

**Marine eukaryote community responses to the climate and oceanographic changes in Storfjordrenna (southern Svalbard) over the past ~14.0 kyr BP: Insights from sedimentary ancient DNA analysis**

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## Community composition

### 1. Phytoplankton

The phytoplankton community consisted of diatoms (11 ASVs), green algae (8 ASVs), and haptophytes (2 ASVs), as well as autotrophic dinoflagellates (10 ASVs). These groups dominated a significant portion of the eukaryote relative abundance (~10 to 40 %). The diatom community comprised families Bacillariaceae, Chattonellaceae, Thalassiosiraceae, and Chaetocerotaceae, with the genera *Thalassiosira* (5 ASVs) and *Chaetoceros* (2 ASVs) dominating the group. The green algae group represented by families Mamiellophyceae (2 ASVs), Chlorophyceae (1 ASV), Prasinodermatophyceae (2 ASVs), and Pyramimonadophyceae (3 ASVs), where haptophytes group, represented by single family; Coccolithophyceae (2 ASVs) (Table S02). The algae group exhibited significant presence at approximately 12.4 Kyr, 10.0 Kyr, 3.3 kyr, and 1.3 kyr BP, with *Micromonas polaris* (ASV13) being the dominant species during these time periods (**Fig. S3**). Autotrophic dinoflagellates represented by the genera *Gymnodinium*, *Nusuttodinium*, *Gonyaulax*, *Prorocentrum*, and *Polarella* (**Table S2**). These autotrophic dinoflagellates were present throughout the entire time period, with varying relative abundance percentage. Notable periods of significant presence occurred approximately 12.2 kyr, 9.2 kyr, and 1.8 kyr BP, with *Polarella glacialis* (ASV16) and *Gymnodinium* sp. (ASV29) dominant at these times (**Fig. S3**).

### 2. Mixoplankton

The mixoplankton community was primarily composed of mixotrophic silicoflagellates (3 ASVs) and dinoflagellates (5 ASVs). In the context of silicoflagellates, the genera *Helicopedinella* and *Pseudopedinella* were identified, with *Pseudopedinella elastica* (ASV03) exhibiting a predominance in term of read abundance throughout the entire time interval. Mixotrophic dinoflagellates consisted of several genera such as *Biecheleria*, *Ansanella*, *Gotoius*, and *Heterocapsa*. However, their occurrence was restricted to specific time periods: ~13.2 kyr BP, ~12.6 kyr to ~10.5 kyr BP, and ~2.2 kyr BP (**Fig. S3, Table S2**).

### 3. Microzooplankton (or planktonic heterotrophic protists)

The microzooplankton group included radiolarians (Polycystina [3 ASVs], and Acantharia [3 ASVs]), pelagic ciliophora (Spirotrichea [6 ASVs], Litostomatea [3 ASVs], and Prostomatea [1 ASV]), heterotrophic silicoflagellates (Pedinellales [3 ASVs]), and heterotrophic dinoflagellates (Peridinales [2 ASVs], and Gymnodiniales [2 ASVs]). These groups were identified as being present at certain time periods within the study area (**Fig. S4**). Notable periods of presence included

heterotrophic dinoflagellates (~2.3 kyr BP), heterotrophic silicoflagellates (~10.2 kyr and 1.3 kyr BP), pelagic ciliophora (~12.4 kyr BP), and radiolarians (~12.4 kyr and 2.0 kyr BP) (**Fig. S4**). The dominant species within these groups were *Islandinium* sp. (ASV22) and *Gyrodinium* sp. (ASV140) from heterotrophic dinoflagellates, *Actinomonas* sp. (ASV32), and *Pteridomonas* sp. (ASV105) from heterotrophic silicoflagellates, *Heteracon* sp. (ASV54) from radiolarians, and *Strombidium* spp. from pelagic ciliophoran (**Fig. S4**). The highest species diversity was recorded in the pelagic ciliophora, with 10 ASVs, including six species belonging to the genus *Strombidium* (**Table S2**).

#### **4. mesozooplankton**

The mesozooplankton group primarily consisted of arthropods (Copepoda [4 ASVs] and Malacostraca [1 ASV]), larvaceans (Appendicularia [2 ASVs]), and one species from the class Sagittoidea, *Sagitta* sp. (ASV910). Within the arthropod group, the genus *Calanus* was frequently recorded throughout the study area. In contrast, *Fritillaria* sp. from the larvaceans was recorded only around ~1.9 kyr BP (**Fig. S4**).

#### **5. Zoobenthos**

In this study, zoobenthos was recorded as one of the groups with the highest species diversity within the eukaryotic community. It encompassed 36 ASVs, primarily represented by macrobenthic species (**Table S2**). Macrobenthos was categorized based on their habitats and feeding strategies in order to examine their activities in relation to the environmental changes observed during the study period. The categories included annelids (Polychaeta [14 ASVs], Sipuncula [1 ASVs], Palaeonemertea [1 ASVs]), ascidians (Ascidacea [8 ASVs]), molluscs (Bivalvia [6 ASVs]), cnidarians (Octocorallia [1 ASVs], Hydrozoa [1 ASVs]), and echinoderms (Holothuroidea [1 ASVs], Asteroidea [1 ASVs], Crinoidea [1 ASVs], Ophiuroidea [1 ASVs]) (**Table S02**). Zoobenthos had a significant presence around ~9.3 kyr BP, as well as between ~12.3 kyr and 12.0 kyr BP (**Fig. S5**). The majority of them were represented by annelids, primarily composed of polychaetas (**Table S2**). These species occurred in high number during specific time intervals, particularly *Barantolla* sp. between ~13.1 kyr and 12.6 kyr BP, *Spiochaetopterus* sp. around ~3.1 kyr BP, and *Rhodine* sp. around ~12 kyr BP (**Fig. S5**). Ascidians exhibited a similar presence to annelids but with a lower relative abundance. However, around ~9.3 kyr BP they were dominated by *Oligotrema* sp. (**Fig. S5**). The genus *Pelonaia* was notable within this group around ~12 kyr BP, being one of the dominant taxa (**Fig. S5**). Cnidarians and echinoderms were present

only during significant time intervals. Notably, the cnidarian *Lateothela* sp. appeared around ~1.3 kyr BP, while echinoderm *Ctenodiscus* sp. was observed around the ~12.0 kyr BP (**Fig. S5**). Molluscs were one of the dominant groups within the zoobenthos community, being particularly abundant around ~12.7 kyr BP, as well as between ~12.2 kyr BP and 12.0 kyr BP, and around ~2.8 kyr BP. This group was primarily represented by *Talochlamys* sp. (**Fig. S5**).

## 6. Parasites

Despite their belonging to different taxonomic groups, the parasite species were categorized into one group for better clarification. The parasite group included: Apicomplexa (2 ASVs), Oomycota (3 ASVs), Mermithidae (1 ASV), and parasitic dinoflagellates (18 ASVs) (**Fig. S6, Table S2**). The Oomycota group included genera *Miracula* and genus *Pirsonia*, which coincided with a strong presence of diatoms, and recorded around ~13.2 kyr BP, ~10.4 kyr BP, ~9.5 kyr BP, and ~3.0 kyr to ~2.3 kyr BP (**Fig. S6**). The parasitic dinoflagellates were mostly composed with uncultured ASVs belonging to the parasitic Syndiniales group. This group had the highest species diversity among the parasites, comprising 18 ASVs. Two subgroups were identified: Dinophyceae dino-group-I (9ASVs), and Dinophyceae dino-group-II (9ASVs). They were dominant throughout the studied time interval, especially around ~1.7 kyr to ~1.4 kyr BP, ~9.2 kyr BP, and ~12.2 kyr to ~12.0 kyr BP (**Fig. S6**).

## 7. Other heterotrophs

### 7.1 Cercozoa

Of the other heterotrophs, the most dominant species group was Cercozoa. This group accounted for a significant proportion of relative abundance (approximately 5% to 99%) throughout the study period, exhibiting the highest species richness (**Fig. S7, Table S2**). The phylum Cercozoa included species from classes Ascetosporea (1 ASV), Phytomyxea (1 ASV), Granofilosea (2 ASVs), Thecofilosea (51 ASVs), and Imbricatea (10 ASVs) (**Fig. S7, Table S2**). Based on the information of regarding ecological roles from molecular adaptation, and closely related species or families within the group, Cercozoa were divided into the three groups: parasitic, predatory, and bacterivorous. Classes Ascetosporea, and Phytomyxea were recognized as parasitic cercozoans. Phytomyxea species were present mainly around ~10.0 kyr to ~8.0 kyr BP, whereas Ascetosporea species were recorded throughout the study period with a notable presence around ~13.3 kyr to ~12.0 kyr BP (**Fig. S7**). Most of bacterivorous cercozoans were Granofilosea (Massiteriidae [1 ASV], Limnofilidae [1 ASV]), Imbricatea (Thaumatomonadida [3 ASVs]; Novel-

clade-2 [3 ASVs]; Marimonadida [4 ASVs]; Paulinellidae [1 ASV]), and Thecofilosea (Ebriaceae [1 ASV]; Botuliformidae [2 ASVs]; TAGIRI1-lineage [7 ASVs]; Protaspa-lineage [27 ASVs]; Ventricleftida [3 ASVs]). The most abundant bacterivorous cercozoan was Limnofilidae, with a notable presence around ~8.0 kyr BP, and ~13.3 kyr to ~12.7 kyr BP. Thaumatomonadida and Marimonadida species were only present around ~2.5 kyr to ~1.3 kyr BP. The Protaspa-lineage, was a highly diverse group that was consistently recorded throughout the study period, except between ~12.3 kyr and ~13.3 kyr BP. TAGIRI1-lineage species were only significantly present around ~2.5 kyr to ~1.5 kyr BP. Ebriaceae and Paulinellidae were most abundant at 2.5 kyr and ~10.5 kyr BP, respectively. Predatory cercozoan included the Thecofilosea Cryothecomonas-lineage (8 ASVs), which was especially abundant around ~2.4 kyr, ~7.5 kyr, ~12.0 kyr, and ~12.7 kyr BP to ~13.1 kyr BP (**Fig. S7**).

## **7.2 Labyrinthulea**

The Labyrinthulea group included the saprotrophic members, including Thraustochytriaceae (3 ASVs), Oblongichytridiaceae (5 ASVs), and Aplanochytriidae (1 ASV). However, the majority of these taxa were identified solely as uncultured ASVs. These groups exhibited a consistent presence throughout the studied time period, with notable peaks in richness and dominance occurring around ~13.3 kyr to ~10.0 kyr BP, and ~2.2 kyr to ~1.7 kyr, and, with high richness (**Fig. 3, 4; Table S2**).

## **7.3 Choanoflagellata**

Choanoflagellates group comprises free-living unicellular eukaryotes that are bacterivorous. They played a pivotal role in marine food webs by acting as a crucial link between microbial communities and higher trophic levels (Buck, 1981; Leadbeater, 2014). We identified five species of Choanoflagellates. However, most of them were uncultured ASVs (Stephanoecidae-Group-H & D Clades), except for *Calliacantha* spp. Choanoflagellates were only present during specific time intervals, and dominated around ~12.7 kyr to ~12.2 kyr BP (**Fig. 4; Table S2**).

## **7.4 Apusomonada**

The Apusomonada group is represented by the genera *Amastigomonas* (2 ASVs) and *Podomonas* (1 ASV), which belong to the class Apusomonadea. *Amastigomonas* dominated during the early Holocene (~10.4 kyr BP), while *Podomonas* became more abundant in the late Holocene (~1.7 kyr BP) (**Fig. 4**).

### 7.5 Marine stramenopiles (MAST)

The MAST group comprised uncultured heterotrophic protists that were identified as bacterivores feeding on small organic particles. This group comprised 15 ASVs, representing four sub group: MAST-1 (2 ASVs), MAST-3 (1 ASVs), MAST-9 (5 ASVs), and MAST-12 (7 ASVs) (Fig. S6 & Tabel S2). The MAST species were predominantly represented by the MAST-9 and MAST-12 sub group, which dominated throughout the studied time period and exhibited high species richness. MAST 9 species showed the highest abundance within the MAST group at ~3.2 to ~2.9 kyr, ~7.5 kyr BP, ~11.5 kyr to ~9.3 kyr BP, and ~13.2 kyr BP. These were dominated by MAST-9D and MAST-9A. Similarly, MAST-12 species account for the majority of the MAST group at ~3.3 kyr to ~2.2 kyr BP, ~7.5 kyr BP, ~10.3 to ~9.5 kyr BP, and ~13.2 kyr to ~13.0 kyr BP. The dominant species was MAST-12A being the dominant species (**Fig. S6**).

### 7.6 Benthic ciliates, & Telonemea

The benthic ciliates group comprised by two species from genera *Holosticha*, and *Trachelophylum*, where mostly recorded in littoral zone. *Holosticha* sp. (ASV87) dominated around Younger Dyars based on the abundance reads (**Table S2**). The group Telonemea consists with phagotrophic flagellates *Telonema* sp. (ASV255), and dominated around Younger Dyars (**Table S2**).