

## **Eukaryotic community structure in sinking particles in the seasonal sea ice zone of the Southern Ocean**

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At the same time as the melting of the sea ice in austral summer, ice-edge blooms occur in the seasonal sea ice zone of the Southern Ocean. Organic matter derived from ice-edge blooms may sink toward the seabed in the form of sinking particles. This carbon transport is called a biological pump, which is strongly affected by ice-edge bloom. It is important to clarify the organisms in the sinking particles to understand the ecosystem in the Southern Ocean. However, as these sinking particles are mostly detritus-like substances, it is sometimes difficult to assume their origin using microscopy. In this study, the community structures of eukaryotes in sinking particles in the seasonal sea ice zone of the Southern Ocean were examined.

The sediment trap was deployed at 63°51.57'S, 109°87.19'E from December 10, 2016 to January 8, 2017. Seven sediment samples (#1: December 10–14, #2: December 14–18, #3: December 18–22, #4: December 22–26, #5: December 26–30, #6: December 30–January 3, and #7: January 3–8) were collected every four days at a 50 m depth and fixed in Lugol's solution. PCR amplification was conducted with the V9 region of 18S rRNA genes using eukaryotic universal primers, 1389F and 510R, and metagenomic DNA extracted from the samples. These PCR products were analyzed using the high throughput sequencing method, and operational taxonomic unit (OTU) clustering and analyses of eukaryotic community structures were conducted.

In total, 512,078 sequences were obtained and clustered into 1,849 OTUs with a 97% similarity. Compared in the time series, the number of OTUs were 339 and 677 in samples #1 and #2, respectively. This increased to 1334–2203 in samples #3 to #6, then decreased to 136 in sample #7. This indicated that species diversity in sinking particles was higher in December than in January. The clonal frequency of Acantharea, a class of Rhizaria, shared 77.6% and 89.8% of total sequences in samples #1 and #2, respectively. In sample #3, the frequency of Acantharea decreased to 32.4%, and the class Dinophyceae and unidentified eukaryotes shared 28.2% and 27.5%, respectively. Clones of unidentified eukaryotes showed no significant similarities with known eukaryotic 18S rRNA gene sequences. Unidentified eukaryotes reached 72%, and Dinophyceae decreased to 7.3% in sample #4. In samples #5 and #6, unidentified eukaryotes (49.7–51.1%), and Dinophyceae (20.3%) were dominant. Polycystinea, another class of Rhizaria, appeared in sample #7, reaching 89%.

The eukaryotic community structure for the entire period mainly consisted of unidentified eukaryotes (31.4%), Acantharea (28.6%), Polycystinea (12.8%), and Dinophyceae (12.4%) at the class level. In our previous unpublished study of protistan community structures in floating sea ices in the same region, classes of Rhizaria (Acantharea and Polycystinea) were hardly detected. They may not inhabit sea ice but reproduce and strongly contribute to the biological pump in the seasonal sea ice zone. In this study, the class Dinophyceae consisted of five orders, and one of the orders, Gymnodiniales, comprised 93% of the total clones of Dinophyceae. This group has been detected frequently in floating sea ice and water columns in this area. Although we have not revealed the community structures at genera/species levels in these three samples, members of the order Gymnodiniales may be a key species in the biological pump in this area. Surprisingly, many fewer clones of the class Bacillariophyceae (diatoms) were detected (3.6%) in trap samples, whereas they have been frequently detected and sometimes, have been recorded as the most abundant class in floating sea ice and water columns in this area. It is possible that diatoms are taken into the food web and contribute less to the biological pump. Several biases in clone analysis might have occurred in this study due to differences in DNA extraction efficiencies and/or copy numbers of 18S rRNA genes between the groups of organisms. Despite this, these results extended our knowledge about biodiversity and the drivers of the biological pump in the seasonal sea ice zone in the Southern Ocean.