# Molecular diversity patterns among various phytoplankton size-fractions in West Greenland in late summer

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## ABSTRACT

Arctic regions have experienced pronounced biological and biophysical transformations as a result of global change processes over the last several decades. Current hypotheses propose an elevated impact of those environmental changes on the biodiversity, community composition and metabolic processes of species. The effects on ecosystem function and services, particularly when invasive or toxigenic harmful species become dominant, can be expressed over a wide range of temporal and spatial scales in plankton communities. Our study focused on the comparison of molecular biodiversity of three size-fractions (micro-, nano-, picoplankton) in the coastal pelagic zone of West Greenland and their association with environmental parameters. Molecular diversity was assessed via parallel amplicon sequencing the 28S rRNA hypervariable D1/D2 region. We showed that biodiversity distribution within the area of Uummannaq Fjord, Vaigat Strait and Disko Bay differed markedly within and among size-fractions. In general, we observed a higher diversity within the picoplankton size fraction compared to the nano- and microplankton. In multidimensional scaling analysis, community composition of all three size fractions correlated with cell size, silicate and phosphate, chlorophyll $a$ (chl $a$) and dinophysistoxin (DTX). Individually, each size fraction community composition also correlated with other different environmental parameters, i.e. temperature and nitrate. We observed a more homogeneous community of the picoplankton across all stations compared to the larger size classes, despite different prevailing environmental conditions of the sampling areas. This suggests that habitat niche occupation for larger-celled species may lead to higher functional trait plasticity expressed as an enhanced range of phenotypes, whereas smaller organisms may compensate for lower potential plasticity with higher diversity. The presence of recently identified toxigenic harmful algal bloom (HAB) species (such as *Alexandrium fundyense* and *A. ostenfeldii*) in the area points out the potential risk for this vulnerable ecosystem in a changing world.

## 1. Introduction

Marine planktonic microorganisms are key players in global primary production, dominating their habitats in terms of abundance and biomass (Zinger et al., 2012) and are, thus, important biological drivers in marine ecosystems (Falkowski et al., 1998; Field, 1998). The eukaryotic microalgal group comprises a large and diverse species complement and is responsible for sustaining major biogeochemical processes and ecosystem functioning. The relationship between species diversity and ecosystem functioning has appeared as a central issue during the last few decades (Loreau et al., 2001; Ptacnik et al., 2008), but the critical issues remain unresolved. Ultimately, high functional diversity is considered to represent an ecosystem that is less susceptible to changes and environmental stress (Loreau et al., 2001). Enhanced diversity may have a positive effect on ecosystem stability (Striebel et al., 2009) but need not be the driver of this interplay. Rather, the

stability of the ecosystem depends on the ability of communities to sustain species and functional groups, which are capable of differential responses to a changing environment (Isbell et al., 2011; Loreau et al., 2001). Under homogeneous environmental conditions, a minimal number of essential species may serve to maintain ecosystem stability, whereas a larger number is probably required for stability in a rapidly changing environment (Loreau et al., 2001). Furthermore, stable environments, such as oligotrophic and tropical oceans, and to a certain degree polar seas, lead to reduced adaptive capability of species, because their reaction norms in terms of phenotypic plasticity have narrow windows (Pachauri et al., 2014; Poulin et al., 2011; Thomas et al., 2012; von Dassow et al., 2015).

Expected anthropocenic environmental change is especially profound in the Arctic (Stocker, 2014; White, 1990) and thus has led to an increased number of scientific studies in this polar region during the last decade (Kilias et al., 2014a; Lovejoy et al., 2002; Madsen et al., 2001; Pančić et al., 2015; Thoisen et al., 2015; Włodarska-Kowalczuk et al., 2012). In general, Arctic and subarctic coastal waters around Greenland, Iceland, Norway, and Svalbard are characterized by ice cover during winter, high water stratification, and periods of low light availability (Hanna et al., 2008; Krauss, 1995; Sha et al., 2014; Straneo and Cenedese, 2015; Swift and Aagaard, 1981). Glacial ice production and sea-ice coverage greatly influence the productivity and seasonal succession of the plankton community, which has been studied in detail in Disko Bay (West Greenland) (Hansen et al., 2012). The presence of sea-ice reduces underwater light levels and leads to light limitation for phytoplankton growth. The combination of sea-ice breakup and glacial melt within the water column results in a very productive phytoplankton spring bloom. This impacts secondary production in the bay and thus provides the basis for traditional artisanal and commercial fishing and hunting activities in the region (Hansen et al., 2012).

Disko Bay marine resources are important to the Greenland economy, comprising one of the most important fishing areas in Greenland, and from where natural seafood products, such as fish, shrimp, and mussels constitute ca. 90% of the total Greenlandic export (Nielsen, 2005). Disko Bay is situated at the southern extent of the sea ice-covered area during winter; since the 1990 s, the bay region has experienced a decrease in ice cover concurrent with higher water temperatures. Given these rapid changes, the bay is particularly well situated for studying species diversity of microorganisms in response to climate change, i.e. temperature, salinity and nutrient regime. Changes in the composition of key species at the base of the food web may lead to a cascade of changes at higher trophic levels and also impact biochemical cycles (Hansen et al., 2012). Ultimately, it is crucial to reveal which species are best able to adapt to changing environmental conditions.

Previous research on phytoplankton community structure in Disko Bay has been focused on diatoms (Dünweber et al., 2010; Krawczyk et al., 2014; Sha et al., 2014) and their primary copepod grazer *Calanus* spp. (Juul-Pedersen et al., 2006; Levinsen et al., 2000; Madsen et al., 2001). The area is characterized by high abundance and diversity of diatom species, especially of the genera *Chaetoceros*, *Fragilariopsis* and *Thalassiosira*. The species distribution is closely related to hydrographic conditions, in particular to surface water temperature and changing ice cover (Hansen et al., 2003; Hasle et al., 1996); in this context glacial melting are expected to alter salinity, stratification and nutrient regimes. However, little is known about molecular diversity and distribution of the eukaryotic marine phytoplankton in the Arctic, including the Disko Bay area of the Greenland coast. Furthermore, Arctic diversity surveys have only rarely investigated different size-fractions of the pelagic phytoplankton or subjected them to differential molecular analysis.

Recent developments in high-throughput next generation sequencing (NGS) technologies and their application in molecular microbial ecology have revealed a high diversity of eukaryotic lineages, many

components of which escape detection by microscopic analysis and which comprise taxa that are difficult to isolate (Vaulot et al., 2008; Zinger et al., 2012). Sequencing ribosomal RNA genes (rRNA) has been integrated as a standard in studies to assess diversity of microorganisms from different environments and target groups (Bik et al., 2012; Gottschling et al., 2012; Medlin and Kooistra, 2010). Amplicon sequencing via NGS allows DNA barcoding approaches for marine phytoplankton (i.e., molecular markers for species/biodiversity assessment) and provides new insights into biodiversity of all planktonic size-fractions (De Vargas et al., 2015; Meyer et al., 2007; Taberlet et al., 2012). In line with the increasing use of NGS technologies, the amount of comparative data (i.e., taxonomically relevant sequence information) has increased exponentially. A high complexity of microbial eukaryotic communities has been revealed, comprising substantially more molecular operational taxonomic units (OTUs), when compared to data sets generated from strain-dependent and Sanger sequencing methodologies (Bittner et al., 2013; Egge et al., 2014; López-García et al., 2001; Moon-van der Staay et al., 2001; Vargas et al., 2015).

Our overall aim was to access the molecular biodiversity of three planktonic size-fractions in the coastal waters off west Greenland, with particular emphasis on microplankton (20–50 μm), nanoplankton (3–20 μm) and picoplankton (0.2–3 μm). This size segregation was invoked to investigate the potential impact of cell size on diversity because cell size is a prime parameter for many physiological and metabolic processes (e.g., larger cells of the microplankton are more morphologically plastic and have a lower cell surface to volume ratio as compared to nano- and picoplankton). Within this context, we also investigated which key environmental parameters may be associated with the observed diversity, particularly with respect to the presence of harmful algal bloom (HAB) species, because they possess a potential risk for ecosystem services and function in a changing environment.

## 2. Materials and methods

### 2.1. Sampling

Sampling was performed during the MSM-21/3 expedition (ARCHEMHAB; http://dx.doi.org/10.1594/PANGAEA. 819731) aboard the *RV* Maria S. Merian at the western coast of Greenland (Uummannaq Fjord, Vaigat Strait and Disko Bay) between July 29 to August 8, 2012 (Fig. 1). Water samples were collected in the epipelagic zone by Niskin bottles mounted on a rosette-sampler with conductivity, temperature and depth (CTD) sensors to measure temperature and salinity (Holinde and Zielinski, 2015). In total, 15 stations were sampled at the surface layer (over the upper 30 m) and, additionally, bottom water (10 m above ocean floor) was collected at eight stations.

Samples for nutrient measurements (nitrate, nitrite, ammonium, phosphate and silicate) were directly taken from the Niskin bottles at seven depths per station, processed without filtration and stored frozen at −20 °C for approximately a month until analysis. Nutrient samples were analyzed with a continuous-flow autoanalyzer (Evolution III, Alliance instruments, Frepillon, France) according to standard sea-water methods. Determination of nitrate and nitrite followed the method of Armstrong et al. (1967), ammonium was determined according to Koroleff (1969), silicate according to Grasshoff et al. (1983), and phosphate according to Eberlein and Kattner (2000).

*In situ* chl *a* concentrations were determined by filtering 1.0–2.0 L of seawater (GF/F glass microfiber filters, Whatman, UK; nominal pore size: 0.7 μm). The filtration unit was covered to exclude light, and sample filters were frozen immediately at −80 °C until laboratory analysis. Pigments were extracted by sonication of filters in 10 mL 90% acetone-water and incubation overnight at 4 °C. The extract was centrifuged at 3020×*g* for 10 min, and the fluorescence of the supernatant was determined at 665 nm (Laboratory Fluorometer TD-700, Turner Design, USA). Chl *a* data are archived in PANGAEA (http://dx.
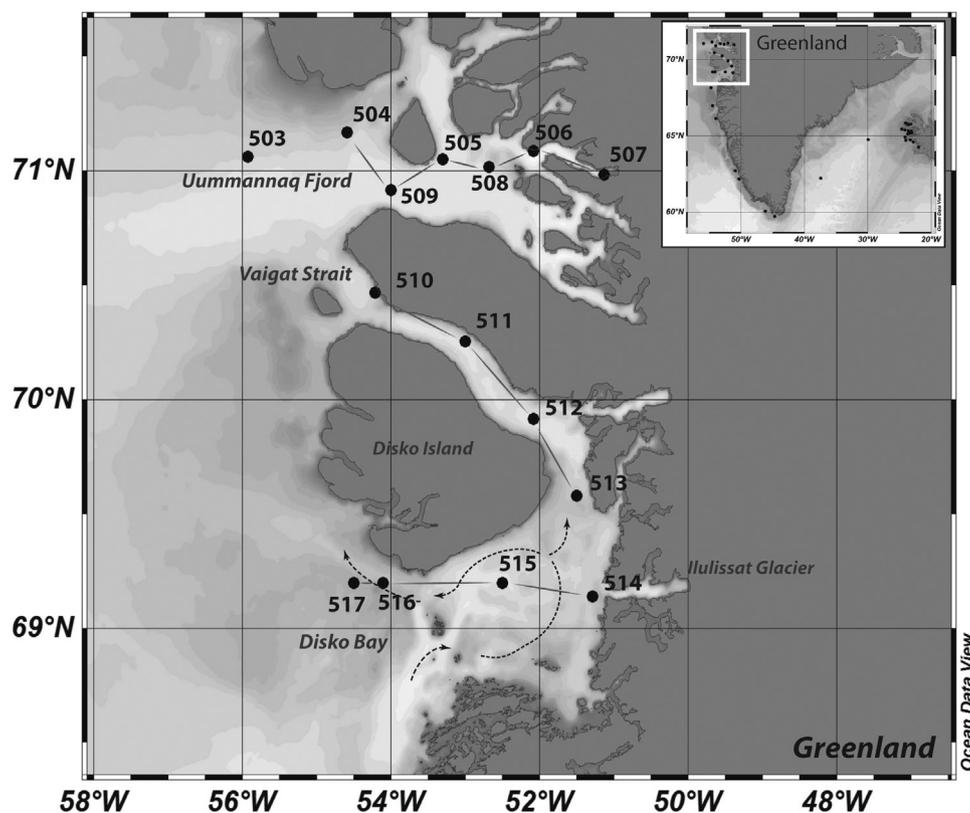
**Fig. 1.** Study site and sampling stations at the West Greenland coast. A scheme of the dominant current system within Disko Bay, modified after Buch (1990), is shown as dashed lines. Stations belonging to one of the three observational transects in Uummannaq Fjord, Vaigat Strait and Disko Bay are connected with solid lines.

doi.org/10.1594/PANGAEA.810651, Zielinski et al., 2013).

Seawater for DNA extraction and toxin analyses was sampled integratively from the surface (1–30 m) with a membrane pump (QBY-25- SS; AGB-Pumpen, Germany). In total, 60 L of sea water per sampling station was passed over a sequential filter tower with different nylon mesh sizes (200, 50 and 20 µm) to obtain different plankton size- fractions. Additionally, vertical net tows (20 µm mesh size, 50 cm net diameter) were performed from 30 m depth to surface. Plankton particulates > 20 µm were subsequently size-fractionated using a filter tower as described above. For nano- and picoplankton size-fractions, 15 L water from three depths (30, 15 and 3 m) was collected with Niskin bottles. Water samples were first pre-filtered (20 µm) and then 1 L of this filtrate was further sequentially size-fractionated by filtration over 3 µm- and 0.2 µm-cutoff polycarbonate membrane filters (Millipore, USA).

Retentates from the filter tower and membrane filters for molecular analyses were backward-rinsed into centrifugation tubes with 0.2 µm-filtered seawater, adjusted with filtered seawater to a total volume of 45 mL and split into three 15 mL aliquots. Cell pellet aliquots were separated for DNA and hydrophilic and lipophilic toxin analysis. Pellets for DNA analysis were resuspended in 400 µL AP1 buffer (Qiagen, Germany), transferred to a vial with 0.2 µm glass beads, shock frozen in liquid nitrogen and kept at −20 °C until further processing.

### 2.2. Determination of phycotoxins in plankton samples

Pellets for determination of hydrophilic paralytic shellfish poisoning (PSP) toxins were homogenized and extracted by reciprocal shaking with 0.03 M aqueous acetic acid followed by centrifugation and filtration of the supernatant prior to analysis, as described in detail in Suikkanen et al. (2013). The PSP toxins were analyzed by ion-pair chromatography on a reverse-phase column coupled to post-column derivatization and fluorescence detection, according to Suikkanen et al.

(2013).

Pellets for determination of lipophilic phycotoxins were treated the same way as for the PSP toxins, but with 100% methanol as extraction solvent. Concentrations of lipophilic toxins, plus the hydrophilic domoic acid (DA), were determined by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Toxins analyzed included domoic acid (DA), gymnodimine (GYM), spirolides (SPX), dinophysis-toxins (DTX) including okadaic acid (OA), pectenotoxins (PTX), yessotoxins (YTX) and azaspiracids (AZA). Methodological details of extraction and LC-MS/MS analyses are given in Krock et al. (2008).

### 2.3. DNA Extraction, amplification and sequencing

For the micro- (20–50 µm) and nanoplankton (3–20 µm) fractions, DNA extraction was performed with the Qiagen DNeasy Plant Kit (Qiagen, Germany). Picoplanktonic DNA was extracted using the Genomic DNA from Soil kit (Macherey-Nagel, Germany). The manufacturer's protocol was followed for both extraction methods. Genomic DNA was eluted from the column, and extracts were stored at −20 °C.

Amplification for subsequent 454-sequencing (micro- and nano-plankton) was carried out in triplicate, targeting the hypervariable LSU D1/D2 region with the primer set Dir-F (5'-ACC CGC TGA ATT TAA GCA TA-3') and Dir2-CR (5'-CCT TGG TCC GTG TTT CAA GA-3') (Scholin et al., 1994). Adaptor sequences were added to the primers to be suitable for the 454-sequencing protocol (Roche). Details in PCR reactions, purification and quality control of amplicons are found in the Supplementary information.

For subsequent 454-pyrosequencing, equal molecular weights of the amplicons were pooled. The pooled amplicons were sequenced on a GS Junior sequencing platform (Roche, Germany).

The picoplankton (0.2–3 µm) sample preparation differed slightly from the methods described above. The same primer set (Dir-F/ Dir2-CR) was used, but the method was modified according to the require-

ment for GS FLX 454-pyrosequencing. Amplification was done in triplicate and pooled amplicons were sequenced on a 454 GS FLX sequencer (Roche, Germany).

### 2.4. Sequence read analysis

In total 35 surface samples across size-fractions and eight bottom water samples were analyzed by 454-pyrosequencing. An analysis pipeline developed in-house (Stecher et al., 2015), for which the backbone consists of scripts from QIIME version 1.8 (Caporaso et al., 2010), was used for sequence-quality filtering and trimming, OTU clustering, chimera detection and preliminary taxonomic classification based on only-sequence-similarity characteristics. The OTUs thereby annotated as alveolates or stramenopiles were classified more accurately by PhyloAssigner version 6.166 (Vergin et al., 2013) with a phylogenetic placement onto reference trees based on 18S/28S concatenated alignments.

Detailed information on reference sequences and tree sets, the quality control of the sequences and OTU clustering are found in the Supplementary information.

The final OTU table was built after removing singletons and doubletons (i.e., OTUs with only one or two reads, respectively, in the dataset) before further analysis, as recommended for spatial differentiation in species diversity, analysis of family or generic category (representing ecotypes or functional groups) (Terlizzi et al., 2009). If there was no match for generic category, the next higher nodal point of the phylogenetic tree with a match was selected for statistical analysis.

### 2.5. Determination and discrimination of potential HAB species

Potentially harmful algal species were identified among classified OTUs. For more detailed species discrimination, qPCR assays were applied for some *Alexandrium* species, with nomenclature according to John et al. (2015). Primers and probes specific for *A. fundyense*, *A. mediterraneum*, *A. minutum*, *A. tamarense*, and *A. tamutum* (Toebe et al., 2013a), as well as *A. ostenfeldii* and *A. pacificum* (Table S1), were accessed for the qPCR assays. All qPCR reactions (1 ng DNA per reaction) were performed in triplicate in a 20 µL assay on a StepOnePlus Real Time PCR System (Applied Biosystems, USA). Quantification was performed as described in Toebe et al., (2013a, 2013b), where efficiency and sensitivity were normalized from standard curves from cultured strains (DNA/cell) and diluted field plankton samples to account for potential matrix influences. Results were normalized to 1 L, corresponding to filtered pump/net tow samples.

### 2.6. Statistical analysis

Statistical analyses were run in the R environment version 3.0.2 (R Development Core Team, 2011) with the package 'vegan' (Oksanen et al., 2013) and 'plyr' (Wickham, 2015). The sequence numbers per sample were not subsampled to the smallest sequence number of any sample, to avoid an increase of potential false positives (McMurdie and Holmes, 2014) by comparison to the relative abundance of counts in samples.

Rarefaction and species (OTU) accumulation curves of each station were calculated in vegan using the function 'rarecurve' and created after quality control and clustering to represent the local species richness and to serve as a reference for the covered diversity in a sample. Sample diversity was estimated by the inverse Simpson's index with the function 'diversity'. The Pielou's evenness index was additionally calculated to differentiate between environments inhabited by an equal number of different species or by a few dominant species. Estimation of sample diversity, taking only species richness or evenness into account, may be highly biased by the sequencing effort. The use of diversity indices, which account for species richness (number of

OTUs/taxa), evenness (relative abundance of sequences within OTU/taxa) and total amount of sequences provide a relatively simple univariate statistic (Countway et al., 2010). The inverse Simpson's index, which was used here, is the reciprocal form of the Simpson's index, is sensitive towards dominant species and can range from 1 to the maximum number of OTUs in each sample and thus increases with increasing diversity (Magurran, 2004).

Non-metric multidimensional scaling (NMDS) with calculated Bray-Curtis dissimilarities was carried out to compare similarities among samples. The OTU table was Hellinger-transformed (double square-root transformation) as recommended for clustering or ordination of species abundance data (Legendre and Gallagher, 2001; Rao, 1995) with the 'decostand' function. Environmental factors were Hellinger-transformed to normalize the dataset and standardized by the factors' total abundance.

## 3. Results

### 3.1. Physical environment and nutrient regime

The upper 10 m of water column in Uummannaq Fjord was characterized by low salinity and showed the lowest salinity (~30) and lowest temperature (~3 °C) within the Uummannaq Fjord transect towards the head of the fjord, close to the Perlerfiup Sermia Glacier (Station 507) (Fig. 2). Stations 504 and 510 are 88 km distant and connected the sampling areas within Uummannaq Fjord and Vaigat Strait. These latter stations exhibited similar salinities, but a temperature difference of 5 °C, with lower temperature at Station 510 (~3 °C). Temperature profiles of the Vaigat Strait indicated considerably colder surface waters than those of Disko Bay. Station 514, near the Ilulissat/Jacobshavn Glacier separating Vaigat Strait and Disko Bay, had the lowest temperature (~0 °C) and highest salinity (~33.5) in surface water along the whole transect. In contrast, the highest temperature (10 °C) and lowest salinity (~30) were measured 50 km away in Disko Bay at Station 515. The more off-shore Stations 516 and 517 were characterized by warmer water (~7 °C) with a high salinity (~33).

In the surface layer (integrated over 3–30 m), low nitrite, phosphate and ammonium concentrations (~0.02–1.1 µM) were measured at all stations (Fig. 3). Nitrate and silicate concentrations were highest towards the head of the fjord (Stations 506 and 507; ~3–9 µM) and close to the Ilulissat Glacier (Stations 513 and 514; ~9–12 µM). Chl *a* concentrations, also integrated over 3–30 m) at the 15 stations were lower within Uummannaq Fjord and along the Disko Bay transect (0.03–1.09 µg L$^{-1}$) compared to within Vaigat Strait (1.1–10.1 µg L$^{-1}$) (Fig. 4).

### 3.2. Molecular biodiversity

Clustering of 14 stations yielded OTU numbers that often correlated with sequencing depth (read numbers or amount of sequences per sample), showing more definable OTUs at stations with higher read numbers (total sequence counts) and *vice versa*. Rarefaction curves calculated to elucidate the grade of sequence saturation for each sample, revealed near-saturation for most samples with the exception of the picoplankton (Fig. S1).

Sequencing of three size-fractions resulted in a wide spectrum of sequencing depth and number of OTUs (4032–37,060 sequences; 110–1165 OTUs, Table S2), after quality control via QIIME, i.e., removal of singletons, doubletons and potential fungal sequences (defined by QIIME). Among the total of 2759 OTUs, some OTUs overlapped between size-classes (Fig. 5). Most OTUs were shared between all three size-fractions (976), followed by shared OTUs between the nano- and picoplankton (419). The number of unique OTUs was greatest for the picoplankton (723), followed by the microplankton (97) and then nanoplankton (80).
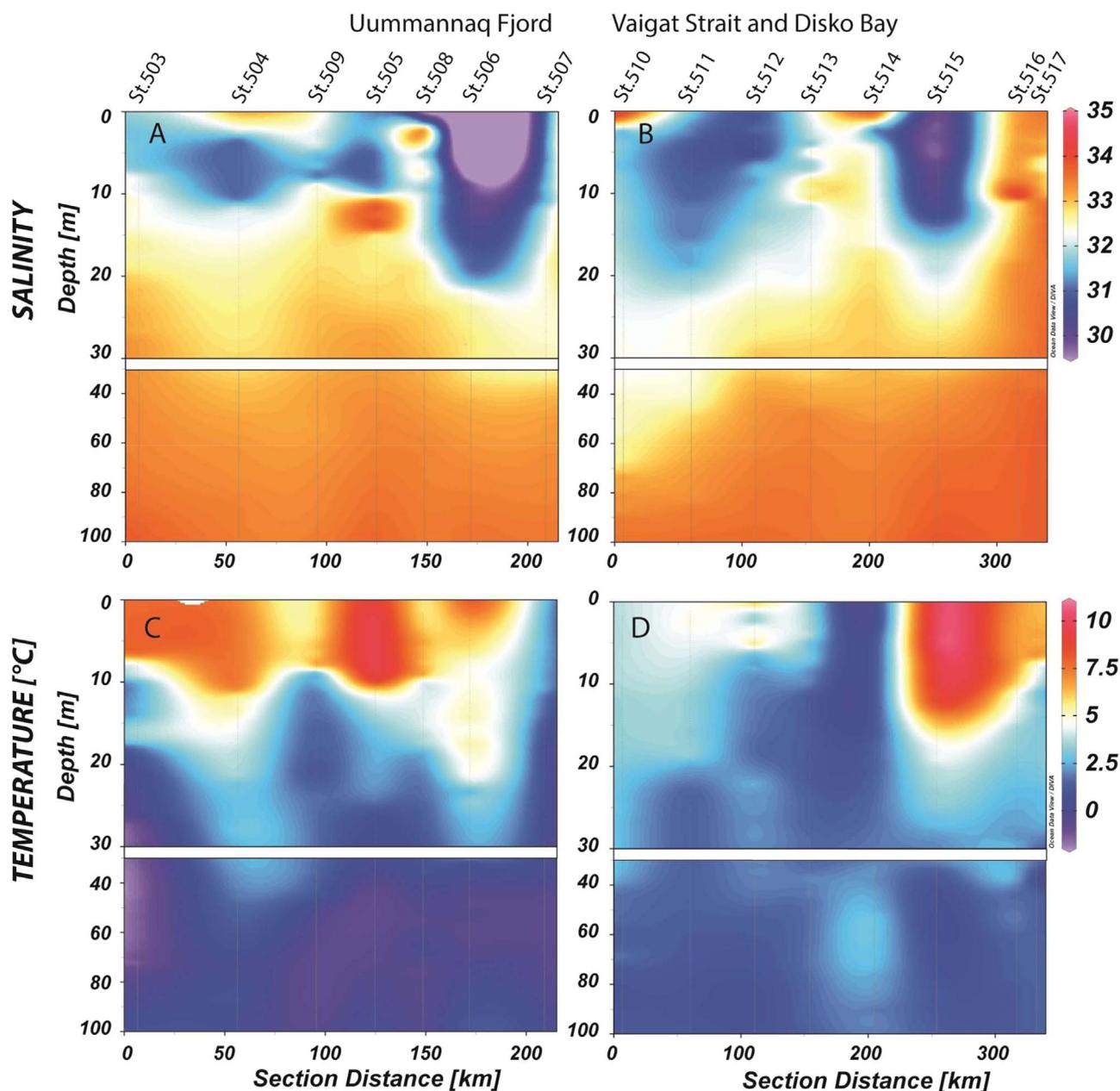
**Fig. 2.** Plot of salinity profile in Uummannaq Fjord (2A) and in Vaigat Strait and Disko Bay (2B); Plot of temperature [°C] profile in Uummannaq Fjord (2C) and in Vaigat Strait and Disko Bay (2D). Plots show the upper 100 m water column of the sampling transects. Note the changes of the depth scale.

Univariate statistics derived as the inverse Simpson's diversity and Pielou's evenness indices were compared among the three areas and among the size-fractions and are shown as boxplots (Fig. 6 and Table S3). For the microplankton, median diversity indices (Fig. 6A) of the samples from Vaigat Strait and Disko Bay were significantly lower (*t*-test: $p \leq 0.05$, Table S4) than the median of the samples from the Uummannaq Fjord. The same applies for the nanoplankton (*t*-test: $p \leq 0.05$). Both larger size-fractions were significantly less diverse than the picoplankton (*t*-test: $p \leq 0.05$), which showed similar median diversity for all three areas. The same relationships were observed for the median evenness of samples (Fig. 6B and Table S3). For the Vaigat Strait, where the highest chl *a* concentrations were found (Fig. 4), the overall lowest median diversity index (microplankton: 2.53; nanoplankton: 3.35) and evenness index (microplankton: 0.37; nanoplankton: 0.44) values were calculated. In contrast, in this region, the highest median diversity (10.02) and evenness (0.66) values were found for the picoplankton.

Similarity estimates among samples based on a Bray-Curtis dissimilarity matrix are presented in NMDS plots (Fig. 7) for the three size-fractions. The NMDS plot (Fig. 7A) revealed a separation of the three size-fractions with a higher overlap between the micro- and nanoplankton (ANOSIM statistics $R = 0.6936$, significance $= 0.001$). Similarities between size-fractions indicated a higher similarity within the picoplankton in all three areas (Uummannaq Fjord, Vaigat Strait and Disko Bay) (Fig. 7B). Fitting the environmental factors to the NMDS ordination resulted in significant ($p \leq 0.05$, Table S5 and S6) linear correlations with ranked distances between the samples (p-fitting for cell size, chl *a*, DTX, silicate, and phosphate) (Fig. 7A). Different environmental parameters correlated significantly ($p \leq 0.05$) to the NMDS ordinations of each size fraction (Fig. 7B). For the microplankton, temperature nitrate, phosphate and DTX correlated significantly ($p \leq 0.05$), whereas for the nanoplankton the most significant associations were with temperature, nitrate, silicate, and the toxins SPX, DTX and PTX. For picoplankton,
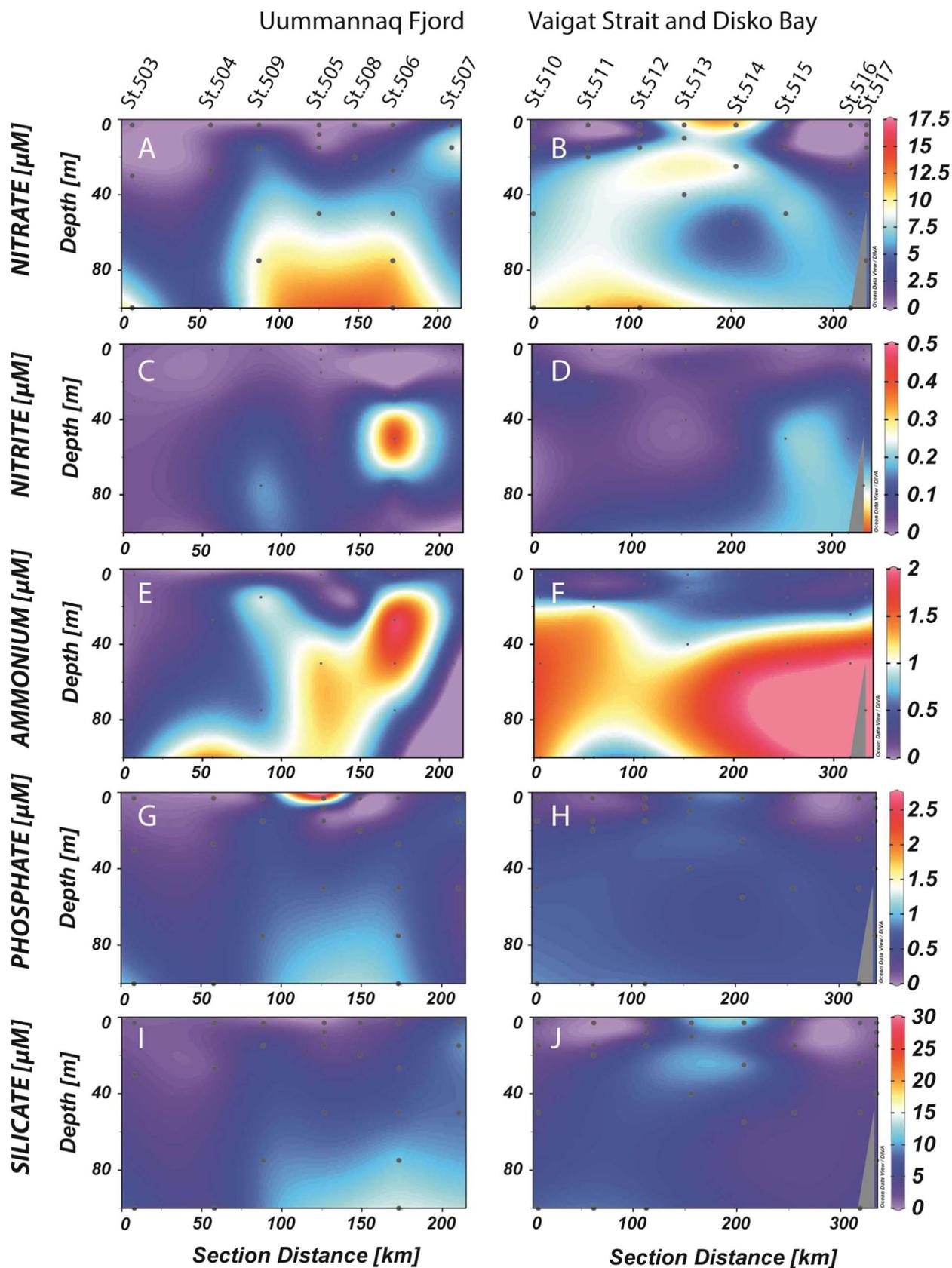
**Fig. 3.** Inorganic nutrient concentrations in the upper 100 m water column for the sampling transects in Uummannaq Fjord (3A, C, E, G, I), and Vaigat Strait and Disko Bay (3B, D, F, H, J). Plots show profile of nitrate [μM] (3A-B), nitrite [μM] (3C-D), ammonium [μM] (3E,F), phosphate [μM] (3G,H), and silicate [μM] (3I-J).

the salinity, nitrate, phosphate, silicate, chl *a* and DTX variables were dominant. Thus, nitrate and DTX were the only environmental parameters that correlated significantly ($p \leq 0.05$) to the NMDS ordinations for all size-fractions. Nitrite, ammonium, PSP toxins and DA did not correlate significantly for any NMDS ordination.
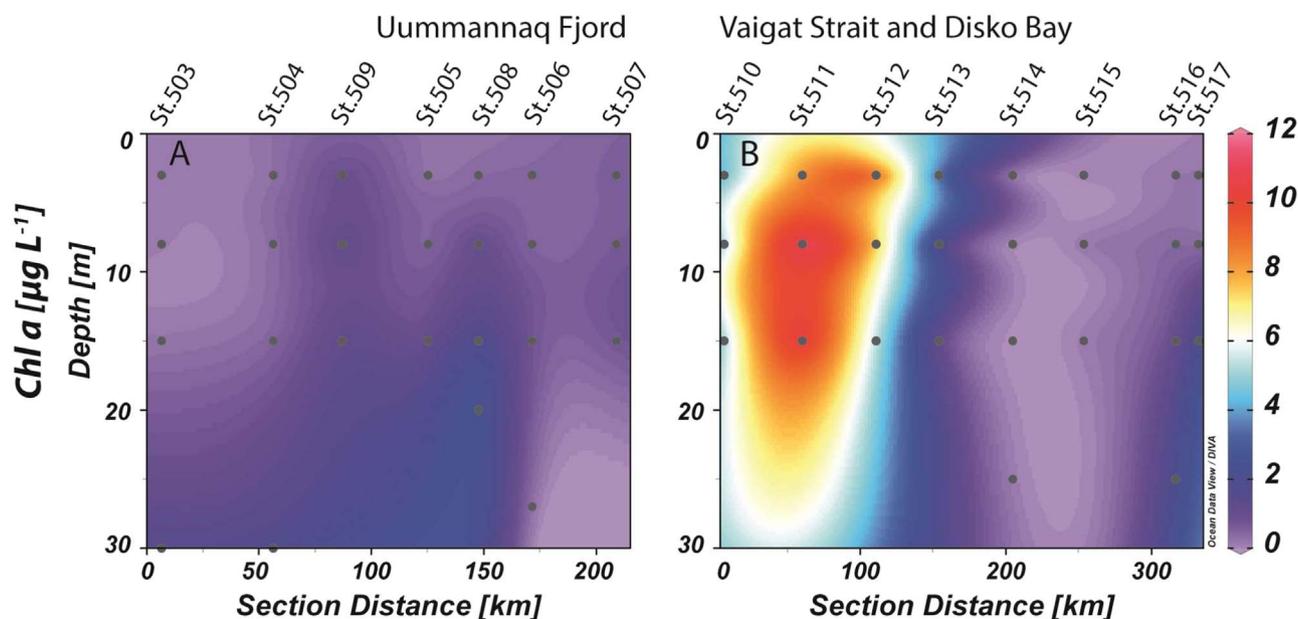
**Fig. 4.** Chlorophyll *a* (chl *a*) concentration [µg L$^{-1}$] in the upper 30 m of the water column of the sampling transects in Uummannaq Fjord (4A), and Vaigat Strait and Disko Bay (4B).



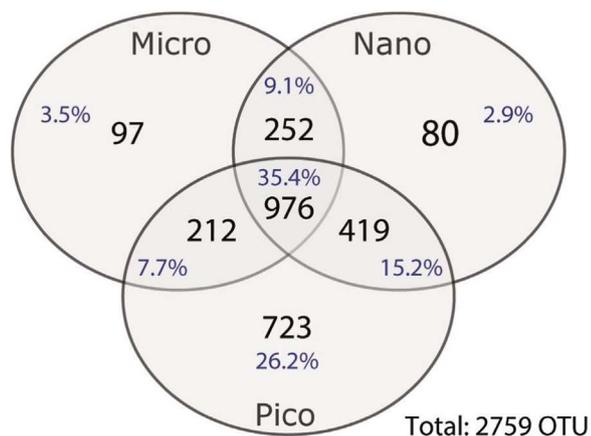**Fig. 5.** Venn diagram of OTU intersections between the three different size-classes (microplankton: 20−50 µm, nanoplankton: 3−20 µm and picoplankton 0.2−3 µm). Numbers represent the number of OTUs with their corresponding percentage [% of total OTU]. OTUs were generated based upon a similarity threshold of 98%.

The relative abundances of different OTUs were grouped accordingly to their affiliations to major phylogenetic groups pre-classified by QIIME (e.g., as Alveolata, Haptophyta, Metazoa and Stramenopila) for each size-fraction (Fig. 8 and Table S7). For all three size-fractions, the dominant groups were stramenopiles and alveolates, covering 83.4% of total community richness. Other groups, comprising 11.7%, and 5.0% of the reads, respectively, either could not be assigned to particular groups or were non-specifically assigned to eukaryotes. The majority of the OTUs could thus be assigned at least to a phylum.

The Dinophyceae alignment, consisting of 640 sequences, was 5748 bp long and comprised 3,518 parsimony informative sites (61%; 5.50 per terminal taxon). Many nodes of the best fitted maximum likelihood (mL) tree (−ln=218,591.63) showed high if not maximal support values, although the backbone exhibited many weakly supported nodes. Most Dinophyceae were retrieved as monophyletic (94LBS; LBS = mL bootstrap support) and segregated into a number of more or less established taxonomic units at the ordinal level, such as Dinophysiales (99LBS), Gonyaulacales, Peridiniales, Prorocentrales and Suessiales (80LBS), whereas other unarmored "Gymnodiniales" were polyphyletic.

The concatenated stramenopile alignment consisted of 1777 sequences and 9212 site columns. The underlying average sequence length was 2338 bp. The 5,817 sites of the 9212 columns were used for phylogenetic inference by RAxML (63%, 3.37 per taxon). Most nodes close to the branch end showed good to maximal support values (≥60), but many inner nodes were poorly supported. Most of the OTUs have been assigned at least to generic category (placements just below terminal node) and represent ecotypes or functional groups; this assignment is therefore not a major issue for our purposes.

Phylogenetic placement on reference trees for alveolates and stramenopiles yielded partial taxonomic assignment down to generic level. For alveolates, 78.7% of their pre-assigned OTUs were subsequently placed within a generic category, covering 78.8% of the richness within this group (Table 1). For stramenopiles, 75.0% of the OTUs were assigned to a genus, covering 84.9% of the richness in this group. The phylogenetic placement of stramenopile and alveolate OTUs on the reference trees resulted in an 82% OTU match for generic categories.

For the microplankton size-fraction (20−50 µm), stramenopiles were the dominant group, especially in Vaigat Strait (≥84%). Within the stramenopiles, most reads identified diatoms, with *Chaetoceros* followed by *Thalassiosira* and *Skeletonema* in abundance (http://dx.doi.org/10.1594/PANGAEA.857403). In the Uummannaq Fjord region, the relative read abundance of *Skeletonema* and *Thalassiosira* switched in comparison to Vaigat and Disko Bay. For Disko Bay, alveolates showed the highest relative abundance among the stations (≥52%), with the dinoflagellates *Protoperidinium* (Peridiniales), *Gyrodinium* (phylogenetic position uncertain) and *Biecheleria* (Suessiales) predominant. For Uummannaq Fjord, we found no clear group distribution pattern, except for the high abundance of metazoans (74% and 84%) at Stations 509 and 505, respectively, and more unassigned reads at the head of the fjord (Station 507; 19.4%). In all other microplankton samples, metazoans and unassigned reads each accounted for < 5% of total abundance at each station.

Within the nanoplankton (3−20 µm), we found a more even and similar distribution of alveolates among the three sampling areas (39.3% ± 19.8%), followed by stramenopiles (49.4 ± 20.2%). Stations 513−515 showed different profiles, with 60−70% of the reads belonging to stramenopiles at Stations 513% and 514% and 90% of the reads belonging to alveolates at Station 515. Compared to the microplankton,
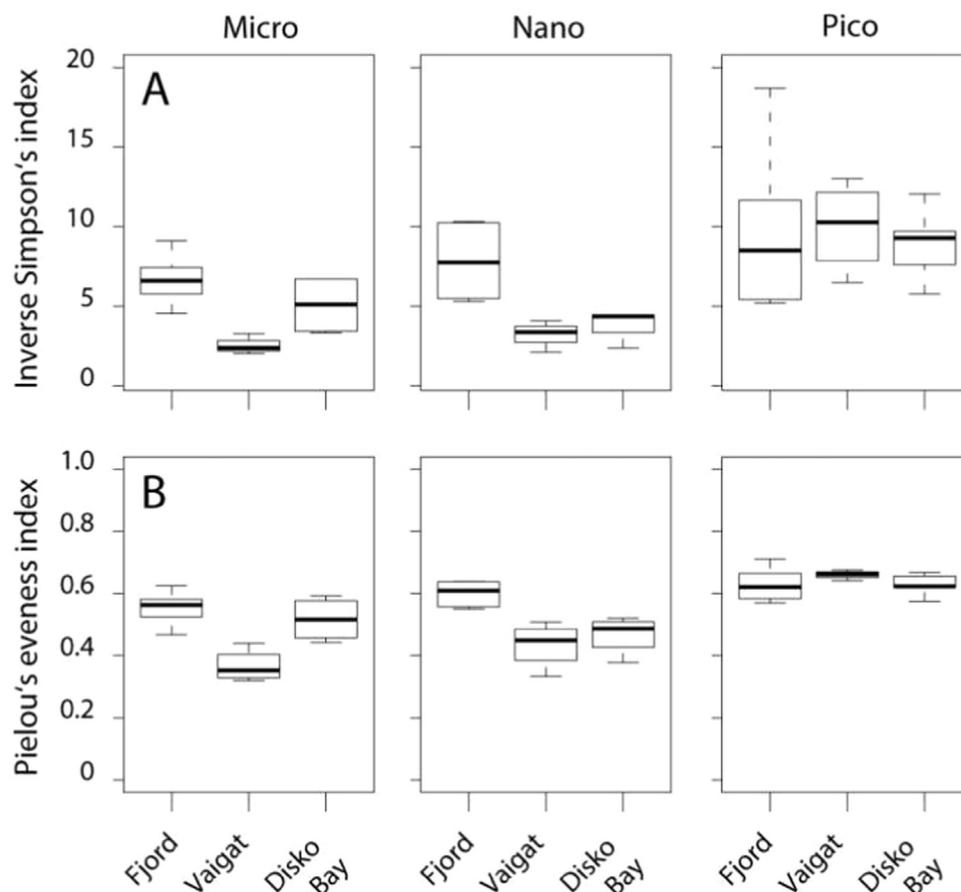
**Fig. 6.** Calculation of the inverse Simpson's index (6A) on the genus level suggests a higher diversity in the picoplankton (0.2–3 μm) size-fraction. Diversity was also significantly ($p \leq 0.05$) higher within the micro- (20–50 μm) and nanoplankton (3–20 μm) size-fractions of Uummannaq Fjord compared with Vaigat Strait and Disko Bay. Boxplots illustrate the 25–75 percentile values (box), median (bar inside the box), standard deviation (whiskers), and outliers (circles). Calculation of Pielou's evenness index (6B) indicates higher evenness in the picoplankton and in Uummannaq Fjord.

we found fewer reads for the diatoms *Chaetoceros* and *Thalassiosira*, but more reads accounted for *Skeletonema* (http://dx.doi.org/10.1594/PANGAEA.857403). The heterotrophic dinoflagellate *Protoperidinium* was less abundant in read number within the Vaigat region compared to the other stations. The dominant group at Station 504 was Metazoa (51%; most of them belonging to Lophotrochozoa and Arthropoda). For all other nanoplankton stations, metazoans accounted for < 3% of reads.

Within and among the stations, the picoplankton (0.2–3 μm) groups were more evenly distributed and showed less differences among samples compared to the micro- and nanoplankton. The main groups were alveolates (18–69%), stramenopiles (≤41%) and Viridiplantae (≤41%). The alveolates contributed more to the community diversity at greater depth (53.2% ± 11.1%) than near the surface (26.6% ± 4.8%). Compared to the two larger size-fractions, a higher number of reads could not be assigned to generic category (picoplankton: 68.5%, micro-/nanoplankton: 15.7%/19.1%), and 43.3% of the reads could only be assigned to kingdom or phylum level.

### 3.3. Association of toxin derivatives with potential HAB species

Toxin profiles (Fig. 9) of plankton of the outer Uummannaq Fjord stations 503–509 most commonly included domoic acid (DA) and spirolides (SPX, each ~50 ± 27% relative toxin concentration), whereas the inner stations 505 - 507 comprised ~90% SPX and ~10% pectenotoxins (PTX, Fig. 9 and Table S8). For the Vaigat Strait stations 510–513, SPX and dinophysistoxins (DTX) were predominant, with SPX concentration decreasing (6 ng NT$^{-1}$ to 0.23 ng NT$^{-1}$) and DTX abundance increasing (0.11 ng NT$^{-1}$ to 1.9 ng NT$^{-1}$) along the

transect. At station 515 and 517 (Disko Bay), SPX dominated the toxin profiles (90–98% relative toxin concentration). Paralytic shellfish poisoning (PSP) toxins were detected (423.6 ng NT$^{-1}$) only at Station 516, where highest SPX concentration was also measured (61.0 ng NT$^{-1}$).

With respect to the molecular diversity of potential HAB species, a low number of OTUs (maximum 373 OTUs per station per taxon) were assigned to *Alexandrium*, *Dinophysis*, *Gonyaulax*, *Gymnodinium*, *Prorocentrum* or *Pseudo-nitzschia* (Fig. 9 and Table S9), but these assignments did not apparently correlate well with toxin composition or concentration in plankton. At Station 516, 373 OTUs were assigned to species of *Alexandrium*, whose presence was also confirmed by microscopy (results not shown, see Tillmann et al., 2016). To differentiate toxic and non-toxic species of *Alexandrium*, qPCR assays were applied for three stations (515, 516 and 517). We detected the usually toxic *A. fundyense* (Group I) and *A. ostenfeldii*, as well as the non-toxic *A. tamutum*, but we found no signals for *A. mediterraneum* (Group II), *A. tamarense* (Group III), *A. pacificum* (Group IV) or *A. minutum* (Table 2).

## 4. Discussion

### 4.1. Evaluation of the marker sequence approach to plankton diversity

Plankton biodiversity analyses with an amplicon sequencing approach are typically based upon the V4 or V9 region of the SSU rRNA (De Vargas et al., 2015; Massana et al., 2015; Stoeck et al., 2010). We decided, however, to focus on the LSU rRNA D1/D2 region, because it
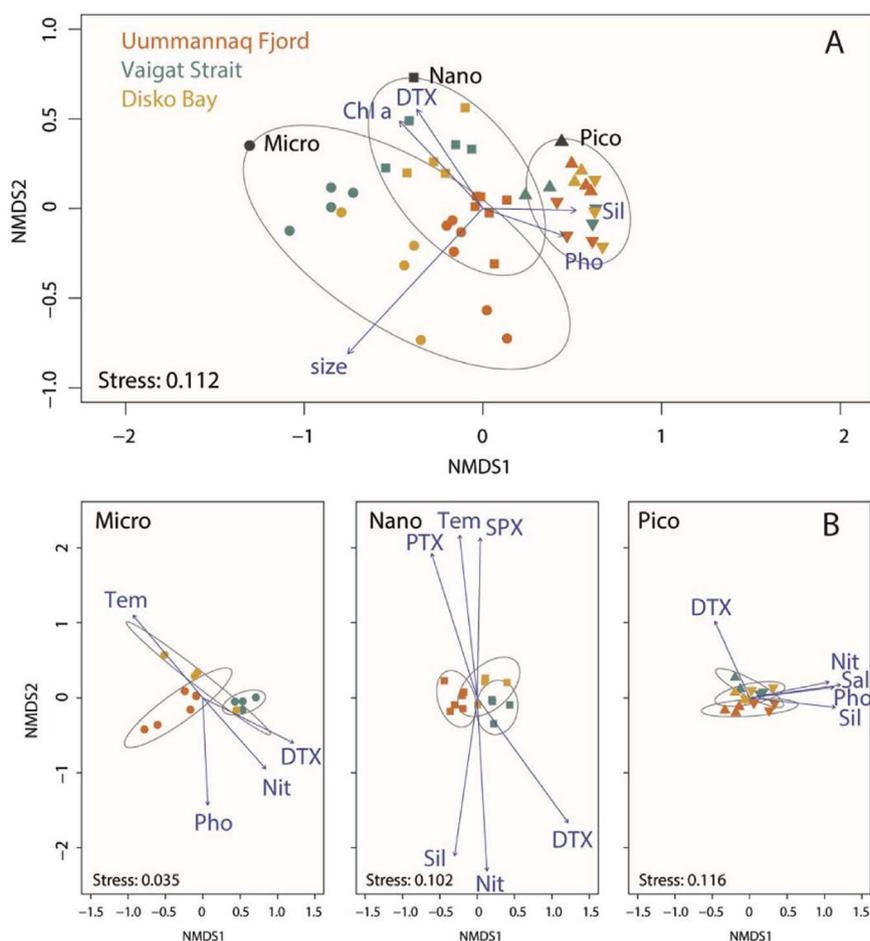
**Fig. 7.** Non-metric multidimensional scaling (NMDS) biplot of a Bray-Curtis dissimilarity matrix of the OTU abundance data with assigned taxonomy at genus level or lower taxonomic assignment. (A) OTU dissimilarity matrix from all three size-classes (microplankton: 20–50 μm, nanoplankton: 3–20 μm and picoplankton 0.2–3 μm). Environmental parameters were fitted onto the ordination plot and showed significance (p≤0.05) for cell size, chl *a*, DTX, silicate and phosphate. (B) OTU dissimilarity matrices for each size class. Significant environmental vectors were fitted onto the ordination plot (DTX = dinophysistoxin, Nit = nitrate, Pho = phosphate, PTX = pectenotoxin, Sal = salinity, Sil = silicate, SPX = spirolides, Tem = temperature). Ellipses were drawn at 95% confidence limit for size-fraction samples (A) and sampling areas (B). Circles = microplankton, squares = nanoplankton, triangles = picoplankton samples; orange = Uummannaq Fjord, green = Vaigat Strait, yellow = Disko Bay.

has been shown to have a higher resolution to differentiate at the species level for many protists, such as diatoms, dinophytes and haptophytes (John et al., 2003; Kretschmann et al., 2015; Medlin et al., 1998; Moniz and Kaczmarska, 2009; Sonnenberg et al., 2007; Toebe et al., 2013a). Some plankton groups (e.g., dinophytes) may be overrepresented compared to others as a consequence of group specific biases such as multiple rRNA copies in the genome and primer specifications (Huber et al., 2009; Kunin et al., 2010). The haptophytes and prasinophytes were rarely detected in our study. Underrepresentation of these groups, based upon sequencing with universal primers for rRNA, is a known problem, and thus specific primers targeting both groups have been used in recent studies (Bittner et al., 2013; McDonald et al., 2007). It is commonly accepted, however, that no ideal universal primer exists. New primers have been designed for the Illumina sequencing platform to minimize such biases for upcoming field investigations.

Methodological errors in 454-sequencing and OTU clustering are also known, and may lead to an over- or underestimation of diversity (Reeder and Knight, 2009). To counter this issue, we have first taxonomically assigned OTUs within QIIME to higher systematic categories. Second, we have been able to reliably assign OTUs to generic category down to genus or even species level, or to higher nodal points in the phylogenetic tree, by accessing PhyloAssigner, which contains the representative diversity of the SSU and LSU rRNA gene regions (http://dx.doi.org/10.1594/PANGAEA.857403). However, an

OTU must not necessarily directly translate to a unique species because within some species intraspecific variances of rRNA copies (ribotypes) have been reported. Several OTUs can therefore correspond to a single species (John et al., 2005; Kremp et al., 2014).

### 4.2. Molecular biodiversity

High-throughput sequencing has facilitated reliable environmental surveys of molecular diversity and is now commonly used to study marine plankton communities (De Vargas et al., 2015; Egge et al., 2013; Sogin et al., 2006; Stoeck et al., 2010). The approach is also possible because a tremendous effort has been made during the past decade to resolve alpha-taxonomy in protists and to link DNA-sequences to species names. Otherwise, any DNA-barcoding approach is condemned to fail (Adl, 2013), although references to OTUs rather than species *sensu stricto* have been used (Schloss and Handelsman, 2005) to access species diversity of communities and thus make studies more comparable. Our comparison of micro-, nano-, and picoplankton reveals many OTUs belonging to more than one size-fraction. The range of a size-fraction to classify microorganisms into micro-, nano-, and picoplankton is largely operational and not based on objective criteria, and may therefore differ among studies (De Vargas et al., 2015; Massana et al., 2015). Our OTU overlap of ~8–15% between size-fractions and ~35% among all stations is somewhat higher than observed in other studies (Kilias et al., 2014b; Massana et al., 2015).
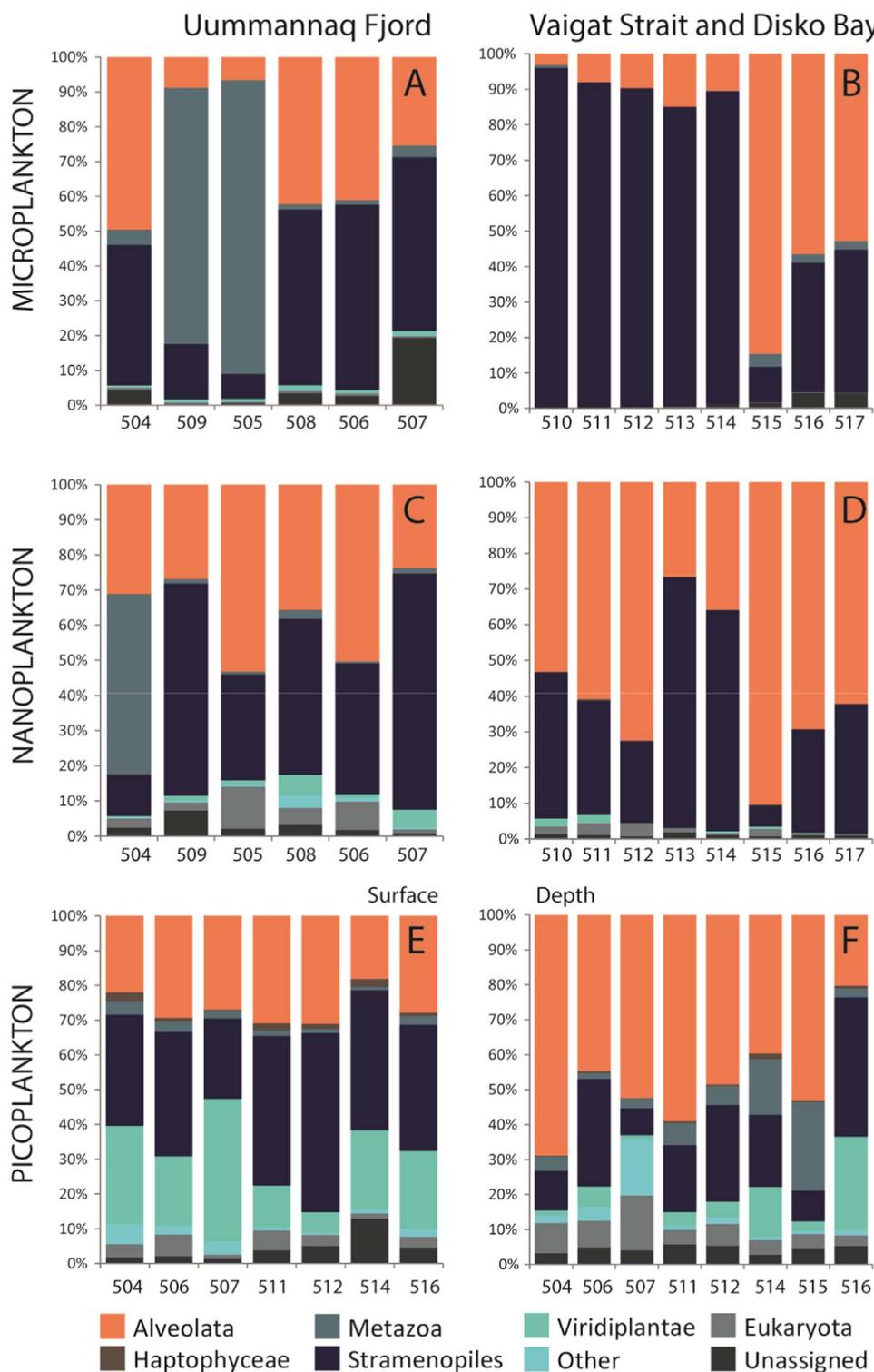
**Fig. 8.** Relative abundance of the main taxonomic groups [% of total reads] for the micro- (8A,B; 20 –50 μm) and nanoplankton (8C-D; 3 – 20 μm) at the sampling Stations 504–507 in Uummannaq Fjord (8A, C), and Stations 510–517 in Vaigat Strait and Disko Bay (8B, D) obtained by 454-pyrosequencing and taxonomic assignment at 98% similarity level. The relative abundance of the main taxonomic groups for the picoplankton (8E-F, 0.2–3 μm) is plotted for near surface (8E) and depth (8F) samples for Stations 504–516. Unassigned = no taxonomic classification; Eukaryota = taxonomic assignment to eukaryote level; Other = other eukaryotic groups ≤1% (Centroheliozoa, Choanoflagellida, Cryptophyta, Euglenozoa, Glaucocystophyceae, Picozoa, Rhizaria).

Explanations may include differences in life history stages (e.g., small gametes or spores) and alternative cell forms, or the ability to form chains or colonies. Although low vacuum pressure was applied during sample fractionation and filtration to minimize cell breakage or disruption of cells in chains, some of the OTU overlap might constitute

artifacts of such handling and thus may influence apparent diversity of each size class.

The high number of unique OTUs within the picoplankton and overall higher diversity as revealed by diversity statistics is in agreement with an overall higher diversity of small eukaryotes (De Vargas

**Table 1**
Effectiveness of phylogenetic placement of OTUs on the reference tree of alveolates (A) and stramenopiles (B).

|   | Level | Counts in level | Counts in level (%) | Sequence abundance | Sequence abundance (%) |
|---|-------|-----------------|---------------------|--------------------|------------------------|
| **A** | Genus | 37 | 78.7 | 173,746 | 78.8 |
|   | Family | 5 | 10.6 | 10,439 | 4.7 |
|   | Order | 4 | 8.5 | 34,391 | 15.6 |
|   | Class | – | – | – | – |
|   | Phylum | – | – | – | v |
|   | **Alveolates** | 1 |  | 7,030 | 3.2 |
| **B** | Genus | 45 | 75 | 190,897 | 84.9 |
|   | Family | 2 | 3.3 | 221 | 0.1 |
|   | Order | 3 | 5 | 959 | 0.4 |
|   | Class | 7 | 11.6 | 17,526 | 7.8 |
|   | Phylum | 2 | 3.3 | 470 | 0.2 |
|   | **Stramenopiles** | 1 |  | 14,719 | 6.6 |

et al., 2015; López-García et al., 2001; Vaulot et al., 2008). Although we observed high sequence read numbers yielding the highest molecular biodiversity (OTUs) in picoplankton samples, we acknowledge that saturation may not have been achieved and thus the diversity may not have been fully resolved in all cases. Among the postulated total marine eukaryotic plankton diversity of ~150,000 OTUs, more than half may be represented by picoplankton (De Vargas et al., 2015). Explanations for the higher diversity may be a reflection of their smaller cell size. The small size of the picoplankton results in a higher surface to volume ratio, and therefore organisms of this fraction may possess and exhibit different metabolic processes and kinetic mechanisms (e.g., nutrient uptake, light utilization) compared to the nano- and microplankton (Litchman et al., 2007). Cell size may be seen as a master trait that shapes ecological niches of phytoplankton (Litchman and Klausmeier,

2008). For example, picoplankton often dominate low nutrient environments, whereas larger cells (e.g., of diatoms) tend to increase in abundance with increasing nutrients, partially because of their lower affinity but higher maximum uptake rates (Kerimoglu et al., 2012; Litchman et al., 2009; Smith et al., 2014). Additionally, small cell size obviously allows fewer morphological variants and this may account for a lower functional trait plasticity (i.e., a range of phenotypes), whereas larger-celled species may show a higher plasticity instead of evolving several different optimal phenotypes (Rengefors et al., 2015). Hence, higher species diversity of picoplankton compensates for potentially higher plasticity of the larger cell-size taxa. Taking the high diversity of the picoplankton community into account, different species may be more specialized and establish different ecological niches. Thus, the community of more specialized species might be able to adapt to a broader range of environmental conditions. The picoplankton community might also adapt faster compared to the microplankton because of the larger effective population size, standing genetic variation and shorter generation times.

### 4.3. Potential linkage of diversity and biogeographical partitioning to environmental parameters

The high spatial and temporal similarity in the environmental regime may reflect the fact that sampling stations were located within a radius of 250 km and were sampled synoptically within one week. Nevertheless, definable differences in diversity and biogeography of taxa distribution within size-fractions were evident from among the study areas.

In general, fjords are characterized by an environmental gradient with high freshwater input, often from glacial influence, yielding high stratification with sharp surface temperature gradients and lower salinity and high nutrient input at the head of the fjord, and open
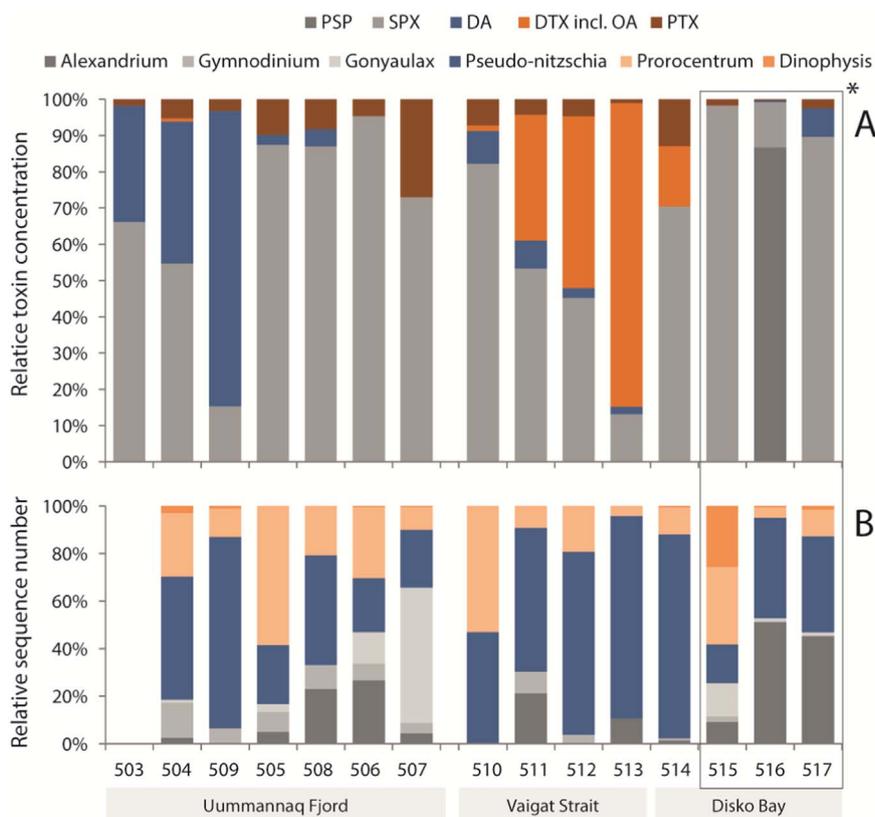


**Fig. 9.** Toxin profile as relative toxin concentration [% of total toxin in ng NT⁻¹] of PSP toxins (PSP), spirolides (SPX), domoic acid (DA) and pectenotoxins (PTX) in Uummannaq Fjord, Vaigat Strait and Disko Bay (9A). OTUs assigned to potential toxin producers in the genera *Alexandrium, Gymnodinium, Gonyaulax, Pseudo-nitzschia, Prorocentrum* and *Dinophysis* are presented as relative sequence number [%] (9B). (*) qPCR yielded positive signal for *A. ostenfeldii, A. tamutum,* and *A. fundyense* (Group I).

**Table 2**
The qPCR analysis [ng μL$^{-1}$] detected three *Alexandrium* species, but yielded no amplification for *A. minutum, A. mediterraneum* (Group II), *A. tamarense* (Group III) or *A. pacificum* (Group IV); NT = Net Tow sample.

| Station | A.fundyense[a] (Group I) | | | A. ostenfeldii[a] | | | A. tamutum | | |
|---|---|---|---|---|---|---|---|---|---|
| | Nano | Micro Pump | Micro NT | Nano | Micro Pump | Micro NT | Nano 31.1 | Micro Pump | Micro NT |
| 515 | – | 22.2 | 24.3 | – | 26.9 | 269.9 | 31.1 | 24.9 | 27.9 |
| 516 | – | 27.7 | 28.0 | 31.6 | 23.5 | 21.5 | 31.9 | 24.4 | 27.0 |
| 217 | – | 38.7 | – | 28.7 | 22.6 | 22.9 | – | 26.2 | 28.8 |

[a] Indicates toxigenic species.

coastal ocean conditions (e.g., higher salinity, increased mixing) beyond the mouth of the fjord. This typical pattern was shown for the Uummannaq Fjord (Figs. 2 and 3). The persistence of this environmental gradient together with the different phytoplankton community might be the reason that no environmental vectors within the non-metric multidimensional scaling (NMDS) analysis could be clearly identified with these species assemblages. In any case, the environmental conditions associated with the surface water mass flowing towards the mouth of the fjord determined the structure of the micro- and nanoplankton community of the fjord and thereby yielded both higher molecular diversity and evenness compared to well-mixed plankton communities and water masses from Vaigat Strait and Disko Bay. Prevailing environmental conditions may shape the phytoplankton community to a certain degree and might allow a community to develop a higher diversity and evenness within a more hydrodynamically stable regime within the fjord and thereby contributing to increased biostability within the ecosystem. A higher molecular diversity and evenness was found for eukaryotic plankton within Uummannaq Fjord and particularly for the picoplankton, which suggests a more stable community capable to buffer responses to slightly changing environmental conditions. The picoplankton might buffer the different environmental conditions in spite of their lower morphological plasticity by means of higher surface/volume ratios for nutrient acquisition. The NMDS (Fig. 7A) suggests a positive influence of silicate and phosphate on picoplankton samples supporting their better capability of nutrient uptake.

The glacial ice-melt runoff from the Ilulissat Glacier results in up to 5 °C lower water temperature adjacent to the glacier. Local hydrodynamic factors and currents transport this cold glacial melt water into the Vaigat Strait (Fig. 1), also presumably advecting the plankton component and resulting in a different community profile (higher diatom OTU abundance) within the microplankton size-fraction in this area. The nutrient input by the upwelling in front of the glacier, and the contribution of silicate by runoff of melt water, may explain the high silicate concentrations at the surface. This interpretation also supports the link to potential growth of diatoms, which incorporate silicate into their cell walls. Unfortunately, we lack detailed information about the biomass for this data set, but high chl *a* in the Vaigat Strait (Stations 510 – 513, Fig. 4), the depletion of nutrients, as well as the lower calculated diversity and evenness indices (Fig. 6) provide indirect evidence of higher phytoplankton biomass production.

On the molecular level, this corresponds to a high OTU abundance of diatoms within the microplankton, even though OTU abundance cannot be directly correlated to biomass. Nevertheless, the chl *a* vector in the NMDS analysis pointing towards micro- and nanoplankton fractions of Vaigat Strait (Fig. 7A) does suggest a higher contribution to the biomass from the bigger cells. The higher number of unknown OTUs within the picoplankton may belong to heterotrophic taxa, which do not contribute to chl *a*. The higher nitrate concentrations at Stations 513 and 514 could also support continued cell growth and could explain the higher relative abundance of diatoms within the nanoplankton at these two stations.

The micro- and nanoplankton community within Disko Bay was defined by a higher temperature and lower nutrients (phosphate and nitrate; Fig. 7B). This might have had a positive effect on dinoflagellate abundance and thus would lead to a lower species diversity and associated lower evenness. Disko Bay represents an intersection of environmental influences from the outer coast, the adjacent fjord and glacial contribution and the Vaigat Strait, including advective processes and associated plankton communities.

There is an ongoing debate as to whether or not protists are truly cosmopolitan, and lack distinct distributions (Fenchel and Finlay, 2004), or also comprise endemic species (Medlin, 2007). In our study, in addition to the evidence of the evenness index values, NMDS indicates a higher similarity among picoplankton samples, relative to nano- and microplankton, but no clear separation of the regions. This conservative picoplankton distributional pattern is in spite of the fact the environmental conditions are differentiated among the regions, especially for Vaigat Strait. We studied diversity in samples collected in close spatial and temporal proximity and have demonstrated clear differences in distribution of micro- and nanoplankton. These findings are supported by other metabarcoding studies (De Vargas et al., 2015; Kilias et al., 2014b; Massana et al., 2015); intraspecific investigations have also demonstrated structured phytoplankton populations (Alpermann et al., 2010; Kremp et al., 2014; Masseret et al., 2009; Tahvanainen et al., 2012). However, the view is not so clear for the picoplankton community. Obviously, regional environmental constraints play a role in community structuring, with a stronger structuring evident for larger organismal size- fractions and increasing dispersal limitation ranging from small cells to larger cells (De Vargas et al., 2015).

### 4.4. Group diversity of the eukaryotic plankton community

The alveolates and stramenopiles were commonly detected in high relative abundance within west Greenland coastal waters. The placement of OTUs on the two concatenated phylogenetic trees not only addresses the issue of over- or underrepresentation of diversity, but also permitted assignment of 78.8% and 84.9% of the OTUs to generic category for alveolates and stramenopiles, respectively (Table 1).

### 4.4.1. Stramenopiles, especially diatoms

For west Greenland, chain-forming diatom species, such as those of *Chaetoceros* (Mediophyceae), *Fragilariopsis* (Bacillariophyceae) and *Thalassiosira* (Mediophyceae) and at lower densities of *Navicula* (Bacillariophyceae), *Pseudo-nitzschia* (Bacillariophyceae) and *Skeletonema* (Mediophyceae), have been previously reported (Krawczyk et al., 2014; Zamora-Terol et al., 2013). The genus *Chaetoceros* is the most abundant, as inferred from molecular sequence data (~100,000 OTUs), especially within Vaigat Strait, supporting previous reports of this genus as one of the most abundant in Arctic-subarctic waters (Berard-Therriault and Poulin, 1999; Østrup, 1895; Throndsen et al., 2007; von Quillfeldt, 2000). However, the *Chaetoceros* lineage is very diverse and includes many species that are difficult to identify. *Chaetoceros* species have been found in more saline open marine waters in the Davis Strait south of 70°N (Krawczyk et al., 2014). *Chaetoceros neogracile* accounts for more than the half of all OTUs associated with *Chaetoceros,* whereas three other species,

namely *C. debilis*, *C. lorenzianus*, and *C. radicans*, were also found in our study. In other studies in central northern Baffin Bay, *Chaetoceros* species have been previously observed to be a prominent part of the micro-phytoplankton community in July to September (Booth et al., 2002; Lovejoy et al., 2002).

*Thalassiosira* species are abundant in the Vaigat region, but only *T. nodulolineata*, an uncommonly recorded taxon, was identified to species level. Off the west coast of Greenland, *Thalassiosira* species, such as *T. nordenskioldii* and *T. poroseriata*, have been reported among the dominant taxa, especially in summer (von Quillfeldt, 2000). Krawczyk et al. (2014) described the diatom distribution from southwest to west Greenland and found that other *Thalassiosira* species were common in coastal waters (mainly Godthabsfjord/Nuuk) and linked to increased surface temperature, typical for summer water stratification in this area. Moreover, there is a clear spring bloom scenario for West Greenland with a relatively high biomass of *Thalassiosira* species based on samples collected during different years and seasons (Zamora-Terol et al., 2013). However, the overall occurrence of *Thalassiosira* species may vary widely due to the annual succession processes.

In our study, we did not detect any OTU related to *Fragilariopsis*, although such species are associated with and often dominant at high latitudes in the entire circumpolar area, particularly during the spring bloom, and are often associated with ice (Lundholm and Hasle, 2010). Krawczyk et al. (2014) observed a small number of cells of *Fragilariopsis* in west Greenland. We may have missed *Fragilariopsis* because of the seasonal mismatch because their study was conducted during June-July 2007, whereas we sampled during July-August 2012.

The genus *Skeletonema* represents a large and diverse lineage of globally distributed diatoms and has been often referred to the *S. costatum* species complex (Kooistra et al., 2008; McDonald et al., 2007; Sarno et al., 2005). In our study, we did not identify to species level, but we observed *Skeletonema* in higher OTU abundance especially in stratified waters at the head of Uummannaq Fjord and at Stations 512–514 from Vaigat Strait to Disko Bay passage influenced by glacial melt water. This result agrees with Ilyash et al. (2014), who also observed *Skeletonema* within Dvina Bay (White Sea) characterized by pronounced freshwater-driven stratification.

### 4.4.2. Alveolates, especially dinophytes

The contribution of alveolate OTUs to the community diversity was particularly high among the nanoplankton. Dominant dinophyte OTUs identified in our study have been previously reported from cold waters. *Protoperidinium* (Peridiniales) is diverse in North Atlantic waters and abundant in biomass particularly during summer (Bachy et al., 2012; Lovejoy et al., 2002; Mertens et al., 2013; Okolodkov, 1998; Okolodkov and Dodge, 1997; Y.B., 1998; Yamaguchi et al., 2006, 2005). Another taxon of uncertain phylogenetic position in the dinophyte tree, but determined as *Gyrodinium nom. cons.* (Christensen, 1987; Silva, 1980), has been collected before in northern Denmark (Hansen and Daudbjerg, 2004) and northern Japan (Takano and Horiguchi, 2004).

All observed OTUs assigned to *Biecheleria* (Suessiales) are associated with *B. baltica* (="*Woloszynskia*" *halophila*), which is a rather well known cold-water species optimally growing at 0–6 °C (Kremp et al., 2005; Moestrup et al., 2009; Warns et al., 2013). However, it has been considered to be restricted to brackish water with salinities of 6 - 30. Although there are freshwater influences at Baffin Bay (Tang et al., 2004), salinity and temperature differ from the reported tolerance spectra for this species (Kremp et al., 2005; Moestrup et al., 2009; Warns et al., 2013). *Biecheleria* species have been recently reported from Hudson Bay (Heikkilä et al., 2014), and also in Arctic sea-ice samples (Bachy et al., 2012), both geographically and ecologically relatively close to our study regime.

### 4.4.3. Other phylogenetic lineages

The high relative abundance of Viridiplantae within the picoplankton comprises Chlorophyta (e.g., Prasinophyceae) and Streptophyta but most OTUs were only assignable to the lineage Viridiplantae. Little is known about these small-sized phytoplankton; nevertheless, within the small nano- and picoplankton, Chlorophyta are especially important in marine waters (Not et al., 2012). Most SSU rRNA sequences recovered from the oceanic environment correspond to the polyphyletic group of Prasinophyceae (Guillou et al., 2004).

Metazoans were especially abundant in microplankton samples of Stations 509 and 505 and the nanoplankton sample at Station 504. This group includes, for example, Arthropoda, Cnidaria or Porifera, and is usually not considered as part of the micro- or nanoplankton, as their organismal size is typically larger. Nevertheless, organismal fragments or gametes of this group may be collected during sampling and subsequently detected by NGS.

### 4.5. Potential HAB taxa within the transect

High temporal and spatial sample throughput offered by NGS provides reliable comparison and confirmation of potentially false classification based only on morphological analysis. For example, if the conventional taxonomy of potentially toxic species is well known, even in cases for which the nomenclature remains controversial, e.g., for genus *Alexandrium* (John et al., 2015), unequivocal identification of the taxon can be confirmed. Among the potentially toxigenic plankton that may cause harmful algal blooms (HABs) when present in high cell numbers, we detected OTUs corresponding to members of the genera *Alexandrium*, *Dinophysis*, *Gonyaulax*, *Gymnodinium*, *Prorocentrum* and *Pseudo-nitzschia* (Table S9). To date OTU sequences can only support interpretations with great uncertainties and quantitative techniques, such as qPCR, must be applied for species verification. Nevertheless, as evidence of how toxins and species presence may be related, at three stations we were able to confirm OTUs corresponding to the non-toxic *A. tamutum,* the toxic *A. fundyense* (Group I) and typically toxigenic *A. ostenfeldii*, with a qPCR assay to reliably differentiate and discriminate between toxic and non-toxic *Alexandrium* species. At Station 516, OTUs assigned to *Alexandrium* species peaked (at 373 of 60,038 total reads per station), which matches with the presence of paralytic shellfish poisoning (PSP) and spirolide (SPX) toxins at this station. However, given the high detection limit of PSP toxins, we might have missed detection of low levels of these toxins at other stations. The *A. tamutum* isolates from Greenland have been confirmed as non-toxigenic, but *A. fundyense* (Group I) isolates were all PSP toxin-producers (Tillmann et al., 2016). The spirolides (SPXs) have a lower detection limit by LC-MS/MS than PSP toxins and hence were detected at all stations. Highest SPX concentrations were detected at Station 516, where *A. ostenfeldii* was also identified from OTU sequences, by subsequent qPCR and from other Greenland and Arctic stations (Tillmann et al., 2014).

For Vaigat Strait, we have shown that higher chl *a* concentrations, and depleted silicate and phosphate concentrations, correspond to a higher relative abundance of diatoms. Nevertheless, OTUs assigned to *Pseudo-nitzschia* (Bacillariophyceae), the potential producer of the neurotoxin domoic acid (DA), were only detected in low read numbers (8–40 of ~28,000–50,000 total reads per station, Table S9). This matches with the lower DA concentrations in plankton from Vaigat Strait compared to Uummannaq Fjord and at Stations 516 and 517. In contrast, no DA was measured at the head of the Uummannaq Fjord (Station 506 and 507) and Station 514, although here we detected higher numbers of OTU reads assigned to *Pseudo-nitzschia,* as well as higher silicate and phosphate concentrations that would support diatom growth. The DA production within *Pseudo-nitzschia* cells is influenced by growth conditions and induced nutrient limitation (of phosphate or silicate), therefore cell concentration does not necessary correlate with toxin concentration (Lelong et al., 2012).

*Dinophysis* (Dinophyceae) is known producer of dinophysistoxins (DTX) and okadaic acid (OA), as well as of pectenotoxins (PTX). The OTUs assigned to *Dinophysis* were detected in low numbers in Uummannaq Fjord and at Disko Bay stations (1–11 of ~15,000–60,000 total reads per station, Table S9). Both DTX and OA have a relatively high detection limit by LC-MS/MS analysis, which might be the reason why we did not detect these toxins at some stations where corresponding OTUs were found (Table S8), but pectenotoxins (PTXs) have a lower detection limit and were detected at all stations. Diatoms were dominant within the Vaigat Strait, and the overall relative low abundances of Dinophyceae within this area may explain the lack of *Dinophysis* signatures.

Although we did not find blooms of any potentially harmful algae, as defined by high cell concentrations, we were able to associate the presence of OTUs identified by molecular diversity probes with toxin signals in the plankton. In this fashion, NGS may be used to detect potentially toxigenic species even in general monitoring or research surveys. In any case, environmental shifts may increase risks for HABs in areas where toxigenic taxa have already been detected. Increasing surface water temperature may have a positive effect on dinoflagellate species and cell abundance and thus increase the potential for HAB events in the Arctic.

## 5. Conclusion

We conclude that the diversity of the small phytoplankton is differently affected by abiotic factors compared to larger cells. The higher diversity of the small cells may compensate for their lower phenotypic plasticity. We suggest the possibility of an advantage of the small cell-size community in response to changing environmental conditions. To address further questions about the ecological function of size-class differentiation, functional analysis (e.g., based on mRNA) must be considered and included in future studies and rRNA-based diversity surveys. In the Arctic, if species composition of plankton communities changes, particularly with respect to an increase of potentially toxic and/or otherwise harmful algae, the livelihood and well-being of coastal communities in Greenland may be profoundly and negatively affected.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dsr.2016.11.002.

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