Arctic Biology Field Course

Qeqertarsuaq 2020

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Section for Freshwater Biology, Department of Biology
University of Copenhagen
Universitetsparken 4, 3rd floor
2100 Copenhagen Ø
Denmark

Section for Terrestrial Ecology, Deptartment of Biology
University of Copenhagen
Building 1, 2nd floor
Universitetsparken 15
2100 Copenhagen Ø
Denmark

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and
Anders Michelsen, Section for Terrestrial Ecology

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Further information about Arctic Station is available here: www.arktiskstation.ku.dk

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Preface

Day 0: We are all so excited to be here and the sheer beauty of the Greenlandic nature is unbelievable and breathtaking in the most literal sense.

Day 1: Today the work in the field started for real. Arctic char were caught, beetle larvae were found, samples were collected and some measured the greenness while others pinpointed their way to analyze the vegetation. We are still waiting to see some whales and are keeping an eye out!

Day 11: It has been a bittersweet day, as we've all had an utterly amazing stay at Arctic Station, but at the same time we're all excited for what is happening next. Still, this is for sure a 'see you later' rather than 'goodbye' (despite the insane amount of mosquitoes)!

These sentences from the diary that the students kept running on Facebook through the course, say it all. The privilege of taking part in the annual Arctic Biology Field Couse with overwhelming landscapes, large contrasts and a fascinating biodiversity is apparent for any of the 12 students.

The most important purpose of the Arctic Biology Field course held at Arctic Station is to get first-hand insight to the Arctic environment, and how it forms the terrestrial and aquatic ecosystems. Furthermore, it is the goal that the students get a detailed understanding of the organization of biological structures and the interaction between organisms. This is obtained through their own research projects. The students are involved in the whole process of defining the research questions, setting up the experimental work, doing the fieldwork, analysing the data and not least writing a comprehensive scientific report which gives the students very important skills. The setting at Arctic Station cannot be better as the arctic landscapes are just outside the front door.

The 2020 version of the Arctic Biology Field course took place from 13th to 23th July and was held despite the pandemic Covid19 which overshadowed everything - everywhere. Fortunately, it became possible to travel to Greenland during summer although under restrictions (Covid19 test and use of facemasks) and by turning Arctic Station into a quarantine site. We took extreme care to obey all Greenlandic and Danish restrictions to make sure that our visit to Qeqertarsuaq was without risk for us and for the local community.

The specific student projects are presented as chapters in this report. The project themes cover terrestrial plant community responses to soil, snow and other climatic factors as well as the occurrence and abundance of freshwater organisms in relation to environmental factors. The report can be obtained as PDF from the Arctic Station website (www.arktiskstation.ku.dk) or from Kirsten S. Christoffersen (kchristoffersen@bio.ku.dk).

Despite long working days, we also found time for excursions to Østerlien and Kuanit as well as a guided walk through the entire village. Furthermore, we had lectures about Arctic Station and the long-term monitoring programmes by the scientific leader Martin Nielsen.

Overall, the course went well and we appreciated the high service level provided by the 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 the Department of Biology.

- and yes; we did see whales eventually ☺

Kirsten S. Christoffersen & Anders Michelsen
Department of Biology
University of Copenhagen, Denmark

1st December 2020

Participants

1. Katrine Skjold Ottesen
2. Sverre Juul Schou
3. Peter Kristian Petersen
4. Kirsten S. Christoffersen (teacher)
5. Kasper Grønbech Andersen
6. Alessandra Bateman Neubert
7. Anders Michelsen (teacher)
8. Tine Seligmann
9. Anna Marie Stevnsvig
10. Sofie Kirstine Westphal Sørensen
11. Christina Fernández García
12. Ida-Marie Stokholm Mollerup
13. Regina Jo Larsen
14. Marta Contreras Serrano




Course diary

Monday 13th July (Day 1)

We have all arrived safe and sound to Arctic station. The trip was long, but we did get to have the enlightenment of experiencing the true meaning of lower face sweat.

It is 9 in the evening local time, but everyone has gone down to admire the icebergs as the Arctic summers cannot be bothered with day/night cycles and the sky is still fully lit. We are all so excited to be here and the sheer beauty of the Greenlandic nature is unbelievable and breathtaking in the most literal sense.

Pictures do not do it justice, so here are some of us and some bones we found instead.

Tuesday 14th July (Day 2)

This morning we all worked on our projects in freshwater and terrestrial ecology in our classroom at the Arctic Station. We had to prepare the tools and apparatus and make sure that we had everything ready for tomorrow. Within the terrestrial ecology we are very excited to start our vegetation analysis and herbivory on the warming experiments along a gradient and under longer snow exposition. The groups working on freshwater are assessing beetle communities, phytoplankton and Arctic charr growth.

During the afternoon, we took a nice guided hike to visit all the experiment locations around the station. Even with a misty weather, the views were stunning! Oh, and before I forget… some of the students got so deeply moved by this trip that made this amazing Greenland nature inspired vegan cake!! Looking forward to start our field data collection routines.

Wednesday 15th July (Day 3)

For some the day starts early with a (very fast) dip in the sea. Today the mosquitos were abundant from the early morning and followed us almost all the way into the arctic water. That should have been a clue to be aware of mosquitos for the rest of the day. Unfortunately not everybody was properly prepared and thus suffered the consequences.

Today the fieldwork started for real. Arctic char were caught, beetle larvae were found, samples were collected and some measured the greenness while others pinpointed their way to analyze the vegetation.

We are still waiting to see some whales and are keeping an eye out!



Thursday 16th July (Day 4)

All groups are well on their way with their projects and work very independently. Kasper and Marta had a big success with their beetle traps. They recorded on video a beetle´s big mistake: to enter their trap! Regina and Ida-Marie have started to analyze the samples they gathered yesterday and added new water samples to the collection today. While Sverre and Katrine were taking the temperature of various plots, an Arctic fox (*Vulpes lagopus*) took Katrine's jacket and ran away with it. Christina, Sofie and Anne visited the experimental plots of the snow fences, and did some routine work advancing with their project. And finally Alessandra and I caught one of the largest Arctic char compared to previous studies from Blæsedalen. To end the day I went snorkeling by the icebergs and came out of the water with two Atlantic wolffish. The others did not expect that!!

Friday 17th July (Day 5)

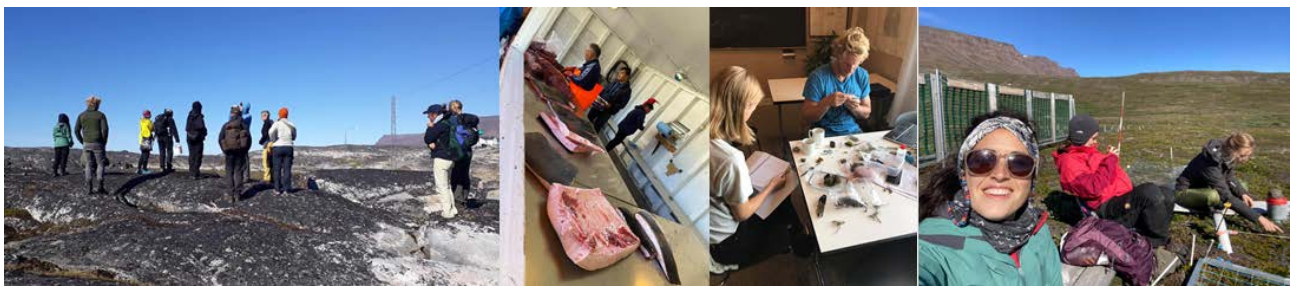
This morning we woke up to a rather windy day, thus the "perfect" day for more of us to join the morning dip at the beach for the first time - as if regular conditions weren't cold enough!

Following this fresh start we were ready for a city walk through Qeqertarsuaq guided by Kirsten. We heard different stories about the culture of the Greenlandic people, and amongst other places saw the beautifully decorated graveyard, and the area were all the sledge-dogs were hangin' during summertime, and enjoyed the coziness of the village. We even got company of an elderly local man at the church.

After coming back from the walk, it was time to get back to business. The terrestrial groups were out in the field collecting plants and were happy to find that the mosquitoes for once were low in numbers. Lots of thanks to the wind! Even happier were Marta and Kasper aka the "Beetle-group", when they went to collect their traps near the city, and found that beetles had been caught in all of the ponds!

Furthermore, all of the groups had lots to do at the station. Plants were carefully identified by Katrine and Sverre as well as by Anna, Sofie and Christina; Peter and Alessandra started on the difficult job of locating and collecting the otoliths of arctic char; Marta and Kasper took a closer look at the beetles whereas Ida-Marie and Regina spent all afternoon filtering the rest of the collected lake samples. Additionally, they were exposed to a small proportion of the world of chemistry when the alkalinity measurements were started.

We have had another fantastic day in Greenland and some of us even saw freshly caught and cut whale meat! At 7 pm it was even more improved by the (as always) delicious dinner. We even had a taste of the Atlantic wolffish captured by Peter yesterday!



Saturday 18th July (Day 6)

To start the day off right, a few students took a fresh dip in the ocean, followed by a nice hot shower at the station. After breakfast a group of students and teachers attended a Greenlandic wedding and congratulated the happy couple. After the ceremony it was time for some field work.

Alessandra and Peter went one last time to "Blæsedalen" to do some final measurement, while the two terrestrial groups (Sverre+Katrine and Sofie+Christina+Anna) continued their hard work by identifying plants and herbivory.

The "beetle-group" (Marta+Kasper) and the "algae-group" (Ida-Marie+Regina) joined forces, for a +20km demanding hike to some distant lakes. On the way, there was an opportunity to climb some steep slopes and get close to the glacier on top of the mountains. After the hike, Marta and Kasper had time to empty some of their traps near the station and found their largest number of beetles and larvae yet, while Ida-Marie and Regina filtered their precious water-samples. Back in the lab Alessandra and Peter were successful in retrieving otoliths from all their specimens.

After a yet a nice dinner, Martin - the scientific leader of Arctic Station - gave an interesting lecture on the history of the station, the monitoring programs and his own scientific work.

Sunday 19th July (Day 7)

After our first week at Arctic station working so hard on our projects, today we all start to feel very very tired. Some people even stayed awake working past midnight (the advantages of the arctic midnight sun). However, we are very happy with our data collection and seeing our own and our mates' progress.

The botany groups have been in the field all day identifying the amazing variety of arctic lichens and assessing herbivory. On the other hand, the fresh water groups have had a bit more relaxed day at the laboratory assessing the arctic char age through the otoliths (the ear stones you see in the picture below that have growth rings), looking at beetle larvae size and filtering phytoplankton water samples.

Off topic, it has been laundry day too and we ate amazing mixed berry muffins to increase our blood sugar levels to keep on working on our data.

Monday 20th July (Day 8)

The life at the station has now fallen into a pleasant routine, which for many include a morning swim out to touch the nearest ice berg, and the water actually seems to get warmer.

Today's work included microscoping algae, vegetation analysis in the field, identification of lichens and mosses, in addition to emptying water beetle traps near a glacier and determining fish age by analyzing otoliths (ear stones), all in all a quite diverse bunch of projects, spanning widely across the realm of living things.

Even so, a thing that got everybody to look up from their work was our first sight of a whale! A fin whale (*Balaenoptera physalus*) swimming in the ocean in front of the station. In the evening we all went for a hike to see the incredible rock formations at Kuannit, where we saw the Kvan-fields (*Angelica archangelica*), a bunch of Black guillemots (*Cepphus grylle*) and a pair of white Greenlandic Gyrfalcons (*Falco rusticolus*).

Tuesday 21st July (Day 9)

It is boat trip time!

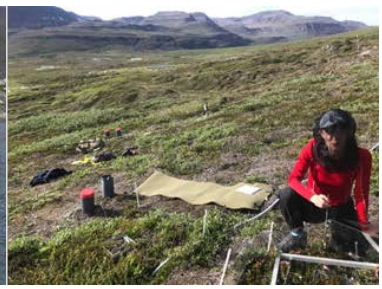
To start the day, some of us went for a quick and cold dip in the icy waters, a good way restart the blood circulation through our skin and enhance the sensitivity of our senses.

After an abundant and nutritious breakfast, we packed our bags and walked to the Qeqertarsuaq harbor to board the Arctic Station boat that would take us to visit the Kangarssuk lake. The fact of boarding the ship was hard for Marta and me at the thought of getting sea-sick again, recalling our journey from Aasiaat to Disko. However, the trip ended up being smooth and very enjoyable for everybody. The disappointment of not seeing the expected whales breaking the sea-water surface was buffered by the sighting of a black Arctic fox calmly making its way along the shore.

Besides two absences, most of the group spent the morning walking around the lake and enjoying the impressive view of the western Disko Island landscape. Meanwhile, Kirsten took her needed water samples and Peter helped her retrieve data loggers lost in the lake's bottom sediment. Marta and Kasper spent the whole time looking for beetle larvae in nearby ponds.

In the afternoon, all groups advanced in their projects, but most importantly, we worked on a preliminary presentation of what each of our projects are about, explaining what we have done until now and how we expect to continue our work. After 2 hours of presentations and group discussions, I can confirm that everybody here has done a great effort and are on the right path towards a great scientific report. Cannot wait to read them all!



Wednesday 22nd July (Day 10)

As our journey gets to an end, all of the projects seem to be in good shape and promise some very interesting results. Katrine and Sverre have been measuring the last environmental parameters from their plots, completing their temperature, NDVI and moisture analyses. Cristina, Sofie and Anna have also had a very productive day and have concluded with all their fieldwork by the snow fences. By now, the plant people (as we call them) have all of their data ready and have just kidnapped Anders for some late-night statistical advice. The freshwater groups have spent most of the day in the lab or working on their data. Ida-Marie and Regina have finished looking at all of their algae samples in the microscope and are finding some congruent and enlightening trends. Peter and Alessandra have been going into the math behind their data regarding arctic char growth rates (and apparently inventing their own calculations for it). Kasper and Marta have mostly struggled with the statistical analyses of their beetle data but have found some interesting significant results.

Our projects keep us busy at the time, but whales have been diving in and out the waters right in front of the station, giving everybody a well-deserved and exciting break from work every now and then. To finish the day, we have had an amazing assortment of Greenlandic "tapas" that Kirsten has prepared for us as a surprise: narwhale blubber, scallops, halibut fish, dried cod snacks, arctic charr and several other Greenlandic delicacies, all marinated with a fancy rosé wine. Some sweet carrot cake has closed up the perfect farewell supper. We are now sitting in the living room having some tea and, as these words are being typed in, one can certainly sense the strong feeling of satisfaction that hard work and adventure bring. Cannot wait to be old and wrinkly and tell my grandsons about these days.

Thursday 23rd July (Day 11)

Today was the very last day as everyone left Arctic Station and Disko. Katrine, Sverre, Anna, Sofie, Marta, Alessandra, and Cristina had to get up early to catch the ferry to Ilulissat at 7 am. Regina, Ida-Marie, Peter, and Kasper were lucky - they were allowed a slow morning as their ferry was not until 9.30. Anders and Tine stayed behind, whereas Kirsten joined the later ferry team. When everyone arrived at Ilulissat, we checked in to our different hostels. Afterwards, some took (several) naps where others went for walks around town and out to see the ice fjord. By the ice fjord many groups of whales turned up to feed on delicious fish and other goodies, which was a very nice surprise!

In the evening we all met up to have dinner and beer before going our separate ways tomorrow. Katrine, Sverre, Anna, Sofie, Marta, Alessandra, and Cristina are staying in Greenland and going on different trips, whereas Regina, Ida-Marie, Peter, and Kasper (along with Kirsten) are going back to Copenhagen. It has been a bittersweet day, as we've all had an utterly amazing stay at Arctic Station. Still, this is for sure a 'see you later' rather than 'goodbye' (despite the insane amount of mosquitoes).

***Pedicularis lapponica*, in arctic plant community at "Blæsedalen", Disko Island, Greenland.**
Photo: Sverre J. Schou

The effects of increased growing season temperature on arctic plant communities along a snow-bed gradient on Disko Island, Greenland

Katrine S. Ottesen & Sverre J. Schou

Abstract:

The Arctic is characterized by short growing seasons, which is expected to be impacted by climate changes with prospected increasing temperatures. By looking at a snowbed gradient with varying growing season durations in combination with warming treatment, the impact can be investigated. The study was designed using a pre-existing fully cross-factored design located along a snowbed gradient with four zones, on Disko Island, Greenland. Each zone had five replicates of open topped chambers (OTC) to simulate global warming with paired control plots. Additionally, two zones were added at the top and bottom of the hill with only control plots. The point-intercept method was used to analyze vegetation coverage and species diversity. In general, variation with different growing season duration was much larger than the difference between treatments with warming and control. No interactive effect was detected. However, the coverage of graminoids increased with almost twice as much for treatments with OTC than for control plots. Only graminoids responded significantly to the OTC-treatment. As graminoids tend to be first movers, this could indicate a change in vegetation composition and the beginning of a succession of species on Disko Island with the increasing temperatures. Growing season duration had a large impact on species abundance and composition. Overall, no rapid change in vegetation communities was detected, however, by continuing the experiment, future studies can show the long-term effects and interactive effects of global warming on vegetation communities on Disko Island.

Keywords: *Tundra, climate changes, vegetation composition, snow cover, biodiversity, warming experiment*

Introduction

The Arctic region is characterized by a short growing season and low temperatures. However, the climate in the region is changing with a fast pace as compared to the rest of the world, with a temperature increase over the last 100 years close to double that of the global average (Solomon et al., 2007). This pattern in the temperature increase is a trend that is very likely to continue (IPCC, 2014). The elevated temperature increase in the Arctic is largely attributed to positive feedback effects, such as the decreasing permafrost layer causing a release of greenhouse gasses, and the decrease in the ice and snow cover, which is lowering the albedo effect of the region and thus making it more heat absorbent (Solomon et al., 2007). The snow cover is providing protection from weather and cold temperature for the underlying vegetation, in addition to determining the length of the growing season for Arctic plants by its duration, thus, making these plant communities directly affected by climate changes and global warming in several ways (Niittynen et al., 2018).

A continuous increase in the temperature of the Arctic region could cause a greening of the Arctic vegetation as a result of increased photosynthesis rate, due to the longer growing season (Schedlbauer et al., 2018). Additionally, these changes could also alter the composition of the Arctic vegetation, and lead to northwards shifting of the circumpolar Arctic bioclimatic subzones (CABS), and succession of northern vegetation zones, getting outcompeted by more southern species that thrives better in a longer growing season (Niittynen et al., 2018; Walker et al., 2005; Weijers et al., 2017). The succession by the more southern vegetation zones, could ultimately lead to a movement towards the north of the tree-line. This trend is indicated by an altitudinal movement of the tree-line in

mountainous areas as a response to climate changes and elevated temperatures (Gatti et al., 2019) in addition to already reported expansion of shrubs within the arctic regions (Tape et al., 2006).

The Arctic is subject to a rather low overall biodiversity, although many niches are understudied. When looking at plants, the Arctic regions also show a very low species diversity. Less than one percent (ca. 2218) of the world's vascular plants are represented in the Arctic, and only 106 endemic species are found in the region. Additionally, there is a large overlap in the species present within the different bioclimatic and vegetation zones (Alaska Geobotany Center, 2008; Meltofte et al., 2013). However, when looking at mosses and lichens, the trend is quite different. About eighteen percent (ca. 900) of the mosses and ten percent (ca. 1750) of the total number of lichens worldwide are found in the Arctic (Meltofte et al., 2013). As many mosses have a wide distribution range, either due to the age of the group and/or their reproduction form, there are very few endemics to the Arctic itself, as many also are present in sub-Arctic mountain ranges (Meltofte et al., 2013). The Arctic lichens, although severely understudied, counts almost 300 species that are endemic or rare outside the Arctic, additionally, lichens are the dominating vegetation in high altitudes as well as the higher CABS (Alaska Geobotany Center, 2008; Meltofte et al., 2013).

As the Arctic region is facing a great rise in temperature and is subject to a short growing season, the ecosystems may be more susceptible to changes in the climate than other parts of the world. It is therefore important to understand how an increase in temperature and lengths of growing seasons will affect species abundance and diversity, both as main factors and in combination.

The aim of this study was to investigate Arctic plant community responses to climate changes, by looking at the species composition of the vegetation, biomass/coverage and the greenness, measured by Normal Difference Vegetation Index (NDVI). To simulate climate changes, we looked at two different factors: increased growing season temperatures and varying growing season duration. The temperature increase was simulated by establishing test plots artificially heated by transparent open topped chambers (OTC). OTC-plots, along with corresponding control plots, were placed along an altitude gradient to obtain different duration of growing seasons due to the varying duration of the snow cover that increases with altitude.

Hypothesis

For this project we tested the two following hypotheses, in order to investigate the effect of climate changes on Arctic vegetation.

Hypothesis A:

H_0 = Increased temperature during the growing season will not affect plant communities along slopes on Disko Island, Greenland, by altering composition, cover and/or greenness.

H_1 = Increased temperature during the growing season will affect plant communities along slopes on Disko Island, Greenland, by altering composition, cover and/or greenness

Hypothesis B:

H_0 = Different snow cover duration will not influence plant communities along slopes on Disko Island, Greenland, by altering composition, cover and/or greenness.

H_1 = Different snow cover duration will influence plant communities along slopes on Disko Island, Greenland, by altering composition, cover and/or greenness.

Methods and Materials

Study site and experimental design

The study was conducted between the 15th and 22nd July 2020 using a pre-existing fully cross-factored design located along a snowbed gradient in Itinneq Kangilleq (Blæsedalen) (69.160 N, 53.270W) approximately 3 km from Arctic Station in Qeqertarsuaq (Godhavn) on Disko Island, Greenland. The site was located in the low-arctic climate zone and the annual air temperature from 1991-2017 was (mean ± SD) -3°C ± 1.8°C (Zhang et al., 2019).

The experiment was established in early July 2014 to study the long-term effects of climate changes. To simulate rising temperatures plots with OTC, made by transparent plastic, were

situated first at three different zones along the snowbed gradient with a corresponding control plot and each with five replicates. An additional zone was added later (Zone 2) in August when snow had melted. The plots are referred to as 'T' for warming with OTC, 'C' for control and 'Zone 2', 'Zone 3', 'Zone 4' and 'Zone 5' descending from high elevation to low. In total, the existing experiment consisted of 40 plots. This study was conducted using an incomplete design as two additional zones were added, each containing five untreated C-plots. 'Zone 1' at the very top of the hill and 'Zone 6' as the lowest zone of the experiment.

Temperature, moisture and NDVI measurements

The temperatures were measured using Ludwig Schneider Pocket Digit Thermometer Typ 12070. The soil temperature was measured by pressing the thermometer two centimeters into the ground, and the surface air temperature was measured by placing the thermometer resting on the ground, without the tip touching it. Both surface air and soil temperature measures were replicated three times for each plot. The soil moisture was measured using a Theta Probe ML2x, by pressing the tip into the ground. This was repeated a total of three times for each plot. The NDVI was measured using a SKYE SKR 100 with a SKYE 660nm/730 nm sensor. The sensor was held approximately 50 cm above ground and the NDVI were measured at two different wavelengths, 660 nm and 730 nm, in the same location. The paired measurements were carried out in a total of three times at three different locations for each plot. Temperature and NDVI measurements were made on two dates, the 16th of July, which was a cloudy day with little sun, and on the 22nd of July, which was a very sunny day, with only a few clouds. Soil moisture was only measured on the 16th of July.

We used the NDVI as an estimate of greenness applying the following equation to calculate the relation between the wavelengths 660 nm (Red) and 730 nm (Near InfraRed (NIR)):

$$NDVI = \frac{NIR - Red}{NIR + Red}$$

Vegetation analysis

The vegetation within each plot was examined using the point-intercept method. A quadratic frame with a grid consisting of 11 x 11 squares was placed in each plot. At each grid point, a pin was vertically lowered making a total of 100 points. Each time the pin touched a plant, moss, liverwort, lichen, litter, rock or bare soil it was noted. If the pin touched the tin squares, added for a different experiment, or a point within them it was not noted, and an additional pin measurement was taken in the grid point made up by the frame and the grid, to compensate for this. All plants were identified to species level, except *Taraxacum sp*., and mosses, liverworts and lichens were identified to the lowest possible taxonomic level. Various floras were used for the identification of vascular plants (Bocher et al., 1968; Rune, 2011) mosses and liverworts (Atherton et al., 2010; Christiansen, 1981) and lichens (Hansen, 1995).

Statistical analysis

Effects of warming (OTC) and location along a snowbed gradient on surface air temperature, soil temperature, soil moisture, NDVI, vegetation cover and vegetation diversity were analyzed for plots in 'Zone 2', 'Zone 3', 'Zone 4' and 'Zone 5' using an analysis of variance with treatment (C and T) and location (zones) as fixed effects (Two-Way ANOVA). Block was included as factor (but did not have significant effects).

A post hoc Tukey's test was used to demonstrate the differences between zones.

The effect of zones was separately analyzed further adding the plots of two additional zones, 'Zone 1' and 'Zone 6' using One-Way ANOVA. *P*-values below 0.05 were considered significant. All statistical analysis was performed using Statistical Package for the Social Sciences version 27 (PASW v 27.0; SPSS Inc., Chicago, Ill., USA).

Illustrations were made using Microsoft Office Excel version 16.37.

Biodiversity analysis

The biodiversity was analyzed using the Shannon-Wiener index method, to measure biodiversity in an ecosystem (Cao, 2002). The more species, the higher the index number, additionally, a more heterogeneous distribution of species in relation

to the amount of different species, will also give a higher score. The Shannon-Weiner index assumes that the population is infinite, and all species are represented equally in the test (Cao, 2002). The equation for estimating the Shannon-Weiner index number, is illustrated below, where "H" is diversity index number, "i" is a given species, "Pi" is the proportion of a given species, weighted from the sum of all hits in the test, and "s" is the number of different species (Cao, 2002; Shannon, 1948).

$$H = \sum_{i=1}^{s} Pi\ ln(Pi)$$

Pielou's evenness was used to describe the abundance of species, from the Shannon-Weiner index (Pielou, 1966). The equation for estimating Pielou's evenness is illustrated below, where "J" is the index number, ranging from 1 to 0, where 1 means that all species are equally represented, and 0 means that one single species has total dominance. "H" is the diversity index number from the Shannon-Weiner index, and Hmax = (LN(S)), where "S" is the number of different species, is the highest possible Shannon-Weiner index number for the test site (Pielou, 1966).

$$J = \frac{H}{Hmax}$$

Results

Temperature, moisture and NDVI:

There were no significant differences in 'Soil Temperature 1 and 2' between treatment ('C' and 'T') (*P*=0.25 and *P*=0.30, Two-Way ANOVA) and location (zone) (*P*=0.18 and *P*=0.27, Two-Way ANOVA). However, 'Air Temperature 1' showed a main effect of both treatment and location (*P*=0.02 and *P*=0.01 Two-Way ANOVA), but the second measurement, 'Air temperature 2', showed no main effect of neither treatment nor location (*P*=0.45 and *P*=0.56 Two-Way ANOVA). 'Soil Moisture' showed a significant difference at various locations along the gradient, but not for treatment (*P*=0.03 and *P*=0.78, Two-Way ANOVA). The same pattern applied for 'NDVI 1 and 2', where significant differences were detected for locations along the gradient (*P*<0.001 and *P*<0.001, Two-Way ANOVA), but not for treatment (*P*=0.31 and *P*=0.55, Two-Way ANOVA)(Appendix 1).

Vegetation diversity and vegetation coverage:

There were significant differences in 'total number of species' and 'total coverage' between different locations along the gradient (zone) (*P*<0.001 and *P*=0.01, Two-Way ANOVA), but not for treatments (C and T) (*P*=0.90 and *P*=0.35, Two-Way ANOVA) (Figure 1 and 2). 'Graminoids' were the only vegetation group that showed a main effect of both treatment and location in the assessment of abundance (*P*=0.01 and *P*=0.02, Two-Way ANOVA) (Figure 3). No significant interaction effects were detected for any of the assessed variables (Table 1, Two-Way ANOVA). When correcting for "block" as a co-variate, it did not alter which functional types that were distributed significantly. Neither was any functional type significantly distributed for "block".

When including Zone 1 and 6 and only analyzing the control-plots, coverage showed significant differences for location for 'Lichens', 'Evergreen shrubs', "Deciduous shrubs', 'Forbs', Lycopods, Ferns and Horsetails' and 'Total Coverage' (P<0.05, One-Way ANOVA), but not for 'Graminoids' and 'Mosses and Liverworts' (*P*=0.75 and *P*=0.17 One-Way ANOVA).

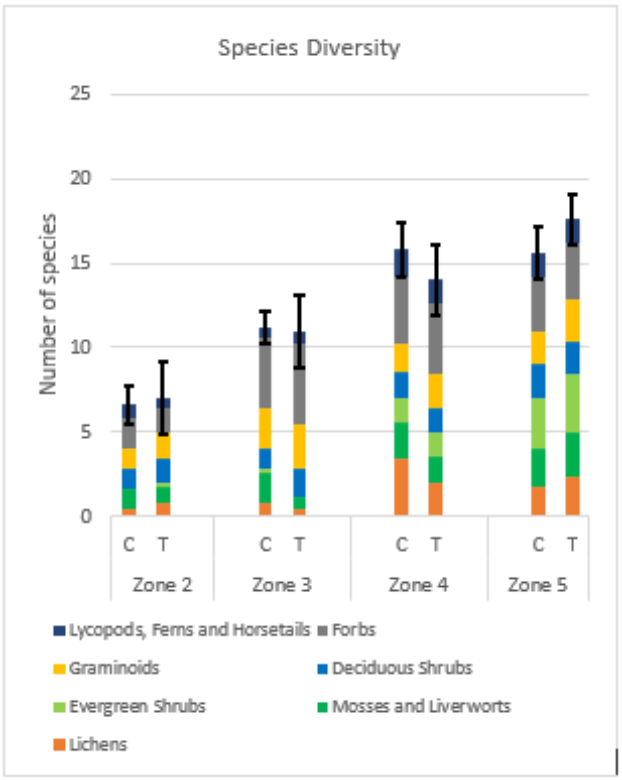


Figure 1: All data is presented as mean with confidence interval for Zone 2-5. Only zones with both 'C' and 'T' plots were analyzed. There was a significant difference between species diversity for all locations, but not for treatment (Appendix 2, Two-Way ANOVA). Species diversity showed a linear increase from Zone 2-5.

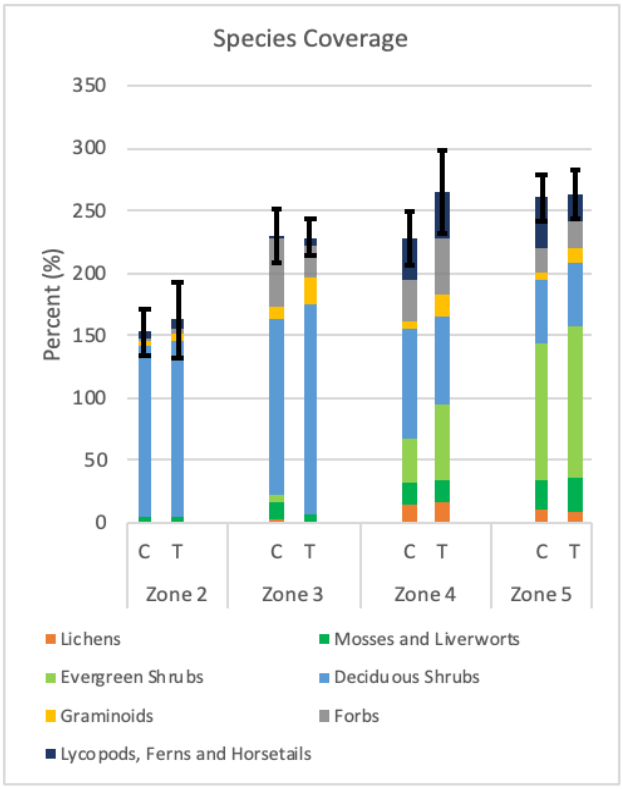


Figure 2: All data is presented as mean with confidence interval. Only zones with both 'C' and 'T' plots were analyzed. There was a significant difference between 'Lichens', 'Mosses and Liverworts', 'Evergreen Shrubs', 'Deciduous Shrubs', 'Graminoids', 'Forbs' and 'Lycopods, Ferns and Horsetails' at different locations but not for treatment (Appendix 2, Two-Way ANOVA). As the only species, 'Graminoids' showed a main effect for treatment and location (*P*=0.01 and *P*=0.02, Two-Way ANOVA) (Figure 3). Species coverage showed a linear increase from Zone 2-5.

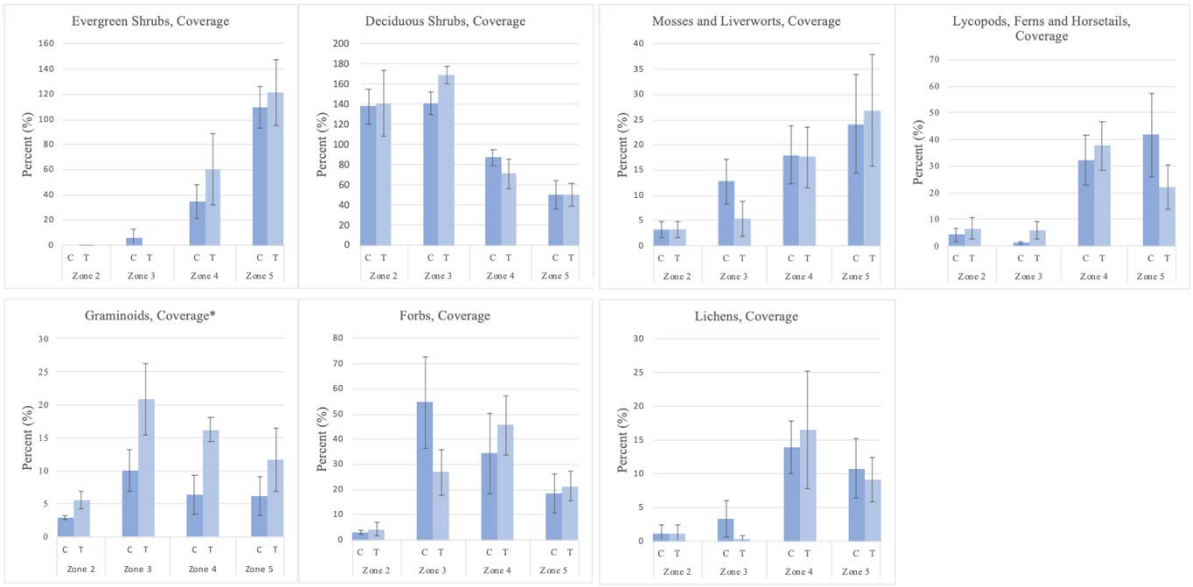


Figure 3: All data is presented as mean with confidence interval. Only zones with both 'C' and 'T' plots were analyzed. *indicate statistical difference for treatment (P<0.05, Two-Way ANOVA). All species showed significant differences for location and only 'Graminoids' showed also significant differences for treatment (*P*=0.01, Two-Way ANOVA).

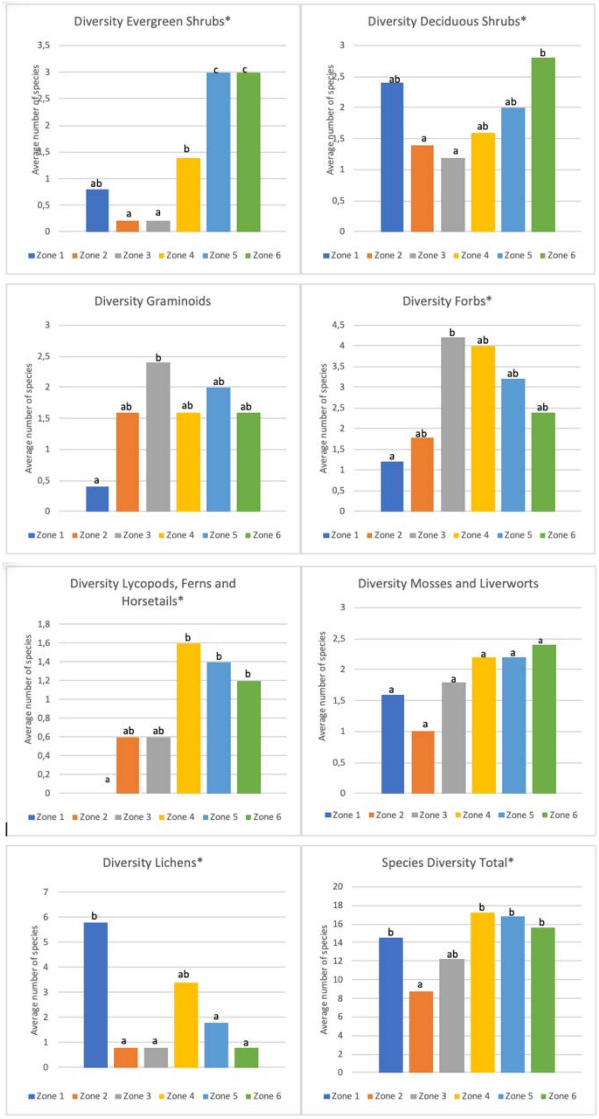


Figure 4: All zones analyzed for 'C' plots. *indicate a significant difference (P<0.05, One-Way ANOVA). [a,b,ab,c] Indicate a significant difference between the groups according to Tukey's post-hoc test. There was a significant difference in species diversity between 'Lichens', 'Evergreen Shrubs', 'Deciduous Shrubs', 'Forbs' and 'Lycopods, Ferns and Horsetails' at different locations (*P*<0.05, One-Way ANOVA), but not for 'Mosses and Liverworts' (*P*=0.46 One-Way ANOVA). There was a tendency for 'Graminoids', but not a significant difference (*P*=0.07, One-Way ANOVA).

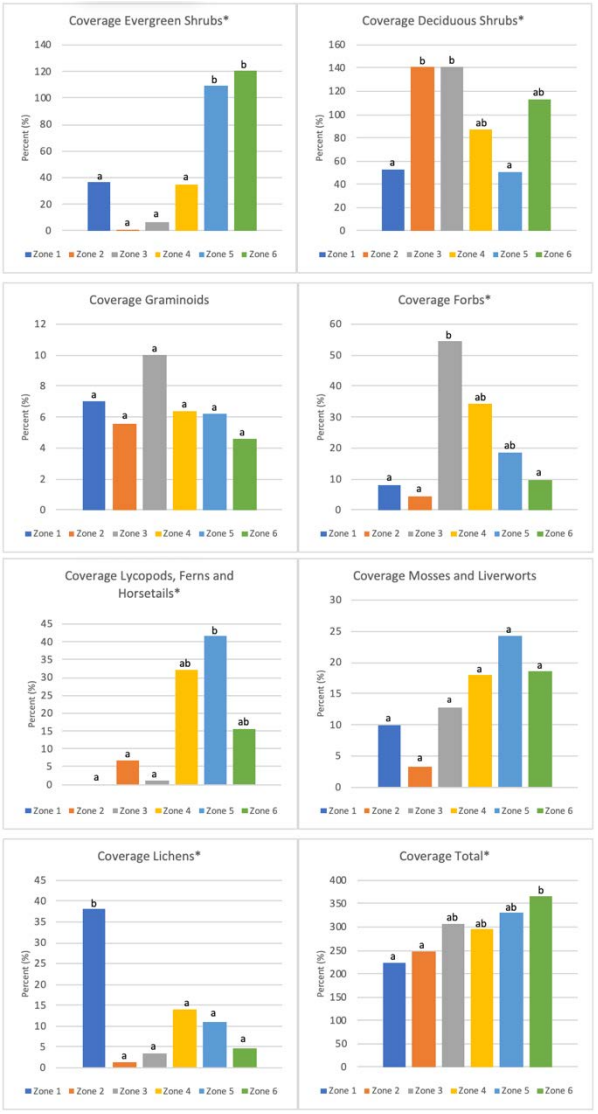


Figure 5: All zones analyzed for 'C' plots. *indicate a significant difference (P<0.05, One-Way ANOVA). [a,b,ab,] Indicate a significant difference between the groups according to Tukey's post-hoc test. There was a significant difference in species coverage between 'Lichens', 'Evergreen Shrubs', 'Deciduous Shrubs', 'Forbs' and 'Lycopods, Ferns and Horsetails' at different locations when not accounting for treatment, but not for 'Graminoids' and 'Mosses and Liverworts' (*P*=0.75 and *P*=0.17 One-Way ANOVA).

Vegetation analysis

A total of eighty-eight different species were found, where seventy were identified to species level, nine to genus and nine to a higher taxonomic level. Evergreen shrubs counted four species, deciduous shrubs four species, graminoids ten species, forbs twenty species, ferns, lycopods and horsetails four species, mosses fourteen species and lichens thirty-eight different species (Appendix 3). When moving down the gradient an increase in biomass/coverage was seen, as well as a trend showing an increase of species number, when looking at Zones 2-5. Zone 1 stood out from the other zones by having the largest number of unique species, and relatively few overlapping species, which was a prevailing tendency among the other zones. Only one species, *Bistorta vivipara*, were present throughout all zones (Appendix 3).

Table 1: A descriptive analysis of the species and vegetation composition for each plot group.

Zone 1C	The uppermost zone was characterized by bare rocks and soil, and a large number of lichens, predominantly *Cetraria* and Stereocaulaceae species. The vegetation, where present, was in 1-2 layers, with moss communities dominated by *Dicranum scoparium* and *Polytrichum* species, additionally this were the only zone where liver worts were found. Litter were also present, but almost exclusively beneath evergreen and deciduous shrubs, largely dominated by *Vaccinium ulignosum* and *Empetrum nigrum*. Only six herbaceous plant species were present, of which two thirds, were only found in this zone, including one graminoid; *Carex rupestris,* and two cushion-forming forbs; *Diapensia lapponica* and *Silene acaulis*.
Zone 2C	This zone was almost exclusively populated with 1-3 layers of *Salix herbacea* with underlying litter, covering the plots. Very few herbaceous plants were present, chiefly *Equisetum arvense*, but practically no mosses and lichens.
Zone 3C	The third zone was very similar to the second, although with far more vascular plants emerging from the *S. herbacea* layers which consisted of 2-4 layers, in addition to patches dominated by 1-2 layers of *Sibbaldia procumbens*. Namely *E. nigrum, Salix glauca, Bistorta vivipara, Taraxacum sp.* and *Pedicularis* species in addition to a mix of sedges and grasses. A moss layer where present sporadically, mainly *D. scoparium* and an unidentified species.
Zone 4C	This zone was characterized by varying types of cover dominated by different species. One with 1-3 layers of *S. herbacea* and underlying litter, a second type consisted of 2-4 layered evergreen shrubs, dominated by *E. nigrum*, with and underlying moss layer dominated by *Aulacomnium palustre,* a third type consisted of 2-4 layers of *Diphiastrum alpinum* with underlying moss or litter layer and a fourth type was dominated by 1-2 layers of *S. procumbens* with underlying litter. Additionally, patches of varying *Cladonia* species were also present throughout the zone. A small amount of graminoids and forb was emerging, mainly from the *S. procumbens* and *S. herbacea* cover, dominantly *B. vivipara* and *Pedicularis* species, where three different species were present.
Zone 5C	This was the highest zone that was overall dominated by evergreen shrubs, in 2-3 layers composed chiefly *E. nigrum* and *Phyllodoce caerulea,* in addition to a lower layer of *D. alpinum* and *Harrimanella hypnoides* and an underlying moss layer dominated by *Racamitrium c.f. canescens* and an unidentified species. *S. glauca* made an extra top layer in large parts of the plots, and patches of 2-4 layers of *S. herbacea* was still present, in addition to sporadic lichen patches of *Cladonia crispata* and *Stereocaulon* species. Vascular plants were found sporadically, mainly *B. vivipara*, *Pedicularis* species and a mix of graminoids, similar that of zone 3c and 4c.
Zone 6C	The lowest control plot was characterized by an almost absence of *S. herbacea*, but covered in a dense canopy of shrubs, with top layers of *Betula nana* and *S. glauca*, followed by middle layers heavily dominated by *E. nigrum, P. caerula* and *Cassiope tetragona*, and a bottom layer of *V. ulignosum* and *Lycopodium annotinum*, in addition to and underlying moss layer, dominated by *Scorpidium sp.* and an unidentified species. Almost no lichens were found, apart from presence of *Cetraria islandica*. A little number of forbs and graminoids were present in this zone, mainly *Pyrola grandiflora* and *Poa pratensis*. *Juncus trifidus* was found only in this zone.
Zone 2T	The uppermost test plots showed a strong dominance of *S. herbacea* in 1-3 layers with approximately ten percent *S. glauca*. A very limited number of vascular plants were present, and these mainly consisted of *E. arvense* and *Poa alpina*. Hardly any mosses or lichens were found in these plots.
Zone 3T	These test plot showed similarity to the level above, by being largely dominated by *S. herbacea*, although with and extra layer of coverage, additionally a sporadic moss layer,

	mainly composed of *A. palustre* were present. A very high diversity of herbaceous vascular plant was present in these plots; six species of graminoids, twelve species of forbs and three species of lycopods and horsetails, of all these *Pedicularis flammea* and *Festuca rubra* were most abundant. Virtually no lichens were observed.
Zone 4T	The prevailing tendency in these plots revealed and almost equal dominance between evergreen and deciduous shrubs, mainly between *S. herbacea* and *E. nigrum.* Additionally, several patches of *D. alpinum* and *S. procumbens* were present. An underlying moss layer covered about twenty percent of the plot area, chiefly consisting of *A. palustre*. Many lichens were also present, mainly *Stereocaulon paschale*, *Peltigera* and *Cladonia* species. A relatively large number of other graminoids and forbs were present, especially *B. vivipara* and *Trisetum spicatum*.
Zone 5T	The lowest of the test plots were characterized by a dominance of layers of evergreen shrubs chiefly consisting of *H. hypnoides* and *P. caerulea*. Species such as *S. herbacea, S. glauca, E. nigrum, C. tetragona* and *D. alpinum*, all contributed significantly to the thick layers of shrubs, in addition, to an underlying twenty-five percent moss cover, namely composed of *A. palustre*, *Polytrichum* species and an unidentified species. Some lichens, mainly *S. paschale* were also present. Apart from a good amount of *B. vivipara* and *Pedicularis* species, very few forbs were registered. The graminoids were well represented, with 6 different species, mainly *Carex* species.

Table 2: This table shows the results of the biodiversity analysis, where "H" is the Shannon-Weiner index value, "Hmax" is the maximum possible index number for the given number of different species, and "J" is Pielous' evenness index number describing the dominance of species. The range of "J" is 0-1, "0" being total dominance of one species and "1" being equal distribution of all species.

	Zone 1c	Zone 2c	Zone 3c	Zone 4c	Zone 5c	Zone 6c	Zone 2t	Zone 3t	Zone 4t	Zone 5t	Zone 2c-5c	Zone 2t-5t
H	2,609	0,573	1,752	2,618	2,605	2,408	0,963	1,454	2,680	2,868	2,342	2,446
Hmax	3,434	2,708	3,332	3,829	3,664	3,526	2,996	3,401	3,611	3,738	4,111	4,159
J	0,760	0,211	0,526	0,684	0,711	0,683	0,321	0,428	0,742	0,767	0,570	0,588

Biodiversity analysis

The Shannon-Wiener index showed very similar scores in Zones 1, 4, 5 and 6, Zone 5T scoring slightly higher than the others and Zone 2C given the lowest index number of the test. There was a prevailing trend of an increasing index number when going down the gradient throughout Zones 2 - 5, but only a slightly higher index number was found for the treated plots, compared to the control plots (Table 2). Pielous' evenness index also showed a trend to increase down the gradient in Zones 2 - 5, and hardly any overall difference between treated and control plots. The highest evenness index numbers were found in plot 1C and 4T, and the lowest index value was found in Zone 2C and 2T, which were also a striking observation when looking at the plots, supporting almost exclusively Salix herbacea specimens (Table 2).

Discussion

This study demonstrates the main and combined effects of growing season duration and an increase in temperature on vegetation diversity and coverage. In general variation with different growing season duration was much larger than the difference between treatments with warming and control. No interactive effect was detected.

Increased growing season temperatures

Our experimental manipulation of climate changes resulted in an average increase in temperature of 1.77 degrees Celsius (Air Temperature 1) and 1.02 degrees Celsius (Air Temperature 2) for plots treated with OTC as compared to ambient temperatures. The

surface air temperature showed a main effect of treatment for the first measurement (Air Temperature 1) (P=0.02 Two-Way ANOVA), but not the second (Air Temperature 2) (P=0.45 Two-Way ANOVA) (Appendix 1). The temperature was measured on two different days with two different weather conditions. As the second measurement was conducted during sunshine, this could have altered the results, possibly resulting in a type two error. As the first measurement was measured during overcast, this measurement was more accurate.

NDVI did not show an effect of treatment (Appendix 1). This is in accordance with a study of Ravn et al., 2020 that did not find a significant difference in NDVI with OTC-warming. The result is also in coherence with the minor effect of treatment in general and no significant difference for both total species composition and biomass. This could additionally be a consequence of the below five mentioned factors.

There was no main effect of treatment on the diversity of any of the overall species groups (Appendix 2)(Figure 1). The species diversity in the Arctic is generally low, and thus it is more difficult to statistically prove a difference, due to the low numbers. This could additionally be a consequence of the below five mentioned factors. In terms of coverage only graminoids showed a main effect of treatment, with an average percent of coverage close to double in plots treated with OTC compared to control plots (Appendix 2; Figure 2 & 3). This corresponds with a study by Shaver et al., 1997 who attribute this to a high tissue turn-over time, and it is further supported by a meta-analysis that showed rapid responsiveness to increased temperatures for graminoids Dormann and Woodin, 2002. However, there can be a number of factors, or a combination of these, explaining why there is only a main effect of treatment on this rapid responsive vegetation type: (1): The experiment is still in an early phase and a marked response is most likely reliant on a long time period of warming treatment. In the future, we might experience significant differences in other functional vegetation types, which have a slower response time than graminoids to increasing temperatures. (2): During the experimental time span the overall ambient temperature has increased (Zhang et al., 2019), which means it has increased for both treatments – resulting in a less effectful temperature increase for OTC. This might limit the effects of the OTC. (3): The temperature indicated a modest difference between treatments as only the first measurement of air temperature showed significant difference between treatments and none of the soil temperature measurements. (4): Slow growing vegetation types and most Nordic plants often have a large temperature tolerance and thus respond little to temperature changes (Grime et al., 2008; Sætersdal and Birks, 1997). The CABS are, however, defined by their growing season temperatures, and show a great variation in species composition and numbers, ranging from around 100 species in CABS A to more than 2000 different species in CABS D (Meltofte et al., 2013), which does provide evidence that different Nordic plants, do vary with temperature (Walker et al., 2005). A study by Niittynen et al., 2018 suggested that various vegetation groups in the Arctic responded differently to increased temperatures, so that vascular plants would generally increase in species diversity, mosses showing different results, but with a trend to increase, where lichens would decrease in species richness, and retreat to high altitudes. (5): According to previous observations and weather monitoring of Arctic Station the melting of the snow has occurred very early this season, resulting in an extensive visual difference between greenness of C and T-plots, which we did not see this season (Brandt, 2017). These previous observations indicate a greater difference in the beginning of the individual growing seasons of the zones. This coincides with a study of Cooper et al., 2011 demonstrating that plant development is greatest affected by the timing of snowmelt directly after melting. This suggests that we possibly could have detected a significant difference between the plots, had the analysis been conducted right after snowmelt.

Overall, this is only slightly in line with the H1 of hypothesis A. However, as graminoids would be the 'first moovers', the significant difference of graminoid coverage, due to treatment, is a minor indication of a shift in the vegetation community and a possible species succession resulting from increasing temperatures of slopes on Disko Island, Greenland.

Changes in snow cover duration

The experiment revealed that 'Air Temperature 1' and 'Soil Moisture' showed a significant change down the gradient from Zones 2-5, however, the results were non-linear as Zone 3 and 4 had the highest temperatures (Appendix 1). Therefore, these differences are expected to be caused and/or influenced by other factors than the snow cover duration, since that theory would only be supported by a linear trend in the significant results. The experiment also demonstrated a number of responses and changes in vegetation communities due to the varying snow cover duration. Total species diversity (Figure 1 and 4) and total species coverage (Figure 2 & 5) exhibited a significant uneven distribution down the gradient. From Zone 2-5 the trend was linear, as the highest diversity was found in Zone 4 and 5, and in addition, Zone 5 had the highest coverage (Figure 1 & 2). This is expected as the growing season duration increases down the slope concurrently with snowmelt and is in accordance with H1 of hypothesis B. The NDVI increased down the gradient with a significant linear trend for both measurements (Appendix 1). As NDVI is a proxy of greenness, this corresponds well with the demonstrated increase in vegetation cover/biomass (Figure 2 & 5).

The species composition of the plant community varied down the slope and were in accordance with the expected findings of site in CABS D with a general cover of vascular plants (Walker et al., 2005). CABS D is characterized by a number of dominant species, such as *B. nana, C. bigelowii, Aulacomnium sp.* and *S. arctica.* This experiment did not encounter any *S. arctica*, but another dominant willow, *S. herbacea* of which most were represented throughout our test site (Appendix 3) (Alaska Geobotany Center, 2008). Especially *S. herbacea* was strongly dominating the plant community in Zone 2 - 3, which were also the zones where the highest level of dominance was estimated (Table 1 & 2). Zone 3-6 showed a strong accordance with characteristics of CABS D vegetation unit S1, "erect dwarf-shrub tundra", with species such as *Empetrum, S. glauca, V. uliginosum, Aulacomnium, Dicranum, Racomitrium, Stereocaulon* and *Cladonia* (Appendix 1) (Table 1) (Alaska Geobotany Center, 2008). The vegetation composition in Zone 2 showed ambiguous trends, as it lacked most of the species usually dominating CABS D zones, except *S. herbacea*, which also dominates certain vegetation subunits in CABS C. However, Zone 2 also lacked the species like *C. tetragona*, which would be characteristic for CABS C, leaving it somewhere in between CABS C and D (Alaska Geobotany Center, 2008; Walker et al., 2005). Zone 1 stood out from the rest and showed more similarities with those characteristics of CABS C and vegetation units usually confined to this bioclimate, with a dominating lichen and moss cover and few vascular plants like *C. tetragona*, *C. rupestris* and cushion forming forbs such as *S. acaulis* (Alaska Geobotany Center, 2008; Walker et al., 2005). Although a general trend in the Arctic regions shows that vegetation biodiversity increases from CABS A-E with temperature and longer growing season, however the relative number of endemic species increases from CABS E-A (Meltofte et al., 2013). Thus, a movement of the lower climate zones towards the North would cause a locally higher biodiversity, but likely cause regional extinction, and therefore an overall lower biodiversity. The experiment described in this article showed a tendency to an increase in biodiversity down the gradient as well as a species composition growing more and more towards a CABS D type of vegetation, rather than a CABS C, which points towards an increased biodiversity correlated to snow cover duration (Table 2)(Figure 4) (Walker et al., 2005). A study by Niittynen et al., 2018 also supported evidence for a

biodiversity-loss at a larger scale as an effect of decreased snow cover duration, as many plant and moss species are confined to snow bed and rely on these to be able to compete with other species. Lichens, however, showed to be little affected by snow cover duration, as they often inhabit windswept areas, where the snow cover has little effect (Niittynen et al., 2018).

As both plant cover, greenness and species diversity (Figure1 and 2)(Appendix 1) increase down the gradient, the results of this study support the hypothesis B, that species composition, cover and greenness all are changing down the gradient with a varying snow cover duration. However, the increased local species diversity could result in an overall lower species diversity (Meltofte et al., 2013).

Climate changes in the Arctic

It is difficult to imagine that a temperature change in the Arctic would not have an effect on the snow cover duration, and vice versa. The missing interactive effect between treatment and location can be due to the limited differences between treatments. If the significant level of the treatment plots increases in the future, as expected, an interaction can possibly be detected. In continuation, the locations are naturally occurring and have had a much longer time to differentiate, while treatment is a manipulated intervention, only existing from 2014 and ecosystems show a great capacity to buffer temperature changes (de Valpine and Harte, 2001; Euskirchen et al., 2006). A study by Niittynen et al., 2018 supports evidence that there are interactions between these factors in terms of species diversity, as the temperature is generally increasing the species richness of plants and mosses. The reduction in snow cover duration is causing plants and mosses confined to snow beds to go extinct, and thus tempering the increase in the species richness, and possibly resulting in an overall decline in biodiversity. Lichens were demonstrated to be little affected by snow cover duration but showed a decline in species richness as a result of increased temperature, however, they showed the largest decrease with combined temperature increase and snow cover duration decrease (Niittynen et al., 2018).

Taken together, the results suggest no rapid changes in vegetation communities with increased temperatures. However, a long-term effect might be possible to detect after a continuation of the experiment and not only for graminoids – like the vegetation composition is very different between zones, which have developed over a long period of time.

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Size and biomass distribution of major phytoplankton groups in ponds and shallow lakes at Southern Disko Island

Phytoplankton sampling in the field (Photo: Kasper Grønbech Andersen)

Size and biomass distribution of major phytoplankton groups in ponds and shallow lakes at Southern Disko Island

Ida-Marie Mollerup, Regina Jo Larsen

Abstract

Ponds and lakes can make up more than 50% of the arctic habitat. Thus, they constitute an important part of the overall terrestrial ecosystem and the freshwater biota contributes to the overall productivity. Phytoplankton is known to be essential organisms in standing waters as they are primary producers and react to changes in the physicochemical properties. Several studies have already investigated the magnitude of the chlorophyll *a* concentrations on a community level and have linked it to distribution of the main taxonomic groups of phytoplankton. However, further distinction of the phytoplankton community into specific size groups i.e., picoplankton, nanoplankton and microplankton, has not yet been done. The aim of this study was to investigate, whether there is a difference in the distribution of biomass and abundance between these size groups within a range of lakes and ponds differing in conductivity and alkalinity. Among the nine sampled lakes and ponds, differences in dominating size groups were observed. However, there was a general dominance of the nano- and picoplankton compared to microplankton. We did not detect any correlations between conductivity, biomass and density nor with alkalinity. This is likely due to other limiting factors such as light, predation, nitrogen, or phosphorous. The most dominating groups were found to be chlorophytes and chrysophytes.

Keywords: *Phytoplankton, microplankton, nanoplankton, picoplankton, Arctic, lakes.*

Introduction

Freshwater environments make up a large proportion of the arctic environment. In fact, lakes and ponds might cover more than 50% of habitat in some areas (Pienitz et al. 2008). Thus, understanding the limnic environments is crucial for the understanding of arctic ecology and makes for an interesting topic.

Arctic lakes and ponds are generally known to be oligotrophic, as their surroundings mainly consist of bedrock and a limited amount of vegetation (Christoffersen 2006; Lizotte 2008). Moreover, the energy that is delivered to arctic ecosystems through heat is significantly lower than temperate and tropical ecosystems, thus potentially preventing establishment of high productive lakes, as phytoplankton metabolism is related to temperature (Raven and Geider 1988, Thomas et al. 2012). As such, lower temperatures might lead to lower productivity - unless adjacent to polluting sources (Thomas et al. 2013). Conductivity is a measure of the water's ability to conduct an electrical current which is related to the ion concentration of the water (Pestryakova et al. 2018)). In lakes and ponds in the Disko Bay area, conductivity has previously shown to range from 6 - 305 µS cm^{-1}. Levels of the photosynthetic related pigment, chlorophyll *a*, have been found to range from 0.4 - 10.2 µg L^{-1} (Christoffersen et al. 2004). A survey of arctic lakes in Canada shows that alkalinity might range from 0.02 - 5.27 meq L^{-1} (Kling 2012). Alkalinity is a measure of the waterbody's ability to neutralize acidification and is equal to the sum of bases in the solution. From alkalinity it is possible to determine the concentration of the dissolved inorganic carbon compounds; CO_2, HCO_3^-, and CO_3^{2-}. The distribution between these compounds is determined by the pH, with CO_2 being most abundant at low pH, HCO_3^- at pH of approx. 6-9, and CO_3^{2-} at higher pH. This distribution is interesting because phytoplankton are able to utilize CO_2 and HCO_3^- but not CO_3^{2-} as a carbon source

(Wetzel, 2001b). The buffering capacity of the water can also be affected by other compounds such as OH^- in waters of high pH.

Arctic lakes and ponds are heavily impacted by their surroundings, as they receive run-off water from thawing glaciers, mountain ice as well as run-off rainwater, which often deliver nutrients and changes of the physicochemical properties. The amount of run-off water is determined by the annual climate, thus arctic lakes can be subject to an immediate change in conditions (Christoffersen 2006).

Phytoplankton are important organisms in pelagic environments, such as the water column of lakes and ponds because they are primary producers and form the first part of the foodweb. Through photosynthesis, they are able to utilize inorganic nutrients to build up biomass that can be further transferred to higher trophic levels through grazing by eg. zooplankton, thus, phytoplankton are essential for all organisms of higher trophic levels (Kaiser et al. 2011). Furthermore, they are able to quickly respond to the surrounding physicochemical changes because of their surface:volume ratio (see later) and thereby serve as bioindicators of the trophic state of the system (Deininger et al. 2017; Winder and Summer 2012). However, despite the general low nutrient availability, the arctic freshwater ecosystems are quite diverse with almost all eukaryotic phytoplankton and cyanobacteria represented to some degree (Lizotte 2008). Dominating phytoplankton is known to consist of chrysophytes, cryptophytes, dinoflagellates, and often diatoms as well (O'Brien et al. 1997; Christoffersen et al. 2004; Pedersen and Nielsen 2019).

Phytoplankton are commonly categorised into three different size fractions; pico-, nano-, and microplankton with sizes of 0.2-2, 2-20, and 20-200 µm, respectively (Fenchel 1987). The small sizes of pico- and nanoplankton results in a higher surface:volume ratio, which enhances the nutrient uptake that occurs by molecular diffusion over the cell membrane. This is an advantage in oligotrophic environments and algae size has been observed to correlate with increasing nutrient content of waters. The autotrophic microflagellates obtain their nutrient supply by eddy diffusion, but many algae of this size class are mixotrophic, thus able to fulfill their nutrient requirements by feeding on either bacteria or phytoplankton. This can be a

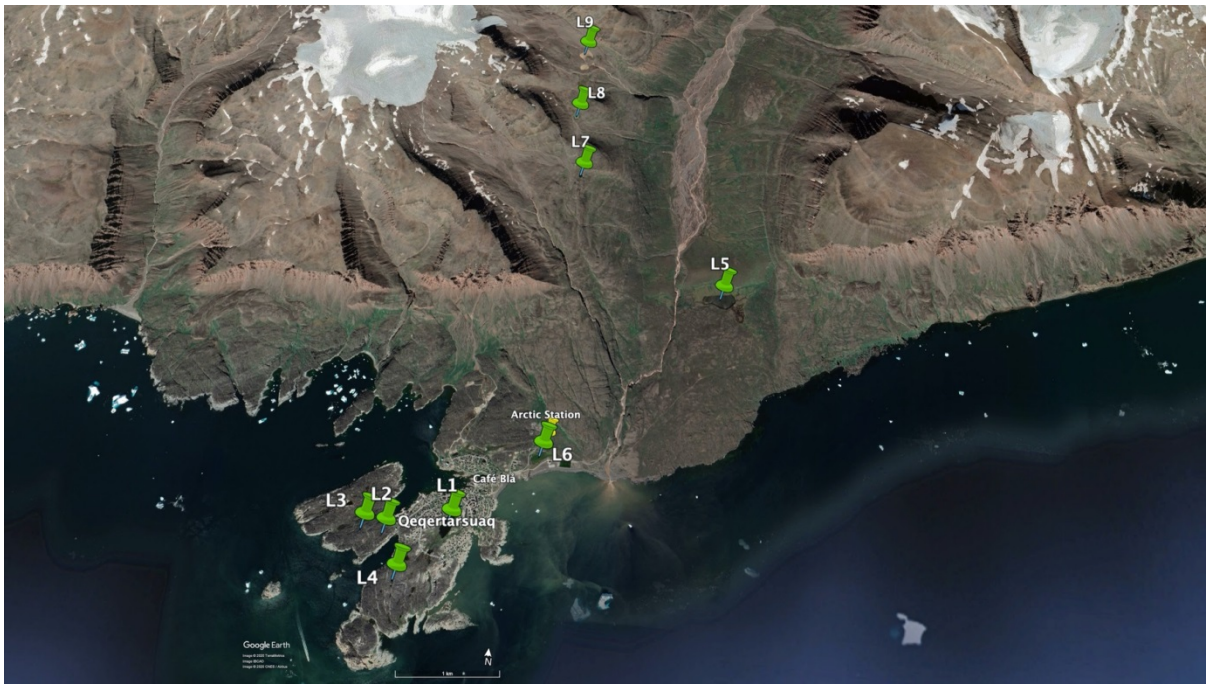

Figure 1 Map of Qeqertarsuaq and the surrounding area (Google Earth Pro 2020). Investigated lakes and ponds are marked with green pins and their respective labels are noted. The yellow pin marks the location of Arctic Station. L1 = Thygesens sø, L5 = Morænesø, and L6 = Stationssø.

significant advantage in environments where inorganic nutrients are limiting (Wetzel 2001c).

Communities of phytoplankton in Greenlandic lakes and ponds have been investigated several times in previous studies (Christoffersen et al. 2004; Christoffersen et al. 2008; Pedersen and Nielsen 2019), but to our knowledge, no studies have investigated the relationship between the size groups and their individual chl *a* concentration in relation to physicochemical properties. As such, the aim for this study is to determine the pico-, nano-, and microplankton ratios in different lakes and ponds in the Qeqertarsuaq area (Southern Disko Island, Greenland), and to determine the major groups found within these communities. As a proxy for phytoplankton biomass, the chl *a* content is often used (Jespersen & Christoffersen 1987) as chl *a* is the primary pigment in all algae (Wetzel 2001c). Additionally, the study aims to answer the following question: Is the distribution of phytoplankton size groups related to physicochemical parameters like conductivity and alkalinity? Conductivity can be used as a proxy for ionic carbon sources and minerals in general, while alkalinity can be used as a proxy for ionic carbon sources as well as the site's general stability, as it indicates how resistant a water body is to acidification (Wetzel 2001a; Wetzel 2001b).

The location of the sample sites chosen for this study (*Figure 1*) were chosen due to their differences in size, depth, altitude, transparency, distance to glacial water source and distance to the sea and human settlements. For simplicity, all sample sites are named L1, L2, L3 etc. even though L2 and L3 are by definition ponds (depth < 3 meters (Rautio et al. 2011)). Actual measurements of the depths were not possible, but visual assessment and general knowledge of some of the sites (Pers. com. K. S. Christoffersen) made it possible to distinguish between lakes and ponds.

It is well known that the arctic lakes will contain an overall low density (cells mL^{-1}) of phytoplankton compared to temperate lakes, since Arctic lakes often are co-limited by nitrogen and phosphorous availability (Thomas et al. 2013). Generally, there is a dominance of small phytoplankton species in nutrient poor environments (Wetzel 2001c), therefore, we will expect the dominating groups to be nano- and picoplankton, as their larger surface:volume ratio might be preferable in nutrient poor environments. We also expect L1 to contain the highest density of phytoplankton, as it is considered polluted due to its location in the town center where there is an increased nutrient inflow from household waste water and sledge dog faeces (Christoffersen et al. 2004). Finally, we expect there to be an overall correlation between phytoplankton biomass (chl *a*) and density.

Methods and Materials

Sampling water from arctic lakes

Triplicate water samples were collected from the nine different sites in or around Qeqertarsuaq. Depending on the topography of the sites, samples were either collected by

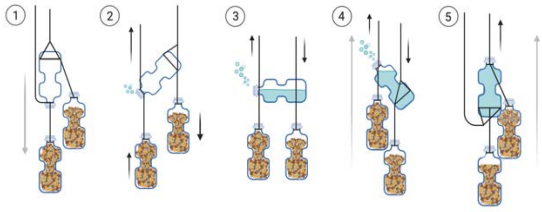

Figure 2. Illustration of the Homemade Sampling Device 2020 (HSD20). (1) Two bottles filled with gravel and sand served as weights. These were mounted to the bottle collecting the water for sampling. The weight mounted to the neck of the sampling bottle weighed a bit more than the weight mounted to the bottom of the sampling bottle. This made sure that the sampling bottle would descend vertically, thereby prohibiting water from entering. (2) Once down at the desired depth, a string mounted to the neck of the sampling bottle was pulled. Simultaneously, a string mounted to the bottom of the sampling bottle was lowered. These two actions allowed air to escape the bottle and water to enter. (3) and (4) This motion was continued to further fill the bottle, and the whole device was at this point slowly raised up through the water column. (5) Once full, the device was raised vertically the remaining way. Motion of strings presented with black arrows. Motion of the overall device presented with light grey arrows. Illustration made with BioRender (https://biorender.com/).

our homemade sampling device (Homemade Sampling Device 2020, HSD20) (*Figure 2*) or by hand. The device was lowered vertically into the water and tipped once close to the bottom (c. 15 cm from bottom). Tipping allowed water to enter. It was then slowly raised up through the water column until nearly reaching the surface and filled completely with water. It was used in sites where lowering of the device was possible from an elevated platform. Samples from shallow sites, accessed by waders, were collected by hand, simulating the HSD20 as much as possible.

Water samples for alkalinity measurements were collected approx. 20 cm below the surface in 200 mL glass bottles, one from each lake. Before collecting the samples, the glass bottles were rinsed with lake water three times in order to dispose of possible contaminants. Once filled with water, the lids of the bottles were attached while still being under water. This precaution prohibited atmospheric air from entering the bottle, as this would affect the alkalinity of the sample.

Filtering water samples

To separate the phytoplankton into three different size groups, each sample was sequentially filtered using a standard filtering setup with a vacuum pump, filter holder with exchangeable filters and vacuum bottle. Generally, 500 mL of all samples were filtered through a 200 µm nylon filter in order to remove any larger particles and zooplankton. Then, 2 mL were taken aside for microscopy investigation. Now 498 mL samples were filtered through a 20 µm nylon filter retaining the microplankton. Once again, 2 mL were taken aside for microscopy investigation. Then 496 mL samples were then filtered using a 2 µm polycarbonate filter (Whatman® Nuclepore™ Track-Etched Membranes) retaining nanoplankton. Another 2 mL were taken aside for microscopy. The remaining sample volume of 494 mL were lastly filtered through a 0.8 µm polycarbonate filter (Whatman® Nuclepore™ Track-Etched Membranes) in order to retain the picoplankton.

Chlorophyll a measurements

Individual filters of 20 µm, 2 µm and 0.8 µm, respectively, were placed in separate falcon tubes using forceps in order to avoid contamination. The tubes were stored in a freezer at -20°C. The freezing process preserved the filters until chlorophyll extraction was done and also destroyed cells, thus making the extraction more efficient. Filters in falcon tubes were frozen for at least 12 hours, after which 10 mL of ethanol 96% was added to all tubes using a dispenser. Falcon tubes containing both filters and ethanol were placed at room temperature in a dark cabinet for approximately 14 hours (Jespersen and Christoffersen 1987). Chl *a* was measured using a Trilogy Fluorometer (Turner Designs 2019a; 2019b). Solutions were flipped upside down three times before a fraction was added to a glass vial for fluorescence measurements. The fluorometer automatically calculated chlorophyll *a* concentrations using the following equation:

$$C_{stand} \times \left(\frac{F_{samp} - F_{blank}}{F_{stand} - F_{blank}}\right) \times \left(\frac{V_{solvent}}{V_{water}}\right)$$

C_{stand}: Concentration of standard, F_{samp}: Fluorescence of sample, F_{blank}: Fluorescence of blank, F_{stand}: Fluorescence of standard, $V_{solvent}$: Volume of solvent, V_{water}: Volume of filtered samples.

Any deviations from the general filtering process were accounted for when measuring chl *a*.

Alkalinity measurements

Dor alkalinity measurements the Inflection Point method (USGS Alkalinity Calculator, https://or.water.usgs.gov/alk/methods.html#ipt) was used. In the laboratory, a standard pH-meter was used. The pH electrode was placed in a pH electrode stand and calibrated using two solutions of pH 4.0 and 7.0, respectively. The electrode was thoroughly rinsed with demineralized water after entering each solution. Thereafter, 50 g (±0.073g) – the equivalent of 50 mL – lake water was weighed off in a 100 mL beaker and used for titration. In the beaker, a magnet was added and the beaker was placed on a magnetic stirrer with

700 revolutions per minute. The electrode of the pH meter was carefully positioned in the water, the whole electrode approximately 1 cm under the surface of the water. The starting pH was measured and noted as well as the temperature of the water. For the titration, 1.0 M of HCl was used. The amount of HCl added to the water was determined by the pH of the water. The lower the starting pH, the closer the samples were to the drop zone, which is where pH drops drastically. Therefore, less HCl was added at the beginning of the titration, as even small amounts of added acid would lead to a high drop in pH. This was to be avoided in order to get precise data. Small concentrations of acid (2-10 µL HCl) were added to samples with a pH of < 6.35 using a precision pipette. Because of the buffering capacity of the water, a larger amount of HCl was added with higher starting pH, as these samples were further from their drop zone. After adding the acid, the pH was noted again. The amount of acid added to the samples was adjusted during the titration according to the previous pH drop. A drop of more than 0.2 was to be avoided, and within the drop zone a drop of no more than 0.1 was preferred. This process was continued until the pH reached approximately 3.5. The first investigated lake (L5) had the Gran method performed three times to estimate the deviation within the same sample. The rest of the lake samples were tested once, and the alkalinity deviation from the triplicates were added to each lake. Once all the data was collected, the information was applied to the USGS Alkalinity Calculator from Oregon Water Science Center, where the actual alkalinity for each lake and pond was calculated (USGS Alkalinity Calculator, https://or.water.usgs.gov/alk/).

Conductivity measurements

Conductivity was measured on leftover water from the samples collected for alkalinity measurements using a hand held field probe (Yellow Spring Instruments, YSI, US), which was calibrated against a standard solution of 1470 µS cm^{-1}. The probe was fully submerged into each water sample and the conductivity (µS cm^{-1}) was noted.

Microscopy investigations

For enumeration and genus investigation, a gridded Sedgewick rafter counting chamber was used. The microscope objective lens enlarged the samples by 100-fold and identification of microplankton was possible. A higher magnification was preferred in order to ID nano- and picoplankton as well, but the available equipment did not allow for this. With a disposable pipette, 1 mL of a sample was added to the Sedgewick rafter and placed under a BX51 Olympus microscope. The number of grids were 20 x 50. A starting line for each analysis was chosen randomly and 5 x 50 grids were counted. Each time an algae was observed, it was identified to the genus level using Nygaard and Kristiansen 2001, Tikkanen & Willén 1992 and algaebase.org (https://www.algaebase.org/).

Statistical analysis

A One-Way ANOVA test was performed using the statistical programme RStudio (RStudio, Version 1.2.5033, © 2009-2019 RStudio, Inc.) to test for differences in total chl*a* concentration between the sites. Additionally, we tested for differences between the chl*a* of phytoplankton size groups within the individual sites, and for differences between the size groups across the lakes. These tests were based on the chl*a* measurements. Subsequently, a TukeyHSD test was used to further investigate where differences were seen.

Results

Analyses of the physiochemical parameters showed differences among the sites (Table 1). The span of conductivity reached from 19 µS cm^{-1} in L8 to 276 µS cm^{-1} in L6. The second highest conductivity was seen in L6 at 112 µS cm^{-1}. The three sites in higher altitude, L7, L8 and L9, showed the lowest conductivities. Quite a big difference in pH was observed among the sites with L1 having the highest value of 9.99 and L3 the lowest of 5.69. L8 and L9 were somewhat similar in pH (6.90 ± 0.01).

Table 1. Overview of the nine sites, their coordinates (Google Earth Pro 2020), measured conductivity, pH and alkalinity. The alkalinity analysis specifies which anion is removed by titration, thus which anion likely has the stabilising ability in the given site. For L5, the results are the mean of three measurements. Standard error (SE) of alkalinity measurements: OH- = 0, CO32- = 0.003, HCO3- = 0.08.

Lake name	Coordinates		Conductivity	pH	Alkalinity		
	Latitudde	Longitudes	(µs cm^{-1})		OH^- (meq L^{-1})	CO_3^{2-} (meq L^{-1})	HCO_3^- (meq L^{-1})
L1	69°14'40.56"N	53°32'20.69"W	112	9.99	0.11	0.25	0.25
L2	69°14'35.22"N	53°33'18.54"W	81	6.35	0	0	0.07
L3	69°14'36.05"N	53°33'38.86"W	53	5.67	0	0	0.02
L4	69°14'21.00"N	53°33'0.62"W	63	6.08	0	0	0.02
L5	69°16'12.34"N	53°28'29.31"W	79	7.68	0	0.003	0.96
L6	69°15'6.87"N	53°31'6.61"W	276	8.82	0.01	0.05	0.72
L7	69°16'58.40"N	53°31'8.09"W	24	7.24	0	0	0.23
L8	69°17'25.33"N	53°31'22.12"W	19	6.91	0	0	0.17
L9	69°17'55.82"N	53°31'23.89"W	20	6.89	0	0	0.21

L6, too, had a relatively high pH of 8.82. L5 and L7 had a pH of 7.68 and 7.24, respectively, whereas L2 and L4 had quite low pH values of 6.35 and 6.08, respectively. Alkalinity measurements ranged from 0.02 meq L^{-1} to 0.96 meq L^{-1} HCO_3^- (the typical freshwater anion). Furthermore, L1, L5, and L6 showed to contain more than one stabilizing anion (Table 1). Significant differences were seen in the One-Way ANOVA tests, which analysed the differences in total chl *a* between lakes and the chl *a* among size groups, both within and between lakes (Figure 3).

Chlorophyll a analyses

A One-Way ANOVA comparing the total chl *a* between the different lakes showed significant differences (F- value = 13.3, $p < 0.05$). The TukeyHSD test revealed no significant difference between L1, L3, L4, L6, L7, L8 and L9, and that L2 and L5 were significantly different from all sites except each other (Figure 3). In L1, biomass measurements of picoplankton were significantly higher those of nano- and microplankton, and no significant difference was observed between the latter. L2 showed no significant difference between the size groups. L3, L4, L6, and L9 showed a

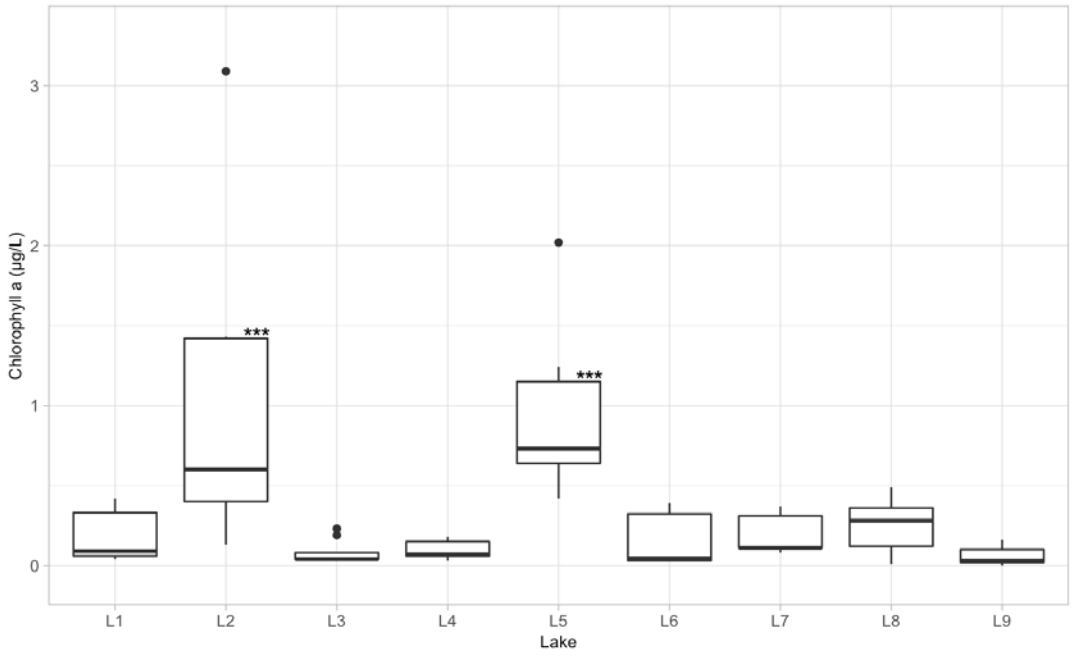

Figure 3. Boxplots calculated on chl *a* measurements from all filtered samples (*Supplementary table 1*), representing the total chl *a* amount in each lake. Significantly different lakes are presented with a significance code. Signif. codes: *p* = 0 '*', *p* = 0.001 '**', *p* = 0.01 '*'. Black dot(s) by L2, L3 and L5 represent outlier data points.**

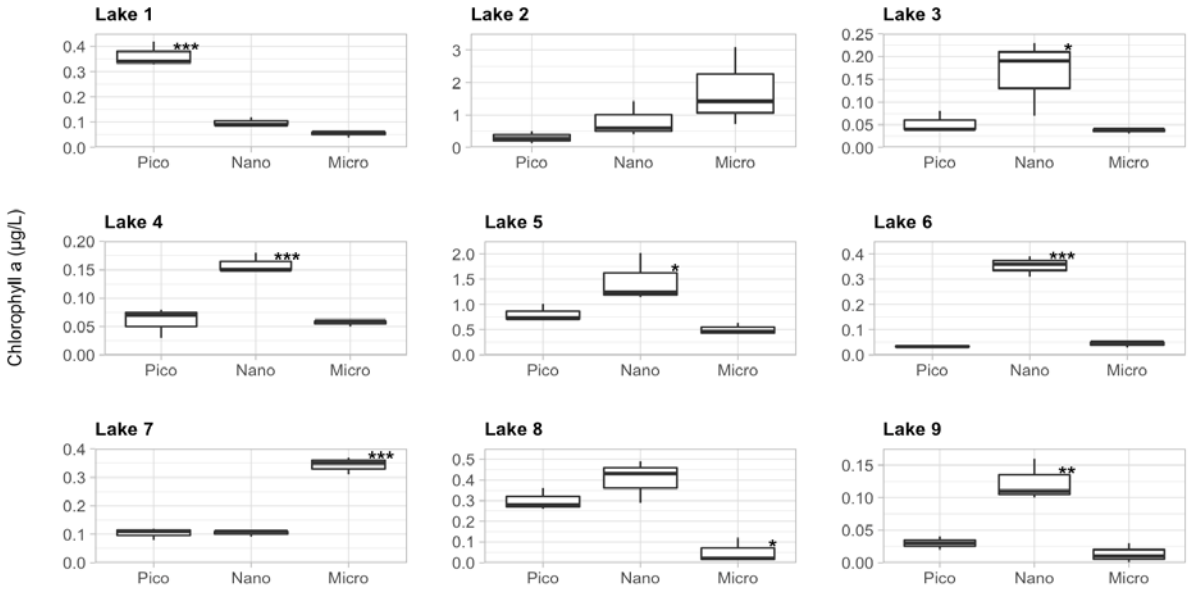

Figure 4. Boxplots based on chl *a* measurements of the filtered size groups pico-, nano-, and microplankton within each lake. Signif. codes: *p* = 0 '*', *p* = 0.001 '**', *p* = 0.01 '*'. If two size groups are significantly different from each other but neither are significantly different from the third group, the smaller significantly different size fraction is presented with the significance code. E.g: In L5, nano- and microplankton are significantly different from each other (p = *), but neither are significantly different from picoplankton. Thus, the smaller size fraction of the two, in this case nanoplankton, is presented with the significance code (*). If one group is significantly different from the two other groups but to a different degree, the group is presented with the smallest of the significance codes. E.g.: In L8, microplankton is significantly different from both pico- and nanoplankton (*p* = * and *p* = **, respectively). Thus, microplankton is presented with the smallest significance code (*) (*Supplementary table 2*).**

significantly higher chl a concentration of nanoplankton compared to micro- and picoplankton, with no significant difference between the latter. L5 showed a significant difference between micro- and nanoplankton, and neither were significantly different from picoplankton.

L7 showed a significantly higher microplankton biomass, while it was significantly lower in L8. Neither L7 or L8 showed a significant difference between pico- and nanoplankton The highest total chl *a* concentration (2.79 µg L^{-1}) was found in L5 which also had the highest biomass of picoplankton and nanoplankton (*Supplementary table 1*). L9 had the lowest biomass concentration, only containing 0.17 µg chl *a* L^{-1}. In general, nanoplankton was the dominating group. However, L2 and L7 were dominated by microplankton (*Supplementary table 1*).

A One-Way ANOVA was used for investigation of significant differences between the groups among the nine lakes. The test showed that there was significance between sites (One-Way ANOVA; Microplankton: *F*-value = 16.25, $p < 0.05$. Nanoplankton: *F*-value = 75.49, $p < 0.05$. Picoplankton: *F*-value = 38.26, $p < 0.05$). A TukeyHSD test later revealed that chl*a* measurements of microplankton in L2 showed to be significantly higher than all other sites, and L5 showed to be significantly higher than all other sites except L7 (and L2), though microplankton only constituted 20% of the total chl *a*. Even though L7 does show a higher chl *a* measurement for microplankton compared to most other sites, it is only significantly different from L2, as mentioned.

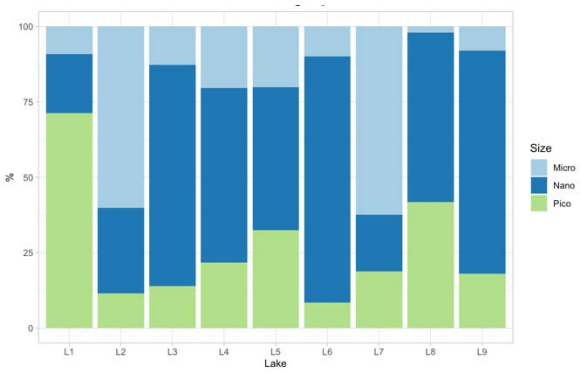

Figure 5. Histogram showing the %-distribution of picoplankton, nanoplankton, and microplankton at each site.

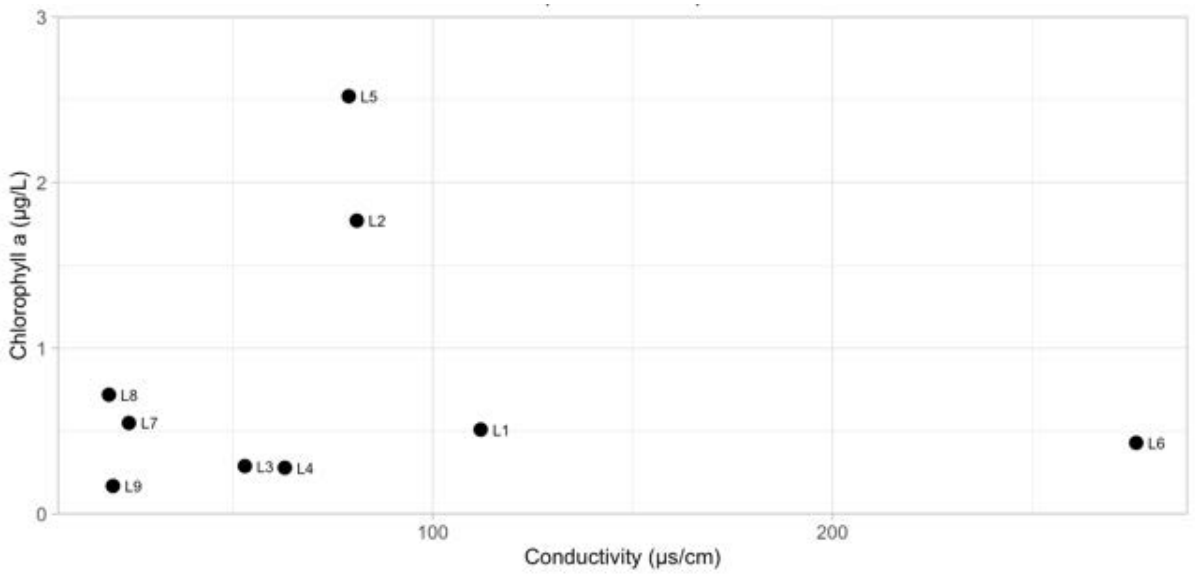

Figure 6. Mean total chl *a* (mean=3)) in relation to conductivity. Points are marked with site labels.

However, L2 and L7 almost share the same percentage distribution (*Figure 5*). For nanoplankton, L5 was significantly different from all other lakes. L2, L6, and L8 were significantly different from the other sites but not from each other. For picoplankton, L1 and L8 were significantly different from all sites but L2 and each other. L1 and L8 also show the largest proportions of picoplankton being 71% and 41%, respectively. L2 was only significantly different from L5.

Plotting total chl *a* in relation to conductivity (*Figure 6*) shows that there is no correlation between chl*a* and conductivity. No correlation between dominating size groups and conductivity was seen (*Table 1*).

Microscopy

Microscopic analyses of L9 proved it to be the least diverse site of all nine sites with only 4 *Chlamydomonas* mL^{-1}. Pico-sized phytoplankton cells were observed in L9, but ID and counting was not possible due to technical limitations (see method section).

Overall, chlorophytes, more specifically the genera *Chlamydomonas*, *Cosmarium*, *Oocystis*, *Staurastrum* and *Westella*, were

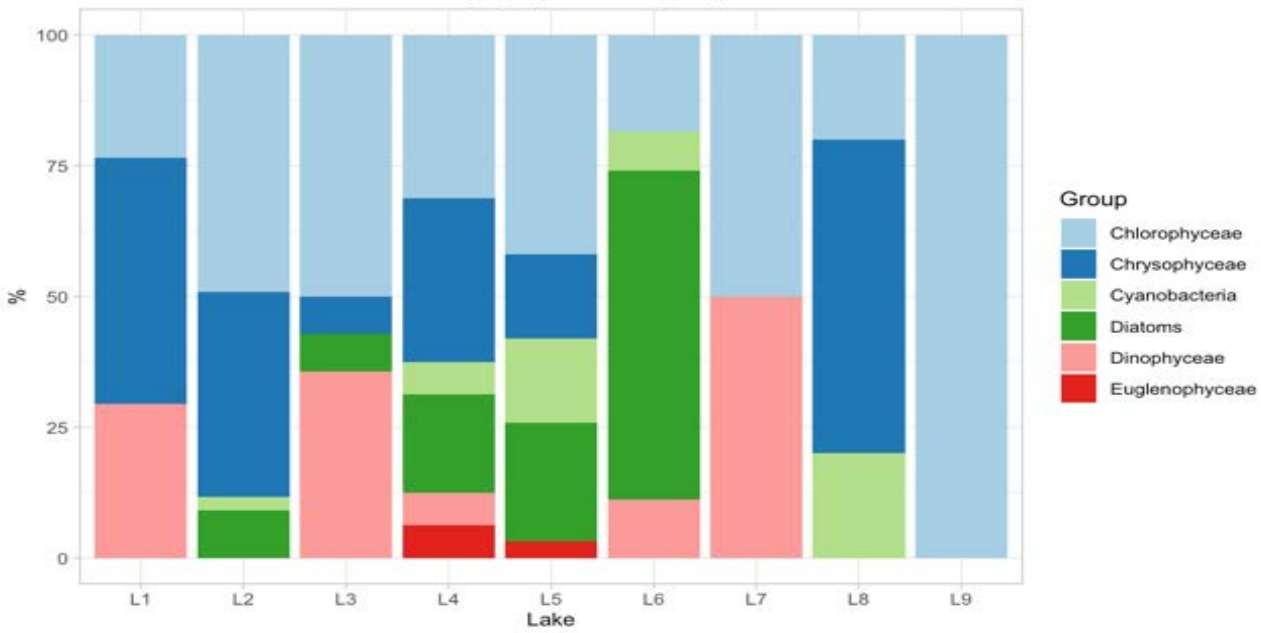

Figure 7. Percentage distribution of taxonomic phytoplankton groups between the nine sites.

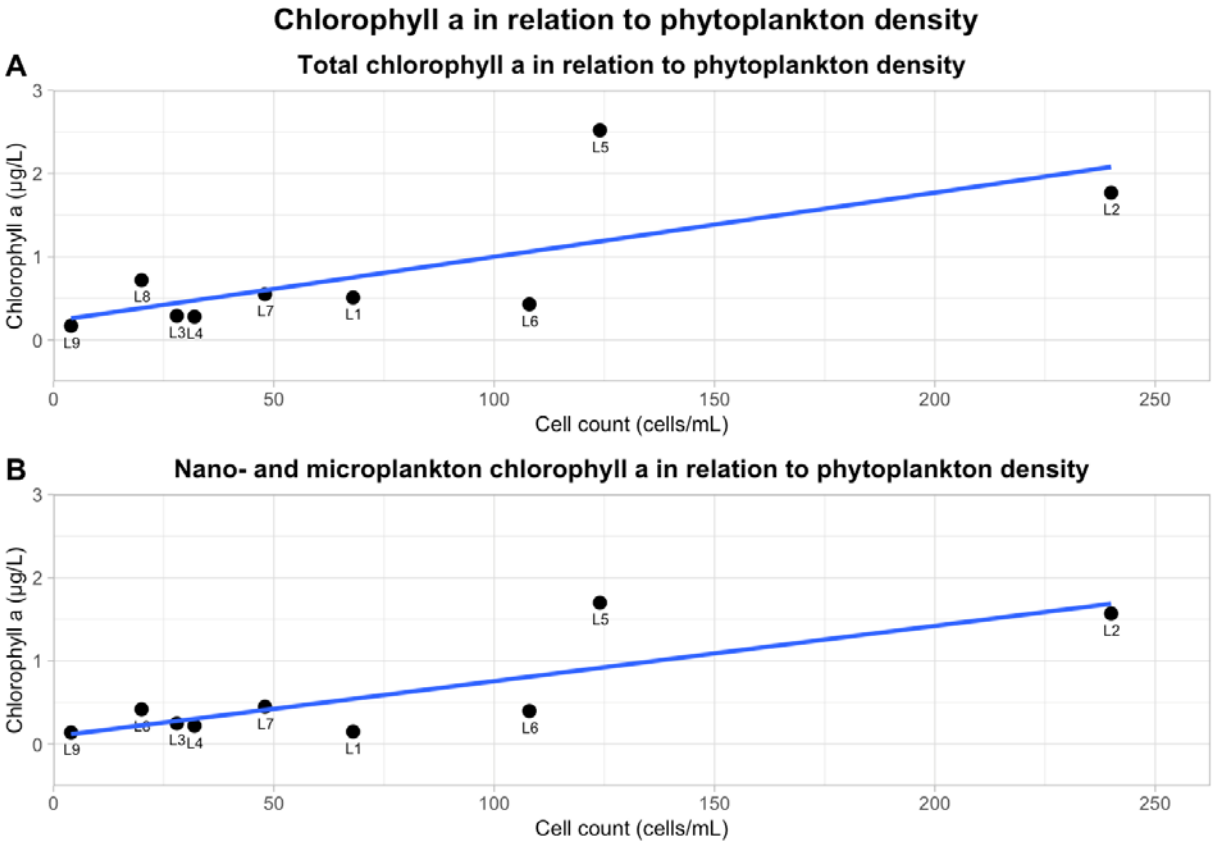

Figure 8. (A) Total chl *a* in relation to phytoplankton density. Adjusted R^2 = 0.44 and p-value = 0.0314. (B) Sum of chl *a* of nano- and picoplankton in relation to phytoplankton counts. Adjusted R^2 = 0.61 and p-value: 0.0070. Linear regression lines in blue and points are marked with site labels. Adjusted R^2 is adjusted to the number of datapoints (unlike R^2) and describes how well the data fits the curve.

dominating, especially in L2. Chrysophytes were the second most dominant group with *Dinobryon* being the most abundant genus overall (*Supplementary figure 1*, *Supplementary table 3*). Diatoms were especially abundant in L5 and L6 (*Supplementary table 3*), with the diatom ratio being the largest in L6.

A slight but not significant correlation between total chl *a* and phytoplankton density is present with an adjusted R^2 value of 0.44 (*Figure 8A*). A correlation between the sum nano- and microplankton biomass, and phytoplankton density is also present with an adjusted R^2 value of 0.61 (*Figure 8B*). Thus, this correlation is slightly stronger than the correlation between total chl *a* and phytoplankton density.

Discussion

The aim of the study was to determine, whether there was a significant difference between the biomass of three main phytoplankton size groups (pico, nano, and micro) in lakes and ponds with different physiochemical conditions and to investigate phytoplankton genera abundance and composition.

Despite a relatively small sample size (nine locations) it was possible to detect differences in the biomass and density of the phytoplankton size groups, both within the lakes and between the lakes. There is also a difference in the total amount of chl *a* between the nine locations which, however, was correlated to neither conductivity nor alkalinity.

As expected, L1 stands out from the other locations by having a high biomass but low species diversity. L1 is located within the town of Qeqertarsuaq which leads to higher nutrient loading from anthropogenic sources compared to the other sites. Furthermore, it is shallow and warms up during summer. Thus,

the conditions in L1 are beneficial to algal growth (Kaiser et al. 2011). The study was carried out in the middle of July, which is also the time of year where we could expect the phytoplankton bloom to just have ended, thus explaining the high pH, as phytoplankton utilize many anions, thus making the pH higher (Wetzel 2001c).

The results from this study show that picoplankton was the least dominating group. Based on our data, we cannot point to a direct explanation for this distribution, For a better understanding, parameters such as inorganic nutrient concentration, light intensity and attenuation as well as controlling biological factors such as grazing should be involved. This was not possible during our study.

Microscopy

The phytoplankton composition included chlorophyceae, chrysophyceae, cyanobacteria, diatoms, dinophyceae, and euglenophyceae which largely corresponds with the findings by Christoffersen et al. (2004) from the same area. Other previous findings have also observed cyanobacteria of the genera *Anabaena* and *Microcystis* (Thomas et al. 2013), however, while we did observe these, they were far from numerous (*Supplementary table 1*). Chrysophytes have previously been observed to be the most dominating group in arctic lakes (Christoffersen et al. 2008; Thomas et al. 2013), and although they were abundant in our samples, they were exceeded by chlorophytes. The past decades, Greenland has experienced rises in temperature which cause earlier melting of snow cover and decrease in its extent (Ciais et al. 2013). Changes in the phytoplankton composition may be expected as the indirect impacts of warming can led to eutrophication processes (Nõges et al. 2011), which might be why we did not observe the same distribution has the previously mentioned studies. However, there may also be more direct explanations to the outcome of the species distribution as the robustness to handling of different algae species vary. Chrysophytes are delicate and may fall apart when stored in bottles, filtered, and pipetted (Pers. com. K. S. Christoffersen). Actually, we did see a considerable amount of remains of phytoplankton which were not possible to identify. As a consequence, an underestimation of chrysophytes – and possibly also of other genera – is probable and will bias our conclusions. While chrysophyceae was not the dominating group, *Dinobryon*, a genus of chrysophytes, was the most abundant genus of all (*Supplementary table 3*). This corresponds with previous studies that have found this particular genus to be a major component of the phytoplanktonic community in oligotrophic lakes. *Dinobryon* is especially efficient at taking up phosphate even at very low concentrations (Wetzel 2001b), such as could be found in arctic lakes. Furthermore, *Dinobryon* is a mixotrophic genus able to feed on bacteria to gain carbon, phosphorous and nitrogen, which is an advantageous ability in nutrient poor environments (Thomas et al. 2013).

The comparison of density and biomass showed a significant correlation but the correlation between the two parameters was not completely linear. This was expected as the chl *a* content varies greatly across cell size an phylum, and, generally, the pigmentation, and thereby the chl *a* content per cell, can vary significantly across taxonomic groups. Thus, explaining the high variance (Wetzel 2001c).

Physicochemical parameters

The conductivity of a lake is dependent on the concentration of charged ions present in the water (Wetzel 2001a) and is highly affected by the content of ionic composition of the soil that the inflow water passes (Thierfelder 1999). Conductivity of L7, L8, and L9 was at the same level but lower than all the other sites. This might be due to fact that these locations are at higher altitudes (c 400-500 meters above sea level) and were fed by runoff from a nearby glacier. Another fact that affects conductivity is related to evaporation and water retention time. As the water in lakes evaporates, the concentration of ions increases, thus increasing the conductivity.

This may be enhanced in lakes where the retention time is high. Water bodies with more frequent inflow of water will sustain the lake with water and prohibit loss by evaporation, thus preventing a decrease in conductivity (Feth 1971). Due to the location of L7, L8, and L9, one might imagine that they experience inflow for a longer period of time than the sites in lower altitudes, as they might have somewhat constant inflow of water from the glacier and mountain snow, which was the case with L9. On the other hand, they might experience less loss of water, as the higher altitude might lead to lower temperatures, which might lead to longer periods of ice cover. Two other sites also had constant inflow; L5 and L6. However, these two sites both showed a relatively high conductivity compared to the last three sites. The conductivity of L6 is probably related to its close position to the sea which probably lead increased occurrence of sea-spray, which contains salts. The remaining sites did not have constant inflow, and once the snow and surface ice melts, their main water source must be rainwater. As a result, they might be more affected by evaporation than L7, L8, and L9.

The higher the alkalinity, the better the lakes are able to neutralise incoming acidic sources, i.e. water from rainfalls (Lake access, https://www.lakeaccess.org/russ/ph.htm). Thus, lakes with low alkalinities are more susceptible to changes when presented with new inputs of acids. Generally, all the sites showed low alkalinities. The higher alkalinities measured in this study were in L1, L5, and L6. L1 had alkalinities at 0.11 meq OH^- L^{-1}, 0.25 meq CO_3^{2-} L^{-1} and 0.25 meq HCO_3^- L^{-1}, which means that OH^-, CO_3^{2-}, and HCO_3^- were removed by titration. The same is applied to L6, which showed alkalinity levels at 0.01 meq OH^- L^{-1}, 0.05 meq CO_3^{2-} L^{-1} and 0.72 meq HCO_3^- L^{-1}. L5 removed both CO_3^{2-} and HCO_3^- by titration, where the other sites only removed HCO_3^-. This is consistent with what is seen in most Danish and other temperate lakes which often contain mostly HCO_3^- (Vestergaard and Sand-Jensen 2000, Wetzel 2001b). OH^- is often present in high pH lakes, which was the case with L1 and L6. Generally, the high OH^- concentrations originate from anthropogenic water run-offs or natural carbonate minerals (Pers. com. Mel Murphy), thus, the higher alkalinity in L1 might be explained by the anthropogenic pollution, whereas the higher alkalinity in L6 might be explained by the inflow of groundwater, which might have collected carbonate minerals from bacterial degradation on its way through the underground (Wetzel 2001b).

Concluding remarks

For optimization of our study, it would have been preferable to measure the content of available nitrogen and phosphorous, as these are typically limiting factors for phytoplankton growth in arctic lakes and ponds. Furthermore, continuous temperature measurements could be relevant as significant daily temperature fluctuations are expected to occur within small lakes and ponds. Lastly, information about ice cover of the sites could be interesting as well. However, these extra elements would all require that the study could span over a longer period of time. When samples were collected, our first notion was to collect them in the middle of the lakes from a boat using the HSD20, but logistical matters prevented us from doing so. Therefore, alternative methods were employed, and the sampling method at each lake or pond was not the exact same. However, we argue that the shallow depth of the lakes and ponds prevent stratification, hence approximately the same phytoplankton will be found anywhere in the water column. In the lab, the identification of algae by microscopy would have been improved if it had been possible to use a higher enlargement, as this would have allowed us to identify smaller organisms.

Despite the limitations for our study we were able to show: This study indicated that, in many cases, there is a significant difference between the distributions of phytoplankton size groups both within and between the sites. There is also a correlation between the measured chl *a* and phytoplankton densities. However, our study showed that no correlation between chl *a* and conductivity nor alkalinity is present. Thus, carbon is not

indicated to be the limiting factor for phytoplankton growth in the arctic lakes and ponds in the Qeqertarsuaq area. Limiting factors might be grazing and/or other nutrients like nitrogen and phosphorous. Unfortunately, and as mentioned, it was not possible to analyse these parameters for logistical reasons.

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Appendix

Supplementary table 1. Unit: µg L^{-1}. Table showing chlorophyll *a* measurements of the three size groups from each lake. Red numbers are outliers diverging the mean and an error occurred previous to the chlorophyll a measurements. *: Filtration was stopped a considerable amount of time earlier than the two other samples from this lake. This can possibly have led to the higher chl *a* concentration. **: Much of the ethanol content was spilled on the table and new ethanol was added to the falcon tubes in order to cover the whole filter again. This has caused a dilution of the chl *a* concentration and explains the low value. *: The falcon tube seemed to contain less than 10 mL but we did not know exactly how much. It was estimated to contain 7.0 mL, but it might have been too low of a volume. ****: High value possibly caused upwelling from the sediment during collection of the water sample.**

Lake	Micro	Nano	Pico	Total
1	0.04	0.09	0.34	
	0.06	0.09	0.42	–
	0.04	0.12	0.33	
avg.	**0.05**	**0.10**	**0.36**	**0.51**
%	**9.15**	**19.61**	**71.24**	**100.00**
2	3.09*	1.43*	0.51*	
	1.42	0.60	0.28	–
	0.71	0.40	0.13	
avg.	**1.74**	**0.81**	**0.31**	**2.86**
%	**60.91**	**28.35**	**10.74**	**100.00**
3	0.04	0.19	0.04	
	0.03	0.07**	0.04	–
	0.04	0.23	0.08***	
avg.	**0.04**	**0.16**	**0.05**	**0.25**
%	**14.47**	**64.47**	**21.05**	**100.00**
4	0.05	0.18	0.08	
	0.06	0.15	0.07	–
	0.06	0.15	0.03	
avg.	**0.06**	**0.16**	**0.06**	**0.28**
%	**20.48**	**57.83**	**21.69**	**100.00**
5	0.42	1.15	0.71	
	0.46	1.24	0.73	–
	0.64	2.02	1.01	
avg.	**0.51**	**1.47**	**0.82**	**2.79**
%	**18.14**	**52.63**	**29.24**	**100.00**
6	0.05	0.39	0.04	
	0.05	0.31	0.03	–
	0.03	0.36	0.04	
avg.	**0.04**	**0.35**	**0.04**	**0.43**
%	**10.00**	**81.54**	**8.46**	**100.00**
7	0.37	0.11	0.08	
	0.31	0.11	0.11	–
	0.35	0.09	0.12	
avg.	**0.34**	**0.10**	**0.10**	**0.55**
%	**62.42**	**18.79**	**18.79**	**100.00**
8	0.01	0.43	0.28	
	0.02	0.29	0.36	–
	0.12****	0.49	0.26	
avg.	**0.05**	**0.40**	**0.30**	**0.75**
%	**6.64**	**53.54**	**39.82**	**100.00**
9	0.03	0.16	0.03	
	0.01	0.10	0.04	–
	0.00	0.11	0.02	
avg.	**0.01**	**0.12**	**0.03**	**0.17**
%	**8.00**	**74.00**	**18.00**	**100.00**

Supplementary table 2. F-values and p values of the One-Way ANOVA analysing the difference between size groups within each site. TukeyHSD test crosses size groups and compares them each, which produces a p-value, describing how different two size groups are from each other. Signif. codes: 0 '*', 0.001 '**', 0.01 '*', 0.05 '.'**

	One-Way ANOVA			TukeyHSD p-values					
Lake	F-value	p		Pico - Nano		Pico - Micro		Nano - Micro	
L1	87.71	0.0000	***	0.0001	***	0.0000	***	0.2332	
L2	2.617	0.1520		0.7193		0.1395		0.3723	
L3	5.684	0.0412	*	0.0797		0.9135		0.0479	*
L4	30.03	0.0007	***	0.0014	**	0.9738		0.0012	**
L5	8.038	0.0201	*	0.0829		0.4631		0.0182	*
L6	165.5	0.0000	***	0.0000	***	0.9029		0.0000	***
L7	115.2	0.0000	***	1.000		0.0000	***	0.0000	***
L8	17.44	0.0031	**	0.2871		0.0156	*	0.0029	**
L9	23.15	0.0015		0.0041	**	0.6280		0.0018	**

Supplementary table 3. Results from Sedgewick rafter counts. The counter chamber consists of 20 x 50 rows, 5 or 10 rows were investigated from each lake, thus, the original count was multiplied by 4 or 2, respectively, to get the results in organisms mL^{-1}. (Unident. = unidentified. It was not possible to identify the algae further than by group).

		Number of organisms pr. mL								
	Genus	**Lake 1**	**Lake 2**	**Lake 3**	**Lake 4**	**Lake 5**	**Lake 6**	**Lake 7**	**Lake 8**	**Lake 9**
Chlorophyceae	*Chlamydocapsa* sp.	4								
	Clamydomonas sp.	8	14		8	16	20	4	4	4
	Closterium sp.		8	2				4		
	Cosmarium sp.		28			4		8		
	Desmatractum sp.		2							
	Euastrum sp.		2							
	Gleotila sp.		8							
	Monoraphidium		2							
	Oosystis sp.					16		4		
	Pandorina sp.		4			8				
	Scendesmus sp.	4	4					4		
	Staurastrum sp.		20							
	Staurodesmus sp.		12							
	Westella sp.			10	2	8				
	Unident. greenalgae 1		10							
	Unident. greenalgae 2		4							
	Unident. greenalgae 3			2						
	Total Chlorophyceae	**16**	**118**	**14**	**10**	**52**	**20**	**24**	**4**	**4**
Chrysophyceae	*Chrysococcus* sp.					8				
	Dinobryon sp.	32	70	2	10	12			12	
	Unident. chrysophytes 1		24							
	Total Chrysophyceae	**32**	**94**	**2**	**10**	**20**	**–**	**–**	**12**	**–**
Cyanobacteria	*Anabeana* sp.						4			
	Chroococcus sp.		2		2	4	4			
	Coelosphaerium sp.					8				
	Microcystis sp.					8				
	Unident. cyanobacteria 1		4							
	Unident. cyanobacteria 2								4	
	Total Cyanobacteria	**–**	**6**	**–**	**2**	**20**	**8**	**–**	**4**	**–**
Diatoms	*Amphora* sp.					4				
	Asterionella sp.						12			
	Diatoma sp.		2			12	4			
	Navicula sp.					4				
	Synedra sp.						52			
	Unident. diatom 1		14	2	4	4				
	Unident. diatom 2		6							
	Unident. diatom 3				2					
	Unident. diatom 4					4				
	Total Diatoms	**–**	**22**	**2**	**6**	**28**	**68**	**–**	**–**	**–**
Dinophyceae	*Peridinium* sp.			6				16		
	Unident. dinoflagellate 1	12			2			8		
	Unident. dinoflagellate 2	4								
	Unident. dinoflagellate 3	4		4			12			
	Total Dinophyceae	**20**	**–**	**10**	**2**	**–**	**12**	**24**	**–**	**–**
Euglenophyceae	*Euglena* sp.				2					
	Phacus sp.					4				
	Total Euglenophyceae	**–**	**–**	**–**	**2**	**4**	**–**	**–**	**–**	**–**
	Total phytoplankton count	**68**	**240**	**28**	**32**	**124**	**108**	**48**	**20**	**4**

Water beetle biology, diversity and distribution around Arctic Station (Qeqertarsuaq) at Disko Island (West Greenland)

Beetle trap deployed in arctic pond (photo: Marta Contreras Serrano)

Water beetle biology, diversity and distribution around Arctic Station (Qeqertarsuaq) at Disko Island (West Greenland)

Kasper Grønbech Andersen and Marta Contreras Serrano

Abstract

Water beetles (*Coleoptera*) have seldom been studied in Greenland, with the majority of available data being relatively scattered. The lack of knowledge and long-term data hinders biodiversity assessments and might lead to incorrect conclusions. In the present study, we aimed to provide insight into water beetle presence in a specific area of Greenland. We used a trapping method to sample for water beetles across twelve ponds and lakes around Arctic Station (Qeqertarsuaq) at Disko Island (West Greenland). A number of physico-chemical parameters were obtained for each pond or lake, including altitude, depth, size, vegetation, shelter, pH, conductivity, thawing time and sediment type. Subsequently, macro- and micro-habitat models were created, defining the habitat preferences for each species collected from the traps. We further attempted to study larval development of *Colymbetes dolabratus*, generating a length-weight (L:W) correlation and analyzing how length relates to thawing time of the lake or pond. Our findings support presence of the two species that had been previously reported in the area: *Hydroporus morio* and *C. dolabratus*. Significantly higher frequency of water beetles was found in ponds as compared to lakes. *H. morio* preferred ponds with shelter and presence of vegetation. Results for *C. dolabratus* imagines were inconsistent due to low catch, but *C. dolabratus* larvae appeared to prefer ponds with presence of vegetation. The length analyses as well as the L:W correlation were less successful and driven by 6 outliers from a single lake. Interestingly, these specimens originated from the lake with higher altitude and later thawing date from all locations examined and were therefore in an earlier developmental stage than the rest of the collected larvae. Finally, we report, to our knowledge, the northernmost location of the water beetle *Gyrinus opacus*, thus redefining the northern limit of the distribution of this species. Our results set a simple but solid method for potential development of future studies of Arctic water beetles.

Keywords: *Water beetles, arctic, freshwater, lake, pond.*

Introduction and state of the art

The freshwater macroinvertebrate fauna of the Arctic region is dominated by larvae of mosquitoes and midges (*Insecta*, *Diptera*) and fairy shrimps (*Crustacia*, *Anostraca*) (Hodkinson et al. 2013; Jensen and Christensen 2003), as it becomes obvious at first sight. As one takes a closer look, water beetles might rapidly become visible, swimming around between the rocks and macrophytes by the shore of lakes and ponds (Böcher et al. 2015; Hodkinson et al. 2013). Four water beetle species (*Coleoptera*) have been reported in Greenlandic freshwaters up to date (Böcher et al. 2015; Jensen and Christensen 2003): two species of the *Dysticidae* family consisting of *Colymbetes dolabratus* (Paykull, 1798) and *Hydroporus morio* Aubé, 1838; one whirligig beetle species, *Gyrinus opacus* Sahlberg, 1819 belonging to the *Gyrinidae* family; and one species of the *Hydrophilidae* family, *Helophorus brevipalpis* Bedel, 1881 that has been recorded only once in 1984.

Overall, little is known about the distribution and biology of water beetles in arctic circumpolar regions (Hodkinson et al. 2013). This lack of knowledge also applies for Greenland and may lead to incorrect conclusions when assessing biodiversity. In general, global biodiversity assessments point to greatest diversity in the tropics and focus

on temperate to tropical regions (Jäch and Balke 2008). Circumpolar assessments of water beetles are, in comparison, lacking, and the few that are available emphasize such issue while attempting to update records from the last hundred years (Gren and Lubecki 2019). In such framework, the Catalogue of Palearctic Dysticidae and Hydrophilidae (Coleoptera) (Nilsson and Hájek 2017; Przewoźny and Fikáček 2017), provides a useful resource to crosscheck one's findings with the historical knowledge of water beetles of a particular country. Unfortunately, Greenland is excluded from such catalogue and thus cannot be of use for our purpose. To our knowledge, previous studies of water beetles on Disko Island (W Greenland) are scarce (Sønnichsen 1973), with the latest review on the subject dating back to the 1988 in "Meddelelser om Grønland" (Böcher 1988), which mostly includes anecdotic and observational reports on the subject. Similarly, the database from the Global Biodiversity Information Facility (GBIF) only has few scattered datapoints of *C. dolabratus* and *H. morio* in Greenland, with none on Disko Island (GBIF 2020a; GBIF 2020b). Long-term population data on individual Arctic species is entirely lacking (Hodkinson et al. 2013), no comprehensive database of Arctic water beetles exists, and rather few, scattered literature is available on this matter across Greenland (Hodkinson 2018).

Water beetle biology in arctic circumpolar regions

Two species of water beetle have previously been reported from Disko Island and thus will be the focus of the present study: *C. dolabratus* and *H. morio* (Böcher 1988). The whirligig beetle, *G. opacus*, has been reported from boreal regions and the lower Arctic. In Greenland, *G. opacus* has been reported from Kap Farvel (southernmost point of Greenland) to Sisimiut District (66º 56' N, 53º 33' W), and it is listed as "northwards scattered" and "only inland", with a northernmost range limit in West Greenland at 67° 27' N, 50° 30' W (Böcher et al. 2015). Given the proximity of the northern range limit of *G. opacus* to our study area at Disko and the lack of consistent sampling for water beetles throughout time, we also consider this species as a potential catch in our study. The three species are typically found in ponds during summer, although they can also inhabit lakes (Böcher et al. 2015).

H. morio (Fig. 1A) is with its small size of 3 - 3.5 mm the smallest water beetle of Greenland. It is always fully winged and able to disperse by flight (Böcher et al. 2015). The diet assumingly consists of chironomid larvae, small crustaceans and rotifers that it hunts in the water column. There is no sign of seasonal migration and the species must therefore be able to tolerate freezing during hibernation. Hibernation in bottom sediment of both imagines and larvae is most likely (Böcher et al. 2015). *H. morio* inhabits any kind of small ponds and marshes, although it is preferably found in shallow ponds with shelter and rich vegetation. The species is high-arctic to boreal circumpolar and in Scandinavia also boreo-alpine. Moreover, *H. morio* has been reported in Scandinavian bogs, tolerating highly acidic waters (Böcher 1988; Foster 1995; Yee 2014).

G. opacus (Fig. 1B) is the only species of the *Gyrinidae* family that is present in Greenland (Böcher et al. 2015). *G. opacus*, with a size of 5.5 to 6 mm, is a predator with specialized divided compound eyes for vision in both air and water. Such adaptation allows for hunting insects caught in the water surface while maintaining the ability to hunt other invertebrates in the water column (Böcher et al. 2015). *G. opacus* is often found skating on the water surface, and therefore prefers stagnant or slowly flowing waters, including both ponds and lakes. Adults hibernate on the bottom and thus have to be able to tolerate freezing to some extent (Böcher et al. 2015). The species is boreal to low-Arctic circumpolar and has been found in Scandinavia at altitudes up to 1000 m (Böcher 1988).

C. dolabratus is with its approximate length of 13-17 mm the largest beetle found in Greenland. Both larvae (Fig. 1D) and imagines (Fig. 1C) are predacious, with a diet assumed to consist of nematocera larvae and crustaceans that they hunt in the water

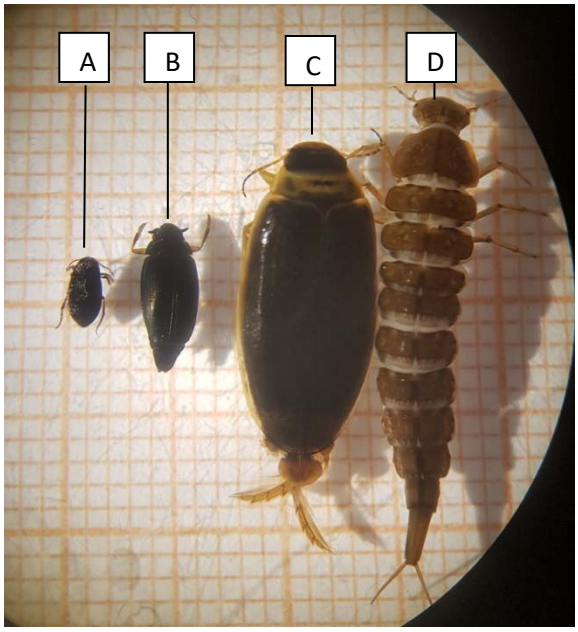

Figure 1. Examples of the three species found in Greenland, including larva of *C. dolabratus*. (A) *H. morio*, (B) *G. opacus*, (C) imago *C. dolabratus*, (D) larva *C. dolabratus*.

column (Böcher et al. 2015). Furthermore, cannibalistic interactions amongst larvae have been reported (Yee 2014). Larval development is divided into three instars, distinguishable by body length, number of abdominal segments and development of mandibles and palpi (Galewski 1968). The larval body length ranges from 4.6 to 8.5 mm for the first instar, 8.5 to 13.9 mm for the second instar and 14 to 25.8 mm for the third instar. Imagines have fully developed wings, thus allowing migration over considerable distances, a characteristic trait for many dytiscid beetles (Yee 2014). Such migration is observed after winter when the surface ice of the lake thaws, allowing for dispersal to new breeding localities including ponds and lakes. Both larvae and adult can therefore be found in ponds and lakes during summer. In late summer, imagines migrate to lakes that do not freeze to the bottom (typically >3m deep) to hibernate (Böcher et al. 2015). During summertime, larvae and imagines can be found simultaneously. South of 67°N, the larval and pupal stage is completed in one season, whereas individuals dispersed further north take two years to complete the lifecycle. This is most likely due to the shorter ice-free season north of 67°N and may lead to simultaneous presence of first-year and second-year larvae, as well as adults (Böcher et al. 2015; Sønnichsen 1973). *C. dolabratus* is vastly eurytopic and can be found in most types of stagnant waters, although it preferably inhabits ponds with rich vegetation (Böcher et al. 2015). Its range covers high Arctic to boreal circumpolar regions, further extending south through Scandinavia where it occupies both *Sphagnum* bogs and lakes or ponds above 1000 m (Böcher 1988).

Aims and hypotheses

In this study, we aim to update the water beetle records from the 1980s and contribute to the knowledge on the diversity and distribution of water beetles near Arctic Station, southern Disko Island, W Greenland. We further aim to determine the environmental variables that could drive water beetle diversity and distribution, as well as discuss whether the species niche presented in "The Greenland Entomofauna" (Böcher et al. 2015) still applies. We use a (i) micro-habitat approach to test which environmental variables drive diversity and distribution within a water body and (ii) a macro-habitat approach to determine those variables determining differential diversity and distribution among water bodies within the study area. Additionally, we aim to generate, to our knowledge, the first length-weight regression for *C. dolabratus* larvae and subsequently analyze larval size development in relation to thawing date of lakes and ponds.

We hypothesize that (i) *G. opacus* might have reached northern latitudes as compared to those exposed in Böcher et al. (2015); (ii) *C. dolabratus* will mostly appear in ponds with rich vegetation, as compared to other habitats; (iii) *H. morio* will be more frequently found in shallow ponds with muddy sediment and presence of vegetation and/or rocks that provide shelter; (iv) *C. dolabratus* and *H. morio* will be more frequently found in ponds as compared to lakes; (v) *C. dolabratus* larvae will follow a gradient of length across altitude, where larvae from lower altitudes will be significantly larger and heavier than those from higher altitudes, in response to

Figure 2. Map of the study site including specific sampling areas (C, N, M, F) and their respective lakes and ponds. Image from Google Earth (Google 2020).

differential thawing/freezing times of the waters they inhabit.

Materials and methods

Study sites

The study was carried out around Arctic Station (Qeqertarsuaq) at Disko Island (69º 15'N, 53º 31'W), on the western coast of Greenland. Four different areas (N, C, M, F) comprising a total of twelve study sites (five lakes and seven ponds, *N* = 12) were selected for sampling (Fig. 2), in an effort to maximize variability among environmental parameters of the different water bodies. Each pond or lake was at least 100 m apart from the others and was considered an independent sampling unit.

Beetle trapping

Fieldwork took place from the 14th to the 20th of July 2020. For each lake or pond, 2 beetle traps were placed in the water close to the shoreline, at specific locations that were considered representative for the whole water body (*n* = 24 locations). From these locations, only *n* = 22 were considered in the final analyses, excluding F3 (see Methods - Statistical analyses for specific reasons).

The deployed water beetle traps (Hulcr 2018) (Fig. 3) were recommended by the entomologist Mogens Holmen (independent Danish consultant). Each trap consisted of a plastic bottle cut in two with the top part inverted and inserted in the bottom part. Slits were cut in the bottom edges of the bottle, to allow air flow. Bait (a raw piece of cod) was placed inside the bottle together with a piece of styrofoam, to keep the air slits above water. Traps were attached to the shore of the lake or pond using tent pegs and strings and left for a period of 2-3 days. Each trap was individually emptied through a fine mesh sieve and into a bucket, in such a way that water beetle imagines and *C. dolabratus* larvae would get retained into the sieve and would then be collected, counted and classified into vials with ethanol 90%. This trapping method was tested beforehand in Denmark to ensure reliable functionality. Positive results were obtained from such testing, with large catches containing water beetles.

Active net-trapping was applied upon arrival to a lake or pond and before trap setting, as

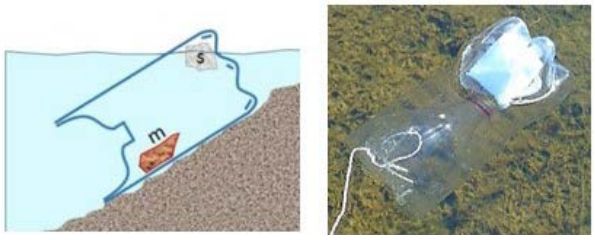

Figure 3. Water beetle trap, diagram to the left (from Hulcr, 2018) and own trap to the right.

an additional part of the lake/pond recognition routine. For such purpose, a simple fine mesh sieve or bare hands were used. Netting was applied for approximately 5 minutes at each site. The outcome from this random collection was not further processed nor is it included in the present report (except for *G. opacus* observations).

Collection of environmental data

For each sampled pond or lake, the following environmental parameters were obtained: size, altitude, depth at point of trap location, sediment, presence/absence of shelter and vegetation, thawing day of the year, pH and conductivity. Size refers to the area of the water body (m^2) and was measured either *in situ* using a handheld GPS and walking around the water body or *a posteriori* using Google Earth's surface measurement tools (Google 2020). Altitude (m) was measured *in situ* using a handheld GPS. Depth at the point of trap location (m) was measured using a graduated stick after placing the trap. Sediment was assessed by eye *in situ* on the day of trap setting and re-assessed on the day of trap emptying: from such observations, a specific percentage of sediment coverage was given to each pond or lake, main sediment was determined and a sediment category was attributed to each trap. Presence/absence of shelter was defined as presence/absence of relatively dense vegetation and/or overlaying rocks providing protection and hiding, and was assessed by observation *in situ*. Furthermore, presence/absence of vegetation (macrophytes, algae) was assessed *in situ* by eye. Water pH and conductivity data were retrieved from Larsen and Mollerup (2020), who performed these analyses simultaneously with our study. Thawing date for each pond or lake was estimated from satellite images retrieved from Satellite Sentinel-2 L2A (Sinergise Laboratory for geographical information systems 2020). Date of thawing was established on the day at which thaw onset was first visible from the satellite; if the sequence of satellite images had gaps between the date of a frozen-surface picture and the date of a thawing-surface picture, the day in between those two dates was taken as thawing date (see Appendix A for an illustration of this). Satellite images from subsequent days were checked to ensure that there was no complete re-freezing of the water surface. For small ponds (*e.g.* ponds at C and N areas), thawing date of the largest pond was determined and assumed to apply to the rest of the ponds in that area. Thawing day was defined as the date of first breaking of ice, allowing for hibernating beetles to replenish their air supply and potentially migrate. Next, thawing date was used to calculate thawing day of the year, where January 1st was day 1 and December 31st was day 366.

Length-weight measurements

In order to get a length-weight (L:W) relationship, 50 (or as many as available, if less were collected) larvae of *C. dolabratus* from each site were measured and weighted individually. To measure body length, each larva was placed on a measuring grid and examined through a stereo microscope. Length from head (excluding mandibles and palpi) to urogomphi (excluding cerci) was recorded, following Mroczyński and Daliga (2016). To measure weight, the larvae were dried in individual tin-foil cups at 105ºC for 15-24 hours. The samples were cooled and kept inside a desiccator in order to avoid ambient moisture to increase their weight. Next, larvae were weighted individually using a precision balance and weight was recorded.

It must be noticed that standard methods for invertebrate weight measurements use specimens that have been kept in water instead of ethanol, as we do here. Our L:W results might be biased as a consequence of ethanol treatment, given that specimens were kept in ethanol for different periods of time (from 1 to 6 days), and thus might not be comparable to other studies.

Statistical analyses

Environmental niche analyses:

Statistical analyses were performed in Rstudio (RStudio-Team 2020), using generalized linear

models with Poisson distribution. Lake F3 was excluded from the analyses because no catch was obtained from it and reasons for that were attributed to factors other than those contemplated in our study: Lake F3 was subjected to constant perturbation from glacier inflow likely generating an unsuitable environment for water beetles (*e.g.* fast currents, dominance of clay sediment). For the remaining *N* = 11 water bodies (total of *n* = 22 traps), two different approaches were used: (i) micro-habitat approach: environmental parameters determining micro-habitat for each trap setting location (*i.e.* depth, presence/absence of vegetation and presence/absence of shelter at location of trap setting) were used in a first model as independent variables modelling the species counts from each individual trap; for this analyses; (ii) macro-habitat approach: environmental parameters defining the macro-habitat for each pond or lake (*i.e.* water pH and conductivity, altitude, size and main sediment of the water body, pond/lake category, and thawing day of the year) were used in a second model as independent variables modelling the species counts from each individual water body (*i.e.* dependent variables were here the sum of species counts of both traps from the same water body).

Independent variables were tested for correlation using Pearson's correlation coefficient test, prior to building the model. From such correlated variables, only one was chosen to build up models, following logic and pursuing lower AIC values. The p-values from the resulting models were used to test the environmental-niche hypotheses (see hypotheses (ii), (iii) and (iv) in section 1.2).

Larval length and weight analyses:

From a total of 309 measured and weighted larvae, 33 specimens were considered damaged as they showed *e.g.* head disattached from body, empty insides, large body length (> 1.9 cm) but very low weight (< 0.01 g), etc. Such damages are presumably attributed to handling, prolonged ethanol treatment and/or larval cannibalism with external digestion (Yee 2014). Therefore, these specimens were removed from further analyses, obtaining a final dataset with *n* = 276 larvae. This was used to establish a length-weight correlation in Rstudio (RStudio-Team 2020). Such L:W correlation was built using power and linear functions in a regression analysis, with 95% confidence intervals (CI).

Next, larval development as related to thawing day of the year and altitude was considered. Rstudio (RStudio-Team 2020) was used to plot the regression between length and thawing day, with 95% CI. A mixed linear model was subsequently used to test correlation between larval length and thawing day of the year. However, such analyses are not presented in this report, nor further statistical analyses were performed, as the correlation was obviously driven by 6 outlier datapoints which, if removed from the tests, led to a total loss of significance. Instead, our discussion for this part remains mostly observational.

Results

A total of 529 beetle specimens were captured, (Fig. 4). Of these, 434 were *C. dolabratus* larvae (82%), 28 were *C. dolabratus* imagines (5.3%) and 67 were *H. morio* imagines (12.7%). The distribution of the catches among traps was rather irregular for the three groups. For *C. dolabratus* imagines, very few specimens were collected per trap (mean catch = 1.1 specimens of *C. dolabratus* imago per trap, max. = 7, min. = 0). For *C. dolabratus* larvae, catches were usually abundant (mean catch = 16.7 specimens of *C. dolabratus* larva per trap, max. = 90, min. = 0). Finally, for *H. morio* catches laid in between the two previous ones (mean catch = 2.6 specimens of *H. morio* imago per trap, max. = 19, min. = 0).

No *G. opacus* were trapped nor observed at any of the sampling sites at Disko Island. However, plenty of specimens of *G.opacus* were observed in a pond at the outskirts of Ilulissat (69º12'09''N 51º06'01''W) on July 23 and again on July 24. A few specimens were collected. To our knowledge, this is the

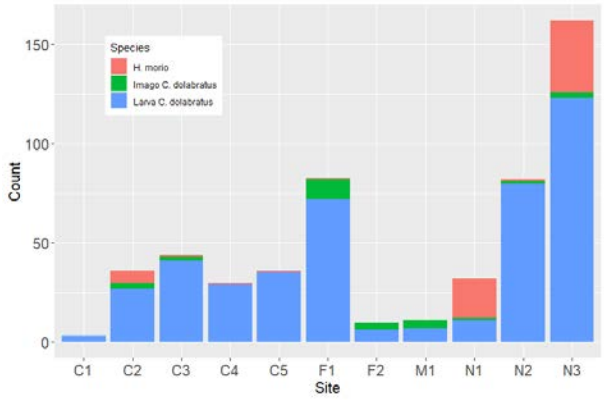

Figure 4. Stacked bar plot including catches of *C. dolabratus* imagines, *C. dolabratus* larvae and *H. morio* for each sampled site (N=11). It becomes clear that larvae of *C. dolabratus* was the most frequent catch.

northernmost range limit at which this species has been reported up to date.

As a general observation, both beetle imagines and larvae were much more visible on sunny days than on cloudy or rainy days.

Environmental niche models

Two different environmental models were applied: a micro-habitat model and a macro-habitat model (see details in the methods section). Such models were built in an effort to describe the distribution of counts of each individual water beetle species and further find a general pattern applicable to all water beetles collected in the study.

Micro-habitat model

The micro-habitat model (Appendix C, Table 1) included presence/absence of shelter, presence/absence of vegetation and depth at site of trap location. This model was significant when the counts of all species were considered together ($p < 0.0001$, AIC = 659.45), and indicated that presence of vegetation was positively correlated to higher counts of all species ($p < 0.0001$). Conversely, depth at site for trap location and presence of shelter did not appear to be significantly correlated to species counts, when all species were analyzed together ($p > 0.05$).

The model was also significant for describing *H. morio* distribution ($p < 0.0001$, AIC = 155.51). Presence of shelter ($p < 0.0001$) (Fig. 5) and presence of vegetation ($p = 0.0046$) were positively correlated to higher *H. morio* counts, while depth was found to be not correlated ($p > 0.05$). When describing counts and distribution of *C. dolabratus* imagines, the model was found to be significant ($p < 0.0264$, AIC = 70.89) but none of the environmental parameters (shelter, vegetation and depth) were significantly correlated to counts of *C. dolabratus* imagines ($p > 0.05$). This was probably a result of the small catch of *C. dolabratus* imagines. Finally, the model was significant for the counts and distribution of *C. dolabratus* larvae ($p < 0.0001$, AIC = 633.84), with presence of vegetation being positively correlated to higher counts ($p < 0.0001$) (Fig. 6) but presence of shelter and depth of the trap being not significantly correlated to larvae counts ($p > 0.05$).

Macro-habitat model

Regarding the macro-habitat model (Appendix C, Table 2), several independent variables had

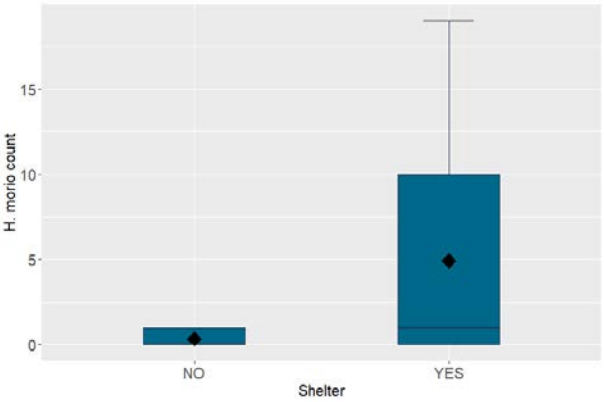

Figure 5. Box plot representing shelter preference for H. morio across all locations (n=22). Error bars represent standard deviation.

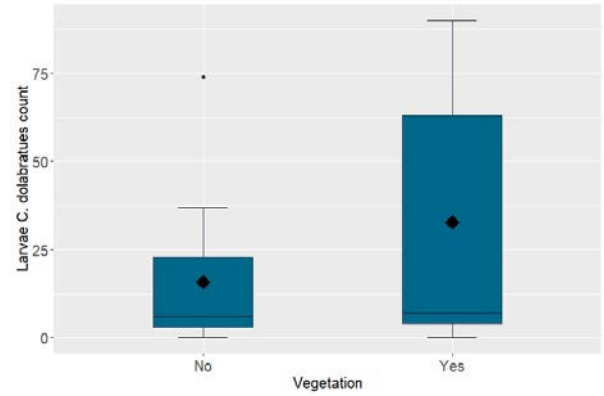

Figure 6. Box plot representing preference of vegetation presence for *C. dolabratus* larvae across all locations (n=22). Error bars represent standard deviation.

to be removed due to correlation with other independent variables in the model. Thawing day of the year had a strong positive correlation with altitude (Pearson correlation coefficient r = 0.996, p < 0.0001). Moreover, altitude values had two outliers, as most samples were taken close to the coast (0-60 m) and just two were taken in upper altitudes (380-450 m), with no intermediate gradient. Thus, altitude was removed from further analyses. Pond/lake category was also found to be significantly correlated with size (r = -0.628, p = 0.0386) and with thawing day of the year (r = - 0.659, p = 0.0273), with ponds having a significantly smaller size and thawing earlier in the year, as compared to lakes. Finally, both conductivity and pH are measures of dissolved ions in a solution (hydrogen ions in the case of pH and electricity-conducting ions in the case of conductivity) and thus could be somewhat correlated. Our analyses did not indicate any significant correlation (r = 0.495, p = 0.122). However, pursuing a logic and a low AIC value for our final model, we decided to remove pH from the analyses and instead keep conductivity. The final macro-habitat model was built using four independent variables that tried to cover as many different aspects of the habitat as possible, without bringing too much autocorrelation into the model: conductivity, thawing day of the year, pond/lake category and main sediment.

The final macro-habitat model was overall not significantly correlated to all species counts, when these were considered as a whole (p > 0.05, AIC = 169.1209). In this model, thawing day of the year was found to be not significantly correlated to all species counts (p > 0.05). However, pond/lake category (Fig. 7), conductivity and main sediment were all found to be correlated to all species counts (p < 0.0001), with ponds with lower conductivity and algae-covered bottoms having the highest counts.

The macro-habitat model was significant for describing *H. morio* counts and distribution (p = 0.000281, AIC = 50.309). All environmental parameters were, in this case, significantly correlated to *H. morio* counts, although

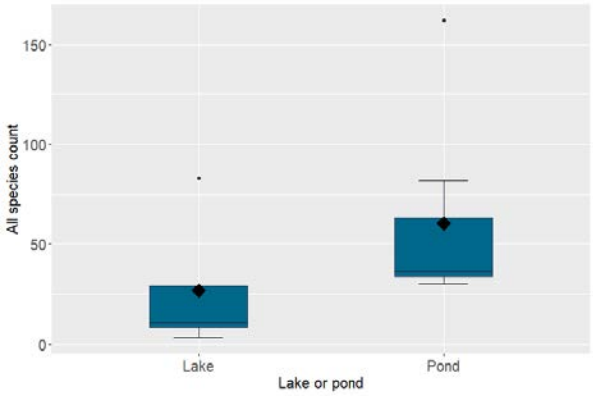

Figure 7. Box plot representing preference of pond versus lake for all species across all locations (n=22). Error bars represent standard deviation.

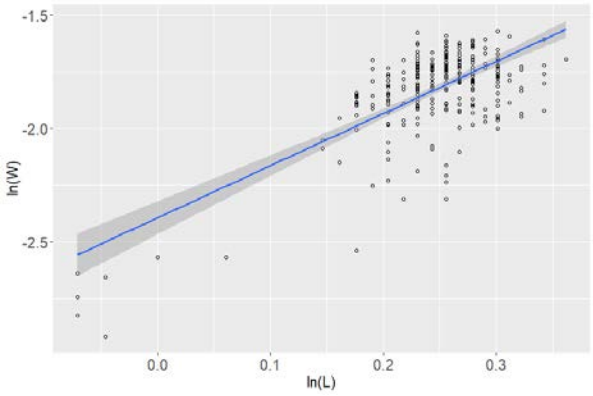

Figure 8. Length-weight linear regression for *C. dolabratus* larvae. The correlation is clearly driven by a few outliers from lake F2.

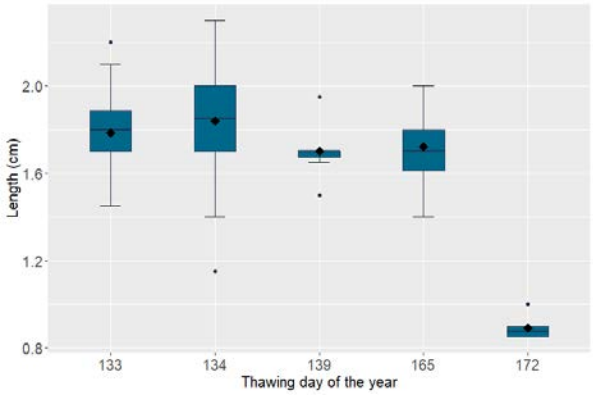

Figure 9. Boxplot diagram for length of *C. dolabratus* larvae, collected in ponds with different thawing days. Error bars represent standard deviation.

sediment, conductivity and pond/lake category (p < 0.0001) showed a slightly stronger correlation than thawing day of the year (p = 0.0002). Overall, the model indicated that *H. morio* prefers ponds with later thawing day of the year, lower conductivity and algae-covered bottoms. Regarding *C. dolabratus* imagines, the model did not seem good

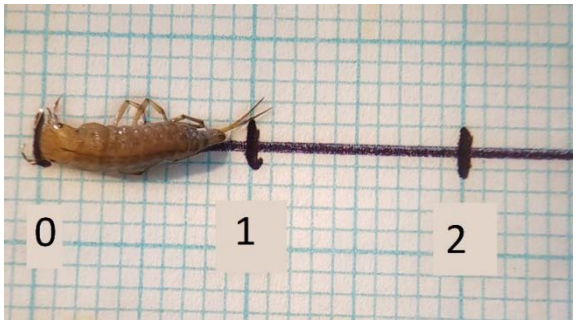

Figure 10. Very small specimen of larva *C. dolabratus*, found in lake F2. Approximate 0.9 cm of length in second instar level

Figure 11. Average specimen of *C. dolabratus* larva. Measuring approximately 1.9 cm, third instar stage.

enough to explain the counts and distribution of the group ($p > 0.05$, AIC = 35.6867), and only main sediment was found to be significant, with *C. dolabratus* imagines preferring algae- over gravel-covered bottoms ($p = 0.0148$). Finally, the model was not significant for *C. dolabratus* larvae ($p > 0.05$, AIC = 177.8195). However, all individual parameters within the model were found to be significantly correlated to larval counts, again with sediment, conductivity and pond/lake category ($p < 0.0001$) having a slightly stronger correlation than thawing day of the year ($p < 0.0082$). According to the model, *C. dolabratus* larvae prefer ponds that thaw later in the season, have lower conductivity and a rocky-bottom sediment.

Larval length and weight analyses

Both the L:W correlation (Fig. 8) and the correlation between larval length at capture and thawing time (Fig. 9) were found to be driven by 6 outlier datapoints corresponding to F2 lake: the lake with latest thawing day (172). These specimens have the shortest body length of the study, ranging from 0.85 to 1.0 cm (Fig. 10), thus being included in the development (Galewski 1968). In contrast, the rest of the larvae from all the other sites with earlier thawing day ranged from 1.4 to 2.3 cm (Fig. 11), thus belonging to the third instar (Galewski 1968) (except for one single specimen from N1 that measured 1.15 cm).

For the W:L correlation, the linear function (Fig. 10) ($R^2 = 0.471$) gave a best fit that the power function (data not shown) ($R^2 = 0.394$).

Discussion

General observations

In general, our findings and observations of Greenlandic water beetles seem to correlate with the descriptions made by Böcher et al. (2015). *C. dolabratus* was observed in most types of water bodies, while *H. morio* was observed in waters sheltered by rocks and/or vegetation. However, with our observation of *G. opacus* on 69º N we can conclude that the distribution of the species has moved considerable northwards since it was last recorded in the area more than 30 years ago, thus confirming hypothesis (i). It is clear that earlier distribution data of Greenlandic water beetles is clustered around villages and harbors, undoubtedly for logistic reasons. With our observation of several *G. opacus* in a single pond within walking distance of the major village of Ilulissat, it is highly unlikely that the species was present in 1988, when the last review of the distribution was completed (Böcher 1988). We suggest that this northward expansion since 1988 could be the result of a slow succession or might even be influenced by warmer climatic conditions. Unintentionally dispersal by humans can however not be outruled as a potential cause.

We observed higher water beetle activity on sunny days as compared to cloudy or rainy days. This observation might be supported by Downes (1964) and Roland (1982), who argue that Arctic arthropods have adapted to a high degree of opportunism in relation to activity periods and further have the ability to absorb and retain heat from the sun, therefore being more active on sunny days.

Finally, the chosen trapping method overall proved to be effective for catching dytiscid beetles. In all ponds and lakes with visual observation of specimens, the traps proved effective in catching the observed species. The traps did however prove less effective in larger, wind-exposed lakes (*e.g.* lake M), where the traps would get thrown around.

Habitat models

Micro-habitat model

According to the micro-habitat model, presence of vegetation seemed to be an important factor determining higher frequency of all water beetles in a specific location within a pond or lake. Statistical analyses indicated that a higher frequency of both *H. morio* and *C. dolabratus* larvae was significantly correlated to the presence of vegetation around trap location. However, such significance was lost for *C. dolabratus* imagines, probably as a result of the small sample size and/or the eurytopic nature of this group (Böcher et al. 2015). Therefore, the initial hypothesis (ii) suggesting that higher counts of *C. dolabratus* would be found in areas with vegetation (Böcher et al. 2015) was confirmed for the larvae but rejected for the imagines. Being predatory consumers, *C. dolabratus* larvae and imagines might benefit from the presence of vegetation in the water column, as this likely contributes to a higher presence of prey and lower conspicuousness for them as predators. Rich vegetation might also be an indicator of a longer growth season and warmer conditions, which are also favorable for both larvae and imagines.

Following our expectations and confirming part of our hypothesis (iii), the micro-habitat model indicated that significantly higher counts of *H. morio* were correlated to presence of shelter and vegetation. This is consistent with previous observations made by Böcher et al. (2015). Conversely, depth of the trap did not significantly correlate to *H. morio* counts. Given the small size of this species, presence of shelter and vegetation is likely important to ensure a more protected environment with low currents, thus allowing for *H. morio* to swim and hunt effectively. According to the literature (Böcher et al. 2015), shallower waters should also contribute to higher frequency of *H. morio*. However, and consistent with our results, we suggest that shallower ponds might also be more exposed to wind and thus present higher instability and turbulence in the water column, hindering the conditions for *H. morio*.

Macro-habitat model

The macro-habitat model used to describe species distribution and counts included conductivity, thawing day of the year, pond/lake category and main sediment of the water body as independent variables. Overall, ponds had a significantly higher frequency of all species grouped together than lakes, confirming our initial hypothesis (iv). A potential explanation for this would be that ponds thaw earlier in the season and their lower volumes of water can heat faster during summer season, as compared to those from lakes. In turn, this would imply a longer growing season, a higher primary productivity and, overall, more resources for water beetles in ponds (Douglas et al. 1994; Payer et al. 2013). Furthermore, newly thawed ponds offer an intraspecific competitor-free environment for *C. dolabratus*, which can be utilized by the species high rate of dispersal, as described by Böcher et al. (2015). Moreover, when all species were grouped together, a significantly higher frequency was found in ponds with algae-covered bottoms, when compared to gravel cover. This supports results of the micro-habitat model, highlighting the importance of presence of vegetation and the high reliability of *H. morio* on presence of shelter. Furthermore, ponds and lakes with algae-covered bottoms had significantly higher frequency of *C. dolabratus* imagines when compared to those with gravel-covered bottoms ($p = 0.0148$). This result might leave the impression that *C. dolabratus* imagines prefer algae-cover over gravel-cover. However, looking closer at the data (Appendix B), such result appears to be a type I error, as more *C. dolabratus* imagines were collected in ponds with gravel-cover, compared to algae-cover. Thus, we do not

conclude any preference for sediment type from such results.

Finally, lower conductivity also showed a significant correlation to higher frequency of all species grouped, as well as to *H. morio* and *C. dolabratus* larvae, independently. The lower conductivity indicates low concentration of ions, including nutrients for primary production. However, none of the ponds and lakes sampled in this study can be classified as polluted, perhaps with the exception of lake C1, that had contamination of dog feces and kitchen sewage. While more beetles were found in waters with lower conductivity, this is unlikely to be a driver for the presence of water beetles, as beetles were found in different ponds covering a wide range of conductivities. It is likewise concluded by Foster (1995) that *H. morio* presence is driven by other factors other than the preference of a certain pH. Nevertheless, we remark that higher conductivity in lakes like C1 (see Appendix B), likely caused by the foresaid pollution, might be a driver of oxygen depletion in the water throughout the long winter, thus making this habitat not suitable for overwintering. In turn, this could also drive lower water beetle abundance in the summer season, supporting our results.

The macro-habitat model had overall some inconsistencies, as proving that there was significantly higher frecuency of *H. morio* in waters with later thawing day of the year. This does not follow logic and probably reflects an artifact of our results.

Length-weight analyses

The length-weight analysis was overall not successful. The method used was not in compliance with standard methods, as the specimens were kept in 90% ethanol for a duration of 1 - 6 days. This resulted in a large uncertainty of the length and weight measurements of the individual specimens. Such inconsistencies become clear in Fig. 8, where the data points are widely scattered. Furthermore, the regression is clearly driven by six outliers corresponding to the F2 lake which, if removed from the regression, lead to a loss of statistical significance. However, with this study we confirm that *C. dolabratus* larvae are efficiently trapped using our method. This creates a good outline for future studies with focus on the L:W correlation.

The correlation between length and thawing day was found to be driven by the 6 same outliers from lake F2 and is therefore instead presented in a boxplot in Fig. 9. This correlation was further prevented by the lack of a clear gradient of thawing days and altitude. The majority of ponds and lakes sampled were located in a gradient of 4-53 m altitude, with only two lakes located above 300 m. This resulted in similar thawing days, with the two lakes located above 300 m as clear outliers. No significant results were obtained, and we cannot therefore accept hypothesis (v). The specimens collected in lake F2 were however noteworthy because of their small size. With body sizes ranging from 0.85 to 1.0 cm, only larvae of the second instar level seemed to inhabit this lake. In contrast, in other lakes and ponds, we observed almost exclusively larvae of the third and final instar level. Whether these are first- or second-year larvae remains unknown to us. Our observations suggest that altitude and thereby late thawing day do affect the size and lifecycle of the *C. dolabratus* larvae, but fail to draw a clear pattern. However, this represents a good outline for future studies, with optimized experimental setup including a clear gradient of altitude and thawing day that might make it possible to document a correlation between larval size and altitude, and better understand the life cycle of arctic water beetles.

Future studies

Our study supports the habitat preferences of *H. morio* and *C. dolabratus* as described by Böcher et al. (2015). The methods we present can be used in an expanded project including even more environmental parameters and potentially enhancing the quality and accuracy of the study. Furthermore, we confirm the possibility of creating a W:L correlation for *C. dolabratus* larvae with specimens from ponds and lakes located near

Arctic Station, if the right methods are applied from the start. With more research on locations of ponds at different altitudes, it seems also plausible to prove a consistent correlation between *C. dolabratus* larval length and altitude. Finally, our observation of *G. opacus* emphasizes the need of updating the distribution of Greenlandic water beetles, for both future use and comparison with earlier distribution data. We present an effective and systematic method of collecting dytiscid beetles that is not widely used in scientific literature. The trap is cost-efficient, easy to make, lightweight and needs no specialized tools or materials. Moreover, the method is not as reliant on sunny weather, as traditional hand-netting is. We can therefore recommend this trap model for future studies of dytiscid beetles.

Overall, achieving a solid knowledge regarding biology, status and trends of Arctic water beetles will contribute to a better understanding of Arctic freshwater biology in general and further add valuable details for water quality assessments, as water beetles might act as indicators of water quality in Arctic ponds and lakes (Hodkinson et al. 2013). Furthermore, consistent studies will enable a comprehensive baseline for studying the effects of climate warming on Arctic biodiversity, as Arctic invertebrate communities are very likely to respond rapidly to increasing temperatures (Callaghan et al. 2004).

Acknowledgements

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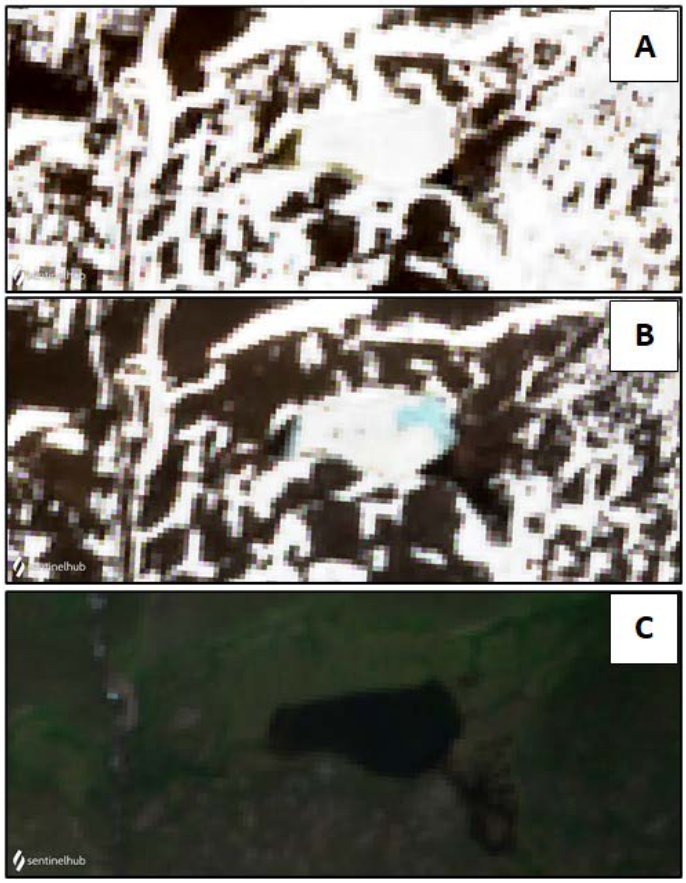
Appendix A – Sentinel Satellite images for thawing dates



Appendix A. Satellite images from lake M, retrieved from Sentinel-2 L2A. A) May 16, 2020; B) May 21, 2020; C) August 9, 2020. Thawing date for this lake was located between dates A and B for which satellite images were missing. Thus, the mean-lower date, May 18, 2020 (day of the year 139), was taken as thawing date.

Appendix B – Specimens collected at each site and macro-habitat parameters

Site nº	Pond/Lake	Thawing day	Altitude (m)	Conductivity (S/m)	Main sediment	Larvae *C. dolabratus*	Imagines *C. dolabratus*	*H. morio*	All species grouped
C1	Lake	133	4	0,0112	Rock	3	0	0	3
C2	Pond	133	24	0,0081	Algae	27	3	6	36
C3	Pond	133	27	0,0053	Rock	41	2	1	44
C4	Pond	135	22	0,0093	Rock	29	0	1	30
C5	Pond	135	24	0,0081	Rock	35	0	1	36
M1	Lake	139	71	0,0079	Rock	7	4	0	11
N1	Pond	134	35	0,0055	Rock	11	1	20	32
N2	Pond	134	37	0,0058	Rock	80	1	1	82
N3	Pond	134	53	0,0049	Rock	123	3	36	162
F1	Lake	165	382	0,0023	Rock	72	10	1	83
F2	Lake	172	445	0,0019	Gravel	6	4	0	10

Appendix C – Tables for the Micro- and macro-habitat models

Table 1. An overview of P-values found when testing the micro habitat model. Both P-values for each variable (shelter, vegetation and depth) and for the whole model (model) are given. The model is tested for individual species and for the species grouped together. Vegetation presence (YES) is compared to vegetation absence (NO) and Shelter presence (YES) is compared to Shelter absence (NO).

	All species	Imagines *H. morio*	Imagines *C. dolabratus*	Larvae *C. dolabratus*
Shelter YES	0,078	<0,0001	0,2428	0,975
Vegetation YES	<0,0001	0,0046	0,1357	<0,0001
Depth (m)	0,367	0,9276	0,0573	0,144
Model	<0,0001	<0,0001	0,0264	<0,0001

Table 2. An overview of P-values found when testing the macro habitat model. Both P-values for each variable (conductivity, thawing day, gravel and rock) and for the whole model (model) are given. The model is tested for individual species and for the species grouped together. Pond YES is compared to Lakes. Gravel and Rock is compared to Algae sediment.

	All species	Imagines *H. morio*	Imagines *C. dolabratus*	Larvae *C. dolabratus*
Pond YES	<0,0001	<0,0001	0,0621	<0,0001
Conductivity	<0,0001	<0,0001	0,0705	<0,0001
Thawing day	0,1693	0,0002	0,1087	0,0082
Gravel	<0,0001	0,989615	0,0148	<0,0001
Rock	0,821	<0,0001	0,0566	0,41421
Model	0,35	0,000281	0,0891	0,48223

A comparison of two morph types of Arctic charr: landlocked from Røde Elv and anadromous from Kuannersuit Sulluat, Disko Island, Greenland

Landlocked Arctic charr (Photo: Alessandra Bateman-Neubert)

A comparison of two morph types of Arctic charr: landlocked from Røde Elv and anadromous from Kuannersuit Sulluat, Disko Island, Greenland

Alessandra Bateman-Neubert & Peter K. Petersen

Abstract

In July 2020, a total of 30 landlocked and 5 anadromous Arctic charr were analyzed. The landlocked specimens were collected from two nearby ponds in Blæsedalen from a small pond (SP) and a big pond (BP) with 14 and 16 individuals, respectively. The (5) anadromous specimen were caught in Kuannersuit Sulluat (S), a fjord at Southern Disko island. The condition index (CI) was calculated for all fish, which allowed the comparison between similar sized fish (SP vs BP), as well as the trend between the landlocked and anadromous fish. Additionally, the stomach content, parasite presence and the degree of egg maturation in females was analyzed. BP fish have significantly higher CI values than SP fish, as well as a more diverse diet and a more mature egg development, suggesting a much more suitable environment. In support of this, two parasites were found in SP fish, suggesting a weaker immune system which could also be related to a more stressful and competitive environment. Furthermore, SP fish had a significantly lower degree of egg maturation than BP fish, suggesting a different reproductive strategy. Inter-annual growth rates were investigated by using otoliths and compared to variations in annual temperatures and precipitation. These correlations did not show any significance, but showed a trend in which warmer mean winter air temperatures could be related to slightly slower growth rates, both for landlocked and anadromous Arctic charr. These trends suggest that fish in Blæsedalen could be resource limited.

Keywords: *Arctic charr, growth rates, otoliths, landlocked, anadromous*

Introduction

The Arctic charr (*Salvelinus alpinus*) is a species with a great potential for the colonization of cold and oligotrophic environments. It is a highly diverse species composed of a number of morphs that are ecologically and phenotypically distinct (Jonsson and Jonsson, 2001; Klemetsen, 2013). It has not only been called the “most variable vertebrate on Earth”, but it is also the only fish species found in the northernmost regions of the Arctic. It includes anadromous populations that migrate to the sea during summer and back to freshwater systems in the early autumn to reproduce and overwinter (Christiansen and Reist, 2013), and landlocked populations that remain within lacustrine systems year-long (Klemetsen, 2013).

Given the differences in life history traits, anadromous morphs will tend to grow bigger as resources are not a limiting factor during the growing season (summer), as they migrate to the sea to feed. However, freshwater systems in the Arctic can be very nutrient poor as these usually receive low nutrient concentrations with the surface runoff of melt water (Christoffersen et al. 2008). Thus, anadromous morphs of Arctic charr may face a great resource shortage when they are in their freshwater spawning sites. Additionally, the energy expenditure needed to reproduce makes that some individuals must skip one year in their reproductive cycle in order to replenish the energy storage lost during their previous reproduction (Dutil, 1986). Instead, non-migratory morphs may have been landlocked for a long time due to the formation of permanent barriers, where they have successfully adapted to the highly

variable and harsh seasonal conditions experienced in the low and high Arctic freshwater systems (Christiansen and Reist, 2013; Klemetsen, 2013). Landlocked populations are constrained to the Arctic's short growing season, after which food availability and temperatures drop, making activities such as reproduction difficult (Gullestad and Klemetsen, 1997*)*. Nevertheless, landlocked populations in Svalbard have also been seen to invest most of their energy storage in a spawning event, obligated to reproduce every two years (Gullestad and Klemetsen, 1997). In arctic ponds and lakes, Arctic charr is seen in two distinct morphs: invertebrate feeders called 'dwarfs' with an adult stage <15 cm fork length (FL), and piscivores called 'giants' or 'cannibals' with an adult stage typically >20 cm FL and can have a mass up to several kilograms (Florø-Larsen et al. 2016; Berg et al. 2010).

The study of landlocked populations gives interesting insights on potential impacts of climate change on Arctic charr, as these have no chance of migrating to ameliorate the changing conditions. Arctic charr, as ectothermic organisms, are especially sensitive to environmental temperatures as their body temperatures vary along with daily and seasonal thermal fluctuations. Any temperature related increase in the basal metabolic rate (BMR) is likely to be translated to an increase in respiration and thus, an increase in energy demands (Schulte, 2015). Furthermore, as the environment warms up, the growing season lengthens (Wilson and Nilsson 2009; Christoffersen et al. 2008), increasing the need for energy to maintain an active lifestyle. If these demands are covered, the expectations are to see faster development rates on ectotherms under higher temperature conditions (Kristensen et al., 2006). Warmer temperatures can also lead to an earlier maturation and generally to a decreased adult body size in ectotherms (Angilletta et al., 2004). Other studies confirm that female Arctic charr under constant temperature conditions above 5°C produce poorer quality eggs and temperatures above 11ºC inhibit ovulation (Gillet, 1991). Thus, small increases of temperature will probably disrupt the general performance of this species, which may be particularly at risk due to its cold-adapted singularity and the faster warming trends seen in Arctic environments.

Otolith analysis has been used in the last decades as a tool to study inter-annual growth rates of fish. Given the positive relationship between the increase in otolith and fish length, researchers are able to calculate the growth of the fish in the previous years by measuring the increments between the annual growth rings (Vigliola and Meekan, 2009). In this project we will apply the otolith method to study landlocked Arctic charr populations in Blæsedalen, a region of braided channels feeding the water flow of Røde Elv, in Disko Island, located off the West coast of Greenland. Previous studies have inferred a slower growth rate in Røde Elv's charr population (69° 16'N, 53° 29'W) as a consequence of a lower resource availability when compared to other locations during the same period of time (i.e. nearby lake Kangarssuk enclosed a charr population with increased growth rates during the same period of time; Kristensen et al., 2006). Later studies by Hedemand, Nielsen and Gai (2016) in Blæsedalen (69°16'44.9"N 53°28'42.3"W), showed that the growth rate could be mostly explained by winter temperatures and showed an increased overall fork length when compared to the ones studied by Mordhorst and Due (1990).

We aim to (i) assess body condition of anadromous Arctic charr and compare life history traits to landlocked populations. Through otolith analyses we further aim to (ii) analyze the inter-annual growth rates of landlocked Arctic charr populations from Røde Elv (in Blæsedalen) and compare our data to a previous study by Hedemand et al., (2016) as well as (iii) assess the influence of climatic variables such as temperature and precipitation on potential shifts in growth rates.

Given the life history traits of anadromous morphs, we expect to see a faster inter-annual growth rate when compared to

landlocked populations. Furthermore, we expect to see differences in presence of parasites, stomach content and degree of egg maturation. When analyzing inter-annual growth rates for landlocked Arctic charr, we expect to find a correlation with temperature fluctuations such as seen in previous studies (Hedemand et al., 2016; Kristensen et al., 2006). Given the nutrient poor environment that characterizes Blæsedalen, we expect to find small fish and with potentially slower growth rates during warmer years, as increased energy demands may not be covered by enough resources in such small landlocked ponds.

Methods and Materials

Study sites

This study was carried out in Blæsedalen where Røde Elv is located on the southernmost part of Disko Island, off the west coast of Greenland (Fig. 1). Røde Elv, without any major anthropogenic disturbances, consists of two different parts; a region of braided channels with pools and rivulets in a large glacial valley called Blæsedalen and the main river which runs out into the sea, around 500m east from to the Arctic Station, Disko Island. The main water input to the Røde Elv River comes from the snowmelt in the catchment. This is connected to several homo-thermic springs that maintain the bottom of pools and rivulets unfrozen all year long, making it possible for the Arctic charr to 'overwinter' (Mordhorst and Due, 1990). The charr population in the delta of Røde Elv has been isolated the last 6000 years due to land formations such as the waterfall located around 1.2 km upstream of the estuary which impedes charr migration (Mordhorst and Due, 1990; Pers. Conv., Kirsten S. Christoffersen, Juli 2020). The chosen sampling sites were based on previous literature and for practical reasons.

The sampling took place in two ponds in Blæsedalen (Fig. 1) between the 14th and 16th of July, 2020. These two ponds were chosen based on the fish abundance (visually determined) and the poor connectivity to the bigger river flow. A larger pond (referred to as Big Pond, 'BP' (69°16'45.4"N 53°28'43.9"W)) consisting of two major basins, a shallower and a deeper part, with a combined estimated water volume of 144845 L and an instant measured temperature of 5.9°C. The smaller pond (referred as Small Pond, 'SP' (69°16'42.5"N 53°28'50.3"W)) with an estimated water volume of 32329 L and with a water temperature of 7.4°C (Pond measurements: Table 1 ~ Appendix). For both ponds the bottom substrate was mostly stones and fine sand/mud. The municipality gave permission to fish in these locations (pers. Comm. Kirsten S. Christoffersen).

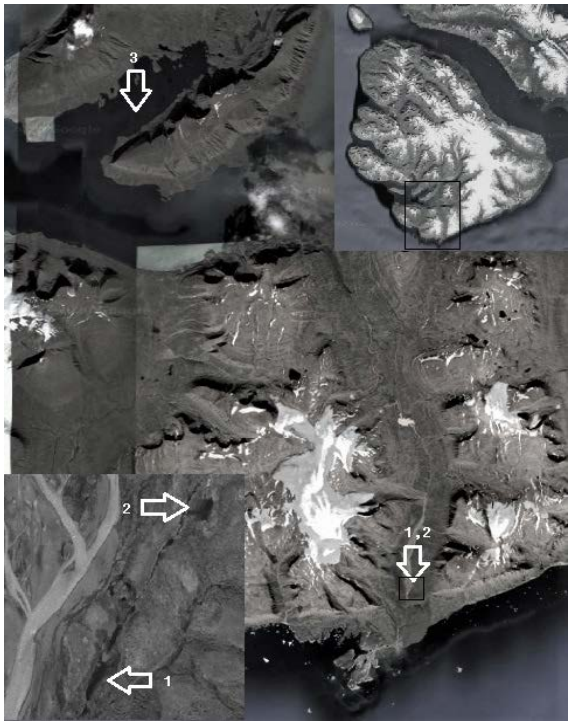


Figure 1. Map of the southern part of the island Disko with the 3 sample sites shown with white numbers. 1: Small pond and 2: Big pond in Blæsedalen, 3: the fjord Kuannersuit Sulluat. Insert right: Disko Island. Insert left: A closeup of the ponds in Blæsedalen. Extracted from Google Earth.

Five additional anadromous fish were caught in Kuannersuit Sulluat (referred as Sea, 'S' (69°30'25.5"N 53°48'05.7"W)), a fjord in the Disko Island, by the crew members of the research vessel 'Porsild' (Fig. 1).

Sampling

The collection of Arctic charr was done with a 10 mm gill net in the SP and 10 mm and 18

mm gill nets in the BP. The mesh sizes were chosen according to the observed fish sizes in the pond. The gill nets were placed in the middle of the pool, using stones as weights to keep the fishing nets tight to the bottom. While fishing, the fish were scared into the net by walking along the shore of the pond and towards the center. The caught fish was put in a marked plastic bag and transported to the laboratory at the Arctic Station.

The GPS location was taken with a smartphone application (GPS Status). Water temperature of the two ponds was measured with a hand-held thermometer. Depth, length and width measurements of the ponds were done through longitudinal and transverse transects using waders, a string and a ruler. The substrate of the ponds was also recorded.

Laboratory and computer analyses

Measurements and dissection

Caught fish went through a protocol of measurements and body analyses before otolith extraction. External body analyses included the description of the general skin coloration patterns, measurements of fork length (FL) -with a ruler to the nearest millimeter (mm)- and weight (W) -with a *Mettler PJ300* weight. The fishes were opened with a combination of a scalpel and scissors making a ventral longitudinal cut (anus to gills). Internal meat color was described, sex of the fish (Male~M; Female~F; Immature~I) was determined by examining the gonad morphology and the egg maturation of females was classified into 3 stages where 1 was the least developed and 3 highest developed, following the indications of Hedemand et al., 2016 (Fig. 2). Stomach content was analyzed and identified to the highest possible taxonomic level in order to understand the feeding preferences of the fish and general food availability in their environments. The presence/absence of parasites was determined through a thorough search of the gills, mouth, skin, superficial muscle tissue and intestines. All analyses were performed with a *Leica WILD M3C* stereomicroscope.

Otolith extraction

A scalpel was used to make a 30° incision just behind the eyes, down through the skull, towards the posterior part of the head following the instructions of Stevenson and Campana (1992). Tweezers and a *Leica WILD M3C* stereomicroscope were used for support to find the otoliths (Fig. 2). The otoliths were cleaned gently with the fingers and stored in small triangular paper bags.

Otolith measurements

Otoliths were placed in a petri dish with some ethanol 96º and on a black background to make the annual growth rings clearer. Both authors independently determined the age of the fishes visually by looking at one otolith at a time. In cases where determination was in disagreement the process was repeated until agreement. Pictures were obtained with the camera of a Samsung Galaxy A3 2017, fixed with a smartphone adapter mount to the *Leica WILD M3C* stereomicroscope set at 40X magnification. Images were measured using a free image processing program (*ImageJ 1.53a, Image J Inc. Java 9.0.1*). To transform the images to the decimal metric system, a picture of 5 mm paper was measured with the

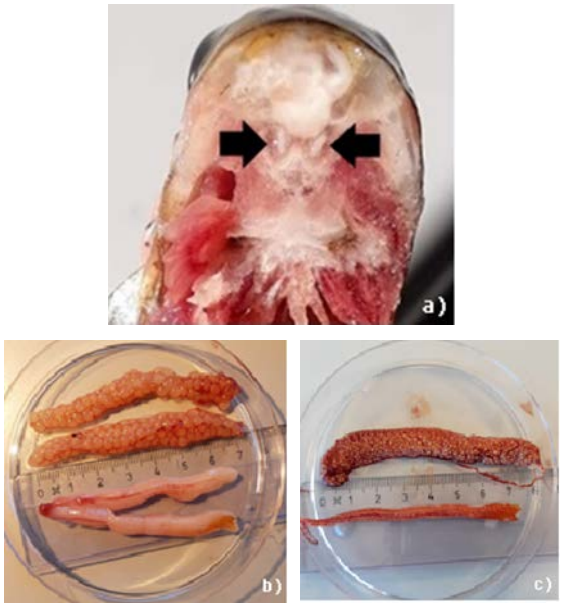


Figure 2. Images taken at the laboratory of a) a dissected head of Arctic charr, where the black arrows show the two otoliths, b) stage 3 ovaries and mature sperm sacs extracted from two landlocked individuals of Arctic charr (BP) and c) two ovary samples of stage 2 (top) and stage 1 (bottom) from anadromous Arctic charr (S).

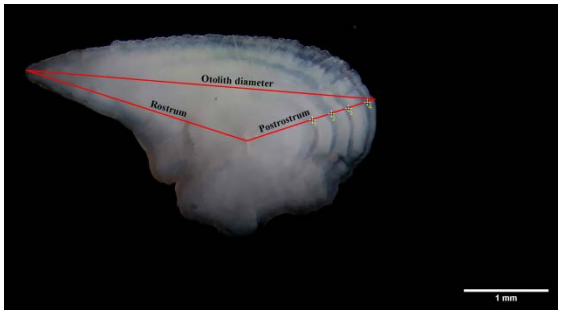


Figure 3. Arctic charr otolith extracted from anadromous fish number 3 (S3). Red lines show otolith diameter, rostrum, post-rostrum and yellow dots indicate 4 annual growth rings from the center to the otolith edge. Extracted from ImageJ 1.53a

program in order to get a pixel/mm ratio (mean of 417 pixels mm^{-1}), which was applied to all the measurements (Hedemand et al., 2016).

A central point was determined in the otoliths to obtain the size of the rostrum and post-rostrum (Fig. 3). The diameter was measured from the external edge of the rostrum to the one of the post-rostrum. Annuli measurements were done on the post-rostrum due to the fragility of the rostrum and definition of annual rings in the post-rostrum (Fig. 3). Age of the fish was determined by counting the dark annuli from the center to the edge of the post-rostrum. Inter-annual growth was obtained by measuring the distance from the central point to the internal region of the first dark band (first year ~ yolk dependent growth), from that point until the start of the next dark band (second year), and so forth until the last annuli. The most recent growth band (2020) was not considered as a year as it has not ended yet. The width of the dark and white bands was measured to get the growth rates of winter (September 1st - May 31st) and summer (June 1st - August 31st) periods, respectively (Vigliola and Meekan 2009).

Calculations

Fulton's Condition Index (CI) is a way to measure the overall health of a fish (more fat, muscles and gonads give a high CI value) and to infer food availability in the ecosystem. It follows a simple formula where W is weight and FL is Fork Length. In order for the CI to be comparable, similar FL ranges were selected. Thus, only the SP fish were compared to the smallest fish caught in the BP.

$$CI = (W\ (g)/FL(cm)^3) * 100$$

In order to estimate the fork lengths of the fish, the calculations suggested by Morita and Matsuishi (2011) were used. First a multiple regression analysis was done between the post-rostrum (O), Age at time of capture (T) and Fork Length (L) of the fish captured at the BP and S (separately), obtaining the following equation:

$$O = \alpha + \beta L + \gamma T$$

A, β and γ are constants used in the following formula, which enables us to estimate the fork length of the fish at a certain age (L_t). Additionally, the fork length at time of capture (L_T), the age at time of capture (T), the otolith length at time t (O_t), the post-rostrum length at age of capture (O_T)and the age at time t.

$$L_t = -\frac{\alpha}{\beta} + \left(L_T + \frac{\alpha}{\beta} + \frac{\gamma}{\beta}T\right)\frac{O_t}{O_T} - \frac{\gamma}{\beta}t$$

Mean growth rates per month were calculated:

$$GR = \frac{L_{t+1} - L_t}{12}$$

In order to compare the annual growth rates to temperature and precipitation seasonal variations, summer was defined as a 3 month period from June 1st to August 31st and the winter as a 9 month period from September 1st to May 31st. Temperature and precipitation data during the time period of 2015 to 2019 was obtained from the Greenland Ecosystem Monitoring Programme (GEM), provided by Asiaq – Greenland Survey, Nuuk, Greenland.

Statistics

A T-test was performed to compare the CI between SP and BP fish at 95% confidence level. A one-way ANOVA was performed to compare the FL and degree of egg maturation between fish caught in SP, BP and S. All

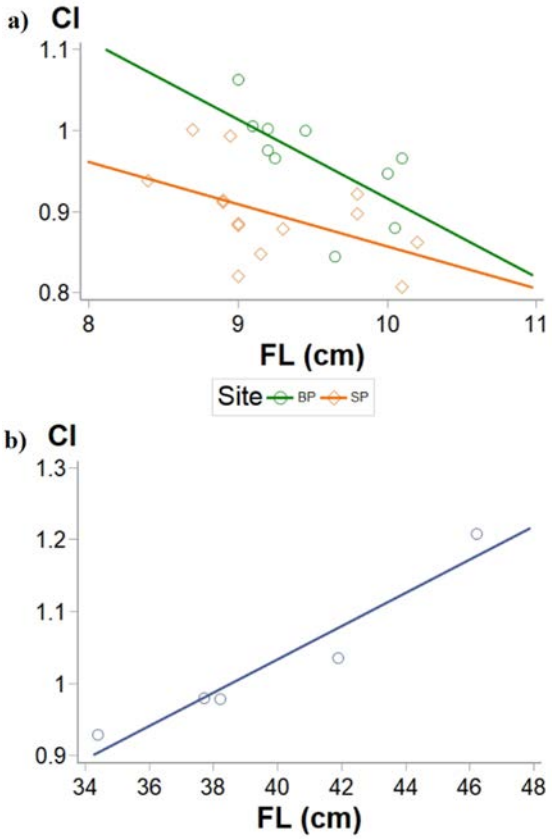


Figure 4. Regression of Condition Index (CI) over Fork Length (FL) of (a) fish ranging between 8.5 and 10.5 cm long, caught in SP (R^2 = 0.2502) and BP (R^2= 0.4165) and (b) fish caught in the Sea (R^2= 0.9233). Extracted from SAS software Version 7.15 HF8, Inc. 2017.

ANOVA analyses were further tested through a Tukey's test under a 95% confidence degree in SAS software Version 7.15 HF8, Inc. 2017.

Linear and multiple regression analysis were performed between post-rostrum (PostR), FL, age, temperature (summer, winter, annual) and summer precipitation. One-way ANOVA was performed to compare annual growth rates of fish between 2016 and 2019, for individuals caught in BP and S.

Results

Measurements and dissection

In July 2020, a total of 35 fish were analyzed; 14, 16, and 5 individuals of Arctic charr were collected from the Small Pond (SP), Big Pond (BP), and Kuannersuit Sulluat (S), respectively. The fork length (FL) of the fish had mean values +/- SD of 92.29 +/- 5.39 mm, 117 +/- 30.32 mm and 396.8 +/- 45.12 mm for SP, BP and S, respectively (SP < BP < S; ANOVA p-value < 0.05; Tukey's p-value <0.05). The weight (W) had mean values +/- SD of 7.08 +/- 1.11 g, 17.33 +/- 13.01 g and 680.26 +/- 316.82 g for SP, BP and S, respectively. SP and BP had no significantly different weights, but were significantly smaller than fish caught in S (ANOVA p-value < 0.05; Tukey's p-value <0.05). Fish ages had mean values +/- SD of 1.71 +/- 0,61 years, 3.81 +/- 1.11 years and 5 +/- 0.71 years for SP, BP and S, respectively (SP < BP < S; ANOVA p-value < 0.05; Tukey's p-value <0.05). Out of 35 individuals, 18 were females, 13 males, 2 immatures and 2 were unidentified, probably immatures as well.

The fishes caught in Blæsedalen were relatively small, rather dark-colored with 10-15 stripes on the dorsal sides of the fish and white ventral skin colour; muscle tissue had a white-transparent colouration. BP fish caught with the 18 mm gill net had a slightly different colouration, presenting small orange dots and light orange ventral skin colour. It wasn't possible to determine the colour of the fish from Kuannersuit due to loss of colour after death, but muscle tissue was light-pink. The stomach content of SP fish was mostly dominated by *Diptera,* mostly *Chironomidae* larvae. BP stomach content had a diet based on *Diptera,* but had a greater diversity: 1 *Araneae* (spider) and 5 *Trichoptera* individuals were found. The stomach content of anadromous fish was mostly unidentifiable,

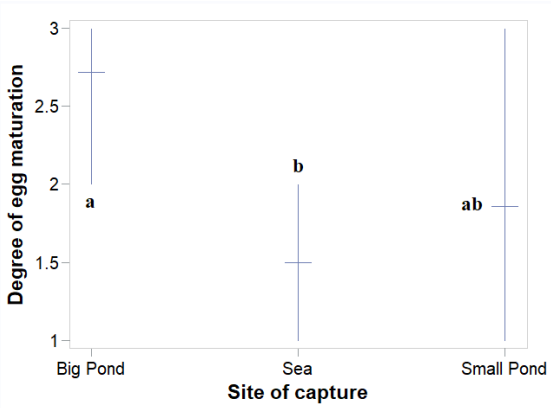


Figure 5 Box-plot showing the degree of egg maturation in females caught in Big Pond, Sea and Small Pond. Big pond females are significantly more mature than females caught in the sea (One-way ANOVA p < 0.05; α = 0.05). Extracted from SAS software Version 7.15 HF8, Inc. 2017.

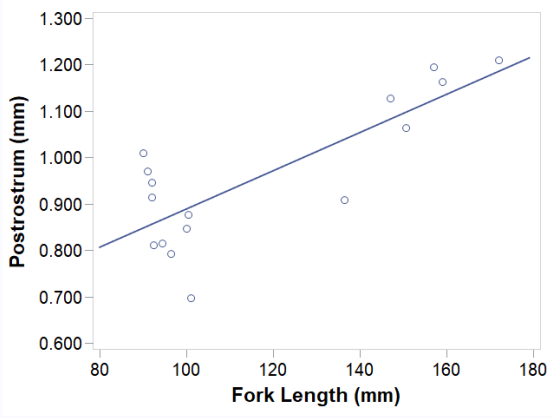


Figure 6 Linear regression of Postrostrum (mm) against Fork Length (mm) for Big Pond fish (R^2= 0.64; p-value < 0.001; α = 0.05). Extracted from SAS software Version 7.15 HF8, Inc. 2017.

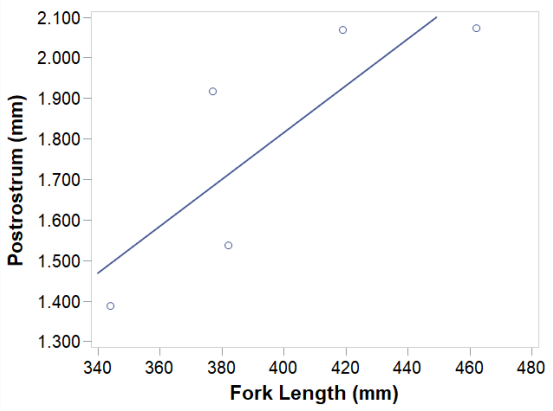


Figure 7. Linear regression of Post-rostrum (mm) against Fork Length (mm) for Kuannersuit Sulluat fish (R^2= 0.68; p-value > 0.05; α = 0.05). Extracted from SAS software Version 7.15 HF8, Inc. 2017.

except for 2 small fish and a shrimp-like crustacean. Few parasites were found; 2 of 14 SP fish presented one *Cestoda* individual in their intestines, BP fish had no parasites and 2 of the 5 Sea fish presented one *Nematoda* individual.

Condition Index

When comparing similar sized fish of FL between 8.0 and 10.5 cm, selected due to their overlapping FL range, the mean values +/- SD of Fulton's Condition Index (CI) were 0.90 +/- 0.056 and 0.97 +/- 0.064 for landlocked charr in SP and BP, respectively. BP had fish of significantly higher CI than fish in SP (T-test p-value < 0.05; Fig. 4a). Fish caught in the sea had a CI between 0.93 and 1.21 with a significantly positive relationship (R^2= 0.9233; p < 0.01), where bigger fish seem to have a better body condition, while pond fish have a negative relationship, significantly in BP fish (p < 0.5).

Degree of egg maturation

The degree of egg maturation in females caught in the BP (2.7 +/- 0.49) compared to those in S (1.5 +/- 0.58) were significantly higher (One-way ANOVA p-value < 0.5; Tukey's test p-value < 0.05). Females in the SP (1.86 +/- 0.9) showed great variation in their

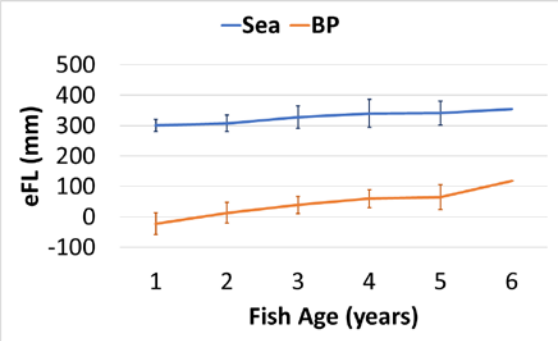


Figure 8 The estimated FL (mm) ± SD, of fish collected in the S (blue) and BP (orange), plotted against fish age (years). Extracted from Microsoft Excel

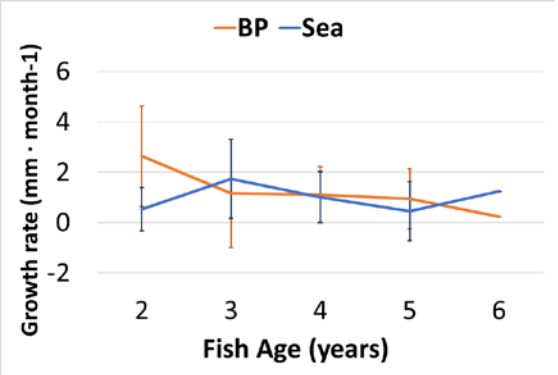


Figure 9. Mean age specific growth rate (mm month^{-1}) ± SD from the S (blue) and BP (orange) fish of ages 2-6. Extracted from Microsoft Excel.

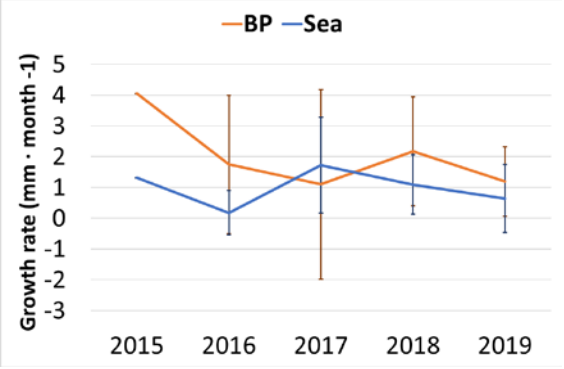


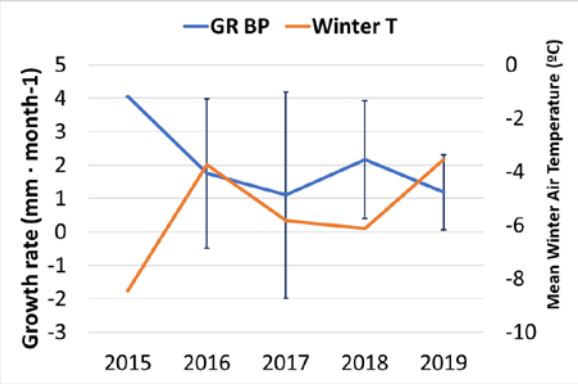
Figure 10. Mean annual growth rate (mm month^{-1}) ± SD of S (blue) and BP (orange) fish plotted against time (2015 to 2019). Extracted from Microsoft Excel.

maturity, resulting in an uncertain degree of maturation (Fig. 5).

Growth rate analysis

Small Pond fish were not used in the growth rate analysis because the young age (1.7 year) of the individuals did not allow to obtain a growth (See summary of fish analyses: Table 2 ~ Appendix). Linear regression between PostR and FL were performed for BP fish and S fish. A high significantly positive relationship was obtained for BP fish (Fig. 6); a multiple regression analysis between PostR, FL and Age was highly significant as well and constants were used for the latter calculations (R^2 = 0.88; p-value < 0.01; α = 0.05). For S fish, a positive but not significant relationship - probably due to few replicates (n=5) - was found between PostR and FL (Fig. 7), as well as in the multiple regression analysis (R^2 = 0.82; p-value > 0.05; α = 0.05). The constants were used for the latter calculations as well.

Back-calculations were used to estimate the fork lengths of all fish caught in BP and S. BP fish show negative FL in their first year of life, which could be due to the accumulated error during the measurements and the low replicates used. However, the increasing trend of the FL with age is sensible enough to calculate the growth rates of the fish in BP (Fig. 8). Instead, the estimated fork length for the sea fish shows a very slow yearly increase, probably not accurate to real fish growth (Fig. 8). Still, the growth rates of the migratory fish have been calculated in order to visualize a growth rate pattern. Mean age specific growth rate shows a decreasing trend in BP and S fish (non-significant; Fig. 9). The 6th year of age is not used in the statistical



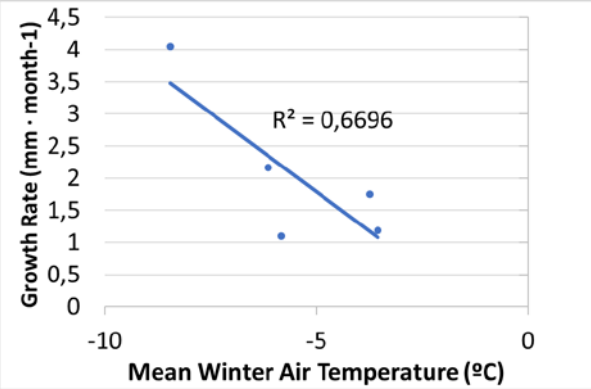
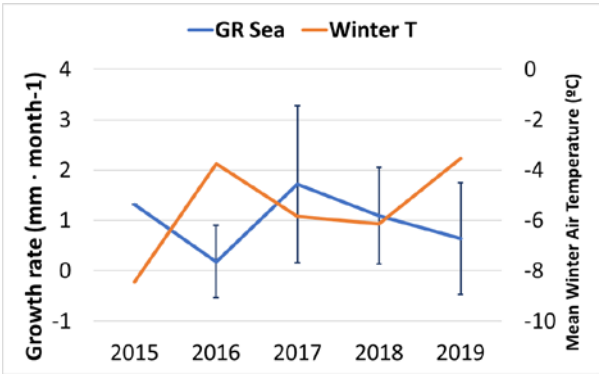


Figure 11 (left). Mean annual growth rate (mm month^{-1}) ± SD in fish from BP and mean winter air temperature (°C) plotted against time from 2015-2019. Blue: GR, Orange: Winter Temperature.
Figure 12 (right). Mean annual growth rate (mm month^{-1}) ± SD in BP fish plotted against mean winter air temperature (°C) (R^2= 0.67; p-value > 0.05; α = 0.05). Extracted from Microsoft Excel



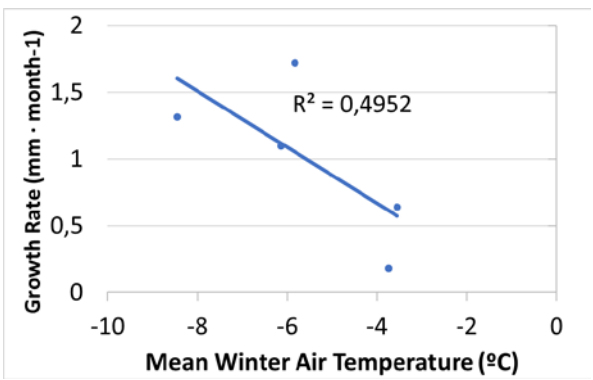


Figure 13 (left). Mean annual growth rate (mm month^{-1}) ± SD in fish from S and mean winter air temperature (°C) plotted against time from 2015-2019. Blue: GR, Orange: Winter Temperature.
Figure 14 (right). Mean annual growth rate (mm month^{-1}) ± SD in Sea fish plotted against mean winter air temperature (°C) (R^2= 0.49; p-value > 0.05; α = 0.05). Extracted from Microsoft Excel.

analyses, as both (BP and S) are based on a single individual.

Mean annual growth rates are not significantly different between years in both BP and S fish (one-way ANOVA p-value > 0.05; Fig. 10). A regression analysis of growth rates against mean annual, winter and summer air temperatures and summer precipitation was made for both BP and S, all of which resulted in non-significant relationships. However, winter temperatures are more positively correlated to the growth rates in BP (Fig. 11, 12) and S (Fig. 13, 14) than annual and summer temperatures and summer precipitation patterns (*Figures 15-26 in appendix*). Both BP and S fish follow a trend where they have slightly higher GR during colder winters, while lower GR seem to be related to higher temperatures.

Discussion

The obtained results show a gradient in size from smaller fish caught in the Small Pond (SP) to bigger fish in the Big Pond (BP), to even larger fish caught in Kuannersuit Sulluat (S). The condition index (CI) was significantly higher in BP fish than in SP fish (Fig. 4a). The bigger CI seen in BP fish suggests a more isometric growth of the fish, probably due to a higher resource availability in the BP when compared to the SP. This can be supported by the general *species–area model* where a larger surface area can hold more species (in terms of richness and abundance) as suggested by Hill et al (1994). In this case, BP is around 4.5 times bigger in water volume than SP, which is related to a usually bigger fish size (Riget et al., 2000). Moreover, the BP probably includes a higher number of niches available, also supported by the higher diversity of food items found in the stomach dissection of the BP fish (Riget et al., 2000). Furthermore, a smaller habitat in SP, which means less space per fish, may increase intraspecific competition for limited food resources, potentially leading to a reduction in fitness (Cuenco et al. 1985), also reflected in the CI value. Another reason to explain the low CI in SP fish is the presence of parasites (Barker et al., 2002), not found in BP fish. However, whether the presence of parasites or the nutrient poor habitat is the factor lowering the CI in SP fish cannot be inferred. The presence of parasites can indicate a weak immune system, result of a nutrient poor diet (Karvonen et al., 2004).

We do not compare the CI index statistically between landlocked and migrating fish populations due to a very different size range. However, it is possible to see that migrating fish CI increases with increasing FL (Fig. 4b), following a normal weight-length relationship, while landlocked fish CI decreases with increasing FL (Fig. 4a), meaning that fish become slender as they grow in length indicating food and space limitation (Cuenco et al. 1985; Riget et al., 2000).

Analyses s of the stomach content in the present study showed that adult *Diptera* and *Chironomidae* larvae appeared in both ponds, while *Trichoptera* and *Araneae* only appeared in the stomach contents of the fish from BP. Bigger fish were found in BP, which probably switch to bigger food items, increasing the variety seen in our stomach analyses (Christoffersen, 2006). D*iptera* and *Chironomidae* larvae were dominating the diet composition of Arctic charr, consistent with results in Hedemand et al. (2016) and Mordhorst and Due (1990).

The degree of egg maturation was significantly higher in the landlocked population from BP in Blæsedalen compared to the anadromous population from the sea, while SP fish had great variation in their maturity level (Fig. 5). When populations of Arctic charr mature sexually at a size of 11–13 cm (or smaller) it is defined as ‘dwarf’ morph (Christoffersen et al., 2008b). While it is not known which reproductive strategy follows each sample of Arctic charr, it is possible to infer that most BP fish were in their reproductive year. Instead, fish caught in Kuannersuit Sulluat showed a lower degree of egg maturation, probably as they are on their way to the breeding grounds (in freshwater). A reason why the landlocked population had more developed eggs could be due to the shorter growing season they are constraint to.

Landlocked fish must spawn before winter freezing events, while migratory fish have a little more time before the river freezes (Berg et al., 2010). An alternative interpretation to these results implies a different resource investment into reproduction. Large migratory fish may invest smaller amounts of yolk into each egg to increase the number of offspring. Instead, smaller landlocked fish may invest more energy storage into fewer eggs to secure offspring survival in a more nutrient poor environment (Winemiller and Rose 1993).

Regression analysis between otolith post-rostrum (PostR) radius (mm) and fork length (FL) showed a significant positive relationship in BP fish and a non-significant positive relationship in SP and S fish. GR of fish in Blæsedalen is based on BP fish; due to the young age of SP fish, these were removed from our analyses. Furthermore, fishes tend to have a greater growth rate during the first years, increasing the variation in data for the years of 2018 and 2019. GR for sea fish are also calculated and used, although multiple regression analyses show non-significant results (due to few replicas ~ n=5). Statistical analysis of growth rates plotted against mean winter, summer, annual air temperature and summer precipitation did not show any significant relationship, but there is a trend in BP fish (Fig. 11-12) and S fish (Fig. 13-14) where growth rates increase during colder years and decrease during warmer years. Hedemand et al. (2016) found that changes in inter-annual growth rates could be mostly be explained by fluctuations in winter temperatures, which could partly support our findings. However, they show that growth rates increase during warmer years, while our results, although not significant, show the contrary. In Arctic environments, warmer years may be translated into longer growing seasons, increasing the time period of activity an individual has to perform (Christoffersen, 2006). However, whether the growth rate accelerates or decelerates depends on the resource availability of the environment fish are found in (Kristensen et al., 2006). Thus, it could be inferred that during the years of 2016 and 2019, when temperatures were warmer, fish have had an increased activity rate, but, instead of having an increase in growth rate as seen in previous studies (Hedemand et al., 2016), the potentially nutrient poor environment where they were found has led them to a reduction of energy investment in their somatic growth during warmer years. Both ponds in Blæsedalen are probably food limited during the summer, due to the low nutrient concentrations that come from surface runoff of melt water. Thus, growth rates of the Arctic charr from Blæsedalen could be limited due to other factors than temperature. The results indicate that biotic and abiotic factors could determine growth in the summer season and are as well subjected to winter fluctuations.

Furthermore, migratory Arctic charr spend the winter at their breeding grounds and migrate to the sea to feed during summer, returning later in the season to reproduce (Christoffersen, 2006). Thus, S fish are probably also subjected to similar winter temperature fluctuations as fish caught in Blæsedalen. However, their overwintering grounds are not known and therefore, their resource availability is also unknown. It is possible that, given the similarity to BP fish in their growth rate patterns in relation to the winter temperature fluctuations among the years 2015-2019, they face similar limitations such as a nutrient poor environment that limits somatic growth during warmer years (when growth rate could be accelerated due to a potentially higher BMR).

Further suggestions

Given the limited time to perform this study, some potential sources of errors must be mentioned.

First of all, the back-calculations resulted in negative estimated fork lengths for BP fish in their first years of life. An obviously problematic result, that may be caused by the low number of data points used for the multiple regression analysis (n=16). Furthermore, similar problems were faced with S fish which, with an even smaller sample size (n=5), showed no significant relationship

in a PostR-FL regression analysis. This result may have accumulated slight numerical errors into the back-calculations that showed a very slow growth rate, much smaller than BP fish, also contrary to what would be expected.

Secondly, the ontogenic related growth was not subtracted before analyzing the potential effect of environmental factors on the growth. This step was not performed due to lack of time and expertise.

Finally, even though some inferences have been made in order to compare our results to previous studies, the comparison is not possible due to the incomplete results in our study. The obtained numerical values for eFL and growth rates cannot be used. However, the patterns and trends have been used to try and elucidate some potential relationships to environmental data.

Conclusion

Analysis of the CI of Arctic charr collected in BP and SP, suggests that the BP morphometry and habitats leads to fish with a potentially greater fitness. Additionally, differences in degree of egg maturation reflect different reproductive strategies and thus, different life history traits, between migratory and non-migratory Arctic charr morphs.

Regression analyses between growth rates and climatic variables (mean annual, summer and winter air temperatures and summer precipitation) show no significant relationships. However, a trend could be inferred where warmer winter temperatures are related to lower growth rates. Although, contrary to previous studies performed in a closeby location, this could reflect that the selected pond of study was resource poor compared to previous studies (Hedemand et al., 2016).

The arctic environment is suffering from exacerbated temperature increases compared to the rest of the world's ecosystems. Landlocked Arctic charr, as ectotherms living in small ponds with few resources may be especially at risk. Warmer temperatures and a longer growing season in these ponds may lead to an increased general activity, but if energy demands cannot be covered, we could expect a general decrease in their growth rates and their fitness as well.

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Appendix

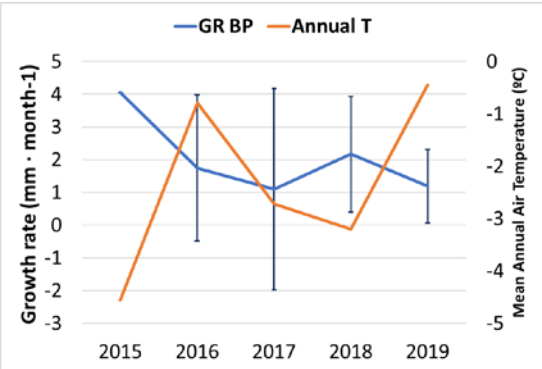
Table1. Mean values of pond measurements

	Depth (cm)	*Lenght (cm)*	*Width (cm)*	*Water temp. (°C)*	*Water Vol. (L)*
Small pond	26.06	2280	544	7.4	32328.73
Big pond (shallow part)	17.55	660	420	5.9	4864.86
Big pond (deep part)	58.84	1300	1830	5.9	139980.36

Table 2. Overview of data of observations, measurements and calculations from the 35 Arctic charr collected in Small pond (SP) and Big pond (BP) in Blæsedalen and Kuannersuit Sulluat (S) in the present study. Abbreviations explanations – MS (mm): Mesh size, FL (mm): Fork Length, W (g): Weight, CI: Condition index, Skin colour (D) & (V): Dorsal and ventral, P (P/A): Parasites (present/absent), P loc.: Parasite location, O Diam. (mm): Otolith diameter, Rost. (mm): Rostrum, PostR (mm): post-rostrum; Year 1 to 6 - S & W (mm): Summer and winter growth rings measured from the center to the otolith edge.

Fish(n)	MS (mm)	FL (mm)	W (g)	CI	Skin Colour (D) & (V)	Meat Colour	P (P/A)	P loc.	Sex	Degree of egg maturation (1,2,3)	Stomach content	O diam. (mm)	Rost. (mm)	PostR. (mm)	Year1 S(mm)	Year1 W(mm)	Year2 S(mm)	Year2 W(mm)	Year3 S(mm)	Year3 W(mm)	Year4 S(mm)	Year4 W(mm)	Year5 S(mm)	Year5 W(mm)	Year6 S(mm)	Year6 W(mm)	Age (capture)
SP1	10	89	6,43	0,91	D: G/O V: W	W	A	-	I	-	Diptera A	1,903	1,133	0,802	0,362	0,41	0,5	0,543									2
SP2	10	90	5,98	0,82	D: G/O V: W	W	A	-	I	-	Diptera A&L	1,383	0,74	0,655	0,316	0,382											1
SP3	10	87	6,59	1,00	D: G/O V: W	W	A	-	F	3	Diptera L	1,512	0,868	0,667	0,311	0,341	0,435	0,462									2
SP4	10	91,5	6,50	0,85	D: G/O V: W	W	A		M	-	Diptera A&L	1,779	1,015	0,798	0,335	0,388	0,526	0,575									2
SP5	10	101	8,32	0,81	D: G/O V: W	W	Cestoda	Int.	M	-	Diptera A&L	1,665	0,933	0,755	0,305	0,365											1
SP6	10	89,5	7,12	0,99	D: G/O V: W	W	A	-	F	1	Diptera A&L	1,659	0,867	0,819	0,362	0,418	0,563	0,6									2
SP7	10	93	7,07	0,88	D: G/O V: W	W	A	-	M	-	Diptera A&L	1,722	1,087	0,683	0,245	0,283	0,363	0,406									2
SP8	10	102	9,16	0,86	D: G/O V: W	W	A	-	F	2	Diptera A&L	1,948	1,177	0,794	0,418	0,458	0,569	0,586									2
SP9	10	98	8,45	0,90	D: G/O V: W	W	A	-	F	2	Diptera A&L	1,849	1,12	0,816	0,236	0,268	0,587	0,608									2
SP10	10	84	5,56	0,94	D: G/O V: W	W	A	-	I	-	Diptera A&L	1,545	0,828	0,739	0,308	0,348											1
SP11	10	98	8,67	0,92	D: G/O V: W	W	A	-	M	-	Diptera A&L	1,753	1,007	0,779	0,365	0,396	0,506	0,534									2
SP12	10	90	6,44	0,88	D: G/O V: W	W	A	-	F	1	Diptera A&L	1,49	0,844	0,702	0,308	0,424											1
SP13	10	89	6,44	0,91	D: G/O V: W	W	A	-	F	1	Diptera A&L	1,298	0,814	0,672	0,414	0,43											1
SP14	10	90	6,46	0,89	D: G/O V: W	W	P Cestoda	Int.	F	3	Diptera A&L	1,647	0,916	0,768	0,308	0,341	0,492	0,536	0,609	0,636							3
BP1	18	172	47,08	0,93	D: G/O w. dots V: w	W	A	-	F	3	Tricoptera L, Dip. A&L	2,623	1,496	1,21	0,34	0,387	0,531	0,566	0,724	0,795	0,875	0,92	1,002	1,034	1,087	1,112	6
BP2	18	157	31,68	0,82	D: G/O w. dots V: w	W	A	-	F	3	Diptera A&L	-	-	1,194	0,272	0,311	0,45	0,493	0,618	0,656	0,788	0,817	0,951	0,992			5
BP3	18	147	30,41	0,96	D: G/O w. dots V: w	W	A	-	M	-	Diptera L	2,617	1,53	1,128	0,499	0,564	0,74	0,767	0,873	0,899	0,964	0,986					4
BP4	18	159	34,57	0,86	D: G/O w. dots V: w	W	A	-	F	2	Diptera A	2,635	1,546	1,163	0,514	0,549	0,68	0,733	0,794	0,816	0,901	0,947	1,003	1,039			5
BP5	18	150,5	28,79	0,84	D: G/O w. dots V: w	W	A	-	F	3	Diptera L	2,644	1,627	1,063	0,517	0,568	0,691	0,743	0,809	0,828	0,889	0,928					4
BP6	10	136,5	22,06	0,87	D: G/O V: W	W	A	-	M	-	Diptera L	2,217	1,347	0,909	0,292	0,371	0,537	0,6	0,703	0,743							3
BP7	10	101	9,96	0,97	D: G/O V: W	W	A	-	I	-	Tricoptera L, Dip. A&L	-	-	0,698	0,339	0,363	0,469	0,502									2
BP8	10	100	9,48	0,95	D: G/O V: W	W	A	-	M	-	Diptera L, Araneae	1,934	1,172	0,848	0,488	0,536	0,645	0,671	0,727	0,763							3
BP9	10	100,5	8,93	0,88	D: G/O V: W	W	A	-	M	-	Diptera A&L	1,849	1,042	0,878	0,334	0,365	0,497	0,543	0,648	0,676							3
BP10	10	92	7,81	1,00	D: G/O V: W	W	A	-	F	3	Diptera A&L	2,333	1,446	0,914	0,542	0,568	0,617	0,641	0,791	0,82							3
BP11	10	92,5	7,64	0,97	D: G/O V: W	W	A	-	M	-	Diptera L	1,91	1,139	0,811	0,303	0,341	0,471	0,503	0,606	0,643							3
BP12	10	96,5	7,59	0,84	D: G/O V: W	W	A	-	M	-	Diptera L	1,826	1,05	0,794	0,338	0,404	0,546	0,585	0,662	0,694							3
BP13	10	90	7,75	1,06	D: G/O V: W	W	A	-	F	2	Trichoptera L, Dip. L	2,175	1,179	1,009	0,497	0,538	0,618	0,67	0,74	0,769	0,882	0,913					4
BP14	10	94,5	8,44	1,00	D: G/O V: W	W	A	-	M	-	Trichoptera L, Dip. L	1,831	1,063	0,816	0,311	0,366	0,515	0,552	0,63	0,658							3
BP15	10	91	7,58	1,01	D: G/O V: W	W	A	-	F	3	Trichoptera L, Dip. L	2,187	1,264	0,971	0,284	0,3	0,436	0,488	0,621	0,651	0,722	0,745	0,792	0,821			5
BP16	10	92	7,60	0,98	D: G/O V: W	W	A	-	M	-	Trichoptera L, Dip. L	2,341	1,421	0,947	0,339	0,363	0,482	0,515	0,642	0,669	0,745	0,77	0,819	0,846			5
S1	-	377	525,06	0,98	-	W/P	A	-	F	2	Not visible	4,489	2,623	1,917	0,761	0,87	1,022	1,096	1,315	1,378	1,535	1,612	1,723	1,759			5
S2	-	419	761,30	1,03	-	W/P	A	-	F	2	Not visible	4,824	2,837	2,07	0,592	0,673	0,843	0,925	1,045	1,093	1,249	1,386	1,501	1,586	1,747	1,797	6
S3	-	382	545,21	0,98	-	W/P	A	-	F	1	Not visible	4,106	2,73	1,538	0,676	0,722	0,913	0,988	1,154	1,194	1,415	1,467					4
S4	-	462	1191,50	1,21	-	P	Nem.	Stom.	M	-	2 Fish	5,093	3,176	2,073	0,692	0,733	0,861	1,047	1,291	1,436	1,609	1,667	1,724	1,8			5
S5	-	344	378,22	0,93	-	W/P	Nem.	Muscle	F	1	Crustacean	3,295	2,009	1,389	0,42	0,455	0,538	0,592	0,755	0,79	0,918	1,014	1,158	1,19			5

Big Pond growth rates against climatic variables.



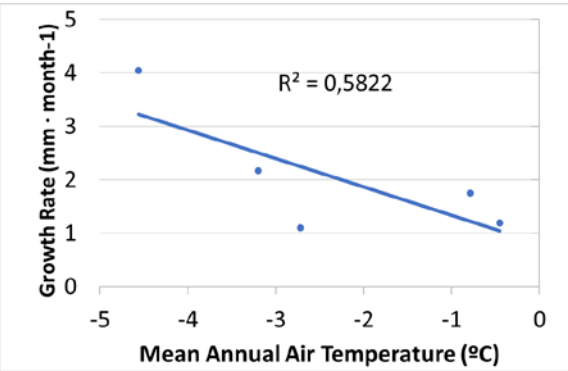
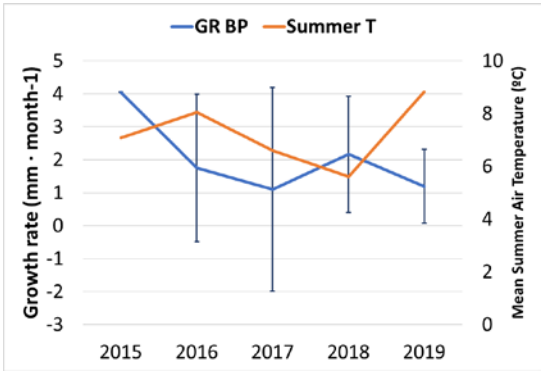


Figure 15 (left). Mean annual growth rate (mm month^{-1}) ± SD in fish from BP and mean annual air temperature (°C) plotted against time from 2015-2019. Blue: GR, Orange: Annual Temperature.
Figure 16 (right). Mean annual growth rate (mm month^{-1}) ± SD in BP fish plotted against mean annual air temperature (°C) (R^2= 0.58; p-value > 0.05; α = 0.05). Extracted from Microsoft Excel.



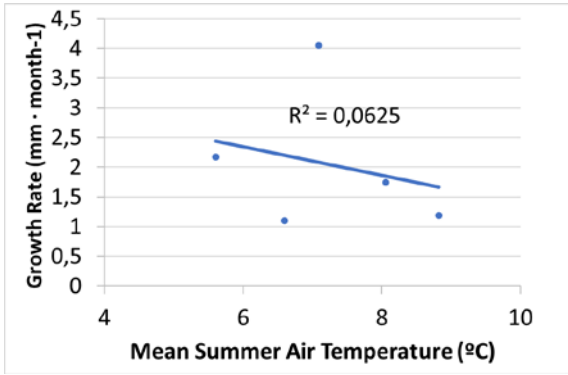
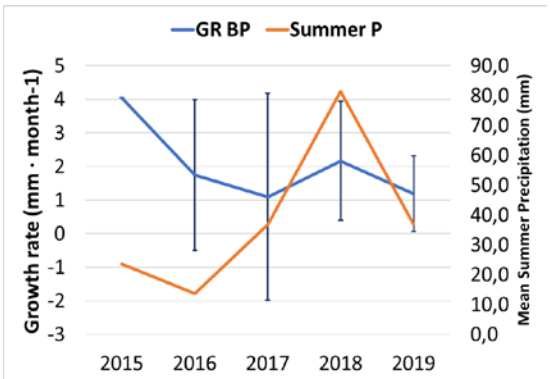


Figure 17 (left). Mean annual growth rate (mm month^{-1}) ± SD in fish from BP and mean summer air temperature (°C) plotted against time from 2015-2019. Blue: GR, Orange: Summer Temperature.
Figure 18 (right). Mean annual growth rate (mm month^{-1}) ± SD in BP fish plotted against mean summer air temperature (°C) (R^2= 0.06; p-value > 0.05; α = 0.05). Extracted from Microsoft Excel.



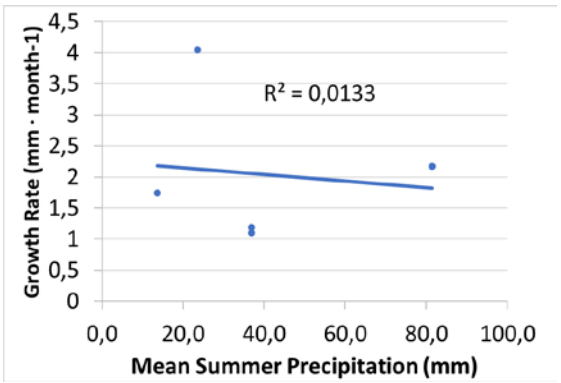
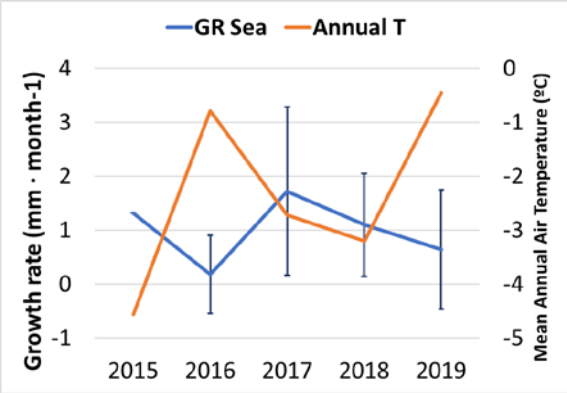


Figure 19 (left). Mean annual growth rate (mm month^{-1}) ± SD in fish from BP and mean summer precipitation (mm) plotted against time from 2015-2019. Blue: GR, Orange: Summer precipitation.
Figure 20 (right). Mean annual growth rate (mm month^{-1}) ± SD in BP fish plotted against mean summer precipitation (mm) (R^2= 0.01; p-value > 0.05; α = 0.05). Extracted from Microsoft Excel.

Sea growth rates against climatic variables.



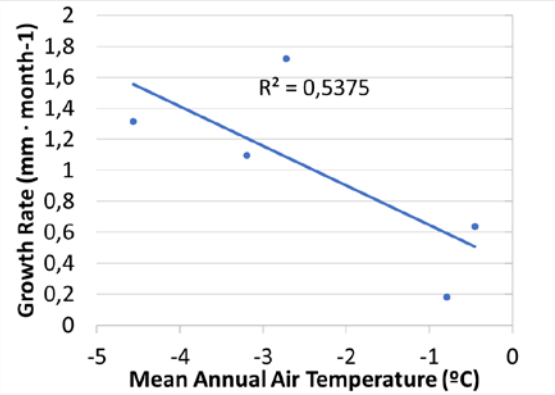
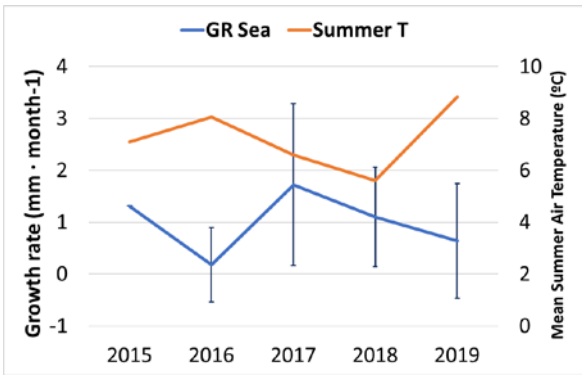


Figure 21 (left). Mean annual growth rate (mm month^{-1}) ± SD in fish from S and mean annual air temperature (°C) plotted against time from 2015-2019. Blue: GR, Orange: Annual Temperature.
Figure 22 (right). Mean annual growth rate (mm month^{-1}) ± SD in Sea fish plotted against mean annual air temperature (°C) (R^2= 0.53; p-value > 0.05; α = 0.05). Extracted from Microsoft Excel.



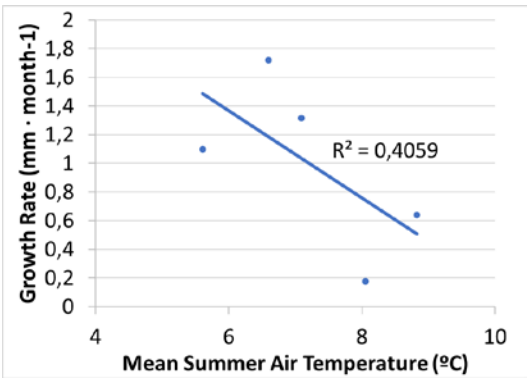
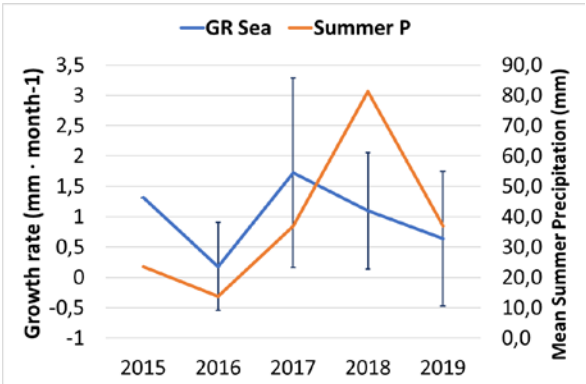


Figure 23 (left). Mean annual growth rate (mm month^{-1}) ± SD in fish from S and mean summer air temperature (°C) plotted against time from 2015-2019. Blue: GR, Orange: Summer Temperature.
Figure 24 (right). Mean annual growth rate (mm month^{-1}) ± SD in Sea fish plotted against mean summer air temperature (°C) (R^2= 0.40; p-value > 0.05; α = 0.05). Extracted from Microsoft Excel.



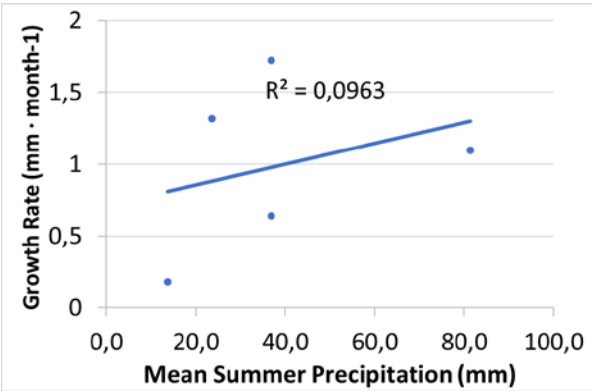


Figure 25 (left). Mean annual growth rate (mm month^{-1}) ± SD in fish from S and mean summer precipitation (mm) plotted against time from 2015-2019. Blue: GR, Orange: Summer Precipitation.
Figure 26 (right). Mean annual growth rate (mm month^{-1}) ± SD in Sea fish plotted against mean summer precipitation (mm) (R^2= 0.09; p-value > 0.05; α = 0.05). Extracted from Microsoft Excel

Heath plant community and invertebrate herbivory responses to climate change in the Arctic

Salix glauca **Blæsedalen, Disko (Photo: Cristina Fernández Garcia)**

Heath plant community and invertebrate herbivory responses to climate change in the Arctic

Cristina Fernández García, Anna Marie Stevnsvig & Sofie Kirstine Westphal Sørensen

Abstract

As the climate is changing alterations in atmospheric summer temperatures and precipitation are expected to have a great influence on the Arctic plant communities and damaging effects from invertebrate activities.This study combines investigation of Arctic plant heath communities and leaf area damage due to invertebrate activities response to altering climatic conditions. The study was carried out at the CENPERM-KU snow fences in Blæsedalen, Disko, West Greenland. The data has been collected from experiments focusing on increased summer temperatures and increased snow cover leading to increased soil temperatures during winter (winter warming) and shorter growing season, as the snow will melt away later and add soil moisture in the spring. The treatments do indeed influence the Arctic heath plant communities. Deciduous shrubs and *B. nana decreased while* gramnoids show a trend of significant increase between the control and winter plots. Summer and winter warming increased NDVI significantly, yet the summer and winter warming interaction did not. The *Salix spp.* leaves showed a tendency for decrease in total leaf area damage with summer warming. Probably due to limited data, *B. nana* did not show any response. In summer warming, flavonoids and anthocyanins in the leaves of *Salix ssp.* decreased significantly. Our results show an indication of better growing conditions in the plots, but more research should be done on the subject as well as on the damaging invertebrate activity.

Keywords: *Arctic plant communities, heath, climate change, Salix glauca, Salix arctophila, Betula nana*

Introduction

Global change is leading to an increase of the atmospheric temperatures and causing rapid changes to the climate with alterations of precipitation and temperature. These changes have an impact on ecosystems around the globe and the Arctic is experiencing the largest effects of climate change worldwide (Mosbacher et al. 2013). As primary producers are directly affected by invertebrate herbivores, which are often the first organisms responding to climatic changes (Barrio et al. 2016), it is the purpose of this study to investigate the impact of climate change on Arctic plant communities. It is expected that climate change will lead to an increase of winter precipitation (hereafter referred to as winter warming or WW) as well as an increase of the atmospheric temperatures (hereafter referred to as summer warming or SW). The winter warming will likely result in higher snow depositions in the Arctic and later snow melt, narrowing the biological window. This means that the snow free period in which flora can photosynthesize, grow and reproduce gets shortened, which can provoke mismatches and shifts in the flora-fauna interactions (Barrio et al. 2016; Blok et al. 2016).

The summer warming will likely result in a higher degree of arthropod herbivory, since invertebrates generally respond positively to warming. For the Arctic invertebrates this may not apply, since species such as the moth *Gynaephora groenlandica* tend to be well adapted to a cold environment, and it may not perform well under warmer conditions (Barrio et al. 2016). *Salix* species in the Arctic are susceptible to gall mites. Not much is known about the effects of increased snow cover (WW) and temperatures (SW) on gall attacks. Studies have shown that mite galls do affect the plants' overall performance, but the impact of galls on *Salix* might decrease with increasing temperature and soil moisture (Mosbacher et al. 2013; Patankar et al. 2013).

As *Salix* species are considered key species of Arctic heaths, due to their abundance, the effect of changing climatic conditions with relation to gall mite attacks should be investigated.

The plants of the Arctic areas are hardy, having adapted to withstand extreme conditions such as low temperatures, prolonged periods with no sunlight and very limited nutrient availability in the soil with Nitrogen being the common limiting factor. Even though this vegetation type is well-adapted to harsh, nutrient poor environments, the Arctic plant communities may not be that competitive under more favourable growing conditions. Climate change will likely cause vegetation shifts in the Arctic due to succession and thus some species might be outcompeted due to an increase of soil moisture, precipitation, atmospheric and soil temperature, and nutrient availability. (Svoboda and Henry 1987).

We hypothesize that climate change will increase water and nutrient availability, boosting the microbial loops, which speeds up litter decomposition, and increases the plant species richness and performance (NDVI, Chlorophyll/Flavonoid content (NBI), Anthocyanin content, etc.). Higher temperatures might increase the number of grazers, hence herbivory related stress on plants, which might lead to an increased production of secondary metabolites such as flavonoids. Therefore, we expect to find bigger and more competitive plant species in the winter warming treatment plots. We also expect to find differences in plant communities between experiments in the dry and the wet heath. As the wet heath is already an environment with high soil moisture content, the extra water addition from the snow melting may lead to a decrease of aerobic soil microbial process rates. This might not translate into large differences in plant communities and leaf damage in the winter warming experiments.

Picture 1: Showing the leeward side of a snow fence in the wet heath. The summer and winter warming interaction plot with an open top chamber on the left and a winter warming plot on the right.

To investigate impacts of climate change on Arctic heath plant communities, species composition and gall mite attacks in the winter and summer warming experiments were analysed. To do this, we utilized snow-fences and open-top-chambers (OTCs), installed during summer 2012 (dry heath) and 2013 (wet tundra) (Blok et al. 2016) at Blæsedalen, Disko Island, to analyse winter and summer warming respectively. The function of the snow-fences is to accumulate snow, protecting the vegetation underneath the snow from the wind and extreme cold atmospheric temperatures.

In the dry heath we expected to find a large abundance of evergreen and deciduous shrubs as the most dominant functional plant groups and bryophytes and lichens to a lesser extent. Key species like *E. nigrum*, *V. uliginosom, B. nana* and *S. glauca* were expected to be evenly distributed throughout the study sites.

In the wet heath we expected to find deciduous shrubs, graminoids, bryophytes and equisetums as the most dominant functional plant groups, with the key species *S. arctophila, C. stans, E. angustifolia*, *T. nitens* and *E. arvense.*

Aim and objectives

The aim of this study is to assess the effect of summer warming and increased snow-cover and winter warming on the relationship

between key Arctic plant species and local herbivores in wet and dry heath. Assessment of plant conditions will be based on analysis of plant community, greenness, chlorophyll, and flavonoid level differences between the sites in Blæsedalen.

The objectives are listed below.

- Plant community description and identification of the most common plants as key species.
- Comparison of species composition and plant functional groups between each treatment
- Assessment of the herbivory through evaluation of foliage damage for each treatment
- Linking soil and plant empirical data (soil moisture, air and soil temperature, plot greenness, NBI, chlorophyll and flavonoids) with species composition and degree of leaf damage.

Vegetation and leaf damage analyses as well as measurements of soil moisture, air and soil temperature and greenness have been carried out for each treatment plot of each snow fence of both the dry and wet heath, whereas the NBI, chlorophyll and flavonoid data was only collected from the dry heath plots.

Methodology

Study site

The study site, Blæsedalen, is located northeast of the Arctic Station on Disko Island in West Greenland at 69 °16'N, 53°27'W. The valley is comprised of mesic tundra with patches of dry and wet heath. The study was specifically undertaken at the snow-fences that are part of the CENPERM-KU climate change research. The fences were established in 2012 (dry heath) and 2013 (wet tundra), and their function is to study the interactive effects of increased snow cover and summer and winter warming. The fences are positioned in sites with manipulated increased snow cover on the leeward site during winter. The fences are 1.5 m tall and 14.7 m in length, with 6 of them located in the dry and 6 in the wet heath. On each site of the fences, 4 different treatment plots were established: a plot with an open top chamber, one with shrubs removed and open top chamber, a plot without a chamber but with shrubs removed, and a control plot with no treatment at all. In this study, only the two plots without the shrubs removed were included, with a few exceptions during data collection for leaf damage.

The plots are located 3-8 m from the fence at the South side, ensuring the increased snow

Picture 2: Point intercept analysis frame on dry heath control plot.

Picture 3: Background data measurements taken in wet heath summer + winter warming plot.

cover effect of the fence on the plots. On the North side the plots are situated 6-11 meters from the fence to negate the effect of the snow-fence on the plots (Blok et al. 2016).

Materials and methods

Vegetation analysis

For vegetation analysis, point intercept analysis was used. The pin-point frame was placed in each treatment area within each plot and it was noted each time species, pieces of litter or substrate types touched the stick at string intercept points.

Herbivory analysis

The assessment of herbivory was done by counting 200 leaves of both *Betula nana* and Salix species and noting if they were completely intact or had damage. There were distinguished between cat. 0: 0 %, cat. 1: 0.01-1 %, cat. 2: 1-5 %, cat. 3: 5-10 %, cat. 4: 10-30 %, cat. 5: 30-50 %, cat. 6: 50-75 % and cat. 7: 75-100 % inspired by Barrio and Kozlov 2015 (Barrio and Kozlov 2015), and also between mite galls and other types of damage. Later on, however, this has been changed into cat. 1: 0 %, cat. 2: 2,5 %, cat. 3: 7,5 %, cat. 4: 20 %, cat. 5: 40 %, cat. 6: 62,5 % and cat. 7: 87,5 % in order to calculate averages of leaf area damage for correlation tests.
For a few plots Salix and/or Betula was present in the corresponding shrub removal plots, but not in the non-shrub removal plots.

Table 1. Plant community change. Average coverage of functional plant groups and key species ± standard errors. P-values of significances (*) and tendencies also shown for all the treatments (C = control/SW = summer warming/WW = winter warming/SWW = summer winter warming). – indicates not measured treatments for these variables or species not found.

	Dry Heath				Wet Heath			
	C	**SW**	**WW**	**SWW**	**C**	**SW**	**WW**	**SWW**
Deciduous shrubs	115.33 ± 15.56	112.50 ± 7.48	69.00 ± 11.58 ***↓ (p = 0.006)**	82.67 ± 13.16	115.33 ± 27.32	121.83 ± 7.48	180.33 ± 27.46 ***↑ (p = 0.044)**	181.67 ± 38.93
Evergreen shrubs	60.83 ± 9.21	75.83 ± 15.81	42.00 ± 11.29	64.33 ± 5.71	6.33 ± 6.33	0.00 ± 0.00	0.17 ± 0.17	0.00 ± 0.00
Forbs	8.00 ± 3.51	2.50 ± 3.51	5.50 ± 2.81	5.00 ± 3.22	5.33 ± 3.44	6.00 ± 1.77	5.67 ± 1.93	9.50 ± 4.16
Graminoids	0.83 ± 0.48	0.83 ± 0.65	2.67 ± 1.15 **↑ (p = 0.073)**	2.17 ± 0.91	104 ± 32.45	76.00 ± 15.98	51.83 ± 10.64	81.50 ± 13.69
Equistetums	0.00 ± 0.00	0.17 ± 0.17	0.50 ± 0.50	3.33 ± 3.33	100.83 ± 15.79	98.50 ± 15.48	107.50 ± 26.66	225.67 ± 47.81
Bryophytes & Lichens	22.50 ± 9.23	14.67 ± 5.58	23.50 ± 1.82	29.67 ± 5.98	16.33 ± 6.68	18.17 ± 3.89	23.50 ± 6.36	4.67 ± 1.74 **↓ (p = 0.055)**
Litter	42.33 ± 4.43	43.33 ± 6.93	46.33 ± 7.28	41.50 ± 6.72	32.67 ± 10.07	24.83 ± 5.67	13.17 ± 5.31	22.00 ± 8.98
B. nana	56.17 ± 15.39	67.83 ± 13.23	24.33 ± 8.10 ***↓ (p = 0.037)**	43.67 ± 12.19	00.00 ± 00.00	00.00 ± 00.00	6.17 ± 6.17	6.67 ± 4.29
S. arctophila	-	-	-	-	57.33 ± 13.93	58.50 ± 9.76	86.83 ± 13.18 **↑ (p = 0.100)**	79.17 ± 19.15
S. glauca	12.33 ± 5.09	4.00 ± 2.41	5.33 ± 3.98	3.67 ± 2.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	15.33 ± 13.26
V. uliginosum	41.50 ± 3.21	40.67 ± 8.99	39.33 ± 5.79	35.33 ± 9.99	0.67 ± 0.67	4.83 ± 4.83	0.50 ± 0.50	1.33 ± 1.33
C. stans	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	17.00 ± 4.34	6.83 ± 3.37	21.00 ± 7.45	27.33 ± 9.38
E. angustifolium	-	-	-	-	31.50 ± 16.68	19.67 ± 7.67	13.50 ± 7.07	29.00 ± 10.48
E. arvense	0.00 ± 0.00	0.17 ± 0.17	0.00 ± 0.00	1.67 ± 1.67	100.33 ± 15.47	96.83 ± 15.10 **↑ (p = 0.063)**	106.00 ± 26.14 ***↑ (p = 0.033)**	225.17 ± 47.89 ***↑ (p = 0.050)**
P. squarrossa	-	-	-	-	2.17 ± 0.83	3.00 ± 1.51	0.50 ± 0.50 ***↑ (p = 0.033)**	0.50 ± 0.34
T. nitens	-	-	-	-	12.67 ± 6.53	11.67 ± 4.60 **↓ (p = 0.095)**	19.67 ± 7.40	1.50 ± 0.96

In these instances, the data was collected from the shrub removal plots or for the controls outside the plots within the fence area instead.

Background data

Air and soil temperature were measured thrice at each treatment plot using a standard thermometer. Soil moisture was also measured at each plot using a Theta probe. Normalized difference vegetation index (NDVI) was taken with a SKR 100 sensor, Skye Instruments, Powys, Wales from app. 0.5 m above each plot site in order to get a measure of greenness. For all dry plot sites, nutrient balance index (NBI), chlorophyll, flavonoids and anthocyanins were measured using a Dualex Scientific instrument on 10 healthy and 10 gall-attacked leaves of *Salix spp*.
As mentioned earlier, not all the non-removal plots contained *Salix spp.*, where removal ones did, and for these we likewise tested on the removal plots or outside the plots for the controls.

Statistical analyses

Effects of snow addition and warming on surface air and soil temperature, soil moisture, NDVI, NBI of *Salix spp.*, chlorophyll and flavonoids, leaf damage on *Betula nana* and *Salix spp.*, composition of plant functional groups and abundance of key species were analysed in Statistica (StatSoft, Inc. (2011). Statistica (data analysis software system), version 10. www.statsoft.com). Results were found by utilization of 2-way ANOVAs and multiple regression analyses. For this report, results are considered statistically significant, if the p-value is ≤ 0.05 and considered to show a tendency, if the p-value is [0.05;0.1].

Results

Plant community change

Dry heath

In the dry heath, the deciduous shrubs were significantly reduced in the winter warming

Table 2. Leaf area damaged and leaf compounds. Average of the total leaf area damaged by galls, others in the *Salix* spp. ± standard errors. P-values of significances (*) and tendencies also shown for all the treatments (C = control/SW = summer warming/WW = winter warming/SWW = summer winter warming). – indicates not measured treatments for these variables or species not found.

	Dry Heath				Wet Heath			
	C	**SW**	**WW**	**SWW**	**C**	**SW**	**WW**	**SWW**
Salix spp. galls	0.71 ± 0.33	0.93 ± 0.48	0.00 ± 0.00	1.00 ± 0.72	0.38 ± 0.19	0.96 ± 0.79	0.06 ± 0.06	6.30 ± 5.43
Salix spp. other	1.91 ± 0.88	0.54 ± 0.17	0.67 ± 0.14	0.33 ± 0.19	1.48 ± 0.85	0.25 ± 0.09 **↓ (p = 0.077)**	0.51 ± 0.23	0.08 ± 0.03
Salix spp. total	2.63 ± 0.72	1.47 ± 0.47	0.67 ± 0.14 **↓ (p = 0.069)**	1.34 ± 0.65	1.86 ± 0.78	1.21 ± 0.75	0.57 ± 0.23	6.38 ± 5.41
NBI (healthy)	19.74 ± 1.91	21.29 ± 0.81	17.91 ± 1.29	19.66 ± 1.23	-	-	-	-
NBI (attacked)	18.71 ± 1.00	19.36 ± 0.35	16.88 ± 1.13	19.51 ± 3.35	-	-	-	-
Chlorophyll (H)	37.87 ± 2.22	39.98 ± 2.28	34.92 ± 1.87	37.50 ± 2.36	-	-	-	-
Chlorophyll (A)	37.13 ± 2.30	35.90 ± 0.52	33.54 ± 2.07	33.84 ± 2.56	-	-	-	-
Flavonoids (H)	1.99 ± 0.03	1.87 ± 0.06 ***↓ (p = 0.027)**	1.98 ± 0.03	1.91 ± 0.01	-	-	-	-
Flavonoids (A)	1.99 ± 0.02	1.82 ± 0.01 ***↓ (p = 0.006)**	1.97 ± 0.03	1.79 ± 0.12	-	-	-	-
Anthocyanins (H)	0.13 ± 0.00	0.11 ± 0.01 ***↓ (p = 0.032)**	0.13 ± 0.00	0.12 ± 0.01	-	-	-	-
Anthocyanins (A)	0.56 ± 0.42	0.14 ± 0.03	0.14 ± 0.01	0.14 ± 0.01	-	-	-	-

treatments (F1,1 = 9,588, p = 0.006*) (Table 1). The graminoids showed a similar tendency in the winter warming plots (F1,1 = 3.588, p = 0.073). Neither of these showed any differences when subjected to summer warming in the open top chambers (OTCs) (F1,1 = 0.194, p = 0.664 and F1,1 = 0.089, p = 0.768 respectively). The abundance of other functional groups, these being evergreen shrubs, forbs and bryophytes and lichens, was also tested for in the dry heath plots. None of the groups showed any significant change between treatments, but interestingly, the abundance of forbs was exactly the same on each side of the snow fences (F1,1 = 0.000, p = 1.000).

Focusing on the key species, there was a significantly lower occurrence of the key species *B. nana* in plots on the leeward side of the fences compared to that of the control sites (F1,1 = 5.007, p = 0.037). No difference was found between summer warming and non-warmed plots (F1,1 = 1.534, p = 0.230).

Other key species showed no significant change in neither the summer nor winter warming plots; these include *S. glauca* (F1,1 = 1.041, p = 0.320 for winter warming; F1,1 = 1.935, p = 0.179 for summer warming) and *V. uliginosum* (1,1 = 0.251, p = 0.622 for winter warming; F1,1 = 1.104, p = 0.750 for summer warming). Likewise, litter showed no statistically significant difference between treatments (F1,1 = 0.028, p = 0.868 for summer warming; F1,1 = 0.089, p = 0.796 for winter warming).

Wet heath

In the wet heath, there was a significantly higher occurrence of the deciduous shrubs in plots on the leeward side of the fences compared to that of the control sites (F1,1 = 4.606, p = 0.044). No difference was found between summer warming and non-warmed plots (F1,1 = 0.018, p = 0.894). Furthermore, the bryophytes and mosses group shows a tendency in decrease of abundance in the interaction between summer and winter warming plots F1,1 = 4.135, p = 0.055.

There was a significantly higher occurrence of the key species *E. arvense* in plots on the

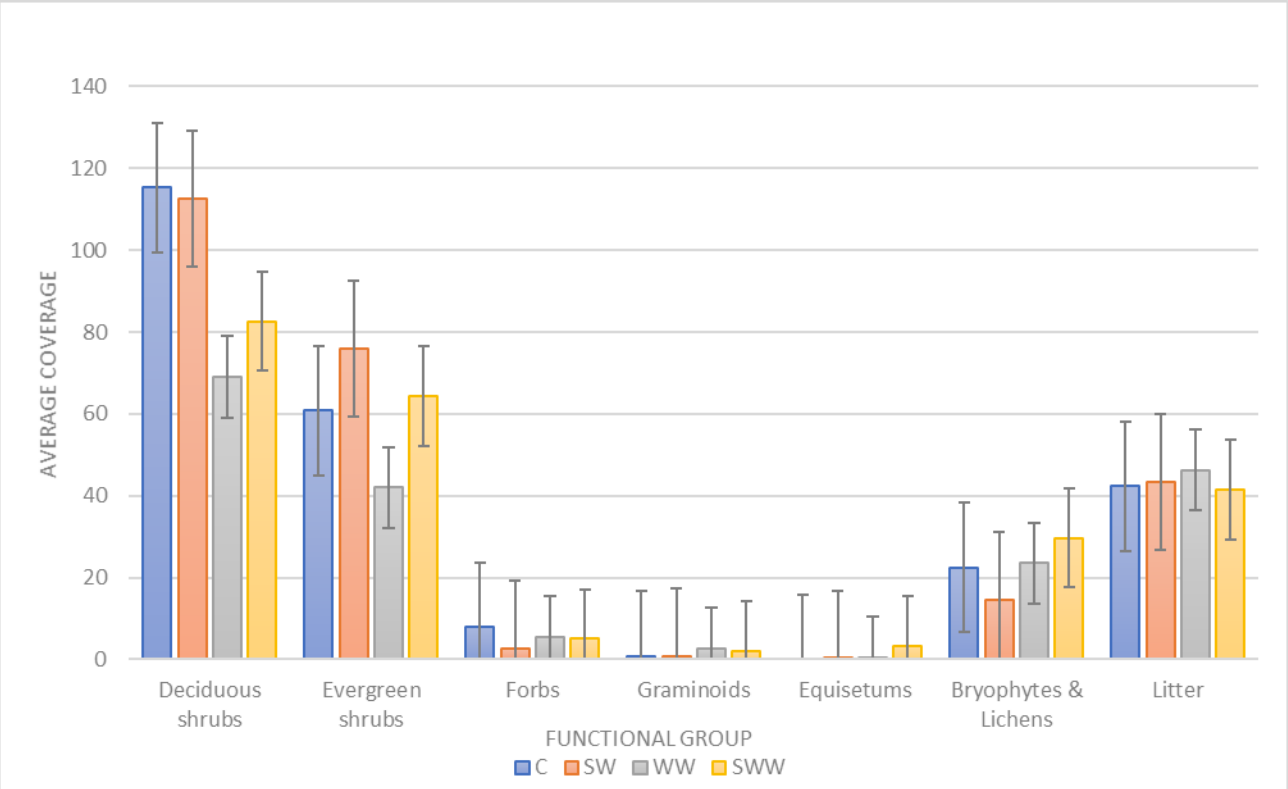


Figure 1. Coverage of plant groups per treatment in the dry heath. C: control, SW: summer warming, WW: winter warming & SWW: summer + winter warming. Functional groups on the x-axis and average coverage on the y-axis.

Table 3. NDVI and abiotic factor. Average of the abiotic factors and NDVI ± standard errors. P-values of significances (*) and tendencies also shown for all the treatments (C = control/SW = summer warming/WW = winter warming/SWW = summer winter warming).

	Dry Heath				Wet Heath			
	C	**SW**	**WW**	**SWW**	**C**	**SW**	**WW**	**SWW**
NDVI	0.42 ± 0.04	0.48 ± 0.01 ***↑ (p = 0.044)**	0.36 ± 0.02 ***↓ (p = 0.016)**	0.40 ± 0.03	0.52 ± 0.03	0.53 ± 0.03	0.55 ± 0.05	0.60 ± 0.04
T (air)	10.99 ± 0.84	11.45 ± 0.28	11.04 ± 0.41	12.35 ± 0.70	12.22 ± 0.20	12.66 ± 0.87	11.09 ± 0.40	12.34 ± 0.43
T (soil)	9.48 ± 0.26	9.56 ± 0.31	9.06 ± 0.15	9.67 ± 0.34	9.93 ± 0.45	9.74 ± 0.42	10.39 ± 0.58	10.28 ± 0.50
Soil Moisture	26.9 ± 2.88	19.23 ± 3.23	25.61 ± 3.10 **↑ (p = 0.085)**	33.77 ± 5.01 ***↑ (p = 0.043)**	75.33 ± 10.04	71.89 ± 3.83 **↓ (p = 0.096)**	83.86 ± 5.94	63.96 ± 5.22

leeward side of the fences compared to that of the control sites (F1,1 = 5.213, p = 0.033). Additionally, a significant increase is seen in the interaction plot with both summer and winter warming (F1,1 = 4.368, p = 0.050). Only a tendency is seen in summer warming (F1,1 = 3.884, p = 0.063).

Other key species showed no significant change in neither the summer nor winter warming plots; these include *E. angustifolia* (F1,1 = 0.151, p = 0.0.702 for winter warming; F1,1 = 0.027, p = 0.871 for summer warming and for the interaction; F1,1=1.503, p = 0.234) and the moss *T. nitens,* which however show a tendency of decreasing in abundance in the summer warming (F1,1 = 00.084, p = 0.775 for winter warming; F1,1 = 3.075, p = 0.095 and for the interaction; F1,1 = 2.467, p = 0.132). *C. stans* show a tendency of increased abundance in winter warming; F1,1 = 3.455, p = 0.078 (summer warming; F1,1 = 0.085, p = 0.774, interaction; F1,1=1.567, p = 0.225). *S. arctophila* also show a tendency of higher abundance with winter warming; F1,1 = 2.972, p = 0.100 (summer warming F1,1=0.050, p = 0.826 interaction; F1,1 = 0.092, p = 0.765). *P. squarrosa* was significantly more abundant in the winter warming treatments (F1,1 = 5.217, p = 0.033), (summer warming; F1,1 = 0.209, p=0.653, interaction; F1,1 = 0.209, p = 0.653).

Herbivory

Dry heath

For herbivory, only a decreasing tendency is seen for total leaf area loss (galls + other) in the winter warming treatment plots (WW; F1,1 = 3733, p = 0.069) (table 2).

Wet heath

A decrease is seen in leaf area damage in *S. arctophila* under the warming treatments (WW; F1,1 = 1.647, p = 0.214, SW; F1,1 = 3.485, p = 0.077, SWW; F1,1 = 0.815, p = 0.378).

Leaf damage on *B. nana* and *S. arctophila* only showed a decreasing tendency in the summer warming plots (SW; F1,1 = 1.647, p = 0.214, WW; F1,1 = 3.485, p = 0.077, SWW; F1,1 0.815, p = 0.378).

Background data

Dry heath

Soil moisture showed a significant interaction, and an increasing tendency in the winter warming treatments plots (WW; F1,1 = 3.285, p = 0.085, SW; F1,1 = 0.004, p = 0.947, SWW; F1,1 = 4.689, p = 0.043). NDVI decreases significantly in the winter warming treatments and increases significantly in the summer warming treatments (WW; F1,1 = 6.903, p = 0.016, SW; F1,1 = 4.638, p = 0.044).

In the healthy *S. glauca* leaves, the flavonoid content in the leaves decreased significantly in the summer warming plots (WW; F1,1 = 0.109, p = 0.747, SW; F1,1 = 6.4, p = 0.027, SWW; F1,1 =

0.508, p = 0.491), this is also the case for the flavonoid content in damaged *S. glauca* leaves

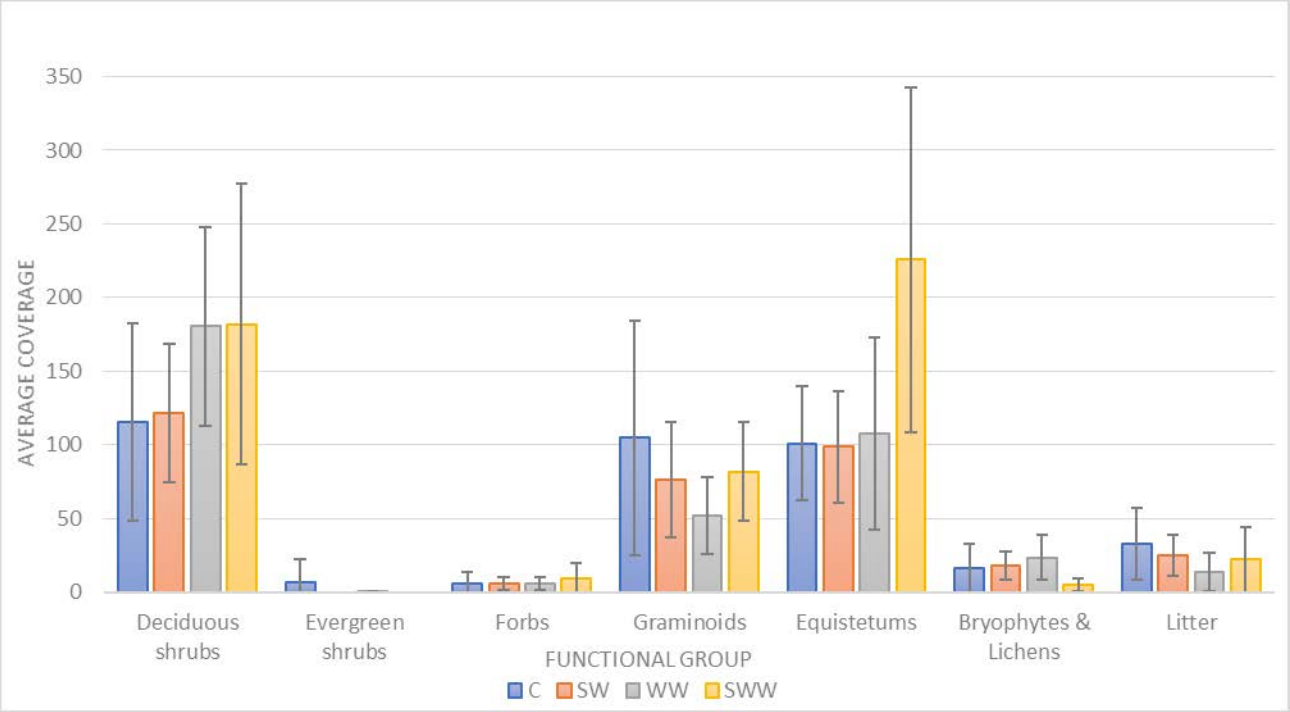


Figure 2. Coverage of plant groups per treatment in the wet heath. C: control, SW: summer warming, WW: winter warming & SWW: summer + winter warming. Functional groups on the x-axis and average coverage on the y-axis.

(WW; F1,1 = 0.39, p = 0.552, SW; F1,1 = 14.75, p = 0.006, SWW; F1,1 = 0.02, p = 0.902). We also tested for anthocyanins, in both damaged and healthy leaves. The only significant result was in the healthy leaves, which decreased with summer warming (SW; F1,1 = 5.988, p = 0.032).

Wet heath

The abiotic factors NDVI, soil and aboveground temperature, soil moisture, NBI, chlorophyll and flavonoid content treatments showed no significant differences between treatment plots in the wet heath. The soil moisture however seems to have a decreasing tendency in the summer warming treatment (WW; F1,1 = 0,002, p = 0.965, SW; F1,1 = 3.059, p = 0.096, SWW; F1,1 = 1,523, p = 0.231).

Discussion

Summer and winter warming effects on plant community composition

Dry heath plant community change

From the analysis of variance (ANOVAs), a significant decrease was found on the deciduous shrubs under the winter warming treatment. This significant decrease was also observed in one of the deciduous key species *B. nana*. In contrast, graminoids show an increased tendency with winter warming. An explanation could be that with higher snow depositions during winter, snow melts later, shortening the biological window in which the plants grow and reproduce. Thus, graminoids seem to have a clear advantage as they are more generalist and capable of fast nutrient uptake outcompeting in turn, deciduous shrubs like *B. nana* that seem to need more time to grow and adapt to environmental changes as nutrients become a limiting factor. This is supported by a tendency found in soil moisture on the plots under the winter warming treatments. The winter and summer warming interaction treatment show a significant increase in soil moisture. These results support the fact that the snow melting will moisturise the soil, speeding up the microbial loop and hence leading to an increase of soil nutrients availability. This has not been studied yet in winter warming, but the information is supported by recent findings about dryer and warmer Arctic future conditions and plant responses (Hicks Pries et al. 2013; Blok et al. 2016). Following this

Picture 1: Mite galls on *S. glauca*.

theory, we would also expect the litter abundance to be significantly lower in the snow cover plots as the microbes will degrade the organic matter faster. Such, however, was not found in our examination. This could be explained by the fact that the litter layer is thinner, though still equally distributed on the top soil surface, which the point intercept analysis does not take into account. The evergreen shrubs, bryophytes and lichens did not show any significant changes or tendencies within the treatments, neither did any of the other key species. This could be due to the treatments not having great enough influence on the groups, or that another method is needed to gain more insight.

Wet heath plant community change

For the wet heath a significant increase was found on the deciduous shrubs under the winter warming treatment. This can be supported by a tendency found under the same treatment for *S. arctophila* as a main shrub of the deciduous key species.

For the big functional group, including lichens and bryophytes, we found a tendency of reduced cover under the combination of both treatments. *P. Squarrosa* decreases significantly under the winter warming treatment. This could be explained by an out shading caused by an increased cover of deciduous shrubs.

Equisetums are usually abundant in the wet heaths, but increasing tendencies and significances were found in the combined treatment. These significant changes seem to be visible mostly due to *E. arvense,* which is considered a key species for the wet heath. What seems to have the biggest effect is an increase on the snow cover during winter. This could be explained, again, by the increase of nutrients during winter when the snow protects the soil and vegetation and through this, speeds up the microbial loop and in turn leaves nutrients readily available to be taken up.

The evergreen shrub, graminoids and forbs did not show any significant changes or tendencies within the treatments, however *C. stans* are the only key species showing a slightly increasing tendency under winter warming treatment. This could be due to the competitive *C. stans* taking advantage of the beforementioned increase in available nutrients.

Summer and winter warming effects on total leaf area damage

No difference was found for the degree of leaf area damage between treatments for *B. nana*. Either there is no difference between invertebrate damaging between plots, or it is due to the much smaller sample size of *B. nana* than *Salix spp.* The latter theory could be excluded by utilizing a different method for investigating herbivory and leaf dama ge on *B. nana*, such as counting more leaves per plot and using a binary approach listing the number of intact and damaged leaves.

Flavonoids were only measured on *Salix spp.* due to the size of the leaf, since the apparatus only works on leaves large and flat enough for the sensors to be completely covered. If a different apparatus was to be used, it would be very interesting to see if *B. nana* exhibited the same very significant reduction in flavonoid content between control and summer warming plots. The leaf area loss has a tendency for decrease between control and winter warming plots. This could be due to an increase in soil moisture in early summer, which may have a negative effect on mites and other invertebrates.

In Mosbacher et al. 2013 they found no effects from environmental manipulations on the severity of gall attacks on *Salix arctica*, and on basis of their stud, they expect no change in gall mite attacks in the High Arctic. Further studies should be done in lower latitudes of the Arctic to conclude that the same applies to these ecosystem types, as they differ from the more Northern ones.

The method of counting leaves and marking the number of leaves in each leaf area damage category is useful for getting an overview of the degree of foliage damage, but it has its critique points; randomization of collection is at times not doable if there are not many plants present. Further, having a range of percentages makes it fairly quick to assess the degree of leaf area damage but when converting the categories, the data might not sufficiently represent the reality. With the limited time, it might have been better to use a binary method of determining leaf damage. This would also negate our need to convert the categories.

Conclusion

The communities of the Arctic heath change under the effect of the treatments. A significant decrease in abundance of deciduous shrubs and *B. nana* and an opposite trend for graminoids was seen between control and winter warming plots.

NDVI increased significantly with summer and winter warning, but not with summer and winter warming combined. Total leaf area damage on *Salix spp*. leaves had a tendency of decreasing with summer warming, Betula showed nothing, likely due to limited data. Flavonoids and anthocyanins in *Salix spp*. leaves decreased significantly with summer warming. All in all, our results could indicate better growing conditions in those plots, but more thorough research should be done before concluding anything.

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