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Stratification and summer protist communities in the Arctic influenced coastal systems of Nunavik (Québec, Canada)

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Phytoplankton and other protists in the 3 µm to 50 µm size fraction are grazed on by zooplankton and form the base of Arctic marine food webs essential for local indigenous communities. Anthropogenic climate change is increasing stratification over much of the Arctic Ocean and surrounding seas, but the influence of stratification on protist communities in more coastal regions along Eastern Hudson Bay, Hudson Strait and Ungava Bay is little known. We used 18S rRNA and rDNA amplicon sequencing during two consecutive summers (2017 and 2018) and detailed water column properties to compare the 3 µm to 50 µm protist communities under contrasting stratification regimes in the Eastern Hudson Bay Complex. We found that the surface mixed layer in Eastern Hudson Bay, which is under the influence of river runoff, was strongly stratified and dominated by mixotrophic and bacterivorous taxa, mostly the dinoflagellates *Heterocapsa rotundata* and *Gymnodiniales* spp., and a more diatom-dominated community at the Subsurface Chlorophyll Maximum (SCM), which persisted in deeper colder and more saline water. The massive sequencing effort retrieved seven putative toxic algae from the upper warmer waters of eastern Hudson Bay. These included *Pseudo-nitzschia* spp. and potentially harmful dinoflagellates, most notably *Alexandrium* sp. The persistent weaker stratification conditions in Hudson Strait and Ungava Bay in summer favored a different diatom community, dominated by *Chaetoceros* spp. and *Thalassiosira* spp., and small photosynthetic flagellates including *Phaeocystis pouchetii* and *Micromonas polaris*. As freshwater input increases and stratification intensifies in the Arctic, our findings suggest the summer dinoflagellate-based community seen in coastal Hudson Bay may also be favored in other regions receiving increased river runoff. These conditions could also favor harmful algal events. The Hudson Strait and Ungava Bay protist communities were found nearer the surface and consisted of diverse species able to profit from ongoing nutrient input due to tidal mixing. These results suggest greater resilience in this and other tidally influenced coastal Arctic Bays lacking larger rivers inputs.

Introduction

Biogeography and phenology of phytoplankton and other planktonic microbial eukaryote species are intimately associated with local nutrient and light conditions determined by mixing and stratification dynamics of the sunlit water column. In recent decades, global warming has fundamentally altered oceanic temperature and salinity, resulting in increasing stratification of the world upper ocean (Li et al., 2020). By inhibiting vertical mixing of the water column, stratification slows the exchange of nutrients and gases, which ultimately decreases marine productivity (Matear and Hirst, 2003; Behrenfeld et al., 2006; Breitburg et al., 2018). While low latitude oceans are mainly stratified by temperature, for Arctic and sub-Arctic seas, by far the most significant determinant of stratification is salinity (Carmack, 2007). Upper water column stratification is particularly pronounced in high latitude regions where rivers discharge, freshening the surface waters and increasing stratification. The strong water column stratification prevents vertical transfer of nutrient-rich deeper water into the euphotic zone (Arrigo et al., 1999; Tremblay and Gagnon, 2009; Ferland et al., 2011). Because of anthropogenic warming, river discharge is increasing along with an accelerated hydrological cycle, impacting Arctic regions including the large Arctic-sub-Arctic Hudson Bay Complex (Stadnyk et al., 2020). An additional concern is that warming conditions could also increase the probability of harmful algal events with impacts on human health and local food chains (Anderson et al., 2021).

Hudson Bay (HB) is the core of the Hudson Bay Complex that connects Arctic modified Pacific origin waters to the Labrador Sea through Hudson Strait (HS) and Ungava Bay (UB). The Hudson Bay catchment covers one third of Canada's land mass and is dominated by large riverine inputs (Déry and Wood, 2005; Macdonald and Kuzyk, 2011). In the greater Hudson Bay a low nutrient, warmer and fresher summer mixed layer sits atop colder saltier waters that are more nutrient rich (Granskog et al., 2011). This surface layer ranges from 30-60 m depth over most of the HB and contains most of the freshwater load from the annual accumulated river runoff of the catchment as well as seasonal sea-ice melt. These large volumes of freshwater feed an anti-clockwise north flowing boundary current along the eastern coastal region of HB (Granskog et al., 2009). The warmer, less-saline and strongly stratified waters limit phytoplankton production of HB in late summer (Harvey et al., 1997; Ferland et al., 2011).

HS+UB regions are influenced by different hydrodynamic processes compared to HB. The HS+UB region drains a much smaller catchment basin and is characterized by strong tidal and meteorological mixing that weakens stratification and increases biological productivity (Drinkwater and Jones, 1987; Straneo and Saucier, 2008). However, although net annual productivity differences between HB and HS+UB are reported, other than historic species lists of mostly phytoplankton from microscopy (Poulin et al., 2011), little is known about the composition of other microbial eukaryotic communities in the two hydrologically contrasting systems. To our knowledge, there have been no direct comparisons of the summer protist communities that occur when stratification is greatest in HB, but nearly absent in the HS+UB region. Here, we addressed this data gap using molecular techniques to identify the protist taxa and in combination with physical oceanographic data sought to gain understanding for the role of stratification in species selection in this high latitude region. We focused our analysis on protist communities in the 3 µm to 50 µm size range, which includes nanoplankton (2-20 µm) and the slightly larger diatoms, dinoflagellates and ciliates, which are prey for zooplankton and commonly reported using light microscopy. As this size fraction would be biased towards taxa that are reported from microscopy, our results should facilitate transition to community-based monitoring by local indigenous communities that live along the coastal Ungava Bay Peninsula. However, as filtration is not perfect, typically some smaller and larger taxa are also retained on the filters, due to filter blockage and cell breakage, respectively, material captured on the filters would provide catalog of taxa suitable for comparison between different systems.

To address which organisms were likely more active as opposed those that persist under the different conditions (Morency et al., 2022), we used amplicon sequencing targeting both 18S rRNA genes (rDNA) and its transcriptional products (rRNA). Although rRNA cannot be directly used to assess the growth and activity of microbes, the variability of rRNA within a species may provide information on the potential of organisms to synthesize proteins in a particular environment (Blazewicz et al., 2013). In addition, given that harmful algal groups are prone to bloom under summer conditions, and climate change scenarios are of concern to local communities (McKenzie et al., 2020), we paid particular attention to the potential presence of harmful algal species in eastern coastal HB and HS+UB under their respective summer stratification regimes.

Materials and methods

The study was carried out in eastern Hudson Bay, the southern reaches of Hudson Strait and along the coast of Ungava Bay (Nunavik, Québec, Canada) (Table 1). Sampling was realized onboard the Canadian research icebreaker CCGS Amundsen during two cruises in early July 2017 and 2018. Seawater was collected directly from 12-L Niskin-type bottles mounted on a rosette system equipped with a relative nitrate (*In-Situ* Ultraviolet Spectrophotometer, ISUS, Satlantic), dissolved oxygen (Seabird SBE-43), chlorophyll fluorescence (Seapoint) and a fluorescent colored dissolved organic matter sensor (fCDOM; Wetlabs ECO). We targeted the surface layer (1 or 2 m) and the subsurface chlorophyll maximum (SCM). The depth of the subsurface chlorophyll maxima (SCM) was determined on the downward cast. For each depth, 6 L of seawater was first prefiltered through a 50 µm mesh to minimize microzooplankton and then filtered onto a 3 µm pore size polycarbonate filter (Millipore, Canada) so the nominal community collected would be in the 3 to 50 µm range. The polycarbonate filters were placed in 2 ml microtubes containing 1.8 ml of RNAlater (Thermo Fisher Scientific, USA). After 30 min at room temperature, filters were transferred to −80°C until nucleic acid extraction. Water samples for nitrate (NO_3^-), nitrite (NO_2^-),

TABLE 1 Environmental data for the Hudson Bay, Hudson Strait and Ungava Bay.

Sample (station_year)	Date (year-month-day)	Lat	Lon	Layer	Temp (°C)	Sal	NO_3^- ($\mu mol\ L^{-1}$)	NO_2^- ($\mu mol\ L^{-1}$)	Si$(OH)_4$ ($\mu mol\ L^{-1}$)	PO_4^{3-} ($\mu mol\ L^{-1}$)	Depth (m)	Fluo ($\mu g\ L^{-1}$)	N^2 ($10^{-3}\ s^{-2}$)	Pycn (m)	rDNA Samples (n° of replicates)	rRNA Samples (n° of replicates)
732_2017	2017-07-07	55.39	-77.99	Surf	9.49	25.35	0	0.07	7.79	0.30	2	0.42	34.51	14	3	2
736_2017	2017-07-08	58.43	-78.30	Surf	6.52	27.60	0.04	0.06	2.65	0.38	3	0.23	27.58	9	2	2
720_2017	2017-07-09	60.70	-78.56	Surf	1.93	30.17	0	0	1.69	0.50	4	0.23	19.78	29	1	1
688_2017	2017-07-10	62.37	-74.66	Surf	3.69	30.96	0	0.04	0	0.25	2	0.37	16.89	14	1	1
684_2017	2017-07-11	61.79	-71.91	Surf	0.88	31.47	0.17	0.05	2.31	0.40	2	2.19	9.88	43	1	1
670_2017	2017-07-12	58.99	-67.94	Surf	0.17	31.22	0.15	0	0.31	0.40	3	0.75	13.64	34	1	1
676_2017	2017-07-12	60.13	-69.06	Surf	1.02	31.12	0	0	0	0.31	2	1.42	13.81	28	1	1
730_2018	2018-07-08	56.18	-76.72	Surf	3.78	23.27	0.39	0.03	12.85	0.43	3	0.78	45.27	19	1	1
731_2018	2018-07-08	55.41	-77.93	Surf	3.34	25.29	0	0	6.89	0.39	2	0.72	37.33	21	1	1
736_2018	2018-07-08	58.42	-78.31	Surf	3.27	26.45	0	0	7.86	0.47	3	0.61	33.28	17	1	1
689_2018	2018-07-11	62.34	-75.53	Surf	-0.22	29.46	0.01	0.04	4.18	0.49	2	2.33	24.43	4	1	1
689_2018	2018-07-11	62.34	-75.53	SCM	-1.52	30.66	0.98	0.09	5.81	0.69	15	2.60	na	na	1	1
732_2017	2017-07-07	55.39	-77.99	SCM	-0.62	31.62	1.50	0	2.84	0.56	30	0.23	na	na	2	1
736_2017	2017-07-08	58.43	-78.30	SCM	-0.56	29.69	0.33	0.06	7.95	0.72	39	7.67	na	na	1	1
720_2017	2017-07-09	60.70	-78.56	SCM	-0.90	30.79	0.96	0.02	7.09	0.82	50	4.71	na	na	1	1
688_2017	2017-07-10	62.37	-74.66	SCM	-0.65	32.05	3.19	0.06	4.44	0.67	25	2.18	na	na	1	1
684_2017	2017-07-11	61.79	-71.91	SCM	0.81	31.56	0.26	0.02	1.90	0.44	20	3.07	na	na	1	1
670_2017	2017-07-12	58.99	-67.94	SCM	-0.62	31.62	1.50	0	2.84	0.56	30	3.61	na	na	1	1
676_2017	2017-07-12	60.13	-69.06	SCM	0.13	31.32	0.19	0	0.03	0.35	29	2.69	na	na	1	1
730_2018	2018-07-08	56.18	-76.72	SCM	3.03	24.05	0.72	0.03	13.38	0.49	16	1.19	na	na	1	1
731_2018	2018-07-08	55.41	-77.93	SCM	-1.03	28.13	3.00	0.07	12.68	0.78	33	0.89	na	na	1	1
736_2018	2018-07-09	58.42	-78.31	SCM	-0.63	29.37	2.48	0.03	14.21	0.90	32	2.75	na	na	1	1

Lat, latitude; Lon, longitude; SCM, subsurface chlorophyll maximum; Surf, surface; Temp, temperature; Sal, salinity; Fluo, fluorescence; Pycn, depth of the pycnocline. N2, Brunt-Väisälä frequency.

phosphate (PO_4^{3-}) and silicate ($Si(OH)_4$) measurements were collected into acid rinsed, then sample rinsed, 15-ml Falcon™ tubes (Corning, USA) after filtration through a 0.22 μm syringe filter. The nutrient samples were analyzed on board the ship using a Bran-Luebbe 3 autoanalyzer (Grasshoff et al., 1999). The stratification parameter was defined with the Brunt-Väisälä (or buoyancy) maximum frequency N^2 calculated for each station in Ocean Data View (v 5.2.1; Schlitzer, 2002). The more stratified the water column, the higher the N_2 value.

DNA and RNA were co-extracted from the filters using AllPrep DNA/RNA Mini kit (Qiagen, Germany) following the manufacturer protocol and V4 region of 18S rRNA gene (rDNA) and 18S rRNA (rRNA) was amplified and sequenced using a combination of universal forward E572F (5'-CYG CGG TAA TTC AG CTC-3') and reverse primers E1009R (5'-CRA AGA YGA TYA GAT ACC RT-3') using the cycling conditions as described in Comeau et al. (2011). 26 Samples from 11 sampling events were sequenced at the Institut de Biologie Intégrative et des Systèmes, Université Laval, Sequencing Plateform (Table 1). Overlapping paired end reads from the fastq files were processed within the qiime2 environment (Caporaso et al., 2010) using dada2 v2019.10 (Callahan et al., 2016). The quality of the forward and reverse reads was estimated using fastqc v0.12.0 (Andrews, 2010). For each run, forward and reverse reads were demultiplexed. Removing of primers, trimming of forward and reverse reads, denoising of low-quality reads, merging and removing of chimeras was performed using the denoise-paired command in dada2 (trim-left-f = 5, trim-left-r = 5, trunc-len-f = 285, trunc-len-r = 260, maxEE = 1). The two denoised runs were merged and taxonomy was assigned to each ASV in mothur using the PR2 database v4.12 (Guillou et al., 2013). Sequences affiliated to Metazoa and chloroplasts were removed from the analysis as well as sequences at the unclassified Phylum level using the R package Phyloseq v1.32.0 (McMurdie and Holmes, 2013). For dinoflagellate taxa detected as potentially harmful, taxonomy was refined by BLASTn against the NCBI nr database (Table S2).

To correct for differential sequencing depth of the samples, data were transformed to relative abundance and ASVs below the threshold of 1×10^{-5} total relative abundance were removed from the resulting matrix table. The rDNA and rRNA ASVs and reads were analysed on the same pipeline in Phyloseq, resulting in a relative abundance table of 856 ASVs. All the subsequent clustering analysis were run in the R environment. A square-root transformation was applied to the relative abundance table and a Bray-Curtis (BC) matrix was calculated. Hierarchical clustering was performed using the "ward.D2" method on the BC matrix. Distance-based redundancy analysis (db-RDA) of microbial communities was performed with standardized environmental variables using the capscale() function in vegan v2.5.6. We adjusted the R^2 results using Ezekiel's formula with the RsquareAdj() function. The adjusted R^2 measures the unbiased amount of explained variation and was used to reduce the number of significant variables using a forward selection and 999 ANOVA permutations. Co-varying variables were removed from the final db-RDA calculation. Pearson correlation between environmental variables and relative abundance of taxa at the "Genus"

classification level was calculated with the stats R package and visualized using ggcorrplot v0.1.4 (Kassambara, 2019). Taxa with significant correlation ($P < 0.001$) with at least one environmental parameter in the rDNA and rRNA dataset were retained for specific correlation analysis. Linear regression models were used on these selected taxa to further test the influence of environmental parameters on the distribution of rDNA and rRNA reads. To assess the distribution of the most abundant taxa, the relative abundance of ASVs were summed at the species level and zscore-transformed ((x-mean) sd^{-1}). When no known species were identified for the group, taxa were annotated at the highest taxonomic level. DNA and RNA sourced datasets were treated separately. To assess significant differences in mean relative abundance between the HB and HS+UB clusters, Student's t-tests were performed with a P-value threshold of 0.05.

Results

Environmental parameters

Temperature and salinity profiles in 2017 and 2018 both show warmer and fresher surface waters in eastern HB compared to surface waters in HS+UB (Figures 1A, B). The warmest temperature was recorded at the surface of station st732 in 2017 (9.49°C) and freshest surface waters were at st730 (salinity = 23.27; Table 1). The upper warmer layer in eastern HB was constrained to the top 20 meters of the water column and contrasted with colder and saltier waters underneath (Figure 1B), for example with a salinity of 31.62 and a temperature of -0.62°C for deeper water of st732. The stratification was associated with the marked SCM with a maximum fluorescence detected at st736 in 2017 (7.67 μg chl *a* L^{-1}). The SCM in eastern Hudson Bay was more developed during the sampling in 2017 than in 2018 with higher chl *a* concentrations as estimated by fluorescence. Surface waters were depleted in nutrients all along the cruise track for both years, contrasting with nutrient rich water in the deepest layers. In the HS+UB, the nitracline was closer to the surface and associated with higher surface chl *a* concentration compared to the HB. Temperature and salinity profiles in the HS+UB indicate a mixed water column contrasting with the stratified water column in the HB. The Brunt-Väisälä frequency maximum (higher values indicate stronger stratification) through the pynocline ranged from 9.88 to 16.89 in HS+UB, and 19.78 to 45.47 in the HB with the highest value at st730 in 2018 (Table 1).

Structure of protist communities

The hierarchical clustering based on the Bray-Curtis distance separated the community sequences into 4 distinct clusters (Figure 2B). The most distant cluster consisted of mostly the rDNA collected from the HB surface in both 2017 and 2018. The vast majority of HB rRNA surface communities formed a separate cluster. The HB SCM rDNA and rRNA samples and st688 in Hudson Strait clustered together. The remaining stations that
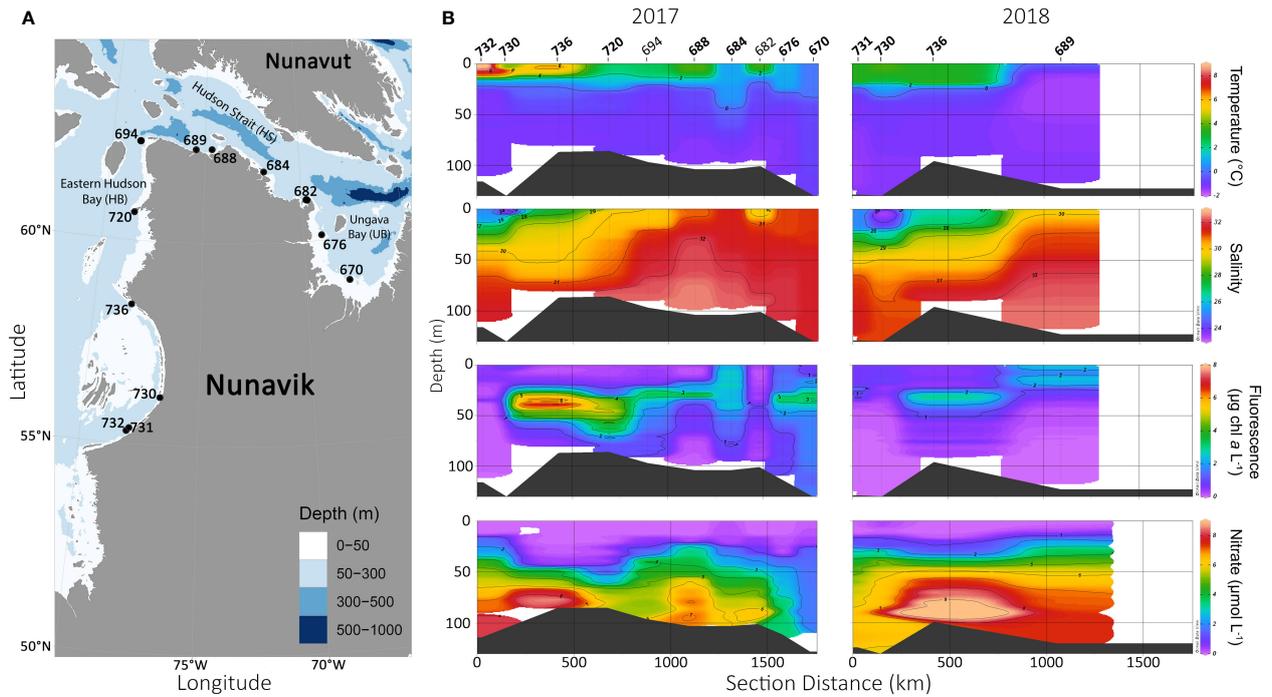
FIGURE 1
Sampling sites and water column properties in Eastern Hudson Bay, Hudson Strait and Ungava Bay. **(A)** Station locations during summer 2017 and 2018. **(B)** Vertical water temperature, salinity, fluorescence and nitrate concentrations from individual stations, the continuum is extrapolated from CTD profiles using Ocean Data View v5.2.1.
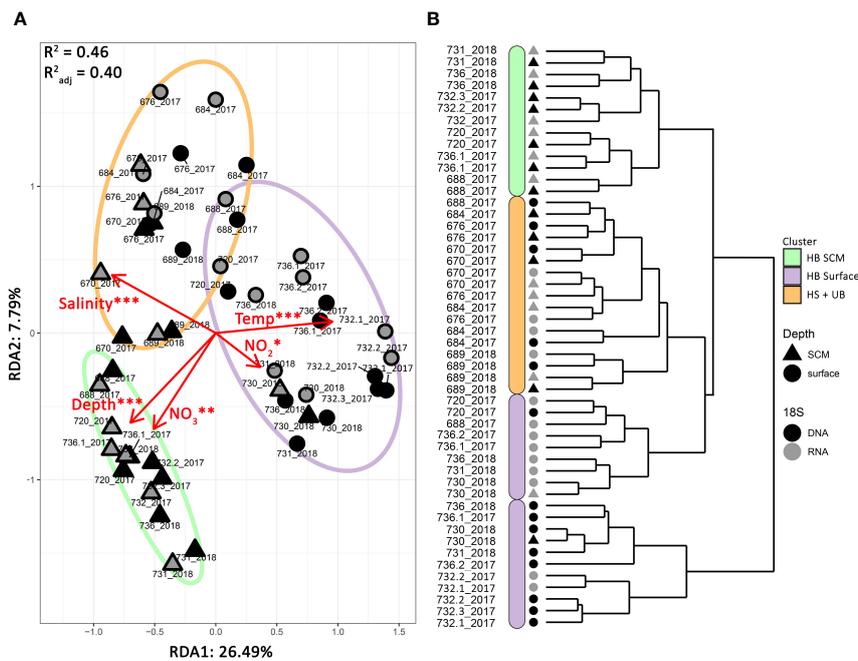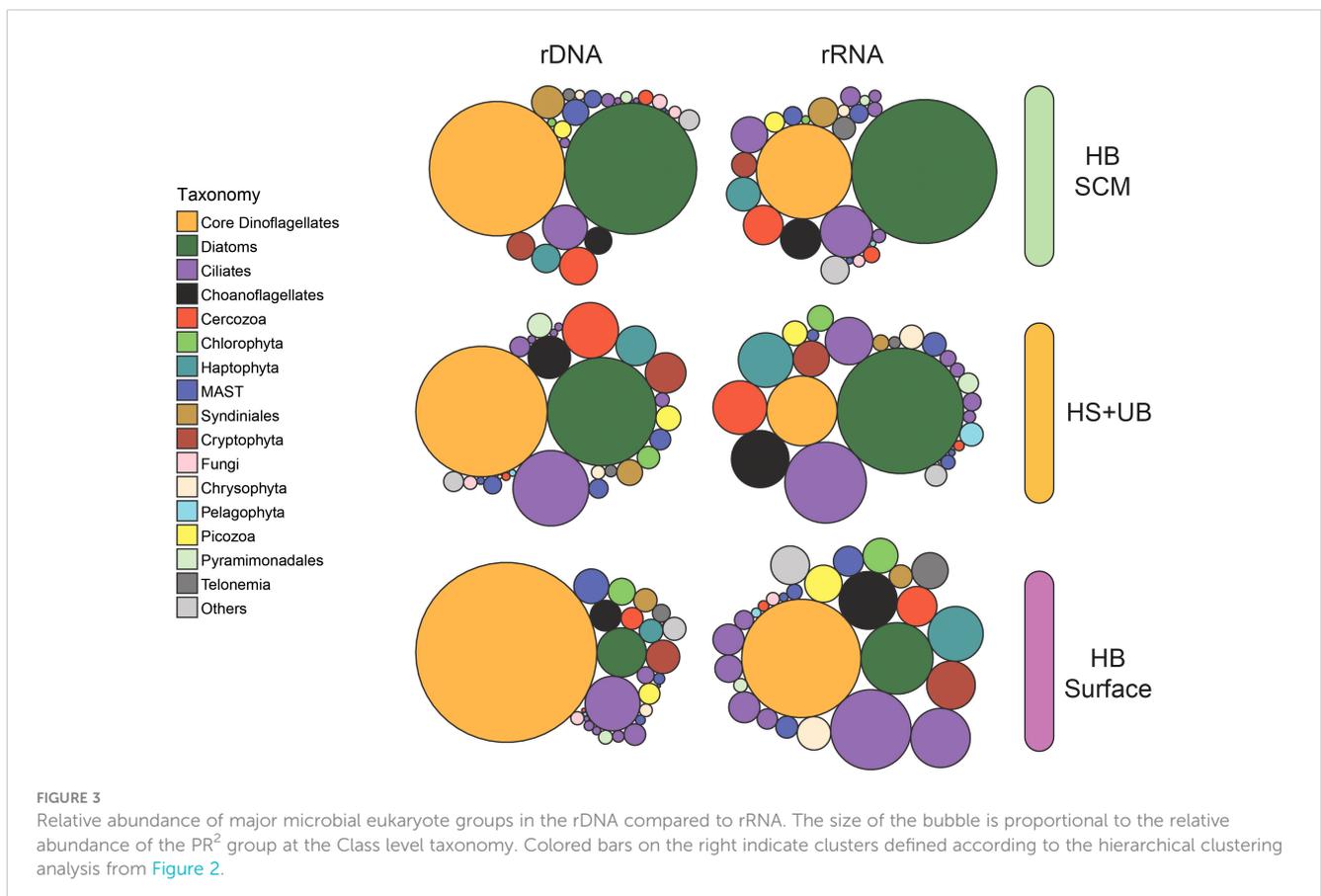

FIGURE 2
Summer protist community structure. **(A)** Ordination plot of distance-based redundancy analysis (db-RDA) of microbial eukaryote communities using a Bray-Curtis distance. Symbol shapes indicate the depth layer, with nucleotide source in grey or black. Statistically significant environmental variables are shown (ANOVA test). *P*-value: *** = 0; ** < 0.001, * <0.01. **(B)** Hierarchical clustering analysis. The dendrograms were constructed with Bray−Curtis distance using the "ward.D2" method. Environmental clusters are designated by colored bars.

were collected along Hudson Strait and the Ungava Bay formed a separate cluster consisting of rRNA and rDNA reads from both surface and SCM of the Hudson Strait and Ungava Bay (collectively referred to as HS+UB). The samples from the same location and depth category tended to cluster together independent of collection year, except for st688_2017, where rDNA clustered within HS+UB while rRNA clustered within HB.

A distance-based redundancy analysis (db-RDA) was used to seek the environmental parameters associated with the spatial repartition of the communities. Together, temperature, salinity, depth, nitrite, and nitrate concentrations explained 40% of the total variance (R_2 adj = 0.40; Figure 2A). Phosphate was also significant but was excluded from the final analysis due to its high positive correlation with nitrate (Pearson's correlation = 0.75; P-value = 0.03) and because nitrate is expected to be the main limiting nutrient for phytoplankton growth in Hudson Bay (Tremblay et al., 2019). The surface waters in the HB clearly separated from HS+UB along the first canonical axis explaining 26.49% of the total variance. Higher temperatures and nitrite concentrations were associated with the surface waters of HB, and saltier conditions were associated with the surface and SCM samples in the HS+UB. Temperature explained most of the variance in our dataset (F = 19.65; P-value < 0.001; Table S1). The second axis, which explained 7.79% of the total variance indicated that communities at the SCM in HB were associated with greater depth and the higher nitrate concentrations that occur below the halocline.

Dinoflagellates, diatoms, and to a lesser extent ciliates accounted for the greatest number of sequences in rDNA and rRNA datasets (Figure 3). The dinoflagellates and diatoms contributed about equal proportions in the rDNA data set of the SCM in HB, with relatively lower representation of dinoflagellates and more diatoms in the rRNA data. The HS+UB communities had a similar pattern with fewer dinoflagellates and more diatoms in the rRNA compared to the rDNA. Dinoflagellate read relative abundance was greatest in the HB surface especially in the rDNA dataset with relatively fewer diatoms in both the rDNA and rRNA compared to HS+UB.

To identify the principal protist groups underlying separation of the communities in the db-RDA (Figure 2A), we used Pearson's correlation between environmental parameters and relative abundance of 252 protist groups that were placed at the same classification level in the PR^2 database (Guillou et al., 2013). Only correlations with P-values less than 0.001 were considered as significant. Among the 252 groups tested, 39 showed at least one significant correlation with an environmental parameter in both rDNA and rRNA datasets (Figure S1). Ciliate reads classified in the *Laboea*, Rimostrombidium group A, Strombidiida group F, as well as the dinoflagellates *Heterocapsa* and *Gymnodinium* were highly correlated with decreasing salinity in both rDNA and rRNA datasets. Increasing temperature was also correlated with increased representation of small photosynthetic taxa such as *Bathycoccus* and *Micromonas* in rDNA and the dinoflagellates *Margalefidinium* in both rDNA and rRNA. Lower temperatures

FIGURE 3
Relative abundance of major microbial eukaryote groups in the rDNA compared to rRNA. The size of the bubble is proportional to the relative abundance of the PR^2 group at the Class level taxonomy. Colored bars on the right indicate clusters defined according to the hierarchical clustering analysis from Figure 2.
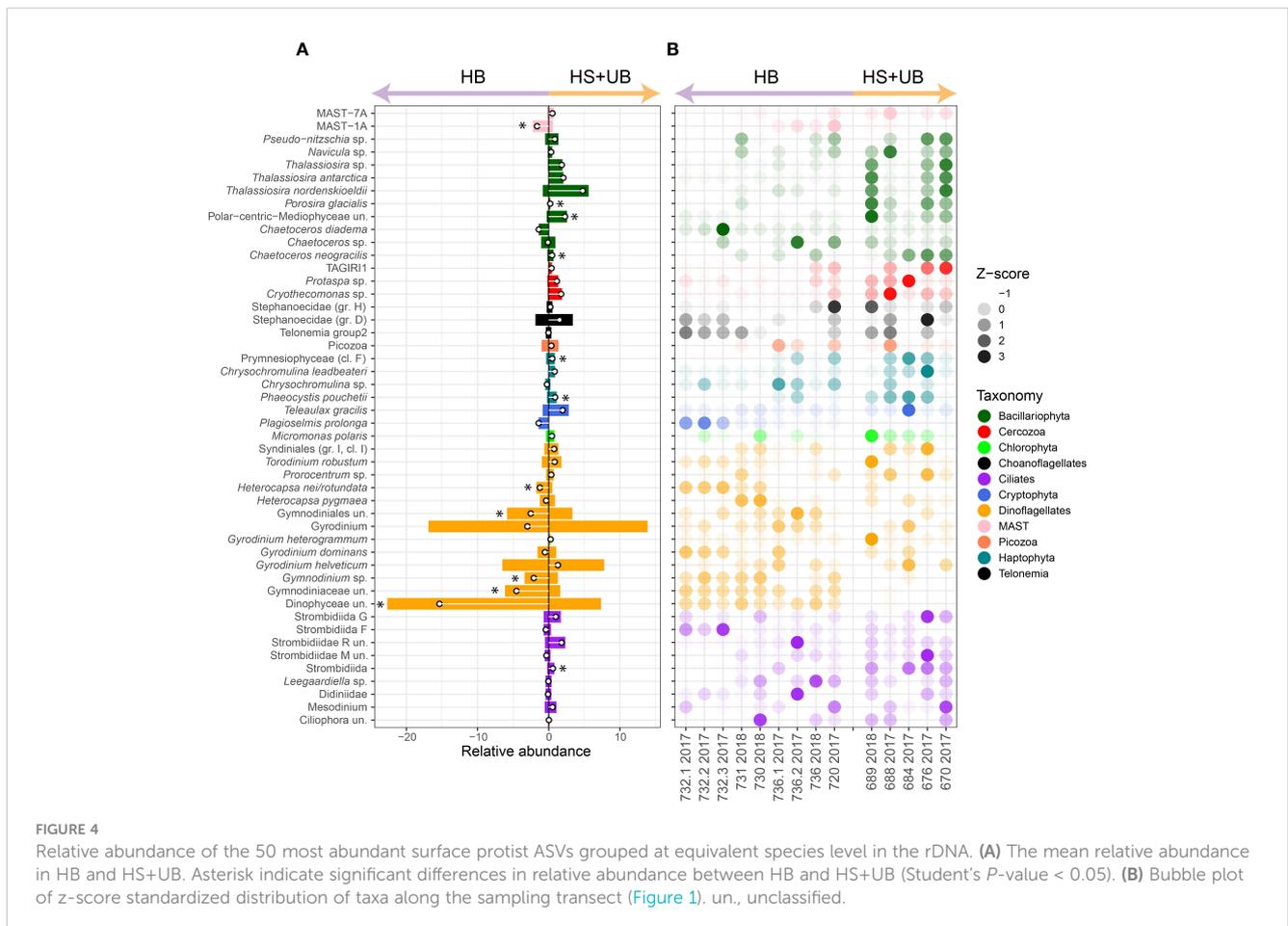
were associated with an increase in diatom relative abundance from the genus *Thalassiosira*. Linear regression models tended to confirm these patterns and showed opposite trends for the diatom *Thalassiosira*, compared to the dinoflagellates and ciliates (Figure S2). Reflecting this, warmer temperatures and lower salinity were associated with significantly higher proportions of Gymnodiniales, *Heterocapsa* and Strombidiida F reads. Again, this contrasted with the diatom reads that increased with salinity and decreased with warmer temperatures. The macronutrients, phosphate, nitrate and silicate, were correlated with several of the groups (Figure S1). Especially, higher nitrate concentrations were correlated with higher proportions of the diatom *Pseudo-nitzschia* in the rRNA dataset.

At a finer taxonomic level, distribution of the top 50 species level taxa also showed the biogeographic patterns in the surface layer (Figures 4A, B). Together, these top 50 taxa represented between 82% and 95% of the total community in surface samples from the rDNA dataset. Most of the diatom species, especially *Porosira glacialis, Pseudo-nitzschia, Navicula* sp., *Thalassiosira antarctica, Thalassiosira nordenskioeldii, Chaetoceros neogracilis* and an unidentified ASV of Mediophyceae were more abundant in HS+UB, with high proportions at st689 in HS and at st676 and st670 in Ungava Bay. Among the diatoms, particularly for sequences classified as *Pseudo-nitzschia* spp., *Thalassiosira* spp.

and *Chaetoceros* spp., reads at the SCM in HB were proportionately higher compared to the surface for both rDNA and rRNA (Figure S3). Prymnesiophyceae from the environmental clade F, and *Phaeocystis pouchetii* showed a significant relative increase in HS+UB compared to HB (Figure 4A). Most of the dinoflagellate ASVs except for taxa from the family Heterocapsaceae, were more abundant in HB. Gymnodiniales and Peridiniales were overrepresented in the rDNA compared to rRNA, (Figure S3). Ciliates tended to have a pattern of increasing relative abundance in HS+UB compared to HB, particularly for ASVs in the Strombidiidae family.

HAB species

Our results based on metabarcoding showed no evidence of any HAB event at the time of sampling, but our targeted search indicated a widespread occurrence of reads from species reported as HABs. *Pseudo-nitzschia* spp. were present at all sampled stations, but especially in the rRNA dataset at the SCM in the HB samples where they may have been particularly active (Figure S3B). Despite their low relative abundance, six putative harmful dinoflagellate species were detected. Among these 6 taxa, *Margalefidinium fulvescens, Protoceratium reticulatum* and *Phalacroma*

FIGURE 4
Relative abundance of the 50 most abundant surface protist ASVs grouped at equivalent species level in the rDNA. **(A)** The mean relative abundance in HB and HS+UB. Asterisk indicate significant differences in relative abundance between HB and HS+UB (Student's *P*-value < 0.05). **(B)** Bubble plot of z-score standardized distribution of taxa along the sampling transect (Figure 1). un., unclassified.

rotundatum had unique best blast hits > 99% of identity and annotation at the species level, while *Tripos, Dinophysis* and *Alexandrium* were not resolved to species based on best blast hits to multiple species (see Figure 5; Table S2). Specifically, the distribution of *Alexandrium* sp. was restricted to HB with a peak at st732 in 2017 where it contributed to more than 4% of the total rRNA reads (Figure 5). The relatively high number of sequences of *Alexandrium* sp. at st732 coincided with the highest water temperature (9.49°C) recorded during our sampling of the area (Table 1; Figure 1B).

Discussion

Marine microbial eukaryotes respond rapidly to changing environmental conditions and can have a direct influence on the energy and carbon transfer to higher trophic levels (Worden et al., 2015). Here we showed that the structure of protist assemblages recovered from 18S rDNA and rRNA distinguished the surface and SCM as two distinct communities in eastern HB, with a single more uniform HS+UB community. The differences in species composition between the eastern HB compared to northern and western stations surrounding the HS+UB were consistent with hydrological conditions in the two marine regions (Figures 1, 2).

For the stations that were sampled during both years, rRNA and rDNA showed little or no differences in the microbial community structure, suggesting our observations were characteristic of summer conditions in this part of the HB complex. Surface temperatures at st688 were the highest in 2017 in HS+UB, which may have influenced the clustering of the rRNA sample in the HB group. Overall, the strength of the stratification was a key driver of microbial eukaryotic community structure and distribution, especially in HB (Table 1). The communities in eastern HB were vertically separated in keeping with greater stratification associated with a fresher warmer summer surface mixed layer above a well-established SCM (Figures 1, 2). The development of an SCM dominated by diatoms in summer is a characteristic feature of the HB (Roff and Legendre, 1986; Ferland et al., 2011; Matthes et al., 2021). The SCM was particularly well-defined in 2017 and was mainly associated with *Thalassiosira* spp. and *Chaetoceros* spp., two genera that also occur at the SCM after sea ice breakup in central HB (Jacquemot et al., 2022).

The low salinity and warm temperature in the surface waters of southeastern HB compared to HS+UB suggested a strong influence of river runoff in the region at the time of sampling, especially in 2018 (Figure 1; Table 1). During our sampling in 2017 and 2018, most of the nutrients at the surface were depleted, with low inorganic nutrient conditions in the surface mixed layer (Figure 1), consistent with the post spring bloom timing of our sample collection. The conditions were favorable for dinoflagellates which accounted for a high proportion of reads at the surface, particularly in the rDNA dataset where they exceeded 70% of the total reads at st732, st731, st730 and st736 (Figures 3; S3A). The presence of multiple gene copies of the 18S rRNA gene in dinoflagellates might result in an overestimation of their relative abundance in metabarcoding studies (Zhu et al., 2005; Medinger et al., 2010). However, variations in their relative abundance across different regions and depths can offer valuable insights into the preferential ecological niche of dinoflagellates. Compared to HS +UB, the dominance of reads associated with specific ASVs of dinoflagellates in HB suggest that some genera or species were particularly successful despite the low nutrient waters, but consistent with being primarily heterotrophic. For example, the marine stramenopiles from the MAST-1A clade, the Peridiniales *Heterocapsa rotundata* and the Gymnodiniales *Gyrodinium* sp. and *Gymnodinium* sp. had high relative representation in the surface
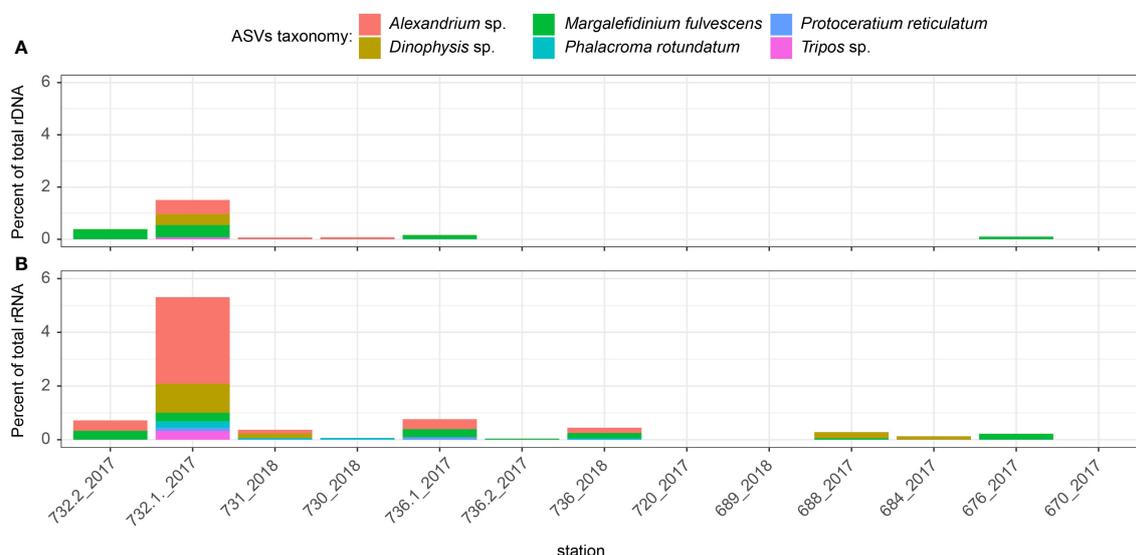

FIGURE 5
Proportion (as %) of potential harmful dinoflagellates detected at surface in rDNA **(A)** and rRNA dataset **(B)**.

waters of HB compared to the HS+UB (Figure 4). Moreover, both rDNA and rRNA were higher for *Heterocapsa* and *Gymnodinium* in the HB than in the HS+UB, suggesting a high potential for growth and acclimatation of these two genera in the warmer waters of the HB surface (Figure S3A). Arctic *Heterocapsa* spp. have chloroplasts (Rintala et al., 2010) but the genus is considered mixotrophic (Millette et al., 2017) and able to photosynthesize and graze on bacteria. While some species of Gymnodiniales are mixotrophic (Li et al., 2000), *Gyrodinium* spp. are mostly heterotrophic dinoflagellates that graze on small phytoplankton and bacteria (Hoppenrath, 2017). Both genera are nearly always reported in Arctic waters in summer (Lovejoy et al., 2006; Kubiszyn and Wiktor, 2016; Onda et al., 2017). The significant correlation of Gymnodiniales, *Gymnodinium* and *Heterocapsa* relative abundance with increasing temperature and decreasing salinity suggest that the warmer and fresher waters in the surface mixed layer were favorable to the growth of these dinoflagellates (Figures S1, S2). This is in keeping with reports of salinity and temperature having the greatest influence on the growth rate and biomass accumulation of diverse dinoflagellate species (Kim et al., 2004; Dhib et al., 2013). In a recent study, Amiraux et al. (2022) showed that the higher concentrations of dinoflagellates in eastern HB resulted in lower but more diverse content of bioactive molecules being transferred to higher trophic levels. Because the open water season is becoming longer and freshwater inputs are expected to increase in HB, our results suggest heterotrophic groups such as MAST-1A and heterotrophic/mixotrophic dinoflagellates from the Order Peridiniales and Gymnodinales may become more prevalent in summer in coastal HB, with potential influence on the local marine food web.

Diatoms in Hudson Strait and Ungava Bay

Samples from the surface and SCM clustered together in HS+UB, suggesting that the communities were driven by similar environmental parameters in the two layers (Figure 2). Unlike HB, the chl *a* fluorescence profiles in the HS+UB showed that phytoplankton were abundant and likely growing in the upper surface waters. The dominance of photosynthetic species is in keeping with a less stratified water column, as nutrients would be entrained into the photic zone (Figure 1; Table 1). Larger diatom species such as *Thalasssiosira* spp., *Porosira glacialis*, and the smaller *Chaetoceros neogracilis* seemed to be favored, but small photosynthetic flagellates including *P. pouchettii* and *M. polaris* were also relatively abundant (Figures 4; S3). Together, these results indicate that environmental conditions at the time of sampling were favorable for the growth of a mixed phytoplankton assemblage in HS+UB. The occurrence of diatoms in the moderately nitrate-depleted surface waters may have been unexpected since nitrate limits the biological production in HB (Kuzyk et al., 2010; Tremblay et al., 2019). However, the weak stratification in the HS+UB suggest that phytoplankton persistence over summer could be supported by regular supply of nitrate from the deeper waters. These pulses of nutrients are favored by hydrodynamic processes driven by wind

and tidal mixing in southern HS (Harvey et al., 1997; Straneo and Saucier, 2008; Ferland et al., 2011). The dominant taxa seen in the metabarcodes was consistent with the notion of ongoing nutrient pulses as *Chaetoceros* and *Thalassiosira* can quickly become dominant and persist in polar waters when nutrients become available (Lovejoy et al., 2002).

Stratification provides suitable habitat for HAB species

Identifying phytoplankton community composition is of concern for inhabitants of Nunavik as local communities consume marine resources, whose quality could be affected by the taxonomic composition of primary producers (Rapinski et al., 2018; Amiraux et al., 2022). Conditions in temperate coastal regions that promote different inshore communities during summer are reasonably well studied and factors leading to different phytoplankton communities include temperature, stratification regimes, intertidal mixing and the extent of allochthonous inputs, which bring in nutrients but can also change light conditions associated with particles and colored dissolved organic material from the land (Peperzak, 2003; Wells et al., 2015; Joli et al., 2018; Boivin-Rioux et al., 2022). High nutrients and stratification due to warmer surface water temperatures can trigger community transition towards dominance by harmful algal bloom forming species (Anderson et al., 2021). The marine Arctic has traditionally been considered unfavorable for toxin-producing marine harmful algal blooms (HABs), but numerous potentially harmful algae species have been reported to occur in Canadian Arctic waters, including Hudson Bay (Poulin et al., 2011; Pućko et al., 2019; McKenzie et al., 2020; Dhifallah et al., 2021). Toxins produced by many of these algae accumulate in filter-feeding marine bivalves and can be transferred up the food chain with severe health consequences for marine wildlife such as sea birds and marine mammals, in addition to humans (Beltrán et al., 1997; Scholin et al., 2000; Hendrix et al., 2021). Of greatest concern in northern waters are domoic acid and saxitoxin produced respectively by species of *Pseudo-nitzschia* and *Alexandrium* that are causative agents of human illness, amnesic and paralytic shellfish poisoning, respectively (Rue and Bruland, 2001). Because HABs are common in temperate and tropical regions, warming Arctic Ocean conditions raise growing concern for the potential expansion of HAB into these regions (Anderson et al., 2021; Hendrix et al., 2021). Moreover, the expected increase of shipping activity associated with the longer open water season, make the coastal HB system particularly vulnerable to the importation of toxic algae from ship ballast and other accidental introductions (Hochheim and Barber, 2014; Andrews et al., 2018).

Our results showed that, although we did not detect any HAB events, Hudson Bay in summer could be a suitable habitat for several toxin producers. *Pseudo-nitzschia* spp. were present at all sampled stations, especially at the SCM (Figure S3B). Not all species of *Pseudo-nitzschia* are toxic but toxigenic representatives have been recorded from northern temperate waters, with some events

leading to the closure of harvesting sites in Eastern Canada (Bates, 1998; Trainer et al., 2012). In the Arctic, the presence of toxic *Pseudo-nitzschia* species has been reported from Arctic regions of the United States (Alaska), Greenland, Canadian and Russian coastal waters (Hansen et al., 2011; Poulin et al., 2011; Harðardóttir et al., 2015; Bruhn et al., 2021). *Pseudo-nitzschia* blooms can be triggered by upwelling events or nutrient inputs from increasing river discharge (reviewed in Trainer et al., 2012).

Five of the six potential harmful dinoflagellates detected in this study; *Protoceratium reticulatum*, *Phalacroma rotundatum*, *Tripos* sp., *Alexandrium* sp. and *Dinophysis* sp., have been previously reported in HB (Pućko et al., 2019; Dhifallah et al., 2021). *Margalefidinium fulvescens* has been documented in the Beaufort and Chukchi Seas (Galaska et al., 2023), but to our knowledge, this is the first record of this species in HB. Previous occurrences of *Alexandrium* sp. were reported throughout the Canadian Arctic and in HB, particularly in Churchill Harbor and in ballast tanks at Deception Bay Harbor near HS in Nunavik (Pućko et al., 2019; McKenzie et al., 2020; Dhifallah et al., 2021). Because of their capacity to form cysts, *Alexandrium* spp. are highly adaptable to new regions and can lay dormant in sediments until environmental conditions are suitable for germination and growth (Jensen and Moestrup, 1997; Anderson et al., 2018). Interestingly, the increased number of sequences of *Dinophysis* sp. and *Alexandrium* sp. at st732 coincided with the highest water temperature (9.49°C) recorded in our sampling campaign (Table 1; Figure 1). Temperature also showed significant positive correlation with relative abundance of *Dinophysis*, *Alexandrium* and *Margalefidinium* genera (Figure S1). Although mechanisms triggering dinoflagellate blooms are not well understood, several studies have brought out the importance of nutrient ratios and short-term warming events (Granéli et al., 2011; Laabir et al., 2011; Du et al., 2016; Clark et al., 2019). Compared to rDNA, rRNA reads associated to *Alexandrium* sp. were detected at a greatest number of stations in HB and their higher relative abundance of rRNA at station 732 suggest that this species has the potential to rapidly synthesize proteins and could respond to favorable conditions.

In the Arctic, the warming ocean has been associated with the expansion of the spatial and temporal windows for harmful algal growth. As continuing sea ice loss and longer open water can result in warmer surface waters, the presence of these harmful algae raises valid concerns about future exposure risks of people living near the coast, as these indigenous communities rely on marine resources for basic food security. Monitoring of HAB species during warmer periods in coastal HB should become a priority to ensure food safety. The utilization of metabarcoding targeting the V4 region of the 18S rRNA enables the detection of various harmful taxa. However, as highlighted by Dzhembekova et al. (2017), the V4 region has limited taxonomic resolution at the species level for some genera, as evidenced by multiple species appearing as best hits for *Dinophysis*, *Alexandrium* and *Tripos* genera (Table S2). As sequencing technology becomes more efficient and affordable, the implementation of long-read sequencing using multiple assays could be a practical solution for rapidly identifying and distinguishing harmful algae species (Hatfield et al., 2020), and would limit the need for trained taxonomists.

Conclusion

This study provides the first extensive characterization of microbial eukaryotes in coastal Eastern Hudson Bay, Hudson Strait and the western side of Ungava Bay using a metabarcoding approach. We found that the stability of the water column drove the composition of protist assemblages at the surface with a dinoflagellate-dominated community in the stratified Hudson Bay and a community enriched in diatoms in the more well mixed waters of Hudson Strait. These results support the view that increasing stratification, under the influence of climate warming would favor a more heterotrophic/mixotrophic food web in coastal Hudson Bay. We showed that metabarcoding on the 18S rRNA gene is a suitable tool to detect the occurrence of several potential harmful microalgae. This study provides a time sensitive reference to track the potential introduction of new non-indigenous microbial species into the Hudson Bay System. Future efforts should move toward making DNA metabarcoding in near real time a standard practice to track harmful species in coastal Arctic regions in summer.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/ , PRJNA627250.

Author contributions

LJ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. J-ET: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing, Data curation. CM: Data curation, Formal analysis, Methodology, Validation, Writing – review & editing. CL: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

Acknowledgments

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1321604/full#supplementary-material

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