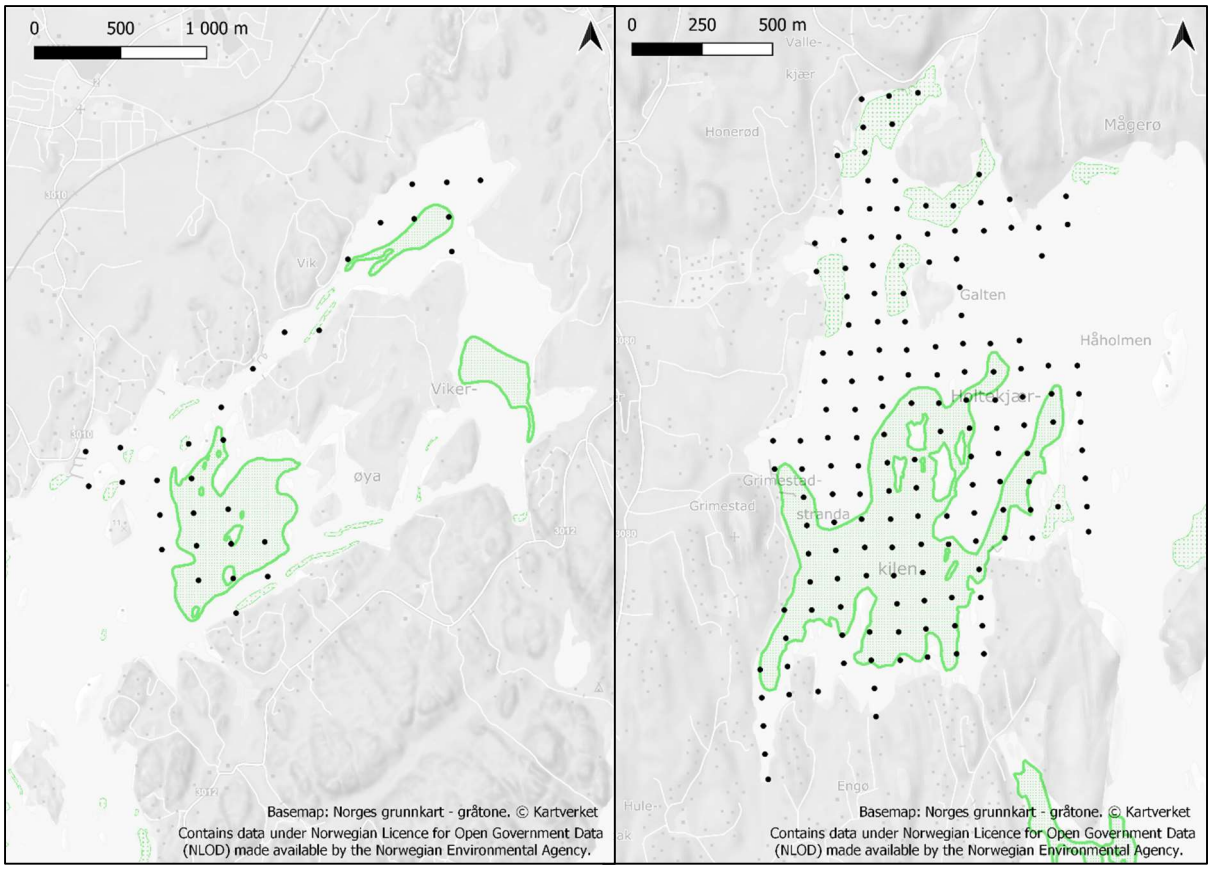

Figure 3 Overview of the study area in Viksfjorden (left panel) and Tjøme (right panel) with previously registered eelgrass meadows (green polygons). Including all sampling stations (black dots) where abundance of A. vermiculophyllum and eelgrass was investigated by video and grapnel in September 2020. Green polygons represent eelgrass meadows from the dataset "Naturtyper – DN-håndbok 19- Naturtype marin -ålegras" made available by The Norwegian Environmental Agency

Tjøme is an island in the municipality of Færder (Figure 2). The shoreline in the area is dominated by beach houses and leisure property. A large *Z. marina* meadow is located in Holteskjærkilen, south in the investigated area (Figure 3, right panel) (Norwegian Environmental Agency). Presence of *A. vermiculophyllum* was detected in Ødegårdskilen at Tjøme in 2012 (Figure 1, red dots) (Husa et al. 2013).

However, very limited presence of *A. vermiculophyllum* was observed in Viksfjorden and Tjøme in August 2020, and therefore a second field work in September 2020 was conducted in inner Oslo fjord to compare and identify environmental factors that might restricts the abundance in the first locations. The methods used were similar so findings of *A. vermiculophyllum* could be compared to those of Viksfjorden and Tjøme. The field work in the inner Oslo Fjord was conducted at sites in Solvikbukta and Slependrenna in Bærum and Asker municipality, respectively (Figure 2). The surroundings are urban, with its proximity to

Oslo and its suburbs. Solvikbukta and Slependrenna are home to several large marinas, which dominates the shore on these sites.

Solvikbukta houses a seagrass meadow containing *Z. marina* (Figure 4, right) (Greipsland et al. 2019)The Norwegian Environmental Agency), and mats of *A. vermiculophyllum* was detected on the site in 2018 (Norwegian Biodiversity Information Centre c). Slependrenna has a large *Z. marina* meadow (Figure 3, left), and previous investigations has detected *A. vermiculophyllum* in the meadow (Rinde et al. 2020).

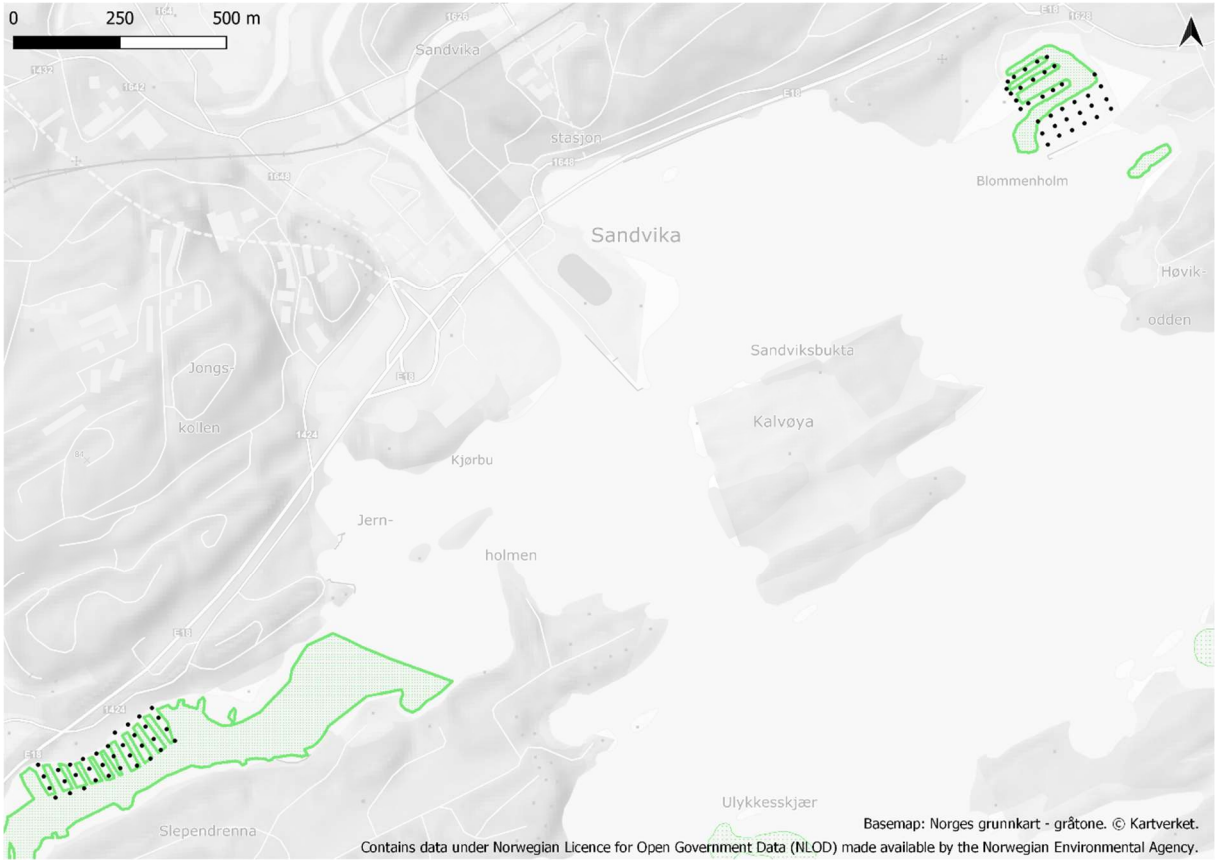

Figure 4 Overview of the study area in Slependrenna (left) and Solvikbukta (right) with previously registered eelgrass meadows (green polygons). Including all sampling stations (black dots) where abundance of A. vermiculophyllum and eelgrass was investigated by video and grapnel in September 2020. Green polygons represent eelgrass meadows from the dataset "Naturtyper – DN-håndbok 19- Naturtype marin -ålegras" made available by The Norwegian Environmental Agency

2.2 Field survey design

2.2.1 Collecting species data

Field work was conducted in late summer 2020 at four locations. As the initial objective for this study was to investigate the presence of *A. vermiculophyllum* in seagrass meadows compared to areas without seagrass, the sites Viksfjorden and Tjøme in outer Oslo fjord were

surveyed in the periods between 17.08.2020-22.08.2020 and 23.08.2020-29.08.2020, respectively. The sample stations were laid out in a grid pattern based on this objective. The surveyed areas hence include a variety of habitats, like shallow and muddy ones, seagrass meadows, as well as exposed deeper waters to detect possible drift of loose laying *A. vermiculophyllum.* Additional field work was done in Solvikbukta on 22.09.2020 and in Slependrenna on 23.09.2020.

The sampling stations in Viksfjorden and Tjøme were originally placed in a grid with 100 m between each sampling station (Figure 3). Due to time restriction from equipment issues fewer stations were covered in Viksfjorden, and to ensure the investigation of a larger area with a wider variety of conditions a 200 m grid was used instead (Figure 3, left panel). In Viksfjorden the field work was conducted from a Zodiac nautic cadet 310 alu, at Tjøme a larger boat was used in addition. The circumstances did not allow for work from a boat in Solvikbukta and Slependrenna. Based on eelgrass data from The Norwegian Environmental Agency (Figure 4), stations on the docks were believed to give sufficient access to the meadows, and hence the data collection was done in an approximately 30 m grid systems varying somehow due to the placement of the docks (Figure 4). Stations further apart was not doable without a boat available.

In Viksfjorden and at Tjøme the stations were determined in a grid system pre fieldwork and a GARMIN GPSMAP64s was used to localize the correct position. For Solvikbukta and Slependrenna the stations were measured and placed along the marinas as the work was done, and the same GPS was used to mark the stations with waypoints.

At each sampling station, a camera rig was lowered to about 0.5 m above the sea floor, in the shallowest areas closer to the bottom, and moved along for 1 minute from the moment it reached its correct position above the sea floor. In Viksfjorden and at Tjøme the rig was dragged slowly by the boat. In Solvikbukta and Slependrenna the rig was operated from the docs in the marina and dragged along by a walking person.

The rig was custom made of plastic pipes with various equipment attached; one drop camera with wire running from the rig along a rope to a screen in in the boat/on land ensured real time observations of the seafloor. The second camera, a recording GoPro Hero Black 7 in a dive housing provided footage for later analyses. The rig also contained a lead weight, and a in most cases a flashlight.

Figure 5 A grapnel: equipment used for sampling flora in the transects.

A rope with a lead wight at the end was lowered to measure the depth at each station. A grapnel (Figure 5) was thrown twice along each transect and the algal- and water plant species were displayed in a white tray while identified to lowest level possible at site. A rough estimate of percentage of each species present in the samples was also done. *A. vermiculophyllum* is known to be hard to visually distinguish from the native *Gracilaria gracilis*. The identification was done under training and supervision from Vivian Husa, and by distinguishing based on more red colour (*G. gracilis*) vs. a more brown colour (A. *vermiculophyllum*), as done and genetically validated by Rinde et al. (2020).

In addition to the ordinary sampling, extra search stations were investigated to look especially for *A. vermiculophyllum*. This was done in Viksfjorden and at Tjøme from boat, or from land when weather or defect equipment prevented original field work from being executed.

2.2.2 Collecting hydrographical data

To obtain data on salinity and temperature a handheld RBRconcerto^3 CTD was lowered down to right above the sea floor. A smaller version of the same model was used in Solvikbukta and Slependrenna.

The location of the hydrography stations was determined on a map before going out for the samples and marked as waypoints with a GARMIN GPSmap62stc while in the field. For Viksfjorden and Tjøme eight stations each were chosen and sampled from on 24.08.2020, while in Solvikbukta and Slependrenna five stations each were chosen and sampled on 22.09.2020 and 23.09.2020, respectively. Hydrography stations are assumed to represent a snapshot of the conditions of the surrounding sampling stations.

Water samples were collected from the hydrographic stations in triplicates, kept cool and dark until fixated in chloroform, approximately 0,2 ml in each sample. The samples were collected by hand in small plastic containers around 50 – 30 cm from the water surface. The samples

were analysed for the nutrients nitrogen dioxide (NO_2), nitrate (NO_3), phosphate (PO_4), and silicon (Si) by the Institute of Marine Research.

2.3 Laboratory experiment design

2.3.1 Sampling for temperature experiment

To investigate how *A. vermiculophyllum* survives after exposure to temperatures of 2°C, and 0°C, an experiment was arranged. Fresh material of *A. vermiculophyllum* was sampled from Solvikbukta at 0.9 m depth, a temperature of 15.9°C, and a salinity of 21.3 PSU, and brought to Bergen for the lab experiment. During the eight hour drive the algae were kept in two closed buckets with seawater inside a Zarges box that contained insulation material and a bucket of ice to ensure a cool environment. After arrival, the algae were distributed in open buckets inside a climate room set to 10°C and kept for a week. The water was changed twice a week throughout the entire experimental period. The lamp in the ceiling was the only source of light during this week (Appendix A). On 01.10.2020 the algae were transported in closed buckets inside an insulated unit by car to a facility in Austevoll where they were kept at approximately 10°C as the experimental set up was arranged the same day.

2.3.2 Temperature-experiment, part one: cold treatment

A selection of *A. vermiculophyllum* thalli were chosen randomly from the containers. Individuals showing clear and large signs of bleaching were discarded, and fragments were cut from healthy coloured areas. The thalli were cut up into fragments and mixed in two containers. 48 fragments each measuring three cm were picked out for the experiment, 24 of them from the "main axis", and 24 from "side branches". As *A. vermiculophyllum* does not have a clear main axis, main axis was defined as the starting point of the thallus in the basal end following the thickest axis further up as it divides.

Side branches were defined as any branching, regardless of being directly from "main axis" or not, but as a rule the side branch fragments were cut from the thinner parts of the thallus (Figure 6). Most fragments also had small side branches. To restrict the size of the fragments and make their growth potential more identical, all side branches longer than one cm were removed from the fragments. Fragments were distributed randomly to one of four temperature treatments: six of each fragment-type in each temperature treatment. Space restriction in the experimental set up made it impossible to have more than six replicates. Epiphytes were removed from the fragments using a flat pincer under a Leica MS5 magnifier with a 1.6 magnification, followed by a quick rinse in fresh water, and a rub with paper towels. Each

fragment was put in a 50 mL plastic centrifuge tube with seawater. The tubes with algae fragments were placed in test tube racks and submerged in as much freshwater as possible without it entering the tubes. The temperature of the water surrounding the tubes was lowered to intended temperatures gradually (Table 1).

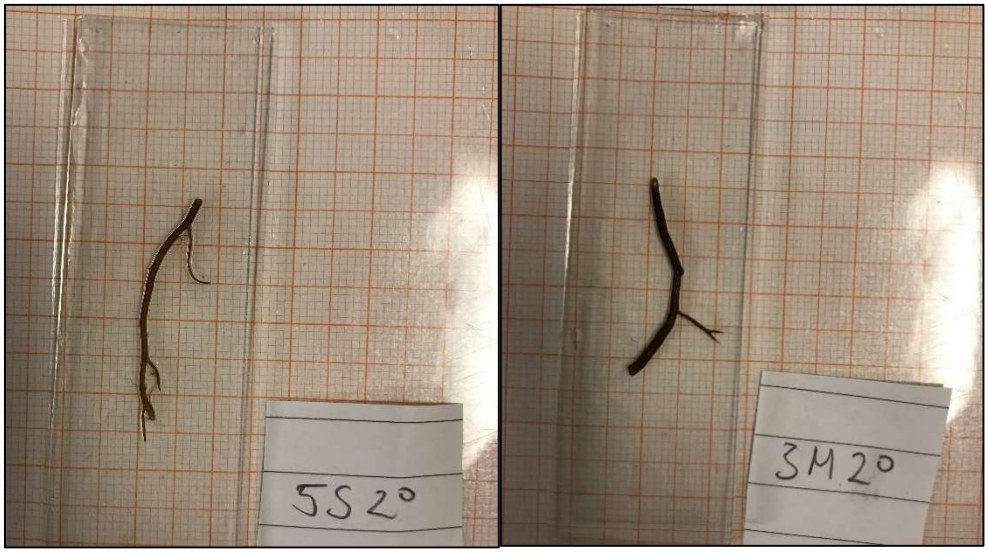

Figure 6 Example of a side branch fragment (left) and a main axis fragment (right) from the temperature experiment, both with all branches below 1 cm kept.

Table 1 Acclimatisation of the fragments to cold temperatures. Dates for adjustment of temperature down to cold treatment conditions. The acclimatisation of the treatment groups was done over individually number of days (underlined) within the timeframe of the seven days shown.

Date	**0°C**	**2°C**	**«Ambient»**	**«Heated»**
01.10.20	Set to 8°C	Set to 8°C	Set to 8°C	Set to 10°C
02.10.20	Set to 6°C	Set to 6°C	Left at ambient temp.	Left at 10°C
03.10.20	Left at 6°C	Left at 6°C	Left at ambient temp.	Left at 10°C
04.10.20	Left at 6°C	Left at 6°C	Left at ambient temp.	Left at 10°C
05.10.20	Set to 4°C	Set to 4°C	Measured to 7.2°C	Measured to 12°C
06.10.20	Set to 2°C	Set to 2°C	Measured to 8.0°C	Measured to 12.4°C
07.10.20	Set to 0°C	Left at 2°C	Measured to 8.4°C	Measured to 12.4°C

The “ambient” treatment was situated in a plastic container with fresh water holding the same temperature as the surrounding climate room. The temperature of this treatment held an average of 8.4°C with a standard deviation of 0.5 throughout the cold treatment period (days lacking temperature measurements and acclimatisation period excluded). Complete temperature range can be found in Appendix B.

The “heated”-treatment was intentionally tried kept at 10°C with a Grant scientific heated Circulator of the model Optima TM TXF200. This proved difficult with this equipment, resulting in an average temperature of 11.7°C with a standard deviation of 0.8 during the cold treatment period. Complete temperature range can be found in Appendix B.

The “2°C”-treatment was kept in a Grant scientific LTC4-kit with a R4 refrigeration unit and a heating controller unit of the model Optima TM TX150. The “0°C”-treatment was kept in a Grant scientific combined refrigerated and heating bath circulator of the model LT ecocoolTM 150, here frost liquid was added as the manual of the equipment instructed to avoid ice-formation. To obtain these low temperatures, the cold-treatment part of this experiment was done inside a climate room.

Due to difficulties with the light set up, the led lamp in the ceiling was the only source of light the first 4 days of the experiment. Giving an intensity of 8-12 µmol photon $m^{-2}s^{-1}$ in a 8:16 light/dark cycle. A light panel (Figure 7) consisting of four CO/TECH Flexible RGB led strips with a spectrum of 460-620 Nm secured light during the rest of the cold treatment. These gave an intensity in the range between 40-50 µmol photon $m^{-2}s^{-1}$, within the same 8:16 light/dark cycle. The same light/dark cycle and similar irradiance has been used to imitate Norwegian winter conditions in previous research on algae (Armitage and Sjøtun 2017). All light intensities in this experiment were measured with a Vernier PAR-sensor of the model LQ2-LE along the top of the tubes containing the algae (Appendix A).

As the light panel was situated in an angel above the algae (Figure 7), giving a slight variation in irradiance within the treatment groups, the tube racks were turned 180° approx. every two days, ensuring each row of tubes the same light condition through a week. Temperature was measured frequently with a YSI ProDSS Handheld Multiparameter Water Quality Meter or a Durac® digital thermometer -40/232°C, to ensure correct temperature. The water in the tubes were changed twice a week. It held the same temperature as the treatment group it was intended for and aerated before added to the tubes.

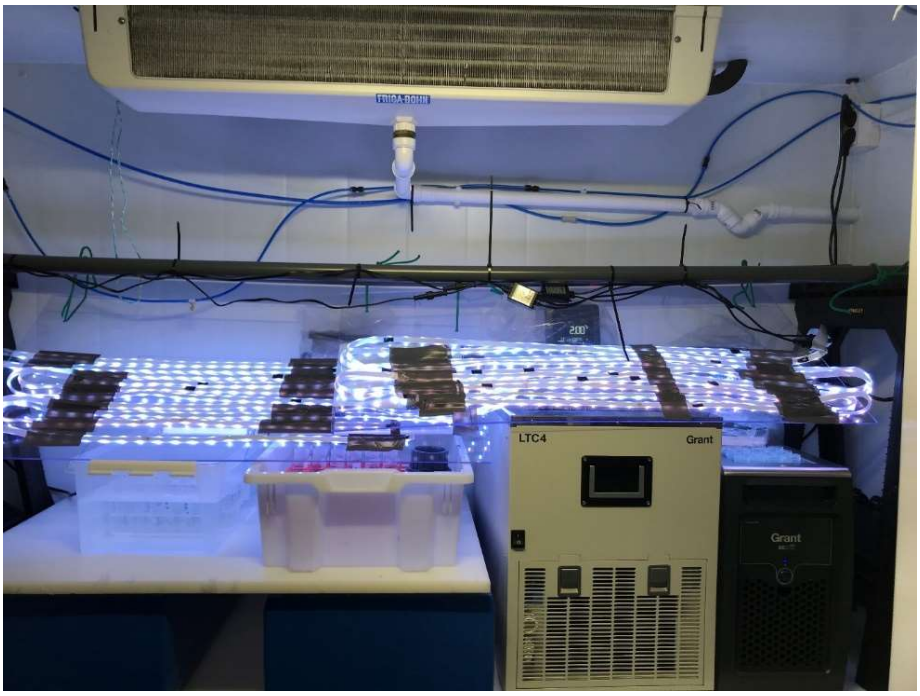

Figure 7 Experimental set up of cold treatments. From left to right: plastic container with water holding ambient temperature, plastic container with water heated by a heating circulator, a combined refrigeration and heating controller unit with 2°C water, a combined refrigerated and heating bath circulator with 0°C water. All containers hold 12 tubes in racks holding the algal fragments. Light panels above.

2.3.2 Temperature-experiment, part two: optimum conditions

After 30 days in the climate room in Austevoll with acclimatisation down to intended "cold"-temperature (Table 1), cold treatment, and acclimatisation back up to "optimum"-temperature (Table 2), the algae were moved back to the climate room in Bergen to investigate their survival through growth and ability to photosynthesise. During the transport they were kept in their tubes with the lid on, inside an insulated unit with water-elements holding the same temperature. Pictures were taken prior to placement in optimum conditions, and again after three weeks, to be used for growth measurements. Each fragment was placed between two microscope slides on top of millimetre paper and taken picture of. The pictures were taken from the approx. same distance each time, as close as one could get and still get a focused picture with the iPhone 7 that was used. The tubes were changed to get rid of any bacterial and algal growth that had formed and filled with fresh seawater from 180 m depth. The algae were then placed within a climate room with a temperature of 15 °C.

Table 1 Acclimatisation of fragments to optimum temperature. Dates for adjustment of temperature up to optimum temperature (15°C). The acclimatisation of the treatment groups was done over individually number of days (underlined) within the timeframe of the nine days shown.

Date	0°C	2°C	«Ambient»	«Heated»
22.10.20	Set to 2°C	Measured to 2.0°C	Measured to 8.2°C	Measured to 11.8°C
23.10.20	Set to 4°C	Set to 4°C	Measured to 8.8°C	Measured to 12.4°C
24.10.20	Not measured	Not measured	Not measured.	Not measured
25.10.20	Not measured	Not measured	Not measured.	Not measured
26.10.20	Set to 6°C	Set to 6°C	Measured to 8.4°C	Measured to 10.9°C
27.10.20	Set to 8°C	Set to 8°C	Set to 8°C	Measured to 11.8°C
28.10.20	Set to 10°C	Set to 10°C	Set to 10°C	Not measured
29.10.20	Set to 12°C	Set to 12°C	Set to 12°C	Set to 12°C
30.10.20	Set to 14°C	Set to 14°C	Set to 14°C	Set to 14°C

Figure 8 Experimental set up of optimum conditions. Treatment groups and replicates placed randomly. Lamps mounted on the wall behind providing light.

Fluorescent lamps were mounted on the wall beside the algae providing a light intensity measuring between 73 and 214 µmol photon $m^{-2}s^{-1}$, depending on the placement of the tubes (Figure 8). In order to give all algae approximately the same light condition throughout the experiment, the tubes were moved around twice a week. Choice of temperature- and irradiance levels were based on previous research on specific growth rate in *A. vermiculophyllum* (Nejrup et al. 2013). The light/dark cycle was set to 16:8 to imitate Norwegian summer conditions as done by Armitage and Sjøtun (2017) , with light between 05:00 and 21:00.

2.3.3 Measurements of maximum photosynthetic yield (Fv/Fm)

In addition to the pictures taken for growth measurements, a Walz underwater chlorophyll fluorometer of the model DIVING-PAM-II was used after the algae had been one week in optimal conditions. Through measuring Fv/Fm (variable fluorescence over maximum fluorescence) the maximum photochemical quantum yield of PSII, or maximum photosynthetic yield of the algae was obtained (Walz, 2018). This provided information on the physiological state of the fragments, and if their ability to photosynthesize had been reduced by stress. In the case of no observed growth in the fragments, or death during the optimum period, this could indicate if they were alive after the cold treatment. Samples were dark adapted for 20 minutes, a few minutes longer than reported in previous studies on *Agarophyton* sp. (Weinberger et al. 2008; Leal et al. 2020), to ensure proper dark adaptation. Measurements were done with the end piece of the fiber optic 7.5 mm from the algae fragment, with a 60° angle and a saturation pulse intensity of 5000 µmol $m^{-2}s^{-1}$, as is the default setting (Walz, 2018).

2.4 Data analysis and statistics

2.4.1 Video analysis of species data

The video footage was analysed using VLC media player, the transects of approximately 1-minute length being analysed continuously.

The species identified from the samples and video-material were categorised as follows. Individuals of *A. vermiculophyllum*, *G. gracilis*, *Z. marina*, and *Z. noltei* were categorized to species level. All other species were categorised in broader groups, the same for cases where the above-mentioned species could not be told apart (Table 3). All species and groups were marked as either “present” or “absent” in the data set for each transect.

Table 2 Explanation of categorisation groups used when determining presence /absence and abundance for the species present in the video material and grapnel samples.

Filamentous algae	**Unidentified loose laying**	**Unidentified water plant**	**Fucus sp.**	**Other**
Filamentous algae such as: *Sphacelaria* sp. *Cladophora* sp. *Ceramium* sp. *Ectocarpus* sp. *Pylaiella* sp. *B.hamifera* *Polysiphonia* spp.	All algae observed that could not be identified but resembled *A.vermiculophyllum* or *G. gracilis*	All water plants that could not be identified to species but resembled *Z.marina, Z. notei,* or *Ruppia* sp.	All species of genus Fucus identified, mainly *F.serratus* and *F.vesiculosus*	*Ruppia* sp. *C. filum* *F. lumbricalis* *Ulva* spp. *A. plicata* *R. confervoides* *S. muticum* *D. japonica* *O. oederi* *P. rotunda* *Spermathamnion* sp. *S. tenella* *S. paradoxus* And all non-identifiable algae not covered by the mentioned categories

To determine the abundance of species present, a semi-quantitative scale adapted from Husa et al. (2004, 2008) with levels ranging from 0 to 3 was used. One extra category; "4" (Table 4) was added to this scale to illustrate cases where the transect were almost completely covered by one species. As the case of *Z. marina* meadows and mats of *A. vermiculophyllum.* The main species of interest *A. vermiculophyllum*, the similar *G. gracilis*, *Z. marina*, and *Z. noltei*, in addition to groups "Unidentified loose laying" were classified after this level of abundance (Table 4).

Table 3 Semi-quantitative scale of species abundance used when analysing the video footage in combination with the data from the grapnel samples. Scale as presented in Husa et al. (2004, 2008), with one additional level of categorisation.

Level	**Explanation of abundance level**
0	Not found
1	Rare, one or a few specimens found
2	Common, many specimens found, estimated to <10% of the total algal biomass
3	Plenty, estimated to > 10% of the total algal biomass
4	Dominant, almost all the area investigated covered and estimated to > 80% of the total algal biomass

The video material was analysed in combination with the species data obtained from the grapnel samples. In cases where a species was clearly identifiable in the video footage but not present in grapnel sample, or a grapnel sample was missing, the species was added to the final data set. In cases where the species could not be identified in the footage, it was added to the final dataset anyway if it was identified from the grapnel sample. In these cases, the species was added as "present" with abundance category 1 (Table 4).

2.4.2 Visualisation of species data

The abundance data was visualised using QGIS (QGIS Development Team, 2021) for the species *A. vermiculophyllum, G. gracilis, Z. marina* and the category "Unidentified loose laying". Maps of presence/absence of *Z. noltei* from sample stations and one search station, *A. vermiculophyllum* from search stations, and all other maps presented in this thesis were also made with QGIS.

2.4.3 Hydrographical data

The triplicates of the nutrients data, in addition to temperature and salinity data from the upper five meters of the CTD-profiles were averaged. Due to variability between the depth of sampling stations and the assigned hydrographical station, the upper five meters were chosen as reasonable middle ground for calculating averages. *A. vermiculophyllum* is yet to be found deeper than five meters in Norwegian waters (Rinde et al. 2020). Each sampling station were assigned to its closest hydrography station using R Studio (R Core Team, 2021), resulting in data on NO_2, NO_3, PO_4, Si, temperature, and salinity for all sampling stations.

2.4.4 Statistical analysis of species data and hydrographical data

Hydrographical data, and depth was used to investigate explanations of the *A. vermiculophyllum* distribution using R studio (R Core Team, 2021). An exploratory data analysis was performed though model selection. Temperature was not included as a predictor variable as the data sampled is a snapshot of late summer temperature and is not representative of the highly fluctuating temperature range throughout the year. Temperatures within the range measured are neither believed to be of any limiting factor on distribution as the same temperature range previously have provided optimum growth rates in Danish *A. vermiculophyllum* (Nejrup et al. 2013). After assessing a correlation plot created with the GGally package (Crowley and Crowley, 2021), NO_2 and Si were excluded as variables before conducting the model selection, as high correlation was observed with other predictor variables. The remaining nutrient variables NO_3 and PO_4 were then log + 1 transformed.

With a response variable (presence/absence of *A. vermiculophyllum*) of binomial distribution and a random effect (site), a general mixed-effect model (glmm) was chosen. The four predictor variables depth, salinity, NO_3 and PO_4 were included in the model selection, where eight models were fitted using the glmer function from the lme4 package (Bates et al. 2015). One null model, one for each of the four predictor variables, one "abiotic model" with depth + salinity, one "nutrients model" with NO_3 + PO_4, and one full model were fitted. All of them contained the random predictor variable site, and none of them contained interaction effects.

The most explaining model given the data was determined by assessing The Second order Akaike Information Criterion; AICc (Hurvich and Tsai 1989), and AICc weights from each model, provided by the package MuMIn (Barton 2020). The pseudo-R squared for glmm's (the conditional delta version), and degrees of freedom were also reported for each model.

2.4.5 Image analysis of temperature experiment data

As the main objective of the temperature experiment was to investigate survival, two sets of pictures were used to obtain growth data on the algae to tell if they had survived the treatment. Pictures of the algae pre optimum and the pictures taken three weeks later, the post optimum, were analysed in the software ImageJ using the calibration tool "set scale" followed by manual outlining of the algae. To obtain information on the survival of the algae, their growth during the optimum treatment was calculated. The post optimum area, measured in cm^2, was subtracted from the pre optimum area for each fragment using Microsoft Excel resulting in growth from each fragment in the three-week period.

2.5.6 Statistical analysis of temperature experiment data

Effect of temperature treatment, and axis type on the growth- and Fv/Fm data were analysed using R software (R Core Team 2021). As both the data sets consisted of two categorical predictor variables (temperature treatment, axis type) and one continuous response variable (growth or Fv/Fm), a linear model was chosen. The assumptions for a linear model were checked by assessing the diagnostic plots of the two data sets. No major violations of assumptions were observed, and the data analysis was carried out using a two-way analysis of variance (ANOVA) for both data sets ($p<0.05$). The treatment effect of temperature treatment and axis on growth, was included in the model as well as the interaction effect of the two. The same was done on the Fv/Fm data set. Boxplots were made to visualise the data using the package ggplot2 (Wickham 2016).

3 Results

3.1 Abundance of *A. vermiculophyllum* in seagrass habitats

3.1.1 Viksfjorden and Tjøme

In Viksfjorden *Agarophyton vermiculophyllum* was detected only on two of the 31 investigated sampling stations (Figure 9, left panel). *Zostera marina* was present on 22 of 31 sampling stations. Both findings of *A. vermiculophyllum* overlapped with presence of *Z. marina* (Figure 9, right panel). On the station where *A. vermiculophyllum* was categorised as "Rare" (Figure 9, left panel) and only one or few specimens were found, *Z. marina* was categorised as "Plenty" (Figure 9, right panel), estimated to > 10% of the station's total algal biomass. On the second station (Figure 9, left panel) *A. vermiculophyllum* was "Common" and many specimens were found. At this station *Z. marina* was also categorised as "Common" and estimated to <10% of the total algal biomass (Figure 9, right panel). *A. vermiculophyllum* was found on 3.1- and 3.7-meters depth respectively at these two stations.

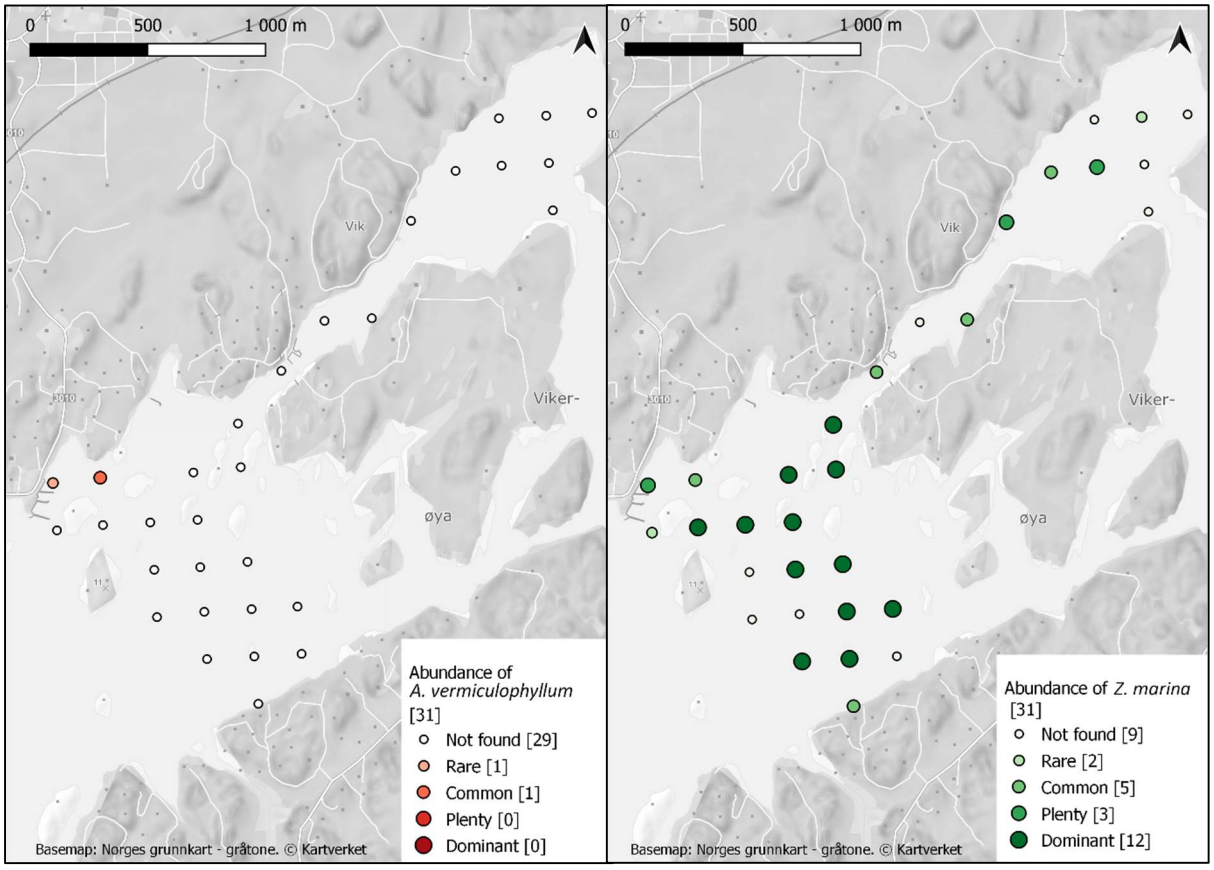

Figure 9 Abundance of A. vermiculophyllum and Z. marina in Viksfjorden. Categorised by abundance. Number of stations shown in brackets. Abundance data based on grapnel samples and video footage combined.

The endangered *Zostera noltei* was detected on two stations (Figure 10) but did not overlap with findings of *A. vermiculophyllum.* The native *Gracilaria gracilis* that resembles *A. vermiculophyllum* was found on one station (Figure 11, left panel). Four stations had observations from the category "Unidentified loose laying"; algae that resembled *A. vermiculophyllum* or *G. gracilis* but could not be identified to species level, raising the possibility of *A. vermiculophyllum* on two additional stations in Viksfjorden.

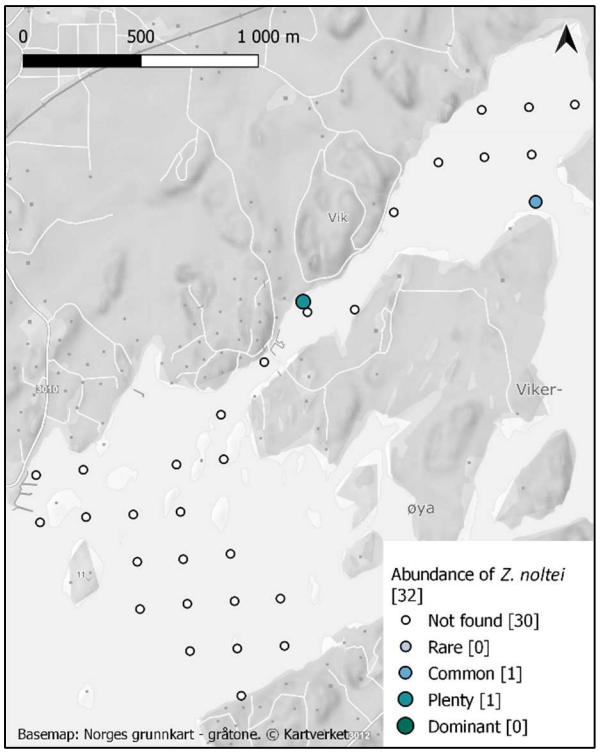

Figure 10 Abundance of Z. noltei in Viksfjorden. On one sampling station (east) and one search station (west)

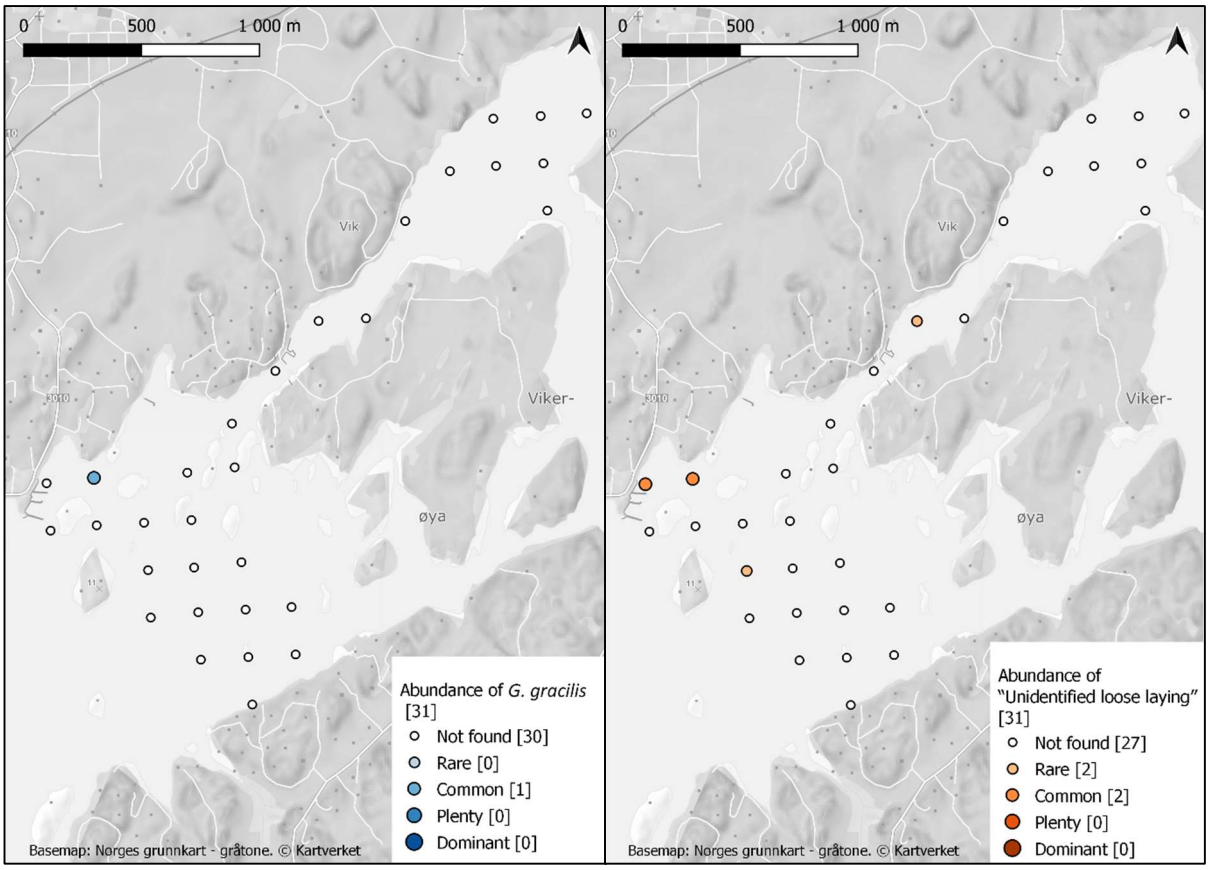

Figure 11 Abundance of G. gracilis and "Unidentified loose laying" in Viksfjorden. Categorised by abundance. Number of stations shown in brackets. Abundance data based on grapnel samples and video footage combined.

"Filamentous algae" such as *Cladophora* sp. and alike (Table 3) were found on all stations where *Z. marina* was present. Overall, the abundance of *Z. marina* dominated the benthic communities in this area while *A. vermiculophyllum* was hardly detected.

At Tjøme *A. vermiculophyllum* was also just detected at two of the sampling stations, of the 158 investigated (Figure 12, left panel). On the northern one (Figure 12, left panel) it was found to be plenty of *A. vermiculophyllum* relative to the total algal biomass, while *Z. marina* was not found (Figure 12, right panel). On the southernmost station (Figure 12, left panel) *A. vermiculophyllum* was classified as "Common", and overlapped with presence of *Z. marina* which was found to be "Rare", with one or a few specimens (Figure 12, right panel*). A. vermiculophyllum* was found on 0.6- and 0.3-meters depth respectively.

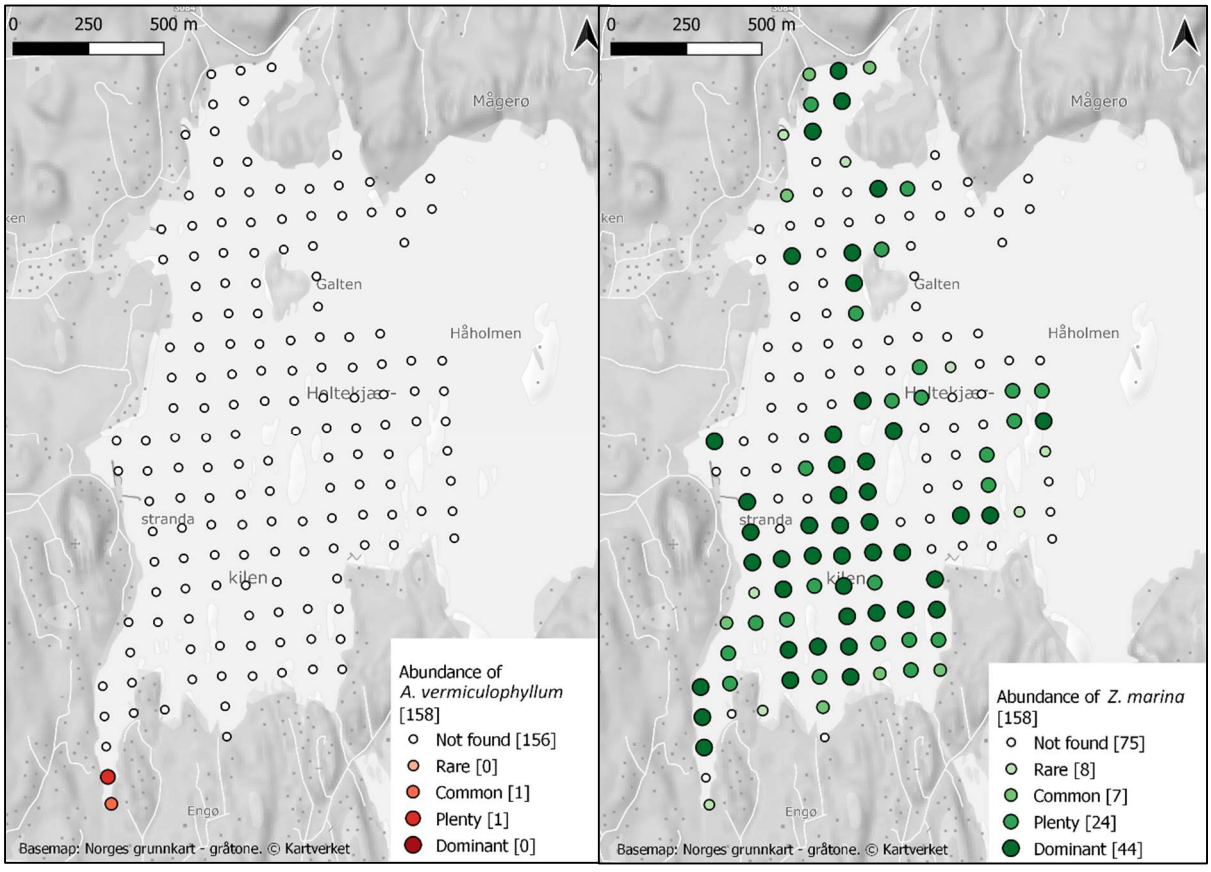

Figure 12 Abundance of A. vermiculophyllum and Z. marina at Tjøme. Categorised by abundance. Number of stations shown in brackets. Abundance data based on grapnel samples and video footage combined.

G. gracilis was present on 15 of the sampling stations in this area (Figure 13, left panel), and "Unidentified loose laying" on 37 stations (Figure 13, right panel). Filamentous algae were present on all stations where *Z. marina* was present with only two exceptions.

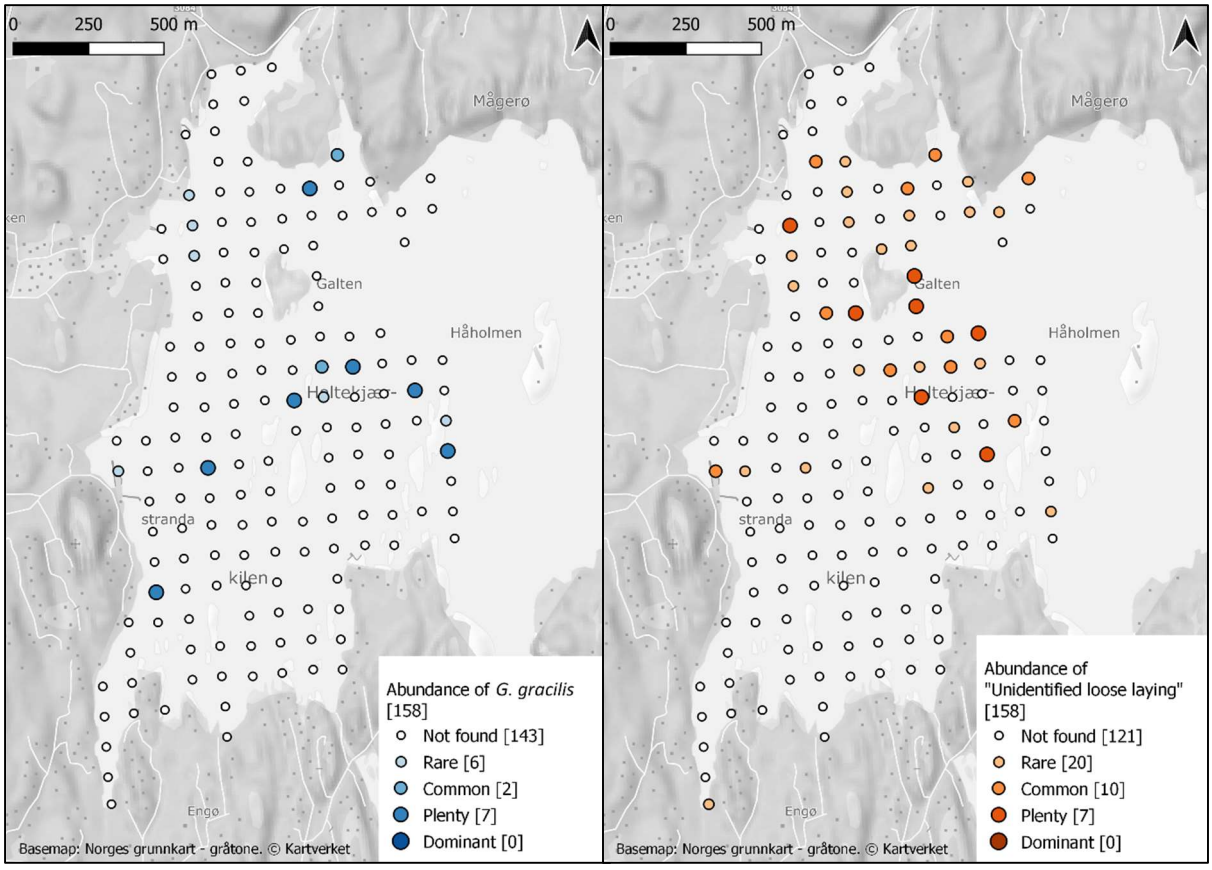

Figure 13 Abundance of G. gracilis and "Unidentified loose laying" at Tjøme. Categorised by abundance. Number of stations shown in brackets. Abundance data based on grapnel samples and video footage combined.

Z. marina was also here found to be dominant on a large proportion of the sampling stations (Figure 12, right panel) while *A. vermiculophyllum* was hardly detected (Figure 12, left panel).

On the target stations in the Viksfjorden-area *A. vermiculophyllum* was found on seven of the 15 investigated (Figure 14, left panel). On the target stations in the Tjøme-area *A. vermiculophyllum* was found on one of the six investigated (Figure 14, right panel).

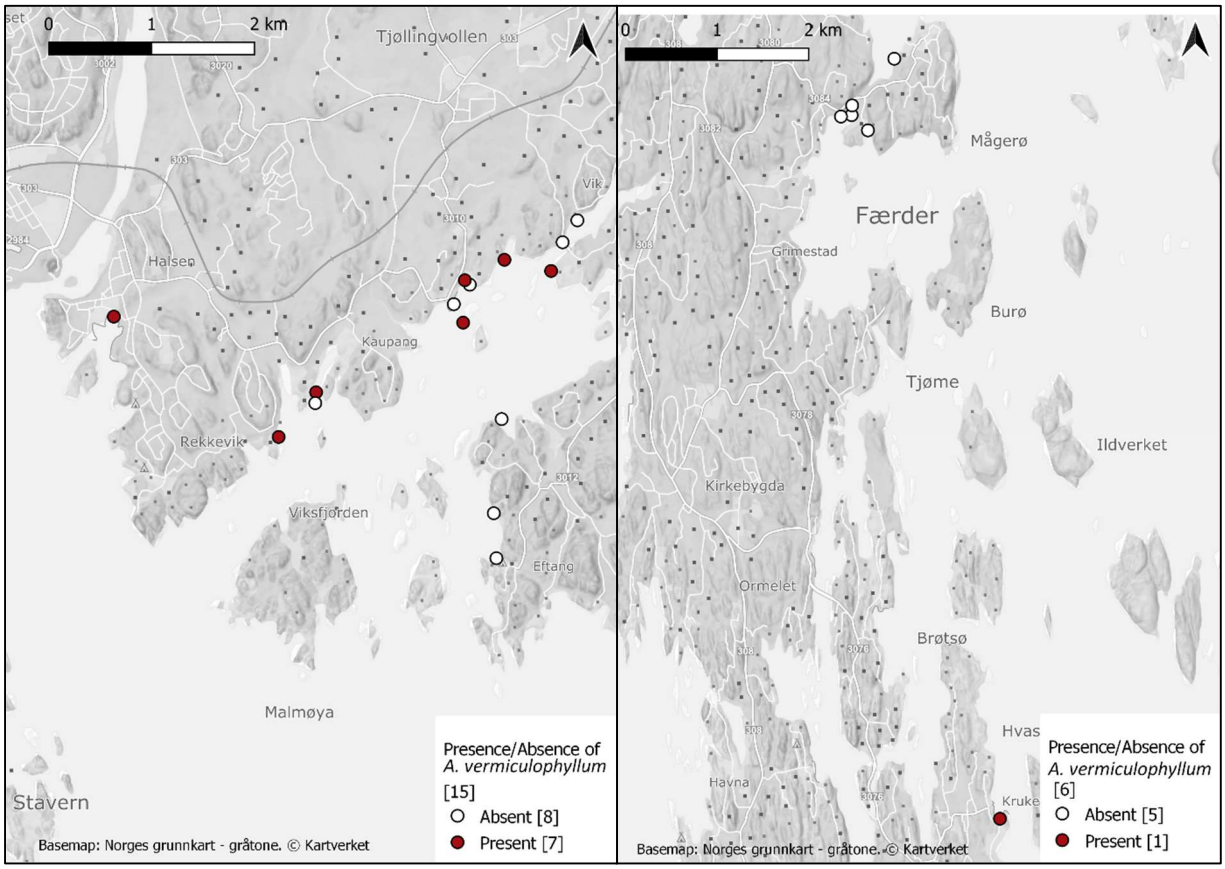

Figure 14 Presence of A. vermiculophyllum on search stations from land or by boat. Viksfjorden-area in the left panel and Tjøme area in the right panel. Presence/absence data based on grapnel samples and/or observations in the field.

3.1.2 Solvikbukta and Slependrenna

In Solvikbukta *A. vermiculophyllum* was found on 20 of the 36 investigated stations (Figure 15, left panel), while *Z. marina* only occurred on six (Figure 15, right panel). In contrast to Viksfjorden and Tjøme *A. vermiculophyllum* was here categorised as "Dominant" on three stations, while the highest abundance of *Z. marina* detected was "Common", hence estimated to contribute under 10% to the total algal biomass on the three stations in question. Five of the sampling stations had overlapping occurrence of *A. vermiculophyllum* and *Z. marina*. Here *A. vermiculophyllum* was categorised as "Rare", "Common", and "Plenty", while *Z. marina* was found to be "Rare" or "Common". In addition, *A. vermiculophyllum* appeared on 15 stations without *Z. marina*, resulting in *A. vermiculophyllum* - *Z. marina* overlap of 25% on the *A. vermiculophyllum* stations. *A. vermiculophyllum* appeared within the depth range of 0.3 to 3.6 meter.

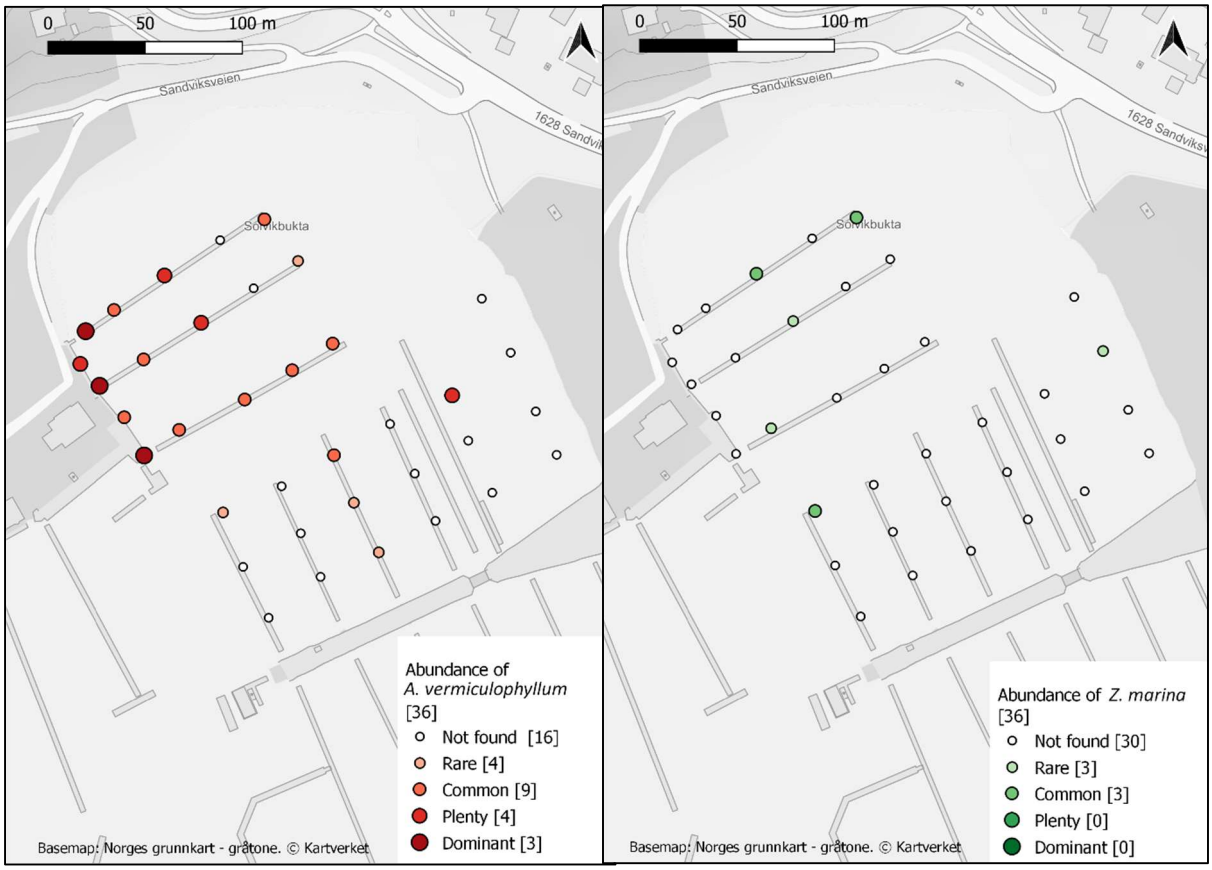

Figure 15 Abundance of A. vermiculophyllum in Solvikbukta. Categorised by abundance. Number of stations shown in brackets. Abundance data based on grapnel samples and video footage combined. All stations placed at docks, although this basemap shows a dislocation of the docks in relation to the stations on the right.

G. gracilis was present on nine of the sampling stations (Figure 16, left panel), and "Unidentified loose laying" on 16 stations (Figure 16, right panel). "Filamentous algae" were present on all stations where *Z. marina* was present.

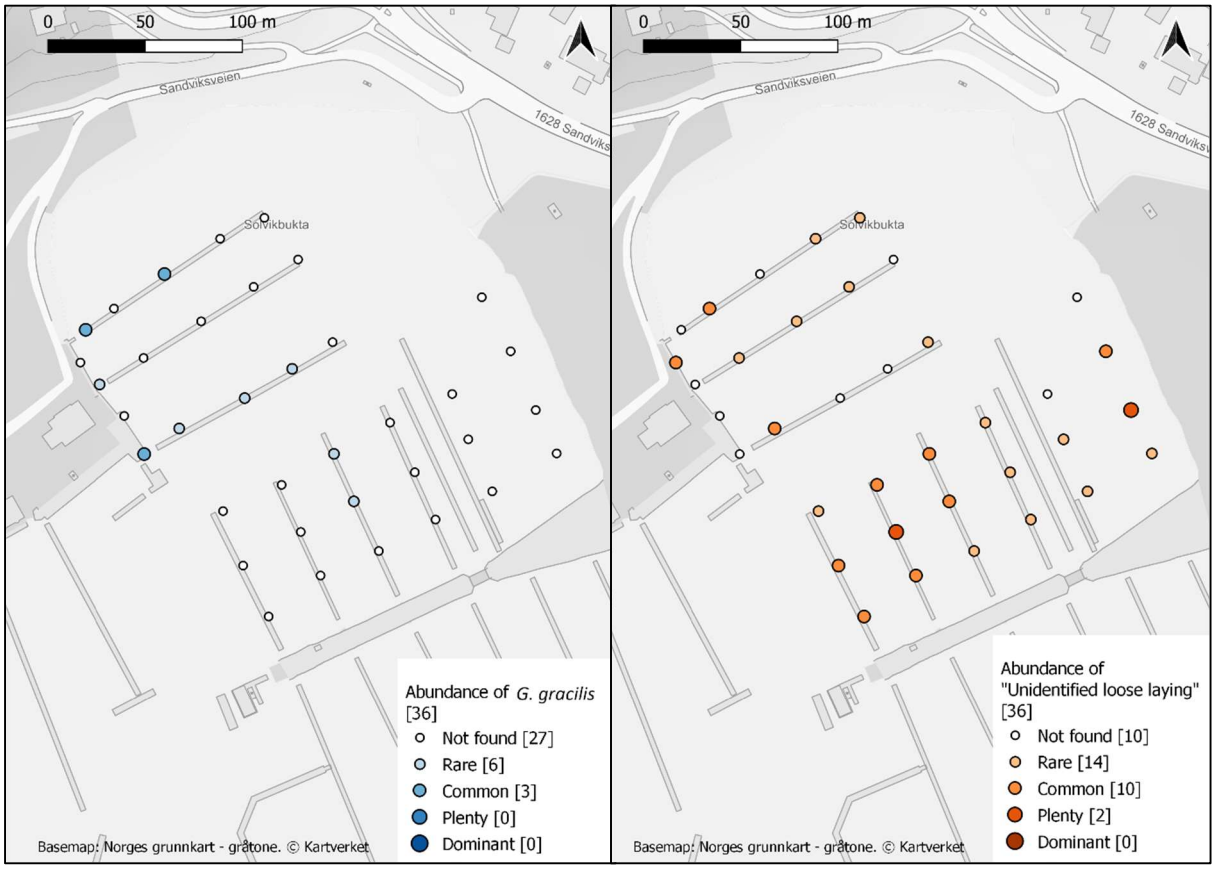

Figure 16 Abundance of G. gracilis and "Unidetified loose laying" in Solvikbukta. Categorised by abundance. Number of stations shown in brackets. Abundance data based on grapnel samples and video footage combined. All stations placed at docks, although this basemap shows a dislocation of the docks in relation to the stations on the right.

A. vermiculophyllum represented more than 10% of the total algae biomass in 44.4% of the investigated stations, while *Z. marina* was to a much lesser degree detected.

In Slependrenna *A. vermiculophyllum* was present on 14 of the 35 sampling stations (Figure 17, left panel), while *Z. marina* occurred on 13 (Figure 17, right panel). *A. vermiculophyllum* occurred on seven stations with *Z. marina*, and seven stations without. Both species had their abundance levels in the "Rare", "Common", and "Plenty" categories, appearing most frequent in the category "Rare", and none in the category "Dominant". *A. vermiculophyllum* had more stations with the abundance level "Plenty", while *Z. marina* more often appeared in the category "Common" (Figure 17). *A. vermiculophyllum* appeared within the depth range of 1 to 3.4 meter.

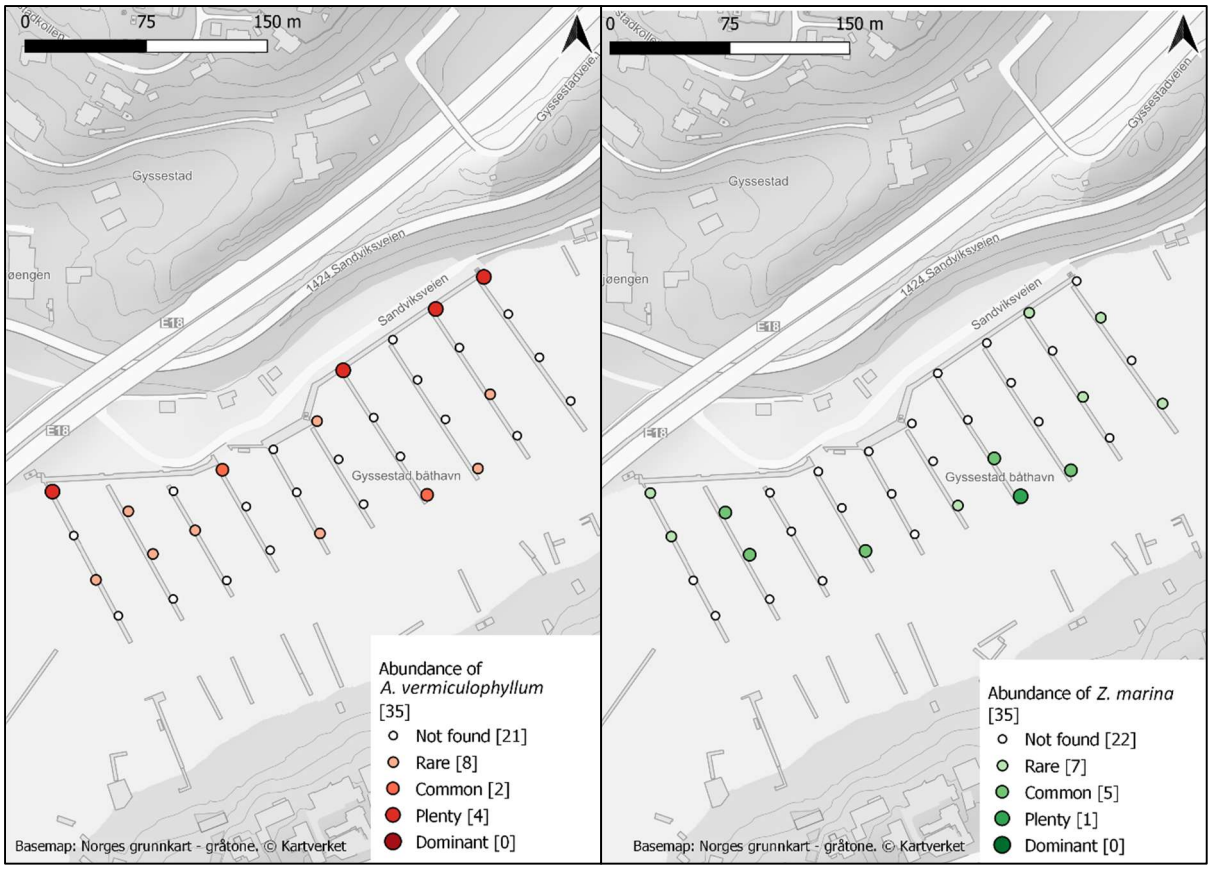

Figure 17 Abundance of A. vermiculophyllum and Z. marina in Slependrenna. Categorised by abundance. Number of stations shown in brackets. Abundance data based on grapnel samples and video footage combined.

G. gracilis was present on 24 of the sampling stations (Figure 18, left panel), and "Unidentified loose laying" on 27 stations (Figure 18, right panel). "Filamentous algae" were also here present on all stations where *Z. marina* was present.

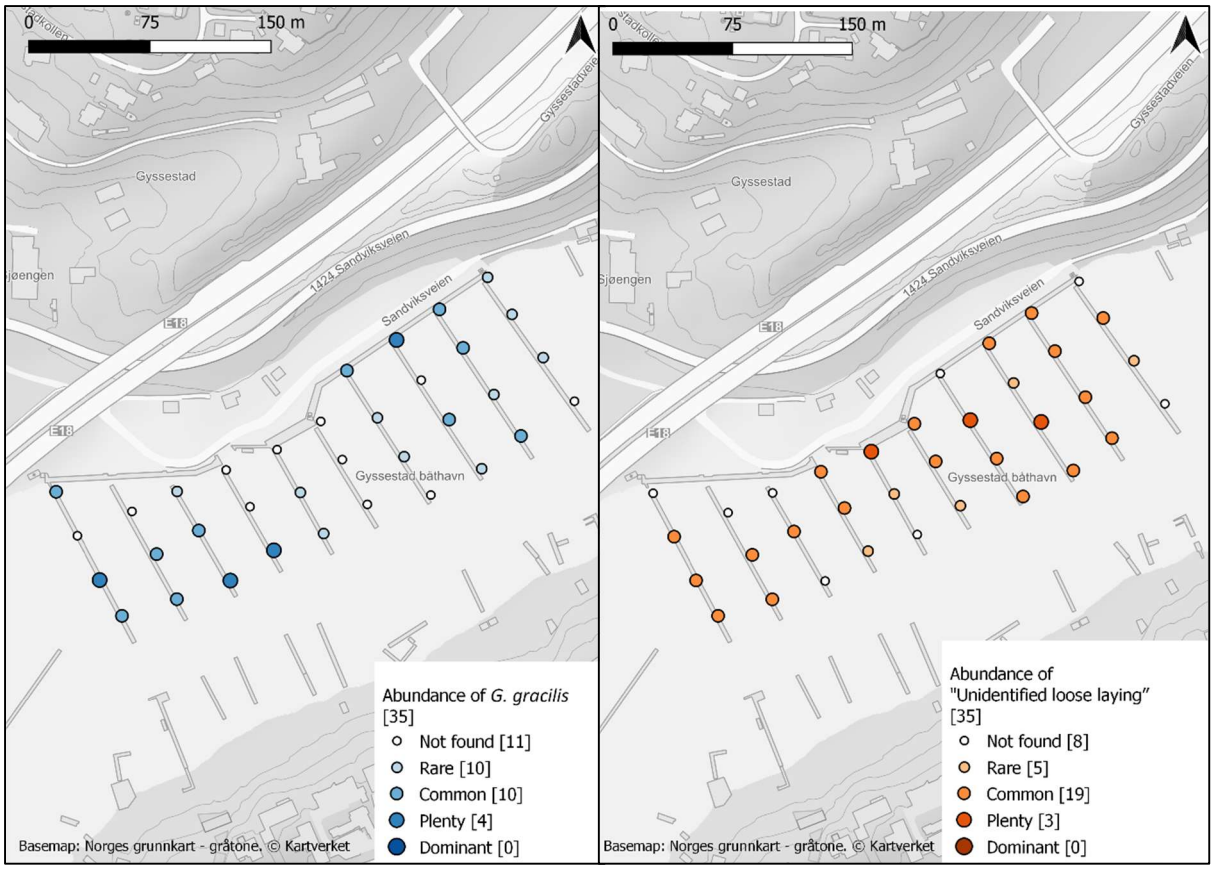

Figure 18 Abundance of G. gracilis and "Unidentified loose laying" in Slependrenna. Categorised by abundance level. Number of stations shown in brackets. Abundance data based on grapnel samples and video footage combined.

On all sampling stations investigated at all sites, *A. vermiculophyllum* was present on 38 of the 260 stations. *A. vermiculophyllum* appeared on more stations without *Z. marina* (8.8%) than with *Z. marina* (5.8%), 23 and 15 stations respectively.

3.1.3 The physical and chemical environment at the investigated sites

In Viksfjorden and at Tjøme temperature was measured in the range of 18.6-20.7°C. In Solvikbukta and Slependrenna temperature was measured in the range of 15.8-16.7°C.

Tjøme had the highest mean salinity (24.22 psu ± 0.33), while Viksfjorden had the lowest (20.08 psu ± 2.83) (Appendix C), however here it showed large variability between the hydrography stations and had values in the range between 16.27 -24.02 psu.

Viksfjorden and Slependrenna had the highest mean values of NO_3 , but also showed larger variability than the two other sites (Appendix C). Tjøme had the overall lowest values and Viksfjorden showed a markedly high mean of Si at 14.0 µmol/l ± 4.57.

3.2 Environmental factors explaining *A. vermiculophyllum* abundance

Eight models were fitted to investigate the most important predictors in explaining the presence/absence of *A. vermiculophyllum* of the environmental variables sampled. The Depth model returned the lowest AICc and is hence the suggested the best model fit of the models tested. This model returned an AICc weight of 0.661 and provides 66.1% probability that this model is the best representation of reality, when presented with these model alternatives (Table 5). The depth model also returned the highest R^2 which provides an absolute value of the variance explained by the model. Depth as a fixed effect and site as a random effect hence is the best explanation of the presence or absence of *A. vermiculophyllum*, with depth as the best predictor of where to find *A. vermiculophyllum*

Table 5 Result of model selection for generalised linear mixed effects models explaining the response variable presence/absence of A. vermiculophyllum. All models with the clustering effect from the random predictor variable "Site". Depth model shows the lowest AICc value and in hence the best fitted model. The AICc weights provide the probabilities of a model being the best representation of reality compared to the other models fitted. The pseudo-R squared for glmm's reports how much of the response variable's variance that is explained by the model.

Model	Predictors	AICc	AICc Weights	df	R^2
Null model	Intercept	150.6877	0.013	2	0.3789617
Depth model	Depth	142.7742	0.661	3	0.6717152
Salinity model	Salinity	151.2751	0.009	3	0.3535076
NO_3 model	NO_3	152.0256	0.006	3	0.3852163
PO_4 model	PO_4	152.2202	0.006	3	0.3687344
Abiotic model	Depth + salinity	144.6002	0.265	4	0.6635771
Nutrients model	$NO_3 + PO_4$	154.0765	0.002	4	0.3811691
Full model	Depth + salinity + $NO_3 + PO_4$	148.5467	0.037	6	0.6667598

3.3 Investigation of survival after exposure to low temperatures

3.3.1 Growth of *A. vermiculophyllum* after exposure to low temperatures

In order to answer the hypothesis that *A. vermiculophyllum* will have lower survival after exposure to the below 5°C treatments the mean growth in the four treatment-groups were tested for significant differences after the three weeks in optimum conditions. The Two-way ANOVA failed to reject H0, and hence did not detect a significant difference between the growth-means. No relationship between the factors temperature and growth [$F(0.9314)=3$, $p = 0.4345$], axis-type and growth [$F(0.6389)=1$, $p = 0.4288$], or the interaction of the two and growth [$F(0.4288)=3$, $p = 0.7335$] were detected.

This means that the four temperature treatments did not cause significant differences in the fragments ability to grow during the following three weeks in optimum conditions (Figure 19).

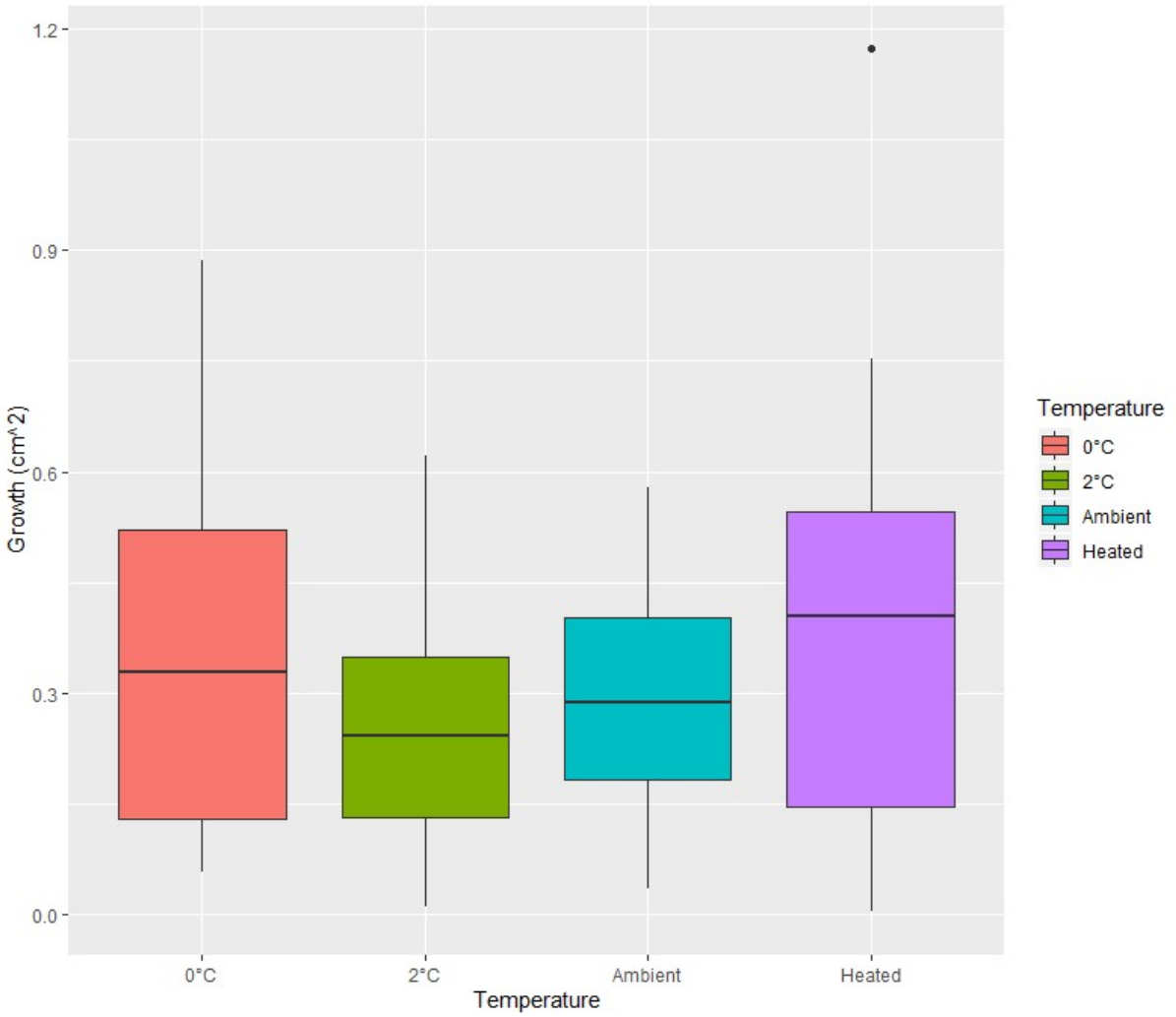

Figure 19 Growth of A. vermiculophyllum (cm^2) during three weeks in optimum condition. Growth (cm^2) on the y-axis, as a function of temperature treatment (0°C, 2°C, "Ambient" = 8,4°C (SD = 0,5), "Heated" = 11,7°C (SD = 0,8)) they were exposed to prior to growth on the x-axis. Axis type of fragments not showed.

Nor axis type had any significant effect on the growth performance of the fragments. None of the fragments grew less than 0.003 cm^2 (Figure 20).

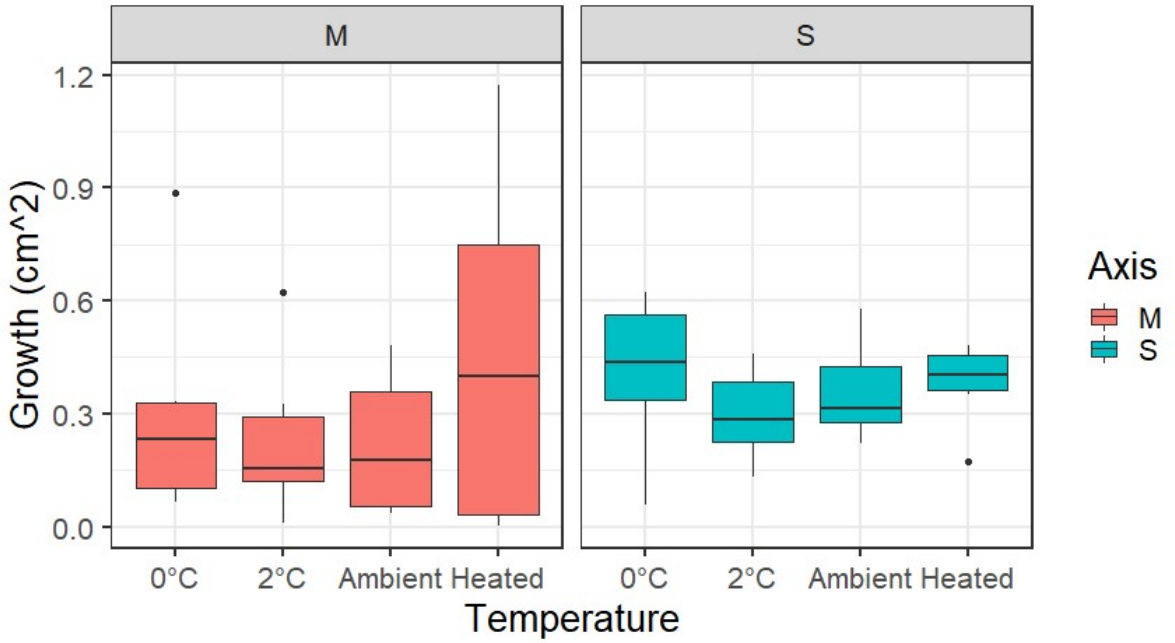

Figure 20 Growth of A. vermiculophyllum (cm^2) during three weeks in optimum condition. Growth (cm^2) along the y-axis, as a function of temperature treatment (0°C, 2°C, "Ambient" = 8,4°C (SD = 0,5), "Heated" = 11,7°C (SD = 0,8)) they were exposed to prior to growth on the x-axis. Sorted by axis-type. M = fragments taken form "main-axis" on thallus, showed in red (left panel). S = fragments taken from "side-branch" on thallus, showed in blue (right panel).

3.3.2 Maximum photosynthetic yield (Fv/Fm) of *A. vermiculophyllum* after exposure to low temperatures

In addition to the information on survival attained from the growth data, the fragments' ability to photosynthesise was measured through Fv/Fm and analysed to get information on their physiological state. The two-way ANOVA failed to reject H0, and hence did not detect a significant relationship between the factors temperature and Fv/Fm [$F(1.0427)=3$, $p = 0.3841$], axis type and Fv/Fm [$F(0.0076)=1$, $p = 0.9311$], or the interaction of the two and Fv/Fm [$F(0.5907)=3$, $p = 0.6247$]. Meaning that the four temperature treatments did not cause significant differences in the fragments maximum photosynthetic yield after one week in optimum conditions (Figure 21).

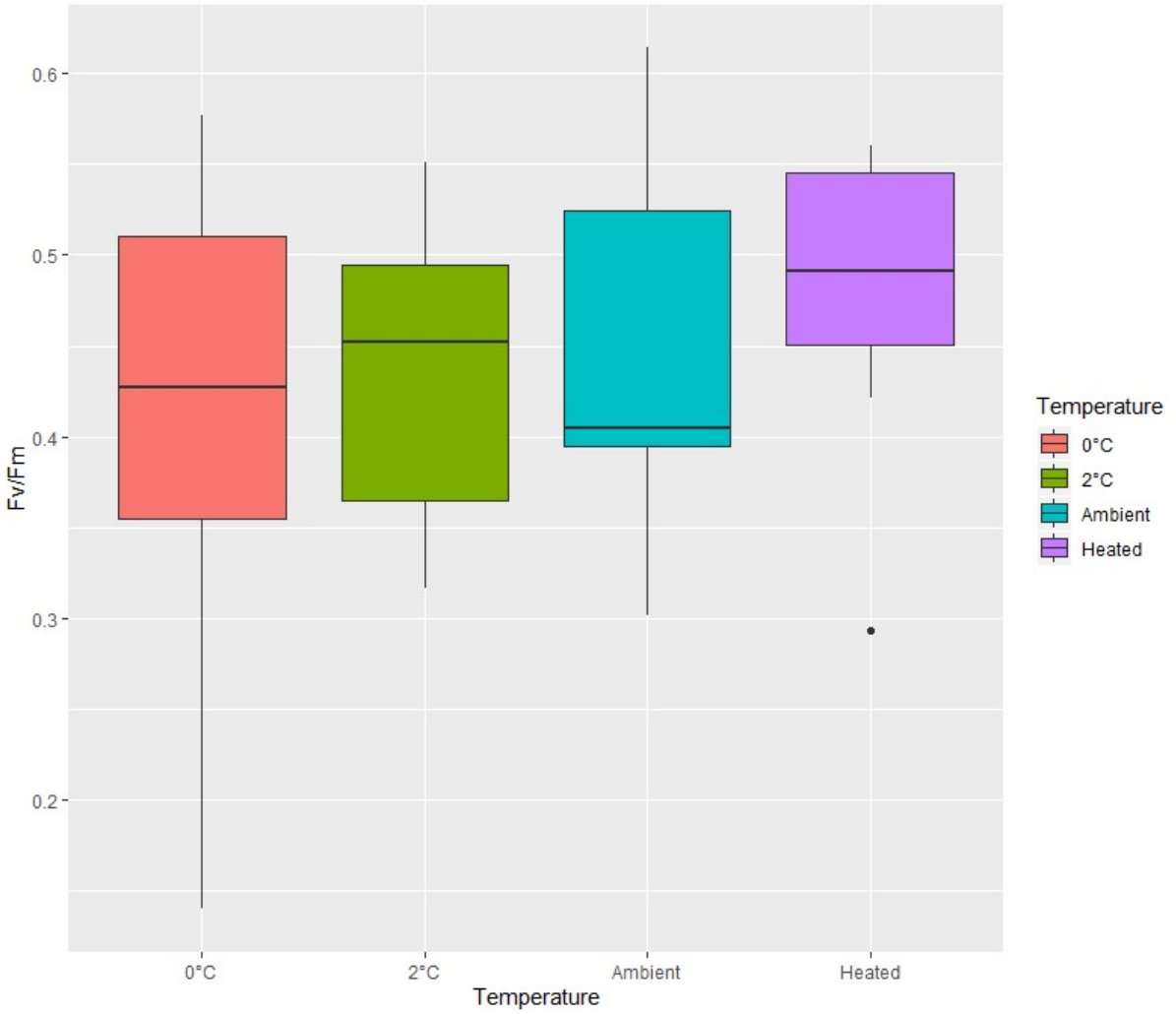

Figure 21 Fv/Fm as an indicator of maximum photosynthetic yield in A. vermiculophyllum after one week in optimum conditions. Fv/Fm along the y-axis, as a function of the temperature treatment (0°C, 2°C, "Ambient" = 8,4°C (SD = 0,5), "Heated" = 11,7°C (SD = 0,8)) they were exposed to in the cold treatment on the x-axis. Axis type of fragment not showed.

Axis type did not have any significant effect on the maximum photosynthetic yield of the fragments. None of the fragments showed values below 0.14 (Figure 22).

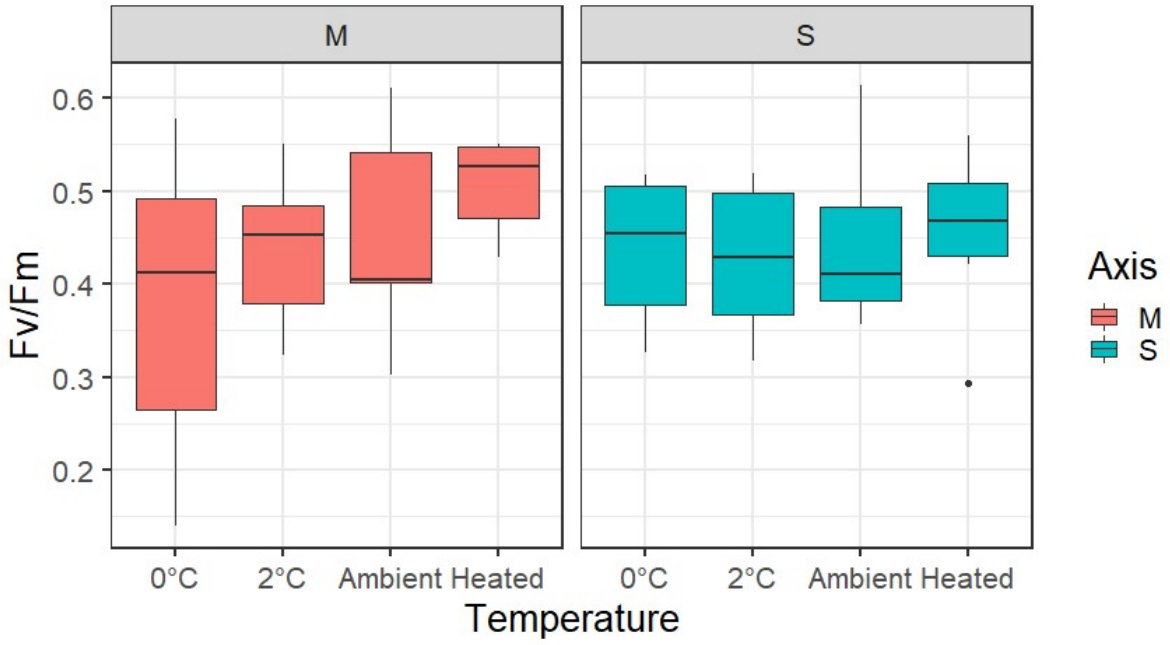

Figure 22 Fv/Fm as an indicator of maximum photosynthetic yield in A. vermiculophyllum after one week in optimum conditions. Fv/Fm along the along the y-axis, as a function of the temperature treatment (0°C, 2°C, "Ambient" = 8,4°C (SD = 0,5), "Heated" = 11,7°C (SD = 0,8)) they were exposed to during the cold treatment on the x-axis. Sorted by axis-type. M = fragments taken form "main-axis" on thallus, showed in red (left panel). S = fragments taken from "side-branch" on thallus, showed in blue (right panel).

Mean Fv/Fm values were 0.41±0.13, 0.43±0.08, 0.45±0.11, and 0.48±0.08 with standard deviation for the 0°C, 2°C, "Ambient", and "Heated" treatment respectively.

4 Discussion

4.1 Abundance of *A. vermiculophyllum* in seagrass habitats

Agarophyton vermiculophyllum was not present on more stations with *Z. marina* than on stations without. Of all sampling stations investigated, *A. vermiculophyllum* was present on 8.8% stations without *Z. marina*, while they appeared together on 5.8% of the stations.

Although presence of *Zostera* species (*Z. marina, Z. noltei*) and *A. vermiculophyllum* are to a large degree reported within the same estuaries and lagoons (Parker et al. 2001; Rueness 2005; Thomsen et al. 2013; Rinde et al. 2020; Vieira et al. 2020; Glenn et al. 2020), reports on problems with consistent overlapping distribution with subsequent damage on *Zostera* species under natural conditions has proven hard to find.

Nyberg (2007) reported *Z. marina* as one of three primary producers with highest biomass that was found together with *A. vermiculophyllum* when investigating sites in Sweden, Virginia (US) and Denmark, but stated that no negative effects of *A. vermiculophyllum* had been documented in Sweden. The same conclusion was drawn by Nyberg et al. in (2009).

In situ experiments investigating the effect of *Gracilaria*-algal mats on seagrass has showed reduction in seagrass densities (Huntington and Boyer 2008; Thomsen et al. 2013; Vieira et al. 2020). A study from California investigated the effect of *Gracilariopsis* sp.- mats in a bay still dominated by *Z. marina* (Huntington and Boyer 2008). The amount of added *Gracilariopsis* sp. was based on the least, the mean and the max weight of *Gracilariopsis* detected during a survey in the bay (0, 325, and 1700 g m^{-2} respectively), and the experiment hence represent a controlled investigation of the effects from representative amounts of the *Gracilariopsis* in this area. Huntington and Boyer (2008) detected a significant negative effect on *Z. marina* shoot density and final growth rate under the highest add on-treatment of *Gracilariopsis* (1700 g m^{-2}) comparing to the two other treatments after the three month long field experiment. They stated shading as the most likely limiting factor. In a field experiment in Denmark, Thomsen et al (2013) found that adding 3 kg wet weight per m^2 of *A. vermiculophyllum* reduced the above ground biomass of Z. marina significantly.

What appears to be the best documented case of *A. vermiculophyllum* and *Zostera* coexistence in Europe is a lagoon in Portugal. Here dense seasonal mats of *A. vermiculophyllum* has been reported in Ria de Aveiro lagoon, where it is one of the main macrophytes (Abreu et al. 2011; Vieira et al. 2020). No field observations on negative effects from *A. vermiculophyllum* on *Z.*

noltei were reported, but a field experiment on cooccurring stressors was conducted in a healthy *Zostera noltei* meadow in the area (Vieira et al. 2020). Vieira et al. (2020) tested interactive effects of *A. vermiculophyllum* abundance (three levels), nutrient levels, and sediment additions. The highest abundance level of *A. vermiculophyllum* gave a significant negative impact on *Z. noltei,* by giving reduced above- and below ground biomass. The shoot density was also negatively affected when the medium *A. vermiculophyllum* abundance was applied together with nutrient enrichment and sediment addition (Vieira et al. 2020), indicating that a multi-stress scenario is the most apparent threat to *Z. noltei* meadows

This has also been the case for *Z. marina* in laboratory studies, where negative tendencies on the seagrass by *A. vermiculophyllum*-addition has appeared at high temperatures (26°C - 30°C) (Martínez-Lüscher and Holmer 2010; Höffle et al. 2011).

Agarophyton vermiculophyllum's effect on seagrasses seems to be density dependent, as negative effects happens in the highest add-ons (Thomsen et al. 2013; Vieira et al. 2020), and or in combination with other stressors (Martínez-Lüscher and Holmer 2010; Höffle et al. 2011; Vieira et al. 2020). Extensive periods of heavy algae-mats hence could lead to decline in local *Zostera* populations.

In Viksfjorden and at Tjøme *A. vermiculophyllum* was only detected on four stations combined, three of them overlapping with findings of *Z. marina.* However, these findings are so rare that they offer little insight into if *A. vermiculophyllum* prefers the habitat that overlaps with that of seagrass. In 2015 *A. vermiculophyllum* was detected in Hølen marina in Larviksfjorden next to Viksfjorden (Norwegian Biodiversity Information Centre a), where one thallus was observed. In 2012 findings of *A. vermiculophyllum* were detected in Ødegårdskilen at Tjøme (Husa et al. 2013), also here only one thallus was detected. During the fieldwork in autumn of 2020 Ødegårdskilen was searched especially thorough, with an extra search station from boat, and by search along the shore. Despite this *A. vermiculophyllum* was not detected there (Figure 14, cluster of white points). These findings suggest that the species has not increased its abundance here in the five and eight years, respectively, that has passed since *A. vermiculophyllum* was first detected in these areas.

Based on observations done by Nyberg (2007) of extensive dispersal done in Sweden in the two subsequent years after their first discovery of *A. vermiculophyllum* 180 km north and south from the original sighting, presence was expected in Viksfjorden and Tjøme as it already had previously been detected in the areas.

Agarophyton vermiculophyllum was present in Viksfjorden and Tjøme (Figure 9 and 12), and in the areas close by (Figure 14), but the detected abundance in this study was low. *A. vermiculophyllum* has showed that it rapidly can become highly abundant in specific areas. In 2008 the first detection of *A. vermiculophyllum* was done in the Venice Lagoon, where it was reported to reach abundances of 8 – 10 kg fw m^{-2} within few years (Sfriso et al. 2020). It is now the most abundant NIS in the area, with an estimated standing crop of 66383 tonnes (Sfriso et al. 2020). In France *A. vermiculophyllum* has been present since the mid-1990s (Mollet et al. 1998), and has reached high abundances in the Bay of Brest (276.5±64.1 g m^{-2}). Another location where it is highly abundant is Ria de Aveiro in Portugal, where its abundance has been reported to reach 2.27±0.40 kg fw m^{-2} (Abreu et al. 2011).

With the very low abundance of *A. vermiculophyllum* observed in Viksfjorden and Tjøme, a second period of field work was conducted to compare and identify environmental factors that might restricts the abundance in the first locations. In Solvikbukta *A. vermiculophyllum* was present on five stations together with *Z. marina*, while on 15 stations it was present without. In Slependrenna their presence overlapped on seven stations, and *A. vermiculophyllum* was present on seven without seagrass. Hence was *A. vermiculophyllum* present more often without *Z. marina* than together on the two sites combined.

The highest abundance detected of *Z. marina* in Solvikbukta was "Common", hence estimated to contribute under 10% to the total algal biomass on the three stations in question (Figure 15). Otherwise, it was categorised as "Rare" with only one or a few specimens found, or not found at all. In Slependrenna *Z. marina* was categorised as "Plenty" on one station, contributing with more than 10% to the total observed algal biomass, on the rest of the stations it was "Common", "Rare", or most frequently not found.

Based on registrations provided by The Norwegian Environmental Agency (Figure 4) access to the seagrass meadows were expected from the docks. However, the low abundance detected of *Z. marina* indicate that they only provided access to the outskirts of the meadow. Rinde et al. (2020) reports that the docks shade, and hence restrict growth of *Z. marina* in Slependrenna, and this could be the reason for the low abundance observed.

In Solvikbukta mats of *A. vermiculophyllum* was detected in 2018 (Norwegian Biodiversity Information Centre b), estimated to 100 thalli. The observation matches the findings in autumn of 2020 where three of the sampling stations had records of *A. vermiculophyllum* categorised as "Dominant" and hence covered the seafloor almost completely (Figure 15). In

Slependrenna *A. vermiculophyllum* has been reported in the *Z. marina* meadow previously (Rinde et al. 2020).

There is the possibility of the *Z. marina* abundance being influenced by the high abundance of *A. vermiculophyllum* (as especially detected in Solvikbukta), but as this thesis only provides a snapshot in time of the species composition in Solvikbukta and Slependrenna, this cannot be answered. Mats of *Gracilaria* spp. were reported within the seagrass meadow in Slependrenna by Rinde et al. (2020), and presence of both *G. gracilis* and *A. vermiculophyllum* were confirmed genetically after the investigations. However, this was deeper than the sampling done in this thesis. Rinde et al. (2020) reported that no conclusion of negative impact on the meadows from *A. vermiculophyllum* could be done, as the observations were done at five meters depth which is the reported lower growth limit of *Z. marina* in the area. They urged further surveillance of *A. vermiculophyllum* to be able to detect possible negative impacts on the meadow.

Although this thesis does not provide evidence that *A. vermiculophyllum* has any negative effect on the seagrass meadows at this point, seagrass is under increasing pressure from other stressors. "Filamentous algae" such as *Cladophora* sp. and alike (Table 3) were found on almost all stations where *Z. marina* was present. The phenomenon of filamentous alga is a known problem especially in Viksfjorden (Moy et al. 2014; Christie and Rinde 2020) and Slependrenna (Rinde et al. 2020), where they grow extensively in highly eutrophicated water and deplete the area of oxygen resulting in harmful hydrogen sulphide in the sediment (Christie and Rinde 2020). This black sediment was also detected in Solvikbukta, Slependrenna and Viksfjorden during this fieldwork.

The abundance of the filamentous alga was not quantified as this was not within the scope of this study, but especially in inner parts of Viksfjorden they were present to the extent that driving a small boat proved difficult. The video material and grapnel samples provide useful information for possible future comparisons of worsening of the situation.

At this point *A. vermiculophyllum* constitutes no threat to *Z. marina* and *Z. noltei* in Viksfjorden and at Tjøme. This thesis unfortunate does not provide further understanding to which degree *A. vermiculophyllum* poses a threat to the *Z. marina* meadows in Solvikbukta and Slependrenna. However, with the current threat from eutrophication and filamentous alga blooms, increasing ocean temperatures and increased extreme heat events (IPCC 2014), a

future add on effect of possible large mats of *A. vermiculophyllum* would not be a positive one, and hence the pressure on this valuable ecosystem should be closely monitored.

4.2 Environmental factors explaining *A. vermiculophyllum* abundance

The model selection showed that the model with depth as the only fixed predictor variable and the random predictor "Site" returned the lowest AICc value and hence is the best model representation of reality given the data. Depth is hence the variable that best explains the presence/absence of *A. vermiculophyllum.* Presence of *A. vermiculophyllum* was detected in stations with the depth range between 0.3 and 3.7 m, while no presence was detected deeper than 3.7 m although the dataset contains stations in the range of 0.3 – 20 m depth (in Solvikbukta and Slependrenna the deepest station was 3.6 m). Based on this it is apparent that the presence of *A. vermiculophyllum* is more likely in shallow water. One explanation for this can be that low light is a restricting factor in the deep. Through experiments Weinberger et al. (2008) concluded that light limitation hinder net growth of *A. vermiculophyllum* below a mean depth of 3 m in the Kiel Fjord. Unfortunately, this thesis does not provide information on light availability in the areas investigated, and a determination of which depth that represents the threshold of long-term net growth here cannot be done.

However, the depth model returned a R^2-value of 0.67, and hence only 67% of the variance in presence of *A. vermiculophyllum* can be explained with this model. The data contains many stations in the shallow depth range that did not show presence of *A. vermiculophyllum*, and other environmental factors must explain the remaining variance.

The salinities measured at the sites (means per site in the range of 20.08-24.22 psu, Appendix C), lies well inside the optimum salinity range of *A. vermiculophyllum* (Yokoya et al. 1999; Kim et al. 2016; Wu et al. 2018). Yokoya et al. (1999) and Kim et al. (2016) reported optimum growth rates in salinities from 15 to 30 psu. Hence there is no reason to believe that the salinities in Viksfjorden and Tjøme (means 20.08 ± 2.83 and 24.22 ± 0.33 psu respectively) should act as a restricting factor on the presence and abundance of *A. vermiculophyllum.*

No strong consensus seems to exist on which environmental variables that is more important for the abundance of *A. vermiculophyllum* in an area. The occurrence of large populations in shallow estuarine areas has led to investigations of nutrients as a likely explanatory factor (Thomsen and McGlathery 2007; Nejrup and Pedersen 2010; Sfriso et al. 2012). Nejrup and Pedersen (2010) investigated two estuaries with different degrees of eutrophication with the

hypothesis that high values of nutrients was the likely explanation of the difference in *A. vermiculophyllum* abundance. However, their results indicated that, nor nutrient loading or grazing pressure significantly influenced growth rate, and hence could not explain the large difference in biomass of *A. vermiculophyllum* between the two sites, where *A. vermiculophyllum* had been introduced around the same time. Also Thomsen and McGlathery (2007) tested levels of nutrient on growth rate, but did not detect any significant difference on the biomass of *A. vermiculophyllum* when exposed to nutrient enrichment compared to no enrichment. In Italy however, high nutrient availability is reported to be explanatory of the establishment and dominance of *A. vermiculophyllum* (Sfriso et al. 2012).

Nejrup and Pedersen (2010) proposes a different explanation that also often occurs in nutrient rich areas; exposure. Or to be precise; the lack of it. This observation is interesting in connection to the data in this thesis. The mean NO_3 concentration in Viksfjorden was higher than all other sites, and the PO_4 mean was second highest after Slependrenna (3.65 ± 2.73 µmol/l and 0.52 ± 0.22 µmol/l respectively, Appendix C). However, the models including NO_3 and PO_4 as predictors was not the most explaining model given this data.

Looking at the map (Figure 2), exposure is a variable that sets these two groups of sites apart. Outer part of Viksfjorden and Tjøme is potentially more exposed to wave and current action as they are closer to Skagerrak, than Solvikbukta and Slependrenna that is situated in the innermost of the Oslo fjord (Figure 2). This might hinder settlement of new populations, even if they are introduced to the area frequently, if the introduced specimens are transported out to deeper waters where light is limiting growth before they have had time to establish a population in the shallow.

Sfriso et al. (2012) however reports a lack of *A. vermiculophyllum* establishment in areas of high-water exchange and low nutrient concentrations in the Venice area, while in other investigated areas that also provides high water exchange but in addition has high nutrient concentrations presence has been recorded.

With the low abundance of *A. vermiculophyllum* detected in Viksfjorden and at Tjøme it is hard to determine the most important factors allowing for abundance, as stochastic events might be important in the time before the populations become more abundant. Exposure as an explanatory factor of lack of establishment of abundant populations of *A. vermiculophyllum*, despite high nutrient levels in an interesting hypothesis and further research should include

exposure as a possible explanatory variable when investigating or modelling distribution of *A. vermiculophyllum*.

4.3 Investigation of survival after exposure to low temperatures

No significant difference between the growth- means or the Fv/Fm-means of the temperature treatments was detected in the two-way ANOVAs. Hence the fragments in the 2°-treatment and 0°-treatment did not show any sign of lower survival after cold-exposure than the two warmer treatments.

4.3.1 Growth of *A. vermiculophyllum* after exposure to low temperatures

Growth was measured after the cold exposure when the fragments had been returned to more ideal conditions for three weeks. This was done as the main objective was to investigate if the fragments were alive after simulating winter conditions. Fragments that showed zero growth, would drag down the mean of their treatment group and cause significantly difference in mean growth. However, this was not observed as all fragments showed some degree of growth once returned to optimum conditions. This would simulate a winter in harsh conditions both light and temperature wise, before returned to spring temperatures and an excess of light.

These findings are in line with the reports of abundant populations of *A. vermiculophyllum* in areas with low water temperatures in winter (Nyberg et al. 2009; Kim et al. 2016). In Connecticut (US) Kim et al. (2016) reports water temperatures down to 0.4 °C and occasionally lower in an area where *A. vermiculophyllum* is highly abundant. Nyberg et al. (2009) report of Swedish winter water temperatures in the range of -2 to 5 °C and presence of ice on the Swedish west coast where *A. vermiculophyllum* is present.

It should be noted that the light intensities simulating winter light (40-50 µmol photon $m^{-2}s^{-1}$) are based on an experiment adapted for winter conditions on the south west coast of Norway (Armitage and Sjøtun 2017). There winters are normally milder than on the southeast coast of Norway where winter are colder and ice cover with added snow occurs to a larger degree. Hence these light intensities might be a little high for simulation winter conditions as they do not account for shading by ice formation. However, Nyberg and Wallentinus (2009) has previously illustrated that regrowth is possible after eight months in complete darkness, so winter light intensities is not likely to be a restriction factor for the *A. vermiculophyllum* populations at the investigated sites.

Low temperatures (below 15°C) have showed to reduce growth rate in *A. vermiculophyllum* while it is exposed to these temperature (Yokoya et al. 1999; Weinberger et al. 2008; Nejrup et al. 2013; Kim et al. 2016), but as long as the temperature rises afterwards (as in the spring) this will likely not constrict the growth and hence abundance of *A. vermiculophyllum* based on the findings of this experiment. No experiments performed in temperatures below 4°C has been detectable during the literature search in this thesis. This is likely due to the fact that experiments with temperatures that low requires equipment suitable for low temperatures. This thesis hence provides insightful additions to the literature on the response of *A. vermiculophyllum* to temperatures at 0 and 2°C in a controlled experiment.

4.3.2 Maximum photosynthetic yield (Fv/Fm) of *A. vermiculophyllum* after exposure to low temperatures

The same logic was applied to the Fv/Fm measurements. This was measured after the cold exposure when the fragments had spent one week in the more ideal conditions. The main objective was to investigate if the fragments were alive after the cold treatment by assessing their capacity of photosynthesis. Fragments that showed very low to zero Fv/Fm, would drag down the mean of their treatment group and cause significantly difference the mean Fv/Fm values between the treatments indicating lower survival. The Fv/Fm mean values of each treatment group lay within the area of 0.41 and 0.48, and no significant differences were detected between the temperature treatments. However, these values are lower than the Fv/Fm values measured by Wienberget et al. (2008) in *A. vermiculophyllum* after an experiment with approx. 2 weeks at various depths (0-5 m). They showed means in the 0.65-0.75 range, somewhat higher than the Fv/Fm values after the cold treatments, and hence could indicate that the maximum photosynthetic yield in of the fragments in this thesis is lowered due to stress.

The Fv/Fm measurements provides data that can be compared in future studies, but they are not obtained as much importance in this thesis as they were an assurance to get information on the physiological state of the fragments should they i.e., have died due to non-controllable factors unrelated to previous temperature treatment later in the optimum period before growth could be measured.

4.4 Evaluation of methods

Preferably the sampling stations at the four locations should have been in identical grids, and access to a boat in Solvikbukta and Slependrenna would have improved this study by

providing stations in the seagrass meadow. This would have provided more insight in the status of abundance of *A. vermiculophyllum* in the meadow.

The distance covered in the one-minute video transects varied as wind and currents affected the boat speed. A more accurate method of measuring distance and area, and hence more accurate quantification of biomass would have improved the study, but this would have required more advanced equipment than what was available. The semi quantitative abundance scale used is relative to the other algae biomass found. The "Common" and "Plenty" categories are almost detached from absolute amount and hence the same classification can result in very different amounts of the algae in question in a transect completely covered by biomass compared to one with almost no algae-biomass. The "Rare" and "Dominant" categories quantify the presence to a larger degree as "Rare" can only be used for one or a few specimens, while the category "Dominant" requires an almost complete cover of the transect by the species/group in question, which nuances stations completely covered by *A. vermiculophyllum* or by a lush *Z. marina*-meadow. This makes the observations in the "Rare" and "Dominant" categories more accurate than the observations in the "Common" and "Plenty" categories and should be noted in a future study comparing abundances to these data.

The "Unidentifiable loose laying"-category contains both observations from video where the grapnel sample did not provide confirmation on presence of *A. vermiculophyllum* or/and *G. gracilis*, as well as in the case where *A. vermiculophyllum* and/or *G. gracilis* ware present on the grapnel but it was impossible to determine which was which on the video. In the latter cases the presence of *A. vermiculophyllum* and/or *G. gracilis* was categorised to "Rare", as it had been confirmed on the grapnel sample, while the biomass of the loose laying algae was categorised in the abundance category "Unidentifiable loose laying". *G. gracilis* and "Unidentified loose laying" are hence presented in the results (Figure 11, 13, 16, and 18), as "Unidentifiable loose laying"-category contains some biomass of *A. vermiculophyllum* that could not be categorised to species, this is especially the case on stations where both *A. vermiculophyllum* and *G. gracilis* were present and impossible to distinguish on video.

The environmental data going into this model selection is a snapshot of the state of the system in late summer, and hence does not provide the robustness that it preferably should. A longer timeseries of i.e., average summer/winter temperatures, salinities and nutrients would provide a truer picture of these environments and might have provided other environmental predictors than depth as the best representations of reality in the model selection. Also, stations along the entire coastline would have been a more robust method in identifying key factors for preferred

habitat. However, this was not possible time, budget (or global pandemic)-wise during this thesis field work in the autumn of 2020.

Due to large variability between the depth of sampling stations and the assigned hydrographical station, the upper five meters were chosen for average salinity, as *A. vermiculophyllum* is yet to be found deeper than five meters in Norwegian waters (Rinde et al. 2020). This however does not fully represent the actual conditions found on the seafloor for the majority of the stations. In an ideal study one would have had the sea floor temperature and salinity for all individual sampling stations. Depth is the only predictor included in the model that was measured on each sampling station, which automatically makes it more explanatory, and contributed to this being the most explanatory factor of presence/absence.

It should be noted that the replicates in this temperature experiment was not performed ideally. Due to the bleaching already quite prominent in some thalli prior to the experiment after one week in storage, fragments within the axis-categories were repeatedly taken from the same thalli, before mixed and assigned randomly to a treatment. This was however not possible to account for in the statistical analysis as the fragments originating from the same individuals were assigned to a temperature at random. These individual differences might have had more effect than the treatments and disturbed the outcome of the experiment.

However, *A. vermiculophyllum* is a species that reproduce asexually by fragmentation, and especially loose laying individuals in invading populations have been shown to mainly reproduce by fragmentation (Krueger-Hadfield et al. 2016). All alga included was in addition collected from a loose laying mat at the same station. This means that there may be very small genetic variation of the sampled material, and hence the decision to use healthy material from the same thalli in several replicates was chosen over the use material showing signs of deterioration. A way to avoid these issues would have been to collect *A. vermiculophyllum* from different sites and include them in each temperature treatment equally, while knowing which replicate originated were. In this manner site, or genetic differences would be a fixed effect and could been accounted for in the statistical analysis.

The two warmest treatments (“Ambient” and “Heated”) were planned to hold temperatures of 6°C and 10°C respectively. This was not possible to maintain though the experimental period as a lot of activity and experiments were conducted in the facility giving more fluctuations in the room temperature than expected. The “Ambient”-treatment did not have any heating or colling device and were following the ambient temperature in the climate room, while the

“Heated”-treatment had a heated head that however did not manage to keep the temperature as low as intended. Temperature and light conditions during transportation and storage, acclimatisation periods and more could have had improvements, however all was done according to the resources and personnel available.

As *A. vermiculophyllum* has a rounded shape with branching in all planes one challenge was to get the entire fragment visible in a picture. This error was tried eliminated by putting the fragments between two microscope slides and placing them in the best possible way to make as much as possible of the fragment surface visible from above. However, this had its limitations, and the growth measurements holds more uncertainty due to this, but as the method was the same for all fragments this has likely no affect om the mean growth of each treatment.

4.5 Concluding remarks

Agarophyton vermiculophyllum was not present on more stations with *Z. marina* than on stations without. It had not had noticeable dispersal in Viksfjorden or at Tjøme and hence proposes no threat to the seagrass communities at this point. In Solvikbukta and Slependrenna the question of threat was not answered as stations were mainly outside of the meadow.

Depth as a fixed effect and site as a random effect provided best explanation of the presence or absence of *A. vermiculophyllum* given the data. Future studies are proposed to include wave exposure as an explanatory variable as no previous studies have provided sufficient distribution explanations based on nutrient availability or salinity as here.

The results from the temperature experiment strengthens the consensus on that A. *vermiculophyllum* has wide tolerance limits both for temperature and light. The low temperatures had no apparent effect on survival, and temperatures down to 0°C hence does not serve as an apparent limitation to further dispersal. Future studies could focus on confirming these results. Monitoring of further spread northwards on the Norwegian coast should be implemented, especially in areas close to seagrass meadows where *A. vermiculophyllum* potentially serves an elevated threat.

5 References

Abreu, M.H., Pereira, R., Sousa-Pinto, I., and Yarish, C. 2011. Ecophysiological studies of the non-indigenous species *Gracilaria vermiculophylla* (Rhodophyta) and its abundance patterns in Ria de Aveiro lagoon, Portugal. Eur. J. Phycol. **46**(4): 453–464. Taylor and Francis Ltd. doi:10.1080/09670262.2011.633174.

Armitage, C.S., and Sjøtun, K. 2017. Can an old alien benefit from rising ocean temperatures? An experimental and field study on the growth and local distribution of Codium fragile subsp. fragile (Chlorophyta). Mar. Biol. **164**(6): 142. Springer Verlag. doi:10.1007/s00227-017-3170-5.

Bekkby, T., Rinde, E., Erikstad, L., Bakkestuen, V., Longva, O., Christensen, O., Isæus, M., and Isachsen, P.E. 2008. Spatial probability modelling of eelgrass (Zostera marina) distribution on the west coast of Norway. ICES J. Mar. Sci. **65**(7): 1093–1101. Oxford Academic. doi:10.1093/icesjms/fsn095.

Bellorin, A.M., Oliveira, M.C., and Oliveira, E.C. 2004. Gracilaria vermiculophylla: A western Pacific species of Gracilariaceae (Rhodophyta) first recorded from the eastern Pacific. Phycol. Res. **52**(2): 69–79. Wiley. doi:10.1111/j.1440-183.2004.00330.x.

Barton, K. 2020. MuMIn: Multi-Model Inference. R package version 1.43.17. https://CRAN.R-project.org/package=MuMIn

Bates, D., Maechler, M., Bolker, B., Walker, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1-48. doi:10.18637/jss.v067.i01.

Christie, H., Norderhaug, M., and Fredriksen, S. 2009. Macrophytes as habitat for fauna. Source Mar. Ecol. Prog. Ser. **396**: 221–234. doi:10.2307/24874274.

Christie, H., and Rinde, E. 2020. Tre ålegrasenger på Skagerrakkysten : Forvarsel om en naturtype i forfall ?

Crowley, E. and Crowley, J. 2021. GGally: Extension to 'ggplot2'. R package version 2.1.2. https://CRAN.R-project.org/package=GGally

Davoult, D., Surget, G., Stiger-Pouvreau, V., Noisette, F., Riera, P., Stagnol, D., Androuin, T., and Poupart, N. 2017. Multiple effects of a Gracilaria vermiculophylla invasion on estuarine mudflat functioning and diversity. Mar. Environ. Res. **131**: 227–235. Elsevier

Ltd. doi:10.1016/j.marenvres.2017.09.020.

Duarte, C.M. 1995. Submerged aquatic vegetation in relation to different nutrient regimes. Ophelia **41**(1): 87–112. Taylor & Francis Group. doi:10.1080/00785236.1995.10422039.

Duarte, C.M. 2002. The future of seagrass meadows. Environ. Conserv. **29**(2): 192–206. Cambridge University Press. doi:10.1017/S0376892902000127.

Duarte, C.M., Marbà, N., Gacia, E., Fourqurean, J.W., Beggins, J., Barrón, C., and Apostolaki, E.T. 2010. Seagrass community metabolism: Assessing the carbon sink capacity of seagrass meadows. Global Biogeochem. Cycles **24**(4). John Wiley & Sons, Ltd. doi:10.1029/2010GB003793.

Enerstvedt, K.H., Lundberg, A., Sjøtun, I.K., Fadnes, P., and Jordheim, M. 2017. Characterization and seasonal variation of individual flavonoids in Zostera marina and Zostera noltii from Norwegian coastal waters. Biochem. Syst. Ecol. **74**: 42–50. Pergamon. doi:10.1016/J.BSE.2017.08.003.

Fredriksen, S., Christie, H., and Andre Sæthre, B. 2005. Species richness in macroalgae and macrofauna assemblages on *Fucus serratus* L. (Phaeophyceae) and *Zostera marina* L. (Angiospermae) in Skagerrak, Norway. Mar. Biol. Res. **1**(1): 2–19. Taylor & Francis . doi:10.1080/17451000510018953.

Freshwater, D.W., Montgomery, F., Greene, J.K., Hamner, R.M., Williams, M., and Whitfield, P.E. 2006. Distribution and identification of an invasive Gracilaria species that is hampering commercial fishing operations in southeastern North Carolina, USA. Biol. Invasions **8**(4): 631–637. doi:10.1007/S10530-005-1809-5.

Glenn, M., Mathieson, A., Grizzle, R., and Burdick, D. 2020. Seaweed communities in four subtidal habitats within the Great Bay estuary, New Hampshire: Oyster farm gear, oyster reef, eelgrass bed, and mudflat. J. Exp. Mar. Bio. Ecol. **524**: 151307. Elsevier. doi:10.1016/J.JEMBE.2019.151307.

Greipsland, I., Roseth, R., Pettersen, R.A., Bechmann, P., Lundsør, E., and Brabrand, Å. 2019. E-18 Lysaker-Ramstadsletta. Forundersøkelser av vannkjemi og biologiske kvalitetselement 2018. NIBIO. Available from https://nibio.brage.unit.no/nibio-xmlui/handle/11250/2592341 [accessed 7 July 2021].

Gurgel, C.F.D., Norris, J.N., Schmidt, W.E., Le, H.N., and Fredericq, S. 2018. Systematics of

the gracilariales (Rhodophyta) including new subfamilies, tribes, subgenera, and two new genera, Agarophyton gen. nov. and Crassa gen. nov. Phytotaxa **374**(1): 1–23. Magnolia Press. doi:10.11646/phytotaxa.374.1.1.

Henriksen, S., and Hilmo, O. (red. . 2015. Norsk rødliste for arter 2015. Artsdatabanken, Norge. Available from https://www.artsdatabanken.no/Files/13973/Norsk_r_dliste_for_arter_2015_(PDF) [accessed 10 July 2021].

Höffle, H., Thomsen, M.S., and Holmer, M. 2011. High mortality of Zostera marina under high temperature regimes but minor effects of the invasive macroalgae Gracilaria vermiculophylla. Estuar. Coast. Shelf Sci. **92**(1): 35–46. Academic Press. doi:10.1016/j.ecss.2010.12.017.

Huntington, B.E., and Boyer, K.E. 2008. Effects of red macroalgal (Gracilariopsis sp.) abundance on eelgrass Zostera marina in Tomales Bay, California, USA. Mar. Ecol. Prog. Ser. **367**: 133–142. doi:10.3354/MEPS07506.

Hurvich, C.M., and Tsai, C.-L. 1989. Regression and time series model selection in small samples. Biometrika **76**(2): 297–307. Oxford Academic. doi:10.1093/BIOMET/76.2.297.

Husa, V., Agnalt, A.-L., Svensen, R., Rokkan- Iversen, K., Steen, H., Jelmert, A., Farestvedt, E., and Petersen, H. 2013. Kartlegging av fremmede marine arter i ytre og indre Oslofjord Utredning for DN 4-2013. Direktoratet for naturforvaltning. Available from https://www.miljodirektoratet.no/globalassets/dokumenter/publikasjoner/oppdragsrapporter/dn-utredning-4-2013_nett_final.pdf [accessed 5 June 2021].

Husa, V., Fredriksen, S., and Sjøtun, K. 2018. Gracilaria vermiculophylla, vurdering av økologisk risiko. Fremmedartslista 2018. Artsdatabanken. Available from https://www.artsdatabanken.no/Fab2018/N/1236 [accessed 1 July 2021].

Husa, V., Sjøtun, K., Brattenborg, N., and Eiliv Lein, T. 2008. Changes of macroalgal biodiversity in sublittoral sites in southwest Norway: impact of an introduced species or higher temperature? Mar. Biol. Res. **4**(6): 414–428. Taylor & Francis Group . doi:10.1080/17451000802232874.

Husa, V., Sjøtun, K., and Lein, T.E. 2004. The newly introduced species Heterosiphonia japonica Yendo (Dasyaceae, Rhodophyta): geographical distribution and abundance at

the Norwegian southwest coast. Sarsia **89**(3): 211–217. Taylor & Francis Group . doi:10.1080/00364820410006600.

IPBES. 2019. Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. doi:10.5281/ZENODO.3831674.

IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. *In* Ipcc. Geneva, Switzerland. Available from http://www.ipcc.ch. [accessed 13 May 2020].

Katsanevakis, S., Wallentinus, I., Zenetos, A., Leppäkoski, E., Çinar, M.E., Oztürk, B., Grabowski, M., Golani, D., and Cardoso, A.C. 2014. Impacts of invasive alien marine species on ecosystem services and biodiversity: A pan-European review. Aquat. Invasions **9**(4): 391–423. doi:10.3391/ai.2014.9.4.01.

Kim, J.K., Yarish, C., and Pereira, R. 2016. Tolerances to hypo-osmotic and temperature stresses in native and invasive species of Gracilaria (Rhodophyta). Phycologia **55**(3): 257–264. Allen Press Publishing Services. doi:10.2216/15-90.1.

Kim, S.Y., Weinberger, F., and Boo, S.M. 2010. Genetic data hint at a common donor region for invasive atlantic and pacific populations of Gracilaria vermiculophylla (Gracilariales, Rhodophyta) (note). J. Phycol. **46**(6): 1346–1349. doi:10.1111/j.1529-8817.2010.00905.x.

Krueger-Hadfield, S.A., Kollars, N.M., Byers, J.E., Greig, T.W., Hammann, M., Murray, D.C., Murren, C.J., Strand, A.E., Terada, R., Weinberger, F., and Sotka, E.E. 2016. Invasion of novel habitats uncouples haplo-diplontic life cycles. Mol. Ecol. **25**(16): 3801–3816. Blackwell Publishing Ltd. doi:10.1111/mec.13718.

Krueger-Hadfield, S.A., Kollars, N.M., Strand, A.E., Byers, J.E., Shainker, S.J., Terada, R., Greig, T.W., Hammann, M., Murray, D.C., Weinberger, F., and Sotka, E.E. 2017a. Genetic identification of source and likely vector of a widespread marine invader. Ecol. Evol. **7**(12): 4432–4447. John Wiley & Sons, Ltd. doi:10.1002/ECE3.3001.

Krueger-Hadfield, S.A., Magill, C.L., Bunker, F.S.P.D., Mieszkowska, N., Sotka, E.E., and Maggs, C.A. 2017b. When Invaders Go Unnoticed: The Case of Gracilaria vermiculophylla in the British Isles. Cryptogam. Algol. **38**(4): 379–400. Muséum

national d'Histoire naturelle, Paris. doi:10.7872/CRYA/V38.ISS4.2017.379.

Leal, P.P., Ojeda, J., Sotomayor, C., and Buschmann, A.H. 2020. Physiological stress modulates epiphyte (Rhizoclonium sp.)-basiphyte (Agarophyton chilense) interaction in co-culture under different light regimes. J. Appl. Phycol. **32**(5): 3219–3232. Springer Science and Business Media B.V. doi:10.1007/s10811-020-02153-w.

Lilley, R.J., and Unsworth, R.K.F. 2014. Atlantic Cod (Gadus morhua) benefits from the availability of seagrass (Zostera marina) nursery habitat. Glob. Ecol. Conserv. **2**: 367–377. Elsevier. doi:10.1016/J.GECCO.2014.10.002.

Martínez-Lüscher, J., and Holmer, M. 2010. Potential effects of the invasive species Gracilaria vermiculophylla on Zostera marina metabolism and survival. Mar. Environ. Res. **69**(5): 345–349. Elsevier. doi:10.1016/j.marenvres.2009.12.009.

Matheson, K., McKenzie, C., Gregory, R., Robichaud, D., Bradbury, I., Snelgrove, P., and Rose, G. 2016. Linking eelgrass decline and impacts on associated fish communities to European green crab Carcinus maenas invasion. Mar. Ecol. Prog. Ser. **548**: 31–45. doi:10.3354/meps11674.

Mollet, J.C., Rahaoui, A., and Lemoine, Y. 1998. Yield, chemical composition and gel strength of agarocolloids of Gracilaria gracilis, Gracilariopsis longissima and the newly reported Gracilaria cf. vermiculophylla from Roscoff (Brittany, France). J. Appl. Phycol. **10**(1): 59–66. Springer. doi:10.1023/A:1008051528443.

Moy, F., Albretsen, J., Bodvin, T., Naustvoll, L.J., and Ohldieck, M. 2014. Truet ålegressforekomst i indre Viksfjord, Larvik (Rapport fra Havforskningen nr. 21 - 2014) | Institute of Marine Research. *In* Institute of Marine Research. Available from https://www.hi.no/en/hi/nettrapporter/rapport-fra-havforskningen/2014/hi-rap_21-2014_alegress_til_web [accessed 12 February 2021].

Nejrup, L., and Pedersen, M. 2010. Growth and biomass development of the introduced red alga Gracilaria vermiculophylla is unaffected by nutrient limitation and grazing. Aquat. Biol. **10**(3): 249–259. doi:10.3354/ab00281.

Nejrup, L.B., Pedersen, M.F., and Vinzent, J. 2012. Grazer avoidance may explain the invasiveness of the red alga Gracilaria vermiculophylla in Scandinavian waters. Mar. Biol. **159**(8): 1703–1712. Springer. doi:10.1007/s00227-012-1959-9.

Nejrup, L.B., Staehr, P.A., and Thomsen, M.S. 2013. Temperature- and light-dependent growth and metabolism of the invasive red algae *Gracilaria vermiculophylla* – a comparison with two native macroalgae. Eur. J. Phycol. **48**(3): 295–308. Taylor & Francis . doi:10.1080/09670262.2013.830778.

Nyberg, C.D. 2007. Introduced marine macroalgae and habitat modifiers - their ecological role and significant attributes (doctoral thesis). Göteborg University. Available from http://hdl.handle.net/2077/4719 [accessed 5 June 2021].

Nyberg, C.D., Thomsen, M.S., and Wallentinus, I. 2009. Flora and fauna associated with the introduced red alga Gracilaria vermiculophylla. Eur. J. Phycol. **44**(3): 395–403. Taylor & Francis Group. doi:10.1080/09670260802592808.

Nyberg, C.D., and Wallentinus, I. 2009. Long-term survival of an introduced red alga in adverse conditions. Mar. Biol. Res. **5**(3): 304–308. doi:10.1080/17451000802428159.

Norwegian Biodiversity Information Centre a, Artskart.artsdatabanken.no 03.06.2021. Observational data from: Norsk botanisk forening, BioFokus. Downloaded from Artskart.

Norwegian Biodiversity Information Centre b, Artskart.artsdatabanken.no 31.08.2021. Observational data for Gracilaria vermiculophylla (Ohmi) Papenfuss from Norsk botanisk forening, so2-algae, catalogue number: 13323809

Norwegian Biodiversity Information Centre c, Artskart.artsdatabanken.no 31.08.2021. Observational data for Gracilaria vermiculophylla (Ohmi) Papenfuss from Norsk botanisk forening, so2-algae, catalogue number: 20382734

Ohmi, H. 1956. CONTRIBUTIONS TO THE KNOWLEDGE OF GRACILARIACEAE FROM JAPAN : II. On a new species of the genus Gracilariopsis, with some considerations on its ecology. Bull. Fac. Fish. Hokkaido Univ. **6**: 271–279.

Olsen, J.L., Coyer, J.A., Stam, W.T., Moy, F.E., Christie, H., and Jørgensen, N.M. 2013. Eelgrass Zostera marina populations in northern Norwegian fjords are genetically isolated and diverse. Mar. Ecol. Prog. Ser. **486**: 121–132. doi:10.3354/MEPS10373.

OSPAR Commission 2009: Background Document for Zostera beds, Seagrass beds, Biodiversity series. Available from https://www.ospar.org/work-areas/bdc/species-habitats/list-of-threatened-declining-species-habitats/habitats/zostera-beds

[accessed 8 September 2021]

Parker, J.D., Duffy, J.E., and Orth, R.J. 2001. Plant species diversity and composition: experimental effects on marine epifaunal assemblages. Mar. Ecol. Prog. Ser. **224**: 55–67. Inter-Research. doi:10.3354/MEPS224055.

QGIS Development Team, 2021. QGIS Geographic Information System. Open Source Geospatial Foundation. URL http://www.qgis.org

Raikar, S. V, Iima, M., and Fujita, & Y. 2001. Effect of temperature, salinity and light intensity on the growth of Gracilaria spp. (Gracilariales, Rhodophyta) from Japan, Malaysia and India. Indian J. Mar. Sci. **30**: 98–104.

R Core Team . 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rinde, E., Christie, H., and Stenrud Brkljacic, M. 2020. Utbredelse og tilstand til en nasjonalt viktig ålegraseng i Slependrenna i Asker og Bærum. *In* 26. Norsk institutt for vannforskning. Available from www.niva.no [accessed 5 June 2021].

Rueness, J. 2005. Life history and molecular sequences of Gracilaria vermiculophylla (Gracilariales, Rhodophyta), a new introduction to European waters. Phycologia **44**(1): 120–128.

Sfriso, A., Buosi, A., Wolf, M.A., and Sfriso, A.A. 2020. Invasion of alien macroalgae in the Venice Lagoon, a pest or a resource? Aquat. Invasions **15**(2): 245–270. doi:10.3391/ai.2020.15.2.03.

Sfriso, A., Maistro, S., Andreoli, C., and Moro, I. 2010. First record of Gracilaria vermiculophylla (Gracilariales, rhodophyta) in the po delta lagoons, Mediterranean sea (Italy) (Note). J. Phycol. **46**(5): 1024–1027. John Wiley & Sons, Ltd. doi:10.1111/j.1529-8817.2010.00893.x.

Sfriso, A., Wolf, M.A., Maistro, S., Sciuto, K., and Moro, I. 2012. Spreading and autoecology of the invasive species Gracilaria vermiculophylla (Gracilariales, Rhodophyta) in the lagoons of the north-western Adriatic Sea (Mediterranean Sea, Italy). Estuar. Coast. Shelf Sci. **114**: 192–198. Academic Press. doi:10.1016/J.ECSS.2012.07.024.

Sjøtun, K., Husa, V., and Peña, V. 2008. Present distribution and possible vectors of introductions of the alga Heterosiphonia japonica (Ceramiales, Rhodophyta) in Europe.

Aquat. Invasions **3**(4): 377–394. Regional Euro-Asian Biological Invasions Centre. doi:10.3391/AI.2008.3.4.3.

Stachowicz, J.J., Terwin, J.R., Whitlatch, R.B., and Osman, R.W. 2002. Linking climate change and biological invasions: Ocean warming facilitates nonindigenous species invasions. Proc. Natl. Acad. Sci. U. S. A. **99**(24): 15497–15500. National Academy of Sciences. doi:10.1073/pnas.242437499.

Surget, G., Lann, K. Le, Delebecq, G., Kervarec, N., Donval, A., Poullaouec, M.-A., Bihannic, I., Poupart, N., and Stiger-Pouvreau, V. 2017. Seasonal phenology and metabolomics of the introduced red macroalga Gracilaria vermiculophylla , monitored in the Bay of Brest (France). J. Appl. Phycol. 2017 295 **29**(5): 2651–2666. Springer. doi:10.1007/S10811-017-1060-3.

The Norwegian Environmental Agency, Dataset: Naturtyper – DN-håndbok 19 – Naturtype Marin -ålegras. Available from https://kartkatalog.miljodirektoratet.no/MapService/Details/naturtyper_marine_hb19 [accessed 14 September 2021]

Thomsen, M.S., and McGlathery, K.J. 2007. Stress tolerance of the invasive macroalgae Codium fragile and Gracilaria vermiculophylla in a soft-bottom turbid lagoon. Biol. Invasions **9**(5): 499–513. doi:10.1007/s10530-006-9043-3.

Thomsen, M.S., Staehr, P.A., Nejrup, L., and Schiel, D.R. 2013. Effects of the invasive macroalgae Gracilaria vermiculophylla on two co-occurring foundation species and associated invertebrates. Aquat. Invasions **8**(2): 133–145. doi:10.3391/ai.2013.8.2.02.

Thomsen, M.S., Staehr, P.A., Nyberg, C.D., Schwaerter, S., Krause-Jensen, D., and Silliman, B.R. 2007a. Gracilaria vermiculophylla (Ohmi) Papenfuss, 1967 (Rhodophyta, Gracilariaceae) in northern Europe, with emphasis on Danish conditions, and what to expect in the future. Aquat. Invasions **2**(2): 83–94. doi:10.3391/ai.2007.2.2.1.

Thomsen, M.S., Wernberg, T., Stæhr, P., Krause-Jensen, D., Risgaard-Petersen, N., and Silliman, B.R. 2007b. Alien macroalgae in Denmark - A broad-scale national perspective. Mar. Biol. Res. **3**(2): 61–72. Taylor & Francis Group. doi:10.1080/17451000701213413.

Tseng, C.K., and Xia, B.M. 1999. On the Gracilaria in the western Pacific and the southeastern Asia region. Bot. Mar. **42**(3): 209–217. De Gruyter.

doi:10.1515/BOT.1999.024.

Tweedley, J.R., Jackson, E.L., and Attrill, M.J. 2008. Zostera marina seagrass beds enhance the attachment of the invasive alga Sargassum muticum in soft sediments. Mar. Ecol. Prog. Ser. **354**: 305–309. doi:10.3354/MEPS07242.

Vieira, R., Martin, A., Engelen, A.H., Thomsen, M.S., and Arenas, F. 2020. Interactive effects of co-occurring anthropogenic stressors on the seagrass, Zostera noltei. Ecol. Indic. **109**. Elsevier B.V. doi:10.1016/j.ecolind.2019.105780.

Walz, H. 2018. DIVING-PAM-II Underwater Chlorophyll Flurometer, Manual for Standalone Use, Fourth Edition, July 2021. Available from https://www.walz.com/downloads/manuals/diving-pam-II/DIVING_PAM_II_04.pdf [accessed 6 September 2021]

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., and Williams, S.L. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc. Natl. Acad. Sci. U. S. A. **106**(30): 12377–12381. National Academy of Sciences. doi:10.1073/pnas.0905620106.

Weinberger, F., Buchholz, B., Karez, R., and Wahl, M. 2008. The invasive red alga Gracilaria vermiculophylla in the Baltic Sea: adaptation to brackish water may compensate for light limitation. Aquat. Biol. **3**(3): 251–264. doi:10.3354/ab00083.

Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Wu, H., Shin, S.K., Jang, S., Yarish, C., and Kim, J.K. 2018. Growth and nutrient bioextraction of gracilaria chorda, G. Vermiculophylla, ulva prolifera, and U. compressa under hypo-and hyper-osmotic conditions. Algae **33**(4): 329–340. Korean Society of Phycology. doi:10.4490/algae.2018.33.11.13.

Yokoya, N.S., Kakita, H., Obika, H., and Kitamura, T. 1999. Effects of environmental factors and plant growth regulators on growth of the red alga Gracilaria vermiculophylla from Shikoku Island, Japan. Hydrobiologia **398/399**(0): 339–347. Springer. doi:10.1023/A:1017072508583.

Appendix

A: Light intensities pre-experiment and during the temperature experiment

	Before experimental set up	**The first days of the experiment**	**During the cold treatment**	**During optimal conditions**
Time period	24.09.2020 - 01.10.2020	02.10.2020 - 05.10.2020	05.10.2020 – 30.10.2020	31.10.2020 – 20.11.2020
Light source	Lamp in the ceiling	Led lamp in the ceiling	Light panel of four CO/TECH Flexible RGB led strips with a spectrum of 460-620 Nm. Above the algae.	Fluorescent lamps on the wall beside the algae
Light intensity	NA	8-12 µmol photon $m^{-2}s^{-1}$	40-50 µmol photon $m^{-2}s^{-1}$,	73 - 214 µmol photon $m^{-2}s^{-1}$
Light/dark cycle	Continuous light	8:16 light/dark cycle	8:16 light/dark cycle	16:8 light/dark cycle

B: Temperatures for the treatment groups "heated" and "ambient" during the cold treatment.

Date	Heated	Ambient
01.10.2020	10°C	Acclimatisation
02.10.2020	NA	NA
03.10.2020	NA	NA
04.10.2020	NA	NA
05.10.2020	12°C	7.2°C
06.10.2020	12.4°C	8°C
07.10.2020	12.4°C	8.4°C
08.10.2020	10.2°C	8.9°C
09.10.2020	12.1°C	8.9°C
10.10.2020	NA	NA
11.10.2020	NA	NA
12.10.2020	12.5°C	9°C
13.10.2020	12.6°C	8.9°C
14.10.2020	NA	NA
15.10.2020	11.7°C	8.4°C
16.10.2020	12.3°C	8.2°C
17.10.2020	NA	NA
18.10.2020	NA	NA
19.10.2020	11.4°C	8.2°C
20.10.2020	11.6°C	8.2°C
21.10.2020	11.6°C	8.3°C
22.10.2020	11.8°C	8.2°C
23.10.2020	12.4°C	8.8°C
24.10.2020	NA	NA
25.10.2020	NA	NA
26.10.2020	10.9°C	8.4°C
27.10.2020	11.8°C	Acclimatisation
28.10.2020	NA	NA
29.10.2020	Acclimatisation	Acclimatisation
30.10.2020	Acclimatisation	Acclimatisation

C: Hydrographical measurements, averaged per site

Nutrient values from all water stations averaged per site, shown with standard deviation. mean salinity at the upper 5 meters averaged per sites with standard deviation

Site	NO_2, µmol/	NO_3, µmol/l	PO_4, µmol/l	Si, µmol/l	Salinity, psu
Viksfjorden	0.27 ± 0.13	3.65 ± 2.73	0.52 ± 0.22	14.0 ± 4.57	20.08 ± 2.83
Tjøme	0.03 ± 0.01	0.02 ± 0.02	0.18 ± 0.09	3.38 ± 0.49	24.22 ± 0.33
Solvikbukta	0.06 ± 0.02	0.16 ± 0.08	0.19 ± 0.03	4.82 ± 0.62	21.38 ± 0.06
Slependrenna	0.08 ± 0.03	1.33 ± 1.74	1.20 ± 1.52	7.15 ± 3.16	21.38 ± 0.09