Arctic Biology Field Course

Qeqertarsuaq 2016

UNIVERSITY OF COPENHAGEN
FACULTY OF SCIENCE

ARKTISK STATION

Arctic Biology Field Course

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Table of Contents

Preface

The purpose of the Arctic Biology Field course is to allow the students to get acquainted with the arctic environment, which they have learned about during courses at University of Copenhagen or elsewhere, and to provide them with an opportunity to get experience with the process of creating a scientific research project from the first idea to the final report. The Arctic Station provides an optimal teaching environment where the students get a genuine understanding of how unique the arctic ecosystems are by first-hand observations and analysis.

The Arctic Biology Field course in 2016 took place from 12th to 22nd July. The overall theme for the course in 2016 was to study responses of arctic ecosystems to global warming. The students are well prepared as they beforehand have defined their group projects, have researched the literature and have prepared/tested methods for sampling and laboratory analysis. Still, the groups were challenged with some obstacles both in the field and during laboratory analysis. However, these problems were solved and field work continued throughout the stay and the samples were analysed alongside in the laboratory.

The themes for group projects were:

1) The impact of homothermic springs on arctic vegetation along the water course that runs in typically dry tundra heath landscapes. The investigation included analyses of abiotic factors (available nutrients, soil moisture and organic matter) and biotic (vegetation coverage and biomass of plants) in specific zones from the stream and into the dry tundra.

2) The inter-annual growth rates of a population of landlocked Arctic charr (*Salvelinus alpinus L.*) in Røde Elv in relation to changed climatic conditions. This was investigated using otolith analyses for back calculation of fish growth and the long term climate record obtained by Arctic Station.

3) The impact of gall mites on the leaves of two arctic willow species (*Salix glauca* and *Salix arctophila*) in relation to wet and dry conditions. This was studied experimentally using an ongoing snow fence experiment with four different treatments (control, warming, snow and snow+warming) where ecophysiological measurements were performed.

Fine weather conditions prevailed during our stay and made field work enjoyable. A few common excursions took place to visit Morænesø, Østerlien and Kuanit. Interactions with other guests at the station and with the two station managers, the housekeeper as well as the crew onboard Porsild, where highly appreciated. We wish to thank them all for their splendid work.

The findings and conclusions based on the student projects are published in the report "Arctic Biology Field Course - Qeqertarsuaq 2016" (ISBN: 978-87-89143-22-4). The report can be obtained as PDF from Kirsten S. Christoffersen (kchristoffersen@bio.ku.dk) or from the Arctic Station website.

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Assessment of vegetation transitions in the homothermic spring habitat by investigation of three Greenlandic springs.

The homothermic spring at Østerlien near Arctic Station (Klara A. Rask, July 2016)

Assessment of vegetation transitions in the homothermic spring habitat by investigation of three Greenlandic springs.

Klara Andrés Rask and Sissel Lindhart Fredsgaard

Abstract

Homothermic springs have an effect on arctic vegetation as the stream runs through the otherwise dry tundra heath landscape. To investigate these effects transects consisting of four zones based on dominating plant species or functional group, were laid out at three homothermic springs on Disko Island. Representative plots in each zone were investigated for abiotic factors, including plant available nutrients, soil moisture and organic matter, as well as biotic factors including vegetation coverage and biomass of plant species or phyla. Soil moisture was decreasing away from the studied streams and showed to be positively correlated with the content of phosphate, being highest in zone 1. Nitrate showed a polynomial tendency with the highest content in zone 2, while ammonium did not show any variation between zones. Zone 1, closest to the stream, was dominated by bryophytes. The highest amount of pteridophytes, mainly *Equisetum arvense*, was found in zone 2. Zone 3 was dominated by *Salix glauca*, while evergreen shrubs primarily were found in zone 4. Vegetation coverage of *S. glauca* and soil organic matter content was highest in zone 3, while the chlorophyll index of *S. glauca* leaves decreased linearly with increasing soil moisture.

Keywords: Homothermic springs, nutrient gradients, vegetation zonation, the Arctic, Salix glauca

Introduction

Geothermal springs with homothermic source water temperatures can be found all over Greenland, where the occurrence of several thousands of such springs has been estimated (Heide-Jørgensen and Kristensen, 1998). Homothermic springs are especially common on the Disko Island off the West coast of Greenland, but are only confined to the Southwestern part of Disko (Hjartarson and Armannsson, 2010) (Fig. 1).

The springs' peculiar lush greenness in this arctic region makes the island's nature unique (see previous page).

Homothermic springs are defined as springs with a constant annual temperature that is 3°C or more (Sørensen, 2012). This makes them a very unique freshwater habitat in the permafrost zone where all springs and minor streams normally freeze during the winter. This gives rise to a distinct flora composing species that in many cases have their northern limits in this area (Heide-Jørgensen and Kristensen, 1999). The reason for this unusual plant community is mainly due to higher soil temperature and prolonged growing season, in the microclimate associated
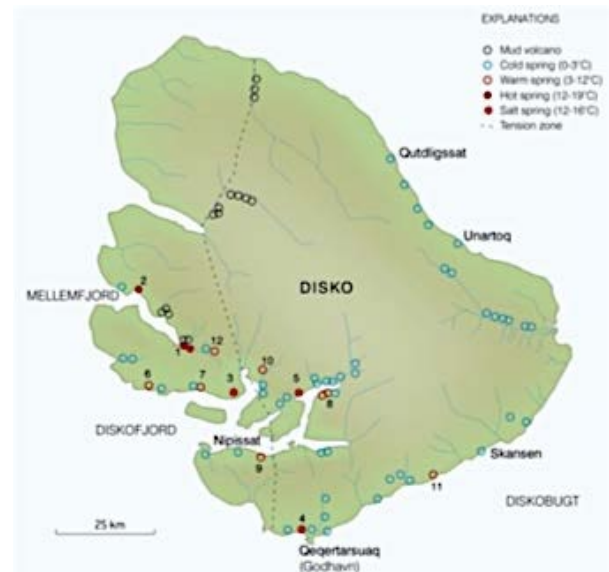

Figure 1. Springs on Disko Island. 1) Puilassoq, 2) Unartukavsak, 3) Unartuarak, 4) Unartorssuaq, 5) Tarajungitsok, 6) Qeqertarssuk, 7) Kuanit, 8) Angujartutit, 9) Unartoq, 10) Avdlaugissat, 11) Qofat, 12) Kildedalen, (Hjartarson and Armannsson, 2010, partly adapted from Kristensen 1987).

with the springs (Heide-Jørgensen and Kristensen, 1999; Jensen, 1999). However these homothermic properties might be expected to affect other vegetation patterns besides biogeographic range. High winter temperatures in the soil near the springs can be expected to boost the microbial activity and increase the decomposition rate, which in turn affects the nutrient balance and plant demography. Enhanced decomposition and nutrient levels are expected to alter the plant community structure and productivity by facilitating higher growth rate and competition advantage for some plant species. It is therefore hypothesised that there is a transition in dominating plant species and biomass from the banks of the spring to the surroundings driven by the spring microclimate, and that this pattern can be divided into zones based on the dominating plant species. Some plant species, such as the competitive *Salix glauca* L., is expected to benefit more than others from the homothermic spring effect (Formica et
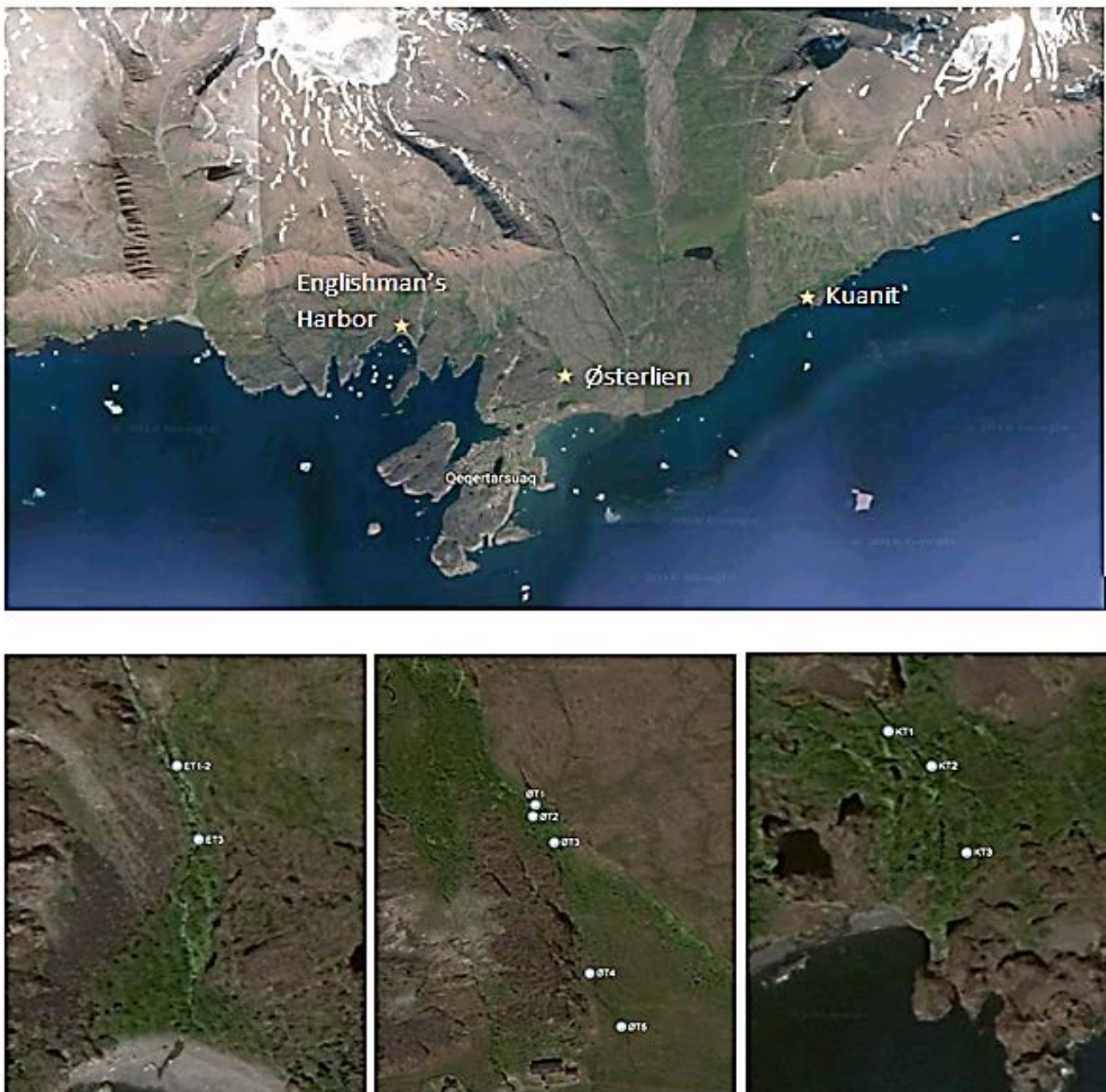

Figure 2. Locations of the study sites on Disko Island, Greenland. Top: Overview showing the three locations: Engelskmands Havn (Englishman's Harbor), Østerlien and Kuanit. Bottom left: Transects 1-2 (same starting point) and 3 at Engelskmands Havn. Bottom middle: Transects 1-5 at Østerlien. Bottom right: Transects 1-3 at Kuanit.

5

al. 2014). *S. glauca* can very effectively sequester nutrients from the soil and allocate them to plant growth (Bowman and Conant, 1994).

By describing the vegetation patterns of three homothermic springs on Disko Island, we aim to assess the diversity and distribution of plants across a gradient away from the springs by comparing zones and locations.

Methods and materials

Fieldwork

Due to the short duration of our stay, study sites were chosen based on the availability of homothermic springs in a walking distance from Arctic Station. The spring studied at Østerlien (Ø) lies close to a rock wall on the top of Østerlien Valley at 74 m.a.s.l. The catchment of the spring is a marsh-like plateau, dominated by willow. The spring at Engelskmands Havn (E) is steep and fast running ending on a rocky beach. The vegetation is lush The third spring is located between numerous cold and homothermic springs running in a basalt setting with a rich vegetation dominated by willow thickets and *Angelica*, which gives the place its' name: Kuanit (K).

To investigate the zonated transition in the vegetation surrounding the homothermic springs, 3 to 5 orthogonal transects (T) were placed in an attempt to cover the area from source to outflow as representatively as possible. Each transect was divided into four zones depending on the dominating plant species: Zone 1 (Z1) being dominated mainly by bryophytes, zone 2 (Z2) by *Equisetum arvense*, zone 3 (Z3) by *S. glauca* and zone 4 (Z4) by tundra heath. In each zone a representative 1x1m plot was chosen. Not all zones were present in all transects, giving a total of 40 plots distributed on 11 transects. Three transects (T) were laid out at Kuanit and at Engelskmands Havn, while five transect were laid out at Østerlien where it was possible to track the stream back to its source (Fig. 2). All sampling was carried out in mid July 2016. For all transects, the GPS position and transect orientation was detected using a compass, as well as other notable field site characteristics. The water temperature belonging to each transect was noted. In each plot the soil moisture was

measured using a ThetaProbe at three random spots. A soil sample for further analyses of soil chemistry was taken using a 5.7 cm-diameter soil core sampler. No soil samples were taken in T2Z2 and T3Z1 at Engelskmands Havn since no soil was present in these zones. A visual estimation of each vascular plant species' coverage was made in each plot, including the phyla Hepatophyta and Bryophyta. A representative 10x10 cm square within the plot was harvested for biomass analyses. The annual shoot growth (cm year^{-1}) of *S. glauca* for the year 2016, 2015 and 2014 on three twigs in each zone were measured using measuring tape. A fresh leaf on the same twigs was chosen for analysis of nitrogen balance index (NBI), and chlorophyll- and flavonol content (µg cm^{-2}) using a Dualex Scientific+™.

Laboratory procedures

In the laboratory at the Arctic Station the top 5 cm of the soil cores were used for further analyses after removal of stones and roots >1 mm in diameter.

The dry soil samples were stored in ziplock bags and brought to Copenhagen where content of organic matter (OM) was examined. Empty crucibles were weighed (a). The samples were dried at 80°C before they were transferred to the crucibles and weighed herein (*b*). The samples were ignited at 550°C for 6 h and reweighed (*c*). OM% was calculated using the formula:

$$OM\ (\%\ of\ dry\ weigh) = \frac{(b-c)*100}{b-a}.$$

For soil nutrient analysis, 5 g fresh soil was transferred to plastic containers and 25 ml ddH$_2$O was added. The containers were shaken for 30 s every 15 min for an hour, *i.e.* five times, and stored at 5°C overnight. The water was filtered using a VWR Mini diaphragm vacuum pump, VP 86 and transferred to 20 ml vials. The soil extracts were stored in a freezer, transported to Copenhagen and stored at -20°C until the nutrient analyses were performed, approx. one month after sampling. The samples were thawed at 5°C overnight. 5.5 ml water sample was transferred to tubes, avoiding settled particles. Content of ammonium, phosphate, nitrate and nitrite was determined using a FOSS Tecator FIAstar 5000 analyzer, according to the protocols "Determination of ammonium in water by FIAstar

5000", "Determination of ortho-phosphate in water by FIAstar 5000" and "Determination of the sum of nitrate and nitrite in water by FIAstar 5000" (Tecator Application Note, 1984).

Aboveground biomass was determined for the 10x10 cm plot samples. The harvested vascular plants and bryophytes from the 10x10 cm plots were sorted in six functional groups: bryophytes,

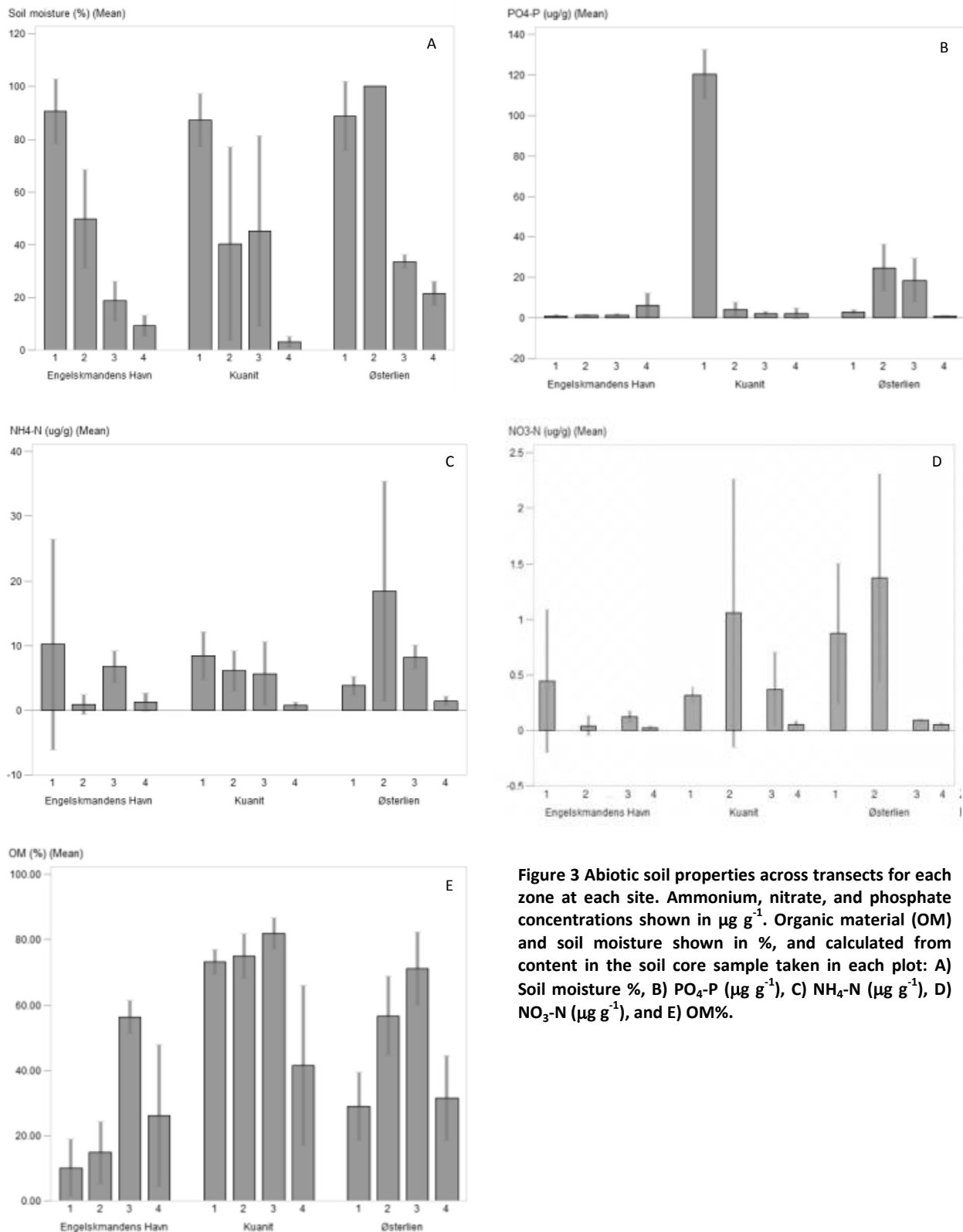**Figure 3 Abiotic soil properties across transects for each zone at each site. Ammonium, nitrate, and phosphate concentrations shown in $\mu g\ g^{-1}$. Organic material (OM) and soil moisture shown in %, and calculated from content in the soil core sample taken in each plot: A) Soil moisture %, B) PO_4-P ($\mu g\ g^{-1}$), C) NH_4-N ($\mu g\ g^{-1}$), D) NO_3-N ($\mu g\ g^{-1}$), and E) OM%.**

pteridophytes, graminoids, evergreen shrubs, deciduous shrubs and broadleaved forbs. All plants were dried at 70°C for at least 24 h and then weighed to gain the dry weight biomass.

Data analyses

To test whether the observed vegetation patterns correspond with and are dependent on the chemical soil properties constituting the springs' microclimate both variance and regression analyses were used. At first the biological and chemical parameters were tested for their difference between zones and locations, using one-way ANOVA and Tukey's Studentized Range test in the statistical program Statistical Analysis Software (SAS). Comparisons were tested significant for results where $p<0.05$ and very significant for $p<0.01$.

Significant differences were then tested for their underlying connection with correlation tests in the Scientific Data Analysis and Graphing Software; SigmaPlot (Σplot). After an estimation of the best-fitted correlation, an either linear or exponential model was tested.

Results

Details of the studied transects are summarized in the Appendix including position and orientation of transects, zone distribution, plot distance from the stream, water temperature, and a general description of the surroundings.

The zone number is significantly linearly correlated with plot distance (linear regression, $R^2 = 0.5125$; $p<0.0001$).

Soil and nutrient variations

Abiotic factors measured varied between zones (Fig. 3). Organic matter content (OM %) showed an increasing tendency from Z1-3, but was lowest in Z4, however OM% also varied between locations (ANOVA: p<0.0001), with the percentage being highest at Kuanit (Tukey's: $p<0.0001$), but not different at Østerlien and Engelskmands Havn (Tukey's: $p>0.05$). Plant available nutrients showed different patterns. Ammonium concentration did not vary between zones (ANOVA: $p<0.05$) while nitrate concentration did vary (ANOVA: $p<0.05$) but only between Z2 and Z4 (Tukey's: $p<0.0001$). Phosphate content did not vary between zones in the model as a whole (ANOVA: $p>0.05$), however the content was very significantly higher in Z1 than Z4 (Tukey's: $p<0.0001$). Soil moisture was positively correlated with PO_4-concentration (linear regression: R^2=0.1354, p=0.0230) (Fig. 7A). Testing a multiple regression model of abiotic factors and zonation, shows that all the abiotic factors in combination is an overall good explanation for the zonation in the spring ecosystem ($p<0.0001$), but the combination of soil moisture and OM% is the best explanatory factor ($p<0.0001$).

Vegetation analysis

Estimated vegetation coverage and biomass samples for six functional plant groups showed that vegetation composition and distribution varied between zones (Fig. 4 and Fig. 5).

The total coverage, *i.e.* the sum of multiple layers of vegetation, differed between zones (ANOVA: $p<0.0001$), Z2 having the highest vegetation coverage of all zones (Tukey's: $p<0.0001$), while total biomass did not differ significantly between zones (ANOVA: $p>0.05$).

Bryophyte coverage differed significantly between zones (ANOVA: $p<0.0001$, Tukey's: $p<0.0001$), except between Z1-Z2 and Z3-Z4. p=0.0016). Coverage of deciduous shrubs, mainly consisting of *S. glauca*, varied significantly between zones (ANOVA: $p<0.0001$), Z3 having highest coverage (Tukey's: $p<0.0001$). The *Salix* biomass did not vary significantly between zones (ANOVA: $p>0.05$), however there was a tendency towards Z3 having the highest biomass (Fig. 5B). Both coverage and biomass of evergreen shrubs were highest in Z4 (Tukey's: $p<0.001$). Neither forbs, nor graminoids showed any significant tendencies. Pteridophytes dominated Z2 significantly regarding coverage (ANOVA: $p<0.0001$, Tukey's, $p<0.0001$), with only a supporting tendency regarding biomass (Fig. 4B; Fig. 5B).
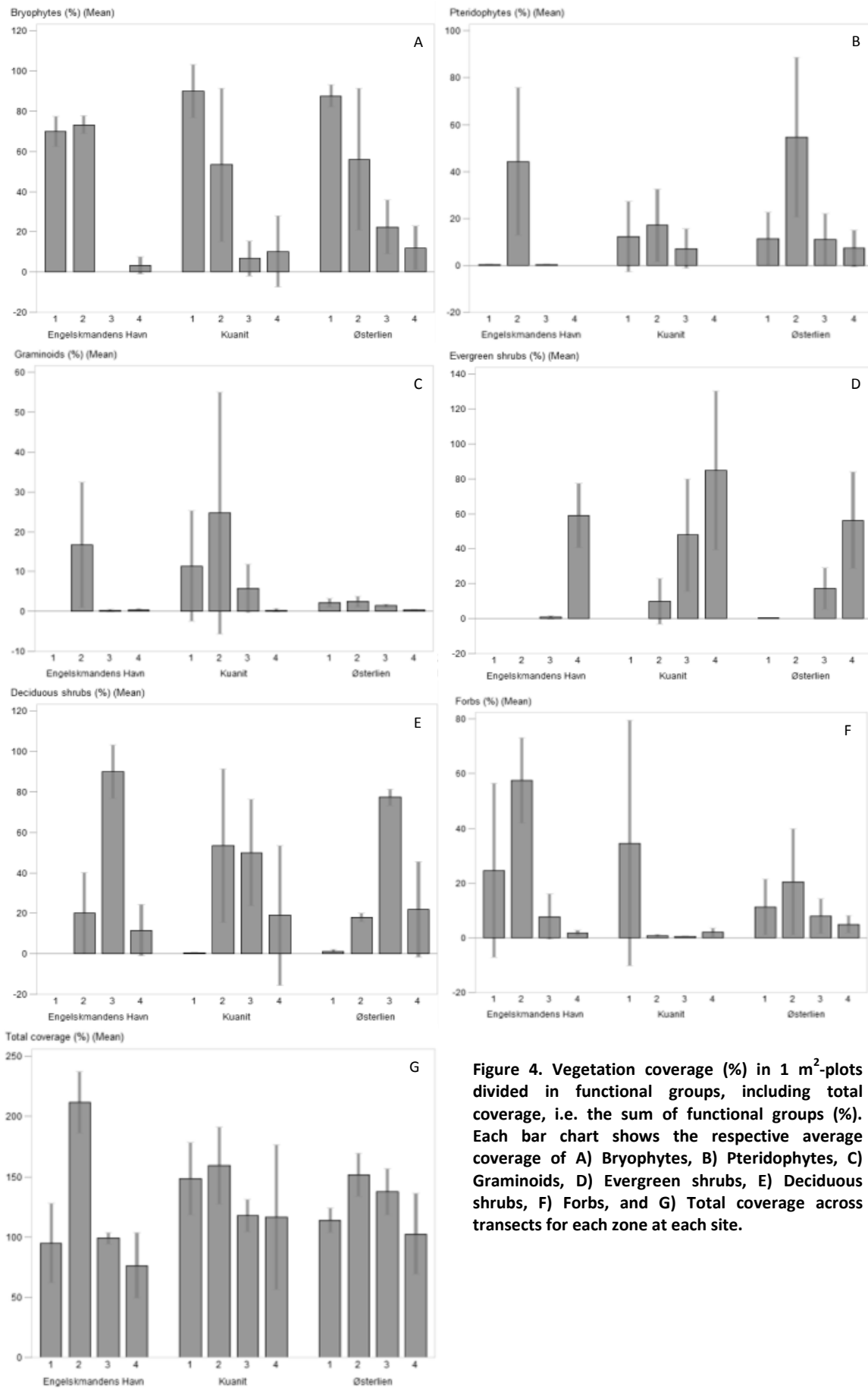
Figure 4. Vegetation coverage (%) in 1 m^2-plots divided in functional groups, including total coverage, i.e. the sum of functional groups (%). Each bar chart shows the respective average coverage of A) Bryophytes, B) Pteridophytes, C) Graminoids, D) Evergreen shrubs, E) Deciduous shrubs, F) Forbs, and G) Total coverage across transects for each zone at each site.

9

Figure 5. Dry biomass (g m^{-2}) of A) Bryophytes, B) Pteridophytes, C) Graminoids, D) Evergreen shrubs, E) Deciduous shrubs, F) Forbs, and G) Total biomass across transects for each zone at each site.

Testing which combination of either biomass or coverage from each functional plant group that gives the best linear model fit shows that both coverage (Model fit: p<0.0001) and bioma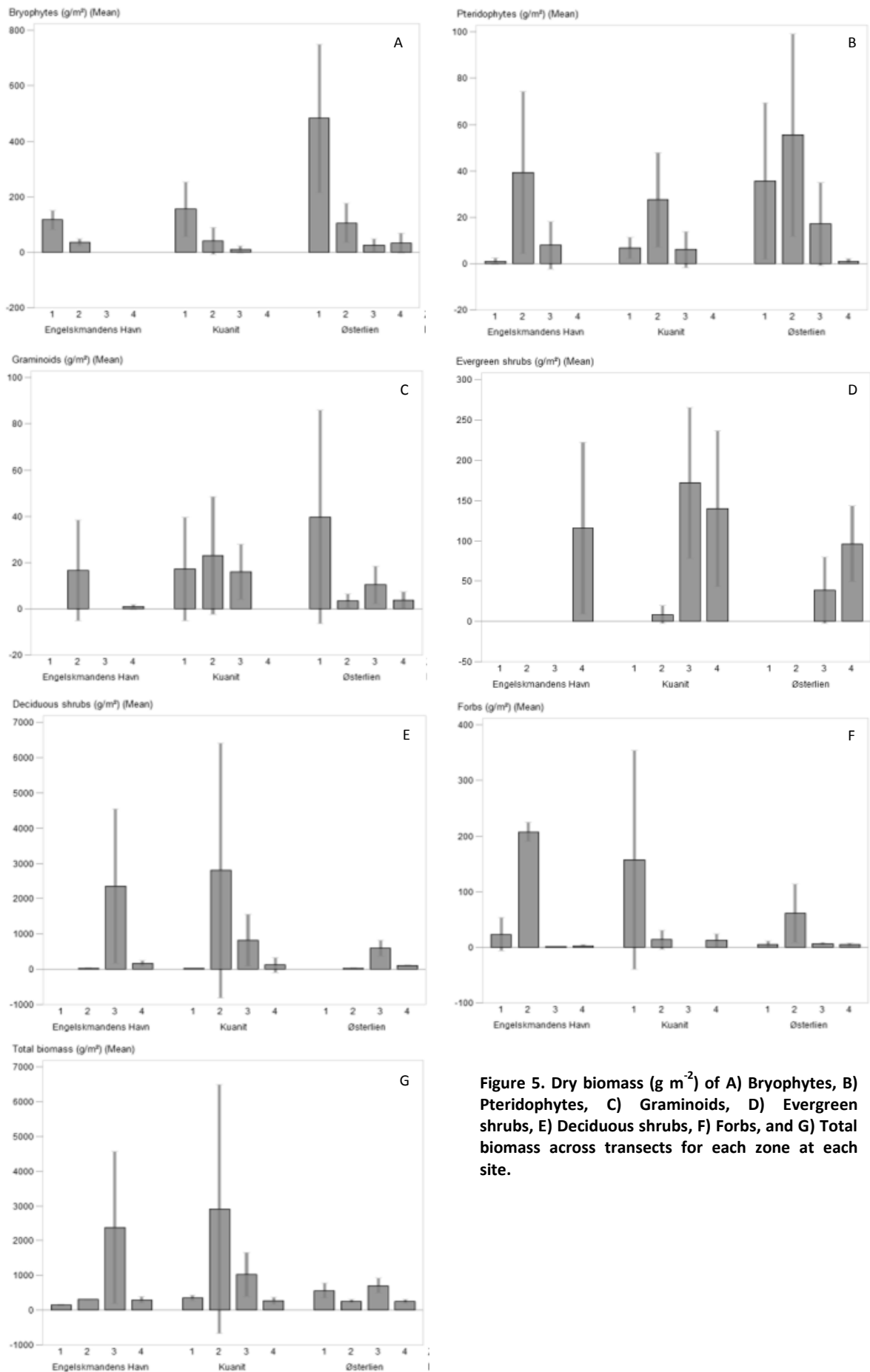ss (Model fit: p<0.0001) are overall good explanatories for the zonation in the spring ecosystem. *S. glauca* and bryophytes are the best explanatories regarding coverage (*Salix glauca* %: *p*=0.0012, bryophytes %: *p*<0.0001), also in combination (*S. glauca* % * Bryophytes %: *p*=0.0017). When it comes to biomass, evergreen shrubs (g m^{-2}) and bryophytes (g m^{-2}) have the best linear fit (*p*=0.0216, *p*=0.0003).

Salix glauca

One of the most dominant species along the springs, *S. glauca*, generally showed differences in distribution and production across zones (Fig. 6). Analysis of *S. glauca* vegetation coverage showed that there is a significant peak in Z3 (Fig. 4E). The *Salix* distribution across zones is furthermore dependent on the concentration of soil nutrients. A combinatory effect of PO_4 and NO_3 shows a good linear fit with the coverage of *S. glauca* (Model: *p*=0.0454, (PO_4 (µg g^{-1}) * NO_3 (µg g^{-1})): *p*=0.0173). A linear model fit shows on the other hand that nutrients have no influence on biomass of *S. glauca,* neither on chlorophyll nor NBI.

Dualex measurements showed no clear trends between zones (ANOVA: *p*>0.05) (Fig. 6). However, *S. glauca* leaf chlorophyll index decreased linearly with increasing soil moisture (linear regression: *Rsqr*=0.2766, *p*<0.0001). Average annual shoot length did not vary between zones (ANOVA: *p*>0.05). Furthermore *S. glauca* vegetation coverage is negatively correlated with the biomass of all plants excluding deciduous shrubs, *i.e.* almost everything except *S. glauca* (linear regression: R^2=0.1240, *p*<0.05). The positive connection between *S. glauca* domination and zone biomass is supported by the exponential growth between the percentage of deciduous shrubs and total biomass (R^2 =0.3492, *p*<0.0001) (Fig. 7B).

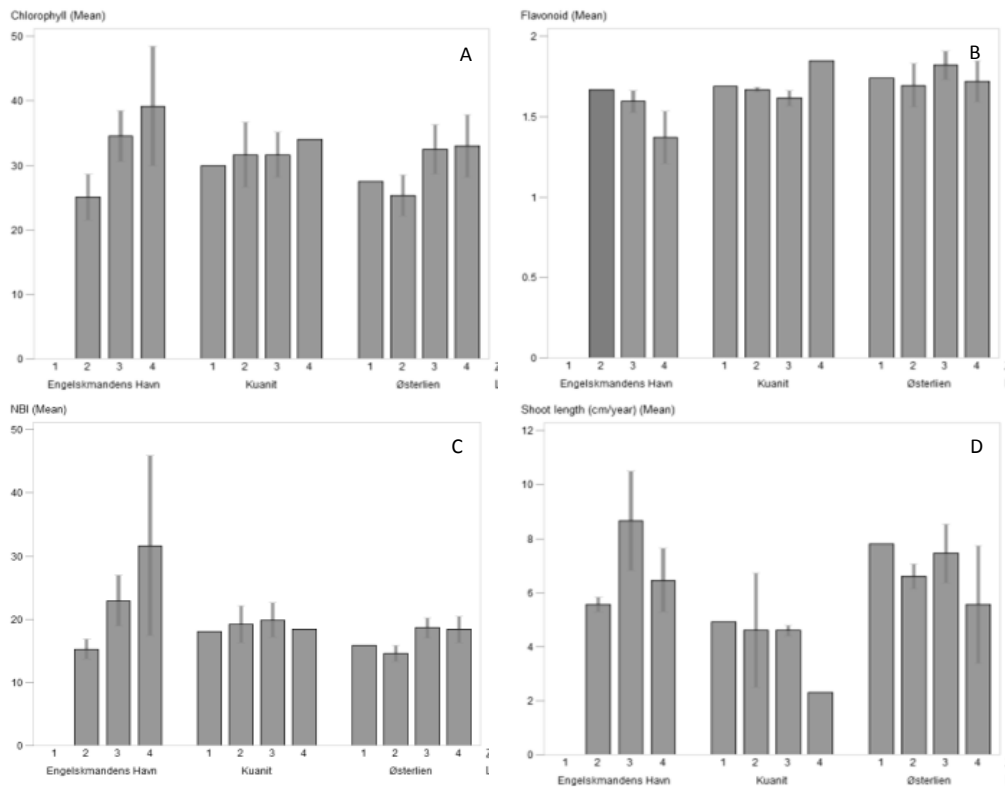**Figure 6. Salix glauca measurements across zones. Average from all locations and transects. Distribution of A) Chlorophyll (µg cm^{-2}), B) Flavonoid (µg cm^{-2}), C) NBI, and D) Shoot length (cm year^{-1}).**
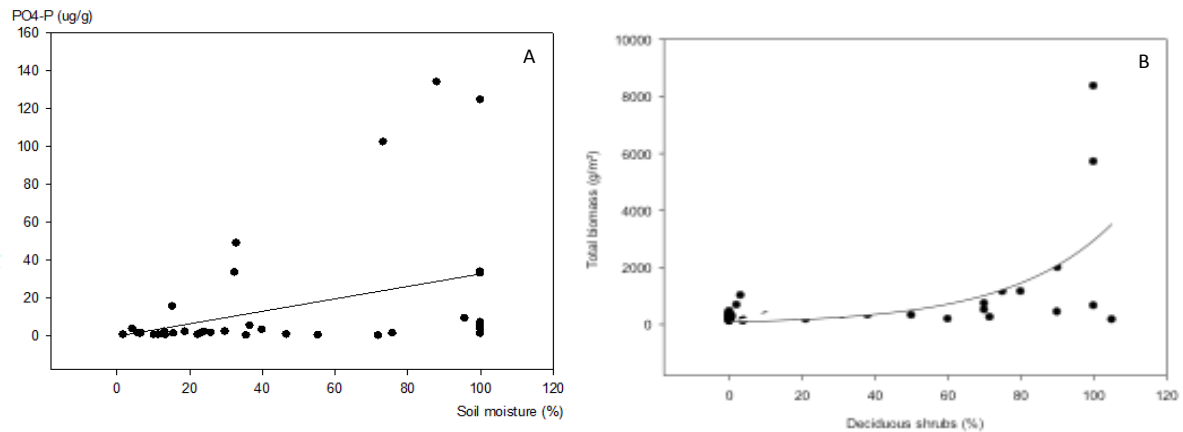
Figure 7 Linear regressions showing the overall zonation trends based on the average from all locations and transects. A) Linear regression showing a significant positive correlation between phosphate content and soil moisture. R^2 = 0.1354; p=0.023; f = -0.2143+0.3297x. B) Exponential correlation between the coverage of deciduous shrubs (%) and the total biomass (g m^{-2}) across zones. R^2 =0.3492; p < 0.0001; f = 84.722 exp(0.0355*x).

Discussion

In general, the studied locations were similar regarding local surroundings. Division of transects into zones was determined by the degree of slope, resulting in shorter zones at steep transects where water runs off and wider zones at flat transects.

Water temperature which was expected to influence the spring microclimate was also similar except for T1-3 at Østerlien (Appendix). This was the only location where the source was found and spring temperature measured at the origin. A possible explanation for the rapid increase in water temperature with distance from the source might be that the spring is fed by meltwater from snow or small glaciers. Possibly, melt water ran beneath the surface and appeared as a source, making a spring that was later on mixed with warmer water from other sources. This geological factor disturbing the homothermic springs as isolated systems may also have a certain influence on other chemical properties than water temperature. If the spring is influenced by melt water and possibly also rock withering, when the water makes its way through the soil, a fair amount of silt might enter the stream, adding nutrients to the water and thus to the soil on the spring banks. Rocks especially are a rich pool of inorganic phosphorous. Therefore, the higher concentration of PO_4 in the soil in Z1 than in Z4 might not only be a consequence of temperature enhanced microbial decomposition. In either

way, it is an interesting effect of the spring on its local surroundings. It is especially noticeable that we found such high values of phosphate in the soil on the banks of the springs, especially at Kuanit (Fig. 3A).

There is not only a significant difference in the phosphate concentration between zones, which has a peak in Z2. Here we might actually be observing a thermal-boosted microbe activity, where the decomposition is enhanced in the closer zones, although slightly limited by waterlogging in Z1.

A good example of a lack in temperature dependency but influence of nutrient is the *S. glauca* distribution across zones, which is significantly dependent on the concentrations of soil nutrients. Actually, both PO_4 and NO_3 in combination show a good linear fit with *S. glauca* coverage. It has been proven earlier by Eckert (2015) that it is not temperature, but nutrients that affect the *Salix* growth. On the other hand, there is also the possibility that the summer water temperature measured in this investigation is rather redundant, because it is in fact the springs' winter temperature that is the most essential.

That the homothermic springs would have effects on the surrounding soil properties, showing a gradual nutrient gradient, is verified from the results found in this study. Both because of a general difference in the abiotic factors measured across zones, but also because of the zone-dependent PO_4-concentration, and the zone differences in the amount of organic matter, soil

12

moisture, and nitrate concentration. The high average OM content measured for Z4 and the relatively low in 1 and 4 across locations is probably due to the difference in the aboveground input of organic matter from the huge pool of biomass coming from *S. glauca* litter. Even though it could be expected that the decomposition of organic matter would be limited by waterlogging in Z1, our results indicate that the *S. glauca* domination in the *Salix* zone (Z3), both when it comes to biomass and coverage, eliminates the water effect on the organic matter pool. Not even the thick layer of moss on the spring banks affects the amount of organic matter, but instead it might be the very local moss bog in Z1, influencing the nutrient binding in the soil, when the exudation of protons from the moss rhizomes binds to the negative soil particles, thereby making the positive ammonium ions outwashable. Furthermore, the bryophytes in Z1 seem to have a positive feedback loop with the soil moisture absorbing and keeping the water, and at the same time thriving because of this plant group's preference for moist habitats.

This domination of one particular plant group in each zone, defining our original division of zones and influencing the zonal vegetation pattern, can be verified to be an important character defining these homothermic spring ecosystems. The division of transects into zones was supported by vegetation analyses with Z1 being dominated by bryophytes, pteridophyte coverage being highest in Z2, mainly consisting of *E. arvense*, Z3 being dominated by S. glauca, and finally the tundra heath zone with the highest amount of evergreen shrubs (Fig. 4D).

But not only do the differences in soil properties influenced by the spring make these clear zone divisions of character plants, the changing plant domination also influences other aspects of the vegetation in the spring habitat. In Z2 there is a high total coverage due to multiple layers of vegetation, with pteridophytes on top, giving light to forbs and moss underneath. This is very unlike *S. glauca*, whose dense vegetation has a very significant negative influence on the biomass of all other plants.

Conclusion

In conclusion, it can certainly be verified that homothermic springs on Greenland constitutes a unique ecosystem with chemical soil properties and vegetation which are very different from the surroundings. This results in a clear zonated transition in dominating plant species and biomass from the banks of the spring to the further surroundings which is driven by the spring microclimate. It is not possible from this study to make general conclusions on whether the enhanced nutrient levels are a consequence of a thermal-boosted microbial activity caused by the geothermal winter heating or simply an effect of the common arctic geological dynamics of ice and snow. However, the conspicuous, tall, green vegetation surrounding the homothermic springs and the presence of southern plant species are clear signs that they are indeed very different from the otherwise sparse arctic vegetation. A temperature-induced gradient which could not directly be verified in this study can only be proved by comparison with non-homeothermic Arctic springs. Even though the nutrient effect is a more interesting phenomenon when taking into account that the arctic plants are to a higher degree constrained by the lack of nutrients than of the cold temperatures (Ulrich and Gersper 1978, Shaver et al. 2001). Especially the nutrient demanding *S. glauca* benefits from relatively richer soils such as those present at homothermic springs. It has before been reported that *S. glauca* responds to an increase in N-availability primarily at the shoot-level by increasing leaf area per shoot (Bowman and Conant, 1994). This increase of *Salix* density has been termed "the greening of the Arctic" (Sturm et al. 2005) and is expected to become more prominent because of climate change. Physiological responses to climate change are in general likely to have important effects on the properties of the ecosystem due to demographic changes, which can lead to shifts in species distribution and relative abundance, composition, and community (Fowbert and Smith, 1994). With our results showing that *S. glauca* coverage has a very significant negative influence on the biomass of all other plant groups, the homothermic spring

vegetation can be a possible indicator of future vegetation patterns.

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Appendix

GPS-position, transect orientation, distance from source, plot position and zone distribution, water temperature in the stream and a general description of each transect at Østerlien (Ø), The Engelskmands Havn (E) and Kuanit (K).

Transect	GPS (DDD° MM.MMM')	Transect orientation (direction from stream)	Distance (m from source)	Plot, zone start-zone end (m from stream)	Water mean temp. (°C)	Description
ØT1	N69°15.366' W053°31.056'	N	0.6	Z1: 0.5, 0-1 Z2: 2.9, 1-4.6 Z3: 10, 4.6-19.8 Z4: 21.6, 19.8-	1.2	T1 was parallel to a W-SW facing rock wall, but slightly slanting upwards. Water flow towards W-SW.
ØT2	N69°15.359' W053°31.060'	W	11	Z1: 0.5, 0-0.5 Z2: 1, 0.5-1.2 Z3: 10.8, 1.2-19 Z4: 22.5, 19-	1.75	Wet, hilly bog like area in stream level (Z1-3) containing boulders creating dry tussock like islands. Otherwise flat until Z4, located on higher rocky ground. Z1-2 were narrow and laid out in 0.5x2m plots.
ØT3	N69°15.343' W053°31.024'	SW	76	Z2: 0.5, 0-1.3 Z3: 2.4, 1.3-3.8 Z4: 6.6, 3.8-	4.1	Flat and wet (Z2-3), then slanting up on dry plateau on rock (Z4). Low rock wall NW of T3 creating a shelf, potentially a snow bed.
ØT4	N69°15.261' W053°30.963'	E-SE	332	Z1: 0.5, 0-1 Z3: 2.5, 1-4.7 Z4: 4.7, 4.7-	No data	Flat temporarily desiccated riverbed below Østerlien Valley. Water ran under ground. T4 lay on a small, steep slope made by the water flow below flat heath tundra. An east facing rock wall west of the stream ran parallel to the water.
ØT5	N69°15.227' W053°30.909'	E-SE	413	Z1: 0.5, 0-1 Z3: 2.5, 1-5 Z4: 6.3, 5-	5.25	The stream at T5 was almost desiccated, yet still contained running water. Otherwise, surrounded by flat tundra heath in level with the stream surface.
ET1	N69°15.723' W053°34.161'	SE	No data	Z1: 0.5 Z2: 3 Z3: 8 Z4: 10	5.7	Almost flat transect (Z1-3), then a steep slope up to dryer ground (Z4).
ET2	N69°15.723' W053°34.161'	NW	No data	Z1: 0 Z2: 2.5 Z3: 4.5 Z4: 9.2	5.7	Transect on opposite side of the stream from ET1. Steep slope from stream and up. Many rocks in the soil.
ET3	N69°15.695' W053°34.151'	SE	No data	Z1: 0 Z2: 2.5 Z3: 5 Z4:10	No data	Valley shielded by rock wall to the east, creating a snow bed (especially in Z4), melted at the day of our visit. Valley characterized by fallen boulders from above and wind from below.
KT1	N69°15.915' W053°26.440'	W (NW)	No data	Z1: 0.5, 0-1.5 Z2: 7, 1.5-14 Z3: 17, 14-	5.4	T1 on a SE faced steep slope. Towards N a high cliff with a waterfall creating some shelter and water spray.
KT2	N69°15.905' W053°26.405'	W	No data	Z1: 1.4, 0-2.1 Z2: 3.6, 2.1-5 Z3: 7.3, 5-9.3 Z4: 11, 9.3-	5.95	Flat transect in stream level (Z1-2), then a rocky slope facing E-SE (Z3-4). The stream might be influenced by other stream systems downstream of T1.
KT3	N69°15.876' W053°26.357'	E (SE)	No data	Z1: 0.5, 0-1 Z2: 1.5, 1-2.5 Z3: 3.5, 2.5-5 Z4: 8, 5-	5.85	The stream at T3 is a distributary to the main stream creating a small flat marshy area (3x3m). Transect flat except for a 0,5m ground increase (Z4). Approx. 100m from the sea coast.

Effects of warming on inter-annual growth of landlocked Arctic charr (*Salvelinus alpinus L.) from Røde Elv, Disko Island, using otoliths as a proxy.

Fishing for Arctic charr in Røde Elv (Kirsten S. Christoffersen, July 2016)

Effects of warming on inter-annual growth of landlocked Arctic charr (Salvelinus alpinus *L.)* from Røde Elv, Disko Island, using otoliths as a proxy

Cecilie K. Hedemand, Søren R. Nielsen & Frederik F. Gai

Abstract

The inter-annual growth rates of a population of landlocked Arctic charr (*Salvelinus alpinus L.*) found in Røde Elv, Disko Island, Greenland, was investigated using otolith analyses. Obtained growth rates were compared to annual variations in temperatures and fork lengths were compared to a previous study in the same area to investigate whether or not temperature has effects on growth. Results showed a positive correlation between annual temperatures and growth rates (r^2 = 0.92, p<0.05, α = 0.05). This relationship could be explained by the winter temperatures (r^2 = 0.97, p<0.05, α = 0.05), but not by the summer temperatures (r^2 = 0.09, p>0.05, α = 0.05). Annual growth rates ranged from 1.9 mm*month^{-1} in the coldest year to 2.6 mm*month^{-1} in the warmer years. Statistical comparisons with earlier studies were not possible due to differences in calculations and locations. However, the results from the present study suggest that fork lengths have increased since the earlier study, indicating possible long-term benefits of warming in the area.

Keywords: Arctic charr, otolith analysis, warming, Greenland, Disko

Introduction

Arctic freshwater ecosystems are complex and diverse with respect to structure and function of the food web. There are few freshwater fishes in the high Arctic region with the Arctic charr (*Salvelinus alpinus L.)* as the most abundant and widespread species (Reist et al. 2006). Most Arctic freshwater ecosystems are influenced by strong seasonality (long, cold winters and short, chill summers), generally low levels of precipitation which mostly falls as snow, high inter-annual variability in temperatures and long-term ice cover (Prowse et al. 2006b), all of which are prominent features of the Arctic climate. As a consequence of global warming, mean annual air temperatures are increasing, and are expected to increase by more than 2°C by the end of this century, with an even greater temperature rise predicted for the Arctic region relative to the global mean (IPCC 2014). According to the "metabolic theory of ecology", an increase in temperature can enhance metabolic rates, which in turn will increase respiration (Brown et al. 2004). Each species has a temperature optimum, and its growth is restrained above a certain threshold value. Fish are poikilotherms and are therefore strongly influenced by increases in temperature in their environment (Clarke and Johnston 1999). The metabolic processes in fish are therefore also highly temperature dependent, and an increase in ambient temperature leading to an increase in the metabolic rate without a sufficient food supply can imply a reduction in growth rates (Russell et al. 1996). Furthermore, the abundance and composition of food resources available to higher trophic levels are likely to be dependent on warming as well (O'Gorman et al. 2016). Freshwater ecosystems are predicted to be particularly sensitive to the impacts of warming due to the constraint in potential species range, and thus species with a freshwater-only life cycle might not be able to relocate to thermal optima, and must seek thermal refugia or adapt to the warmer environment in order to survive (Hoffmann and Sgró 2011; O'Gorman et al. 2016). Failure to conform will eventually lead to a loss of fitness.

The mean annual air temperatures on Disko had increased significantly from 1991 (Hansen et al. 2003). According to previous studies of fish conducted at Disko Island, the growth rates of

the cold adapted Arctic charr in Røde Elv have shown to be sensitive to changes in temperature (Kristensen et al. 2006, Mordhorst and Due 1990). Growth rates obtained in the years 1982-1989 (Mordhorst and Due 1990) were significantly higher than growth rates obtained between 1995 and 2003 (Kristensen et al. 2006).

The thin growth bands of otoliths in many fish species, corresponds directly to annual growth, which is also the case for the Arctic charr. Studies of otoliths have become an important tool for understanding and measuring growth in fish (Pannella 1971). Comparing the growth rates of Arctic charr to mean annual temperatures can therefore be used as a proxy for future climate change in the Arctic.

In this study we aim to use otoliths in landlocked Arctic charr from Røde Elv, Disko Island, in order to determine annual growth rates and to relate growth rates to mean annual temperatures obtained at a nearby weather station as well as summer- and winter temperatures from Disko Island during the lifespan of the fishes, to establish to what extend these have an effect on the growth rates of the Arctic charr population. Further, we aim to compare the obtained results to previously published data on growth rates of the Arctic charr (Mordhorst and Due 1990) in order to investigate long-term effects on growth rates from the expected intensified warming in the Arctic in the years 1982-2016. Results are only compared to this one study, due to possible differences in populations from other studies. We hypothesised that an increase in temperature has negative effects on Arctic charr growth rates, and that intensified warming has led to further decreases in growth rates since 1982.

Methods and Materials

Røde Elv, Disko Island was chosen as sampling site due to its populations of homogenous and landlocked charr as described in Kristensen et al. 2006. The specific location at which the Arctic charr were caught, was not the same as in Kristensen et al. 2006, but in a pool connected to the main stream (69°16'44.9"N 53°28'42.3"W) (Fig. 1). Collection sites of Mordhorst and Due

(1990) and Kristensen et al. (2006) are also shown in Fig. 1.

Most of the charr were caught using a modified multi-mesh gill net, which is designed to catch different size classes representative of a population. The original net was 40 meters long with 12 different mesh sizes from 6.25 to 43 mm. The net was split into smaller segments in order to match the size of the small pool where the fish were caught. They were then placed in the middle of the clear pool, where fish abundance was visually determined to be high. Furthermore, one third of the charr were caught by angling using small flies as bait.

The specimens were taken back to the laboratory, where physical and morphological measurements were conducted. The fish were measured for weight, fork length, gender, level of female gonadal maturation (1, 2 or 3: 1 being the
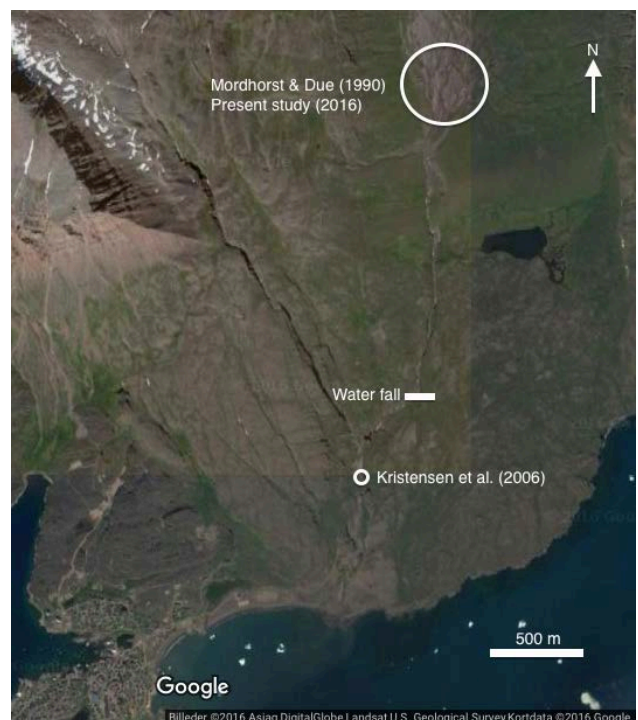

Figure 1. Map of southern Disko Island, showing the sample spots in 1990 (Mordhorst and Due 1990), 2004 (Kristensen et al. 2006) and the present study (2016).

least developed, 3 being highly developed), colour of flesh and abdominal skin, readily identifiable stomach contents and macroscopic parasites. The otoliths of the charr were removed by opening the top of the skull using scalpels, clearing away the brain by forceps or pipette after which the otoliths could be retrieved,
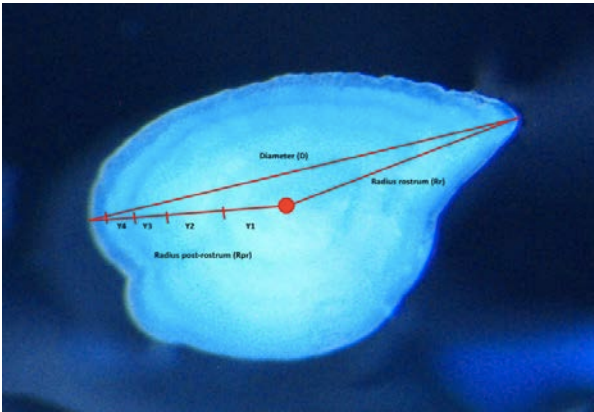
Figure 2. Arctic charr otolith from specimen #17. Lines show diameter and radii used for calculations.

cleansed in water and stored in zip-lock plastic bags.

For analysis the otoliths were placed in a droplet of tap water on a microscope slide with the sulcus of the otolith facing downwards. Digital images of the otoliths were obtained using an Olympus SZX-12 stereomicroscope fitted with a digital Olympus DP20 camera. All images were taken in a magnification of 20X. In order to measure distances on the otoliths and between growth bands, we used a digital image analysis tool (Image J 1.44k, Image J Inc. 2010). Distances in this program are measured in pixels and were converted to mm by taking a photo of a ruler in 20X magnification. Using Image J the distance of 1 mm was measured 10 times which gave a mean of 401.2 pixels mm^{-1}. A centre point on the otoliths was determined in order to obtain the radius from centre to rostrum (Rr) and post rostrum (Rpr) and the otolith diameter (length) (D) (Fig. 2). The distance from the centre point to the onset of the first transparent zone is defined as the first year of growth (Y1) (Godiksen 2010). From the beginning of the first transparent zone to the beginning of the next is defined as the second year of growth (Y2) and so forth. The most recent growth band was not included, since the annual growth for 2016 has not yet ended.

Calculations

Back calculations of fork lengths were done using measurements of otolith radii as well as fork lengths and age at time of capture (Morita and Matsuishi 2001; Nielsen 2011). This back calculation includes the allometrically uncoupled

growth effects of age and fork lengths on Arctic charr otoliths, assuming the relationship:

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

where O = otolith length, L = fork length and t = fish age, all at time of capture. α, β and γ are constants obtained through multiple linear regression analysis of the 3 factors (www.vassarstats.net). Thus, assuming that these growth effects of the former relationship affects the Arctic charr throughout their lifespan, the constants can be used in back calculation of fork lengths in the following equation from Morita and Matsuishi (2001), corrected as suggested by Nielsen (2011):

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

where T = age at time of capture, t = age at time t, L_t = fork length at age t, L_T = fork length at time of capture, O_t = otolith radius at age t and O_T = otolith radius at time of capture. Logistic curve fitting was applied on all individual growth curves in order to avoid maternal ontogenetic effects, as fish growth is dependent on the age and life stage of the individual fish.

Growth rates of all individuals were obtained using the relationship:

$$Gt = L_{t+1} - L_t$$

Mean growth rates for the specific years were calculated.

Long-term temperature data was obtained from the Disko Basis monitoring program from Arctic Station (AWS-1). We defined the winter season as the period from September 1st to May 31st, and summer from June 1st to August [3rd].

Statistics

Linear regression analysis (www.graphpad.com) was used to find correlations between temperature, growth rates, precipitation, fork length and postrostrum radius.

Using one-way ANOVA, including a TUKEY post-hoc test (www.graphpad.com) we compared the annual growth rates obtained in this study from 2012 to 2015.

Table 1. Overview of fish number, fork lengths and age.

# Fish	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Length (mm)	155	146	130	136	145	218	125	122	104	144	121	124	124	118	137	115
Age (y)	5	5	4	4	5	9	3	4	4	4	4	4	5	4	5	3

# Fish	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Length (mm)	132	135	122	112	127	115	120	105	95	82	87	83	78	77	80	80	70
Age (y)	4	4	5	5	4	4	4	4	3	3	4	4	3	3	3	4	2

Results

The fork length of the fish ranged from 70 mm to 218 mm (Tab. 1) and weighed from 3.06 g to 66.36 g (Tab. 1 and Appendix). Fish age ranged from 2 to 9 years (Tab. 1).

Of the 33 individuals, 20 were males and 13 were females (Tab. 1 and Appendix). Ventral skin colour varied from deep orange to pearlish white (Tab. 1 and Appendix). We found no correlation between skin colour and age, or between skin colour and sexual maturity. These data were not investigated further and is not presented in this study. Dissection revealed completely uncoloured meat, with the exception of the biggest individual, which had a distinct orange tint. Macroscopic parasites were found in 4 of the 33 individuals (Tab. 1 and Appendix). Further dissection gave insights into foraging habits of the population, which revealed a diet primarily consisting of Diptera (mosquitoes) and a few Trichoptera (Tab. 1 and Appendix).

Linear regression analysis between otolith postrostrum radius and fork length was found to be highly significantly positive ($r^2 = 0.75$, $p<0.0001$, $\alpha= 0.05$) (Fig. 3) and the constants used in the further calculations. From estimated fork lengths obtained from back-calculation, the relationship between fork length and age could be depicted alongside results from previous studies (Fig. 4). It turned out that the present study found higher fork lengths at all ages (age 1 to 8). From age 6 to 9, however, the results of the present study were based on one single individual and are therefore not included in the statistical analyses. This data is available in the appendix (Fig. 1-4 and Appendix).

Mean annual growth rates of the Arctic charr from 2008 to 2015 showed increased growth (approx. 1.5 mm*month[-1]) until 2013 (approx. 2.8 mm*month[-1]), where it remained the same until 2014 and decreasing rapidly in 2015 (approx. 1.83 mm*month[-1]) (Fig. 5). Growth rates from the years 2008 to 2011 were based upon one single individual and will not be part of the statistical analyses throughout this study. Growth rates from 2013 and 2014 were significantly higher than in 2015 ($p<0.001$, $\alpha = 0.05$), whereas no significant differences were found between growth rates from 2012 compared to 2013, 2014 or 2015.
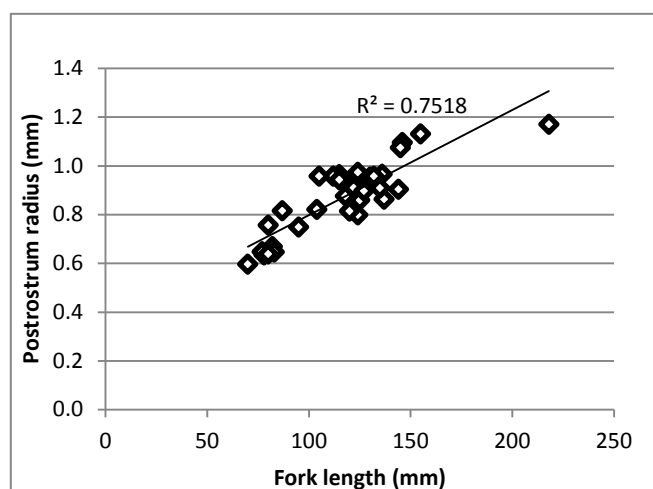

Figure 3. Linear regression of otolith postrostrum radius (mm) correlated with fork length (mm) ($p<0.0001$, $\alpha = 0.05$)
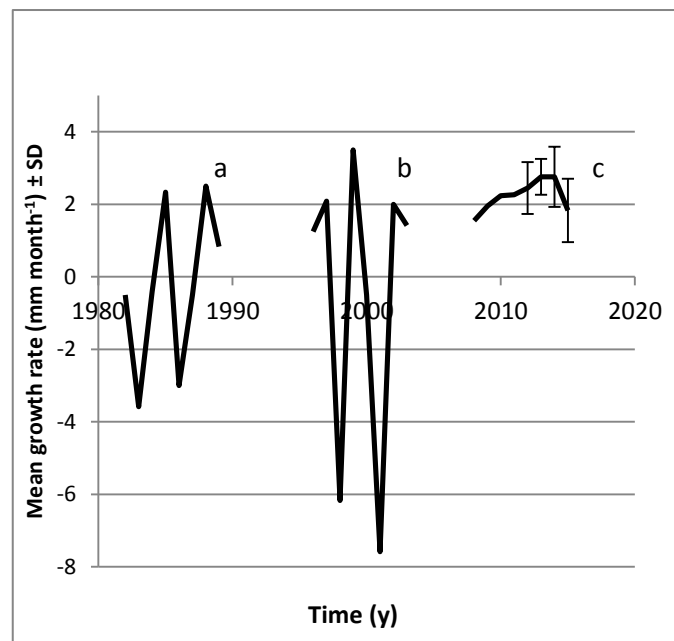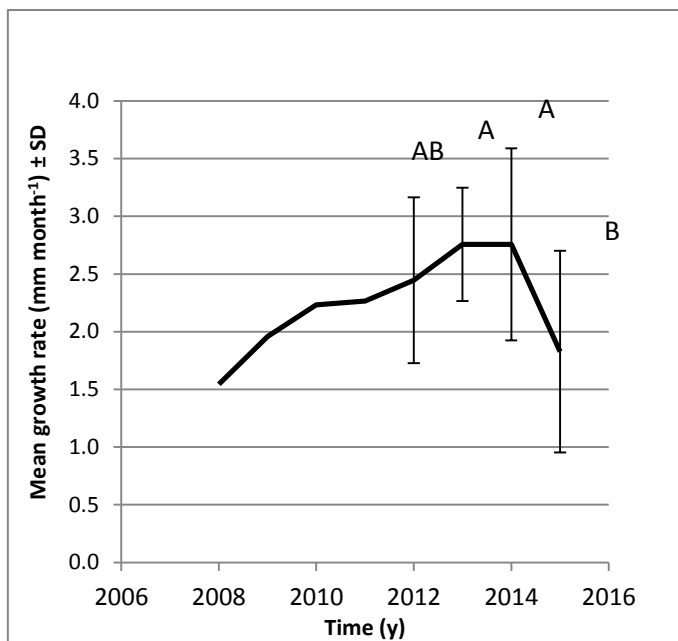
Figure 5. Mean annual growth rates (mm month^{-1}) ± SD of Arctic charr in the years 2008-2015. Only data from 2012-2015 was included in the statistical analyses, as data from 2008-2011 was based on one individual. Means with the same letter are not significantly different, and thus growth rates from 2013 and 2014 are significantly different from 2015 ($p<0.001$, $\alpha = 0.05$), while growth rates from 2012 are not significantly different from the other years.

Figure 5. Growth rates (mm month^{-1}) ± SD plotted against time in years of data obtained in 1990 (a), 2004 (b) and the present study, 2016 (c).

Visual comparison of growth rates from the present study and the two previous studies shows very high fluctuations of positive and negative growth rates in both the study from 1990 (Mordhorst and Due 1990) and the study from 2004 (Kristensen et al. 2006) (Fig. 6a and

6b), whereas more stable, positive growth rates were found in this study (Fig. 6c). The results however derive from different methods and calculations, and are therefore not statistically comparable.

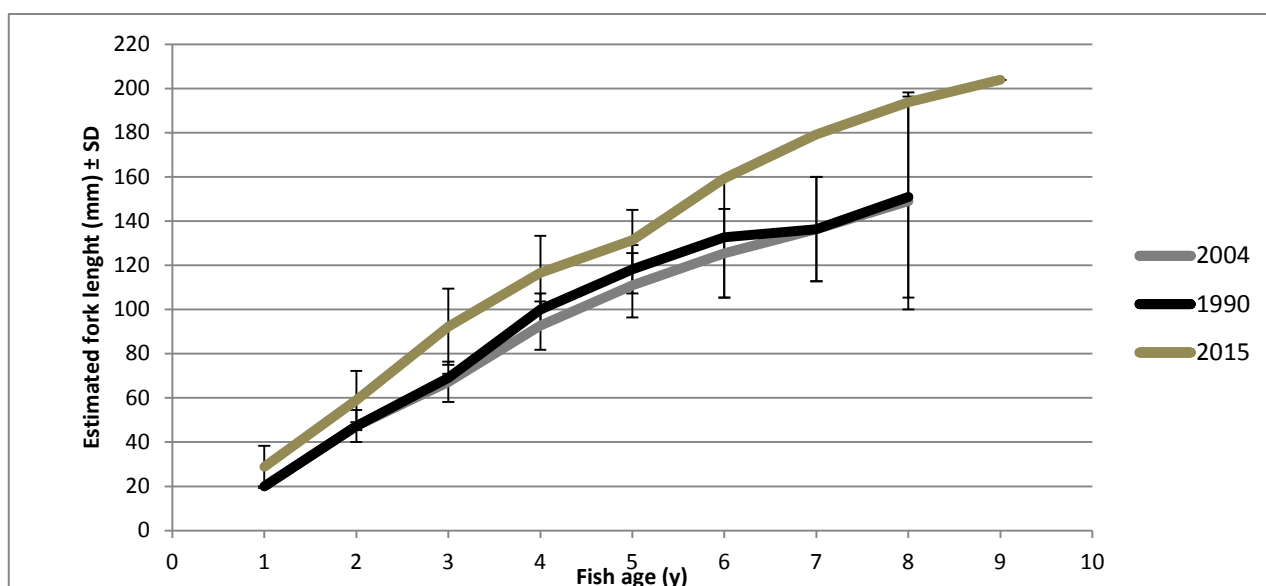Age specific growth rates were highest in the

Figure 6. Estimated fork length (mm) ± SD plotted against fish age (y) of data obtained in 1990 (Mordhorst and Due 1990), 2004 (Kristensen et al. 2006) and 2016 (the present study).
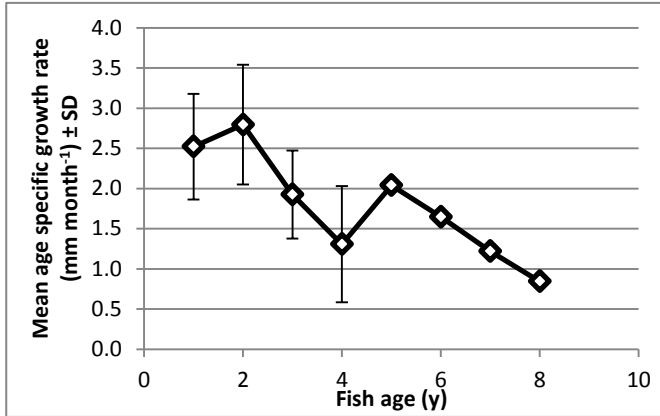
21

Figure 9. Mean age specific growth rate (mm month^{-1}) ± SD of ages 1 to 8.

young fish. Age 1 and 2 were significantly different from age 3 and 4 (p<0.05, α = 0.05). Age specific growth rates then decrease through age 3, which is significantly different from age 1, 2 and 4 (p<0.05, α = 0.05), until age 4, which is significantly different from 1, 2 and 3 (p<0.05, α = 0.05) (Fig. 7).

A significant relationship was found between

growth rates and annual air temperatures (Fig. 8) (p<0.05, α = 0.05, r^2 = 0.92). This significant relationship was enhanced when focusing on the growth rates in comparison with only mean winter temperatures (Fig. 9) (p<<0.05, α = 0.05, r^2 = 0.97). No significant relationship was found between growth rates and mean summer temperatures (Fig. 10) (p>0.05, α = 0.05, r^2 = 0.09), nor between growth rates and mean annual precipitation (Fig. 11) (p>0.05, α = 0.05, r^2 = 0.14).

Mean annual temperatures have increased during the last decades (r^2 = 0.54), starting from a mean temperature of − 6 °C in 1982 to − 4.5 °C in 2015 (Fig. 12).

Discussion

Contradictory to the main hypothesis of this study, our results show a significant positive linear correlation between mean annual temperature and growth rates (Fig. 8). These
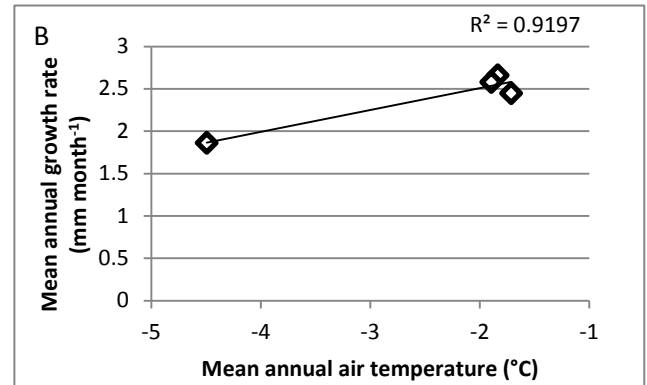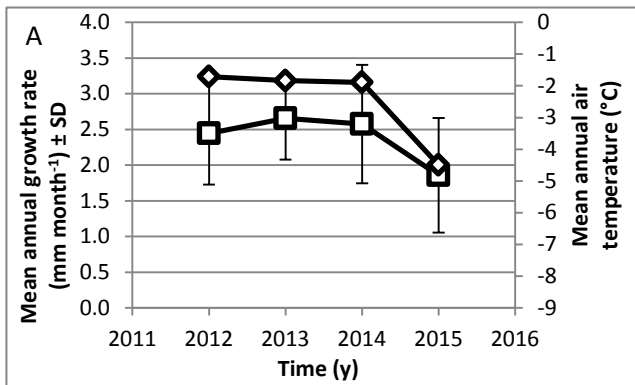

Figure 8. A) Mean annual growth rate (mm month^{-1}) ± SD (□) and mean annual air temperature (°C) (◇) plotted against time from 2012-2015. B) Mean annual growth rate (mm month^{-1}) ± SD plotted against mean annual air temperature (°C) (p<0.05, α = 0.05).
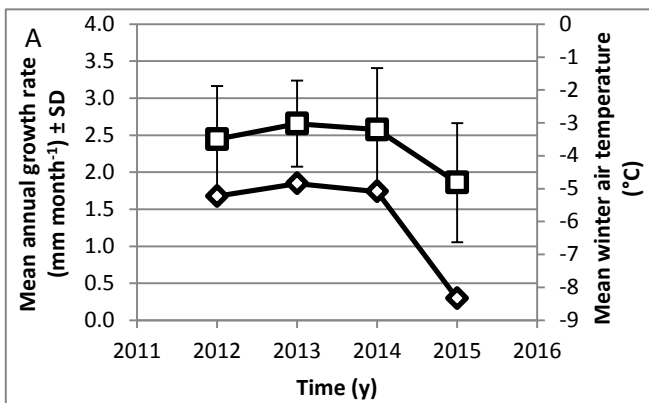
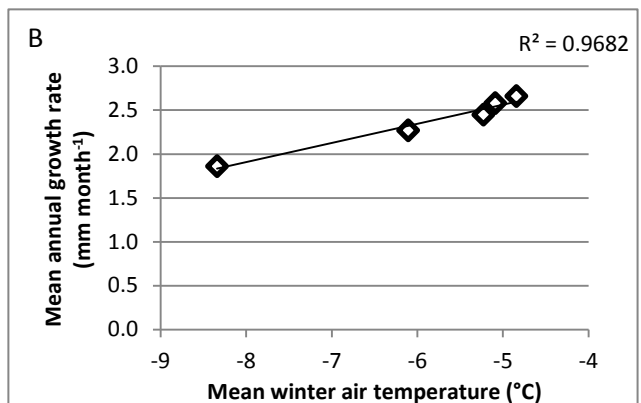**Figure 8. A) Mean annual growth rate (mm month^{-1}) ± SD (□) and mean winter air temperature (°C) (◇) plotted against time from 2012-2015. B) Mean annual growth rate (mm month^{-1}) ± SD plotted against mean winter air temperature (°C) (p<<0.05, α = 0.05).**

22

Figure 11. A) Mean annual growth rate (mm month^{-1}) ± SD (□) and mean summer air temperature (°C) (◇) plotted against time from 2012-2015. B) Mean annual growth rate (mm month^{-1}) ± SD plotted against mean summer air temperature (°C) (p>0.05, α = 0.05)

Figure 11. a) Mean annual growth rate (mm month^{-1}) ± SD (□) and mean annual precipitation (mm) (◇) plotted against time from 2012-2015. b) Mean annual growth rate (mm month^{-1}) ± SD plotted against mean annual precipitation (mm) (p>0.05, α = 0.05).
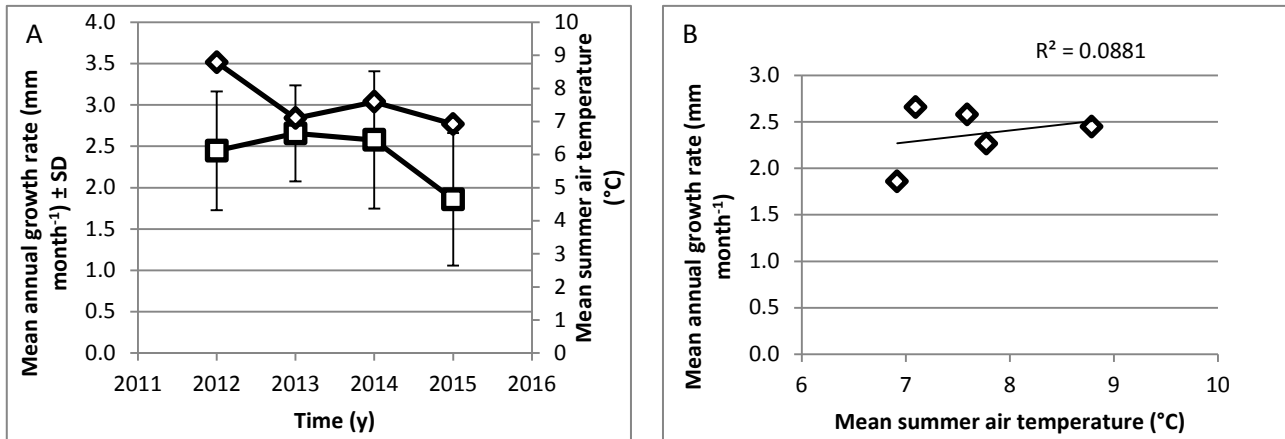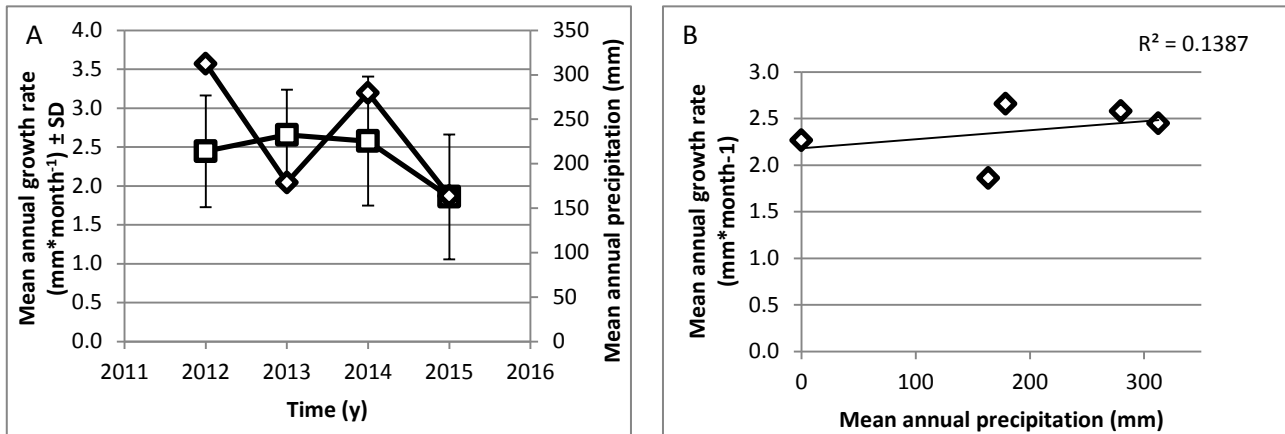
findings are consistent with results from 1990 from the same population (Mordhorst and Due, 1990), where growth of Arctic charr was positively correlated to increasing temperatures. The factor explaining this correlation was found to be mean winter temperature (Fig. 9) whereas mean summer temperature did not significantly explain the observed correlation (Fig. 10). This means that growth rates of the Arctic charr from Røde Elv are determined by temperature only in the winter season, and indicates that physiological or environmental conditions other than temperature are more significant in determining growth in the summer season. Great versatility in feeding strategies and specific food objects are found between different species of the Arctic charr (Fraser et al. 2007). Studies of the stomach contents of the charr in the present study (July) showed that the primary food source

was Diptera, which are abundant during the summer season and we therefore suggest that food is a limiting the growth during the summer. Furthermore, microscopy of otolith growth rings showed that approx. ⅔ to ¾ of the yearly growth increment was during the summer period (Fig. 2), where the abundance of Diptera is high. In a warmer Arctic, where spring arrives earlier and earlier (Høye et al. 2007), the mosquito season is prolonged, and studies from Western Greenland have shown that mosquito mortality is lowered in a warmer environment as well (Culler et al. 2015). The findings in this study suggest that the charr is still living below its temperature optimum and thus was not stressed by temperature directly. However, as the optimum temperature for fish growth can only be found when there is no food limitation (Jobling 1981), this remains unclear. Therefore we hypothesize that the charr

of Røde Elv have the potential to increase growth in the summer season if the food supply would indeed increase.

As our study confirms, lowered temperature has a negative effect of the growth of Arctic charr. The question is, however, if it is the physiological effects of a decrease in temperatures in the winter that lowers the growth rate or if the lowered temperatures cause other challenges for the Arctic charr. Research has shown that growth of Arctic charr is lowered in winters with exceptionally low temperatures (Michaud et al. 2010), as is also the case in the present study when observing the drop in growth rates during the cold winter of 2015 (Fig. 5). However, it also seems possible that a colder winter would isolate the charr from food sources coming from above, such as Diptera, further into the spring, by delaying the melting of the ice cover. This would be the case especially in some of the slower, still pools where most of our samples were taken.

In addition to mean annual temperatures, growth rates were also compared to precipitation data. However no significant correlation was found (Fig. 11). Modelling has shown that precipitation will increase in the Arctic region (Peterson et al. 2006) and more minerals and nutrients will be washed into the stream, changing the biological systems in Røde Elv and it seems reasonable to suggest that this could easily have a significant biological impact on the Arctic charr, even just by increasing the amount of water in the system. Since no correlation between precipitation and growth rates was found, it is possible that the slower streams and

pools of the sample site is not as highly influenced as the main stream, or that in the case of rainfall, the "mudding" of the waters are too temporary to have a significant impact.

Data from the present study is only compared with data from 1990 (Mordhorst and Due, 1990), since these arise from the same geographical location. This site is most likely entirely geographically isolated from the site used by Kristensen et al. (2006). The two sites are a couple of kilometres apart, and separated by waterfalls too steep to overcome (Fig. 1). Kristensen et al. (2006) argue that the change from a positive to a negative correlation between temperature and growth rate from 1990 to 2004 are due to the energy resources in Røde Elv not being able to sustain high metabolic rates caused by increasing temperatures, which in turn decreases the fitness of the fish and therefore the growth rates. This interpretation, however, does not take the difference in sampling sites into consideration. The sample site of the present study and Mordhorst and Due (1990) consisted of a vast array of smaller streams and pools accessible for the charr, in addition to the main stream. These life conditions might prove superior to those downstream the waterfall, used by Kristensen et al. (2006), where the current is strong and where calm pools are scarce or non-existent. Such conditions might result in lower fitness and thereby lower growth rates of this population.

The mean annual temperatures in the study area have increased throughout the last decades (Fig. 12). Comparing growth rates from the present study to earlier findings was, however,
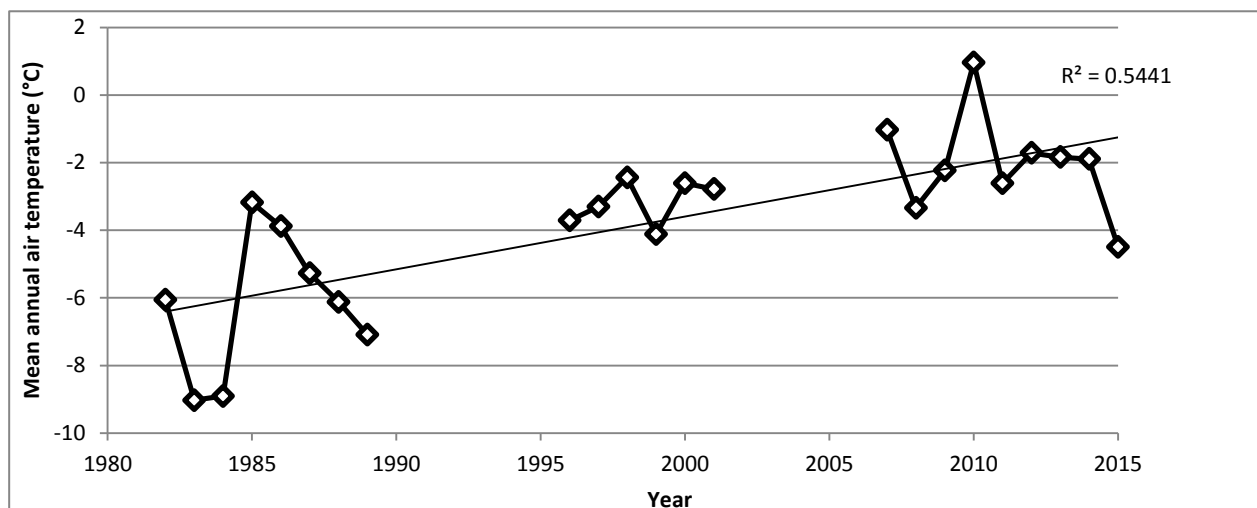

Figure 12. Mean annual air temperatures (°C) for Aasiaat (1982-1989), Qeqertarsuaq (1996-2001) and Qeqertarsuaq (2007-2015).

not possible, since the published growth rates are derived from a faulty modelling function for back calculation (Morita and Matsuishi 2001) – the same model used in the present study, however corrected as suggested by Nielsen (2011). This was apparent when observing the highly fluctuating and negative growth rates from Kristensen et al. (2006) (Fig.6), which was also inconsistent with their estimated fork length results (Fig. 4), as neither of these are decreasing on a yearly basis.

The comparison of data is further complicated by the fact that Kristensen et al. (2006) have used air temperature data from Aasiaat and not from Qeqertarsuaq at Disko Island for the years 1982-1990, when comparing growth rates to annual temperatures (Fig. 4). Obviously, this was done in an attempt to create a larger temporal picture, which is justifiable. It does however add an inconsistency, which is very hard to account for. The distance between the two weather stations is approx. 70 km and differences in topography and orientation might cause meteorological differences in mean annual air temperature, questioning the very idea of comparing these data to the recent.

With this in mind, it was not possible to make a statistical comparison of fork length data from data obtained in 1990 (Mordhorst and Due 1990, Kristensen et al. 2006). Back calculations of fork lengths are however always based on the measured lengths of the fish, and therefore some visual comparison can be made from Fig. 4. Comparing the results from the present study to the earlier findings, it is apparent that fork lengths from this study are somewhat higher at all ages, indicating an increase in annual growth since 1990, possibly due to the higher temperatures in the area (Fig. 4).

As an extension of this study, further analysis of the otoliths could be conducted, comparing each summer and winter band, making detailed comparison of the growth seasons possible. For this to be feasible, additional time with microscopy must be anticipated, and possibly each otolith would require polishing in order to distinguish the individual seasons. Furthermore, if raw data from 1990 to 2004 was to become available, the same mathematical functions that were used in this study could be applied and thus

a proper statistical comparison could be made. This could in turn provide information about the long-term effects of warming on Arctic charr in Røde Elv.

Further studies of stomach contents, including sampling throughout the summer season, could provide an interesting insight into feeding habits, which could give an understanding of the food subject dynamics of the Arctic charr over the growth season, instead of the momentary picture provided by this study.

Acknowledgements

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Appendix

Figure 1. Mean annual growth rate (mm month^{-1}) ± SD (□) and mean annual air temperature (°C) (◇) plotted against time from 2008-2015.

Figure 2. Mean annual growth rate (mm month^{-1}) ± SD (□) and mean winter air temperature (°C) (◇) plotted against time from 2008-2015.
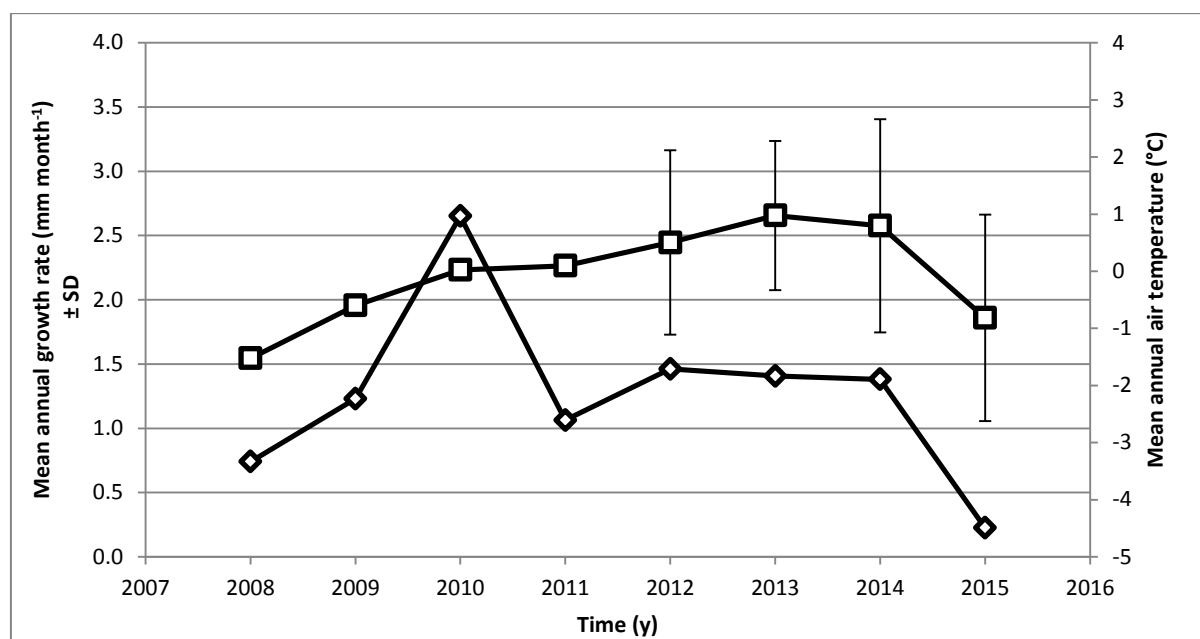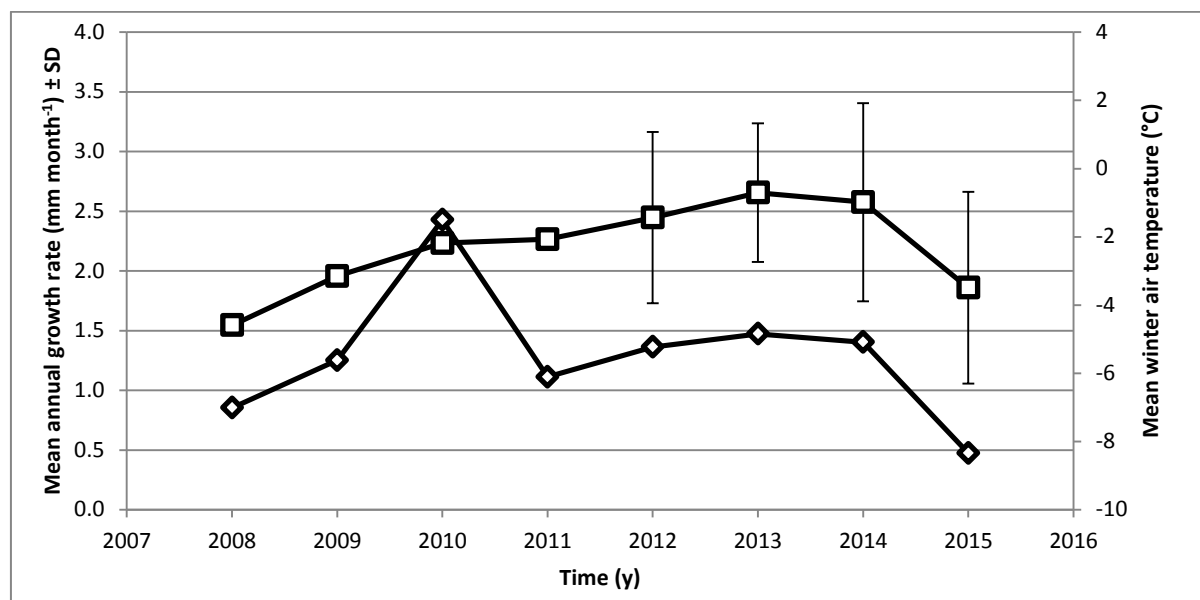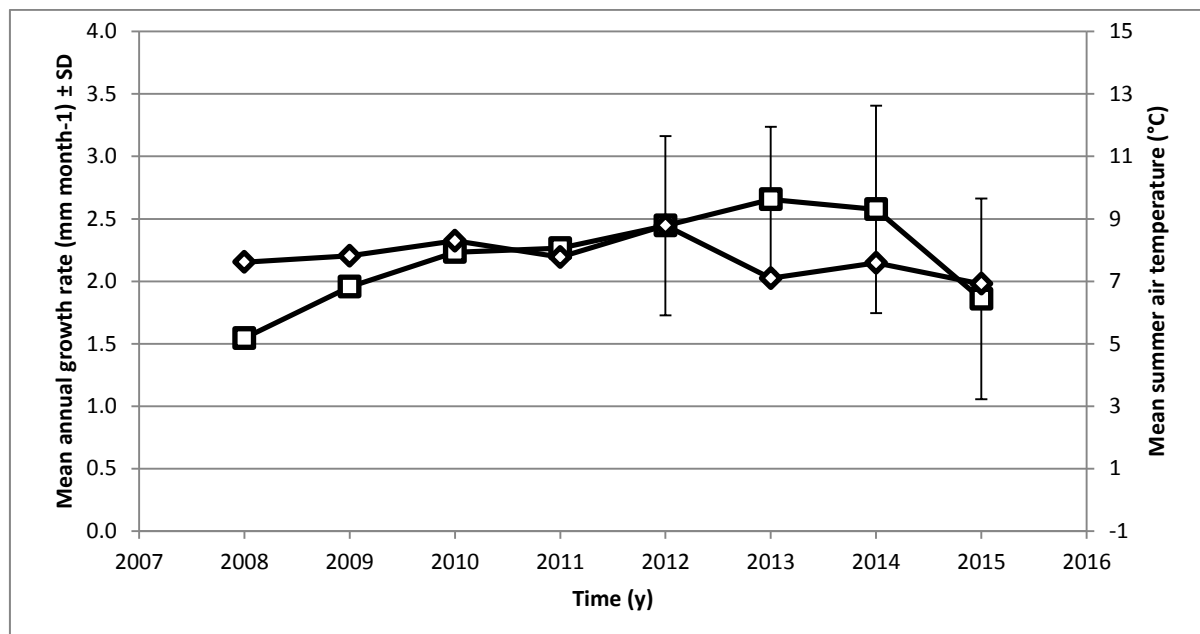
Figure 3. Mean annual growth rate (mm month^{-1}) ± SD (□) and mean summer air temperature (°C) (◇) plotted against time from 2008-2015.
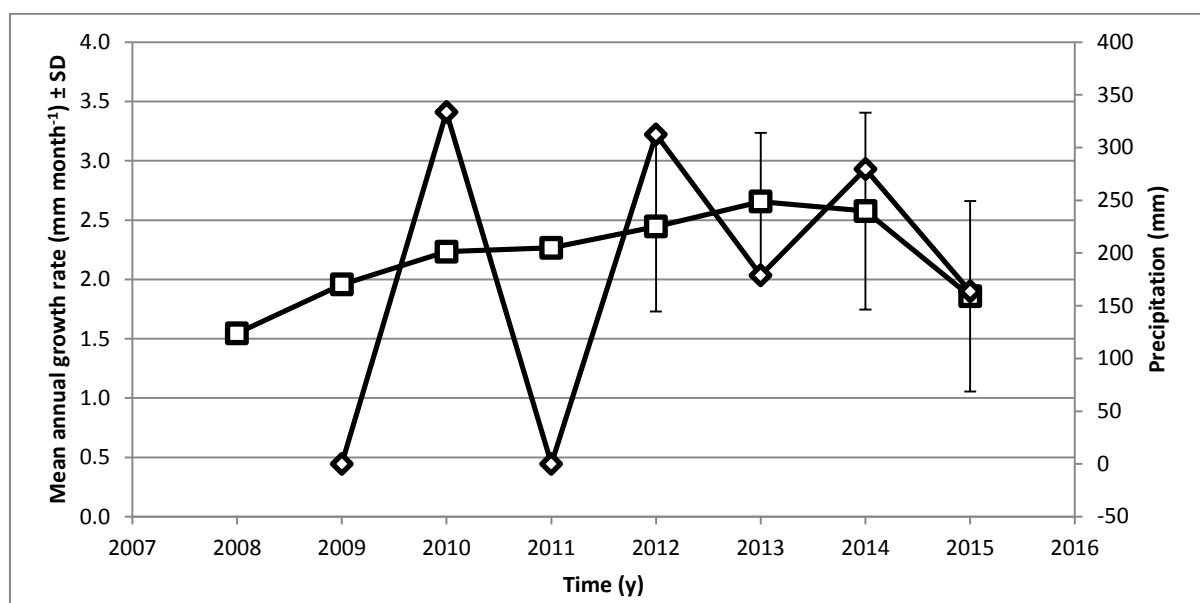

Figure 4. Mean annual growth rate (mm month^{-1}) ± SD (□) and mean annual precipitation (mm) (◇) plotted against time from 2008-2015.

# Fish	Length (mm)	Weight (g)	Macroscopic parasites	Colour skin	Colour meat	Stomach content	Sex	Degree of egg maturation (1,2,3)	O diameter (mm)	R Rostrum (mm)	R Postrostrum (mm)	Year 1 (mm)	Year 2 (mm)	Year 3 (mm)	Year 4 (mm)	Year 5 (mm)	Year 6 (mm)	Year 7 (mm)	Year 8 (mm)	Year 9 (mm)	Age (capture)
1	155	27.48	0	Orange+	White	Diptera	M	-	2.61	1.51	1.13	0.24	0.41	0.64	0.75	0.92					5
2	146	23.9	6 (cestoda)	Orange+	White	Diptera	F	3	2.62	1.61	1.09	0.29	0.55	0.72	0.84	0.90					5
3	130	16.81	0	Orange -	White	Diptera	F	3	2.08	1.24	0.95	0.22	0.39	0.72	0.83						4
4	136	19.45	0	Orange+	White	-	M	-	2.39	1.47	0.97	0.27	0.47	0.65	0.83						4
5	145	27.32	0	Orange+	White	Diptera	M	-	2.61	1.57	1.07	0.32	0.64	0.77	0.87	0.96					5
6	218	66.36	0	White	Yellow-orange	Diptera	F	3	2.81	1.74	1.17	0.24	0.35	0.47	0.62	0.69	0.79	0.85	0.91	1.03	9
7	125	17	0	Orange -	White	Diptera	M	-	2.01	1.23	0.86	0.31	0.52	0.71							3
8	122	16.39	0	Orange -	White	-	M	-	2.13	1.21	0.93	0.23	0.42	0.62	0.76						4
9	104	10.77	0	White	White	-	M	-	1.74	0.95	0.82	0.23	0.35	0.63	0.76						4
10	144	23.27	0	Orange+	White	Diptera + Tricoptera	F	2	2.20	1.32	0.90	0.26	0.44	0.59	0.72						4
11	121	16.02	1	Orange-	White	Diptera	F	2	2.23	1.35	0.92	0.24	0.40	0.64	0.78						4
12	124	15.57	0	Orange-	White	Diptera	F	3	2.09	1.35	0.80	0.20	0.45	0.59	0.70						4
13	124	17.46	0	Orange	White	Diptera + Tricoptera	M	-	2.27	1.31	0.97	0.26	0.41	0.52	0.70	0.87					5
14	118	16.18	0	Orange-	White	Diptera	M	-	1.90	1.05	0.88	0.26	0.42	0.60	0.74						4
15	137	21.59	0	Orange-	White	-	F	2	2.28	1.49	0.86	0.22	0.43	0.55	0.68	0.77					5
16	115	13.49	0	Orange-	White	Diptera	M	-	1.97	1.07	0.96	0.31	0.50	0.76							3
17	132	17.77	0	Orange	White	Diptera + Tricoptera	M	-	2.11	1.19	0.96	0.29	0.57	0.72	0.86						4
18	135	22.74	0	White	White	Diptera	M	-	2.09	1.22	0.91	0.41	0.57	0.72	0.86						4
19	122	15.56	0	White	White	-	F	1	2.14	1.28	0.91	0.27	0.48	0.64	0.77	0.86					5
20	112	13.64	0	Orange-	White	Diptera	M	-	2.30	1.37	0.96	0.24	0.44	0.56	0.67	0.78					5
21	127	19.83	0	Orange-	White	-	F	2	2.05	1.18	0.90	0.14	0.34	0.58	0.74						4
22	115	14.06	0	White	White	Diptera	F	1	2.25	1.36	0.94	0.30	0.56	0.67	0.79						4
23	120	14.93	0	Orange-	Wihte	-	F	2	2.07	1.04	0.81	0.21	0.35	0.50	0.65						4
24	105	11.73	0	Orange-	White	Diptera + Tricoptera	M	-	2.25	1.35	0.96	0.27	0.59	0.68	0.83						4
25	95	7.18	0	White	White	Diptera	F	1	1.70	1.07	0.75	0.31	0.45	0.63							3
26	82	6.03	0	Orange-	White	Diptera	M		1.54	0.90	0.67	0.37	0.48	0.59							3
27	87	6.84	0	Orange-	White	Diptera	F	1	1.96	1.21	0.81	0.24	0.34	0.50	0.62						4
28	83	5.6	0	Orange-	White	Diptera	M		1.65	1.00	0.65	0.19	0.31	0.42	0.55						4
29	78	5.7	0	Orange-	White	-	M		1.43	0.84	0.63	0.21	0.34	0.49							3
30	77	4.75	0	Orange-	White	Tricoptera	M		1.48	0.86	0.65	0.27	0.39	0.52							3
31	80	4.86	0	White	White	Diptera	M		1.75	1.02	0.76	0.32	0.50	0.62							3
32	80	4.42	1	Orange-	White	-	M		1.57	0.97	0.64	0.27	0.40	0.47	0.56						4
33	70	3.06	1	White	White	Diptera	M		1.42	0.86	0.60	0.26	0.42								2

Table 1. Specific observations, measurements and calculations from the 33 Arctic charr caught in Røde Elv in the present study. Years 1, 2, 3 etc. are distances from postrostrum to the yearly growth bands.

Effect of gall mites on photosynthesis and stress in two species of Salix
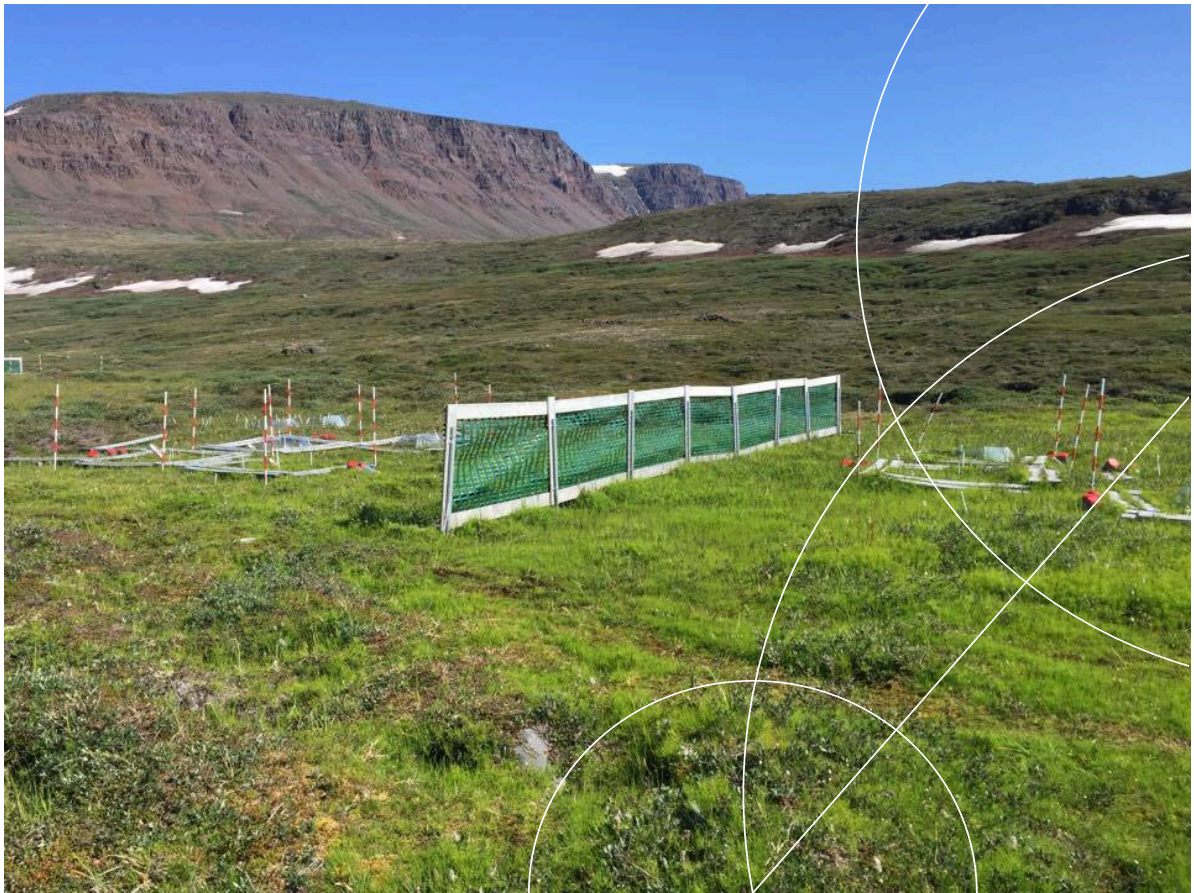
Snow fence experimental area in Blæsedalen (Nanna Devantier, July 2016)

Effect of gall mites on photosynthesis and stress in two species of Salix

Nanna Devantier & Michelle Strecker Svendsen

Abstract

Global change has a more pronounced effect on the Arctic region compared to temperate ones. This will have massive consequences for the arctic environment and may cause significant ecological changes that can alter the trophic interactions between herbivores and plant communities. This study examined the impact of eriophyoid gall mites on the leaves of two Arctic willow species (*Salix glauca* and *Salix arctophila*) in an Arctic tundra located on Disko Island, Western Greenland. The study was done as an experimental study conducted on an area with snow fences located in dry and wet biomes. In each snow fence four different treatments were conducted; control, warming, snow and snow+warming (12 snow fences, 48 plots). A set of ecophysiological measurements was done in situ. For both ungalled and galled leaves the chlorophyll and flavonoid concentration and the ratio between them (nutrient balance index) were measured. The galled leaves showed a significant decline in flavonoid concentration but had the highest nutrient balance index (NBI). The highest abundance of both species was found in the wet site, but the highest percentage of infested leaves was found on the dry site. This suggests that *Salix* on the wet site have the highest amount of nutrients available and therefore are more capable of producing secondary metabolites and other defense compounds. To fully understand the effect of gall mites on *Salix* species more studies are required. Our findings suggest that gall mites have significant impact on photosynthetic processes that most likely will affect the overall functions of the Arctic willow.

Keywords: Gall mite, Arctic, herbivory, climate change, Salix arctophila, Salix glauca

Introduction

Arctic terrestrial ecosystems are often associated with low productivity, short growing season, harsh weather conditions, drought and low nutrient availability. However the Arctic environment is currently facing chances due to climate change. The predicted changes will include warmer weather, due to increased CO_2 levels in the atmosphere, increased precipitation, melt of glaciers and ice caps and a prolonged growing season. The climatic changes will by time lead to greater changes in the ecosystems, weather and environment (Callaghan et al. 2004). This will result in massive changes at high speed and can cause a problem for organisms that are adapted to the cryospheric environments and niches.

A great amount of the world's freshwater, and associated carbon is locked in the icecap of the Arctic. The combination of a warmer and longer growing season will lead to the permanent melt of ice and permafrost. As a result of the climate changes there will follow a release of a huge part of the carbon pools available there. The more ice that melts, the more carbon is released and so this is a self-reinforcing effect of climatic changes. Certain plants, animals and insects might benefit from these changes while others might have to adjust to the new conditions and interactions and some might go extinct due to it. Another consequence of a longer, warmer and more wet growing season, is that the microbial activity in the ground increases and release more nutrients to the soil, which might be beneficial to most plants by increasing biomass and leaf nutrient status (Callaghan et al. 2004). This will also decrease the layer of organic litter aboveground, which might alter both the belowground and the aboveground processes and cycling in ways we cannot predict precisely (Chapin et al. 2012).

The Arctic tundra environment is characterized by permafrost and a shallow active layer that thaws and refreezes every year, and an annual cycle of mostly constant sunlight or constant darkness. This is a challenge for the organisms that live and thrive there (Thomas et al. 2008). The plants in the Arctic have adapted to these

conditions through thousands of years. These adaptations include shallow root formations, which prevent larger plants and trees from growing in the Arctic region and the ability to perform photosynthesis during the summer season and save the nutrients and energy throughout the winter season (Born and Böcher 1999). The plants adapt to periods of drought, with very little precipitation, frozen ground and very low water content in the air and wind. The drought also results in fewer available nutrients in the soil and therefore increases the competition from neighbor plants.

Climate change causes higher temperature, precipitation and nutrient availability in the soil and therefore affects the ecological processes for plants (Michelsen et al. 1995).

Warming will create an increase in chlorophyll concentration. This will lead to an increase in carbon assimilation but will at the same time increase the water consumption by shrubs. In dry tundra sites this can lead to a decrease in soil moisture and therefore lead to enhanced competition for water (Michelsen et al. 1995).

With higher nutrient availability there will be an increase in growth mainly allocated as photosynthetic structures, leaves. However an increase in nutrients in the soil can lead to an increased supply of nitrogen to the plants. This makes it possible for the plant to produce secondary metabolites, such as flavonoids, as a defense against UV-radiation and herbivores (Wink 1997). This is according to the carbon-nutrient balance hypothesis that states that more nutrients lead to an increase in growth and might also an increase in the allocation of nitrogen-based defense compounds while carbon-based compounds may be reduced by the enhanced growth (Hansen et al. 2006). An increase in temperature stimulates the production of defense compounds, which are secondary metabolites (Ramakrishna and Ravishankar 2011).

Climate change can affect the plant-herbivore interaction in several ways. One of many stress factors for plant growth and activity is herbivory. It has a huge impact on both the individual plant and the entire ecosystems (Crawley 1997). The plant produces secondary metabolites as a defense against herbivores. Climate change can

have indirect effects on herbivores through these secondary metabolites (Rosenthal and Berenbaum 1992). The plant-herbivore interactions will most likely differ with future climate change. Enrichment in CO_2 will affect plant tissue quality and cause an increased mortality and thus lower the abundance, richness and diversity of herbivores (Cornelissen 2011). In contrast the temperature will have a positive impact on herbivore abundance. The combined effect for herbivores will therefore be specie-specific and will depend on the development of defense strategies in the plant. A higher precipitation and CO_2 level will increase the production of secondary metabolites and decrease the amount of parasitic herbivores on the plant (Cornelissen 2011).

On *Salix*, mites form parasitic buds on the leaves called galls. Gall mites belong to the order Arthropods and the family Eriophyidae (Böcher 2001). They are very small animals (approx. 0.25 mm) and only have two pairs of legs (Böcher 2001). The end of their body is long and wide. They live on different plant species where they form malformations on the leaves. These malformations are called galls. On *Salix* they form red buds on the leaves. In Greenland there are at least 10 species but the field is highly understudied and many more might exist (Böcher 2001). Because they are so small, it was not possible to identify which gall mites was present on the *Salix* leaves. The difference species do practice the similar functions on the leaves. Because our main focus is on the plant it is not necessary to know the specific gall mite species.

The level of stress in the plant can be detected using several parameters. For this study it was chosen to measure flavonoid and chlorophyll concentration and the ratio between them, because these are directly linked to level of stress in the plant.

This study of galls on leaves of *Salix* aims to study the stress tolerance and level of stress in selected plants in manipulated conditions designed to imitate the climate in 10 years. Our project is based on the interaction between mite and plant, and we aim to examine whether there is a difference in photosynthesis and stress level and -tolerance in the leaves from galled leaves vs. ungalled leaves under the climatic ongoing

experiment in Blæsedalen that include climatic factors as increased temperature and increased precipitation.

In this study we examine chlorophyll and protective leaf pigments for two Arctic willows (*Salix glauca* and *Salix arctophila*) that are found on a dry and wet tundra site, respectively. We hypothesize that the galls on leaves of both *Salix* species will cause a significant reduction in the production of leaf pigments. This reduction will be expressed as a lowered production of secondary metabolites in galled leaves, as the plants will not have carbon in excess to protect themselves against herbivory with secondary metabolites. Furthermore we hypothesize that the highest abundance of galls will be found on the dry site. This is due to the expectation that more nutrients are available for the plants on the wet site.

Materials and methods

Study site

The fieldwork for this study was conducted in Blæsedalen on Disko (Disko Island) from July 12th to 21st. Disko is located in the Baffin Bay on the coast of West Greenland (69°15'N, 53°34'W). Blæsedalen is a valley created by glaciers and it stretches 30 km northwards to Disko fjord. The experimental site is located by the coast of Disko

Figure 1. Bioclimatic subzones in Arctic. From Bhatt et al. 2013.

bugten (bay) in the south.

Disko is located in the bioclimatic zone D (Fig.1) and is defined as low Arctic (Fig. 1; Gloud et al. 2002; Yurtsev 1994; Bhatt et al. 2013). The mean annual temperature is -3 °C (1992-2012) and July is the warmest month of the year with a mean temperature of 7.9 °C. The mean annual precipitation is 436 mm, where 42% falls as snow. The growing season lasts from approximately July to August, though yearly variations do occur (Blok et al. 2015).

In 2012 six snow fences were established as part of the CENPERM project on dry tundra-heath dominated by *Salix glauca* and *Betula nana*. In 2013 another six snow fences were established at the same site but this time on wet tundra with poor drainage. This site is dominated by *Salix arctophila*, *Carex stans* and *Polygonum viviparum*.

For our study we used twelve snow fences in total. At each snow fence there are four treatments; control, warming, snow and snow+warming. This makes a total of 48 plots. Our experiment is of a factorial design with a randomized distribution of snow fences within each site. The snow fences are placed towards the northern wind so that the snow accumulates on the southern side of the fence. This creates a warming of the soil in the late winter. At the southern side the two treatments, snow and snow+warming are located. The warming treatment is made with Open Top Chambers (OTC). These are standardized and increase the temperature inside by 2.5 ℃ during the growth season (Blok et al. 2015).

Each plot is 150 x 150 cm. The warming treatment was conducted by OTC and these only cover the inner area of the plots. Thus all measurements were taken inside the OTC. On plots without warming the entire plot was used for measuring.

The measurements were carried out on two species of willow, *Salix arctophila* and *Salix glauca*. *S. arctophila* was found on the wet site and *S. glauca* was found on the dry site. The measurements were done on both ungalled and galled leaves.
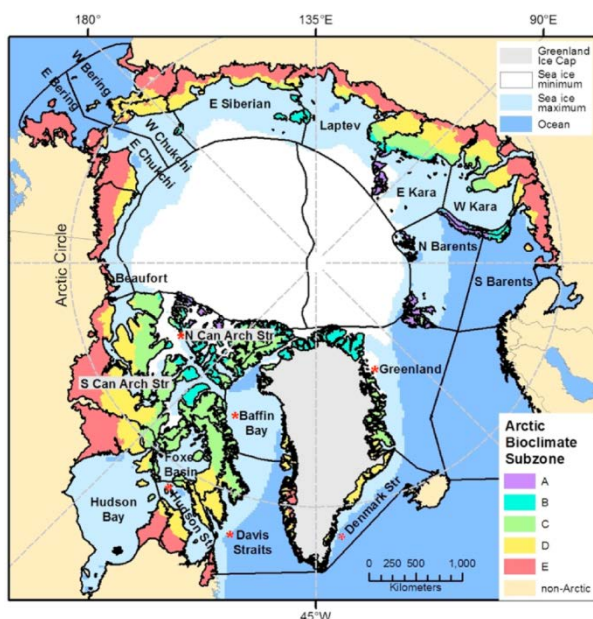
Study plant species

Salix arctophila (Salicaceae) is a dwarf shrub. It grows to a height of 2-12 cm and has lying branches. The leaves are elliptic to egg-shaped with a length of 10-50 mm. The leaves are green and glossy on top and somewhat bluish underneath. They are always hairless. *S. arctophila* is common in wet areas such as bogs, marshes and along streams. It is only found in Southern and Western Greenland and has its most northern distribution at 71 °N (Rune 2011).

Salix glauca (Salicaceae) is a high shrub and can reach a height of 250 cm. The leaves are broadly egg-shaped or elliptic blades with a length between 20 and 75 mm long. The leaves are bluish-green and are hairy on both side, though sometimes almost hairless above. It is very common in Greenland with a northern distribution of 75 °N (Rune 2011).

Sampling methods

The aim of the fieldwork was to find the plant stress parameters for ungalled and galled *Salix* leaves. This was done by measuring chlorophyll concentration, flavonoid concentration and NBI. The physiological measurements were done in situ in July 2015. In each plot five galled and five ungalled leaves from each species were chosen. The leaves were handpicked and chlorophyll, flavonoid and NBI were measured using a Dualex Scientific+TM Polyphenol and Chlorophyll-meter.

In each plot the percentage of willow was estimated. This was done with an observational approach. Of the total amount of willow the amount of galled leaves was estimated.

The soil moisture and temperature were measured in all plots. The soil moisture was measured by using a ThetaProbe ML2x (Delta-T Devices Ltd) and the temperature was measured at approx. 2 cm depth by using a soil probe-thermometer. For both measurements three replicates were taken in each plot and afterward a mean was calculated.

Chlorophyll concentration

The chlorophyll concentration was correlated with the amount of chlorophyll *a* in the leaves. Chlorophyll *a* is used by plants in photosynthetic

processes such as light harvesting and energy conversion (Yu et al. 2014). The concentration of chlorophyll *a* is correlating with the rate of photosynthesis in plants (Fleischer 1935; Emerson 1929). It is therefore possible to use the chlorophyll concentration as an indicator of stress. If the herbivore pressure is very high, the plant will use energy to produce secondary metabolites, thus lowering the level of photosynthesis (Yu et al. 2014).

Flavonoid concentration

Flavonoids are carbon-based secondary metabolites. They serve mainly as UVB protection for the plant but do have other purposes as well, for example recruiting plant pollinators and seed dispersers (Wilkey-Shirley 2002). In the Arctic it is essential to have protection against UVB since the intense UVB radiation can otherwise easily lead to photo damage of photosystem II (Wilkey-Shirley 2002).

Nutrient Balance Index

Nutrient Balance Index (NBI) is the ratio of the chlorophyll and flavonoid concentration. The plant will produce flavonoids as a response to herbivore and a low NBI is therefore often interpreted as a stress indicator.

Statistical analyses

The snow fences were placed completely randomly within the two sites, but the plots were restricted to the snow fences.

For statistical analysis, the program PAST 3.0 was used.

For soil temperature and moisture a two-way ANOVA test was chosen to examine the differences between the treatments and between the two sites. No transformations were needed. If the result was significant a Tukey pairwise post-hoc test was performed to find the direction of the difference.

This method was used to examine the chlorophyll, flavonoid and NBI concentration as well.

To compare the galled and ungalled leaves a one-way ANOVA test was chosen. No transformation was needed for this either.
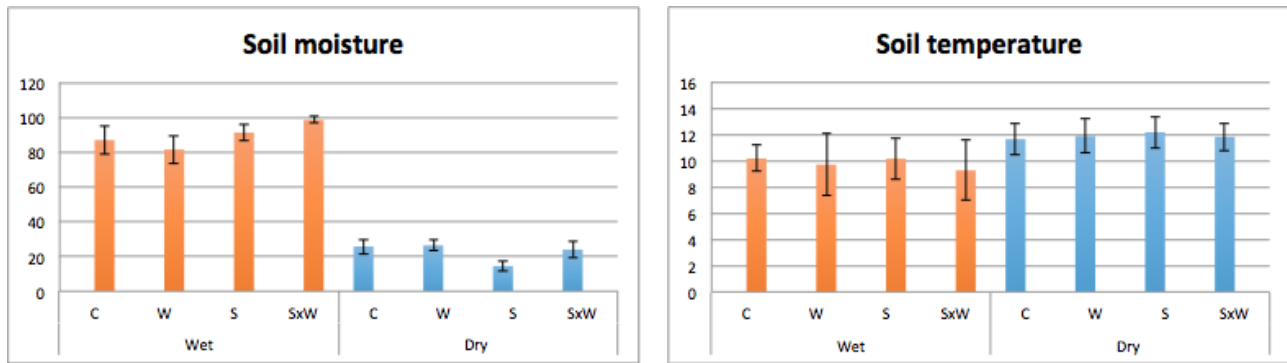
Figure 2. Mean soil moisture and temperature (±SD) in the four different treatments (control, warming, snow, snow+warming) at the wet and dry tundra-site.

The following significance levels were used: trend: p<0.1, significant: p<0.05, highly significant: p<0.001.

Results

Soil moisture and temperature

To create an overview on the water content and the temperature in the soil two simple bar charts were created (Fig. 2, Tab. 1). The percentage of water was highest on the wet site, while the temperature was highest on the dry site. The most separated mean was found for the soil water content. The mean soil moisture was 89.5% at the wet site and 22.6% at the dry site. For the soil temperature the mean was 9.8 °C at the wet site and 13.4 °C at the dry site.

The result from the ANOVA test showed that there was significant higher soil water content ($F=491.7$, $p=4.392*10^{-24}$) on the wet site compared to the dry site. No significance was found between the four treatments ($F=1.514$, $p=0.2255$).

For the soil temperature the ANOVA test showed a significant difference between the sites ($F=4.806$, $p=0.03424$), but not between the four treatments ($F=0.671$, $p=0.5748$). Opposite to the water content the highest temperature occurred on the dry site.

Plant stress measurements

To test the differences between the chlorophyll concentrations on the leaves of both *Salix* species, an ANOVA test were run on the data. For the ungalled leaves no significance were found between the treatments ($F=1.589$, $p=0.2086$) but there were significant differences between the dry and wet site ($F=10.6$, $p=0.002425$). This showed that the chlorophyll concentration was significantly higher on the dry site (Fig. 3).

Table 1. Mean soil water content and soil temperature (±SD) for the treatments and the sites (n=6).

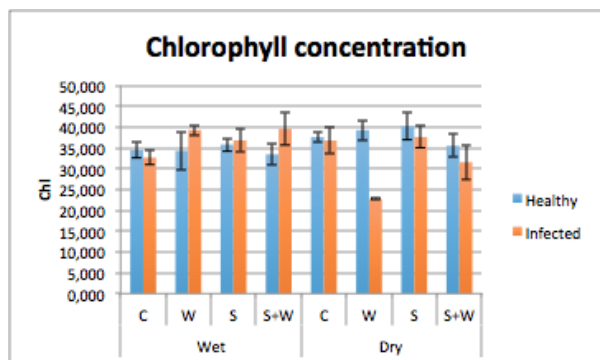|		Wet	Dry
Soil water content (%)	C	86.98 (±8.45)	25.75 (±4.37)
	W	81.53 (±8.04)	26.39 (±2.95)
	S	91.27 (±4.61)	14.53 (±2.88)
	S+W	98.51 (±1.83)	23.99 (±4.68)
Soil temperature (°C)	C	10.17 (±0.98)	11.64 (±1.23)
	W	9.70 (±2.35)	11.88 (±1.28)
	S	10.15 (±1.59)	12.17 (±1.21)
	S+W	9.26 (±2.26)	11.82 (±1.06)

Figure 4. Chlorophyll concentration (µg cm^{-2}) (±SD) in four different treatments (control, warming, snow, snow+warming) on wet and dry tundra (n=6).

Figure 3. Linear model of chlorophyll concentration (µg cm^{-2}) for ungalled leaves at wet and dry tundra (n=6). Black: control, red: snow treatment, blue: warming treatment, green: snow+warming treatment.

The ANOVA test for the galled leaves showed that the chlorophyll concentration did not vary significantly across the treatments ($F=1.608$, $p=0.2137$). There was a trend towards higher chlorophyll concentration on the wet site ($F=2.977$, $p=0.09732$).

The chlorophyll concentration for ungalled and galled leaves was compared in a bar chart (Fig. 4). This showed no clearly difference between ungalled and galled leaves. This was supported by a one-way ANOVA test ($F=0.1932$, $p=0.6615$).

The ANOVA test for the ungalled leaves showed a significant higher flavonoid concentration at the wet site ($F=12.72$, $p=0.001021$). Only a tendency was found between treatments ($F=2.219$, $p=0.1022$). The trend showed a higher flavonoid concentration in the snow treatment on the dry site and a lower concentration in the warming treatment on both sites.

For the galled leaves the ANOVA test showed no significant difference between the sites
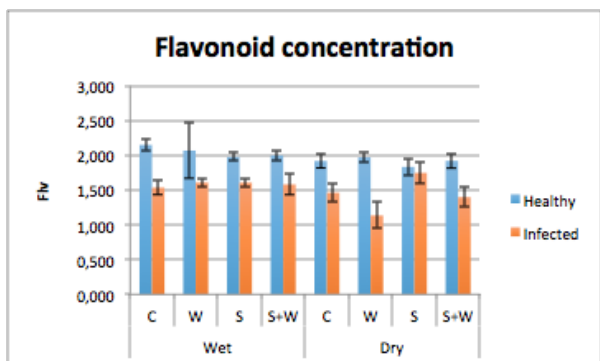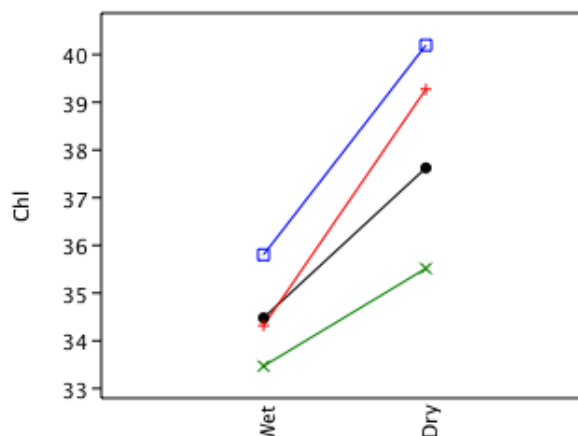
($F=2.009$, $p=1692$) or between the treatments ($F=1.187$, $p=0.3356$).

A bar chart was done to create an overview over the flavonoid concentration in ungalled and galled leaves of both *Salix* species (Fig. 5). It shows that the concentration in general is higher for the ungalled leaves than for the galled. This observation was supported by a one-way ANOVA test ($F=96.25$, $p=4.336*10^{-15}$). The Tukey's test showed this as well ($p=0.0001142$).

The ANOVA test for NBI on ungalled leaves showed a significant difference on both treatments ($F=3.794$, $p=0.01812$) and sites ($F=29.46$, $p=3.744*10^{-6}$). NBI was highest on the dry site and the warming treatment at both sites was higher than the other treatments.

The test clearly showed a significant higher NBI on the dry site and in the warm treatment.

An ANOVA test was done on NBI for galled leaves as well. This showed no significant difference between treatments ($F=0.258$,
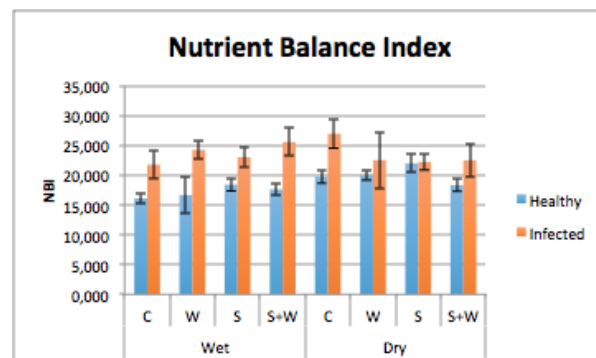

Figure 5. The flavonoid concentration (µg cm^{-2}) (±SD) in four different treatments (control, warming, snow, snow+warming) at wet and dry tundra (n=6).

Figure 6. The NBI (±SD) for ungalled and galled leaves in the different treatments at the wet and dry site (n=6).
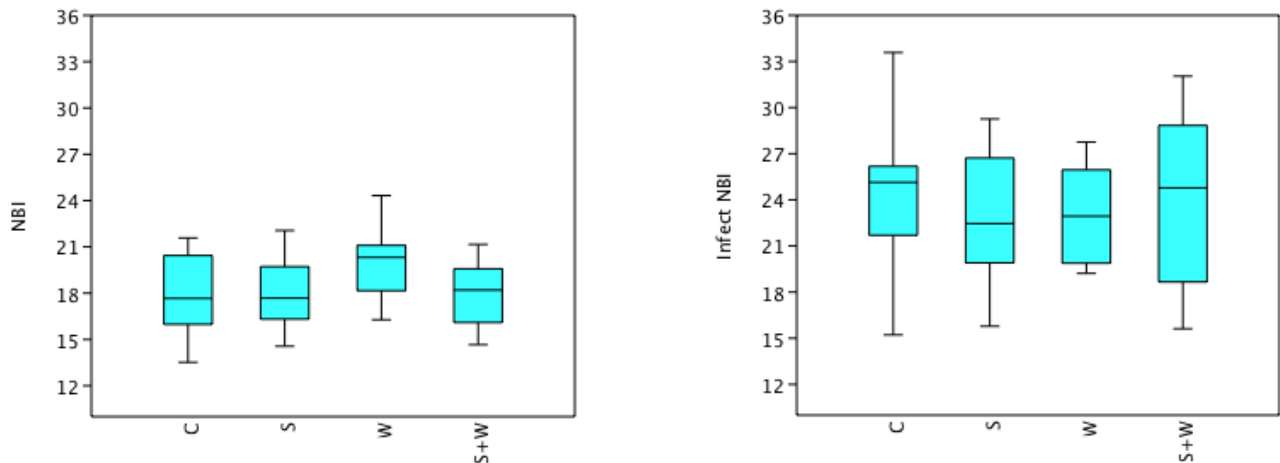
36

Figure 7. NBI concentration (±SD) of ungalled (left) and galled (right) leaves against the four treatments: Control (C), snow (S), warming (W) and the combination of snow+warming (S+W) (n=6).

$p=0.8549$) or sites ($F=0.05439$, $p=0.8176$).

NBI for ungalled and galled leaves were compared in a bar chart, which illustrates that the NBI is higher in galled leaves compared to ungalled leaves (Fig. 6).

This was supported by a one-way ANOVA test, which showed a significant difference between NBI for galled and ungalled leaves ($F=47.5$, $p=1.479*10^{-9}$). The Tukey's test showed a higher NBI for galled leaves ($p=0.0001142$).

To illustrate the results from the ANOVA test two box plots are shown below (Fig. 7).

For each treatment an estimate of the percentage of *Salix* was performed. The ANOVA test showed that there was a significant difference in the amount of *Salix* from the dry and wet site ($F=34.35$, $p=7.414*10^{-7}$), with the wet site having the highest percentage of *Salix*. No significant difference was found between treatments ($F=0.871$, $p=0.4641$)(Fig. 8).

Out of the total cover of *Salix*, an estimate over the percentage of galled leaves was done.

The percentage of galled leaves in the different treatments was compared with each other in a bar chart (Fig. 9). This showed that a higher amount of galled leaves at the dry site compared to the wet site. The ANOVA test for the amount of galled leaves there were on *Salix* followed the same pattern as the percentage of *Salix* in the plots. There was a significant difference between the sites ($F=13.6$, $p=0.0006721$), with the dry site having the highest percentage of galled leaves. Only a trend was found between the treatments ($F=2.502$, $p=0.07306$). The trend showed a lower percentage of galled leaves in the snow treatment at the wet site compared to the other treatments. The trend also showed a higher percentage in the warming treatment at the dry site compared with the other treatments.
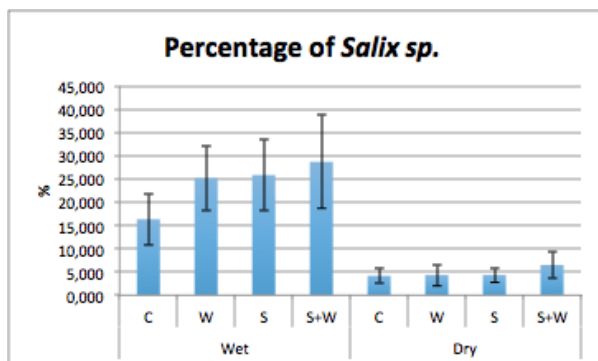

Figure 8. The percentage cover of Salix (±SD) in the wet and dry tundra (n=6). Four different treatments are applied: control, warming, snow and the combined effect of snow and warming (s+w).
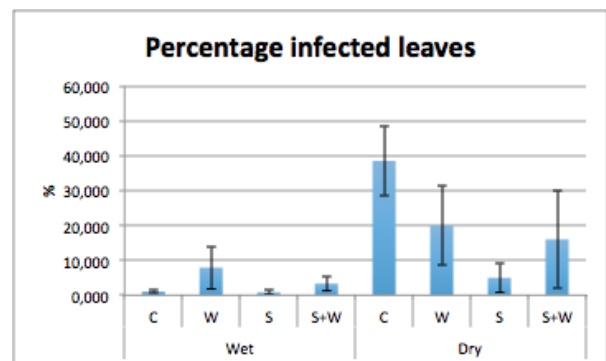

Figure 9. The percentage of galled leaves (±SD) in the different treatments at the wet and dry site (n=6).

Discussion

This study has shown that galls on leaves of *Salix* decrease the secondary metabolites. Our results showed a significant reduction in the flavonoid concentration for the galled leaves on both *Salix* species. This support our hypothesis of mite galling causing a decrease in secondary metabolites such as protective leaf pigments.

Our results showed that the highest soil water content was found in the wet site which is not surprising. The temperature was found to be lowest in the wet area which makes sense because it takes more energy to heat up soil containing a high percentage of water compared to dry soil. The highest percentage of *Salix* was found in the wet area. In the wet area we expect to find a higher microbial activity and this creates a higher amount of available nutrients, which is also mentioned by Semenchuk et al. (2015). This might cause the higher abundance of *Salix* on the wet site compared to the dry.

In contrast to what we would expect, we did not find increased water content in the snow treatments. These results are the same as Blok et al. (2015) who at the same site did not find any significant effect of increased snow cover on soil water content. This can be due to an increased plant growth in the snow treatments (Chapin et al. 2012). However, it is worth mentioning that the snow fences were located on a gentle slope and water therefore has the possibility to drain away from the plot. This may also be the reason why an increase in water content was not found on the snow treatments.

For the secondary metabolites, we found a significantly higher amount of galled leaves in the snow fences located in the dry habitat compared to the wet area. It thus seems like the plants in the wet area have fewer leaves infested with galls compared to the plants in the dry area. This fits our hypothesis. In a wet area we would expect more nitrogen to be available to the plant (Callaghan et al. 2004). If the plants have more nitrogen available they can produce a greater amount of secondary metabolites and thus have a better defense against the gall mites (Mosbacher et al. 2006). If the nutrients in the dry area are more sparse, the plant will need to

prioritize how much energy to put into growth and how much to put into defense.

Plants produce flavonoids as a protection against herbivory. The subject is still very poorly understood but it seems that the flavonoids make the leaves less attractive for herbivores by altering the taste. When a gall mite produces a gall on a leaf, this galling will take up nutrients such as nitrogen from the leaf. This reduces the physiological capacity in the leaf and therefore reduces the production of secondary metabolites. Our results (Fig. 5, Fig. 9) show precisely this trend. In the galled leaves the flavonoids concentration is lower than in the ungalled leaves.

No significant differences were found for the chlorophyll concentration in galled leaves between the wet and dry site. The same applied for the chlorophyll concentration between treatments, though with a lower chlorophyll concentration for galled leaves at the warming treatments on the dry site. A depression of the efficiency of photo system II has been observed in other gall-infested plants (Huang et al. 2011). This indicates that the efficiency of photosystem II is not affected by the galls on the leaves. The lower level of chlorophyll for galled leaves in the warming treatment at the dry site suggests that there is a decrease in the photosynthetic activity. This indicates that the galls will decrease the photosynthetic activity with an increasing effect with warmer climate (Mosbacher et al. 2006).

NBI was greater for galled leaves than for ungalled leaves and follows our results from the flavonoid and chlorophyll concentration. NBI is the ratio between the chlorophyll and flavonoid concentration. Our results show that the chlorophyll concentration remains the same while the flavonoid concentration is highest for ungalled leaves. This will therefore give the lowest value of NBI for ungalled leaves.

We expected a higher difference amongst the four treatments. The snow treatment imitates a higher precipitation and warming of the soil in late winter. The warming treatment imitates a warming of the soil and air temperature. We would have expected significant deviations from the control plot, but did not find any. It would therefore be interesting to have data from several years, thus making a multi-year study. If

we had data from multiple years it might reveal trends that we are not able to see from our short-term study.

Borner et al. (2008) showed that not even six years of snow treatment is enough to find significant differences from control plots. The snow fences in this study have only been in place for two years, so this might be an explanation to why we do not see a more pronounced difference between treatments.

We compared two different species of *Salix* in the wet and the dry sites, as we did not have the same species in both sites. This might have interfered with our results if the two species react differently to stress.

To get a broader picture of the performed study, it would also be favorable to include data from several years in a row. This would hopefully show whether the plants are in the process of adapting to climate change and if a steady state will occur at any point. Other things that could have improved our data is to include factors like stomatal conductance, Fv/Fm, P index and the C/N ratio, which all also may contribute with information on the level of stress and adaptation.

Lastly, it would be very interesting to look at how all these factors affect the plant as an individual and as part of a community. How do the galls, the defenses against and the adaption to galls affect e.g. growth rate, offspring, survival rate etc. for the plants?

We admit as well that it would be more suitable to perform a three-way ANOVA test on our data instead of a two-way ANOVA test. We have three parameters that we compare; site, snow and OTC. It would therefore be more accurate to use the three-way ANOVA test. If this study was to be conducted again this should therefore be applied.

The two *Salix* species in this study represent the functional group: deciduous shrubs. This group is predicted to expand in arctic ecosystems due to climate change (Patankar et al. 2013). A greater expansion of *Salix* might lead to the expansion of gall mites as well. It is interesting to study if this might lead to a negative feedback on the expansion of *Salix*.

Salix species are subject to many different stress factors, both biotic and abiotic. The biotic stress factors are poorly understood. Our study shows that there is a significant decrease in physiological parameters on leaves infested by galls, but further studies are needed. Based on our results we therefore suggest that gall mites have an important impact on the function of the entire plant.

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Contents

Humpback whales feeding near Arctic Station (Kirsten S. Christoffersen, July 2016)