

# *Arctic Biology Field Course*

*Qeqertarsuaq 2017*



UNIVERSITY OF COPENHAGEN  
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***Qeqertarsuaq 2017***

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For further information about Arctic Station: [www.arctiskstation.ku](http://www.arctiskstation.ku).

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## Preface

The purpose of the Arctic Biology Field course is to give students an authentic impression of the environment and biota in the Arctic ecosystem, which they have been acquainted with during courses at University of Copenhagen or elsewhere. It is also a very important aspect to provide an opportunity to get first-hand experience with the process of creating a scientific research project from the initial “wild” ideas to the final results and conclusions. The Arctic Station provides an optimal teaching environment where the students are in the field within minutes and can get a genuine understanding of how unique the arctic ecosystems are by own observations and analysis.

The Arctic Biology Field course in 2017 took place from 11<sup>th</sup> to 20<sup>th</sup> July. The overall theme for the course was to study responses of arctic ecosystems to human impact and specifically to global warming. The students were prepared as they had defined their group projects beforehand, had researched the literature and had tested the methods that they were going to use for sampling and analyses. As always when working with natural populations in their environment, this is not necessarily straight forward and ad hoc modifications are often needed. However, the obstacles both in the field and during laboratory were solved and all groups obtained excellent data for their reports.

The subjects for group projects were:

- Human impacts on cetacean behaviour with focus on disturbance by boats in Greenland
- The influence of climate change on Disko Island’s macro algal communities
- The impact of microplastic on blue mussels (*Mytilus edulis*)
- Growth rates of Arctic charr populations (*Salvelinus alpinus*) in relation to climate conditions
- Vegetation cover and physiological responses in plants along a snow bed gradient exposed to warming
- The effect of warming on CO<sub>2</sub> flux along a natural vegetation gradient in an arctic tundra landscape

We were blessed with fine weather conditions during our stay which made the field work enjoyable. Several common scientific excursions took place to visit Morænesø, Østerlien and Kuanit. Besides, we had a guided walk through the entire village, took part in the Sunday church ceremony and had a lecture by the scientific leader (Regin Rønn) about the monitoring activities Arctic Station as well as his research projects.

Overall, we had a splendid course and highly appreciated the interactions with other guests at the station and with the two station managers, the housekeeper as well as the crew on board Porsild. We wish to thank them all for their support.

The findings and conclusions based on the student projects are published in the report “Arctic Biology Field Course - Qeqertarsuaq 2017” (ISBN: 978-87-89143-23-1). The report can be obtained as PDF from Kirsten S. Christoffersen ([kchristoffersen@bio.ku.dk](mailto:kchristoffersen@bio.ku.dk)) or from the Arctic Station website.

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

## Participants



1. *Anders Michelsen (teacher)*
2. *Tine Seligmann*
3. *Charlotte A. B. Køhnke*
4. *Thomas A. Arkwright*
5. *Camilla H. Scharff-Olsen*
6. *Mads Schultz*
7. *Cathrine Kallestrup*
8. *Lasse Brandt*
9. *David H. Blitz*
10. *Liv A. Nobel*
11. *Louise C. Flensburg*
12. *Paula M. Gonzalez*
13. *Kirsten S. Christoffersen (teacher)*
14. *Marc Allentoft Larsen*
15. *Samuel Black*

## Course diary

### July 10<sup>th</sup> (Monday)

Ready – set – go! Now it is here – the day for departure to Greenland, which we have all been waiting for!!! The group met at Copenhagen airport a 7 AM with our teachers Kirsten and Anders. This was a bit early in the day for us but we were all full of excitement anyway. After a brief stop in Kangerlussuaq Airport, some went to Ilulissat and some went to Aasiaat after over an hour of delay. We all got on the plane even though the small airplanes looked very full. We got something to drink and a cookie. Well arrived in the destination airports we got a warm welcome by the mosquitoes. We went by taxi to the harbor and waited a bit for the boat to arrive. The mosquitoes found us immediately but as soon we went on the boat, they were gone. The trip crossing the Disko Bay was great - we were very lucky to see whales, seals and beautiful icebergs.

We arrived at Qeqertarsuaq, Disko Island at 6 o'clock after a long but nice trip. Here we got a warm welcome by the mosquitoes (again) and Akaaraq (the station manager). We found our sleeping "stables" and got a nice dinner. Now we were ready for 10 amazing days at Disko ☺ !!!

David & Louise

### July 11<sup>th</sup> (Tuesday)

As the jetlag did not seem to bother anyone, the breakfast was ready at 8, when we all gathered in the dining room. Freshly made oatmeal and buns were preparing all of us for a meeting and the first real day for project work. After a quick introductory meeting, we were all dressed and ready for a hike to the closest study sites surrounding the Arctic station. The tour started out with a small introduction to the methane measuring station right next to the Arctic station, as these measurements needs a stable power source, they have to be situated in the vicinity of the station, with power cables wrapped in fox-proof covers. Next stop presented the bridge over the river Røde Elv, opening the view to Blæsedalen, or if you will the "Windy Valley" as Anders nicely translated it. The view across this valley with massive brown-red mountain walls on one side, followed by soft descending green hills, covered with light green spots from the water running down the mountain, to the magnificent, glittering icebergs on the blue sea, was beautiful. The path to the plant sites along the snow fences presented a cold wind, giving reason to the name Blæsedalen, but as the sun was still shining from a blue sky, only the wind jackets came on. We continued to the dry vegetation sites, followed by the wet vegetation sites and finally arrived higher up on the side of the mountain which was very warm and pleasant due to the shelter provided by the mountain side. The view was great from up there! And we spotted three whales swimming between the icebergs towards the bay area. We then started the descend to the lake Morænesøen, which has no fish in it however it did have a pair - possibly a breeding pair, of red throated diver. From here, some people went back to the site they had to study and the rest continued back to the field station along a deep gorge where the Røde Elv was going through. Along the gorge there were some great views of waterfalls together with the ocean and



icebergs in the background. The general discussion over dinner was about the high abundance of flies indoor as well as the high abundance of mosquitoes outdoors! As we are writing this, Lasse and David have gone to catch some wolf fish, as we are hoping to cook them during the week. The rest of us are gathered around the station in small groups talking projects and relaxing after a long day.

Mads & Camilla

### *July 12<sup>th</sup> (Wednesday)*

The day started nice and early with large breakfast made by Thomas, Camilla and Tine. Shortly after we all gathered to get a quick presentation and discussion of our current intentions with the projects. It was good to hear the stages people were at and the enthusiasm we all had for our projects. Saying out loud exactly what we planned to do also strengthened the plans of action for the day. For many, today was a day spent collecting their first part of data, while for others it was a day spent better on preparing or visiting potential sites for the work that was to come. After the meeting we all went our separate ways to get on with our separate tasks, while some of us hiked off to the mountains, others went down to the harbor or off to the rocky coast, all with excitement. The day was long and tiring but the work was good, we made lots of progress counting hundreds of flowers and recording hours' worth of whale dives for our respective projects. The work was arduous but the satisfaction of progressing with our projects easily outweighed our tired eyes and minds. Before we knew it, dinner was upon us and we were enjoying a tasty Danish inspired dish with meatballs in curry with rice. The evening was then left available to ourselves to do with what we wanted. Some of us continued working into the night on our projects, something made possible by the non-stop Arctic sunlight. Though even with the possibility of working all night, there was great satisfaction in typing up collected data into Excel and the feeling of accomplishment that came from that. Overall while the day provided many challenges, such as keeping a straight mind with counting tiny flowers or not losing hope with the mist hiding the whales, there was undoubtedly a feeling of great pride getting work done and seeing everyone hard at work, there is no better incentive. The day ended with a well-deserved sleep, with the knowledge of an early beginning to a new day of data collection."

Thomas & Catherine

### *July 13<sup>th</sup> (Thursday)*

Thursday was an early start for the aquatic project teams, who ate breakfast at the small hour of 6.30 am. Primed for the day ahead after a bowl of salty porridge the group, comprised of 4 students (Sam, Marc, David, Mads) and Kirsten, wedged themselves into the stations pick-up truck and headed for the RV Porsild berthed in Qeqertarsuaq harbor. After some minor paper issues the boat set sail for Fortuna Bay in perfect conditions, weaving between several large ice-bergs and two fin whales along the coastline. The teams were sailed ashore and headed their separate ways – one to a lake with arctic char and another to the rocky shore. Both teams had successful days, one catching a record 11 Arctic Char and the other managing to complete two rather exposed underwater transects (macro algae). Although equally beautiful the



journey home was less eventful and once back at the station both teams enjoyed a more horizontal orientation.

Back onshore, the terrestrial groups spent most of the day in Blæsedalen continuing the work from yesterday. Lasse and Cathrine finished the flower counting – which they were probably glad about after counting around 10.000 flowers. They have now started on measuring the chlorophyll and flavonoids on the plants in all the fields. Charlotte and Liv have finally finished the CO<sub>2</sub> measurements after 3 days work with some computer problems and have now moved on to measure NDVI and plant stress. Lunch was eaten in the sun with icebergs and jumping whales on the horizon. Thanks to the incredible culinary skills of the marine plastic group who had spent the day in the lab preparing samples and completing calculation, by 7pm everyone was enjoying the wonderful combination of pasta and lasagna, including oven baked aubergine (eggplant), fresh red pepper and grilled cheese. With loose ends to tie up, data to type in and fish to catch out, everybody went their separate ways after dinner as the ice-bergs continued to drift past the windows of the Arctic station.

Sam & Liv

### July 14<sup>th</sup> (Friday)

Our day started with breakfast as usual about 3 minutes past eight. The buns which were served were definitely the best served yet. Then we had the morning meeting at 8:45 AM where all the groups presented their schedule for the day. The fishing group went to their last location and did unfortunately not catch any fish. The whale team went back to the library and had 4 hours without seeing any whales at all. The micro plastic group were in the library and picked up some water for their experiment at the harbour in the morning for continuing their experiment. The macro algae group were at home for most of the day and went out just before low tide to pick up some samples, they were not home for dinner and the water was very cold. Lastly, the two plant groups went to Blæsedalen once again, to do plant samples etc. We found that the snow bed had melted a meter since day one. Furthermore this was the very first day with rain and very cold weather conditions.

The dinner group had caught 14 cod the day before and two wolf fish, which they used to make incredibly delicious “fiskefrikadeller” with potatoes and parsley sauce. At 20:30 PM Ludovika made a presentation about her PhD project and her new Post Doc experiments in Blæsedalen. Afterwards we all hung out in the living room, some made excel sheets, and others were facebooking, and the rest played a very nice card game about Arctic Stations around the Arctic. Most of the course participants went to bed early, because we were all very tired and excited after four days of hard work and so many adventures. We are all looking very much forward to day number 5. We will sign out now and go to bed. Goodnight.

Lasse & Charlotte



### *July 15<sup>th</sup> (Saturday)*

We woke to a grey and cold day at Disko Island. For some, the air temperature was not cold enough so people decided to go for a swim among the icebergs. The macroalgae group went out early to take samples in Torskebugten, the rest of the groups went for a nice city walk led by Kirsten. After the walk the whole group split up to continue with their projects. The fish group went back to their first location to try to get lucky with the catch. The microplastic group started a new experiment by feeding the mussels some tasty micro plastic particles. The whale group went to their usual cosy lab corner to spot whales. The plant groups continued with their measurements.

At 7 pm the “fruit de la mer” had moved to Arctic Station and the dinner was served in a sophisticated way consisting of 3 courses, handpicked mushrooms as an entrée, a massive pile of snow crab served with the local herb Kvan as the main course and for dessert “risengrød” á la Nissebanden. The food was digested to a lecture by Regin Rønn – the scientific leader of the station.

Paula & Marc

### *July 16<sup>th</sup> (Sunday)*

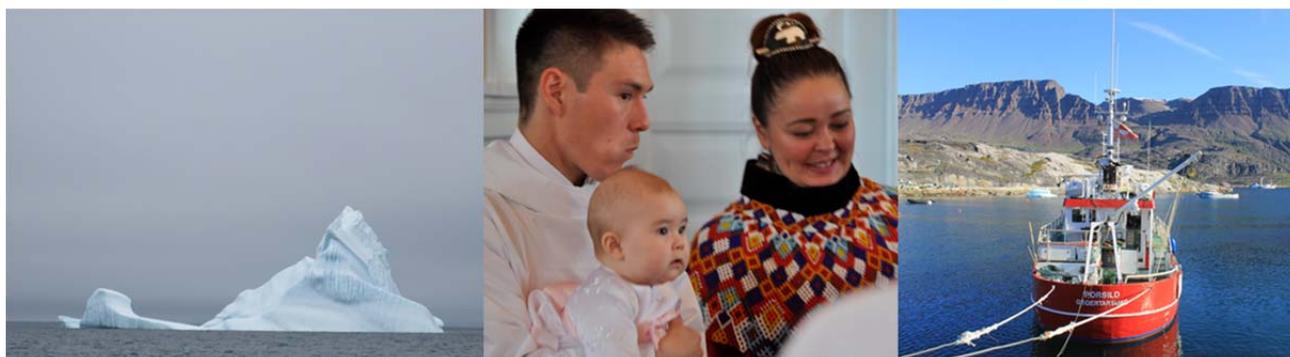
After breakfast we started off the day with a baptism in the church. The sermon was beautiful, although we didn't understand a single word, and we got to see the national garments of the local people. After the baptism some people went to Kuanit and some went to work on their projects. Kuanit was very beautiful with basalt formations, lush vegetation and homothermic springs.

For dinner Kirsten presented a very special Greenlandic snack which we had as a starter: narwhal mattak. We were told about the how it can be served (with “aromat”) and why it is healthy. Everyone (almost) loved it! Mattak is the skin and blubber of the whale which is served raw. It is very tasty. The main course consisted of a nice Greenlandic lamb with veggies and salad. After dinner some people went to work on their projects while others rested.

Camilla & David

### *July 17<sup>th</sup> (Monday)*

The day started early, as most of us were going out on the scientific vessel Porsild. Breakfast at 7.30 and leaving the Arctic Station at 8.15. When we arrived at the harbour the Captain was a bit worried as the sea was turbulent and the trip to Laksebukten might turn out to be quite rough. It was that bad and upon arrival Sam and Marc was transported to the shore and started to dive for the macro algae. The rest of us stayed on the boat and some of us started to fish for cod. David and Mads fished for Arctic Char but with little luck. For almost an hour no one caught anything until suddenly... four big cods passed under the boat and were hooked by Lasse, Kirsten and Cathrine. The catch was timed with the return of Marc and Sam and the boat went home in sunny weather. Upon arrival Mads, Lasse, Cathrine and David went fishing next to



the “whale graveyard”.

The dinner, Mexican á la Marc and Sam, was half an hour earlier than usual as most of us went for a walk towards the glacier. The glacier adventuring teams started as two groups traveling to two different spots of the same glacier on Lyngmarksfjeldet. The team going the marked (blue) way reached the top and walked around on the glacier for a while before going down again. The team going the unmarked easterly way walked together to the foot of the glacier before splitting up into two groups; one turned back down while Marc and Sam crossed the glacier and returned via the marked trail. People came back between 1 and 4 in the night, taking advantage of the midnight sun.

Cathrine & Mads

### *July 18<sup>th</sup> (Tuesday)*

The day began with an interesting start as many of us had taken the long and gruelling glacial hike the night prior, and so had returned at a variety of different times throughout the early morning. We therefore arrived at different times for breakfast but all met for a briefing which addressed the impending departure from the station in two days' time. Close to the end, people set off for what was for some of us, the last day of sampling. The motivation of collecting the last bit of data was in everyone's minds as putting an end to what was often physically and mentally demanding work came to an end. Only the data analysis would be left and then starting the report could occur soon after. Unfortunately for some the lack of sampling days was quite intimidating as time was of the essence, but careful planning could assist in this pressure.

A tasty dinner caught by the group's best fishermen/women was prepared with love by Tine and Anders before everyone had a chance to sit and relax before presenting their projects and the process they had currently made. Everyone had clearly progressed a lot with their projects and could produce some nice preliminary data that suggested many different things that could easily be investigated ready for the reports. It was very interesting to see the stage that everybody was at and good to see everyone had progressed so much. The expectations for tomorrow are high but the end of the course is near and people are beginning to prepare for the return home to come.

Thomas & Liv

### *July 19<sup>th</sup> (Wednesday)*

Fairly tired from several busy days, the group ate a slow breakfast as the rain battered against the windows. With the majority of the teams opting for a dry day it was only Lasse and Cathrine who decided to brave the storm and make their way to some fieldwork in Blæsedalen. The rest of the teams worked indoors counting samples and formulating their collected data. The previous evening's dinner of freshly caught cod was again enjoyed at lunch time by everyone except Lasse and Cathrine who arrived sometime later soaked to the skin. In the afternoon the Arctic Charr group, David and Mads, set out on an optimistic



trip with the goal of catching more Arctic Charr in the nearby Røde Elv. Now flowing at an alarming rate, Marc, Charlotte, Sam and Tine watched curiously as David and Mads attempted to cast their homemade net down the deep red torrent. Although unsuccessful today, the fruits of the Arctic Charr groups fishing efforts were enjoyed by everyone at dinner, accompanied by mashed potato and cheese, salad and hollandaise sauce. Later on we all enjoyed a fantastically baked carrot cake made by Charlotte whilst sipping a coffee, reading our books or playing backgammon. With the impending final day drawing ever closer we all dread the thought of leaving the cosy confines of the Arctic Station and hope that one day we will all return to enjoy yet another slice of Charlotte's carrot cake.

Charlotte & Sam

### *July 20<sup>th</sup> (Thursday)*

The last morning! These 10 days had gone too fast. For some of us the morning started with an early fresh swim in the arctic water, followed by our last delicious breakfast all together. After breakfast, we all, sadly, packed our belongings and a group of us prepared to leave for Aasiaat mid-afternoon, while the other group had more time to enjoy Disko until they departed for Ilulissat later in the afternoon. Almost all of us went to the harbour, to say goodbye to the people leaving first, all were very sad to be leaving Greenland and such a wonderful group of people. The boat trip to Aasiaat was very bumpy, but some of us still managed to fall asleep. We arrived at Aasiaat and headed directly to our charming hotel. After getting a bit lost through the city we managed to find a nice place to eat and then headed to the bar and enjoyed some time together. We headed back to sleep, next day we had our last stop before Copenhagen, Kangerlussuaq, and we were feeling excited that we had one more day to enjoy Greenland.

The people going to Ilulissat had to wait a little before their boat arrived. Regins family was coming to visit him, so we had a chance to meet them. Just after, we found some seats on the boat and waved to Kirsten and Regin. As soon as we left the summer harbour, hefty waves hit our boat and continued to do it throughout the ride to Ilulissat. We all agreed on that the 10 days at Arctic Station had been some of the most wonderful days of our lives and we were all hoping to see it again someday. When we finally arrived in Ilulissat, we all walked together from the docks to the town centre, Louise was going to Ice cap Hotel to find her parents, Sam was going to find his place and Charlotte and Lasse was going to find a place to put up their tents. Just outside Ilulissat there are lots of dogs with puppies, so Lasse had to drag Charlotte along because of her love for small puppies. Finally after many minutes, and conversations about puppies, we walked to the "camping site" close to the Icefjord. Here we ate our "kebab stew". Charlotte was still talking about puppies while Lasse put up his tent. At around midnight we saw a polar fox and the sky was flaming red.

Lasse & Paula



*Human impacts on cetacean behaviour with focus on disturbance by boats in Greenland*



**Diving whale (photo: Colourbox)**

# *Human impacts on cetacean behaviour with focus on disturbance by boats in Greenland*

*Thomas Arkwright and Camilla Hjorth Scharff-Olsen*

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## **Abstract**

Human presence has been linked to cetacean disturbance for many years, with the presence of boats and other vessels considered disturbance stimuli that elicit anti-predator or aversive behaviours by whales. Whale watching is becoming an increasingly common practice of ecotourism and is expected to increase within Disko Bay at Qeqertarsuaq in the West of Greenland. Using observer based measures of dive duration and frequency, as well as incorporating a theodolite to measure distance, speed and surfacing points, individual humpback and fin whales were observed for comparative changes in diving and spatial behaviours when in the presence and absence of boats. Results varied between diving and spatial behaviours as well as on a species level. Dives were found to be significantly different in the presence of boats for humpbacks in that longer long dives and fewer short dives were done in the presence of boats, whereas fin whales only exhibited significantly fewer short dives in the presence of boats with duration of long dives not varying significantly. Dive duration for humpbacks did not differ in the presence or absence of boats or across different boat sizes; however it did differ for boats travelling at different speeds. Distance travelled by humpbacks did differ significantly in the presence of boats, as well as when boats were of different sizes and between boat speeds. However, no significant differences in the speed travelled by humpbacks occurred when in the presence of boats of different sizes or speeds. Fin whales exhibited significant difference in dive duration in the presence of boats, despite dive duration not differing significantly between boats sizes and speeds. No significantly different distances or speeds were travelled by the fin whales in the presence of boats. The varied outcomes of the study provide possible insights into the effects of boats on whale behaviour and whether disturbance occurs and may subsequently impact individual whale energy stores and thus, fitness, for the upcoming seasonal migration to lower latitudes. Furthermore, understanding the extent to which the whales visiting Disko Bay respond to human presence, helps better address both the historical impacts of subsistence hunting within the area, as well as consider consequences of increased whale related tourism in the future and the most utilitarian way to approach it.

**Keywords:** *Humpback whale, fin whale, disturbance, boats, behaviour.*

## **Introduction**

Over the years, whale watching tourism has grown and become a huge industry. In 2008 alone, whale watching tourism was conducted in 119 countries worldwide, with an estimate of 13 million people participating, thus generating a total of 2.1 billion US dollars globally (O'Connor et al., 2009). In Greenland, the number of tourists participating in whale watching has also increased from 2500 people in 1998 to 3250 in 2007 (O'Connor et al., 2009). Most of these whale watching tours are concentrated on the central west coast of Greenland, in the Disko

region (e.g. Ilulissat, Qeqertarsuaq ), however tours are also conducted in other areas of Greenland such as in the south, e.g. Qaqortoq and Nanortalik, and the in the east e.g. Kuummiut and Tasiilaq (O'Connor et al., 2009). Thus, there could be socioeconomic benefits related to an increase in the Greenlandic whale watching industry, as well as other benefits such as opportunities to generate awareness to the general public and therefore aid in the conservation of the whales. However, it is known that whales are affected by anthropogenic activities and the noise that occurs in relation to these, such as drilling, military activities, boat

traffic, whale watching and fisheries (Bejder et al., 2006; for a review see Parsons, 2012). However, there are different opinions as to whether it is possible for the whales to become habituated to these activities and the noise related to them or if they are negatively affected by it, and in that case, to what extent they are affected e.g. change/alter their natural behaviour (Bejder et al., 2006), a decrease in reproductive rate/success and/or foraging thus potentially decreasing the individuals survivability etc. (Lusseau and Bejder, 2007; for a review see Parsons, 2012).

Amongst the potential behavioural changes in whales due to human influences, changes in movement dynamics are likely. Excluding large scale migratory movements, the local scale spatial dynamics of whales may alter circumstantially. Factors such as abundance of prey resource in feeding grounds will typically result in directional movement of whales to areas in the water column where prey biomass per volume of water is highest (Laidre et al., 2010). Alternatively, predation of orcas (*Orcinus orca*) on baleen whales has been well-established to elicit anti-predator behaviours, of which one is the flight response that involves “high-speed swimming away” (Ford and Reeves, 2008). This aversive behaviour simply demonstrates how an external factor may result in unidirectional movement of a whale and alter its swimming speed. There is still debate on the topic of anthropogenic presence as a behavioural stimulus of baleen whales, particularly with whale based tourism, where tour boats have been suggested to prompt anti-predator aversive behaviours in the whales such as; increased dive time, lower percent surface time and change in swimming direction (Scheidat et al., 2004; Stamation et al., 2010). Others argue that large vessels and small boats are not considered a threat to the whales, and that some instances even describe whales approaching the vessels and in a welcoming manner (Evans, 1996). This ambiguity further supports the need to better understand movement based behaviours in the context of human derived disturbances. A clear issue with movement studies on cetaceans is the presence of the observers themselves potentially

biasing any recorded data on account of their own proximity to the focal animals. Land based observational techniques through the use of a theodolite have allowed for behavioural observations without observer based disturbances, by using trigonometry, the observer can calculate the position of a whale and thereafter the speed and distance of which it travels from a suitable vantage point (Würsig et al., 1991).

The current study aims to investigate the effect of human presence on the diving and movement behaviours of two baleen whale species, the humpback whale (*Megaptera novaeangliae*) and the fin whale (*Balaenoptera physalus*) around Qeqertarsuaq in Disko Bay, western Greenland. Humpback whales are well distinguished by their disproportionately large pectoral fins as well as the tubercles found at different places on their body, with typical mean length of mature male and female humpbacks at 9.9 m and 9.7 m (Clapham and Mead, 1999). With their long and slender bodies, fin whales are much larger than the humpbacks and as the second largest whale species they may reach lengths of 27 m (Mizroch et al., 1984).

During the summer, both species migrate to high latitude waters as production of prey species is high, where they then feed using their large baleen plates to filter water and capture small fish and crustaceans. With such concentrated numbers of feeding individuals, the whales are more vulnerable to hunting and whale tourism as well as other human activities that may influence behaviour (Mizroch et al., 1984; Clapham and Mead, 1999).

With the considerable potential of Greenlandic whale based tourism arising in the near future, there is a strong need for understanding the impact that boats and vessels may have on the behaviour of visiting whales at Disko Bay, especially as the site is of high importance to the species that periodically feed there (Hoyte, 1995). In order to understand the effect of human presence on the whales, we will measure both the diving and spatial movement behaviour of whales in Disko Bay in both the

presence and absence of boats. We expect the presence of boats will elicit measurable behavioural responses both in diving and movement dynamics. We hypothesize that in the presence of boats: i) diving behaviour will change in form of fewer short dives and longer duration of long dives, since we expect that the whales will avoid exposure to boats on the surface with vertical avoidance behaviour. We also hypothesize that: ii) movement dynamics will change through increased swimming speed of whales and longer distances being travelled, on account of the whales considering passing boats to be a disturbance, we would expect the whales to move further away and at a fast pace from the boats. Our hypotheses follow our expectations that both species of whale will be disturbed by any boats present, and thus will respond with the avoidance behaviours described above.

## *Methods and Materials*

### *Study site*

Observations took place in the library building of Arctic Station, Qeqertarsuaq, Disko, Greenland. The library, which is located 14.5 meters above sea level, has windows parallel to the bay area, thus creating an ideal observatory for spotting whales and operating the theodolite. In addition to this, Udkiggen (the traditional whale watching tower) was used on one day and here we have observations of one individual (ID 1) but only for dive data since the theodolite use could not be permitted due to technical difficulties (fig 1). Observations took place from 9:30 AM to 6:45 PM every day and some days, when possible, the evenings were included from 8:00 PM to 10 PM. A sampling boundary was decided prior to observations based on, visible structures including rock formations and building structures that occur at the limits of observer visibility. The horizon limit was determined based on observer ability, thus whales deemed too far for reliable observations would not be monitored.

### *Diving measurements*

Using both a telescope and binoculars, whales were spotted from the observation point.

Upon sightings, a focal individual would be selected. Using a Samsung Galaxy S5 Mini mobile phone with the Dictaphone app, an audio recording would be initiated upon the next surface of the focal individual, where after each surfacing was noted and given a surfacing number in a chronological fashion, so that dives and theodolite measures could be correlated by number. Factors such as weather and sea state were also noted for the benefit of the study when necessary, as well as the species of the focal individual. Each time a boat appeared in the sampling area, its presence was noted on the Dictaphone as well as its size (characterized as: small, medium or large) and speed (characterised as: stationary, slow, medium or fast). Characterizations of speed and size were decided based on observer knowledge of types of boats in the area and their intended purpose which may affect their travel speed. The status of the boat would be noted until it had left the sampling area, at such instances, its absence was noted on the Dictaphone. Focal individuals would be monitored for up to an hour when more whales were present, in order to gain sufficient observations of multiple whales. However, when no other whales were present, the whale was monitored until no longer visible. A focal whale that was deemed no longer visible had either left the sampling boundary, swam behind a large gathering of icebergs, or partaken in a dive that occurred for a time of >25 minutes. The >25 minute limit of non visibility was decided based on the fact of being the average time it takes before new whales enter the sampling boundaries and thus additional data could be lost on account of monitoring a whale that may have left the sampling area. Focal individuals had their dive durations characterized as either long or short, whereby long dives occurred for >60 s and short dives for <60 s as seen in the study by Boye et al. (2010). An indication of long dives is typically associated with the visibility/orientation of the fluke; diagrams were also referred to beforehand (fig S1). The Dictaphone recording was left to run for the sample of each individual whale, so that dive duration may be calculated. Recordings were only ended when sampling of the individual in question had ceased.

### *Spatial displacement and locomotive behaviour*

Spatial movement and displacement of the whales in relation to boats were measured by use of a TOPCON Electronic Total Station GTS-6 (theodolite). Theodolite calibration was achieved as described in the TOPCON Electronic Total Station GTS-6/6B manual, and a reference point was decided prior to sampling, based on a distinguished landmark on the edge of the sampling boundary. From the lookout point, the first surfacing point of the focal individual was found with the theodolite and the horizontal and vertical coordinates noted as well as the given number of surface. The same would occur for the individuals' following surfaces until no longer observed. Just as with the diving measurements, boats whenever present, were noted, thus the length of the dives and swimming speed could be estimated both in the presence and absence of boats, when including the information from the Dictaphone. When possible, theodolite measures of boat movements were taken in relation to the focal whales, however, the task proved too difficult to perform at the same time as measuring the whale surfacings for two observers, and so was abandoned.

### *Data analysis*

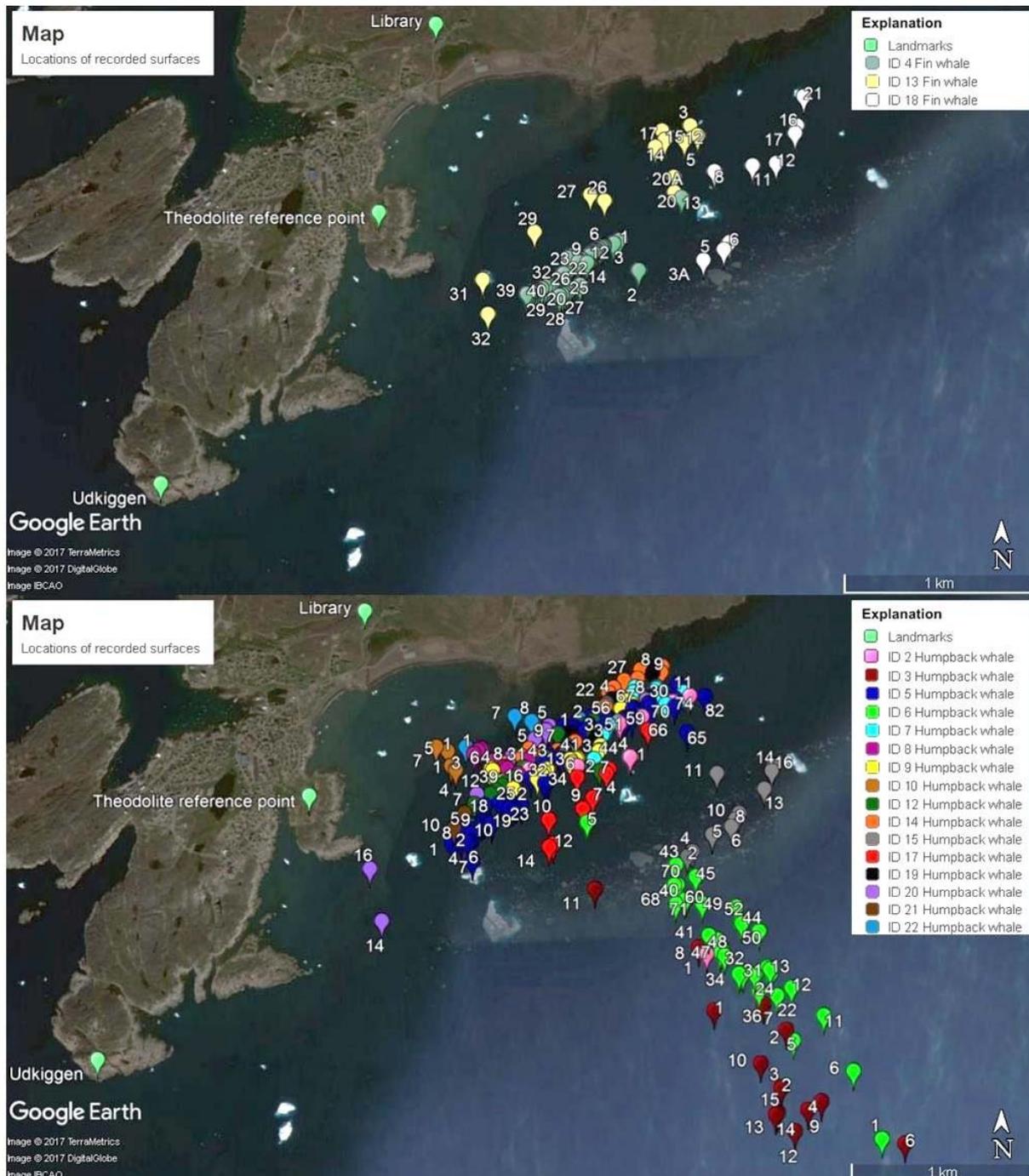
For the benefit of the visual and statistical analysis, spatial and dive data were organised in Microsoft Excel 2007 and 2013. In order to fill in dive data, each Dictaphone recording was replayed with each surfacing noted as its time of occurrence based on the start time of the recording. The time in between each surfacing could be noted and the dive duration could be calculated accordingly. As spatial data were often covered across numerous dives, a decision had to be made for the analysis as to whether boats could be considered present for one travel measure that included dives both in the presence and absence of a boat. In such cases, boats would be considered present as behaviours may have been affected within the short time period that the boat was present, and engine acoustics may still be within range despite the boat having just left the observers visual range. A similar situation arose, whereby boats changed speed over numerous dives that were listed under one

spatial movement. During such instances, the more conservative (slower) speed was used, to avoid data bias under the assumption that faster boats with outboard engines produce more noise and may therefore have greater impact on whale behaviour; as suggested by Cantor et al. (2010). Collected data was analysed using RStudio version 1.0.44 (2016) with the following packages "coin", "zoo", "TH.data", "sandwich", "modeltools", "car" and "multcomp". Data was first checked for a normal distribution using a Shapiro-Wilk test. A Levenes' test for equal variances was then used for the non-normally distributed data centred on the mean, as is followed of a typical Levenes' test. The Levene's test for equal variances revealed significant p-values for nearly all the tested variables. The status of boats (present or absent), boat size, and boat speed all produced significant outcomes when they were tested with total dive duration and dive length, only boat speed with dive length provided a non-significant outcome (Table S1). Therefore only variances between boat speed and dive length can be considered equal, the remaining variables do not have equal variances and thus cannot be trusted for further analysis of variances. As data were not normally distributed and did not have equal variances, a non-parametric test was run in form of a Mann-Whitney U test. The data is divided by species and tested separately in the presence and absence of boats. There were too little data to do statistics on the spatial data for fin whales with varying boat speed and boat size - only three boats were recorded, all small sized and with two boats in medium speed and one fast going boat.

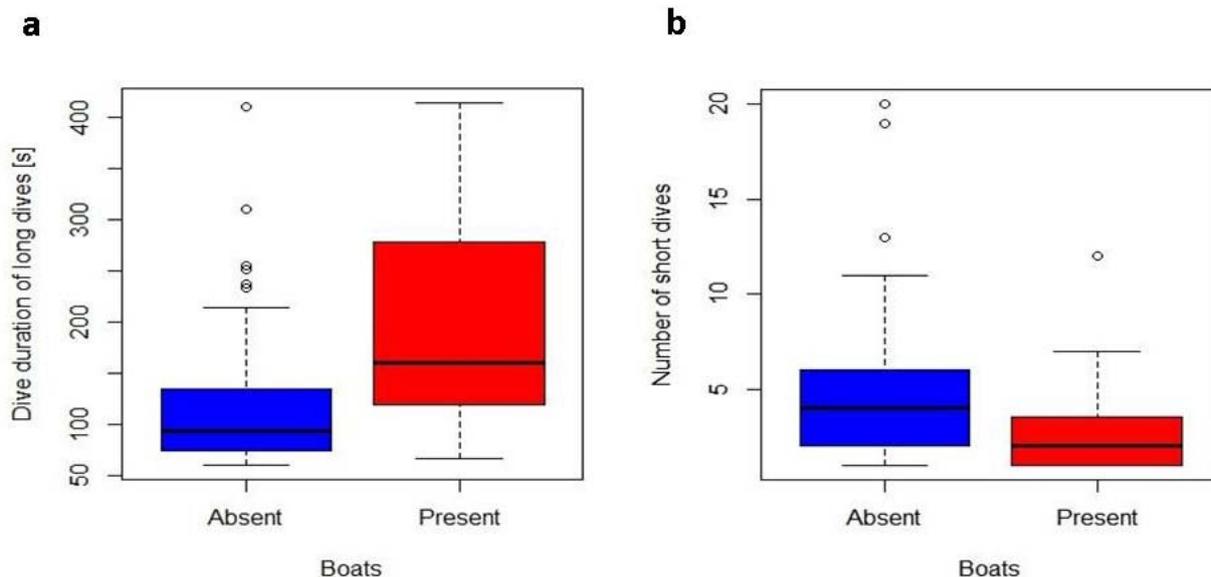
Maps were constructed in Google Earth (Earth-version 7.1.7.2606; program downloaded and coordinates accessed the 23<sup>rd</sup> of July 2017) to visualize the movement patterns of the whales within the sampling area. By using Google Earth the following Universal Transverse Mercator (UTM) coordinates were found for the library of the Arctic Station: 22 W 400364.44 7684552.92; and for the landmark/mast used for setting the theodolites horizon to 0.0: 22 W 400040.43 7683588.57. According to Google Earth, there is 1017.24 meters in a straight line between the two locations.

By using the horizontal and vertical coordinates found by the theodolite, when the whales surfaced and the landmark (a mast), it was possible to estimate where the whales surfaced by using the following trigonometric

functions: The tangent equation was used to find the missing angles and distances to the observed whale:



**Figure 1** Map of the all spatial movement patterns of a, fin whales (*Balaenoptera physalus*) and b, humpback whale (*Megaptera novaeangliae*) in the western part of Disko Bay which can be viewed from the library of the Arctic Station (pale green) at Qeqertarsuaq, Disko, Greenland. The numbers indicate the actual number of each surface, while the different colours represent different individuals. Another landmark is a mast (light green) that was used as a reference point for the theodolite.



**Figure 2** Boxplots of **a**, the dive duration of the long dives [s] in the absence (n=75) and presence (n=19) of boats (U=320;  $p < 0.05$ ; adjusted for ties) and **b**, the number of short dives (U=875;  $p < 0.05$ ; adjusted for ties) in the absence (n=20) and presence (n=20) of boats, for humpback whales (*Megaptera novaeangliae*).

Opposite cathetus =  $\tan(V) \times \text{adjacent cathetus}$  (1)

The law of cosines was used to find the distance travelled by the whale between two surfacing points after a deep/long dive:

$$a = (b^2 + c^2 - 2bc \cdot \cos(A)) \quad (2)$$

The speed travelled by the whale was estimated with the following equation:

$$\text{Speed [m s}^{-1}] = \frac{\text{distance travelled [m]}}{\text{time [s]}} \quad (3)$$

Spatial data were not recorded for ID 1 due to difficulties with the tripod for the theodolite - the height could not be adjusted on the tripod so the theodolite could not be correctly operated. Additionally, for ID 11 and ID 16 the theodolite ran out of battery.

It was not always possible to record all the surfaces spatially; either due to the whale being out of sight (behind icebergs), difficulties spotting the individual, locating it with the theodolite to get the correct coordinates in time before it dived again, and/or simply due to writing efficiency (when writing down the coordinates). Additionally, if the observer, who was counting

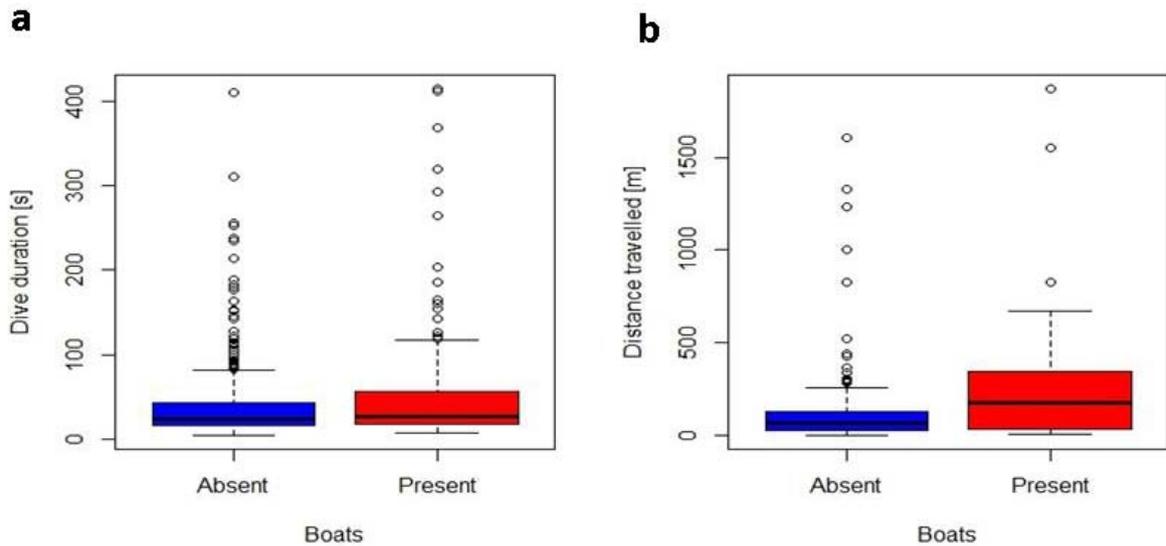
the number of surfaces, were insecure about the last counted number we skipped some numbers to be sure not to count the same number twice.

## Results

We observed and got data from a total of 22 whales, of which 18 individuals were humpback whales and the remaining four individuals were fin whales. Observations accounted for a total of 8 hours and 36 min over seven days (12<sup>th</sup> of July 2017 - 18<sup>th</sup> of July 2017) (fig S2). For humpback whales a total of 484 dives were recorded, 94 long dives and 390 short dives, with 77 dives in total for which a boat was present (fig S3). Furthermore, a total of 174 dives were recorded for fin whales, 33 long dives and 141 short dives (fig S3), whereby 31 dives had a boat present. The maps visualize the spatial movement of the whales, there seem to be some areas that are more used than others (fig 1; fig S4-S14 for maps of individuals).

### Humpback whales

There was a significant difference in the duration of the long dives in the presence of boats compared to when boats were absent (fig 2a) as well as for the number of short dives in the



**Figure 3** Boxplots of a, the total dive duration [s] of humpback whales (*Megaptera novaeangliae*) between two surfacings in the absence (n=407) and presence (n=77) of boats (U=14056;  $p > 0.05$ ; two tailed; adjusted for ties) and b, the distance travelled [m] between two surfacings of humpback whales in the absence (n=208) and presence (n=38) of boats (U=2501;  $p < 0.01$ ; two tailed).

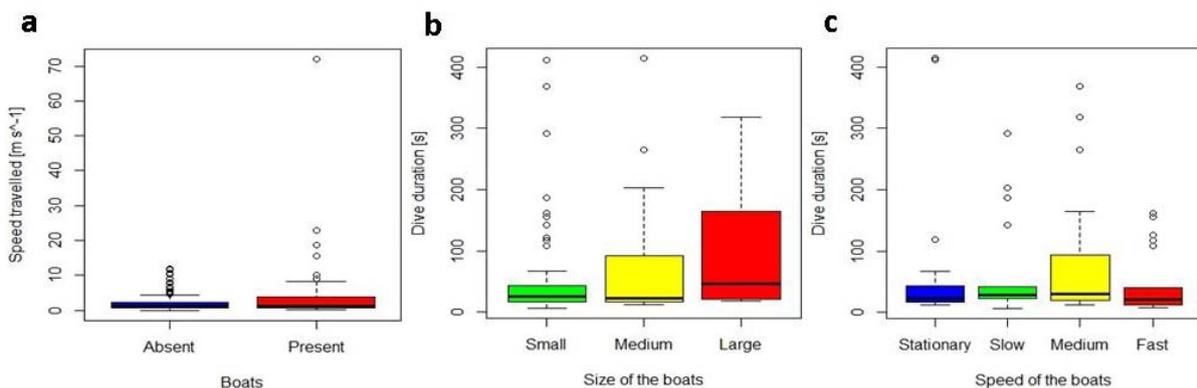
presence of boats compared to when boats were absent (fig 2b).

There was no significant difference between the dive duration of the whales between two surfacings [s] when a boat is absent or present (fig 3a).

However, there was a significant difference

between the distance travelled by the whales between two surfacings [m] when a boat is absent compared to when a boat is present (fig 3b).

No significant difference between the speed of which the whale travels between two surfaces [ $m s^{-1}$ ] when a boat is absent or present was found (fig 4a). In addition to this, the dive



**Figure 4.** Boxplots of: a, speed traveled by the humpback whale (*Megaptera novaeangliae*) [ $m s^{-1}$ ] in relation to the absence (n=184) and presence (n=38) of boats (U=3189.5;  $p > 0.05$ ; two tailed; adjusted for ties); b, dive duration of humpback whales [s] between two surfacings in relation to boat sizes: small (n=53), medium (n=19) and large (n=5) (small and medium: U=470.5;  $p > 0.05$  two tailed; adjusted for ties. Medium and large: U=34;  $p > 0.05$  two tailed; adjusted for ties) and c, the dive duration [s] between two surfacings of humpback whales in the presence of stationary (n=17), slow (n=18), medium (n=20) and fast (n=22) going boats (stationary and slow: U=121.5;  $p > 0.05$  two tailed; adjusted for ties. Stationary and medium: U=127;  $p > 0.05$  two tailed; adjusted for ties. Stationary and fast: U=221.5;  $p > 0.05$  two tailed; adjusted for ties. Slow and medium: U=162;  $p > 0.05$  two tailed; adjusted for ties. Slow and fast: U=258;  $p > 0.05$  two tailed; adjusted for ties. Medium and fast: U=302;  $p < 0.05$  two tailed; adjusted for ties).

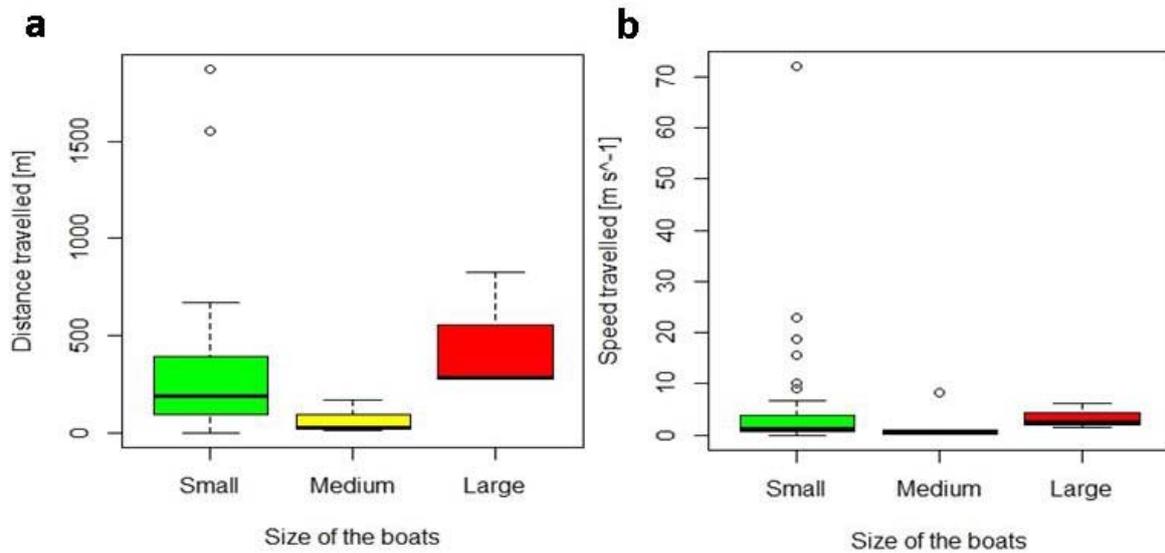


Figure 5 Boxplots of: a, the distance travelled [m] between two surfacings of humpback whales (*Megaptera novaeangliae*) in the presence of small (n=29), medium (n=6) and large (n=3) sized boats (small and medium: U=141; p<0.05 two tailed. Small and large: U=26; p>0.05 two tailed. Medium and large: U=0; p<0.05 two tailed.) and, b, the speed travelled by the humpback whales [m s<sup>-1</sup>] in relation to the different boat sizes; small (n=29), medium (n=6) and large (n=3)(Small and medium: U=130; p>0.05 two tailed. Small and large: U=31; p>>0.05 two tailed. Medium and large: U=3; p>0.05 two tailed).

duration did not differ between different sizes of boats: small and medium; small and large; as well as between medium and large (fig 4b).

There is a significant difference in the dive duration between boats travelling at a medium speed and boats travelling at a fast speed (fig 4c).

However, no significant difference between the dive durations and the remaining boat speeds, namely: stationary and slow; stationary and medium; stationary and fast; slow and medium; as well as slow and fast (Table S3).

A significant difference in the distance travelled between two surfacings of the whale [m] and the size of the boat was found, when it was; a small and medium sized boat; medium and large sized boat (fig 5a). On the other hand, there was no significant difference in the distance travelled between two surfaces of the whale and the size of the boat if it was a small and large boat. There was also no significant difference in the speed of which the whales travel [m s<sup>-1</sup>] between the different boat sizes; small and medium, small and large or medium and large (fig 5b).

Alternatively, a significant difference was found for the distance travelled between two surfaces of the whales and the speed of boats, when the boats are; stationary and medium; medium and fast (fig 6a). Nevertheless, there is also a non-significant difference between the distance travelled between two surfaces of the whales and the speed of boats, when the boats are: stationary and slow, stationary and fast, slow and medium or slow and fast.

No significant differences were found for the speed traveled between two surfacings of the whales [m s<sup>-1</sup>] and the speed of the boats when: stationary and slow, stationary and medium, stationary and fast, slow and medium, slow and fast, and medium and fast (fig 6b) (Table S5)

### Fin whale

There was no significant difference found in the duration of the long dives for fin whales in the presence of boats compared to when boats were absent (fig 7a). However, significantly fewer short dives did occur when in the presence of boats compared to when boats were absent (fig 7b).

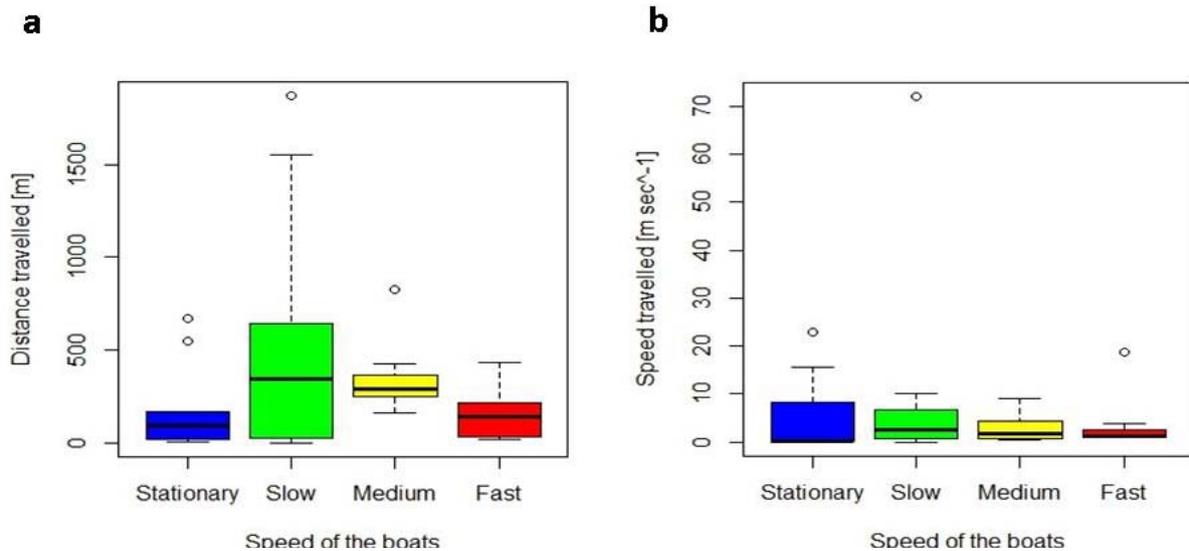


Figure 7 Boxplots of a, the distance travelled [m] between two surfacings of humpback whales (*Megaptera novaeangliae*) in the presence of stationary (n=9), slow (n=9), medium (n=8) and fast (n=12) going boats (speed) (Stationary and slow: U= 27; p>0.05 two tailed. Stationary and medium: U=15; p<0.05 two tailed. Stationary and fast: U=39; p>>0.05 two tailed. Slow and medium: U=35; p>>0.05 two tailed. Slow and fast: U=66; p>>0.05 two tailed. Medium and fast: U=81; p<0.01 two tailed) and, b, the speed travelled by the humpback whales [m s<sup>-1</sup>] in relation to the different boat speeds: stationary (n=9), slow (n=9), medium (n=8) and fast (n=12)(Stationary and slow: U=29; p>>0.05 two tailed. Stationary and medium: U=24; p>>0.05 two tailed. Stationary and fast: U=34; p>0.05 two tailed. Slow and medium: U=44; p>>0.05 two tailed. Slow and fast: U=252; p>>0.05 two tailed. Medium and fast: U=1; p>>0.05 two tailed).

A significant difference was found for the dive duration of the fin whales between two surfacings [s] in the absence and presence of boats (fig 8a). Yet there was no significant difference in the dive duration [s] between the boat sizes small and medium (fig 8b).

In relation to dive duration and the speed travelled by the boats, there were no significant outcomes obtained when the speed was: stationary and slow, stationary and medium, stationary and fast, slow and medium, slow and fast or medium and fast (fig 9a).

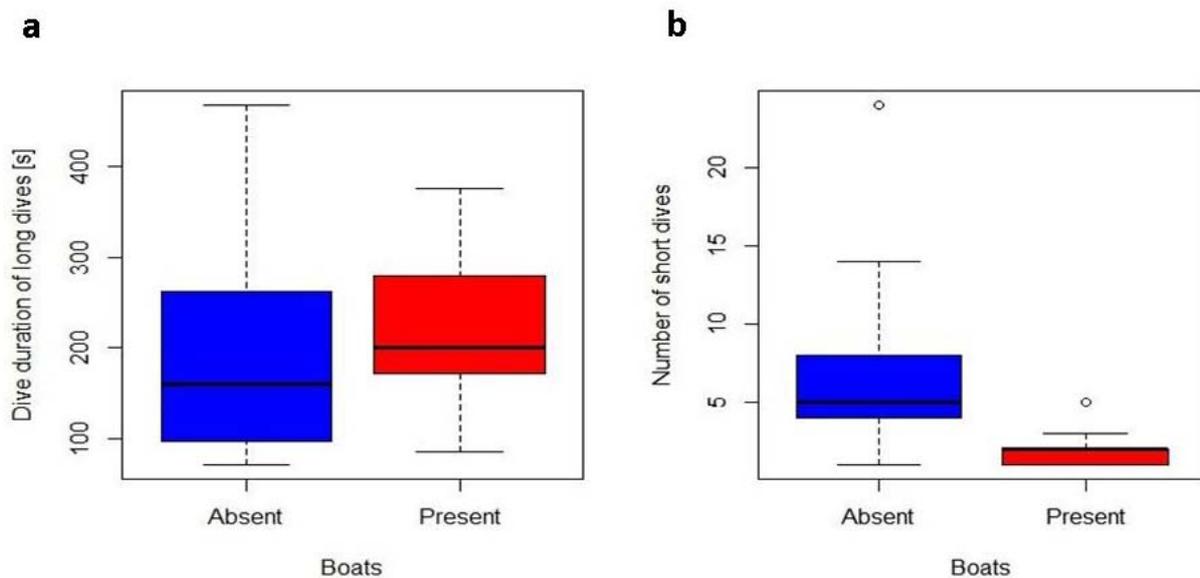


Figure 6 Boxplots of a, the dive duration of the long dives [s] in the presence (n=12) and absence (n=20) of boats (U=85; p>>0.05), and b, the number of short dives in the presence (n=24) and absence (n=24) of boats (U=142.5; p<<0.05 adjusted for ties), for fin whales (*Balaenoptera physalus*).

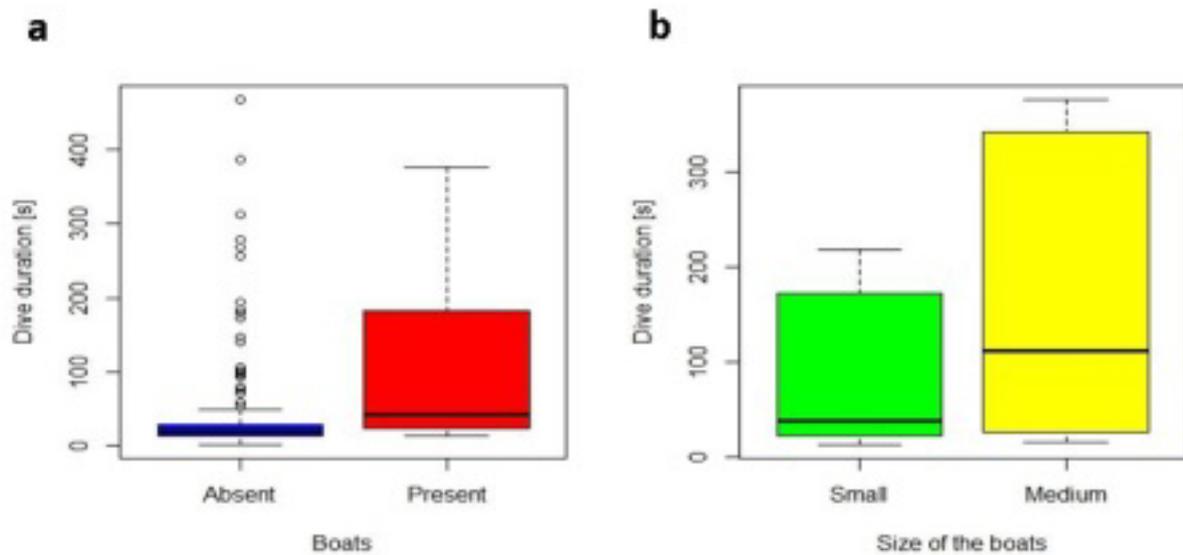


Figure 8 Boxplots of: a, the dive duration [s] between two surfacings of fin whales (*Balaenoptera physalus*) in the absence (n=407) and presence (n=143) of boats (U=1079; p<0.01 two tailed, adjusted for ties), and b, the dive duration [s] between two surfacings of fin whales in relation to small (n=21) and medium (n=10) sized boats (U=78.5; p>0.05 two tailed; adjusted for ties).

Furthermore, there were no significant differences in the distance travelled between two surfacings of the whale in the absence and presence of boats (fig 9b). Additionally there were also no significant difference in the speed travelled by the whale between two surfacings [ $m s^{-1}$ ] in the absence and presence (fig 9c; Table S6-8).

unequal sightings of the two species across the sampling days. This outcome is perhaps not so unusual as the observations were focused in the bay area that is in close proximity of the beach and at shallower depths than further out. Humpback whales have been known to display habitat preference for shallower depths than most baleen whales. They are typically found to prefer depths of between 0-100 m around West Greenland in response to the large occurrences of prey species that occur around the inshore

## Discussion

The unevenness of data is accountable to the

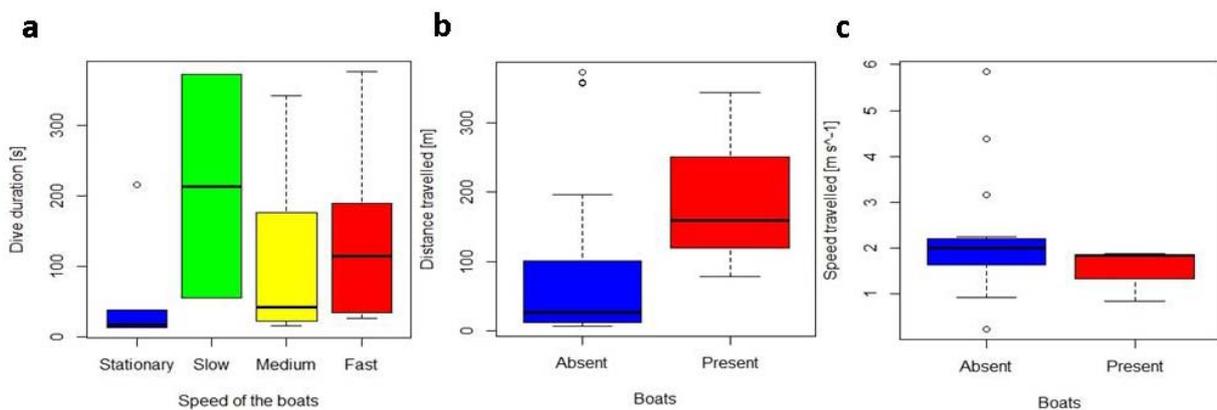


Figure 9 Boxplots of: a, the dive duration [s] between two surfacings of fin whales (*Balaenoptera physalus*) in relation to: stationary (n=6), slow (n=2), medium (n=13) and fast (n=10) going boats (Stationary and medium: U=21; p>0.05 two tailed; adjusted for ties. Stationary and fast: U=12.5; p>0.05 two tailed, adjusted for ties. Slow and fast: U=13; p>>0.05 two tailed. Medium and fast: U=48.5; p>0.05 two tailed, adjusted for ties), and b, distance travelled by fin whales [m] between two surfacings in relation to the presence (n=3) or absence (n=56) of boats (U=30; p>0.05 two tailed), and c, speed travelled by the fin whales [ $m s^{-1}$ ] in relation to the presence (n=3) or absence (n=17) of boats (U=36; p>>0.05 two tailed).

surface waters (Heide-Jørgensen and Laidre, 2007; Laidre and Heide-Jørgensen, 2012). In addition to this, estimated abundances of humpbacks in West Greenland have increased to roughly 3 times as much as the 360 whales estimation given in Larsen and Hammonds's (2004) study, with a distinct rate of increase in abundance of humpbacks being recorded in their West Greenlandic summering grounds (Heide-Jørgensen et al., 2007; Heide-Jørgensen et al., 2012). Despite numerous studies recording fin whale occurrences within coastal West Greenland, fin whales are often better associated with deeper offshore waters like that of their preferred feeding area in Store Hellefiske bank that accommodates their larger size and more energetically taxing deepwater lunge feeding strategies (Heide-Jørgensen et al., 2010; Laidre et al., 2010; Laidre and Heide-Jørgensen, 2012). Failings to see any fin whales in the Disko Bay area were a prominent outcome of the study by Heide-Jørgensen et al., (2007). They accounted this to the time of sampling within the season, a factor that is perhaps applicable to the current study, which focused observations in much earlier summer months. Though both whale species feed on capelin (*Mallotus villosus*) that frequently spawn in coastal areas like Disko Bay where numerous individuals were observed washed up on the beach within the current study, it is more often humpbacks that will target the fish in these areas, further supporting the more prominent presence of humpbacks within the data set (Mizroch et al., 1984; Laidre et al., 2010).

The large difference in the number of short dives compared to long dives was found for both whale species observed in the current study. The most frequent dives performed by the whale species were the short dives which poses no surprise due to the fact that almost all diving cetaceans perform successions of shallow dives before undergoing a longer deep dive (Berta et al., 2006) (fig S1). In fact, humpbacks observed within the current study also spent prominently short amounts of time on the surface, much like the average <40 seconds described in other studies (Winn and Reichley, 1985; Heide-Jørgensen et al., 2012 ). These shallow dives occur several times before the whale partakes in

a longer travel or foraging dive, which can be subjected to the fact that like all mammals, cetaceans are aerobic organisms that require consistent access to air in order to survive (Berta et al., 2006). It is on this basis that cetaceans associate highly with the surface, typically entering deeper waters for the purpose of feeding, improved travel, sleeping and even reducing metabolic costs (Berta et al., 2006).

Maps that were generated from the recorded whale surfacings (fig 1 and S4-S14) indicate some general pattern of spatial movement of the whales in the study area. From our observations we noticed that the area is both used for occasional feeding (we observed gulp feeding by several humpbacks across different days) but also just for transit. Based on our observations it seemed to be approximately the same place that feeding took place across the days. Clear indications of both feeding and transit could be insinuated from both observations and data obtained. In that feeding behaviours typically involved longer dives and less linear movements much as can be observed in e.g. figure S4. Alternatively travelling behaviour involved surface swimming in a linear fashion consisting of numerous and frequent short dives (reference) similar to such examples in the current study as for example figure S11.

### *Effects of boats on diving behaviour*

#### *Humpback whales*

The difference between humpback whale diving behaviours and human influence in form of boats varied quite noticeably. Despite this, both a longer duration of long dives (fig 2a) and fewer short dives (fig 2b) were exhibited by the humpbacks when in the presence of boats. Therefore the whales are spending more time underwater than on the surface when boats are nearby, thus when addressing the hypothesis of the current study, our outcome for changed diving behaviour in form of fewer short dives and longer long dives can be supported by the output we received for humpback whales in relation to boat presence. On a larger scale however, no significant difference between dive duration and

the presence of boats was found, a small numerical difference could be observed however, in that dive duration was typically longer when in the presence of boats despite not being significantly different (fig 3a). Furthermore, the total duration of dives did not differ significantly in relation to different boat sizes being present (fig 4b). Baleen whales often respond to the presence of boats through inconspicuous behaviours that may easily be overlooked, or ignoring the vessels entirely. It is often only erratic or unpredictable boat movements, or alternatively, rapid and direct travel towards the whale that prompt more pronounced avoidance behaviour (Richardson and Würsig, 1997). However, studies on humpback whale behaviours in response to whale watching vessels have also shown shifts in diving behaviour whereby vessels approaching whales in close proximity elicited dive responses, even increasing mean dive durations much like we observed within the current study (Corkeron, 1995; Stamation et al., 2010). It should be noted however that the aforementioned findings were from studies that focused on pods as opposed to individuals as measured in the current study. Additionally both studies included more extensive measures of proximal distance of the vessels to the whales, as most behaviours occurred when vessels broke the 100m regulated watching distance, whereas the current study had no reliable measure of the exact proximity of vessels to the whales. Therefore the contrast in the outcome of the current study may be on account of the number of whales observed at one time, as well as the absence of an exact measure of distance of boats from the whales, that may perhaps have greater influence on diving behaviour than first thought. Interestingly, a significant outcome was found in that total dive duration was significantly different when boats were travelling at medium speed than at fast speeds (fig 4c). The finding supports the hypothesis that boats altered whale diving behaviour through increasing the duration and of long dives when a boat travelling at medium speed was present when compared to a fast travelling boat. It is possible that boats travelling at medium speeds better mimic the speed travelled by hunting vessels that are stalking or in pursuit of the whales, especially seeing as

humpbacks are not a fast swimming species, travelling at around 7.9-15.1 km h<sup>-1</sup> (Clapham and Mead, 1999). Typically fast going boats are small vessels travelling with intention or purpose of reaching a destination, and thus proximate whales may view such boat speeds as innocuous. Nevertheless, the result was quite striking, as it is customarily thought that boats approaching whales at slower speeds are less invasive on the whales and results in less aversive behaviours than those travelling at faster speeds (Cantor et al., 2010).

### *Fin whales*

Diving behaviour equally varied for fin whales in relation to boats, with the hypothesis being realised in that diving behaviour changed by longer long dives being performed (fig 7a) and fewer short dives (fig 7b) as well as an overall longer duration of dives (fig 8a). Changes in diving behaviour have been observed for fin whales in the Mediterranean when in the presence of small vessels, whereby reduced surfacings and decreased percentage of surfacings were recorded (Jahoda et al., 2003). Increased dive durations have also been noted amongst other baleen whales when in the presence of boats, particularly under circumstances of harassment under close proximity as described for humpback whales (Scheidat et al., 2004). At Qeqertarsuaq (Disko Island) in 1985, due to low population numbers humpbacks became a protected species, while minke whale (*Balaenoptera acutorostrata*) and fin whales remain hunted under aboriginal subsistence to the present day (Caulfield, 1993). On account of this hunting pressure, it is entirely possible that fin whales may adopt more pronounced avoidance behaviours in the presence of boats, as boats may represent hunting vessels to the whales, therefore evoking anti-predator aversive responses in form of increased dive duration. On the other hand, diving duration was not significantly different for any of the different boat sizes (fig 8b) or boat speeds (fig 9a) that were noted throughout the study. As a result, the hypothesis on suspected change of dive behaviour in form of changed dive durations in relation to boats could not be validated in these

cases. The lack of significant findings for dive duration with different boat sizes or boat speeds may simply be a product of the inadequate data on the fin whales described previously. Equally the significant outcome for dive duration as well as duration of long dives and number of short dives with boat presence referenced prior, should also be handled with caution as, insufficient data may have skewed the observed outcomes of the fin whale analysis.

### *Effect of boats on spatial behaviour*

#### *Humpback whales*

A significant difference was seen for the distance travelled by a whale when in the presence or absence of boats in that when in the presence of boats, the whales travelled longer distances, as hypothesised (fig 3b). Frankel and Clark (2000), found within their study on the impact of Acoustic Thermometry of Ocean Climate (ATOC) projects on Humpback behaviour, that the acoustic stimuli provoked an increased underwater travel distance of the whales, confirming responsive behavioural changes. It is possible that the noise produced by the vessel traffic within the current study may have stimulated the same increased travel response of the observed whales. Especially as small boats were the most common size recorded, and typically have outboard engines that have been documented to promote higher frequency and more intense behavioural responses by whales (Corkeron, 1995; Cantor et al., 2010).

Similarly, the hypothesised change in distance travelled was also observed when analysed with boat size, in that travelled distance in the presence of small and medium sized boats, as well as medium and large sized boats were found to be significantly different (fig 5a). Distance travelled by the humpbacks when in the presence of medium sized boats was the source of the significant variation as travel distance in the presence of small and large sized boats were not found to be significantly different. As previously mentioned it could be possible that variation in motor noise levels may be a determining factor, in that only outboard motors

of small boats or the more powerful inboard motors of large boats are sufficient loud to stimulate behavioural response. The inboard but less powerful motors of medium sized boats may not be loud enough to evoke behavioural response in the humpbacks. A similar outcome was observed by Cantor et al. (2010), whereby they obtained significant behavioural difference for whales in the presence of small compared to large boats, whereby lower frequency and magnitude responses were obtained for large vessels than small. As no medium sized boat category was incorporated within their study, it is possible that results may have correlated with the current study when boat sizes overlapped with the subjectively categorised medium boat sizes of the current study. However, as only one large boat was recorded within the current study, outcomes should be treated with caution.

Interestingly the hypothesis of increased distance travelled in relation to boats was supported yet again when comparing whale travel distances in the presence of stationary and medium, and medium and fast travelling boats (fig 6a). It is generally seen that stationary boats are not perceived as a threat by baleen whales, whereas boats at medium speeds, that have prolonged exposure to the whales may be viewed as threats (Richardson and Würsig, 1997). Alternatively, fast boats may pass the whales at speeds that may be interpreted by the whales as uninterested. This may be further supported by the fact that both medium and slow travelling boats did not differ significantly in invoking longer travel distances by the whales, with slow boats triggering the largest variety in travel distances by the whale (fig 6a). As previously stated, boats travelling at medium and slow speeds may better replicate the speeds travelled by hunters that have historically stalked or chased the whales. Especially as the >48 year lifespan of the species may mean an individual measured in the current study, was present when hunting in the area was not so tightly regulated (Clapham and Mead, 1999). However, care should be taken in concluding on the behaviours exhibited, as measures of the different boat sizes and speeds are unequal across sampling.

Speed adopted by the humpbacks did not differ significantly in the presence of boats (fig 4a), neither did it significantly differ across the respective boat sizes (fig 5b) or boat speeds observed in the current study (fig 6b). As a result, the hypothesized increase in swimming speed in relation to boats cannot be supported. Based on Ford and Reeves's (2008) study on anti-predator strategies of baleen whales in response to killer whale attacks, humpback whales are typical "fight" strategists, in that rather than partaking in high speed fleeing behaviour they instead choose to remain in a close group and act aggressively. In contrast to this, studies on humpback whales in both Ecuador and as nearby as Godthaabsfjord in West Greenland have documented increased swimming speed as a response to whale watching vessels (Scheidat et al., 2004; Boye et al., 2010). While findings in locomotive responses of humpback to perceived threats clearly varies, it is difficult to confirm whether humpbacks perceive boats as threats at all and thus whether anti-predator or aversive behaviours are necessary. Typically the whale watching vessels described in the latter studies may have been perceived as threats based on the nature of whale watching boats to harass whales through direct approaches or unpredictable changes in speed. On the other hand most vessels within the current study merely passed the location of the whales, with few explicitly diverting course or focusing attention on the whales themselves, and so may not have elicited anti-predator responses.

### *Fin whales*

Unlike the humpback whales, the fin whales did not exhibit any significant difference between travel distances in the presence of boats (fig 9b). Although, just as with the humpbacks, no significant difference was obtained for the speed travelled by the whales in the presence or absence of boats (fig 9c). The absence of any significant difference in spatial behaviours in relation to boats means the hypothesised increased speed and distance in presence of boats was not observed, thus the hypothesis should not be accepted. On account of the data, it is possible that all outcomes obtained for the fin whales are a product of the lack of data

collected on them. However should the results be trusted, it could be argued that the lack of significant spatial behaviour in the presence of boats may be a reflection of activity budgets adopted by the whales. Behaviours exhibited by the whales may be budgeted under temporal dynamics in form of activity states, whereby specific behaviours may be exhibited in the presence of vessel traffic at one time and yet may be different upon the next time set of exposure (Lusseau et al, 2009). For example, while no significant outcome for horizontal aversive behaviours were observed when boats were present, vertical avoidance may have occurred instead, particularly as dive duration was significantly longer in the presence of boats. Therefore it is possible that the activity state of the whales was budgeted as vertical as opposed to horizontal avoidance behaviours within the current study. Care should be taken when making assumptions on the behaviours exhibited, as while dive behaviour is attributed to aversion, it is also a frequent component of fin whale feeding behaviour. It can therefore be difficult to evaluate the effects of boat presence within Disko Bay as the summer production of prey species was transpiring, meaning any changes in locomotive behaviours may be manifestations of feeding or foraging behaviours.

### *Concluding remarks*

Changing behaviours in the face of human disturbance has been constantly challenged across time, factors such as critical habitats or individual energetic state may limit or permit certain individuals from averting disturbance (Bejder et al., 2006). The current study should make no rash assumptions on the motives of focal individuals observed, despite this, it could be argued that human impacts in form of boat presence may alter cetacean behaviours and should be considered in the developmental future of Disko Bay. When interpreting the outcome of the study, one should consider that data collected was not normally distributed on account of the limited sampling time and uneven measures of behaviours in the absence of boats than in the presence of them. In fact, the disproportionate number of significant

relationships between measured variables for the humpback whales is less likely a biological outcome, and more likely a simple product of unequal observations on humpbacks than fin whales. With further limitations on account of observer visibility by obstructing features such as large icebergs and unfavorable weather conditions as well as large waves, the importance of adequate observation equipment is accentuated. Replications of the study should both consider lengthening the study duration and including a higher number of observers, as two observers not only created difficulties in spotting focal individuals, but also prevented the potential use of collecting data on the spatial movements of boats present during observations, that may have better represented the interactions between boats and whales. Future studies could also investigate the effect of multiple vessel presence on cetacean behaviour as the disruptive potential may be higher as escape routes for the cetaceans are blocked by the vessels, unlike the current study where only one vessel was noted at a time. However based on our findings, we believe that humpback whales are disturbed by the vessel traffic within coastal Disko Bay and therefore thought should be given on alternative travel routes so that disturbance on the humpbacks during feeding may be minimized. It is possible that fin whales also experience a degree of disturbance based on our outcomes, however their tendency to feed further out perhaps places their risk of disturbance by boats as lower than the humpbacks. As both species of whale rely heavily on these feeding grounds for building up energy stores for their subsequent seasonal migration to lower latitude breeding grounds, disturbance by boats at the Disko Bay feeding grounds may have fitness depriving impacts on individual fitness. Considerations should therefore be made for minimizing disturbance of both species, in order to mitigate the potential fitness depriving effects that boats may cause by disturbing the whales during their feeding season.

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## Appendix

**Supplementary table S1.** Outcome for Levene's test of equal variance on the tested variables

Variable 1	Variable 2	DF	F value	P value
<i>Status of boats (present or absent)</i>	Dive length (coded)	1	7.684	0.005**
<i>Boat size (coded)</i>	Dive length (coded)	1	4.116	0.044*
<i>Boat speed (coded)</i>	Dive length (coded)	1	0.170	0.680
<i>Status of boats (present or absent)</i>	Total dive duration (s)	147	2.145	3.701e-10***
<i>Boat size (coded)</i>	Total dive duration (s)	64	3.265	3.322e-05***
<i>Boat speed (coded)</i>	Total dive duration (s)	64	2.368	0.001**

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*'

**Supplementary table S2.** Statistical results of the Mann-Whitney U tests for humpback whales (boat presence with movement behaviours).

Variables tested (for humpback whales)	W (not adjusted for ties)	p-value (not adjusted for ties)	Z(adjusted for ties) (No ties=NT)	Exact p-value (adjusted for ties)(no ties=NT)	Mean of the tested variables in absence (A) and presence (P)	Median of the tested variables in absence (A) and presence (P)
<i>Absence (n=407)/presence (n=77) of boats with total dive duration [sec]</i>	14056	0.152	-1.435	0.152	(A)39.993 (P)66.792	(A)24 (P)26
<i>Absence (n=208)/presence (n=38) of boats with distance travelled by whale[m]</i>	2501	0.000	NT	NT	(A)116.622 (P)294.273	(A)63.388 (P)171.681
<i>Absence (n=184)/presence (n=38) of boats with speed of the whale [m sec<sup>-1</sup>]</i>	3189.5	0.396	0.850	0.397	(A)1.912 (P)5.360	(A)1.236 (P)1.208

**Supplementary table S3.** Statistical results of the Mann-Whitney U tests for humpback whales (boat size with diving behaviours).

Variables tested (for humpback whales)	W (not adjusted for ties)	p-value (not adjusted for ties)	Z(adjusted for ties) (No ties=NT)	Exact p-value (adjusted for ties)(no ties=NT)	Mean of behaviour in presence of different boat sizes (small=(S), medium(M), large(L))	Median of behaviour in presence of different boat sizes (small=(S), medium(M), large(L))
<i>Boat size (small (n=53)/medium (n=19)) with dive duration [sec]</i>	470.5	0.678	-0.422	0.678	(S)58.698 (M)77	(S)26 (M)23
<i>Boat size (small (n=53)/large (n=5)) with dive duration [sec]</i>	89.5	0.239	-1.191	0.244	(S)58.698 (L)113.8	(S)26 (M)46

<i>Boat size (medium (n=19)/large (n=5)) with dive duration [sec]</i>	34	0.355	-0.960	0.358	(M)77 (L)113.8	(M)23 (L)46
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**Supplementary table S3.** Statistical results of the Mann-Whitney U tests for humpback whales (boat speed and diving behaviours).

<b>Variables tested (for humpback whales)</b>	<b>W (not adjusted for ties)</b>	<b>p-value (not adjusted for ties)</b>	<b>Z(adjusted for ties) (No ties=NT)</b>	<b>Exact p-value (adjusted for ties)(no ties=NT)</b>	<b>Mean of behaviour in presence of different boat speeds (stationary=(ST), slow(SL),medium(M),fast(F))</b>	<b>Median of behaviour in presence of different boat speeds (stationary=(ST), slow(SL),medium(M),fast(F))</b>
<i>Boat speed (stationary (n=17)/slow (n=18)) with dive duration [sec]</i>	121.5	0.306	-1.041	0.306	(ST)75.353 (SL)65.444	(ST)22 (SL)28.5
<i>Boat speed(stationary (n=17)/medium (n=20)) with dive duration [sec]</i>	127	0.195	-1.312	0.1945	(ST)75.353 (M)85.25	(ST)22 (M)30.5
<i>Boat speed (stationary (n=17)/fast (n=22)) with dive duration [sec]</i>	221.5	0.335	0.978	0.3357	(ST)75.353 (F)44.5	(ST)22 (F)20.5
<i>Boat speed (slow (n=18)/medium (n=20)) with dive duration [sec]</i>	162	0.608	-0.527	0.6073	(SL)65.444 (M)85.25	(SL)28.5 (M)30.5
<i>Boat speed (slow (n=18)/fast (n=22)) with dive duration [sec]</i>	258	0.106	1.632	0.1047	(SL)65.444 (F)44.5	(SL)28.5 (F)20.5
<i>Boat speed (medium (n=20)/fast (n=22)) with dive duration [sec]</i>	302	0.040	2.066	0.03847	(M)85.25 (F)44.5	(M)30.5 (F)20.5

**Supplementary table S4.** Statistical results of the Mann-Whitney U tests for humpback whales (boat size with movement behaviours).

Variables tested (for humpback whales)	W (not adjusted for ties)	p-value (not adjusted for ties)	Z(adjusted for ties) (No ties=NT)	Exact p-value (adjusted for ties)(no ties=NT)	Mean of behaviour in presence of different boat sizes (small=(S), medium(M), large(L))	Median of behaviour in presence of different boat sizes (small=(S), medium(M), large(L))
<i>Size of boat (small (n=29)/medium (n=6)) with distance travelled [m]</i>	141	0.0162	NT	NT	(S)326.648 (M)54.836	(S)186.383 (M)22.711
<i>Size of boat (small (n=29)/large (n=3)) with distance travelled [m]</i>	26	0.286	NT	NT	(S)326.648 (L)460.186	(S)186.383 (L)279.734
<i>Size of boat (medium (n=6)/large (n=3)) with distance travelled [m]</i>	0	0.024	NT	NT	(M)54.836 (L)460.186	(M)22.711 (L)279.734
<i>Size of boats with (small (n=29)/medium (n=6)) with travel speed by the whales [<math>m\ sec^{-1}</math>]</i>	130	0.062	NT	NT	(S)6.324 (M)1.717	(S)1.214 (M)0.375
<i>Size of boats with (small (n=29)/large (n=3)) with travel speed by the whales [<math>m\ sec^{-1}</math>]</i>	31	0.457	NT	NT	(S)6.324 (L)3.338	(S)1.214 (L)2.444
<i>Size of boats with (medium (n=6)/large (n=3)) with travel speed by the whales [<math>m\ sec^{-1}</math>]</i>	3	0.167	NT	NT	(M)1.717 (L)3.338	(M)0.375 (L)2.444

**Supplementary table S5.** Statistical results of the Mann-Whitney U tests for humpback whales (boat speed with movement behaviours).

Variables tested (for humpback whales)	W (not adjusted for ties)	p-value (not adjusted for ties)	Z(adjusted for ties) (No ties=NT)	Exact p-value (adjusted for ties)(no ties=NT)	Mean of behaviour in presence of different boat speeds (stationary=(ST), slow(SL),medium(M),fast(F))	Median of behaviour in presence of different boat speeds (stationary=(ST), slow(SL),medium(M),fast(F))
<i>Speed of boats (stationary (n=9)/slow (n=9)) with distance travelled [m]</i>	27	0.258	NT	NT	(ST)179.4 (SL)553.107	(ST)92.37 (SL)342.328
<i>Speed of boats (stationary (n=9)/medium (n=8)) with distance travelled [m]</i>	15	0.046	NT	NT	(ST)179.4 (M)347.845	(ST)92.37 (M)288.585
<i>Speed of boats (stationary (n=9)/fast (n=12)) with distance travelled [m]</i>	39	0.31	NT	NT	(ST)179.4 (F)150.587	(ST)92.37 (F)138.004
<i>Speed of boats (slow (n=9)/medium (n=8)) with distance travelled [m]</i>	35	0.963	NT	NT	(SL) 553.107 (M)347.845	(SL)342.328 (M)288.585
<i>Speed of boats (slow (n=9)/fast (n=12)) with distance travelled [m]</i>	66	0.422	NT	NT	(SL) 553.107 (F)150.587	(SL)342.328 (F)138.004
<i>Speed of boats (medium (n=8)/fast (n=12)) with distance travelled [m]</i>	81	0.01	NT	NT	(M) 347.845 (F)150.587	(M)288.585 (F)138.004
<i>Speed of boats (stationary (n=9)/slow (n=9)) with the speed of the whale[m sec<sup>-1</sup>]</i>	29	0.340	NT	NT	(ST)5.352 (SL)10.761	(ST)0.269 (SL)2.617
<i>Speed of boats (stationary (n=9)/medium (n=8)) with the speed of the whale[m sec<sup>-1</sup>]</i>	24	0.277	NT	NT	(ST) 5.352 (M)2.819	(ST)0.269 (M)1.703
<i>Speed of boats (stationary (n=9)/fast (n=12)) with the speed of the whale [m sec<sup>-1</sup>]</i>	34	0.169	NT	NT	(ST) 5.352 (F)3.0113	(ST)0.269 (F)1.208
<i>Speed of boats (slow (n=9)/medium (n=8)) with the speed [m sec<sup>-1</sup>]</i>	44	0.481	NT	NT	(SL)10.761 (M)2.819	(SL)2.617 (M)1.703
<i>Speed of boats (slow (n=9)/fast (n=12)) with the speed of the whale[m sec<sup>-1</sup>]</i>	52	0.917	NT	NT	(SL)10.761 (F)3.011	(SL)2.617 (F)1.208
<i>Speed of boats (medium (n=8)/fast (n=12)) with the speed of the whale [m sec<sup>-1</sup>]</i>	48	1	NT		(M) 2.819 (F)3.011	(M)1.703 (F)1.208

**Supplementary table S6.** Statistical results of the Mann-Whitney U tests for fin whales (boat presence with movement behaviours).

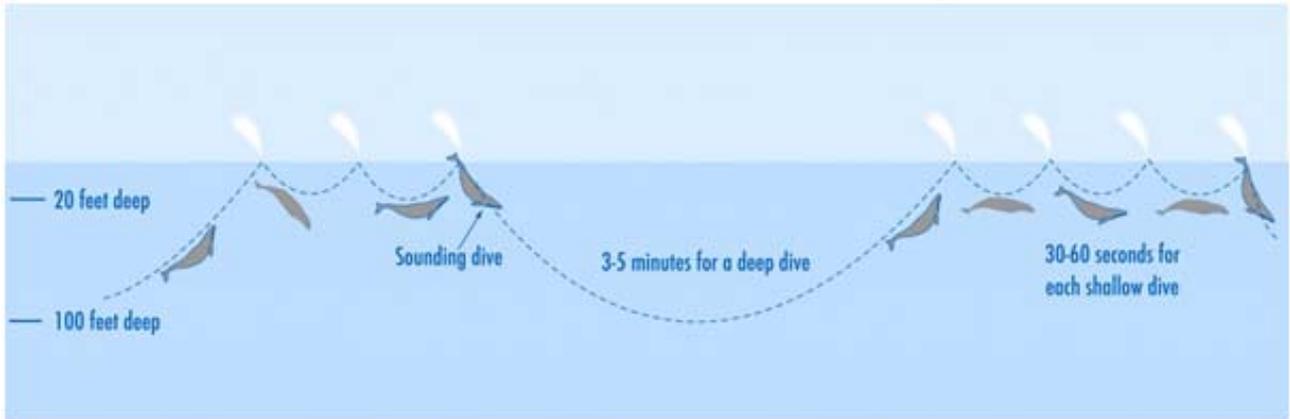
Variables tested (for fin whales)	W (with ties in the ranks)	p-value (with ties in the ranks)	Z(exact test - taking ties in the rank into account)(no ties=NT)	Exact p-value (taking ties in the rank into account) (no ties=NT)	Mean of the tested variables in absence (A) and presence (P) of boats	Median of the tested variables in absence (A) and presence (P) of boats
<i>Absence (n=407)/presence (n=143) of boats with total dive duration[sec]</i>	1079	0.008	-4.477	0.004	(A)43.070 (P)108.8065	(A)19 (P)42
<i>Absence (n=56)/presence (n=3) of boats with distance travelled between two surfaces [m]</i>	30	0.065	NT	NT	(A)72.474 (P)193.737	(A)26.294 (P)159.205
<i>Absence (n=17)/presence (n=3) of boats with speed of the whale travelling between two surfaces [m sec<sup>-1</sup>]</i>	36	0.305	NT	NT	(A)2.192 (P)1.511	(A)1.997 (P)1.828

**Supplementary table S7.** Statistical results of the Mann-Whitney U tests for fin whales (boat size with diving behaviour).

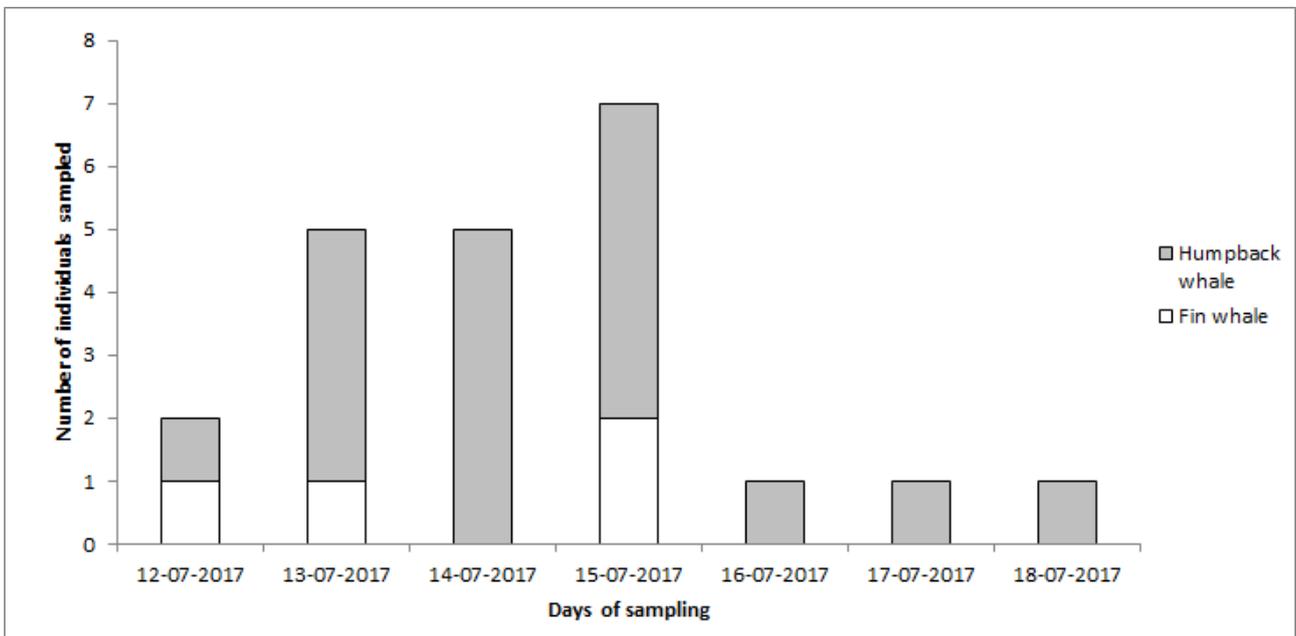
Variables tested (for fin whales)	W (not adjusted for ties)	p-value (not adjusted for ties)	Z(adjusted for ties) (No ties=NT)	Exact p-value (adjusted for ties)(no ties=NT)	Mean of behaviour in presence of different boat sizes (small=(S), medium(M), large(L))	Median of behaviour in presence of different boat sizes (small=(S), medium(M), large(L))
<i>Boat size (small (n=21)/medium (n=10)) with total dive duration [sec]</i>	78.5	0.272	-1.120	0.272	(S)83.476 (M)162	(S)38 (M)111.5

**Supplementary table S8.** Statistical results of the Mann-Whitney U tests for fin whales (boat speed with diving behaviours).

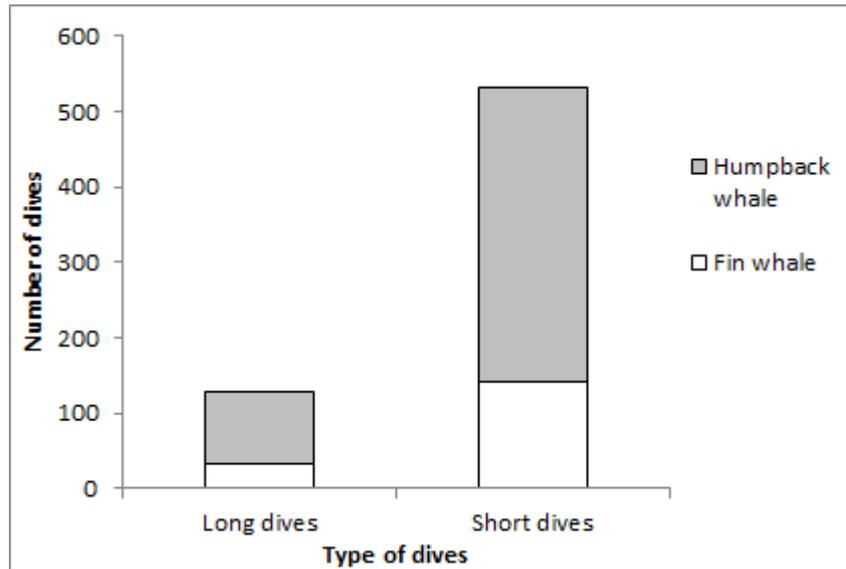
Variables tested (for fin whales)	W (not adjusted for ties)	p-value (not adjusted for ties)	Z(adjusted for ties) (No ties=NT)	Exact p-value (adjusted for ties)(no ties=NT)	Mean of behaviour in presence of different boat speeds (stationary=(ST), slow(SL),medium(M),fast(F))	Median of behaviour in presence of different boat speeds (stationary=(ST), slow(SL),medium(M),fast(F))
<i>Boat speed (stationary (n=6)/slow (n=2)) with dive duration [sec]</i>	1	0.131	-1.677	0.107	(ST)53 (SL)213.5	(ST)18 (SL)213.5
<i>Boat speed (stationary (n=6)/medium (n=13)) with dive duration [sec]</i>	21	0.125	-1.580	0.122	(ST)53 (M)101.385	(ST)18 (M)42
<i>Boat speed (stationary (n=6)/fast (n=10)) with dive duration [sec]</i>	12.5	0.065	-1.901	0.060	(ST)53 (F)131	(ST)18 (F)114.5
<i>Boat speed (slow (n=2)/medium (n=13)) with dive duration [sec]</i>	21	0.202	1.360	0.219	(SL)213.5 (M)101.385	(SL)213.5 (M)42
<i>Boat speed (slow (n=2)/fast (n=10)) with dive duration [sec]</i>	13	0.606	NT	NT	(SL)13.5 (F)131	(SL)213.5 (F)114.5
<i>Boat speed (medium (n=13)/fast (n=10)) with dive duration [sec]</i>	48.5	0.321	-1.024	0.320	(M)101.385 (F)131	(M)42 (F)114.5



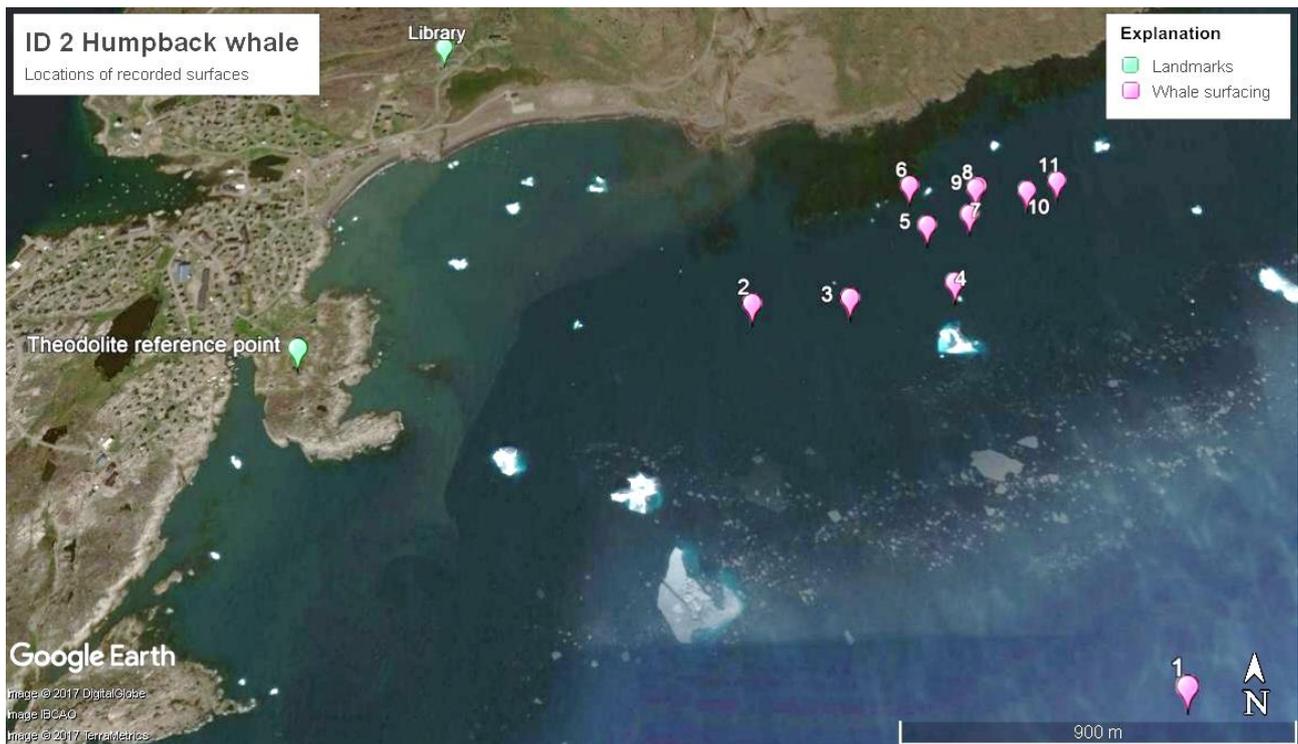
Supplementary Figure 1. Illustration of typical cetacean diving behaviour. <https://aquarium.ucsd.edu/blog/whale-watching-the-tale-of-a-whales-trail>



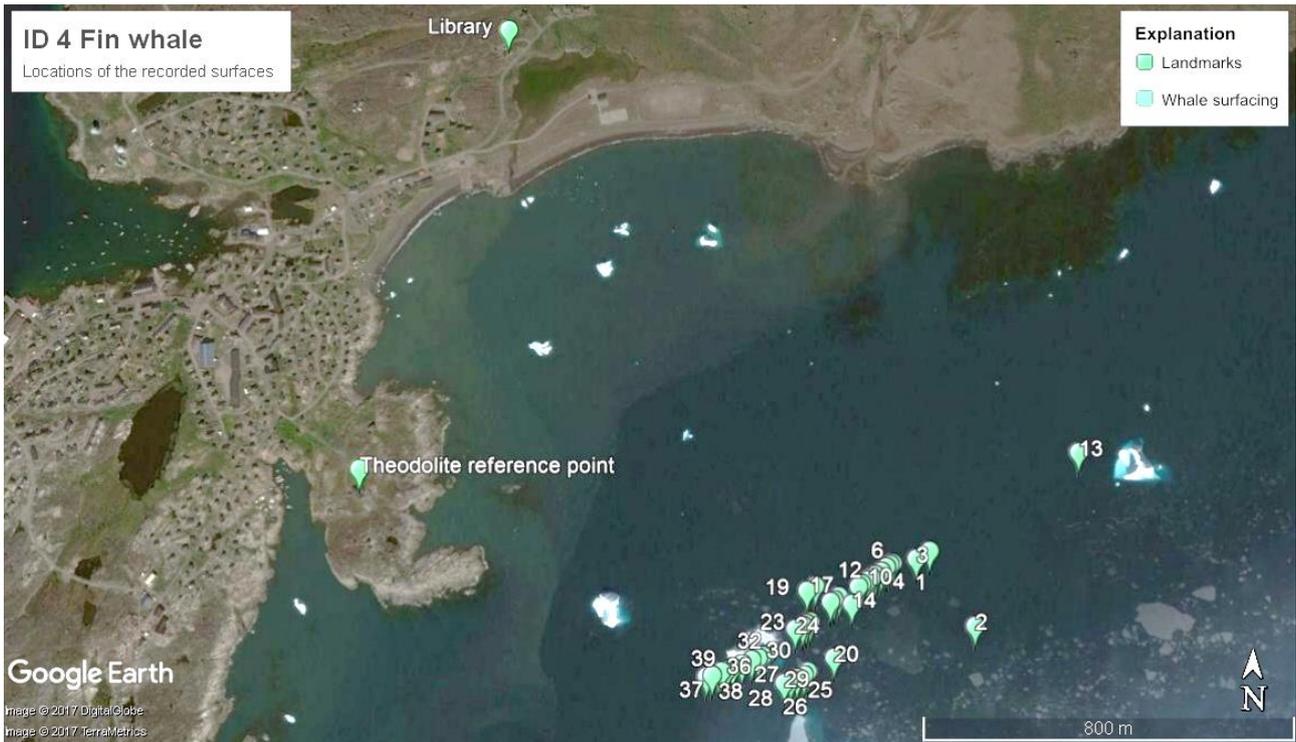
Supplementary Figure S2. Barplot of the number of whales sampled across the 7 sampling days, with humpback whales in grey and fin whales in white.



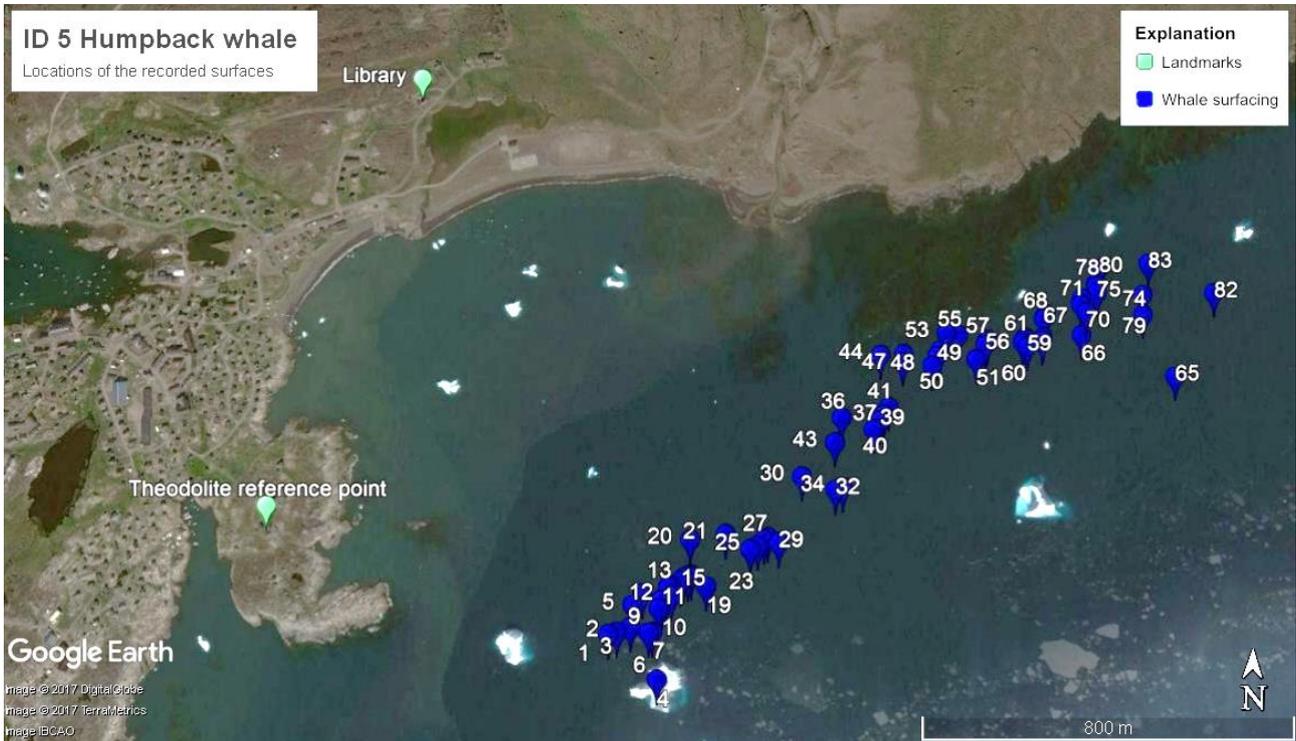
Supplementary Figure S3. Barplot of the number of dives performed by both whale species and the type of dive performed, characterised as either long (>60 s) or short (<60 s).



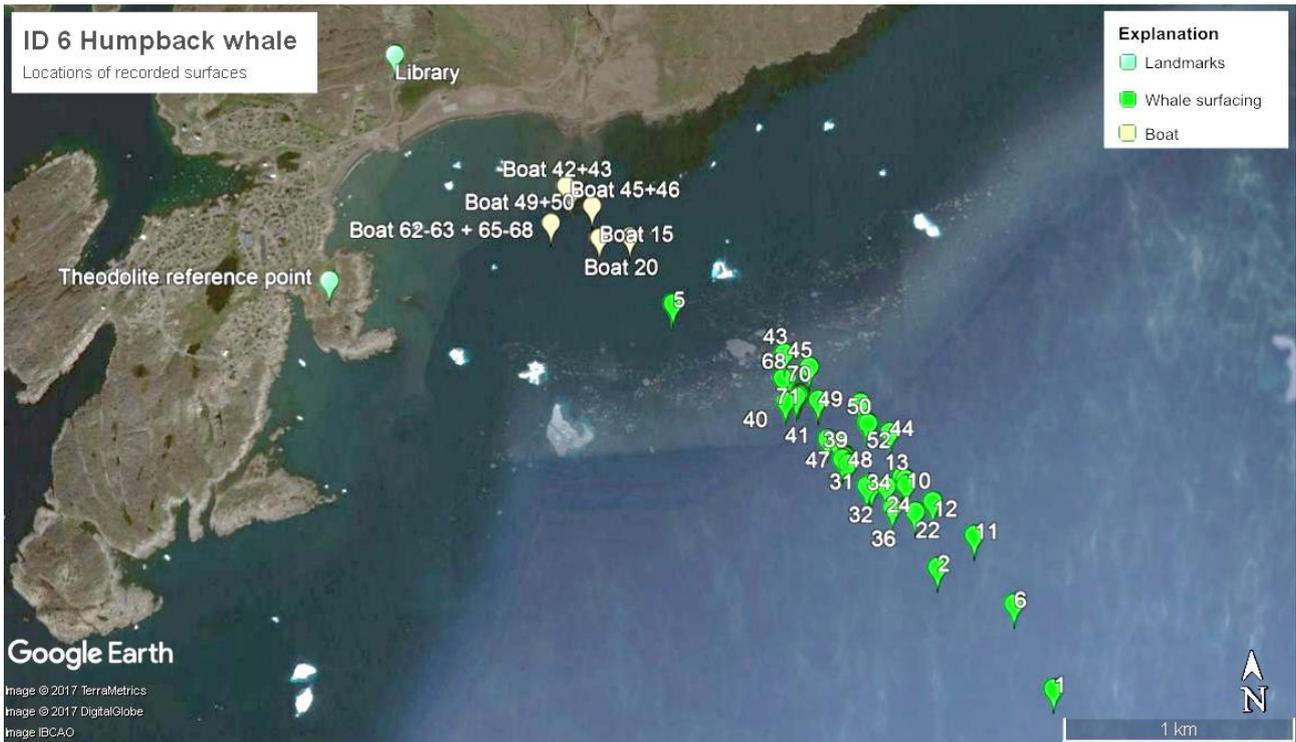
Supplementary Figure S4. Map of the spatial movement patterns of a humpback whale (*Megaptera novaeangliae*) (ID 2; light pink) in the western part of Disko Bay which can be viewed from the library of the Arctic Station (light green) at Qeqertarsuaq, Disko, Greenland. The numbers indicate the actual number of each surface. A mast (light green) was used as a reference point for the theodolite.



Supplementary Figure S5. Map of the spatial movement patterns of a fin whale (*Balaenoptera physalus*) (ID 4; light grey) in the western part of Disko Bay viewed from the library of the Arctic Station (light green) at Qeqertarsuaq, Disko, Greenland. The numbers indicate the actual number of each surface. A mast (light green) was used as a reference point for the theodolite.



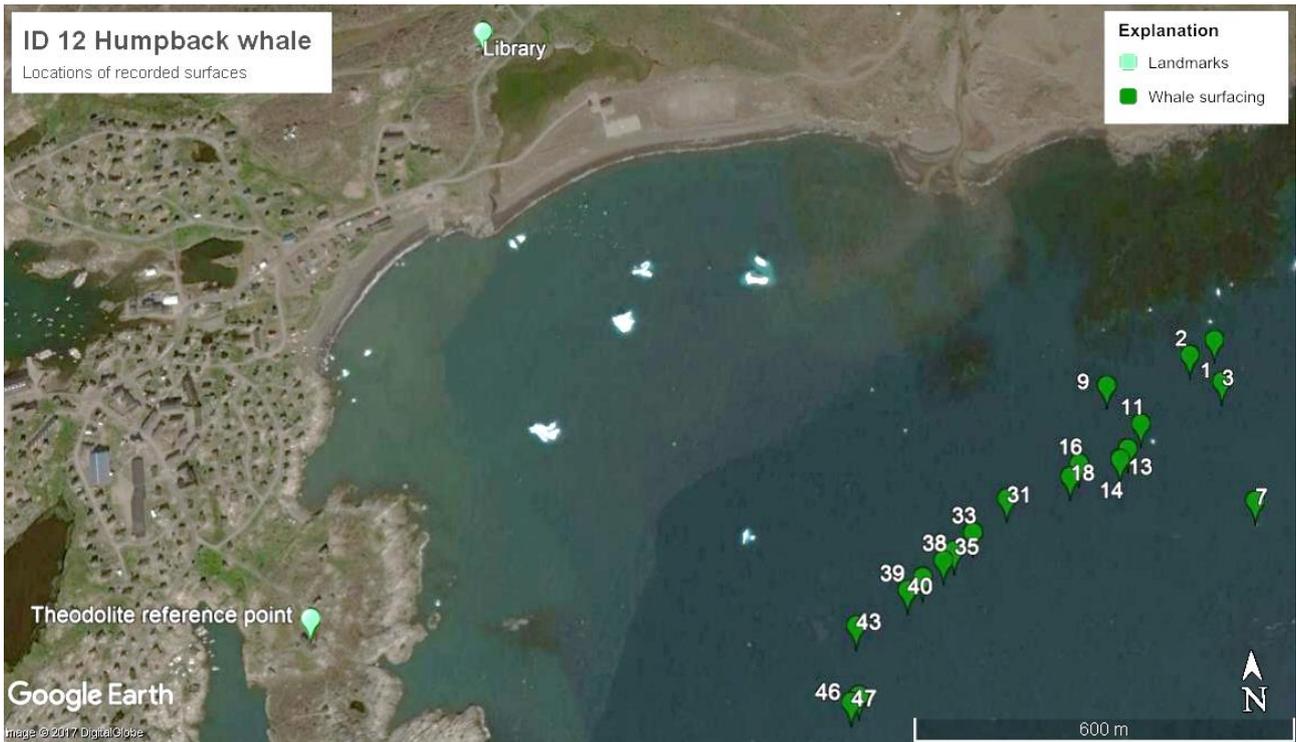
Supplementary Figure S6. Map of the spatial movement patterns of a humpback whale (*Megaptera novaeangliae*) (ID 5; dark blue) in the western part of Disko Bay viewed from the library of the Arctic Station (light green) at Qeqertarsuaq, Disko, Greenland. The numbers indicate the actual number of each surface. A mast (light green) was used as a reference point for the theodolite.



**Supplementary Figure S7.** Map of the spatial movement patterns of a humpback whale (*Megaptera novaeangliae*) (ID 6; green) and a boat (light grey) in the western part of Disko Bay viewed from the library of the Arctic Station (light green) at Qeqertarsuaq, Disko, Greenland. For the whale (green), the numbers indicate the actual number of each surface and for the boat the numbers indicate at which surfaces, where the boat was present in the given situation. A mast (light green) was used as a reference point for the theodolite.



**Supplementary Figure S8.** Map of the spatial movement patterns of a humpback whale (*Megaptera novaeangliae*) (ID 9; yellow) in the western part of Disko Bay viewed from the library of the Arctic Station (light green) at Qeqertarsuaq, Disko, Greenland. The numbers indicate the actual number of each surface. A mast (light green) was used as a reference point for the theodolite.



**Supplementary Figure S9.** Map of the spatial movement patterns of a humpback whale (*Megaptera novaeangliae*) (ID 12; dark green) in the western part of Disko Bay viewed from the library of the Arctic Station (light green) at Qeqertarsuaq, Disko, Greenland. The numbers indicate the actual number of each surface. A mast (light green) was used as a reference point for the theodolite.



**Supplementary Figure S10.** Map of the spatial movement patterns of a fin whale (*Balaenoptera physalus*) (ID 13; light yellow) in the western part of Disko Bay viewed from the library of the Arctic Station (light green) at Qeqertarsuaq, Disko, Greenland. The numbers indicate the actual number of each surface. A mast (light green) was used as a reference point for the theodolite.



**Supplementary Figure S11.** Map of the spatial movement patterns of a humpback whale (*Megaptera novaeangliae*) (ID 14; orange) in the western part of Disko Bay viewed from the library of the Arctic Station (light green) at Qeqertarsuaq, Disko, Greenland. The numbers indicate the actual number of each surface. A mast (light green) was used as a reference point for the theodolite.



**Supplementary Figure S12.** Map of the spatial movement patterns of a humpback whale (*Megaptera novaeangliae*) (ID 17; red) in the western part of Disko Bay viewed from the library of the Arctic Station (light green) at Qeqertarsuaq, Disko, Greenland. The numbers indicate the actual number of each surface. A mast (light green) was used as a reference point for the theodolite.



Supplementary Figure S13. Map of the spatial movement patterns of a fin whale (*Balaenoptera physalus*) (ID 18; white) in the western part of Disko Bay viewed from the library of the Arctic Station (light green) at Qeqertarsuaq, Disko, Greenland. The numbers indicate the actual number of each surface. A mast (light green) was used as a reference point for the theodolite.



Supplementary Figure S14. Map of the spatial movement patterns of a humpback whale (*Megaptera novaeangliae*) (ID 20; light purple) in the western part of Disko Bay viewed from the library of the Arctic Station (light green) at Qeqertarsuaq, Disko, Greenland. The numbers indicate the actual number of each surface. A mast (light green) was used as a reference point for the theodolite.

*The influence of climate change on Disko Island's macroalgal communities*



**Macroalgae (photo: Samuel Black)**

# *The influence of climate change on Disko Island's macroalgal communities*

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## **Abstract**

As the Arctic Ocean warms at twice the rate of the global ocean significant melting of perennial sea ice, northward range shifts and species extinctions are observed. With a tight coupling to ocean temperatures and a key role in ecological systems, macroalgal communities act as a useful tool when examining how marine systems may respond to a changing climate. Although known to be both culturally and ecologically important, only few research initiatives have focused on the macroalgal communities around Qeqertarsuaq on Disko Island on Greenland. Resultantly this study aimed to examine, describe and review macroalgal species composition, relative abundance, zonation and historical data in order to develop an understanding of southern Disko Island's macroalgal communities and how they may respond in response to climate change. The latter was achieved by carrying out a series of quadrats along two 10 m transects across a total of 6 different locations in order to identify the species present, their relative abundance and the overall zonation patterns present. Once identified and collated, broad comparisons of the sample locations were elucidated through the use of kite diagrams and a Jaccard coefficient similarity measure. Historical perspectives were enabled by collating data from previous literature. Overall, the populations species composition and zonation patterns were found to be moderately similar, falling into three distinct groups largely controlled local physical parameters such as sediment loading and exposure to wind, wave and ice scouring. Slope profile also likely exerting a controlling factor at each location. The results also showed a discernable absence in red algal species at each location compared to historical data. A suggested reason is increased UV radiation exposure due to decreased sea ice cover, with changes likely to be in species composition rather than class composition. Highly exposed locations appeared to be more similar to historical data in species composition suggesting difficulties in reproduction and adaptation by new macroalgae species. Further changes in community structure driven by northward range shifts are predicted to increase as warming continues.

**Keywords:** *macroalgae, zonation, climate change, exposure, ice scouring*

## **Introduction**

Currently the Arctic Ocean is warming at twice the rate of the global ocean (Pachauri et al., 2014). Even under conservative global warming scenarios this rapid warming is predicted to continue, with a warming of around 5 °C estimated for the year 2100 (Christensen et al., 2013). As subarctic isotherms shift poleward and significant melting of perennial sea ice occurs, many species extinctions and northward range shifts are observed (Burrows et al., 2011).

Marine intertidal communities are particularly sensitive to such rising temperatures given their tendency to occupy the upper limits of their temperature tolerance (Tomanek, 2010). As a result it is predicted that a rapidly warming Arctic will drive large changes within arctic marine intertidal communities (Jueterbock et al., 2016). Macroalgal communities represent a pragmatic tool for examining such change for a number of reasons. Firstly, macroalgae distribution is tightly coupled to ocean temperatures (Jueterbock et al., 2013) and secondly, macroalgal species are thought to hold

an important function in ecosystem structuring by providing protection, habitat and food for other biota within the littoral and sub-littoral zones (Harley et al., 2012).

Recent research has documented an array of northward expansions of southern macroalgal species into sub-Arctic and Arctic regions (Fredriksen et al., 2014; Wiencke & Amsler, 2012; Hawkins et al., 2009; Blanchette et al., 2008), with the potential to cause distinct changes in ecosystem function and community structure (Jueterbock et al., 2013). Whilst such expansions are dependent on climatic conditions, dispersal of larvae and pelagic transport via ocean currents are also important factors (Høgslund et al., 2014). Given its direct connection with the North Atlantic through the Irminger Current and North Atlantic drift, the west coast of Greenland is thought to be an important gateway to the Arctic for macroalgal species expanding their range northwards. Despite this, the macroalgal communities of Western Greenland remain largely poorly studied (Wulff et al., 2009).

Acknowledged by the International Union for Conservation of Nature (IUCN) as an 'Ecologically and culturally Biologically Significant Area' and proposed as an Arctic marine area of heightened ecological and cultural significance, the Disko Bay area in Southwest Greenland is considered to be an important area for breeding, overwintering and migrating for a variety of seabirds, marine mammals, fish and crustaceans (AMAP/CAFF/SDWG, 2013). Although largely dominated by four genera, *Fucus*, *Agarum*, *Laminaria* and *Desmarestia*, macroalgal communities in Disko Bay have been shown to be highly diverse relative to other Arctic regions. Overall, a total of 79 species of algae have been identified by recent research in the area (Boertmann et al., 2013). The importance of macroalgae in the Disko Bay area is also captured within traditional knowledge, with local fishermen observing the relationship between diverse kelp forests and good cod fishing (Krause-Jensen & Duarte, 2014). Recent research suggests that the predominant control on macroalgal abundance appears to be the availability of suitable substrate although there was a distinct

negative relationship between sea urchins (*Strongylocentrotus droebachiensis*) abundance and total macroalgae cover (Boertmann et al., 2013). As intense grazers of kelp, high densities of urchins are capable of clearing areas of kelp forests leaving behind barren ground. Such areas have been reported in the littoral zone on the west coast of Disko Island, although the true extent of their influence on macroalgal vegetation is poorly understood (Hansen & Schlutter, 1992).

It is highly likely that climate change will influence the macroalgal communities of Disko Bay. This will be predominantly driven by a lengthened growing season as sea ice extent continues to decline alongside the northwards expansion of Atlantic species (Arctic Station Long Term Monitoring Program, 2017). It is also proposed that decreased Arctic sea ice cover will increase the annual productivity of *Strongylocentrotus droebachiensis*, which could have a dramatic influence on macroalgal abundance (Müller et al., 2009).

Given their function as 'ecosystem engineers' and their importance within local traditional knowledge, examining the macroalgal communities of Disko Bay is essential, particularly as the area becomes a potential 'hot spot' for extractive industries. As within any management action plan regarding the protection of vital marine resources, a monitoring effort to establish the current distribution of macroalgal communities in the area is an important prerequisite. Although well-studied in recent years (Rasmussen, 2016; Boertmann et al., 2013; Mosbech et al., 2007; Brandt et al., 1999; Hansen, 1998; Hansen & Schlütter, 1992), few research initiatives have focused on establishing a broad overview of the macroalgal populations around Qeqertarsuaq on Disko Island. Resultantly this study aims to examine and describe the macroalgal species composition, relative abundance and zonation patterns at a variety of locations on southern Disko Island. Through the use of data from 1990 to present, the study also aims to make a broad inference regarding how macroalgal communities in the area are changing with respect to climate change.



Figure 1 The six locations on southern Disko Island. Source: Google Earth.

## Methods

### Sampling locations

A total of six sample locations on southern Disko Island were visited between the 10<sup>th</sup> - 20<sup>th</sup> of July 2017 (figure 1). The chosen sites, Winter Harbour (WH), Rockpools (RP), Qeqertarsuaq Harbour (QH), Torskebugten (TB), Fortuna Bay (FB) and Laksebugten (LB), were based on previous literature (appendix 1) (Rasmussen, 2016; Boertmann et al., 2013; Hansen, 1998; Hansen & Schlütter, 1992). The locations' exposure was estimated using a coast type classification scheme as described in Pedersen's book 'Grønlands Havalger' (Pedersen, 2011). The scale ranges from a coast type 1, defined as a coastline sheltered from wind, wave and ice scouring, to a coast type 4, categorized as a location with a high exposure to wind, wave and ice scouring. All sites exhibited a clear zonation pattern with upper, middle and lower zones.

### Sampling protocol

Sample locations were visited at low tide at each location to ease sample collection and to obtain a reference level to enable sample comparison at different locations. The tide data was obtained from an online harmonic tide clock

and tide predictor software (Flater, 1998). At each location two 10 m transects were carried out. The 5 m mark on the cable was placed at the low tide level in order to obtain a transect sampling 5 m above and 5 m below the low tide level (figure 2). At every meter along the transect a 0.2 m<sup>2</sup> quadrat containing 81 squares was completed (figure 3). The height above or below the low tide was also measured at every meter using a measuring stick and a clinometer. To make samples comparable to each other a mean low water (MLW) was calculated based on the tidal height at low water for all of the sampling days. The slope profile measurements at each transect were then calibrated relative to the calculated MLW by accounting for the difference between the MLW and the low water value. The relative abundance of each species was estimated by counting the number of squares that each species was present within, e.g. 32 squares of *Fucus distichus*, 16 squares of *Chordaria flagelliformis* and so on until all species were counted within the quadrat before a percentage value was calculated. Quadrats below the water level were completed using snorkeling gear. The information was relayed to the recorder onshore. Unidentifiable algal taxa were counted, handpicked, noted with a sample ID and



Figure 2 Top 5 m of 10 m transect at Qeqertarsuaq Harbour.

placed in a labelled plastic bag to be identified in the lab using a stereoscope.

#### Laboratory, analyses and statistics

Species were identified using Grønlands Havalger (Pedersen, 2011). If species identification could not be determined by eye a small sub-sample was placed under a microscope or a stereoscope and identified based on a variety of different morphological characteristics. If still unidentifiable a picture was taken and sent to an Arctic macroalgal expert for confirmation. Once completed, the data was tabulated and incorporated into a kite diagram to display each transects' slope profile, species composition, relative abundance and zonation at each location.

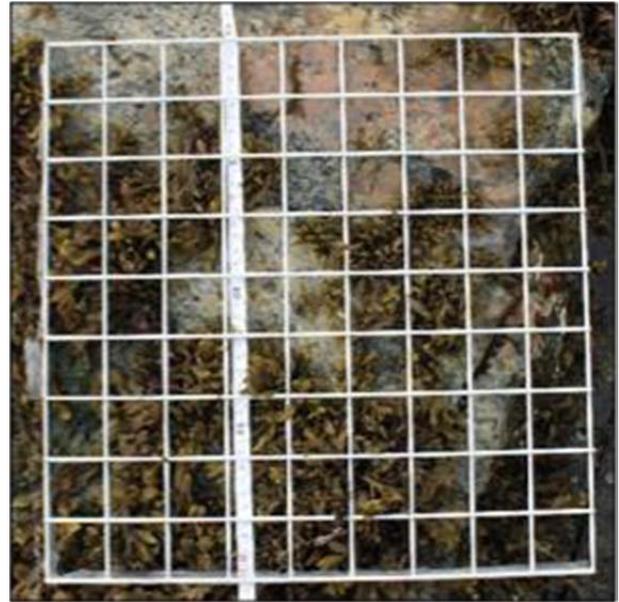


Figure 3 Example of quadrat at Qeqertarsuaq Harbour.

A broad comparison of the sample locations based on the presence and absence of macroalgal species (appendix 2) was achieved by conducting a Jaccard coefficient similarity measure in PAST-paleontological statistics, ver. 2.17c (Hammer et al., 2008; Jaccard, 1912).

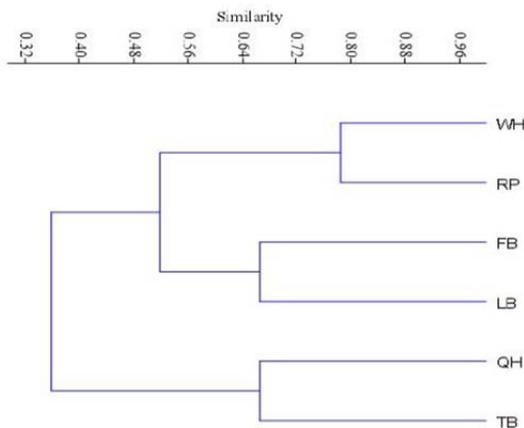
#### Results

##### Overall location comparison

Table 1 shows the total number of species found at each location within each class of macroalgae. The most species were found at location LB (14), consisting predominantly of brown algae (10) and a lesser number of green algae (3) and red algae (1). The fewest number of species were found at location QH (7), again mostly comprised of brown algae (5) and two

Table 1 Macroalgal class and number of species found at each location. WH = Winter Harbour. RP = Rockpools. QH = Qeqertarsuaq Harbour. TB = Torskebugten. FB = Fortuna Bay. LB = Laksebugten.

Macroalgal Class	Location					
	WH	RP	QH	TB	FB	LB
Rhodophyceae	0	0	0	1	0	1
Phaeophyceae	8	9	5	4	7	10
Chlorophyceae	5	3	2	3	4	3
<b>Total number of species</b>	13	12	7	8	11	14



**Figure 4** Jaccard coefficient similarity measure based on presence/ absence data with a similarity value ranging from 0% to 100%. E.g. populations with exactly the same species composition would be 100% similar.

green algal species. As an overall trend, it is observed that the six locations are dominated by brown algae and one species of red algae at location LB and TB.

The Jaccard coefficient similarity measure highlights that, in terms of overall species composition, the 6 sample locations cluster into three groups (Figure 4). The first and most similar group, with a similarity of 78%, is comprised of WH and RP. FB and LB form a second group with a similarity of 67% to one another and a similarity of 52% with WH and RP. QH and TB form the third group with a low similarity (34%) to the first two groups and a 65% similarity to one another.

### Species composition and slope profiles

The kite diagrams illustrate the diversity of species compositions, zonation patterns and slope profiles within the 12 transects across the 6 locations (figure 5). Overall it appears that comparable zonation patterns are observed between the two transects at each location despite slight variations in the dominant species present. The highest macroalgal abundance was found at QH whilst the lowest abundance was present at LB.

At least one or more of the brown fucoid species such as *Fucus distichus*, *Fucus vesiculosus* and *Fucus evanescens* appear to be the most dominant species group, occurring in both high abundances and wide zonation patterns across all

locations, particularly in the upper tidal zone above 0.5 m above. Other phaeophyceae species such as *Pylaiella littoralis* and *Dictyosiphon foeniculaceus* also appear in high abundances and are largely confined to the middle and lower tidal zones, with the latter species particularly abundant at FB and LB. Green algal species such as *Acrosiphonia arcta* and *Spongomorpha aeruginosa* appear to be most abundant in the middle and lower tidal zone except in the presence of *Dictyosiphon foeniculaceus*. The only red algal species, *Clathromorphum compactum*, shows a distinct zonation pattern of low relative abundance in the lower tidal zone occupying bare areas or in conjunction with *Dictyosiphon foeniculaceus*. Given the diversity of slope profiles both in height above and below MLW across the 12 transects, making unambiguous inferences regarding the relationship between species composition, zonation patterns and the slope profile is challenging.

## Discussion

### Zonation and coast type

Given the wide range of micro-environmental gradients and habitats occurring over variety temporal and spatial scales across the intertidal zone, a certain degree of variation in species composition and zonation patterns is unsurprising (Coyer et al., 2011). However, although based on a limited window of observation, several important factors known to heavily influence high-latitude macroalgal communities such as optical conditions and availability of substrate do appear to be fairly constant across the six locations (Quartino et al., 2013). Therefore, broadly speaking, the observed variation between the locations is a likely a result of varying magnitudes of wind, wave and ice scouring. Sediment loading may exert a large control on the macroalgal populations at WH and RP given the location's proximity to the nearby Røde Elv, known to transport large quantities of red mud during late spring and after heavy rainfall events (Hansen, 1964).

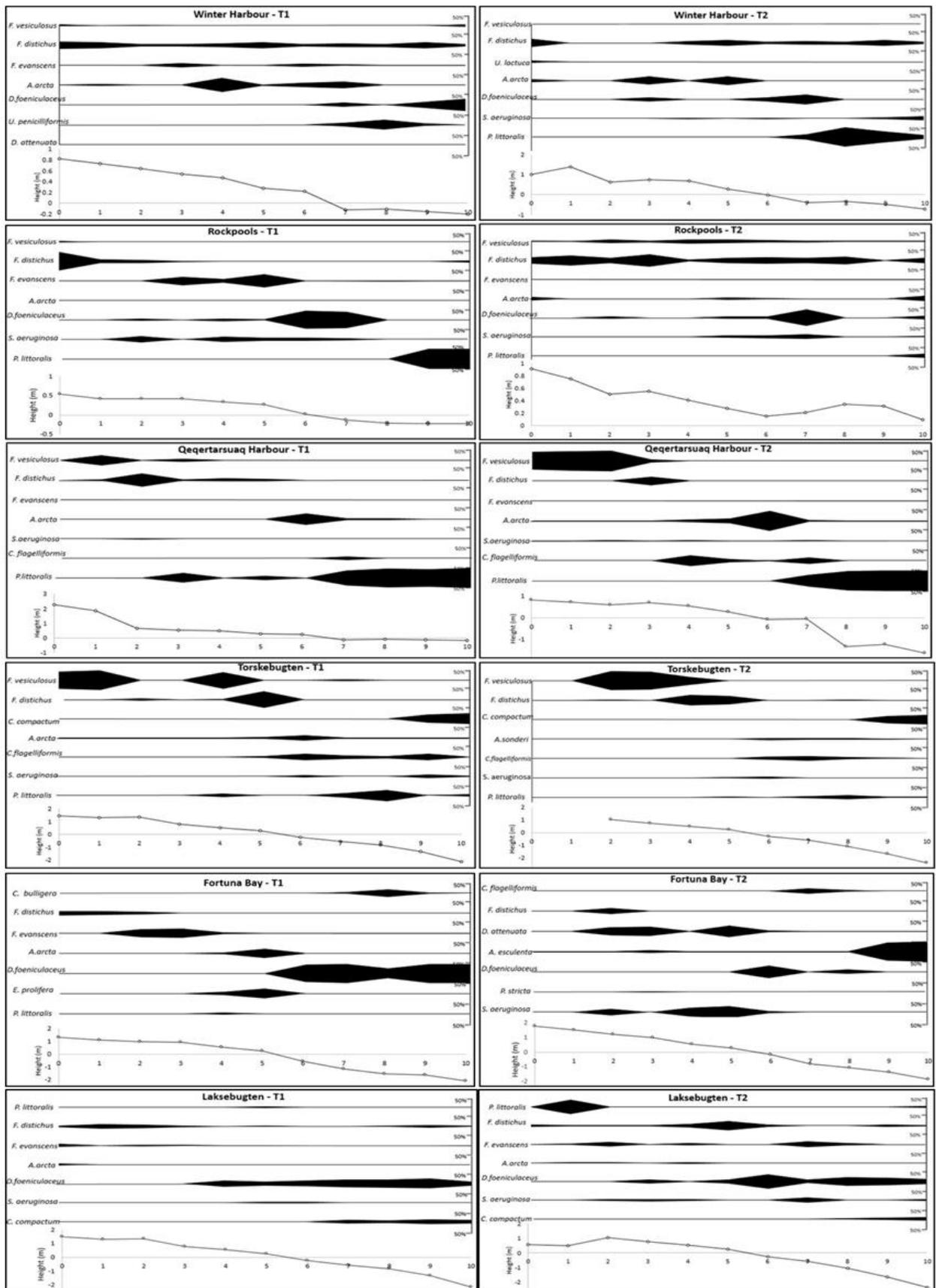


Figure 5 Kite diagrams and slope profiles for each transect at each location. The 7 most dominant macro algal species at each transect were selected. Size of kite is indicative of relative abundance at each quadrat. Slope profiles are all relative and adjusted by a mean low water value for the sampling days.

sampling a given tidal range at each location, a 10 m transect was performed, potentially creating a bias at location with steeper slope profiles where a greater tidal amplitude was sampled. Adding to this, further bias may arise from macro-scale variations in topography such as cracks and crevices, which are known to offer vital protection to macroalgal species (Adey & Hayek, 2005).

### *Historical comparison and climate change perspective*

One species of red algae was found in only two out of the six locations, TB and LB. Only the coralline red algae species *Clathromorphum compactum* was found at the lower zone, starting from around -100 cm from the average low tide. This is notable when compared to previous species composition analyses at the same six locations (Rasmussen, 2016; Boertmann et al., 2013; Hansen, 1998; Hansen & Schlütter, 1992). Previous literature shows a higher abundance in red algae throughout revisited locations. At location RP, a previous study by Hansen & Schlütter, 1992 showed that two red algae species were present at RP, whilst a similar observation was made by Hansen et al. 1998 barring a difference of one species. Furthermore, at TB a previous study observed a total of five red algae species (Hansen & Schlütter, 1992) whilst only one was observed in the present study. More recently, a brief assessment 2016 identified the presence of two red algae species at location

FB (Rasmussen, 2016). Historically higher abundance of red algae could indicate a change in species composition and algae community structure. One study from 2003 suggested that increased solar UV radiation might be harmful to red macro algae. The study focused on four red algae species present in both Antarctic and Arctic regions. By treating the algae with UV radiation their results showed damages and disruption of the chloroplasts. The study furthermore suggests that macro algae that grow in the deeper sublittoral zone could be more sensitive to UV radiation because of lack of natural UV protection (Poppe et al., 2003).

Data on sea ice cover in end-March based on raw data collected by the Arctic Station Long Term Monitoring Program since 1991 shows a slight decrease in sea ice cover over the past 24 years. Decreasing sea ice cover could affect the solar UV radiation through the water column because of reduction in reflectivity and increased UVB radiation exposure to the water surface (Bais et al., 2015). However, the lack of red algae findings in the present study could be biased from the limited sampling range. As half of the plots were carried out above low tide and in the upper littoral zone, many of these samples were dominated by fucoids and did not represent the full species spectrum of the lower littoral zone. The trend in sea ice cover is not significant, but the chart shows alarming drops, down to 5% in the period 2004-2012. This could however, be the

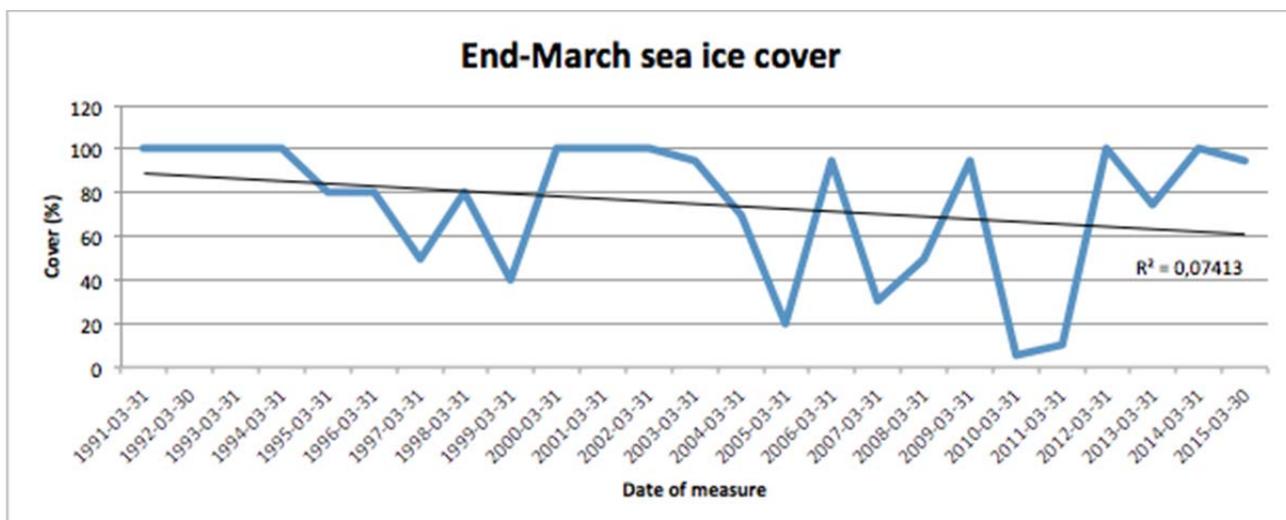


Figure 6 End-March sea ice cover from Disko Bay from 1991-2015. Source: Arctic Station Long Term Monitoring Program, 2017.

consequence of malfunction in the measuring instruments.

One clear conclusion from the present study is the dominance of a few species of brown algae within the sampled populations. The same pattern is also observed within historical literature, showing not only the same dominant class but also an almost identical number of species present within both brown and green algal classes. Only Hansen (1998) observed a few more species within both classes, which is probably due to a more comprehensive survey. This indicates that the green and brown algae class composition have not change much over the past 27 years. Although based on only a number of previous studies focused on Disko Island's macroalgal communities, the results indicate that green and brown macroalgal species are more resilient than the red algal species with respect to climate change. The present study shows that 35%-70% of the species found were the same species found in the previous literature depending on the location. Furthermore, the more exposed locations seemed to be more similar to previous studies with a 70% similarity at LB and a 35% similarity at QH. The reason for this may be, that it takes more time and is physically harder for new species to adapt and reproduce at high exposure areas compared to protected areas where the algae does not have to allocate its resources for hydrodynamic drag (Hurd, 2000). The dominance of brown algae is exemplified by *Fucus distichus*, which was found at each location not only in the present study but also throughout the previous literature (from 1990 until now). This pattern is not surprising according to a new study made in 2016. According to this study, *F. distichus* and presumably many species like this are likely to push their northern distribution limit more poleward due to change in temperature. This could cause unpredictable changes in community structure and marine ecosystems (Jueterbock et al., 2016).

One surprising observation was the absence of the brown algae *Ascophyllum nodosum* at all locations within both the present and the 2016 study. Results of a recent study examining the

species in the Arctic and sub-Arctic suggest that warming has a positive effect its growth-rate and that the stimulating effect on productivity may continue into the future (Marbà et al., 2017). Having extensively sampled the species in 2009 at Qeqertarsuaq within the study, the absence of *Ascophyllum nodosum* from the four locations at Qeqertarsuaq within the present study could indicate that such stimulation of the species productivity by warmer waters is restricted to those already established as opposed to encouraging colonisation of new areas. Furthermore, macroalgal communities are strongly affected by the change in weather and temperature, which can cause rapid and pronounced changes that cannot be captured by annual sampling efforts.

In summary, the study has shown that the species composition, relative abundance and zonation patterns at six different locations on southern Disko Island are shaped by a variety of interdependent physical parameters such as sediment loading in the water column and exposure to wind, wave and ice scouring. The study also shows that, with respect to overall species composition, macroalgal communities in the area have remained broadly similar over a 24 year period. The main exception to this conclusion is our observation of a distinct lack of red algal species and the brown algae *Ascophyllum nodosum*. As the effects of climate change become more pronounced in the Disko Bay area, it is imperative that future studies focus on establishing not only a wider and more comprehensive understanding of Disko Island's macroalgal communities but also those further north in order to examine and predict the potential impacts of climate change upon Greenland's marine ecosystems.

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## Appendix

### Appendix 1 – Sample Location Information

Location	Date of sampling	Position	Tide	Coast type	Substrate type
Rock Pools	07.07.17/1 6.07.17	69°14'27.31"N 53°32'00.92"W	0.3m/ 0.52m	1,5	Bedrock and boulders
Winter Harbour	07.07.17/1 6.07.17	69°14'32.40"N 53°31'57.20"W	0.3m/ 0.52	2	Bedrock with cracks
Fortuna Bay	13.07.17	69°15'53.8"N 53°48'39.9"W	0.19m	3	Bedrock with cracks
Qeqertarsuaq Harbour	14.07.17	69°14'47.02" N 53°32'54.45" W	0.24m	1	Bedrock, boulders and sand
Torskebugten	15.07.17	69°15'21.04" N 53°33'29.67" W	0.27m	1,5	Bedrock, boulders and sand
Laksebugten	17.07.17	69°18'59.6"N 53°56'11.6"W	0.3m	3	Bedrock and boulders

Appendix 2 - Macroalgal species composition (Y = Present. N = Absent) at 6 locations. WH = Winter Harbour. RP = Rockpools. QH = Qeqertarsuaq Harbour. TB = Torskebugten. FB = Fortuna Bay. LB = Laksebugten.

Species Present	Locations					
	RP	QH	WH	TB	FB	LB
<b>Rhodophyceae</b>						
<i>Chlathromorphum compactum</i>	N	N	N	Y	N	Y
<b>Phaeophyceae</b>						
<i>Alaria esculenta</i>	Y	N	Y	N	Y	Y
<i>Agarum clathratum</i>	Y	N	Y	N	N	Y
<i>Chordaria flagelliformis</i>	N	Y	N	Y	N	N
<i>Coelocladia arctica</i>	N	N	N	N	N	Y
<i>Coilodesme bulligera</i>	N	N	N	N	Y	Y
<i>Delmarea attenuata</i>	Y	N	N	N	Y	Y
<i>Dictyosiphon foeniculaceus</i>	Y	N	Y	N	Y	Y
<i>Fucus distichus</i>	Y	Y	Y	Y	Y	Y
<i>Fucus evanescens</i>	Y	Y	Y	N	N	Y
<i>Fucus vesiculosus</i>	Y	Y	Y	Y	N	N
<i>Pilaylla littoralis</i>	Y	Y	Y	Y	Y	Y
<i>Saccharina latissima</i>	Y	N	Y	N	Y	Y
<b>Chlorophyceae</b>						
<i>Acrosiphonia arcta</i>	Y	Y	Y	Y	Y	Y
<i>Acrosiphonia sonderi</i>	N	N	N	Y	Y	Y
<i>Enteromorpha prolifera</i>	N	N	N	N	Y	N
<i>Monostroma grevillei</i>	N	N	Y	N	N	N
<i>Spongomorpha aeruginosa</i>	Y	Y	Y	Y	Y	Y
<i>Ulva lactuca</i>	Y	N	Y	N	N	N
<i>Urospora penicilliformis</i>	N	N	Y	N	N	N

*An assessment of microplastic impact on blue mussels (*Mytilus edulis*) from Qeqertarsuaq harbor, Disko Bay (Greenland)*



**Macroplastic pollution found at the local harbor in Qeqertarsuaq (photo: Louise C. Flensburg)**

# *An assessment of microplastic impact on blue mussels (*Mytilus edulis*) from Qeqertarsuaq harbor, Disko Bay (Greenland)*

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## **Abstract**

Microplastic pollution in the marine environment is a recent growing problem and the consequences in the ocean ecosystems are not well understood. If microplastics are ingested by filter feeders it can cause serious problems, like reduction of feeding leading to reduce growth, and they can act as vectors transporting the microplastic into higher trophic levels of the food web. In this study we wanted to assess the effects of microplastic on the clearance rate of blue mussels (*Mytilus edulis*) sampled in Qeqertarsuaq main harbor (Disko Island). Furthermore, we also did a pilot sampling of plastic pollution in Disko Bay. We performed a feeding experiment with *Rhodomonas salina* and microplastics (50  $\mu\text{m}$ ) on mussels, in laboratory experiments that lasted for one hour. Experiment 1 with an algae concentration of 4,000 cells  $\text{ml}^{-1}$ ; Experiment 2 with an algae concentration of 4,000 cells  $\text{ml}^{-1}$  and a microplastic concentration of 0.09  $\text{mg m}^{-3}$ ; and Experiment 3 with a microplastic concentration of 0.09  $\text{mg m}^{-3}$ . We found reductions in the concentration of algae and microplastic over time, which imply that the mussels must feed on microplastic particles. Furthermore, we found that the clearance rate in the experiment with microplastic alone was significantly higher (7.7  $\text{L h}^{-1}$ ), than filtration rates obtained when microplastic and algae were present together (1.9  $\text{L h}^{-1}$ ). Thus, the clearance rate of *R. salina* was reduced when microplastic is present. This may raise some serious concern for the ecological consequences. The reduced clearance rates could cause changes in behavior, fitness and growth of the blue mussel - which are likely to affect the food web. From a single sampling event in Laksebugten we found a concentration of 0.0048 particles of microplastic  $\text{L}^{-1}$  in the surface waters. Our study has showed that plastic pollution is present in Disko Bay and that blue mussels are likely to feed in such particles. Further research is needed to determine the extent of the plastic pollution of Disko Bay and the consequences hereby.

**Keywords:** *clearance rate, microplastic, mussels, plastic pollution.*

## **Introduction**

The worldwide production of plastics has increased considerably since the middle of the 20<sup>th</sup> century and consequently, its accumulation as waste in the marine environment (Andrady, 2011). Plastics are therefore a growing source of marine and freshwater pollution and a cause of marine animal mortality (Derraik, 2002; Gregory, 2009). It is estimated that 99% of seabirds by 2050 will have ingested plastic during their life cycle and that the weight of plastics in oceans may exceed fish biomass (WEF, 2016; Wilcox *et al.*, 2015).

Once in the ocean plastic undergoes gradual degradation into smaller fragments via physical, chemical and biological processes. These small plastic particles are called secondary microplastics (MP). Primary MP consist of manufactured raw and small plastic material that enter the ocean via runoff from the land (Andrady, 2011). This kind of MP is used in facial scrubs, for medical application and synthetic clothing (Thompson *et al.*, 2004; Browne *et al.*, 2007; Masura *et al.*, 2015).

There is not a standard definition for MP, some define it as plastic particles smaller than 5.0

mm in size (Lavender & Thompson, 2014) while others define it as smaller than 300  $\mu\text{m}$  (Enders *et al.*, 2015). The lower boundary size for MP is not defined either (Lavender & Thompson, 2014). The accumulation of MP and the consequences it has on the ecosystem and the marine biota are not clear and it is a highly debated theme right now.

Due to their slow degradation, MP can become a serious ecological threat if ingested by the marine biota (Lenz *et al.*, 2015). The accumulation of MP in the biota can be a problem itself but the plastics can also absorb toxic compounds from the water, like persistent organic pollutants (POP) and heavy metals, which can later accumulate in the organisms that ingest them and these pollutants can be carried into the food web (Rochman, 2013; Lenz *et al.*, 2016). Since MP has similar size range as planktonic organisms, they are available to an array of marine invertebrates that normally would not be affected by large marine plastic debris (Wright *et al.*, 2013). A number of organisms, both vertebrates and invertebrates have been found to ingest MP. Examples include representatives of numerous organism groups with different feeding mechanisms; including detritivores, deposit feeders and filter feeders (Besseling *et al.*, 2013).

MP can accumulate in epithelial cells of the digestive system (von Moos *et al.*, 2012) and has the potential to pass from the digestive tract to the circulatory system of the organisms that ingested it (Browne *et al.*, 2008). The extent and impact of MP ingestion in wildlife is not well understood at this time, but some studies have shown that ingestion of MP can affect organisms by increasing their energy consumption (respiration). This can be linked to increased stress (Smolders *et al.*, 2002), and will lead to a reduction of their feeding, survival, fecundity and could even cause them death (Besseling *et al.*, 2013). However, most of these studies use concentrations of MP far above the levels found in the marine environment, so some of the effects observed in these experiments may not have the same extent under natural conditions (Lenz *et al.*, 2016).

It is also uncertain how MP particles are distributed worldwide. Ocean currents can move plastics from the source point to very distant locations, like the Arctic, and accumulate MP in some areas (Nerland *et al.*, 2014). Recent studies indicate that there is an accumulation of MP in arctic waters (Cózar *et al.*, 2017), but the knowledge about the extent and distribution of MP in the Arctic is still very limited. The abundance of MP in the North Atlantic Ocean has been determined to be between 13-501 particles  $\text{m}^{-3}$  (Enders *et al.*, 2015).

The arctic marine ecosystem and environment is under a great pressure from pollution, habitat destruction, human activity, climate change, etc. The marine arctic food web is very active in a short period in the summer, where some species are of high importance for the food web. The blue mussel (*Mytilus edulis*) is a common and abundant mussel in arctic waters and is an important food source for many different species, as birds or larger animals, like the walrus (Nielsen & Maar, 2007). A blue mussel of approximately 3 cm has a filtration rate of around 2-3 L of seawater every hour (Clausen & Riisgård, 1996). Therefore, these suspension feeders are at risk of exposure and ingestion of MP. The blue mussel is a selective filter feeder, ingesting only algae and particles of approximate size and shape (Defosse & Hawkins, 1997), and excreting other particles via pseudofaeces, which is material that has been cleared from suspension, but never ingested (Gosling, 2003). Even though they are selective filter feeders, several laboratory studies have observed ingestion of MP by blue mussels (Avio *et al.*, 2015). If the MP accumulates in the blue mussels, they can act as a vector transporting the MP into higher trophic levels in the food web (Farrell & Nelson, 2013).

In this study, we wanted to assess the potential effect of MP on the clearance rate (CR) of blue mussels (*Mytilus edulis*) sampled in a harbor at Disko Island (low Arctic). We hypothesized that CR obtained by blue mussels would increase with increasing MP concentration in the water. Additionally, we hypothesized that

MP was detectable in our study area, but at a very little amount, between 0-1 particles L<sup>-1</sup>.

## Methods and Materials

### Study area

This study was performed at Disko Island, in central west Greenland (69°15'N, 53°34'W) (Fig. 1). The mussel sampling area was located in the main harbor of Qeqertarsuaq, which is located on the south coast of Disko Island. The surface water sampling area was located in the area of Laksebugten. The study was performed from the 10<sup>th</sup> of July to the 20<sup>th</sup> of July 2017. All experiments and analyses were performed at the Arctic Station situated just outside Qeqertarsuaq (Fig. 1).

### Mussel experiments

Sampling of the blue mussels was conducted in the main harbor of Qeqertarsuaq. The mussels were located at approximately one-meter depth on rocks. The mussels were transported to the Arctic Station, and were kept in a plastic container with 3 L of seawater and an oxygen

pump, for at least 24 hours, which in theory would allow complete gut clearance of the mussels (Myrand *et al.*, 2002). The water in the container was changed every day to prevent reuptake of pseudofaeces. The mussels were kept without food, and were thus 'starved' when we started the feeding experiments. For the whole experimental period, the mussels were kept in a cooling container with a constant temperature of 5 °C. The seawater used for the whole experiment was collected from the main harbor, to provide the mussels with similar conditions to the natural environment, with the same temperature (5-6 °C) and salinity (33-32.5 ppt). The seawater was filtered through a 20 µm net, which removed unwanted particles (debris, sediment particles or other MP particles) and any food sources (plankton).

We wanted to investigate if MP is ingested by blue mussels and to what extent it would change the CR. To do that we performed three different experiments; Experiment 1 with an algae concentration of 4,000 cells ml<sup>-1</sup>; Experiment 2 with an algae concentration of 4,000 cells ml<sup>-1</sup> and a MP concentration of 0.09 mg m<sup>-3</sup>; and



Figure 1 a) Map of Greenland presenting the Disko Bay area (Andersen *et al.*, 2014). b) study area (Google maps, 2017).

Experiment 3 with a MP concentration of 0.09 mg m<sup>-3</sup>. The three experiments had the same exact setup.

The algae used as food source was a culture of *Rhodomonas salina*, obtained from Helsingør, University of Copenhagen (Denmark), grown in F/2 media. The culture was kept at a temperature of 5-6 °C in a cold room with a light source and an oxygen pump working at all time. An algae concentration of 4,000 cells ml<sup>-1</sup> was chosen because it ensured maximum filtration (Pascoe *et al.*, 2009; Riisgård *et al.*, 2006; Riisgård *et al.*, 2011). The MP source was commercially produced plastic beads with a diameter of 50 µm. The concentration of 0.09 mg m<sup>-3</sup> was chosen to match the average concentration of MP in the North Pacific Subtropical Gyre (Goldstein *et al.*, 2012), which is a hotspot for MP. This concentration corresponds to 1375 beds L<sup>-1</sup> of our MP.

Before the experiment was started, three mussels with similar sizes (between 3.3-3.9 cm) were picked randomly from the container where all the mussels were kept for starvation. The three mussels were then placed into three separate containers with 3 L of filtered seawater and an air pump to mix the water and to prevent particles from sinking down. The mussels were left undisturbed for 30 minutes to make sure that they were not stressed or affected by the handling. We observed if the individual mussels were active and behaved naturally before we started and experiment. When running the experiments, we investigated one mussel at a time to make sure that we measured the exact CR for that specific mussel. When an experiment was started, we added the algae and/or MP from stock solutions and made a gently manual stirring of the water using a glass rod. The experiments lasted for 1 hour and each container was manually stirred two times clockwise rounds with a glass rod every 5 minutes to prevent particles from sinking down. Water samples of 10 mL were taken at every 0, 15, 30, 45 and 60 minutes. These water samples were preserved with Lugol solution and analyzed under the microscope with a Sedgewick-Rafter chamber in order to count

the algae cells and the MP in the water. The entire volume was counted.

After each experiment, we performed the controls with the same setup as the actual experiments but without mussels. Samples of the controls were only taken at 0 and 60 minutes, and were later analyzed as stated before.

#### ***Analysis of the data and statistical methods***

The CR was determined using the usual clearance formula:  $CR = V/(n \cdot t) \cdot \ln(C_0/C_t)$ . Where V is the volume of the tank (L), t is the time (h), n is the number of mussels, C<sub>0</sub> is the initial concentration of particles and C<sub>t</sub> is the terminal concentration of particles (Riisgård *et al.*, 2011).

The CR was calculated for each sampling time (15, 30, 45 and 60 minutes) for each mussel and the average and standard deviation was calculated. For the initial concentration of particles, we used the average concentration obtained in the controls, since these were not affected by mussel feeding.

A one-way ANOVA test was performed, using the XLSTAT software, to analyze if there was a significant difference between the different CRs. When significant differences were recorded, further testing was needed to know between which CRs there was a difference and between which ones there was not a difference. For this, we used the Ryan procedure or REGWQ test to perform a multiple comparison with a grouping test. REGWQ tests each individual experiment CR between each other and shows between which of them there is a significant difference, and between which experiments there is not a significant difference. In contrast, the ANOVA test shows if there is a significant difference between all of the experiments, but not specifically between which ones.

#### ***Plastic pollution in the study area***

We performed a visual investigation of the area around the main harbor of Qeqertarsuaq, looking for large plastic pollution and documenting it by taking pictures.

We also looked for MP pollution in the local environment. We took a surface water sample in Laksebugten, to assess the abundance of MP. The sampling was done by dragging a plankton net with a mesh size of 80  $\mu\text{m}$  and a diameter of 25 cm through the surface water over a distance of 21 m. The total amount of water that passed through the net was 1030 L.

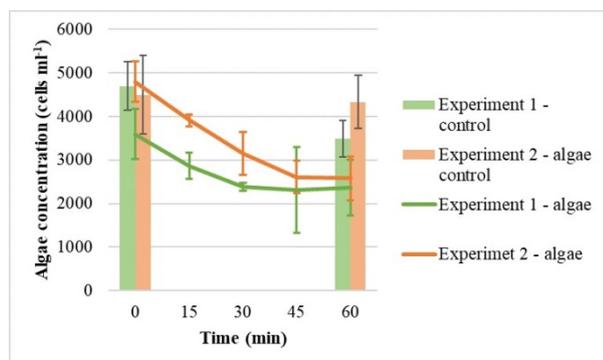
The concentrated seawater was further concentrated on a 20  $\mu\text{m}$  net and inspected by microscopy for presence of MP. When a piece was observed, we used a glass pin to feel if the compound was solid or not. We also looked for unnatural color. From this we could state that the particles were most likely MP.

## Results

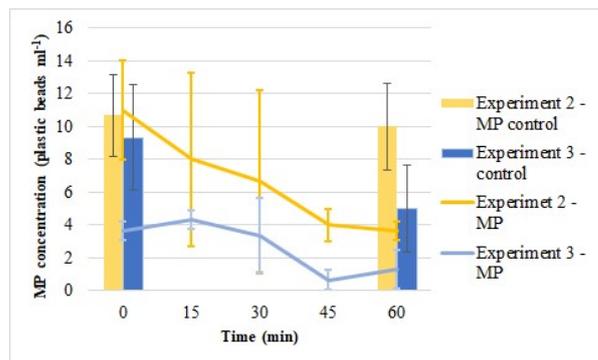
### Mussel feeding experiments

The average concentration of algae over time decreased in experiment 1 and 2 (Fig. 2) with and without mussels (controls). Both the controls and the treatments results have a high standard deviation, which make the results less confident.

The average concentration of MP in experiment 2 and 3 decreased over time (Fig. 3) with and without mussels. As before, both the controls and the treatments results have a high standard deviation, which make the results less confident.



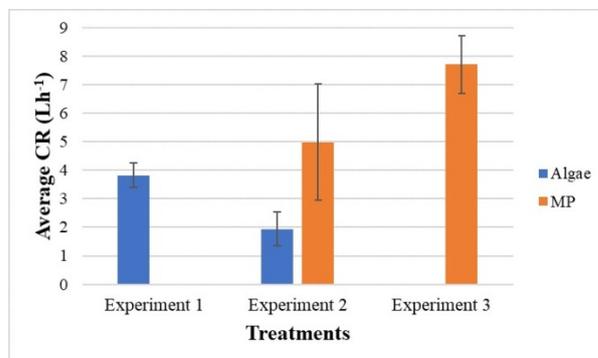
**Figure 2** Average algae concentration (cells  $\text{ml}^{-1}$ ) over time (min) in the feeding and control experiments. Bars represent the average algae concentration for the controls, lines represent the average algae concentration for the treatments and the error bars are the standard deviation ( $n=3$ ).



**Figure 3** Average MP concentration (plastic beads  $\text{ml}^{-1}$ ) during time (min) in experiments with and without (control) mussels. Bars represent the average MP concentration for the controls, lines represent the average MP concentration for the treatments and the error bars are the standard deviation ( $n=3$ ).

The CR values from the three experiments (Fig. 4) were significantly different according to the ANOVA test ( $F = 12.260$ ,  $d.f = 3$ ,  $p=0.002$ ). The CR in Experiment 3, with MP, is the highest of all the experiments, being  $7.7 \text{ L h}^{-1}$ . The CR for MP in Experiment 2 (Algae + MP) is the second highest, being  $4.9 \text{ L h}^{-1}$ , but it also has a high standard deviation ( $\pm 2.04$ ), which decreases the confidence of this value. Experiment 1 with algae alone, has a CR of  $3.8 \text{ L h}^{-1}$ , and the lowest CR is found in Experiment 2 (Algae + MP) in the algae clearance rate, being this  $1.9 \text{ L h}^{-1}$ .

With a further statistical analysis, the REGWQ test (table 1) shows a significant difference between the CR of experiment 3 and, the CR of algae in experiment 2 ( $p=0.002$ ), the CR of experiment 1 ( $p=0.010$ ) and the CR of MP in experiment 2 ( $p=0.023$ ). There is also a significant



**Figure 4** Average CR ( $\text{L h}^{-1}$ ) between the different treatments. Bars represent the average CR for the different experiments and the error bars are the standard deviation ( $n=3$ ).

difference between the CR of algae and MP in experiment 2 ( $p=0.034$ ). There is no significant difference in the CR of algae in experiment 1 and MP in experiment 2, and between both algae CR in experiment 1 and 2.

**Table 1** The outcome of REGWQ test with the p-values for the different treatments comparisons. Highlighted yellow represents the significantly different results

Contrast	Pr > Diff
Experiment 3: MP vs Experiment 2: algae	0.002
Experiment 3: MP vs Experiment 1: algae	0.010
Experiment 3: MP vs Experiment 2: MP	0.023
Experiment 2: MP vs Experiment 2: algae	0.034
Experiment 2: MP vs Experiment 1: algae	0.268
Experiment 1: algae vs Experiment 2: algae	0.090

In other words (table 2), the CR of the experiment 3 is significantly different to all the other treatments (group A). Experiment 2 MP and

experiment 1 CR are not significantly different (group B). The CR of experiment 2 MP is significantly different to experiment 2 algae (group C). Experiment 1 and experiment 2 algae have no significant differences (group C).

**Table 2** REGWQ test grouping. The table shows the different grouping made by the REGWQ test and the significant difference between the experiments CR and the CR means.

Category	LS means	Groups		
Experiment 3: MP	7.720	A		
Experiment 2: MP	4.989		B	
Experiment 1: algae	3.827		B	C
Experiment 2: algae	1.942			C

#### *Local pollution with macroplastic*

Macroplastic pollution was found in the local environment, specifically in the main harbor of Qeqertarsuaq where the blue mussels were sampled (Fig. 5). The pollution was primary fishing gear and nets, ropes and oil barrows.



**Figure 5** Macroplastic pollution found on the local harbor in Qeqertarsuaq. The pollution on the pictures are primary from fishing gear/nets (1), buoys (2), oil barrels (3), plastic boxes (4) and ropes (5).

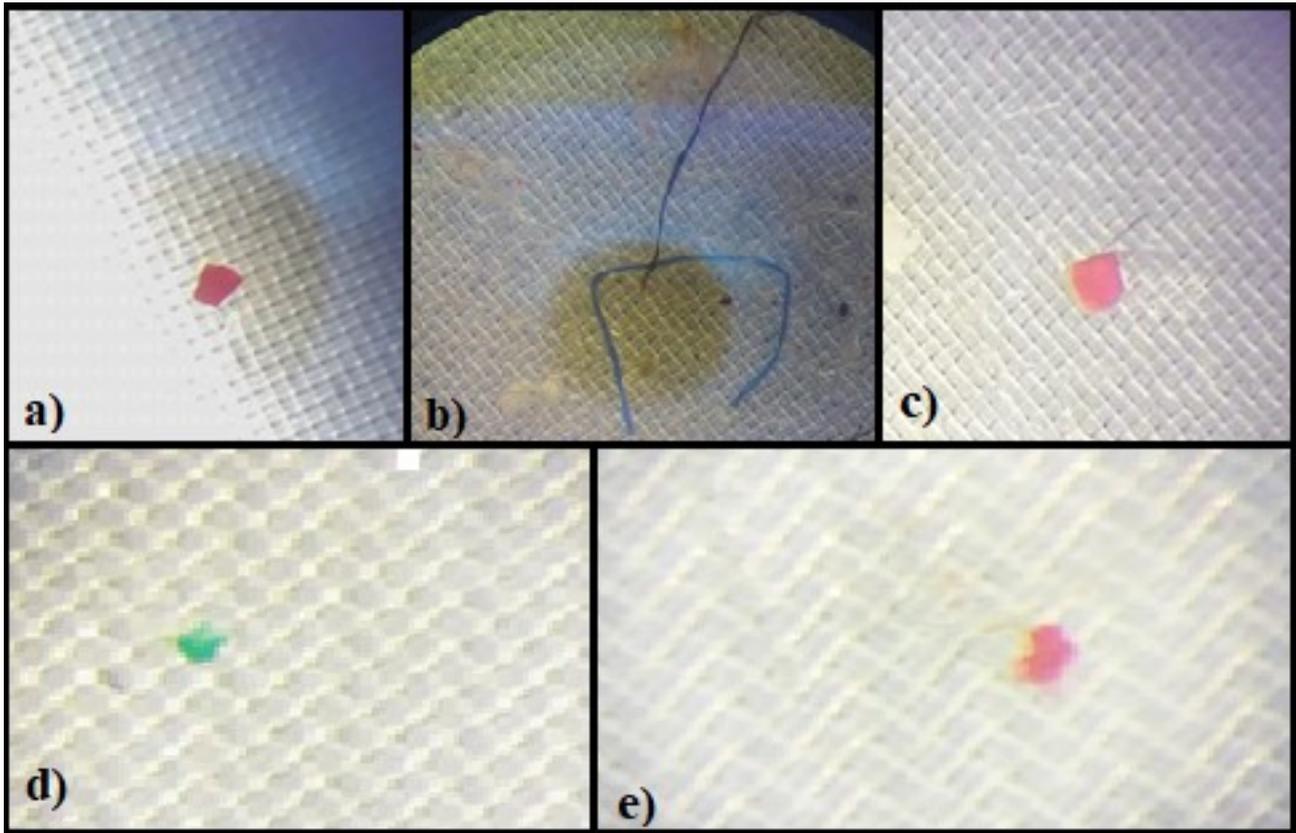


Figure 6 Microplastic pieces found in the surface water sample. The different MP pieces were: a, c and e) red particles of approximately 60-80  $\mu\text{m}$  in diameter, b) blue fiber of 720-800  $\mu\text{m}$  long, and d) a green particle of approximately 40  $\mu\text{m}$  in diameter.

### *Microplastic pollution in the surface waters*

Several pieces of MP were found in the surface water from Laksebugten (Fig. 6). A total of 5 particles of MP per 1030 L of water was found, which equals a final concentration of 0.0048 particles of MP  $\text{L}^{-1}$  in Laksebugten surface waters. The particles (range from 40-800  $\mu\text{m}$ ) found in the samples varied in color and shape, being three of them red, one blue and one green. Most of the particles were small bits of plastic, while one of them most likely was a fiber.

### *Discussion*

The experiment with the blue mussels, showed that MP (50  $\mu\text{m}$  in diameter) is filtered by the mussels and, furthermore, that this filtration affects the CR of the water and the edible food particles of *R. salina*.

The CR obtained in Experiment 1 with algae alone is comparable with published values (Clausen & Riisgård, 1996). The CR in experiment

3 is significantly higher than in Experiment 1 which could indicate that the mussels can sense the particles in the water and start filtering. They keep filtering for food, and have to increase the filtering because there are not enough particles in the water. It could also be the case that the blue mussels catch the particles but because it is of low quality is rejected and excreted as pseudofaeces, which they will clear from the water, explaining the drop in MP concentration (Gosling, 2003). A clear change of behavior in the mussels was observed when the MP was added to the water. They went from a passive/inactive (starvation) behavior to being highly active (Jönsson, 2016). The change of behavior could also mean that the mussels are uncomfortable with MP in the water. In experiment 2, we can see that the CR of algae is not significantly different from the CR of algae in experiment 1, which could mean that the mussels clear the water at a similar rate as under normal conditions, without MP. Experiment 2 contains

algae and MP, so maybe the mussels clear the water at a similar rate as with algae, because they do find quality food in this experiment, so they do not need to filtrate the water as many times as in experiment 3. Furthermore, we observed that the presence of MP may have a negative effect on the CR of algae in experiment 2. This may reduce the CR on more edible particles such as *R. salina*.

The mussels will in periods with low food availability enter a starvation mode closing the shells together and saving energy. The mussels will frequently scan the water and check for food availability and start filtering, when it passes the lower threshold limit which is located around 1000 cells ml<sup>-1</sup>. Here the mussel gets enough energy to sustain itself and the filtering processes (Riisgård *et al.*, 2011).

The uptake of MP by the blue mussels may have a broad range of consequences. They detect the MP in the water and spend a lot of energy, as seen in Experiment 3, on filtering the water. This could have consequences, especially in the arctic environment where there are extended periods of starvation in the cold winter seasons (Riisgård & Larsen, 2015). If the mussels spend energy filtering the water all year round instead of saving energy for the spring blooms, this could affect the fitness and growth of the mussels (Besseling *et al.*, 2013) which in turn can have consequences for the environment and the ecological food web.

Another concern of MP being ingested by the mussels is the amount of toxins that can bind to MP and eventually be accumulated in the mussels. Mussels have been shown to act as vectors transporting MP and toxin up the food web (Tanaka *et al.*, 2013). Mussel industry is a very important income on a global scale (Trevail *et al.*, 2015), so this could have an economic impact on mussel harvesting by humans and also for the animals that depend on the blue mussel.

In our study, we experienced a variation in the start concentration of MP and algae in all experiments. Some of the variation can be caused by the mussels eating part of the algae and MP before we took the first samples at time 0. Other

reasons could be current and stirring not being enough for dispersing the algae and MP equally, which can give some uncertainties in the results for determining the concentration of MP and algae. We used a Sedgewick-Rafter chamber to determine the concentration of algae and MP, which can cause errors in the counting and we observed that the particles tend to clump together near the corners of the chamber, which leads to variation in the counting. Normally in feeding experiments a particle counter by means of a laser beam is used. This method is far more precise and would save a lot of time.

A slight decline in recovery of the particles was also observed in the controls which could have some of the same explanations as mentioned above, but one thing specifically here could be current issues. We had oxygen pumps and manual stirring which may not have been efficient enough and the particles would have sunk down over time. Another problem with the controls was that we had to perform these after the experiments, instead of at the same time, due to the lack of air pumps, so the conditions could have slightly changed. The variation in the concentrations of algae and MP occurs throughout the experiments including the controls and means that the data needs to be taken with some caution. Taken into consideration the limitation we had at the field station where we did the experiment this is also what we could expect.

Another thing that could be improved would be to perform more samplings over time and/or add more replicates. We can see that there is some variation between the measurements from different mussels. Furthermore, when filtering the water for the experiment we used a 20 µm net. This remove most of the unwanted particles in the water, but we cannot know for sure if the water was completely free from particles which could act as food source. But we can assume that the concentration that might be added to the experiment is equal in all experiment since we use the same methods for filtering the water.

The macroplastic pollution found in Qeqertarsuaq harbor is most likely local and can

over time be broken down to MP (Andrady, 2011). The extent of plastic pollution in Disko Bay is still unknown but it seems likely that coastal waters are affected. Macroplastic pollution is harmful to the environment, being a known fact that many animals such as birds, seals and whales ingest plastic causing them a higher mortality (Derraik, 2002; Gregory, 2009). It has been estimated that 80% of all plastic pollution in the marine environment world-wide comes from terrestrial systems (Andrady, 2011). The remaining 20 % comes from marine activities (shipping, industrial fishing, etc.) (Cole *et al.*, 2011). The local plastic pollution we found in the Qeqertarsuaq harbor was a mixture of industrial materials and household waste from the local dumping grounds and may contribute to MP in the ocean over time by producing secondary MP (Cole *et al.*, 2011). It seems very likely from our observations and other studies in the area (Enders *et al.*, 2015) that MP is present in the Disko Bay environment. Recent studies indicate that this concentration is not only from local sources, but also transported from other places to the area by global currents and is therefore accumulated in the arctic (Cózar *et al.*, 2017). The concentration of MP in the North Atlantic has been determined to be around 0.013-0.513 particles L<sup>-1</sup> (Enders *et al.*, 2015). In our pilot sample in Laksebugten we got a concentration of MP of 0.0048 particles L<sup>-1</sup> in the surface water. This indicates that MP is present in the Disko environment, but the sample we took was too small for a valid conclusion. Furthermore, more advanced methods than microscopy is needed to identify and count MP.

To conclude, our study has shown that MP is present in the marine waters in Disko Bay and that MP is eaten or at least filtered by the mussels. We have shown that the CR of valuable food particles such as *R. salina* may be reduced in the presence of MP. We also observed a significantly higher CR in mussels with MP than on the algae.

For further research into this important subject we suggest conducting a study on the extent of MP pollution in the environment taken surface water samples, sediment samples and

beach samples to investigate the pollution in the area. It would also be very interesting to investigate if the mussels or other organisms have MP in them to see if they accumulate MP in them or can eliminate it by pseudofaeces and to what extent. A long-term experiment with MP would also make it possible to look for changes in fitness and growth of the mussels.

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*Growth rates of Arctic charr populations (Salvelinus alpinus) in the Disko area, West Greenland*



Arctic charr (photo: Kirsten S. Christoffersen)

# *Growth rates of Arctic charr populations (*Salvelinus alpinus*) in the Disko area, West Greenland*

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## **Abstract**

Climate change has increased air temperatures in the Arctic regions and is projected to keep increasing temperature further; this is suggested to have an impact on organisms living there. In this study, the growth rates of three populations of Arctic charr (*Salvelinus alpinus*) have been estimated, to detect if the increasing air temperatures have an impact. The three populations included two landlocked and one anadromous population, from respectively Røde Elv (Disko), Kangersuk (Disko) and Laksebugten (Disko Bay). Growth rates were calculated by performing back calculations of annual growth rings obtained from otoliths and suboperculum, respectively. A significant difference in growth rates between two landlocked populations was found. The populations from Røde Elv and Laksebugten did not show a correlation with temperatures, but for the landlocked population of Kangersuk the growth rates determined from the subopercula, showed a significant correlation with mean summer temperature. This shows inter-annual temperatures can have an effect on growth of charr populations, either directly physiologically or indirectly via other temperature driven factors leading to a more beneficial habitat. The growth rates from otoliths showed no significant correlation with climate data. From a methodological point of view, it was found that the suboperculum was easier to retrieve and to read, however no statistically different results were obtained.

**Keywords:** *Salvelinus alpinus*, Growth rates, Otolith, Suboperculum, Disko.

## **Introduction**

The Arctic region experience a rapid and severe change in climate compared to more temperate and tropical environments. Temperatures in the Arctic have increased as much as 3-4°C during the past 50 years (ACIA 2004) and is predicted to increase by 5.2°C by the end of this century (IPCC 2013). The warmer climate also resulted in significantly more precipitation (ACIA 2004) and more of this in the form of rain, bringing earlier onset of snowmelt (ACIA 2004). These overall Arctic data are backed by local climate increase at 3°C for July at lake Toolik, Alaska, measured throughout 16 years (McDonald et al. 1996) and 2.5°C increase for the Disko area measured from 1982-2015 (Hedemand et al. 2016), however, these local increases might be a response to short terms fluctuations in temperature. These temperature

increases can affect fish populations and communities in different ways. Some habitats will open up for species, enabling them to increase their distributional range northward (Sharma et al. 2007; Hein et al. 2011) and some will be driven further north, either as a response to temperature requirements or species competition (Dulvy et al. 2008; Hein et al. 2012). The Arctic charr has a circumpolar distribution and can occur as the only fish species or live sympatrically with other species such as brown trout or pike (Amundsen 2009; Hein et al. 2012; Jensen et al. 2012). The Arctic charr is the most northern distributed freshwater fish species, with a northern distributional border of Ellesmere Island (Reist et al. 1995),

Arctic charr can live both as landlocked and anadromous populations. Landlocked populations live their entire life in the same

freshwater system, these are landlocked either as a life strategy or because they are secluded from marine areas by a physical barrier, like a waterfall or dry land. Anadromous populations spend parts of their life in the sea and parts in freshwater systems. These populations migrate to the sea during the summer to eat and migrate back into the freshwater systems during winter, where they spawn (Nordeng 1977). Because of this, the populations utilize different foraging areas, thus it is expected, the populations do not grow at the same rate nor to the same size. Arctic charr is poikilothermic and growth will hence be influenced by the ambient water temperature, as metabolism acts in accordance (Pörtner et al. 2008). Regional temperature patterns have been suggested as an important factor for Arctic charr growth (Knopp et al. 2012), as it has been shown to have a direct effect on lakes in freshwater systems previously studied in the Disko area (Kristensen et al. 2006). Fish have an optimum temperature, described as the temperature where most growth is theoretically possible (Fry 1947). This optimum is of course dependent on availability of food. If the optimum temperature for Arctic charr is met, growth can react in different ways. Under the circumstances of no food limitations, a growth spurt would be expected, though with food limitation, we would expect lesser growth, as more food would be required for system maintenance due to the increased metabolism. Fish will adapt to their immediate habitat temperatures (Fry 1947), the optimum temperature for Arctic charr, are therefore influenced by what population it is measured from. Optimum temperatures for Arctic charr has been measured on Swedish populations ranging from 14.1°C to 16.3°C (Larsson et al. 2005), but the most recent temperature optimum has been found on anadromous Arctic charr from the Disko area to be just 7.5°C (Hansen et al. 2016), these findings show a geographical adaptation. We assume a similar adapted optimum temperature for both anadromous and landlocked populations, as the anadromous fish live some of their life in freshwater systems exposed to similar air temperatures as the landlocked individuals. Furthermore, the Arctic charr is a generalist with no especially selected trait towards temperature

preference (Siikavuopio et al. 2014), at least when exploiting different niches in a system. Since the Arctic charr have most of their growth period during summer (Amundsen et al. 2009), it would be expected that summer air temperatures close to the optimum temperature would show a positive response in growth for populations not exposed to food limitation.

A method widely recognized for measuring growth and aging fish, is the reading of otoliths (Pannella 1971; Watkins et al. 2015). The otolith structure is part of the inner ear, it is constructed of layers of calcium carbonate. The layers are continually building, with a faster build up in times when increased growth occurs (Pannella 1971). This growth-influenced, continued build-up of layers makes it possible to differentiate growth seasons. As Arctic charr grows less during winter (Amundsen et al. 2009), the visual growth rings created, will depict the annual growth. One year will consist of a dense, short band of winter growth, followed by a wide band of summer growth. On the Arctic charr, the otoliths are quite small, with a 1:80 otolith length to fish length relation (Härkönen 1986). Obtaining the otoliths and analysing them can thus be difficult, even with samples sometimes being lost during retrieval (Watkins et al. 2015). For other fishes, it has been examined if other hard structures are just as good for aging and measuring growth, it was found that both gill covers, scales and dorsal spines also give an accurate age or growth estimation (Thoresen 1996; Jackson et al. 2007; Watkins et al. 2015). The suboperculum (SO), found as the lower of the gill covers, have been used as a systemic measure for aging fish (Thoresen 1996), however not used specifically on Arctic charr, the size of the suboperculum is quite large compared to the otoliths, for Arctic charr, and retrieval is hence easier and faster. The suboperculum is large enough to conduct a quick, in-field aging. The suboperculum method might prove for Arctic fishes exposed to climate change to have an advantage over otoliths as temperature rises. For back calculating of growth measurements to be reliable, there must be an exact correlation between structure measured and the size of the fish at a given age (Mosegaard et al. 1988; Thoresen 1996; Vigliola et al. 2009).

The otolith growth rate is, as the metabolism also controlled by temperature, but the increase in growth does not stop at the optimum temperature for somatic growth, instead it keeps increasing (Mosegaard et al. 1988). Thus, increasing temperature in the Arctic could mean potentially altering the measurement reliability for otoliths; it has not to our knowledge been tested if the suboperculum has increased growth above the optimal temperature for somatic growth.

While it is reasonable to expect a correlation between air temperature and water temperature, at least during summer where the freshwater systems in the Disko area have no ice cover, lake morphology can play a large role in the actual temperature experienced by Arctic charr. Deeper lakes can in theory provide cold refuge for larger sized Arctic charr, which has lower temperature preferences than juvenile and smaller individuals (Pörtner et al. 2008; Siikavuopio et al. 2013), high current in streams might as well decrease the heating effect on the water thereby making shallow waters such as fast running streams have different temperatures than expected. Because of this, temperature data needs to be very specific for the individual freshwater systems. As this is not possible due to no long-term data on the freshwater systems sampled from in this study, we will assume that climate change effect depicted in air temperatures will be sufficient to show any climate driven response in fish growth.

In this study, we aim to examine two populations of landlocked Arctic charr and one anadromous population. The populations will be compared by growth rate (GR) and back calculated total lengths (TL). The growth rates of Arctic charr in the Disko area will be measured with regards to difference between populations and how they are affected by inter-annual change in air temperatures. The growth rates will be conducted on both suboperculum and otoliths, to give an indication of the usefulness of the two different methods.

We expect to find generally lower growth rates in the landlocked populations than in the

anadromous, as we expect food limitations for the anadromous fish are not an issue. We also expect to find a difference between the two landlocked populations, as the habitats differed in morphology, one being a stream and the other being comprised of two lakes combined by a small stream. These characteristics are expected to provide a difference in food availability and temperature range. These expectations are partly build on previous findings, where specimens from the stream location, Røde Elv, were found to be smaller than the specimens from the lake location, Kangersuk (Kristensen et al. 2006).

## *Methods and Materials*

### *Sampling*

Two locations with landlocked populations where studied: a lake in Killiit (Fortune Bay; 69°16'7.66"N, 53°49'32.56"V) (referred to as Kangersuk) and the upper parts of the river Røde elv above the waterfall (Blæsedalen; 69°16'37.56"N, 53°28'58.00"V) (referred to as Røde Elv). In addition, anadromous charr were sampled in Laksebugten (69°19'8.27"N, 53°54'35.00"V (*Figure 1*).

The Kangersuk system consisted of two lakes, one with a depth larger than 3 m, surface area: app. 61554 m<sup>2</sup> (Google Earth Pro) and one shallow (average depth: 36 cm (Christiansen et al. 2015), surface area: app. 44124 m<sup>2</sup> (Google\_Earth\_Pro) connected by a small stream. The shallow lake freezes solid during the winter, whereas the deep lake has an unfrozen water column below 1-2 m ice during winter (pers. comm. K. S. Christoffersen) It is rather likely the shallow lake serves as feeding grounds during summer for the population living in the deep lake (pers. comm. K. S. Christoffersen).

Laksebugten is an open bay area with oceanic saline water. The inner part connects to a stream leading to an inland freshwater system.

Røde Elv consists of two rather different parts; a plateau in the valley containing shallow streams and pools (max depth < 1 m), where the water is slow to moderately moving (average

water velocity:  $0.13 \text{ m s}^{-1}$  (Pedersen et al. 2015). The catchment of this plateau is mostly glacier melt-off and is thought to be nutrient poor. The other part is found below the plateau and after a series of waterfalls. The watercourse becomes deeper ( $> 1 \text{ m}$ ) and the water velocity is visibly higher (not measured).

In Røde elv, the fish were caught using gill nets with a mesh size of 10 and 18 mm. The nets were placed in the outlet of small pools where the fish were expected to live. The fish were subsequently chased into the nets by walking behind them or throwing rocks into the water. In Kangersuk the fish from the deep lake were caught by angling with small spinner lures and flies as well as gill nets with 28 and 42 mm mesh sizes. In the small stream connecting the deep lake with the shallow lake fish were caught using a gill net with a mesh size of 10 mm. As no fish were caught in Laksebugten by us, 3 fish heads from the location were supplied by Kjeld Akaaraq Mølgaard (station manager, Arctic Station, UCPH).

The fish were then brought to the laboratory

for weighing and length measurements. Otoliths were extracted by removing the gill arches and cracking the braincase open by snapping the head backwards. Gill covers were extracted using a scissor, cutting the whole operculum off, and then simply removing the suboperculum by pulling the skin off lightly, separating the suboperculum (SO) from the operculum.

The task of sex determination was not undertaken, as analyses of data from a previous study conducted in the area (Hedemand et al. 2016) did not show any significant difference between males and females (*Appendix 3*).

Each otolith and SO were then aged independently by each author. Contradicting results were discussed and re-estimations performed if necessary. Otoliths were then placed in ethanol with the sulcus facing down and examined by stereo microscopy with 16 times magnification on a dark background. Pictures were obtained with the camera of an iPhone 5S.

SO were measured by angling it in a light beam with a dark background to reveal the



Figure 1 An overview of the three sampling sites at Disko Island. West Greenland (Google earth pro).

annuli. Images of SO were obtained by scanning with an Epson Perfection V850 pro. To visually detect the annuli on the scanner, small black dots were placed on each annulus with a small permanent marker. Measurements were carried out with a digital analysis tool (imageJ). To be able to scale in imageJ a known distance of 2.5 cm on the SO scans and 1 cm on the otolith pictures were measured 10 times to get an average pixel/mm ratio. This pixel/mm ratio was then applied for all measurements. On the otoliths a centre point was established to determine the radius from the centre to the post rostrum (Rpr).

From the starting point of each annulus, the first year corresponds to the first annulus and so forth. The most recent growth band was not included since the fish were still growing.

### Calculations

To test whether SO and otoliths are a good estimate for growth rate estimates and back calculations each SO length and otolith Rpr was plotted against total length of each fish. Because SO length and otolith length and fish TL was significantly correlated ( $p < 0.0001$ ,  $\alpha = 0.05$ ) we found support for utilizing the nonlinear body-proportional hypothesis (nBHP). This hypothesis implies that the length of the SO

and the length of the fish is always proportionate (Morita et al. 2001). Back calculations of suboperculum/otoliths were done using the nonlinear body-proportional hypothesis:

$$L_t = \left( \frac{SO_t}{SO_T} \right)^k L_T$$

Where  $L_t$  is the back calculated length of the fish at age  $t$ ,  $L_T$  is the length of the fish at capture time,  $SO_t$  is the length of the suboperculum/otolith Rpr at annulus  $t$ ,  $SO_T$  is the length of the suboperculum/otolith Rpr at capture time and  $k$  is a constant.  $k$  is given by log transforming data and fitting an exponential trend line (Thoresen 1996).

$$L = a \cdot SO^k$$

Where  $L$  is length of the fish,  $a$  is a constant,  $SO$  is the length of the suboperculum/otolith Rpr and  $k$  is the slope of the regression  $\log(L)/\log(L_{SO})$ .

Growth rates were calculated using the formula:

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

Where  $G_t$  is the growth rate in  $\text{cm yr}^{-1}$ ,  $L_t$  is the length at annulus  $t$  and  $L_{t+1}$  is the length of

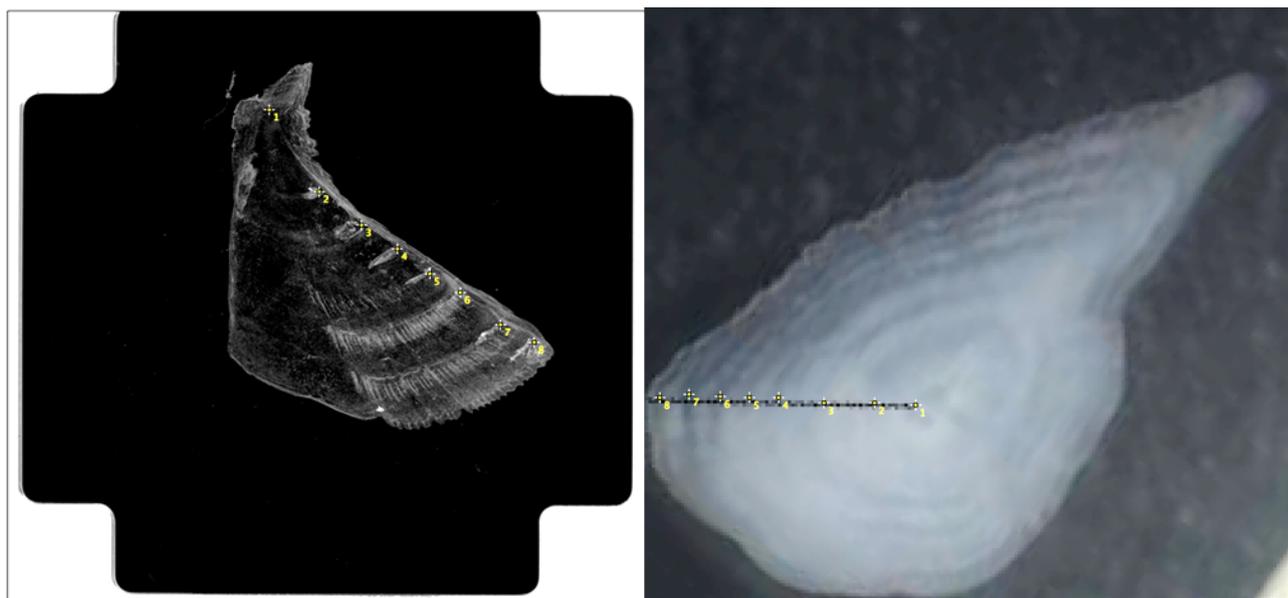
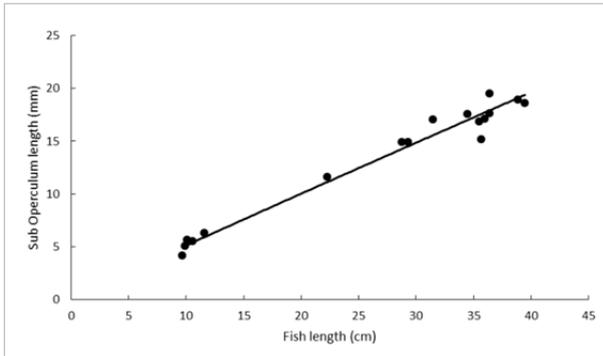


Figure 2 Suboperculum (top) and otolith (bottom) of a seven-year-old *Arctic charr*. Annulus are displayed as small yellow dots. Dot 1 indicates the start point of measurements (= year 0). Dots 2-8 are the corresponding years.



**Figure 3** Correlation of fish total length and suboperculum length.  $r^2 = 0.977$ ,  $P < 0.0001$ ,  $\alpha = 0.05$ .

the fish at annulus  $t+1$ .

Mean growth curves for specific ages and years were calculated. Since we did not have the true length of the fish from Laksebugten we calculated the lengths using a formula that estimates the size of an Arctic charr in relation to the otoliths (Härkönen 1986).

$$FTL = -102.7 + 124.7 \cdot OL$$

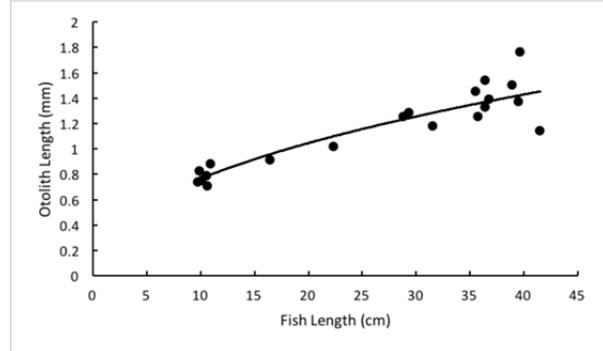
Where FTL is the fishes TL and OL is the length of the otolith. To compare with climate data, temperature data for the years 2008-2016 were obtained (The Arctic Station Long Term Monitoring Program 2017). Summer period was defined as June 1<sup>st</sup> to August 31<sup>st</sup>. Winter was defined September 1<sup>st</sup> to May 31<sup>st</sup>.

### Statistics

All statistical tests were performed in PRISM 7 software (Graphpad Prism v. 7). To compare growth curves and growth rates between populations a one-way ANOVA with a following TUKEY-test was conducted. To find correlations between growth rates in specific years and climate data a correlation matrix was calculated with corresponding Pearson R values.

### Results

In total 23 fish were caught. Of these 14 fish were from Kangersuk, aging between 2 and 6 years and having a TL of 10.5 – 39.5 cm. In Røde elv 6 fish were caught ranging between 2 and 5 years and having a TL of 9.7 – 16.4 cm. The fish heads from Laksebugten were estimated to have

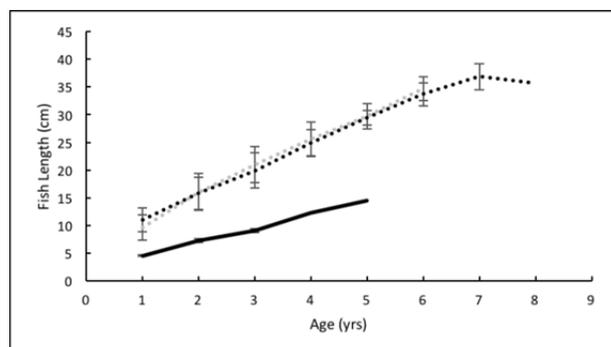


**Figure 4** Correlation of fish total length and otolith length.  $R^2 = 0.879$ ,  $P < 0.0001$ ,  $\alpha = 0.05$ .

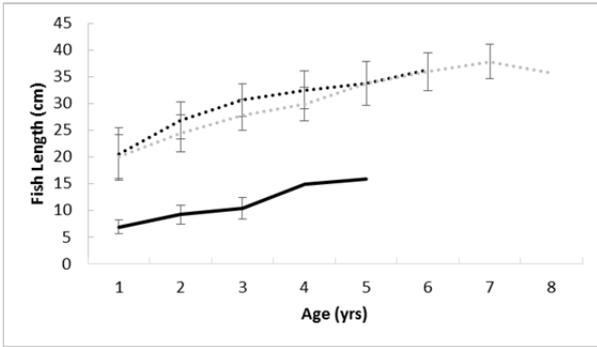
a TL of 36.8 – 41.5 cm and being 7-8 years old. In *Appendix 1* and *appendix 2*, a list of measurements, ages and back calculation lengths is provided.

Correlation analysis between SO length and total fish length (*Figure 3*) was found to be significantly positive ( $R^2 = 0.977$ ,  $P < 0.0001$ ,  $\alpha = 0.05$ ) showing a relationship between gill cover length and fish length. Correlation between fish TL and otolith Rpr revealed a positive relationship ( $R^2 = 0.879$ ,  $P < 0.0001$ ,  $\alpha = 0.05$ ) (*Figure 4*).

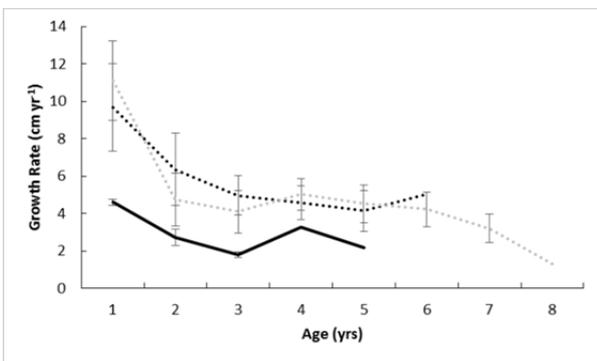
Analysis of back calculated TL by SO (*Figure 5*) revealed no significant difference between the landlocked population of Kangersuk and the anadromous fish caught in Laksebugten. Between Kangersuk and Røde elv a significant difference was found ( $P = 0.047$ ,  $\alpha = 0.05$ ). A significant difference between the landlocked population of Røde-elv and Laksebugten was also found ( $P = 0.0092$ ,  $\alpha = 0.05$ ).



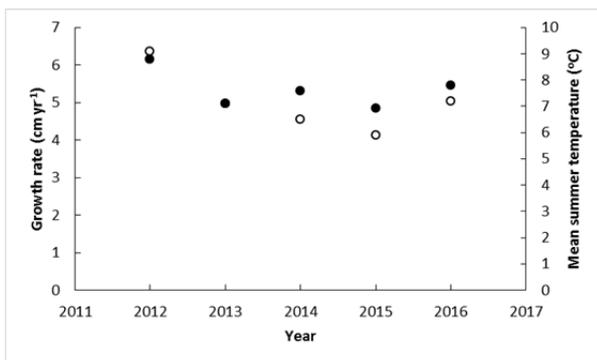
**Figure 5** Back calculated lengths from suboperculum of the different populations of fish. Solid black line = Røde elv, dotted black line = Kangersuk and dotted grey line = Laksebugt. Mean with  $\pm SD$ .



**Figure 6** Back calculated lengths from otoliths of the different populations of fish. Solid black line = Røde elv, dotted black line = Kangersuk and dotted grey line = Laksebugt. Mean with  $\pm$ SD.



**Figure 7** Growth rates from suboperculum of the different populations of fish. Solid black line = Røde elv, dotted black line = Kangersuk and dotted grey line = Laksebugt. Mean with  $\pm$ SD.



**Figure 8** Growth rates by suboperculum ( $\text{cm yr}^{-1}$ ) plotted against year (black dots) and mean summer temp ( $^{\circ}\text{C}$ ) plotted against year (white dots).

Back calculated TL by otoliths (*Figure 6*) showed no significant difference between the populations of Laksebugt and Kangersuk. A

significant difference were found between the population of Røde elv and both the population of Kangersuk ( $P=0.0001$ ,  $\alpha=0.05$ ) and Laksebugt ( $P<0.0001$ ,  $\alpha=0.05$ ).

Growth rates calculated by SO for each population were not found to be significantly different from each other when means of year 1-5 was tested (*Figure 7*). When year 2-5 were tested, significant differences in GR were found between the Røde Elv population and both the population from Laksebugten ( $P=0.0058$ ,  $\alpha=0.05$ ) and the Kangersuk population ( $P=0.0018$ ,  $\alpha=0.05$ ), but not between the Kangersuk population and the fish from Laksebugten. Mean annual mean summer and mean winter temperatures were tested for correlations to the growth rates of each population of the corresponding years. Testing against growth rates at all ages showed no significant correlation. Testing for correlation without growth rates from year 1 showed a significant correlation between mean summer temperatures and the corresponding growth rates of the Kangersuk population ( $P=0.034$ ,  $\alpha=0.05$ ) (*Figure 8*). No further correlations were found by removing year 1 growth rates.

Growth rates calculated by otoliths (*Figure 9*) for each population showed no significant correlations when year 1-5 was tested. When year 2-5 were tested, significant difference was found between the fish of Laksebugten and the population of Røde Elv ( $P=0.0439$ ,  $\alpha=0.005$ ); between the populations in Røde Elv and Kangersuk a tendency towards a difference ( $P=0.0645$ ,  $\alpha=0.05$ ) was found, however, this was not significant. Mean annual, mean summer and mean winter temperatures were tested for correlation to each population's growth rate of the corresponding years, both with growth rates from year 1 and without growth rates from year 1 - no significant correlation was found.

No significant difference was found between the results measured on otoliths and the results measured on SO (*Figure 10* and *Figure 11*).

## Discussion

### On reading hard structures

Age determination of Arctic charr performed from annual rings on the otoliths and subopercula proved to have both forces and weaknesses. Although aging fish by otoliths and measuring the length of each annual ring is often used methods on Arctic charr (Mosegaard et al. 1988; Riget et al. 2000; Kristensen et al. 2006; Hedemand et al. 2016), we found locating the initial annual growth ring on the otoliths particularly difficult. Otolith formation occurs by continual growth on all parts of the otolith surface, which means the center will increase its diameter to all sides, which makes it hard to distinguish any annual growth rings in the thick part (referred to as masking). Masking of the initial annual growth ring on the SO occurred as well, though the structure is not nearly as thick, as more growth is directed caudal, than in the width, meaning it had a higher readability. As masking made it difficult to determine the first year(s), high standard deviations in the back calculations are more evident in the initial living years of the individuals. Measuring the outer (younger) annual growth rings of the otoliths got easier, the closer they got to the edge. These findings are supported by (Watkins et al. 2015), who found that confidence in otolith readings increases with fish age and further that fish younger than 5 years are easier aged by instead reading their scales. We did not have any fish older than 8 years, thus the measuring of otoliths and subopercula cannot be compared for fish with a long lifespan. A method for ensuring higher readability of the otoliths, would have been polishing them, thereby overcoming the masking obstacle of initial annual growth rings, as was done by (Kristensen et al. 2006). During a previous study (unpublished) attempts to polish redfish otoliths, which are larger in size, ended up shattering them and thereby destroying the samples (pers. obs. M. Schultz). Therefore, the polishing was not done for this study in order to keep the sample size intact. Furthermore, the handling time for retrieving and reading otoliths were longer and more difficult for otoliths than for SO. These findings suggest SO to be the

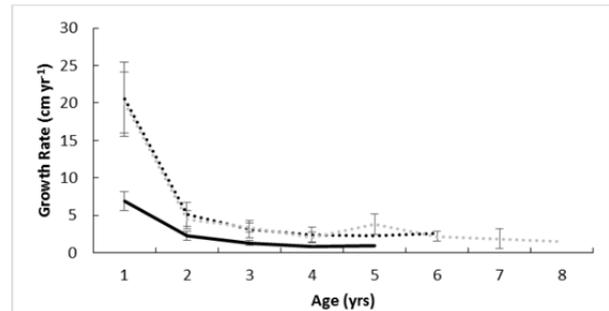


Figure 9 Growth rates from otoliths of the different populations of fish. Solid black line = Røde elv, dotted black line = Kangersuk and dotted grey line = Laksebugt. Mean with  $\pm$ SD.

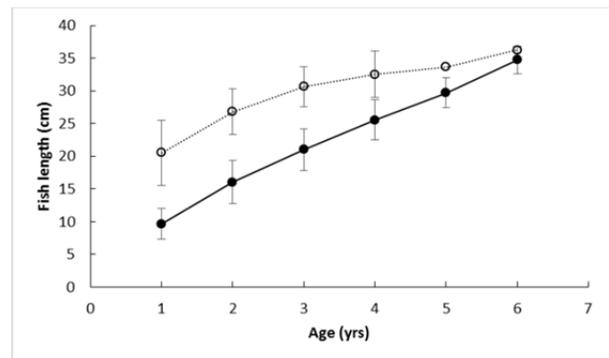


Figure 10 TL for Kangersuk fish plotted against age. White dots = otolith measurements and black dots = suboperculum measurements. Means with  $\pm$ SD.

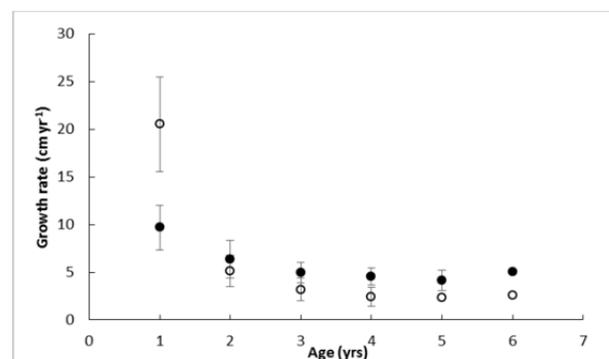


Figure 11 Growth rates for Kangersuk fish plotted against age. White dots = otolith measurements and black dots = suboperculum measurements. Means with  $\pm$ SD.

preferred structure to analyse in future studies of Arctic charr, if the individuals in question are expected to be younger than 8 years.

### Populations and their habitats

Otolith readings have been found to be unreliable when used on individuals living at temperatures above their temperature optimum (Mosegaard et al. 1988), this is however not

considered to be an obstacle for the populations sampled in this study, as the highest measured summer mean was at 8.8°C and the larger landlocked individuals, which would have the low temperature optimum of 7.5°C (Pörtner et al. 2008; Siikavuopio et al. 2013; Hansen et al. 2016), were all caught in the deep lake of the Kangersuk system, where they would in theory have the opportunity to take refuge in deeper colder waters. The upper part of Røde Elv, where our smaller specimens were caught, had a max temperature during July 2015 of 4.4°C (Pedersen et al. 2015). For the same period, the mean air temperature was approximately 9°C (The Arctic Station Long Term Monitoring Program 2017). There is no water temperature data available for the Kangersuk system; however it can be assumed that the shallow lake and thus the outlet stream have a higher temperature than the deeper lakes.

The specimens caught in the Kangersuk system can be categorized into two. The larger individuals caught in the deep Kangersuk lake and the smaller individuals caught in the stream part of the Kangersuk system. It can be assumed, as method of catch was by angling with spinner lures, that the large individuals were at least partly piscivorous. These individuals have the possibility of preying on smaller Arctic charr as well as benthic invertebrates or zooplankton species (Ethelberg-Findsen et al. 2015), hence, we do not assume food limitations to be an issue for these. For the smaller individuals of the system, the prey availability is also unknown, however, they have been found to feed heavily on chironomid larvae (Ethelberg-Findsen et al. 2015).

The anadromous specimens caught in Laksebugten, are not assumed to experience food limitations, as they migrate to avoid this. The Røde Elv specimens live in water, which largely consists of melt-off from an overlying glacier, and mountain springs. As they were all significantly smaller than the two other populations, we assume they experience some levels of food limitations and suggests as (Kristensen et al. 2006), that the upper part of Røde Elv does not offer the most optimal habitat for growth. The

upper part of Røde Elv contain chironomidae, presumably providing the food for the Arctic charr population in this system, as these do not seem to be large enough to live as piscivores (Malmquist et al. 1992).

### *On back calculations*

The type models used for the back calculations of growth rates are numerous (Morita et al. 2001). Back calculating the inter-annual growth of the different populations was in this study carried out with the nonlinear body proportional hypothesis (nBPH) (Morita et al. 2001). The nBPH was used due to findings of the otoliths and suboperculum showing a high fit to a nonlinear function. For the hard structures to be used for back calculating growth, they need to show significant correlation between fish length and structure size (Mosegaard et al. 1988; Morita et al. 2001; Vigliola et al. 2009) this is as well the result (SO and TL:  $P < 0.0001$ ,  $\alpha = 0.05$ ; otolith and TL:  $P < 0.0001$ ,  $\alpha = 0.05$ ). A non-linear regression was fitted to the plots of TL against structure size resulting in a high fit for SO ( $R^2 = 0.977$ ) and a lower fit for otoliths ( $R^2 = 0.87$ ). This indicates that the length of the SO is more dependent on the fish TL than the otolith length. Assuming this close relationship, we estimate that the back calculated TL with the nBPH model is closer to the true length of the fish at a given age when using the SO. In this case, for otoliths, other factors, such as the effect of age, must be included to ensure a more precise back calculation (Morita et al. 2001). Other studies found that when using a biological intercept, the back calculated lengths were more consistent with observed lengths (Campana 2011; Ashworth et al. 2017).

The specimens from the Laksebugten population were analysed by lengths obtained from otolith diameter, which might give different results than if true measures had been used.

### *Comparing the populations*

The nPBH model showed the back calculated TL for the fish in Røde Elv were significantly different from those of Kangersuk (SO  $P = 0.047$ ,  $\alpha = 0.05$ ; Otolith  $P < 0.0001$   $\alpha = 0.05$ ). This is the

same as found by (Kristensen et al. 2006). The anadromous population of Laksebugten also showed to be significantly different from the Røde Elv population (SO  $P=0.0092$ ,  $\alpha=0.05$ ; otolith  $P<0.0001$ ,  $\alpha=0.05$ ), there were however no significant difference between Laksebugten and the Kangersuk population.

Back calculating of the different populations found a TL for one year olds in the Røde Elv population to be under half as long, as the Kangersuk and the Laksebugten populations. In (Kristensen et al. 2006), the fork length (FL) found by sampling conducted in 2004, for 1 year olds is approximately 2.5 cm for Røde Elv and 7.5 cm for Kangersuk, while (Hedemand et al. 2016) found 1 year olds in Røde Elv to have a FL of around 3 cm. Though TL is bound to be higher than FL as more of the fish is measured, the much higher findings of our study do not seem to fit the general of previous studies in this area, the length calculated in in this study does rather fit the 2 year old individuals from both previous studies, which is around 5 cm for the samples from 2004 (Kristensen et al. 2006) and 6 cm for samples collected in 2016 (Hedemand et al. 2016). In a strictly visual, non-statistical comparison of samples collected in 1990, 2004 and 2016, Hedemand et al. (2016) showed an increasing length for the 1 year olds, suggesting that our findings would potentially be higher than previous findings. Some of our individuals do however fit the previous findings (*Appendix 1 and 2*), suggesting that the back calculated measurements for 1 year olds can be corrupted by masking of the first annulus.

Statistical analysis of the growth rates calculated for ages 1-5 for the three populations did not show any significant difference. As discussed above, the first year was hard to read, resulting in a very high uncertainty regarding the initial annulus lengths. We therefore decided to remove the first years and then analyse the differences of the ages 2-5. This showed significant difference between the Røde Elv population and Kangersuk (SO  $P=0.0018$ ,  $\alpha=0.05$ ) as well as the Røde Elv population and Laksebugten population (SO  $P=0.0058$ ,  $\alpha=0.05$ ; otolith  $P=0.0439$ ,  $\alpha=0.05$ ).

### ***Growth rates and climate change***

Statistical analysis of growth rates compared against mean winter and annual air temperature did not show any significance. The mean summer temperatures did however show a positive correlation with the Kangersuk population (SO  $P=0.034$ ,  $\alpha=0.05$ ). The Kangersuk population is, as described above, not assumed to be food limited as the growth rates are comparable to the anadromous population, the temperature optimum is hence able to have an effect on growth rates. This finding is comparable to the previous studies from the same population (Kristensen et al. 2006, Hedemand et al. 2016). The temperature correlated growth rate cannot with certainty be attributed to physiological optimised conditions as the water temperature is not measured. The correlation to temperature can potentially be affected by other factors, such as food availability or a longer growth season due to earlier onset of snowmelt. More to it is that food availability is not always a matter of the present years mean temperature, as earlier years might have a large impact as well. It should hence be considered, that annual temperatures might have an indirect environmental effect as well as a direct physiological effect (Michaud et al. 2010).

### ***Methodological remarks***

The study presented above have shown many difficulties in treating the data, as discussed readings were difficult due to masking of the initial annual growth rings. For future research in line with this study, we therefore suggest that a standard distance from the centre to the initial growth ring is determined based on several young individuals, where lesser masking occurs. This standard distance, should then be used to aid the readings of older individual's hard structures, as well as when determining growth increments. Note that the standard distance should not be used directly to measure initial growth rings, but merely as a guide to find the initial growth rings.

### ***Conclusion***

The study showed no direct indication that mean annual air temperature was the main determining factor of inter-annual growth in the

Arctic charr populations present in Røde Elv, Kangersuk and Laksebugten, nor did the winter temperatures. The growth season or the summer, did however show a correlation between mean temperatures and the growth rates of the Kangersuk population. The direct reason to this correlation cannot be found without additional research of the system in regards to food availability and actual water temperatures.

It was shown via back calculations, that the stream-based, landlocked population of Røde Elv were significantly smaller than the landlocked Kangersuk population and the anadromous Laksebugten population. As the Kangersuk and Laksebugten populations did not show any significant difference, it is suggested, that food limitation is a factor in the Røde Elv population. This was based on the assumption that the anadromous population does not experience any shortage of food in the growing season occurring in summertime. As the Kangersuk population did not differ from these, the fact that the Røde Elv population is landlocked cannot be the determining factor. As described, temperature for Røde Elv was quite low, even during warm periods in the summer, thus the small size of specimen living here, cannot be appointed the need for colder waters in shallow habitats.

The investigation of the different methods of aging and determining growth rates, were proven to favour the method using suboperculum for age reading, when using the nonlinear body proportional hypothesis, at least when working with Arctic charr 8 years or younger. The added element of the suboperculum being far easier to work with and retrieve, further underlines the advantages of using it instead of the otoliths.

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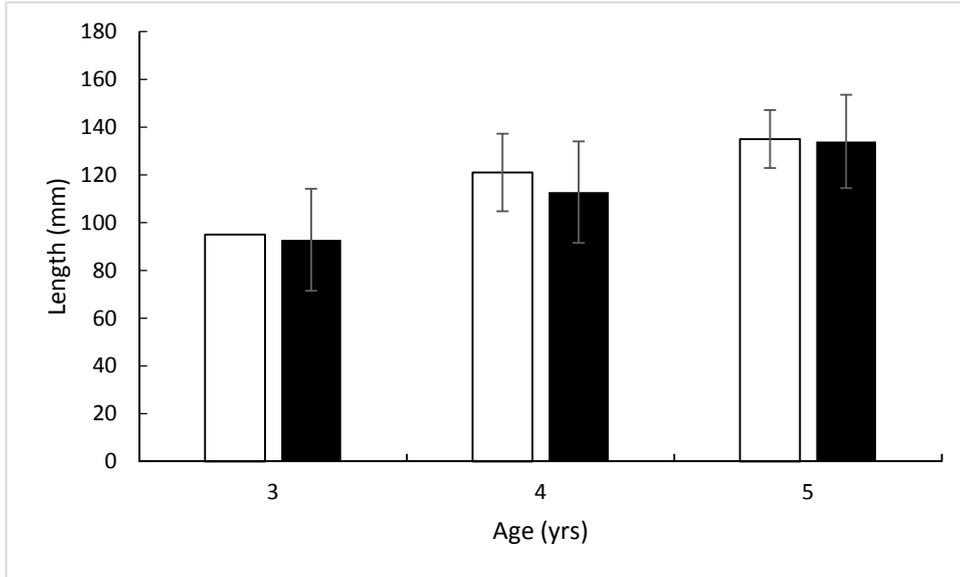
## Appendix

Location	Landlocked/ Migrating	Number	Weight (g)	Length (cm)	Tag name	Age of capture by Suboperculum (yr)	Length of suboperculum (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)	K
Kangersuk	Landlocked	1	291.10	31.5	S1	5	17.072	5.199	7.815	9.777	12.18	14.73	-	-	-	0.93
Kangersuk	Landlocked	2	331.7	35.5	S2	4	16.835	4.927	10.65	13.423	15.2	-	-	-	-	0.74
Kangersuk	Landlocked	3	393.3	38.9	S3	6	18.915	3.687	7.422	10.363	12.39	15.35	17.73	-	-	0.67
Kangersuk	Landlocked	4	464.6	36.4	S4	5	19.482	5.143	8.053	10.732	13.75	17.02	-	-	-	0.96
Kangersuk	Landlocked	5	416.2	36.4	S5	6	17.619	2.503	5.866	8.754	11.4	13.27	16.268	-	-	0.86
Kangersuk	Landlocked	6	362.2	34.5	S6	6	17.557	4.006	6.094	8.355	10.73	13.8	15.978	-	-	0.88
Kangersuk	Landlocked	7	479.8	39.5	S7	6	18.571	2.826	5.852	8.748	11.25	14.54	17.431	-	-	0.78
Kangersuk	Landlocked	8	362.6	36	S8	6	17.111	2.481	5.726	7.254	9.532	13.27	15.749	-	-	0.78
Kangersuk	Landlocked	9	274	29.3	S9	5	14.899	3.387	6.596	8.165	10.49	13.12	-	-	-	1.09
Kangersuk	Landlocked	10	382.9	35.7	S10	6	15.168	3.558	5.496	7.397	10.54	12.46	14.387	-	-	0.84
Kangersuk	Landlocked	11	244.3	28.8	S11	5	14.915	4.152	6.905	9.113	11.68	14.26	-	-	-	1.02
Kangersuk	Landlocked	12	77.5	22.3	S12	3	11.595	4.196	7.916	10.274	-	-	-	-	-	0.70
Kangersuk	Landlocked	13	8.4	10.5	S13	-	-	-	-	-	-	-	-	-	-	0.73
Kangersuk	Landlocked	14	10.7	11.6	S14	2	6.268	1.966	4.768	-	-	-	-	-	-	0.69
Røde Elv upper part	Landlocked	1	11	10.6	RØ1	3	5.477	2.045	3.809	4.814	-	-	-	-	-	0.92
Røde Elv upper part	Landlocked	2	8.1	9.7	RØ2	3	4.126	1.666	2.864	3.756	-	-	-	-	-	0.89
Røde Elv upper part	Landlocked	3	7.9	9.9	RØ3	3	5.014	2.045	3.196	4.247	-	-	-	-	-	0.81
Røde Elv upper part	Landlocked	4	8.6	10.1	RØ4	3	5.657	1.981	3.898	5.054	-	-	-	-	-	0.83
Røde Elv upper part	Landlocked	5	11.7	10.9	RØ5	3	6.28	2.152	3.995	5.106	-	-	-	-	-	0.90
Røde Elv upper part	Landlocked	6	34.7	16.4	RØ6	5	8.794	1.824	3.057	4.238	6.185	7.551	-	-	-	0.79
Laksebugten	Migrating	1	875.6	41.5	M1	7	22.807	5.302	8.693	11.391	13.84	15.94	19.109	21.17	-	1.23
Laksebugten	Migrating	2	732.8	39.6	M2	7	23.971	5.491	7.542	9.254	12.78	16.11	19.33	22.22	-	1.18
Laksebugten	Migrating	3	547.1	36.8	M3	8	21.463	3.62	6.006	8.834	12.31	15.79	17.975	19.71	20.63	1.10

### Appendix 1. Measurements of the 23 fish. Years 1-8 is the length of the SO for each fish.

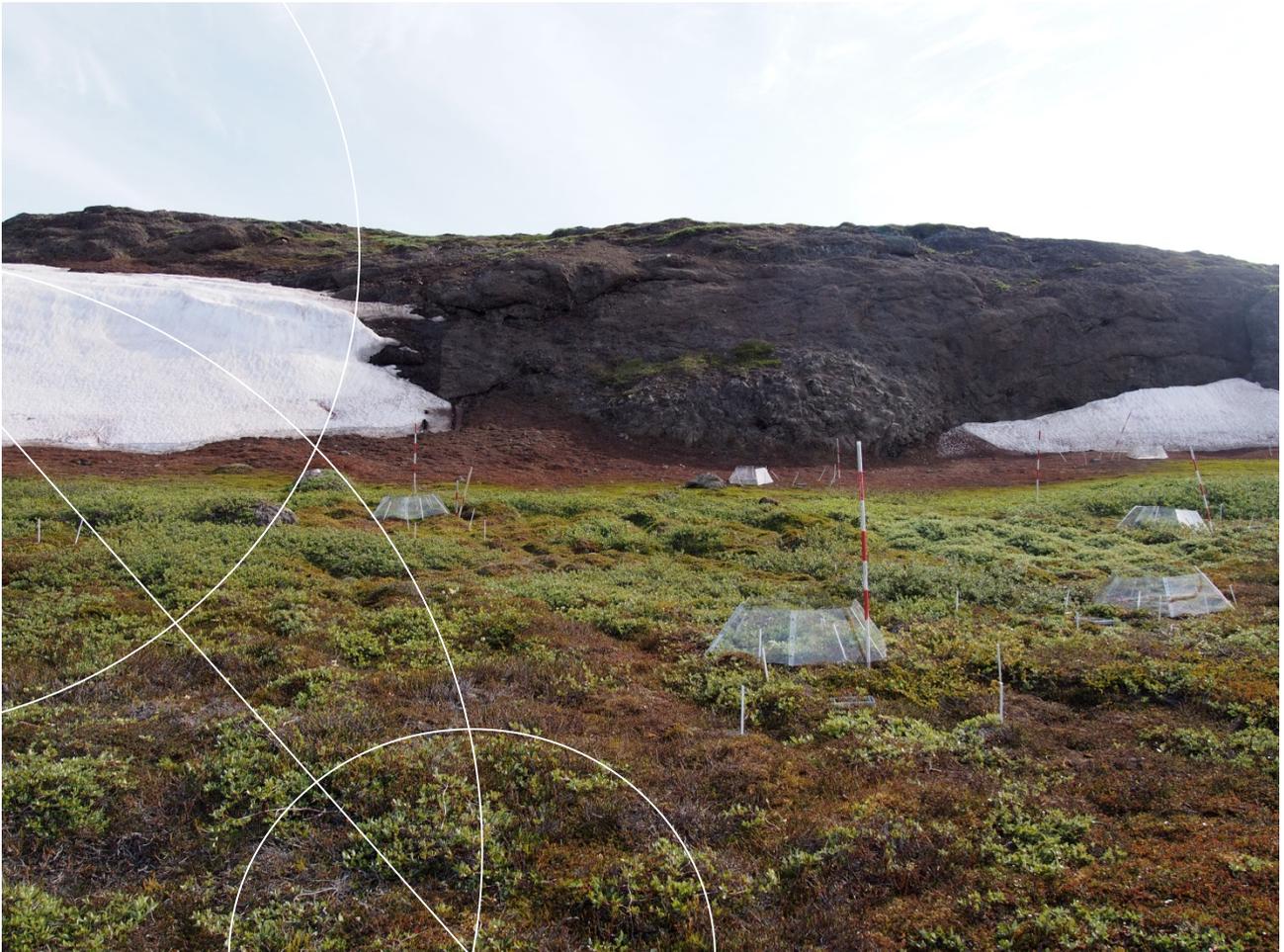
Location	Landlocked /Migrating	Number	Weight	Length	Tag name	Age of capture by otolith	Radius of Postrostrum (mm)	Year 1 (mm)	Year 2 (mm)	Year 3 (mm)	Year 4 (mm)	Year 5 (mm)	Year 6 (mm)	Year 7 (mm)	Year 8 (mm)
Kangersuk	Landlocked	1	291.10	31.5	S1	4	0.382	0.712	0.94	1.068	-	-	-	-	-
Kangersuk	Landlocked	2	331.7	35.5	S2	3	0.747	1.129	1.308	-	-	-	-	-	-
Kangersuk	Landlocked	3	393.3	38.9	S3	6	0.467	0.795	0.872	0.989	1.136	1.313	-	-	-
Kangersuk	Landlocked	4	464.6	36.4	S4	4	0.706	0.973	1.251	1.465	-	-	-	-	-
Kangersuk	Landlocked	5	416.2	36.4	S5	4	0.503	0.738	1.059	1.238	-	-	-	-	-
Kangersuk	Landlocked	6	362.2	34.5	S6	-	-	-	-	-	-	-	-	-	-
Kangersuk	Landlocked	7	479.8	39.5	S7	4	0.407	0.737	0.914	1.146	-	-	-	-	-
Kangersuk	Landlocked	8	362.6	36	S8	-	-	-	-	-	-	-	-	-	-
Kangersuk	Landlocked	9	274	29.3	S9	3	0.718	0.872	1.093	-	-	-	-	-	-
Kangersuk	Landlocked	10	382.9	35.7	S10	3	0.743	0.994	1.183	-	-	-	-	-	-
Kangersuk	Landlocked	11	244.3	28.8	S11	4	0.644	0.867	1.045	1.126	-	-	-	-	-
Kangersuk	Landlocked	12	77.5	22.3	S12	2	0.513	0.893	-	-	-	-	-	-	-
Kangersuk	Landlocked	13	8.4	10.5	S13	1	0.614	-	-	-	-	-	-	-	-
Kangersuk	Landlocked	14	10.7	11.6	S14	-	-	-	-	-	-	-	-	-	-
Røde Elv upper part	Landlocked	1	11	10.6	RØ1	2	0.353	0.613	-	-	-	-	-	-	-
Røde Elv upper part	Landlocked	2	8.1	9.7	RØ2	3	0.254	0.447	0.645	-	-	-	-	-	-
Røde Elv upper part	Landlocked	3	7.9	9.9	RØ3	3	0.382	0.557	0.724	-	-	-	-	-	-
Røde Elv upper part	Landlocked	4	8.6	10.1	RØ4	3	0.269	0.551	0.656	-	-	-	-	-	-
Røde Elv upper part	Landlocked	5	11.7	10.9	RØ5	3	0.335	0.582	0.767	-	-	-	-	-	-
Røde Elv upper part	Landlocked	6	34.7	16.4	RØ6	5	0.294	0.525	0.669	0.751	0.85	-	-	-	-
Laksebugten	Migrating	1	875.6	41.5	M1	7	0.355	0.564	0.7	0.839	1.013	1.149	1.334	-	-
Laksebugten	Migrating	2	732.8	39.6	M2	7	0.659	0.836	1.026	1.141	1.55	1.674	1.712	-	-
Laksebugten	Migrating	3	547.1	36.8	M3	8	0.269	0.447	0.635	0.727	0.891	1.063	1.212	1.314	-

### Appendix 2. Measurements of the 23 fish. Years 1-8 is the length of the Rpr for each fish.



**Appendix 3. White bars = males, black bars =female. P=0.89. Data from Hedemand et al. 2016.**

*Analysis of vegetation cover and physiological responses in plants along a snowbed gradient exposed to warming with open-top chambers*



**Open Top Chambers in front of a snow fan in Blæsedalen (Photo: Liv Alexa Nobel)**

# *Analysis of vegetation cover and physiological responses in plants along a snowbed gradient exposed to warming with open-top chambers*

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## **Abstract**

The arctic regions are known for their short growing seasons and low temperatures, but with the current climate changes, that has shown to be more pronounced in the Arctic, longer growing seasons and higher temperatures are part of the future. This study tries to predict the future changes in vegetation cover and physiological responses in plants as the growing season prolongs and the temperature rises by doing a vegetation analysis, a flower count, measuring NDVI, chlorophyll and flavonoid content in the dominant *Salix herbacea* and measuring leaf length of *S. herbacea* in 36 plots, warmed and control, over a gradient of increasing growing season. We found that *S. herbacea* was the overall most dominant species along the gradient. Moving down the gradient, the abundance of *S. herbacea* declined and the abundance of *Diphysastrum alpinum* increased. Our two NDVI measurements showed an increase in the NEAR SNOW-zone from the first to the second and a decrease in the END-zone from the first time measured to the second time but showed no difference between warming treatment and control. We found leaf length in *S. herbacea* to decrease when we moved down the gradient, and an increase in leaf length of *S. herbacea* in the warming treatment plots.

**Keywords:** *climate change, experimental warming, vegetation cover, plant physiological response*

## **Introduction**

An increased emission of greenhouse gasses during the past century has led to a rise in global temperatures. Expectations of increased global surface temperatures in this century have been estimated to be between 2.6°C and 4.7°C (IPCC, 2013). Because of these changes in temperature, the arctic climate is expected to change. The rise in temperature will lead to an increased snow-melting rate and are therefore contributing to a longer growing season for plants something that could lead to a greening of the arctic. Between 1982 and 2012, measurement of The Normalized Difference Vegetation Index (NDVI) have been made, these show that less than 4% of the Pan-Arctic area have browned, while one third of the Pan-Arctic area have shown a substantial greening (cited in (IPCC, 2014) from (Xu et al., 2013)).

Sixty-one sites with warming experiments in tundra regions worldwide have shown that a warmer climate leads to an increased

proliferation, growth and cover of deciduous shrubs and graminoids, whereas cover and species diversity of lichens and bryophytes decreases (Elmendorf et al., 2012).

Snow cover extent and snow cover duration are two very important factors controlling plant growth in the Arctic. Snow acts as an insulator and therefor protects underlying plants and soil from extreme temperatures and harsh winds and when the snow melts it will contribute to higher soil moisture content (Petraglia et al, 2014). Because of climate change and a higher temperature in the Arctic, it is thought that snow duration will be shorter and that earlier snowmelt dates will be experienced in the future. These changes could alter the growth, development and flowering cycles of the plants (Petraglia et al, 2014).

Typically in the Arctic tundra, plant communities and vegetation are distributed along an environmental gradient. Wind exposed ridges face severe weather conditions with low



**Figure 1** Examples of the vegetation with the 1m x 1m grid used to do the pin-point analysis and flower count. a is NEAR SNOW, b is TOP, c is MED and d is END

snow cover, low winter temperatures (due to lack of insulation effect when snow is not present), strong winds and unreliable water supply. Depressions tend to have a shorter growing season because snow accumulation will be higher here. Furthermore depressions will have higher soil moisture content (Gaae et al, 2011).

Depressions forming snowbed areas are common in the arctic, and may form at the lee side of exposed ridges, behind which a snowpack may build up. These areas will have a longer lasting snow cover than the surroundings. In such areas the amount of snow accumulated increases when moving up the gradient towards the snowpack. The melting of the snow and the resulting snow-free season is different along the gradient. Because of this, the lower end of the gradient will experience a longer growing season than zones closer to the snowbed, and the timing of the of the snowmelt is therefore a major and crucial factor controlling the plant communities and plant growing conditions (Petraglia et al, 2014).

The aim of this study was to shed light on the consequences of climate changes for plants in the Arctic. Furthermore the goal was to investigate the impact of the length of the growing season. This was done by making a vegetation analysis along an elevation gradient and adding an artificial warming effect, with open-top chambers, to see if these factors change the species composition and their allocation of

nutrients. Lastly we wanted to give an estimate of how successful each species are at reproducing, measured as flowering.

### *Hypothesis*

Our hypothesis is divided into two; what we expected to happen along the gradient and what we expected with the warming treatment. With the warming treatment we expected less vascular plant diversity in the warmed plots and less lichens and mosses. We expected a more dense cover of graminoids and dwarf shrubs and a higher chlorophyll and flavonoid content. We also expected an increased vegetation cover and an increased amount of flowers. Along the gradient, with increasing distance from the snowpack and decreasing elevation we expected more vascular plant diversity and less lichens and mosses. We expected more graminoids and dwarf shrubs and higher chlorophyll and flavonoid content. We also expected an increased vegetation cover and an increased amount of flowers.

### *Methods and Materials*

#### *Field site description*

In Blæsedalen, 69°21'N 53°30'W, on Disko Island, Greenland, a field experiment simulating the direct effects of climate warming has been running since early July 2014 (with the addition of 6 "NEAR SNOW" plots added in August 2015). The experiment is located along a snowbed gradient, where the melting snow provides moisture to the soil and furthermore transports nutrients along with the water from upper parts of the gradient to the lower part of the gradient. The gradient consists of paired plots with open-top chambers and associated controls. The gradient is divided into four; NEAR SNOW, with 3 replicates and TOP, MED and END, all with 5 replicates, and all with paired control and open-top chamber plots, leading to 36 plots in total. Mean annual air temperature is -2,9°C and mean annual precipitation is 273 mm (Lindwall et al., 2016). The vegetation is primarily dominated by graminoids, such as *Poa alpina*, *P. alpigena* and *Carex bigelowii* and dwarf shrubs, such as *Empetrum nigrum*, *Salix herbacea*, *Salix glauca*

and *Betula nana*. Furthermore lichens and bryophytes were found in most of the plots.

### **Vegetation analysis**

Vegetation analysis was made to show vegetation composition and species distribution. We used pin-point analysis with the a rectangular grid tool consisting of 81 squares and 100 pin-point corners, see Fig 1. We placed a knitting needle in each corner of each square and noted which species the needle touched. This was done in all of the corners of the grid tool and repeated in all of the 36 plots. All plants were identified at species level, except mosses and lichens, and except *S. herbacea* and *S. glauca*, which were divided into female, male and unknown. Litter was based on estimates over the grid and not pin-point analysis.

### **Flower count**

To estimate the abundance of flowering plants in the plots, we used a grid tool to help us keep track of the plants and flowers. The rectangular grid tool consisted of 81 squares used for pin-point analysis. All flowering plants were counted and for *S. herbacea* and *S. glauca* the flowering plants were divided into males, females and unknown. This was done in all 36 plots.

### **Flowering success**

Flowering success is the rate between percentage vegetation cover and flowers counted of that same species. It gives an estimate of how successful each species are at reproducing themselves through flowering.

### **Chlorophyll, flavonoid, Nitrogen Balance Index (NBI) and NDVI measurements**

Eight leaves from *S. herbacea* was collected from non-flowering plants from each plot (only if the plots had more than 250 leaves pr. plot) and Nitrogen Balance Index (NBI), flavonoid and chlorophyll content was measured with a Force-A Dynamax Dualex on the 8 leaves collected pr. plot. NBI is the chlorophyll to flavonoid ratio and chlorophyll and flavonoids have the unit  $\mu\text{g}/\text{cm}^2$ . Normalized Difference Vegetation Index (NDVI) was measured using a SKYE SKR 100 with a SKYE 660/730 nm sensor at a distance of 45 cm to the

plot. We measured the NDVI three times at two different wavelengths (660 nm and 730 nm) on each plot and the relation between the two wavelengths was used to estimate the greenness of the plots.

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

Where NIR (Near InfraRed) is wavelength 730 and Red is 660. The wavelengths were measured as  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

### **Statistics**

Statistical analysis of NDVI, NBI, chlorophyll content, flavonoid content and leaf length of *S. herbacea* were all performed using SAS Enterprise 6.1. They were all analyzed using repeated calculations of factorial two-way ANOVA - linear models, with elevation and treatment as variable factors. Significance level was set as  $P < 0.05$  and marked with \*. Furthermore Tukey's test was performed to show both difference with elevation and treatment, this is shown with letters above the graphs. Means that share letters are not significantly different. Data from vegetation analysis, flower count and flower success rate have been sorted by elevation and treatment and an average was calculated. Standard deviation was also calculated and added to the graphs that were all made in Microsoft Office Excel. Flowering success was calculated as the percentile vegetation cover divided with the flowers counted of that same species.

## **Results**

### **Vegetation analysis**

Multiple layers of plant leaves can result in multiple hits in the pin-point analysis, which can lead to sum-values that exceed 100%. NEAR SNOW plots were clearly dominated by litter in both control (98.3%) and treatment (95%) plots. Treatment plots were also dominated by unknown *S. herbacea* (8.3%). See fig 2A. The top control plots were found to be dominated by unknown *S. herbacea* (50,6%), litter (31,8%) and female *S. herbacea* (17,2%), whereas in the .

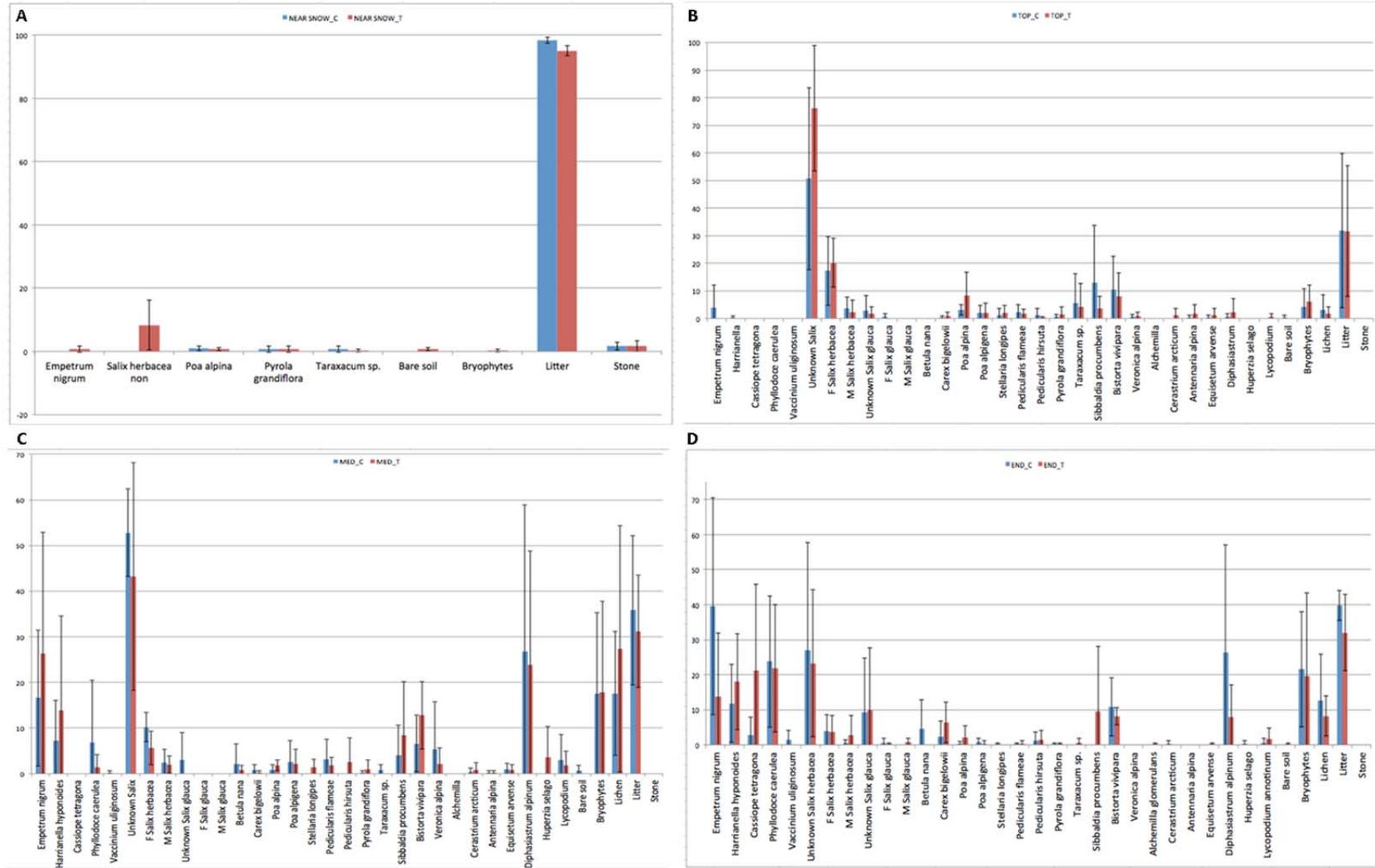


Figure 2 Vegetation analysis in control (C) and warming treatment (T) plots. Values are shown as % cover and standard deviation bars are included. A: NEAR SNOW elevation, B: TOP elevation, C: MED elevation, D: END elevation.

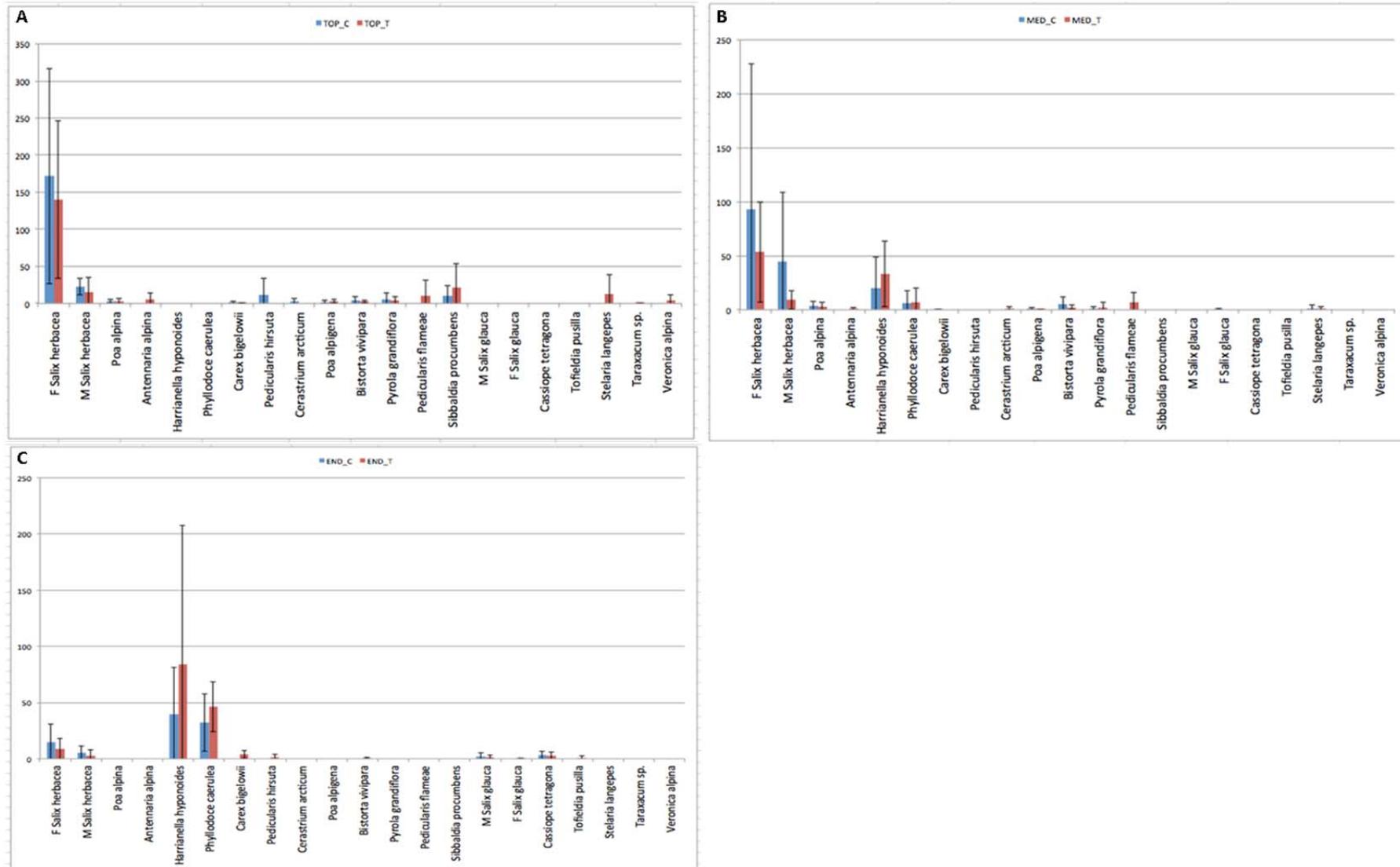


Figure 3 Flower counts in control (C) and warming treatment (T) plots. Values are shown as average flowers counted and standard deviation bars are included. A: TOP elevation, B: MED elevation, C: END elevation.

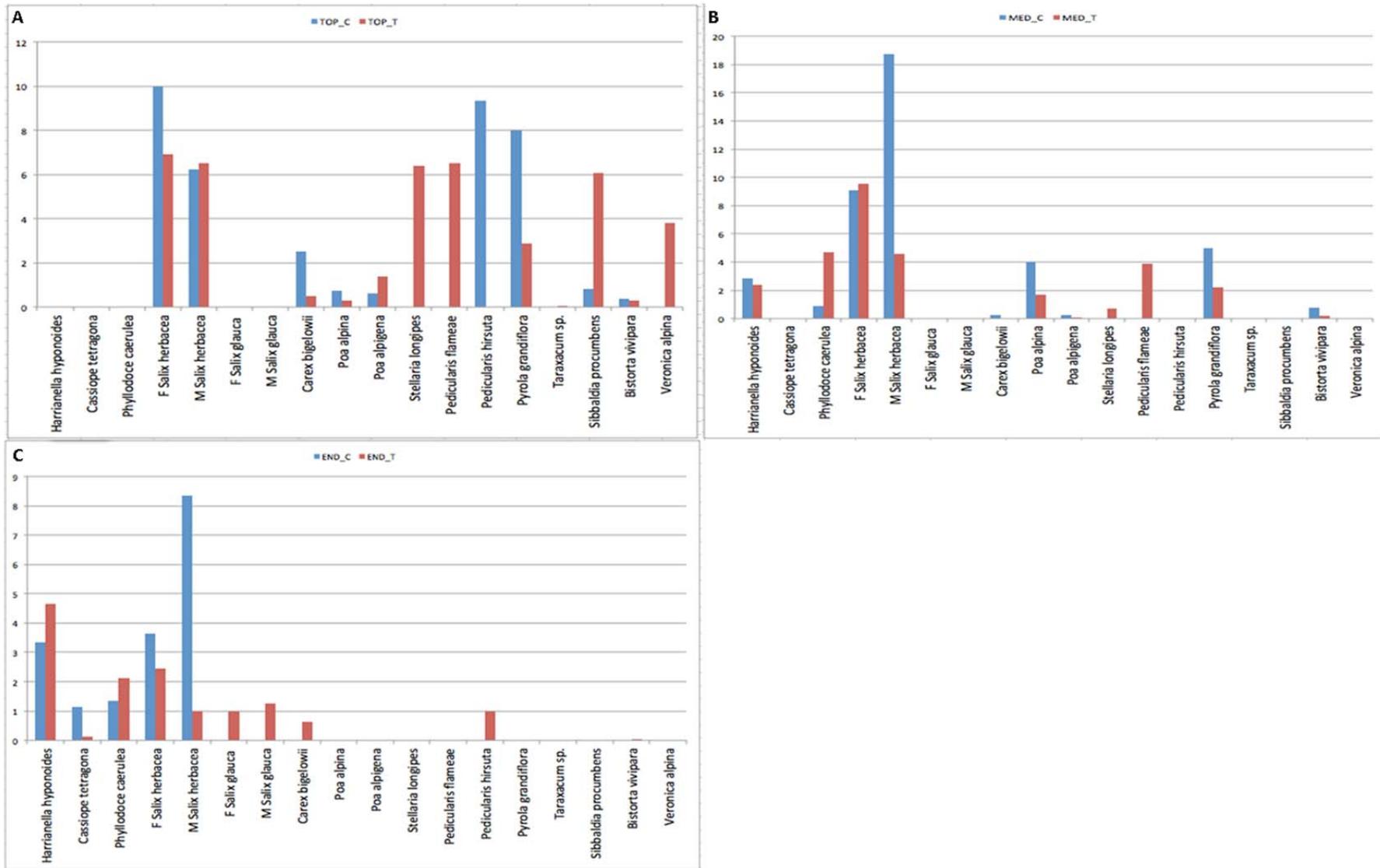


Figure 4 Flowering success rate in control (C) and warming treatment (T) plots. Values are shown as the rate between flowers counted and the fractional vegetation cover of the species. A: TOP elevation, B: MED elevation, C: END elevation.

treatment plots unknown *S. herbacea* (76,2%), litter (31,6%) and female *S. herbacea* (20,2%) were dominating. See fig. 2B. MED control plots were dominated by *S. herbacea* (52.8%), litter (35.8%) and *Diphasiastrum alpinum* (26.8%). The treatment plots in MED elevation were also dominated by *S. herbacea* unknown (43.2%), litter (31.25%) and *Diphasiastrum alpinum* (23.8%). See fig. 2C. The END control plots were dominated by litter (39.8%), *Empetrum nigrum* (39.6%), *S. herbacea* unknown (27%) and *Diphasiastrum alpinum* (26.4%). The treatment plots in END elevation were dominated by litter (32%), *S. herbacea* unknown (23.2%), *Phyllodoce caerulea* (21.8%) and *Cassiope tetragona* (21.2%). See fig. 2D

### Flower count

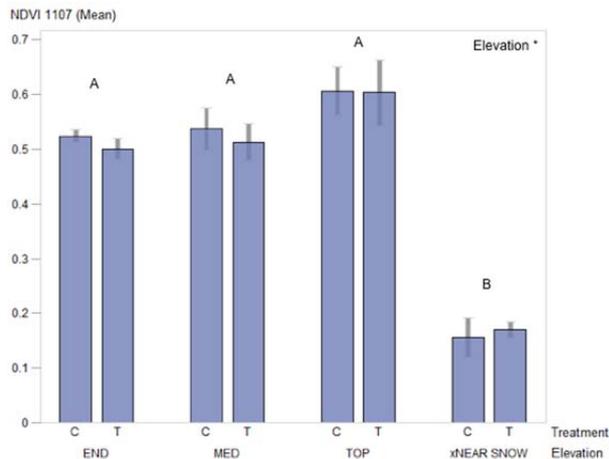
Values above are counted flowers. An average was calculated for each gradient with the associated treatment. As no flowers were present in the NEAR SNOW plots we have no data from these plots. TOP control plots were solely dominated by female *S. herbacea* (171.6). The treatment plot in the TOP elevation was also dominated by female *S. herbacea* (140). See fig. 3A. The control plots at the MED gradient were dominated by female *S. herbacea* (92.8), male *S. herbacea* and *Harrimanella hyponoides*. MED treatment plots were clearly dominated by female *S. herbacea* and *Harrimanella hyponoides*. See fig. 3B. END control plots were dominated by female *S. herbacea*, *Harrimanella hyponoides* and *Phyllodoce caerulea*. Treatment plots in END zone were dominated by *Harrimanella hyponoides* (84) and *Phyllodoce caerulea* (46.2). See fig. 3C.

### Flowering success

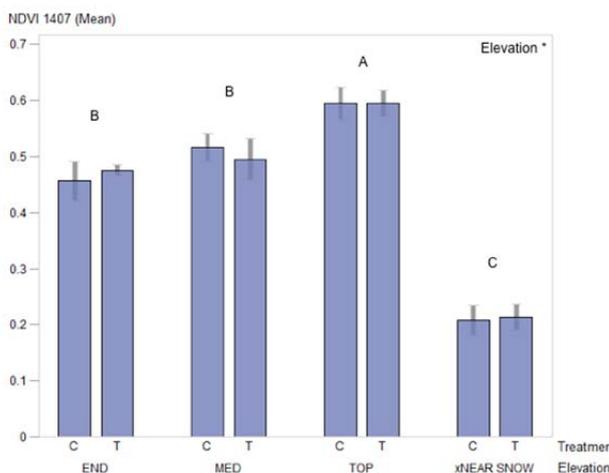
The flowering success is an estimate of how many of the plants found at each elevation that had flowers at the specific time we did the flower

**Table 1 Species diversity listed with elevation and treatment.**

Elevation	Treatment	Nr. of species
NEAR SNOW	Control	3
NEAR SNOW	Treatment	6
TOP	Control	23
TOP	Treatment	21
MED	Control	25
MED	Treatment	25
END	Control	25
END	Treatment	25



**Figure 6 NDVI measured on July 11<sup>th</sup>**

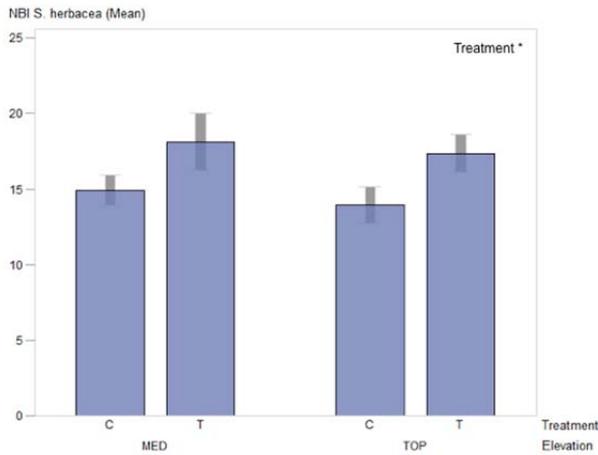


**Figure 5 NDVI measured on July 14<sup>th</sup>**

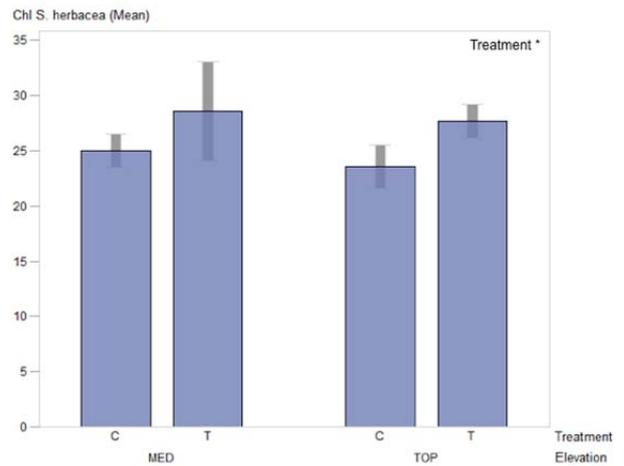
counting. As no flowers were present in the NEAR SNOW plots we have no data from these plots. In the TOP elevation plots the female *S. herbacea* had the best flowering success in both control and treatment plots. In the treatment plots rate in both control and treatment plots, though female *S. herbacea* was successful in flowering rate in the control plots. See fig. 4B. In the treatment plots *Phyllodoce caerulea* and especially *Harrimanella hyponoides* show high flower success rates. See fig. 4C.

### Species diversity

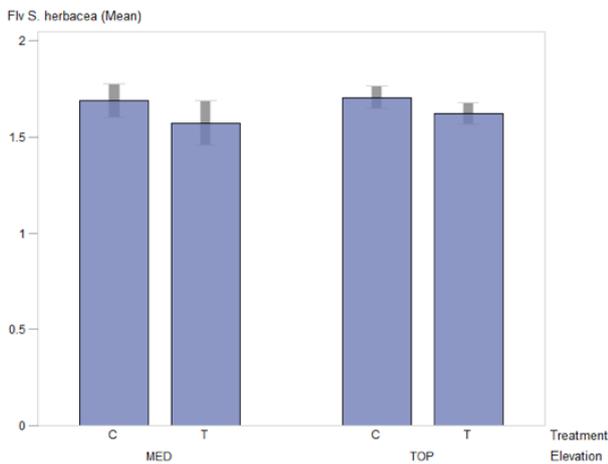
The species diversity is the same across all elevations except for the NEAR SNOW plots and does not differ with treatment either.



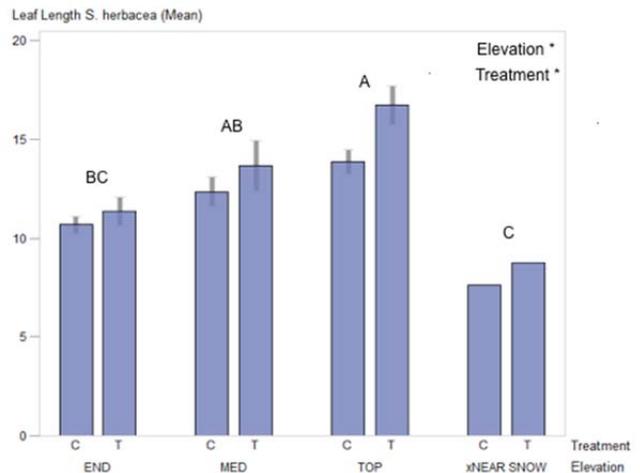
**Figure 7** Mean NBI for *Salix herbacea* measured on 8 leaves in each plot with more than 250 leaves available (4 measurements in MED control, 2 in MED treatment and 5 in TOP control + treatment).



**Figure 8** Mean chlorophyll content of *Salix herbacea* measured on 8 leaves in each plot with more than 250 leaves available (4 measurements in MED control, 2 in MED treatment and 5 in TOP control + treatment).



**Figure 9** Mean flavonoid content of *Salix herbacea* measured on 8 leaves in each plot with more than 250 leaves available (4 measurements in MED control, 2 in MED treatment and 5 in TOP control + treatment).



**Figure 10-** Average leaf length of *Salix herbacea*, measured on 10 leaves in each of the plots except NEAR SNOW, where only one control and one treatment plot was measurable

The Normalized Difference Vegetation Index (NDVI) measured on the 11th of July was not found to be significantly different with warming treatment but was found to be significantly different with elevation between NEAR SNOW and the rest ( $P < 0.0001$ ).

NDVI measured on the 14th of July was found to be significantly different along the gradient as NEAR SNOW was lower than the rest and TOP was higher than the rest ( $P < 0.0001$ ). No significance was found with the warming treatment. NDVI changed from the first to the

second measurement as it decreased in the END gradient ( $\sim 0.5$  to  $\sim 0.45$ ) and increased in the NEAR SNOW gradient ( $\sim 1.5$  to  $\sim 2.0$ ).

Nitrogen Balance Index (NBI) in *S. herbacea* was significantly higher with treatment than in control plots both at TOP and MED gradient. No significance was found between MED and TOP zones along the gradient.

Chlorophyll content in *S. herbacea* was significantly higher in warmed plots than in control plots both at TOP and MED gradient. No

significant difference was found between MED and TOP zones along the gradient.

Flavonoid content in *S. herbacea* was not significantly different with warming treatment nor was it significantly different between MED and TOP zones along the gradient.

Leaf length in *S. herbacea* was found to be significantly higher with warming treatment along the entire gradient (except NEAR SNOW) and was significantly different between the elevations as well. It was not possible to measure leaf length in more than one plot in the NEAR SNOW plots, this was due to the short growing season and because of this, no standard deviation bars are seen here.

## Discussion

### Changes in vegetation along the gradient:

#### Vegetation analysis, flower count and flowering success:

The vegetation goes from solely *S. herbacea* domination to a more diverse plant community as we move down the gradient. NEAR SNOW and TOP has a clear *S. herbacea* dominated vegetation whereas MED and END are increasingly more dominated by other species such as *Diphasiastrum alpinum*, *Empetrum nigrum*, lichens and bryophytes. This might be because of *S. herbacea*'s ability to survive under a very short growing season, something also found in previous studies (Sedlacek *et al.*, 2015). The flower counting confirms the thesis that *S. herbacea* is being outmatched by other, more competitive plants, as we move down the gradient. *S. herbacea* is a species that thrives with a short growing season and seem to have few other competitors in NEAR SNOW plots. But in the presence of other plants that come with a prolonged growing season, *S. herbacea* cannot maintain its dominance and other plants, like *Diphasiastrum alpinum* increase in presence (at least from TOP to MED). This is also confirmed by the flowering success rate as *S. herbacea* gets a lower success rate as we move down the gradient. We do see an increase in the total

amount of flowers counted as we move down the gradient, as we also hypothesized, though only from TOP to MED. From MED to END we see a small decrease though the number of flowers counted is still higher than the TOP gradient. We also expected an increase in species diversity along the gradient, but there was no significant change in the number of species present at each level of the gradient though we did not look into the distributions of functional groups as these might have changed as seen in other experiments (Elmendorf *et al.*, 2012a). Lichens and bryophytes actually decreased with elevation, the opposite of what we expected. This might be because the nutrient availability was greater at the top of the gradient and the lichens and bryophytes were outmatched by vascular plants. This theory also matches why graminoids and dwarf shrubs do not decrease with elevation as we otherwise expected.

#### Normalized Difference Vegetation Index (NDVI):

The NDVI measurements showed a slight response along the gradient, as both measurement days were significantly different with elevation. The TOP gradient had the highest NDVI and decreased down the gradient though only significant from TOP to MED. The NEAR SNOW gradient did not follow this tendency, but showed the lowest measures of NDVI, this might have to do with the time of the year we did the measurements. The snow had only very recently disappeared from the NEAR SNOW plots and the vegetation had not yet appeared, something also visible in the increase in NDVI at NEAR SNOW plots from the 11<sup>th</sup> of July to the measurements taken the 14<sup>th</sup> of July. We did not expect this positive response with elevation as we hypothesized an increase in vegetation cover as we moved down the gradient. This might have to do with the increased water and nutrient availability found closer to the snowbed, something confirmed by previous studies suggesting that water and nutrient availability is more important than the length of the growing season (Gamon *et al.*, 2013). The finding of a decrease in NDVI with the gradient contradicts our hypothesis of an increased vegetation cover. This is because we expected the water and

nutrient availability to increase as we moved downwards from the snowbed. This was disproved when we arrived to see the plots with our own eyes, and this might be the reason why many of our hypotheses did not correlate with our findings.

#### ***Chlorophyll content, Flavonoid content and Nitrogen Balance Index (NBI) in Salix herbacea:***

Chlorophyll, flavonoid and NBI did not show any significant response with elevation, this might suggest that nutrient and water availability does not determine the allocation of Nitrogen and Carbon in the leaves. But as we were only able to measure on two of the 5 vegetation zones along the gradient our results are not very representative to say anything about the responses along the gradient. We expected an increase in both chlorophyll and flavonoid content, this we did not see, but this might again be because of the few measurements we were able to do.

#### ***Leaf length of Salix herbacea:***

Leaf length shows the same tendency as NDVI; that is to increase significantly with increasing elevation (NEAR SNOW being the exception again). This contradicts previous studies that found smaller leaves with earlier snowmelt (Wheeler *et al.*, 2016), but our findings might suggest that nutrient availability might have a greater influence at leaf length than growing season.

#### ***Warming responses***

##### ***Vegetation analysis, flower count and flowering success:***

We hypothesized, and other studies have shown, that warming affects the diversity in a negative way, leading to a decrease in species diversity (Walker *et al.*, 2006). When looking at table 1 there is clearly no difference in diversity between the treatment and control plots. We also expected that warming would favor dwarf shrubs and graminoids instead of, for example, lichens and mosses. When looking at fig. 1 (a, b, c, d), bryophytes and lichens show no decline in their abundance and no real tendency are seen

between warming treatment and control in the abundance of dwarf shrubs. Other studies have shown that the abundance of graminoids was positive correlated with warming and that the warming had a negative effect on the abundance of dwarf shrubs (Elmendorf *et al.* 2012a; Walker *et al.*, 2006). For mosses, soil moisture seems to be the controlling factor on how they will handle the climate warming (Elmendorf *et al.*, 2012a; Elmendorf *et al.*, 2012b). And lichen seems to decrease with warming (Walker *et al.*, 2006) One plausible reason why our data didn't show these results could be due to the relatively short timeline of the experiments. These have been going on for 4 summers and there is a possibility that with more time, a change in the plant community could take place. *Harrianaella hyponoides* had an increased flower success in the warmed plots both in MED and END. Both male and female *S. herbacea* experienced a decline in number of flowers in the warmed plots, and could show that the control plots were more favorable environment for this species. See fig. 2 (a,b,c). This is contradicting to our hypothesis. We expected that the dwarf shrubs would enhance their flowering patterns with warming. Female *S. herbacea* had almost 30% flowering success in the TOP plots and less than 5% in MED and END plots.

##### ***Normalized Difference Vegetation Index (NDVI):***

None of the vegetation zones showed any significant NDVI response to the warming treatment. This might suggest that the vegetation cover is limited by nutrients and water and not by temperature. We hypothesized that the vegetation cover increased with warming treatment, but this does not correlate with the data we collected. This might have something to do with the fact that we measured rather early in the season and the warming response might be significant later on in the season.

#### ***Chlorophyll content, Flavonoid content and Nitrogen Balance Index (NBI) in Salix herbacea:***

Chlorophyll and NBI showed significant increase with warming treatment, this might suggest that the leaves in the warmed plots allocate more N towards growth, and less

towards protection in the form of flavonoids, than the leaves found in the control plots. The flavonoid content did not show any significant response with warming treatment suggesting that the plants in the warmed plot have more nitrogen present and a higher growth rate, as they have a higher chlorophyll content. The increase in chlorophyll corresponds with our hypothesis but we also predicted an increase in flavonoids with warming treatment, something we did not find in our measurements.

#### *Leaf length of Salix herbacea:*

The leaf length measurements supports the idea that plants in the warmed plots seem to have more nutrients available as the leaves that have received warming treatment was significantly longer than the leaves in the control plots.

#### *Conclusion*

The standard deviations seen on our graphs are, in general, high because the communal vegetation types differed greatly between the different replicates of the gradient. With even more replicates this problem could not have been avoided but greatly diminished. We initially planned to make a third NDVI measurement, but due to bad weather and no more time this was not possible. The third NDVI would have shown us just how much the NEAR SNOW plot had greened in just 10 days. *S. herbacea*, the overall most dominant species along the entire gradient, also proved to be the species most vulnerable to the prolonged growing season that is expected to occur with the future climate changes. As *S. herbacea* declines in dominance *Diphysastrum alpinum* takes over and is more present as we move down the gradient. NDVI showed a change from the 1st to the 2nd measurement as it decreased in the END-zone and increased in the NEAR SNOW-zone. If we had a third measurement the difference between the gradient zones would probably have increased even more. Leaf length of *S. herbacea* decreased as we moved down the gradient, this also correlates with the fact that *S. herbacea* seems to thrive with a shorter growing season, as there are fewer competitors. It also suggests that nutrient

availability is more important for *S. herbacea* than a longer growing season. Bryophytes and lichens were not seen to decline with the warming treatment. Because the experiment only has been running for four summers, there is a possibility that this is not enough time for a measurable change in the plant community composition. The NDVI measurements showed no significant difference between control and warming, this could be due to that our measurements was done early and the warming of the plots possibly could happen later. Another suggestion could be that temperature is not a limiting factor for vegetation cover but water and nutrients could be. There was a significant difference in chlorophyll concentration between warmed and control plots. A reason for this could be that leaves in the warmed plots allocate N towards growth rather than protection. We found that the leaf length of *S. herbacea* in the warmed plot was significantly different from the control plot. The leaves had an increased length in the warmed plots and a reason for this could be that the rise in temperature increases the growth potential.

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*The effect of warming on CO<sub>2</sub> flux along a natural vegetation gradient in an arctic tundra*



**Open-Top Chamber in Blæsedalen (Photo: Liv Alexa Nobel)**

# *The effect of warming on CO<sub>2</sub> flux along a natural vegetation gradient in an arctic tundra*

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## **Abstract**

Climate changes affect ecosystems worldwide and are proven more and more significant in the Arctic. This study is investigating the effect of warming on the CO<sub>2</sub> flux in an arctic tundra on Disko Island, West Greenland. A natural vegetation gradient created by a snowbed nearby was used to investigate ecosystem processes in vegetation zones with different length of growing season. Furthermore, warming was achieved with open top chambers. Warming did not affect net ecosystem exchange (NEE) or gross ecosystem production (GEP) significantly, but ecosystem respiration (ER) increased with elevated temperatures. Furthermore, the length of the growing season influenced normalized difference vegetation index (NDVI) while indices of plant physiological performance were not directly influenced by warming.

**Keywords:** *CO<sub>2</sub>, warming, vegetation gradient, Arctic*

## **Introduction**

Terrestrial ecosystems are complex structures and are based on interactions between a wide range of organisms. Furthermore, several abiotic factors affect them. These interactions and controlling factors all help to define the ecosystem.

Right now, we see a changing environment due to the huge changes in the climate. These changes have shown to be exceptionally strong in the Arctic region. A general higher temperature is foreseen in the Arctic with the most pronounced increase in the autumn and winter periods. Furthermore, an increase in precipitation during the winter is also predicted (Serreze et al. 2000).

Changes in temperature have a direct effect on snow cover and the period of snowmelt. The presence of snow influences the abiotic factors and soil processes. Furthermore, the length of the growing season for the plants and thereby also the plant community composition is determined by the presence of snow (Petraglia et al. 2014).

Several studies show that warming in arctic tundra influence the vegetation either directly or indirectly (Hobbie & Chapin, 1998; Lindwall, Svendsen, Nielsen, Michelsen, & Rinnan, 2016; Welker, Fahnestock, Henry, O'Dea, & Chimner, 2004)

Hobbie and Chapin (1998) found that warming had no effect on NEE but influenced both ER and early season GP. Welker et al. (2004) found stronger effects on NEE and GEP more than on ER in heaths in Canadian high Arctic. Furthermore, Lindwall et al. (2016) found that warming had an increasing effect on GEP in wet tundra. The changes in winter precipitation and overall temperature might alter the litter decomposition rates, which could lead to changes in carbon (C) cycling rates in tundra ecosystems. With these changes, the growth of tundra shrubs is expected to proliferate by increasing their stature and cover. It is possible that the increased plant growth could lead to an increase in the soil microbial activity as plants and shrubs in particular change the thermal conditions of the soil during winter and summer, whilst producing an increased amount of litter and act as snow accumulating objects (Blok et al. 2016).

The rising temperature will also affect the mineralization of the large stocks of soil organic matter (SOM) stored in the active layer. The biological processes as decomposition are accelerated and more nutrients might become available and could thereby result in a higher primary production (Chapin et al. 1995; Semenchuk et al. 2015). Consequently, assimilation of CO<sub>2</sub> from the atmosphere by plants will increase, as will plant growth. Earlier studies have furthermore shown that the warming of soils during the cold season increases decomposition and overall C fluxes (Semenchuk et al. 2015).

The current experiment was carried out in an arctic alpine tundra in a snowbed area on Disko Island, West Greenland. The arctic tundra is characterized by the slow rate of many processes like decomposition and is due to low temperatures and the length of the period with snow and frozen soils. The tundra is dominated by plants capable of growing in soil with low nutrient availability and with a short growing season, e.g a wide range of dwarf shrubs, mosses and lichens. Plants growing in areas with low nutrients often allocate their resources to produce secondary compounds. This leads to low nutrient content/more recalcitrant plant litter, which again slows down the decomposition rate. Due to the amount of recalcitrant material, arctic tundra soils show high microbial immobilization, which makes it difficult for plants to obtain inorganic nutrients. Studies show that organic nitrogen compounds have an important role in plant nutrient uptake (Stark 2007).

The aim of this study is to investigate the effect of warming on the CO<sub>2</sub> flux (NEE, ER and GEP). This has been done in arctic environments before (Chapin et al. 1995; Hobbie and Chapin 1998; Welker et al. 2004) but in our experiment, the plots are located along a natural vegetation gradient created by a snowbed uphill at the study site. The vegetation gradient is reflecting vegetation zones with different length of growing season. The plants at the End of the gradient (furthest

away from the snowbed) will have a longer growing season than Top.

## *Methods and materials*

### *Study site*

The experiment took place in Blæsedalen valley (69°16'N, 53°27'W) (D'imperio et al. 2017) on the south-tip of Disko Island, in West Greenland. In late June 2014, 18 plots were established along a natural vegetation gradient in a snowbed area.

The formation of the gradient is due to snow accumulating on a slope uphill during winter time and the slow melt of snow throughout the summer creates alterations in the vegetation states along the gradient (changes in growing season length and timing). Each field is divided into a control (C) and a warmed (T) plot (Open Top Chambers (OTC's)) and provided with a 20x20 metal square base placed randomly in the plots. The gradient is divided into 5 blocks and 4 vegetation zones; Near snow, Top, Medium and End. There are 5 replicates in each zone except the "Near snow" zone, which only has 3 replicates in blocks 2, 3 and 4. These were established in early July 2015, the following year.

### *Field work*

The experiment was performed from 11-18th of July 2017. A vegetation analysis was conducted in the chamber frames and the method used was a pin point analysis in 49 grids. Normalized Difference Vegetation Index (NDVI) was measured inside the chamber frames, on the fourth day and again on the eight during the experiment, using a Skye 660/730 nm sensor.

CO<sub>2</sub> flux (NEE and ER) was measured using a polycarbonate flux chamber placed on a metal square frame inserted into the soil. An EGM-4 CO<sub>2</sub> infrared gas analyzer measured the CO<sub>2</sub> concentration every 1.6 seconds during a 10-minute period in each plot (5 minutes measurements of NEE and 5 minutes of ER) of which the middle three minutes of each period were used for calculating the flux.

The chamber was equipped with a fan for air circulation to ensure more precise measurements. Water was filled in the gas chamber and the chamber frames to ensure air tight connection of frame and chamber. NEE were measured at the ambient light level and ER were measured by covering the plexiglas chamber with an opaque cloth. GEP were calculated afterwards by subtracting ER from NEE. Same method was used by Hobbie & Chapin (1998) in a tussock tundra in Alaska. Whilst measuring the CO<sub>2</sub> flux, photosynthetically active radiation (PAR) was measured using a PAR sensor placed horizontally on the ground outside the gas chamber. Also soil moisture at 0-6 cm depth were measured using a Theta probe and the soil temperature measurements were at 2 and 5 cm depth using a soil thermometer. The CO<sub>2</sub> measurements and the parallel examinations of soil moisture and temperature conditions took place two times during our 10-day experiment.

Chlorophyll and flavonoids were measured on plants with some of the biggest leaves, *Bistorta vivipara* (*B. vivipara*) and *Salix glauca* (*S. glauca*) using a Dualex instruments, which gives a spectroscopic measurement of pigment and flavonoid concentrations to give an idea of the nutrient availability in the area, and how the plants are using resources for growth and protection.

Furthermore, we measured PI and Fv/Fm (plant stress), to determine whether the plants are stressed by the effect of warming and the availability of nutrients, water content or other abiotic factors. In each plot (C/T) 6 clips (12 clips in total) were attached to the two species *B. vivipara* and *S. glauca*, and left closed for at least 30 min to exclude all sunlight before measuring Fv/Fm and PI with a Handy-PEA. This was repeated after three days.

### Data and statistical analysis

The percentage vegetation cover was calculated by using the equation:

$$\% \text{ vegetation cover} = \frac{\text{Species abundance} * 100}{49}$$

where the species abundance is the number of times the specific plant is observed in the pin point analysis using 49 grid points.

To test whether our measurements varied between zones (elevation: Near snow, Top, Medium, End) and treatment (C/T) we used a statistical test that tests for variance. At first a Levene's test was used to test for variance homogeneity, followed by a two-way ANOVA to investigate the main factors warming and elevation, and followed by a Tukey's test where a difference found in relation to elevation ANOVA could be further specified to specific elevations (Near snow, Top, Medium and End). Alpha=0.05 was used as the significance level but we also reported tendencies when (0.05 ≤ p ≤ 0.1). To optimize the model the interaction part was removed if p > 0.15. This was done to lower the degrees of freedom used for the model, in cases where the interaction had no influence.

## Results

### Normalized difference vegetation index

NDVI differed between zones (ANOVA:  $p < 0.0001$ ), and between treatments (ANOVA:  $p = 0.0156$ ) and showed a tendency towards a treatment effect at the second measurement round (ANOVA:  $p = 0.0732$ ) (Figure 1 A2). Near snow differed from the remaining zones by being lower, and furthermore Top was significantly higher than End for the first measurements (Tukey's test:  $p < 0.05$ ).

### Vegetation analysis

The vegetation composition and distribution differed between zones along the vegetation gradient (Table 1). The most dominant species were *Salix herbacea* which were found in 35/36 plots. Moss in 27/36 and *B. vivipara* in 25/36 plots. Furthermore, *Poa alpigena* was found in 19/36 plots and *Diphysastrum alpinum* in 14/36 plots. Not all species were represented in all plots, and some had a low frequency but a high percentage cover when present. At the same time, it is worth to mention that the analysis was made in a smaller area in the plots. There was a higher percentage vegetation cover for

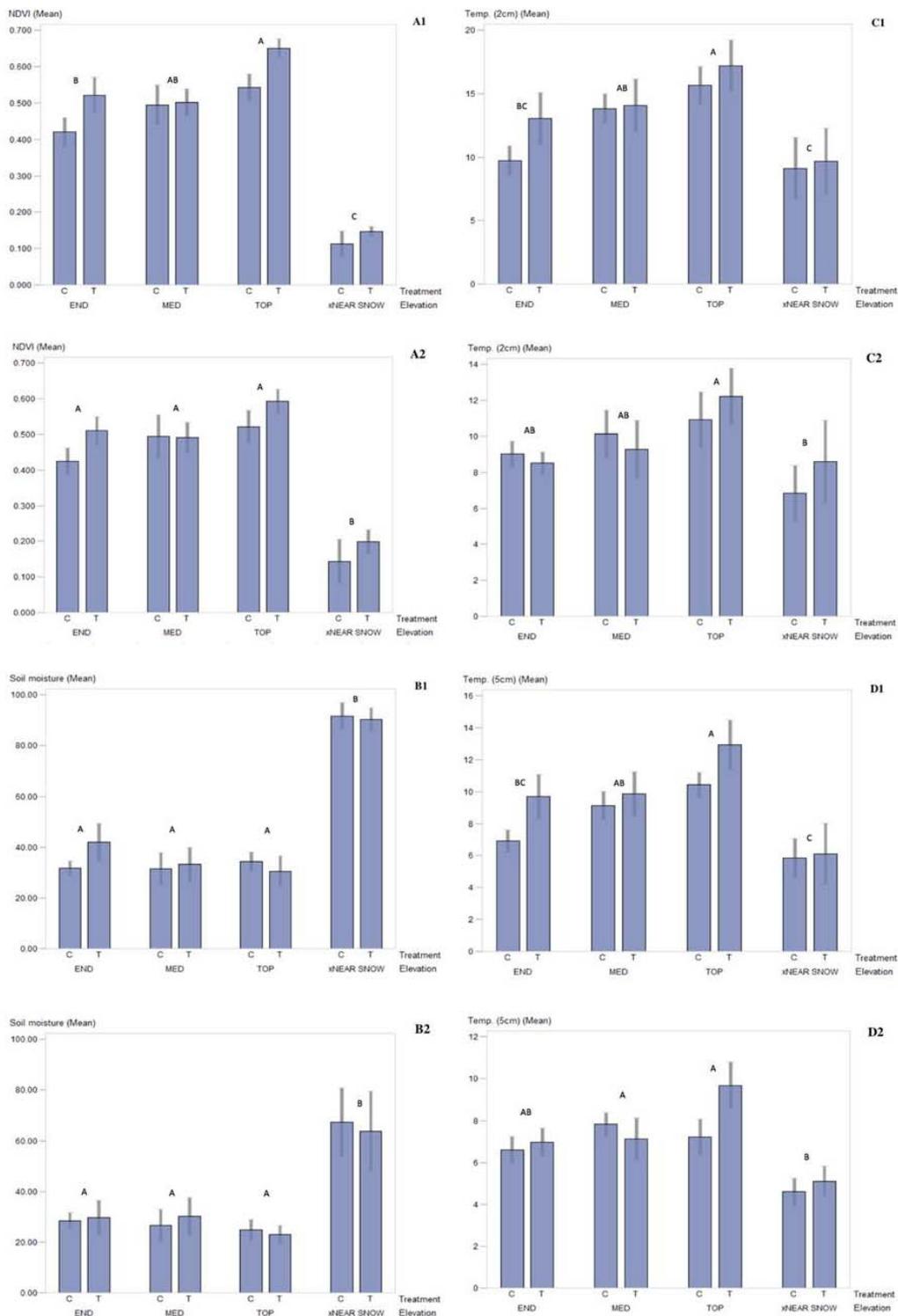


Figure 1 shows the average value for the different parameters with SE for the vegetation zones and treatments (C: control; T: warming). First and second measurement is in separate graphs expressed by the numbers 1-2. Results from the Tukey's test is shown over each bar (A-B) ( $p < 0.05$ ). Bars that do not share letter are not significantly different. Graph A shows NDVI. Graph B show soil moisture content in 6 cm depth [vol%]. Graph C show temperature in 2 cm depth ( $^{\circ}\text{C}$ ) and D show temperature in 5 cm depth ( $^{\circ}\text{C}$ ).

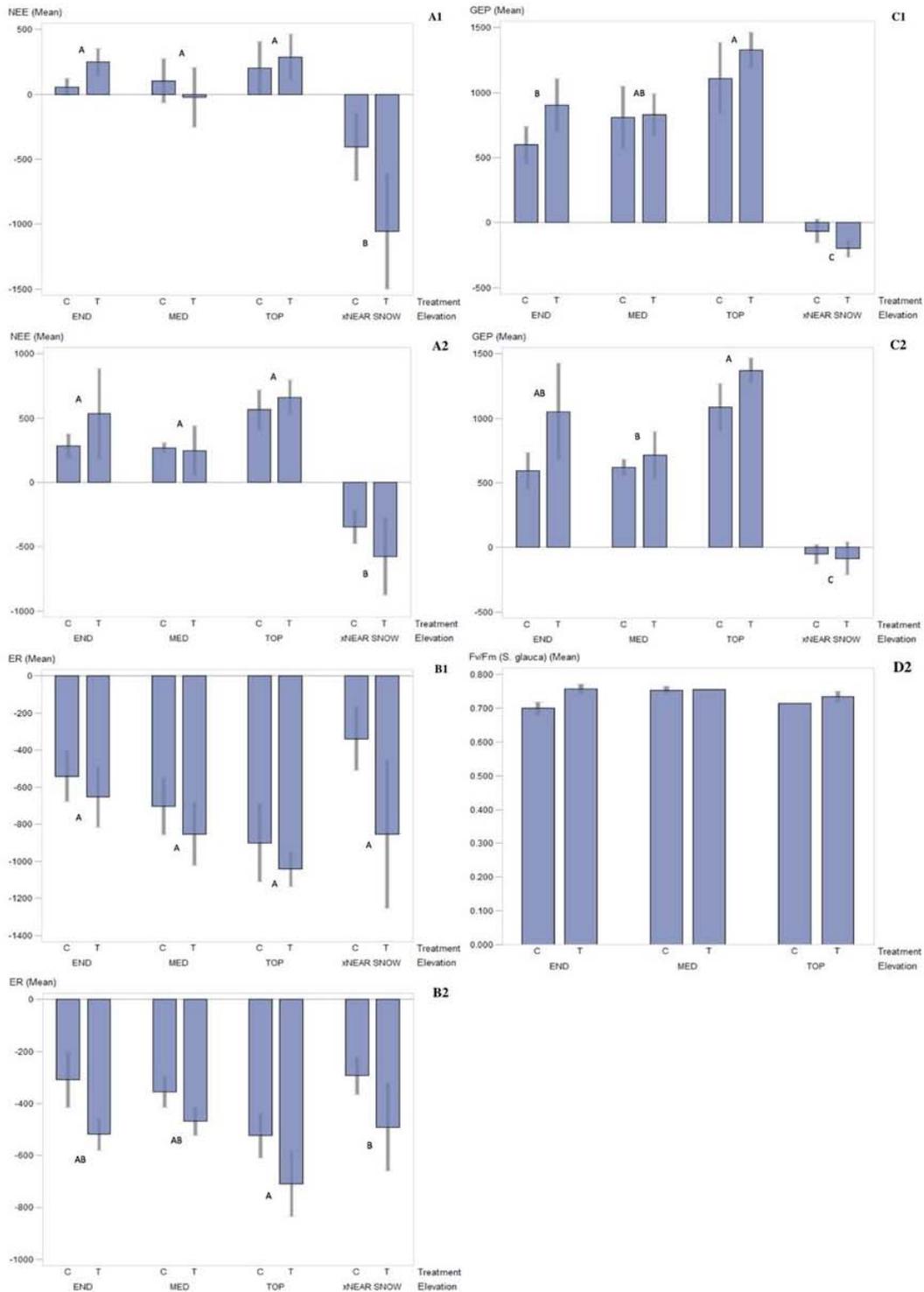


Figure 2 shows the average value for the different parameters with SE for the vegetation zones and treatments (C: control; T: warming). First and second measurement is in separate graphs expressed by the numbers 1-2. Results from the Tukey's test is shown over each bar (A-B) ( $p < 0.05$ ). Bars that do not share letter are not significantly different. Graph A shows NEE [ $\text{mg CO}_2\text{-C m}^{-2} \text{ hour}^{-1}$ ]. B shows ER [ $\text{mg CO}_2\text{-C m}^{-2} \text{ hour}^{-1}$ ], C shows GEP [ $\text{mg CO}_2\text{-C m}^{-2} \text{ hour}^{-1}$ ] and D shows Fv/Fm for *S. glauca*.

Table 1 is a table of the vegetation analysis showing the percentage vegetation cover of each species found in the 20x20 aluminum square base. It is an average of the 5 (3 in Near snow) replicates. C: control; T: warming

Plot		NEAR	NEAR	TOP	TOP	MED	MED	END	END
Treatment		C	T	C	T	C	T	C	T
Scientific name	Nickname								
<b>Evergreen shrubs</b>									
<i>Empetrum nigrum</i>	Revling	0	0	0	0	0,4	0	9,0	0
<i>Phyllodoce caerulea</i>	Blålyng	0	0	1,5	0	3,7	6,5	7,3	43,3
<i>Harrianaella hyponoides</i>	Moslyng	0	0	0	0	0	0,41	15,9	2,4
<b>Deciduous shrubs</b>									
<i>Salix glauca</i>	Blågrå pil	0	0	0	0	0	0	4,9	14,3
<i>Salix herbacea x polaris</i>	Dværg-pil	21,1	34,0	63,8	69,4	59,6	42,0	20,4	12,7
<b>Graminoids</b>									
<i>Carex bigelowii</i>	Star	0	0	2,0	2,4	4,1	1,2	2,9	6,1
<i>Poa alpina</i>	Fjeld Rapgræs	0	0	1,5	0	1,2	0,8	0	0
<i>Luzula multiflora</i>	Frytle	0	0	0	0,7	0	1,6	0	0,4
<i>Poa alpigena</i>	Rapgræs	0	0	2,0	13,6	1,6	4,5	5,3	0,4
<b>Forbs</b>									
<i>Minuartia biflora</i>	Topblomstret norel	0	0	0	0	0	0	0	1,2
<i>Stellaria longipes</i>	Stilk fladstjerne	0	0	1,5	0,3	1,2	1,6	0	0
<i>Pedicularis flameae</i>	Brand troidurt	0	0	3,1	0,3	2,4	2,9	1,6	0
<i>Pyrola grandiflora</i>	Sommer konval	0	0	0	0	1,2	0	0,4	0
<i>Taraxacum sp.</i>	Mælkebøtte	0	0	0,5	6,8	0,4	0	0,4	0,4
<i>Cerastrium arcticum</i>	Hønsetarm	0	0	0,5	0,3	0,4	0	0	0
<i>Antennaria</i>	Kattefod	0	0	0	0,3	0	0	0	0
<i>Bistorta vivipara</i>	Topspirende pileurt	0	0	10,2	5,1	8,6	14,7	14,7	9,4
<i>Veronica alpina</i>	Ærenpris	0	0	0	0	0	0	0	0
<i>Sibbaldia procumbens</i>	Trefingerurt	0	0	13,3	0	10,6	9,0	0	0
<i>Pedicularis hirsuta</i>	Lådden troidurt	0	0	0	0	0	0	0	0,4
<i>Equisetum arvense</i>	Agerpadderokke	0	0	0	1,4	0	0	0	1,6
<i>Lycopodium annotinum</i>	Femradet ulvefod	0	0	0	0	0	0	0	5,7
<i>Diphasiastrum alpinum</i>	Bjerg ulvefod	0	0	10,7	9,5	9,0	11,4	26,5	13,9
<b>Others</b>									
	Bare soil	0	0	2,0	0,7	1,6	0,4	0	0
	Moss	4,1	2,0	16,8	14,6	14,3	28,2	8,2	21,6
	Lichens	0	0	6,6	0,7	5,3	9,8	2,9	8,2
	Litter	93,9	97,3	86,2	72,8	89,0	77,1	91,8	80,4
Species diversity		3	3	16	16	18	16	15	17

evergreen plants in the End plots, than in the rest of the plots.

The most abundant plant group in the plots were graminoids. However, the group with highest percentage vegetation cover is deciduous shrubs and moss were present in all vegetation zones with a percentage vegetation cover at 2.0-28.1%. *Diphysastrum alpinum* (*D. alpinum*) were only present in 14/36 plots but had a high percentage vegetation cover at 9.0-13.9% when present. *Sibbaldia procumbens* were present in even fewer plots (6/36) but like *D. alpinum* it had a high percentage vegetation cover when present (9.0-13.3%).

### **Soil moisture and temperature**

The abiotic factors measured such as temperature and soil moisture content varied between zones and treatments, Figure 1 (B-D). Soil moisture content for both measurements varied between zones (ANOVA:  $p < 0.0001$ ) but not between treatments. Near snow was the only vegetation zone that was different from the others, with higher soil moisture content (Tukey's:  $p < 0.05$ ). Along the gradient, the temperature measurements at 2 cm depth also varied between zones (ANOVA:  $p = 0.0010$  and  $p = 0.0260$ ). Top differed from End and Near snow. Furthermore, Near snow and Medium also differed along the vegetation gradient (Tukey's  $p < 0.05$ ). The temperature was lowest in Near snow, and decreased along the gradient. Data analysis for the second measurement round showed a difference between Near snow and Top, Near snow had a lower temperature than Top (Tukey's  $p < 0.05$ ). A different pattern was seen at the temperature in 5 cm depth, with difference between zones (ANOVA:  $p = 0.0002$  and  $p = 0.0013$ ) including a difference between treatments for the first measurement round. The treated plots had a higher temperature than controls (ANOVA:  $p = 0.0292$ ). The Tukey's test showed the same pattern as the first measurement round in 2 cm depth (Tukey's:  $p < 0.05$ ) in the four zones. In the second measurement round there was no difference

between treatments, but Near snow differed from Top and Medium, by having a lower temperature than Near snow (Tukey's:  $p < 0.05$ ).

### **Chlorophyll/flavonoids, NBI and plant stress**

Chlorophyll, flavonoids, NBI and plant stress did not differ between zones or treatment (ANOVA:  $p > 0.05$ ). However, plant stress (Fv/Fm) measurements on *S. glauca* differed between treatments for the first measurement. Control plots were lower than the plots with open top chambers (ANOVA:  $p = 0.0456$ ).

### **CO<sub>2</sub>**

CO<sub>2</sub> fluxes all differed between zones. Net Ecosystem Exchange (NEE) differed between Near snow and the remaining zones along the vegetation gradient. Near snow were the only one with a negative value (ANOVA:  $p < 0.0001$  and Tukey's:  $p < 0.05$ ). A tendency was seen for ecosystem respiration (ER) between zones (ANOVA:  $p = 0.0634$ ) in the first measurement round and a significant effect in the second (ANOVA:  $p = 0.0181$ ). Top had higher respiration, than Near snow (Tukey's:  $p < 0.05$ ). Furthermore, a difference between control and warming (ANOVA:  $p = 0.0448$  and  $p = 0.0033$ ), with higher respiration in the warmed plots.

Gross Ecosystem Production (GEP) showed a difference amongst the various zones (ANOVA:  $p < 0.0001$ ). Near snow differed from the remaining zones, by having a negative value, Top was higher than End and the same pattern was seen in measurement two (Tukey's:  $p < 0.05$ ). In the second measurement round warming had an increasing effect on GEP (ANOVA:  $p = 0.0710$ ).

## **Discussion**

### **Vegetation analysis**

The vegetation analysis was made in a 20x20 frame (used for the CO<sub>2</sub> chamber) and the vegetation in the frame was clearly different from the larger part of the

experimental plots. Another group made a vegetation analysis in the plots (not in the chamber frames) and found on average 7.75 more species. This may be due to a larger area investigated, but may also indicate that the chamber frame itself has an influence on the vegetation, and thereby on CO<sub>2</sub> fluxes measured. No effect of treatment was found on the amount of species in the various plots (Table 1). The Near snow zone had, because of the gradually retreating snowpack, a shorter growing season and had therefore by the time of our experiment no plants with leaves emerged. But during our stay we saw a change in these plots with more plants/buds emerging, because of more snow melting away/retreating.

It was not possible to see a pattern in the vegetation analysis when looking at growth form or percentage vegetation cover along the gradient, except that evergreens showed a higher percentage vegetation cover in the End plots, compared to remaining plots (Table 1).

#### ***Plant stress***

No significance was found for plant stress in almost all plots. Fv/Fm for both *S. glauca* and *B. vivipara* were between 0.7-0.8 (see appendix). This indicates that the plants were not stressed when exposed to warming.

Fv/Fm for *S. glauca* were significant for treatment, with a lower value (0.724) in the control field than in the warmed plots (0.749) (Figure 2D). This means that plants in the control plots are more stressed than plants in the warmed plots. The opposite was expected because warming can dry out the soil and damage the photosynthetic system by heating the leaves. It is worth to mention that the average difference is 0.025 and none of the plants seemed affected or stressed.

#### ***Chlorophyll/Flavonoids/Nitrogen balance index***

No significant difference was found for chlorophyll and flavonoids measurements for neither *S. glauca* nor *B. vivipara*. The values for chlorophyll for *B. vivipara* were in the

range of 32-56 µg/cm<sup>2</sup> and *S. glauca* 26-39 µg/cm<sup>2</sup> (see appendix). Semenchuk et al. (2015) measured chlorophyll on *Salix polaris* which ranged from 10-50 µg/cm<sup>2</sup> (Semenchuk et al. 2015).

It could have been expected to see a difference in chlorophyll/flavonoids in the End plots compared to the other plots, because of the higher percentage vegetation cover of evergreen plants (Table 1). Evergreen plants often invest their nutrients differently than deciduous shrubs do. Deciduous shrubs need to invest a lot of nutrients in leaf production, where evergreen plants normally produce more secondary compounds for protection against threats like weather and herbivores.

#### ***NDVI***

We found that NDVI was significant in both elevation and treatment. A higher NDVI was measured in the warmed plots compared to the control. Warming stimulates plant growth and decomposition which both leads to more plant biomass/more plant cover. Figure 1 A1 shows lowest NDVI in Near snow (no real vegetation yet), and highest in Top (with small new plants). Medium and End had lower NDVI probably due to the more developed vegetation, which contains more woody shrubs and therefore are less green. The second measurements showed a significance in elevation and a tendency in treatment. Only Near snow was significant (Figure 1 A2). That means that the rest of the plots are now equally green, which make sense because the second measurement round were done later in the growing season. From this we can conclude that warming speeds up leafing in plants in the beginning of the growing season.

#### ***Soil moisture and temperature***

The soil moisture differed between the different zones. Near snow plots had a higher soil moisture content due to the melting of the snowpack above. The same pattern was found in the second measurement (Figure 1B).

The temperature in 2 cm depth was significant in elevation ( $p=0.0010$ ) and the same was the case at the second measurement of temperature at 2 cm depth ( $p=0.0260$ ). Looking at Figure 1 C1, Near snow was clearly different from the Top, which had a much higher mean temperature. This can possibly be explained by the longer presence of snow in the plots closest to the snowbed. At the End of the gradient the temperature was almost as low as the Near snow plots. Also, there was no significance between the temperature at Medium and Top (Figure 1 C1). This might be correlated with the soil moisture content (Figure 1B), given that the soil moisture is higher at the End of the gradient because of the water running down the slope and accumulating there, when compared to Top and Medium. The soil moisture might have a more cooling effect on the temperature in the “wet” plots and it is a much longer process for the sun to heat up wet soil than dry soil. Furthermore, the lower temperature at the End of the gradient might be explained by a higher canopy cover that shadows the ground and thereby results in lower soil temperatures. The higher temperatures at the Top gradient might be because of it being more exposed to the sun due to the higher elevation on the slope, when compared to End that was placed in sort of a “dent”. When looking at the second measurement round, Near snow is not significant from either Medium or End, but still significant from Top, which in general has a higher mean temperature, especially in some of the warmed plots (Figure 1 C2). Considering that more snow melts away over time, a gradual increase in temperature could be expected in all the plots along the gradient at the second measurement round, as an effect of more snow melting away during our ten-day measurement period. Instead we saw a decrease in temperature in the second measurements (Figure 1 C2), and taking the weather changes at the day of the second measurement round into account, it could be a result of the weather, since the temperature in the Top layers of the soil are more affected by the air conditions and a higher canopy. Since the soil moisture results also show a

relatively high decrease (Figure 1 B) when compared to the second measurement round, the soil moisture content might have a more cooling effect than first anticipated.

The temperature measurements at 5 cm depth showed a significance in both elevation ( $p=0.0002$ ) and treatment ( $p=0.0292$ ) (Figure 1 D1), whereas the second measurement only showed a significance in elevation ( $p=0.0013$ ) (Figure 1 D2).

The pattern observed at 5 cm depth are much the same as at 2 cm depth, except for the mean temperatures which in general are lower at 5 cm depth, when compared to those at 2 cm. When looking at our measurements in 5 cm depth, we also found significance in the experimentally warmed plots. This might be because of the measurements were less affected by the surrounding air conditions, as the measurements in 2 cm depth that showed no significance in the warmed plots.

### *CO<sub>2</sub> flux*

The net ecosystem exchange (NEE) differed between vegetation types (elevation) and Near snow were the only plots with a CO<sub>2</sub> loss from the ecosystem (Figure 2A). This can be explained by the lack of plants in the Near snow plots. At the first day of measuring, one of the plot sticks were still covered in snow and no vegetation were found in any of those plots. The treatment had no significant effect on NEE. The same results were found at the second measurement round (Figure 2 A2). At that time plants had started to grow in the Near snow plots but not in the CO<sub>2</sub> chamber frames. A study by Welker et al. (2004) showed that their measured values of NEE indicated that the three ecosystems (wet, mesic- and dry tundra) worked as a net CO<sub>2</sub> sources in the early summer, but later after a two weeks’ period all worked as a CO<sub>2</sub> sinks. Our NEE values indicate that the study site generally serves as a CO<sub>2</sub> sink in July, and that it in the beginning of the growing season might have been a source, given that the Near snow plots (almost still covered in snow by the time of the first measurements), during the time of our experiment was a CO<sub>2</sub> source (Welker et al. 2004).

The ecosystem respiration (ER) differed between treatments and tended to do so for elevation. Higher respiration was observed in the warmed plots (Figure 2 B1).

The second measurements showed significance in both treatment and elevation. The respiration was significantly higher in the warmed plots due to the effect of warming on biological processes (Figure 2 B2). Warming enhances biological processes, such as decomposition. Furthermore, the warmed plots potentially had more plant biomass that could add to respiration. Hobbie and Chapin (1998) found a higher root N concentration in warmed plots, which will require a higher maintenance respiration. Although we did not measure the roots it could still be part of an explanation to the higher respiration in warmed plots (Hobbie and Chapin 1998).

ER were significantly lower in Near snow compared to the Top, probably due to the lower temperature inhibiting the microbial activity. Top plots had the highest respiration explained by the shorter period of unfrozen soil where decomposition has been possible. This means that more of the easy degradable material is available for the microbes, which can lead to a higher respiration rate.

Gross ecosystem production (GEP) was significantly different between vegetation types (elevation), and Near snow had 0 photosynthesis due to the lack of plants. Highest photosynthesis was found in the Top plots and from there GEP gradually decreased towards the End (Figure 2 C1). A reason for the lower photosynthesis in the End plots could be the longer expected life span of the leaves of the plants, which in many cases are evergreens. They had a longer growing season due to earlier snowmelt and are therefore probably not in need of spending as much energy in production of photosynthetic active leaves.

For the second round of measurements, the same was found, and furthermore there was a tendency towards an effect of warming (Figure 2 C2), which could have an influence on plant productivity and therefore might

affect photosynthesis positively. Lindwall et al. 2016 found a significance in GEP for warming in the season average, but not for the July measurements (Lindwall et al. 2016). Furthermore, Welker et al. 2004 established that in a dry tundra, warming resulted in a higher GEP in the early and mid-summer, leading to a higher NEE in a warmed dry tundra, compared to a tundra under ambient conditions (Welker et al. 2004).

## *Conclusion*

In relation to the aim of the experiment no evidence was found during our 10 days' measurements in July, that warming should influence NEE or GEP. The opposite can be concluded for ecosystem respiration (ER), which showed to be affected by the elevated temperatures. In general, warming increased the ecosystem respiration. From the experimental setup, it is not yet possible to say whether the changes in respiration is due to a direct effect of warming on plants or an effect on the life in the soil matrix. Further investigations could be done to investigate the change in soil nutrients or the influence of warming on the microbial community. The greenness of the plots was found significant corresponding to the length of the growing season at the specific zone along the slope (no plants in Near snow, new plants in Top and older plants in Medium and End).

The measurements done on the vegetation did not show any effect from elevated temperatures as plants were neither stressed nor different in nutrient utilization.

Furthermore, it would make sense to further investigate the influence of the plant community composition and if the appearance of certain species influences the CO<sub>2</sub> flux. It could also be interesting to extend the experiment to get data from a whole season or even a couple of seasons. This could e.g. give an overview of the ecosystem dynamics affecting whether the ecosystem changes to be a CO<sub>2</sub> sink or source.

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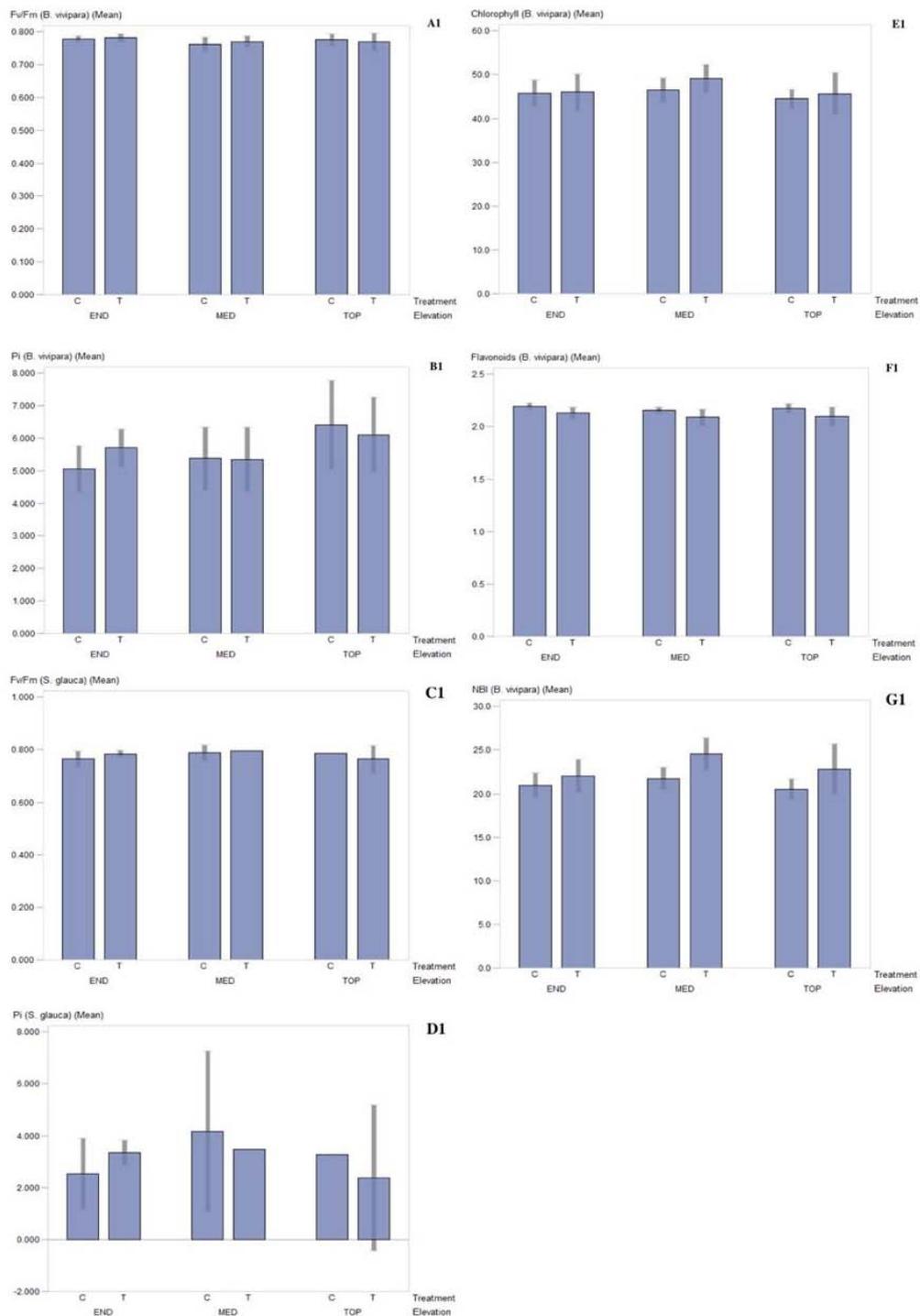
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## Appendix

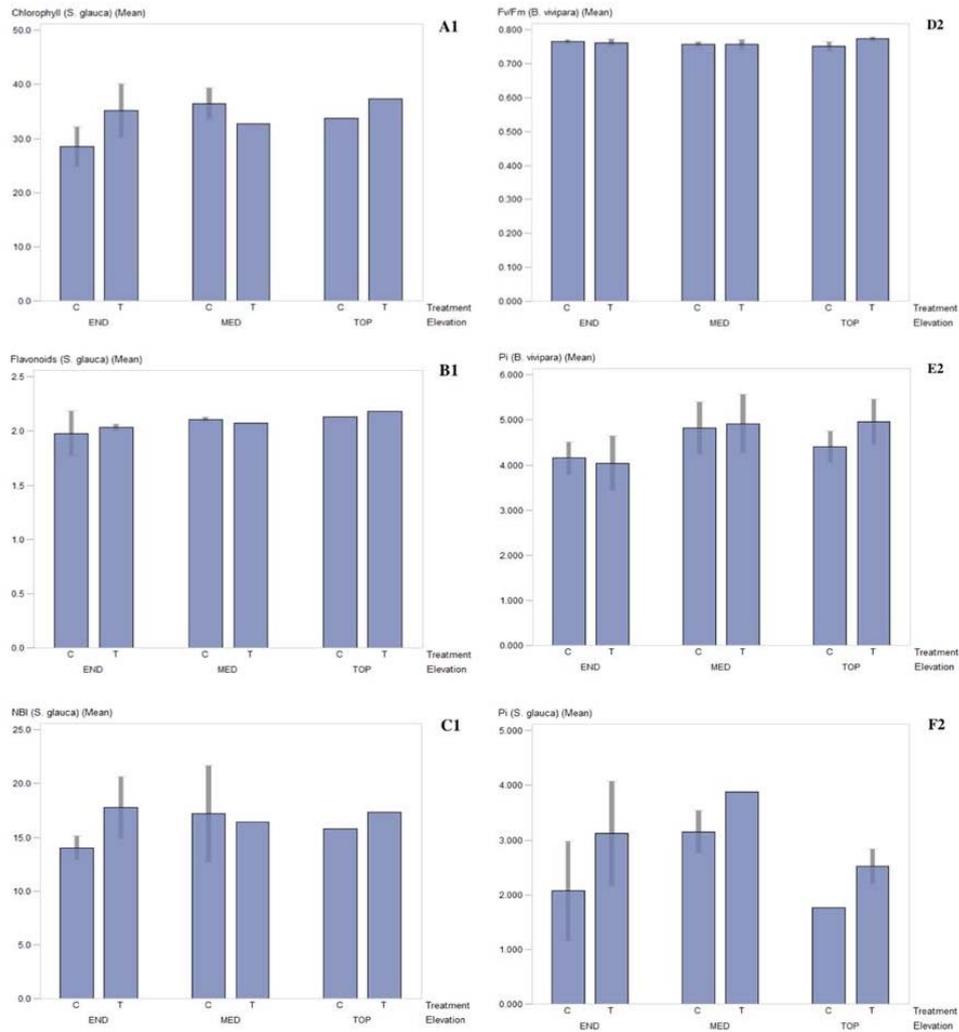
### Appendix 1

Graphs showing non-significant measurements. 1 and 2 referring to the number of measure. (C: control; T: warming). A) Fv/Fm *B. vivipara* B) PI *B. vivipara* C) Fv/Fm *S. glauca* D) PI *S. glauca* E) Chlorophyll *B. vivipara* F) Flavonoids *B. vivipara* G) NBI *B. vivipara*



## Appendix 2

Graphs showing non-significant measurements. 1 and 2 referring to the number of measure. (C: control; T: warming). A) Chlorophyll *S. glauca* B) Flavonoids *S. glauca* C) NBI *S. glauca* D) Fv/Fm *B. vivipara* E) PI *B. vivipara* F) PI *S. glauca*.



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