Polar Biology (2019) 42:347–356
https://doi.org/10.1007/s00300-018-2426-y

ORIGINAL PAPER

The effect of melting treatments on the assessment of biomass and nutrients in sea ice (Saroma-ko lagoon, Hokkaido, Japan)

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Received: 1 December 2017 / Revised: 10 October 2018 / Accepted: 22 October 2018 / Published online: 2 November 2018

Abstract

Melting of sea-ice samples is an inevitable step in obtaining reliable and representative measurements for biogeochemical parameters such as inorganic nutrients and particulate matter. The impact of the sea-ice melting procedure has been previously evaluated for biological parameters such as chlorophyll *a* and cell abundance. For nutrient and biomass concentrations in sea ice, it is generally considered to be best practice to melt samples fast; however, no systematic evaluation exists in literature. The impact of melting temperature and buffer addition to avoid osmotic shock was tested on ice samples in Saroma-ko Lagoon on the northeastern coast of Hokkaido, Japan. The focus was on inorganic nutrient concentrations (NO_3^-, NO_2^-, PO_4^-, NH_4^+, $Si(OH)_4$) and particulate organic carbon and nitrogen concentrations and their isotope ratios. Coherent small changes have been observed for the parameter related to nitrogen, suggesting marginal cell lysis of a specific part of the microbial community. When differences are statistically significant, they are close to the uncertainty of the measurements and small in regard to the expected natural variation in sea ice. Our study suggest a minimal effect between melting treatments on biomass (POC, PN, and Chl *a*) and nutrient measurements in diatom-dominated sea ice and should be repeated where the sympagic community is dominated by flagellates.

Keywords Sea ice · Direct melting · Buffered melting · Nutrients · Particulate matter · Chlorophyll a · Sea of Okhotsk

Introduction

Sea ice is a semisolid matrix permeated by a network of channels and pores that are variably connected with underlying seawater. To investigate the biogeochemistry of the sea ice, it is important to obtain representative samples without altering the in situ conditions of the parameters of interest. However, the destruction of the ice matrix is inevitable, inducing drastic changes in both salinity and temperature (Garrison and Buck 1986; Garrison et al. 1986). Comparing different melting procedures during a field study, Rintala et al. (2014) concluded that fast melting of brackish sea-ice samples without the addition of a buffer resulted in more accurate results for biological parameters (e.g., biomass, primary production, biological assemblages,…). This contrasts with other work in more saline ice (Antarctic and Arctic), indicating that rapid changes in temperature and salinity should be avoided to prevent osmotic shock on the most delicate organisms (Garrison and Buck 1986; Mikkelsen and Witkowski 2010). The effect of melting procedures on inorganic nutrient concentrations was not discussed by Rintala et al. (2014) or any of these publications.

According to McMinn et al. (2009), the direct melting of sea ice in the dark is advised for physicochemical parameters and this approach is used for nutrient samples in most studies though no specific test has been made. The melting should be done fast (< 6 h) to avoid biological processes that

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00300-018-2426-y) contains supplementary material, which is available to authorized users.

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can alter nutrient concentrations. However, melting without buffer to avoid osmotic shock can lead to cell lysis and the release of internal inorganic nutrient pools. This would result in a decrease of particulate matter and an increase of nutrient concentrations. Rapidly changing conditions during melting could also induce stress to living cells which could lead to efflux of intracellular nutrients. To assess microbial growth, it is common to use a buffer such as filtered seawater or artificial seawater (Kottmeier and Sullivan 1987; Meiners et al. 2004; Kaartokallio et al. 2007; Mikkelsen and Witkowski 2010). When adding a seawater buffer, the volume and nutrient concentrations of the added buffer need to be quantified precisely for later dilution correction of the nutrient levels in the sea-ice sample (Kristiansen et al. 1992). Sea-ice brines are expected to be significantly different from the underlying seawater. Therefore, the addition of local seawater could change the biogeochemical conditions (nutrient limitation,…) and boost or stress the sea-ice microbial community during melting.

Although inorganic nutrient concentrations can be considered as essential parameters to understand sea-ice biogeochemical cycles, no methodological comparisons have been conducted so far to properly assess the effect of different melting procedures. In this study, the effects of melting temperature and salinity on several biogeochemical parameters were tested in spring sea ice from the Saroma-ko lagoon (Japan): Chl *a*, particulate organic carbon (POC), particulate nitrogen (PN), nitrate, nitrite, phosphate, silicic acid, and ammonium. Isotope signatures give valuable information on the sources of fixed N being used by the microbial community (e.g., new vs. regenerated primary production) (Altabet 1988) and intertwined biogeochemical processes (e.g., the co-occurrence of nitrate assimilation and nitrification) (Munro et al. 2010; Fripiat et al. 2014). Isotope ratios for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were therefore measured on POC and PN, respectively. Four different melting protocols were evaluated: (1) melting at room temperature, (2) melting at room temperature with a salinity buffer addition, (3) melting at low temperature, and (4) melting at low temperature with salinity buffer. No large differences were observed between the four treatments though some cell lysis cannot be excluded. More studies are required, especially in areas where the sympagic community is dominated by flagellates.

Materials and methods

The methodology evaluation of the different melting procedures was done on March 2, 2016 in Saroma-ko lagoon (surface area, 149 km^2; mean depth, 14.5 m) on the northeast coast of Hokkaido, Japan. The lagoon is connected to the Sea of Okhotsk by two inlets and consists mainly of seawater with a freshwater input from the Saromabetsu River (Shirasawa and Leppäranta 2003; Nomura et al. 2009). Generally, sea-ice formation starts at the beginning of January and covers the whole lagoon between early February and early April with a thickness of 34–60 cm (Shirasawa et al. 2005).

A procedure as described by Rintala et al. (2014) was used to collect samples for the different melting protocols. Sea ice was sampled at three locations to reduce the effect of spatial variability: site 1 was located at GPS coordinates 44°07.329′N 143°57.004′E, site 2 was 10 m north and site 3, 10 m south. At each location, 10 ice cores were sampled using a Kovacs Mark-II ice corer with 9 cm internal diameter and placed in plastic bags (Fig. 1). Temperature of the snow on sea ice (1 cm depth from top of snow), atmosphere, and underlying seawater (1 m depth from bottom of ice) was measured with a temperature sensor (Testo 110 NTC, Brandt Instruments Inc.). Sea-ice samples were transported in a dark container to a laboratory in Napal Kitami, which was located near the sampling site. Two cores from each of the three sites (6 cores total) were selected randomly and crushed with a rubber hammer in smaller pieces (< 2.5 cm) outside of the laboratory (about 0 °C) and pooled in large plastic containers (40 L). This yielded five replicates (A–E) each consisting of six cores from the three sampling sites (2 cores per site). The crushed ice mixtures were then homogenized and divided in four subsamples using large plastic spoons and placed in 5 L plastic buckets.

The homogenized subsamples were melted with one of the four melting procedures (Fig. 1). Samples were melted in the dark at room temperature (20 °C) or at low temperature (6.4 ± 2.6 °C). The salinity buffer used in protocol (2) and (4) was artificial seawater, prepared by dissolving the following in Milli-Q water (Milli-Q Advantage A10 Q-POD® Dispenser with ELIX 5 UV 100V 50/60HZ): NaCl: 49.20 g L^{-1}, KCl: 1.34 g L^{-1}, $CaCl_2$ $2H_2O$: 2.72 g L^{-1}, $MgSO_4$ $7H_2O$: 12.58 g L^{-1}, $MgCl_2$ $6H_2O$: 9.32 g L^{-1}, $NaHCO_3$: 0.36 g L^{-1}. The salinity of the artificial seawater buffer was 62.1. A measured volume (1.5–2.2 L) was added to the buffered samples, and nutrient concentrations in the buffer were assessed as described below, to correct for any seawater contamination. Prior to use, all equipment had been cleaned with HCl (1 M, 24 h) and rinsed with Milli-Q water.

During and after melting, the ice samples were shaken regularly to homogenize and reduce sample warming. Bulk salinity was measured using a conductivity sensor (Cond 315i, WTW Wissenschaftlich-Technische Werkstätten GmbH). For later analysis of inorganic nutrients (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, $Si(OH)_4$), samples were filtered over 0.22 µm syringe filters (SLGV033RS Millex PVDF) in duplicate 15 ml tubes and frozen at − 25 °C. Nutrient concentrations were measured spectroscopically at Hokkaido University (Japan) using a QuAAtro 2-HR system (Seal Analytical Inc., Mequon, WI, USA) for ammonium (NH_4^+),

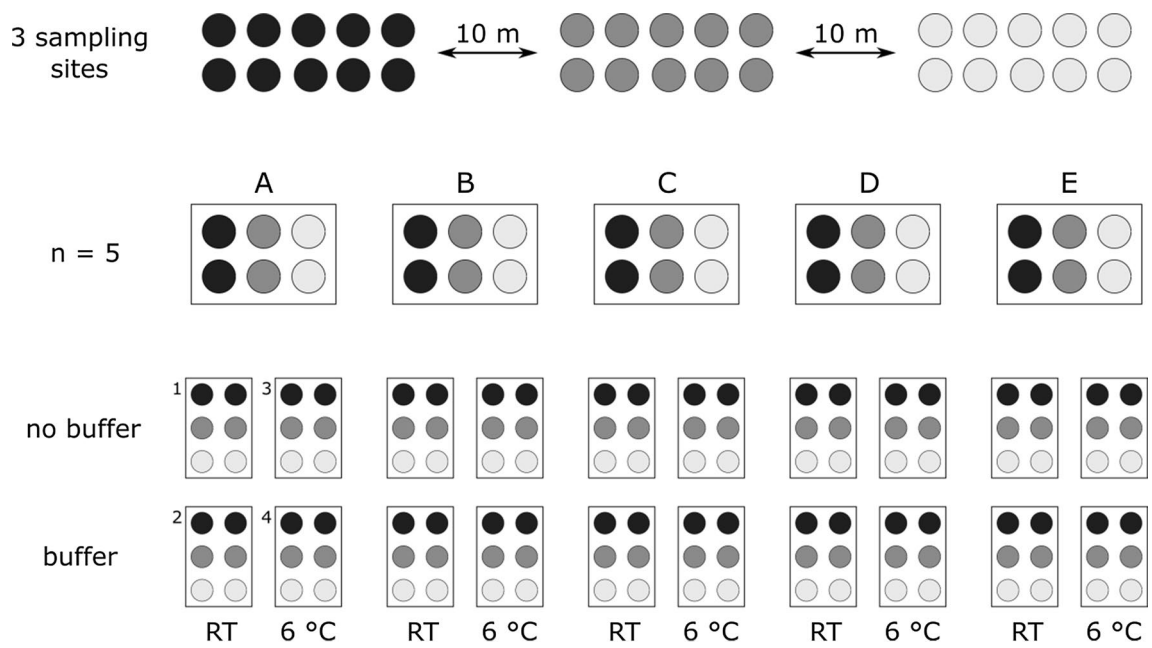


Fig. 1 Sampling setup, based on Rintala et al (2014). Ice cores were obtained from 3 different sampling sites (black, gray, and white circles). They were then pooled randomly, crushed, and placed in five 40 L containers (**a**–**e**) to be homogenized. Each pooled replicate was divided in four 5 L containers and melted following one of the melting protocols tested: (1) direct melting at room temperature (20 °C), (2) buffered melting at room temperature (20 °C), (3) melting at 6 °C, (4) buffered melting at 6 °C

nitrate (NO_3^-), and nitrite (NO_2^-). Nitrate and nitrite measurements were repeated at the Vrije Universiteit Brussel (Belgium) using a QuAAtro39 auto-analyzer (Seal Analytical Inc., Mequon, WI, USA) together with silicic acid ($Si(OH)_4$) measurements. The relative standard deviation (RSD), based on the duplicate analysis at different labs, was calculated. For nitrate and nitrite, the median RSD values were 5.9 and 4.9%, respectively. For silicic acid determination, samples were thawed slowly (4 °C) over a prolonged period (2 weeks). This method has been proven suitable for the recovery of the polymerized forms of silicic acid that form as a result of sample freezing (Dore et al. 1996). Phosphate (PO_4^{3-}) concentrations were in the nanomolar range and were measured manually using a 1-m Liquid Waveguide Capillary Flow Cell, halogen light source (HL-2000-FHSA), and a USB+2000 detector (all Ocean Optics) at the Vrije Universiteit Brussel. All standards for nutrient analysis had adjusted salinities of 3.5 for sea-ice and 32 for seawater samples.

Samples for particulate organic carbon (POC) and particulate nitrogen (PN) and their respective isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$, in per mil versus VPDB and atmospheric N_2, respectively, and expressed as follows for $\delta^{13}C = (((^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{VPDB} - 1) \times 1000))$) were filtered (1 L) over 0.7 µm precombusted (5 h at 450 °C) GF/F filters (25 mm, Whatman). Carbon and nitrogen isotope data were normalized using certified reference materials IAEA-CH6 and IAEA-N2, respectively. Filters were dried (60 °C) and stored in the dark at room temperature until analysis at the Vrije Universiteit Brussel. For analysis, they were packed in silver cups after acid fume treatment to remove carbonates and analyzed using an Elemental analyzer (EUROVECTOR) coupled with an isotope ratio mass spectrometer (Delta V, Thermo). Filters were measured in duplicate on different days and the relative standard deviation was calculated. The median RSD were 4.1% for particulate organic carbon concentrations and 6.6% for particulate nitrogen. For POC and PN isotope ratios, the reproducibility rates were 0.38 and 0.43‰, respectively.

For Chl *a* measurements, water samples (400–500 mL) were filtered through 25 mm Whatman GF/F filters. Pigments on the filters were extracted in dimethylformamide (Suzuki and Ishimaru 1990) for 24 h at approximately − 25 °C. After returning to the laboratory in Hokkaido University, concentrations of pigments were determined using a fluorometer (Model 10AU, Turner Designs, Inc., San Jose, CA, USA), following the methods described by Parsons et al. (1985).

At the three sampling sites, an additional core was taken for ice depth profiles of temperature and salinity. Immediately after sampling, ice temperatures were measured by inserting a needle-type temperature sensor (Testo 110 NTC, Brandt Instruments Inc.) in holes drilled into the core. Ice cores were sliced on site into 0.1 m thick sections with a handsaw and the samples were placed in plastic bags. The sections were melted without salinity buffer at 6 °C. For the

ice core collected at site 1, chlorophyll *a* was measured. In addition, cell counting for ice algae community assemblage was examined with a microscope (Olympus, BH-T, Tokyo, Japan) 10× oculars and 40× objective. This was done for the center (20–30 cm depth) and bottom (40–50 cm depth) ice section of the ice core from site 1, as well as for two samples from melting replicate B4 (buffered melting at 6 °C). The underlying seawater was collected at a depth of 1 m from the bottom of sea ice using a Teflon water sampler (GL Science Inc., Tokyo, Japan). For ice texture analysis, an additional ice core was taken at site 1. The ice core was sliced into 0.003-m thick sections in the cold room (– 16 °C) at Hokkaido University, and the ice crystallographic structures were examined by illuminating the 0.003-m thick sections between crossed polarizers (Langway 1958).

Calculations

Samples that were melted with addition of artificial seawater were corrected for the added-volume of buffer. To calculate the dilution factor, the volume of artificial seawater (V_{AS}) was measured and the volume of ice sample (V_{Ice}) was estimated using Eq. (1).

$$S_{AS} \cdot V_{AS} + S_{Ice} \cdot V_{Ice} = S_{total}\left(V_{Ice} + V_{AS}\right), \quad (1)$$

where V_{AS} is the known volume of artificial seawater added; V_{Ice} the unknown volume of ice sample; S_{Ice} the measured salinity of sea ice (mean = 3.6, S.D. = 0.2); S_{AS} the salinity of the artificial seawater (= 62.1); and S_{total} the final salinity measured in the samples after melting. Equation 1 is then solved for V_{ice} (Eq. 2).

$$\frac{V_{AS} \times \left(S_{AS} - S_{total}\right)}{S_{total} - S_{Ice}} = V_{Ice}. \quad (2)$$

The dilution factor is then estimated using Eq. (3).

$$\frac{V_{AS} + V_{Ice}}{V_{Ice}} = \text{dilution factor}. \quad (3)$$

Addition of nutrients from the artificial seawater to the samples was corrected by measuring nutrient concentrations in the artificial seawater and taking into account the amount of nutrients added to the samples based on the volume added. Calculations were done using Eq. (4) where $[Nut]_{total}$ is the nutrient concentration measured in the samples, $[Nut]_{AS}$ is the concentration in the artificial seawater, and *V* is the volume of the different pools.

$$\frac{V_{total} \cdot [Nut]_{total} - V_{AS} \cdot \left[Nut_{AS}\right]}{V_{Ice}} = [Nut]_{Ice}. \quad (4)$$

A factorial two-way ANOVA with replicates was conducted for each of the measured variables to compare the effect of temperature and buffering and the interaction between the two factors. The factor of temperature consisted of two levels (+ 6 °C, 20 °C) and buffering included whether or not artificial seawater was added. Statistical significance was tested at the 0.05 significance level.

Results

Physicochemical conditions

Average ice thickness at the three sampling sites was 51 cm (3 cm freeboard) with an average snow cover of 3.4 cm. The ice texture analysis indicated that the top 40 cm of the ice was granular ice while the lower 9 cm was columnar, separated by a 2 cm mixture of granular and columnar ice.

Snow temperature (1 cm depth) was about – 2.9 °C, while air temperature was slightly lower at the time of sampling (– 5.5 °C). Sea-ice temperatures were relatively high with lowest observations at the top of the ice (−2 °C), increasing downwards to – 1 °C (Fig. 2). Salinity of the sea ice was within the range observed at Saroma-Ko lagoon (Nomura et al. 2011) and highest at the surface (4–6), decreasing to 3 with increasing depth (Fig. 2). This is just slightly below sea-ice salinities observed in Arctic and Antarctic sea ice (Kovacs 1996). Temperature of the underlying seawater at a depth of 1 m was – 1.2 °C and salinity was 30.8.

Chl *a* concentrations were low at the ice surface but increased deeper in the ice reaching 37 µg L^{-1} at the bottom section. The concentration in the underlying seawater was low at 0.55 µg L^{-1}. The sea-ice algal community was clearly dominated by diatoms (95%, cell counts) and the remaining were cryptophytes and dinoflagellates. A more detailed list of observed microorganisms can be found in Online Resource 1. These results correspond with previous studies that examined the Saroma-ko Lagoon (Robineau et al. 1997; McMinn et al. 2008).

Impact of the melting protocol

For each parameter and melting procedure, five replicates were measured. Mean values ($n = 5$) and standard deviation (SD) for each melting procedure are shown as histograms in Fig. 3 (mean ± SD). Results of the two-way ANOVA with replicates for each parameter are shown in Table 1 as *F* value and *P* value ($\alpha = 5\%$) for addition of buffer (artificial seawater), temperature, and interaction between temperature and buffer addition. *F* critical for each parameter tested was $F(1, 16) = 4.49$ based on the degrees of freedom (1 and 16) for $\alpha = 5\%$.

For the samples melted with a buffer, the final salinity was 31.5 (SD = 0.6), being close to the salinity expected from the brines in the bottom sections where most sea-ice

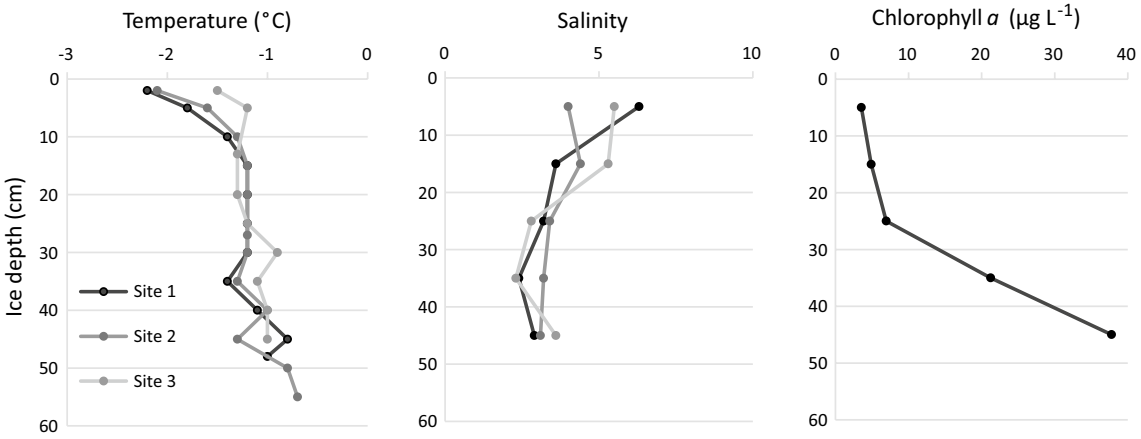


Fig. 2 Sea-ice depth profiles for temperature (°C) and salinity at the three sampling sites and chlorophyll *a* (µg L^{-1}) from site 1

algae were encountered, and the dilution factor ranged from 1.83 to 1.96 (1.91 ± 0.04). Samples melted without buffer had a final salinity of 3.6 (SD = 0.2). The temperature at which sea-ice samples are melted had a large impact on the time required for melting. Samples at room temperature without artificial seawater addition (protocol 1) took slightly over 25 h for complete melt, while at 6 °C (protocol 3) melting required almost 60 h. The addition of the buffer reduced the melting time to about 21 h and 42 h at room temperature (protocol 2) and 6 °C (protocol 4), respectively.

Particulate organic carbon and nitrogen

For particulate organic carbon (POC), there was no significant effect between the melting protocols. The main effect for temperature yielded an *F* ratio of $F(1, 16) = 0.54$, $P > 0.05$ indicating no significant difference between melting at 6 °C (66.2 ± 7.2 µmol L^{-1}) and room temperature (63.4 ± 10.0 µmol L^{-1}). There was also no significant difference ($F(1, 16) = 2.56$, $P > 0.05$) between buffered (67.8 ± 9.8 µmol L^{-1}) and direct melting (61.8 ± 6.3 µmol L^{-1}). Also POC $\delta^{13}C$ (‰) was not affected significantly by melt temperature $F(1, 16) = 0.16$, $P > 0.05$, at 6 °C (− 26.6 ± 0.5‰) and room temperature (− 26.7 ± 0.5‰). Buffer addition also had no significant effect $F(1, 16) = 1.32$, $P > 0.05$ on POC $\delta^{13}C$, with (− 26.5 ± 0.5‰) and without artificial seawater addition (− 26.8 ± 0.5‰).

Particulate nitrogen (PN) concentration was affected by buffer addition yielding an *F* ratio of $F(1, 16) = 8.53$, $p = 0.01$. This indicates a significant effect such that samples with buffer addition (8.0 ± 1.0 µmol L^{-1}) are marginally higher than samples melted without artificial seawater (6.9 ± 0.6 µmol L^{-1}) but close to analytical uncertainty (RSD = 6.6%). Results for PN $\delta^{15}N$ also presented a significant effect of buffer addition, although still being marginal ($F(1, 16) = 8.60$, $P = 0.01$), with a PN $\delta^{15}N$ for direct melting (5.5 ± 0.5‰) being higher than buffered melting (4.8 ± 0.5‰). There was no significant effect of melting temperature on PN concentrations, $F(1, 16) = 2.15$, $P > 0.05$, melting at 6 °C (7.2 ± 1.0 µmol L^{-1}) and 20 °C (7.7 ± 0.9 µmol L^{-1}) and PN isotope signatures, $F(1, 16) = 0.76$, $P > 0.05$, melting at 6 °C (5.2 ± 0.6‰) and 20 °C (5.0 ± 0.7‰).

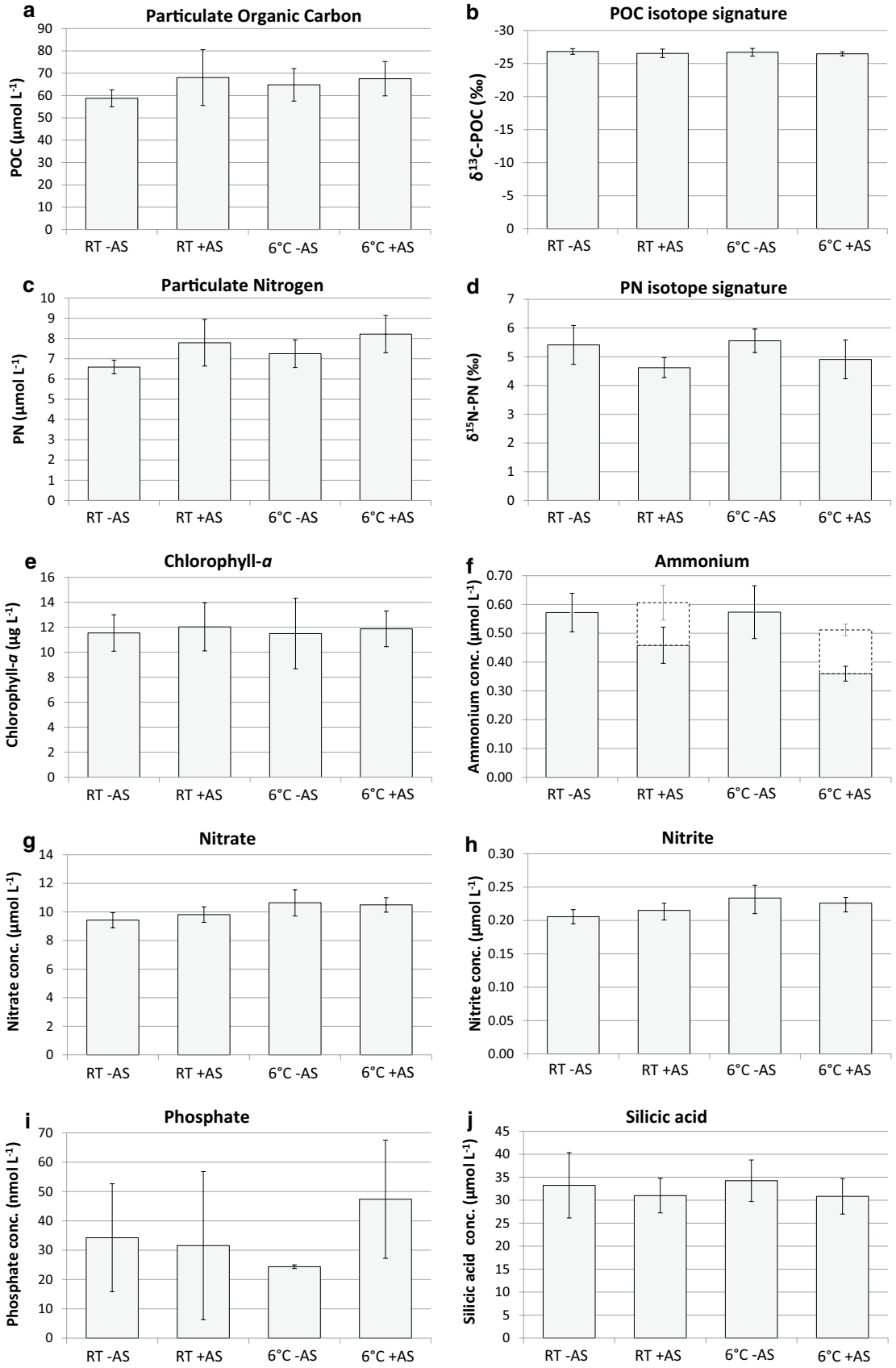
Chlorophyll *a*

The two-way variance analysis showed no significant effect on Chl *a* concentration (µg L^{-1}) for any factor. The effect for the melt temperature yielded a F ratio of $F(1, 16) = 0.01$, $P > 0.05$ with no difference between melting at 6 °C (11.7 ± 2.2 µg L^{-1}) and room temperature (11.8 ± 1.6 µg L^{-1}). Buffer addition yielded an *F* ratio of $F(1, 16) = 0.23$, $P > 0.05$, without AS (11.5 ± 2.1 µg L^{-1}) and with AS addition (12.0 ± 1.6 µg L^{-1}).

Inorganic nutrients

There is a significant effect of buffer addition on ammonium concentrations ($F(1, 16) = 30.65$, $P < 0.0001$) with the average ammonium concentration of direct melting (0.57 ± 0.08 µmol L^{-1}) being higher compared to buffered melting (0.41 ± 0.07 µmol L^{-1}). The absolute difference in ammonium concentration between the direct melting treatment and buffered melting is limited and only 0.16 µmol L^{-1}. The main effect of melt temperature yielded an *F* ratio of $F(1, 16) = 2.72$, $p > 0.05$, and no significant difference between 6 °C (0.47 ± 0.13 µmol L^{-1}) and room temperature (0.52 ± 0.09 µmol L^{-1}) was noted.

For nitrate concentrations, the main effect on buffer addition yielded an $F(1, 16) = 0.17$, $P > 0.05$, implying no significant difference between direct (10.0 ± 1.0 µmol L^{-1}) and buffered melting (10.2 ± 0.6 µmol L^{-1}). For melt temperature, there was a statistically significant effect (*F*(1,


a
Particulate Organic Carbon
POC (µmol L-1)
b
POC isotope signature
δ13C-POC (‰)
c
Particulate Nitrogen
PN (µmol L-1)
d
PN isotope signature
δ15N-PN (‰)
e
Chlorophyll-a
Chlorophyll-a (µg L-1)
f
Ammonium
Ammonium conc. (µmol L-1)
g
Nitrate
Nitrate conc. (µmol L-1)
h
Nitrite
Nitrite conc. (µmol L-1)
i
Phosphate
Phosphate conc. (nmol L-1)
j
Silicic acid
Silicic acid conc. (µmol L-1)
RT -AS
RT +AS
6°C -AS
6°C +AS

◂**Fig. 3** Histograms depict the mean value ($n=5$) and standard deviation (error bars) for ten biogeochemical parameters processed with four different melting protocols: (1). RT without buffer, (2). RT with buffer, (3). 6 °C without buffer, (4). 6 °C with buffer for **a)** Particulate organic carbon conc. (µmol L^{-1}), **b)** Particulate organic carbon isotopic signature (‰), **c)** Particulate nitrogen conc. (µmol L^{-1}), **d)** Particulate nitrogen isotopic signature (‰), **e)** Chlorophyll a conc. (µg L^{-1}), **f)** Ammonium conc. (µmol L^{-1}), **g)** Nitrate conc. (µmol L^{-1}), **h)** Nitrite conc. (µmol L^{-1}), **i)** Phosphate conc. (nmol L^{-1}), **j)** Silicic acid conc. (µmol L^{-1}). Dotted histograms for ammonium are based on an ammonium concentration of 0.10 µmol L^{-1} in the artificial seawater (see Discussion)

16) = 10.70, $P=0.005$), such that samples melted at 6 °C (10.6 ± 0.7 µmol L^{-1}) had higher nitrate concentrations than those melted at room temperature (9.6 ± 0.5 µmol L^{-1}). Though this effect is significant, the difference is limited to 1 µmol L^{-1}, and close to the analytical precision (RSD = 5.9%).

The results for nitrite are similar to those observed for nitrate. There is no significant difference ($F(1, 16) = 0.02$, $P>0.05$) between direct (0.22 ± 0.02 µmol L^{-1}) and buffered melting (0.22 ± 0.01 µmol L^{-1}). The main effect for melt temperature yielded an $F(1, 16) = 11.10$, $P=0.004$, with the nitrite concentration significantly higher for samples melted at 6 °C (0.23 ± 0.02 µmol L^{-1}), compared to room temperature (0.21 ± 0.01 µmol L^{-1}). Nitrite concentrations in the ice were very low (mean = 0.22 µmol L^{-1}). Although there is a significant difference between melt protocols, the absolute difference is limited (< 0.03 µmol L^{-1}) and close to analytical uncertainty (RSD = 4.9%).

Phosphate concentrations are very low, situated in the nanomolar range (< 100 nmol L^{-1}). The main effect on buffer addition yielded an $F(1, 16) = 0.10$, $P>0.05$ with no significant difference between direct (30.5 ± 14.8 nmol L^{-1}) and buffered melting (39.5 ± 23.1 nmol L^{-1}). There is no significant ($F(1, 16) = 1.12$, $P>0.05$) difference between melting at 6 °C (38.7 ± 19.3 nmol L^{-1}) and room temperature (32.9 ± 20.9 nmol L^{-1}).

There is also no significant effect on the silicic acid concentration between the four melting protocols. The main effect for the melt temperature yielded a F ratio of $F(1, 16) = 0.04$, $P>0.05$ indicating no significant difference between melting at 6 °C (32.5 ± 4.4 µmol L^{-1}) and room temperature (32.1 ± 5.5 µmol L^{-1}). For buffer addition, the F ratio was 1.59, $P>0.05$, with no difference between direct (30.9 ± 3.6 µmol L^{-1}) and buffered melting (33.7 ± 5.6 µmol L^{-1}).

Discussion

For most parameters in this study (POC, δ^{13}C-POC, Chl *a*, PO_4^{3-}, $Si(OH)_4$), there is neither significant impact of melting temperature nor buffered melting. For the parameters related to nitrogen, statistically significant differences are reported between treatments (PN, δ^{15}N-PN, NH_4^+, NO_3^-, NO_2^-) but they remain low in comparison to the analytical precision, the actual values, and the variability usually encountered in sea-ice environments (Fripiat et al. 2014, 2015, 2017).

Both nitrate and nitrite are significantly affected by the melting temperature, resulting in slightly higher concentrations (< 1 and 0.03 µmol L^{-1}, respectively) for samples melted at low temperatures (Table 1 and Fig. 1). For these nutrients, the highest mean concentration was observed with treatment 3 (salinity-buffered 6 °C melting) which showed the highest variability. Nitrite and nitrate are, respectively, the intermediate and product of nitrification which is considered a photo-inhibited process (Guerrero and Jones 1996; Merbt et al. 2012). Nitrification is also known to play an important role in sea ice (Fripiat et al. 2014; Baer et al. 2015). Keeping the samples for prolonged time in the dark would prevent photosynthetic assimilation but could favor nitrification. The longer melting time for samples at low temperatures (42–60 h) could allow nitrification to continue for longer, and may explain the slightly higher concentrations observed for nitrite and nitrate in these samples. However, this was not observed in ammonium concentrations and such differences remain close to the analytical uncertainties (RSD = 5.9 and 4.9%, 2 SD is 1.1 and 0.02 µmol L^{-1} for NO_3^- and NO_2^-, respectively) implying that this effect remains marginal.

The addition of a buffer, used to avoid osmotic shock, had a statistically significantly effect on three parameters. Particulate nitrogen concentrations were slightly lower (by 1 µmol L^{-1}) in samples melted without buffer, whereas PN δ^{15}N and ammonium concentrations were higher (by 0.7‰ and 0.16 µmol L^{-1}, respectively). Rintala et al. (2014) observed a significant effect on the total biomass concentrations between treatments. Highest concentrations were observed for melting with filtered seawater addition (FSW) and lowest for direct melting or addition of filtered artificial seawater with added nutrients (F/2 3). Since the FSW and F/2 3 had a similar salinity, it was concluded that the difference in biomass between the procedures was not due to osmotic differences. Growth promotion by FSW or inhibition of algae growth by a constituent in the F/2 3 medium was considered (Rintala et al. 2014). Growth promotion by the addition of artificial seawater instead of cell lysis by direct melting could also play in our study but this was not observed in our POC and Chl *a* concentrations. Also, the salinity difference between direct melting and buffered melting was only 3 in the study of Rintala et al. (2014) due to the use of brackish sea-ice samples while in our study it was around 28.

The result of decreasing PN and increasing ammonium concentrations in directly melted samples could indicate that some particulate matter is lost due to osmotic shock and cell

Table 1 The results for the two-way analysis of variance for the different parameters are shown as *P* values

Parameter measured	Buffer		Temperature		Interaction	
	F (1, 16)	*P* value	*F* (1, 16)	*P* value	*F* (1, 16)	*P* value
Particulate organic carbon (µmol L^{-1})	2.56	0.13	0.54	0.47	0.77	0.39
δ^{13}C-POC (‰)	1.32	0.27	0.16	0.70	0.01	0.91
Particulate nitrogen (µmol L^{-1})	**8.53**	**< 0.05**	2.15	0.16	0.10	0.76
δ^{15}N-PN (‰)	**8.60**	**< 0.05**	0.76	0.40	0.09	0.77
Chlorophyll a (µg L^{-1})	0.23	0.63	0.01	0.91	0.00	0.95
Ammonium (µmol L^{-1})	**30.65**	**< 0.05**	2.72	0.12	2.86	0.11
Nitrate (µmol L^{-1})	0.17	0.68	**10.70**	**< 0.05**	0.84	0.37
Nitrite (µmol L^{-1})	0.02	0.88	**11.10**	**< 0.05**	2.17	0.16
Phosphate (nmol L^{-1})	0.10	0.76	1.12	0.31	1.79	0.20
Silicic acid (µmol L^{-1})	1.59	0.23	0.04	0.85	0.07	0.80

The effect of two factors and their interaction was tested. The factor of temperature compared melting at room temperature (RT) and low temperature (+ 6 °C). The factor for buffering compared direct melting and buffered melting with addition of artificial seawater (Buffer). *F* critical for each parameter tested was $F(1, 16) = 4.49$ based on the degrees of freedom (1 and 16) for $\alpha = 5\%$. Values for *P* smaller than 0.05 (bold) indicate a significant difference between the two levels of this factor and parameter on a significance level of 5%. There was no significant effect on any of the parameters for interaction by the two factors

lysis. Nutrients that are stored within cells are then released in solution. Several studies (Dortch et al. 1984; Lomas and Glibert 2000; Kamp et al. 2015) reported that especially flagellates and ciliates can store large amounts of intracellular ammonium within their cytoplasm. For samples that are melted without buffer, the increase in ammonium (0.16 µmol L^{-1}) cannot account for the loss in PN (1 µmol L^{-1}). Possibly other intracellular components that are rich in nitrogen such as free amino acids and proteins are released as dissolved organic matter (Dortch et al. 1984). The higher N content in free amino acids make that the effect of cell lysis would be more clear on PN in contrast to POC and Chl *a*. The increase in PN δ^{15}N indicates that a fraction of the microbial community is likely more prone to be lost during melting. Regenerated primary production (i.e., likely flagellates) have a lower δ^{15}N compared to new primary production (i.e., diatoms) (Altabet 1988; Fawcett et al. 2011). This is due to the lower isotope signature of ammonium that is released by heterotrophic processes and zooplankton. This isotopically lighter ammonium is than used as a nitrogen source for regenerated production. Hence, the results for PN and PN δ^{15}N would fit with a loss of a proportion of the microbial community due to cell lysis.

The impact of cell lysis on the algal community were observed by Garrison and Buck (1986) and Mikkelsen and Witkowski (2010). They found differences in the composition of the microbial community because especially the fragile members (e.g., flagellates) were affected by cell lysis. As a result, organisms with a more ridged cell membrane (e.g., diatoms) were over-represented in samples that were melted fast (20 °C) without a salinity buffer. However, no data on nutrients and biomass concentrations are given in these studies. We also note that Mikkelsen and Witkowski (2010) compared different cores from a 1 m^2 area and their results could instead be affected by some unaccounted spatial variability.

Few studies discuss the effect of ice sample melting on measured nutrient concentrations. Thomas et al. (1998) observed no correlation between inorganic nutrient concentrations and Chl *a* in rotten Antarctic sea-ice samples and concluded there was no substantial release of intracellular nutrients. In contrast, Cota et al. (1990) found a correlation between inorganic nutrients and Chl *a* concentrations in Arctic bottom fast ice. This concurrent accumulation was suggested to be the result of intracellular nutrient pools being released upon melting without a salinity buffer. However, recent studies (Fripiat et al. 2014, 2015) show that nitrification can play a large role in sea ice. Hence, coinciding high nutrient and biomass concentrations are not necessarily due to the release of intracellular nutrients but can also be the result of increased remineralisation.

The effect of cell lysis appears to be marginal in this study as it represents 13% (1 µmol L^{-1}) of the PN pool; it is not recorded in both Chl *a* and POC; and the changes in PN concentrations are close to the analytical uncertainty (RSD = 6.6%, 2 SD = 1.1 µmol L^{-1}). In addition, it is low in comparison with the expected variability of concentration and δ^{15}N observed in sea ice for which concentrations vary over several orders of magnitude and PN δ^{15}N ranges from 0 to more than 5‰ (Fripiat et al. 2014, 2017).

Though no ammonium was deliberately added to the artificial seawater, we measured a concentration of 0.26 µmol L^{-1} in this water after the experiments, indicating that some ammonium contamination took place. Ammonium concentrations in the buffered ice samples were corrected for this contamination of artificial seawater addition as shown

in Eq. (4). However, in case this ammonium contamination of the artificial seawater occurred after the experiment, the ammonium concentrations in the samples would be over-corrected. This means that the true concentration in these samples could actually be higher and closer to those in directly melted samples (Fig. 3f: dashed histograms). We therefore cannot exclude that there is no significant difference between the different melting protocols for ammonium concentrations. The correct quantification of nutrients added to the samples through buffer addition might be challenging for some parameters such as ammonium. It also illustrates what are the risks associated with buffer addition to sea-ice samples. By using filtered seawater, other 'unknown' contaminates (e.g., dissolved organic matter) might be added to sea-ice samples which could also impair results.

Conclusion

There are small differences between the four different melting procedures tested in this study for some parameters and an effect of cell lysis on PN for directly melted samples cannot be excluded. When statistically significant, the differences are, however, close to the analytical precision of the measurements and small compared to the variability observed in sea ice. Although our study suggests a minimal effect between melting treatments on biomass and nutrient measurements in sea ice, this experiment should be repeated for sea-ice environments where the sympagic community is dominated by flagellates instead of diatoms. The former are believed to be more susceptible to osmotic shock and cell lysis. Therefore, differences attributed by the different melting treatments in this study could be minimized due to the low abundance of these flagellates.

Acknowledgements We express our heartfelt thanks to Dr. Aoki Shigeru for their support in the fieldwork. This research was supported by funds from the Japan Society for the Promotion of Science (grant numbers 15K16135 and 17H0471507), International Antarctic Institute, Institute of Low Temperature Sciences, and BEPSII (SCOR). This study is a contribution to SCOR Working Group 152 ECV-Ice (Measuring Essential Climate Variables in Sea Ice).

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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