Arctic Biology Field Course

Qeqertarsuaq 2019

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Preface

Nothing compares to one's own unfiltered experience in real-time and on-site! This is precisely what the Arctic Biology Field Course offers to the students – and it leaves an everlasting impression.

For exactly that reason, the purpose of the Arctic Biology Field course at Arctic Station is to provide the students with an authentic first-hand experience of the arctic environment and how it shapes the terrestrial and aquatic ecosystems. Through performing their own research projects, the students get a detailed understanding of the organization of biological structures and how they are influenced by environmental factors. Furthermore, the whole process of defining the research questions, setting up the experimental work, doing the field work, analysing the data and not least writing a comprehensive scientific report gives the students very important skills.

The Arctic Station itself motivates because of its optimal teaching and research conditions and because the field sites are just a short walk or a short sail-trip away. Even just stepping outside the doors provides stunning views of foraging whales in the bay, a goose family walking by or flowering plants. Altogether, it leads to a genuine understanding of how unique the arctic ecosystem is, but also how sensitive to disturbances.

Our Arctic Biology Field course in 2019 took place from 7th to 18th July. The overall theme for the course was to study responses of aquatic ecosystems to environmental conditions including human impact and global warming. Two projects studied marine phytoplankton, one project focused on algae on glacier snow and ice, one project studied antibacterial capacity of freshwater and marine phytoplankton and one project was dedicated to aquatic plants in ponds and lakes.

The students were well prepared and had everything in place so they could initiate their field work almost immediately upon arrival. Still, ad hoc modifications of methods, sites and time schedules had to be made but this is indeed also an important part of performing research. By the end of the 10th day all groups had a nice dataset for their reports

The specific subjects for group projects were:

- A screening for antimicrobial properties of microalgae in Arctic marine- and freshwater environments
- Macrophyte biomass and production in arctic lakes and ponds in West Greenland
- Biovolume of snow algae in different glacial habitats at Lyngmarksbræen, Disko; and the effect of red snow on snow surface albedo
- Picoplankton distribution in Disko Fjord and Disko Bay (West Greenland)
- Phytoplankton community composition and abundance along a salinity gradient in Disko Fjord

Besides the strong focus on the student projects, we allowed time for excursions to Østerlien and Kuanit as well as a guided walk through the entire village. We were invited twice by local people for the famous Greenlandic "kaffemik" and enjoyed the fantastic food and cakes. Some of us even went to the Sunday church ceremony and a village stroll afterwards. Furthermore, we had lectures about Arctic Station and the long-term monitoring programmes by the scientific leader Martin Nielsen and about toxic marine phytoplankton by Nina Lundholm.

Overall, the course was very successful and enjoyable. We appreciated the interactions with other guests at the station and not least the high service-level provided by two station managers as well as the crew on board Porsild. We wish to thank them all for their support.
The course would not have been possible without support from The Faculty of Science and Dept. of Biology which supports the course economically.

The findings and conclusions of the student projects are published in this report "Arctic Biology Field Course - Qeqertarsuaq 2019" (ISBN: 978-87-89143-24-8). The report can be obtained as PDF from the Arctic Station website (http://arktiskstation.ku.dk/english/) or from Kirsten S. Christoffersen (kchristoffersen@bio.ku.dk).

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14. Maria Wagner Jørgensen

Course diary

Day 1

Today was our first full day in Greenland, and what a day!
We are now accommodated at the historical Arctic Station, which dates back more than 100 years. To our pleasant surprise the weather here has surpassed all expectations, and it has already been discussed among the group, if we brought too much clothes.

We woke up to the sun and blue skies, which was perfect for our guided tour around the town of Qeqertarsuaq, or Godhavn in Danish. The tour was led by Kirsten and Nina, who took us around the two harbors, the church, the dump site and much more. Before our tour we had a quick lesson in Greenlandic culture, including the mandatory hello ("Aluu") when meeting people on the street. As we were walking down the street, we crossed the house of the Station Manager, Akaaraq and his wife, Nalu, who invited us in for "kaffemik" (a social gathering involving coffee and a lot of cake). Akaaraq and Nalu were kind enough to show us around their house, as well as, showing us the traditional Greenlandic outfit, a Narwhal tooth and the skull and horns of a musk ox.

Later in the day the different marine and freshwater projects were initiated. Most of the people doing marine projects boarded the research ship, Porsild and sailed to Disko Bay. Most of the freshwater groups stayed at the Arctic Station to coordinate the days ahead, while one group ventured up the mountain side behind the station, to look for aquatic plants within its streams.

The day is now at an end, and we sit in the iconic living room, glancing at the Disko Bay, where we yesterday spotted one whale after another. Now it's time to go to bed, as another exciting day awaits tomorrow.

Simone & Jonas

Day 2

We started the day with our standard morning meeting after breakfast and then a lovely hike (guided by Kirsten and Nina) in the area around the station. We went to Østerlien, Blæsedalen and Moræneso (a lake). It was such a fine day with blue skies, yet still a bit windy, so we weren't eaten alive by mosquitoes!

After lunch each group worked on their individual projects. Some went further hiking up to Lyngmarksbræen to sample for red snow algae. This is also known as the beautiful phenomenon ''Watermelon snow'' which exhibits a bright red color on the top of the snow.Others did lab work, with a gorgeous view of icebergs and whales. Lucky as can be, we just celebrated being finished with work, by having cake (as every scientist thrives after). Now we will go glance over the Disko Bay, with a beer in one hand and a pair of binoculars in the other.

Camilla & Cecilie



Day 3

We had to get up early, as the marine groups had to go onboard Porsild (the research vessel) for a trip to Mellemfjord, which is a four-hour trip one way. Due to currents, wind and foggy weather conditions the return is delayed by 3 hours and is expected to around 11 pm tonight, but a least with a ship full of water samples.

One of the freshwater groups went looking at ponds behind the station, to characterize the aquatic plant abundance and growth. The ponds were mainly filled with mosses, but we also found Common Mare's-tail (*Hippuris vulgaris*) and Slender-leaved Pondweed (*Stuckenia filiformis*).
The weather today has been very foggy, which led to perfect fishing conditions, resulting in 5 fish caught within half an hour: 3 Uvak (*Gadus ogac*) and 2 Sculpin (*Myoxocephalus* sp.). We also witness a huge iceberg fall apart right in front of our eyes! What a day!

Jonas

Day 4

Today, we started a bit later than usual due to the long boat-trip the day/night before. After breakfast, the snow algae group hiked to Lyngmarksbræen to take more samples of the red snow and to place sensors on the glacier to measure diurnal changes in temperature and light. Meanwhile at the station, the freshwater group collected mosses and aquatic plants at several nearby lakes and ponds. Lots of filtering and looking at algae in the microscope for the marine groups. After dinner, some went for an evening swim in the bay and more filtering was on the agenda for the marine groups. Soon it will be beer o'clock time and an early good night for a lot of us.

Helena & Camilla

Day 5

A day all about lab work. Some groups were counting snow algae while others measured annual buds on mosses to determine the yearly growth. Another group collected water samples from two different lakes (Moræne sø and Thygesens sø) and went back to the lab to filter the water to start phytoplankton cultures.

In the evening we observed a humpback whale eating close to shore. Besides we had a lecture about toxic diatoms and their interactions with copepods.

Julie & Katrine

Day 6

The marine group had to get started rather early as they were going onboard Porsild once again, to sample from a transect with the starting point at "Permanent Station" (the site where the Arctic Station marine monitoring program are sampling every month). On the way out, we were so lucky that a few humpback whales where foraging just 40 m away from us. It´s amazing to get so close.

The sampling went great, and we had a lot of fun with the staff at Porsild. When arriving at home around 18:00, all water samples were examined in microscopes, and filtration of the water had to be conducted right after dinner and lasted until the early hours of the upcoming day. Most people spent time in the lab this evening. Unfortunately, some people at the station (it is fully booked) are coming down with flu like symptoms. Fortunately, everyone was being nice and supportive and the unlucky few worked as much as they could in their conditions.

Cecilie & Ellen

Day 7

Yesterday, half the groups went to church in the morning, to witness a true Greenlandic service. Unfortunately, no extraordinary events were happening, so they didn't get to see them in their traditional clothing, however, it was quite impressive, nonetheless. After lunch, some went on a walk to the beautiful destination Kuannit, a spot along the coast were volcanic rock formations (basalt) are exposed and the slopes are filled with lush shrubs. One very large plant, kvan (*Angelica archangelica)*, was fond in massive stands. This plant was used by pioneers and locals to palliate the scarcity of vitamin rich food. Today it is still highly utilized by local people because of its sweet and rather special taste. Later, some went to Kaffemik at the Porsild's captain Eric's 65th birthday, where they got to taste whale meat among other delicacies. Dried narwhal, raw beluga skin and blubber were served on a buffet, as well as marinated fish (the one eaten by humpback whales) and usual sweets and cakes. In the afternoon and the evening, all the teams were in the lab analyzing their samples. Some were using microscopy to quantify phytoplankton diversity, some were counting red snow algae and others were using dry weighted mosses to investigate the productivity of the sampled mosses from ponds behind the station. The day ended with "hygge" and (more) cake.

Rafael & Freja

Day 8

This day started out with the usual morning meeting, followed by a wonderful view of the humpback whales hunting and feeding, right outside our windows. Most people spent their time in the lab; counting algae and sorting mosses were the main activities. We had wonderful weather and an amazing view from lab over the sea, with countless icebergs as well as whales swimming by once in a while. Hours in the lab go by very fast, so all of a sudden, dinner was served. After dinner we had a lecture from Martin Nielsen, the Scientific Leader of Arctic Station, who told us of himself, his projects and all of the monitoring programs going on in the area, and their importance. After the lecture, some people went to the lab again, others played cards, and finally, a cake was served. The day ended with a view over the sea and again watching the whales feeding (we are kind of obsessed with them).

Cecilie

Day 9

The day started with breakfast prepared by Julie and Kirsten. Some had a morning meeting at 8:30 to



discuss final experiments and analyses that needed to be made. Most of us went to see Akaaraq to buy Arctic Station merchandise, including t-shirts, caps, books and mugs with the Arctic Station logo on.

Two groups sailed to Fortuna Bay with the research vessel Porsild, where one group searched for aquatic plants in two lakes close to the shore while the other group went fishing for Arctic Charr. The rest of the day, the groups prepared their project presentations for the evening session. After dinner, each group presented their work and the results they had managed to produce so far. While most of us had a lot more to do with regard to data analysis, it was interesting to see how much could be done in so few days of field and lab work, and the challenges each group had faced and how they solved and/or adapted to the situation. After the presentations, we discussed how the report should end up with regard to content and format. Later we had coffee and cake, and some went down to the beach to enjoy the view… with a beer of course.

Jonas

Day 10

Today was our last day on the Arctic station, as we have to leave early tomorrow morning; we need to finish off this incredible stay!

The day started as every other day, with breakfast but no morning meeting and no one was heading to the field today. Instead we all had to finished analyses and then to clean up the equipment we had used the last many days, pack our own stuff and get last minute things done. While cleaning up actually was a quick business, many spent the day doing hikes, walking to the store or just relaxing and breathing in the last of Qeqertarsuaq.

In the afternoon we had planned a football match against the local womens team, but unfortunately no one showed up, so we ended up playing a bit around ourselves, until we got distracted by a bunch of whales just 50 m from the shore. We celebrated the win in high fashion with an extravagance meal prepared by Kirsten and Nina. The menu was snow-crabs with green salads, freshly made bread and homemade kvan (*Angelica archangelica*) and garlic butter. Everyone was invited including the captain and sailors from Porsild, Akaaraq & Nalu and Martin & Birgitte. One of the sailor's wife had brought her homemade traditional pearl necklaces, for everyone to buy. All was sold out in no time. We ended the evening with nice kaffemik in our living room. Tomorrow we have to get up early to catch the morning ferry. We are going to Ilulissat and most of us have planned for various activities and other places to see. For all of us, this is not a goodbye to Greenland; it's a see you later!

Jonas



A screening for antimicrobial properties of microalgae in Arctic marine- and freshwater environments

Collecting water samples in the field (Photo: Katrine J. Nielsen)

A screening for antimicrobial properties of microalgae in Arctic marine- and freshwater environments

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Abstract

Microalgae represent a natural reservoir of bioactive compounds with a potential for having antimicrobial properties. The global dispersal of antibiotic resistance creates an interest in developing alternative treatments and finding novel sources of antimicrobial agents. Several studies have successfully extracted compounds produced by algae with antibacterial effect, which may be of relevance in the medical industry.

This study examines microalgae and their derivatives from Arctic marine and freshwater environments as a possible reservoir for inhibitory components against bacteria. Sampling were executed in/or nearby Qeqertarsuaq and in Disko Fjord off the west coast of Greenland. The water samples were filtered and divided into two cell size-fractions (3 µm-20 µm; 20 µm-50 µm) and the algae were cultivated for three days. Subsequently, all samples were divided into supernatants and pellets, to examine extra- and intracellular compounds. The samples were inspected in a well diffusion assay investigating the inhibitory effect of diffusible compounds. Samples showing antimicrobial potential were further analyzed in a microplate growth inhibition assay.

In the well diffusion assay, both marine and freshwater samples displayed inhibition potential against *Escherichia coli,* visualized as clearing zones. Significant differences of clearing zones and size-fractions or components were found at three and one location, respectively. No significant correlation was found between location and clearing zone. Samples from one location, out of six, were significantly different from the respective controls. However, in the microtiter growth inhibition assay, samples from same location showed no inhibition potential. No precise conclusion can be achieved regarding the inhibition potential of Arctic algal species given the outcome of this screening.

Keywords: *antimicrobial properties, antibiotic activity, Arctic microalgae, Escherichia coli, Bacillus cereus.*

Introduction

The impact of antibiotic resistance on human health makes the scientific community search for new possible treatments and compounds with antimicrobial effects (Blaskovich et al. 2018). Microalgae have been of huge interest in the search for antimicrobials, given their biodiversity, short generation time and their relatively simple growth needs (Falaise et al.; Pérez et al. 2016). Since 1944, when Pratt et al. (1944) discovered antimicrobial activity of the green algae *Chlorella* targeting both Gram-positive and Gram-negative bacteria, numerous studies have proven effective in finding antimicrobial compounds produced by algae (Pratt et al. 1944; Bhagavathy et al. 2011; Mudimu et al. 2014; Pane et al. 2015). These studies emphasize the possibility of finding new antimicrobial agents produced by algae all over the world.

Antibacterial agents

In order to treat diseases caused by bacteria, antibiotics are used to eradicate infectious species or to inhibit their growth within a host. The antibacterial agents have several modes of action including inhibition of cell wall synthesis, protein synthesis, nucleic acid replication and translation, or damage of the

bacterial plasma membrane (Madigan et al. 2015).

Natural compounds derived from microorganisms, such as bacteria and fungi, are the main sources of antibiotics (Bérdy 2005; Wohlleben et al. 2016). Bacterial and fungal secondary metabolites with antimicrobial activity are usually produced during the stationary phase, as a product of defense when nutrient levels are low (Bérdy 2005; Madigan et al. 2015). Additionally, several studies have discovered algal derivatives to be a potential source of antimicrobial agents (Leflaive and Ten-Hage 2007; Bhagavathy et al. 2011; Mudimu et al. 2014; Pane et al. 2015). Microalgae are known to synthesize a variety of secondary metabolites, including allelopathic compounds and toxins. Allelopathic compounds can vary in function from inhibition of photosynthesis to cellular paralysis. Whereas toxins often cause inhibition of enzymes or interference with membrane receptors of the cell (Leflaive and Ten-Hage 2007). Algal primary metabolites such as proteins and vitamins have displayed antimicrobial effects against human pathogens as well (de Jesus Raposo et al. 2013; Falaise et al. 2016). Additionally, fatty acids (saturated and unsaturated), exopolysaccharides and pigments such as chlorophyll and β-carotene have all shown inhibitory effect against bacteria. The potency of these components does, however, vary depending on the concentration of the extracts, the extraction method and the solvent (Bhagavathy et al. 2011; Falaise et al. 2016).

Antibiotic resistance

In parallel with the development of new antibiotics and the following human consumption, and thus selective pressure, bacteria have evolved multiple resistance mechanisms to combat the antimicrobial agents and their mode of action. Mechanisms of drug resistance involves modification and inactivation of the drug, alteration of the target enzyme, exclusion of the drug by using specific- or multidrug-resistance efflux pumps, among others (Wilson et al. 2011). Useful genes which improve survival during selective pressure may be passed vertically or arise spontaneously by genetic mutations. Apart from transmitting genes encoding resistance vertically, bacterial species in close proximity also have the opportunity to transfer mobile genetic elements by horizontal gene transfer (HGT) (Munita et al. 2016).

As increased human consumption of antimicrobial agents selects for resistance among bacteria, multiresistant species have evolved. A species of concern includes *Escherichia coli* which, besides being part of the intestinal microbiome, can appear as a human pathogen during certain circumstances. Many strains of *E. coli* may cause a variety of enteric diseases or extra-intestinal infections (Kaper et al. 2004; Poirel et al. 2018). Another species showing resistance towards a highly important class of antibiotics (β-lactams) include *Bacillus cereus* which mainly is associated with food poisoning (Bottone 2010).

The emergence of multiresistant bacterial strains causes global concern because treatment options have become limited and inefficient. Currently even simple infections may be fatal especially to immunocompromised patients (Hughes and Karlén 2014; Farha and Brown 2015). This have generated a renewed interest in the development processes of alternative treatments and sources of antimicrobials, among others the utilization of algae derivatives.

Arctic algae

It has been estimated that >1700 freshwater and terrestrial algal species are found in the Arctic and >2300 algal species are found in Arctic marine waters. In low Arctic regions, the majority of freshwater phytoplankton consists of different species within green algae, chrysophytes, diatoms and cyanobacteria (CAFF 2013). The diversity abundance of planktonic eukaryotes in Arctic marine waters is dependent on the location and the identification process. High throughput sequencing highlights

dinoflagellates, ciliates and cryptophytes as the dominating groups, whereas identification through microscopy highlights diatoms as the major group (CAFF 2013).

In order to survive the extreme competitive environments in the Arctic, microalgae have evolved several defense strategies to cope with bacteria, fungi and viruses. The defensive compounds often include various metabolites (Falaise et al. 2016).

Species of green algae, diatoms, chrysophytes and cyanobacteria have all shown to possess antimicrobial potential (Qin et al. 2013; (Seraspe et al. 2012; Tuney et al. 2007; (Ghasemi et al. 2007; Falaise et al. 2016). These algal groups are in particular represented in Arctic marine and freshwaters (CAFF 2013). Several studies investigating algal species have used different extraction methods and solvents to examine the antibacterial potential of algal derivatives.

Marine: extracts derived from *Thalassiosira* sp. have been inhibiting the growth of both *Staphylococcus aureus* and *Bacillus pumilus*. Extracts were from continuously grown algal cultures. Different extraction techniques differed in efficacy, with sonication as the most effective method (Qin et al. 2013). Another study investigating extracts of *Chaetoceros* sp. showed antibacterial properties against human pathogens such as

Figure 1. Map showing the six different sampling locations in Disko Fjord and on mainland. The map was constructed using Google Maps.

S. aureus, B. subtilis and *E. coli.* The most effective antibacterial activity was obtained when using methanol as extraction method and hexane as the solvent. When extracts were diluted in aqueous suspensions, no antimicrobial activity were achieved, which indicate that the bioactive compounds were non-polar (Seraspe et al. 2012). Tuney et al. (2007) discovered *in vitro* antibacterial activity of algal bulk samples containing *Navicula* sp., *Thalassiosira* sp., *Melosira* sp., *Pseudo-nitzschia* sp., *Ceratium* sp., *Chaetoceros* sp. and others when using different organic compounds in the extraction method such as methanol, acetone, ethanol and diethyl ether. Diethyl ether displayed improved inhibition potential of the extracts and especially against Gram-positive bacteria (Tuney et al. 2007).

Freshwater: species in green algal genera including *Chlamydomonas* and *Scenedesmus* have been effective in producing antimicrobial substances targeting a variety of human pathogens. In fact, both the supernatants and the methanol intracellular extracts from the algal cultures, grown in 15 days, have displayed antimicrobial activity (Ghasemi et al. 2007).

These results indicate a potential source of new antimicrobials within Arctic marine - and freshwater algae.

The hypothesis of this study claims that Arctic microalgae within marine and freshwater environments have antimicrobial properties. One may anticipate dissimilar antimicrobial potential between intra- and extracellular compounds produced by algae and between different size-fractions of algal species. Based on previous studies finding numerous algal species producing antimicrobial compounds (Pratt et al. 1944; Ghasemi et al. 2007; Tuney et al. 2007; Seraspe et al. 2012; Qin et al. 2013), samples containing microalgae were divided into two size-fractions (3 µm-20 µm; 20 µm-50 µm).

This study aimed to investigate whether antimicrobial activity could be achieved from raw microalgal cultures, sampled at different

locations within and around the Disko Island, West Greenland.

Methods and materials

Sampling was performed in three lakes at Disko Island and at three locations in Disko Fjord, at the west coast of Greenland. The three lakes were located in or nearby Qeqertarsuaq, whereas the marine samples were collected from Disko Fjord at three different distances from a glacial outlet (Figure 1).

Sampling and filtering

Collection of water samples were done by compiling 10 L of water from six different locations; Moræne sø (69°16'11.5"N 53°28'26.9"W), Thygesen sø (69°14'41.1"N 53°32'21.9"W), Stationssøen (69°15'06.7"N 53°31'06.7"W) and from three different locations in Disko Fjord (Marine L1:69° 32'24.4"N 53°33'29.1"W, Marine L2:69° 27'29.6" N 53°42'17.3" W Marine L3:69° 28'14.2"N 54°06'03.0"W).

Marine samples were collected at 30 m depth due to higher chlorophyll concentrations (suppl. Figure 1). Freshwater samples were collected approximately 10 cm below the surface to reach the upper photic zone where most pelagic primary production occurs. Thus, avoiding the assemblage of surface water (Fluet-Chouinard et al. 2016).

The water samples were divided into pseudo-triplicates and were filtered using 50 μm filters.

The water samples were first filtered through a 20 μm filter and next, through a 3 μm polycarbonate filter (Whatman® Nuclepore™ Track-Etched Membranes). When 50 mL was left in the funnel, the water containing particles >3 μm and <20 μm were transferred into 250 mL Nunc flasks using plastic pipettes. 100 mL growth media were added to the suspensions (cf. 2.2). Particles accumulating on the 20 μm filters, thus in size range >20 μm and <50 μm, were backwashed with 100 mL of growth media and collected in 250 mL Nunc flasks. The sampling resulted in bulk algal samples containing cells in two size fractions: 3 μm-20 μm and 20 μm-50 μm.

Controls

Filtered marine- and freshwater were used as controls. Water samples from each freshwater location and one marine location were collected in triplicates and processed using 0.2 μm or 0.8 μm filters (both Whatman® Nuclepore™ Track-Etched Membranes). Filtration through 0.2 μm were used to avoid most bacterial cells, whereas the 0.8 μm filter contained small bacterial cells. This resulted in three control groups: water samples containing growth media filtered using 0.8 μm filters and 0.2 μm filters, and water samples consisting of 0.2 μm filtered water.

Culturing of algae

Due to inefficient filtration equipment, 0.8 μm filtered water were used to prepare growth media instead of 0.2 μm water. Growth media were added to the bulk cultures. 1 L freshwater growth media were composed of 0.8 μm filtered freshwater along with 69 mL of Bold 1NV medium, which among others consists of biotin- and thiamine vitamin solutions (suppl. Table 1). pH was adjusted to a value between 5.5 and 6.5 in the suspensions before further use.

1 L marine growth media were prepared by using 0.8 μm filtered marine water along with L1-medium which among others consists of micronutrients and vitamin solutions (Guillard and Hargraves 1993).

All samples were stored in a refrigerated incubator at 9 °C with a light intensity of 165 μmol photons m^{-2} s^{-1}, to imitate natural conditions. Due to limited equipment in the experimental setup, light intensity and temperature were adjusted to obtain growth conditions suitable for both fresh- and marine algal cultures. The suspensions were shaken twice a day to enable aeration of the cultures thus decreasing the pH, and incubated for minimum three days. The incubation periods were limited to three days due to lack of time.

Identification
Samples were examined in a Sedgewick rafter counting cell using a light microscope. Morphological characteristics were used for identification to microalgal genus level.

Preparation of bacterial cultures
Stock cultures of *Escherichia coli* SP548 and *Bacillus cereus* SP1236 were obtained from the Section of Microbiology at University of Copenhagen. Prior to the experiment the susceptibility towards different antibiotics were tested, to avoid antibiotic resistance and misleading outcomes of the experiment. In order to prepare overnight cultures a scrap of the frozen bacterial stocks was taken using an inoculation needle, and were subsequently immersed into 5 mL liquid LB media. The cultures were incubated at 37°C for 12 hours and 100 µL were poured onto solid LB agar medium to create a bacterial lawn. To inspect for antibiotic sensitivity several antibiotics having different targets were being tested in a well diffusion assay. Following antibiotics were tested; Ampicillin (100 µg ml^{-1}), tetracycline (20 µg ml^{-1}) and kanamycin (50 µg ml^{-1}).

Both strains showed sensitivity against tetracycline with the greatest clearing zone, and were thus used as a control group in future analysis.

Algal extracts
Algal extracts were subdivided into supernatant suspensions and pellet derivatives.

Supernatants and pellets were prepared with inspiration from the study of Mudimu et al. (2014). All suspensions were concentrated due to low cell densities. The bulk cultures were poured into 15 mL Falcon tubes and centrifuged at 1343 G (using Heraeus Multifuge 3S) in 5 min to facilitate stratification. 2 mL of the supernatants were pipetted into 2 mL eppendorf tubes and pellets were transferred with a pipette into 1.5 mL eppendorf tubes. Supernatants and pellets were stored at -20°C until further examination. Freezing were used as the extraction method of cells present in the pellet.

Well diffusion assay
Well diffusion assays were performed to screen for diffusible compounds with antimicrobial activity (Balouiri et al. 2016). Antimicrobial activity of algal derivatives, that diffuses into the agar medium and inhibits the growth of the bacterial species of interest, were quantified by observing clearing zones.

On separate LB agar plates aliquots of 100 µL of overnight culture containing the bacterial isolate were inoculated to create bacterial lawns. Subsequently, three wells in each plate were created by using a vacuum pump. The tube of the vacuum pump was soaked in ethanol and held over a Bunsen burner, to sterilize the tube before use. Aliquots of 70 µL of thawed pellet diluted in 600 µl phosphate-buffered saline (PBS), undiluted supernatant or control samples were poured into separate wells. PBS was used as solvent due to its non-toxic effects on bacterial cultures (Liao et al. 2003). Different dilutions of tetracycline were prepared and used as reference (40 µg ml^{-1}, 20 µg ml^{-1}, 10 µg ml^{-1}, 5 µg ml^{-1} and 2.5 µg ml^{-1}).

The plates were incubated at 24°C for 24 hours and determination of inhibition was identified by clearing zones. When determining the inhibition capacity of algae derivatives, clearing zones were measured with a ruler.

Microplate growth inhibition assay
Samples showing effective inhibition of *E. coli*, were further examined in a microplate growth inhibition assay. Dilutions of *E. coli* were being tested to examine the inhibition capacity of the minimum concentration of *E. coli*. Visible clearance of wells was qualified as inhibition of bacterial growth.

The microplate growth inhibition assays were carried out using 96-well microtiter plates. Each well contained 120 µl of suspension in total. The grouping was as follows: overnight cultures of *E. coli* in dilutions: $5 \cdot 10^5$, $5 \cdot 10^4$,

$5{\cdot}10^3$ and $5{\cdot}10^2$, were mixed with liquid LB broth, tetracycline or algae derivatives in a 3:1 ratio. Liquid LB medium, PBS and tetracycline in concentrations 40 µg ml^{-1}, 20 µg ml^{-1}, 10 µg ml^{-1}, 5 µg ml^{-1} and 2.5 µg ml^{-1} were used as controls. The samples were incubated at 37 °C for 24 hours, before being examined.

Data analysis

Data analysis were performed using R studio (version 1.1.463; Rstudio Team 2016) and were executed on data obtained from the well diffusion assay containing *E. coli* SP548 (suppl. Figure 2).

One-way ANOVA tests were performed on samples within locations and their respective controls, to test if any significant difference were found between the mean of the clearing zones of the samples and their respective controls at a given location. Furthermore, a Tukey's honestly significant difference (HSD) test was performed on the marine data, to examine if any means of the samples differed significantly from the means of the controls, and to obtain the P-value of these comparisons. Samples were found to be significantly different from their respective controls at a given location using an α of 0.05 as a cut-off for significance.

One-way ANOVA tests were likewise performed on all means of samples containing pellet and supernatant within each location,

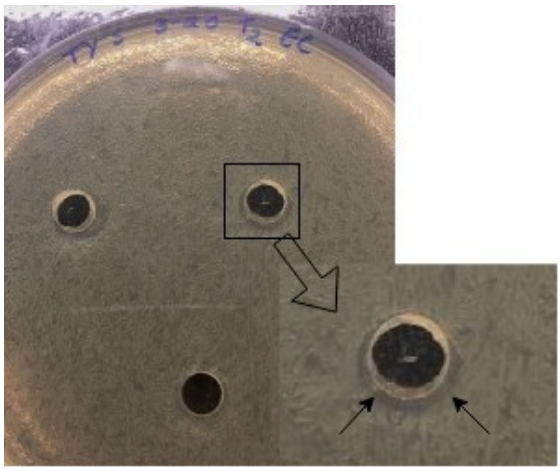

Figure 2. A result from the well diffusion essay. The derivatives were from Thygesen sø containing the supernatant from the size fraction 3-20 µm, triplicate no. 2. Visible clearing zones were observed around each well.

and on all means of samples containing cells in the size-fraction 3-20 µm and 20-50 µm within each location. These ANOVA tests were performed to examine if any significant difference was found between sample compound and clearing zone and size fraction and clearing zone, respectively.

A one-way ANOVA test was furthermore used to examine if any correlation was found between location and means of clearing zone.

Results

Algal composition

The lake bulk samples were in general dominated by diatoms and mobile small green algae. In the Moræne sø, green algae including *Scenedesmus* sp*., Pediastrum* sp*.* and diatoms such as *Asterionella* sp*., Melosira* sp., and *Tabellaria* sp. were prevalent. However, also pennate diatoms as *Synura* sp. and few cryptophytes were observed. Both Thygesen sø and the Stationssøen displayed similar patterns with regard to prevalent algal groups and genera. Nevertheless, pennate diatoms as *Navicula* sp*.*, and *Gomphonema* sp., were frequent in Thygesen sø, whereas *Chlamydomonas* sp*.* and *Anabaena sp.* were frequent in the Stationssøen.

Samples collected from marine waters were homogeneous in genus composition and dominated by *Thalassiosira* sp. and *Chaetoceros* sp. and different dinoflagellates*.* Also, diatoms like *Leptocylindrus danicus*, *Leptocylindrus minimus* and *Pseudo-nitzschia* sp*.* were prevalent, and their dominance tended to increase with further distance from the glacial outlet in Disko Fjord. The salinity of marine samples at the different localities was as follows: Marine L1: 38, Marine L2: 35 and Marine L3: 36; whereas the salinity of all lakes was 0.

Well diffusion assay

All agar plates were examined for visual clearing zones around each well. Visible clearing zones were only obtained for *E. coli*, whereas no clearing zones were obtained for *B. cereus*, except wells containing tetracycline

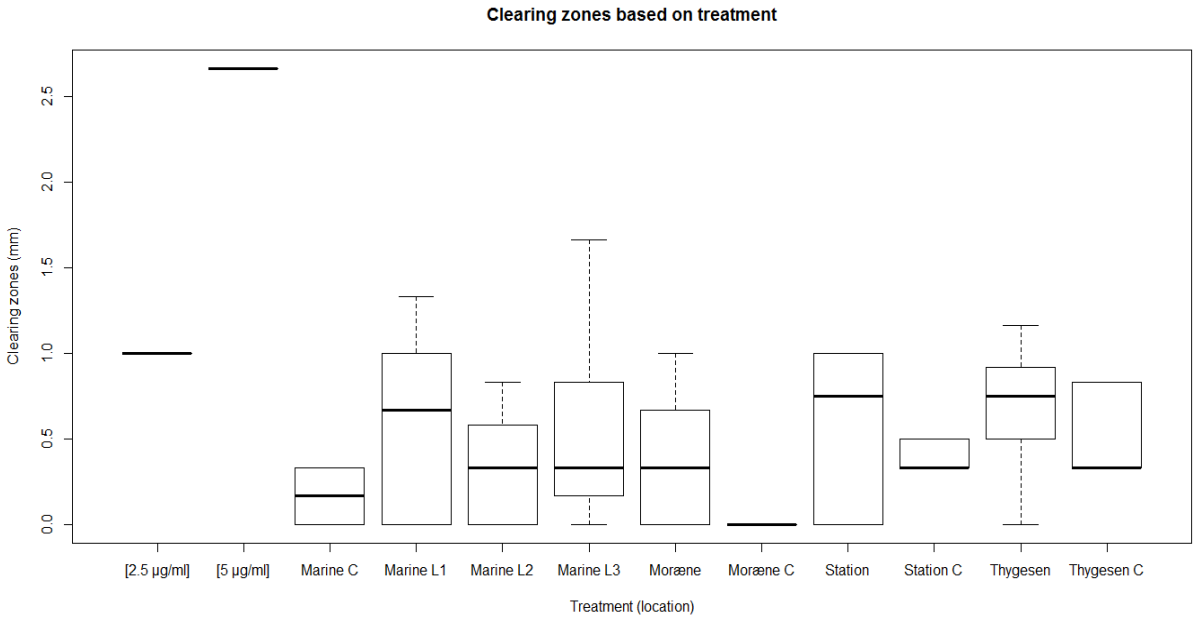

Figure 3. Means of clearing zones from different treatments, measured in mm, as a result of the well diffusion assay on *E. coli* SP548. Treatments, Stationssøen samples (Station), Stationssøen controls (Station C), marine location 1 samples (Marine L1), marine location 2 samples (Marine L2), marine location 3 samples (Marine L3), marine controls (Marine C), Moræne sø samples (Moræne), Moræne sø controls (Moræne C), Thygesen sø samples (Thygesen), Thygesen sø controls (Thygesen C), Tetracycline (2.5 µg ml^{-1}) and Tetracycline (5 µg ml^{-1}).

in the following concentrations; 40 µg ml^{-1}, 20 µg ml^{-1}, 10 µg ml^{-1} and 5 µg ml^{-1}. All visible clearing zones were measured with a ruler (Figure 2).

A boxplot of all clearing zones measured in mm based on each treatment, were created to visualize the results of the well diffusion assay, as seen in Figure 3. Marine L1, the Stationssøen and Thygesen sø showed to be the most effective against *E. coli* with an average mean clearing zone at approximately 0.6 mm. However, the average mean clearing zone of 0.6 mm indicates lower inhibition potential compared to the clearing zone produced by 2.5 µg ml^{-1} tetracycline. The remaining locations showed a reduced inhibitory effect with an average mean clearing zone at <0.5 mm. However, huge standard deviations emphasized in the difference and uncertainty in inhibition capacity among samples from the same location. Filtered water samples (Thygesen C, Moræne C, Station C and Marine C) and different tetracycline concentrations (2.5 µg ml^{-1} and 5 µg ml^{-1}) were used as standard of reference.

No significant difference were detected between the mean clearing zone of the marine samples (Marine L1, Marine L2, Marine L3) and the marine control (Marine C) as P-values > 0.05. The clearing zone mean of the Stationssøen samples were likewise not significantly different from the clearing zone means of the controls, with a P-value of 0.197. Also, the clearing zone mean of Thygesen sø samples were not significantly different from the clearing zone mean of the controls, with a P-value of 0.212 (P>0.05). However, the clearing zone mean of samples from the Moræne sø were significantly different from the controls. The P-value obtained was 0.0025 (P<0.05). All P-values are presented in Table 1.

To examine if any correlation was found between size-fraction or content (pellet or supernatant) and clearing zones ANOVA tests were performed using samples containing supernatants and pellet and samples within 3-20 µm and 20-50 µm, respectively.

At three locations, out of six, a significant difference in circumference of clearing zone was found between samples containing pellet and samples containing supernatant (suppl.

Table 2; suppl. Figure 4). Whereas one location out of six showed significantly different in means of clearing zones between samples within the size-fraction 3-20 µm and 20-50 µm (suppl. Table 3; suppl. Figure 5).

Despite the fact that the majority of all samples were not significantly different from their respective controls, an ANOVA test was performed to test whether the visible clearing zones depended on the location. The null hypothesis stated no correlation between the dimension of clearing zone and the location (H0; All means were equal). Whereas, the alternative hypothesis claimed a correlation between location and dimension of clearing zone (H1; All means were not equal). The ANOVA test resulted in a P-value of 0.327, which indicated no significantly difference between any of the samples. This result suggested no correlation between clearing zone and location, and one may thereby reject the alternative hypothesis.

In summary, the clearing zone means of samples from one location out of six were significantly different from its respective controls. Significant correlation between size-fraction or content of sample (pellet or supernatant) and clearing zone were found at some locations. Besides, no correlation between location and clearing zone were found.

Microplate growth inhibition assay

Triplicates showing inhibition potential against *E. coli* in the well diffusion assay were furthermore examined in the microplate growth inhibition assay (suppl. Figure 2). All control samples indicating antimicrobial potential against *E. coli* in the well diffusion assay were included in the microplate growth inhibition assay as well (suppl. Figure 2). Samples from both marine- and freshwater displayed inhibition potential against *E. coli.*

One sample from the Moræne sø containing 20 µm-50 µm cells revealed an inhibition potential of $5{\cdot}10^3$ *E. coli* bacterial cells mL^{-1}. Likewise, one sample from the Stationssøen containing 20 µm-50 µm cells showed inhibition potential against $5{\cdot}10^3$ bacterial cells mL^{-1}. However, some control samples from the latter location displayed similar tendencies.

Two samples from Thygesen sø comprising size-fractions 3 µm-20 µm pellet, and 20 µm-50 µm supernatant, showed inhibition potential against $5{\cdot}10^4$ cells mL^{-1} and $5{\cdot}10^3$ cells mL^{-1}, respectively. Several samples from Marine L1 and L2 showed inhibition potential of $5{\cdot}10^4$ bacterial cells mL^{-1}. Whereas no samples from Marine L3 displayed any inhibition potential (suppl. Figure 3).

In summary, results obtained from the well diffusion assay were somewhat inconsistent with results obtained from the microplate growth inhibition assay. The highest inhibition capacity observed in the microplate growth inhibition assay was $5{\cdot}10^3$ bacterial *E. coli* cells mL^{-1}.

Table 1. Table visualizing the P-values from each location and its respective control. * represent a significant difference between the mean of clearing zones of Moræne and its respective control, Moræne C.

Location	P-value
Marine C-Marine L1	0.0668
Marine C-Marine L2	0.7873
Marine C-Marine L3	0.1626
Station C-Station	0.1972
Moræne C-Moræne	0.0026*
Thygesen C-Thygesen	0.2123

Discussion

Despite the fact that several samples induced clearing zones indicating inhibition potential in the well diffusion assay, many samples were not significantly different from their respective controls in clearing zone circumference. Only one locality, the Moræne sø, was significantly different from its respective control group in the well diffusion assay. Nevertheless, samples from the same location did not exhibit any sign of inhibitory

activity against *E. coli* SP548 in the microplate growth inhibition assay. Likewise, the ANOVA test did not detect any significant differences among locations in the well diffusion assay, indicating no correlation between location and clearing zone. Additionally, no consistent result was obtained concerning correlation between size-fraction or compound and clearing zone in all samples.

In overall terms, the inhibition potential of algal cultures examined were difficult to determine due to contradictory results and control groups implying antibacterial effect in the two assays.

No inhibition potential of *B. cereus* SP1236 was observed in the well diffusion assay. Resistance mechanisms such as exclusion of algal components by using specific or multidrug-resistance efflux pumps could be one explanation for the obtained results.

Several controls collected from the locations showed inhibition potential against *E. coli* in both the well diffusion assay and in the microplate growth inhibition assay.

The different inhibitory effects displayed by control samples and replicates, may be due to different chemical and physical conditions during cultivation, such as dissimilar light intensities or temperatures. Distinct mixing patterns of the suspensions and thus sedimentation of algae may likewise have contributed to the varying results, because the sedimentation process of algae increases the pH due to an altered CO_2/HCO_3- balance when CO_2 gets limited (Lavens and Sorgeloos 1996). As a consequence of different chemical and physical conditions during cultivation, the algal biomass were probably dissimilar in each replicate thus showing different inhibition potential.

Several collected algal species, including *Chlamydomonas* sp., *Scenedesmus* sp., *Thalassiosira* sp. and *Chaetoceros* sp. have previously shown effective in inhibiting growth of various bacterial species (cf. 1.3). A consideration of interest could be to cultivate the algal cultures for longer time e.g. 15 days as Ghasemi et al. (2007), and thereby observe if the cultivation period are of importance concerning the concentration of bioactive compounds. It would likewise be interesting to culture the algae under stress conditions, such as nutrient depletion. Thereby initiate stationary phase and potentially induce the production of bioactive compounds with antimicrobial activity, as a product of defense (Wohlleben et al. 2016). Exposing the algae cultures to decreased light intensity may as well induce distinct behavior.

Additionally, algal cultures could be exposed to bacterial components and one could observe if the production of antibacterial substances were induced.

Extracts of *Chaetoceros* sp. dissolved in aqueous solutions displayed no antibacterial potential in the study by Seraspe et al. (2012). However, when using hexane as the solvent the authors observed that the extract had antimicrobial effect. In this study, pellets samples were diluted with PBS. Nevertheless, it would have been interesting to use hexane as solvent and investigate whether the solvent had an effect on the outcome. However, the natural bactericidal effect of hexane must be taken into account when preparing the samples (Aono et al. 1994).

Previous studies have used methanol as an effective method of extracting antimicrobial compounds from algal cultures (Ghasemi et al. 2007; Seraspe et al. 2012). Methanol extraction could have been a method of interest. However, the natural inhibition potential of methanol on bacterial cultures had to be considered (Wadhwani et al. 2009).

A study by Qin et al. (2013) proved sonication as the most effective method for extracting antimicrobial compounds produced by algae. Thus, it would have been interesting to use sonication as the extraction method and investigate if different results would have been obtained.

One study observed antimicrobial properties using supernatants obtained from cultures of *Chlamydomonas* sp. and *Scenedesmus* sp.

(Ghasemi et al. 2007). Despite the fact that species of *Chlamydomonas* and *Scenedesmus* were present in the examined algal cultures, no significant antibacterial activity were observed, which may be due to the suspensions being too dilute.

In addition to the extraction methods and the use of solvents the assays had certain disadvantages as well.

Among others, the well diffusion assay was dividing the samples within rough categories, using a lawn of an undefined concentration of bacteria. Additionally, clearing zones were not that accurate since measured with a ruler. However, the method was simple and had low cost.

The microplate growth inhibition assay was affected by manual undertaking and accuracy. The results were influenced by this approach due to the risk of errors during the preparation of solutions and while pipetting. Additionally, the results were determined using the unaided eye and thereby risking neglecting growth in the wells (Balouiri et al. 2016). Alternatively, one could have quantified the bacterial growth in the wells by using spectrophotometry.

Likewise, the microplate growth inhibition assay had a low cost and was relatively quick and simple. The method has high throughput potential regarding the ability to test excessive quantities of microorganisms and antimicrobial agents simultaneously.

Both assays were selected in the experimental progress due to the fast generation of results.

Another limitation of the study includes the unknown algal dry/wet weight of each sample, which could have contributed with information about the algae biomass and thereby the required concentration necessary for inhibition of bacterial species. However, the dry weight would include detritus and organic matter which may imply a misleading estimate of algae quantity in each sample. Likewise, the algal biomass could be quantified by manual counting or by measuring the chlorophyll content.

This study can serve as a pilot study screening for Arctic algae in marine- and freshwater environments displaying antibacterial activity. The composition of Arctic marine algae is known to be dominated by diatoms, when using microscopy as the identification method, whereas the algae composition in Arctic freshwater environments are dominated by green algae, chrysophytes, diatoms and cyanobacteria (CAFF 2013). The freshwater and marine samples in this study, contained a variety of algae dominating in these environments which emphasize the relevance of the screening.

Concluding remarks

Arctic algal cultures in marine and freshwater environments demonstrated inconsistent antibacterial properties during the screening. Significant difference between size-fractions or content of sample (pellet or supernatant) and clearing zone circumference were found at three and one location, respectively. No location mean of clearing zone were significantly different from one another in the well diffusion assay, which indicate no correlation between location and antibacterial properties. One location out of six was significantly different from its respective control. Nevertheless, the tested samples from this location did not show any inhibition potential in the microplate growth inhibition assay. No conclusion can be obtained regarding the inhibitory properties of the algal cultures given the outcome of this screening.

By using other solvents, size-fractions or extraction methods, different results may have been obtained.

Despite the outcome, previous studies have demonstrated antibacterial activity among algae present in Arctic waters. This emphasizes the potential of finding antibacterial properties of Arctic algae in future research.

Acknowledgement

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Appendix

Supplementary Table 1: Recipe for 1 L total Bold 1NV medium.

To approximately 900 mL of dH20, add each of the components in the order specified (except vitamins) while stirring continuously. Bring the total volume to 1L with dH20.
For 1.5 % agar medium add 15 g of agar into the flask; do not mix. Cover and autoclave medium When cooled add vitamins (For agar medium add vitamins, mix, and dispense before agar solidifies). Store at refrigerator temperature.

No.	Component	Amount	Stock solution (conc.)	Final conc.
1	$NaNO_3$ (Fisher BP360-500)	10 mL/L	10 g/400 mL d H_2O	2.94 mM
2	$CaCl_2$-$2H_2O$ (Sigma C-3881)	10 mL/L	1 g/400 mL d H_2O	0.17 mM
3	$MgSO_4$-$7H_2O$ (Sigma 230391)	10 mL/L	3 g/400 mL d H_2O	0.3 mM
4	K_2HPO_4 (Sigma P 3786)	10 mL/L	3 g/400 mL d H_2O	0.43 mM
5	KH_2PO_4 (Sigma P 0662)	10 mL/L	7 g/400 mL d H_2O	1.29 mM
6	NaCl (Fisher S271-500)	10 mL/L	1 g/400 mL d H_2O	0.43 mM
7	P-IV Metal solution	6 mL/L		
8	Vitamin B12	1 mL/L		
9	Biotin Vitamin Solution	1 mL/L		
10	Thiamine Vitamin Solution	1 mL/L		

Supplementary Table 2.

P-values as a result of difference between Pellet samples (P) and Supernatant samples (S) within the different locations.* representing samples being significantly different (P-value < 0.05).

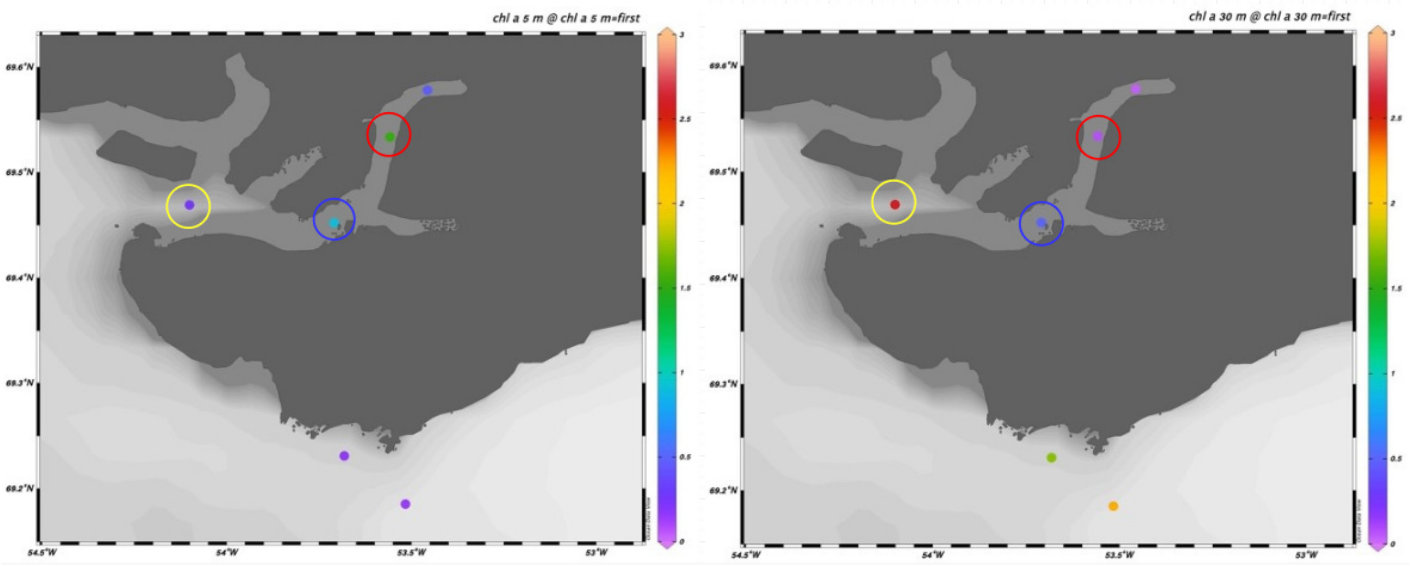
Location	P-value
Marine L1 (S) – Marine L1 (P)	0.4150
Marine L2 (S) – Marine L2 (P)	0.3510
Marine L3 (S) – Marine L3 (P)	0.0037*
Thygesen (S) – Thygesen (P)	0.0095*
Moræne (S) – Moræne (P)	0.0388*
Station (S) – Station (P)	0.8650
Means of all stations (S) – Means of all stations (P)	0.549

Supplementary Table 3.

P-values as a result of difference between 3-20 µm samples (3-20 µm) and 20-50 µm samples (20-50 µm) within the different locations. .* representing samples being significantly different (P-value < 0.05).

Location	P-value
Marine L1 (3-20 µm) – Marine L1 (20-50 µm)	0.7870
Marine L2 (3-20 µm) – Marine L2 (20-50 µm)	0.0712
Marine L3 (3-20 µm) – Marine L3 (20-50 µm)	0.6960
Thygesen (3-20 µm) – Thygesen (20-50 µm)	0.4790
Moræne (3-20 µm) – Moræne (20-50 µm)	0.0388*
Station (3-20 µm) – Station (20-50 µm)	0.1200
Means of all stations (3-20 µm) – Means of all stations (20-50 µm)	0.0103*

Supplementary Figure 1.
Chlorophyll concentrations (in µg/L) measured at marine sampling sites. Marine Location 1, 2 and 3 is represented with a red, blue and yellow ring, respectively. Left map representing the chlorophyll content at 5 m depth, right map representing the chlorophyll content at 30 m depth. Maps were created by Ellen Salamon.



Supplementary Figure 2.

Results of the well diffusion assay containing *E. coli*. All samples showed no inhibition potential towards *B. cereus* in the well diffusion assay. Dark grew represents all triplicates within a sample showing inhibition whereas light grey represent control samples showing inhibition. Inhibition is a representative of clearing zones in mm in the bacterial lawn. T.no. represents triplicate number, P/S represent pellet (P) or supernatant (S) and C represents control samples. Mean represents the mean clearing zone (in mm) of three wells.

Location	T.no	P/S	Size fraction	Mean
Marine L1	T1	P	3-20	1.3
Marine L1	T2	P	3-20	0
Marine L1	T3	P	3-20	0
Marine L1	T1	P	20-50	0
Marine L1	T2	P	20-50	1
Marine L1	T3	P	20-50	0.67
Marine L1	T1	S	3-20	0
Marine L1	T2	S	3-20	1
Marine L1	T3	S	3-20	1
Marine L1	T1	S	20-50	0.3
Marine L1	T2	S	20-50	1
Marine L1	T3	S	20-50	0.67
Marine L2	T1	P	3-20	0
Marine L2	T2	P	3-20	0.5
Marine L2	T3	P	3-20	0.3
Marine L2	T1	P	20-50	0.67
Marine L2	T2	P	20-50	0
Marine L2	T3	P	20-50	0.3
Marine L2	T1	S	3-20	0
Marine L2	T2	S	3-20	0.3
Marine L2	T3	S	3-20	0
Marine L2	T1	S	20-50	0.83
Marine L2	T2	S	20-50	0.83
Marine L2	T3	S	20-50	0
Marine L3	T1	P	3-20	0
Marine L3	T2	P	3-20	1.67
Marine L3	T3	P	3-20	1
Marine L3	T1	P	20-50	1.167
Marine L3	T2	P	20-50	0.67
Marine L3	T3	P	20-50	0.3
Marine L3	T1	S	3-20	0.3
Marine L3	T2	S	3-20	0.3
Marine L3	T3	S	3-20	0
Marine L3	T1	S	20-50	0.3
Marine L3	T2	S	20-50	0.3
Marine L3	T3	S	20-50	0
Marine	C	-	0.2 media	0.167
Marine	C	-	0.8 media	0.3
Marine	C	-	0.2 water	0
Stationssøen	T1	P	3-20	0
Stationssøen	T2	P	3-20	1
Stationssøen	T3	P	3-20	0
Stationssøen	T1	P	20-50	1
Stationssøen	T2	P	20-50	1
Stationssøen	T3	P	20-50	0.3

Location	T.no	P/S	Size fraction	Mean
Stationssøen	T1	S	3-20	1
Stationssøen	T2	S	3-20	0
Stationssøen	T3	S	3-20	0.67
Stationssøen	T1	S	20-50	1
Stationssøen	T2	S	20-50	0
Stationssøen	T3	S	20-50	0.3
Stationssøen	C	-	0.2 media	0.3
Stationssøen	C	-	0.8 media	0.3
Stationssøen	C	-	0.2 water	0.5
Thygesen sø	T1	P	3-20	0.67
Thygesen sø	T2	P	3-20	0.67
Thygesen sø	T3	P	3-20	0
Thygesen sø	T1	P	20-50	0
Thygesen sø	T2	P	20-50	0.83
Thygesen sø	T3	P	20-50	0.67
Thygesen sø	T1	S	3-20	0.83
Thygesen sø	T2	S	3-20	1.67
Thygesen sø	T3	S	3-20	0.3
Thygesen sø	T1	S	20-50	1
Thygesen sø	T2	S	20-50	1
Thygesen sø	T3	S	20-50	0.83
Thygesen sø	C	-	0.2 media	0.3
Thygesen sø	C	-	0.8 media	0.83
Thygesen sø	C	-	0.2 water	0.3
Moræne sø	T1	P	3-20	0.3
Moræne sø	T2	P	3-20	0.3
Moræne sø	T3	P	3-20	0
Moræne sø	T1	P	20-50	0.83
Moræne sø	T2	P	20-50	1
Moræne sø	T3	P	20-50	0.5
Moræne sø	T1	S	3-20	0
Moræne sø	T2	S	3-20	0
Moræne sø	T3	S	3-20	0.67
Moræne sø	T1	S	20-50	0
Moræne sø	T2	S	20-50	0
Moræne sø	T3	S	20-50	0.67
Moræne sø	C	-	0.2 media	0
Moræne sø	C	-	0.8 media	0
Moræne sø	C	-	0.2 water	0
Tet. 40 µg/ml	-	-	-	9
Tet. 20 µg/ml	-	-	-	5
Tet. 10 µg/ml	-	-	-	4.3
Tet. 5 µg/ml	-	-	-	2.67
Tet. 2.5 µg/ml,	-	-	-	1

Supplementary Figure 3.

Results of the microplate growth inhibition assay. Grey cells represent bacterial growth, white cells represent no bacterial growth (clearance). C indicate control samples (only *E. coli*). TET 1, 2, 3, 4, 5 represent tetracycline in concentrations 25 μg/ml, 20 μg/ml, 15 μg/ml, 10 μg/ml, 5 μg/ml, 2.5 μg/ml, respectively. M 1, 2 and 3 represent Marine location 1, 2 and 3, St. represents Stationssøen, Mo. represents Moræne søen and Thy. represents Thygesen sø. T 1, 2, 3 represent triplicate 1, 2 and 3. P and S represents pellet and supernatant. 3-20 and 20-50 represent size fraction 3-20 μm and 20-50 μm.

Plate 1.

C:5x10^5 cells/mL	C:5x10^4 cells/mL	C:5x10^3 cells/mL	C:5x10^2 cells/mL	C:5x10^5 cells/mL	C:5x10^4 cells/mL	C:5x10^3 cells/mL	C:5x10^2 cells/mL
M1,T2,S,20-50	M1,T2,S,20-51	M1,T2,S,20-52	M1,T2,S,20-53	M2, T1,P,3-20	M2, T1,P,3-20	M2, T1,P,3-20	M2, T1,P,3-20
M1,T3,S,20-50	M1,T3,S,20-51	M1,T3,S,20-52	M1,T3,S,20-53	M2, T2,P,3-20	M2, T2,P,3-20	M2, T2,P,3-20	M2, T2,P,3-20
M1,T1,S,20-50	M1,T1,S,20-51	M1,T1,S,20-52	M1,T1,S,20-53	M2, T3,P,3-20	M2, T3,P,3-20	M2, T3,P,3-20	M2, T3,P,3-20

Plate 2.

C:5x10^5 cells/mL	C:5x10^4 cells/mL	C:5x10^3 cells/mL	C:5x10^2 cells/mL	C:5x10^5 cells/mL	C:5x10^4 cells/mL	C:5x10^3 cells/mL	C:5x10^2 cells/mL
M3,T1,P,20-50	M3,T1,P,20-50	M3,T1,P,20-50	M3,T1,P,20-50	M, C, 0.2 med.	M, C, 0.2 med.	M, C, 0.2 med.	M, C, 0.2 med.
M3,T2,P,20-50	M3,T2,P,20-50	M3,T2,P,20-50	M3,T2,P,20-50	M, C, 0.8 med.	M, C, 0.2 med.	M, C, 0.2 med.	M, C, 0.2 med.
M3,T3,P,20-50	M3,T3,P,20-50	M3,T3,P,20-50	M3,T3,P,20-50				

Plate 3.

C:5x10^5 cells/mL	C:5x10^4 cells/mL	C:5x10^3 cells/mL	C:5x10^2 cells/mL	C:5x10^5 cells/mL	C:5x10^4 cells/mL	C:5x10^3 cells/mL	C:5x10^2 cells/mL
St, T1, P,20-50	St, T1, P,20-50	St, T1, P,20-50	St, T1, P,20-53	Thy C,0.2 H2O	Thy C, 0.2 H2O	Thy C, 0.2 H2O	Thy C, 0.2 H2O
St, T2,P,20-50	St, T2,P,20-50	St, T2,P,20-50	St, T2,P,20-53				
St, T3,P,20-50	St, T3,P,20-50	St, T3,P,20-50	St, T3,P,20-53				

Plate 4.

C:5x10^5 cells/mL	C:5x10^4 cells/mL	C:5x10^3 cells/mL	C:5x10^2 cells/mL	C:5x10^5 cells/mL	C:5x10^4 cells/mL	C:5x10^3 cells/mL	C:5x10^2 cells/mL
St C, 0.2 med.	St C, 0.2 med.	St C, 0.2 med.	St C, 0.2 med.				
St C, 0.2 H2O	St C, 0.2 H2O	St C, 0.2 H2O	St C, 0.2 H2O				
St C, 0.8 med.	St C, 0.8 med.	St C, 0.8 H2O	St C, 0.8 med.				

Plate 5.

C:5x10^5 cells/mL	C:5x10^4 cells/mL	C:5x10^3 cells/mL	C:5x10^2 cells/mL	C:5x10^5 cells/mL	C:5x10^4 cells/mL	C:5x10^3 cells/mL	C:5x10^2 cells/mL
Mo,T1,P,20-50	Mo,T1,P,20-50	Mo,T1,P,20-50	Mo,T1,P,20-50	Thy,T1,P,3-20	Thy,T1,P,3-20	Thy,T1,P,3-20	Thy,T1,P,3-20
Mo,T2,P,20-50	Mo,T2,P,20-50	Mo,T2,P,20-50	Mo,T2,P,20-50	Thy,T2,P,3-20	Thy,T2,P,3-20	Thy,T2,P,3-20	Thy,T2,P,3-20
Mo,T3,P,20-50	Mo,T3,P,20-50	Mo,T3,P,20-50	Mo,T3,P,20-50	Thy,T3,P,3-20	Thy,T3,P,3-20	Thy,T3,P,3-20	Thy,T3,P,3-20

Plate 6.

C:5x10^5 cells/mL	C:5x10^4 cells/mL	C:5x10^3 cells/mL	C:5x10^2 cells/mL	C:5x10^5 cells/mL	C:5x10^4 cells/mL	C:5x10^3 cells/mL	C:5x10^2 cells/mL
Thy,T1,P,20-50	Thy,T1,P,20-50	Thy,T1,P,20-50	Thy,T1,P,20-50	Thy,T1, S,3-20	Thy,T1, S,3-20	Thy,T1, S,3-20	Thy,T1, S,3-20
Thy,T2,P,20-50	Thy,T2,P,20-50	Thy,T2,P,20-50	Thy,T2,P,20-50	Thy,T2, S,3-20	Thy,T2, S,3-20	Thy,T2, S,3-20	Thy,T2, S,3-20
Thy,T3,P,20-50	Thy,T3,P,20-50	Thy,T3,P,20-50	Thy,T3,P,20-50	Thy,T3, S,3-20	Thy,T3, S,3-20	Thy,T3, S,3-20	Thy,T3, S,3-20

Plate 7.

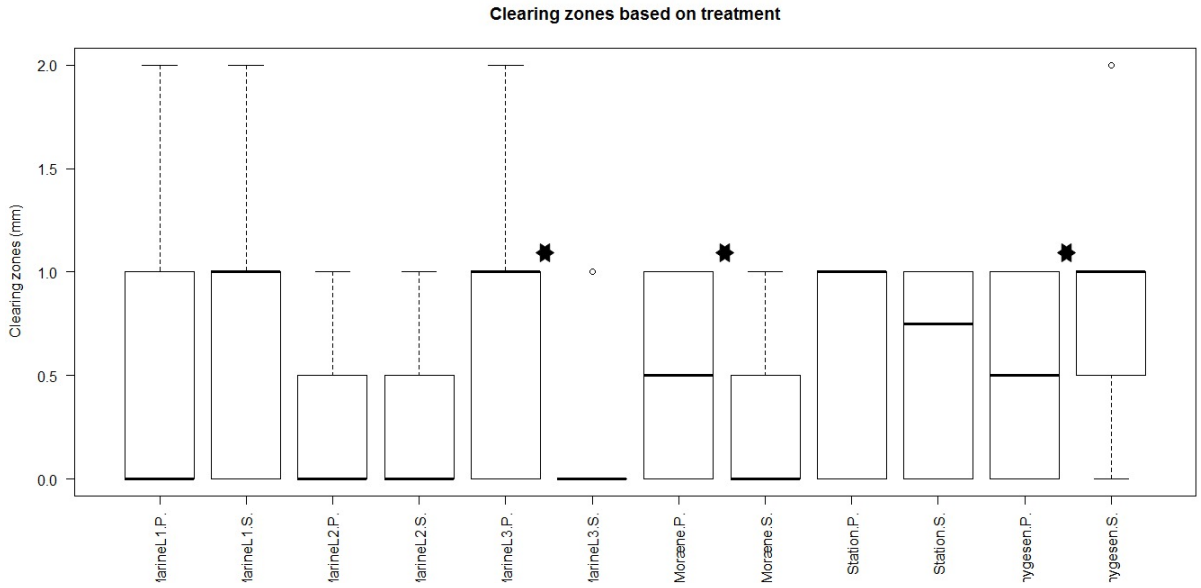
C:5x10^5 cells/mL	C:5x10^4 cells/mL	C:5x10^3 cells/mL	C:5x10^2 cells/mL	C:5x10^5 cells/mL	C:5x10^4 cells/mL	C:5x10^3 cells/mL	C:5x10^2 cells/mL
Thy C,0.2 med.	Thy C,0.2 med.	Thy C,0.2 med.	Thy C,0.2 med.	Thy,T1,S,20-50	Thy,T1,S,20-50	Thy,T1,S,20-50	Thy,T1,S,20-50
Thy C,0.8 med.	Thy C,0.8 med.	Thy C,0.8 med.	Thy C,0.8 med.	Thy,T2,S,20-50	Thy,T2,S,20-50	Thy,T2,S,20-50	Thy,T2,S,20-50
				Thy,T3,S,20-50	Thy,T3,S,20-50	Thy,T3,S,20-50	Thy,T3,S,20-50

Plate 8.

C:5x10^5 cells/mL	C:5x10^4 cells/mL	C:5x10^3 cells/mL	C:5x10^2 cells/mL	C:5x10^5 cells/mL	C:5x10^4 cells/mL	C:5x10^3 cells/mL	C:5x10^2 cells/mL
TET 2	TET 2	TET 1	TET 1				
TET 3	TET 3	TET 2	TET 2				
TET 4	TET 4	TET 3	TET 3				
TET 5	TET 5	TET 4	TET 4				
TET 6	TET 6	TET 5	TET 5				

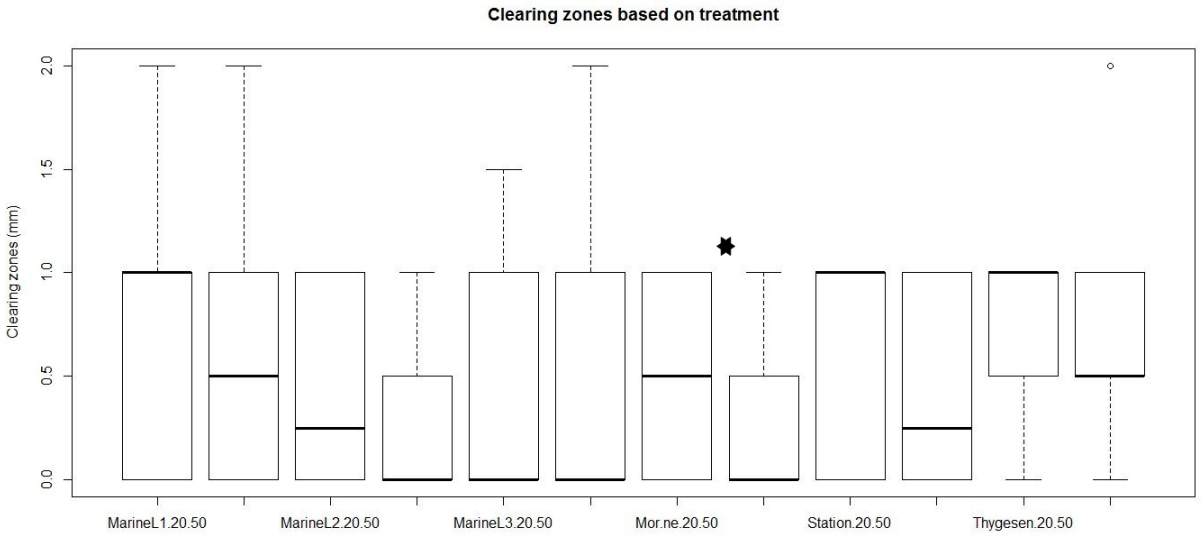
Supplementary Figure 4.

Means of clearing zones from different treatments; Pellet (P) and Supernatants (S) measured in mm, as a result of the well diffusion assay using E. coli SP548. Stars representing locations which were significantly different in clearing zone from samples containing pellet and samples containing supernatants (supplementary Table 2).



Supplementary Figure 5.

Means of clearing zones from different treatments; 3-20 µm samples (3-20) and 20-50 µm samples (20-50) within the different location measured in mm, as a result of the well diffusion assay using E. coli SP548. Stars representing locations which were significantly different in clearing zone from 3-20 µm samples and 20-50 µm samples (supplementary Table 3).



Macrophyte biomass and production in arctic lakes and ponds in West Greenland

Fieldwork in Stationssøen (photos: Kirsten S. Christoffersen)

Macrophyte biomass and production in arctic lakes and ponds in West Greenland

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Abstract

The differences between aquatic macrophytes and the factors affecting these in arctic lakes and ponds have yet to be fully understood. This study investigated differences between the aquatic macrophytes in arctic freshwater ecosystems, by examining three lakes and four ponds in West Greenland, with focus on mosses and aquatic plant species. The study was done, to gain greater insight to factors affecting macrophytes in arctic lakes and ponds, as well as, the importance regarding coverage, biomass, and productivity. This was examined by determining the coverage of both aquatic plants and mosses. In ponds the coverage was determined by three transects each with three plots of along the transect. In the lakes the coverage was determined, at every 2nd meter in the littoral zone with no fixed number of transects. In ponds all mosses within the plots where collected and examined in order to determine biomass and primary production. As plants where only found in one pond, however not in the transects, no plants where collected. In lakes, moss was sampled randomly when present, whereas aquatic plants where only registered but not collected. The moss was sampled, to compare productivity with the moss sampled from the ponds.

The results showed 4.5 times higher moss coverage and variance in ponds compared to lakes, whereas the lakes had the highest plant coverage and variance observed. Moss coverage showed uniform distribution in both lakes and ponds, with variance:mean ratios close to zero, with plant coverage likewise dispensing uniform distribution in the lakes.

Moss biomass ratio showed higher in lakes compared to ponds, but only in 2019 was a significant difference observed, though 2018 also showed big differences.

The dry weight (DW) biomass from the ponds showed a higher DW in the first pond, however, when the average DW per percentage cover per m^2 was assessed, it showed the highest values in the third pond.

This study shows how primary production is highly affected by the stability of the environment. It indicates that with greater depth and higher water volume less rapid environmental changes in physical parameters in lakes can facilitate the colonizing of less resilient species, however, also cause higher exposure to aquatic plants, as the macrophytes are exposed to mechanical disturbances such as wind, affecting their anchorage and stems.

Keywords: Aquatic macrophytes, Arctic lakes, Arctic ponds, Productivity, Biomass.

Introduction

The Arctic region has in earlier studies been referred to as "the world's largest wetland" due to the high numbers of ponds and lakes, which are prominent in the Arctic landscape (Kling, 2009; Rautio et al., 2011; Wrona et. al., 2013).

The definition and separation between lakes and ponds can vary and are often arbitrary. Typically, ponds are defined as shallow small water bodies, where the shallow depth allows the light to penetrate the entire bottom surface and permit an extensive coverage of aquatic macrophytes. Lakes are often defined as deeper and/or bigger than ponds, where sunlight cannot reach down to the deepest area (Rautio et. al., 2011).

Lakes and ponds in the Arctic are subjected to large seasonal fluctuations throughout the year and optimal conditions for growth in these freshwater systems are highly controlled by the physical and chemical conditions (Christoffersen et al., 2008a). Nutrient concentrations in arctic lakes and ponds are often very low and they will typically be categorized as oligotrophic (Wrona et al. 2013). The low concentrations are a result of the inflowing water, that originates from the melting ice and snow runoff, which contains small amounts of important, limiting nutrients, such as nitrogen and phosphorous. Due to the cold climate arctic ponds and lakes will generally be ice-covered approximately 8-10 months of the year, and more shallow water bodies, such as ponds, will freeze solid during the winter (Christoffersen et al., 2008a, b). In the winter-periods, the ice-cover will be covered by snow which will prevent light to penetrate the water column and therefore affect the light availability (Christoffersen et al., 2008a, b). In the summer-periods, arctic lakes and ponds will be exposed to 24-h daylight due to midnight sun (Rautio et al., 2011). The shallow ponds will heat up more quickly than the lakes which can enhance the productivity, but temperatures can increase rapidly, and the ponds can potentially dry out during the summer, whereas the lakes are more stable (Rautio et al., 2011). These chemical and physical conditions create a harsh environment for the organisms inhabiting such ecosystems, with primary production inhibited by limiting nutrients and the length of ice-cover. The organisms must cope with low nutrient content, polar nights and

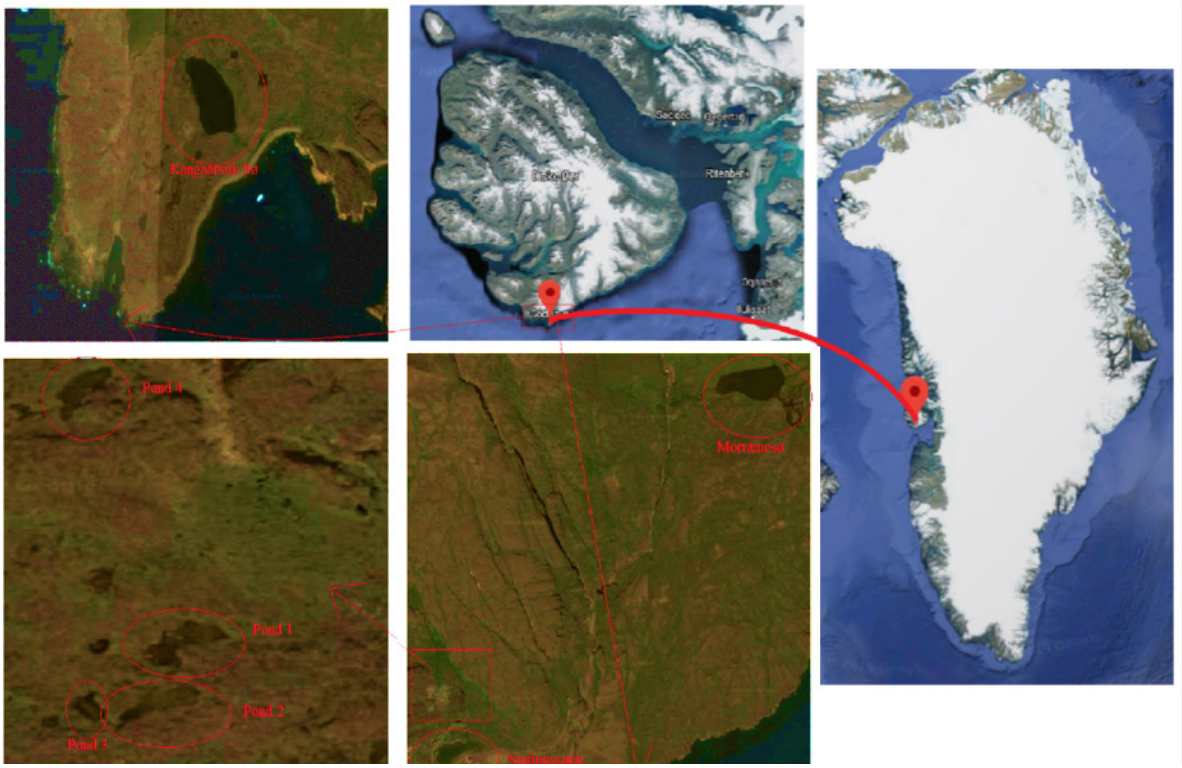

Figure 1. Map showing the location of the four ponds and the three lakes (maps from Google Earth).

withstand freezing, all factors which will challenge the abundance and productivity of macrophytes established in the lakes and ponds in the Arctic.

In the Arctic the distribution of vascular freshwater plants is limited, and mosses are the most frequent macrophyte present in the lakes and ponds (Sand-Jensen et al., 1999; Chambers et al., 2008) Aquatic mosses are slow growing and require small amounts of nutrients and light, which makes them suitable for the harsh climate in the Arctic with short growing season and the nutrient-poor environments (Riis & Sand-Jensen 1997; Sand-Jensen et al., 1999). The vascular macrophytes are typically seen in the littoral zone were light is available, and the abundance decreases with enhanced depth where they will be replaced with stress-tolerated moss species. Even though the aquatic mosses are slow-growing they can contribute significantly to the pool of organic detritus due to the low temperatures and nutrient availability which will decrease the microbial activity in arctic ponds and lakes (Sand-Jensen et al. 1999).

In this study we wanted to estimate the moss coverage, abundance, biomass and productivity in arctic ponds and to compare with the productivity of mosses in lakes,. The overall aim was to investigate how much the vegetation can differ in arctic freshwater ecosystems and thus achieve a better understanding of what factors affect the macrophytes in arctic lakes and ponds. We hypothesized that **(1)** Moss coverage would be higher in ponds compared to lakes due to the earlier ice- and snowmelt and thus to more light, and therefore prolong the growing season. **(2)** Higher plant coverage in lakes compared to ponds due to more stable conditions and **(3)** higher moss production in lakes compared to ponds. The production of the mosses would be described as the annual growth per season. Several moss species have an apical growth, which can reconstruct information about the growth history. The technique has been used by Riis et al (2014) and Thiemer et al. (2018), where they have been investigating annual growth of *D. trifarius* to receive the historic growth in lakes and ponds in East Greenland.

Methods and Materials

Sites

The study was conducted in 3 lakes and 4 ponds in Qeqertarsuaq, West Greenland (69°25’33’’N, 53°51’73’’W) in July 2019 (Fig. 1).

The different locations examined included ponds ranging from 134 to 580 m^2 and lakes ranging from 24,052 to 63,537 m^2 in size. The area of the ponds and lakes were determined using orthophotos from NunaGIS (Nunagis.gl). The average depths were calculated based on bathrymetric maps (Morænesø and Kangaarssuk sø) provided by K. S. Christoffersen or estimated on location.

Table 1. Surface area and average depth of the lakes and ponds examined.

	Surface area (m^2)	Total coverage (%)
Stationssøen	24052	16
Kangaarsuk sø	50154	26
Morænesø	63537	0
Pond 1	580	92
Pond 2	390	56
Pond 3	135	73
Pond 4	344	55

During winter the examined ponds are known to freeze completely due to the shallow depths whereas the lakes will have a body of water underneath the ice-cover. In the spring, the ponds are known to thaw earlier than the lakes due to the lower water volume.

Sampling

Moss coverage was estimated from 3 transects across the pond using a square of 0.32 m^2 which was thrown randomly 3 times at each transect. All mosses within the square were collected for later estimation of productivity (see procedures below). None of the ponds examined had aquatic plants present within the transects so only the moss included If any aquatic plants were seen in the studied pond, a value of 1% was given to the aquatic plant coverage.

The sampling in lakes was done by walking towards the middle of the lake, sampling at approximately every second meter. A square of 1.3 m^2 was randomly thrown at each sampling location and the coverage was determined for both aquatic plants and mosses. To avoid differences in light availability between lakes and ponds, only the littoral zone was examined, determined as the interface between land and the open water, in which light could reach the bottom (Wetzel, 1990). The ponds were shallow, causing light to reach the bottom throughtout the entire area. The lakes were deeper compared to ponds (lakes: 0.75-3 m, ponds 0.1-0.35 m), creating zones in which light could not reach the bottom, these zones were excluded, and when reffering to lakes, only the littoral zone is meant, unless otherwise noticed. Due to lower coverage and higher area in lakes a bigger square was used. Aquatic plant taxa were determined to species using Schou et al. (2017) and Rune (2011).

Moss productivity and biomass

To determine moss productivity and biomass the collected samples were cleaned manually using tap water to remove silt and mud. As the majority of the moss was of the genus *Drepanocladus* (Thiemer et al., 2018), all other moss genera were discarded to avoid differences in water content between different genera. After the removal of silt and mud, the mosses were left in a strainer for approximately one hour, before the entire sample was weighed to determine the wet weight. Due to the apical growth pattern of *Drepanocladus spp.*, we were able to determine the growth history of the mosses that were collected, this was possible due to the shoot architecture with descrete annual segments (Riis and Sand-Jensen 1997; Bisang et al. 2008; Riis et al. 2014). A subsample was used to determine dry weight (DW) and productivity (= biomass) for the present year (2019), the year before (2018) and all previous years. As the mosses could be different ages, large subsamples were picked, to get a normal distribution around the sample mean. The DW was determined by drying the subsamples at 105°C for at least 24 hours.

Moss productivity in the selected ponds and lakes could be described as the accumulated DW biomass per growth season. Moss biomass of the ponds was calculated using the moss coverage percentage and the size of the pond. By comparing the moss productivity in the lakes and ponds, we investigated the ratio of the production from this year (2019), last year (2018) and the rest, to observe if there were any differences between the two ecosystems.

Data analysis

Prior to analysis, data for moss and aquatic plant coverage was logit transformed to obtain a Gaussian distribution. In the statistical testing we used t-test when the data had a Gaussian distribution and was homoscedastic. In cases where data were non-Gaussian distributed or showed heteroscedastic a Welch t-test or a Wilcoxon rank sum test were used, respectively.

Moss and aquatic plant coverage were compared between ponds and lakes, respectively, using a student's t-test or Wilcoxon rank sum test with moss or aquatic plant coverage as the dependent variable and type (pond vs. lake) as the independent variable. Furthermore, homogeneity of moss and plant coverage distribution in lakes and ponds were assessed, respectively, by using the mean and variance of each study site, examining the mean:variance ratio to describe the distributional pattern. Ratios > 1 indicating a clumped distribution, ratios = 1 indicating a random distribution, and ratios < 1 indicating a uniform distribution.

Moss productivity as the ratio of shoots from 2019, 2018 and the rest (all other years) were analyzed using a t-test or Wilcoxon rank sum test, with ratio of shot from the different years as the dependent variable, and type as the independent variable.

Total moss biomass in ponds was calculated and fitted against surface area and total coverage, respectively, to investigate for any

correlations using a linear regression. Prior to analysis total moss biomass was log-transformed to obtain a Gaussian distribution.

Results

Moss and plant coverage

Moss coverage was higher in ponds than in the littoral zone in lakes, averaging 67% (range: 55-83%, Table 2) and 14% (0-22%, Table 2) respectively. Tests showed significantly different moss coverage between ponds and lakes (t-test, t(5)= -5.63, p = 0.01), with a 4.5 times higher moss coverage in ponds, compared to lakes (Fig. 2).

Plant coverage was highest in litteral zones in lakes averaging 5% (18%, Table 2), whereas no plants were present within the plots for pond sampling, although few individuals of two different plant species (i.e. *Hippuris Vulgaris* and *Rununculus pygmaeus)* were observed in pond 4. Furthermore, plant coverage was significantly different in lakes and ponds (Wilcoxon rank sum test, W = 12, p = 0.03), indicating higher plant coverage in lakes compared to ponds (Fig. 2).

Moss coverage showed a tendency towards higher variance in ponds compared to lakes (table 2), but was not significantly different (t-test, t(4)=-1.73, p = 0.16). Furthermore, the variance:mean ratio (Table 2) showed close to zero in both lakes and ponds, indicating a uniform distribution and no distributional differences in moss coverage between arctic lakes and ponds.

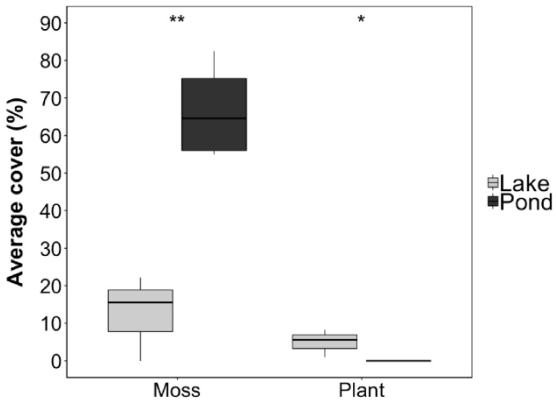

Figure 2. Average cover of moss and aquatic plants in lakes (n=3) and ponds (n=4), respectively. Grey boxes indicate lakes and black boxes indicates ponds. Error bars indicate the highest and lowest values, respectively. Coverage of moss was compared using a t-test, while plant coverage was not normally distributed, thus using a Wilcoxon-test. Significance levels: '*' 0.001, '**' 0.01, '*' 0.05, '.' 0.1.**

Table 2. Mean and variance of moss and plant coverage and the ratio btween these parameters, in Stationssøen, Kangaarsuk sø, Morænesø and the four different ponds that were studied.

	Moss coverage (%)			Plant coverage (%)		
	Mean	Variance	Ratio	Mean	Variance	Ratio
Stationssøen	15.6	875	0.02	5.6	123	0.05
Kangaarsuk sø	0	0		1	36	0.03
Morænesø	22.2	1278	0.02	8.3	263	0.03
Pond 1	82.5	1053	0.08	0	0	
Pond 2	56.3	2060	0.03	0	0	
Pond 3	72.8	1857	0.04	0	0	
Pond 4	55	2406	0.02	1	0	

Due to few or no aquatic plants present in any of the ponds, we were unable to compare distributional patterns of aquatic plants in arctic lakes and ponds. The aquatic plant coverage showed a variance:mean ratio (Table 2) close to zero, indicating a uniformly spread distribution.

Moss production

The ratio of moss production in the lakes was different from that of ponds (Fig. 3), with a higher proportion of the biomass in new shoots (2019) and shoots from last year (2018) for lakes compared to ponds (Table 3). The yearly production of moss shoots (2019) showed significant difference between lakes and ponds (t-test, t(4)=-6.2, p = 0.003). The

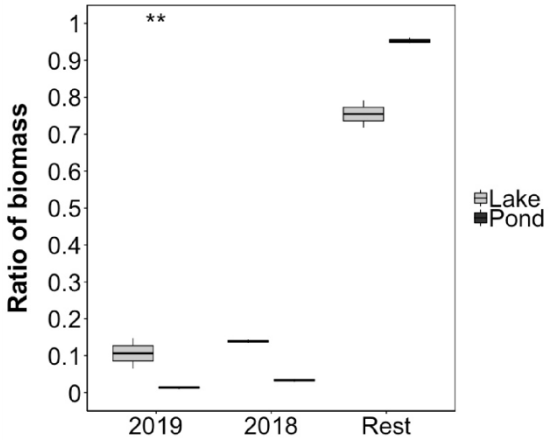

Figure 3. Ratio of moss biomass production in 2019, 2018 and all previous years, respectively, the sample spans two lakes (one of the three lakes were without presence of moss) and four ponds. The leftmost boxes for each year represent the lakes and the rightmost the ponds. Error bars indicate the highest and lowest values, respectively. Only 2019 showed a significant difference between lakes and ponds (p = 0.003)

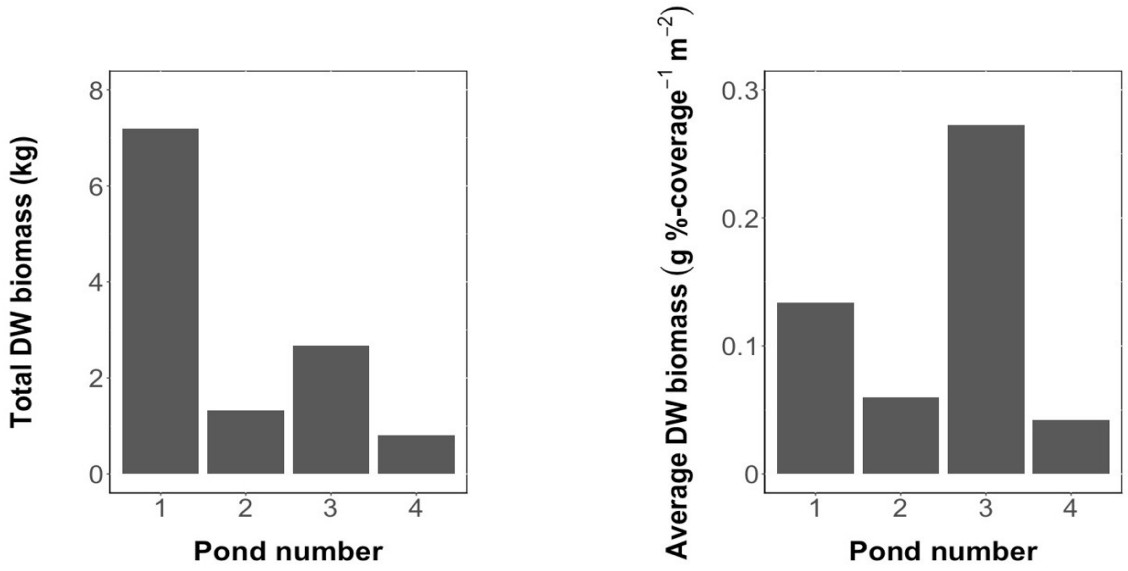

Figure 4. Left: Total moss biomass in kg DW in each of the four examined ponds. Right: Average moss DW biomass per percentage per m^2 in grams, in each of the four examined ponds

yearly production of 2018 showed no significant differences between lakes and ponds (Wilcoxon rank sum test, W = 0, p = 0.13). Nor was there any difference in the Rest (Wilcoxon rank sum test, W = 8, p = 0.13). Thus, indicating faster relative production of mosses in lakes than ponds

Moss biomass

Total moss biomass and average biomass in ponds were estimated using the mean coverage of each pond, the average DW and the surface area of the pond or lake. Total DW biomass showed no significant correlation with surface area ($F(1,2) = 0.42$, $p = 0.58$), indicating no effect of surface area on the DW biomass of ponds. Total DW biomass showed a significant correlation with the total moss coverage in ponds ($F(1,2) = 56.7$, $p = 0.017$)

Table 3. Ratio of moss production biomass for the year 2019 and 2018, as well as the remaining years (rest), for each of the location sites. Kangaarsuk sø was removed due to no moss present.

	Ratio of biomass		
	2019	2018	Rest
Stationssøen	0.15	0.13	0.72
Moræneso	0.07	0.14	0.79
Pond 1	0.01	0.03	0.96
Pond 2	0.02	0.04	0.95
Pond 3	0.02	0.04	0.95
Pond 4	0.01	0.03	0.95

with a R^2 of 0.97, indicating the moss biomass was determined by the moss coverage. Large variation was seen, with pond 1 having the highest biomass, approximately 7-fold higher than that of the lowest (pond 4) (Fig. 4 left). Average g moss DW biomass per percentage cover per m^2 was assessed for each pond (Fig. 4 right), indicating grams of DW moss in a square-meter with a coverage of 1%. This calculation was done to compare moss biomass between ponds as there was big variation among the ponds; the highest being pond 3, nearly 6.5-fold higher than the lowest (pond 4).

Discussion

The aim of this study was to examine differences in moss and plant coverage in arctic lakes and ponds, as well as determining the productivity of mosses and the total biomass of mosses in ponds.

Moss coverage was found to be significantly higher in ponds compared to lakes in our study and thus supports hypothesis 1. This is likely due to the extreme environment in ponds with freezing of the entire water column during winter and desiccation or extreme diurnal fluctuations during summer which makes it difficult for other aquatic plants to inhabit the system (Thiemer et al., 2018; Sand-Jensen et al., 1999). Thus, limiting

the distribution of macrophytes to only the few very resilient species, which are capable of withstanding the extremes, but also rewards with an earlier snow and ice melt in the ponds. Furthermore, higher moss coverage in ponds compared to lakes, could be due to lower water depth in ponds. The ponds were characterized by being shallow with sunlight reaching the bottom throughout the ponds, whereas the lakes were deeper with sunlight not necessarily penetrating all the way to the lake bottom. This could indicate that sunlight is a limiting factor for moss coverage in lakes.

Lakes had higher coverage of aquatic plants than ponds. This is in accordance with hypothesis 2, which predicts higher presence of aquatic plants in lakes, due to the more stable environment compared to ponds, caused by differences such as depth and a higher water volume (Rautio et al., 2011), thus making it possible for the lesser resilient species to colonize. Furthermore, with increasing area an increasing wind impact is expected, thus creating wave disturbance which can limit the establishment of macrophytes (Van Zuidam & Peeters, 2015). The higher mechanical disturbance by wave exposure will possible affect mosses to a higher degree, as they don't have roots to anchorage, thus possibly causing biomass loss by breaking of stems (Schutten et al., 2004, 2005). Furthermore, higher plants would be expected to be affected by mechanical disturbance, due to breaking of anchorage and roots, which would not be the case for mosses consequently resulting in different niches among higher aquatic plants and mosses (Schutten et al., 2004, 2005). If mosses were to be affected by wind events to a higher degree than plants, we would expect differences in spatial distribution patterns. Populations being negatively affected by wind would be expected to have a clumped distribution in areas with lower wind. This pattern was not seen, most likely due to multiple factors affecting this, with especially the substrate and morphometric of the lakes and ponds having an effect on both the species present, but also the effect of wind. Further studies need to be performed to investigate this hypothesis.

A higher richness in aquatic plant species in lakes could be caused by lakes having higher surface area, thus higher chance of seeds reaching the lake, or higher amounts of birds foraging, which could transfer seeds between lakes (Brochet et al., 2009; Lovas-Kiss et al., 2019).

The estimated moss production in 2019 was significantly higher in lakes compared to ponds (hypothesis 3) but there was no significant difference in the ratio of 2018. The higher ratio of 2019 shoots in the lakes could be due to better growing conditions in lakes, with higher nutrient concentrations than the very oligotrophic ponds (Riis et al., 2010). The harsher environment in ponds will most likely affect the productivity of mosses, causing lower productivity, which is circumvented in lakes due to the higher water volume. Nevertheless, over a full growing season the moss production in the ponds could have similar production. This could be supported by moss production in lakes being faster in the beginning, but that the moss production season in ponds is longer and the production over a complete growing-season being the same. The faster production in mosses in lakes of 2019 shoots could be due to lakes exposed to higher wind events during spring, thus blowing away the snow from the lake surface, consequently allowing light to penetrate the ice (Greenbank, 1945), making it possible for macrophytes to grow earlier than in ponds. Additionally, with increasing age a higher accumulation of biomass is expected, though this might not affect the ratio of biomass prior to 2018 significantly, as the mosses will allocate the nutrients towards the newest part of the shoots and weight in the older parts will decrease (Sand-Jensen et al., 1999). This, if proven, could indicate that growing conditions for mosses in lakes are better than in ponds and thus able to grow more during the growing season.

In the ponds examined, the total moss biomass showed great variation, and therefore, contrary to expectations, no

significant correlation was found with surface area of the ponds. A positive relation between pond surface area and moss biomass would be expected. Total moss DW biomass showed significant correlation with total moss coverage, indicating that total moss coverage is determining total moss biomass. This is surprising as a high coverage wouldn't necessarily indicate high biomass. Smaller ponds could be expected to have higher coverage, as there is less area to fill out, whereas larger ponds could be expected to have a lower coverage, but in total a higher biomass, due to the larger area and higher water volume leading to more nutrients. The insignificance of surface area of ponds on moss biomass, could be due to the morphometric of the ponds, with bigger ponds not necessarily being deep as well. For further analysis we propose that the volume of the ponds is used as a parameter as well in order to analyze the significance of the water volume compared to the pond surface area, and thereby account for the morphometric of the ponds.

To be able to compare the moss biomass between ponds, the average DW biomass per percentage per m^2 (ADWB) was calculated, this removes the differences in coverage and surface area, making it comparable. Pond 3 showed the highest ADWB while also being the smallest pond (Table 1), while pond 2 and 4 had approximately the same surface area and coverage. Thus, indicating big variation in moss biomass in arctic ponds, most likely due to differences in physiochemical parameters, but this was not examined further. Further studies could look into the physio-chemical parameters, for a full understanding of the effect of these parameters over a full growing season.

In conclusion, we have found substantial differences in macrophyte coverage in arctic lakes and ponds. Moss species primarily colonizing ponds in which they are affected to a lesser degree of wind disturbance, compared to lakes, but challenged by an extreme environment in which intense diurnal temperature fluctuations during summer or even desiccation is common, as well as facing being frozen during winter. Aquatic plant cover showed almost non-existent in arctic ponds, with the exception of one pond, though present in arctic lakes, but only to a minimal. The minimal abundance of aquatic plants in the Arctic is probably assigned to the rough environment, not only because of the long and dark winter, but also the stress induced from 24-hour sunlight during summer (Demmig-Adams & Adams, 1992). Moreover, the spreading of seeds is of mere chance, and is higher with more foraging birds and other plants in the vicinity, with the relatively small species pool in Greenland (CAFF, 2019) and the very northern sampling location it is most likely helping to reduce the chances of seeds spreading through these ways.

Moss production were higher in lakes than in ponds for the shoots of 2018 and 2019, though only 2019 showed a significant difference. This could be explained by the more stable conditions in lakes during the early spring and summer, this hypothesis could be tested by introducing data for weather conditions and snow/ice melt dates. We did not have enough time to perform this test but will suggest it to be included in future studies.

The results obtained in this study could also have been influendced by low sampling sizes, affecting the tests and data comparison, forcing the use of a more conservative test. Investigating this could be foundation for further studies.

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Biovolume of snow algae in different glacial habitats at Lyngmarksbræen, Disko; and the effect of red snow on snow surface albedo

Snow algae and field work (photos: Camilla S. Kampen and Helena M. Nørgaard)

Biovolume of snow algae in different glacial habitats at Lyngmarksbræen, Disko; and the effect of red snow on snow surface albedo

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Abstract

Red snow is blooms of cryophilic algae found in polar and high alpine regions, usually observed during late spring and summer. Snow algae have an important impact on the physicochemical conditions of the glacial biome that is defined by low nutrients, moisture, high irradiance and UV exposure, as well as freeze- thaw cycles. It is possible for the algae to thrive in the glacial environment due to the physiological adaptations. Examples of these adaptations can be found in the complex lifecycle of the algae where encystment results in the production of secondary pigments, lipid storage and production of unsaturated fatty acids. Snow algae have been reported to lower the surface albedo of the snow and deplete nutrients during blooms. The overall purpose of the study was to investigate the biovolume of different glacial habitats: snow on the glacier, snowfields not connected to the glacier during melting period and cryoconite holes. This study focused on the biovolume as well as their impact on surface albedo at the different sites. Our findings indicate that cryoconite holes had the highest biovolume, most likely due to accumulation from supraglacial runoff. There was a significant negative correlation between biovolume of snow algae and snow surface albedo. While the correlation was not based only on the biovolume of algae as the samples were not pure, it is clear that the biovolume has impact on albedo. Lastly, logger data of diurnal variation in light intensity and temperature verified that snow algae experience varying light intensity and temperature throughout a 24 hr period even during polar summer nights.

Keywords: *Snow algae, biovolume, red snow, albedo, green snow.*

Introduction

Red snow is a phenomenon caused by blooms of cryophilic algae (also known as snow algae), mainly from the phylum Chlorophyta (Remias et al., 2005). Snow algae are found in places with long seasonal or perennial snow cover, such as alpine or polar snowfields characterised by low temperature and nutrient availability, high irradiance and exposure to freeze-thaw cycles (Anesio et al., 2017; Dial et al., 2018). Snow algal blooms are not mainly due to a high proliferation rate, but rather, to the snow melting and the algae accumulating on the snow surface (Hoham & Duval, 2001; Thomas et al., 2008).

Glacial biome

Snow algae can be found on the snow surface of a glacier or snowfield as well as in cryoconite holes (Uetake et al., 2010). Here, the low temperature limits availability of liquid water. Snow becomes large-grained with repeated freeze-thaw-cycles, which decreases the ability of the snow surface to retain water which again limits the ability of water even further (Dial et al., 2018). Microorganisms living on surfaces of glaciers and snowfields are exposed to high irradiance from the highly reflective surface of the snow. The ratio of reflected light from incoming light, known as albedo, is high in pure snow. Light absorbing impurities on the snow or ice surface lower the albedo, creating small pools of water known as cryoconite holes, where snow algae populations can establish (Anesio, 2017).

Snow algae life cycle and adaptations

Snow algae are able to live in these seemly harsh conditions due to physiological adaptations

throughout their lifecycle where the transformation for motile reproducing stage, to a dormant cyst (Hoham & Duval, 2001; Remias, 2012). For the dominant snow algae genus, *Chlamydomonas*, the vegetative stage occurs in spring and early summer when the snow begins to melt and nutrients becomes available. The cells are green and motile, allowing them to travel in the water column for the right temperature and irradiance exposure (Remias, 2012). During summer, the cells accumulate sugars, lipids and secondary carotenoids. The transformation to a cyst with production of more secondary pigments (carotenoids) protects the cells from UV and turns the cells red (Gorton and Vogelmann, 2003; Remias, 2012; Bidigare et al., 1993). A high proportion of unsaturated fatty acid makes the membrane more fluid which prevents the cells from freezing (Müller et al., 1998).

The secondary pigments used by the snow algae is one of the adaptations used for protection and as a coping mechanism against the low temperatures, extreme irradiation and desiccation stress due to freezing events found in the hostile environments of many arctic and cold regions. The main secondary pigment found in the snow algae, is keto-carotenoid astaxanthin (and esterified derivatives). Before turning red the cells are mostly coloured by chlorophylls and primary carotenoids. When turning red some studies found that the cells have close to five times more astaxanthin than chlorophyll a. (Remias et al., 2016)

Reduced surface albedo caused by red snow

The high density of snow algae that are accumulated on the snow surface are one of many key players in lowering the glacial albedo and thereby increasing the melt of it. The red pigmentation found in the algae darkens the surface and thereby lowering the albedo, which can have an increasing effect on the glacial melt. (Lutz et al., 2016; Dial et al.,, 2018). Previous studies (Ganey et al., 2017; Lutz et al., 2014; Lutz et al., 2016) have reported that algae are responsible for reduced albedo. One study (Lutz et al., 2016) estimated that snow algae lower surface albedo by 13% during an entire melting season. Another study (Lutz et al., 2014) estimated albedo reduction of up to 20% from a single time-point measurement.

Purpose and hypotheses

The main purpose of this study was to determine the biovolume of snow algae in different glacier habitats at Lyngmarksbræen (Disko Island) which previously have had massive algae formation, as

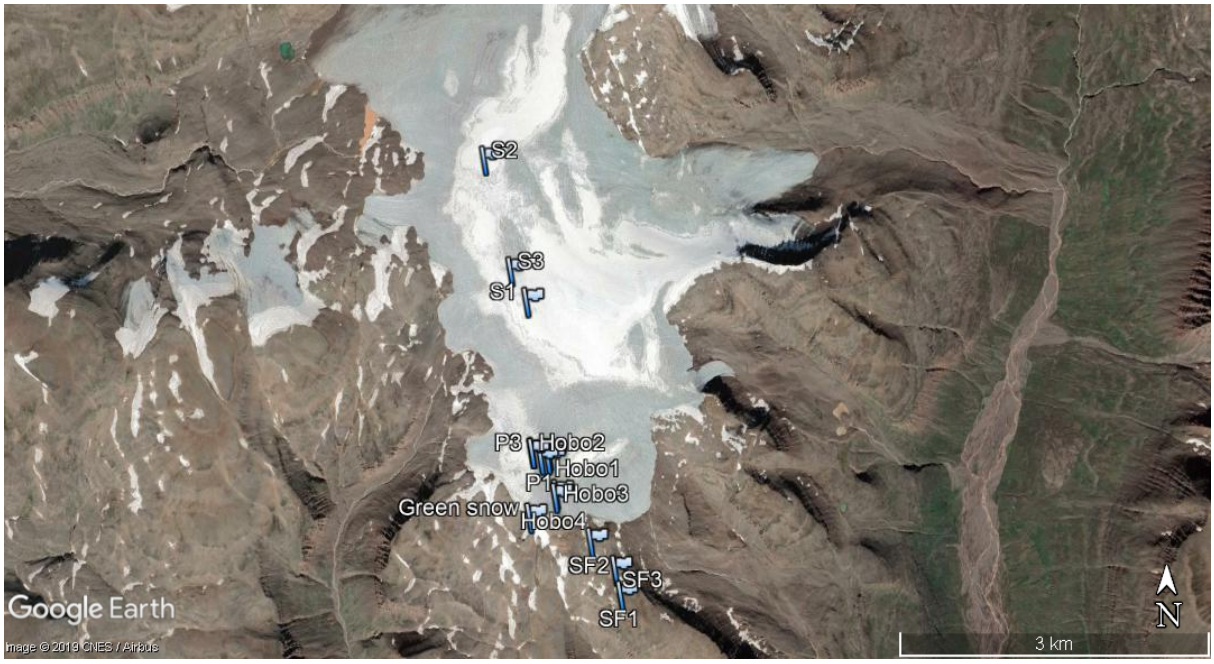

Photo 1. Map of Lyngmarksbræen on Disko Island, W Greenland with points representing our sampling and measuring sites. S= Snow, P= Cryoconite holes, SF= snowfield, HOBO1-4: HOBO-loggers. Source: Google Earth

well as to investigate the effect that the snow algae mayhave on surface albedo. We studied three types of environments where snow algae were prevalent: snow on the glacier, cryoconite holes and snowfields not connected to the glacier. It was expected that the biovolume was highest in cryoconite holes. Cryoconite holes have been extensively studied (Anesio et al., 2017; Uetake, 2010) which reports limited microbial diversity yet high microbial biomass (Takeuchi, 2001). Unlike the snow on the glacier or on snowfields, snow algae in cryoconite holes are not limited by water availability and nutrients is accumulated in the holes (Takeuchi, 2001). Furthermore, the diurnal changes in temperature and light intensity was investigated. A possible explanation for the transformation to cysts has been linked to exposure to freeze-thaw cycles (Remias et al., 2010; Hoham, 1975). Lastly, red snow affects the surface albedo of snow, thereby contributing to snowmelt in the summer months. Therefore, we wished to determine if there was a significant difference in surface albedo in seemingly clean snow compared to red snow.

Methods and materials

Sampling location

The samples were collected from 7th -18th July 2019 on and around Lyngmarksbræen on Disko Island, Greenland (Photo 1). Three main location types were sampled and 3-5 snow samples and measurements were taken each location. Transportation to reach the red snow patches on the glacier was by means of walking and by dog sledding.

The locations types were: 1) snowfields not connected to the glacier, 2) snow on the glacier and 3) cryoconite holes on the glacier. For each location type, we sampled at three different areas for respectively 1), 2) and 3). Furthermore, vegetative cells of snow algae were found, also known as green snow, therefore samples of green snow were also taken (photo 2).

Field measurements of light and temperature

Albedo was measured at each sampling spot with two PASCO wireless light sensors, one facing up and the other facing down. The measurements were conducted for 30 seconds and repeated three times. The temperature of the upper snow or water surface was measured using a handheld thermometer. The temperature was registered at 2.5 cm below the snow and/or water surface. Slope and cardinal direction was determined with Clinometer App, by placing the phone directly on the snow surface.

HOBO-loggers were placed on at different places on Lyngmarksbræen and one sensor was placed on soil as reference. The loggers measured light intensity (lux) and temperature (°C) over a 48 hour period to examine diurnal variations. The above measurements were carried out before the sampling thereby not disturbing the surface. The diurnal variation measured at different location on the glacier surface has to be seen as relative to each other because all except one of the located HOBO- sensors tipped over and therefore not giving clear results of air temperature and light intensity above the glacier surface and therefore should be seen as relative to each other. The diurnal variation shows the freeze- and thaw-cycles that the snow algae are exposed and adapted to.

Field sampling of snow algae

Samples of snow and water where gathered in 50 ml Falcon tubes from the three different locations and with 3-5 replicates per location. The Falcon tubes were dragged horizontally by hand along the snow surface of red and green snow patches until the tube was filled. No attempt was made to compress the snow. The samples were stored dark until return at the laboratory (2-3 hours) and then stored in a refrigerator at approx. 5°C. The samples were later used for identification of species present as well as for density and size measurement in the laboratory. The snow type was determined by the manual: *The guidelines and sampling procedures for the Geographical Monitoring Program-Geobasis Disko* (Sigsgaard, 2018) (appendix 3) and table from a previous study to determine liquid water in snow (Techel and Pielmeier, 2011) (appendix 2) where picture comparisons was

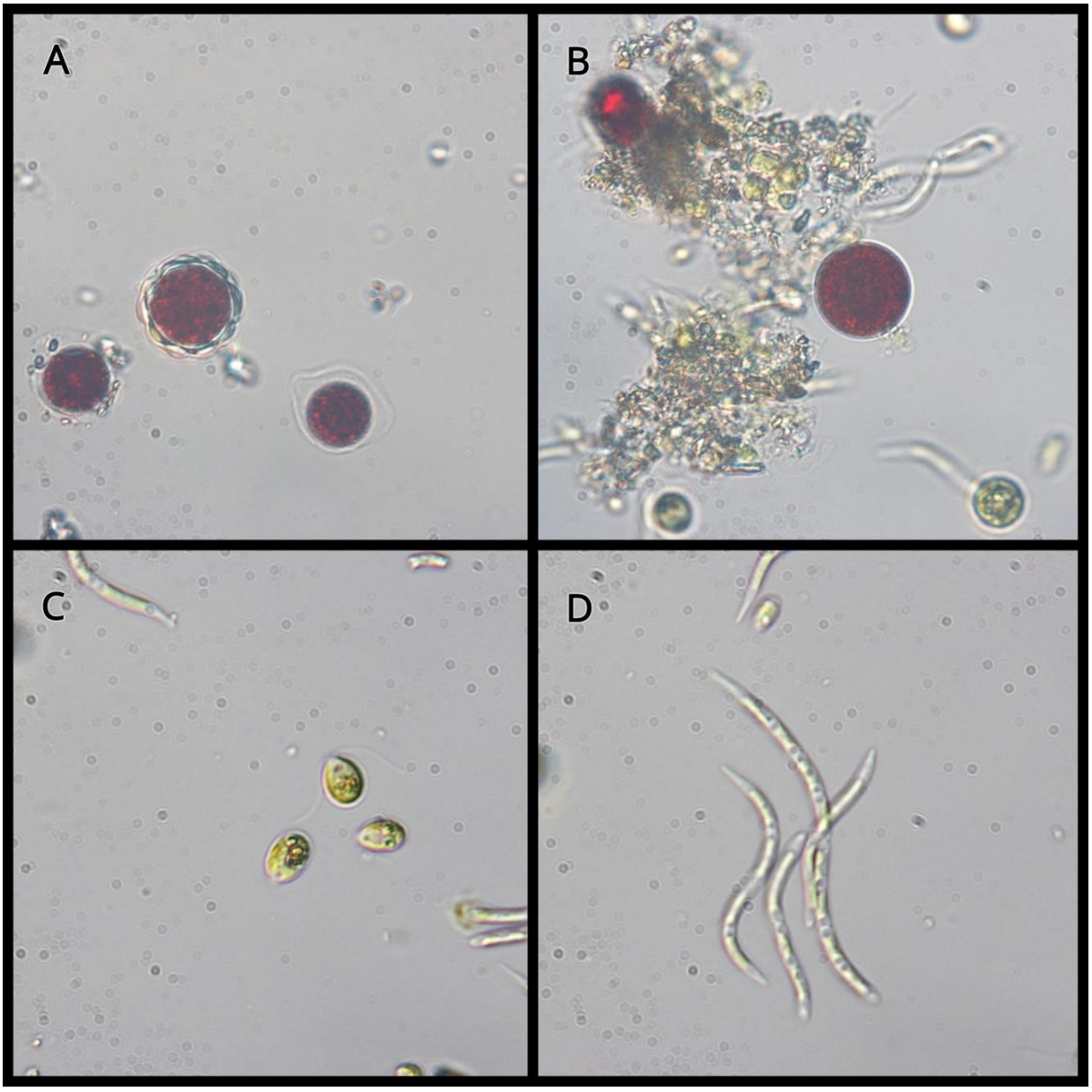

Photo 2. Light microscopy photographs of morphological types in algae samples : A: red algae with and without outer mucilage in the immotile cyst phase, possibly *Chlamydomonas nivalis* B: Red algae in immotile cyst phase of *C. nivalis* and green circular algae in the vegetative phase, C: Green algae in the vegetative phase, some still with flagella for movement, most likely vegetative stage of *C. nivalis* and D: Green algae, possibly *Koliella sp.*.

used to estimate the water density of the given snow.

Laboratory work

In the laboratory the samples were thawed and brought to room temperature to determine pH my means of a Bluelab pH probe meter calibrated the same day.The total volume of melted snow/water was measured to the nearest 0.5 ml. Biovolume of the algae was determined based on count of cell density and cell size and were done as follows: Cell count of the snow or cryoconite water samples was accomplished with a Leica and Nikon microscope using a 1 ml Sedgewick counting chamber. A min. of 50 cells were measured in each sample to determine mean cell size and measuring the cell diameter.

Data analysis and statistics

The biovolume was calculated assuming a spherical shape of the cells with the formula: $V = \frac{4}{3} \cdot \pi \cdot r^3$. The biovolume unbranched chains of barrel shaped cells assumed cylinder shape: V= $\pi \cdot r^2 \cdot h$. The mean volume of the cells was multiplied by the mean number of cells in the respective replicates for each sampling site.

Sparkvue® software was used to extract light data from PACSO® wireless light sensors. Albedo was calculated by taking the ratio of reflected to incident radiation (400-700 nm range). HOBO-logger measurements on the glacier were treated as relative values from the soil logger as all the loggers placed on the snow were tipped over. Therefore, the logger data from the HOBOs could not be used to determine the actual atmospheric variation in temperature and light intensity.

Statistical differences in albedo and biovolume between sites was determined with a One-way ANOVA, followed by a Tukey's HSD. Correlation between algal biovolume and snow surface albedo was determined with a Pearson's correlation test. R studio software was used for data analysis and visualization of data. P-values <0.05 were defined as significant.

Results

Microscopy revealed several different morphological types of snow algae. Due to the encystment of the majority of the red snow algae samples, it was difficult to determine the species in the sample. However, the oval green algae in the green snow and the round cyst in the red snow was identified to be *Chlamydomonas nivalis*, and the long green algae in the green snow to be *Koliella sp* based on previous literature on snow algae species (Kol, 1968) (photo 2).

When examining the mean biovolume of snow algae at the different sites (Figure 1), it was found, that the highest biovolume was at the cryoconite holes (C) sites. Green snow (GS) had the second highest biovolume while the biovolume of snow algae on the snow on the glacier (S) and the snowfields (SF) not connected to the glacier, was lowest and almost the same level. Lastly, the lowest biovolume of snow algae was at the clean snow (CS) sites, which were taken as reference samples

The measurements of the mean at the different sites showed that CS had the highest albedo and was significantly higher than the rest of the sites (Figure 2). Surface albedo at SF and GS was very similar. The site with the second highest albedo was S followed by the SF and the GS very close to that site. The site with the lowest albedo was the C. All the results of the albedo measurements were significantly different (P=<0.01), except SF-

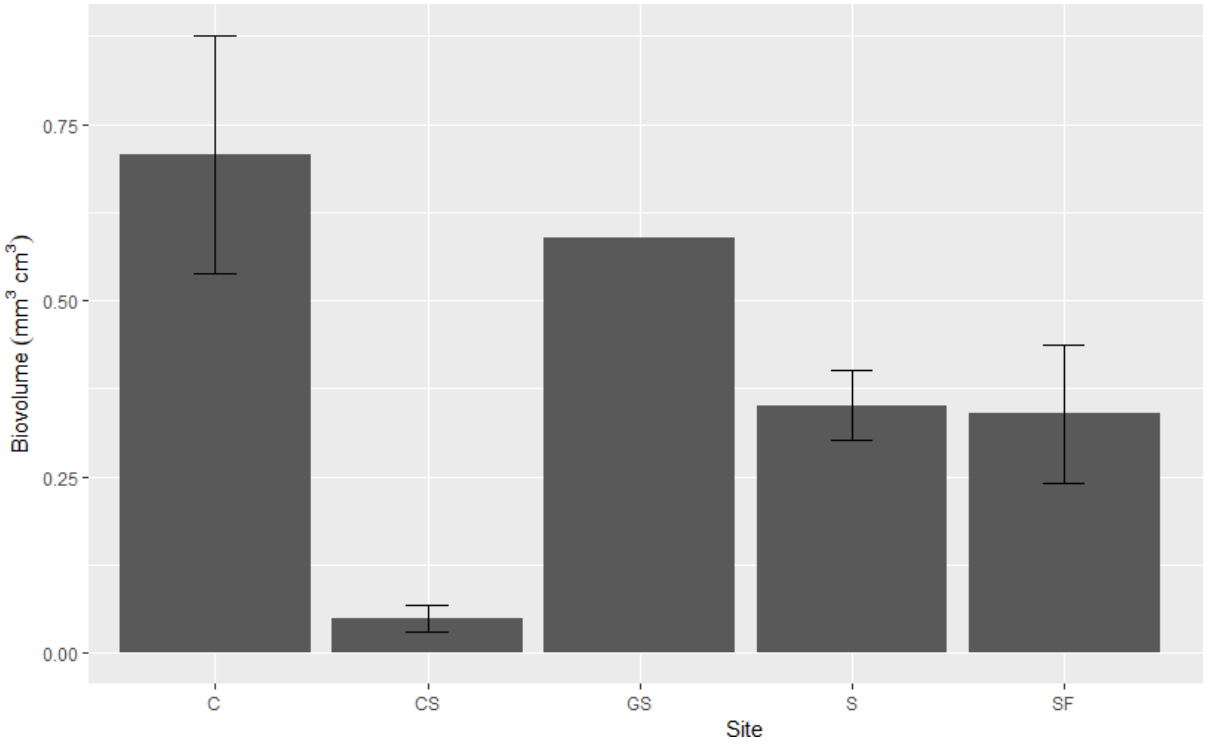

Figure 1. Biovolume (mm^3cm^{-3}) ±SE of snow algae in different environments: C= cryoconite holes, CS= clean snow, GS= green snow, S= red snow, and SF= snowfield. Each bar represents the average of five individual measurements with error bars shown.

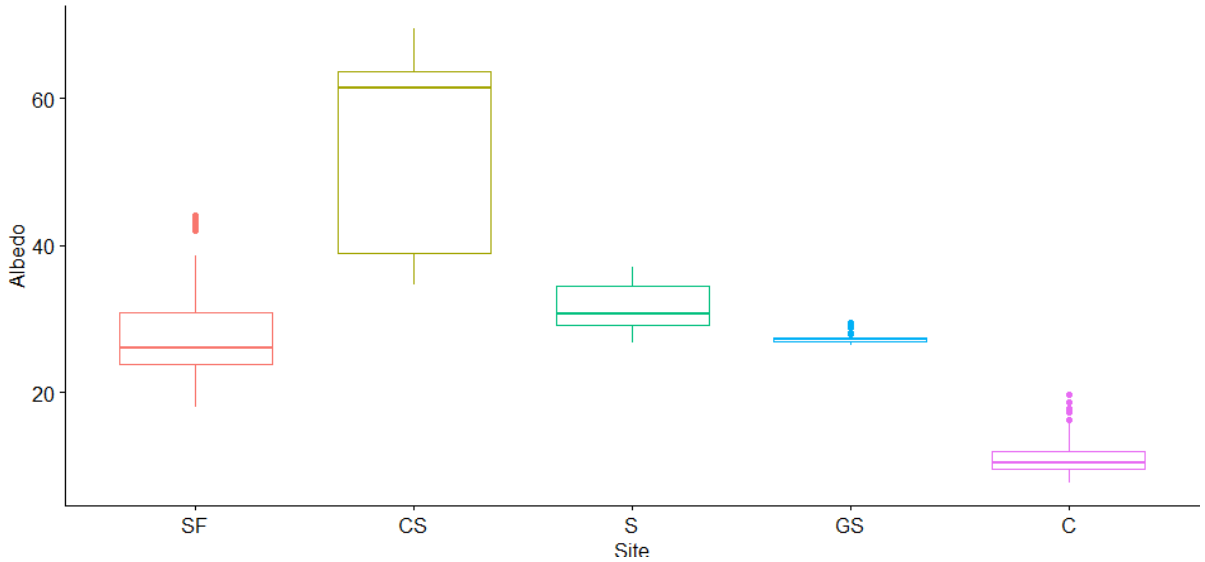

Figure 2. Surface albedo at the different sites: C= cryoconite holes, CS= Clean snow, GS= green snow, S= red snow, and SF= snowfield. All sites had significantly different surface albedo (p=<0.01) except SF-GS.

GS (P=0.09).

It was also found, that the site with the highest albedo was the clean snow found on the glacier. The site with the second highest albedo was found in CS, which also had the lowest biovolume. At the site of SF, the albedo was lower than CS but higher than the albedo found in the site of the green snow, though was the bio volume higher in the GS than that found at the SF. The site with the lowest albedo and highest biovolume was found at the sites of the C. The correlation between surface albedo and biovolume of snow algae was significant (p=0.015; Pearson correlation: -0.95).

The diurnal temperature and relative light measured over 36 hours at the different sites illustrated that, the temperature and light intensity fluctuated daily with the highest level at 12:00 in the afternoon and lowest at 00:00 at night (Figure 4 and figure 5). This data was considered as relative values of the soil temperature.

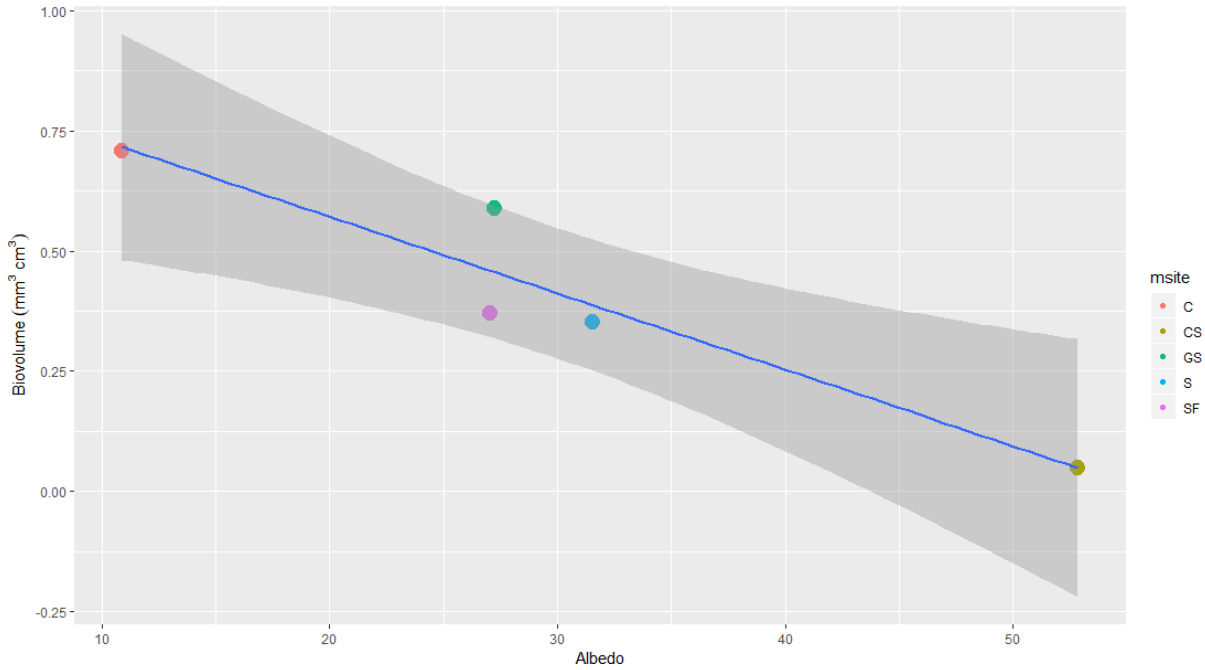

Figure 3. Mean albedo of different habitats compared to the biovolume with trend line (y=-0.0159x+ 0.8898) in blue and standard error marked by grey shadow: C= cryoconite holes, CS= clean snow, GS= green snow, S= red snow, and SF= snowfield (P=0.01, Pearson's coefficient: - 0.95).

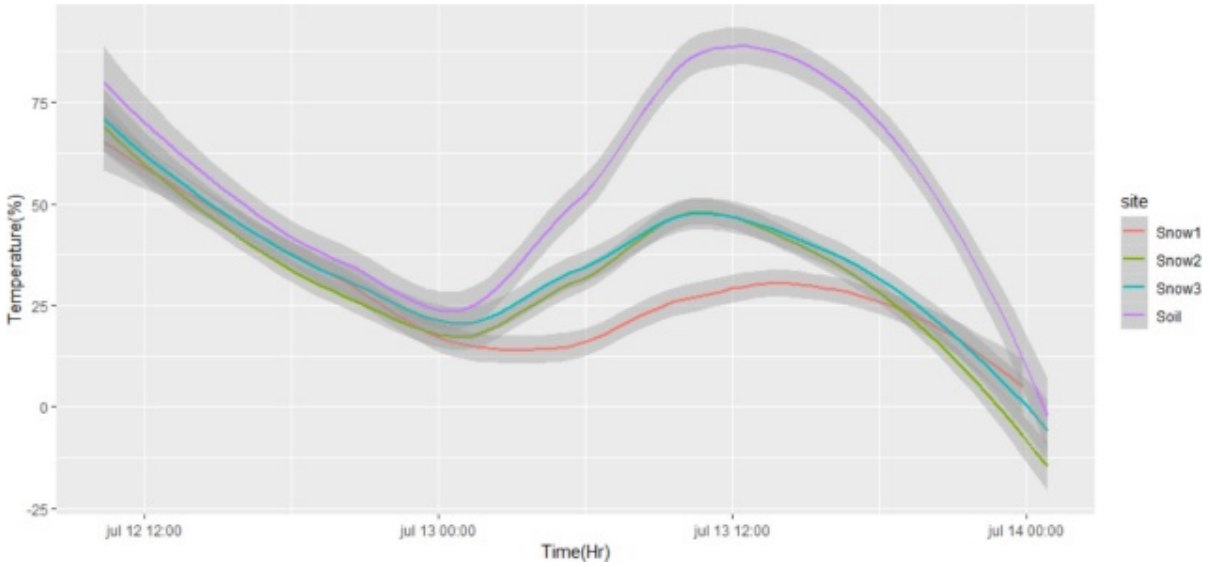

Figure 4. Relative diurnal temperature (°C) at different sites (see table 1 in appendix 1) measured with HOBO loggers. Sites Snow1-Snow3 were placed on the glacier should be considered relative values of the temperature from the Soil site (grey shadow= standard error).

Discussion

The aim of this study was to determine the biovolume of snow algae of different habitats in the glacial biome. As expected, the cryoconite hole sites had the highest biovolume of algae. Advantageously, snow algae in cryoconite holes are not limited by water, thereby not at risk of desiccation. Cryoconite sites were typically found on a plateau at the bottom of a slope on the glacier. Therefore, it is likely that algae, and other debris, are transported by supraglacial streams and deposited into the cryoconite holes and accumulated over time. Liquid water also indicates that the nutrients on the glacier are available, unlike snow, where nutrients can be unavailable due to it being frozen. This is most likely due to the dryness of the snow (appendix 3) as has been reported in a previous study (Lutz et al., 2015).

The mean albedo was highest in CS (52%) and lowest in C (11%) most likely due to the large amount of light absorbing impurities e.g. soot,

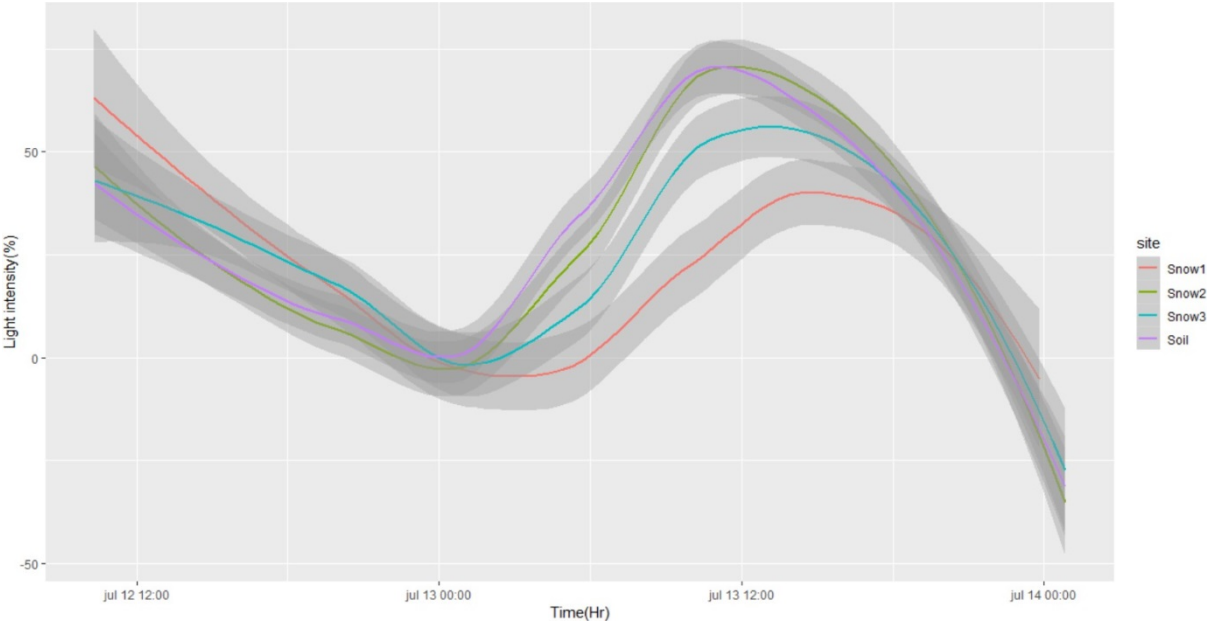

Figure 5. Relative light intensity (Lux) data from HOBO loggers at different sites taken with HOBO loggers. Sites Snow1-Snow3 were placed on the glacier should be considered relative values of the Soil temperature (grey shadow=standard error).

dust and black carbon (Huovinen, 2018). leading to a low albedo. The same has been recorded by other studies (Takeuchi, 2001)

The results from the Pearson correlation test between algal biovolume and surface albedo indicates that there is a correlation between the biovolume of snow algae and the surface albedo ($p=0.015$), which supports our hypothesis and expectations. This correlation between biovolume of algae and surface albedo suggests snow algae to be a primary factor for reducing albedo of the snow's surface. Reduced albedo can result in increased snow melt rates as the material absorbs the light energy and is partly converted to heat energy and thereby melting the snow (Dial et al., 2018). At the sites where albedo was measured, the area was not pure algae in the snow. As discussed earlier, light absorbing particles were present and would also affect the surface albedo (Huovinen, 2018). Therefore, these results cannot quantify the exact effect of snow algae on surface albedo. It can however give an indication of its impact. Samples categorised as 'clean snow' had the highest albedo, which was expected due to no light absorbing impurities present at first sight. From a visual inspection in the field, the snow samples defined as 'clean snow' did not hint a red colour, however the microscopy work revealed there to be snow algae present (figure 1), although significantly fewer than the other sample sites. The clear snow sites seemed to have drier snow (appendix 1), which also affects the surface albedo (Painter et al., 2016).

Unexpectedly, green snow was found on the edge of the glacier on 13th July. Green snow appears when the snow algae are in the vegetative state, and this is usually found in the beginning of the melting season (Hoham and Duval, 2001; Remias et al. 2005). High irradiance, UV exposure and low nutrient availability have been suggested as the main triggers for snow algae to transform into cysts (Remias et al., 2005). The green snow was at the foot of a tall hill, which might explain its presence. The hill overshadows the patch of snow where the green snow was found. This would lower the high irradiance and UV exposure while red snow algae were exposed to near constant sunlight in summer in the arctic. The pH in the green snow was also higher than the red snow (appendix 1).

Interestingly, the green snow did not affect the correlation between biovolume and snow surface albedo. It could be expected that the green colour affected the surface albedo differently than the red snow. Red colour absorbs more light than green (Dial et al., 2018). As discussed earlier, the samples collected were not purely algae but contained other light absorbing impurities, which were also present in the green snow.

Results from the diurnal measurements of temperature and light intensity confirmed our hypothesis, that snow algae experience considerable variation throughout a 24 h period. Even though there was midnight sun, during the measurements, there was still variation as a result of the positioning of the sun. This affects how much light and what temperature the different locations, and algae, are exposed to. (McKinn et al., 2003). As mentioned earlier, diurnal variations in light and temperature is likely a important factors of the encystment of algae. High irradiance and UV exposure, as well as continuous freeze-thaw cycles are serious stress factors (Remias, 2005; Seckbach, 2013)

While red snow clearly has an impact on the physical properties of glaciers (e.g. reducing surface albedo which has potential to increase snow melt rates (Lutz et al., 2016), accumulation of snow algae on the snow surface can affect the chemical environment of the glacier by the carbon and nutrient cycling of algae. High photosynthesis rates of snow algae contribute majorly to supraglacial carbon fixation (Seckbach, 2016).

Snow algae experience little competition for space and nutrients in their habitats, which is why one species forms monospecific blooms that can be found year after year. Red snow has an ecological significance as many organisms feed from the algae such as copepods and ciliates. Several *Paramecium sp*. were found in the snow samples for this study. Other organisms such as psychrophilic bacteria and snow fungi also benefit from the algae's ability to cycle nutrients (Remias, 2012). There are various nutrient

sources e.g. dust, pollen, organic debris deposited on the snow by precipitation, wind or animals (Seckbach, 2013). It has previously been reported that the spatial distribution of snow algae patches is correlated to spatial variability of the nutrient loads on the snow pack. Furthermore, proximity to bird colonies that enrich nutrient concentrations has been previously reported to impact the distribution of snow algal blooms (Seckbach, 2016). It has also been reported that snow algal blooms cause nutrient depletion of especially NO_3-N, NH_4-N and SO_4^{2-} (Seckbach, 2016) and links well with previous studies that N- limitation triggers the production of secondary carotenoids (Thomas et al., 2008; Remias et al., 2005).

With ongoing climatic changes towards warmer winters, it may be speculated that a rise in the red snow algae density on and around the Lyngmarksbræen glacier could happen in the future. This rise in global temperatures could lead to a longer melting period and subsequently exposing snow algae to more available liquid water, prolonging the vegetative and reproductive stage. Increased N-deposition in the arctic (Forsius et al., 2010) and an increase in available nutrients from liquid water could further facilitate a longer proliferation periods. (Lutz et al., 2016)

With a longer period of liquid water and increased nutrient availability for growth in the the snow algae can form the red cyst stage later in the season when the irradiation becomes too high throughout the summer. These blooms can then further make more water available due to the lowering of the albedo on the snow surface, which could then lead to a higher density in snow algae on and around the glacier (Lutz et al., 2014).

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Appendix 1: Physicochemical properties of sites

Physicochemical properties of each site. C= cryoconite holes, S=snow, SF= snowfield, GS= green snow

Sample site	Surface temperature (°C)	Slope (°)	Direction	Snow type	pH	Elevation (m)	Coordinates
C1	0,7	NA	South	NA	5,6	857	N69° 17.602' W53° 35.672'
C2	0,6	NA	East	NA	5,4	846	N69° 17.622' W53° 35.560'
C3	0,7	NA	West	NA	5,6	858	N69° 17.634' W53° 35.797'
S1	0,5	4	Northwest	6/4,5	5,9	888	N69° 18.393' W53° 35.891'
S2	0,6	5	South	6/4,5	5,4	893	N69° 19.118' W53° 36.514'
S3	0,7	3	West	6/4,5	6,3	876	N69° 18.547' W53° 36.119'
SF1	1	15	West	6/4,5	5,9	788	N69° 16.921' W53° 34.544'
SF2	1,1	7	South	6/4,5	5,6	792	N69° 17.189' W53° 34.970'
SF3	0,7	10	North	6/4,5	5,4	755	N69° 17.048' W53° 34.625'
GS	1,1	14	East	06-apr	7,7	808	N69° 17.312' W53° 35.833'

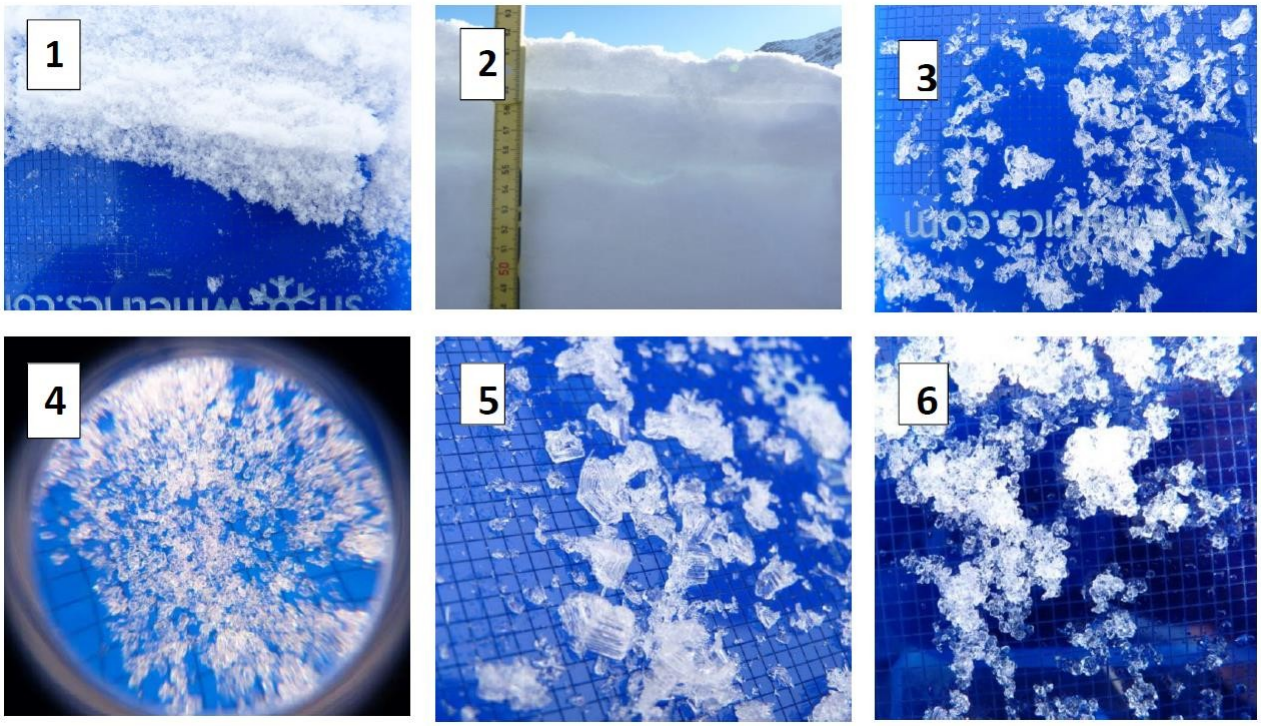
Appendix 2: Liquid water content guide

Guide to determine liquid water content in the field from handheld snow observations. The index (mWC) is a qualitative estimation of liquid water content and θ is the volume fraction of liquid water (Techel and Pielmeier, 2011).

Wetness Content	Index (mWC)	Description	θ [vol. %]
Dry	1	$t_s \leq 0.0\,^{\circ}C$. Disaggregated snow grains have little tendency to adhere to each other when pressed together.	0
Moist	2	$t_s = 0.0\,^{\circ}C$. The water is not visible, even at 10× magnification. When lightly crushed, the snow has a tendency to stick together.	0–3
Wet	3	$t_s = 0.0\,^{\circ}C$. The water can be recognized at 10× magnification by its meniscus between adjacent snow grains, but water cannot be pressed out by moderately squeezing the snow in the hands.	3–8
Very Wet	4	$t_s = 0.0\,^{\circ}C$. The water can be pressed out by moderately squeezing the snow in the hands, but an appreciable amount of air is confined within the pores.	8–15
Soaked	5	$t_s = 0.0\,^{\circ}C$. The snow is soaked with water and contains a volume fraction of air from 20 to 40 %.	>15

Appendix 3: Snow type guide

Guide to determine snow crystal type from Geobasis monitoring programme (Sigsgaard, 2018)



Picoplankton distribution in Disko Fjord and Disko Bay (West Greenland)

Collecting water samples aboard Porsild (photo: Nina Lundholm)

Picoplankton distribution in Disko Fjord and Disko Bay (West Greenland)

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Abstract

Knowledge about the Arctic is important for predicting future scenarios of the world's changing climate. Picoplankton dominates the arctic phytoplankton community for substantial parts of the year and could be increasing in importance with the changing climate. An investigation of the correlation between environmental parameters and absolute and relative picoplankton biomass distribution was carried out. The area investigated was around Disko Island in West Greenland, with four stations in Disko Fjord and two in Disko Bay (including the permanent station). The Chlorophyll *a* (Chl *a*) content, used as proxy for biomass, was measured for samples taken at 5 and 30 m depth, and filtered into three different size fractions: picoplankton (0.8 - <3 µm), nanoplankton (3 - <20 µm) µm and microplankton (>20 µm). Salinity and temperature were measured and recorded. The range of total Chl *a* was 0.00-1.56 µg L^{-1}. The highest Chl *a* measured for picoplankton was 0.7 µg L^{-1} at 30 m in the mouth of the Disko Fjord. The relative amount of picoplankton ranged from 4.4% at 30 m up to 55.4% at 5 m, with both measurements from Disko Bay. Stations were significantly different in amount of Chl *a* picoplankton biomass, but not with regard to the relative amount of Chl *a* picoplankton biomass. The relative amount of Chl *a* picoplankton biomass varied significantly between depths, while that was not the case for absolute amount of Chl *a* picoplankton biomass, indicating that the change in relative Chl *a* biomass was due to changes in Chl *a* biomass of the other two size fractions. No significant distribution differences were found for absolute and relative picoplankton biomass between transects, nor between inner and outermost stations for relative Chl *a* picoplankton biomass to total Chl *a* biomass in both the Disko Fjord and Disko Bay. Furthermore a negative correlation was found between total Chl *a* biomass and relative Chl *a* picoplankton biomass. Temperature correlated positively to the relative amount of picoplankton Chl *a* biomass, but due to covariation between the environmental parameters the results should only be considered as an indication. The findings support previous studies from the Arctic.

Keywords: Phytoplankton size-fractions, picoplankton distribution, Chlorophyll *a* biomass, Disko Bay, Disko Fjord.

Introduction

Phytoplankton in the ocean play an important role as primary producers, and are responsible for up to ~ 50% of the world's primary production (Finkel et al. 2009). Changes in primary production within the arctic region, may have a profound impact on the ecosystem compared to other regions, as there are fewer trophic levels (Simo-Matchim et al. 2016; Wassman and Reigstad 2011). The changing climate has been predicted to have the largest and fastest impact in the Arctic (Trembley et al. 2015). The changing parameters include higher air temperatures, increased precipitation, increased stratification, intensified freshening, altered ocean circulation and decreasing ice cover (Li et al. 2009; Finkel et al. 2009; Coupel et al. 2015). The biological pump exports carbon to the deep sea and keeps it out of contact with the atmosphere over longer periods of time. The changes mentioned above can each affect the biological pump which is partially responsible for the ocean being a long-term carbon sink/source for atmospheric carbon (Finkel et al. 2009). The amount of carbon exported depends partially on the community size structure of the phytoplankton. Smaller

phytoplankton increase the rate of fast carbon turnover through the microbial loop, while larger phytoplankton increase the sedimentation rate of carbon (Finkel et al. 2009). In a global climate perspective, it is therefore important to understand what affects the community size structure (Hilligsøe et al. 2011, Finkel et al. 2009)

The phytoplankton community size structure is divided into three major size groups: picoplankton (0.2–2 µm), nanoplankton (2-20 µm) and microplankton (>20 µm) (Marañón et al. 2012). The size definitions, however, change slightly from study to study. In this study, the size groups are defined as picoplankton (0.8-3 µm), nanoplankton (3-20 µm) and microplankton (>20 µm). The sinking rate of the cells vary with size, with the smaller ones being more resistant to sinking (Li et al. 2009; Hilligsøe et al. 2011). Smaller phytoplankton have a larger surface-area-to-volume and therefore have an improved ability to take up nutrients, and they are more efficient at harvesting light in the water (Li et al. 2009; Raven 1998). Consequently, smaller phytoplankton are better equipped for low light and low nutrient situations (Marañón et al. 2012). Small-sized phytoplankton are predicted to increase in number with a warmer climate, according to the theory that body sizes shrink as climates warm (Peter and Sommer 2012). If relative abundance of picoplankton increases in the ocean it could create a cascading negative effect on the carbon sink (Finkel et al. 2009).

There is an ongoing discussion about which environmental factors are controlling the distribution and abundance of picoplankton (Marañón et al. 2012; Mousing 2013). Some of the environmental factors assessed are temperature, nutrient availability and salinity (Agawin et al. 2000; Morán et al. 2010, Hilligsøe et al. 2011; Tremblay et al. 2015). Specifically, temperature has caused debate to whether it independently affects the size fractions due to its anti-correlation with nutrient supply (Marañón et al. 2012). Increasing temperatures increase metabolic rates and therefore nutrient demand, with small phytoplankton cells being more equipped for the competition. Increasing temperatures, however, also enhance stratification and therefore create nutrient depletion, resulting in interaction between the two parameters (Mousing 2013). Temperature can also affect the growth rates and grazing pressure on different size classes (Mousing 2013). Salinity similarly creates the physical and chemical environment for the phytoplankton. It causes stratification due to altered water densities. Furthermore, salinity can have an impact on phytoplankton physiology by changing the synthesis of osmoticums, which are needed to avoid osmotic issues potentially resulting in cell damage (Finkel et al. 2010; Simo-Matchim et al.2016).

Picoplankton is well adapted to the arctic environment and dominates the community for large parts of the year. However extensive knowledge on the picoplankton distribution and what shapes it in the Arctic is lacking (Metfies et al. 2016). To investigate the picoplankton distribution in the Arctic, the present study looked at the biomass of different phytoplankton size fractions by measuring chlorophyll a (Chl *a*) at two different depths. Furthermore, environmental parameters such as salinity, temperature, and nutrients were measured. The chosen study areas were Disko Fjord and Disko Bay. Disko Fjord is situated on the west coast of Disko Island, Greenland with no glacier flowing directly into the fjord, but with freshwater inputs from the Steenstrup Glacier. It is influenced by Atlantic water that is considered to be relatively warm. The saline water from the West Greenland Current enters the fjord as a deep current, while fresh turbid water flows out on the surface during most of the summer (Andersen 1981; Schmid and Piepenburg 1993). The other study site, Disko Bay, is affected by meltwater associated with the Jakobshavn Glacier and also by the West Greenland Current. The surface salinity measured in summer-time seems to have lowered over time, as a result of increased freshwater input from the glacier (Hansen et al. 2012). Upwelling of the West Greenland Current in summertime happens in both sites (Andersen 1981). By looking at both areas it is possible to compare sites to see if the higher impact of freshwater plays a role in the distribution pattern.

The hypothesis of this study is that the relative biomass of picoplankton is lower in the fjord compared to the Disko Bay due to higher nutrient concentrations associated with glacier input. The lower salinity associated with glacier input may also explain the pattern in biomass distribution. Furthermore, a difference in the picoplankton

biomass between the beginning of the fjord and the mouth, and closer and further from shore in the Disko Bay could also be visible due to different effects of nutrients. At last the temperature might be able to explain some of these patterns too.

Methods and Materials

Sampling

The sampling took place on the 10th and the 13th of July 2019 using the RV "Porsild". A Niskin sampler (KC Denmark A/S Research Equipment) were used to sample at six different stations at 5 and 30 meters to get an overview of the photic zone, expecting the Chl max to be around 30 m. The sampling was done in the Disko Fjord (station D1, D2, D3, D4) and in the Disko Bay (P1, P3 – also known as the permanent station) (Figure 1). Station P2 was also sampled but due to time limitations not used in further analysis. Each sample consisted of water from three different Niskin samplings to get a more representative sample. Since the boat moved during sampling the water samples were not all collected at the exact same position, however the longitude/latitude used in this study is from where the CTD data was measured. The sample was put into a 10-liter dark blue carboy via a funnel and the temperature and salinity were immediately measured. A CTD (SBE 19plus V2) measured salinity, temperature and fluorescence through the whole water column at each station. The samples were put into a cooling box with cooling elements right after sampling and kept there until transferred into a cooling container. The samples were only outside a cooling-controlled area when transported between the harbor and the Arctic Station.

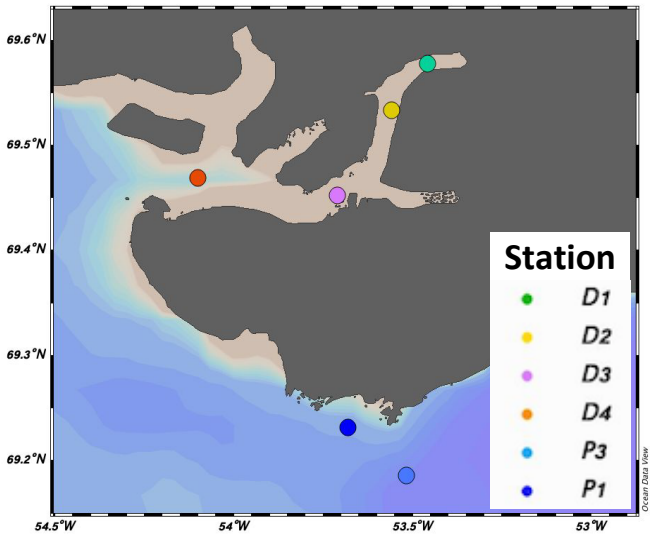

Figure 1. Map showing a part of Disko Island, the Disko Bay (P1 and P3) and Disko Fjord (D1, D2, D3 and D4), including the 6 stations were the sampling took place.

Filtration

All samples were filtered in triplicates 22-30 hours after sampling. A test showed no effect on Chl *a* content if a sample waited overnight before filtering and extraction . Three liters were filtered for each replicate. The water used for filtration was kept in a temperature-controlled container. The filtration of the microplankton fraction took place using a nylon mesh (20 µm). A test had been performed to see if including the >200 µm fraction would have a large effect on the Chl *a* measurement. The fraction did not seem to have a large effect and was therefore included to also measure potential chains of phytoplankton. The nylon mesh was backwashed into a 50 ml falcon tube using 0.8 µm filtered seawater. The water that had passed through the 20 µm nylon mesh was kept in a beaker. The beaker was used to transfer the water in a cooler from the container to the lab, together with the falcon tube containing the microplankton fraction. In the lab, the microplankton fraction was filtered onto a Glass microfiber filter GF/F diameter 47 mm (Frisenette) by either of two filtrations setups: A PALL life sciences Vacuum/Pressure Pump with a Pall Corporation Product No. 15402 filter tower, or a KNF Neuberger Vacuum/Pressure Pump with a Combisart sartorius 16828-CS filter tower. The water from the beaker was filtered through a 3.0 µm Nuclepore Track-Etch Membrane (Whatman) or a 0.8 µm Nuclepore Track-Etch Membrane (Whatman). It should not have an impact on the Chl *a* measurement that different filters were used for the filtration (Chavez et al. 1995). Between 0.6-2 liters of water were used for the >3.0 µm fraction and 0.5-1.5 liters of water for the >0.8 µm depending on the speed of the filtration due to time limitations of the experiment.

Measurement of chlorophyll a

Chl *a* measurement can be used as a faster method to estimate the biomass of phytoplankton compared to using microscopic count and mean volumes. Furthermore, microscopic counting is especially difficult when looking at the smallest phytoplankton size fractions. Chl *a* measurements are, however,

only a proxy for the biomass. Although the amount of chlorophyll per cell might vary, there is a strong overall correlation between Chl *a* content and phytoplankton biomass (Sandu et al 2003). Additionally, phytoplankton biomass can be a measure for abundance (ICPDR 2001).

To extract Chl *a,* filters were put into 15 ml falcon tubes using forceps. The tubes were covered in tinfoil to avoid light degradation. 10 ml of 96% ethanol was added to each tube. All filters were soaked in ethanol for 12-24 hours in a dark refrigerator at 5°C (Jespersen and Christoffersen 1987). Before measuring Chl *a*, all samples were flipped upside down 10 times and centrifuged at 2000 pm for 2 min in a MULTIFUGE 3 s Heraeus (Kendro Laboratory Products) to avoid filter bits. Using a VWR mini pipette, the extracted Chl *a* was put into round glass vials and measured using a Trilogy fluorometer (Turner designs). The chlorophyll *a* concentrations were calculated using the following equation (Turner Designs 2019):

Chl *a* concentration =

$$Cstand \times \left(\frac{(Fsamp - Fblank)}{(Fstand - Fblank)}\right) \times \left(\frac{Vsolvent}{Vwater}\right)$$

Cstand: Concentration of standard
Fsamp: Fluorescence of sample
Fblank: Fluorescence of blank
Vsolvent: Volume of solvent (Ethanol 96%)
Vwater: Volume of water filtered for the sample

The limit of detection on the Trilogy laboratory fluorometer for Chl *a* Extraction (Non-Acidification) measurements was 0.025 µg/L (Turner Designs 2019).

Nutrient analysis

At each station, a nutrient sample was taken at 5 and 30 m, and put into a 50 ml falcon tube. The sample was immediately transferred to the cooling box and put into a freezer (-20°C) upon arrival at the lab. The samples were unfortunately not able to be analyzed and could therefore not be included in the analysis.

Microscopy

A qualitative survey of the species in the >20 µm fraction was performed for each station (with the exception of station 1) using a BX51 Olympus microscope. Either a net sample or a filtered sample was used. The samples included both fixed and live samples.

Programs

The CTD data was processed using SeaTermV2 and converted by SBEDataProcessing-Win32. Ocean Data View was used to plot the data.

Statistics

The Chl *a* values from the picoplankton size fraction had to be transformed by using log10 because it did not seem to be homoscedastic when tested with Levene's test for equality of variance. Furthermore, a histogram of the data showed that the Chl *a* values from the picoplankton size fraction was not normally distributed. To avoid issues with the value zero when transforming the data, the lowest Chl *a* measurement above zero, 0.01 µg/L^{-1}, was used for all zero measurements. When transforming the data with log10 a normal distribution was observed. A One-way ANOVA using SPSS statistics was used to look at picoplankton in relation to the stations, depths and transects. Furthermore, the relative amount of picoplankton Chl *a* compared to total Chl *a*, was calculated in percent. This percentage was then logit transformed using a logit function in R, from the package 'boot', to obtain something closer to normal distribution (Canty and Riply 2017). However, since the data was skewed and had a significant p-value (p-value = 0.00) in the Shapiro-Wilk normality test, the non-parametric test Kruskal-Wallis was used to see if the samples were significantly different. The post hoc test Dunn's test for looking at stochastic dominance was used with the Bonferroni adjustment to avoid type 1 errors, by using the package 'dunn.test' in R (Dinno 2017). The Kruskall-Wallis tests, Mann Whitney U tests and Spearman-Rank correlations were all performed in SPSS statistics (IBM corp. 2019). The data was checked for monotonic relationships before performing Spearman-Rank correlations.

Table 1. Table with the station data (max depth, CTD max depth and position), including the measured temperature, salinity and depth for each sample.

Location	Latitude	Longitude	Depth (m)	Temperature (°C)	Salinity	CTD max depth (m)	Max depth (m)
Station D1	69.57800	-53.45690	5	5.9	31	80	106.8
			30	3.2	37	80	106.8
Station D2	69.53355	-53.55767	5	6.5	32	80	113.3
			30	2.4	38	80	113.3
Station D3	69.45218	-53.70955	5	6.3	33	80	113.7
			30	3.4	35	80	113.7
Station D4	69.46903	-54.10050	5	6.3	33	150	176.7
			30	2	36	150	176.7
Station P1	69.23080	-53.68133	5	5	34	120	143.3
			30	1.4	35	120	143.3
Station P3	69.18520	-53.51658	5	6.6	35	300	348
			30	1.6	35	300	348

Results

Temperature

The six stations had a temperature range between 5.0-6.6 °C at 5 m, with the highest temperature at station P3 and the lowest at P1 (Table 1). At 30 m, the range was 1.4-3.4 °C (Table 1), with the highest temperature at station D3 and the lowest at station P3. At ~ 50 m, the temperature at all stations had dropped to under 2 °C (Figure. S1.a).

Salinity

The salinity at 5 meters ranged between 31-35, with the lowest at station D1 and the highest at P3 (Table 1). At 30 m it was between 35-38 with the lowest at stations D3, P1 and P3 and the highest at station D2 (Table 1). At ~ 7 m all stations had a salinity >35 (Figure S1.b). The four stations from the Disko Fjord (D1, D2, D3 and D4) had different salinities in the first ~ 5 m, while station P1 and P3 in the Disko Bay seemed to have a relatively stable salinity gradient all through the water column (Figure S1.b).

Fluorescence data

The fluorescence peaked for Station D1 and D2 at ~ 3 m (Figure S1.d). Station D3 had its highest fluorescence at ~ 9 m but did not peak as high as the other stations (Figure S1.d). Station D4 had the highest peak observed, and peaked twice, ~ 15 and ~ 30 m (Figure S1.d). Station P1 and P3 peaked at ~ 20 m (Figure S1.d).

Chl a

The total Chl *a* measurement at 5 m was in the range of 0.14-1.52 µg L^{-1} with the lowest at station P3 and the highest at station D2 (Figure 2). For 30 m, the range was 0.03-2.58 µg L^{-1} (Figure 2), with the lowest at station D1 and the

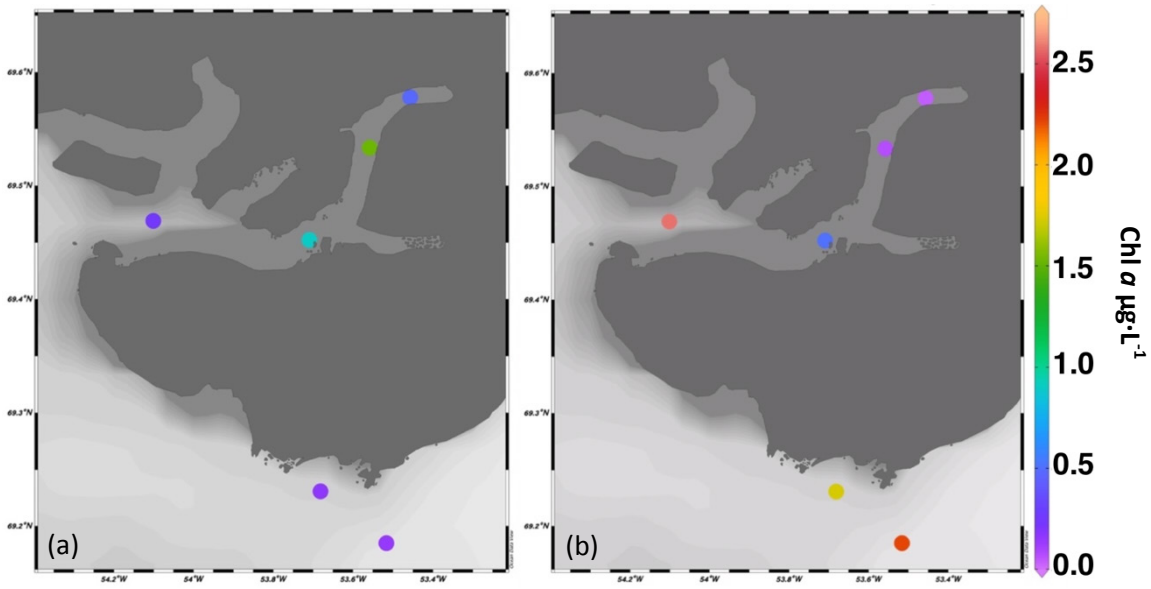

Figure 2. Maps showing the total Chl *a* at each station. (a) depicting the total Chl *a* at 5 m for all size fractions and (b) the total Chl *a* at 30 m for all size fractions.

highest at station D4 (Figure 2).

The microplankton size fraction at 5 m ranged between 0.00-0.06 µg L^{-1}, with D1 having the lowest and D4 the highest (Figure 3). At 30 m it ranged between 0.00-1.56 µg L^{-1} with the highest at P3 and the lowest at station D1 and D2 (Figure 3).

The nanoplankton size fraction at 5 m ranged between 0.04-1.14 µg L^{-1}. The highest value at station D2 and the lowest at station P3 (Figure 3). At 30 m the range was 0.03-1.67 µg L^{-1}. Station D4 had the highest value and station D1 the lowest (Figure 3).

The picoplankton size fraction ranged from 0.07-0.32 µg L^{-1}, with station D3 having the highest

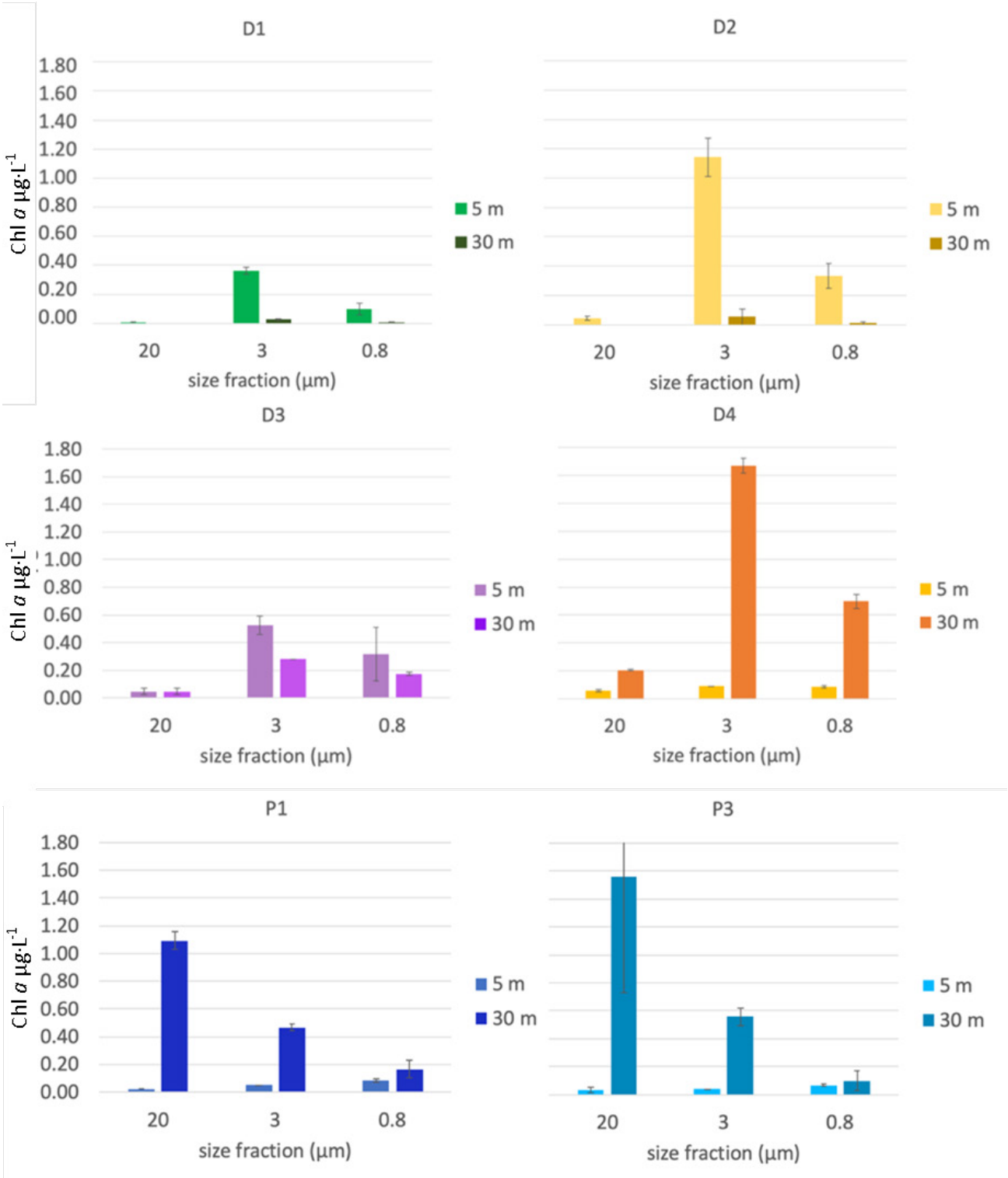

Figure 3. Bar graphs showing the Chl *a* µg·L^{-1} for each size fraction at 5 and 20 me including error bars depicting the standard deviation. Microplankton = >20 µm, nanoplankton = 3-20 µm and picoplankton = 0.8-3 µm.

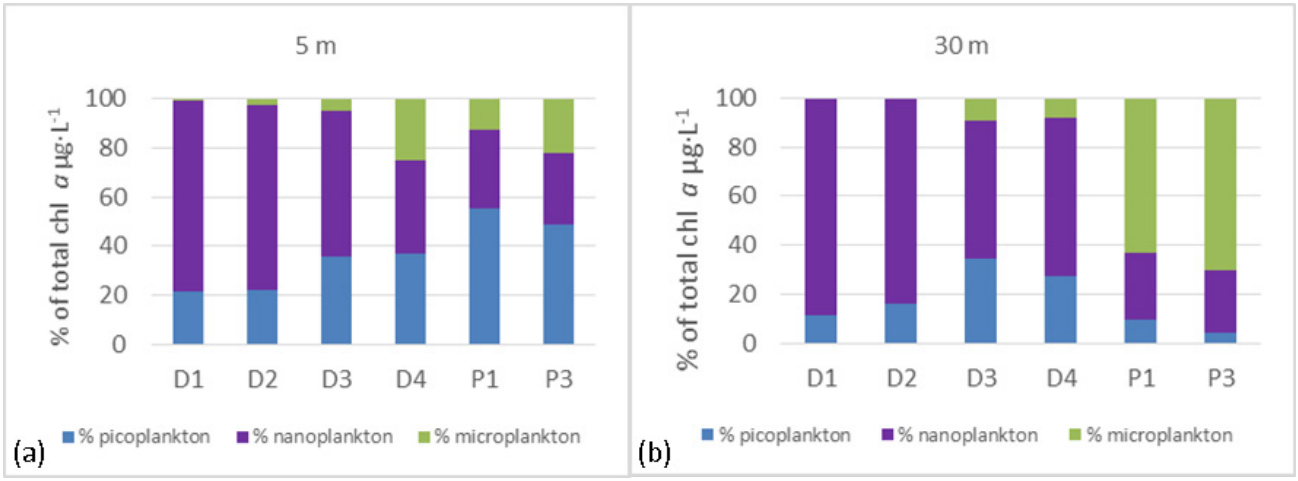

Figure 4. Bar graphs showing the percentage (%) of Chl *a* µg·L^{-1} for each size fraction at 5 and 20 m out of the total Chl *a* µg·L^{-1} .

value and station P3 the lowest (Figure 3). At 30 m the range was between 0.00-0.7 µg L^{-1}, with the highest at D4 and the lowest at station D1 (Figure 3).

The highest percentage of picoplankton relative to the total was observed at P1 with 55.4% at 5 m. The lowest at 5 m was 21.4% at station D1 (Figure 4). The lowest overall at 30 m was 4.4% at station P3 (Figure 4). The highest observed at 30 m was 34.7 % (Figure 4).

Microplankton diversity

Microplankton observed at station D2 was primarily athecate dinoflagellates and *Dinophysis* spp. At station D3 also the genera *Thalassiosira, Chaetoceros, Leptocylindrus, Protoperidinium* and athecate dinoflagellates were observed. At station D4, *Thalassiosira* spp., *Chaetoceros* spp., *Leptocylindrus danicus*, centric diatoms and *Protoperidinium* spp. were prevalent. Station P1 and P3 had similar species present such as *Thalassiosira* spp., *Chaetoceros* spp., *Leptocylindrus danicus* and *Eucampia zodiacus*.

Statistics

The absolute amount of Chl *a* picoplankton biomass was significantly different between the stations (One-way ANOVA p-value: 0.02). Differences between depths and between the two transects were not significantly different (One-way ANOVA p-value: 0.23; p-value: 0.77).

The relative amount of Chl *a* picoplankton biomass was not significantly different between transects, and furthermore not between stations (Kruskall-Wallis p-value: 0.97; p-value: 0.48). The relative amount of Chl *a* picoplankton was, however, significantly different between depths (Kruskall-Wallis p-value: 0.00). A Kruskall-Wallis test looking at the relative amount of Chl *a* picoplankton biomass from each sampling was found to be significantly different (Kruskall-Wallis p-value: 0.00) A post hoc test was furthermore carried out (Dunn-test) on the Kruskall-Wallis test. This shows where in the dataset the differences would be found. The main differences were between the two depths (5 m and 30 m) at station P1 and P3 or in between the Disko Bay stations (P1 and P3) and the Disko Fjord stations (D1, D2, D3 and D4). The differences were mostly found between the 5 and 30 m samples.

The relative amount of picoplankton biomass to total phytoplankton biomass were compared between station D1 and D4, and P1 and P3, and none of them showed significant differences in their distributions (Mann Whitney U -test p-value: 0.07; p-value:0.33). The relative amount of Chl *a* picoplankton biomass was significantly positively correlated with the Disko Fjord transect spanning from the innermost stations and out to the mouth of the fjord. This was, however, only the case at 5 m (P-value: 0.04; correlation coefficient: 0.76), not at 30 m (P-value: 0.36).

When comparing the relative amount of Chl *a* picoplankton biomass to environmental and physical parameters, temperature was found to be significantly positively correlated (P-value: 0.00, correlation coefficient: 0.6) while depth (P-value 0.00, correlation coefficient: -0.52) and total Chl *a* biomass (P-value: 0.01, correlation coefficient: -0.44) were significantly negatively correlated. Salinity was not tested since the

relationship to the relative amount of Chl *a* picoplankton biomass was not monotonic. When testing for correlation between environmental parameters, temperature was found to be significantly negatively correlated with salinity and depth (P-value: 0.00, correlation coefficient: -0.61; P-value: 0.00, correlation coefficient: -0.87). Salinity and depth were significantly positively correlated P-value: 0.00, correlation coefficient: 0.81).

Discussion

Chl a data from the arctic

The range of total Chl *a* measured at the stations, 0.00-1.56 µg L^{-1}, agree with previous measurements in the area. Elferink et al. (2016) found the total Chl *a* in Disko Bay to be 0.03–1.09 µg L^{-1}, and Metfies et al. (2016) measured 0.08-4.50 µg L^{-1} in the Fram strait, Nansen and Amundsen Basin. In 2010, another arctic field course project measured total Chl *a* at two stations almost identical to the ones in this study. Around the position of station D4, they measured 0.40-1.03 mg Chl *a* m^{-3} between 10-45 m (Lett et al. 2010). For station P3 (permanent station), the 2010 study measured 0.56-1.84 mg Chl *a* m^{-3} between 10-45 m (Lett et al. 2010). In this study the range was 0.24-2.58 µg L^{-1} for station D4 and 0.14-2.21 µg L^{-1} for station P3. These values are fairly similar, although this study has slightly higher values. The microplankton species seen in the qualitative survey fit with the quantitative results found in the study by (Devantier et al. 2019) done at the same stations and depths.

The fluorescence measurements indicated that the deeper sample might not have been at the Chl *a* max depth as was originally intended. Rather, the 30 m sample was under or at the Chl *a* max at all stations (Fig. S1.c) Only station D4 seemed to have been sampled in a peak. This could explain why station D4 had the highest total Chl *a* measured overall, as well as the highest nanoplankton and picoplankton Chl *a* biomass measured at 30 m in this study. The differences in the relative amounts would probably not be as affected by whether the samplings were taken at the Chl *a* max or not. It was difficult to determine where the Chl *a* max was while sampling, because the CTD data had to be processed on a computer on land before it could be analyzed. We had, based on our trial sampling and CTD data, decided to sample at 30 m.

Chl a distribution and the effect of environmental parameters

The absolute amount of Chl *a* of the picoplankton fraction was only significantly different between stations. The absolute Chl *a* biomass of picoplankton did not differ significantly between 5 and 30 m, while the relative amount of Chl *a* picoplankton biomass did. It indicates that the change in absolute Chl *a* is greater in the other size fractions than in the picoplankton size fraction itself. This trend has been observed in previous studies, as mentioned in Marañón et al. (2001). This is supported when looking at the correlation between the relative abundance of picoplankton and the total Chl *a* at each station, where a significant negative correlation was observed (not shown). In previous studies looking at larger data sets from the world's oceans, an increase in the picoplankton fraction have been found to be the case when the overall Chl *a* decrease (Marañón et al. 2012; Agawin et al. 2000). One reason could be that when the overall environmental conditions are favorable, the picoplankton loses their advantage in being adapted to nutrient and light poor environments (Agawin et al. 2000). The relative Chl *a* picoplankton biomass was also found to be significantly correlated with the environmental and physical parameters tested, these being depth and temperature. However, since the environmental parameters including salinity were significantly correlated with each other, it is difficult to distinguish which parameter that could be responsible for the distribution, and to what extent - having in mind that correlation does not necessarily mean causation. However, other studies have found temperature to be a significantly describing factor. Lee et al. (2014) found temperature to be positively correlated to picoplankton abundance in the Arctic. Morán et al. (2010) found that 73% of relative picoplankton's contribution to the total Chl *a* biomass is explained by temperature and Hilligsøe et al. (2011) also argued that temperature could independently explain some of the relative contribution of small phytoplankton. Mousing (2013) also supported this view pointing out that temperature has a nutrient dependent and independent effect. But Marañón et al. (2012) argued that it is the

parameters that covary with the temperature that affects the picoplankton, not the temperature itself. Marañón et al. (2012) also pointed out that a substantially large data set is needed to be able to distinguish between the effects of each environmental parameter on the abundance and distribution of phytoplankton; mentioning that nutrient availability and utilization might not be strongly correlated when focusing on smaller areas. According to these ideas, there could be an issue with this current study since the amount of data and stations presented might be too small to draw significant conclusions.

A significant difference in the relative picoplankton distribution was hypothesized between the Disko Fjord and the Disko Bay. This was, however, not the case. The range of the Disko Bay relative abundance at 5 m and 30 m, were varying more than for the Disko Fjord when assessing the data, but the median turned out not to be significantly different. After comparing the innermost Disko Fjord station (D1) with the outermost station (D4) no significantly different distribution was observed, and the same was the case for P1 and P3 when they were compared. These tests all together disprove the hypothesis of difference between the Fjord and the Bay as well as between the near shore station (P1) versus the outer station (P3) and from the innermost fjord station (D1) to the outer fjord station (D4). Similarly did Simo-Matchim et al. (2016) not see any significant difference in total Chl *a* biomass or small phytoplankton Chl *a* biomass between stations in and between fjords in the Canadian High Arctic. Simo-Matchim et al. (2016) did find significant differences in the environmental parameters between the stations, but not in the Chl *a* biomass.

The correlation between station distance from the innermost part of the fjord and the relative picoplankton Chl *a* biomass was significant and positive at 5 m but not at 30 m. What seems to be different between these stations is the salinity which has a gradient from 31-33 at 5 m (Table 1). When furthermore looking at the CTD salinity graph (Figure S2.b.), it is evident that these relatively low salinities are only present in the uppermost part of the water column ~10 m, and not at 30 m. All though the salinities at 30 m varies as well. However, since salinity did not have a monotonic relationship with the data and can be found to correlate with nutrients (Coupel et al. 2015), it is therefore not possible to conclude anything about the effect of salinity based on the current data. Li et al. (2009) did, however, find freshening, to benefit the smaller size fractions due to stratification because nutrients decrease. Freshwater content could have been interesting to investigate as done in Coupel et al. (2015) to know what amount of the freshening that is due to ice melting and river discharge. It could also be relevant to investigate whether distinct water masses were present, as it was found to best describe the abundance of picoplankton in Metfies et al. (2016).

Nutrient influence on phytoplankton size-fractions

The nutrient samples taken during this study were unfortunately not able to be processed. Other studies in Disko Bay have, however, measured nutrient concentrations. Three of these studies investigated locations at, and around, the permanent station (P3). These surveys all took place in the summertime including; mid-July 1996 (Hansen et al. 2012), July/August 2012 (Elferink et al. 2017), and end-July 2016 (Kroon and Sigsgaard 2017) (Table S2). The data show that the nutrient concentrations did not seem to vary substantially between 1996, 2012, and 2016. The concentrations increased with depth and/or peaked around 10 m. In all studies, the nutrient concentrations were considered low (Elferink et al. 2017; Hansen et al. 2012; Kroon and Sigsgaard 2017). This indicates a photic zone depleted of nutrients at this time of year (Kroon and Sigsgaard 2017). The increase in nutrient levels with depth, scarce as they are, could be an explanation as to why the Chl *a* concentration found in this study at station P1 and P3 (Disko Bay) were higher at 30 m than at 5 m (Figure 3). Furthermore, the fact that picoplankton had lower relative biomass contribution at 30 m compared to at 5 m (Figure 4) could point to nutrient concentrations being a determining factor, as picoplankton is known to have an advantage in nutrient scarce environments (Marañón et al 2001). Microplankton Chl *a* biomass increased more than picoplankton between 5 and 30 m (Figure 3). Theoretically, the microplankton could be more nutrient limited than the picoplankton at 5 m, due to their different needs in nutrient concentrations. This would have allowed the

microplankton to have a higher growth potential that could be used when more nutrients become available (Agawin et al. 2000).

Concentrations of nitrite + nitrate >1 µM N should theoretically allow for larger phytoplankton size classes to fulfill their growth potential enough to outcompete picoplankton (Agawin et al. 2000). In contrary concentrations of <1 µM N, picoplankton has an advantage (Agawin et al. 2000). This idea fits well with the observations from this study combined with previous nutrient concentrations from Elferink et al. (2017). They measured <1 µM N at shallow depths, while this study measured the relative amount of Chl *a* picoplankton biomass to be ~50% at 5m (Figure 4). Additionally, they measured up to ~2.1 µM N nitrite + nitrate around 30 m, where the Chl *a* microplankton biomass from this study was over 60% and Chl *a* picoplankton biomass was less than 10%. These patterns could support the idea of nutrients being a determining factor for their distribution. However, this is all speculation as the nutrient data were not available for the current study.

In Disko Fjord, no data was accessible on specific concentrations of the different nutrients. One study investigated Iron (Fe) concentrations and found them to be high. They moreover found relatively low concentrations of dissolved organic carbon (DOC) (1-4 mg/L) (Markussen et al. 2014). Low DOC could potentially indicate low amounts of nutrients. Conditions in fjords affected by glacier input are, however, difficult to know, as they vary substantially in productivity (Hopwood et al 2019). The productivity depends partially on the geometry of the fjord as well as differences in wind, wave and shelf forcing. Shelf forcing is due to shelf density differences causing a pressure gradient between the shelf and the fjord (Jackson et al. 2018; Hopwood et al. 2019). The idea that both Disko Fjord and Disko Bay seem to be nutrient depleted could explain why there was no significant difference between the Chl *a* biomass at each location.

In summary, the hypothesis that the relative biomass of picoplankton was lower in the Disko Fjord compared to the Disko Bay due to higher nutrient concentrations associated with glacier input, had to be rejected. The same was the case for the difference in the picoplankton biomass between the beginning of the fjord and the mouth, and closer and further from shore in the Disko Bay. Previous nutrient data suggests that there is nutrient depletion in the summer at both locations, which could explain why no differences were apparent. Salinity in relation to the glacier melt could not be properly investigated since the data did not live up to the assumptions for the statistical tests used in this study. Temperature could possibly explain some of the distribution patterns, but due to the covariation between environmental parameters as well as the relatively small data set, this can only be considered as an indication and cannot be concluded. Future investigations should include more stations to enhance the data set, nutrient measurements, and a detailed analysis of the water masses to be aware of distinct water masses and their freshwater inputs. This could help getting a better understanding of the picoplankton Chl *a* biomass relative amount and distribution in the Disko Fjord and Disko Bay.

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Appendix

Table S1. Measurements of Chl *a* from all size fractions, including replicates, average and standard deviation. Limit of detection (LOD): 0.025 µg L^{-1}. N/A: not applicable.

Depth (m)	Size fraction (µm)	Chl a (µg·L^{-1}) replicate 1	replicate 2	replicate 3	Average	Standard deviation	Depth (m)	Size fraction (µm)	Chl a (µg·L^{-1}) replicate 1	replicate 2	replicate 3	Average	Standard deviation
Station D1							Station D4						
5	>20	LOD	0	0	0	0	5	>20	0.07	0.06	0.05	0.06	0.01
	3-20	0.39	0.36	0.34	0.36	0.03		3-20	0.09	0.09	0.09	0.09	0
	0.8-3	0.06	0.1	0.14	0.1	0.04		0.8-3	0.08	0.09	0.09	0.09	0.01
30	>20	0	0	0	0	0	30	>20	0.21	0.2	0.21	0.21	0.01
	3-20	LOD	0.03	0.03	0.03	0		3-20	1.61	1.71	1.69	1.67	0.05
	0.8-3	LOD	0	0	0	0		0.8-3	0.75	0.65	0.7	0.7	0.05
Station D2							Station P1						
5	>20	0.06	0.04	0.03	0.04	0.02	5	>20	LOD	LOD	LOD	N/A	N/A
	3-20	1.01	1.27	1.15	1.14	0.13		3-20	0.05	0.05	0.05	0.05	0
	0.8-3	0.41	0.24	0.35	0.33	0.09		0.8-3	0.1	0.08	0.08	0.09	0.01
30	>20	0	0	0	0	0	30	>20	1.08	1.04	1.16	1.09	0.06
	3-20	0.07	0.04	0.05	0.05	0.02		3-20	0.47	0.49	0.44	0.47	0.03
	0.8-3	0	LOD	LOD	0	0		0.8-3	0.24	0.12	0.14	0.17	0.06
Station D3							Station P3						
5	>20	0.07	0.04	0.03	0.05	0.02	5	>20	0.05	LOD	0.03	0.04	0.01
	3-20	0.6	0.48	0.49	0.52	0.07		3-20	0.04	0.04	0.04	0.04	0
	0.8-3	0.54	0.17	0.24	0.32	0.2		0.8-3	0.07	0.06	0.07	0.07	0.01
30	>20	0.04	0.03	0.07	0.05	0.02	30	>20	1.93	2.13	0.61	1.56	0.83
	3-20	0.28	0.28	0.28	0.28	0		3-20	0.52	0.63	0.52	0.56	0.06
	0.8-3	0.18	0.18	0.16	0.17	0.01		0.8-3	0.11	0.02	0.16	0.1	0.07

Table S2. Nutrient data from studies that have taken place in Disko Bay. N/A: not applicable

Study	Elferink et al. 2017	Hansen et al. 2012	Kroon and Sigsgaard 2017
Nutrient	Nutrient concentrations from 0-30 m		
Nitrate	~ 0-2 µM	~ 0-2 µmol L^{-1}	~ 18-80 µg N L^{-1} (0.29-1.29 µmol L^{-1})
Phosphate	~ 0.25-0.4 µM	~ 0-0.2 µmol L^{-1}	~ 1.5-12.5 µg P L^{-1} (0.02-0.13 µmol L^{-1})
Ammonium	~ 0.25-0.75 µM	N/A	~ 10-23 µg N L^{-1} (0.55-1.28 µmol L^{-1})
Nitrite	~ 0-0.1 µM	N/A	N/A
Silicate	~ 0.2-0.4 µM	N/A	N/A

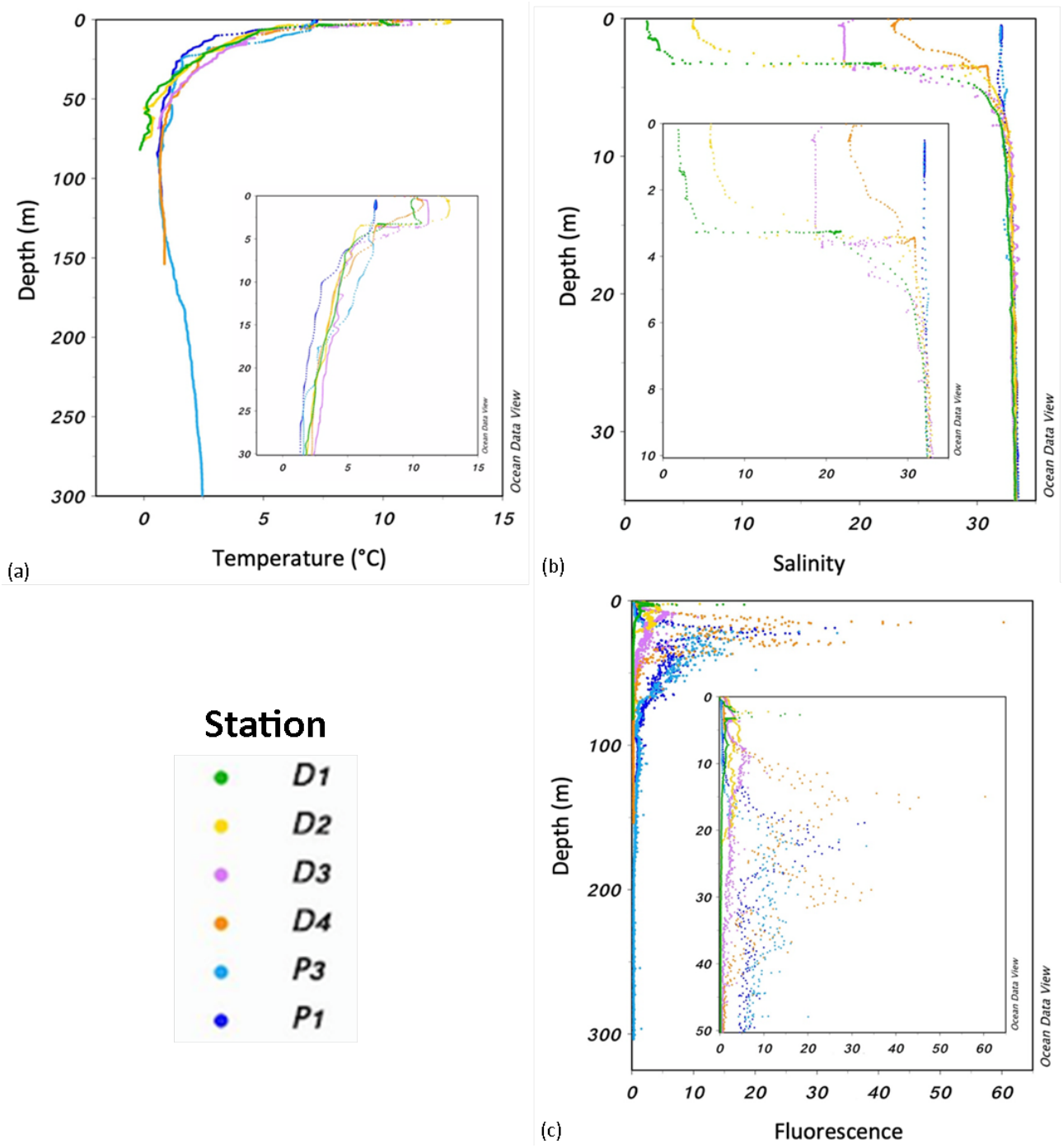
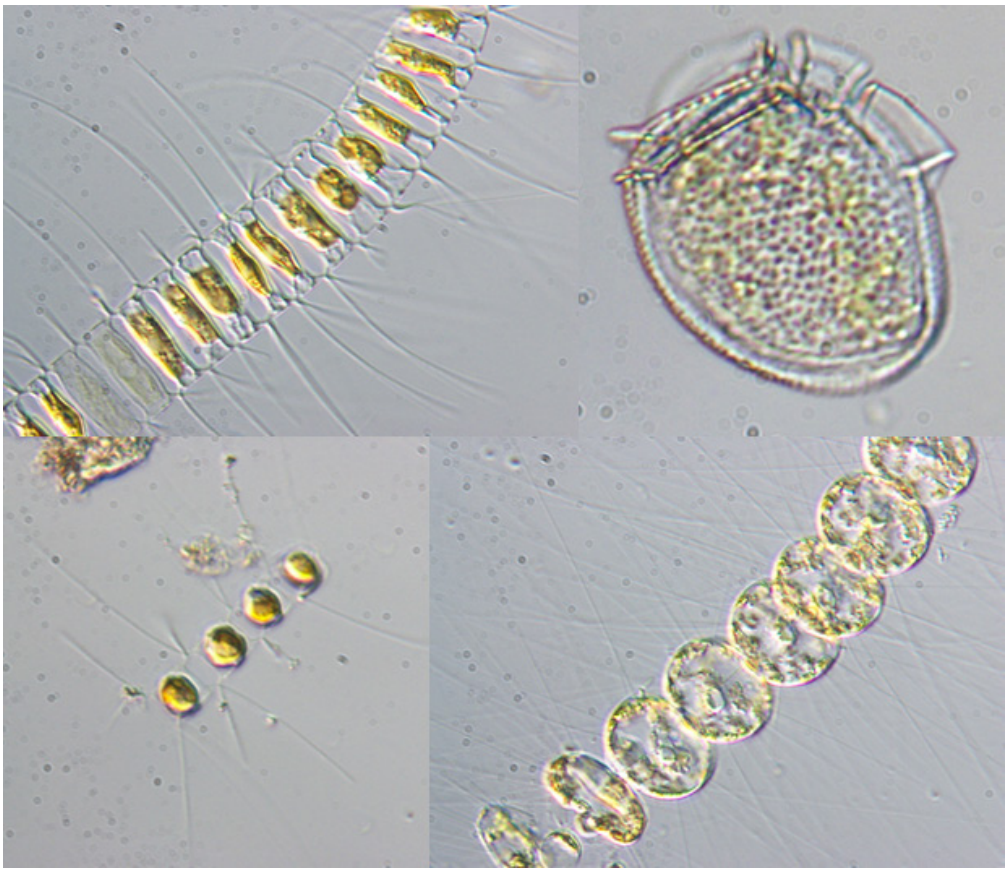


Figure S1. CTD data from the sampling. (a) Temperature at the 6 stations all through the water column, including a zoom for the upper 35 m. (b) Salinity at the 6 stations all through the water column, including a zoom for the upper 35 m. (c) Fluorescence at the 6 stations all through the water column, including a zoom for the upper 50 m.

Phytoplankton community composition and abundance along a salinity gradient in Disko Fjord



Phytoplankton from Disko Fjord (Photos: all group members)

Phytoplankton community composition and abundance along a salinity gradient in Disko Fjord

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Abstract

Arctic waters are undergoing drastic changes. The physical oceanography is modified by a climate warming twice as fast as the global average, leading to substantial sea ice and glacier melting, modifying the dynamics of the water column. Consequently, modifications of ecosystem structures are expected, because taxa diversity and distribution are altered by the changing physical features. A main parameter changing with ice melt is salinity; decreasing in shallow waters due to important input of fresh water from glacial runoff. Fjords at the base of a glacier are characterized by a significant outward increasing salinity gradient.

This study focuses on investigating the microphytoplankton (200-20 µm) abundance, diversity and taxa proportions along a salinity gradient transect in Disko Fjord. A comparative survey was carried out in the open water off the coast of Qeqertarsuaq. A growth experiment with four different salinity conditions was set up to explore the potential effects of salinity on the microphytoplankton community. In the salinity survey, an exponential outward increase in abundance of microphytoplankton was recorded. Despite similar diversity, community composition changed along the fjord. The salinity experiment showed the abundance and composition remained unchanged among the manufactured salinity treatments. The different results in the experiment and the survey suggest a prevalence of other factors than salinity, such as stratification, nutrient depletion, or light limitation, accounting for the changes in abundance and community composition in the Fjord.

Keywords: Phytoplankton, Climate change, Salinity, Glacial melts, Freshening of arctic waters

Introduction

Global climate change has increased the near-surface air temperature in the Arctic approximately twice as much as the average global increase in recent decades. The phenomena of Arctic areas being affected to a higher degree than the global average is known as *Arctic amplification*. Arctic amplification is likely caused by positive feedback loops, which increases the global warming potential (Screen and Simmonds 2010). Melting of the glaciers serves as such a positive feedback loop. Melting of snow and ice alters the albedo on the surface, shifting it towards lower albedo, which in turn increases the melt rates and runoff from the glaciers (Overpeck 1997). This increased ice melt and river runoff from glaciers has resulted in a freshening of the upper Arctic waters over the previous years (Comeau et al. 2011; Straneo et al. 2011). This is mainly caused by the increased summer air temperatures in the Arctic, which yields a massive net retreat of the glaciers (Straneo et al. 2011). The glacial melt accounts for approximately 50% of the total ice loss in Greenland (measured 2000-2010) (Straneo et al. 2011). Freshening of the water may affect the ecology in various ways. The glacial outlets which runs into the fjords, freshens the fjords in the upper layers, and increase the stratification in the water by creating a halocline (Comeau et al. 2011). Stratification is characterized by a stronger pycnocline (density gradient), causing a barrier for vertical mixing, which in turn, decreases the upward transport of nutrients (Comeau et al. 2011). An increase of sea surface temperatures can also lead to additional stratification due to thermal expansion of the water (Guinder and

Molinero 2013). Freshening and warming of surface water, will lead to a stronger halocline and thermocline and hence increase upper water stratification (Guinder and Molinero 2013). This increased stratification could potentially cause differential effects on upper and lower waters, and organisms in the top layers of the water column could be subjected to an increasingly more varying habitat, potentially causing shifts in community composition, abundance or diversity of phytoplankton (Comeau et al. 2011).

Marine phytoplankton provide some important ecosystem services. They are responsible for 50% of the global primary production and their role in the global carbon cycle is vital (Guinder and Molinero 2013; Falkowski and Raven 2013). Being at the basis of the food chain, phytoplankton shape energy fluxes through the food web. They contribute to the ecosystem by biogeochemical cycles and are responsible for movement of carbon from the euphotic zone to the deeper oceanic layers (Finkel et al. 2010; Guinder and Molinero 2013). Also, phytoplankton blooms serve as a major source of primary production in the ecosystems. These blooms function to uphold the higher trophic levels of the pelagic food webs (Falkowski and Oliver 2007). They are found to respond to changes in nutrient availability and light and are controlled by temperature, radiation and water column stability, which may change with salinity. All of which changes through the seasons and are exposed to climate changes (Guinder and Molinero 2013). All of the above, state the importance of phytoplankton, and in a changing world it is crucial to know the response of the phytoplankton to global changes.

With an increasing concern for global climatic changes, phytoplankton response to climate changes has been widely studied (e.g., Huertas et al. 2012., Wiltshire et al. 2008, Guinder et al. 2010, Sarmiento et al. 2004, Boyce et al. 2010). Marine plankton are generally good indicators of climate changes as they mostly are short-lived, and their population sizes are therefore less influenced by previous years. Hence, there is coherence between an environmental change and the dynamics of the plankton community. Furthermore, plankton distribution often changes remarkably as they are free floating. They are able to make quick responses to temperature changes and current systems in the ocean, when they extend or reduce their distribution (Taylor et al. 2002; Hays et al. 2005).

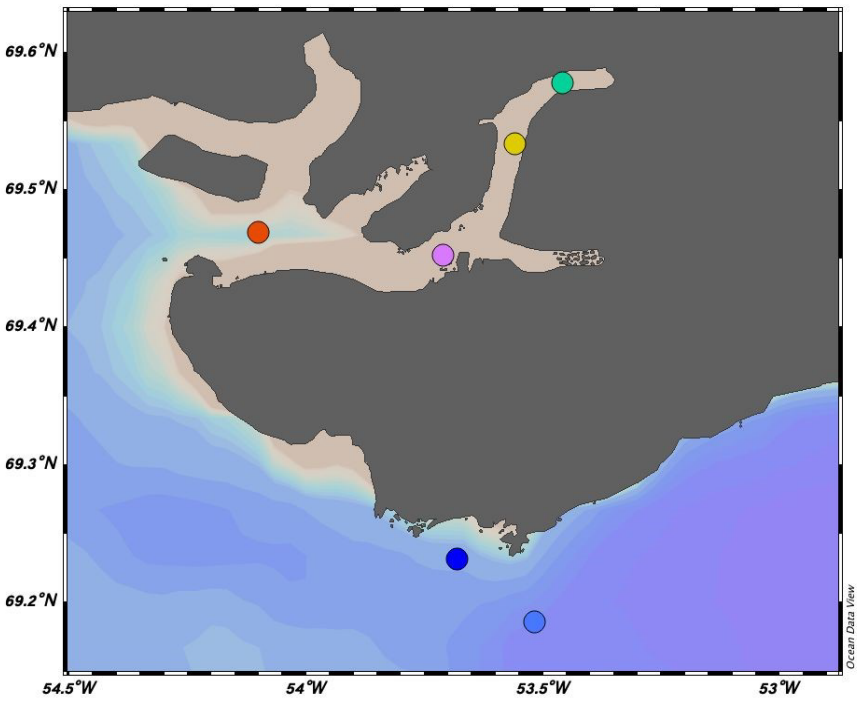

Figure 1. Disko Fjord transect (green = D1, yellow = D2, purple = D3, red = D4) and Ocean transect (light blue = O1, dark blue O3). Colours showing locations for deployment of the CTD, each corresponding to a transect location (Salamon 2019).

In this study we examine how climatic changes, leading to increasing ice melts, could potentially impact the composition of phytoplankton communities. The study was executed in Western Greenland off Disko Island with the purpose of investigating the microphytoplankton (200-20 µm) community composition, abundance and diversity at different salinities, due to increasing concerns about freshening of arctic marine waters. Furthermore, a controlled laboratory

experiment was executed to test the isolated effect of salinity on the phytoplankton community from Disko Fjord.

This study hypothesises an increasing diversity and abundance of phytoplankton along a growing salinity gradient, since we assume fewer taxa are able to cope with a changing salinity and most taxa are adapted to a constant marine stable level of salinity.

Methods and Materials

Study site

In order to perform the salinity survey, two transect locations were chosen – one transect performed in Disko Fjord, starting at a glacial output where a salinity gradient was expected, and a control transect off Disko Island, where no salinity gradient was expected (Fig 1), both sampled at two depths. The Disko Fjord transect started closest to the glacial output and sampling was carried out at four stations, D1, D2, D3, and D4, with varying distances between stations, along a transect of 38,4 km. The location of the first transect station was decided based on salinity of the surface water at different distances to the glacial output. The surface sample at D1 had a salinity of 2, indicating that freshwater was affecting the area. During sampling, the weather was calm. When moving away from the glacial outlet, the visibility of the water increased. The ocean transect took place off Disko Island starting at Permanent station (Fig 1). The transect was approximately 5.5 km in the ocean with icebergs present. Weather conditions were calm, and the water visibility was high.

Water for a salinity treatment experiment was collected at station D4 at 30 m, because this depth was expected to have maximum chlorophyll level and full salinity.

Salinity survey

Due to practical problems, CTD data was retrieved after sampling, which means that the halocline was not defined before the two depths were decided. Hence, the data are not specifically from above and beyond the halocline as intended, but underneath and within the halocline (Fig. S2). This should be accounted for.

Sample collection. At each station a CTD was employed to provide a salinity and temperature profile of the water column. Using a Niskin water sampler, water samples were taken in triplicates and mixed in 25 L carboys, in order to get a more homogenous sample. An additional live sample was collected at each station with a 20 µm plankton net (from 35 m to surface) and evaluated prior to counting. Temperature and salinity of each sample were measured on board with a handheld thermometer and refractometer.

Filtering. 20 L of water from each sample from every location and depth was filtered. A 200 µm prefilter was used to remove zooplankton and a 20 µm filter to collect phytoplankton > 20 µm by backwashing into 50 mL falcon-tubes. Phytoplankton in chains bigger than 200 µm might therefore be missing. Before every filtering, the filters, buckets, volumetric flask and funnel were rinsed with 20 µm filtered seawater. The samples were fixed with seven drops of Lugol's solution.

Salinity treatment experiment

Sample collection. At station D4, 50 L of water was collected at 30 m using a Niskin sampler. The water was cooled until reaching the cooling lab.

Experimental setup. The live sample was prefiltered through a 200 µm net soon after sampling to remove zooplankton. This water sample (salinity 34) was used to create four different salinity treatments in triplicate subsamples in the lab. Triplicate subsamples were fixed to show initial community composition. Treatments consisted of live sample diluted with the same volume of different proportions of filtered seawater and filtered freshwater, in total 750 mL. The four salinity levels were: 26; 28; 31 & 34. These salinities were chosen to represent a salinity gradient similar to what was seen in Disko Fjord with the addition of climate change scenarios (salinities 26, 28). Because the collected sample had very low phytoplankton density, 22 mL of the plankton net

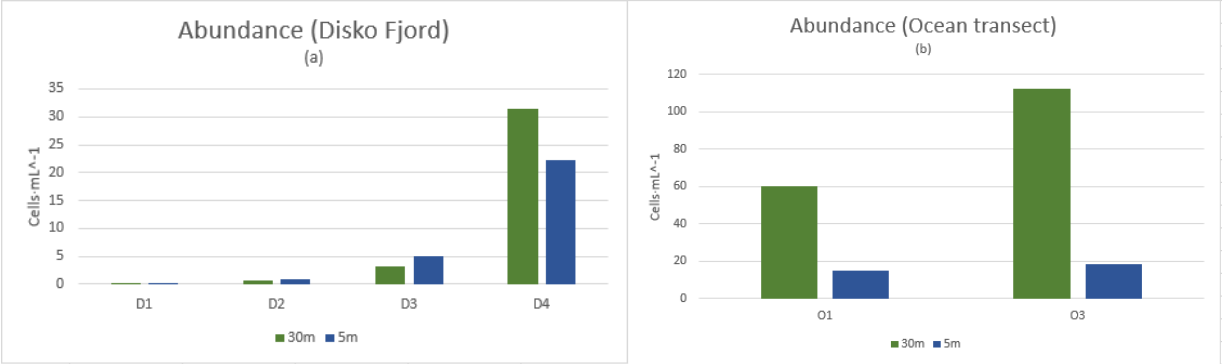

Figure 2. Abundance of cells within each station (D1-D4 (a), O1 and O3 (b)), estimated as cells mL-1. Calculations for abundance can be found in the appendix.

sample (from D4), was added to each flask. The flasks where strapped on a rotating plankton wheel in a cooling container at a temperature of 5°C. Fluorescent light bulbs provided light (100-110 µmol photons m^{-2} s^{-1}). After 12 hours, L-medium containing silicate (Na_2SiO_3), nitrate ($NaNO_3$), vitamins, micronutrients & phosphate (NaH_2PO_4) (Guillard 1975) was added to all flasks. Once a day, the flasks were switched around to even out light conditions. After 4 days and 10 hours (106 hours in total) the flasks were taken off the wheel and fixed with 30 drops of Lugol's solution.

Counting cells in salinity survey and salinity experiment

Richness and abundance estimation. Prior to counting, light microscopy was used to evaluate species composition of the samples. The taxa most commonly appearing in the samples across all stations were chosen as representatives of the algae community composition when counting: *Protoperidinium* spp., *Ceratium* spp., *Dinophysis* spp., *Thalassiosira* spp, *Chaetoceros* spp., *Leptocylindrus* spp. along with 'other centric diatoms', giving a total of seven different taxa/groups to count. In order to estimate the density of cells within the different groups, a Sedgewick Rafter Cell Counter (Karlson et al. 2010) was used. 400 cells (Enevoldsen and Unesco 2003) belonging to one of the above-mentioned groups were counted. Abundance was calculated as cells mL^{-1}.

Richness estimated as number of groups represented, was estimated for each site. Last, a Shannon Index of diversity (Spellerberg and Fedor 2003) was calculated using both abundance and richness estimations.

Data analysis

Data analysis was performed in RStudio (version 1.1.463), graphs and figures in Excel (version 16.27). Results for the salinity survey were tested by running several linear models to see whether cell density (abundance) and community diversity (Shannon Index) could be predicted by either salinity or distance to glacial outlet/shore. Prior

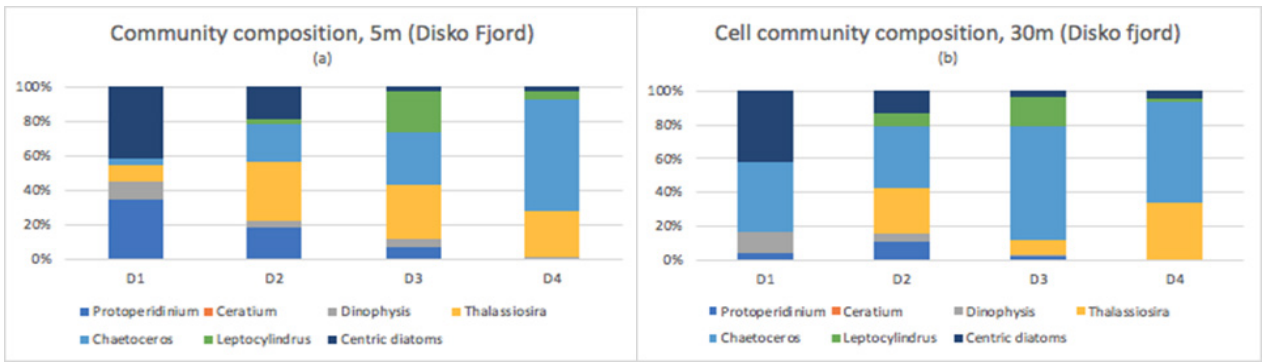

Figure 3. Cell community composition at stations D1-D4 for 5(a) meters and 30(b) meters respectively, shown in percentages of what types of algae appeared in the samples. Other algae were also found. However, the presented algae represent the more abundant algae in the samples.

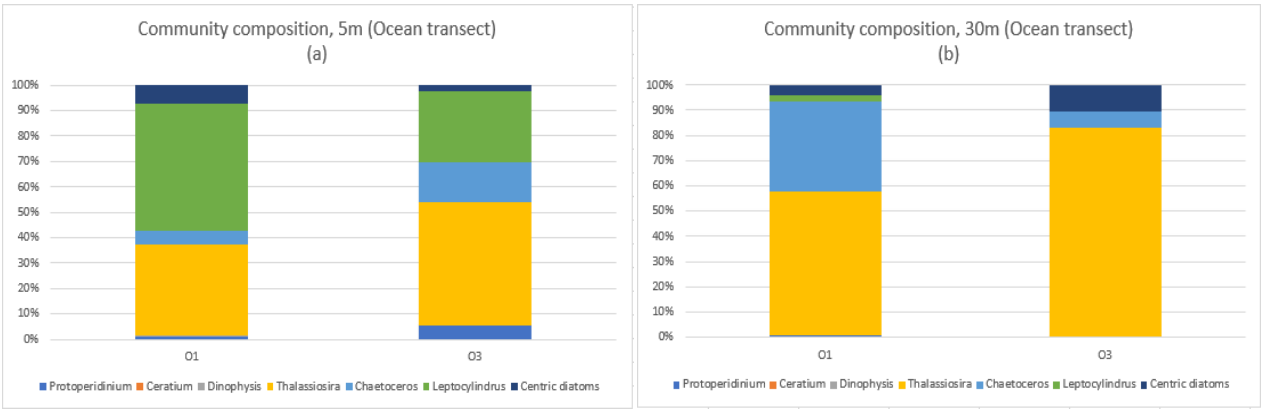

Figure 4. Community composition in the samples taken at 5(a) meters and 30(b) meters respectively. Colours indicate the relative abundance of each group based on a calculation of cell density.

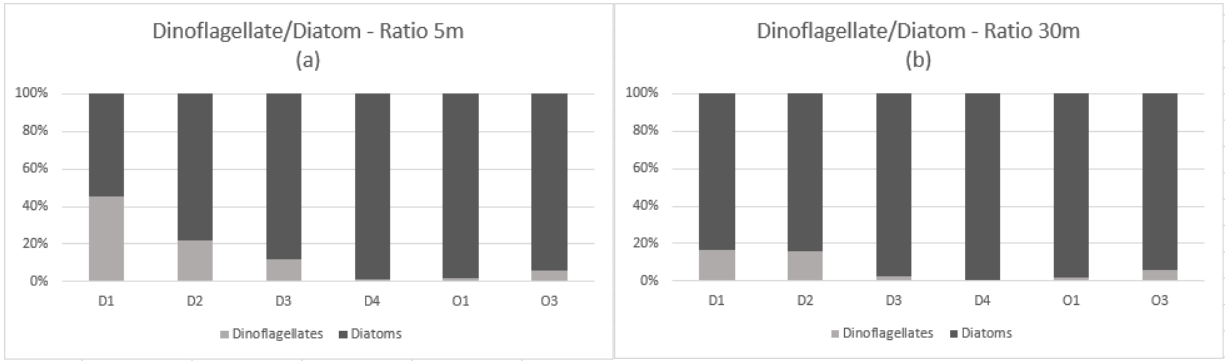

Figure 5. Dinoflagellates/Diatom ratio at each station (D1-D4(a), O1 and O3(b))

to the analysis abundance data was log transformed to assume normality.

The salinity treatment experiment was analysed using a two-way ANOVA to estimate, whether the difference in abundance and diversity could be explained by salinity treatment. A Tukey HSD post-hoc test for multiple comparisons was carried out to determine the source of significance.

Finally, the ratio between dinoflagellates and diatoms was calculated by dividing the percentage of dinoflagellates with percentage of diatoms present in each sample. Dinoflagellates counted were *Protoperidinium* spp., *Ceratium* spp. and *Dinophysis* spp., while diatoms were represented by *Chaetoceros* spp., *Thalassiosira* spp., *Leptocylindrus* spp. and other centric diatoms.

Results

Salinity survey

The salinity survey showed a general increase in phytoplankton abundance moving from station D1 and out through Disko Fjord at both 5m and 30m (Fig. 2a). The samples along the ocean transect showed a similar trend with a slightly higher abundance at the furthest station (Fig. 2b), but generally with a much higher abundance than in Disko Fjord, especially at the deeper sample.

Community composition of the six groups of phytoplankton measured in this study showed some variation between depths within each station but displayed more variation among stations. *Protoperidinium* spp., *Dinophysis* spp. and other centric diatoms were relatively more abundant at the inner stations (dominating at D1), whereas *Thalassiosira* spp., *Chaetoceros* spp., and *Leptocylindrus* spp. seemed to have a higher relative abundance at the outer stations

Table 1. Results of linear models carried out on the data from the Salinity Survey transects at Disko Fjord.

Linear models of algae abundance and diversity					
Depth	Dependent variable	Independent variable	Multiple R-squared	F-statistics	P-value
	Salinitet	Distance to outlet	0.7609	6.366	0.1277
	Log (Abundance)	Salinity	0.9324	27.60	0.0344*
	Log (Abundance)	Distance to outlet	0.9206	23.18	0.0405*
	Diversity (shannon index)	Salinity	0.0736	0.159	0.7288
5	Diversity (shannon index)	Distance to outlet	0.4964	1.972	0.2954
	Salinitet	Distance to outlet	0.3494	1.074	0.4089
	Log (Abundance)	Salinity	0.2607	0.705	0.4894
	Log (Abundance)	Distance to outlet	0.8957	17.18	0.0536
	Diversity (shannon index)	Salinity	0.7473	5.914	0.1355
30	Diversity (shannon index)	Distance to outlet	0.4668	1.751	0.3168

(Fig. 3a+b). In contrast, *Chaetoceros* spp. was relatively abundant throughout all the stations at 30m depth.

Community composition at the ocean transect showed more variability between depths than between stations. *Leptocylindrus* spp. was highly dominant at 5m, but barely present at 30m. At 30m, *Thalassirosira* spp. showed the highest relative abundance at both stations (Fig. 4a+b)

The dinoflagellate/diatom ratio showed, overall a larger proportion of diatoms compared to dinoflagellates. Relatively, more dinoflagellates were present in 5m (Fig. 5a) than 30m (Fig. 5b) at inner Disko Fjord stations. Dinoflagellates were decreasing relatively, going out of Disko Fjord, however a slight increase in relative amount of dinoflagellates was seen from O1 to O3 (Fig. 5a+b).

The Shannon Index showed no trend neither at the Disko Fjord transect nor the Ocean transect (Fig. 6a+b).

Both salinity and distance to glacial outlet were tested in several linier models as predictor values for both abundance and diversity at both 5m and 30m (Table 1). A significant linear relationship was found between log transformed abundance as a function of salinity ($R^2 = 0.9324$, $F = 27.60$, $P = 0.0344$), as well as between log abundance and distance to the glacial outlet at 5m ($R^2 = 0.9206$, $F = 23.18$, $P = 0.0405$). No other models showed significant results. Furthermore, distance to glacial outlet and salinity was tested for correlation and was non-significant for both sample depths.

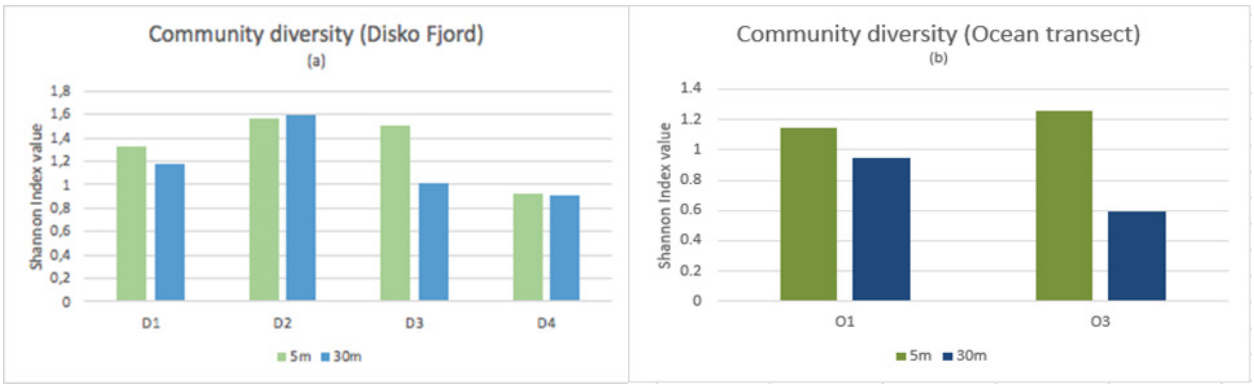

Figure 6. Community diversity at each station (D1-D4(a), O1, and O3(b)), estimated using Shannon Index.

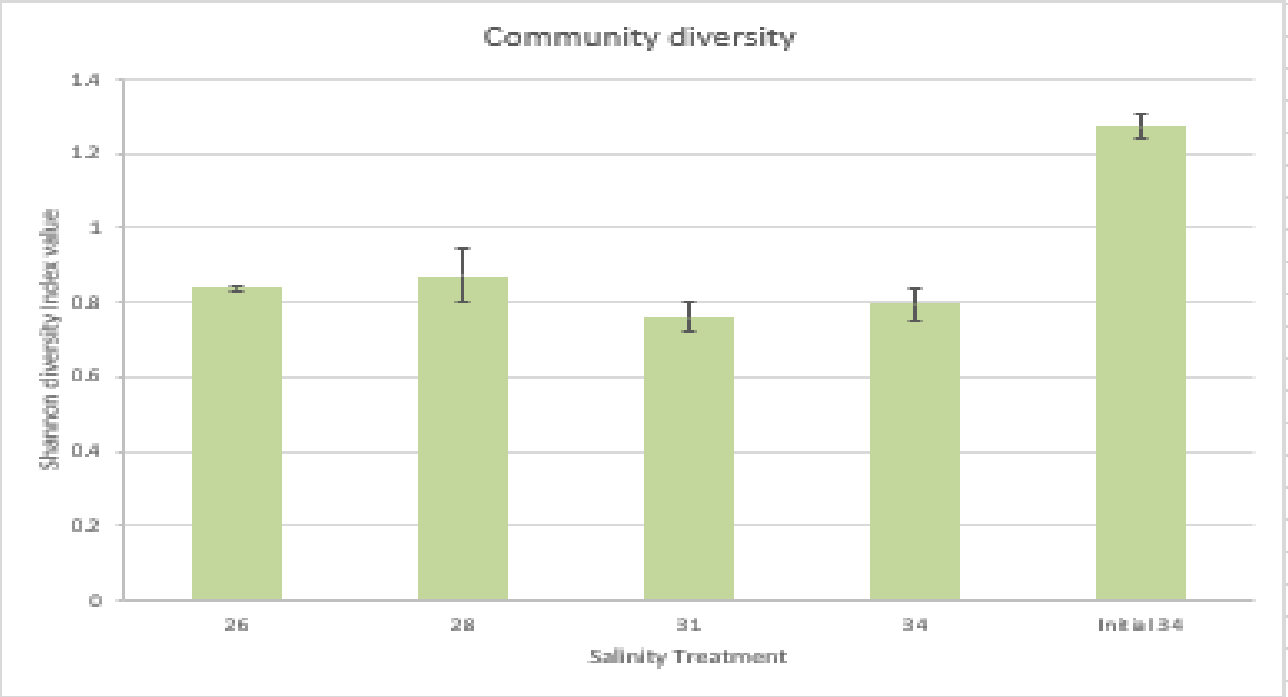

Figure 7. Estimation of diversity in the experiment. Shannon index values is calculated as a proxy for diversity. The graph shows the different salinity treatments and their corresponding diversity index value.

The CTD data showed a strong halocline at around 3m for all stations. Additionally, the halocline seemingly got less stratified moving out the fjord. No clear halocline was observed at the ocean transect. Furthermore, a clear surface salinity gradient was observed between the four stations at Disko Fjord (Fig. S2).

Salinity treatment experiment

Shannon Index diversity was significantly correlated to salinity treatment (ANOVA, *F = 4.031, P = 0.000164*) (Table S3). The post-hoc results found that the initial treatment was significantly different from every other treatment (*P = 0.0002-0.0015*), but the diversity between non-initial treatments was non-significant (Fig. 7).

In all four treatments, *Chaetoceros* spp. and *Thalassiosira* spp. were highly abundant after the 106 hours (Table S2). No significant differences were found between the cell density in the treatments (Table S3), possibly due to high standard deviation (Fig. 8). The diversity in the initial sample was high but the abundance low compared to the four treatments.

The community composition was not differing between the four salinity treatments. The major part of the samples contained *Chaetoceros* spp. The initial sample, however, contained more *Thalassiosira* spp. than the treatments. *Protoperidinium* spp*., Ceratium* spp*., Dinophysis* spp*., Leptocylindrus* spp. and other centric diatoms were either present to a limited extent or not at all.

Discussion

Since the 1950'ies a pattern of changing salinity has emerged, high salinity areas are becoming more saline, while low salinity regions (such as polar regions) are becoming increasingly fresher due to higher precipitation and increasing ice melts. However, due to high regional variability and little data, the extent and consequences are still uncertain (Intergovernmental Panel on Climate Change 2014).

A transect in Disko Fjord glacial outlet was hypothesised to experience a salinity gradient. The gradient would then affect the phytoplankton diversity, in a way where the diversity and abundance of the phytoplankton community would increase with an increase in salinity, when moving away from the glacial outlet. However, a linear model showed no

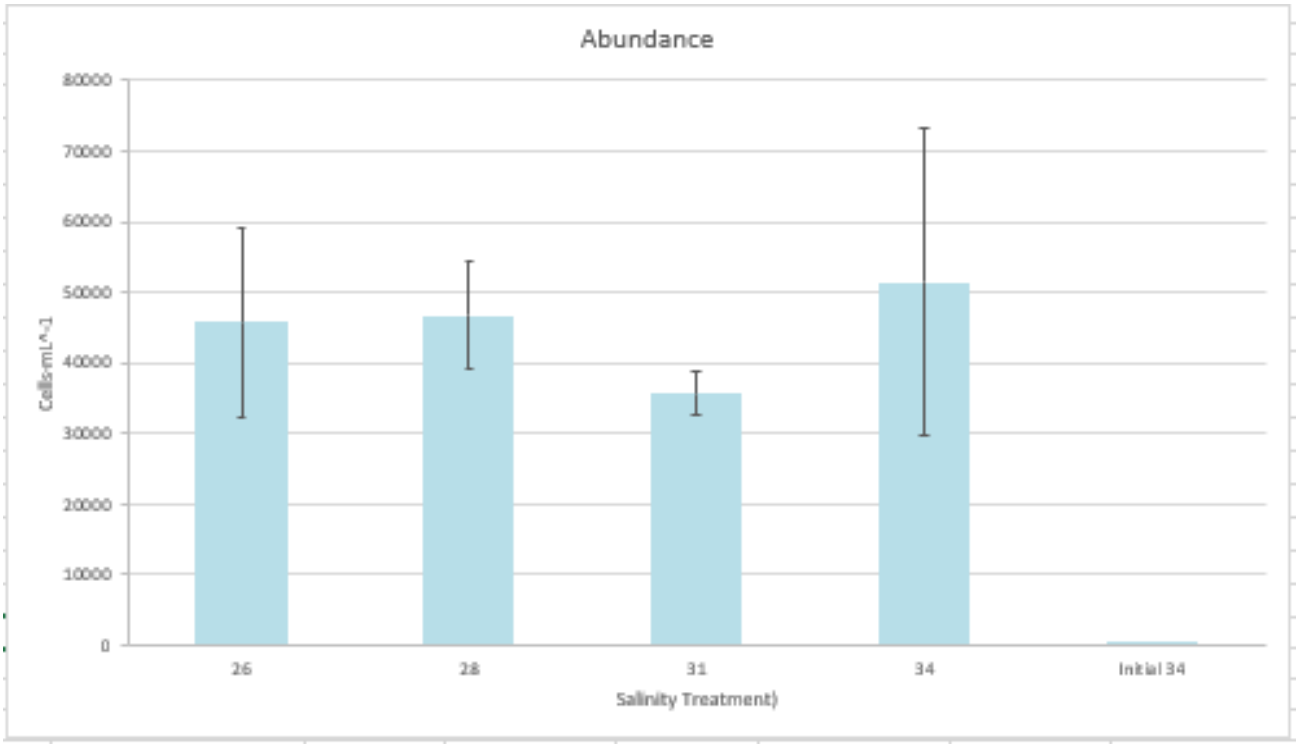

Figure 8. Estimation of abundance in the experiment. The graph shows the different salinity treatments and their corresponding amounts of cells mL^{-1}.

significant linear relationship between distance to the glacial outlet and salinity at either depth (Table 1), indicating that distance to the glacial outlet is not a complete predictor of salinity, although the model showed a salinity gradient in Disko Fjord.

Increasing abundance along a salinity gradient

When comparing the cell density within Disko Fjord to the ocean transect, there is a distinctively higher cell density in the open waters. There is also a significant increase in cell density when moving towards open water in Disko Fjord (Fig. 2a+b). This is supported by the linear models, which showed that at 5 m there is a significant relationship between abundance and salinity, as well as between abundance and distance to glacial outlet. At 30 m there was no significant relationship with salinity, although a tendency was observed, probably due to stratification of the water column with more saline water at the bottom.

The results indicate that abundance in the surface is partly determined by salinity, but probably also affected by other factors, which are correlated to distance from the glacial outlet. These other parameters might be an increase in light penetration. When sampling, there was a clear increase in visibility when moving away from the glacier. The poor visibility might be caused by materials brought into the fjord from the glacial melt outlet (Syvitski 1989). This low light penetration might also be the cause of the higher abundance at 5m than at 30m for the innermost stations (D1-D3). Station D4, as well as the two ocean stations show a higher cell abundance at 30 m compared to 5 m depth. A better light penetration at these stations might favor higher phytoplankton growth at 30m, while still having access to nutrients, which is typically mixed at the bottom of the water column. This hereby favours phytoplankton growth at around 30 m (Hill and Cota 2005). Ocean transect might be affected by precipitation runoff from Disko Island. Other factors may account for the increased abundance at O3 at 30 m, such as increased vertical mixing in the water column.

No significant difference in cell abundance was found between the different salinity treatments

(Fig. S3), indicating no effect of decreased salinity on cell abundance in the experimental set-up. However, an increase in cell density from the initial sample compared to the treatments was found. This suggests that the addition of nutrients, combined with 106 hours of cell proliferation, and optimal light conditions, created an increase in cell abundance of the sample. This indicates that phytoplankton communities might be nutrient- and light-limited in the field study. Hence, we cannot conclude that cell density is related to salinity in the experiment.

Increasing diversity along a salinity gradient

The relative community composition throughout Disko Fjord (Fig. 3), stays within the same distribution at 5 m and 30 m on each station. *Thalassiosira* spp*., Leptocylindrus* spp., and *Chaetoceros* spp. show the same patterns at 5 m and 30m throughout D2-D4, however *Chaetoceros* spp. are more dominant compared to *Thalassiosira* spp. at D3 30 m, which differs from 5 m. *Chaetoceros* spp. and *Thalassiosira* spp. are both known to be common in arctic regions *Chaetoceros* spp. show a higher density in Disko Fjord, which might be caused by a higher specific light absorption than *Thalassiosira* spp. (Sakshaug et al. 1991), however this is species dependent. Comeau et al. (2011) found that *Chaetoceros* spp. are dominant when affected by ice melt. However, the current study shows that *Chaetoceros* spp. are not dominating very close to freshwater exposure at 5 m but increases along the transect. *Chaetoceros* spp. have a very short dividing time, making them good competitors, when nutrients are easily available (Comeau et al. 2011). However, without nutrient analysis, no final conclusions cannot be drawn. 'Other Centric Diatoms' are present to a larger degree at both 5m and 30 m at D1 than in any other station. The high presence of 'Other Centric Diatoms' at D1, could be due to the fact that diatoms are known to have a relatively high growth rates (Daugbjerg et al. 2015), as well as being the primary source of new production (Goldman 1993). With incoming water, an opportunity to grow rises, which might give an advantage to fast growing phytoplankton.

Dinophysis spp. is not found, but when moving towards more open waters, they are found in higher densities close to the glacial outlet. *Dinophysis* spp. are found to have optimal conditions in low turbulence waters due to hydrodynamic calm, which also induces stratification. This is supported in the present study (Fig. 3a+b) (Aubry et al. 2000).

The ocean transect shows similar community composition between the respective depths at the two stations (Fig. 4a+b). The insignificant changes between O1 and O3, indicates that there is not a clear change in the phytoplankton composition, when moving away from shore, in areas without a salinity gradient. However, a shift in community composition is found between 5 and 30 m, on both stations which might be explained by better light conditions and nutrient availability, which might increase competition within the community.

The diatom/dinoflagellate ratios all have a higher proportion of diatoms, with varying proportions of dinoflagellates (Fig. 5a+b). Except for station D1 (45% dinoflagellates vs. 55% diatoms), the diatom/dinoflagellate ratio showed that dinoflagellates are becoming relatively less abundant in comparison to diatoms, going out the transect. It should also be noted that initial microscopy evaluation of the Disko Fjord stations hinted at an increasing number of athecate dinoflagellates, when moving towards the glacial outlet, however, these were not counted in the study.

Other studies have found that a decrease in salinity is followed by a decrease of some phytoplankton groups in arctic waters. Comeau et al. (2011) examined the 2007 sea-ice minimum, which led to a significant decrease in marine heterotrophic stramenopiles, a group that includes diatoms. Similarly, the current study showed that *Chaetoceros* spp. and the overall proportion of diatoms increased from station D1 to D4, with increasing salinity at 5 m (Table 1), and remains the same from D1 to D4, at overall stagnant salinity at 30 m. The loss of taxa, recorded by Comeau et al. (2011), might be caused by a more defined stratification caused by

decreased salinity in the upper layers (Guinder and Molinero 2013), reducing water mixing and thus input of taxa that are less opportunistic in terms of growth rate and not adapted to the conditions (Comeau et al. 2011).

No significant difference in diversity was found in the linear models for the transect from Disko Fjord, which is similar to the results of other studies (Comeau et al. 2011). However, this study only includes the seven most common phytoplankton groups in the area, which creates a richness bias.

While no significant difference was found between treatments in the salinity experiment, the initial sample did show significantly higher diversity compared to the four salinity treatments in the experiment. The lack of significant difference between treatments, suggests that at least *Thalassiosira* spp. and *Chaetoceros* spp. are unaffected by the low salinity treatments. However, based on the current study, it is difficult to tell whether the other phytoplankton groups were simply outcompeted due to the competition or whether salinity played an additional role in removing these groups. Since all treatments showed similar community composition after 106 hours incubation, it indicated that salinity is not the determining factor for diversity. It is possible that nutrient availability or light limitations are responsible for much of the diversity found in the transect.

It is, however, important to note that the total diversity within each sample is not displayed here. In order to be able to count within the given time, a few groups had to be chosen in order to represent the community composition. Furthermore, one must keep in mind that this study is focusing on microalgae, since these were identifiable in the microscope. This could mean that other changes in community composition, abundance, and diversity could be present throughout transect, but would remain undiscovered in the present study.

Concluding remarks

With an increasing concern for the consequences of climate changes, there is an urgent need to understand potential implications of a warming Arctic.

Salinity may influence phytoplankton community composition. This is seen in the significant relationship between abundance and salinity at 5 m. Salinity had no significant effect on species richness and Shannon Index. Other factors may account for species composition, abundance and diversity. Light penetration and nutrient availability appear to account better for the change in community composition, abundance and diversity, although this was not investigated in the current study. It should be noted that the changes found in this study might only represent some of the changes caused by glacial runoff. Studies looking at different size fractions and trophic levels also report changes caused by freshening of the ocean such as smaller size fractions becoming more prominent (Comeau et al. 2011), which would go undetected by the current study.

The experiment did not show significant importance of salinity. A potential future study could include more parameters to find additional factors accounting for algal diversity from a glacial outlet, such as murky water induced by glacial outlet sediments. The experimental setup gave the phytoplankton optimal light and nutrient conditions, parameters that are not considered in the survey. Furthermore, the depth of the halocline and thermocline should be known before sampling, in order to precisely estimate the depths of sampling to correctly sample below and above the pycnocline.

The limited reaction to relatively low saline treatments found in the present study could also be due to the nature of the sample habitat. Phytoplankton found in water close to glacial outlets could have adapted to high saline variability due to large seasonal variability. Thus, the species present here, could be more tolerant of salinity changes showing little reaction (Brand 1984). A similar salinity treatment experiment with abrupt changes in salinity, found the diatom *Pseudo-nitztchia australis* able to tolerate salinities down to 20 (Ayache et al. 2018). This suggests that the phytoplankton community

might be more tolerant to low salinities than first anticipated, at least in the short term. However, it could be possible that growth may be slowed in altered salinity, or that competitiveness might be compromised. A study comparing both an instant change in salinity in addition to a study allowing for acclimation and adaptation might be an interesting angle for future studies.

The results of this study indicate that glacial runoff does indeed seem to affect phytoplankton community composition, abundance and diversity. However, a decrease in salinity does not appear to have an extensive impact on community changes at the levels tested in this study. A combination of the salinity survey to the experimental salinity treatments, suggest that future studies should look at additional factors, such as potential for light penetration and nutrients, determining phytoplankton community changes.

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We would like to give our thanks to University of Copenhagen, for the opportunity to go to Arctic station, Disko island, Greenland, by following the course Arctic Biology Field Course and for providing the supplies for our projects. We would also like to give our thanks to Nina Lundholm for all her guidance on the ship, as well as in the lab and with planning the field work. Finally, we give our thanks to Arctic Station, Disko Island, and its staff, for providing facilities in the lab as well as providing the opportunity to go out on the sea by ship, to collect our samples and with all their help on the boat.

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Appendix

Supplementary figures and tables

Prior to sampling, a pilot study was executed in order to estimate the depths at which to sample from. Due to temperature measurements of the sampled water, which indicated a thermocline, depths of 5m and 30m were chosen. 30m depth was decided in order to potentially reach maximum primary production (Hill and Cota 2005).

Table S1. Coordinates taken during sampling. Due to incoming tides as well as wind and current conditions, the boat drifted to some extent at each station at the transect in Disko Fjord. For this reason coordinates were taken throughout sampling creating a zone corresponding to each station.

Station	Sample depth	Coordinates
Disko fjord D1	30m	69° 34.680' N 53.27.414' W
Disko fjord D1	30m	69° 34.814' N 53.27.601' W
Disko fjord D1	30m	69° 34.569' N 53.27.761' W
Disko fjord D1	5m	69° 34.602' N 53.27.867' W
Disko fjord D1	5m	69° 34.709' N 53.27.400' W
Disko fjord D1	35m (plankton net)	69° 34.760' N 53.27.042' W
Disko fjord D2	30m	69° 32.013' N 53.33.460' W
Disko fjord D2	30m	69° 32.184' N 53.33.333' W
Disko fjord D2	30m	69° 32.244' N 53.33.291' W
Disko fjord D2	5m	69° 31.959' N 53.33.498' W
Disko fjord D2	5m	69° 32.013' N 53.33.429' W
Disko fjord D2	35m (plankton net)	69° 32.142' N 53.33.356' W
Ocean station 1	5m, 30m and 35m	69° 13.848' N 53.40.880' W

Station	Sample depth	Coordinates
Disko fjord D3	30m	69° 27.131' N 53.42.573' W
Disko fjord D3	30m	69° 27.204' N 53.42.145' W
Disko fjord D3	30m	69° 27.259' N 53.41.887' W
Disko fjord D3	5m	69° 27.126' N 53.42.956' W
Disko fjord D3	5m	69° 27.190' N 53.42.683' W
Disko fjord D3	35m (plankton net)	69° 27.296' N 53.42.173' W
Disko fjord D4	30m	69° 28.142' N 54.06.030' W
Disko fjord D4	30m	69° 28.130' N 54.05.998' W
Disko fjord D4	30m	69° 28.085' N 54.05.957' W
Disko fjord D4	5m	69° 28.024' N 54.05.948' W
Disko fjord D4	5m	69° 28.002' N 54.05.951' W
Disko fjord D4	35m (plankton net)	69° 27.935' N 54.06.101' W
Ocean station 3	5m, 30m and 35m	69° 11.112' N 53.30.995' W

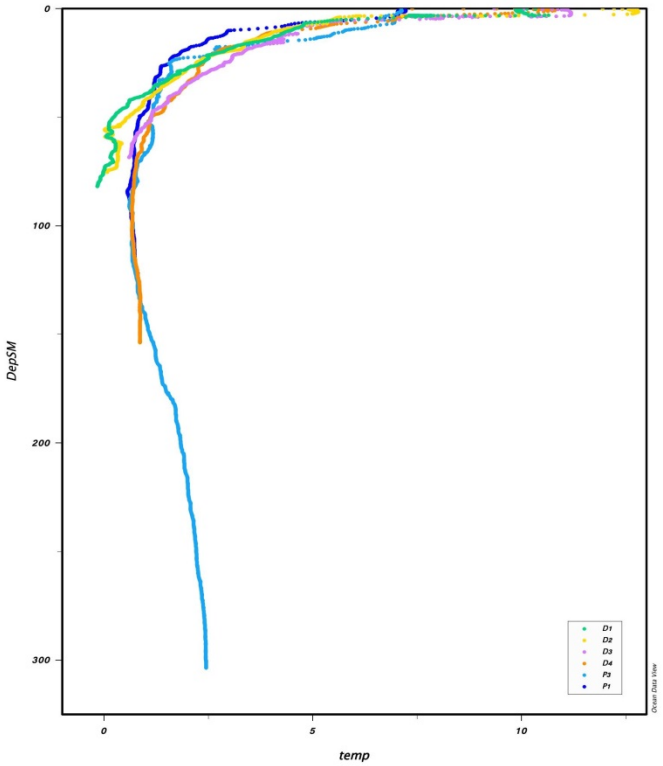


Figure S2. Graph showing temperature variation in the water column. Each colour corresponds to a location on one of the transect locations (fig. 1) (Salamon 2019).

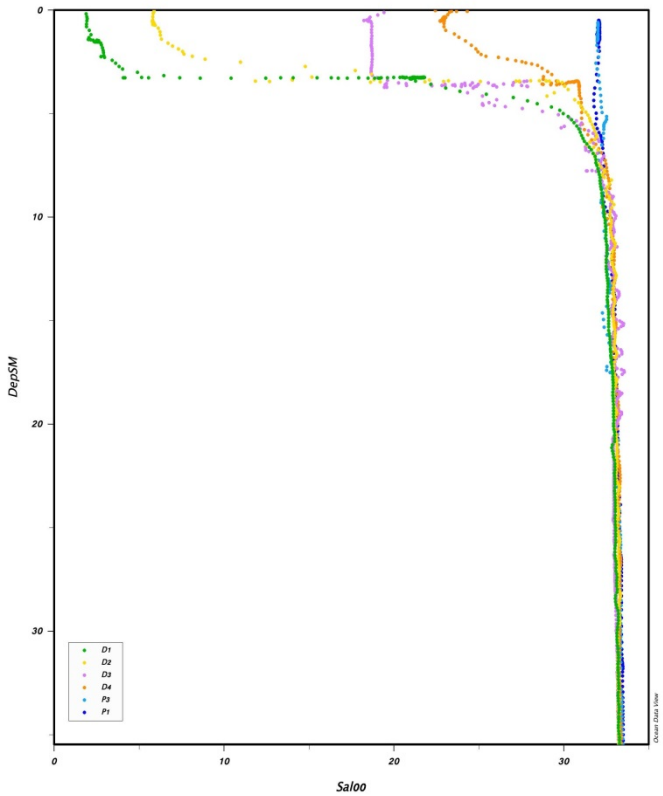


Figure S3. Graph showing salinity variation in the water column. Each colour corresponds to a location on one of the transect locations (fig. 1) (Salamon 2019) .

Table S2. Mean cell density (abundance), richness, as well as Shannon Index score, for each counted algae group at the 5 different salinity treatments.

Phytoplankton mean density (cell pr. mL) for each experimental treatment

Treatment	Protoperidinium	Ceratium	Dinophysis	Thalassiosira	Chaetoceros	Leptocylindrus	Other Centric diatoms	Abundance	Richness	Shannon Index
26	0±0	0±0	0±0	2422.2±828.2	42466.7±12356.4	633.3±388.4	211.1±118.2	45733.3±13539.4	3.3±0.3	0.84±0.009
28	0±0	0±0	0±0	3466.4±1308.3	43111±6394.8	0±0	2333.3±2020.7	4041.5±7569	2±0	0.87±0.069
31	0±0	0±0	0±0	924.2±685.7	34634.2±3753.1	66.7±57.8	57.1±25.8	35682.3±3131.7	2.7±0.3	0.76±0.038
34	15.2±13.1	0±0	0±0	2201±873.4	49144.5±21200.8	0±0	0±0	51360.7±21777.5	2.3±0.3	0.79±0.042
Initial 34	1.7±0.7	0±0	1.7±0.15	29.6±7.95	79.8±8.6	3.5±0.5	8.6±0.9	124.8±3.6	6±0	1.27±0.033

Table S3. Results of the two ANOVA analysis of variance test performed on the Salinity treatments results testing abundance and diversity respectively. Only diversity showed significance as a predictor value for treatment.

ANOVA Comparison of algae abundance, and algae diversity

Response variable	*Predictor variable*	*Df*	*F-value*	*p-value*
Abundance				
	Treatment	4	2.243	0.137
Diversity				
	Treatment	4	4.031	**0.000164*** **

Table S4. Multiple comparison p-values from the post-hoc Tukey test performed on the salinity treatment experiment data. *indicate significance.

Tukey multiple comparisons of means 95% family-wise confidence level

Treatment	26	28	31	34	Initial 34
26	-	0.9875	0.8037	0.9658	0.0008***
28	-	-	0.5404	0.7956	0.0015***
31	-	-	-	0.9890	0.0002***
34	-	-	-	-	0.0004***
Initial 34	-	-	-	-	-

Table S5. Table showing the raw data collected at each station on the transect at Disko Fjord as well as abundance, richness and Shannon index score.

Data from four stations with two depths at Disko Fjord

Location	*Distance to shore/outlet*	*Max depth at station (m)*	*Sample depth (m)*	*Temperature (ºC)*	*Salinity (‰)*	*Abundance (cells mL^{-1})*	*Richness*	*Shannon Index*
D1	4100	106.8	5	5.9	31	0.092	5	1.322
D2	10700	113.3	5	6.5	32	0.923	6	1.563
D3	22200	113.7	5	6.3	33	4.984	6	1.5
D4	38400	113.7	5	6.3	33	22.3	6	0.929
D1	4100	106.8	30	3.2	37	0.014	4	1.182
D2	10700	113.3	30	2.4	38	0.573	7	1.595
D3	22200	113.7	30	3.4	35	3.193	6	1.010
D4	38400	176.7	30	2	36	31.537	5	0.906

Calculations for cell count

Cells/mL in backwashed dilution:

(1)

$$\frac{No.\,cells\ counted}{No.\,chambers\ counted} \cdot 1000\ chambers$$

Total amount of cells in 20L

(2)

$Cells\ \cdot\ mL^{-1}\ (equation\ 1)\ \cdot Volume$ of backwashed dilution (mL)

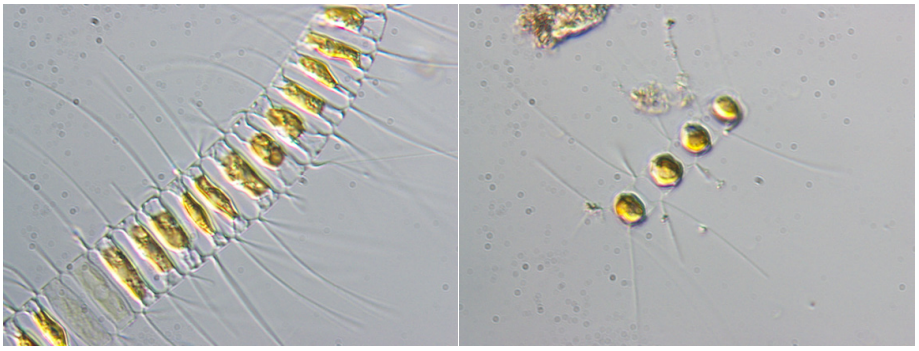
Cells·mL^{-1} in the sea

(3)

$$\frac{Total\ amount\ of\ cells\ (equation\ 2)}{20.000\ mL}$$

Photographs of representative species within each selected group counted

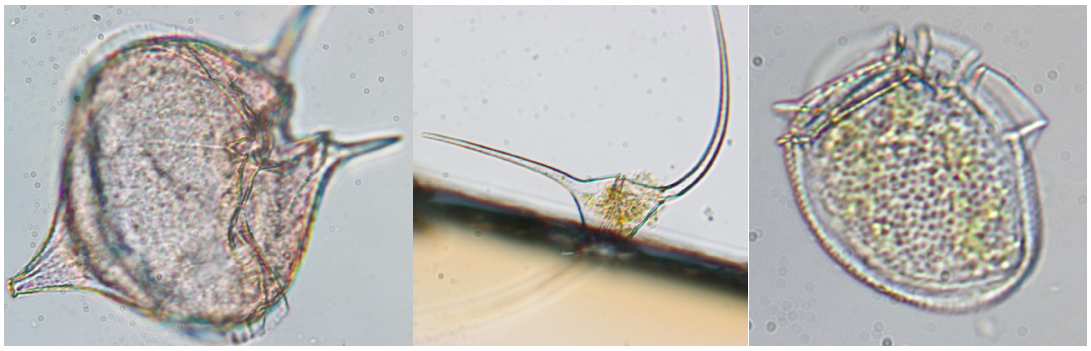
Chaetoceros spp.



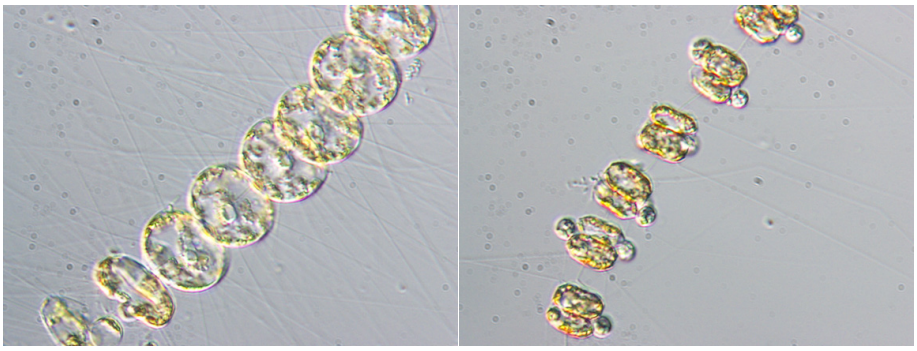
Protoperidinium spp.

Ceratium spp.

Dinophysis spp.

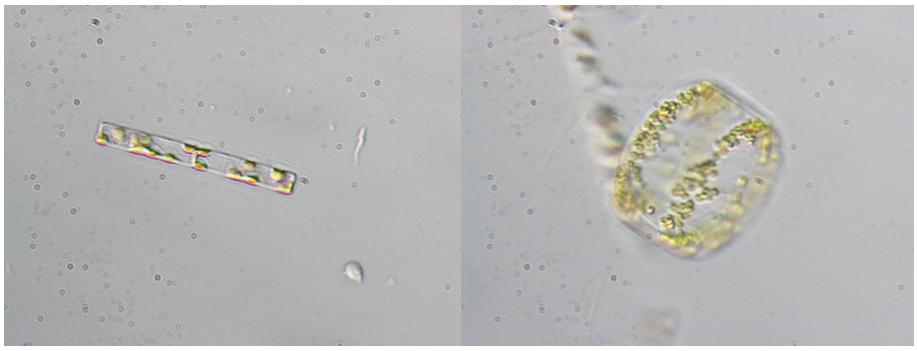


Thalassiosira spp.



Leptocylindrus spp.

Other centric diatoms



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