How to process sea ice for chlorophyll-based photosynthesis measurement of the ice algae?

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Since sea-ice algae contribute ~10–25% of the annual primary production of polar seas (e.g., Lizotte et al., 2001), they play crucial roles in ecosystems and biogeochemistry in sea ice and marginal ice zones (e.g., Smith and Nelson, 1986). It is thus important to better understand the photosynthetic activity and primary productivity (*PP*) of ice algae, and how these differ when measured by conventional ^{14}C or oxygen evolution methods (e.g., Meguro, 1962; Campbell et al., 2019). However, these methods both require bottle incubation of melted ice (i.e., invasive), which have led to either under- or over-estimation of the ice algal *PP* (e.g., Rintala et al., 2014; Campbell et al., 2019). Alternatively, active chlorophyll *a* (chl *a*) fluorescence (ChlF) has also been widely used to understand the photophysiological properties of phytoplankton. Such fluorometric methods can instantaneously measure the photosynthetic activity of photosystem II (PSII) without any bottle incubation (i.e., non-invasive). However, melting procedures can still affect the photosynthetic activity of PSII (e.g., ice shaving: McMinn et al., 2010; and buffered overnight melt: Petrou et al., 2012). This study aims to clarify how sea ice samples should be processed for ChlF measurement. Samples were obtained from natural landfast sea ice in Saroma-ko Lagoon, Japan and artificial sea ice from a purpose-designed ice tank. The bottom 10 cm of the landfast ice was collected using an ice corer in March 2018. The polar diatom *Fragilariopsis cylindrus*, isolated from Antarctic pack ice off Casey Station in 2017, was incubated in artificial sea ice at 30 μmol photons m^{-2} s^{-1} for 9 days. Artificial ice samples were collected using a small hand corer. Active ChlF was measured with a Satlantic Fluorescence Induction and Relaxation (FIRe) or a Chelsea Fast Repetition Rate (FRR) fluorometer for natural and artificial ice samples, respectively. These fluorometers allowed the estimation of the maximum quantum yield of PSII photochemistry (F_v/F_m), functional absorption cross-section of PSII (σ_{PSII}), and turnover time of PSII (τ), which are used for *PP* estimation. The following four treatments were prepared: (1) crushed ice, (2) fast direct melting, (3) direct overnight melting, (4) buffered overnight melting (ice : filtered in situ seawater = 1 : 1). Interestingly, both samples showed consistent behaviours in each fluorescence parameter. F_v/F_m values of the overnight melted samples were significantly higher than those of the crushed ice and fast direct melted samples in both natural and artificial sea ice. This suggests that overnight melting could overestimate the F_v/F_m values. This might be caused by increases in nutrient and/or CO_2 availability after ice melt (Rintala et al., 2014). The crushed ice samples of both natural and artificial ice showed significantly lower σ_{PSII} than the other treatments. The strong scattering of the crushed ice interfered with the algal fluorescence, probably due to the uneven distribution of ice algae on the ice and/or a longer optical pathlength in the measuring cuvette of the fluorometers. Also, the crushed ice samples showed the highest τ values among treatments, possibly due to the optical disturbance. Overall, overnight melting causes an overestimation of the photosynthetic activity of PSII, while direct measurement of ice samples is also unfavourable due to the considerable light scattering of sea ice during measurement. This study implies that fast melt procedures (e.g., ice shaving: McMinn et al., 2010) would be preferable for ChlF measurement. In the presentation, we will also report the results of ChlF measurement at 77 K to address more detailed photosynthetic responses in the ice tank experiments.

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