

## Reconstruction of key ecological responses leading to the most recent dead zone formations in Japan Sea through ancient DNA metabarcoding

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Semi-closed ocean basins are very sensitive to climate changes as recorded in frequent formations of sediments with clearly laminated layers, which was caused by the lack of O<sub>2</sub> available to benthic organisms. This is so-called “dead zone formation” and caused by water stagnation after channel closure and/or nutrient inflow through upwelling and channel opening. In contrast to the hydrologic causes, it remains poorly understood about organismal causes such as enhanced primary production and heterotrophic consumption of O<sub>2</sub>. We investigated ancient eukaryotic DNA obtained from a thinly laminated layer deposited in the Japan Sea across the most recent global warming event after Younger Dryas (TL-1). Metabarcoding of 18S rRNA gene sequences clarified that across the transition from oxygenated to anoxic bottom water conditions, dominant protist populations shifted through five stages:

Stage1: Under oxygenated bottom conditions, the most dominant taxonomic group was Marine Stramenopiles Group 9 (MAST-9). MAST-9 encompasses diverse uncharacterized planktonic species, 18S rRNA gene sequences of which were detected from both anoxic and oxic water bodies in Pacific and Atlantic Oceans, Mediterranean Sea and South China Sea.

Stage 2: Before the onset of dead zone formation, the sharp decline of MAST-9 was accompanied with the expansion of marine diatom species of the genus *Skeletonema*, many members of which are known to be a cause of red tide around the world.

Stage 3: The dead zone formation was synchronized with the expansion of marine radiolarians of the order Chaunacanthida, the members of which are encrusted within SrSO<sub>4</sub>.

Stage 4: Stages 2 was repeated, whereas the expansion of the Chaunacanthida radiolarians was not evident.

Stage 5: The uncharacterized MAST-9 that was dominant before the dead zone formation was gradually recovered towards oxygenated bottom conditions.

From these results, it is suspected that the expansion of Radiolaria species encrusted with SrSO<sub>4</sub> triggered dead zone formation by the accelerated downward transport of dead cells and subsequent heterotrophic consumption of O<sub>2</sub>. From bioturbated sediment samples from a maximum depth of 31 mbsf with a depositional age of 100 ka, the Chaunacanthida sequences closely related to that from the laminated sediments have been previously detected. It is therefore suggested the record of the Chaunacanthida species can be reconstructed from sediments with great depth and age.

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