Arctic Biology Field Course

Qeqertarsuaq 2023







UNIVERSITY OF COPENHAGEN FACULTY OF SCIENCE

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Title:	Arctic Biology Field Course, Qeqertarsuaq 2023				
Published by:	Arctic Station University of Copenhagen 3953 Qeqertarsuaq Greenland	Freshwaterbiological Section University of Copenhagen Universitetsparken 4, 3 rd floor 2100 KBH Ø Denmark			
Publishing year:	2025				
Edited by:	Kirsten S. Christoffersen, Freshwaterbiological Section				
ISBN:	978-87-89143-30-9				
ISSN:	2597-0984				
Front page photo:	Kirsten S. Christoffersen				
Citation:	The report can be cited in full:				
	Arctic Biology Field Course, Qeqertarsuaq 2023. Christoffersen, K.S. (Ed.), 2025. Arctic Station, University of Copenhagen, p. 1-7				
	or in part:				
	<i>Authors(s)</i> , 2025. <i>Title of paper</i> , In: Arctic Biology Field Course, Qeqertarsuaq 2023. Christoffersen, K.S. (Ed.), 2025. Arctic Station, University of Copenhagen, pp. x-x.				

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Preface

The Arctic Biology field course held at Arctic Station in July 2023 was indeed successful and very intense. The 14 highly motivated students had spent hours developing their group-based research project before going to Greenland and we had held a series of meetings to prepare everything. Detailed plans were made for sampling, lab analysis, data handling and writing reports, which of course filled all the days – but still allowed us to have excursions to scenic landscapes, supplementary lectures, meeting with local people and creation three healthy meals every day.

Lots of challenges appeared during the intense 10 days at Arctic Station, such as rather cold and wet weather conditions, but nothing so bad that we couldn't beat in one way or another. Thus, all projects were successful and produced nice results. These are presented in this report. A short summary of each project is provided below.

Resampling of Lakes in Disko, Greenland – This study revisited four lakes at Disko Island, comparing their physical and chemical properties to data from 1962 and 2004. Despite expectations to increased conductivity due to climate change, no significant changes were observed, likely due to an unusually cold summer and delayed snowmelt. However, heavy precipitation led to reduced transparency in three lakes, reinforcing predictions about increased runoff effects. Overall, the lakes' conditions remained similar to previous decades, influenced by annual climate variability.

Spatial Dimorphism of the Arctic Three-Spined Stickleback – This study examined morphological differences in Three-Spined Stickleback populations from four locations on Disko Island. Significant variations were found in lateral plate numbers, body size, gill raker length, and dorsal spine length between marine and freshwater populations. Freshwater sticklebacks displayed fewer lateral plates, while marine individuals exhibited larger body sizes and gill rakers. Differences were partly attributed to local salinity and calcium levels, consistent with patterns seen in other regions.

Benthic Algae Composition in Arctic Streams – Benthic algae communities in six Arctic streams were analyzed to assess differences in composition and biomass. Despite environmental variability, biofilm biomass remained consistent, likely due to extreme weather conditions prior to sampling. However, a strong correlation between diatom abundance and temperature suggests climate change impacts on Arctic stream ecosystems, highlighting the importance of seasonal monitoring.

Zooplankton Communities in Arctic Lakes – The zooplankton species composition in three lakes was reassessed against data from 1962 and 2004. Fewer species were found in 2023, and chlorophyll concentrations, used as a proxy for phytoplankton abundance, were lower than previous years. The delayed arrival of spring likely influenced the zooplankton and phytoplankton communities, suggesting ongoing environmental shifts due to climate change.

Kirsten S. Christoffersen & Sanne M. Moedt

The teachers

Participants



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Resampling of Lillesø, Mellemsø, Morænesø, and Kangarssuk Lake in Disko, Greenland decades after the first studies - status on abiotic factors

Victoria Westmark Sønnichsen & William Himmelstrup

Abstract

The present study has investigated the physical and chemical properties of Lillesø, Mellemsø, Morænesø, and Kangarssuk lake in Southwest Disko Island, Greenland during July of 2023. The results of the study were compared to data published in 1962 and 2004, hypothesizing that climate changes may have altered the abiotic conditions over the years. Each lake was visited once, except Morænesø, which was visited once during partial ice-cover and revisited when the ice-cover had melted. Sampling took place from a boat at the deepest accessible ice-free spot of the lakes. Temperature, specific conductivity, oxygen concentration and saturation, pH, Secchi depth, and particulate organic matter (POM) contents were determined for each lake. In addition, a conductivity, temperature, and depth (CTD) profile was generated. No stratification was observed in any of the lakes. While general patterns of a changing climate imply increases of the mean temperature and precipitation in the Arctic region, this study found that values of conductivity were comparable to previous years, thus failing to observe the predicted increase in conductivity following the increased mean temperatures of the area. However, the summer of 2023 was unusually cold, and snowmelt was late so this was taken into consideration. Furthermore, following heavy precipitation prior to the sampling of the lakes, the Secchi depth of three out of four lakes was lower compared to previous years, partially corroborating the prediction that more precipitation would lead to more runoff and thus, a lower transparency in general. In conclusion, it appears that the physical and chemical conditions of each lake resemble those found in previous decades, and that interannual weather variation contributes to shaping the environmental conditions in the Disko region.

Keywords: Freshwater ecosystems, Disko Island, abiotic factors, water chemistry, CTD-profile, POM.

Introduction

In the face of climate change, the Arctic region is experiencing accelerated effects in comparison to the global average (Rantanen et al., 2022). Meteorological data from Aasiaat, located 65 km south of the Arctic Station on Disko Island in Western Greenland (Fig. 1), show that the 30year annual average temperature from 1961-1990 was -4.9 °C, while the corresponding value in 1991-2020 was -3.7 °C (Fig. 2) (DMI, 2020). Thus, resulting in a 1.2 °C increase in the annual average temperature between the two periods (DMI, 2020). Furthermore, the mean annual precipitation in 1961-1990 was 304 mm, while the corresponding average for 1991-2020 was 330 mm, showing a 27 mm increase. Thus, meteorological data support that there is an increase in both the mean temperature and the amount of precipitation in areas close by the Arctic Station as has been found elsewhere (Rantanen et al. 2022). These factors are likely to affect the ecosystems of the region.



Figure 1 Map of study area on Disko Island, West Greenland. Red dots and numbers indicate approximate location of sampling sites (1-5), and a blue square indicates the approximate location of Aasiaat (5), the source of the weather data. 1: Lillesø, 2: Mellemsø, 3: Morænesø, 4: Kangarssuk lake. 5: Aasiaat. The map is modified from Yde & Knudsen (2007).

Disko Island is home to a number of freshwater lakes. In Arctic lakes, the maximum ice thickness reaches approximately 2 meters (Røen, 1962), and therefore shallow lakes may freeze solid, while deeper lakes remain ice free in the deeper water layer year round. Lillesø, Mellemsø, Morænesø, and Kangarssuk lake are freshwater lakes (>2 m) in the south western region of Disko Island (Fig. 1), which are covered by ice most of the year, typically about 8-10 months (Christoffersen et al., 2008). The chlorophyll levels increase in late spring as the primary producers start to grow when the solar irradiance can penetrate through the gradually thinner ice layer, thus making a head start of the short ice free season for production in Arctic lakes (Rigler, 1978). Furthermore, while nutrient levels (eg. total phosphorus) are typically low (Jeppesen et

al., 2003) which limits the phytoplankton growth, temperature has also been demonstrated to control the rate of production (Rigler, 1978).

Lillesø and Mellemsø were first studied in 1902 by Porsild who made general observations on the properties of the lakes (i.e. depth profiles) and the vegetation of the catchment area (Porsild, 1902). Since then, a study during the Arctic Field Course of 2004 (Christoffersen et al., 2004) investigated the water chemistry and physical properties of Lillesø and Mellemsø, also including data from Morænesø. Similar studies of the three lakes were conducted by Røen (1962), who also included data from Kangarssuk lake and made descriptions of the lakes' size and reassessed the max depths of the lakes reported by Porsild.



Aasiaat - 30 Years Weather data 1961-1990 16 60 Annual mean temperature (°C): -4.9 Annual mean precipitation (mm): 304 45 0 30 1 O -16 -32 15 -48 0 Jan Feb Mar Maj Jun Jul Okt Nov Dec Apr Aug Sep Mean temperature Sum of precipitation • Mean of minimum temperature • Mean of maximum temperature

Figure 2 Graph of the minimum (blue), mean (turquoise), and maximum (red) monthly temperatures (°C) and mean monthly precipitation (mm) in Aasiaat, Greenland over two periods of 30 years. The lower graph contains data from 1961 to 1990, and the upper graph contains data from 1991 to 2020. Annual means have been added and the graph legends have been translated to English from the original graphs acquired from DMI (2020).

The nutrient turnover in lakes is based on the contents of organic matter in the ecosystem which can be divided into two types of input. The autochthonous input refers to organic matter generated by within-lake processes, while the allochthonous input is carried to the lake from the terrestrial environment and inflow from streams (Vincent & Laybourn-Parry, 2008). The latter type of input tends to be higher in the Arctic region compared to in Antarctica, due to

more terrestrial vegetation in the catchment area in the Arctic (Vincent & Laybourn-Parry, 2008). Furthermore, the relative importance of the two types is linked to the size and shape of the lake and the nature of the catchment area (Hideyuki, 2009).

The aim of this study was to describe the present physical and chemical conditions of Lillesø, Mellemsø, Morænesø, and Kangarssuk lake and compare the findings to the previous studies from 1962 and 2004 to evaluate if any changes have occurred and to identify potential causes. POM has not previously been examined in these lakes. Thus, POM will be estimated and evaluated in relation to the properties of the lakes, and the collected data on POM will furthermore provide a baseline, which can be used for future reference.

We hypothesize that the physical and chemical factors of Lillesø, Mellemsø, Morænesø, and Kangarssuk lake have changed since the studies published 61 and 19 years ago due to the ripple effects of the warming of the Arctic region. Based on the generally known implications of warming the following predictions were formulated for this study:

1) The conductivity and POM values of the lakes will be higher than in the previous studies as a result of increased productivity following a higher mean temperature of the area. Furthermore, lakes with higher contents of POM are expected to have lower Secchi depths.

2) The transparency of the lakes will be lower than in the previous studies due to increased runoff from the catchment areas following increased precipitation.

Methods and materials

Sample locations

All four sampled lakes are found on Disko Island in Western Greenland (Fig. 1). The characteristics of each lake were described by Porsild (1902) and since then also by Røen (1962). The following presentations of the lake characteristics are based on Røen's data. The appendices of depth profiles from Lillesø and Mellemsø (Appendix 7 & 8) are from Porsild's (1902) original sketches, which deviate slightly from Røen's (1962) depth estimates. Lillesø and Mellemsø are located in Disko Fjord about 2 km apart. Lillesø is located at an altitude of 24 m, the lake is approximately circular with a diameter of approximately 300 m, and the max depth is 12.5 m (Appendix 7). Mellemsø is located 35 m above sea level and the lake is 1 km long and 300 m wide. The approximate max depth is 27 m (Appendix 8). Morænesø is located north of the moraine in

Blæsedalen outside of Qeqertarsuaq. The lake is 500 m long and 150 m wide with a max depth of approximately 5 m (Appendix 9), but the majority of the lake has a depth of 3 m. The final lake of the study is located at Kangarssuk, Fortunebay, only 200 m from the sea at an altitude of 10 m. The lake is 800 m long and 300 m wide with a max depth of approximately 7 m (Appendix 10), but most of the lake is 4 to 4.5 m deep.

Sampling of the lakes

Lillesø, Mellemsø, and Kangarssuk lake were visited once, while Morænesø was visited twice in order for the ice to melt completely before taking samples at mid lake position. Samples were taken in the period from the 12th of July to the 19th of July in 2023. In order to evaluate the physical and chemical properties of the lakes, a number of parameters were sampled. By a small rubber boat, we sailed to the deepest ice-free position which was determined using a Hondex PS-7 Portable Depth Sounder (Honda Electronics, Japan). Here we deployed a CastAway-CTD (SonTek, USA) which sampled the depth (m), water temperature (± 0.1°C), and conductivity (± 1 % µS/cm), down to the bottom. While out on the boat, oxygen concentrations $(\pm 0.2 \text{ mg/l})$, temperature, and specific conductivity (hereafter referred to as conductivity) were also measured approximately 0.5 m below the surface and 0.5 above the bottom with a Pro Plus Multiparameter Instrument (YSI Inc, USA). The Secchi depth (m) of the lakes was then determined by deploying a Secchi disc and tying knots on the line at the specific depth, and the length of the line was then measured on land to estimate the transparency. The disc was only deployed once at each location. Three separate samples of water were collected in a 10 L bucket from the boat for POM analysis. The pH value was also measured from the bucket using a Checker® HI98103 pH Tester (Hanna Instruments, USA). Pictures of the lakes and catchment area were taken with a Canon EOS 6D Mark II for photo documentation of the ice cover and catchment area of the lakes (Appendix 1-6).

POM analysis

Prior to fieldwork, filters for POM were dried at 105 °C for 60 minutes, weighed on a METTLER AT261 scale (DeltaRange ®, Struers, Denmark), and packed in aluminum foil packets. In the field, 1 L of lake water from each of the three buckets was filtered through Whatman GF/C filters with a particle retention of 1.2 μ m and a diameter of 47 mm. The water was filtered using a handheld vacuum filtration unit. The filters were folded in half and wrapped in aluminum foil. Filters were stored at -18 °C and later dried at 60 °C overnight after which they were weighed on the same scale that was used initially. The weight of the dried filters was subtracted from the weight of the filters with POM in order to estimate the POM contents of each lake.

Acquiring and analyzing of weather data

Weather data were acquired from the Danish Meteorological Institute's online weather archive of climate normals (DMI, 2020). Here, data from





Figure 3. Graph of the minimum (blue), mean (turquoise), and maximum (red) monthly temperatures (°C), and bar plot of the mean monthly precipitation (mm) in Aasiaat, Greenland in the first seven months of 2023. The graph legends are translated to English from the original graphs acquired from DMI (2023).

1961-1990 and 1991-2020 in Aasiaat, Greenland were found and tables showing the mean annual values of temperature and precipitation were also available. The annual means from the tables were added onto the graphs in Figure 2. The exact values from each month were acquired from the interactive graphs, which can be accessed via the links in the reference list. The weather from Aasiaat was used as an approximation of the weather in South Disko, where the lakes are located, as this location had continuous data from 1961, and data from the study year, 2023, was also available. The values that were presented in the introduction, showing how much the temperature and precipitation had increased, were found by subtracting the most recent 30-year period's average from the earliest period's average. Recent weather data from the first seven months of 2023 in Aasiaat was also acquired (Fig. 3) from the online weather archives (DMI, 2023). These data were used to support the immediate observation that it had been a particularly cold and wet summer this year.

Statistical analyses

Two sample t-tests ($\alpha = 0.05$, two-tailed) were performed for pairwise comparison of the POM content in the lakes, and a bar plot visualizing mean POM of each lake was generated. A linear regression analysis was performed on Secchi depth as a product of POM and the graph is included in the report. Further statistical analyses were not performed on temperature, pH, Secchi depth, or conductivity. Instead, these parameters were presented in a table (Table 1) to ease the comparison with previous years. All statistical analyses, data visualizations, and tables of lake parameters in the report were made in R using RStudio software.

Results

In spite of it being mid-July at the time of sampling, Lillesø and Mellemsø were partly covered by ice. Hence, it was not possible to reach the planned mid lake position for the measurements. Furthermore, while Langesø was originally thought to be part of the study, it was excluded from the project, as it was almost 100 % ice covered. Instead, we included the ice free Morænesø and Kangarssuk lakes, and all parameter measurements for the four lakes were taken at the deepest (ice free) position accessible by boat. Here, triplicates of POM filtrates were also collected by boat in Lillesø, Mellemsø and Kangarssuk lake, but in Morænesø they were collected by walking out in waders. However, one pseudo replicate was collected in Lillesø due to time limitations. In the following paragraphs, the results of this study and weather data for 2023 will be presented leading up to the comparison with previous years in the discussion.

Water temperature, conductivity, oxygen, and pH levels

The average water temperature of the four lakes ranged between 3.4°C and 8.2°C (Table 1) with Mellemsø being the coldest and deepest (Appendix 8) and Morænesø the warmest and most shallow lake (Appendix 9). The coldest lake, Mellemsø, had an influx of meltwater from snow (Appendix 4). No thermal stratification was observed in any of the lakes (Fig. 4). Nonetheless, there was a slight temperature variation in Lillesø from the surface of 4.8°C to the bottom of 3.8°C (Table 1), in spite of the lake being partially covered by ice (Appendix 1).

The conductivity varied from 25.2 μ S/cm (Mellemsø) to 59.4 µS/cm (Morænesø) (Table 1) between the lakes. Mellemsø displayed a significant increase in conductivity from the epilimnion to the hypolimnion (25.2 μ S/cm to 49.0 μ S/cm) (Table 1) shifting at approximately 5.2 m (Fig. 4). The CTD profiles show the general temperature and conductivity patterns down the part of the water column that was sampled (Fig. 4). Therefore, the profiles of Lillesø and Mellemsø only represent part of the actual lake profiles, as the deepest spot was inaccessible due to the ice. The general pattern for oxygen content was higher values at the surface than at the bottom, and the values ranged between 10.1 mg/L (Mellemsø) and 15.7 mg/L (Lillesø) (Table 1).

Table 0.1. Secchi depth, pH, specific conductivity, water temperature, and oxygen concentration and saturation at the surface and bottom of the deepest accessible spots (sample depth) of Lillesø, Mellemsø, Morænesø, and Kangarssuk lake. Coordinates and sampling dates are reported. Units are given under the test-parameter. 'Conductivity' refers to the specific conductivity. 'Depth' refers to the sampling depth, not maximum depth.

Lake	Coordinates	Date	Secchi Depth (m)	pН	Sample Depth (m)	Conductivity (µS/cm)	Water Temperature (°C)	O2 (mg/L)	O2 (%)
Lillesø surface	69° 31' 10" N, 53° 43' 08" W	12.07.2023	1.2	8.7	0.2	38.8	4.8	15.7	118.0
Lillesø bottom		12.07.2023			5.5	40.4	3.8	12.1	93.0
Mellemsø surface	69° 31' 14" N, 53° 41' 52" W	12.07.2023	2.7	6.7	0.2	25.2	3.4	15.2	115.0
Mellemsø bottom		12.07.2023			12	49.0	3.3	10.1	85.4
Morænesø surface	69° 16' 09" N, 53° 28' 25" W	19.07.2023	1.94	6.3	0.2	59.3	8.3	12.8	103
Morænesø bottom		19.07.2023			3.5	59.4	8.0	11.8	100
Kangarssuk lake surface	69° 16' 07" N, 53° 49' 37" W	18.07.2023	1.8	5.9	0.2	42.7	6.2	14.1	114.0
Kangarssuk lake bottom		18.07.2023			6.9	43.0	6.0	13.8	110.8



Figure 4. CTD profiles from the deepest accessible spots of Lillesø, Mellemsø, Morænesø and Kangarssuk lake. Depth is reported in meters, temperature is reported in °C, and specific conductivity is reported in μ S/cm. The limits of the x-axis are comparable between the lakes. The y-axis representing sample depth varies between lakes.

Table 0.2. Mean POM (g/L) based on triplicate measurements from surface water of Lillesø, Mellemsø, Morænesø, and Kangarssuk lake. Standard deviation is reported. 'n' = sample size. The parenthesis indicates that one of the samples was a pseudo-replicate.

Lake	РОМ	Standard Deviation	n
Lillesø	0.0038	0.0007	(3)
Mellemsø	0.0012	0.0004	3
Morænesø	0.0023	0.0005	3
Kangarssuk	0.0012	0.0001	3

Mellemsø showed the largest variation between the surface and bottom (10.1 mg/L to 15.2 mg/L). Furthermore, all lakes were oxygen supersaturated in the surface waters (>100 %) (Table 1). The pH values of the lakes spanned from 5.9 (Kangarssuk) to 8.7 (Lillesø) (Table 1).

POM

Mean POM values varied from 0.0012 g/L (Mellemsø and Kangarssuk) to 0.0038 g/L (Lillesø) (Table 2, Fig. 5). Two sample t-tests showed significant differences in mean POM levels between all lakes except between Kangarssuk lake and Mellemsø (Table 3), which had the same mean POM concentrations (0.0012 g/L). The Secchi depth of the lakes varied from 1.2 m (Lillesø) to 2.7 m (Mellemsø). Additionally, there was a slight negative correlation of -0.8 between the Secchi depth and the mean POM concentrations of the lakes (Fig. 6, $R^2 = 0.633$). However, the negative correlation was not significant (p = 0.2042), likely due to the low number of samples.

Weather conditions of the area in 2023



Figure 5. Bar chart of POM concentrations in Lillesø, Mellemsø, Morænesø and Kangarssuk Lake. POM is reported in g/L. Error bars showing the standard error are included.

Lake Comparison	t	df	P value
Lillesø/ Mellemsø	-6.018	4	0.004*
Lillesø/ Morænesø	-3.105	4	0.036*
Lillesø/ Kangarssuk	-6.929	4	0.002*
Mellemsø/ Morænesø	-3.013	4	0.039*
Mellemsø/ Kangarssuk	-0.158	4	0.882
Morænesø/ Kangarssuk	-3.755	4	0.020*

Table 0.3. Results of two sample t-tests (a = 0.05, two-tailed) comparing the POM contents of Lillesø, Mellemsø, Morænesø, and Kangarssuk lake. Significant results are demarcated with '*'.

In the Disko area, the late spring and summer of 2023 (Fig. 3) were unusually cold and wet compared to the climate normals from 1991-2020 and 1961-1990 (Fig. 2). In July of 2023, the mean temperature was 5.5 °C. In comparison, the recent 30-year average (1991-2020) for July was 6.8 °C. Thus, July this year was 1.3 °C colder than

usual. Furthermore, in 2023 the mean precipitation in June was 45.3 mm and in July 2023 it was 114.5 mm. In comparison, the 30year average from 1991-2020 for June was 21.7 mm and for July it was 25.7 mm. Thus, June received 23.6 mm more, and July received 88.8 mm more precipitation than usual.



Figure 6. Linear Regression Model of mean POM and Secchi Depth of the lakes. Secchi depth is reported in meters and POM (particulate organic matter) in g/L. The R²-value and correlation coefficient are reported in the top right corner. The grey band indicates the 95 % confidence interval for the regression line.

Linear Regression Model of POM and Secchi Depth

Discussion

Conductivity and water temperature over time

In the previous studies on the conductivity performed in 1955 and 1956 by Røen (1962) and the Arctic Field Course in 2004 (Christoffersen et al., 2004), the depth, ice conditions of the lake, and month of sampling varied in the studies making direct comparison difficult. The study from 2004 reported the average temperature and conductivity of the water column but did not state the specific date of sampling; just that it was during July and August. Thus, additional information about the ice conditions and sample month are only reported only for Røen's conductivity measures. The following paragraphs will compare the parameters in each lake in turn across the three studies.

In July of 1956, Røen (1962) sampled Lillesø, which had a few small ice floes, and it was most likely sampled at a depth that was reached using waders. Here, the conductivity was 38 μ S/cm. In 2004, the lake was 10.9 °C and the conductivity was 34 μ S/cm averaged over a depth of 11 meters (Christoffersen et al., 2004). In the present study, there was also partial ice cover on the 4.8 °C lake, and the conductivity was 38.8 μ S/cm at the surface and 40.4 μ S/cm at 5.5 m depth. Thus, the current values were very much in accordance with previous years in spite of temperature variations.

On the same day in July of 1956, Røen also sampled Mellemsø, which was also covered by an ice-sheet in the middle of the lake. Sampling again presumably by waders yielded a conductivity of 27 μ S/cm. The conductivity in 2004 was 35.3 μ S/cm and the lake was 6.6 °C averaged over a depth of 17 m. The present measure of conductivity at the surface was 25.2 μ S/cm (3.4 °C), which was again comparable to Røen's conductivity near the bottom, 49 μ S/cm at 12 m, was relatively higher than the value from 2004. However, taking the average of the surface and bottom values yields a conductivity of 37.1 μ S/cm, which is then comparable and again very similar to the average of 35.3 μ S/cm in 2004.

Røen reported that Morænesø had a large ice floe in the middle of the lake in mid-June of 1955, and here the conductivity was 58 μ S/cm at a depth reached using waders. The results from 2004 reported a conductivity of 61 μ S/cm and a temperature of 9.9 °C, while the present conductivity was 59.3 and 59.4 μ S/cm at the surface and at a depth of 3.5 m respectively with a temperature of 8.3 °C. Once again, the measures of conductivity varied minimally compared to previous years.

Finally, Kangarssuk lake was sampled later in the season, in September of 1956, by Røen with no ice on the lake, and the conductivity was 62 μ S/cm. No data was available from 2004. However, our measures of conductivity were 42.7 and 43.0 μ S/cm at the surface and at a depth of 6.9 m respectively when the lake was 6.2 °C. A possible explanation for the roughly 20 μ S/cm higher conductivity of Kangarssuk lake in September of 1955 compared to now might be that the primary production tends to have peaked by September, and the lake will have warmed slightly during summer. Thus, the organic matter decompositions levels and thereby conductivity levels at the bottom are expedited to be higher. Hence, the comparison is not representative of a change over time, but rather seasonality.

The conductivity values that were measured did not match the prediction, that the conductivity values would be higher due to a warming of the Arctic region, which would lead to a higher turnover rate. However, this specific year was rather cold, and the ice conditions of the lakes were similar to Røen's reports, which may explain the similarity of values across the more than 60 years. Even in the warmer period of 2004, the lakes' conductivity values were fairly comparable to present values, indicating relatively stable levels.

While the N and P concentrations were not determined, the conductivity is a measure of the ions and thereby the nutrients that are available

in the system. In general, all of the sampled lakes had relatively low conductivity values, which is typical of Arctic lakes due to the inflowing water largely originating from melted snow and ice (Christoffersen et al., 2008). This is also apparent when the four sampled lakes of this study are compared to a eutrophic lake like Thygesens Sø in Qeqertarsuaq (96 μ S/cm). Here, the conductivity is higher due to the lake's location adjacent to the town center with nutrition inputs from wastewater and neighboring built-up areas (Christoffersen et al., 2004; Røen, 1962).

The lack of variation in the conductivity down the water column in Lillesø, Morænesø, and Kangarssuk (Fig. 4) observed in this study might be a product of the shallow depth at which the CTD was deployed; 4.8 m, 3.5 m, and 5.8 m respectively. In comparison, Mellemsø showed a 23.8 µS/cm increase in conductivity from the surface to the bottom at 12 meters (Table 1). This is typically observed due to the microbial turnover on the bottom, which releases nutrients and thereby ions to the bottom waters, which have limited mixing with the surface water in deeper lakes. The observed shift from low conductivity to high occurred at a depth of 5-6 m in Mellemsø. Hence, the other profiles were hardly deep enough to detect a change from low to high conductivity, if such a change was present. Furthermore, Morænesø and Kangarssuk lake are relatively shallow systems in general (Appendix 9 and 10), and thereby mixing of the water column is more prevalent than in deeper lakes (Padisák & Reynolds, 2003). Thus, we cannot exclude the possibility that a similar shift in conductivity could be present in Lillesø, as the deepest point sits at 12.5 m (Appendix 7).

Water temperature profiles in 2023

In the yet ice-covered Lillesø and Mellemsø, the temperature variations down the water column were very small and showed no signs of stratification (Fig. 4), which is typical for Arctic lakes as a result of the cold climate (Andersen et al. 2004). Morænesø and Kangarssuk lake did not have any ice cover at the time of sampling, but showed similar trends to Lillesø and Mellemsø, as the lakes had not been ice-free long enough for the surface layer to show any notable warming. However, had sampling at the deepest spots been an option in the deepest lakes (Mellemsø and Lillesø), then a greater variation in temperature from top to bottom could possibly have been observed. The reason that the coldest lake was Mellemsø is likely a result of the partial ice cover at the surface, and meltwater input from the mountains. Furthermore, Mellemsø is by far the biggest and deepest lake and therefore the surface area to volume ratio is lower compared to the other lakes, slowing the relative warming of the water. Conversely, the warmest lake, Morænesø, was also the shallowest, making warming more rapid.

pH and oxygen cconcentration

All of the pH measurements are with a reasonable doubt regarding the reliability of the instrument. Four pH measurements were collected from Morænesø, which showed three different results (7.0, 6.3, and 6.1), rendering doubt about the reliability of the instrument. Therefore, the reported pH values of the study should be interpreted and used with caution in potential future comparisons. Thus, further discussion of this parameter is omitted.

Oxygen supersaturation occurred in the surface waters in all of the sampled lakes, which can be attributed to the primary production. The large amount of sunlight during the summer period at such high latitudes increases the primary production, which contributes to the supersaturation of the lakes. Mellemsø had an influx from a melt water stream close to the sampling site which also supplied the lake with dissolved oxygen from mixing and turbulence.

When comparing the oxygen concentration with the values from the 2004 report, similar values were observed from Lillesø, Mellemsø and Morænesø, while there was no comparison data from Kangarssuk lake. The oxygen in 2004, was 15.5 mg/L in Lillesø compared to the current level of 15.7 mg/L (118%). Mellemsø had a content of 14.3 mg/L in 2004 and currently 15.2 mg/L (115%), while Morænesø went from 13.5 mg/L to 12.8 mg/L (103%). The differences in oxygen concentrations from top to bottom in this study (Table 1), can be explained by the photosynthetic activity in the epilimnion producing oxygen during light hours, and the microbial turnover at the bottom consuming oxygen, resulting in lower values in the deeper layers of the lakes.

POM and transparency of the lakes

Previous studies of abiotic factors from these lakes have not included measurements of POM, and we therefore do not have any data for comparison to detect possible temporal changes that may have occurred. Thus, mean POM will only be compared between the lakes of this study (Fig. 5). Furthermore, the values can be used as a reference for future studies. Mean POM levels from the highest to the lowest of the lakes were Lillesø with 0.0038 g/L, Morænesø with 0.0023, and then Mellemsø and Kangarssuk lake with the same content of 0.0012 g/L. As Lillesø was unconditionally the smallest lake, its surface area to volume ratio is higher and thereby there is a foundation for a relatively higher contribution of allochthonous input of POM from the catchment area which may explain the higher levels in Lillesø.

We predicted that the transparency would be lower in all of the sampled lakes, as a result of increased runoff from the catchment areas following generally increased precipitation in the area (Fig. 2). Using Secchi depths as a proxy for transparency, the data was compared over time. In comparison to 2004 (Christoffersen et al., 2004), the present study found a decrease of the Secchi depths in Lillesø (from 5 m to 1.2 m) and Morænesø (4.5 m to 1.94 m). Mellemsø showed a slight increase in transparency (2.5 m to 2.7 m), considering that our samples were collected during a rather cold period (3.3°C at 12 m) compared to the measurements from 2004 (6.6°C at 17 m). The higher water temperatures in Mellemsø in 2004 would likely result in an increased primary production, lowering the transparency down the water column, as a result of higher particle density in the water. Furthermore, the precipitation in late spring and summer of 2023 was very high compared to previous years (Fig. 2 & 3). This is likely to have led to an increase in the allochthonous input, with more organic matter being transported from the catchment area to the lakes. This could

explain the lower values of transparency in Lillesø (1.2 m) and Morænesø (1.94 m). No transparency data for comparison was available for Kangarssuk lake.

In relation to transparency, the final prediction was that lakes with higher contents of POM would have lower Secchi depths. While the regression analysis was not significant due to the small sample size, the negative correlation of -0.8 did point towards there being a pattern of more POM leading to lower transparency of the lakes (Fig. 6). Thus, if the meteorological tendencies of warming in the region around the Arctic Station continue (Fig. 2), studies in future are likely to find relatively lower transparency values than those observed in this study.

Conclusion

In summary, the study reached the following in regard to the two predictions that were formulated initially. The first prediction, that conductivity and POM values would be higher than in previous years, could not be supported on the basis of results from the study. However, while the mean annual temperature has been increasing, significant interannual variation with cold summers like this year may explain the observed deviation from the prediction. The second part of the first prediction, that lakes with higher contents of FPOM would have lower Secchi depths could not be fully corroborated, due to the correlation test value not being significant. However, the study did find a negative correlation which indicates that such a pattern likely exists, had the sample size been larger. The second prediction, that the transparency would be lower due to increased precipitation, was partially reached, since two out of the three lakes that had available comparison data showed lower Secchi depths, likely attributed to the heavy precipitation of 2023.

In conclusion, the study found that the physical and chemical conditions of Lillesø, Mellemsø, Morænesø, and Kangarssuk lake mostly resembled those found in previous decades in spite of the documented warming and increased precipitation in the Disko region. In addition, interannual weather variation like this cold and wet summer of 2023, is contributing to shaping the abiotic factors of the lakes.

Acknowledgements

The authors would like to thank the Faculty of Science and the Department of Biology for supporting and enabling the Arctic Field Course of 2023 both financially and for providing the research equipment. We would like to thank the Arctic Station staff, especially Céline Albert and Akaaraq Mølgaard for assistance during our stay in Qeqertarsuaq, as well as the researchers working at the Arctic Station during our stay for the exciting presentations, they held for us about their fieldwork. We would like to give an immense thanks to our course coordinator Kirsten S. Christoffersen and Ph.d. student Sanne Mariël Moedt for enabling the sampling out on the lakes, assisting with the laboratory work, and guiding our writing process. Lastly, we want to thank our fellow participants of the course for contributing to an overall great experience.

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Appendices



Appendix 1. Lillesø partially covered in ice. (Photo: Camilla Mortensen)



Appendix 2. Part of the catchment area of Lillesø (Photo: William Himmelstrup)



Appendix 3. Mellemsø partially covered in ice. (Photo: Camilla Mortensen)



Appendix 4. Part of the catchment area of Mellemsø. Input of meltwater from snow to the lake. (Photo: William Himmelstrup)



Appendix 5. Morænesø and catchment area. Picture from before the ice melted. (Photo: Victoria Westmark Sønnichsen)



Appendix 6. Kangarssuk lake and stream outlet running into the ocean. (Photo: Victoria Westmark Sønnichsen)



Appendix. 7 Depth chart of Lillesø, Diskofjord. Depth in meters, plots are made by dropping weights from a kayak. Max depth 15 m. Remeasurement of max depth by Røen (1962) re-estimated it to 12.5 m (Drawing: Morten Porsild, 1902)



Appendix. 8 Depth chart of Mellemsø, Diskofjord. Depth in meters, plots are made by plumbing the depths of the lake from a kayak. Max depth 36 m. Remeasurement of max depth by Røen (1962) re-estimated it to 27 m (Drawing: Morten Porsild, 1902).



Appendix. 9 Depth chart of Morænesø, Qeqertarsuaq. Depth in centimeters. Max depth is approximately 4.5 m. (Unpublished data kindly provided by S. Funder, GEUS.)



Appendix 10. Depth chart of Kangarssuk lake, Fortunebay. Depth in meters. Max depth is approximately 6.9 m. The depth chart is from Sheard and Bergddon (2023) pp. 97-114 In: Arctic Biology File course report 2012.

Spatial dimorphism of the Arctic Three-Spined Stickleback (Gasterosteus aculeatus) in selected lakes at Disko Island

Johan Frugaard Poll, Magnus Husen, Freja Engberg Willumsen & Mohammed Naushad Khumri

Abstract

The Three-Spine Stickleback (Gasterosteus aculeatus) has become a model organism for adaptive radiation studies, and determining underlying factors of such, remains a challenge for biologists. In post-glacial isolates, the populations repeatedly founded by anadromous and marine individuals, have undergone rapid morphological diversification amongst sub-populations. To understand the spatial dimorphism of the number of lateral plates, body size and length, dorsal spine length and gill raker length, both abiotic and biotic mechanisms need to be considered. During colonization of freshwater environments, the Three-Spined Stickleback has undergone parallel reduction in the number of lateral plates that has been the basis of this study. The aim of this study is to describe and analyse the spatial dimorphism of the Arctic Three-Spined Stickleback from 4 different locations on Disko Island, Greenland. A total of 210 Stickleback individuals from both marine (n=82) and freshwater (n=128), were used in the study to compute our results. The results showed a significant difference in the number of lateral plates amongst the locations, where the freshwater populations (SF, AS & ML) displayed the lowest number, compared to the marine (QQ) individuals. The results also showed a significant difference in body weight (g) and length (mm) and mean relative gill raker length (mm) between the locations, all being greatest in the marine environment. Dorsal spine length (mm) diverged from our expectations and was greatest in the freshwater lake, AS. These results seem to be a recurring phenomenon in populations outside the Arctic and are influenced in part by the local salinity and calcium levels. The link between the morphological traits and age was also investigated, mainly for the QQ population, however, age information was too scarce to make statistical analyses between the populations.

Keywords: Arctic, Gasterosteus aculeatus, Eco-morphology, Adaptation, Greenland

Introduction

The Three-Spined Stickleback (Gasterosteus aculeatus) is a small (max 11 cm) ray finned, scale-less teleost found across the northern hemisphere where it occurs in marine coastal areas and freshwater environments (Bell & Foster 1995). The Three-Spined Stickleback (hereafter stickleback) has been recorded further and further north, and it is continuously found in new habitats. Reports show that the species has been present in Greenland since before 1896 (Bay, 1896; Bergersen, 1996). The range of G. aculeatus is circum-arctic and temperate, encompassing the coastal waters of Eurasia, Iceland, eastern Asia (north of ~35°N) and Northern America (north of ~ 30°N) including parts of

Greenland. They are found in freshwater habitats extending as far north as northwestern Norway, the northern and northwestern regions of Iceland, and also in the southern to midwestern part of Greenland (Bergersen, 1996; Wooton, 2009). One study regarding sticklebacks living in arctic freshwaters, has recently found populations in freshwater as far north as locations on Svalbard (Svenning *et al.* 2015).

The marine populations prefer shallow coastal waters, while the freshwater populations occur in small, vegetated streams, vegetated lakes and riverbanks over sandy and muddy substrates. Some populations live anadromous and in July-August the juveniles from these populations move towards the sea; At the same time freshwater juveniles move to deeper water bodies (Ostlund-Nilsson *et al.*, 2006). The freshwater form is usually brown or greenish, while the anadromous morphs are bluish/black to silvery green. During the spawning period in the summer, males develop an orange/red colouration on their ventral side, and bluegreen eyes, differentiating them from the females (Morrow *et al.*, 1980).

The stickleback is equipped with a narrow peduncle with an accompanied caudal fin used for sub-carangiform locomotion, accompanied with a pair of pectoral fins for labriform swimming in close quarters (Moyle et al., 1982). Preceding the peduncle is the dorsal fin, and 2-4 dorsal spines with individual triangular fin membranes, with the 2nd dorsal spine usually being the largest (Kottelat et al., 2007; Kristjánsson et al., 2004; Morrow et al., 1980; Moyle et al., 1996). The single spined pelvic fin is followed by the anal fin, which is located behind the dorsal fin (Spence et al., 2013). All the individuals have lateral bony plates that occur in 3 different morphs, low (3-7), partial (6-20) or complete (19-30), (Fig. 1). Studies have shown that sticklebacks vary in number of lateral plates, and thereby morphs, when comparing marine and freshwater environments (Spence et al., 2013; Ostlund-Nilsson et al., 2006; Wiig et al., 2016). The calcareous dorsal spines, in addition to the lateral plates, are used as a defence mechanism, and become erect to limit predation success (Ostlund-Nilsson et al., 2006).

The stickleback is equipped with two sets of 5 ceratobranchials with long slender gill rakers, for food retention/filtration. They feed on larvae, zooplankton, macro-invertebrates, and have been reported to feed on their own fry and eggs as well, only gape size-limited in its prey choice (Ostlund-Nilsson *et al.*, 2006). Sticklebacks have been shown to vary in size and number of gill rakers, resembling either the limnetic plankton feeder, or benthic macro predator (Hall, 2005). Gill rakers are highly polygenic adaptive traits whose development can be predictable based on genetics (Glazer *et al.*, 2014). Genetic studies have shown that the heritability of gill raker

length is relatively high and suggest that the morphological differences among populations are more likely the result of genetic selection pressure rather than acclimatization (Schluter, 1996). This is confirmed further by a study that sought to identify signatures of genomic selection for sticklebacks across a freshwaterbrackish gradient in the coastal Dutch-Belgian lowlands. Fish harbouring marine waters, generally have a longer relative gill raker length, in addition to significantly longer gill rakers in fish harbouring more saline waters, they found that even under the presence of gene flow, outlier loci associated with morphological traits such as gill raker length was detectable (Koniinendiik et al., 2015). Suggesting that it is not salinity directly impacting physiological processes that determine gill raker length but rather factors associated with the salinity. One of which being the species composition and availability of prey species. Analysis of isotopic signatures of fish tissue have shown that sticklebacks with limnetic feeding strategies have longer gill rakers than benthic individuals. Gut content analysis has shown that limnetic exhibit foraging that favours copepods. Hence it is theorized that longer gill rakers are an adaptation to filter ingested zooplankton as with limnetic feeding morphs, whereas shorter gill rakers are associated with a diet consisting of macroinvertebrates as found in the benthic morphs (Matthews et al., 2010; Moyle et al., 1982).



Figure 7. Variation of the 3 plate morphs (low, partial and complete) regarding distribution of lateral plates. The arctic stickleback has up to 30 plates on each side anterior to posterior. The complete morph (A) with 19-30 bony plates, the partial lacking one or more plates in the midsection (B) with 6-20 plates and the low (C) with 3-7 plates lacking plates in the posterior region. Picture from Wiig et al., (2016).

As a part of the sticklebacks diet, they consume copepods, which in freshwater habitats can carry pseudophyllidean cestode of *S. solidus*. This parasite enters the sticklebacks through the oral cavity, and then undergoes plerocercoid development in the abdomen, before it enters avian hosts that feed on sticklebacks (Hopkins & Smith, 1951).

The sticklebacks tolerance for salinity and its wide adaptability to diverse environmental conditions makes them an intriguing subject for scientific studies (Bell & Foster, 1994). Previous studies have provided insights into the spatial morphological variations within the species, particularly focusing on traits such as lateral plates and gill raker morphology, over an abiotic gradient such as salinity (Ostlund-Nilsson et al., 2006; Spence et al., 2013; Moyle et al., 1982). However, knowledge is still limited regarding these traits within the Arctic region and ecosystems (Klepaker & Østbye, 1999). Throughout this paper the following hypotheses will be analysed and discussed:

- We expect a positive linear correlation between length and weight of the individuals, due to an allometric relationship. In addition, we expect to see a significant variation in length and weight between the locations, with those that access to the sea reaching larger sizes (Spence et al., 2013).
- 2. We expect a significant difference in the mean number of lateral plates between the individuals from the 4 locations, due to differences in available Ca+ for development of calcareous structures, between fresh and marine waters (Spence *et al.*, 2013; Wootton, 1976).
- We expect a positive linear relationship between 2nd spine length and body length, due to constant growth of calcium structures throughout the individual's lifespan. We expect to see a significant difference in mean 2nd dorsal spine length between locations due to differences in available Ca+ for calcareous structures, between fresh and marine waters (Spence *et al.*, 2013).

- We expect a positive allometric relationship between gill raker length and body length, due to constant growth. Additionally, we expect a significant difference in relative gill raker length between locations as a response to different diet niches, as found by Day *et al.* (1994).
- We expect to see a positive relationship between age (based on otolith analysis) and total body length, and to see a similar trend in both the dorsal spine length and length of gill rakers.
- 6. Lastly, we expect to see a negative correlation between body length, and number of parasites in individuals from a parasite infested lake, as a product of an ontogenetic shift in diet over time with increasing gape size, decreasing the consumption of copepods carrying *Schistocephalus solidus*. But we don't expect parasites in the individuals from QQ due to physiological restrictions of *S. solidus* to saline water.

Methods and materials

Collection of fish

The sticklebacks were sampled during the summer of 2022 and 2023 from four locations. Qeqertarsuaq (QQ) (69.249079, -53.532059), Seniffik (SF) (69.3551077, -52.9791608) and Mellem Sø (ML): (69.522017, -53.696858) and Stationssø near Arctic Station (AS) (69.251878, -53.518018) (Fig. 2). Sampling was done using hand-held nets as well as homemade plastic bottle minnow traps, baited with raw shrimp. All collected sticklebacks were transported back to the lab in a bucket of water for further analysis. The fish were stored frozen and thawed for further analysis.

An addition batch of fish sampled in 2022 by a German research team was included. The sites vary with different sample sizes; SF (n=84), QQ (n=82), AS (n=32) and ML (n=12), (Table 1).



Figure 8. GIS map, showing locations of sampling; Arctic Station Lake (AS: 69.251878, -53.518018), Mellem Lake (ML: 69.522017, -53.696858), Qeqertarsuaq town lake (QQ: 69.249079, -53.532059) and Seniffik lake (SF: 69.3551077, -52.9791608), in West Greenland on Disko Island

External measurements, lateral plates, and dorsal spines

Before initiating dissection, preliminary measurements were conducted. These include wet weight, length from the anterior of the mandible to the posterior part of the caudal fin (body length) and length from the anterior of the mandible to the posterior of the operculum (head length), (Table 1).

Using a teasing needle on the left lateral side of the specimens, the extent and number of lateral plates was determined mechanically, in relation to the three plate morphologies: low, partial and complete. Additionally, for each individual, the number of dorsal spines was noted, and the length of the 2nd spine was measured. The dorsal spine length was also calculated as a relative spine length to the length of the individual, (Table 1).

Gill rakers

To access the gill rakers the operculum was removed using a sharp scissor and forceps, leaving the gills exposed. Incisions were made above the left epibranchial and below the left hypobranchial to remove the gill arch of interest. The samples were placed in a petri dish under the stereo microscope, to cut out the 1^{st} left ceratobranchial (gill arch). On the ventral side of the arch the length of the 2^{nd} , 3^{rd} and 4^{th} gill raker, was determined using the program Fiji 1.53C built on ImageJ (Schindelin *et al.*, 2012). The mean relative gill raker length to the length of the fish was calculated for each individual, (Table 1).

Otoliths

Due to the small size of the fish, the typical method of removing the top part of the head, to expose the brain cavity, was not possible. Instead, otoliths were extracted by making a dorsoventral cut through the head of the fish, that would expose the otic capsule after extraction of the brain. The largest otolith (sagittae) was extracted from both sides when possible. The extraction was carried out under a microscope using forceps and a teasing needle. The extracted otoliths were thin enough for age determination to be carried out using a stereomicroscope at 60X magnification, alternating between a backlit view and additional side lighting. Age determination for each individual was done according to the classification of Jones & Hynes (1950) and supported by methods from Waldron et al. (2001). Aging of the fish has been carried out a minimum of three times for each individual to maximize accuracy.

Sex determination

The sex of the sticklebacks was determined through identification of various discerning characteristics. Males were distinguished by their distinctive attributes during the breeding season, which included blue irises, red lateral surface coloration, and a pronounced reduction in dorsal melanism (Ostlund-Nilsson *et al.*, 2006). Conversely, females were identified by the presence of eggs. We defined individuals without the presence of eggs or secondary male characteristics as males. The sex ratio of each location was also determined.

Parasites (S. solidus)

While sex was determined, the bow of the fish was cut open, allowing a direct angle of view for determining the presence of parasites. The parasites were removed and

Table 0.1. Results from the measurements of interest	for the Three-Spined Stickleback from the 4 locations
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Sample site information					
Location:	Siniffik (SF)	Arctic station lake (AS)	Qeqertarsuaq harbour (QQ)	Mellem Lake (ML)	
n:	84	32	82	9	
Mean length (mm):	44.34±8.50	52.93±11.08	81.76±8.98	42.08±2.56	
Mean mass (g):	1.29±1.20	1.78±1.16	5.93±1.72	0.76±0.19	
Plate morph(s):	L	L/P	С	L	
Mean # of lateral plates:	5±1	22±6	26±2	4±1	
Mean relative 2 nd spine length (mm):	0.08±0.01	0.09±0.01	0.30±0.04	0.08±0.01	
Mean relative gill raker length (mm):	0.02±0.004	0.02±0.004	0.02±0.003	0.01±0.004	
Location salinity (ppt):	0-0.02	0	20	0	
Sex ratio (m/f):	1.24	1.36	0.54	2	
Note: Mean # of lateral plates are rounded numbers. Relative 2nd spine length and gill raker length are relative to the total length of the individual.					

counted, and the number of located parasites was noted for all infested individuals.

Salinity

Salinity (%) was determined for each of the sites using water samples from each location and analysing it using a portable refractometer (PCE-0100, accuracy: 1%), the values were later converted to ‰, by dividing with 10, (Table 1).

Statistical analysis

All statistical tests were performed using <u>R</u> version 4.3.1 (Beagle Scouts).

We tested variations in the external morphology and gill raker morphology of the different populations using a general linear model (GLM; one-way ANOVA and Student t-tests), to determine if there was a significant difference between the means of the locations. We compared several covariate factors such as: size, weight, #plates, relative spine length and relative raker length against the fixed factor of location. These tests underwent further evaluations using the Tukey HSD (post hoc) Test for multiple comparisons.

Additionally, we used descriptive statistics of co-variation (Spearman rank correlation), to compute potential correlations between body length; parasites and raker size; body length. Here we tested if there was a correlation between the body length and the number of parasites they carried, and whether there was a correlation between body length and mean gill raker length.

When comparing age groups by otolith analysis, we compute a chi-square test of independence, to determine possible associations between sex distribution and age groups.

Results

The 210 individual sticklebacks collected from the 4 different locations, varied in several different morphological traits. Sample site information regarding mean values of the covariate factors is represented in Table 1.



Figure9. Scatterplot of body length (mm) and body mass (g) (n=210). Each dot represents the intercept between body length and body mass of a single individual. Color coding indicates location of origin. Fitted linear trendline from the Spearman's rank correlation, with accompanying confidence interval = 0.95 (grey area). The trendline equation, results from the Spearman's ranks correlation test (R value and level of significance) are indicated on the left Box plots showing median, quartiles, maximum/minimum values and outliers, of the various covariate factors (size, weight, mean relative spine length and mean relative gill raker length), with accompanied letters of significance from the Tukey HSD (post hoc) Test for multiple comparisons, between locations, are found in Appendix 1.

Weight and length

A Spearman's rank correlation (Fig. 3) revealed a positive correlation between body length and body weight (r (210) =0.96, p=<0.001).

Mean weight of individuals from all the locations varied from 0.76 g at ML to 5.93 g at QQ (Table 1). A one-way ANOVA revealed a significant difference in the mean weight of the individuals between at least two locations (F $_{3,206}$ = 181.3, p = <0.001). A Tukey HSD Test for multiple comparisons revealed a significant difference in mean in between locations: AS-QQ (p =<0.001, 95% C.I. = 3.39, 4.92), SF-QQ (p =<0.001, 95% C.I. = -5.20, -4.08) and QQ-ML (p = <0.001, 95% C.I. = 4.09, 6.25), (App.1). This shows that the location with the highest mean weight (QQ) differed significantly from the other locations,



Figure 11. Scatterplot and accompanying linear trendline with grey confidence interval (0.95) showing a positive linear correlation, between the total body length (mm) and length of the 2nd dorsal spine (mm). Each point represents the intercept between body length and 2nd dorsal spine length, of a single stickleback individual. Results from the Spearman's ranks correlation test (n, R and level of significance) are shown in the top left corner and shows a significant correlation between body length (mm) and 2nd dorsal spine length (mm)



Figure 10. Tukey-style box plot of number of lateral plates for individuals from each of the 4 locations (AS, ML, QQ and SF). Sample size is indicated, and the boxplots indicate median, quartiles (Q1 and Q3), interquartile range (IQR (Q1-Q3)) and maximum and minimum values within \pm 1.5 * IQR. Values outside the \pm 1.5 * IQR are outliers. Letters of significance indicate which locations are significantly different in mean number of lateral plates of the sticklebacks.

while the other locations did not differ significantly in mean weight.

Mean body length of all individuals from all locations varied from 81.76 mm at QQ to 42.08 mm at ML (Table 1). There was a significant difference in the mean body length between at least two locations, revealed via an ANOVA (F 3,207 = 274.4, p = <0.001). Further analysis via a Tukey HSD Test revealed significant difference in mean body length between locations: ML-AS (p=0.002, 95% C.I. = -18.43, -3.27), AS-QQ (p=<0.001, 95% C.I. = 24.03, 33.64), SF-AS (p=<0.001, 95% C.I. = -13.38, -3.80), QQ-ML (p=<0.001, 95% C.I. = 31.80, 46.57) and SF-QQ (p=<0.001, 95% C.I. = -41.01, -33.85), (App.1). These results show that there is significant difference in mean length between all locations except the two with the lowest mean lengths, ML (42.08) and SF (44.34).

Number of lateral plates

Plate morphs and mean number of lateral plates varied between locations from low at ML (4) and SF (5) to a mix of partial and complete at AS (22), to complete in QQ (26 plates) (Table 1).

An ANOVA revealed a significant difference between mean number of lateral plates

between at least 2 locations (F $_{3,137}$ = 496.2, p = <0.001). A Tukey HSD Test found significant differences between locations: ML-AS (p=<0.001, 95% C.I. = -19.71, -14.53), AS-QQ (p=<0.001, 95% C.I. = 2.18, 5.87), SF-AS (p=<0.001, 95% C.I. = -18.79, -15.26), QQ-ML (p=<0.001, 95% C.I. = 18.66, 23.63) and SF-QQ (p=<0.001, 95% C.I. = -22.65, -19.45), (Fig. 4). These data show that only the locations with the lower plate morphs (ML-SF) were not significantly different in mean number of lateral plates.

Dorsal spine length

The 2^{nd} dorsal spine length (mm) ranges from 2-9 between all individuals (Table 1), the use of descriptive statistics of co-variation revealed a positive correlation between body length and 2^{nd} dorsal spine length (r (209) = 0.92, p=<0.001), (Fig. 5).

After correcting for body length to compute relative dorsal spine length (2^{nd} dorsal spine length / body length) an ANOVA between the locations revealed a significant difference in mean spine length between at least two locations (F_{3,207} = 17.16, p=<0.001). To test for multiple comparisons, we used a Tukey HSD Test, which revealed significant differences in mean 2^{nd} dorsal spine length between locations: ML-AS (p=<0.001, 95% C.I = -0.02, -0.01), AS-QQ (p=<0.001, 95% C.I = -0.02, -0.01) and SF-AS(p=<0.001, 95% C.I = -0.02, -0.01), (App. 1).

Otoliths

51 sticklebacks had their otoliths extracted and age determined to be one, two or three years old (in their 2nd or 3rd summer), (Jones & Hynes, 1950). Knowing the age of these individuals allowed us to test the sex and morphological traits of interest (e.g length, weight, dorsal spine length) across age groups and sex, using a Chi square test and GLM.

The distribution of age for male and female was not significantly different according to a Chi square test (X^2 (1, N = 43) = 0.28, p = 0.6). between age groups. Student T-tests comparing mean length and weight across

age groups (one- and two-year-olds) revealed a significant difference in length (F $_{1,49}$ = 15.55, p=<0.001) and weight (F $_{1,49}$ = 13.81, p=<0.001 between the age groups. Additional Student T-tests revealed no significant difference in mean length between males and females of either one (F $_{1,20}$ = 0.04, p=0.84) or two-yearolds (F $_{1,25}$ = 0.68, p=0.42). A significant difference was revealed in mean weight between the two sexes for the one-year-olds (F $_{1,20}$ = 9.69, p=0.004), but for the two-yearolds the difference in no longer significant (F $_{1,25}$ = 0.54, p=0.47).

An interesting link was found between the age of a fish and the length of the dorsal spines. An ANOVA test comparing the length of the 2nd spine for one-year olds vs. two-year-olds revealed a significant difference in length (F $_{1,49}$ = 8.25, p=0.006). However, when the length of the 2nd spine is normalized for the length of the individual, the tendency was less pronounced, though still significantly different for the one- and two-year-olds (F $_{1,49}$ = 3.62, p=0.063). As the fish get older their spines get longer, but at a continuously slower rate as the relative spine length



Figure 12. Scatterplot and accompanying linear trendline with grey confidence interval (0.95) showing a positive linear correlation, between the total body length (mm) and mean gill raker length (mm). Each point represents the intercept between body length and mean gill raker length, of a single stickleback individual, calculated as the mean of the 2nd - 4th ventral gill rakers on the left ceratobranchial. Results from the Spearman's ranks correlation test (n, R and level of significance) are shown in the top left corner and shows a significant correlation between body length (mm) and mean gill raker length (mm).


Figure 13. Scatterplot and accompanied linear trendline with grey confidence interval (0.95) between the total body length (mm) and number of parasites. Each point represents the intercept between body length and number of parasites, of a single stickleback individual from either SF, AS or ML. Results from the Spearman's ranks correlation test (n, R and level of significance) are shown in the left side and shows a non-significant correlation between body length (mm) and number of parasites

decreases with the age of the fish.

Unfortunately, the limited number of otoliths secured for SF (5n), AS (15n) and ML (0n), respectively, prevents statistical analysis based on age between the different populations, and the relationship between age and gill raker length.

Gill raker length

The mean gill raker length ranged from 0.13-2.29 (Table 1), and a Spearman's rank correlation (Fig. 6) revealed a positive correlation between body length and mean raker length (r (209) =0.78, p=<0.001). After correction for body length, an ANOVA was computed to assess the difference in mean relative gill raker length (mm) (mean gill raker length / body length). The ANOVA revealed a significant difference in the means between more than two locations (F 3, 102 =14.7, p= <0.001). A follow up Tukey's HSD Test found the mean value of relative gill raker length was significantly differed between locations: ML-AS (p=0.001, 95% C.I =-0.01, <-0.01), QQ-ML (p=<0.001, 95% C.I =<0.01, 0.01), SF-ML (p= 0.006, 95% C.I =< 0.01, 0.01) and SF-QQ (p=<0.001, 95% C.I =-0.01, <-0.01), (App. 1).

Number of parasites

Parasites were found in individuals from SF, ML and AS, in varying amounts per individual. Most infested fish had one (66%), or two (16%) parasites, while the individual with the most parasites had 44. A Spearman's rank correlation revealed no correlation between body length and number of parasites at SF, AS and ML locations combined (r (75) =0.19, p=0.097), (Fig. 7)

Discussion

The results showed a significant positive correlation between body length and body weight of individuals from the 4 locations (Fig. 3). The significant size difference of the individuals from QQ compared to individuals from the other lakes, and we have been able to determine that age is not the main factor behind the difference. It is likely the result of several factors, the main ones being nutrient availability and temperature. QQ was the only lake with no ice cover when visited in 2023, indicating a longer growth period. Being ectothermic organisms, temperature increase or decrease will directly affect the metabolism and therefore growth rate of the sticklebacks (Allen et al., 1982; Kuparinen et al., 2011). Another factor is the nutrient level of the lakes, which undeniably influences the growth of the fish, and of the population, as more food results in bigger and more frequent spawning (Wootton, 1977). QQ is in a village, while the other three lakes have little to no influx of human wastewater and thus less added nutrients, and additionally it is the only saltwater lake in our study. Increased temperatures and nutrient levels in QQ presumably result in a more beneficial growth period, leading to a bigger population of sticklebacks that are both longer and heavier than the fish from the other lakes.

Spence *et al.* (2013) found that calcium is important for growth in sticklebacks; In low calcium and low salinity waters, individuals with low plate morphs have a higher growth rate than that of individuals with a complete plate morph. The complete morph grows slower at lower levels of calcium probably as a result of a higher demand for than the partial or low morphs to grow. The response in growth rate might be connected to the stickleback's ability to alter the growth rate of their plates if calcium is limited. In general, there is a higher calcium availability in marine environments (Wootton, 1976; Wootton, 1998). With increasing salinities and calcium concentration, all plate morphs exhibit increased growth rates, with the complete morphs growth rate increasing beyond that of the low plate morphs (Spence *et al.*, 2013).

In accordance with this our results show individuals from QQ (high salinity) to be significantly larger (length and mass) than individuals from the other locations, thus exhibiting the same pattern of increased growth rate with increasing salinities. The same trend is seen for individuals from AS that were found to be significantly larger than individuals from SF and ML in length, which could potentially be explained by the research on increased growth in more saline environments. AS lake was located near the ocean and connected to the shore by an outlet, giving the potential of an influx of seawater during high tide and storms. Influx of seawater would serve as a calcium source. This could in turn increase growth rates in AS, and therefore begin to explain how AS individuals had significantly higher mean length than individuals from ML and SF. The increased growth of individuals from lakes with more salt (QQ and AS) compared to the pure freshwater lakes (SF and ML) correspond to the pattern shown by Spence et al. (2013), with overall growth rates increasing with increasing salinities (App.1).

Individuals from QQ had the highest mean number of lateral plates overall, and the ones from AS had a higher number of lateral plates than the ones from ML and SF. This aligns with the plate morphs being complete for QQ, partial/complete for AS and low for both ML and SF. This tendency could potentially be explained by an increased availability of essential micronutrients like calcium in the two lakes (QQ, AS) compared to the two others, which is in accordance with previous studies, that have found that differences in calcium availability can have significant implications for the plate quantity and quality (size) of sticklebacks of populations from Norway (Wiig *et al.*, 2016). Thus, supporting our results of the difference in number of plates between locations, with the marine location only having complete plate morphs and the freshwater locations almost only having low morphs, and could begin to explain why we see a mix of complete and partial plate morphs only at the AS location where potential influx of seawater could periodically increase the Ca concentration.

As it was not possible to age all the fish, making definitive conclusions based on age was impossible. Knowledge of the aged individuals, however, adds a good perspective to the discussion of morphological characteristics and how they differ between the populations. With our limited data it was possible to see and confirm the positive relationship between age and length, and age and the length of the 2nd dorsal spine. We see that as the fish get older the dorsal spines grow, but the relative growth slows down as the fish get older, however sexual dimorphism may play a greater role here than we can predict from the obtained data (Kitano et al., 2007).

The mean relative length of the 2nd dorsal spine was significantly higher for fish harbouring AS than for the remaining three locations. In contrast to lateral plates, there seems to be no significant difference in mean relative dorsal spine length between saline and freshwater environments. These results go against several of our other results and are perhaps subject to underlying factors such as genetics, possible hybridisation, or phenotypic plasticity that we are unable to analyse (Bell & Foster, 1994).

As expected, we found a positive correlation between mean gill raker length and body size, making our results consistent with that of previous studies on this allometric relationship (Almeida *et al.*, 2013). The mean relative gill raker lengths decrease in the following order: QQ>AS>SF>ML, (App.1). QQ, the lake with the highest salinity, harboured fish with significantly longer gill rakers relative to their body size, than fish in the freshwater lakes, with the exception of AS. If we acknowledge the idea of a limnetic and benthic feeding strategy in our lakes, it looks as if the QQ (longer gill rakers) population are more in the limnetic feeding morph (Moyle *et al.*, 1982). This indicates that populations such as QQ and AS feed more on zooplankton than populations of SF and ML feeding more from benthic macroinvertebrates.

A noticeable amount of the specimens, from ML, SF and AS, were infested with one or more parasites of the species Schistocephalus solidus. Although the Spearman's rank correlation revealed no correlation between body length and number of parasites at these locations, as we had hypothesized (Fig. 7). Our hypothesis stated a negative correlation between body length and number of parasites, as a product of being gape size limited to small prey, such as copepods carrying the parasite when smaller. But this was not the case in our samples. Previous studies have described the physiological and behavioural alterations in G. aculeatus infested with S. solidus. These include reduced growth rate, increased oxygen consumption and retarded maturation (Lester, 1971). A result of this is a potential sampling bias in this study due to the fact that infected individuals exhibit a reduced predator avoidance response, meaning that our sampling method, particularly when using handheld nets, favours infested individuals over non infested ones (Giles, 1983). It is therefore possible that the specimens from ML and SF are not fully representative of the populations in the two lakes, respectively. The reason for why there were no occurrences of infested individuals from QQ, is S. solidus not being able to tolerate the saline water in the lake. The development of S. solidus eggs remains unaltered in salinities <12.5‰, but the eggs are unable to hatch at salinities >20‰, as found in QQ (Simmonds, 2016).

The arctic is undergoing rapid changes because of climate change, and that will likely impact the arctic biota, including the stickleback, in profound ways (Blois *et al.*, 2013). Calcium availability in the marine environment for example is expected to decline as a consequence of climate change. The increased concentration of carbonic acid will lower the pH and thereby shift the current equilibrium to have more disassociated bicarbonate than carbonate, making less carbonate available for calcifying organisms to incorporate into calcium carbonate structures (Guinotte & Fabry, 2008). Reduced calcium carbonate availability due to factors such as ocean acidification and altered carbonate ion concentrations will impact the sticklebacks' ability to develop and maintain their lateral plates. In a temporal perspective, this could potentially lead to thinner or less developed lateral plates and lower growth rates for both complete and low morphs (Wootton, 1976; Spence et al., 2013). When it comes to freshwater systems, the impacts of climate change on the availability of calcium is not as evident. Expected trends such as increased runoff, thawing of upland permafrost, increased acidic deposition, ambiguous presence of buffering minerals etc. are partly contradicting forces in terms of calcium availability, and it is uncertain which ones will trump the others (Kopáček et al., 2017, Moiseenko et. al., 2022). Additionally, these trends show extreme variability depending on local factors (Camarero et al., 2009). It is therefore not possible to predict how climate changes will impact the calcium availability for arctic sticklebacks in freshwater systems on a broader scale.

This study has investigated the spatial dimorphisms of the arctic three-spined stickleback in 4 different lakes on Disko Island. As expected, weight, length, #lateral plates and relative gill raker length were highest in specimens from the most saline lake, QQ. In contrast, the relative length of the 2nd dorsal spine length was greatest in specimens from AS. Furthermore, a substantial number of specimens from the three freshwater lakes were infested with S. solidus, whereas none were found in specimens from QQ due to the parasite's intolerance to saline water. The specimens infested with these parasites had significantly shorter body lengths, possibly due to reduced growth rates caused by

parasitism. There are more factors to consider and analyse than the scope of this study allows, in order to explain the morphological differences that have been observed. Nevertheless, this study has illustrated morphological variation across a saline gradient in an arctic setting, that aligns with findings of previous studies done on specimens from alternate climates.

Acknowledgements

The authors would like to thank Kirsten Seestern Christoffersen for supervision and help in the field and in the laboratory - as well as creating an inspiring Arctic Biology Field Course together with Sanne Moedt. Furthermore, we would like to thank the University of Copenhagen and especially Arctic station, the staff at Arctic Station for making the field trip an educational and very pleasant experience. We would also like to thank the crew on Porsild for making the sampling efficient and enjoyable. Finally, we would like to thank the German team lead by Lasse Sander from Alfred Wegener Institute, who caught a great number of individuals in 2022 and kindly donated their results for our use.

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Appendix 1: Among-locations variation in body length (mm), body mass (g), mean relative gill raker length (mm) & Relative dorsal spine length (mm). Boxplot of trait values from AS, ML, QQ and SF. Different letters indicate significant differences based on Tukey HSD tests, sample size for each location at each test is indicated.

Appendices

8

A comparative study of benthic algae composition and biomass in Arctic streams, with regards to differing chemical and environmental conditions (Disko Island, Greenland)

Victoria MacCormac, Ida Clausen Galle, Louise Lund Frandsen and Clara Ege Andersen

Abstract

Benthic algae are key components of aquatic ecosystems, playing a crucial role in nutrient cycling, primary production, and overall ecosystem health. They are the main primary producer in arctic streams, with increasing dominance towards the North due to more nutrient-poor conditions in the water column, making their presence a key factor. Their biomasses and compositions can however vary significantly across regions with differing environmental conditions. The aim of this study was to identify and investigate potential differences in the composition and biomass of benthic algal communities in six arctic streams on Disko Island, Greenland, during July 2023. These streams differed in certain chemical and environmental variables of relevance to algae growth within the two major stream types investigated, namely glacier-fed and spring-fed streams. Results indicated that compositions of diatoms, green algae and cyanobacteria varied between the streams, but that biofilm biomass did not. Biofilm biomass was limited potentially due to extreme weather conditions and stream velocities prior to collection. This emphasizes the importance of monitoring seasonally. A statistically significant correlation between *diatoms* (μ g chl-a cm⁻²) and *temperature* (°C) (R= 0.79, p=9.9*10⁻⁵ < α = 0.05) was however found, which suggests a climatic impact on at least one of our variables with predicted climate change outcomes.

Keywords: Benthic algae, biofilm communities, Arctic streams, climate change

Introduction

In Arctic streams, life is dominated by microbial biofilms, which drive most of the aquatic ecosystem's respiration and primary production (Pastor et al., 2021). Biofilm is a key component in the food web of Arctic streams, because it is grazed by a number of small macroinvertebrates (Friberg et al., 2001). Biofilms, which consist of assemblages of different microorganisms (archaea, fungi, bacteria, cyanobacteria, algae, protozoa and small metazoans) are usually embedded in an extracellular matrix of polymeric substances. The compositions and biomasses of biofilm in streams are regulated by a series of abiotic factors such as light availability, stream velocity, nutrient composition and availability, temperature, stream depth and width, pH, substrate composition, and climatic

conditions (Riddle et al., 2008; Quesada et al., 2008).

In the Arctic, streams are less affected by shade and thus light limitation is mainly linked to the depth of the stream (Gudmundsdottir et al., 2011). Moreover, the compositions and biomasses of the microorganisms are also heavily influenced by the surface of the substrate on which the biofilm is attached. A sandy streambed will cause instability and low biomass due to the constant disturbance of the substrate, whereas bedrock constitutes a stable substrate, which causes a higher biomass of biofilm. (Fischer et al., 2002; Romaní et al., 2004; Teissier et al., 2007)

The velocity of a stream is the main driver determining the balance between sedimentation and resuspension of particulate organic carbon. High velocity erodes biofilm, decreases sedimentation and increases resuspension (Acuña et al., 2016). Aquatic biofilms integrate a variety of responses to environmental changes and chemical stressors. Their short generation times result in short-term functional and structural responses, making them useful as early indicators of disturbances (Sabater et al., 2007). Current global trends in flow regime reported an increasing frequency and intensity of extreme hydrological events (Acuña et al., 2016; New et al., 2001; Hirabayashi et al., 2008). In the Arctic it is especially after heavy rainfall or snowmelt that biofilm is exposed to high velocity due to floods involving catastrophic removal of most of the biomass (Acuña et al., 2004; Romaní et al., 2016).

The Arctic is warming more than six-fold faster than the global average (Huang et al., 2017). The Intergovernmental Panel on Climate Change predicts an increase of global warming and changing patterns of rainfall frequency and distribution. In the case of global warming, it is predicted that mean surface temperatures will increase about 2.6-4.8°C by 2100 (IPCC, 2013), and river water temperatures will increase consequently (Mohseni and Stefan, 1999). The consequences of such changes for aquatic biofilm structure and function is difficult to predict mainly due to biofilms being complex and dynamic assemblages of microorganisms (Romaní et al., 2016; Pastor et al., 2021).

Temperature is one of the major factors affecting different key physiological mechanisms such as respiration, primary production, and growth. In rivers and streams, changes in temperature are affecting biofilm the function, competition, and biomass as well as community structures such as trophic relationships (Zoppini et al. 2010, Romaní, 2016).

A study from 2021 by Pastor et al. included four of the six streams investigated in this study. The focus of their study was temperature-induced changes in biofilm organic matter utilization, working with enzymes. For example, warming might increase the content of polymeric substances in the biofilm, increasing its threedimensional structure (i.e., thickness) and suitable habitat matrix for heterotrophic communities with shading and organic matter accumulation. (Pastor, A., 2021).

By studying the environmental and chemical conditions of these streams, and by aligning them with predicted climate change outcomes, insight is gained into the possible futures of these biofilms and their stream ecosystems.

This report focused on benthic algae, which play a crucial role in biofilm, being the main primary producers in arctic streams with increasing dominance towards the North



Figure 1. Map of stream locations and pictures of each stream (1/S, 2/E, 3/L, 4/Z, 5/K, 6/C). Red-marked streams were not sampled. Blue-marked streams were sampled.

(Pastor, A., 2020).

The project aimed to conduct a comparative study on benthic algae in six streams on Disko Island, Greenland, emphasising the influences of varying environmental and chemical factors on their composition and biomass as of July 2023. The study aimed to understand to what extent the compositions and biomasses of benthic algae in small rivers and streams on Disko Island were affected by variations in chemical and environmental conditions, and to discuss how these variations aligned with predicted climate change outcomes. It was hypothesised, that the six streams would vary in their compositions and biomasses of benthic algae due to chemical and environmental variations such as riparian vegetation cover, light availability as well as water pH, temperature, and velocity .

Methods and materials

Study sites

Sampling was done at Disko Island, Greenland between July 10th and July 20th 2023. Disko is located 69°N, on the west coast of Greenland (Aastrup et al, 2016). The island is 8,578 km². It is primarily made up of tertiary basalts, which were first created during the tertiary period, where large amounts of lava and volcanic ash erupted from the ground and covered the entirety of Disko Island. These eruptions occurred multiple times and as the lava hardened, it became what we know as basalts (Jakobsen et al, 2000). The volcanic ash or dust hardening became what we know as tuff-layers, which are less resistant than the basalts. There is also the iron and aluminium rich laterite, which is made from leaching of the basalts (Barfod et al, 1962). These make up the mountains on Disko Island that range from 800-1900 m (Aastrup et al, 2016).

The goal was to re-sample six specific streams within walking distance from Arctic Station. However, due to aforementioned weather conditions, a bridge connecting to two of the streams had washed away, so these were unreachable. Two other streams near Sinifik were therefore selected as replacements. The six streams sampled (blue) as well as the two not sampled (red) were located as shown on *Figure 1*.

The streams are either glacier-fed, spring-fed or a mixture where spring water runs into the glacier-fed stream. Streams *3/L* and *6/C* are glacier-fed. Stream *5/K* is spring-fed. The rest are mixed.

At each stream location (1/S, 2/E, 3/L, 4/Z, 5/K, 6/C) three sampling sites (A, B and C) were chosen approximately 2 m apart. Each site was processed in upstream order, beginning at the downstream end of the stream and moving upward, in order to avoid disturbance between sites.

Chemical and environmental analysis

Chemical and environmental variables were classified or measured at each stream location. Measured variables included the depths and widths at three replicated sampling site (*A*, *B* and *C*). These were measured using a ruler and/or marked rope. The mean depths and widths were then calculated for each stream.

Vegetation height (cm) of plants and shrubs surrounding each stream was also measured. This was done by throwing a rock a couple of meters at random five times to minimise human bias. The highest point of vegetation where the rock landed was measured using a ruler. The mean vegetation height was then calculated for each stream.

Temperature (°C) of the stream water was also measured for each stream location. This was done using a *Vivatavia* groundthermometer.

Velocity was measured by following the travel time of a plastic vial $\frac{3}{2}$ filled with water over a 5 m stretch marked by a rope. Travel time per 5 m was measured using a mobile phone stopwatch (*Apple Inc.*), and *Velocity* (m s⁻¹) was then calculated.

A water sample was collected for measuring *pH, Conductivity* (μ S cm⁻¹), and *Specific*

Conductivity (µS cm⁻¹ at 25 °C) back at the lab. *pH* was measured using a *Hanna Instruments CHECKER1* pH-meter. Conductivity and Specific Conductivity was measured using an *YSI EC* conductivity-meter.

Classified variables included riparian vegetation cover (%), which was estimated at each stream location site (1A-6C).

Also classified at each site was the substrate composition. This was estimated at each site (1A-6C) with a percentage for the amount of bedrock (= > 4 m in diameter)(%), boulder (=0.256-4 m in diameter)(%), cobble (= 64-256 mm in diameter) (%), gravel (=2-64 mm in diameter) (%), sand (= 0.06-2 mm in diameter) (%) and silt (= < 0.06 mm in diameter)) (%). The mean percentage of each was then calculated for each stream. The predominant substrate composition type was then noted.

Further descriptions of the landscape and classifications of stream-type, *glacier-fed* and *spring-fed*, were also noted down at each stream. The presence of potential grazers was also noted here.

BenthoTorch analysis

A bbe BenthoTorch (hereinafter BenthoTorch) uses the in vivo fluorescence of algal cells to determine the intensity of the chlorophyll-a in a biofilm. This is used as a proxy for the amounts of green algae, cyanobacteria and diatoms (μ g chl-a cm⁻²) within a biofilm community.

Biofilm collection

At each site 3-4 stones of approximately 330 cm^2 were selected as being representatives of each site. These stones were placed in trays labelled *A*, *B* and *C*, photographed, and finally biofilm pigment compositions were analysed using the *BenthoTorch*. Contact with measured light-facing sides was held minimal during these proceduresto minimise human error. *BenthoTorch* measurements were taken on each of the three stones for every tray, and mean amounts of green algae, cyanobacteria and diatoms (µg chl-a cm⁻²) were then calculated per tray.

Next, a toothbrush was used to brush all of the biofilm from the light-facing surfaces of each of the stones into the tray. For this a 60 mL syringe was used to wet and wash the stones in an attempt to get everything off the light-facing surfaces. Water from the stream was used for this. The resulting biofilm slurries in the trays were collected and labelled plastic bottles and brought back to the lab for further analyses. This was repeated for the three sites at each stream location.

Dry weight analysis

On the day of collection the biofilm slurries were filtered using pre-weighed GFC filters, making sure to stir the bottle first. The filters were folded once, placed in aluminium foil, and frozen at -18°C until all the samples could be dried at the same time.

Thawed filters were dried at 60°C for 10 hours. Once dried, all the filters were weighed. For this a *Sartorius* lab scale with four digit precision was used. The surface area of the stones was calculated from the photographs using the free software *ImageJ*.

Calculations of the dry weight of the biofilm per stone area (mg cm⁻²) for each tray were done by using eq. (1).

$$D = \left(\frac{\left(\frac{w_2 - w_1}{(\frac{a}{A})\%}\right)\%}{S}\right) 1000$$
⁽¹⁾

D = dry weight of biofilm per stone area (mg cm⁻²), w_1 = weight before (g) w_2 = weight after (g), *a* = filtered biofilm slurry (mL), *A* = total volume of biofilm slurry (mL) and *S* = surface area of stones in tray (cm²). Og 1000?

Chlorophyll analysis

For chlorophyll analysis 2 mL of well-shaken slurry from each sample was filtered onto a GF/F filter using vacuum, and then the filters were submerged in 5 mL of ethanol in a Falcon tube to extract the chlorophyll. They were placed in the dark for over 12 hours. A *Turner Designs, Inc., Trilogy Laboratory Fluorometer* (hereinafter *fluorometer*) was then used to measure the chlorophyll-a content. The *fluorometer* was pre-calibrated and provided chlorophyll concentrations as μ g mL⁻¹ of slurry. The surface area of the stones was then used again for calculating *Chlorophyll-a* (μ g chl-a cm⁻²) on the stones using the eq. (2).

$$C = \frac{VA}{S}$$

Where $C = \text{Chlorophyll-a} (\mu \text{g chl-a cm}^{-2})$ on the stones in each box, V = Volume of chlorophyll-a per mL in sample ($\mu \text{g mL}^{-1}$), A = total volume of biofilm slurry (mL) S = surface area of stones in tray (cm²).

Microscopy

Slurry samples from each stream (1A-6C) were refrigerated and left for sedimentation. 1 mL slurry from a sedimented sample was placed onto a glass slide using a pipette. This was then mounted by placing a cover slip over the

sample and carefully applying light pressure with a plastic or wooden object to ensure the removal of any air bubbles. Morphological characteristics were used for identification of present algae on every slide down to genus level using an Olympus bx51 microscope at 400x magnification. Multiple sighting frequencies were noted in order to determine the genus of the presumed predominant algal species at each stream location. Approximately three drops from each sample (1A-6C) were examined. We used the keys: Freshwater algae: identification, enumeration and use as bioindicators (Bellinger and Sigee, 2015), Quick-guide to common filamentous algae New Zealand fresh waters (Niwa, 2019) and Quick-quide to common diatom genera New Zealand fresh waters (Niwa, 2019) to identify the benthic algae in our samples.

Data analysis

Data was analysed using RStudio (version 2023.06.1+524, © 2023 Posit Software, PBC formerly RStudio, PBC).

Analyses were conducted comparing results per stream location (1A-6C). A Shapiro-Wilk test indicated that data was not normally distributed, and a Kruskal-Wallis H-test was therefore chosen to test for a statistically significant difference between the six stream

Table 1. Overview of measured environmental variables (Mean Stream Width±SD (n=3), Mean Stream Depth±SD (n=3), Mean Riparian Vegetation Cover±SD (n=3), Mean Vegetation Height (n=5), Predominant SubstrateType (Bedrock (= > 4m in diameter), Boulder (=0.256-4m in diameter), Cobble (= 64-256 mm in diameter), Gravel (=2-64 mm in diameter), Sand (= 0.06-2 mm in diameter) and Silt (= < 0.06 mm in diameter)) for each stream (1/S, 2/E, 3/L, 4/Z, 5/K, 6/C).

(2)

Stream	Mean Stream Width±SD (cm)	Mean Stream Depth±SD (cm)	Mean Riparian Vegetation Cover±SD(%)	Mean Vegetation Height (cm)	Predominant Substrate Size
1/S	110±35	17±7	62±20	35.00	Cobble
2/E	240±114	11±3	77±12	19.00	Cobble
3/L	733±153	11±3	0±0	1.00	Bedrock
4/Z	163±21	21±3	68±18	63.00	Cobble
5/K	118±29	22±8	25±5	26.00	Boulder
6/C	85±17	11±4	40±26	14.00	Sand

locations with regards to measured variables. Results indicating a statistically significant difference (P<0.05) were then followed by Dunn-Bonferroni post-hoc test to identify which stream locations were statistically significantly different from one another (adjusted P<0.05). As data was positively skewed, Spearman's Rank Correlation was then applied to all variables with a statistically significant difference as indicated by the Kruskal-Wallis test, in order to measure the strength and direction of association between two ranked variables. Linear Regression Analysis was applied to the identified variables with a statistically significant correlation, in order to interpret the relationship between the variables.

Linear Regression Analysis between BenthoTorch green algae measurements and fluorometer results was additionally applied in order to test the strength of the BenthoTorch measurements as a proxy for algae composition.

Results

Chemical and environmental variables

The water temperatures ranged from 4°C to 10°C (see *figure 2*), where we found a significant difference between streams (*Kruskal-Wallis*, H-statistic = 17.00, p = 0.004, df = 5) (see *appendix table 6*). Statistically significant differences (*Dunn-Bonferroni*, p.adj<0.01) were seen between streams 3/L with the lowest temperature and stream 2/E with the highest measured temperature (see

appendix table 7).

The specific conductivity ranged from 44 to 79.1 μ S cm⁻¹ at 25 °C (see *figure 2*) where a significant difference (*Kruskal-Wallis,* H-statistic = 17.00, p = 0.004, df = 5) was found between the sites (see *appendix table 6*). The lowest measured specific conductivity was found in stream *6/C* and the highest in stream *3/L*, with significant differences between them (p<0.001) (see *appendix table 7*).

The velocity ranged from 0.438 m s⁻¹ at stream 6/C to 0.883 m s⁻¹ at stream 4/Z (see *figure 2*), with a statistically significant difference found between the streams (*Kruskal-Wallis,* H-statistic = 17.00, p = 0.004, df = 5) (see *appendix table 6*). Significant differences (p<0.01) were found between streams 4/Z and 6/C (see *appendix table 7*).

pH also varied with statistical significance (*Kruskal-Wallis*, H-statistic = 17.00, p = 0.004, df = 5) (see *appendix table 6*). The *pH* measured in the six streams ranged between 8 at stream 5/K and 7.2 at stream 3/L (see *figure 2*). We found a significant difference between streams 3/L and stream 5/K (p<0.01) (see *appendix table 7*). Stream 5/K was springfed and stream 3/L was glacier-fed. Looking at *appendix table 7* we see that the only results that could be explained by a difference in water source is *pH*, because there is a significant difference between these two streams, which differ entirely in their stream source type.

The mean vegetation heights (n=5) were also observed as having statistically significant

 Table 2. Table with an overview of comparable data from September 2018 (Pastor, A., et al. (2021)) (Stream = stream location name, Velocity, Width, Depth, Temperature, Conductivity and Chlorophyll-a.

Stream	Velocity (m s-1)	Width (em)	Depth (em)	Temperature (°C)	Condictivity (μ S cm-1)	Chlorophyll-a (µg chl-a cm-2)
3/L	0.00	130	16	1.1+/-0.5	56.00	0.47±0.23
1/S	0.15	50	14	1.6+/-0.5	63.00	0.48±0.15
2/E	0.37	NA	NA	2.5+/-0.7	64.00	1.16±0.19
5/K	0.11	130	5	3.5+/-0.3	102.00	0.35±0.06



Figure 2. Line plots of some chemical and environmental variables (Temperature, Conductivity, Specific Conductivity, pH, Velocity) for each stream (1/S, 2/E, 3/L, 4/Z, 5/K, 6/C).

differences between the streams (*Kruskal-Wallis,* H-statistic = 17.00, p = 0.004, df = 5) (see *appendix table 6*). Highest mean vegetation height was recorded as 65 cm for stream 4/Z, and lowest as 1 cm for stream 3/L (see *table 1*). There was a statistically significant difference between these streams (p<0.01) (see *appendix table 7*). Stream 4/Z was *mixed* and stream 3/L was glacier-fed.

Classified environmental variables also showed statistically significant differences between the streams. These were with regards to the widths of the streams (*Kruskal-Wallis*, H-statistic = 12.87, p = 0.025, df = 5), riparian vegetation covers (*Kruskal-Wallis*, Hstatistic = 13.08, p = 0.023, df = 5), and with regards to certain substrate sizes, specifically (Bedrock (= > 4m in diameter)% (Kruskal-Wallis, H-statistic = 15.68, p = 0.008, df = 5), Boulder (=0.256-4m in diameter)% (Kruskal-Wallis, H-statistic = 11.55, p = 0.041, df = 5), Gravel (=2-64 mm in diameter)% (Kruskal-Wallis, H-statistic = 11.99, p = 0.035, df = 5), Sand (= 0.06-2 mm in diameter)% (Kruskal-Wallis, H-statistic = 13.05, p = 0.023, df = 5) and Silt (= < 0.06 mm in diameter)% (Kruskal-Wallis, H-statistic = 13.35, p = 0.020, df = 5) (see appendix table 6). Recorded results of these can be seen in tables 1 and appendix table 5.

Grazers were observed at sites 6/C, 5/K, 4/Z, 2/E and 1/S. These grazers predominantly consisted of gnat larvae, with the largest number of observations at stream 6/C.

Some stream results (*3/L, 1/S, 2/*E and *5/K*) were comparable with data from a previous study done in September 2018 (Pastor, A. et al., 2021), hereunder velocities, widths, depths, temperatures, conductivities. *Chlorophyll-a* results were also comparable with some of our *BenthoTorch* and *fluorometer* results. Results from this 2018 study are shown in *table 2*.

BenthoTorch

An overview of mean *BenthoTorch* results for each stream (n=3) can be seen in *figure 3* for each stream. These results indicated a statistically significant difference between the streams both for green algae measurements (*Kruskal-Wallis*, H-statistic = 14.04, p = 0.015, df = 5), cyanobacteria measurements (*Kruskal-Wallis*, H-statistic = 13.96, p = 0.016, df = 5), and for diatom measurements (*Kruskal-Wallis*, H-statistic = 15.70, p = 0.008, df = 5) (see *appendix table 6*).



Figure 3. Boxplot of Algae (µg chl-a cm-2) composition as indicated by BenthoTorch measurements per stream (1= Stream 1/S, 2= Stream 2/E, 3= Stream 3/L, 4= Stream 4/Z, 5= Stream 5/K, 6= Stream 6/C), as shown for each algae type (Cyanobacteria, Diatoms and Green Algae)

Statistically significant differences (p<0.05) were observed between streams 1/S and 4/Z, and 3/L and 4/Z for green algae. For cyanobacteria these were observed between streams 1/S and 2/E, 2/E and 3/L, 2/E and 4/Z,

as well as streams 2/E and 5 (p<0.05). For diatoms such differences were observed between streams 2/E and 3/L (p<0.05) (see appendix *table 7*).



Figure 4. Linear plot of a Spearman's Correlation Test for Mean diatom per stream site (1A-6C) (n=3) and Temperature, indicating a strong positive correlation. Results indicate a statistically significant positive correlation (P-value= $9.9*10^{-5}$, alpha = 0.05, R=0.75).

Spearman's Rank Correlation was done with these BenthoTorch results, and chemical and environmental variables. Results of this only indicated a statistically significant correlation between the mean diatom results, and temperature of the streams (R= 0.79, p=9.9*10⁻⁵ < α = 0.05), as seen in *figure 5*. Linear Regression Analysis of this indicated a strong positive correlation between these two variables as indicated by *figure 4*.

Spearman's Rank Correlation was applied to all variables with a statistically significant difference as indicated by the Kruskal-Wallis test. These results are shown in a correlation matrix (figure 5). This plot illustrates the strength and direction of a potential correlation between algal biomass (based on chl-a concentrations) and the measured chemical and environmental variables, and could potentially be used to identify the causation of variation as shown by the Dunn-Bonferroni post-hoc results. We see that only the relation between Diatoms (µg chl-a cm⁻²) and Temperature(°C) (R= 0.79, p=9.9*10⁻⁵ < α = 0.05) provides a statistically significant positive correlation between dependent (algal biomass) and independent (chemical and environmental) variables.

Fluorometer

Figure 6 represents a plot of the *fluorometer* Chlorophyll-a results. These can be seen alongside mean *BenthoTorch* green algae results (n=3), and mean *BenthoTorch* summed algae results (n=3). Summed algae results were calculated by summing the mean *BenthoTorch* measurements of each algae type (green algae, cyanobacteria and diatom) per stream. Results here visually follow the same trend, but the *BenthoTorch* results were generally lower.

Kruskal-Wallis test results indicated a statistically significant difference between streams and these *fluorometer* Chlorophyll-a results (Kruskal-Wallis, H-statistic = 13.41, p = 0.020, df = 5) (see *appendix table 6*). Dunn-Bonferroni post-hoc results indicated that this significant difference was between streams 3/L and 4/Z (p<0.05) (see appendix *table 7*). These correspond to being the lowest and highest values measured, respectively, as presented in *figure 6*.

Linear Regression Analysis between BenthoTorch green algae results and fluorometer results in order to test the strength of the BenthoTorch measurements, can be seen in figure 7. A statistically significant strong positive correlation (Pvalue= $1.2*10^{-7}$, alpha = 0.05, R=0.92) can be seen, suggesting that BenthoTorch results resemble fluorometer results.

Dry weight

It is worth noting that dry weight is an indirect measurement for algal biomass as it is a measure of the total weight of the biofilm, including the non-algal biomass and inorganic matter.



Figure 5. Correlation matrix applied to all variables with a statistically significant difference as indicated by the Kruskal-Wallis test. The results are obtained with a Spearman's Rank Correlation on stream sites (1A-6C) as data was skewed (n=18). An 'X' indicates that the paired variables are not statistically correlated (p<0.05). The number on the intersection between variables indicates R for the paired variables. Colour hues give a visual indication as to whether the correlation is positive (green), or negative (orange). (Bedrock (= > 4m in diameter), Boulder (=0.256-4m in diameter), Cobble (= 64-256 mm in diameter), Gravel (=2-64 mm in diameter), Sand (= 0.06-2 mm in diameter) and Silt (= < 0.06 mm in diameter))

The mean results of these dry weight measurements per stream (n=3) can be seen in *figure 8*. Not much can be deduced from these results because we did not find a significant difference between our six streams (*Kruskal-Wallis*, H-statistic = 8.79, p = 0.118, df = 5) (see *appendix table 6*).

Microscopy

According to the *BenthoTorch* results (see *figure 3*), green algae should have been seen in every stream sample; these were, however,

only observed in streams 2/E, 4/Z, 1/S and 6/C as seen in table 3. Diatoms were also measured with the BenthoTorch in every stream, but only at very low numbers for stream 1/S and 4/Z. This compares well with the observed microscopy results from stream 2/E, 1/S and 6/C, where the highest numbers of diatoms were found as well. It does, however, not compare with stream 5/K where no algae was observed under the microscope. Finally, cyanobacteria, which were measured at stream 2/E and 6/C, aligned somewhat well



Figure 6. Plot of Algae (µg chl-a cm⁻²) (n=3) per stream (1/S, 2/E, 3/L, 4/Z, 5/K, 6/C) as indicated by Fluorometer results (greed, solid) mean BenthoTorch green algae results (n=3) (red, dotted), and mean BenthoTorch summed algae results (n=3) (dashed, purple). Error bars indicate mean±standard deviation

with the microscopy results, which found cyanobacteria in stream 6/C. None where however found in stream 2/E.

Microscopy results also show similarities between the streams. Cf. *Cylindrocapsa* was the most frequent green algae in all of the four streams where green algae were observed, while *Meridion* sp. was the most frequent diatom in all four streams where diatoms were observed. The similarities in the amounts of species within the three groups, ranking from the most diatoms to fewest cyanobacteria, is also noteworthy, as a clear pattern is seen.

Meteorological data

The weather in West Greenland was quite unusual this year, 2023, as lakes were still covered or some-what covered with ice in July as well as major floods that have caused bridges being flushed away as well as landslides. As seen in *table 4* it is clear to see that in 2023 far more rain has fallen in the months of May, June and July than there has been the previous eight years. In terms of temperature (see *table 4*), it is harder to see a clear distinction between 2023 and the previous eight years though the temperature in 2023 has generally been a bit cooler.



Figure 7. Linear plot of a Spearman's Correlation Test of BenthoTorch summed algae results and Chlorophyll-a Fluorometer measurements per stream location (1A-6C), on (Disko Island, Greenland) in July 2023. Results are here transformed with log(x+1), as data was positively skewed. Results indicate a statistically significant positive correlation (P-value=1.2*10⁻⁷, alpha = 0.05, R=0.92).



Figure 8. Mean Dry weight of biofilm on stones (n=3) for each Stream (1/S, 2/E, 3/L, 4/Z, 5/K, 6/C). Error bars indicate mean±standard deviation.



Figure 9. The four most frequent algae based on microscopy results. A: cf. Cylindrocapsa. B: Unidentified Cyanobacteria. C: Meridion sp. D: Unidentified Diatom.

Table 3. Overview of the different species of green algae, cyanobacteria and diatoms, respectively, observed under the microscope for each stream (1/S, 2/E, 3/L, 4/Z, 5/K, 6/C). Most frequently observed genuses are noted, as well as a numeric indication (Most frequent genus) of how many different species were observed (Number of species found).

Stream	Number of green algae species found	Most frequent green algae genus	Number of cyanobacteria species found	Most frequent cyanobacteria genus	Number of diatom species found	Most frequent diatom genus
S/1	0	NA	0	NA	1	Meridion
E/2	1	cf. Cylindrocapsa	0	NA	12	Meridion
L/3	1	cf. Cylindrocapsa	0	NA	NA	NA
Z/4	1	cf. Cylindrocapsa	0	NA	5	Meridion
K/5	0	NA	0	NÁ	NA	NA
C/6	7	cf. Cylindrocapsa	1	Unidentified	12	<i>Meridion</i> and an unidentified

Table 4. Overview of meteorological data (DMI) showing precipitation and temperature for the months May through August in Aasiaat. Mean± standard deviation results regard years 2014-2022 (n=9), which are being compared to results from 2023. Note: that the temperature and precipitation form august 2023 only represents the first 10 days of the month, since this project ended on the 15 august.

Month	Temperature mean±SD 2014-2022 (°C)	Temperature 2023 (°C)	Precipitation mean±SD 2014-2022 (mm)	Precipitation 2023 (mm)
May	-0.7±1.9	-1.3	14.6±12.8	59.2
June	3.7±1.2	1.2	13.6±9.9	45.3
July	6.9±1.1	5.5	34.8±27.5	114.5
August	6.3±0.4	6.0	56.4±38.4	4.2
September	2.8±1.2	NA	38.3±40.3	NA

Discussion

This study aimed to investigate how six arctic streams located on Disko Island (Greenland) differ in composition and biomass of benthic algae, and how they are affected by variations in chemical and environmental conditions, and to discuss how these variations align with predicted climate change outcomes.

Variation in composition and biomass of the benthic algae due to chemical and environmental variations

As mentioned, velocity is an important factor for algae, as it can be a determining factor for their ability to attach themselves and thrive in the stream (Zoppini et al., 2010; Acuña et al., 2016; Romaní et al.,2016.). A higher velocity could explain the lower *Chlorophyll-a* concentration (μ g chl-a cm⁻²) in the results (see *figure 3* and *table 2*), as they might have been washed away if they lack the proper attachment.

Compared with the study done by Pastor et al. (2021), a higher velocity is generally seen in this study's results ($0,68\pm0,17 \text{ m s}^{-1}$), than in Pastor et al.'s ($0,16\pm0,16 \text{ m s}^{-1}$). The differences in velocities between the two studies might be due to more than one combining factor, such as the increase in precipitation alongside the start of the glacier melting and late snowmelt this year. Another reason for the differences in velocities between the two studies might also be due to seasonal changes. This could be due to the streams in the study by Pastor et al. (2021) being sampled in September 2018 and this study's streams being sampled in mid-July. Temperature is also important in regards to benthic algae biomass and composition in streams (Luís et al.2013, Zoppini et al. 2010, Romaní, 2016). In regards to this, a positive relationship between temperature and diatoms was observed.

A higher stream temperature in this study's sampling $(6,25\pm2,63 \text{ °C})$ was recorded, than in the study $(2,18\pm1,06 \text{ °C})$ from 2021 (Pastor, A., et al. 2021). This could again be due to seasonality as temperatures often are lower in September than in July (see *table 4*).

In benthic stone samples, a number of small macroinvertebrates were observed. Biofilm serves as an important food source for macroinvertebrates. The only stream where no macroinvertebrates were observed was stream 3/L, which also had the highest velocity. Stream 3/L was a glacier-fed stream, and stream 2/E was a mixed stream of both glacial and spring water. An earlier study from Disko Island by Friberg et al. (2001) found a contrast in the amount of macroinvertebrates from the glacier-fed streams compared to the more stable groundwater streams. This may also be the case for this study's sampling sites, especially considering the high amount of precipitation and snowmelt in end of June / beginning of July 2023 on Disko Island (Table 4) that biofilm is exposed to high velocity, meaning floods involving catastrophic removals of most of the biofilm (Acuña et al., 2004; Romaní et al., 2016).

Microscopy

When comparing the microscopy results to the *BenthoTorch* data, an alignment was expected. Less algae was in general observed under the microscope than the *BenthoTorch* data would otherwise indicate, although this was to be expected as it could not be expected to observe all measured algae under the microscope. This was due to lack of time to go through the entire sample, the fact that the samples were quite murky, providing lots of hiding places for the algae, and quite possibly a lack of skill in looking through a microscope. Another factor explaining the differences could be a lack of skill in brushing off the biofilm in the field, thus not collecting every measured algae. *BenthoTorch* measurements were repeated on the rocks following the brushing, where small amounts of green algae were sometimes left behind. This aligns with the green algae being the one that was observed the least in comparison to the measurements. One could hypothesise that the green algae had better attachment in the biofilm, but this would require much further analysis to confirm.

Benthic algae in the future - a perspective on the impact of climate changes

There is no doubt that global warming and climate change will continue to have an effect on the arctic climate and especially the Arctic's hydrology (Huang et al., 2017; Yamanouchi and Takata, 2020; Box et al., 2019). However, little is known about to what extent and how severe the consequences will be for all organisms that reside in the Arctic. It is already known that the Arctic, which usually has been a predominantly snow-type precipitation regime, has shifted regimes to a predominantly rain-type precipitation (Bintanja & Andry, 2017). More precipitation and glacial meltwater will mean more runoff to freshwater systems and therefore an increase in velocity in streams. Freshwater streams are very unstable and therefore a more vulnerable ecosystem than most other freshwater systems which means that organisms which have adapted to these environments, will be put under further pressure (Pastor et al., 2021, Romaní et al., 2016; Riddle et al. 2008). As such the effects of an increase in precipitation, and therefore the velocity, in the streams of the Arctic will mean a higher rate of biodiversity loss for certain benthic algae species as well as a lower biomass when conditions become extreme (McCrystall et al., 2021).

In addition to precipitation and velocity, an increase in water temperature in streams will also have a big impact on benthic algae in the future, as supported by this study. The results showed a positive correlation between temperature and diatoms (R= 0.79, p= $9.9*10^{-5}$ < α = 0.05), which supports that with an increase in temperature (°C), amounts of *diatoms* (µg chl-a cm⁻²) will increase. An increase in water temperature may also result in alonger growth season as well as an earlier onset for the benthic algae (Ernakovich et al., 2014). This could also be a possible explanation as to why a positive correlation is seen between an increase in temperature and benthic algae.

In general, increasing temperatures and more extreme weather in the form of rain may have a major effect on the biomasses and compositions of benthic algae in freshwater streams in the Arctic (Gudmundsdottir et al., 2011; McCrystall et al., 2021). The data from this study and results from Disko Island support how an increase in precipitation, as well as glacier and snow melt has pronounced effects on the growth and establishment of benthic algae, in that results show signs of physical losses in comparison with prior studies (see Table 2 and Table 4). These low findings may however, also be due to other unforeseen variables, so further seasonal study is recommended.

In summary, the six streams on Disko Island varied in some chemical and environmental variables, and also in their compositions of benthic algae. The only correlation, however, between the benthic algae compositions and the chemical and environmental variables, was between temperature (°C) and *diatoms* (µg chl-a cm⁻²). This somewhat supports the hypothesis that the six streams would vary in their compositions and biomasses of benthic algae due to chemical and environmental variations such as riparian vegetation cover, pH, temperature, velocity and light availability.

It was further hypothesised that the late onset of summer and the heavy precipitation would affect the algal biofilm. When comparing chlorophyll-a measurements with the 2018 study (see *table 2*) this hypothesis was supported. This study is useful in showing the effects of more extreme environmental conditions. These results are also a clear testament as to why it is important to monitor seasonally, as this was a particularly odd season. With seasonal monitoring, a clearer picture of benthic algae in arctic streams would be seen, but this is but a moment in time.

Acknowledgements

The authors would like to thank Kirsten Seestern Christoffersen for planning and running this field course and making sure everything went smoothly. We would also like to thank the regular staff and the people participating in the Arctic field course 2023. Finally, we would especially like to thank Sanne Mariël Moedt for supervising and guiding us throughout the project design, setup, fieldwork and lab work.

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Appendices

Table 5. Mean±SD values of Substrate Composition (%) (n=3) for each type of substrate (Bedrock (= > 4m in diameter), Boulder (=0.256-4m in diameter), Cobble (= 64-256 mm in diameter), Gravel (=2-64 mm in diameter), Sand (= 0.06-2 mm in diameter) and Silt (= < 0.06 mm in diameter)) as observed at each stream (1/S, 2/E, 3/L, 4/Z, 5/K, 6/C).

Stream	Mean Bedrock±SD(%)	Mean Boulder±SD(%)	Mean Cobble±SD(%)	Mean Gravel±SD(%)	Mean Sand±SD(%)	Mean Silt±SD(%)
1/S	0±0	15±13	42±3	35±13	8±3	0±0
2/E	13±14	5±5	38±20	23±10	3±6	13±6
3/L	42±19	32±8	18±13	3±3	2±3	3±6
4/Z	0±0	12±10	52±14	27±15	5±0	5±0
5/K	22±13	45±22	25±5	8±6	0±0	0±0
6/C	0±0	3±3	27±12	2±6	42±19	5±0



Figure 10. Figures 10A, 10B, 10C: Bar graphs of mean composition of Algae per Tray (A,B,C) (n=3) as indicated by BenthoTorch measurements for every stream (1= Stream 1/S, 2= Stream 2/E, 3= Stream 3/L, 4= Stream 4/Z, 5= Stream 5/K, 6= Stream 6/C), as shown for each algae type (Green Algae, Cyanobacteria and Diatoms). Error bars indicate mean±standard deviation, indicating the variation between the stones of each tray.

Table 6. Overview of Kruskal-Wallis test results for every measured variable. Variable= measured variable, Unit= unit of measured variable, n= number of stream locations, H_statistic= Kruskal-Wallis H test statistic, df= degrees of freedom, p= p-value. Highlighted variables indicate p<0.05, which supports that for the specified variable, streams are statistically significantly different from one another. (Bedrock (= > 4m in diameter), Boulder (=0.256-4m in diameter), Cobble (= 64-256 mm in diameter), Gravel (=2-64 mm in diameter), Sand (= 0.06-2 mm in diameter) and Silt (= < 0.06 mm in diameter))

Variable	Unit	п	H_statistic	df	р
Mean Green Algae	μg chl-a cm-2	18	14.04	5	.015*
Mean Cyanobacteria	μg chl-a cm-2	18	13.96	5	.016*
Mean Diatoms	μg chl-a cm-2	18	15.70	5	.008**
Chlorophyll-a Fluorometer	μg chl-a cm-2	18	13.41	5	.020*
Temperature	°C	18	17.00	5	.004**
Specific Conductivity	μS cm-1 at 25 $^{\circ}C$	18	17.00	5	.004**
pH		18	17.00	5	.004**
Velocity	m s-1	18	17.00	5	.004**
Mean Vegetation Height	cm	18	17.00	5	.004**
Width of River	cm	18	12.87	5	.025*
Depth of River	cm	18	9.12	5	.104
Riparian Vegetation Cover	%	18	13.08	5	.023*
Bedrock	%	18	15.68	5	.008**
Boulder	%	18	11.55	5	.041*
Cobble	%	18	8.59	5	.127
Gravel	%	18	11.99	5	.035*
Sand	%	18	13.05	5	.023*
Silt	%	18	13.35	5	.020*
Stone Surface Area	cm2	18	3.61	5	.606
Dry Weight of Biofilm on Stones	mg cm2	18	8.79	5	.118

Note. * p < .05, ** p < .01, *** p < .001

Table 7: Overview of significant Dunn-Bonferroni post-hoc test results for measured variables, where differences were significant (Adjusted p<0.05). Variable = measured variable, Streams= compared stream locations, Dunns_Test_Statistic =Dunn-Bonferroni post-hoc test statistic, p= p-value, Adjusted_p_value = Bonferroni adjusted p value, Significance_Level = significance level as indicated below. These variables indicate p<0.05, which supports that the specified streams are statistically significantly different from one another with regards to the prescribed variable.

Variable	Unit	Streams	Dunns_Test_Statistic	р	Adjusted_p_value	Signicifance_Level
Mean Green Algae	µg chl-a cm-2	1_4	3.01	.003**	0.04	*
Mean Green Algae	µg chl-a cm-2	3_4	3.01	.003**	0.04	*
Mean Cyanobacteria	µg chl-a cm-2	1_2	2.94	.003**	0.05	*
Mean Cyanobacteria	µg chl-a cm-2	2_3	-2.94	.003**	0.05	*
Mean Cyanobacteria	µg chl-a cm-2	2_4	-2.94	.003**	0.05	*
Mean Cyanobacteria	µg chl-a cm-2	2_5	-2.94	.003**	0.05	*
Mean Diatoms	μg chl-a cm-2	2_3	-2.94	.003**	0.05	*
Chlorophyll-a Fluorometer	µg chl-a cm-2	3_4	2.99	.003**	0.04	*
Temperature	°C	2_3	-3.48	<.001***	0.01	**
Specific Conductance	μS cm-1 at 25 °C	3_6	-3.48	<.001***	0.01	**
pH		3_5	3.48	<.001***	0.01	**
Velocity	m s-1	4_6	-3.48	<.001***	0.01	**
Mean Vegetation Height	cm	3_4	3.48	<.001***	0.01	**
Width of River	cm	3_6	-3.14	.002**	0.03	*
Riparian Vegetation Cover	%	2_3	-3.02	.003**	0.04	*
Sand	%	5_6	3.11	.002**	0.03	*
Silt	%	1_2	2.99	.003**	0.04	*
Silt	%	2_5	-2.99	.003**	0.04	*

Note. * p < .05, ** p < .01, *** p < .001

Re-visiting the zooplankton communities in Lillesø, Mellemsø and Morænesø at Disko Island, Greenland.

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Abstract

Climate change is having an amplified effect on the Arctic compared to other parts of the world and average temperatures are rising considerably. It's therefore vital to collect reproducible data to monitor changes in the Arctic ecosystems. The rising temperatures affects a lot of different species in freshwater lakes and the surrounding vegetation. In this re-sample study we investigate the species composition of plankton communities in three different lakes at Disko in Greenland; Lillesø, Mellemsø, and Morænesø. To look into possible changes of species composition, the data was compared to data from 1962 and 2004.Our data showed fewer zooplankton species in 2023 than found in previous years. Furthermore, the chlorophyll concentration was measured as a proxy of phytoplankton abundance, which showed lower concentrations compared to 2004 and 2017. In 2023 spring arrived later than previous years which might have influenced the composition of zooplankton and phytoplankton communities as well as the concentration of chlorophyll.

Keywords: Zooplankton, Arctic, Lakes, Re-sample, Vegetation

Introduction

Earth is becoming warmer, and the consequences of this will touch every part of the food chain, even the smaller but crucial organisms like zooplankton will be affected. The annual surface temperature has increased 1.1 °C above the annual temperature from 1850 – 1900, which will likely keep increasing as global greenhouse gas emissions have continued to increase due to human activities like land-use, lifestyle, consumption, and production (IPCC 2023). The effects of climate change are already happening in the Arctic and can be measured by increasing temperatures, resulting in less sea ice formation and the loss and gain of species.

Increasing temperatures alter seasonal cycles. Of all the lakes in the world, 25% are located in the Arctic (Lehner et al. 2004). Arctic lakes can be turbid due to glacial runoff or clear by not receiving direct glacial water. This difference plays an important role with



Figure 1. Schematic overview of the interactions between the classic, benthic, and microbial food-web in Arctic lakes (From: Jeppesen et al. 2021 with permission from the Pulisher).

regards to the physical, chemical, and biological conditions within the lakes (Jeppesen et al. 2021).

Generally, the biological communities in these Arctic lakes are characterized by having few species and low productivity (Rautio et al. 2011; Christoffersen et al. 2008b). Phytoplankton found in a clear lake include diatoms, dinoflagellates, cryophytes, and cyanobacteria, but the overall primary productivity is low compared to a nutrientrich lake. During the the polar night no photosynthesis is happening, and the process is being induced as soon as the light penetrates the snow and ice in spring. Phytoplankton is an important food source for zooplankton, which in turn acts as a crucial component of Arctic lakes (Rautio et al. 2011). Zooplankton allows income of nutrients to the lower trophic levels in the freshwater ecosystems, whilst guaranteeing food to the upper levels, as depicted in figure 1. A change in the biodiversity of any of these levels will affect the predator-prey interaction. This will lead to a degradation of the complete aquatic

ecosystem (Jeppesen et al. 2021).

Lakes located in the Arctic are ice dominated, resulting in limited dispersal and a lower resource availability creating a less species rich environment (Christoffersen et al. 2008a). Most zooplankton, including cladocera and some copepods, are sensitive to low food concentrations and to low water temperatures. Therefore, only few species are found in these lakes.

Cladocera includes the *Daphnia*, which can be found in spring after the ice melts and hatching from a resting egg to feed on phytoplankton.

With less food available in the Arctic, the Arctic *Daphnia* has developed a lower food threshold for growth, compared to the temperate *Daphnia*. Due to this adaptation, not all *Daphnia* overwinter as a resting egg, but some will stay as an adult, living off the accumulated fat reserves and thereby surviving the less food during winter. In the presence of fish, *Daphnia* might be



Figure 2. Overview of the lakes where the samples were taken from. Lillesø and Mellemsø, located close to Kangerluk, were reached by boat and then by foot. Morænesø, located near Arctic station was reached by foot.

substituted by smaller species like Bosmina.

Rotifers form often the most abundant zooplankton group in Arctic lakes. They will either produce resting eggs during winter or overwinter as adults like some of the *Daphnia*.

As freshwater ecosystems are found throughout most of the Arctic, future temperature increases, and permafrost thawing will have a large impact on the fine balance currently exciting in Arctic lakes and surrounding vegetation. Depending on the size of the lake, some freshwater areas might be expanding, receiving more nutrients from the tundra, whilst other areas might face landscape erosion, leading to drainage and loss of the lake (Rautio et al. 2011). Increasing temperatures will also lead to longer ice-free periods and a water column stabilisation during summer, leading to changes in the species composition, due to different tolerance to temperature (Jeppesen et al.

2021).

Changes in vegetation structure and plant communities surrounding freshwater can have a direct effect on the influx of water from the mountains and into the streams and lakes, and thereby play a role in the balance of the ecosystem in the lake. Monitoring vegetation surrounding lakes, can therefore act as an indirect indicator of changes within the lakes, like they observed in a study by Hobbie et al. (2017)

Preserving zooplankton and monitoring the species composition of zooplankton in Arctic lakes is crucial to assure a well-functioning ecosystem and food-web. This study therefore aims to resample lakes that has been assessed several times over the last approx. 60 years with focus on zooplankton communities in order to relate the present community compositions with the ones described in the past. To understand how climate changes may



Figure 3: Showing the vegetation sample sites of the three lakes. The samples were collected from the shore of the lake 1, 5 and 10 meters respectively.

have affected the zooplankton composition it is necessary to include historical weather data and finally to understand food conditions for the present zooplankton population we have included measurements of the phytoplankton communities.

Based on the current knowledge of the Arctic and its response to climate change, we have hypothesised that the chlorophyll concentration has increased due to a shorter period with snow cover, resulting in light not being a limiting factor and optimising growth conditions for phytoplankton. Another hypothesis was to find a similar diversity of zooplankton communities in the lakes this summer as were found in previous studies by Røen et al. (1962) and Christoffersen et al. (2004). This is because we were expecting a similar composition of phytoplankton as observed in previous years.

We also hypothesized, that the species composition of water plant changed in relation to the distance from the lakes edge. We expected that mosses and lichens grew closer to the lakes edge and an increase in shrubs and taller vegetation with increased distance from the lakes.

Methods and Materials

Study sites

Three freshwater lakes were included: Lillesø, Mellemsø and Morænesø (figure 2). Lillesø and Mellemsø were visited on the same day, and they were reached by boat. The two lakes were located around 500 to 1000 meters from the coast and are surrounded by low mountains. The samples from Morænesø



Figure 4. Boxplot of chlorophyll measurements fror lillesø, mellemsø and moræne sø. were taken two days later than the other two. It was located around 3000 meters from the Arctic station and reached by foot. There was still part ice on all the lakes (Appendix 1) which limited the opportunities to take samples from all around the lakes.

Chlorophyll

In each lake triplicate water samples were collected in a bucket from a rubber boat. After the water was collected it was mixed well in the bucket and then 1 litre of the water from each sample was filtered through a GF/C filter in the field. The filtration process used a vacuum filtering unit and a hand pump. The filters were then folded in the middle, wrapped in aluminium foil, and kept cold and dark until placed in a freeze (within max 8 h). After a few days the filters were thawed and placed in Falcon tubes with 5 ml of 96% ethanol. The tubes were left in a dark cabinet overnight. The samples were shaken, and a small amount of the extract was transferred to a glass cuvette and analysed by a Turner Fluorometer previously calibrated against a chlorophyll a standard.

The data was tested with an ANOVA test using R studio with a significance level at 0.05.

Plankton diversity

Phytoplankton bulk samples were collected with a 20 μ m plankton net which were thrown at least 15 times from different areas of the lake shoreline. Zooplankton samples were collected in the same way with a 200 μ m plankton net. The samples were stored in glass bottles and fixed with Lugols solution. The zooplankton was identified to genus or



Figure 5. Daphnia Pulex resting egg found in Morænesø July 2023.

Table 1. Overview of the zooplankton found in Lillesø, Mellemsø and Morænesø in 1962 and 2023 respectively. Moreover, the
zooplankton found in the 17 lakes investigated in 2004 are also shown. All the x's mark the species found and the dots mark
where the species are not found

Locality		Lille-1962	Mellem-1962	Moraene-1962	2004	Lille-2023	Mellem-2023	Moraene-2023	
Cladocera	Daphnia pulex			x	х			x	
	Ceriodaphnia quadrangula		x		х				
	Alona affinis	x							
	Macrothrix hirsuticornis		x						
	Candona candida	x	x						
	Candona falcata		x						
	Polyphemus pediculus			x					
	Acroperus harpae		x	x					
	Simocephalus vetulus		x	x	х				
	Bosmina coregoni				х				
	Bosmina longirostris	x			х		x		
	Scapholeberis mucronata				х				
	Alona rectangula	x			x			x	
	Alona quadrangularis	x	x		х		x		
	Alonella nana		x		х				
	Chydorus sp.				х				
	Eurycerus glacialis			x	х		x	x	
	Harpaticoidea				х				
Copepoda	Cyclops sp.	x	x	x	х		x	x	
	Diaptomus sp.	x	x		х		x		
Rotifera	Filinia sp.				х				
	Trichocerca sp.				х				
	Lecane sp.				x				
	Keratella cochlearis				x				
	Keratekka ssp.				x				
	Euchlanis sp.				x				
	Brachionus sp.				х		x		
	Lepadella sp.				х				
	Polyarthra ssp.				x				
	Asplanchna priodonta				х	x	x		
	Conchilus sp.				х				
	synchaeta sp.				х				
	Notholca labis				х		x		
	Notholca sp.				х				
Triopsidae	Lepidurus arcticus			x	х			x	
	Numbers of species	7	10	7	29	1	8	5	

species using a stereolup. The phytoplankton were identified to family using a microscope. Both Phytoplankton and Zooplankton were identified using different identification keys. These were by Nygaard (2001), Harding et al. (1974), Scourfield et al. (1966), and Böcher et al. (2005).

Vegetation

To determine the vegetation around the lakes a string of four meters was used to create a square which measured one square meter in total. For each lake three squares were made respectively 1, 5 and 10 meters (figure 3) from the shore of the lake.

Every single plant within the square was photographed with a phone and Normander (2022) was used to identify the vegetation down to genus and species level where possible.

Results

Chlorophyll

Out of the three sampled lakes, the chlorophyll concentrations at Lillesø were

highest measured at 3.2 μ gL⁻¹ and the lowest concentrations at Morænesø with a measurement of 0.73 μ gL⁻¹ as seen in figure 4.

There was a large variation among the triplicate samples from Lillesø which ranged from 0.96 to $3.2 \ \mu g L^{-1}$. The reason for this is unknown. The ANOVA test had a P-value of 0.385, meaning that there is no significant difference in the chlorophyll levels of the three investigated lakes.

Zooplankton and phytoplankton

The number of zooplankton species differed between the lakes as only species was found in Lillesø, 8 in Mellemsø, and 5 in Morænesø (table 1). In Lillesø we only found Asplanchna priodonta. The samples from Mellemsø were dominated by rotifers, mostly Asplanchna priodonta but we also found Brachionus sp. and Notholca labis.

Furthermore, we found 2 species of copepods; *Cyclops sp.* and *Diaptomus sp.* Lastly, we found 3 species of cladocera; *Bosmina longirostris, Alona quadrangularis* and *Eurycerus glacialis.* In Morænesø the samples were dominated by *Cyclops sp.*, but we also found *Lepidurus arcticus* and 2 different species of cladocera; *Alone rectangula, Eurycerus glacialis* and *Daphnia pulex.*

The phytoplankton were specified to family and compared with the data from 2004 (table 2). In Lillesø we found the families; *Chlorophyceae, Dinophyceae, Chrysophyceae, Diatomophyceae*. In Mellemsø, where most families were observed, we found; Chlorophyceae, Nostocophyceae, Dinophyceae, Chrysophyceae and Diatomophyceae. Lastly, in Morænesø we found; Nostocophyceae, Dinophyceae, Chrysophyceae, Diatomophyceae.

Vegetation

The vegetation composition around all three lake locations was very similar. It was composed of bryophytes, lichens, deciduous plants, smaller evergreens, forbs and a few graminoids. The species diversity varied slightly from each location and distance from the lake (Table 3).

However, genus such as Salix sp. and Bryophyta sp. were present at all three locations. From the transects made, the vegetation around Morænesø was the most species rich and Mellemsø was the least species rich. Five species and 8 genera were identified at Morænesø. At Lillesø 5 species and 5 genera were identified and at Mellemsø, 3 species and 5 genera were identified. Forbs such as *Pyrola sp.* and Ranunclulus lapponicus were mostly found in 1-5 m from the lakes edge whereas graminoids and such as Carex sp. and lichens such as Stereocaulon sp. and Cladonia *borealis* were found 5-10 meters along the transect. Bryophytes and evergreen shrubs such as Empertrum hermaphroditum and *Cassiope tetragona* were found along the whole transect; 1.5 and 10 meters from the lakes edge.

Weather data

Locality	2004	Lille-2023	Mellem-2023	Moraene-2023	
Chlorophyceae	x	x	x		
Prochlorotrichaceae	×				
Nostocophyceae	×		×	x	
Cryptophyceae	×				
Dinophyceae	×	×	x	x	
Chrysophyceae	×	x	×	x	
Diatomophyceae	×	x	×	x	
Prymnesiophyceae	x				

Table 2. Overview of the phytoplankton families found in the investigated lakes from 2004 and 2023. The x's mark where the families are found and the dots mark where the species are not found. For 2004 the phytoplankton found are a total from all the 17 investigated lakes.



Figure 6. Graph showing the average temperature for Ilulissat in June in the last 70 years (1950-2020; blue) plus this year (2023; orange). The temperatures are shown at the y-axis and the x-axis shows the year. The dotted line shows that the temperature is increasing throughout the years. The orange dot shows that the average temperature for June in 2023 is way lower than the past 20-30 years. (Cappelen 2021)



Figure 7: Graph showing the average temperature for Ilulissat in July in the last 70 years (1950-2020; blue) plus this year (2023; orange). The temperatures are shown at the y-axis and the x-axis shows the year. The dotted line shows that the temperature is increasing throughout the years. The orange dot shows that the average temperature for July in 2023 is similar or slightly lower than the past 20-30 years. (Cappelen 2021)

The average temperatures for both June and July have increased through the last 70 years (figure 6 and 7). Comparing this data with the average temperature from this year (2023) it is clear that the average temperature for June is lower than the last 20-30 years. It is slightly different for July where the average temperature for 2023 is below the trendline. However, it is close to the previous measurement (Cappelen 2021).

Discussion

Climate change is having an amplified effect on the arctic ecosystems and with rising average temperatures, the growing season is expected to increase (IPCC 2023; Cappelen 2021). It was expected that the chlorophyll



Figure 8: Bar-plot of the chlorophyll concentrations from Lillesø, Mellemsø, and Morænesø. The x-axis shows the chlorophyll concentrations, and the y-axis shows the two years.

concentration had increased due to a shorter period with snow cover, eliminating light as a limiting factor and making the growth conditions better for algae with chlorophyll. However, we found the chlorophyll concentrations to be lower than in observed in 2004. A reason for this is that the summer was delayed due to large amounts of snow and rain until June 2023 and all lakes being partially covered by ice when the samples were taken (Appendix 1). Due to a delayed spring the summer has also been delayed and the growing season has become shorter. This is the opposite of what we expected and highlights the difficulties of comparing data in an ecosystem with such extreme seasonal variability.

Because we only have one measurement of the chlorophyll levels in the lakes from 2004, it is not possible to do statistical tests to compare the results from 2004 with the

Vegetation 2023	1m	5m	10m
Lillesø	Betula nana	Cassiope tetragona	Cassiope tetragona
	Cassiope tetragona	Bryophyta sp.	Empetrum hermaphroditum
	Empetrum hermaphroditum	Peltigera aphtosa	Salix sp.
	Pyrola sp.	Pyrola sp.	Sphagnum sp
	Ranunculus lapponicus	Salix sp.	
	Sphagnum sp.	Stereocaulon sp.	
Mellemsø	Bryophyta sp.	Cladonia borealis	Carex sp.
	Salix sp.	Bryophyta sp.	salix arctophila
		Salix arctophila	Sphagnum sp.
		Sphagnum sp	Stereocaulon sp.
		Stereocaulon sp	
Morænesø	Carex sp.	Bryophyta sp.	Carex sp.
	Empetrum hermaphroditum	Carex sp.	Bryophyta sp.
	Equisetum sp	Empetrum hermaphroditum	Cassiope tetragona
	Pyrola sp.	Equisetum sp.	Cladonia borealis
	Salix sp.	Pedicularis sp	Empetrum hermaphroditum
	Sphagnum sp.	Pyrola sp.	Equisetum scirpoides
		Salix sp.	Peltigera aphtosa
			Salix sp.
			Stereocaulon sp

Table 3: Overview of the vegetation transects and species observed at Lillesø, Mellemsø and Morænesø at 1,5 and 10m from the lakes edge.
Table 4. Overview of vegetation described by (Røen, 1962) for Lillesø, Mellemsø and Morænesø

Røen vegetation (1962)	
Lillesø	Hipperus sp.
Mellemsø	Angelica sp.
Morænesø	Hippuris sp.
	Batrachium
	Bryophyta sp.

results from 2023, but the mean chlorophyll level in 2023 is lower than the measurements from 2004 in all three lakes (see barplot in figure 8).

Considering the variance in the results from 2023, the difference between 2004 and 2023 is not big in Lillesø and Morænesø. This is however not the case for Mellemsø, where the chlorophyll level was measured to 7.8 μ gL⁻¹ in 2004 and the mean from 2023 was only 1.7 μ gL⁻¹.

Christoffersen et al. (2008b) showed correlation between low water temperature and chlorophyll in arctic lakes at Zackenberg over 9 years. The difference in chlorophyll concentration from 2004 to 2023 in Mellemsø can be explained by this temperature difference, as it was measured to be 6.6 C° in 2004 and only 3.4 C° in 2023 (Table 5). For Morænesø there are data from recent years to compare with (Sigsgaard 2018). The average concentration of chlorophyll for Moræne sø in 2023 was 0,96 µgL⁻¹ which is a lower amount than the concentration measured back in 2017 at 4,7 µgL-1 in July, this indicates the late arrival of spring in 2023 (Sigsgaard 2018).

The ANOVA test did not show any significant differences in chlorophyll levels between the 3 lakes. This could be because all three lakes had only just started their primary production and haven't reached their full potential yet. Earlier studies in Zackenberg have shown that a late ice- out can limit the phytoplankton photosynthesis due to lack of light and nutrients (Christoffersen et al. 2008b). The chlorophyll levels from 2023 are very low in general, resulting in low food availability for the zooplankton of the lakes. This can explain why we see fewer species of zooplankton in 2023 than in past years. It could however also be because our samples look more like spring samples than summer samples.

Zooplankton and phytoplankton

We found far less Zooplankton than in 1962 and 2004 when 24 and 29 different species were found, respectively. In both 1962 and this year (2023) the highest species count was in Mellemsø. It is not possible to compare this directly with those found in 2004 as this data is based on species found in a total of 17 lakes.

Overall, fewer Cladocera species were found this year than in 1962 by Røen and by Christoffersen et al in 2004. This is not as hypothesised as it was expected to find the same species if not more due to the climate change, where warmer temperatures could lead to more nutrients from thawing permafrost, and thereby more favourable conditions for the plankton (Jeppesen et al. 2021). The average temperature for Ilulissat, over the last 70 years has increased over time (Cappelen, 2021). Ilulissat is located fairly close to Qequetarsuag and so the temperatures at the sampling sites are expected to be relatively close. As the average temperatures have been rising it has caused

Table 5. pH and water surface temperature data from Lillesø, Mellemsø and Morænesø from 1962, 2004 and 2023. Data taken from Røen (1962), (Christoffersen et al., 2004), and data collected by William Himmelstrup

• • • • • • • • • • • • • • • • • • • •			
Lillesø	рН	Temperature (surface)	
1962	-	8,9	
2004	7,49	10,9*	
2023	8,7	4,8	
Mellemsø	рН		
1962	-	5,4	
2004	8,25	6,6*	
2023	6,7	3,4	
Morænesø	рН		
1962	-	8,8	
2004	7,55	9,9*	
2023	6,3	8,3	

shorter winters and longer summers. As the winters become shorter (IPCC 2023), it was expected that the zooplankton would have had longer time to develop, breed, and hatch and would have been easy to find in the lakes. However, this was not the case when overall fewer species were found than past years. By the time our team reached the lakes, they were still partly covered in ice (Appendix 1) which is not normal for July. Comparing the temperature in the lake with data from 1962, it is much colder than it was then. Especially, in Lillesø and Mellemsø the temperatures were around four and two degrees colder, respectively, than in 1962 (Table 5). Other than that, it was colder than in 2004, as well (Table 5). Considering the life cycle of the zooplankton, especially the Cladocera, longer winters and colder temperatures make them hatch from their eggs later (Jeppesen et al. 2021). We found a resting egg from a Daphnia pulex in Morænesø (Figure 5) where we also found adult Daphnia pulex.

Finding resting eggs so late in the growing season could be an indication of the conditions not being optimal and the water temperatures being too low for resting zooplankton eggs to hatch. However, Morænesø was the lake with the least temperature differences compared to previous years. Another reason for not finding as many zooplankton species this year could, as mentioned previously, be due to the lack of food available for the zooplankton to feed on.

Most phytoplankton families found are the same, but in 2004 three more families were found within the 17 lakes they investigated. In both Lillesø and Morænesø 4 different families were found and in Mellemsø 5 different families were found. The samples contained families that are common to find in clear arctic lakes like dinoflagellates, diatoms and chrysophytes including the very common *Dinobryon.* But they also contained fewer common families like pelagic cyanobacteria (*Nostocophyceae*) and chlorophytes (Jeppesen et al. 2021).

We found most phytoplankton and zooplankton groups/species in Mellemsø and

Table 6 Chlorophyll measurements from Lillesø, Mellemsø,
Morænesø in µgL ⁻¹

Sample no.	Lillesø	Mellemsø	Moræne
1	0.96	1.19	0.96
2	3.2	1.89	0.73
3	1.27	2.01	1.18
Mean	1.81	1.7	0.96

least in Lillesø. One reason for Lillesø not being very species rich could be down to the low temperatures. Both in 1962 and 2004 the lake temperature was 8,9 and 10,9 degrees whereas we measured the surface temperature of the lake to be 4,8 degrees in 2023 (Table 5). This is a considerable difference and is likely to have an effect on the species richness to some extent.

Vegetation

The degree of summer warming is having a profound effect on tundra vegetation, increasing productivity over much of the Arctic (Elmendorf et al., 2012). Obtaining statistically significant trends in a changing Arctic ecosystem is extremely difficult due to high annual and seasonal variability (Hobbie et al., 2017). It is therefore vital to develop an understanding of the tundra vegetation over time and create reproducible data for future re-sampling and comparison. Therefore, even though we focused on the biotic factors within the sampled lakes, we felt it was important to note the surrounding vegetation of the lakes sampled for future reference.

Røen (1962) was the last to briefly describe the surrounding vegetation of Lillesø, Mellemsø and Morænesø. He described the presence of the aquatic plants *Hipperus* at both Lillesø and Morænesø and *Batrachium* at Morænesø. We didn't find any *Hippuris* or *Batrachium* at any of our resampling lakes, however we concentrated on the vegetation around the edge of the lake and didn't observe any aquatic plants at any of the sample sites. As the spring and summer of 2023 have slightly deviated from the norm (Cappelen, 2021). Mellemsø and Lillesø were still largely covered by ice when samples were taken on the 15th of July 2023. Røen (1962) described the lakes to be either ice free or with relatively little ice cover upon sampling in July 1955 -56.

This is an indication of the spring being much later this re-sample year, which could explain the absence of aquatic plants and have an effect on the vegetation surrounding the lakes. Røen (1962) described the "luxuriant vegetation of Angelica " around the edges of Mellemsø. In our resampling project we haven't identified Angelica within our transects surrounding Mellemsø (Table 3) and a reason for this could be due to late start of the growing season and some species taking longer to establish themselves. At Morænesø, sphagnum sp. and other bryophytes were identified within the vegetation transects this year, where Røen (1962) also described the presence of mosses at Morænesø.

Even though Røen (1962) hadn't mentioned many of the species we found along the transects surrounding the sampled lakes, they are common for an ecosystem situated between the low and high arctic. Both (Elmendorf et al., 2012; Hobbie et al., 2017) studied tundra and ecosystem vegetation changes in response to climate change and summer warming and sampled plots at similar latitudes with similar descriptions to the vegetation surrounding the lakes on Disko Island. Surrounding all three lakes we found byrophytes, lichens, deciduous plants, smaller evergreens, and shrubs as well as forbs and graminoids which (Elmendorf et al., 2012; Hobbie et al., 2017) also identify in their plots.

A reason for finding fewer forbs with increased distance from the lake could be due to the increase in competition for space and the larger and more extensive vegetation has an advantage.

We found fewer species at Lillesø and Mellemsø than at Morænesø which are situated in the middle branch of Disko Fjord, Kangerluk, where the temperatures and weather conditions may have varied slightly from Morænesø situated at the edge of a moraine in Blæsedalen. The vegetation surrounding Morænesø may have been free of snow for a longer period of time allowing for a more species rich community with increased vegetation growth compared Lillesø and Mellemsø in Disko Fjord.

Conclusion

Due to the later spring and ice still partly covering the lakes in mid-July, the onset of phytoplankton production has possibly been affected. The chlorophyll concentration, and therefore the phytoplankton production, was lower than that of 2004 and 2017, respectively. This is the opposite of what we hypothesised.

We found a lower diversity of species richness of zooplankton compared to Røen and Christoffersen which is not as hypothesised.

However, this is believed to be caused by the long winter and therefore, some of the zooplankton species could still be in resting states.

As we hypothesised, we found a pattern in the distribution of vegetation in relation to the distance from the lakes edge. In a changing climate, we hope this data can be used for future reference of change at these three locations.

Carrying out field work on Disko Island in July 2023 has been a clear example of how variations in the arctic weather and seasonal changes make it difficult to collect significant data. Re-sampling zooplankton and phytoplankton as well as identifying surrounding vegetation is an important part of developing an understanding of the changing arctic climate and what we can expect for the future.

Acknowledgements

We would like to thank Kirsten S. Christoffersen and Sanne M. Moedt for their guidance throughout the course in Arctic Field Biology and in producing a report. We would also like to thank Victoria Westmark Sønnichsen and William Himmelstrup for allowing us to use some of their data in our rapport. Lastly, a big thank you to everyone at the Arctic Station in Qeqertarsuaq for an incredible experience on Disko Island.

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Appendices



Appendix 1: Picture of the three lakes. Here it is possible to see how much the lakes are covered with ice. Lillesø, Mellemsø and Morænesø from left to right.

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