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RNA outperforms DNA-based metabarcoding in assessing the diversity and response of microeukaryotes to environmental variables in the Arctic Ocean

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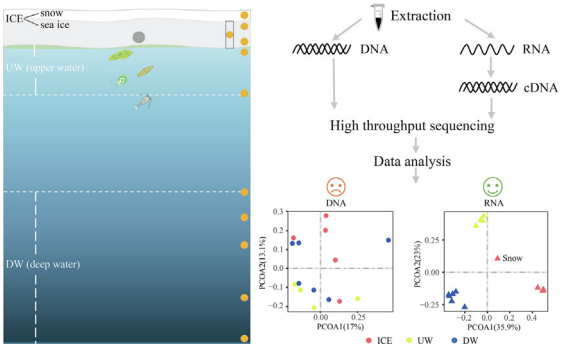
HIGHLIGHTS

- RNA and DNA revealed the distinct composition of microeukaryotes.
- The alpha diversity indices revealed by RNA increased with depth.
- RNA:DNA ratio revealed the relative activity of major microeukaryote groups.
- RNA responds more sensitively to environmental variables than DNA.

GRAPHICAL ABSTRACT



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ABSTRACT

The Arctic Ocean (AO) has a harsh environment characterized by low temperatures, extensive ice coverage, and periodic freezing and melting of sea ice, which has provided diverse habitats for microorganisms. Prior studies primarily focused on microeukaryote communities in the upper water or sea ice based on environmental DNA, leaving the composition of active microeukaryotes in the diverse AO environments largely unknown. This study provided a vertical assessment of microeukaryote communities in the AO from snow and ice to sea water at a depth of 1670 m using high-throughput sequencing of co-extracted DNA and RNA. RNA extracts depicted microeukaryote community structure and intergroup correlations more accurately and responded more sensitively to environmental conditions than those derived from DNA. Using RNA:DNA ratios as a proxy for relative activity of major taxonomic groups, the metabolic activities of major microeukaryote groups were determined along depth. Analysis of co-occurrence networks showed that parasitism between Syndiniales and dinoflagellates/ciliates in the deep ocean may be significant. This study increased our knowledge of the diversity of active microeukaryote communities and highlighted the importance of using RNA-based sequencing over DNA-based sequencing to examine the relationship between microeukaryote assemblages and the responses of microeukaryotes to environmental variables in the AO.

1. Introduction

Due to their large size range, diverse trophic modes, and rapid metabolisms, microeukaryotes (single-celled eukaryotes, protists) play diverse ecological roles in the marine biogeochemical cycles (Caron et al., 2017).

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Consequently, changes in microeukaryotic communities will have profound effects on the structure and function of an ecosystem. In comparison to other oceans, the Arctic Ocean (AO) has an inclement environment characterized by low temperatures, mass ice coverage, and the annual freezing and melting of sea ice. Its complex hydrological conditions offer diverse marine habitats for microorganisms.

In the AO, sea ice is an important habitat for many species (Mock and Thomas, 2005; Bluhm et al., 2018). Microorganisms thriving within the brine channels are subjected to spatially and temporally changing physical and chemical variables, including temperature, light, and salinity. Snow is also a harsh environment, which is characterized by low temperatures and intense ultraviolet irradiation (Brown et al., 2015). However, there is growing evidence that sea ice and snow can harbor diverse and active microbial populations (Harding et al., 2011; Hell et al., 2013). Due to salinity stratification and the covering of both multiyear and first-year ice, the AO is proposed to be more quiescent than any other ocean. Previous studies suggest that changes in the vertical structure of the water column will cause shifts in the microbial communities (Tremblay et al., 2009; Xu et al., 2017; Giner et al., 2020; Sun et al., 2023) and possibly alter the biogeochemical cycling (Worden et al., 2015). In the central AO, microeukaryotes are the predominant primary producers, whereas marine planktonic cyanobacteria are uncommon (Marquardt et al., 2016). However, previous studies have focused more on upper water (Lovejoy et al., 2006; Comeau et al., 2011; Kilias et al., 2014; Xu et al., 2020), and relatively few studies have been conducted on microeukaryotic assemblages of the deep ocean.

Over the past several decades, the AO has experienced a sharp decline in sea-ice volume and extent (Kwok and Rothrock, 2009; Stroeve et al., 2012; Simmonds, 2017). Thick and old multiyear ice has gradually disappeared and been replaced by young and thin first-year ice. It has been predicted that an ice-free AO during summer is likely to occur by the midpoint of the twenty-first century (Wang and Overland, 2012). As a result, significant environmental changes are anticipated to occur in the seawater column. For example, the freshwater input increased stratification of the upper water, which will have a profound effect on nutrient transport into the euphotic zone from the deep (Comeau et al., 2011). In the meantime, a rise in surface water temperatures would alter circulation patterns and exacerbate ocean acidification. The presently occurring climate-driven changes across the AO are believed to affect microeukaryotic communities in numerous ways. For example, it has been reported that pico-sized algae flourish as the AO freshens (Li et al., 2009), and AO primary production continued to increase in the last several decades (Arrigo and van Dijken, 2015).

Surveys on the AO microeukaryotes have been conducted for many years, most of which were based on the traditional, e.g., microscopy-based technique (Booth and Horner, 1997; Gradinger, 1999; Melnikov et al., 2002). The culture-independent approaches, e.g., the sequencing-based techniques, have allowed for a rapid, low-cost, and large-scale survey of microorganisms, including microeukaryotes. Recent years have seen an increase in microbial diversity surveys in high-latitude oceanic regions using high throughput sequencing (Stecher et al., 2015; Hardge et al., 2017; Xu et al., 2022; Sun et al., 2022). DNA can be found in active, dormant, and dead cells (Hansen et al., 2007) and also persists as extracellular DNA (Nielsen et al., 2007), reflecting the "total" diversity. Extracellular RNA is unstable and has been proposed to be a representation of the "active" community (Blazewicz et al., 2013). It has been proposed that the use of both molecules would provide valuable information regarding the species present in a particular environment (Logares et al., 2014).

In the present study, samples taken during the 2019 summer expedition of IBRV ARAON allowed us to explore the microeukaryotic diversity of various environments in the AO by high throughput sequencing of the coextracted environmental DNA and RNA. Our main objectives were to (1) characterize the diversity and vertical distribution of microeukaryotes in the covering snow, the sea ice, and the underlying sea waters; (2) compare microeukaryotic communities shown by DNA and RNA; and (3) determine if microeukaryotic communities revealed by RNA respond more sensitively to environmental variables than those revealed by DNA.

2. Materials and methods

2.1. Sample collection and measurements of environmental parameters

During the 2019 summer expedition of IBRV ARAON, ten seawater samples, four ice-core samples, and one snow sample were collected at three stations (Fig. 1). A whole ice core (ca. 120 cm thick) sample, the top 1/2 of an adjacent ice core sample (ca. 60 cm thick), and an under-ice seawater sample (0 m) were collected at station IC1 (79.50°N, 160.99°W). At station

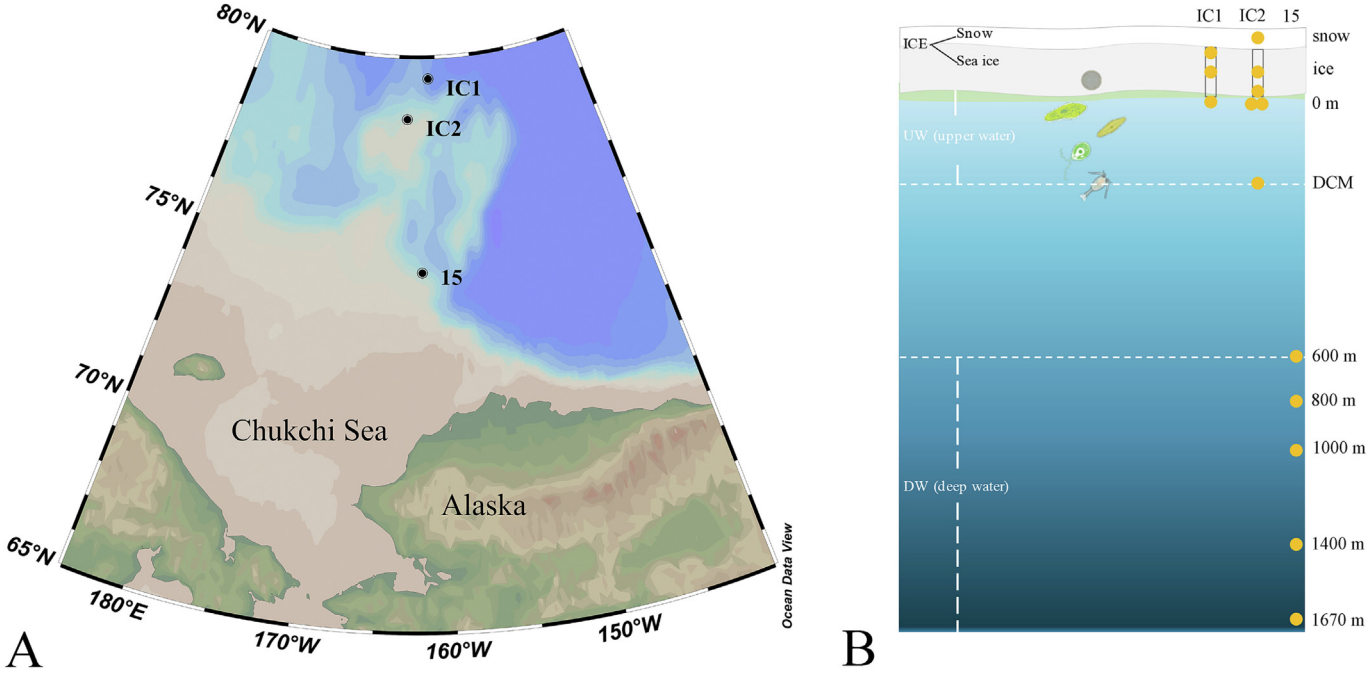

Fig. 1. The map of sample stations (A) and each dot in (B) represent a sample retrieved from a specific depth.

IC2 (78.45°N, 163.67°W), a snow sample, a whole ice core sample (ca. 120 cm thick), the bottom 1/2 of an adjacent ice core sample (ca. 50 cm thick), and four seawater samples (two under-ice samples, one at 42 m, and one at 600 m, respectively) were collected. At station 15 (74.52°N, 161.93°W), samples were collected at depths of 600, 800, 1000, 1,400, and 1,670 m.

The snow was collected using a sterile bucket. Ice-core samples were extracted using a MARK II coring system (Kovacs Enterprises, USA, 9 cm in diameter) and stored in polyethylene (PE) bags. Niskin bottles attached to a CTD profiler with sensors for measuring temperature and salinity were used to collect seawater samples. For seawater samples, 5 L of seawater collected from each depth were prefiltered through 200 μm mesh (Nitex) and then filtered onto a 0.45 μm pore size membrane (Millipore, USA) using a filtration unit connected to a vacuum pump at a rate of 0.5–1 L/min. Snow and ice-core samples were immediately prefiltered through 200 μm mesh (Nitex) and collected with 0.45 μm pore size membrane filters after thawing at 4 °C using the same filtration unit. Samples were filtered within 30 min after being retrieved from the ocean, and then flash-frozen in liquid nitrogen and kept at −80 °C until nucleic acid extraction.

To measure the concentration of chlorophyll *a* (Chl *a*), samples were filtered through Whatman GF/F filters, which were later extracted in methanol at 4 °C for 24 h before being analyzed using a fluorometer (Turner Designs, USA). Samples for inorganic nutrients (NO^{3-} + NO^{2-}, $PO4^{3-}$, and SiO_2) were collected using the Niskin bottles, kept frozen, and measured using an auto-analyzer (QuAAtro, Seal Analytical, USA) according to the manufacturer's instructions.

2.2. Nucleic acid extraction and sequencing

DNA and RNA were coextracted using the AllPrep DNA/RNA kit (Qiagen, USA) by applying an initial mechanical disruption followed by chemical lysis (Xu et al., 2017). RNA was then reverse transcribed into cDNA with the QuantiTect® Reverse Transcription Kit (Qiagen, USA). The eukaryotic universal primers TAReukFWD1 and TAReukREV3 were used to amplify the V4 hypervariable regions of SSU rRNA and its gene (380 bp) (Stoeck et al., 2010). The PCR conditions used were as follows: an initial incubation for 5 min at 94 °C, followed by 30 cycles of 60 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C, and a final extension step of 10 min at 72 °C. Three PCR reactions were run for each sample, and products from the same sample were pooled and purified using the MiniElute Gel Extraction Kit (Promega, China). Sequencing was performed using a paired-end (2 × 250 bp) approach on the Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA).

2.3. Data processing and statistical analyses

FLASH was used to assemble the paired-end reads from the raw DNA fragments (Magoc and Salzberg, 2011) according to the criteria of Li et al. (2021). Mothur was used to screen sequences, and only those lengths between 300 and 500 bp were retained. Using the UNOISE algorithm embedded within USEARCH v.11, ZOTUs (zero-radius operational taxonomic units) were clustered (Edgar, 2010). The taxonomic identities of each ZOTU were determined using SINTAX (Edgar, 2016) against the PR2 database v4.11.1 (Guillou et al., 2012). The unassigned or non-Eukaryota-affiliated ZOTUs as well as singletons were deleted. 2,301,792 reads from 15 DNA samples and 15 RNA samples were clustered into 8278 ZOTUs. The ZOTU table was randomly subsampled to the minimum number of reads per sample (36,234 reads) to enable sample comparisons. The final ZOTU table contained 8160 ZOTUs and 1,087,020 reads. Sequences are available at the NCBI under accession number PRJNA914059.

The alpha diversity indices, including the ZOTU richness, Shannon, and phylogenetic diversity (PD), were calculated in QIIME (Caporaso et al., 2010). Differences in alpha diversity indices between sample groups were evaluated using the Kruskal-Wallis test. Bray Curtis and unweighted UniFrac distances were calculated in R (R Core Team, 2015) and subsequently used as inputs for the principal coordinates analysis (PCoA) to visualize the ordination of microeukaryotic communities. The clustering of sample groups was assessed with the analysis of similarity (ANOSIM) using 999 permutations. Visualizations of Bray Curtis distance matrices were also generated in R using dendrograms based on average hierarchical clustering. Paleontological Statistics (PAST) was used to test the relationships between communities and environmental factors using mantel tests (Hammer et al., 2001).

To evaluate the relative metabolic activity of major microeukaryote assemblages, RNA: DNA ratios for each ZOTU were calculated. ZOTUs found only in DNA or RNA datasets were deleted (Xu et al., 2017). The Kruskal-Wallis test was used to assess differences in RNA:DNA ratios between sample groups for each major microeukaryote assemblage.

2.4. Network analysis

To reduce the complexity of the datasets, only ZOTUs including ≥ 20 reads and occurring in ≥ three samples in the DNA and RNA datasets, respectively, were retained for network construction. 290 and 787 ZOTUs were used for the network analyses of DNA and RNA datasets, respectively. All possible pairwise Spearman's rank correlations (r) between these ZOTUs were calculated in R (Ju et al., 2014). Only robust ($r > 0.8$ or $r < -0.8$) and statistically significant ($P < 0.01$) correlations were included in network analyses (Hu et al., 2017). Network visualization and modular analysis were done in Gephi following Ma et al. (2016). Ternary plots were generated in R. Keystone species were chosen for both the DNA and RNA datasets based on their high degree (> 100) and low betweenness centrality (< 5000) in co-occurrence networks (Berry and Widder, 2014; Banerjee et al., 2018).

3. Results

3.1. Environmental parameters

Temperatures ranged from −2.5 °C to 0.86 °C. Snow, ice, and the upper seawater (0 m and 42 m) all had temperatures below 0 °C. The deep water temperature (600–1,670 m) was slightly higher (Table S1). The average salinity of the ice was 1. The salinity of seawater was 27.8 at 0 m, 30.8 at 42 m, and ca. 34.87 between 600 and 1,670 m (Table S1). The highest Chl *a* concentration was found at the bottom of the ice (ca. 1.19 μg/L) rather than the deep chlorophyll maximum layer (ca. 42 m, DCM, 0.87 μg/L) (Table S1). Inorganic nutrients were only detectable in seawater samples. NO*x* (nitrate + nitrite) concentrations were close to zero at 0 m. Nutrients increased with depth, except for PO_4^{3-} and SiO_2, which peaked at DCM (Table S1).

3.2. Beta diversity and community compositions

Rarefaction curves showed that the sequencing of most samples tended to be saturated (Fig. S1). The PCoA Ordination of all samples demonstrated distinct patterns of clustering for the DNA and RNA datasets, respectively. Two PCoA plots of the RNA dataset showed the same clustering pattern: samples were divided into three groups: the ICE group (5 samples), which included snow and ice-core samples; the UW group (4 samples), which included samples from upper water (0 m and 42 m); and the DW group (6 samples), which included samples from deep water (600 m to 1670 m) (Fig. 2B, D; S2B). The ANOSIM test supported the grouping (Fig. 2B, $R^2 = 0.96$, $p < 0.001$; Fig. 2D, ANOSIM: $R^2 = 0.96$, $p < 0.001$). Nonetheless, in the two PCoA plots of the DNA dataset, samples were dispersed without any discernible patterns (Fig. 2A, $R^2 = 0.07$, $p = 0.207$; Fig. 2C, $R^2 = 0.10$, $p = 0.139$) and the dendrogram showed an identical pattern (Fig. S2A).

Regarding both sequence proportion and ZOTU richness, four supergroups, Alveolata, Stramenopiles, Rhizaria, and Opisthokonta, dominated the microeukaryotic community in both datasets (Fig. 3). There were minor differences between the community composition of individual DNA samples, except that in samples 2SNOWD and 15_1670D, Spirotrichea

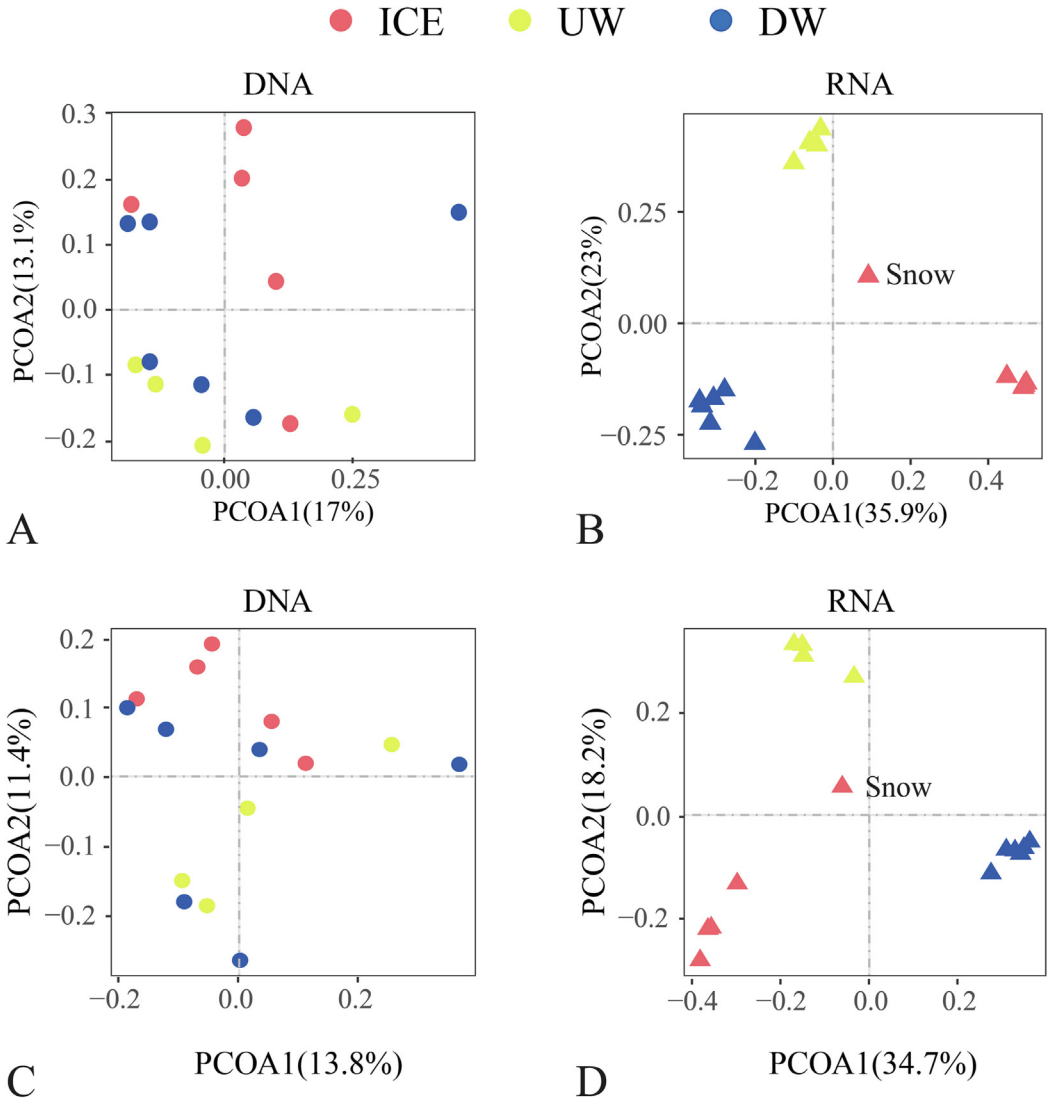

Fig. 2. Principal coordinates analysis (PCoA) based on Bray-Curtis (A, B) and unweighted UniFrac distances (C, D). ICE: snow and ice-core group; UW: upper water group, including samples from 0 m and 42 m; DW: deep water group, including samples from 600 to 1670 m.

and Polycystinea sequences accounted for more than half of the total, respectively (Fig. S3A). On the contrary, significant differences between the three sample groups in the RNA dataset were found, i.e., Bacillariophyta dominated the ICE group, Spirotrichea the UW group, and Dinophyceae the DW group (Fig. 3C). Cercozoa (primarily Filosa-Sarcomonadea) accounted for ca. 53 % of total sequences in the snow sample (Fig. S3C). Approximately one-third of the sequences belonged to unclassified Opisthokonta in sample 1CORE, followed by Ciliophora and Bacillariophyta. Bacillariophyta (ca. 75 %), predominantly pennate diatoms, dominated sample 2CORER. Metazoa (primarily Nematoda and Platyhelminthes) preferred the bottom of the ice core. Ciliophora, mostly Spirotrichea, predominated in the upper water samples (Fig. 3C). Except for sample 15_600R, which was characterized by a high contribution of Metazoa (mainly Ctenophora), the community composition was similar in the DW group. In terms of the ZOTUs richness, Bacillariophyta, Spirotrichea, and Dinophyceae were major contributors to all samples, but their contributions varied among samples (Fig. S3D).

The RNA dataset demonstrated a community structure that was significantly correlated with salinity ($R = 0.75$, $p < 0.001$) and temperature ($R = 0.46$, $p < 0.001$) by mantel test. However, the DNA dataset failed to reveal significant correlations between salinity ($R = 0.13$, $p = 0.13$), temperature ($R = 0.001$, $p = 0.45$) and the community.

3.3. Alpha diversity indices

The alpha diversity indices of DNA samples lacked discernible patterns (Fig. 4). In the RNA dataset, ZOTU richness increased significantly with increasing water depth (Fig. 4). Comparing DNA to RNA, it was clear that the DNA dataset was more diverse than the RNA dataset (Fig. 4). According to the Spearman test, temperature and salinity were significantly correlated with RNA diversity, not DNA diversity (Table S3). Of the ZOTUs, 2002 and 479 were shared among the three groups in the DNA and RNA datasets, respectively (Fig. S4).

3.4. Relative metabolic activity of major microeukaryote assemblages

To determine changes in the relative metabolic activity of major taxonomic groups between ICE, UW, and DW, the RNA:DNA ratios of each ZOTU were calculated. CONThreeP, Litostomatea, Telonemia, Arthropoda, and Cercozoa did not show statistically significant differences among the three groups (Fig. 5). Bacillariophyta exhibited higher relative activity in ICE. The lowest activities of Chrysophyceae, Pelagophyceae, and MAST were found in ICE. Spirotrichea, Mamiellophyceae, Cryptophyta, Haptophyta, Acantharea, and Dictyochophyceae showed higher activity in the UW than in the ICE and DW. The highest relative activities of

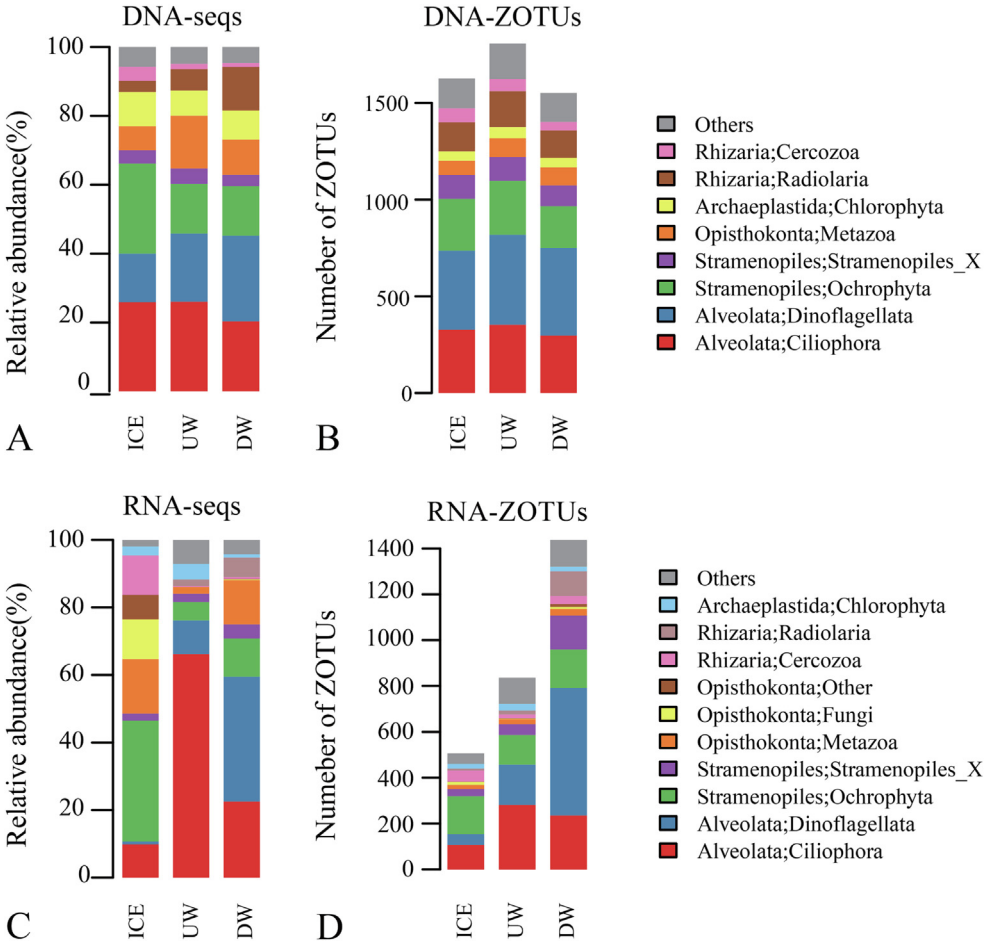

Fig. 3. Proportions of sequences (A, C) and ZOTU richness (B, D) of microeukaryotes in the three sample groups (ICE, UW, and DW) retrieved from DNA (A, B) and RNA (C, D) datasets, respectively.

Oligohymenophorea, Dinophyceae, Syndiniales, Ctenophora, and Polycystinea were found in DW (Fig. 5).

3.5. Co-occurrence networks

Metacommunity co-occurrence networks were built based on correlation relationships between ZOTUs (Fig. 6). The DNA network had 290 nodes and 343 edges, while the RNA network had 787 nodes and 31,303 edges. The RNA network had a higher average degree, network diameter, network density, and average clustering coefficient than the DNA network (Table S4).

Ciliophora (30 %), Dinoflagellata (14.1 %), and Bacillariophyta (11 %) were the three highest contributors to the DNA network (Fig. 6A). The DNA network was parsed into five modules, with the most prevalent accounting for < 10 % of all nodes (Fig. 6C). Primary contributors to the RNA network were Ciliophora (23.6 %), Dinoflagellata (15.6 %), Syndiniales (12 %), Bacillariophyta (10.6 %), and Stramenopiles_X (9.5 %) (Fig. 6B). The RNA network was parsed into four major modules, which collectively comprised 95 % of all nodes (Fig. 6D). Ternary plot analysis showed that most modules in the RNA network were specific to a particular group but not in the DNA network (Fig. 7). In the RNA network, module A, which belonged primarily to UW, was represented mainly by Ciliophora and comprised 34.6 % of all nodes. DW Modules B and C accounted for 45.6 % of all nodes, which were represented mainly by Dinoflagellata, Syndiniales, and Stramenopiles_X. Module D, which belonged to the ICE, contained ca. 14.6 % of all nodes, the majority of which were Bacillariophyta (Fig. 7).

In the RNA dataset, 236 ZOTUs were designated as keystone species, whereas no keystone species were found in the DNA dataset. The recovered keystone species included members of the Syndiniales (71 ZOTUs), Stramenopiles_X (41 ZOTUs), and Dinophyceae (36 ZOTUs), followed by Ciliophora (19 ZOTUs), Radiolaria (19 ZOTUs), Chrysophyceae (10 ZOTUs), and other groups (40 ZOTUs). All of these keystone species were discovered in modules B and C.

4. Discussion

Fewer studies have used RNA-based sequencing than DNA-based sequencing to examine the molecular diversity of microeukaryotes in the AO (Stecher et al., 2015; Comeau et al., 2019; Xu et al., 2020; Sun et al., 2022). Studies that used both nucleic acid sources were even rarer. In the current study, the co-extracted environmental DNA and RNA, followed by the high throughput sequencing, allowed us to characterize and compare the biodiversity, composition, metabolic activity, and co-occurrence relationships of microeukaryotic assemblages from a variety of habitats in the AO, including snow, ice, and seawater from the surface to a depth of 1,670 m. The microeukaryote communities derived from the RNA responded more sensitively to the environmental variables than those derived from the DNA. Our study emphasized the use of the RNA-based approach in characterizing the biodiversity, community composition, and response of microeukaryotes to environmental variables in diverse AO habitats.

4.1. Changes in alpha diversity indices with depth

In the present study, the alpha diversity indices discolosed by RNA increased with increasing water depth (Fig. 4). Some microeukaryotic lineages may be unable to survive in the harsh environment of snow and ice,

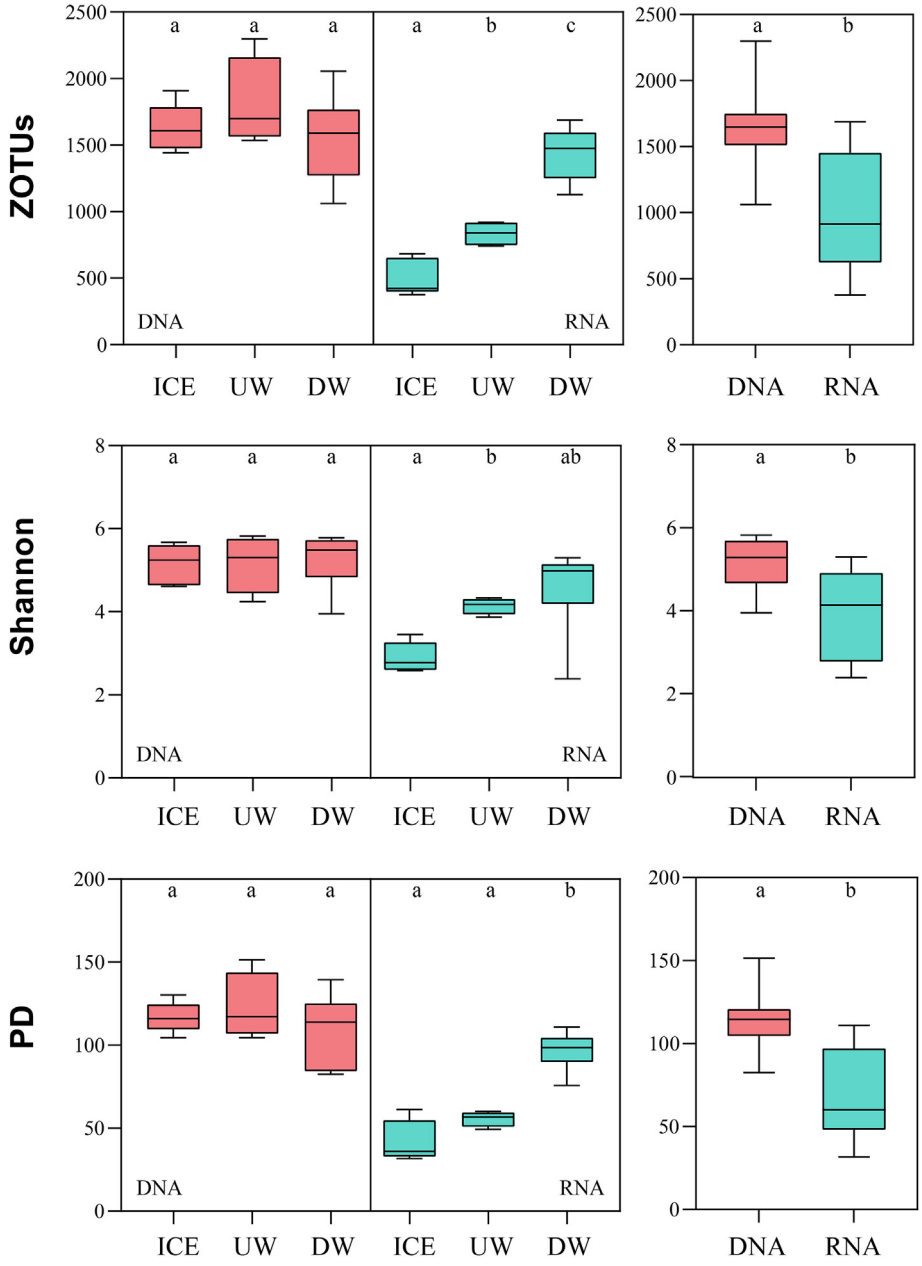

Fig. 4. Comparison of alpha diversity indices (ZOTUs Richness, Shannon, and PD) between the three sample groups (ICE, UW, and DW) as revealed by DNA and RNA datasets, respectively. The upper and lower boundaries of each box represent the 75th and 25th quartile values, respectively, while the lines within each box represent the median values. Bars without shared letters indicate significant differences at the level of $p < 0.05$ according to the Kruskal-Wallis test.

which includes limited living space, low temperature, high ultraviolet radiation, and the gradual depletion of nutrients. As a result, it is not surprising that snow and ice contained fewer microeukaryotes than upper water. A trend of increasing diversity of microeukaryotes with depth was observed in northern Svalbard (Meshram et al., 2017). At station ALOHA in the North Pacific Subtropical Gyre, aphotic zone protist diversity was higher than euphotic zone protist diversity (Ollison et al., 2021). Contrarily, surveys conducted along the water columns of the South China Sea and Mariana Trench showed that the microeukaryote diversity in upper water was significantly higher than that in deep water (Xu et al., 2017; Zhu et al., 2020). In the summertime of AO, melt ponds form due to the melting of snow and ice. It was discovered that the microeukaryote diversity in melt ponds is considerably lower than in seawater (Xu et al., 2020). Some melt ponds will eventually merge with the underlying seawater, which may contribute to the observed decline in the diversity of microeukaryotes in the upper seawater. Compared to snow, ice, and upper water, the deep water in this study was warmer and saltier (Table S2), suggesting that it may provide a more favorable environment for microeukaryotes. In addition, the highest number of unique ZOTUs found in deep water in the RNA dataset may indicate an unexplored diversity of active microeukaryotes in the deep AO (Fig. S4).

4.2. Vertical distribution of microeukaryotic community structure

The snow sample in the RNA dataset was dominated by Basidiomycota and Cercozoa-affiliated sequences (Fig. S3C). Basidiomycota are widely distributed in cold environments, especially polar regions (Buzzini et al., 2017). In other Arctic snow samples, Cercozoa has been identified as major grazers of other microorganisms (Comte et al., 2018). Even though snow represents a different environment from sea ice, the snow sample

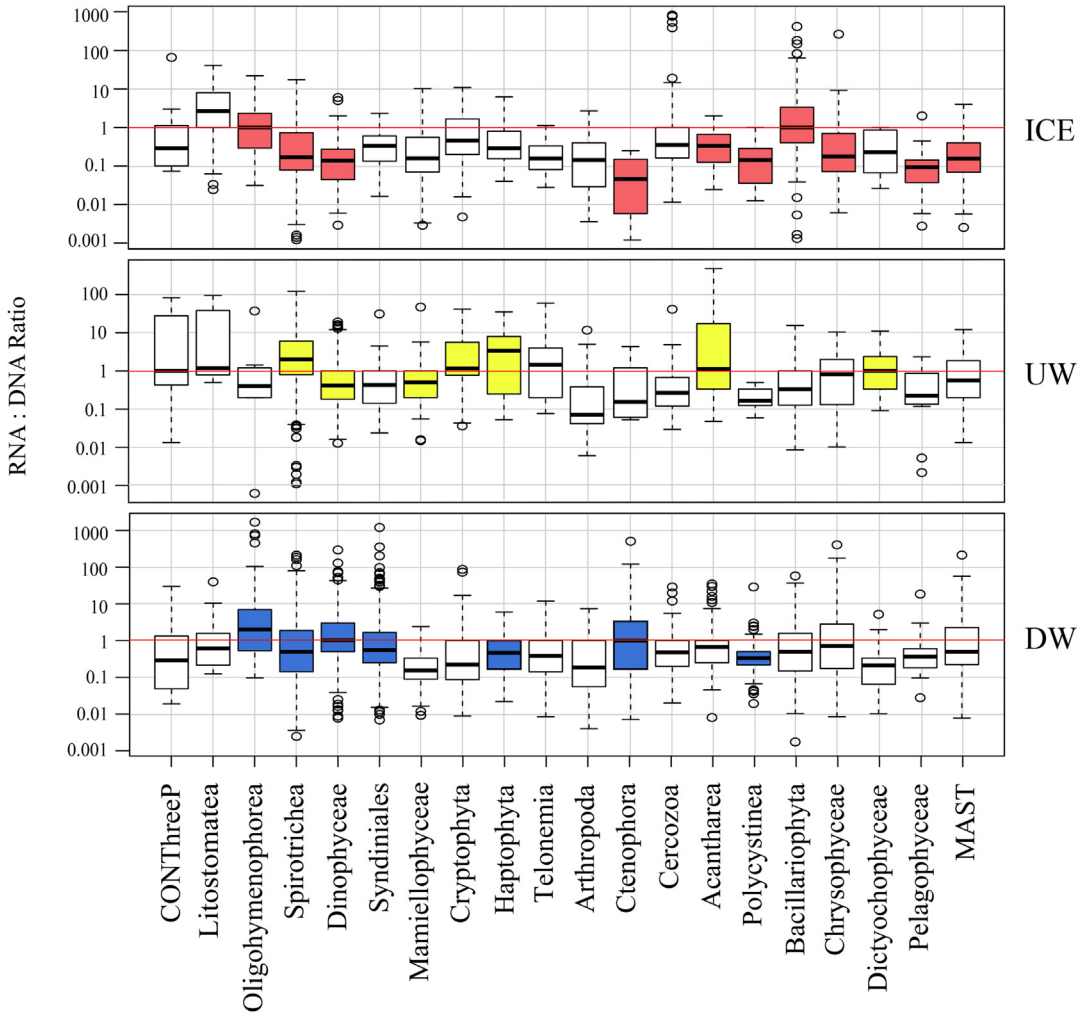

Fig. 5. Comparison of the relative metabolic activity of major taxonomic groups among ICE, UW, and DW using RNA: DNA ratios as a proxy. The red line indicates a ratio of 1. Within each taxonomic group, bars without shared colors indicate significant differences at the level of $p < 0.05$ according to the Kruskal-Wallis test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was clustered with the sea ice samples (Fig. S2B), which may be due to the exchange of microbial species between the lower snow layer and the underlying sea ice surface (Maccario et al., 2019).

Bacillariophyta (Diatom) dominated the ice-core microeukaryote community in the RNA dataset (Fig. 3C). Diatoms were reported to dominate the ice microeukaryotic community and are major primary producers in the AO (Różańska et al., 2008; Fernández-Méndez et al., 2015). They prefer the bottom portion of the ice and the interface between ice and seawater, providing aquatic grazers with a concentrated food resource (Arrigo, 2014; Campbell et al., 2018). Numerous unclassified Opisthokonta sequences belonging to ZOTU 9 were found in sample 1CORE (Fig. S3C). To determine the taxonomic identity of ZOTU 9, its representative sequence was blasted against the GenBank nucleotide database. It turned out that the uncultured Chytridiomycota had the highest similarity (96.57 %) (data not shown). It was reported that this clade of fungi dominated fungal communities beneath the ice when the snowpack was low (Hassett and Gradinger, 2016). Co-occurrence of Chytridiomycota and diatoms was also reported in the Bering Sea (Hassett et al., 2017). Chytridiomycota can alter community structure and primary production patterns via perturbation of parasitic or saprotrophic interaction networks (Kilias et al., 2020).

Ciliophora dominated the upper water microeukaryote community, which is consistent with previous findings using the metabarcoding method (Meshram et al., 2017; Xu et al., 2020, 2022) and microscopy observation (Sherr et al., 2013), serving as both major phytoplankton grazers in the AO. The presence of phototrophic groups, such as diatoms, in the deep water may be attributable to their attachment to rapidly sinking particles, such as fecal pellets, which have occasionally been detected in other deep sea environments (Agusti et al., 2015; Xu et al., 2018). Dinophyceae and Chrysophyceae affiliated sequences were prevalent in the deep water in the present study, which is possibly due to their mixotrophy ability (Bachy et al., 2011; Faure et al., 2019; Unrein et al., 2014). One study reported that at the end of the Polar Night, a remarkable diversity of dinoflagellates was found in the seawater (Bachy et al., 2011), indicating that mixotrophy is an important survival strategy in the presence of darkness (Jones, 2000).

4.3. Relative metabolic activity of major microeukaryote groups

The ratio of RNA:DNA has been used as a proxy for microbial metabolic activity (Charvet et al., 2014; Giner et al., 2020; Massana et al., 2015; Hu et al., 2016; Xu et al., 2017; Giner et al., 2020; Zhu et al., 2020). However, it has rarely been used to assess the activity of microeukaryotes in the AO. Bacillariophyta exhibited higher relative activity in ICE than in UW and DW (Fig. 5), which is consistent with previous findings that diatoms were the major contributor of primary production in the sea ice of the AO (Różańska et al., 2008; Fernández-Méndez et al., 2015). Higher activities of Mamiellophyceae and Haptophyta were observed in the UW compared to the ICE and DW, which is consistent with previous research indicating that these two groups were major primary producers in the upper waters of the AO (Lovejoy et al., 2007; Metfies et al., 2016; Bachy et al., 2022). Spirotrichea (Ciliophora) was also found to be more active in the UW

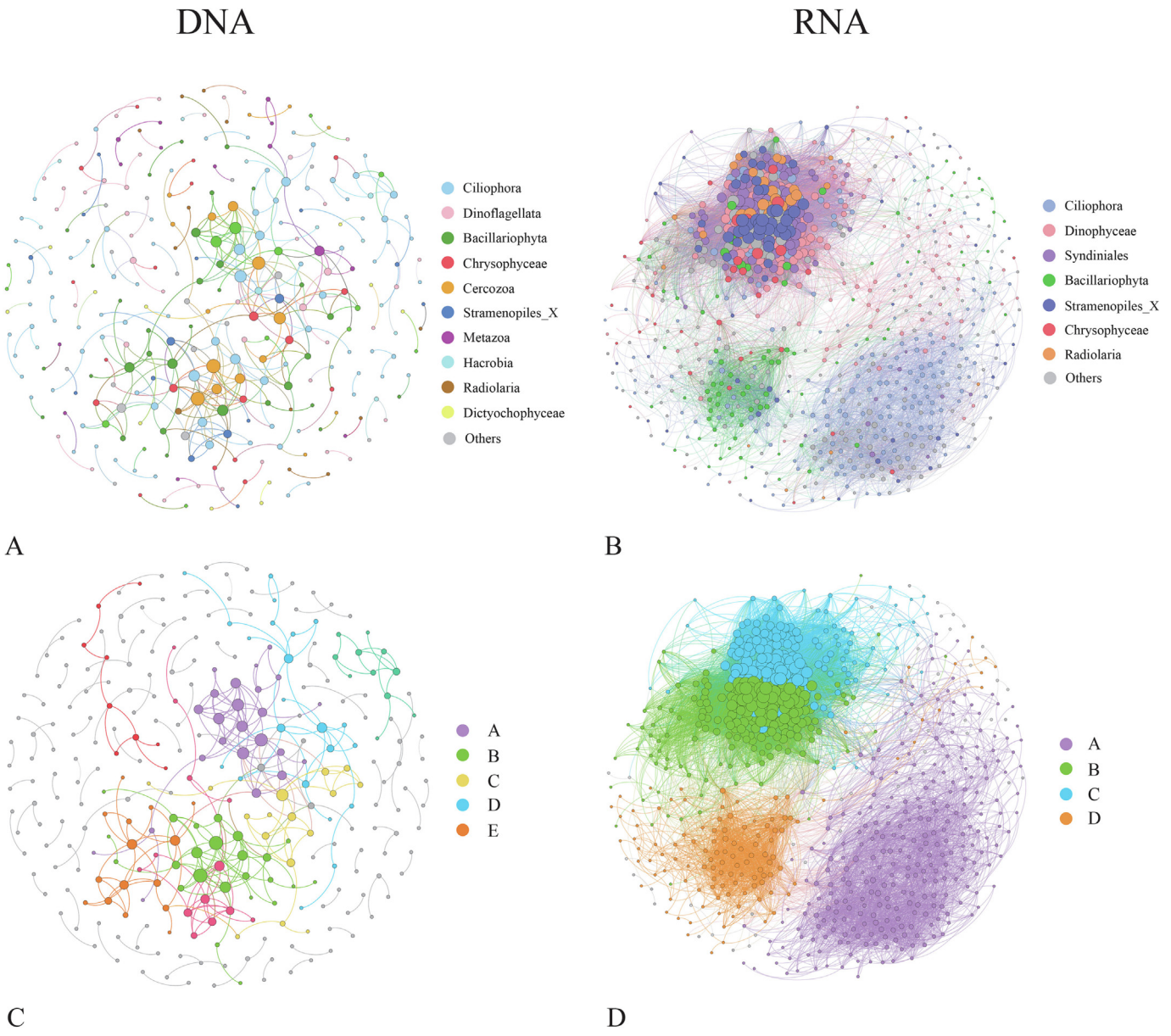

Fig. 6. The co-occurrence patterns of ZOTUs in the DNA (A, C) and RNA datasets (B, D). The nodes were colored according to taxonomic identities (A, B) and modularity classes (C, D). A connection stands for a strong (Spearman's $r > 0.8$ or $r < -0.8$) and significant (P-value <0.01) correlation. The size of each node is proportional to the number of connections (i.e., degree). In the DNA network, only modules representing >5 % of all nodes were shown.

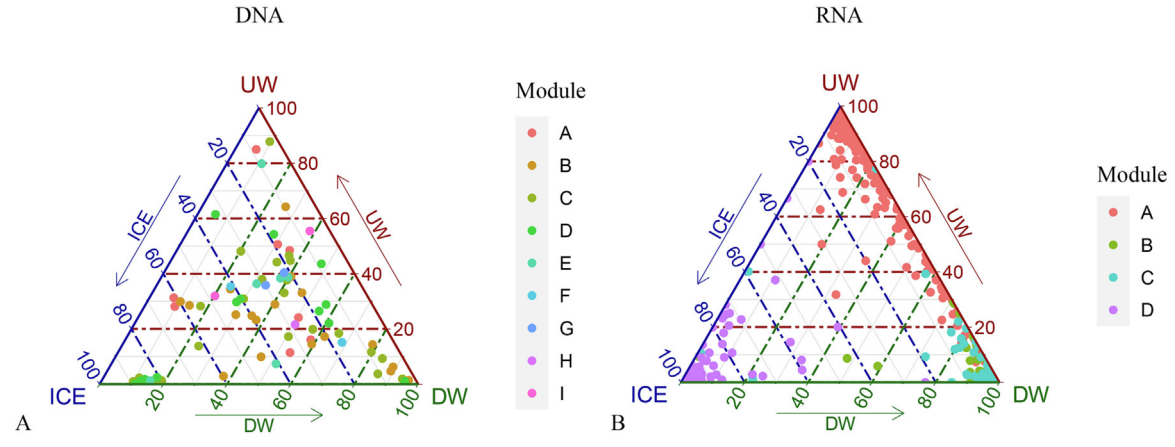

Fig. 7. Ternary plots showing the distribution of ZOTUs affiliated with different modules in three identified sample groups. Each dot represents an individual ZOTU.

(Fig. 5). Spirotrichea consists primarily of naked oligotrichs and is the dominant herbivore in the upper waters of AO (Sherr et al., 2013). Many heterotrophic groups exhibited their highest relative activity in mesopelagic waters, according to previous studies (Xu et al., 2017; Giner et al., 2020). In the present study, members of Oligohymenophorea had the highest activity in DW, which may act as prokaryotes grazers in the deep ocean (Ollison et al., 2021). Dinophyceae is one of the major mixotrophic groups in the ocean (Burkholder et al., 2008), and the highest activity of this group found in DW (Fig. 5) suggested that they may engage in phagotrophy in the deep, light-depleted water. The parasitic group Syndiniales was found to have the highest activity in DW (Fig. 5), which may indicate that parasitism may also be important in the polar deep ocean ecosystems (Guillou et al., 2008).

4.4. Co-occurrence networks among major microeukaryotic groups

Prior studies on the biodiversity of microbial communities focused more on the richness and abundance of species than on their interactions. However, uncovering interactions among species can lead to a deeper understanding of ecosystem function and services (Wilsey and Polley, 2004; Finn et al., 2013). In recent years, network-based approaches have been increasingly used to investigate co-occurrence patterns among microorganisms in complex environments (Ma et al., 2016; Anderson et al., 2020; Sun et al., 2023). However, it should be noted that, while network-based approaches may aid in the discovery of potential interaction relationships among microorganisms in a given community, the true interaction between species should be validated further.

In the present study, the RNA network showed closer relationships and interactions between taxa than the DNA network, which was supported by the network-level properties indicating that the RNA network had a higher number of nodes and edges, a higher average degree and clustering coefficient, and a lower average path length (Table S4). Modularity may be indicative of synergistic relationships, competitive interactions, and niche differentiation. Closely related taxa tended to be highly interconnected (Zhou et al., 2010). The modules of the RNA network were unique to a specific sample group, i.e., ICE, UW, or DW (Fig. 7), indicating that species within the same sample group are more closely related. In contrast, the networks shown by the DNA dataset did not disclose such a pattern.

The presence of keystone taxa, such as Syndiniales, in the DW group of the RNA dataset and the positive relationships found between Syndiniales and dinoflagellates and Syndiniales and ciliates in this study may indicate the possibility of parasitism in the deep ocean. Studies have found that Syndiniales are capable of infecting a variety of dinoflagellates and ciliates and are highly affected by the density of their hosts (Park et al., 2004; Anderson et al., 2020). Meanwhile, positive associations between Syndiniales and ciliates may also indicate predator-prey relationships, as there is evidence that ciliates consume dinospores of Syndiniales (Johansson and Coats, 2002). However, the close relationships between Syndiniales and dinoflagellates/ciliates found in the present study need further study to validate their real interactions in situ.

4.5. RNA-based approach depicts the response of microeukaryotes to environmental variables more accurately

Early studies demonstrated systematic differences between the environmental DNA- and RNA-derived microbial communities for both prokaryotes and microeukaryotes (Martinez et al., 2006; Lanzén et al., 2011; Xu et al., 2017). In the present study, alpha diversity indices and community composition revealed by RNA were highly correlated with temperature and salinity, whereas the DNA dataset failed to disclose such correlations. According to prior studies, temperature and salinity are significant environmental factors that influence the dynamics of AO microbial communities (Kalenitchenko et al., 2019; Xu et al., 2020; Sun et al., 2022). The DNA-derived microeukaryote communities were sparsely distributed in the PCoA plots, and no clustering pattern was observed in our study, which may be due to interferences from extracellular DNA and/or dormant cells (cysts) (Garrison and Buck, 1985; Różańska et al., 2008). In addition, the RNA network showed higher modularity and closer interspecies relationships than the DNA network. Our findings are consistent with previous reports, showing that RNA-based diversity surveys tend to be less biased (Not et al., 2009) and respond more sensitively to environmental variables (Charvet et al., 2014).

5. Conclusion

The present study discovered that the community diversity shown by RNA responds more sensitively to environmental conditions than that shown by DNA in diverse AO environments. The RNA-based dataset outperformed the DNA-based dataset in revealing the alpha diversity, community composition, and co-occurrence relationships of active microeukaryotes in diverse AO habitats. The RNA-based co-occurrence network found close ties between ZOTUs retrieved from similar habitats as well as a number of keystone species that may indicate co-occurrence relationships between taxa in a particular habitat, such as the possible parasitism between Syndiniales and dinoflagellates in deep water. The RNA:DNA ratios can be used as a proxy for the relative metabolic activity (e.g., protein synthesis during population growth and acclimation) to explore and compare the activity of major microeukaryote taxonomic groups in various habitats in the AO.

CRediT authorship contribution statement

Hejun Kong: Writing – original draft, Formal analysis, Investigation. **Eun-Jin Yang:** Resources, Investigation, Funding acquisition. **Nianzhi Jiao:** Resources, Funding acquisition. **Youngju Lee:** Investigation. **Jinyoung Jung:** Investigation. **Kyoung-Ho Cho:** Investigation. **Jong-Kuk Moon:** Investigation. **Jee-Hoon Kim:** Investigation. **Dapeng Xu:** Conceptualization, Supervision, Writing – review & editing, Resources, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162608.

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