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Corresponding Author:	Arnout Roukaerts Vrije Universiteit Brussel Brussel, BELGIUM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Vrije Universiteit Brussel
First Author:	Arnout Roukaerts
First Author Secondary Information:	
Order of Authors:	Arnout Roukaerts
	Daiki Nomura
	Florian Deman
	Hiroshi Hattori
	Frank Dehairs
	François Fripiat
Funding Information:	Japan Society for the Promotion of Science (15K16135) — Dr Daiki Nomura
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Suggested Reviewers:	Janne-Markus Rintala, Dr. University of Helsinki janne.rintala@helsinki.fi Rintala made a similar evaluation on biological parameters.
	Klaus Meiners, Dr. Australian Antarctic Division Klaus.Meiners@aad.gov.au

	Works on both Arctic and Antarctic sea ice ecology and biogeochemistry
	Lisa Miller, Dr Institute of Ocean Sciences Lisa.Miller@dfo-mpo.gc.ca Works on sea ice biogeochemistry
Opposed Reviewers:	
Order of Authors Secondary Information:	

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

Roukaerts Arnout[1], Nomura Daiki[2, 3], Deman Florian[1], Hattori Hiroshi[5], Dehairs Frank[1], Fripiat Francois[4]

1 Analytical, Environmental and Geo-Chemistry & Earth System Sciences, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium
2 Faculty of Fisheries Sciences, Hokkaido University, 3–1–1, Minato–cho, Hakodate, Hokkaido 041–8611, Japan
3 Arctic Research Center, Hokkaido University, Kita–21 Nishi–11 Kita–ku, Sapporo, Hokkaido 060–0814, Japan
4 Max Planck Institute for Chemistry, Hahn-Meitner-Weg 1, 55128 Mainz, Germany
5 Tokai University, 5–1–1–1, Minamisawa, Minami–ku, Sapporo, Hokkaido 005–8601, Japan

Keywords: Sea ice, direct melting, buffered melting, nutrients, particulate matter, chlorophyll a, Sea of Okhotsk

Abstract

Melting of sea ice samples is often an inevitable step in obtaining reliable and representative measurements for biogeochemical parameters such as inorganic nutrients and particulate matter. For biological parameters such as chlorophyll *a* and cell abundance, the impact of sea ice melting has been previously evaluated. For nutrient and biomass concentrations in sea ice it is generally recommended to melt samples fast, though no systematic evaluation exists in literature. The impact of melting temperature and buffer addition to avoid osmotic shock was tested on ice sampled 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. Results show no clear effect of melting temperature nor buffer addition on the parameters measured. 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 and nutrients measurements in diatom dominated sea ice and should be repeated where primary production is dominated by flagellates.

Introduction

Sea ice is a semisolid matrix permeated by a network of channels and pores that are variably connected with underlying seawater. To study 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 often inevitable, inducing drastic changes in both salinity and temperature (Miller et al. 2015). Comparing different melting procedures during a field study, Rintala et al. (2014) concluded that fast melting 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 works 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).

According to McMinn et al. (2009), the direct melting of sea ice in the dark is advised for physicochemical parameters. This should be done fast (<6 h) to avoid biological processes that can alter nutrient concentrations. However, melting without buffer to avoid osmotic shock can lead to cell rupture and the release of internal inorganic nutrient pools. Commonly used buffers are filtered local seawater that is sampled close to the sea ice sampling site or artificial seawater (Kottmeier et al. 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 correction.

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 effect of melting temperature and salinity on several biogeochemical parameters were tested in spring sea ice from the Saroma-ko lagoon (Japan), including: Chl-*a*, particulate organic carbon (POC), particulate nitrogen (PN), nitrate, nitrite, phosphate, silicic acid and ammonium. 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 more studies are required, especially in areas where primary production is driven by flagellates.

Material and Method

The methodology evaluation of the different melting procedures was done on March 2nd 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 consist 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 through 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 (Figure 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) were measured with a temperature sensor (Testo 110 NTC, Brandt Instruments Inc.). Sea ice samples were quickly transported in a dark container to a laboratory in Napal Kitami, which was located near the sampling site. Two cores from each of the 3 sites were selected randomly and crushed with a rubber hammer in smaller pieces (< 2.5 cm) outside of laboratory (about 0 °C) and pooled in large plastic containers (40 L). This yielded five replicates (A - E) each consisting of two cores from the three sampling sites. 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 (Figure 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: 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 10 % HCl (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^+), nitrate (NO_3^-) and nitrite (NO_2^-). Nitrate and nitrite measurements were repeated at the Vrije Universiteit Brussel (Belgium) using a QuAAtro39 auto-analyser (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 was 5.7 and 5.2 % respectively. For silicic acid determination, samples were thawed slowly over a prolonged period. 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 (Whatman). Carbon and nitrogen isotope data was 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 10.4 % for particulate organic carbon concentrations and 7.4 % for particulate nitrogen. For POC and PN isotope ratios, the reproducibility was 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 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 for the centre (20-30 cm depth) and the bottom (40-50 cm depth) ice section of the sea ice (replicate B4: buffered melting at 6 °C) with a microscope (Olympus, BH-T, Tokyo, Japan). 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 Equation 1.

$$S_{AS} \cdot V_{AS} + S_{Ice} \cdot V_{Ice} = S_{total} (V_{Ice} + V_{AS}) \quad \text{Equation 1}$$

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 (= 3.5); S_{AS} the salinity of the artificial seawater (= 62.1) and S_{total} the final salinity measured in the samples after melting. Eq 1 is then solved for V_{ice} (Eq 2).

$$\frac{V_{AS} \cdot (S_{AS} - S_{total}) \cdot}{S_{total} - S_{Ice}} = V_{Ice} \quad \text{Equation 2}$$

The dilution factor is then estimated using Equation 3.

$$\frac{V_{AS}}{V_{AS} + V_{Ice}} = dilution\ factor \quad \text{Equation 3}$$

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 Equation 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.

$$\frac{V_{total} \cdot [Nut]_{total} - V_{AS} \cdot [Nut]_{AS}}{V_{Ice}} = [Nut]_{Ice} \quad \text{Equation 4}$$

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 39.6 cm of the ice was granular ice while the lower 8.5 cm was columnar, separated by a 1.9 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 (Figure 2). Salinity of the sea ice within the range observed at Saroma-Ko lagoon and highest for the surface (4 to 6), decreasing to 3 with increasing depth (Figure 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 %) and the remaining were cryptophyte and dinoflagellate. 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 Figure 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 (α = 5 %) for addition of buffer (artificial seawater), temperature and interaction between temperature and buffer addition.

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 algae were encountered, and the dilution factor ranged from 1.81 to 2.33 (2.08 ± 0.17) . Samples melted without buffer had a final salinity of 3.5 (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.

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196

197 **Particulate organic carbon and nitrogen**

198 For particulate organic carbon (POC), there was no significant effect between the melting protocols.
199 The main effect for temperature yielded an F ratio of F(1, 16) = 0.54, p > 0.05 indicating no significant
200 difference between melting at 6 °C (66.2 $\pm$ 7.2 µmol L^{-1}) and room temperature (63.4 $\pm$ 10.0 µmol L^{-1}).
201 There was also no significant difference (F(1, 16) = 2.56, p > 0.05) between buffered (67.8 $\pm$ 9.8
202 µmol L^{-1}) and direct melting (61.8 $\pm$ 6.3 µmol L^{-1}). Also POC $\delta^{13}C$ (‰) was not affected significantly by
203 melt temperature F(1, 16) = 0.16, p > 0.05, at 6 °C (-26.6 $\pm$ 0.5 ‰) and room temperature (-26.7 $\pm$ 0.5
204 ‰). Buffer addition also had no significant effect F(1, 16) = 1.32, p > 0.05 on POC $\delta^{13}C$, with (-26.5 $\pm$
205 0.5 ‰) and without artificial seawater addition (-26.8 $\pm$ 0.5 ‰).

206 Particulate nitrogen (PN) concentration was affected by buffer addition yielding an F ratio of F(1, 16)
207 = 8.53, p = 0.01. This indicates a significant effect such that samples with buffer addition (8.0 $\pm$ 1.0
208 µmol L^{-1}) are marginally higher than samples melted without artificial seawater (6.9 $\pm$ 0.6 µmol L^{-1}).
209 Results for PN $\delta^{15}N$ also presented a significant effect of buffer addition, although still being marginal
210 (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
211 melting (4.8 $\pm$ 0.5 ‰). There was no significant effect of melting temperature on PN concentrations,
212 F(1, 16) = 2.15, p > 0.05, melting at 6 °C (7.2 $\pm$ 1.0 µmol L^{-1}) and 20 °C (7.7 $\pm$ 0.9 µmol L^{-1}) and PN
213 isotope signatures, F(1, 16) = 0.76, p > 0.05, melting at 6 °C (5.2 $\pm$ 0.6 ‰) and 20 °C (5.0 $\pm$ 0.7 ‰)

214 **Chlorophyll *a***

215 The two-way variance analysis showed no significant effect on chl-*a* concentration (µg L^{-1}) for any
216 factor. The effect for the melt temperature yielded a F ratio of F(1, 16) = 0.01, p > 0.05 with no
217 difference between melting at 6 °C (11.7 ± 2.2 µg L^{-1}) and room temperature (11.8 ± 1.6 µg L^{-1}). Buffer
218 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
219 (12.0 ± 1.6 µg L^{-1}).

220 **Inorganic nutrients**

221 There is a significant effect of buffer addition on ammonium concentrations (F(1, 16) = 30.65, p <
222 0.001) with the average ammonium concentration of direct melting (0.57 ± 0.08 µmol L^{-1}) being higher
223 compared to buffered melting (0.41 ± 0.07 µmol L^{-1}). The absolute difference in ammonium
224 concentration between the direct melting treatment and buffered melting is limited and only 0.16
225 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}).

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 ($F(1, 16) = 10.70$, $p < 0.05$), 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 = 5.7 %).

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 $F(1, 16) = 11.10$, $p < 0.05$, 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 below analytical uncertainty (RSD = 5.2 %).

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 neither 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}) or 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 no significant impact of melting temperature nor buffered melting. For some parameters, 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; Fripiat et al. 2015; Fripiat et al. 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 2.1 and Figure 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 favour nitrification. The longer melting time of samples at low temperatures 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 seen in ammonium concentrations and such differences remain close to the analytical uncertainties implying that this effect remains marginal.

The addition of a buffer, that was 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 $\delta^{15}N$ and ammonium concentrations were higher (by 0.7 ‰ and 0.16 µmol L^{-1}, respectively). Lower PN and higher ammonium concentrations could indicate that some particulate matter is lost due to osmotic shock and cell lysis, with part of the intracellular nutrient stock being released in solution. However, such effect appears to be marginal in regard of the expected variability of concentration and $\delta^{15}N$ observed in sea ice (Fripiat et al. 2014; Fripiat et al. 2017). Mikkelsen and Witkowski (2010) observed a large cell loss for the fragile members of the algal community (e.g., flagellates) due to cell lysis during direct melting at room temperature. We also note that Mikkelsen and Witkowski (2010) compared different cores and their results could instead be affected by some unaccounted spatial variability. Rintala et al. (2014) observed a significant effect on total biomass concentrations between the treatments with biomass being highest with filtered seawater addition (FSW) and lowest for direct melting or addition of filtered artificial seawater (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. Rather the growth promotion by FSW or inhibition of algae growth by a constituent in the F/2 3 medium was considered (Rintala et al. 2014). It is thus possible that also in our study, growth was promoted by the addition of artificial seawater. Standard deviations for POC and PN were also larger for samples melted with AS (figure 2.3a, 2.3c). No significant effect of melting temperature or buffer addition was observed for chl-*a* by Rintala et al.

(2014) suggesting there was no cell lysis. This corresponds with our observations with no significant difference in chl-*a* concentrations (figure 2.3e).

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 some ammonium contamination. Ammonium concentrations in the ice samples were corrected for this contamination of artificial seawater addition as shown in Equation 4. However, in case this ammonium contamination of the artificial seawater occurred after the experiment, the ammonium concentrations in the samples would be overcorrected. The true concentration in these samples would actually be higher and closer to those in directly melted samples. We therefore cannot exclude 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 risk associated with buffer addition to sea ice samples.

Conclusion

There are no clear differences between the four different melting procedures tested in this study. When statistically significant, the differences are close to the analytical precision of the measurements and small compared to the variability observed in sea ice. Although our study suggest a minimal effect between melting treatments on biomass and nutrient measurements in sea ice, this experiment should to be repeated for sea ice environments where primary production is dominated by flagellates instead of diatoms. The former are 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.

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Figure 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.

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

Figure 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 concentration (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 a ammonium concentration of 0.10 μmol L^{-1} in the artificial seawater (see discussion).

Table 1: The results for the two-way analysis of variance for the different parameters are shown as p-values. The effect of two factors and its interaction were 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) = 8.53 based on the degrees of freedom (1 and 16) for α = 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.

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Figure 1

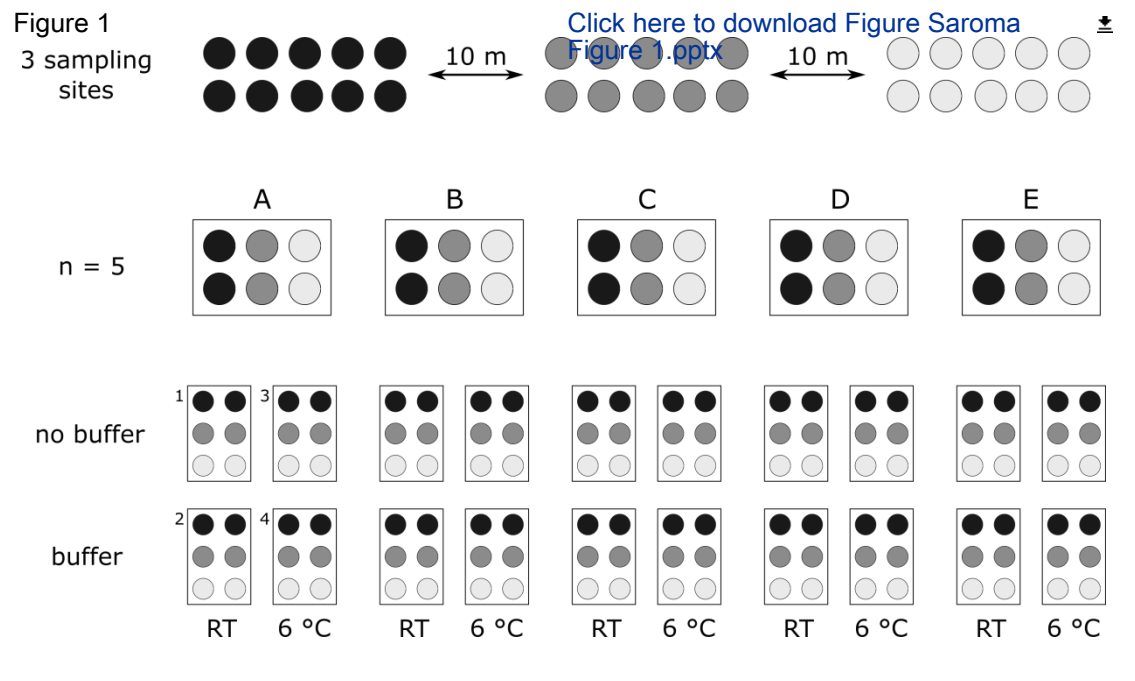

Figure 2

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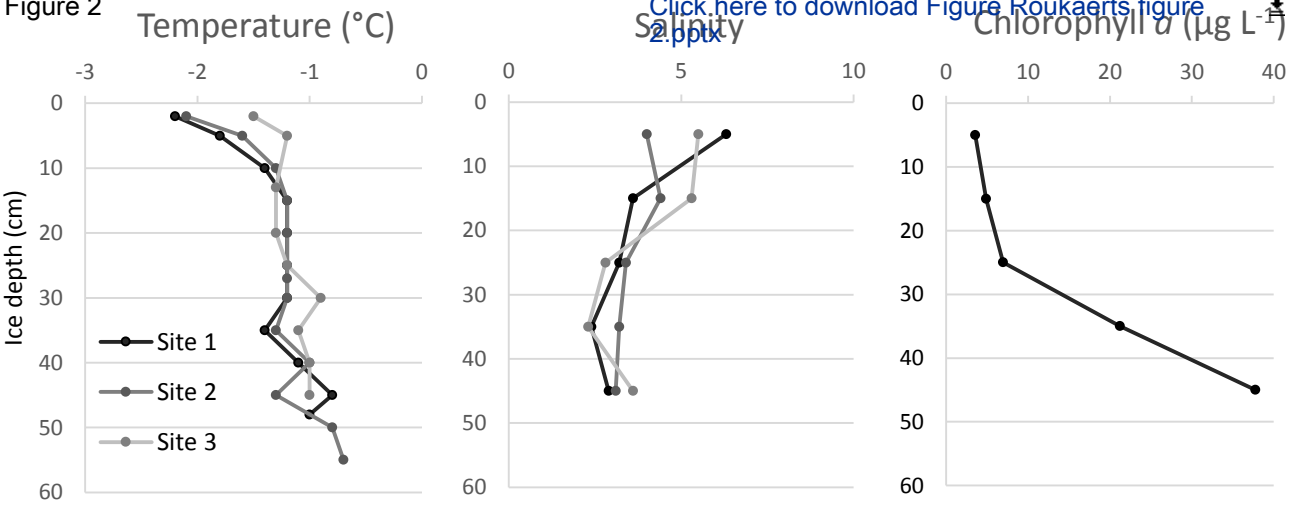

Figure 3

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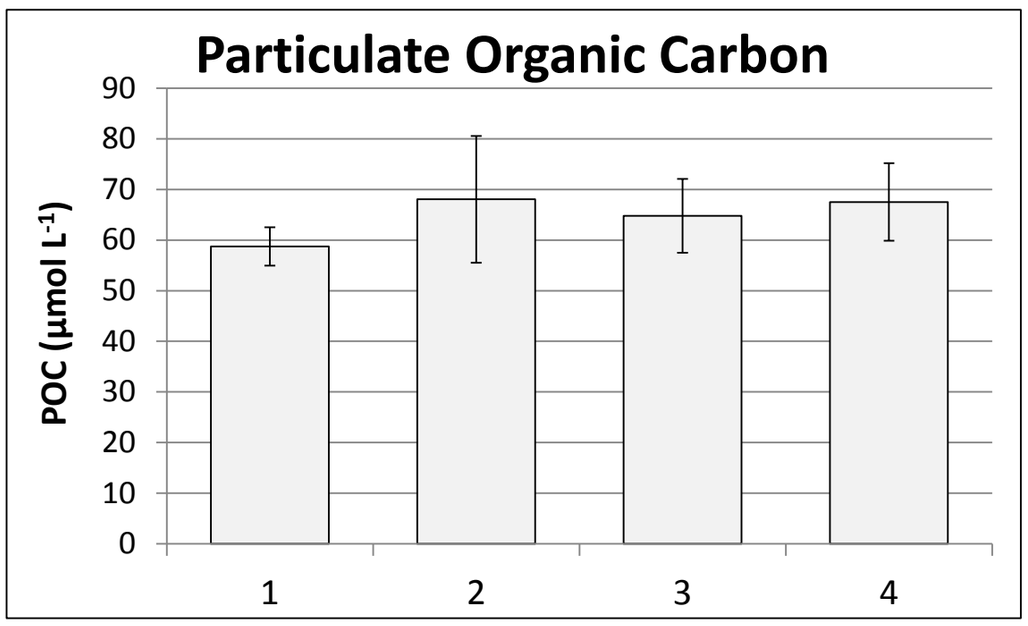

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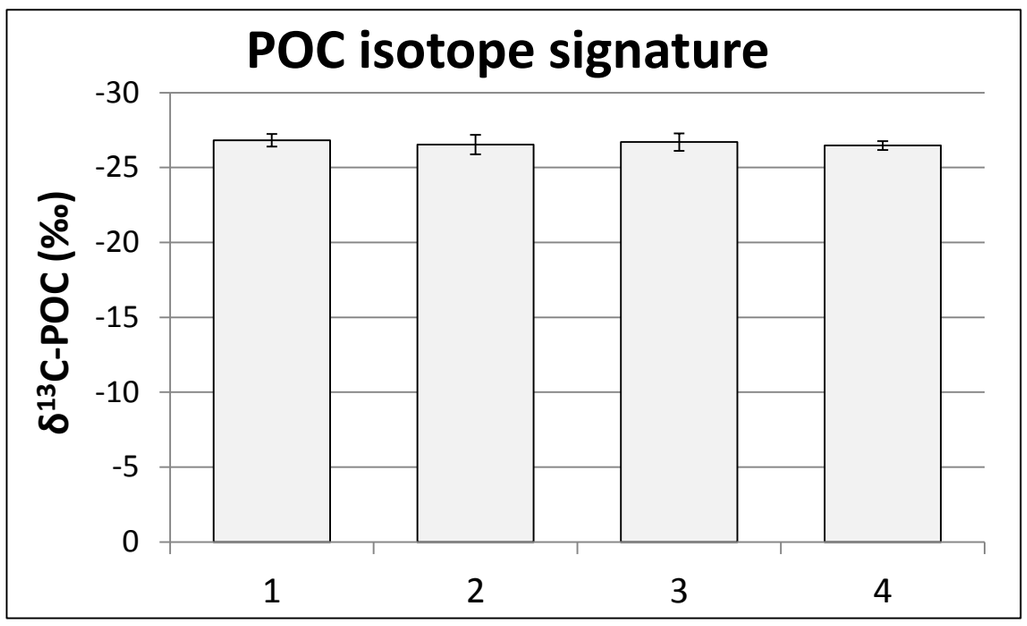

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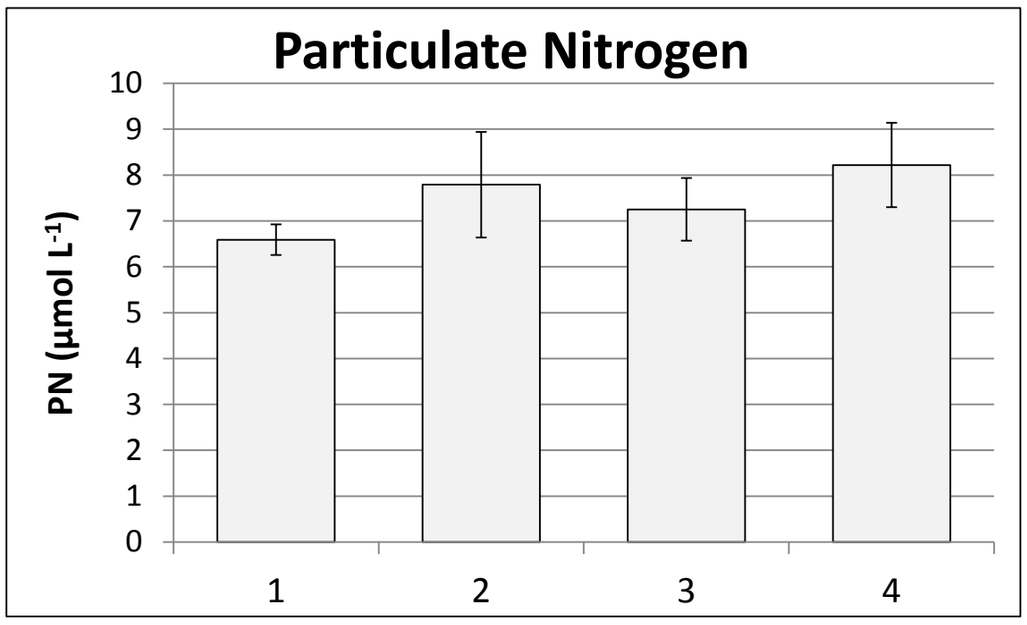

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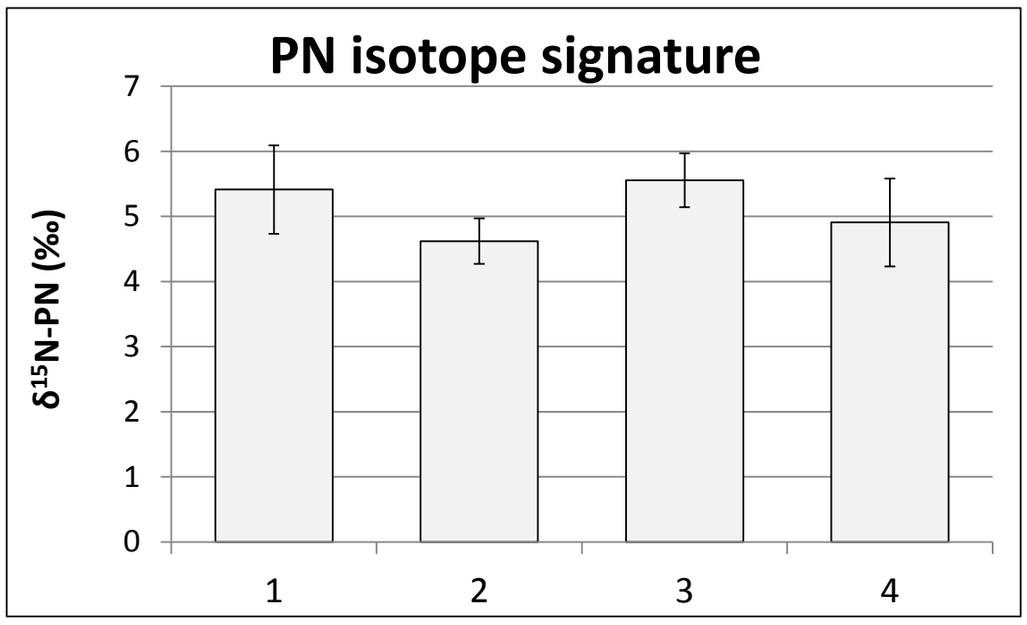

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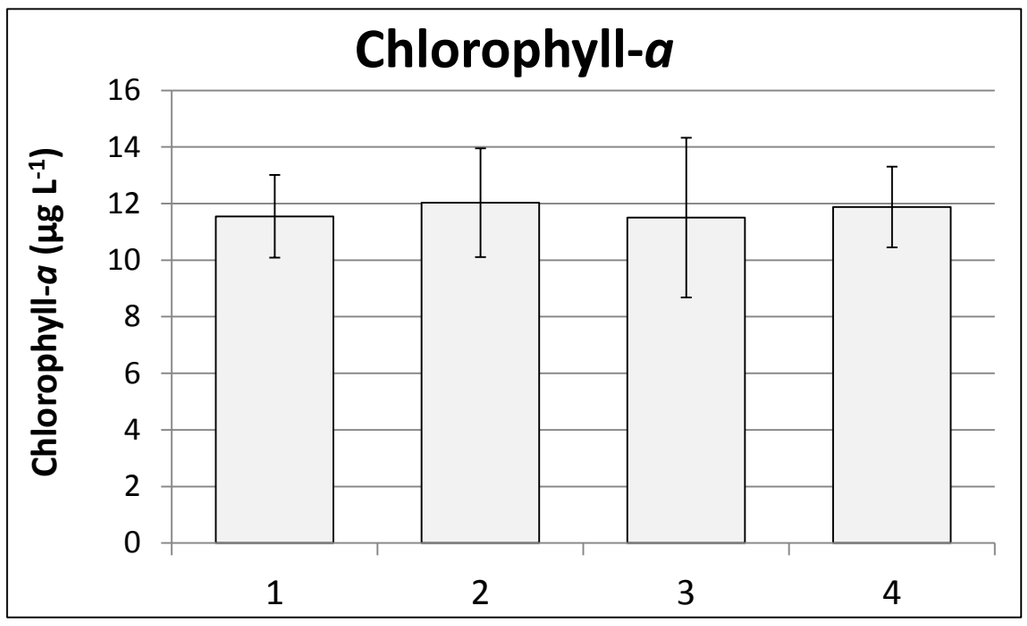

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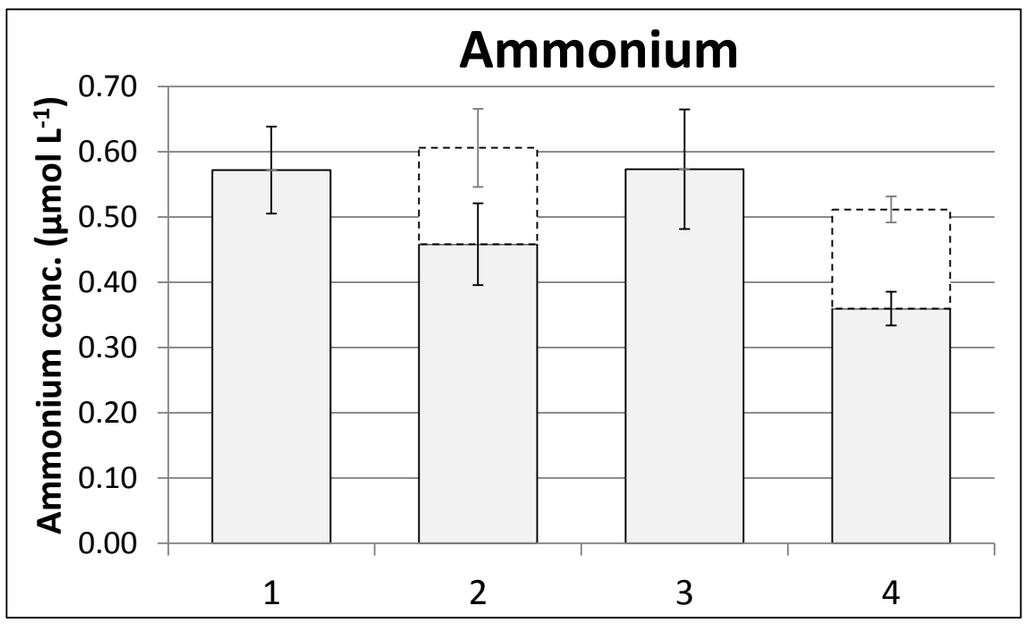

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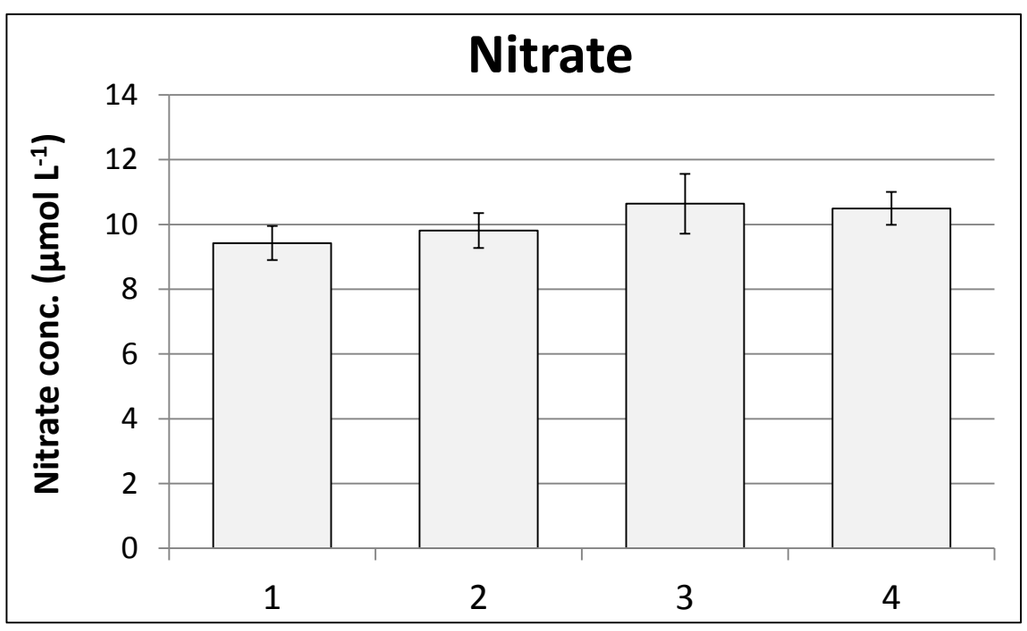

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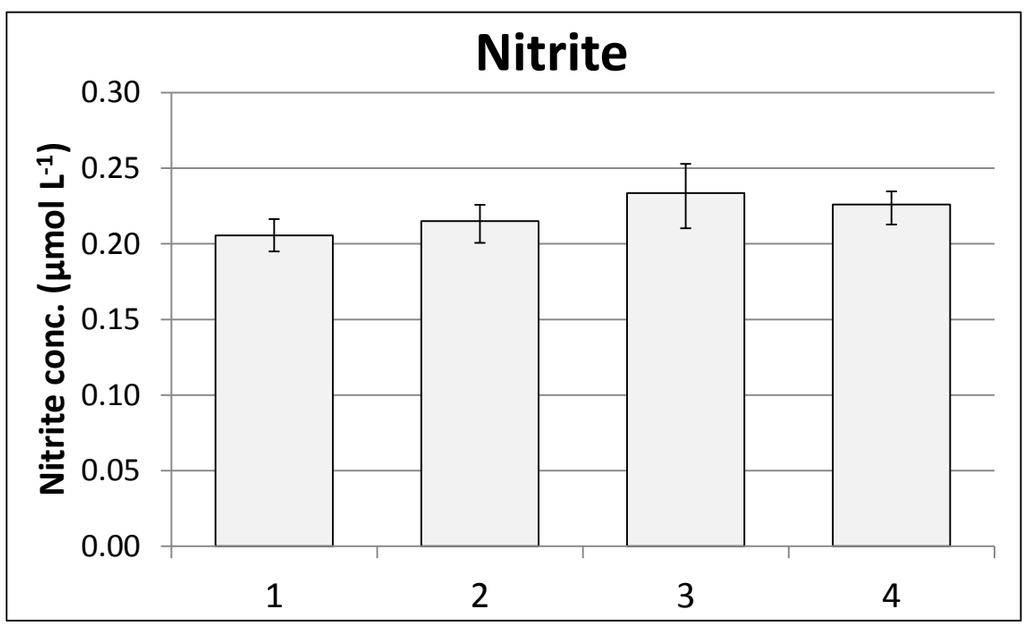

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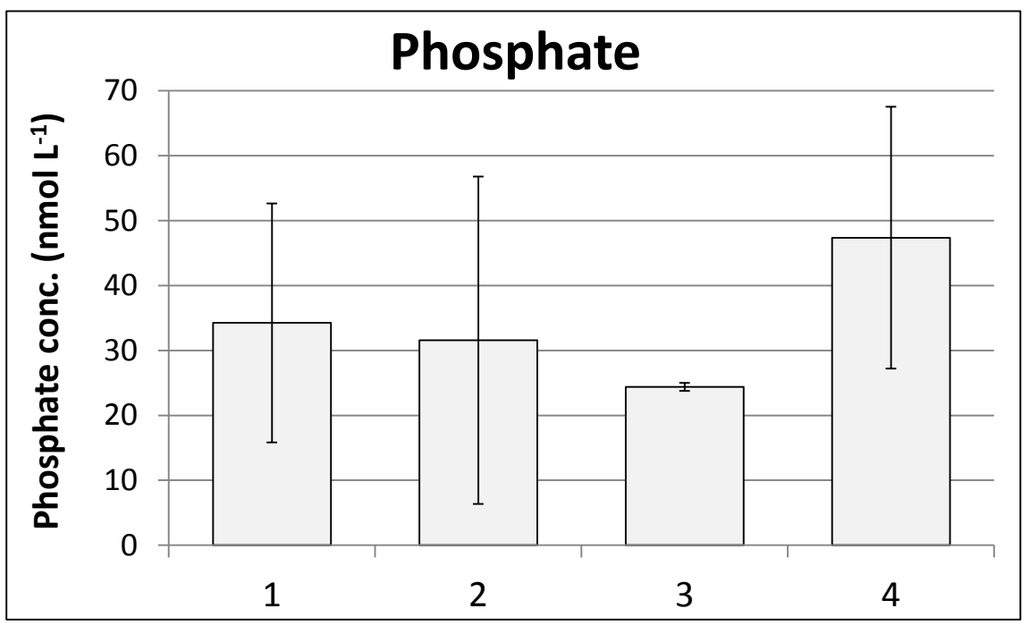

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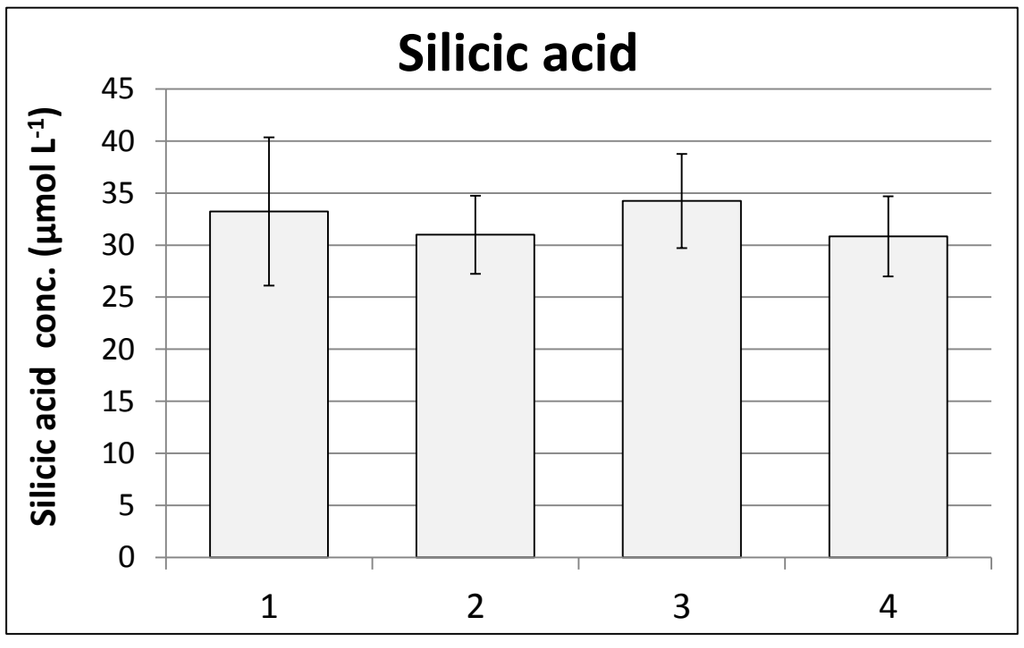

Table 1

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

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