

Photosynthetic strategies of an ice diatom to low Fe availability as estimated through ice tank experiments with 77 K chlorophyll fluorometry

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Sea ice is one of the largest biomes in polar oceans. Ice algal communities contribute 2–25% of annual primary production (PP) in sea ice zones (e.g. Lizotte, 2001; Arrigo, 2017). Also they seed extensive ice-edge blooms, which account for almost 50% of PP during ice melt seasons (Deppeler et al., 2017). Diatoms often dominate ice algal communities and flourish at the bottom of sea ice (e.g. Meiners et al., 2018). Ice diatoms as exclusive energy sources for sea ice biota, enhance the survival and reproduction of the key species of the Southern Ocean, Antarctic krill *Euphausia superba* (Kawaguchi et al., 2016; Kohlbach et al., 2019). Also ice diatoms form aggregates in ice matrix, which efficiently sequester carbon (Boetius et al., 2013). Ice diatoms are thus significant ecological and biogeochemical drivers in polar oceans.

Ice diatoms are important populations in sea ice; however, Pankowski and McMinn (2008) reported that ice diatoms were iron (Fe)-depleted in Antarctic pack ice. Although sea ice forms in iron-replete coastal polynyas, Fe easily runs out due to high Fe requirement of diatoms and brine exchange with Fe-limited waters in the Southern Ocean. In addition, ice diatoms have to regulate the photosynthesis to multiple co-stressors in sea ice (e.g. low temperature, high brine salinity and low light availability). Low Fe availability reduces the photosynthetic plasticity, so ice diatoms have to deal with the multiple co-stressors even under low Fe conditions.

Here, we incubated the model ice diatom *Fragilariopsis cylindrus* in a novel low-Fe ice tank. The low-Fe ice tank enables Fe-manipulated incubation of diatoms in artificial sea ice for an extended period (>10 days). The ice diatom *F. cylindrus* was incubated in the ice tank filled with 70 L of Aquil* medium (Sunda et al., 2005) at high and low Fe concentrations (H-Fe: 400 nM; L-Fe: 15 nM) and at 150 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. The two independent ice tank incubation experiments were conducted for 12 days. After sampling pre-freeze seawater, freezing cycles started to form artificial sea ice slowly for 3 days. Once ice formed, ice samples were recovered every 3 days for 9 days. After all ice sampling, the remained ice in the tank completely melted (Melt samples, hereafter). Intense white light (1000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) was exposed to the Melt samples to assimilate an ice melt environment in the field, i.e. ice algae are released from sea ice to sun-lit open waters (Light samples, hereafter). To understand the physiology and potential abilities of photosynthesis, we applied, for the first time, the low temperature chlorophyll fluorescence technique at 77 K (−196 °C) to ice algae. The 77 K fluorescence, excited at 390 nm, from ice algal and planktonic *F. cylindrus* was measured in liquid nitrogen with a custom-built system connected with a spectrofluorometer (Prášil et al., 2009; Hill et al., 2012).

For ice and seawater samples, we detected peaks at (1) 680 nm from uncoupled light harvesting antennae, (2) 695nm from the photosystem II (PSII) light-harvesting chlorophyll binding protein CP47, and (3) 750 nm from photosystem I (PSI). The obtained peak heights were normalized with the maximum peak height in each measurement. The L-Fe treatment had more uncoupled light harvesting antennae and less CP47, indicating low transfer of absorbed energy by loosely-coupled light harvesting antennae (D'Haens et al., 2015; Yoshida et al., 2018). Also, PSI relative to PSII (PSI:PSII ratio) was lower in the L-Fe treatment, which is known as a low-Fe acclimation strategy reducing Fe-costly PSI (e.g. Geider and LaRoche, 1994). Additionally, uncoupled antennae greatly increased in the L-Fe treatment, whereas those in the H-Fe little changed, suggesting that the Fe-starved cells were more fragile to light stress. CP47 increased in the H-Fe treatment, but it decreased in the L-Fe treatment. This response was reasonable because it would be harmful to transfer energy to the Fe-stressed photosynthetic machinery. Light exposure increased the PSI:PSII ratio in the H-Fe treatment. This increase might alleviate the higher excitation pressure by increasing PSI. In conclusion, ice diatoms could contribute to ice-edge blooms; however, Fe-starved cells could be vulnerable to light stress when they are released from sea ice. Fe starvation would thus reduce the photosynthetic competence of ice diatoms.

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