The occurrence of a dinoflagellate species *Gyrodinium rubrum* in sea ice in the Indian sector of the Southern Ocean

Ryo Matsuda[1], Masayoshi Sano[2,3], Keigo D. Takahashi[4], Shintaro Takao[5], Ryosuke Makabe[2,4,6], Michiyo Yamamoto-Kawai[6], Masato Moteki[2,6], Norio Kurosawa[1]

[1]*Graduate School of Science and Engineering, Soka University*
[2]*National Institute of Polar Research*
[3]*(Present address) Atmosphere and Ocean Research Institute, The University of Tokyo*
[4]*The Graduate University for Advanced Studies, SOKENDAI*
[5]*National Institute for Environmental Studies*
[6]*Tokyo University of Marine Science and Technology*

Introduction

The biological carbon pump is a crucial for carbon sequestration in the deep sea. A better understanding of the complex processes of the biological carbon pump is essential for understanding the global carbon cycle. Previously, we identified faecal pellet-like dinoflagellates (FLD) in the Southern Ocean as *Gyrodinium rubrum* and *G. heterogrammum* (Matsuda et al., in prep). In the Weddell Sea, a phagotrophic athecate dinoflagellate that grows larger (50 μm × 50 μm) was observed in both the floating sea ice and the adjacent water column in the coastal area (Buck et al., 1990). By contrast, FLD of a size similar to ellipsoidal faecal pellet found in sinking particles has never been reported in offshore waters. Based on their appearance, these might have been classified as faecal pellets, not living dinoflagellates. As a result, their role in the food web and carbon cycle may have been significantly overlooked. FLD cells excrete faecal pellets with a sinking rate of 225 ± 139 m d^{-1}, which is comparable with that of faecal pellets generated by metazoans, such as Antarctic krill and copepods. These FLD contributes up to 20% of total particulate organic carbon (POC) flux. These results suggest that FLD have an important role in carbon export.

Recently, Li et al. (2021) demonstrated that the association between sea ice and plankton assemblage is critical for carbon cycles (Li et al., 2021). Using the high-throughput sequencing technic, *G. rubrum* has been frequently detected in the Ryder Bay after sea ice melts (Piquet et al., 2011). These results imply that *G. rubrum* cells are released from sea ice. If *G. rubrum* inhabits in sea ice and spreads geographically via sea ice advection, it potentially has a role in carbon export in the Southern Ocean. In this study, we investigated the occurrence and abundance of *G. rubrum* in sea ice from the Indian sector of the Southern Ocean.

Material and Methods

Field observations were conducted in the Indian sector of the Southern Ocean, off Cape Darnley, off Lützow-Holm Bay, off Totten Glacier from December 2019 to March 2020. During cruises on the icebreaker *Shirase* as part of the 61st Japanese Antarctic Research Expedition, the training vessel *Umitaka-maru* of the Tokyo University of Marine Science and Technology, and the cruise KH20-1 on R/V *Hakuho-maru*, brash ice and newly formed ice (total of 96 samples from 21 stations) were collected using a plankton net with a cover, small fishing nets with a stick, or a stainless steel cage. All sea ice samples were stored at −20°C in the dark until laboratory analysis. The cell abundance of *G. rubrum* was examined by quantitative polymerase chain reaction (qPCR). A 200 g sea ice sample from each station was melted in 800 mL of 3% NaCl artificial seawater. The sea-ice meltwater was filtered and DNA extracted from the filters using the DNeasy PowerWater Sterivex Kit. To develop species-specific primers and a TaqMan probe set, the internal transcribed spacer 1 and 2 regions of *G. rubrum* cells sampled in the Southern Ocean were determined. qPCR was performed using the species-specific primers and a TaqMan probe set on the QuantStudio 1 real-time PCR system (Thermo Scientific) under the following thermal cycling conditions: 95°C for 20 s, followed by 40 cycles of 95°C for 1 s and 60°C for 20 s. Genomic DNA was extracted from the 30 cells of *G. rubrum* cells, and a mixture of the genomic DNA was used as a positive and standard control. The sea-ice meltwater was filtered through a Whatman GF/F filter for analyses of chlorophyll a (Chl. a), POC, and particulate organic nitrogen (PON). The Chl. a concentration was measured using a pre-calibrated fluorometer (10-AU, Turner Designs, USA) after filtration and pigment extraction. POC and PON concentrations were measured using an elemental analyser connected to an isotope-ratio mass spectrometer (Thermo Scientific), after filtration and drying.

Results and Discussion

Using qPCR, *G. rubrum* was found at 13 stations (36 samples), including 4, 1, and 31 sea ice samples from the waters off Cape Darnley, Lützow-Holm Bay, and the Totten Glacier, respectively. *G. rubrum* was observed in all regions, with cell

abundances ranging from 0.0 to 3.4 cells/L (< 1 cells/L in most of the sea ice samples). The cell abundance in samples off the Totten Glacier was 0.25 ± 0.69 cells/L, compared with 0.13 ± 0.085 cells/L in other regions. At station "GPS", where a drifter with a sediment trap was deployed in our previous study, the cell abundance ranged from 0.0 to 0.2 cells/L. The cell abundance at several stations off Cape Darnley and the Totten Glacier was similar to that at station "GPS". No significant correlations between cell abundance and environmental parameters (Chl. a concentration, POC, or PON) were observed. In our previous study, the FLD flux at 60 m depth, which was identified as *G. rubrum*, increased as the sea ice concentration decreased from December 2019 to January 2020, although the cell abundance in most the of sea ice samples was lower than 1 cell/L. The increased *G. rubrum* flux could be supported by its abundance not only in the sea ice but also in the surface water. Its abundance in the surface water needs to be evaluated to reveal the relationship between sea ice melt and *G. rubrum* flux.

In this study, we detected *G. rubrum* in sea ice from off Cape Darnley, Lützow-Holm Bay, and the Totten Glacier by qPCR analysis. However, no significant correlation between the abundance of *G. rubrum* and environmental parameters was observed. Previous studies detected *G. rubrum* in the water column in Ryder Bay, Kerguelen Plateau, Antarctic Peninsula, and in the Scotia Sea in the Southern Ocean using high-throughput sequencing of seawater DNA (Piquet et al., 2011; Georges et al., 2014; Duret et al., 2019; Liu et al., 2022). This is the first observation of *Gyrodinium* cells in the Indian sector of the Southern Ocean.

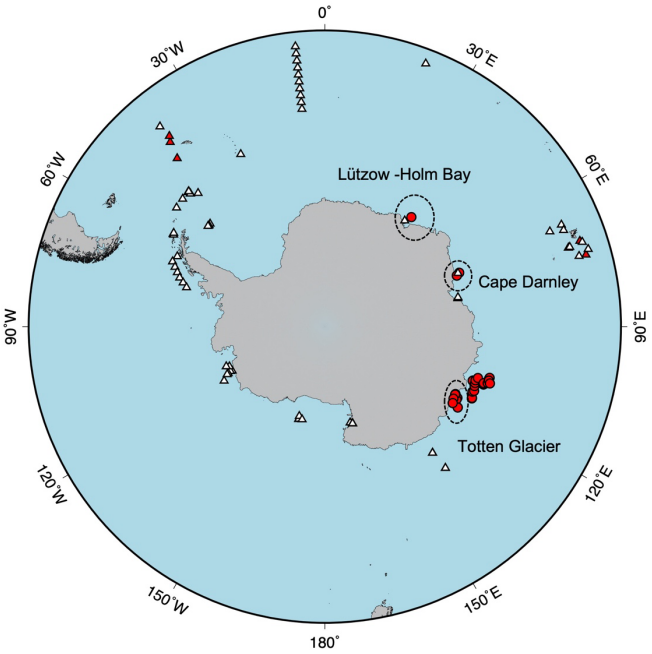

Fig. Occurrence of *Gyrodinium* species in the Southern Ocean
(circle, this study; triangle, studies published from 1989 to 2022, red, *Gyrodinium rubrum*; white *Gyrodinium* species.)

References

Buck KP, Bolt PA, Garrison DL, Phagotrophy and fecal pellet production by an athecate dinoflagellate in Antarctic Sea ice. Mar Ecol Prog Ser, 60, 75–84, 1990.

Duret MT, Lampitt RS, Lam P, Prokaryotic niche partitioning between suspended and sinking marine particles, Environ Microbiol Rep, 11, 386-400, 2019.

Georges C, Monchy S, Genitsaris S, Christaki U, Protist community composition during early phytoplankton blooms in the naturally iron-fertilized Kerguelen area (Southern Ocean), Biogeosci, 11, 5847-5863, 2014.

Lin Y, Moreno C, Marchetti A, Ducklow H, Schofield O, Delage E, Meredith M, Li Z, Eveillard D, Chaffron S, & Cassar N. Decline in plankton diversity and carbon flux with reduced sea ice extent along the Western Antarctic Peninsula. Nat Com, 12, 1–9, 2021.

Liu C, Zhang X, Wang X, DNA metabarcoding data reveals harmful algal-bloom species undescribed previously at the northern Antarctic Peninsula region. Polar Biol., 2022.

Piquet AMT, Bolhuis H, Meredith MP, Buma AGJ, Shifts in coastal Antarctic marine microbial communities during and after melt water-related surface stratification, FEMS Microbiol Ecol, 76, 413-427, 2011.