

# Freezing and melting stress on the photophysiology of ice algae: *Ex situ* incubation of ice algae using an ice tank

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Sea-ice algae contribute 10–25% of the annual primary production of polar seas (e.g., Lizotte et al., 2001). They are also a major energy source for crustaceans such as Antarctic krill. During melting, ice algae are released from the sea ice, and can become a seed population for large-scale ice-edge blooms. Large fluctuations in temperature, salinity, and light availability, associated with freezing and melting of sea ice, can significantly change the photosynthetic physiology of ice algae. It is, however, difficult to assess the photophysiology of ice algae due to limited access to the sea ice zone, unavailability of satellite imagery, and limited capability for time-series measurements (e.g. Steiner et al., 2016). To overcome these difficulties, we developed a purpose-designed ice tank, enabling incubation of ice algae in the laboratory for extended periods. Here, we aim to clarify the effect of multiple co-stressors (i.e., freezing temperature and high brine salinity in a freezing event; and less saline meltwater and sudden high light exposure during ice melting) on the photophysiology of ice algae in a series of ice tank experiments. The polar diatom *Fragilariopsis cylindrus*, isolated from Antarctic pack ice, was incubated in the ice tank under high-light (HL; 150  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) and low-light (LL; 30  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) conditions in the EDTA-buffered Aquil medium for 20 days. Photophysiological states of ice algae were monitored with variable chlorophyll *a* fluorescence using fast repetition rate fluorometry, pigment composition, using ultra-high performance liquid chromatography (Suzuki et al., 2015), and gene expression of *psbA* and *rbcL*, which encode the D1 protein of photosystem II (PSII) and the large subunit of RuBisCO, respectively (Krell et al., 2007; John et al., 2007). When algal cells were frozen into the ice, the maximum quantum yield of PSII ( $F_v/F_m$ ) sharply decreased compared to that obtained prior to freezing. This freezing stress on PSII was possibly due to the damage of PSII reaction centers. Concomitantly, we observed the accumulation of chlorophyllide *a*, a breakdown product of chl *a*. Also, high brine salinity might suppress the reduction capacity downstream of PSII (i.e., quinones and plastoquinone pool) (Ralph et al., 2007). We found gene expression of the *rbcL* gene was highly upregulated, suggesting a strategy to acclimate to the cold ice environment (Young et al., 2015). When the ice melted and the cells were exposed to a high light,  $F_v/F_m$  sharply decreased, while non-photochemical quenching ( $\Phi_{\text{NPQNSV}}$ ) was enhanced, in particular in the LL treatment. These results suggest that ice algae employed safety valves (i.e., non-photochemical quenching) to maintain their photosynthetic capability during the sudden environmental changes. The *psbA* gene was also upregulated after light exposure, indicating accelerated *de novo* synthesis of D1 protein. Our results imply that sea ice algae are well adapted to seawater environments by modifying their photosynthetic competence; a key factor contributing to algal bloom formation in the polar seas.

## References

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