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Inter-fjord variations in species composition in Svalbard as revealed by eDNA metabarcoding: evidence of "Atlantification"?

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PREFACE

This thesis is the final product of my MSc in Ecology at the University of Life Sciences (NMBU). This past year have brought me many new experiences and knowledge. I want to thank my lovely supervisors and everyone that has contributed to my thesis for all their guidance, support, and encouragement through this whole process. I wish to thank my main supervisor Thrond for excellent help with my statistics, and for all feedback and encouragement. I want to thank everyone at Havforskningsinstituttet that took me in and made me feel welcomed, and that made this project possible. Thanks to Tanja for all your help with the laboratory work and your patient in explaining the procedures. Thanks to my co-supervisor Jon-Ivar for all guidance and feedback, and especially thanks for bringing me along for the cruise, it was such a wonderful experience. Finally, I want to thank friends and family that has supported me through this whole process, I could not have done this without you.

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ABSTRACT

Marin ecosystems faces mass extinction from climate change, and the Arctic marine ecosystems have experienced non-linear change the last decade. “Atlantification”, movement of warm and saline water being moved northward by the West Spitsbergen Current (WSC), has led to alteration of the Arctic marine environment and northward movement of Atlantic and sub-Arctic fish species. Knowledge of species distribution is critical to ecological management and conservation biology. Effective management requires the detection of populations, and biodiversity monitoring is necessary to provide baselines for policies. Recently, there has been considerable interest in the detection of short species-specific environmental DNA (eDNA) fragments to allow aquatic species monitoring within different environments due to the potential of greater sensitivity over traditional survey methods which can be invasive, time-consuming and costly. As well as have limitations in detecting species of low densities and is usually based on visual detection and counting.

Water was sampled from two distant fjords in Svalbard, the high-Arctic Rijpfjorden and the sub-Arctic Kongsfjorden to compare patterns of fish biodiversity between the areas. Because of the distant location of the fjord there was expected to be observed difference in species composition, and “Atlantification” was expected to have had a larger impact on the species composition in Kongsfjorden than in Rijpfjorden, whereas Rijpfjorden was expected to be dominated by Arctic species. eDNA was isolated from 1 L per 3 replicates of seawater sampled close to the sea floor from a total of seven stations divided into three sampling groups, three in Kongsfjorden, three in Rijpfjorden and one north of Rijpfjorden. A 170 bp fragment of the mitochondrial 12S ribosomal RNA (rRNA) was used as target region for species detection. Using next-generation DNA sequencing of PCR amplicons, 46 Molecular Operational Taxonomic Units (MOTUs) and 13 fish species were detected.

There was found significant differences between temperature, salinity, and depth between all three sampling groups. Although, the significant difference in temperature and salinity was between Rijpfjorden Inner and the two other groups, while significant difference in depth was found between Kongsfjorden and the two Rijpfjorden groups. Despite this there was found no significant difference in biodiversity between the three sampling groups, neither for within-group dispersal or for the mean of the groups. This shows that there was considerable species overlap between the sampling groups, but Atlantic and sub-Arctic species were more

dominating in Kongsfjorden than in Rijpfjorden, while the inner parts of Rijpfjorden were dominated by Arctic species. The data supports the hypothesis that the fish community in Kongsfjorden was dominated by north Atlantic and sub-polar species, while in Rijpfjorden it was more dominated by Arctic species. This indicates that Rijpfjorden might be more isolated from the “Atlantification” processes, but Kongsfjorden may serve as an analogue for the future of the northern fjords in Svalbard.

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INTRODUCTION

Climate change and the rapid decrease of biodiversity are some of the most critical challenges the Earth is facing in the Anthropocene. Carbon emissions from human activities are affecting the marine environment in a multitude of ways, including ocean warming, acidification, and oxygen loss, changes in nutrient cycling and primary production (Intergovernmental Panel on Climate Change [IPCC], 2019). Marin systems faces mass extinction from climate change (Penn & Deutsch, 2022), and the already rapid loss of biodiversity has large impact on the oceans ability to provide essential ecosystem services (Worm et al., 2006).

Arctic marine ecosystems are especially affected by climate change and have experienced changes in temperature and acidity twice as fast as the global average (Hoegh-Guldberg & Bruno, 2010). A "borealisation" of the arctic (Fossheim et al., 2015), or more specifically "atlantification" of the European Arctic is altering the arctic community composition (Fossheim et al., 2015; Kortsch et al., 2015). The term "Atlantification" refers to the increasing influence of Atlantic Water in the Arctic Ocean, were warmer and saltier Atlantic Water is extending its reach northward into the Arctic Ocean (Polyakov et al., 2017). This encroachment of Atlantic Water into the Arctic represents an essential step towards a new Arctic climate state, where the inflow of Atlantic Water will have an increasingly greater role (Polyakov et al., 2017).

Warming has led to a latitudinal abundance shifts in marine species, with a decrease in abundance in equatorial regions and an increase towards the poles (Hastings et al., 2020), as many species as experienced poleward shifts in their fundamental niches and possibly an increase in abundance in Arctic marine ecosystems (Bryndum-Buchholz et al., 2019; Doney et al., 2012). Indeed, boreal species that currently exist as outlier/border populations in the Arctic or at the edge of the Arctic are predicted to shift their distribution northward and/or increase in abundance (Parmesan, 2006). This invasion can lead to displacement of local species and might result in local extinctions, and a community-wide turnover on a large spatial scale is expected in marine fish (Cheung et al., 2009). A rapid "Atlantification" of the highly migratory, generalist fish into the Arctic Ocean has already been observed to effectively alter the arctic marine food webs (Fossheim et al., 2015; Kortsch et al., 2015). Arctic species such as *Boreogadus saida* (polar cod) will likely be more restricted because of warming and loss of sea ice (Hop & Gjøsæter, 2013), and are expected to be, at least, partly

replaced by species of sub-Arctic and Atlantic origin such as *Gadus morhua* (Atlantic cod), *Mallotus villosus* (capelin), *Clupea harengus* (Atlantic herring) , *Melanogrammus aeglefinus* (haddock) and *Scomber scombrus* (Atlantic mackerel) as they are extending their distribution northwards (Berge et al., 2015; Drinkwater, 2005; Hop & Gjøsæter, 2013; Olsen et al., 2010).

A major issue in the effort of decelerating biodiversity loss is the lack of knowledge on the magnitude of marine species and their distribution (Appeltans et al., 2012). Especially has Arctic biodiversity has long been poorly documented and is now facing rapid transformation due to ongoing climate change (Meltofte, 2013). To be able to detect change and find patterns there is a requirement to have information on current and past biodiversity, and continuous monitoring of marine biodiversity provides a baseline for management. Biodiversity assessment, as an important part of conservation management, should ideally be accomplished with non-invasive methods without influencing the structure and functioning of ecosystems.

Traditional monitoring techniques remain problematic when it comes to monitoring biodiversity. Largely from being invasive and damaging to environments (Jones, 1992), as well as being selective when it comes to specific trawl gears catchability (Fraser et al., 2007), but there are also many problems connected to field observations in marine environments. Traditional sampling methods are typically highly dependence on physical identification of species by visual surveys and is therefore strongly influenced by human ability to recognise distinct morphological characteristics, with difficulties associated with correct identification especially for cryptic species or juvenile life stages and is becoming more problematic as taxonomic expertise is in continuous decline (Thomsen & Willerslev, 2015). Because of these limitations, new approaches for monitoring biodiversity and species distribution have been developed, and the use of environmental DNA (eDNA) metabarcoding has proved to be an efficient and easier method (Djurhuus et al., 2020; Harrison et al., 2019; Rees et al., 2014; Thomsen & Willerslev, 2015).

All organisms shed and excrete genetic material in the form of tissue, mucus, and cells into their environment. eDNA is this genetic material collected from environmental samples, such as soil, sediments, and water (Günther et al., 2018; Thomsen & Willerslev, 2015). There are uncertainties associated with the use of eDNA, especially the ecology of eDNA, including the effect of environmental conditions on eDNA shedding, decay and degradation, transportation,

and detection (Barnes & Turner, 2016; Rees et al., 2014). There are several factors that affect the abundance of eDNA in the environment. In marine ecosystems, eDNA is diluted into a larger volume of water and exposed to pronounced hydrodynamics (e.g., tides, currents) and variation in abiotic conditions (e.g., salinity, temperature), which is likely to affect eDNA transport and degradation (Thomsen et al., 2012). The rate of eDNA shedding/release will depend on the abundance of organisms and the ecology of species (metabolism, behaviour etc.), while the eDNA degradation rate depends on environmental factors such as temperature and microbial activity, among others (De Souza et al., 2016). Colder temperature, low UV-B levels, and alkaline conditions have shown to decrease degradation rates of eDNA, and eDNA will therefor possibly be detectable for a longer period of time in habitats with these characteristics (Strickler et al., 2015). Related to the longer possible detection time, cold Arctic water might provide more time of dispersal over larger distances than what is detected at temperate latitudes (Junen et al., 2019; Port et al., 2015), potentially providing data with lower local fidelity then found in temperate habitats. Despite this, studies have shown clear horizontal and vertical eDNA heterogeneity in Arctic ecosystems (Lacoursière-Roussel et al, 2018), although less is known about eDNA dispersal and degradation relationships in the Arctic than at temperate latitudes.

This method therefor provides a way to analyse genetic material from a sample collected in nature and use species-specific DNA fragments found in the samples as proxy for the presence of species, as well as community composition (Andruszkiewicz et al., 2017), and species abundance (Salter et al., 2019). Because this method allows for simultaneous examination of organisms across multiple trophic levels and domains of life, from microorganisms to vertebrates, it can give important insight into biodiversity and shifts in marine communities, as well as interactions between trophic levels (Djurhuus et al., 2020; Sigsgaard et al., 2015).

eDNA is an emerging tool in conservation and is becoming increasingly more utilised. Although the term eDNA has been around since 1987, then used to study microbes in sediment samples (Ogram et al., 1987), it is relatively new when used in the marine environment (Thomsen et al., 2012). This method has been used in monitoring of seasonal variations in community structure biodiversity (Sigsgaard et al., 2017) and monitoring of endangered species (Bonfil et al., 2021), as well as the potential of early detection of invasive species (Pochon, Bott, Smith, & Wood, 2013). The method itself is seen as non-invasive, less

time-consuming, and less costly than traditional methods, as there is less need for long periods of observation in the field (Sigsgaard er al., 2015). It shows high comparability with traditional survey methods when compared to more traditional monitoring methods such as trawling, especially in detecting fish diversity (Thomsen et al., 2012), and has shown to coincide and even detect higher species richness (Afzali et al., 2021; Fraija-Fernández et al., 2020), showing that this method has the potential to be used to draw valid ecological conclusions that can contribute to biodiversity monitoring efforts (Fraija-Fernández et al., 2020). In Norway, large institutions such as the Institute of Marine Research (IMR) and Norwegian Environment Agency (NEA) is incorporating eDNA as an important tool for their future conservation and management projects (Aasgaard, 2019). And the Ministry of Climate and Environment's budget for 2020 included funds allocated for further development of this method, as they recognise the importance it can have on conservation efforts (Klima- og Miljødepartement, 2019).

The choice of genetic marker used in a metabarcoding eDNA study depends on the group of organisms of interest (fish, invertebrates etc.). Mitochondrial DNA (mtDNA) markers are the most commonly used because of its advantages. Eukaryotic cells have a much higher abundance of mtDNA than nuclear DNA, making it optimal in detection at low abundance in environmental samples. In this study, a 170 bp fragment of the mitochondrial 12S ribosomal RNA (rRNA) was applied. This genetic marker has proven efficient in species identification of marine vertebrates, including fish (Girish et al., 2004; Miya et al, 2015; Yang et al., 2014).

The main objective of this study was to compare patterns of fish biodiversity between two distant fjords in Svalbard using eDNA metabarcoding. More specifically, species richness and alpha diversity within each station was obtained, and beta diversity between the two fjords was analysed. Due to the different locations of the fjords in Svalbard, it was expected to observe a difference in the species composition between them. Because of the increasing effect of “Atlantification” one the coast of Svalbard it was expected to find more north Atlantic and sub-polar species in Kongsfjorden, while Rijpfjorden was assumed to show a higher number of Arctic species.

MATERIAL & METHOD

Description of the sampling areas

The ocean climate around Svalbard is closely connected to the large-scale circulations of water masses between the North Atlantic and the Arctic Ocean as Atlantic Water is transported northward by the West Spitsbergen Current (WSC) along the continental shelf of Svalbard. In the northern part of the archipelago, the WSC diverges into two different branches, the Yermak Branch flows northwards into the Arctic Ocean, while the Svalbard Branch flows eastwards (Aagaard et al., 1987).

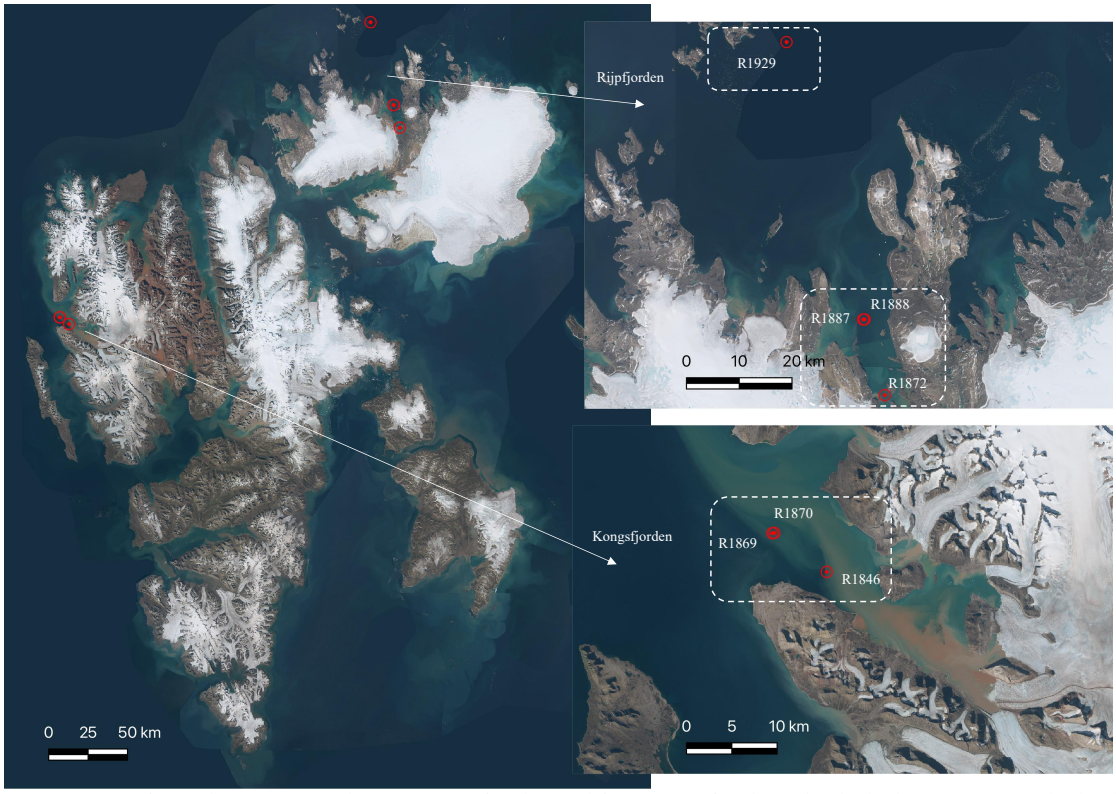

Figure 1 Map of sampling stations; three in Kongsfjorden and four in Rijpfjorden. The dashed squares encircle the three groups of stations. The stations connected to Rijpfjorden is divided into the two groups Rijpfjorden Outer and Rijpfjorden Inner. Satelitte basemap was retrieved from www.geonorge.no/

Kongsfjorden

Kongsfjorden (78°58'5"N 11°59'38"E) is located at the west coast of Svalbard on the Archipelago largest island Spitsbergen, and outer parts of the fjord have a depth up to 350-400 meters (Figure 1). This is a sub-Arctic fjord which open up into the West Spitsbergen Shelf through a common mouth with Krossfjorden and is subject to intermittent exchange between the fjord and offshore Atlantic waters. The fjord is typically dominated by cold, fresh Arctic waters for much of the year, with summertime intrusions of relatively warm, saline

Atlantic-derived waters by the WSC (Cottier et al., 2005), as well as run-off from surrounding glaciers (Torsvik et al., 2019). The watermasses in the fjord is influenced by two different ocean currents. WSC, a branch of the North Atlantic Current, transports large amounts of relatively watm and saline Atlantic Water, and a costal current on the shelf transports cold and less saline Arctic Water (Svendsen et al., 2002). This results in a mixture of Atlantic and Arctic fauna in the fjord community, but the composition varies between years depending on the advection of Transformed Atlantic Water, which represents a mix of the Atlantic and Arctic Water masses (Hop et al., 2002; Willis et al., 2006). In recent years, Kongsfjorden has been strongly influenced by the WSC with increasing temperatures and declining sea ice cover (Cottier et al., 2019; Tverberg et al., 2019). The "Atlantification" has had a large impact on both temperature and salinity in inner Kongsfjorden in the last decade from enhanced advection of Atlantic Water from the WSC (De Rovere et al., 2022).

Rijpfjorden

Rijpfjorden (80°10´N,22°15´E) is the northernmost fjord in the Svalbard archipelago, and is located on the island Nordaustlandet (Figure 1). The fjord is relativly shallow (max. 240 m deep) and oriented South-North and opens towards the Arctic Ocean on the broad shallow shelf with no sill. Rijpfjorden is considered a high-arctic fjord, and the watermasses are characteristics by cold Arctic water and the ice cover last for at least nine months a year (October-June) , and due to the wide and shallow shelf north of Svalbard there is a less direct contact between Rijpfjorden and the WSC (Ambrose et al., 2006; Wallace et al., 2010). However, there are occurrences of intermittent influence of Atlantic water to the system (Falk-Petersen et al., 2008; Wallace et al., 2010). Only a non-significant increase in temperature has been observed in the later years which has not interfered with the seasonal ice cover of the fjord (Cottier et al, 2019).

eDNA sampling and filtration

The samples were collected on a cruise in 2018 (18th August-5th September) as part of the MAREANO project. eDNA water samples was collected from a total of 7 stations, 3 in Kongsfjorden and 4 in Rijpfjorden. The eDNA water samples was collected as close to the seafloor as possible (~10 m above), using a CTD (Conductivity, Temperature, and Depth) with 12 Niskin bottles attached (Sea-Bird Scientific, Bellevue, WA). Water samples was filtered on board the ship through Sterivex^{TM} filter units (Merck KGaA, Darmstadt, Germany)

with pore size 0.22 µm, using a peristaltic pump (Figure 2). 1L in 3 independent replications was filtered per sampling station. 2 liters was collected per replicate from 3 different Niskin bottles. 1 L per replicate was first pumped through the tube before the filters was attached and 1 L was filtered through the filters per replicate. 1 filtration blank were created per station in the same manner as the water samples using deionized water to control for contamination in the filtering process. Before putting filter into falcon tubes for storage, excess water was removed from the filter using a syringe to blow air trough. Air blanks were sampled by filtering 2 x 50 ml of air through a filter using the same syringe as used on the other filters from the same station. The samples were stored in freezers on board at -20°C and transferred to -80°C freezer at land for long-term storage to prevent degradation of eDNA until the time of DNA isolation. In addition to water samples the CTD also collected measurements of temperature, sampling depth, and salinity for each station, which was used as environmental variables. Chlorine was used onboard as disinfection method for all filtering and sampling equipment to avoid contamination of samples. Filtering tubes and water canisters used for the collection of water from the CTD was decontaminated with chlorin between each new station.

Figure 2 Picture for demonstrating the sampling procedure. Filtering of water samples from cruise onboard Kristine Bonnevie March 2022. Here special plastic bags were used instead of using water canisters to collect water from the CTD.

Laboratory practices

Measures against contamination of samples

Since eDNA samples containes only trace amount of DNA, strict laboratory protocols must be established to avoid contaminating the samples with DNA from other sources. The

laboratory work was done in a sterile-room and so the eDNA laboratry is decontaminated before use by saturating the air with hydrogen peroxide. Strict use of lab coats that never leave their designated rooms and always use of clean gloves. Instruments and cabinets used during isolation and PCR are treated with UV lights for 30 minutes before use. As well as having separate storage fridge and freezers for reagents and eDNA samples.

eDNA isolation from Sterivex filters

DNA was isolated from the Sterivex filteres using the DNeasy PowerWater Sterivex Kit (QIAGEN, Hilden, Germany), closely following the manufaktorers protocol with some modifications (**Appendix A**). Heating and use of power beads (step 12 and 13) was exluded as this was not deemed necessary for homogenisation. The isolated DNA, 100 µl per sample, was stored at -80°C in a designated eDNA freezer. A total of 41 water samples, filter blanks, air blanks, and eDNA lab air and extraction blanks were extracted.

PCR amplification, library preparation, and sequencing

The mitochondrial 12S rRNA genes in the samples was amplified using a QIAGEN Multiplex PCR Kit (1000) (QIAGEN, Hilden, Germany), closely following the manufacturer's protocol. The primer used for the amplification is a previously published 12S primer set specifically designed for fish, the MiFish-U primer set (Miya et al., 2015). These primers amplify fragments of ~172 base pair (bp) from the mid region of the mitochondrial 12S rRNA gene (MiFish-U-F, 5′-GTGGTAAAACTCG TGCCAGC-3′; MiFish-U-R, 5′-CATAGTGGGGTATCTAATCCCAGTTTG-3′). A one-step PCR process was used, where all components of the primer was added in the beginning (i.e., including the A-adapter, and the barcodes), rather than a two-step process. This is to limit the possibilities of contamination, as there was no safe way to open the samples to add A-adapter and barcodes on a later stage. DNA metabarcoding multiplexing was performed using 96-well microplates. The positive control sample was taken from aquarium water at Polaria. The PCR reaction per well had a total volume of 20 µl, including: 10 µl of 2x QIAGEN Multiplex Master PCR Mix; 0.16 µl of bovine serum albumin (BSA) (20 mg/ml); 5.84 µl of RNase-Free Water (dH_2O); 1 µl of pooled 12S primer (5 µM), and 3 µl of DNA template. The PCR was performed on a ProFlex PCR system (Thermo Fisher Scientific, Waltham, MA), and the program comprised of an initial denaturation step of 95°C for 10 min, 40 cycles of denaturation of 95°C for 30 sec, anneal step of 60°C for 30 sec, and elongation step of 72°C for 30 sec, and a final

elongation step of 72°C for 5 min. In total, six replicates were performed for each of the 48 sample.

All amplicons were analysed on QIAxcel Advanced System (QIAGEN, Hilden, Germany), using a Qiaxcel DNA High Resolution Kit (1200) (QIAGEN, Hilden, Germany). This kit used an alignment marker with a range of 15 bp – 3 kb. 2 µl of amplicons was added to 8 µl of QX Dilution buffer, compared to a SizeMarker with a range of 50 – 800 pb, diluted with 2 µl of marker in 38 µl of QX DNA Dilution buffer. A layer of QX Mineral Oil was added on top of each well for preservation purposes and to extract possible air bubbles from the solution underneath. Only the PCR products around 300 bp was of interest, as the fragments closer to 400 bp most likely originates from prokaryotic organisms and are results of co-amplification. The samples of interest from each av the replicates were subsequently pooled together, now 18 µl from each well. 100 µl was then pooled from each of the replicate pools into one library.

The library was run through 2% agarose gel with SYBRsafe DNA Gel Stain (Thermo Fisher Scientific, Waltham, MA) to extract desired DNA fragments. The solution added to the gel wells contained 12 µl of the pooled library and 4.5 µl of 10x Track it loading dye. The 50 bp ladder consisted of 1.5 µl of 10x Track it loading dye, 3.5 µl RNase-Free Water, and 1 µl ladder. In total 7 wells were used, 6 for library and one for ladder. The gel ran for 45 min at 76 V. The desired sequences (i.e., length of ~300 bp) was extracted from the gel stripes, and the DNA was isolated using GeneJet Gel Extraction and DNA Cleanup Micro Kit (Thermo Fisher Scientific, Waltham, MA), using manufacturer's protocol C: "DNA extraction from gel protocol". The 6 gel extractions were divided into two Eppendorf tubs during isolation. 20 µl Elution Buffer was used, rather than 10 µl, and the solution was stored in a designated fridge overnight. The eluates were combined after for a PCR cleanup following manufacturer's protocol B: "PCR cleanup, dimers removal protocol". 20 µl Elution Buffer was used, rather than 10 µl. The concentration of the libraries was decided using Qubit dsDNA HS assay (Thermo Fisher Scientific, Waltham, MA). The library (5 µl) had a concentration of 2.63 ng/µl. The concentration in pM was calculated using 290 bp as expected size of amplicons when converting to molarity. The library had a concentration of 13740 pM and was diluted to 100 pM using 682 µl RNase-Free Water (dH_2O). Emultion PCR and loading of the library to the chip was performed on an Ion Chef ™ System (Thermo Fisher Scientific, Waltham, MA). The input library had to have 25 µl library with a concentration of 30 pM, so the library was

again diluted by using 7.5 µl of 100 pM library, 13.5 µl of RNase-Free Water, and 4 µl Ion S5 Calibration Standard (Thermo Fisher Scientific, Waltham, MA). The beginning of the bioinformatic pipeline was completed by the Ion Chef System after the initial sequencing. All primer components were removed so just the amplicon was left, and low-quality sequences was filter out.

The bioinformatic pipeline was conducted using OBITools v1.01.22 software (Boyer et al., 2016). This software package was specifically designed to handle analysis of large next-generation sequencing data and was therefore well suited to use for DNA metabarcoding context. The filtering and processing of the sequences was based on the same method conducted by Sales et al. (2021). The filtering process included *obigrep* filtering sequences with specific length (i.e., 140 to 190 bp), and all sequences including N (un-assigned nucleotide) was removed (*deprelication*), and *obiuniq* grouped unique sequences. *Uchime denovo* (*vsearch v2.15.2*) was then used to remove chimeric sequences and *SWARM v2.0* clustered sequences into Molecular Operational Taxonomic Units (MOTUs) with the d value 3. *Obigrep* was used to filter only the clusters with a size greater than or equal to 2. Taxonomic assignment of MOTUs was performed using *Ecotag* against a locally curated database of mitochondrial 12S rRNA gene sequences and additional taxonomic information. In addition, the R package *LULU* was used for a post-clustering correction, removing erroneous MOTUs to improve the biodiversity metrics (Frøslev et al., 2017).

Each 12S MOTU of interest was manually check for a better match using BLAST search, and best IDs were changed to reflect a higher percent match if one was found. The threshold used to ensure reliability of the taxonomic assignment was 97% for species level, 95% for genus level, and 90% for family level. Reads from blanks, PC, and other not suitable samples was removed before down-stream statistical analysis. The biological replicates per station was collapsed to minimize the stochasticity of the samples within a station, and data coming closer to the true mean. Finally, the function *tax_glom* in the r-package *phyloseq* (McMurdie & Holmes, 2013) was used to merge identical MOTUs with the same highest taxonomic classification. This reduced the amount of MOTUs as the result is only one MOTU per scientific name and corrects for wrongly representation of diversity.

Statistical analysis

Statistical analyses were performed using R (v4.1.1, R Core Team, 2021) in RStudio (v1.4.1103, Rstudio Team, 2021), mainly utilising the R-package *phyloseq* v1.38.0 (McMurdie & Holmes, 2013) and *vegan* v2.5-7 (Oksanen et al., 2020). The stations were grouped into three groups: Kongsfjorden, Rijpfjorden Inner, and Rijpfjorden Outer (Figure 1). The group Rijpfjorden Outer contained only one station (R1929) as this station was deemed too far outside the fjord system to be implemented in a group with the other Rijpfjorden stations. The two inner fjord groups (Kongsfjorden and Rijpfjorden Inner) were compared to study the difference between the fjords. The Inner fjord groups were then compared to the Outer Rijpfjorden group to see wether there were differences between the communities in the fjord and the outer areas.

The collinearity between the environmenal variables was analysed using the function *cor* in the r-package *stats* to see which could be used in combination to describe the fjord effect. For the environmental variables an Analysis of Variance Model was performed using the function *aov* in the r-package *stats*, to analyse whether there were differences in temperature, depth or salinity between the different groupings. It was not possible to analyse the difference within the three groups, because the ANOVA could not compute the variance due to the lack of replicates.

Taxonomic diversity within categories was expressed with the Shannon Diversity Index (Shannon, 1948).

$$Shannon\ Index\ (H) = -\sum_{i=1}^{s} p_i \ln p_i$$

The differences in the Shannon Index between the different groupings was not tested as the amount of data was not seen as sufficient to draw any real conclusions.

The abundancy data was transformed using Hellinger as this method is recommended for ecological data with numerus zero values, as it gives low weights to variables with low counts and many zero values (Legendre & Gallagher, 2001). This method tranformes the abundancy data of a taxa in relation to the other taxa as a value between 0 and 1. The Hellinger transformed abundancy data was visualised in a heatmap using the function *heatmaply* in the r-package *heatmaply* (Galili et al., 2017), the dendrograms represent similarity between the

samples and species. Analysis of variance using the dissimilarity indices Bray-Curtis as distance matrices was performed to analyse the beta diversity. The two tests *betadisper* and *adonis* in the r-package *vegan* was used to test for homogenously dispersal within the groups and for difference in composition between the groups respectively. *Fdr* was used as adjustment for significance level as it is found to reducing false positives as well as minimizing false negatives (Jafari & Ansari-Pour, 2019). All three groups was tested against eachother, as well as a test for the fjords where the two Rijpfjorden groups were combined, and a one where the two inner groups combined was tested against Rijpfjorden Outer.

Nonmetric Multidimensional Scaling (NMDS) plots was created using the function *metaMDS* in the r-package *vegan* to visualise association between the fjords, species and potential variables that show significant correlation. NMDS ordination was chosen because of its ability to work with a variety of similarity matrixes, including Bray-Curtis dissimilarity matrix which was used here because of its ability to handle null values in datasets. Two dimensions was used when plotting, and a stress value <0.1 was considered a good ordination with little risk of drawing false interferences (Clarke, 1993). The different environmental variables were tested for significant effect with NMDS using the function *envfit* in the r-package *vegan*. This function fits environmental vectors or factors onto an ordination.

A Similarity Percentage (SIMPER, Clarke, 1993) analysis was performed using the function *simper* in the R package *Vegan*. This function discriminates species between two groups using Bray-Curtis dissimilarities to detect which species contributes to the differences between the groups. Only the ten highest ranked taxa was included because of declining contribution to the dissimilarity. Due to the SIMPER analyses being affected by taxa with high variance within a group (Warton et al., 2012), the differences between the relative average abundance between the paird groups was important to acknowledge, not just the contribution to the dissimilarity between the pairings. Three simper analyses were performed, one with all three groups as contrasts, one with Rijpfjorden and Inner and Konsgsfjorden combined and Rijpfjorden Outer as contrasts, and one with the two Rijpfjorden groups combined and Kongsfjorden as contrasts. In addition to the SIMPER analyses an Indicator Species Analysis was performed using the function *multipatt* in the r-package *indicspecies* (De Cáceres & Legendre, 2009) to look for significant associations between taxa and the different groups.

RESULTS

Data quality, overall taxonomic composition, and community assemblage

A total of 15,327,381 raw reads was obtained from the chip after the initial quality control and filtering done by the Ion Gene Studio S5 system. After further quality control by and OBITools a total of 11,294,496 (73.69 %) were retained for further manual quality check/control. After LULU, manual quality control and removal of reads from blanks, PC and samples with low number of reads, the total number of reads used for down-stream analyses were 7,204,316, comprising a total of 52 MOTUs. Where some of the MOTUs represent the same species/genus/family.

After OBITools there was a total of 11,294,496 reads retained, divided on 642 MOTUs. Using the locally curated database, 99.03 % (11,185,080 reads) of the reads were classified as *Actinopterygii*, and <1 % as *Elasmobranchii*. Only 1 MOTU was classified as *Elasmobranchii* and was only detected in the PC sample. The remaining reads (<0.01 %) were classified as *Mammalia*. The *Mammalia* species detected was *Sus scrofa* (54.84 %), *Homo sapiens* (37.11 %), *Erignathus barbatus* (<0.02 %), *Phoca groenlandica* (0.36 %), *Balaenoptera musculus* (2.69 %), and *Delphinapterus leucas* (4.98 %). A total of 38,500 reads (0.34 %) was specifically assigned to *Homo sapiens*. Only 0.05 % of the reads (5658 reads) were not classified into at least class level.

After LULU clustering, manual quality control with BLAST, and removal of "non-applicable" species and samples the total number of reads were 10,723,721, clustered in 46 MOTUs. The average number of reads per sample was 372,864, (sd=284,000). "Non-applicable" refered to species with distribution outside of the Svalbard area (i.e., *Salmo trutta*, *Coregonus sp.*) or not classified as *Actinopterygii*, as well as reads from blanks, PC and samples with a low number of reads (R1846_320_1; 61 reads, and 1872_327_2;304 reads). For the two stations that had one replicate removed each, it was assumed enough support for both sites as there was two other replicates for both sites with sufficient number of reads.

The final abundancy data used in down-stream statistical analyses consisted of 100 % reads classified as *Actinopterygii* (**Appendix B**). All reads were assigned to at least order level

taxonomy, where <99.99 % of reads were assigned to level family (only 1 MOTU consisting of 3 reads was not), and 77.21 % of the reads was identified on species level (8,280,177 reads). A total of 13 species was detected in the 28 MOTUs in the final abudancy data, and all species had more than 10 reads. Three species made up more than 50% of the reads (58.68 %); *Borgeogadus saida* and *Clupea harengus* had the highest number of reads, both above 2 000 000 reads, while *Anisarchus medius* had above 1 000 000 reads. Only one species, *Gadus morhua*, had reads below 38,000 reads (19 reads). The other species detected was: *Cyclopterus lumpus*, *Lophius piscatorius*, *Hippoglossoides platessoides*, *Gymnocanthus tricuspis*, *Micromesistius poutassou*, *Gaidropsarus argentatus*, *Mallotus villosus*, *Lumpenus lampretaeformis*, and *Careproctus reinhardti*.

The samples seemed not to cluster around what sampling group they were sampled from, but rather clustered around the most dominant species in the samples, where *B. saida* dominated in one cluster and *A. medius* was most dominate in the other major cluster (Figure 3). For the clustering of species, *B. saida* represented a whole cluster by itself, while *A. medius* dominated in the other cluster. *B. saida* was present in all of the stations and is the most abundant species in 4 of the 7 stations and these are stations across the two fjords. While *A. medius* was most abundant in the Outer Rijpfjorden sample.

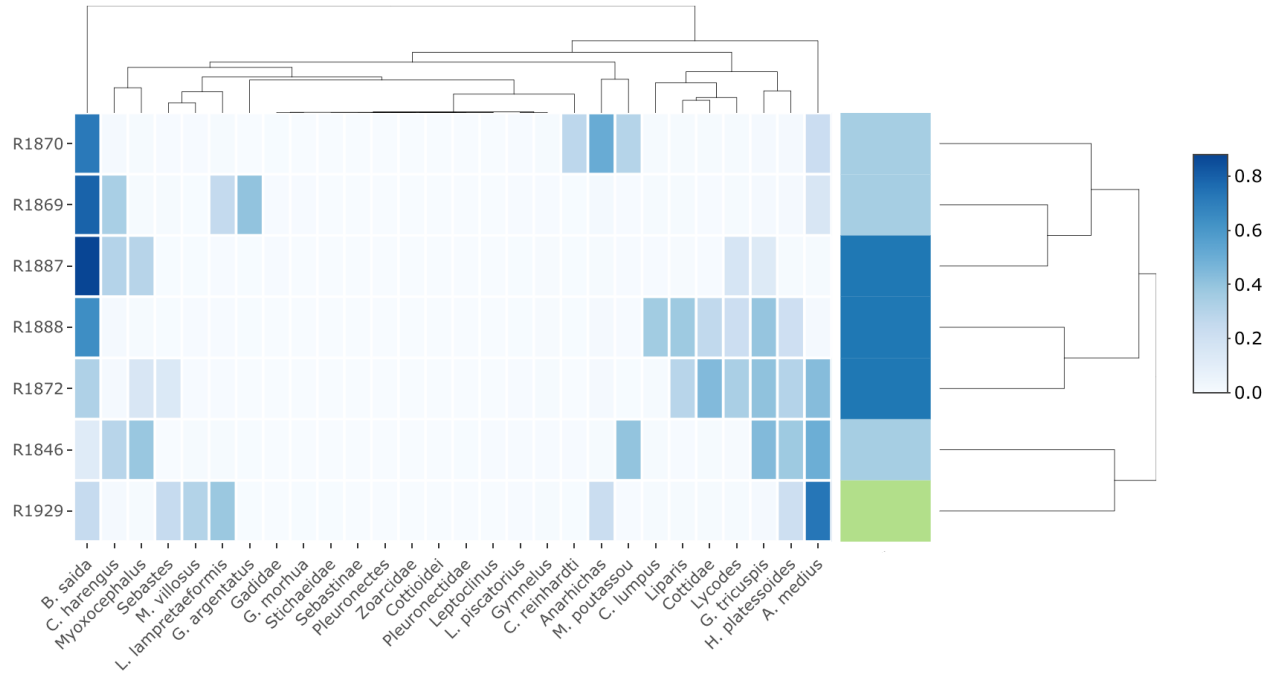

Figure 3 Heatmap illustration Hellinger tranformed abundance of a taxa, the Hellinger values are indicated in the scaled bar furthes to the right. The green-blue-bar show which of the three sampling group the station belongs to. The dark blue represents Rijpfjorden Inner, light blue represent Kongsfjorden, and green represents Rijpfjorden Outer.

Whether a detected taxa was present or absent in a station was illustrated in figure 4.

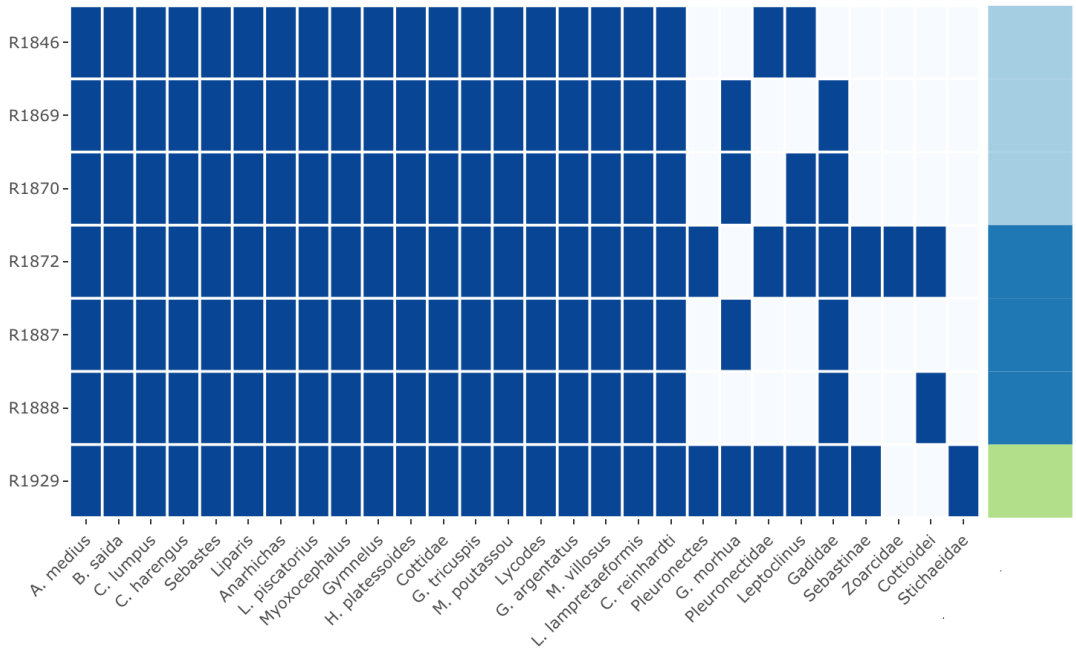

Figure 4 Illustration of which taxa is present in the different samples. Blue square represent the presence of a species in a sample, while white square indicates absence of DNA from the taxa in the sample. The colourbar to the right show which group the sample belong to. The dark blue represents Rijpfjorden Inner, light blue represent Kongsfjorden, and green represents Rijpfjorden Outer.

Environmental variables

The temperature was higest in Rijfjorden Outer, while the lowest temperatures of the sampling stations was found in Rijpfjorden Inner (Figure 5). The salinity level was highest in Rijpfjorden Outer, and lowest in Rijpfjorden Inner. Kongsfjorden had temperature and salinity level in between Rijpfjorden Inner and Rijpfjorden Outer.

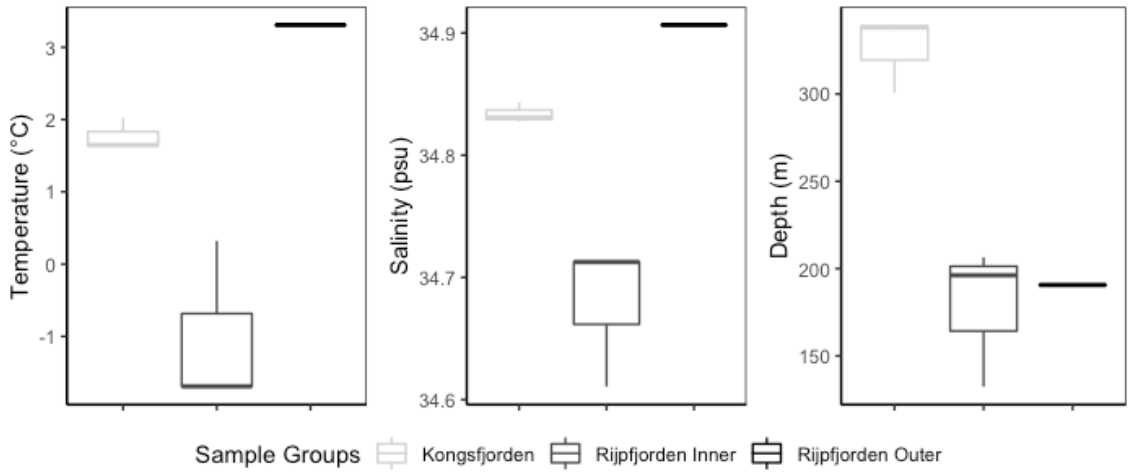

Figure 5 Boxplots of environmental variables. From left temperature, salinity level, and depth measured with CTD at the time of water sample for the three sampling groups: Kongsfjorden, Rijpfjorden Inner and Rijpfjorden Outer. Lower and upper fences are 25th and 75th percentiles, and median is indicated by the bold horizontal line between. Whiskers represent the highest and lowest value.

All the environmental variables had significant different distributions among the three sample groups (ANOVA; $p<0.05$, **Appendix C, A**). There was also a significant difference between all environmental variables when only including the two inner fjord groups ($p<0.05$, $p<0.01$ for depth) (**B**). When comparing the combined inner and outer Rijpfjorden groups to Kongsfjorden (**C**) there was found significant results for depth ($p<0.01$), but not for salinity or temperature. There was found no significance in any of the environmental variables when comparing the inner and outer Rijpfjorden (**D**), but there was a trend between the two groups for salinity and temperature. There was found no significant results when comparing the combined inner fjord groups with the outer fjord sample. This indicates that the main difference in the environmental variables is found between the inner fjord groups. Because of these significant differences in the environmental variables between the groups, these were not used as covariates in combination with sampling group effect in analyses of species diversity.

When testing for collinearity between the environmental variables for fjord effect on diversity, the collinearity was only deemed low enough between temperature and depth to be used together ($r_{sp}=0.4$), but too high between salinity and depth ($r_{sp}=0.6$) and temperature and salinity ($r_{sp}=0.8$).

Species richness & Alpha diversity

One station in Rijpfjorden Inner and the station in Rijpfjorden Outer had the highest species richness with 26 observed taxa each (Figure 6), while the lowest number of taxa detected at a station was 21. The stations were characterised by a Shannon Index between 0.8 and 2.0. The two sampling groups Rijpfjorden Inner and Rijpfjorden Outer was found to have an over all higher species richness and biodiversity than Kongsfjorden, but there was also a larger variability between stations in Rijpfjorden Inner as it contained the station with the highest and the lowest observed Shannon Index, as well as the stations with the highest and lowest species richness. Although there were larger differences in species richness between the sampling groups, there was no tendencies for larger differences in biodiversity.

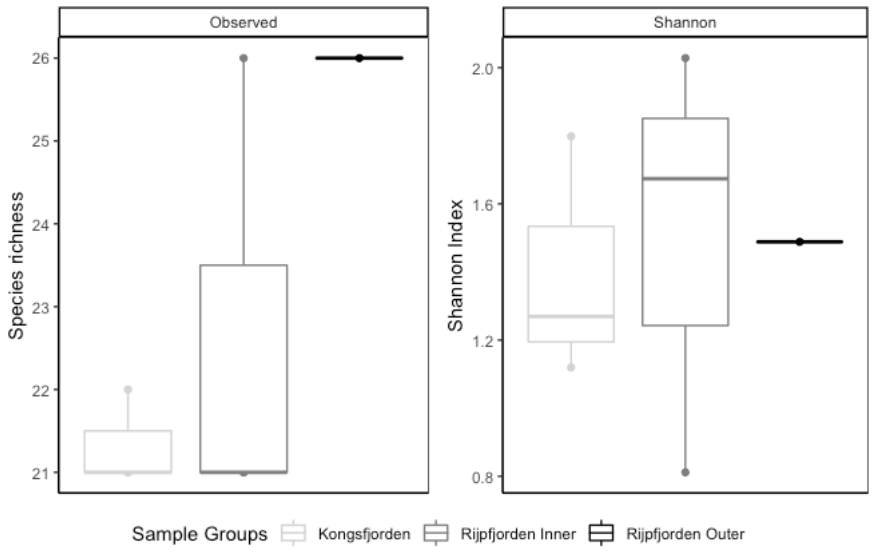

Figure 6 Species richness (left) and Alpha diversity presented using the Shannon Index (right) per station grouped by the three sampling groups. Lower and upper fences are 25th and 75th percentiles, and median is indicated by the bold horizontal line between. Whiskers represent the highest and lowest value.

When exploring relationships between the Shannon Index and environmental variables, biodiversity was found to tend to decrease with increasing depth, as well as with salinity. While it was found to tend to increase with increasing temperature (Figure 7).

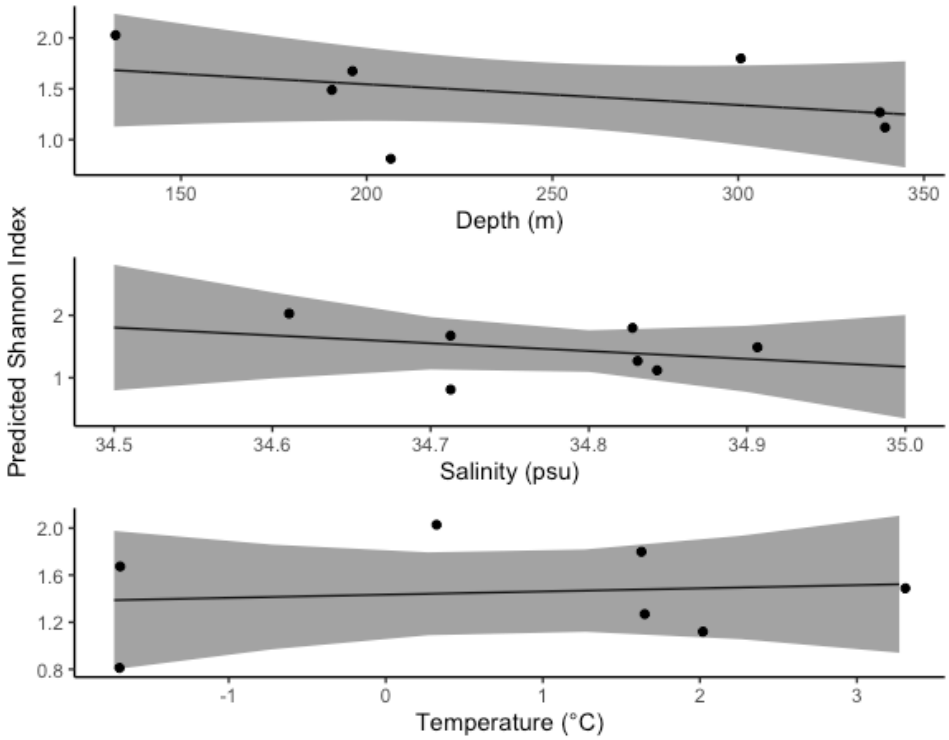

Figure 7 Prediction plots for linear regressions between Shannon Index and the three environmental variables, depth, salinity, and temperature. The confidence bands represent 95% confidence interval.

Beta diversity

Ordination analyses

The *envfit*-analyses revealed significant effect of temperature ($p<0.05$) on the fish community composition, but not for depth and salinity (Table 1). According to the *envfit* output, temperature explained 86 % of the variation.

Table 1 Output from envfit-test of environmental variables in the ordination plot. The analysis was performed 10 times, and standard deviation (SD) is presented. Significant p-value was marked in bold.

Vectors/Factors	R2	Mean p-value	p-value SD
Depth	0.4398	0.302	0.014
Temperature	0.8564	**0.031***	0.003
Groups	0.5034	0.149	0.011

The anovas for significant difference between the groupings in the NMDS plot found no significant differences between any of the groupings, neither for the dispersal within the

groups or for the mean of the groups (Figure 7, **Appendix D**). The stress value for the plot was below the fair stress value (i.e., <0.1).

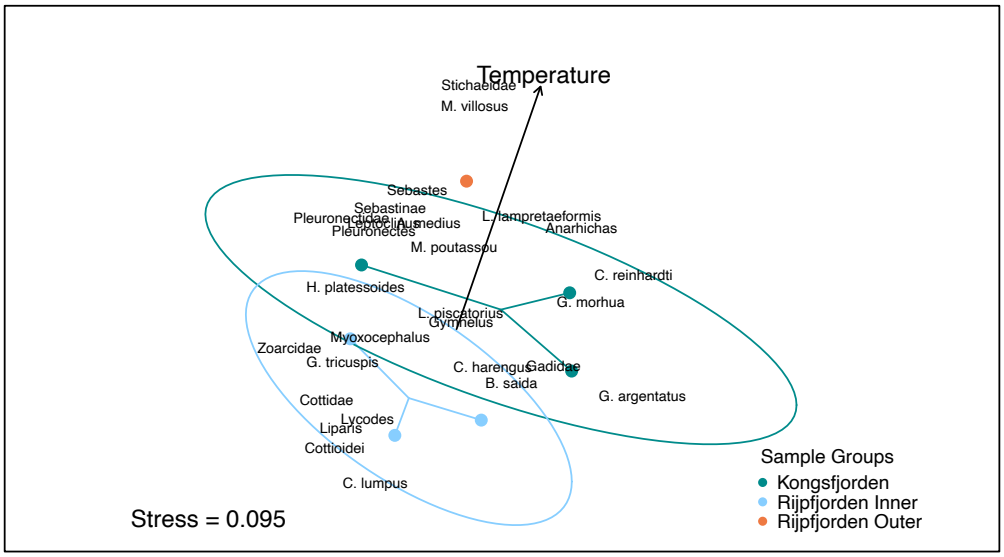

Figure 8 Nonmetric Multidimensional Scaling (NMDS) plot for sampling stations grouped by Kongsfjorden, Rijpfjorden Inner, and Rijpfjorden Outer based on Hellinger transformed abundance data, and Bray-Curtis dissimilarity index with 2 dimensions. Fitted environmental variable, temperature, is represented by black vector. The ellipses represent 95% confidential interval of community composition in NMDS space for each sampling area. Stress value is indicated.

Species such as *G. morhua* and *L. piscatorius* showed to have higher association with Kongsfjorden than to the groups Rijpfjorden Inner and Rijpfjorden Outer, while *M. villosus* is highly associated to Rijpfjorden Outer. The ellipses overlaped for the different groups indicating that there was an overlap in associated species between the groups, e.g., the species *B. saida* and genus *Myoxocephalus* was situated in the overlap of Kongsfjorden and Rijpfjorden Inner.

SIMPER analysis

Based on simper analyses, the species with the highest contribution in all of the pairwise comparisons was *B. saida* and *A. medius* (Table 2), with decreasing contribution of the below taxa. These two species contributes between 19.45 % and 27.86 % to the dissimilarity in all pairings. The species found to have highest avergae relativ abundance in Rijpfjorden Outer was the species *A. medius* (0.731), *M. villosus* (0.311), and *L. lampretaeformis* (0.383), and the Genus *Sebastes* (0.247) and *Anarhichas* (0.230). *M. villosus* had an especailly large difference in avergae relativ abundance compared to Kongsfjorden (0.006) and Rijpfjorden Inner (0.004), and contributed to 10.08% of the dissimilarity between Kongsfjorden and

Rijpfjorden Outer, and 8.73 % between Rijpfjorden Inner and Rijpfjorden Outer. *H. platessoides* (0.216) also had the highest connection to Rijpfjorden Outer, but not such a large difference to Rijpfjorden Inner (0.185) and Kongsfjorden (0.134). The species *B. saida* was found to have a higher average relative abundance in the inner fjord areas (0.579), especially in Rijpfjorden Inner (0.614), but also relative high in Kongsfjorden (0.544). But *B. saida* was well represented in all three groups. The genus *Myoxocephalus* was also found to have the highest avergae relative abundance in the inner fjord areas (0.145), but no large differences between Kongsfjorden (0.135) and Rijpfjorden Inner (0.155). The species with the highest avergae relative abundance in Rijpfjorden Inner was the species *G. tricuspis* (0.312), and the geners *Lycodes* (0.243) and *Liparis* (0.224), and the family *Cottidae* (0.239). *M. poutassou* seems to be the species with the highest avergae relative abundance in Kongsfjorden (0.237), but *C. harengus* was also found to have higher average relative abundance in Kongsfjorden (0.215) than the other groups.

Table 2 Output from the two SIMPER analyses of the three groupings of samples as contrasts. The output indicates which taxa had the largest contribution to the dissimilarity (cumsum) between the three groups: Kongsfjorden, Rijparfjorden Inner, & Rijparfjorden Outer. Avg. abund. constras shown show the average relativ abundance of the taxon for the first (A) and the second (B) contrast in the pairing. Only the 10 highest ranked taxa are included in this table because of the declining contribution to the dissimilarity.

Taxa	Average	SD	Ratio	Avg. abund. contrast A	Avg. abund. contrast B	Contribution (%)	Cumulative (%)
Contrast: Kongsfjorden & Rijpfjorden Inner							
B. saida	0.066	0.049	1.316	0.544	0.614	10.72	10.72
A. medius	0.053	0.031	1.735	0.295	0.153	8.73	19.45
G. tricuspis	0.052	0.033	1.596	0.156	0.312	8.52	27.97
Lycodes	0.049	0.012	4.265	0.007	0.243	8.04	36.01
M. poutassou	0.048	0.037	1.307	0.237	0.004	7.91	43.92
Cottidae	0.046	0.037	1.252	0.008	0.239	7.53	51.45
Liparis	0.044	0.034	1.294	0.007	0.224	7.16	58.61
Myoxocephalus	0.038	0.029	1.275	0.135	0.155	6.16	64.77
Anarhichas	0.037	0.055	0.672	0.179	0.013	6.02	70.79
H. platessoides	0.036	0.028	1.313	0.134	0.175	5.93	76.72
Contrast: Kongsfjorden & Rijpfjorden Outer							
A. medius	0.094	0.043	2.201	0.295	0.731	14.65	14.65
B. saida	0.083	0.051	1.618	0.544	0.245	12.90	27.55
M. villosus	0.064	0.004	14.694	0.006	0.311	10.08	37.63
L. lampretaeformis	0.061	0.028	2.106	0.091	0.383	9.52	47.15
Sebastes	0.051	0.002	20.616	0.004	0.247	8.00	55.15
Anarhichas	0.050	0.009	5.454	0.179	0.230	7.87	63.02

M. poutassou	0.048	0.042	1.146	0.237	0.004	7.46	70.48
C. harengus	0.042	0.037	1.127	0.215	0.015	6.58	77.06
H. platessoides	0.039	0.007	5.410	0.134	0.216	6.20	83.26
G. argentatus	0.029	0.051	0.583	0.137	0.004	4.64	87.90
Contrast: Rijpfjorden Inner & Rijpfjorden Outer							
A. medius	0.121	0.059	2.064	0.153	0.731	16.81	16.81
B. saida	0.079	0.066	1.211	0.614	0.245	11.05	27.86
L. lampretaeformis	0.078	0.009	8.737	0.005	0.383	10.74	38.60
M. villosus	0.063	0.007	8.599	0.004	0.311	8.73	47.33
G. tricuspis	0.059	0.028	2.127	0.312	0.013	8.17	55.50
Lycodes	0.047	0.013	3.679	0.243	0.009	6.48	61.98
Anarhichas	0.045	0.006	8.079	0.013	0.230	6.16	68.14
Cottidae	0.044	0.040	1.096	0.239	0.010	6.12	74.26
Liparis	0.042	0.037	1.125	0.224	0.008	5.81	80.07
Sebastes	0.042	0.019	2.171	0.049	0.247	5.75	85.82
Contrast: Kongsfjorden + Rijpfjorden Inner & Rijpfjorden Outer							
A. medius	0.108	0.048	2.224	0.224	0.731	15.80	15.80
B. saida	0.081	0.053	1.540	0.579	0.245	11.91	27.71
L. lampretaeformis	0.069	0.021	3.266	0.048	0.383	10.18	37.89
M. villosus	0.064	0.005	11.678	0.005	0.311	9.36	47.25
Anarhichas	0.047	0.007	6.327	0.096	0.230	6.96	54.21
Sebastes	0.046	0.013	3.486	0.027	0.247	6.81	61.02
G. tricuspis	0.044	0.039	1.114	0.234	0.013	6.43	67.45
C. harengus	0.032	0.036	0.908	0.163	0.015	4.75	72.20
H. platessoides	0.031	0.018	1.677	0.155	0.216	4.51	76.71
Myoxocephalus	0.028	0.035	0.815	0.145	0.009	4.14	80.85
Contrast: Kongsfjorden & Rijpfjorden Inner + Rijpfjorden Outer							
B. saida	0.070	0.048	1.444	0.544	0.522	11.29	11.29
A. medius	0.063	0.037	1.726	0.295	0.298	10.25	21.54
M. poutassou	0.048	0.036	1.331	0.237	0.004	7.80	29.34
G. tricuspis	0.046	0.036	1.270	0.156	0.237	7.48	36.82
Anarhichas	0.040	0.047	0.850	0.179	0.067	6.49	43.31
C. harengus	0.038	0.032	1.182	0.215	0.087	6.08	49.39
H. platessoides	0.037	0.024	1.559	0.134	0.185	6.00	55.39
Lycodes	0.037	0.024	1.529	0.007	0.184	5.98	61.37
Cottidae	0.035	0.038	0.923	0.008	0.182	5.60	66.97
Myoxocephalus	0.034	0.032	1.089	0.135	0.118	5.57	72.54

Indicator species analysis

Out of the total of 28 taxons there was no taxon with significant association with a group, but there was found a trend for the genus *Lycodes* with a low, but not significant p-value (p=0.058) and high association (stat=0.938) to the group Rijpfjorden Inner (Table 3).

Table 3 Output from indicator species analysis with alpha=1 using Hellinger transformed abundance data. The taxa was listed in descending order with the strongest association on top. A higher stat value ment that the taxon was more strongly associated. The number next to group indicates how many species are ideintified as indicators for that group. Trends in assocation for a taxon to a group was indicated by ".".

	stat	**p.value**
Group Kongsfjorden		#sps. 5
M. poutassou	0.748	0.186
C. harengus	0.510	0.776
C. reinhardti	0.503	0.202
G. argentatus	0.498	0.976
Gadidae	0.270	0.971
Group Rijpfjorden Inner		#sps. 7
Lycodes	0.938	0.058 .
Liparis	0.746	0.358
Cottioidei	0.728	0.430
Cottidae	0.722	0.421
G. tricuspis	0.578	0.523
Zoarcidae	0.500	1.000
C. lumpus	0.497	0.800
Group Rijpfjorden Outer		#sps. 8
Stichaeidae	1.000	0.138
M. villosus	1.000	0.138
Sebastes	0.929	0.138
L. lampretaeformis	0.901	0.138
A. medius	0.840	0.174
Sebastinae	0.832	0.138
Pleuronectes	0.701	0.572
Gymnelus	0.569	0.516
Group Kongsfjorden+Rijpfjorden Inner		#sps. 2
B. saida	0.582	0.599
Myoxocephalus	0.461	0.682
Group Kongsfjorden+Rijpfjorden Outer		#sps. 5
L. piscatorius	0.702	0.349
G. morhua	0.660	0.357
Anarhichas	0.551	0.477

L. maculatus	0.470	0.747
Pleuronectidae	0.370	0.913
Group Rijpfjorden Inner+Rijpfjorden Outer		#sps. 1
H. platessoides	0.23	0.972

DISCUSSION

Water samples was taken from two different fjords to study the fish community in the fjord and whether there were differences between them. There was found significant differences between temperature, salinity, and depth between all three sampling groups. Temperature and salinity were significantly higher in Kongsfjorden and Rijpfjorden Outer compared to Rijpfjorden Inner, while the sampling depth was significantly deeper in Kongsfjorden compared to the stations in Rijpfjorden Inner and Outer. Despite these differences in the abiotic environment, there was no significant difference in biodiversity between the three sampling areas, neither for the within-group dispersal or for the mean of the groups, and there was considerable overlap of species between the three areas.

Environmental variables

The physical environment in the three sampling groups was found to be significantly different from each other for depth, temperature, and salinity. The measurements from the CTD were found to correspond to the assumption of characteristics of the water mass for the fjords. Kongsfjorden had higher salinity levels and higher temperature than Rijpfjorden Inner (Figure 5). This corresponds well as the water mass in Kongsfjorden is characterised by both Atlantic Water and Arctic Water (Svendsen et al., 2002), with evidence for increased influx of warmer and more saline water into Kongsfjorden in the later years (De Rovere et al., 2022). Rijpfjorden has a water mass dominated by Arctic Water, these stations were found to have lower saline levels and colder water than in Kongsfjorden. On the other hand, Rijpfjorden Outer had the highest measured temperature and salinity of all the stations. This might be a result of the Svalbard Branch of the WSC flowing at the stations location, bringing large amount of warm and saline Atlantic water eastward along the north of the archipelago (Aagaard et al., 1987), and it is well within the Pérez-Hernàndes et al. (2017) definition of Atlantic Water with a temperature threshold >1°C and salinity >34.9. The depth and water mass characteristics in this station also corresponded with the occurrence of Atlantic Water masses in the water column in the same area (Cokelet et al., 2008). Although there might be

other reasons for the higher temperature and salinity level at this station. These differences in physical environment lend support to expectations of differences in the species assemblage between the different sampling groups.

Species richness and biodiversity

Rijpfjorden Inner and Rijpfjorden Outer had a generally higher species richness and biodiversity compared to Kongsfjorden. But Rijpfjorden Inner was found to have larger variability in species richness and Shannon Index values between sampling stations compared to Kongsfjorden. The linear regressions between Shannon Index and environmental variables showed that biodiversity tended to increase with increasing temperature, while it showed tendencies to decrease with increasing salinity and depth (Figure 7). These linear regression models correspond with other modelling of marine biodiversity on a worldwide scale, where biodiversity was found to decrease with depth and increase with temperature (Gagné et al., 2020). Rijpfjorden Inner and Kongsfjorden had opposite environmental states, where Rijpfjorden Inner had the lowest temperature and salinity level as well as a generally higher species richness and biodiversity, while Kongsfjorden had higher temperature and salinity levels than Rijpfjorden Inner but had lower species richness and biodiversity. The environmental effect should be interpreted with some caution due to low power, as well as low environmental variation, especially for the salinity values. The variation in salinity is very small and may be too low to drive variations in species composition, while the differences in temperature and depth are more profound and may have larger effect on the detected variations. In addition, the salinity and temperature levels only represent a snapshot of the local environmental state, and larger variations at a spatial and temporal scale might be more relevant for environmental effect on species composition.

The difference in species richness and biodiversity between Kongsfjorden and the two other sampling groups in the Rijpfjorden area, might be a result of the large difference in reads per station. There was a lot less reads detected in the stations in Kongsfjorden than in Rijpfjorden Inner and Rijpfjorden Outer stations. Average reads for the stations in Kongsfjorden was 377,591 (sd=111,850), while in Rijpfjorden Inner the average reads were 1,579,405 (sd=453,946), and the reads for the Rijpfjorden Outer station was 1,213,424. Whether this is because Kongsfjorden had a lower amount of eDNA or the processing of the samples after sampling, is unknown.

The ecology of eDNA could play a role in the difference in detected DNA. The degradation rate of eDNA is dependent on environmental factors such as temperature and microbial activity among other (De Souza et al., 2016), and colder water conditions have shown to decrease degradation rates (Strickler et al., 2015). This may have led to longer preservation of eDNA in the water in Rijpfjorden Inner than in Kongsfjorden, resulting in higher number of reads detected. The same has been observed in the Arctic by Lacoursière-Roussel et al. (2018), where a greater amount of species richness was found in water samples collected in the winter under sea ice cover, than in the summer samples. Temperature is an important environmental factor to considerer when using eDNA as higher temperature increased the amount of eDNA released by fish, making fish abundance/biomass is better reflected at higher temperatures (Lacoursière-Roussel et al., 2016). This could be a factor contributing to higher abundance and biodiversity observed in Rijpfjorden Outer.

The sampling depth might be a factor that affected the number of reads. Kongsfjorden is a deeper fjord than Rijpfjorden and subsequently the eDNA in Kongsfjorden was sampled much deeper. There were significant differences in sampling depth between all the three sampling groups, but the main difference in depth was between the combined Rijpfjorden groups and Kongsfjorden. As biodiversity declines with increasing depth, as well as the uncertainty of degradation rate and the vertical distribution of eDNA, this might have had an effect on the amount of eDNA detected between the three sampling groups. The influence of depth in these samples are unknown as there was only sampled from one depth per station, and vertebrate eDNA in surface water samples and samples taken close to the seafloor have shown differences in detected species, indicating vertical heterogeneity (Andruszkiewicz et al., 2017; Lacoursière-Roussel et al., 2018).

Beta diversity

A major objective in this study was to assess variations in the fish community assemblage in the two fjords systems in Svalbard, Kongsfjorden and Rijpfjorden, as well as the area of the station Rijpfjorden Outer. There was found no significant differences in biodiversity between the three sampling groups; Kongsfjorden, Rijpfjorden Inner and Rijpfjorden Outer, and there was considerable species overlap between the sampling groups (Figure 8). Still, there was taxa/species that showed a larger association to one or more of the sampling areas.

The most dominant species in the abundance data was *B. saida*, *C. harengus* and *A. medius*. *B. saida* and *A. medius* was found to be the two species with the largest contribution to dissimilarity between the sampling groups (Table 2). It is important to note that because the simper analysis is highly affected by taxa with high variance the contribution to the dissimilarity is not always accurate, transformation of data can to some degree reduce, but not fully correct for the mean-variance trend effecting the result of the SIMPER analysis (Warton et al., 2012). This might explain why the two most abundant species, *A. medius* and *B. saida*, was also found to be the two species with the largest contribution to the dissimilarities in all the different pairings (Table 2). Even though not all the groupings seemed to have a large difference in the average relative abundance within the different groups, e.g., *B. saida* was found to contribute 10.72 % of the dissimilarity between Kongsfjorden and Rijpfjorden Inner, but the difference in the average relative abundance was not that large between the two groups, 0.544 and 0.614 respectively.

B. saida was well represented in all three sampling groups but had a higher average relative abundance in the two inner fjord groups (Kongsfjorden and Rijpfjorden Inner) than in Rijpfjorden Outer (Table 2, Figure 8), where Rijpfjorden Inner had a higher average relative abundance than in Kongsfjorden. In shallower areas such as on the shelf *B. saida* is only found sparsely among the ice, but instead tends to be found near the bottom (Mecklenburg et al., 2018). This can be a reason that there is so much DNA obtained from *B. saida* as the eDNA was sampled close to the seabed.

B. saida (polar cod) is a key stone arctic species with high ecological value, that is common in all parts if the Arctic Ocean (Hop & Gjøsæter, 2013), and for some groups spawning occurs around Svalbard (Eriksen et al., 2020). *B. saida* is categorised as an Endangered (EN) species by Artsdatabanken (2021) due to large decline in population size. This decline is most likely induced by the retreating sea ice and increased water temperature in the Arctic (Eriksen et al., 2020; Huserbråten et al., 2019), and *B. saida* are reportedly becoming rare in Kongsfjorden (Brand & Ficher, 2016). Despite this, it was one of the most dominant species detected and is an abundant species in fjords in Svalbard (Renaud et al., 2012), and is still a dominant prey in several predators' diet (Bengtsson et al., 2020; Brand & Ficher, 2016).

Species composition – evidence of "Atlantification"?

Kongsfjorden

Micromesistius poutassou was the species with the highest average relative abundance in Kongsfjorden, but *C. harengus* was also found to have a higher average relative abundance in Kongsfjorden than in the other groups, but was also associated to Rijpfjorden Inner (Figure 8). *G. morhua* (Atlantic cod) had highest association to Kongsfjorden and was detected in two of the three stations in Kongsfjorden, but was in no way a dominant species in the community as previously observed (Brand & Ficher, 2016). An unknown *Myoxocephalus* species had a high average relative abundance in the inner fjord groups, with only a small difference between Kongsfjorden and Rijpfjorden Inner. An issue is that 12S rRNA sequences cannot distinguish some closely related species. Although there was detected *Myoxocephalus* spp. using MiFish metabarcoding, the species in this genus was impossible to distinguish based on the target 12S rRNA region. Both *Myoxocephalus scorpius* (shorthorn sculpin) and *Myoxocephalus quadricornis* (fourhorn sculpin) are common species around Svalbard (Eriksen et al., 2012; Pethon, 2019), were *M. scorpius* is a dominant species in the shallow waters of Kongsfjorden (Brand & Ficher, 2016).

Micromesistius poutassou (blue whiting) has a large distribution in the Atlantic, but also around Svalbard and the Barents Sea, and has newly been found in the ring seal diet in small quantities on the west coast of Spitsbergen for the first time (Bengtsson et al., 2020). *C. harengus* (Atlantic herrring) was detected in all stations and was one of the three species representing >50% of the total number of reads in the abundance data, but was found to have the highest association to Kongsfjorden. *C. harengus* is an Atlantic species and is widly distributed both in the north Atlantic and in the Barents Sea (Pethon, 2019). *Careproctus reinhardti* (longfin snailfish) was also associated with Kongsfjorden, and is an Arctic deep-water species (Pethon, 2019).

The most dominant species observed in Kongsfjorden was typical Atlantic species, but the overall species assemblage consisted of a mixture of Atlantic, Arctic and "something in between" species. This corresponds with the assumed situation of Kongsfjorden. As Kongsfjorden is situated on the west coast of Spitsbergen and has a water mass influenced by a mixture of Atlantic Water and Arctic Water (Svendsen et al., 2002). "Atlantification" has led to increased encroachment of Atlantic Water in Kongsfjorden because of enhanced advection of Atlantic Water from the WSC (De Rovere et al., 2022; Pavlov et al., 2013). This

have consequently affected the fish community assemblage in Kongsfjorden, which harbours both Arctic and Atlantic fauna (Bengtsson et al., 2020; Brand & Ficher, 2016; Descamps et al., 2019). Kongsfjorden has drifted in an Atlantic direction over the last decade, and Atlantic species such as *M. villosus*, *C. harengus*, *G. morhua*, and *M. aeglefinus* has become increasingly more important predator diet items than for example *B. saida* in the later years (Descamps et al., 2019; Vihtakari et al., 2018).

Rijpfjorden Inner

The species found to show highest connection to Rijpfjorden Inner was *Gymnocanthus tricuspis*, and an unknwon *Lycodes* sp. and *Cottidae* sp., and unknown *Liparis* spp (Table 2), as well the species *Cyclopterus lumpus* (Figure 8). An unidentified *Myoxocephalus* sp. was also found to have high association to Rijpfjorden Inner in addition to Kongsfjorden. An unknown *Cottidae* sp. had largest average relative abundance in Rijpfjorden Inner. In addition another *Cottidae* species *Gymnocanthus tricuspis* (Arctic staghorn sculpin) was found to have its largest average relative abundance in Rijpfjorden Inner as well as highest association (Table 2, Figure 8), but was also associated to Kongsfjorden. *G. tricuspis* is a true Arctic species and is common along the whole coast of Svalbard (Eriksen et al., 2012; Pethon, 2019). *C. lumpus* (lumpsucker) has a wide distribution in the Atlantic Ocean, and has the later years expanded its range to the northern parts of the Barents Sea (Pethon, 2019). An unidentified *Zoarcidae sp.*, as well as a *Lycodes* sp., was found to have higher connection to Rijpfjorden Inner. *Zoarcidae* has a year-round residency in Svalbard and is considered an arctic family (Eriksen et al., 2012; Pethon, 2019). The MOTUs assigned to *Zoarcidae* and *Lycodes* had the issue where 12S rRNA sequences could not distinguish closely related species using MiFish metabarcoding, the species in this family and genus was impossible to distinguish based on the target 12S rRNA region. Some of the common species that resides in Svalbard within this family is *Lycodes vahli*, *L. frigidus* and *Zoarces viviparus* (Eriksen et al., 2012; Pethon, 2019).

The genus *Liparis* is generally associated to polar waters (Eriksen et al., 2012), and an unknown *Liapris* sp. was found to have higher association to Rijpfjorden Inner. It was not possible to distinguish between several closely related species in this genus based on the target 12S rRNA region. The species *Liparis fabricii*, *Liparis montagui* and *L. l. bathyarcticus* (subspecies of *L. liparis*) have known distribution in Svalbard (Pethon, 2019). But in comparison to more traditional sampling methods typically highly dependent on physical

identification, the taxonomy of *Liparidea* species is relatively unknown due to the rarity of many species and difficulties in identifying distinct morphological characteristics (Eriksen et al., 2012).

Rijpfjorden is located North-East on the archipelago and as a high-Arctic fjord the water mass is mostly influenced by Arctic water and is less affected by Atlantic water moving northward by the WSC (Ambrose et al., 2006; Wallace et al., 2010). Consequently, the fish community was expected to be dominated by typical Arctic species. Rijpfjorden Inner was found to mainly be dominated by species and genus in families that has year-round residency in Svalbard and therefore considered Arctic (Eriksen, 2012; Pethon, 2019). This indicates that there might be less encroachment of Atlantic Water in Rijpfjorden and that it still has not experienced the same alteration in species assemblage that Kongsfjorden has over the last decade (Descamps et al., 2019; Vihtakari et al., 2018).

Rijpfjorden Outer

The species found to have the highest connection to the Rijpfjorden Outer area was the species *A. medius*, *M. villosus*, and *L. lampretaeformis*, and an unnidentified *Sebastes* sp. and *Anarhichas* sp., but the species in these genera was impossible to distinguish using MiFish metabarcoding based on the target 12S rRNA region. *H. platessoides* also had the highest connection to Rijpfjorden Outer, but also quite high in Rijpfjorden Inner and Kongsfjorden. A very low amount of reads was assigned to an unidentified *Stichaeidae* sp. at this station, but other species within this family was detected, amoung them *L. lampretaeformis* and *A. medius*. *A. medius* (stout eelblenny) is an Arctic species with wide distributed, and is generally only found in waters with temperature lower than 0°C, but has been found in warmer waters in Svalbard areas (Pethon, 2019). *L. lampretaeformis* (snakeblenny) is widly distributed in the North-Atlantic (Pethon, 2019). *L. lampraetaeformis* and *A. medius* are both common species near Spitsbergen archipelago (Eriksen et al., 2012). *M. villosus* (capelin) is a sub-Arctic species found in the North Atlantic and adjacent Arctic water (Mecklenburg et al., 2018), and is found in all waters north for the polar circle (Pethon, 2019), but is extending its distribution northward that may lead to alteration of the Arctic food web (Hop & Gjøsæter, 2013).

Even though Arctic species was most dominant in the species assemblage in Rijpfjorden Outer, this sampling station was closer to Kongsfjorden in temperature and salinity compared

to Rijpfjorden Inner (Figure 5, Figure 8). This might indicate that Rijpfjorden Inner was more isolated from the larger processes along the coast of Svalbard. But that true Atlantic species has not intruded into the north-eastern parts of the archipelago in the same way they have in Kongsfjorden.

These results support the already observed northward shift in Atlantic fish species distribution due to increasing temperatures of the Arctic Ocean, and the "Atlantification" process. With a higher number of Atlantic species observed in Kongsfjorden than found in Rijpfjorden Inner. But these results only provide a snapshot of reality as they are based only a one-time sampling, and therefore do not show variability in species assemblage between e.g., years or seasons. Marine fish distribution often changes based on behaviour ecology and the local species assemblage might be altered based feeding behaviour, migration and spawning, and other ecological factors. In addition, information on the amount of Atlantic water on the west coast of Spitsbergen and its inter-annual variations would be needed to have a better understanding of the potential relationship between species assemblage and the effect of Atlantic Water influx.

Caveats

In addition to the previous mentions of the low variability in the environmental factors, especially the salinity levels that may be too low to drive variations in the species distribution, and the measurements only provide a snapshot of the abiotic conditions of the sampling areas, and do not show spatial and temporal variations. All this considered, there are some weaknesses to the methodology. The low number of samples makes for uncertainties in predictions, although small samples contain a wide range of fish species (Thomsen et al., 2012). eDNA is also known to very stochastic, even though the biological replicates were combined to limit this. There are still unknown issues connected to this. Due to the Hellinger transformation of the data, all associations are based on relative abundance. As well as there being some issue where the targeted 12S rRNA region could not distinguish between closely related species within certain families and genera, creating issues in identifying the exact species assemblage in the different sampling areas. Especially if this is true for families where species are typically too elusive to be able to detect by traditional sampling methods.

Future perspectives

Marine environments are experiencing large changes as a result of climate change, where species are shifting their distribution poleward (Hastings et al., 2020), and the marine system as a whole are in danger of a mass extinction (Penn & Deutsch, 2022). Biodiversity monitoring is important to create a baseline for future management and rate of change. Anthropogenic impacts and the need for systematic conservation planning have further motivated the analysis of diversity patterns and processes at regional to global scales (Margules & Pressey, 2000). Arctic is experiencing a no linear effect of climate change and global warming (Hoegh-Guldberg & Bruno, 2010), and has led to "Atlantification" (Fossheim et al., 2015; Kortsch et al., 2015). Svalbard is an area of interest as an analogue of change as the archipelago is located in a transition between boreal and Arctic biogeographic zones. Especially has Kongsfjorden been considered a fjord of high interest as it is a particularly suitable site for exploring the possible impacts of climate change, as it is highly affected by both the Atlantic water influx by the WSC and melting of tidal glaciers, as both factors are linked to climate variability (Svendsen et al., 2002).

There are uncertainties connected to how this change in fish community will affect the local ecology. The northward movement of species has already altered diet of several Svalbard native species (Barnes & Turner, 2016; Descamps et al., 2019; Hop & Gjøsæter, 2013; Kortsch et al., 2015; Vihtakari et al., 2018). As increasing temperatures leads to northward movement of *G. morhua*, *B. saida* was found to replace the Atlantic cod's normal diet in areas where the two species overlapped (Orlova et al., 2009), illustrating new predator-prey relationships. But so far, the Arctic marine food webs have shown resilience to the climate change, although this resilience might be short-lived (Griffith et al., 2019).

CONCLUSION

As climate change and warming are driving biodiversity loss and altering species distribution, biodiversity monitoring is important to create baselines for management and to understand the effect these changes have on the ecosystem. The Arctic systems are experiencing change much faster and larger than other systems, and the biodiversity in the Arctic is less studied than other ecosystems. The samples of eDNA from two distant fjord in Svalbard reveals differences in species assemblage and indications of "Atlantification" process along the western coast of Svalbard, where there are indications that the northern fjords are yet highly

affected and show signs of being more isolated from the larger processes. The species community and climate state in the fjords on the west coast of Spitsbergen might serve as an analogue for the future of the northern fjords, and the further northern parts of the Arctic Ocean. Further research of biodiversity is needed, were spatial and temporal variations need to be addressed. A better understanding of the ecology of eDNA in Arctic environments as well as the effect of the environmental factors on degradation and persistence in the environment is needed to better monitor biodiversity and the rate of change. And continuous monitoring should be done to forecast regime shifts and resilience in the high-Arctic fjords in Svalbard, but also the future of the Arctic Ocean.

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APPENDICES

Appendix A – Protocol for isolation of genomic DNA from water samples

EXTRACTION PROTOCOL FOR STERIVEX FILTERS
EDNA EXTRACTION USING QIAGEN DNEASY POWERWATER STERIVEX KIT

Protocol: Detailed

Important points before starting

- We recommend you use Sterivex filter units (Millipore cat. no. SVGPL10RC). If you have non–Luer-style Sterivex filters, please refer to the "Troubleshooting Guide", page 17, or contact technical services for recommendations.
- Add Solution ST1A to the bottle labeled Solution ST1B, and mix well.
- Warm Solutions MBL and MR at 65°C for 5–10 min to dissolve precipitates prior to use. Solutions MBL and MR must be used while still warm.
- Shake Solution PW to mix before use.

Procedure

1. Filter water sample through a Sterivex filter unit. Remove as much of the remaining liquid as possible using a syringe containing air. Cap both ends with the inlet and outlet caps.
   Note: For long-term storage, Sterivex filter units should be stored capped without excess liquid at –30 to –15°C.
   Note: We do not recommend adding SET (sucrose/EDTA/Tris) buffer to Sterivex filter units for storage. SET buffer is not required for this protocol and may interfere with DNA extraction and inhibitor removal. Refer to the "Storage with SET Buffer" section in the "Troubleshooting Guide" (page 17) for more information.
2. Remove the inlet cap and add 0.9 ml of Solution ST1B using a pipette tip. Insert pipette completely into inlet so that pipette tip is visible inside the unit, just above the membrane.
   Note: Solution ST1B is a cell-release solution that helps pull microbes from the membrane into the solution so that they can be lysed. After Solution ST1A is added to the bottle labeled Solution ST1B, it should be stored at 2–8°C.
3. Re-cap the inlet and secure the Sterivex filter unit horizontally, with the inlet facing out, to a vortex adapter.
4. Vortex at minimum speed for 5 min.

5. While still attached to the vortex adapter, rotate the Sterivex filter unit 180 degrees from the original position. Vortex at minimum speed for an additional 5 min.
6. Set the Sterivex filter unit with the inlet facing up and remove the inlet cap. Add 0.9 ml of Solution MBL using a pipette tip. Insert pipette completely into the inlet so that the pipette tip is visible inside the unit, just above the membrane. Re-cap the inlet.
   Note: Solution MBL is a strong lysing reagent that includes a detergent to help break cell walls and will remove non-DNA organic and inorganic materials. It is also part of the Inhibitor Removal Technology (IRT). When cold, this solution will form a white precipitate in the bottle. Heating to 65°C will dissolve the components without damaging them. Solution MBL should be used while it is still warm.
7. Incubate the Sterivex filter unit at 90°C for 5 min. Ensure heat is evenly distributed.
   Note: Do not heat at higher temperatures or for longer than 5 min.
   Note: For samples containing easy-to-lyse organisms or where less DNA shearing is desired, this step can be omitted. Refer to the "Alternative Lysis Methods" section, page 18, in the "Troubleshooting Guide".
8. Cool the unit at room temperature for 2 min. Ensure that the caps are on tightly.
9. Secure the Sterivex filter unit horizontally, with the inlet facing out, to a vortex adapter.
10. Vortex at maximum speed for 5 min. Set the Sterivex filter unit with the inlet facing up and remove the inlet cap.
    Note: Vortexing at maximum speed helps to further free microbes and lyse cells within the Sterivex filter membrane.
11. Pull back the plunger of a 3 ml syringe to fill the barrel with 1 ml of air, and then attach it to the inlet of Sterivex filter unit. Push air into the unit until there is resistance, and then release the plunger. Continue to pull back on the plunger to remove as much of the lysate as possible. Detach the syringe from the Sterivex filter unit.
    Note: Lysate containing both intact and lysed microbes is removed from the Sterivex filter unit for further processing.
12. Add the lysates to 5 ml glass PowerBead Tubes. Secure the PowerBead Tubes horizontally to a vortex adapter.

Note: For samples containing easy-to-lyse organisms or where less DNA shearing is desired, steps 12 and 13 can be omitted. Refer to the "Alternative Lysis Methods" section, page 18, in the "Troubleshooting Guide".

13. Vortex at maximum speed for 5 min. Centrifuge at 4000 x *g* for 1 min.
14. Transfer all the supernatant to a clean 2.2 ml collection tube.
    Note: Placing the pipette tip down into the beads and against the bottom of the tube is required. Pipet more than once to ensure removal of all the supernatant. Any carryover of beads will not affect subsequent steps. Expect to recover ~1.5 ml of supernatant. The supernatant is separated and removed from sample debris and beads at this step.
15. Add 300 µl of Solution IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.
    Note: Solution IRS is another part of the Inhibitor Removal Technology® (IRT) and is a second reagent to remove additional non-DNA organic and inorganic materials, including humic acid, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.
16. Centrifuge the tube at 13,000 x *g* for 1 min. Avoiding the pellet, transfer the supernatant to a clean 5 ml collection tube.
    Note: The pellet at this point contains additional non-DNA organic and inorganic materials. For highest DNA yield and purity, avoid transferring any of the pellet.
17. Place a tube extender firmly into an MB Spin Column.
    Note: The tube extender serves as an MB Spin Column extender that allows the one-step addition of all sample lysate (4.5 ml) without the use of a midi or maxi column and centrifugation.
18. Attach the tube extender/MB Spin Column unit to a VacConnector and VacValve on the QIAvac 24 Plus Manifold.
19. Add 3 ml of Solution MR to the collection tube that contains supernatant. Vortex to mix.
    Note: Solution MR is a high-concentration salt solution. DNA binds tightly to silica at high salt concentrations. Solution MR adjusts the salt concentration to selectively allow for the binding of DNA to the MB Spin Column filter membrane, but non-DNA organic

and inorganic materials that may still be present at low levels are prevented from binding.

20. Load the entire 4.5 ml of supernatant into the tube extender/MB Spin Column.
21. Turn on the vacuum source and open the VacValve of the port. Allow the lysate to pass through. After the lysate has passed through completely, close the VacValve of that port.
    Note: The DNA is selectively bound to the MB Spin Column filter membrane while contaminants pass through.
22. While keeping the MB Spin Column attached to the VacValve , remove the tube extender from the MB Spin Column and discard.
    Note: The tube extender is discarded so that the MB Spin Column can be washed
23. Add 0.8 ml of ethanol into the MB Spin Column. Open the VacValve. Allow the ethanol to pass through the column completely. Close the VacValve.
    Note: The ethanol prewash helps remove residual contaminants, to result in higher DNA purity and yield.
24. Add 0.8 ml of Solution PW to the MB Spin Column. Open the VacValve and allow Solution PW to pass through the column completely. Continue to pull a vacuum for another minute to dry the membrane. Close the VacValve.
    Note: Solution PW is an alcohol-based wash solution used to further clean the DNA that is bound to the MB Spin Column filter membrane. This wash solution removes residual salt and other contaminants while allowing the DNA to stay bound to the MB Spin Column filter membrane.
25. Add 0.8 ml of ethanol to the MB Spin Column. Open the VacValve and apply a vacuum until the ethanol has passed through the MB Spin Column completely. Continue to pull a vacuum for another minute to dry the membrane. Close the VacValve.
    Note: Ethanol ensures complete removal of Solution PW, to result in higher DNA purity and yield.
26. Turn off the vacuum source and open an unused port to vent the manifold. If all 20 ports are in use, break the vacuum at the source.

27. Remove the MB Spin Column and place in a 2.2 ml collection tube. Centrifuge the tube at 13,000 x *g* for 2 min to completely dry the membrane.
    Note: The second spin removes residual ethanol. It is critical to remove all traces of ethanol because it can interfere with downstream DNA applications such as PCR, restriction digests, and gel electrophoresis.
28. Transfer the MB Spin Column to a new 2.2 ml collection tube and add 100 µl of Solution EB or sterile, DNA-free, PCR-grade water to the center of the white filter membrane.
    Note: Placing EB (sterile elution buffer) in the center of the small white membrane will ensure the entire membrane is wet. This will result in a more efficient and complete release of the DNA from the filter membrane. As Solution EB passes through the MB Spin Column filter membrane, the DNA that was bound in the presence of high salt is selectively released by Solution EB (10 mM Tris), which lacks salt. Solution EB contains no EDTA. If DNA degradation is a concern, sterile TE may also be used instead of Solution EB for elution of DNA from the MB Spin Column.
    Note: Alternatively, sterile, DNA-free, PCR-grade water may be used for DNA elution from the MB Spin Column at this step.
29. Centrifuge at 13,000 x *g* for 1 min at room temperature. Discard the MB Spin Column. The DNA is now ready for any downstream application.
    Note: We recommend storing DNA frozen (–30 to –15°C* or –90 to –65°C†). To concentrate DNA, refer to the "Troubleshooting Guide" on page 17.

* For freezers that are set at –20°C.
† For freezers that are set at –80°C.

Appendix B - MOTU and abundance table

MOTU ID	TAXONOMIC RANK	SCIENTIFIC NAME	R1846	R1869	R1870	R1872	R1887	R1888	R1929	TOT. NR. OF READS
T12S_000000003	species	*Anisarchus medius*	81785	7479	25362	295589	222	213	649225	1059875
T12S_000000017	species	*Boreogadus saida*	4776	188672	258128	162866	1578116	465718	73080	2731356
T12S_000000028	species	*Cyclopterus lumpus*	16	20	32	127	56	148911	92	149254
T12S_000000038	species	*Clupea harengus*	27753	34790	100	374	189726	171	260	253174
T12S_000000063	genus	*Sebastes*	1	16	9	30345	22	15	73956	104364
T12S_000000088	genus	*Liparis*	9	20	39	133409	82	158243	85	291887
T12S_000000097	genus	*Anarhichas*	39	79	129927	378	237	178	64150	194988
T12S_000000162	species	*Lophius piscatorius*	9	6	11	33	17	13	24	113
T12S_000000184	genus	*Myoxocephalus*	48834	27	45	38734	181310	82	94	269126
T12S_000003138	genus	*Gymnelus*	11	6	31	75	90	49	71	333
T12S_000003144	species	*Hippoglossoides platessoides*	45617	67	98	145911	239	49573	56364	297869
T12S_000004416	family	*Cottidae*	24	10	39	308363	72	80129	118	388755
T12S_000004498	species	*Gymnocanthus tricuspis*	64842	43	66	262739	34630	177928	211	540459
T12S_000004777	species	*Micromesistius poutassou*	52722	9	45576	20	42	19	21	98409
T12S_000009472	genus	*Lycodes*	14	16	28	178830	58431	55281	92	292692
T12S_000015951	species	*Gaidropsarus argentatus*	2	49287	2	8	14	6	16	49335
T12S_000016053	species	*Mallotus villosus*	28	4	10	31	15	19	117285	117392
T12S_000017513	species	*Lumpenus lampretaeformis*	30	19815	25	47	39	22	178151	198129
T12S_000035229	species	*Careproctus reinhardti*	5	4	38375	8	24	11	12	38439
T12S_000041055	family	*Gadidae*	0	2	1	0	2	0	3	8
T12S_000048617	genus	*Pleuronectes*	0	0	0	2	0	0	1	3
T12S_000050527	family	*Gadidae*	0	0	0	0	1	0	0	1
T12S_000100015	family	*Gadidae*	0	0	0	0	0	0	0	0
T12S_000227172	species	*Gadus morhua*	0	5	2	0	2	0	7	16
T12S_000283895	family	*Pleuronectidae*	2	0	0	0	0	0	0	2
T12S_000290569	genus	*Leptoclinus*	9	0	1	6	0	0	6	22
T12S_000325976	family	*Gadidae*	0	1	0	0	2	0	0	3
T12S_000327594	family	*Gadidae*	0	1	0	0	4	1	0	6
T12S_000337345	family	*Gadidae*	0	1	1	0	7	1	0	10
T12S_000350115	family	*Gadidae*	0	1	0	0	1	0	1	3
T12S_000351804	family	*Gadidae*	0	1	1	0	1	0	0	3
T12S_000370662	family	*Gadidae*	0	0	1	0	3	0	0	4
T12S_000380962	family	*Gadidae*	0	0	1	0	3	0	0	4
T12S_000445991	subfamily	*Sebastinae*	0	0	0	1	0	0	1	2
T12S_000485665	family	*Gadidae*	0	0	0	1	2	0	0	3
T12S_000532414	genus	*Liparis*	0	0	0	1	0	1	0	2
T12S_000538325	family	*Zoarcidae*	0	0	0	2	0	0	0	2

T12S_000556016	family	*Pleuronectidae*	0	0	0	1	0	0	1	2
T12S_000567156	suborder	*Cottioidei*	0	0	0	1	0	2	0	3
T12S_000586505	family	*Gadidae*	0	0	0	0	1	0	0	1
T12S_000617146	family	*Gadidae*	0	1	0	0	4	0	0	5
T12S_000748961	species	*Cyclopterus lumpus*	0	0	0	0	0	2	0	2
T12S_000821244	genus	*Liparis*	0	0	0	1	0	2	0	3
T12S_000892463	family	*Stichaeidae*	0	0	0	0	0	0	2	2
T12S_000896054	family	*Stichaeidae*	0	0	0	0	0	0	3	3
T12S_000969605	family	*Stichaeidae*	0	0	0	0	0	0	2	2

Appendix C – Result ANOVA for environmental variables

Result from Analysis of Variance (ANOVA) for the environmental variables between the different groupings: A) Between all three groups; B) Between Kongsfjorden and Rijpfjorden Inner; C) Between Kongsfjorden and the combined Rijpfjorden groups; D) between the two Rijpfjorden groups. The significant results were indicated in bold, and the significance code used was: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

A) Between all three groups

Salinity					
Source	**Df**	**Sums Sq**	**Mean Sq**	**F Model**	**Pr(>F)**
Groups	2	0.05548	0.027742	15.68	**0.0128***
Residuals	4	0.00708	0.001769		
Temperature					
Source	**Df**	**Sums Sq**	**Mean Sq**	**F Model**	**Pr(>F)**
Groups	2	19.038	9.519	13.56	**0.0165***
Residuals	4	2.808	0.702		
Depth					
Source	**Df**	**Sums Sq**	**Mean Sq**	**F Model**	**Pr(>F)**
Groups	2	36020	18010	17.18	**0.0109***
Residuals	4	4193	1048		

B) Between Kongsfjorden and Rijpfjorden Inner

Salinity					
Source	**Df**	**Sums Sq**	**Mean Sq**	**F Model**	**Pr(>F)**
Groups	1	0.03613	0.03613	20.42	**0.0107***
Residuals	4	0.00708	0.00177		
Temperature					
Source	**Df**	**Sums Sq**	**Mean Sq**	**F Model**	**Pr(>F)**
Groups	1	11.648	11.648	16.59	**0.0152***
Residuals	4	2.808	0.702		
Depth					
Source	**Df**	**Sums Sq**	**Mean Sq**	**F Model**	**Pr(>F)**
Groups	**1**	32766	32766	31.26	**0.00502****
Residuals	4	4193	1048		

C) Between the combined Rijpfjorden groups and Kongsfjorden

Salinity

Source	**Df**	**Sums Sq**	**Mean Sq**	**F Model**	**Pr(>F)**
Groups	1	0.01654	0.016542	1.797	0.238
Residuals	5	0.04602	0.009204		
		Temperature			
Source	**Df**	**Sums Sq**	**Mean Sq**	**F Model**	**Pr(>F)**
Groups	1	4.979	4.979	1.476	0.279
Residuals	4	16.868	3.374		
		Depth			
Source	**Df**	**Sums Sq**	**Mean Sq**	**F Model**	**Pr(>F)**
Groups	**1**	35907	35907	41.69	**0.00133****
Residuals	4	4306	861		

D) Between Rijpfjorden Inner and Rijpfjorden Outer

		Salinity			
Source	**Df**	**Sums Sq**	**Mean Sq**	**F Model**	**Pr(>F)**
Groups	1	0.03894	0.03894	11.22	0.0788 .
Residuals	2	0.00694	0.00347		
		Temperature			
Source	**Df**	**Sums Sq**	**Mean Sq**	**F Model**	**Pr(>F)**
Groups	1	14.060	14.060	10.37	0.0844 .
Residuals	2	2.711	1.356		
		Depth			
Source	**Df**	**Sums Sq**	**Mean Sq**	**F Model**	**Pr(>F)**
Groups	1	113	113.2	0.07	0.816
Residuals	2	3223	1611.3		

Appendix D – Result from Multivariate homogeneity of group dispersaion analysis and Permutational multivariate analysis of variance

Results of Multivariate homogeneity of group dispersion analysis (betadisper function) and Permutational multivariate analysis of variance (adonis function): A-B) between the three groups Kongsfjorden, Rijpfjorden Inner and Rijpfjorden Outer; C-D) between the two fjords where the two Rijpfjorden groups are combined; E-F) between only the two inner fjord group Kongsfjorden and Rijpfjorden Inner

A) Multivariate homogeneity of group dispersions analysis between the three groups.

Source	Df	Sums Sq	Mean Sq	F Model	Pr(>F)
Groups	2	0.099831	0.049915	2.5305	0.1949
Residuals	4	0.078902	0.019726		

B) Permutational multivariate analysis of variance (PERMANOVA) between the three groups.

Source	Df	Sums Sq	Mean Sq	F Model	R2	Pr(>F)
Groups	2	0.51445	0.2572	1.5159	0.43116	0.124
Residuals	4	0.67874	0.1697		0.56884	
Total	6	1.19319			1.00000	

C) Multivariate homogeneity of group dispersions analysis between the two fjords, where the two Rijpfjorden groups are combined.

Source	Df	Sums Sq	Mean Sq	F Model	Pr(>F)
Groups	1	0.000003	0.0000026	1e-04	0.9923
Residuals	5	0.125631	0.0251262		

D) Permutational multivariate analysis of variance (PERMANOVA) between the two fjords, where the two Rijpfjorden groups are combined.

Source	Df	Sums Sq	Mean Sq	F Model	R2	Pr(>F)
Groups	1	0.18113	0.18113	0.89487	0.15181	0.531
Residuals	5	0.01206	0.20241		0.84819	
Total	6	1.19319			1.00000	

E) Multivariate homogeneity of group dispersions analysis between only the two inner fjord groups.

Source	Df	Sums Sq	Mean Sq	F Model	Pr(>F)
Groups	1	0.010241	0.010241	0.4967	0.5198
Residuals	4	0.082477	0.020619		

F) Permutational multivariate analysis of variance (PERMANOVA) between only the two inner fjord groups.

Source	Df	Sums Sq	Mean Sq	F Model	R2	Pr(>F)
Groups	1	0.24201	0.24201	1.4262	0.26284	0.2
Residuals	4	0.67874	0.16968		0.73716	
Total	5	0.92075			1.00000	

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