Prof. Daniel Cziczo
Co-Editor of Atmospheric Chemistry and Physics

Dear Dan,

Listed below are our responses to the comments from the reviewers of our manuscript. For clarity and visual distinction, the referee comments or questions are listed here in black and are preceded by bracketed, italicized numbers (e.g. ***[1]***). Authors' responses are in red below each referee statement with matching numbers (e.g. ***[A1]***). We thank the reviewers for carefully reading our manuscript and for their helpful suggestions!

Sincerely,

Allan Bertram
Professor of Chemistry
University of British Columbia

Anonymous Referee #1

Summary

This study evaluates the abundance and characteristics of ice nucleating particles (INPs) in Canadian Arctic waters during the summer of 2014. Nucleation in the immersion freezing mode was quantified for microlayer and bulk (subsurface) seawater samples. Analysis of samples from eight process stations reveal that both bulk and microlayer samples contained elevated INP concentrations compared to an ultrapure water control, as well as station blanks (sample passed through a 0.02 μm filter). Contrary to previous experiments (Wilson et al. 2015), the results do not indicate an enrichment of INPs in the microlayer relative to the bulk samples. The concentration of INPs varies considerably between the eight geographically diverse process stations. The authors correlate INP concentrations with an array of 6 other variables (DMS concentration, bacterial and phytoplankton cell counts, temperature, pH, and salinity), finding that salinity provided the strongest and only statistically significant relationship with INP concentration. Finally, filtration and heating experiments suggest such INPs were between 0.02 μm and 0.2 μm in diameter and thermolabile, suggesting INPs were organic in composition and may have consisted of femtoplankton, cell fragments, or cell exudates/lysates.

General Comments

In all, this paper makes an important contribution to the field by helping to quantify the range of variability in INP concentration sourced from Arctic marine waters. The authors' effort to compare their results with other recent measurements is particularly laudable, and highlights the need for subsequent studies that contrast how oceanic variables, laboratory protocol, and sampling techniques ultimately affect measured INP concentrations. As such, this paper sets the stage nicely for further developments in the field, and therefore is well qualified for publication within ACP. Below, I offer a few questions and comments to strengthen the paper and clarify the results:

Scientific Comments

[1] Page 3, Lines 8 – 9: The authors report that the Niskin bottles were washed with large amounts of seawater before sampling at 0.5 meters below the surface commenced. At the same time, the results suggest that the microlayer samples did exhibit significant INP enrichment relative to the bulk seawater, in contrast to Wilson et al. 2015. Were the Niskin bottles rinsed on-site in the zodiac? If so, is it possible that the organic enriched microlayer was disturbed in the rinsing process, mixing INPs to subsurface waters and muddling the distinction between the two samples that would ordinarily exist?

[A1] To address the referee's comment we provided more information on the how the Niskin bottles were rinsed. Specifically the following text was added to the manuscript (Section 2.1):

"The Niskin bottle was not cleaned with isopropanol before sampling, but the inside of the bottle was rinsed with a large amount of seawater by lowering and leaving it in the seawater with the top and bottom lids open for about a minute before sending down the messenger to close the lids for sample collection. Sampling with the Niskin bottle and the hand-held glass plate were done on opposite sides of the zodiac to minimize the effect of sampling with the Niskin bottle on the microlayer."

[2] Page 3, Lines 33 – 35: Heating seawater to high temperatures (100 ˚C) and gradual cooling can cause salts, especially carbonates, to precipitate from solution (e.g. Anderson, 2005; Jones 1967; Harrison et al. 1980). Were precipitates observed during the thermodegradation heating tests? If so, have the calculations for Corrections for Freezing Temperature Depression (Section 2.4.4) taken this change in salinity/alkalinity into account? If precipitates did form and were unnoticed, then the corrected temperatures reported in Figure 4 may in fact be lower limits.

[A2] No precipitate was observed during the thermo degradation heating tests. Based on references suggested by the referee (Jones, 1967 and Harrison et al. 1980), if precipitate did form, the mass of the precipitate would be small compared to the total mass of dissolved material in seawater. Hence, the effect of precipitate on salinity would be small and would not significantly change the corrections for freezing point depression. Please let us know if we have misunderstood this comment.

[3] Page 5, Lines 15 – 17: A strong anticorrelation between salinity and ice nucleation efficacy was observed across the 8 process stations' samples. Studies have found that ice rafting and melting spurs cell growth (e.g. iron fertilization in the Southern Ocean (Duprat et al. 2016) and possibly phosphorus addition in the Arctic (Perrette et al. 2011)). Although there was only a moderate correlation between cell count and T10 value (or better in the microlayer – Table S2), was there a correlation between salinity and cell count? This would suggest that nutrient addition from melt water might be spurring cell growth and possibly INP production, with interesting implications for future Arctic and Greenland ice loss.

[A3] There exists a strong positive correlation between bacterial abundance and salinity in the microlayer ($R = 0.76$, $p = 0.039$). To address the referee's comment the following text has been added to Section 3.1:

"Also interesting, a strong positive correlation was observed between salinity and bacterial abundance ($R = 0.76$, $p = 0.039$). Consistent with these results, Galgani et al. (2016) observed a higher concentration of bacteria in the open sea (which had a higher salinity) compared to melt ponds (which had a lower salinity)."

Clarification and Technical Comments

[4] Page 1, Line 22 – 23: "INPs were ubiquitous in the microlayer and bulk seawater with freezing temperatures in the immersion mode as high as -14 °C," or something similar to indicate mode of activation.

[A4] The authors changed the wording in the abstract as suggested.

[5] Page 2, Line 10 – 11: "Modeling studies have also suggested that marine INPs may offset the magnitude of anthropogenic aerosol forcing by influencing cloud formation (Yun and Penner, 2013)." This is vaguely worded. How specifically do marine INPs reduce the negative anthropogenic forcing?

[A5] The authors have been more specific in wording this sentence in the revised manuscript. The following is the revised sentence added in Section 1:

"Modelling studies show that natural marine INPs may contribute to more ice formation in mixed-phase clouds, thereby reducing the magnitude of the total top-of-atmosphere anthropogenic aerosol forcing by as much as 0.3 W/m^2 (Yun and Penner, 2013)."

[6] Page 3, Line 23 – 25: The original (Vali 1971) notation makes it more clear that INP concentration is a function of temperature: use [INP(T)] instead of [INP]. This notation more clearly denotes that INP activation is temperature-dependent.

[A6] The term [INP] has been replaced with [INP(T)] throughout the manuscript.

[7] Page 16, Figure 2: What are the temperature uncertainties on a typical data point?

[A7] The caption for Figure 2 has been updated to report the uncertainty in temperature (±0.3 °C).

[8] Page 18, Figure 4: Since the data from Schnell (1975) and Schnell and Vali (1975) are so sparse, they would be easier to see if they were plotted over the other data.

[A8] The authors have updated Figure 4 according to this suggestion.

[9] Page 18, Figure 4: How are the reported uncertainties in [INP(T)] calculated?

[A9] In the original manuscript the upper and lower limits for [INP(T)] values were determined by assuming individual droplets in the freezing experiments could contain multiple INPs and only one INP, respectively, and the reported [INP(T)] values were midpoints between these upper and lower limits. In hindsight, this was not clear in the submitted manuscript nor was this the most appropriate way to describe our experimental data. In the revised manuscript, reported [INP(T)] values are calculated by assuming individual droplets in the freezing experiments contain multiple INPs, consistent with the discussion in Section 2.2.1. Upper and lower limits to [INP(T)] were calculated based on the limited number of nucleation events observed in the freezing experiments (Koop et al. (1997)). These upper and lower limits take into account the statistical uncertainty in the freezing experiments. The method used to calculate the uncertainties has been added to the caption of Figure 4 to make this clear.
Fortunately, the conclusions in our manuscript are not sensitive to the method we used to calculate [INP(T)] values or their uncertainties. Nevertheless, the method used to calculate

[INP(T)] values and their uncertainties in the revised manuscript is now clear and most appropriate.

[10] Supplement Pages 4-6, Figures S1–S3: It would be helpful if the x and y axes sizes and marks (latitude and longitude labels) were consistent between the sample-site plots and the chlorophyll a plots. That way, chlorophyll concentrations at sampling sites could more easily be determined.

[A10] As suggested, the x and y axes sizes and marks in these figures have been adjusted so they are consistent between the sample-site plots and the chlorophyll a plots (see Supplement).

Works Cited

Anderson, R.A. (ed.), Algal Culturing Techniques, Elsevier Academic Press, Burlington, Massachusetts, 578 pp, 2005.

Duprat, L.P., Bigg, G.R. and Wilton, D.J., Enhanced Southern Ocean marine productivity due to fertilization by giant icebergs, Nature Geoscience, 2016.

Harrison, P.J., Waters, R.E. and Taylor, F.J.R., A broad spectrum artificial sea water medium for coastal and open ocean phytoplankton. Journal of Phycology, 16(1), pp.28-35, 1980.

Jones, G.: Precipitates from Autoclaved Seawater, Limnology and Oceanography, 12(1), 165–167, 1967.

Perrette, M., Yool, A., Quartly, G.D. and Popova, E.E., Near-ubiquity of ice-edge blooms in the Arctic, Biogeosciences, 8(2), p.515, 2011.

Anonymous Referee #2

General Comment

Overall my comments are rather minor on this paper. It is a nice addition to the literature on the sources of ice nucleating particles from ocean seawater, and on expectations for enrichment or not in the sea surface microlayer. As detailed in my specific comments, I wonder if there is a reason to rule out non-colligative effects on freezing for explaining salinity variations, I felt it unfortunate that total organic carbon measurements were not included in order to compare with the Wilson et al. (2015, Nature) study, and I feel it would be nice to see the full influence of the heating studies on the temperature spectra of INPs. There is overall perhaps too much emphasis on 10% freezing conditions. Nevertheless, this is an excellent example of the suite of data that one might like to have when simultaneously collecting atmospheric samples over oceans. Minor revisions are recommended. Specific questions/comments for potentially addressing are listed below.

Specific Comments

Introduction

[11] Page 1, lines 37-38: “Homogeneous ice nucleation becomes increasingly important below approximately -33◦C. . .” This statement struck me as odd. Why -33◦C specifi- cally? Use of such a value seems to beg also listing a droplet size and a time scale. In fact, there are abundant observations in the literature of supercooled water present at this temperature and down to 4 or more degrees below this.

[A11] To address the referee’s comments we have added the justification for the choice of -33 °C in the revised manuscript (Section 1). The revised sentence is as follows:

"Homogeneous ice nucleation becomes increasingly important below approximately -33 °C for typical cloud sizes and atmospheric cooling rates (Herbert et al., 2015; Koop and Murray, 2016), but INPs can trigger ice formation in clouds at higher temperatures."

[12] Page 2, lines 13-14: It seems likely that the transfer to the atmosphere also remains a highly uncertain process on the basis of recent studies, although it is not a topic in this paper.

[A12] To address the referee's comments, this sentence has been modified to make it clear that the transfer to the atmosphere is also uncertain. The following is the revised sentence in Section 1:

"Nevertheless, our current understanding of the properties, concentrations, and spatial and temporal distributions of INPs in the microlayer and bulk seawater, as well as their transfer to the atmosphere, remains limited, leading to uncertainties when predicting their impacts on climate and the hydrological cycle."

Experimental

[13] Page 3, line 7: I was curious that there was no apparent pre-sterilization for microbial contamination. Does isopropanol assuredly do that?

[A13] Isopropanol has been used in previous pre-sterilisation protocols (Csuros, M., Environmental Sampling and Analysis for technicians, Lewis Publishers, NY, 1994). This has been made clear in the revised manuscript (Section 2.1).

[14] Page 3, line 20: Should blanks be in quotes? The reason is that this cannot be a true blank. There are literature reports of sub-20 nm particles acting as INPs. I think you will refer to these as "blanks".

[A14] As suggested we have used quotes when referring to blanks throughout the manuscript and also point out that the "blanks" may still contain sub-20 nm particles that can act as INPs. Specifically, we have added the following text to Section 3.1:

"The "blanks" may still contain some INPs, since some particles < 0.02 μm in diameter can act as INPs (Dreischmeier et al., 2017; O'Sullivan et al., 2015)."

[15] Page 3, line 21: Can you state a conductivity level on the DI water?

[A15] In the revised manuscript the conductivity level has been stated in Section 2.2.1.

[16] Page 4, Line 5: Did you happen to test the filters after rinsing with ultrapure water?

[A16] The filters were not tested after rinsing with ultrapure water. Is the referee concerned that the filters contain INPs?

[17] Page 4, line 8: First, it would seem appropriate to state that the water activity correction is an average one based on fits, since uncertainties commonly occur. The authors may also wish to discuss how other elements in the seawater that induce non-colligative freezing effects might stymie this approach. So, for example, what if seawater contained AFPs?

[A17] To address the referee's comments in the revised manuscript we have pointed out the approximations in the water activity correction in Section 2.2.4 by using the following sentence:

"The freezing temperature correction was calculated using the median freezing temperature of each sample and then applied to the rest of the droplet freezing temperatures within that sample."

We also pointed out that the water activity correction does not consider non-colligative freezing effects, although non-colligative freezing effects have not been observed in previous immersion studies with sodium chloride solutions or seawater. The following text was added to the end of Section 2.2.4:

"The water activity corrections do not consider non-colligative effects; however, non-colligative effects have not been observed in previous immersion freezing studies with sodium chloride solutions (Alpert et al., 2011a, 2011b; Knopf et al., 2011; Zobrist et al., 2008) or seawater (Wilson et al., 2015)."

Results and Discussion

[18] Page 5, line 13: What is significant about this arbitrary T10 value chosen? Should not correlations be checked for a range of fractions or at single temperatures? Also, were any TOC measurements made? This seems a missed opportunity to correlate with the relation suggested in Wilson et al. (2015).

[A18] Unfortunately, we did not have reliable measurements of TOC from the cruise. To address the referee's comment, in the revised manuscript we checked for correlations at an additional fraction, specifically T_{50}. This information was indicated in the main text with the following sentence:

"A similar trend was observed for T_{50}-values, where T_{50} represents the temperatures at which 50% of droplets had frozen (Table S2)."

[19] Page 5, line 20: Note the extra space at the end of this sentence. Also, can you rule out non-colligative freezing effects that scale with salinity? I have no reason to understand why this would be so, since I know of no such studies for seawater. It could be useful to show a plot of the relation you are discussing, in the supplemental material, if not in the main manuscript. Then it might be clear if the correlation shows any bias that could be explained by a constant "delta" on the freezing temperature.

[A19] We can not rule out non-colligative freezing effects, but on the other hand, non-colligative freezing effects have not been observed in previous immersion freezing studies with sodium chloride solutions (Alpert et al., 2011a, 2011b; Knopf et al., 2011; Zobrist et al., 2008) or seawater (Wilson et al., 2015). To address the referee's comment, the following information has been added to Section 3.1:

"Another possible explanation for the strong negative correlation between salinity and freezing temperatures is a non-colligative effect not accounted for in the corrections for freezing temperature depression discussed in Section 2.2.4. However, as mentioned in Section 2.2.4, non-colligative effects have not been observed in previous immersion freezing studies with sodium chloride solutions (Alpert et al., 2011a, 2011b; Knopf et al., 2011; Zobrist et al., 2008) or seawater (Wilson et al., 2015)."

In addition, correlation plots between T_{10} and the variables investigated have been added in the supplement (Figure S1).

[20] Page 6, lines 12-13: I suspect that additional studies could also indicate which method is closer to correct, or if a new and more elaborate method might be warranted.

[A20] We are not sure if the referee would like us to add additional text about this point.

[21] Page 7, lines 3-4: The conclusion made here provides a reason to show full temperature spectra for sizing analysis. Would differences stand out at certain temperatures? Or at lower levels of freezing?

[A21] To address the referee's comments, we have added a figure to the supplement (Figure S5) that shows the full temperature spectra for the sizing analysis.

[22] Page 7, lines 7-9: I am curious if there are known things that are non-microbial or nonproteinaceous that are denatured by the heat level used. Is there an expectation that the composition of exudates would be unstable at 100◦C? I do not know the answer, just asking, as O'Sullivan et al. (2015, Scientific Reports) does not suggest anything other than microbial fragments and proteins as being particularly heat sensitive.

[A22] We are not aware of studies that have investigated the stability of exudates to heat. In our discussion where we suggested exudates as the possible source of INPs we assumed that exudates are unstable/denature at 100 °C. However, we recognised that this is not a certainty and we have changed this sentence to reflect this point in Section 3.2.2. Below is the original sentence followed by the modified sentence:

"Potential sources of the INPs observed in this study include ultramicrobacteria, viruses, phytoplankton exudates, or bacteria exudates, all of which could be denatured by heat, but are less than 0.2 µm in size (Ladino et al., 2016; Wilson et al., 2015)."

Modified sentence:

"Potential sources of the INPs observed in this study include ultramicrobacteria, viruses, phytoplankton exudates, or bacteria exudates (Ladino et al., 2016; Wilson et al., 2015)."

Summary and conclusions

[23] Page 7, line 18: "Biological materials" seems too broad or non-specific of a category. They are heat labile biological materials, which might imply something more (i.e., comment just above)?

[A23] In the revised manuscript, the term biological material has been modified to heat labile biological materials throughout the manuscript.

Figures

[24] Figure 6: Question - if only 15 to 20 drops are used, how are frozen fractions below 5

[A24] 15 to 20 drops were used in a single freezing experiment, but for each sample, freezing experiments were performed 3 times, resulting in 45-60 freezing events per sample. This point

has been made clear in the revised manuscript with the following sentence added to the caption of Figure 2:

"Each set of line and markers represents the results for 3 repeat experiments of a sample or "blank", adding up to a total of between 45 to 60 freezing events in each set."

[25] Figure 7: It might be interesting to see separate plots for each filtering size as a function of temperature, as in other plots. This would highlight if any differences occur at low freezing fractions at the warmest temperatures and how things vary with processing temperature. In that manner, the full exclusion of a role of larger particles in the bacterial size range might be better supported.

[A25] See response [A21].

Ice nucleating particles in Canadian Arctic sea-surface microlayer and bulk seawater

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Correspondence to: Allan Bertram (bertram@chem.ubc.ca)

Abstract:

The sea-surface microlayer and bulk seawater can contain ice-nucleating particles (INPs) and these INPs can be emitted into the atmosphere. Our current understanding of the properties, concentrations, spatial and temporal distributions of INPs in the microlayer and bulk seawater is limited. In this study we investigate the concentrations and properties of INPs in microlayer and bulk seawater samples collected in the Canadian Arctic during the summer of 2014. INPs were ubiquitous in the microlayer and bulk seawater with freezing temperatures in the immersion mode as high as -14 °C. A strong negative correlation (R = -0.7, p = 0.02) was observed between salinity and freezing temperatures (after correction for freezing depression by the salts). One possible explanation is that INPs were associated with melting sea ice. Heat and filtration treatments of the samples show that the INPs were likely heat-labile biological materials with sizes between 0.02 µm and 0.2 µm in diameter, consistent with previous measurements off the coast of North America and near Greenland in the Arctic. The concentrations of INPs in the microlayer and bulk seawater were consistent with previous measurements at several other locations off the coast of North America. However, our average microlayer concentration was lower than previous observations made near Greenland in the Arctic. This difference could not be explained by chlorophyll *a* concentrations derived from satellite measurements. In addition, previous studies found significant INP enrichment in the microlayer, relative to bulk seawater, which we did not observe in this study. While further studies are needed to understand these differences, we confirm that there is a source of INP in the microlayer and bulk seawater in the Canadian Arctic that may be important for atmospheric INP concentrations.

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1 Introduction

Ice can form in clouds by homogeneous or heterogeneous ice nucleation. Homogeneous ice nucleation refers to ice nucleation in the absence of a foreign substrate, while heterogeneous ice nucleation refers to ice nucleation initiated by a foreign substrate or an ice-nucleating particle (INP). Homogeneous ice nucleation becomes increasingly important below approximately -33 °C for typical cloud sizes and atmospheric cooling rates (Herbert et al., 2015; Koop and Murray, 2016), but INPs can trigger ice formation in clouds at higher temperatures. Therefore INPs in the atmosphere can affect Earth's climate and the hydrological

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cycle by altering the microphysics, radiative properties, and lifetime of clouds (DeMott et al., 2010; Lohmann, 2002; Lohmann and Feichter, 2005; Tan et al., 2016).

Field and laboratory studies have shown that the sea-surface microlayer and bulk seawater contain INPs, and that these INPs can be emitted to the atmosphere by the bubble bursting mechanism (Alpert et al., 2011a, 2011b; Blanchard, 1964; DeMott et al., 2015; Fahlgren et al., 2015; Fall and Schnell, 1985; Knopf and Forrester, 2011; Prather et al., 2013; Rosinski et al., 1988; Schnell, 1977; Schnell and Vali, 1975, 1976; Vali et al., 1976; Wang et al., 2015; Wilson et al., 2015). The sea-surface microlayer (herein referred to as the microlayer) is the interface between the ocean and the atmosphere. The thickness of the microlayer is < 1 mm (Liss and Duce, 1997), and the physical and chemical properties of the microlayer are different from the bulk seawater (Zhang et al., 2003). For example, the concentration of organic material is often enhanced in the microlayer compared to the bulk seawater (Wurl et al., 2009).

Modelling studies have suggested that the ocean can be a dominant source of INPs in the atmosphere when dust concentrations are low (Burrows et al., 2013; Vergara-Temprado et al., 2017; Wilson et al., 2015). Modelling studies show that natural marine INPs may contribute to more ice formation in mixed-phase clouds, thereby reducing the magnitude of the total top-of-atmosphere anthropogenic aerosol forcing by as much as 0.3 W/m^2 (Yun and Penner, 2013). Nevertheless, our current understanding of the properties, concentrations, and spatial and temporal distributions of INPs in the microlayer and bulk seawater, as well as their transfer to the atmosphere, remains limited, leading to uncertainties when predicting their impacts on climate and the hydrological cycle.

Prior to our work, five studies had examined INPs in bulk waters around North America and near Greenland (Fig. 1), but only one quantified INPs in the microlayer in the immersion mode (Wilson et al., 2015). The immersion mode refers to heterogeneous freezing caused by INPs immersed in liquid droplets, which is the mode most relevant for mixed-phase clouds in the atmosphere (Murray et al., 2012). Our work adds more measurements to the limited data on INPs in the microlayer and bulk seawater, contributing to a better understanding of how the properties and concentrations of INPs in the microlayer vary with location and time.

We investigated the concentrations and properties of INPs in the microlayer and bulk seawater samples in the immersion mode collected in the Canadian Arctic (Fig. 1) during the summer of 2014. The Arctic was chosen for these studies because 1) clouds in this region have been found to be especially sensitive to atmospheric concentrations of INPs (Harrington et al., 1999; Jiang et al., 2000), 2) there have not been previous studies of the freezing properties of the microlayer or bulk seawater in this region, and 3) as sea ice continues to decrease in the Arctic, the microlayer and bulk seawater may become more important sources of INPs in this region.

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2 Experimental

2.1 Sampling locations and collection methods

All samples were collected during July and August 2014 from the eastern Canadian Arctic on board the Canadian research icebreaker CCGS *Amundsen* as part of the Network on Climate and Aerosols: addressing key uncertainties in Remote Canadian Environments (NETCARE) project. The locations of the eight stations sampled in this study are shown in Fig. 1 while Table 1 describes sampling times and specific geographic coordinates of these stations. Supplementary details, including notes and photographs taken at each station during sampling are provided in Table S1.

The microlayer samples were collected using a glass plate sampler (Harvey and Burzell, 1972) from the upwind side of a small inflatable, rigid-hull boat, at least 500 m away from the CCGS *Amundsen* to avoid contamination. The glass plate was immersed

vertically and withdrawn at a slow rate (between 3 to 5 cm/s) and allowed to drain for less than 5 s. The microlayer that adhered to the plate from each dip was scrapped off from one side of the glass plate with a neoprene wiper blade into a 1L high-density polyethylene (HDPE) Nalgene bottle. For each microlayer sample, approximately 500 – 1000 mL was collected, requiring 115-185 dips. Based on the amount of material collected, the number of dips and the area of the plate, the thickness of the layer collected ranged between 60 and 220 µm. Bulk seawater samples were collected at the same times and locations as the microlayer samples using a Niskin bottle deployed from the downwind side of the zodiac. Samples were collected at 0.5 m depth and transferred to 1L HDPE Nalgene bottles. After collection, the Nalgene bottles containing both the microlayer and bulk samples were kept cool in an insulated container. Upon returning to the ship, the samples were homogenised by gently inverting them at least ten times and then sub-sampled into smaller bottles for subsequent analyses.

The glass plate, neoprene wiper blade and all Nalgene bottles were cleaned with isopropanol and ultrapure water and rinsed with approximately 10 mL of the seawater sample before use. Isopropanol has been used in previous pre-sterilisation protocols (Csuros, 1994). The Niskin bottle was not cleaned with isopropanol before sampling, but the inside of the bottle was rinsed with a large amount of seawater by lowering and leaving it in the seawater with the top and bottom lids open for about a minute before sending down the messenger to close the lids for sample collection. Sampling with the Niskin bottle and the hand-held glass plate were done on opposite sides of the zodiac to minimize the effect of sampling with the Niskin bottle on the microlayer.

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2.2 Ice nucleation properties of the samples

2.2.1 Droplet freezing technique and INP concentrations

INP concentrations as a function of temperature were determined using the droplet freezing technique (DFT; Koop et al., 1998; Vali, 1971; Whale et al., 2015; Wilson et al., 2015). Sub-samples of the microlayer and bulk seawater were stored in Nalgene bottles frozen at -80 °C for a maximum of nine months before INP analysis. A previous study suggests that freezing seawater samples does not significantly change the freezing properties of the samples (Schnell and Vali, 1975). Each microlayer and bulk seawater sample was completely thawed and homogenised by inverting at least ten times. Between 15 to 20 droplets of the sample, with volumes of 0.6 µL each, were deposited onto a hydrophobic glass slide (HR3-215; Hampton Research, Aliso Viejo, CA, USA) using a pipette. The slides were put into an airtight cell (Parsons et al., 2004), attached to a cold stage and analysed by the DFT as detailed in Wheeler et al. (2015). The droplets were cooled at a constant rate of 5 °C/min from 0 °C to -35 °C. Each experiment was repeated three times using three different slides. "Blanks" were determined by filtering the microlayer and bulk samples through a 0.02 µm Anotop 25 filter. Ultrapure water (distilled water further purified with a Millipore system, 18.2 MΩcm at 25 °C) was also analysed for INPs using the DFT for comparison.

The concentration of INPs, *[INP(T)]*, was determined from each freezing experiment by the following equation (Vali, 1971):

$$\left[INP(T)\right] = -\ln\left(\frac{N_u(T)}{N_o}\right)N_o \cdot \frac{1}{V} \quad (1)$$

Where $N_u(T)$ is the number of unfrozen droplets at temperature T, N_o is the total number of droplets used in the experiment and V is the volume of all droplets in a single experiment. This equation accounts for the possibility of multiple INPs contained in a single droplet.

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2.2.2 Heating tests

The freezing temperatures of the microlayer and bulk samples were also measured after they had been heated to 100 °C (Christner et al., 2008; Schnell and Vali, 1975; Wilson et al., 2015). This temperature was chosen because some biological

materials have been shown to lose their ice nucleation activity following heating to 95 °C (Christner et al., 2008), possibly due to denaturation of the tertiary structure of ice nucleating proteins (Hill et al., 2016). Samples of microlayer and bulk seawater were put into polypropylene tubes, sealed with lids, and heated to 100 °C in a heating block (Accublock, Labnet, S/N: D1200) for an hour, then cooled to room temperature for approximately 30 minutes before freezing measurements.

2.2.3 The size of the INPs

Following Wilson et al. (2015), the microlayer and bulk seawater samples were passed through filters with three different pore sizes (Whatman 10 µm pore size PTFE membranes, Millex –HV 0.2 µm pore size PTFE membranes, and Anotop 25 0.02 µm pore size inorganic Anopore™ membranes). These filtered samples were subsequently used in the freezing measurements.

2.2.4 Corrections for freezing temperature depression

Since the microlayer and bulk seawater samples contained salts, the measured freezing temperatures were adjusted for the presence of the salts. Using measured salinities and the water activity based approach (Koop and Zobrist, 2009) hypothetical heterogeneous freezing temperatures for salt-free conditions were obtained (salinity = 0 g/kg). The freezing temperature correction was calculated using the median freezing temperature of each sample and then applied to the rest of the droplet freezing temperatures within that sample. For details see Supplement, Section 1. The salinities of the microlayer and bulk seawater samples were measured within 10 minutes of sample collection using a hand-held salinity probe (SympHony; VWR, Radnor, PA, USA) which had been calibrated against discrete seawater samples analysed on a Guildline Autosal 8400B. The correction for the presence of salts based on the measured salinities ranged from 2.0 to 2.8 °C. Hypothetical heterogeneous freezing temperatures for salt-free conditions is more relevant for mixed phase clouds, where freezing typically occurs in dilute aqueous droplets with low salt concentrations (i.e., where water activity tends toward unity). The water activity corrections do not consider non-colligative effects; however, non-colligative effects have not been observed in previous immersion freezing studies with sodium chloride solutions (Alpert et al., 2011a, 2011b; Knopf et al., 2011; Zobrist et al., 2008) or seawater (Wilson et al., 2015).

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2.3 Phytoplankton and bacterial abundance

Duplicate 5 mL sub-samples were fixed with 20 µL of 25% Grade I glutaraldehyde (0.1% final concentration; Sigma-Aldrich G5882) and kept frozen at -80 °C until analysis by flow cytometry, within 7 months of collection (Marie et al., 2005). Cyanobacteria were identified by orange fluorescence from phycoerythrin (575 ± 20 nm). Heterotrophic bacteria samples were stained with SYBR Green I and measured at 525 nm to detect low and high nucleic acid content (Belzile et al., 2008). Archaea could not be discriminated from bacteria using this protocol; therefore, hereafter, we use the term bacteria to include both archaea and bacteria. Photosynthetic eukaryotes were identified by red fluorescence of chlorophyll (675 ± 10 nm). In each sub-sample, microspheres (1 µm and 2 µm, Fluoresbrite plain YG, Polysciences) were added as an internal standard as described by Tremblay et al. (2009). Analyses were performed on an Epics Altra flow cytometer (Beckman Coulter), fitted with a 488 nm laser (15 mW output; blue), using Expo32 v1.2b software (Beckman Coulter).

2.4 Dimethlysulphide (DMS) measurements

Concentrations of DMS were measured on board the ship within approximately two hours of sampling. The samples were analysed by gas chromatography following purging and cryo-trapping according to protocol described in Lizotte et al. (2008).

2.5 Statistical analysis

Pearson correlation analysis was applied to many of the variables measured in this study to compute correlation coefficients (R). Here we use the scheme from Dancey and Reidy (2002) where correlations with R values of 0.1-0.3, 0.4-0.6 and 0.7-0.9 are classified as weak, moderate and strong, respectively. P values were also calculated to determine if the correlations were statistically significant at the 95 % confidence level ($p < 0.05$).

3 Results and Discussion

3.1 INPs in the microlayer and bulk seawater

The fraction of droplets frozen in the immersion mode for both the unfiltered microlayer and bulk seawater samples are shown in Fig. 2. In this figure the "blanks" refer to the freezing properties of the sample after 0.02 µm filtration. The "blanks" may still contain some INPs, since some particles < 0.02 µm in diameter can act as INPs (Dreischmeier et al., 2017; O'Sullivan et al., 2015). The freezing properties of the "blanks" (after correction for freezing point depression by the salts) are similar to or lower than the freezing properties of ultrapure water, which are also shown in Fig. 2. The fraction-frozen curves for each station fall at warmer temperatures than their respective "blanks", indicating that the microlayer and bulk seawater samples from all stations contained INPs. Box plots of the T_{10}-values for the "blanks", the microlayer and bulk seawater samples are shown in Fig. 3, where T_{10} represents the temperatures at which 10% of droplets had frozen. Figure 3 shows that the interquartile range of freezing temperatures for the samples is higher than the interquartile range of freezing temperatures for the "blanks", further illustrating that INPs were present in the microlayer and bulk seawater samples.

The freezing curves varied significantly from sample to sample (Fig. 2). To understand this variability we investigated correlations between the T_{10}-values for the bulk seawater samples and the chemical and physical properties of the bulk seawater (DMS concentration, bacterial and phytoplankton abundance, seawater temperature, pH and salinity). Correlation coefficients were not statistically significant ($p > 0.05$), except in the case of salinity (Table 2 and Fig. S1 in Supplement). A strong negative correlation ($R = -0.7$, $p = 0.02$) was observed between salinity and the T_{10}-values (corrected for freezing depression by the salts). This suggests that more INPs were found in less saline waters. A similar trend was observed for T_{50}-values, where T_{50} represents the temperatures at which 50% of droplets had frozen (Table S2). One possible explanation is that the INPs were associated with melting sea ice. Materials such as algal aggregates, sea ice diatoms and extracellular polymeric substances can be released into the ocean upon sea ice melting (Assmy et al., 2013; Boetius et al., 2015; Fernández-Méndez et al., 2014) and might be potential sources of the INPs observed in this study. Also interesting, a strong positive correlation was observed between salinity and bacterial abundance ($R = 0.76$, $p = 0.039$). Consistent with these results, Galgani et al. (2016) observed a higher concentration of bacteria in the open sea (which had a higher salinity) compared to melt ponds (which had a lower salinity). Another possible explanation for the strong negative correlation between salinity and freezing temperatures is a non-colligative effect not accounted for in the corrections for freezing temperature depression discussed in Section 2.2.4. However, as mentioned in Section 2.2.4, non-colligative effects have not been observed in previous immersion freezing studies with sodium chloride solutions (Alpert et al., 2011a, 2011b; Knopf et al., 2011; Zobrist et al., 2008) or seawater (Wilson et al., 2015).

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The concentration of INPs as a function of temperature, *[INP(T)]*, for the microlayer samples analysed in this study is shown in Fig. 4A. Also included in Fig. 4A are results from Wilson et al. (2015) for the microlayer samples they collected at the locations shown in Fig. 1A. Concentrations of INPs in microlayer samples at stations 2, 9, and 10 overlap with the INP concentrations observed by Wilson et al. (2015) in the Atlantic. However, the INP concentrations in the microlayer measured by Wilson et al. (2015) to the east of Greenland are higher than the concentrations measured here.

Figure 4B shows the concentrations of INPs as a function of temperature for the bulk seawater samples. Also included in Fig. 4B are results from other studies (see Fig. 1B for locations) that measured INPs in samples of bulk seawater or samples containing a mixture of the microlayer and bulk seawater. The range of concentrations observed in our studies agrees well with the range observed by Schnell and Vali (1975), Schnell (1977) and Wilson et al., (2015) (both Arctic and Atlantic). Note, the bulk seawater freezing data from Wilson et al., (2015) was at the detection limit of their instrument; therefore, their INP concentrations for bulk seawater should be considered upper limits.

A strong positive correlation ($R = 0.9$, $p = 0.002$) between the freezing properties of the microlayer and the freezing properties of the bulk seawater was observed in the current study. Shown in Fig. 5A is a correlation plot between the T_{10}-values from the microlayer and bulk seawater samples. The data points, except for one, fall upon the 1:1 line, if the uncertainties in the measurements are considered. In contrast, Wilson et al. (2015) found significantly more INPs in the microlayer than in bulk seawater (Fig. 5B) in both their Arctic and Atlantic samples. Figure 5 also shows correlation plots for bacterial abundance in the microlayer and bulk seawater for this study (Fig. 5C) and from Wilson et al. (2015) (Fig. 5D). Similar bacterial abundances were observed in the microlayer and bulk seawater in the current study, whereas Wilson et al. (2015) found a higher bacterial abundance in the microlayer compared to the bulk seawater in most samples (Fig. 5D).

The differences between the results in the current study and the results from Wilson et al. (2015) may be, in part, related to sampling techniques. In the current study, the bulk seawater was sampled from a depth of 0.5 m while Wilson et al., (2015) sampled from a depth of 2-5 m. In addition, in the current study the glass plate technique used collected a layer that was up to 220 µm thick, while Wilson et al. (2015) used a hydrophilic Teflon film on a rotating drum fitted to a remote-controlled sampling catamaran which collects a microlayer of thickness between 6 to 83 µm (Knulst et al., 2003). Other studies have shown that different sampling techniques lead to different measured enrichments of the microlayer. Aller et al. (2017) compared the enrichments of the microlayer determined with the glass plate and a hydrophilic Teflon film on a rotating drum. They observed an enrichment (by a factor of approximately two) of bacteria in the microlayer when using the rotating drum, but no enrichment when using the glass plate technique. In addition, they observed an enrichment of transparent exopolymer material in the microlayer when using the rotating drum, but a smaller enrichment was observed when using the glass plate technique. Note that Aller et al. (2017) allowed seawater to stand in a 250 gallon tank for one hour before sampling the microlayer with a glass plate whereas the microlayer sampled with the rotating drum was taken directly from the ocean. Additional studies are needed to determine if the methodology used to sample the microlayer and bulk seawater strongly influences measured INP concentrations.

The differences between the results in the current study and the results from Wilson et al. (2015) may also be related to differences in the state of the ocean at the time of sampling. To investigate this we compared monthly average chlorophyll *a* concentrations for both studies. As illustrated in Figs. S2-S4 (Supplement) a clear difference between chlorophyll *a* concentrations in the current study and the Wilson et al. (2015) study was not observed.

Wind speed could also affect the stability of the microlayer and explain differences between results from the current study and the Wilson et al. (2015) study. Previous studies suggest that a microlayer may be stable up to the global average wind speed of 6.6 m/s (Wurl et al., 2011). During the current study, sampling was carried out at wind speeds ranging from 0.7 m/s to 6.7 m/s, while Wilson et al., (2015) carried out sampling at wind speeds ranging from 1.2 m/s to 5.9 m/s. The similar wind speeds in both

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studies and the fact that almost all sampling was carried out with wind speeds less than the global average suggests that the observed differences in INP concentrations is not due to wind speeds.

3.2 Properties of the INPs

3.2.1. Heat-labile biological material

The fraction frozen curves of samples before and after heating to a temperature of 100 °C are shown in Fig. 6. For 7 out of 8 of the microlayer samples, and all of the bulk samples, the fraction-frozen curves are shifted to colder temperatures after heating. These results suggest that the INPs in most cases are heat-labile biological material, consistent with previous measurements of the properties of INPs in the microlayer (Wilson et al., 2015) and bulk seawater (Schnell and Vali, 1975, 1976; Schnell, 1977).

3.2.2 Size of INPs

The T_{10}-values as a function of filter pore size (0.02 µm, 0.2 µm and 10 µm) are shown in Fig. 7. For over half the samples (microlayer samples at Stations 4 and 5, bulk samples at Station 6 and bulk and microlayer samples at Stations 7, 9, and 10) the sizes of the INPs were clearly between 0.02 µm and 0.2 µm, as the T_{10}-values significantly decreased when the samples were passed through a 0.02 µm filter but not when passed through a 0.2 µm filter. For the other samples (bulk samples at Stations 4 and 5, microlayer samples at Station 6, and microlayer and bulk samples at Stations 2 and 8), the uncertainties were too large to draw a clear conclusion about the effect of filtration. Plots of the fraction of droplets frozen versus temperature for samples filtered with a 0.02 µm, 0.2 µm and 10 µm filter are shown in Fig. S5 (Supplement) and are consistent with the results shown in Fig. 7.

The 0.02 - 0.2 µm size range for the INPs identified here is consistent with previous studies of INPs in the microlayer or bulk seawater. Wilson et al. (2015) concluded that INPs in the microlayer were between 0.02 µm and 0.2 µm in size. Rosinski et al. (1986) found that ice freezing nuclei in aerosol of marine origin were below 0.5 µm in size. Schnell and Vali (1975) found ocean-derived ice nuclei to be below 1 µm in size.

The size of whole cell marine bacteria or phytoplankton (excluding femtoplankton) is typically greater than 0.2 µm (Burrows et al., 2013; Sieburth et al., 1978), hence whole cell marine bacteria are unlikely to be the source of the INPs identified here. Furthermore, correlations between INP concentrations and bacterial or phytoplankton abundance were not statistically significant (p-values > 0.05; see supplemental Table S3). This is consistent with the suggestion that whole cells are not the source of the INPs. Potential sources of the INPs observed in this study include ultramicrobacteria, viruses, phytoplankton exudates, or bacteria exudates (Ladino et al., 2016; Wilson et al., 2015).

4 Summary and conclusions

Concentrations of INPs in the microlayer and bulk seawaters at eight different stations in the Canadian Arctic were determined. Results showed that the INPs were ubiquitous in the microlayer and bulk seawater and that freezing temperatures as high as -14°C were observed in both the microlayer and bulk seawater. A strong negative correlation (R = -0.7, p = 0.02) was observed between salinity and freezing temperatures (after correction for freezing depression by salts). One possible explanation is that INPs were associated with melting sea ice. The concentration of INPs in the bulk seawater was in good agreement with concentrations observed in bulk samples at several other locations in the Northern Hemisphere. The concentrations of INPs in the microlayer were consistent with concentrations observed by Wilson et al., (2015) off the coast of North America. Heating the

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samples substantially reduced the INPs' activity, suggesting that heat-labile biological materials were the likely source of that activity. Filtration of the samples showed that the INPs were between 0.02 µm and 0.2 µm, implying that the ice-active heat-labile biological material was likely ultramicrobacteria, viruses, or extracellular material, rather than whole cells.

We conclude that the concentrations and properties of INPs in the microlayer and bulk seawater in the Canadian Arctic are similar to other locations previously studied. However, there were some important differences. On average the concentration of INPs in the microlayer in the current study was lower than the average concentration of INPs measured by Wilson et al., (2015). These differences could not be explained by chlorophyll *a* concentrations from satellite measurements. In addition, similar concentrations of INPs in the microlayer and bulk seawater were observed here, while Wilson et al., (2015) observed significant enrichment of INPs in the microlayer compared to the bulk seawater. The differences may be related to sampling techniques, but they could also be due to the oceanic state during sampling. Further studies are needed to understand how measured concentrations of INPs in the microlayer and bulk seawater depend on sampling techniques. Further studies are also needed to understand how measured concentrations of INPs in the microlayer and bulk seawater depend on oceanic variables, particularly changing sea ice distributions.

As sea ice in the Arctic continues to decrease, the microlayer and bulk seawater could play a larger role in the overall atmospheric INP population in this region. Future modelling studies are needed to determine the magnitude of the effect this INP source has on cloud microphysics in the Arctic region and how it might change as sea-ice distributions change.

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Acknowledgements

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Station number	Sampling start time (UTC)*	Location
Station 2	23rd July 2014 17:10	74°36'935N 94°43'663W
Station 4	30th July 2014 22:10	76°19'882N 071°10'329W
Station 5	31st July 2014 21:00	76°16'568N 074°36'063W
Station 6	3rd Aug 2014 12:20	81°21'743N 064°11'399W
Station 7	4th Aug 2014 18:40	79°58'672N 069°56'051W
Station 8	5th Aug 2014 19:20	79°04'673N 071°39'205W
Station 9	11th Aug 2014 20:00	69°10'009N 100°44'018W
Station 10	12th Aug 2014 18:50	68°55'897N 105°19'809W

*Sampling took 45-90 minutes to complete.

Table 1 – Sampling times and geographic coordinates for the eight stations investigated during July-August 2014 in the Canadian Arctic.

Chemical and physical properties	T_{10}-value		
	R	**p**	**n**
Dimethylsulphide concentration	-0.6	0.074	8
Bacterial abundance	-0.4	0.189	6
Phytoplankton abundance	-0.5	0.138	6
Temperature	0.1	0.381	8
pH	-0.1	0.372	8
Salinity	**-0.7**	**0.020**	**8**

Table 2 - Correlation analyses between chemical or physical properties of bulk seawater and T_{10}-values for the bulk seawater samples. Numbers in bold represent correlations that are statistically significant ($p < 0.05$).

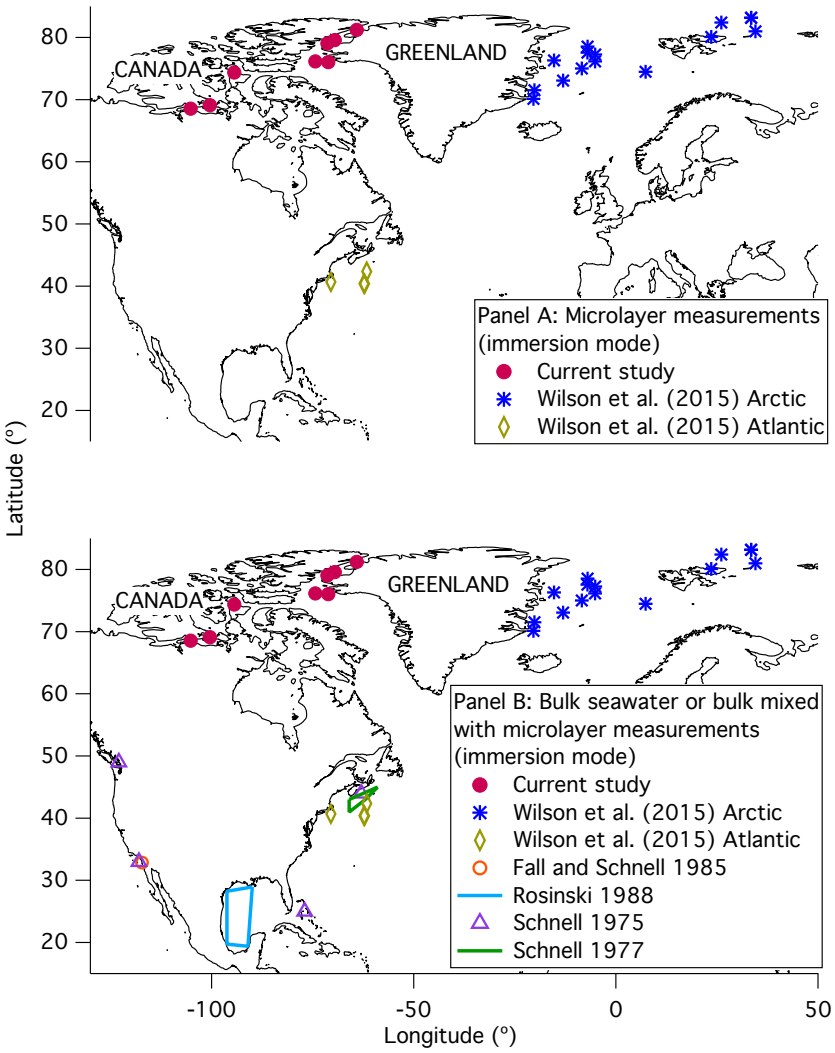

Figure 1 - Panel A: locations of current and previous studies of INPs (immersion mode) in the microlayer. Panel B: locations of current and previous studies of INPs (immersion mode) in bulk seawater or mixtures of bulk seawater and microlayer. Dates and coordinates for samples in the current study can be found in Table 1.

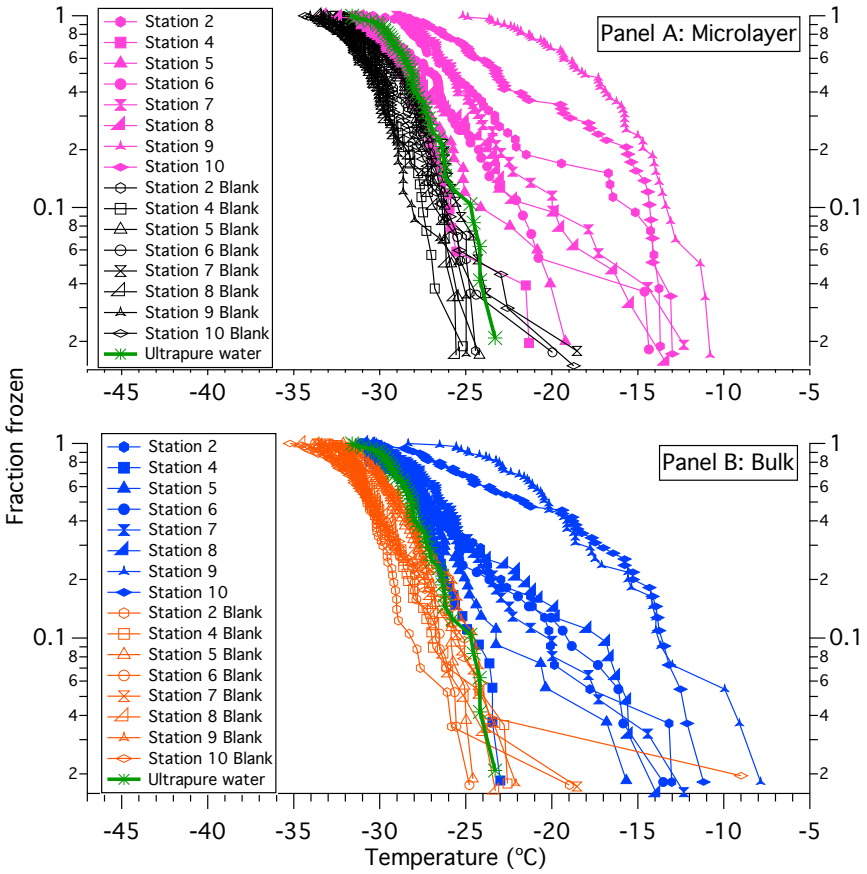

Figure 2 - Fraction of droplets frozen (in the immersion mode) versus temperature. Panel A and Panel B correspond to the microlayer and bulk seawater, respectively. Each set of line and markers represents the results for 3 repeat experiments of a sample or "blank", adding up to a total of between 45 to 60 freezing events in each set. Also included are the respective "blank" samples and ultrapure water. Each data point corresponds to a single freezing event in the experiments. All microlayer and bulk seawater freezing points have been corrected for freezing point depression to account for dissolved salts in seawater (Section 2.2.4). The uncertainty in temperature is not shown but is ± 0.3 °C.

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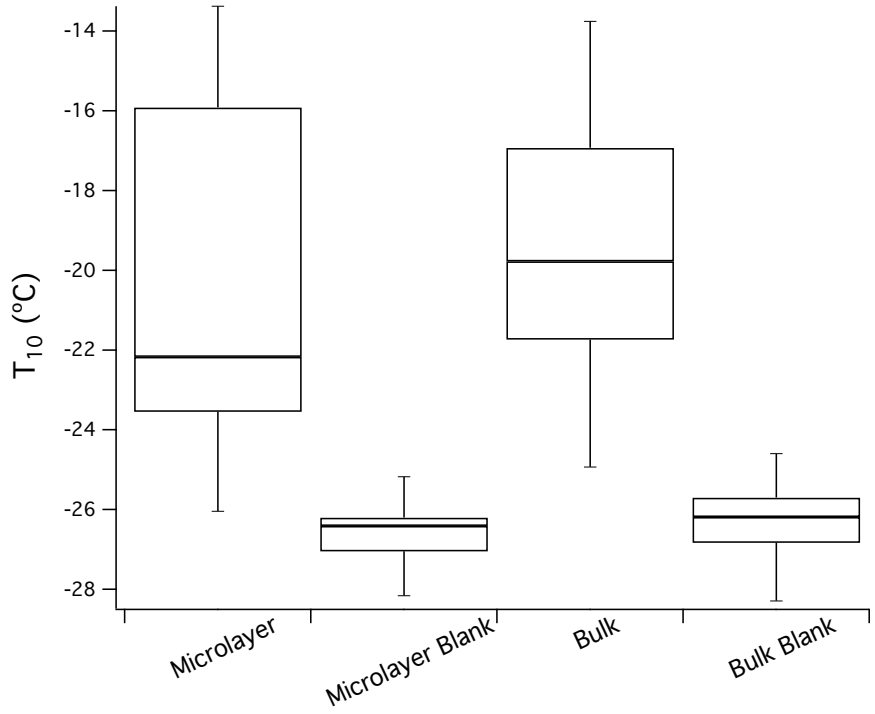

Figure 3 - Temperature at which 10% of droplets had frozen (T_{10}) for microlayer and bulk seawater samples. All data have been corrected for freezing point depression. Boxes represent the 25th, 50th and 75th percentiles, and whiskers represent the minima and maxima.

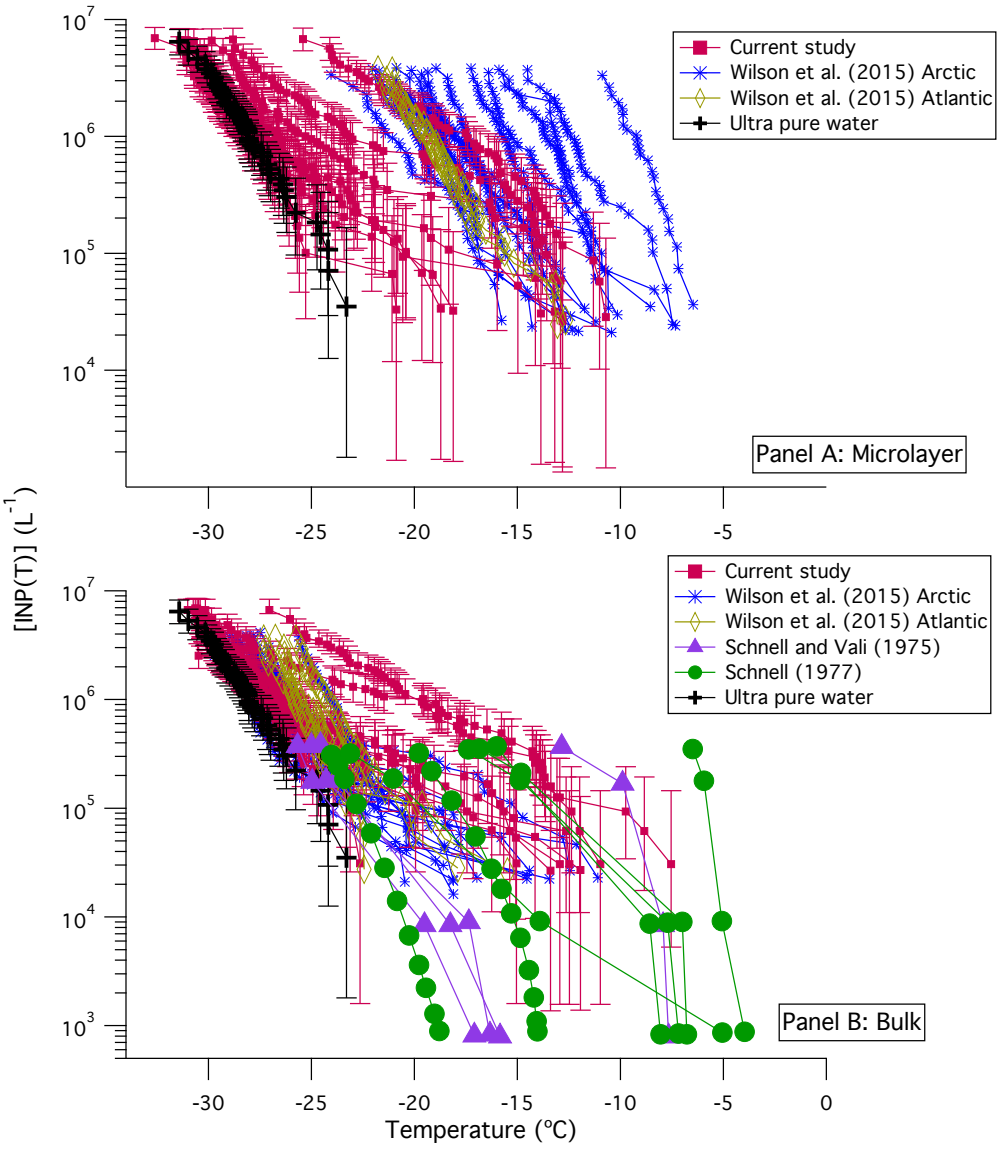

Figure 4 - The concentrations of INPs, [INP(T)], in the microlayer (Panel A) and bulk seawater samples (Panel B). All data, including those from other studies, are corrected for freezing point depression. Upper and lower limits to [INP(T)] (L^{-1}) associated with the current study describe the statistical uncertainty due to the limited number of nucleation events observed in the freezing experiments (Koop et al. (1997)).

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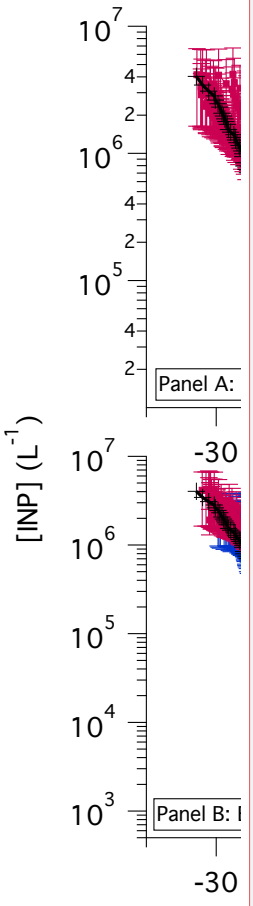

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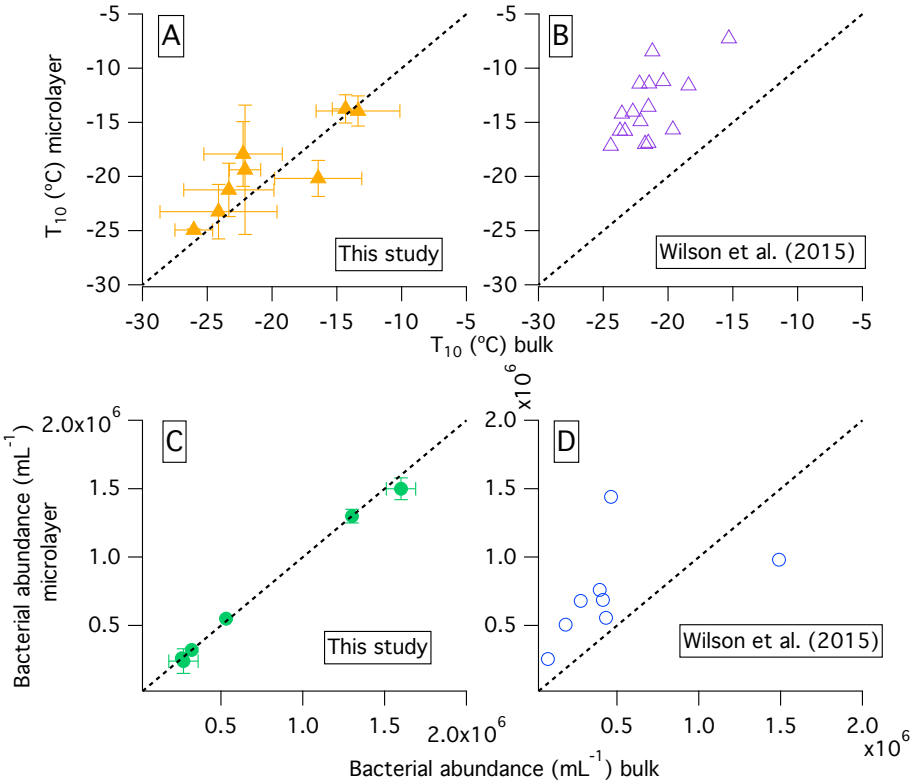

Figure 5 - Correlation plots with a 1:1 line for reference. Panel A: freezing temperatures for microlayer and bulk seawater samples in this study. T_{10} represents the freezing temperatures at which 10% of the droplets had frozen. All error bars represent the 95% confidence intervals of the T_{10}-values from 3 replicate experiments. All data have been corrected for freezing point depression. Panel B: T_{10}-values for microlayer and bulk seawater samples from Wilson et al. (2015). All data have been corrected for freezing point depression. The reported T_{10} values for their bulk samples should be considered upper limits, since their bulk freezing data were at the detection limit of their instrument. Panel C: bacterial abundance in the microlayer and bacterial abundance in the bulk seawater in this study. There was only one reliable microlayer sample from station 7 for bacterial abundance; therefore, the percentage error for this station was assigned the maximum percentage error from the other bacterial abundance. Panel D: bacterial abundance in the microlayer and bacterial abundance in the bulk seawater from Wilson et al. (2015).

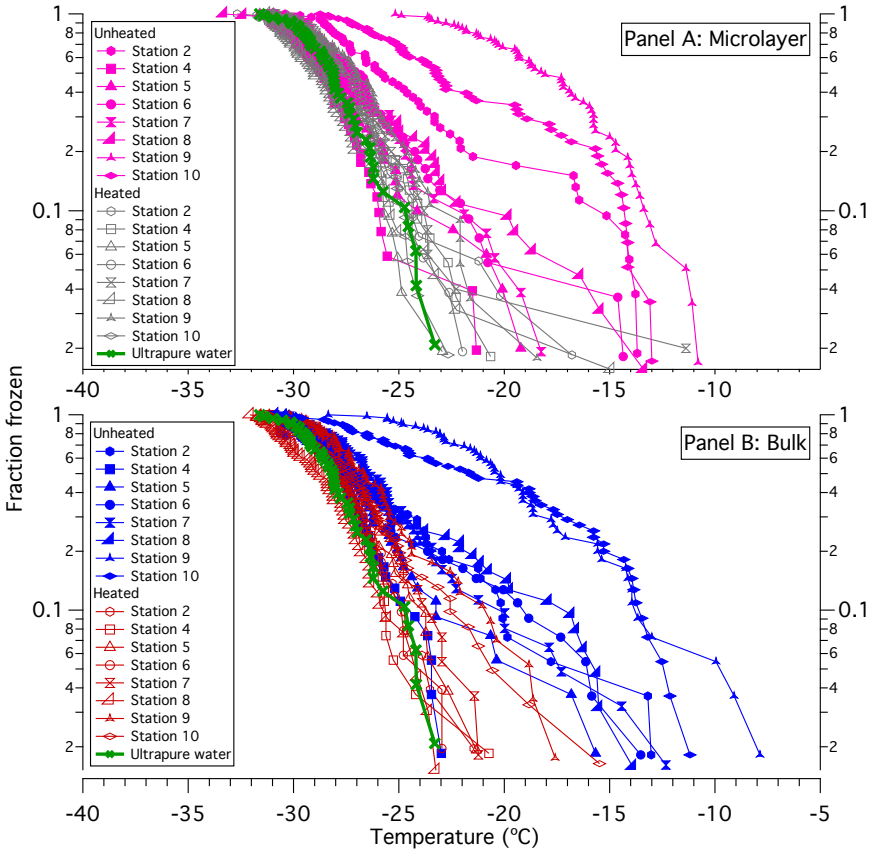

Figure 6 - Effect of heating on the fraction frozen for unfiltered samples from microlayer (Panel A) and bulk seawater (Panel B). Each data point corresponds to one droplet freezing event, and all data have been corrected for freezing point depression. The uncertainty in temperature is not shown but is ± 0.3 °C.

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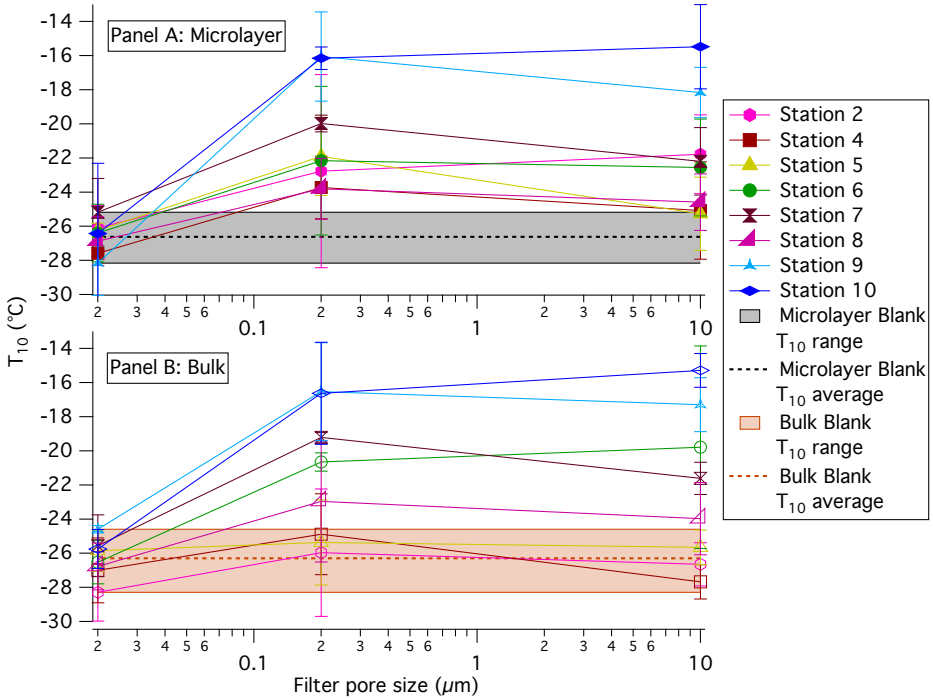


Figure 7 - Temperature at which 10% of the droplets froze (T_{10}) as a function of filter pore size in microlayer samples (Panel A) and bulk seawater samples (Panel B). Filter pore sizes were 10, 0.2, and 0.02 µm. Error bars are the 95% confidence intervals of the T_{10} from 3 replicate experiments. All data have been corrected for freezing point depression.

Supplemental Information

S1 Corrections for freezing temperature depression

The water activity of the sample was calculated from the salinity of the sample and using the online Extended AIM Aerosol Thermodynamics Model (http://www.aim.env.uea.ac.uk/aim/aim.php; Friese and Ebel, (2010); Wexler and Clegg, (2002)). Then the water activity of a salt solution in equilibrium with ice at the median freezing temperature of the sample was determined. From the difference of these two water activities, the freezing temperature in the absence of salts was calculated. For further details see Fig. 1 in Koop and Zobrist (2009).

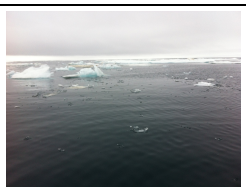
Station	Photos	Notes	Station	Photos	Notes
2		Behind iceberg and sheltered from wind. Sunny day, relatively flat sea surface. Macroalgae spotted approx. 75m away from sampling area. Wind speed: 4.6 m/s.	7		A little wavy, close to ice. Wind speed: 6.7 m/s.
4		Very flat, calm, glassy looking open water. No icebergs in sight. Wind speed: 1.4 m/s. Slick	8		Approx. 200m away from ice island. Partly cloudy. Calm and glassy sea surface. Wind speed: 0.7 m/s. Slick
5		Wavy, open water. Foggy. Wind speed: 3.1 m/s.	9		Overcast and raining. ~15m away from ice with brown material (possible animal faeces). Flat, calm and glassy sea surface. Wind speed: 2.4 m/s.
6		Uniform sea surface, near ice. Overcast. Wind speed: 2.4 m/s.	10		Glassy sea surface. Macroalgae floating approximately 5 m away. Partly sunny. Wind speed: 4.6 m/s. Slick

Table S1 - Conditions at sampling stations.

Chemical and physical properties	**T_{50}-value**		
	R	**p**	**n**
Dimethylsulphide concentration	-0.4	0.167	8
Bacterial abundance	-0.2	0.319	6
Phytoplankton abundance	-0.3	0.268	6
Temperature	0.2	0.313	8
pH	-0.2	0.293	8
Salinity	**-0.8**	**0.006**	**8**

Table S2 - Correlation analyses between chemical or physical properties of bulk seawater and T_{50}-values for the bulk seawater samples. Numbers in bold represent correlations that are statistically significant ($p < 0.05$).

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Biological variable	Microlayer T_{10}-value			Bulk seawater T_{10}-value		
	R	**p**	**n**	**R**	**p**	**n**
Phytoplankton abundance	-0.7	0.058	6	-0.5	0.138	6
Bacterial abundance	-0.7	0.071	6	-0.4	0.189	6

Table S3 - Correlation analysis between phytoplankton and bacterial abundance in the microlayer and bulk seawater and T_{10}-values for the microlayer and bulk seawater.

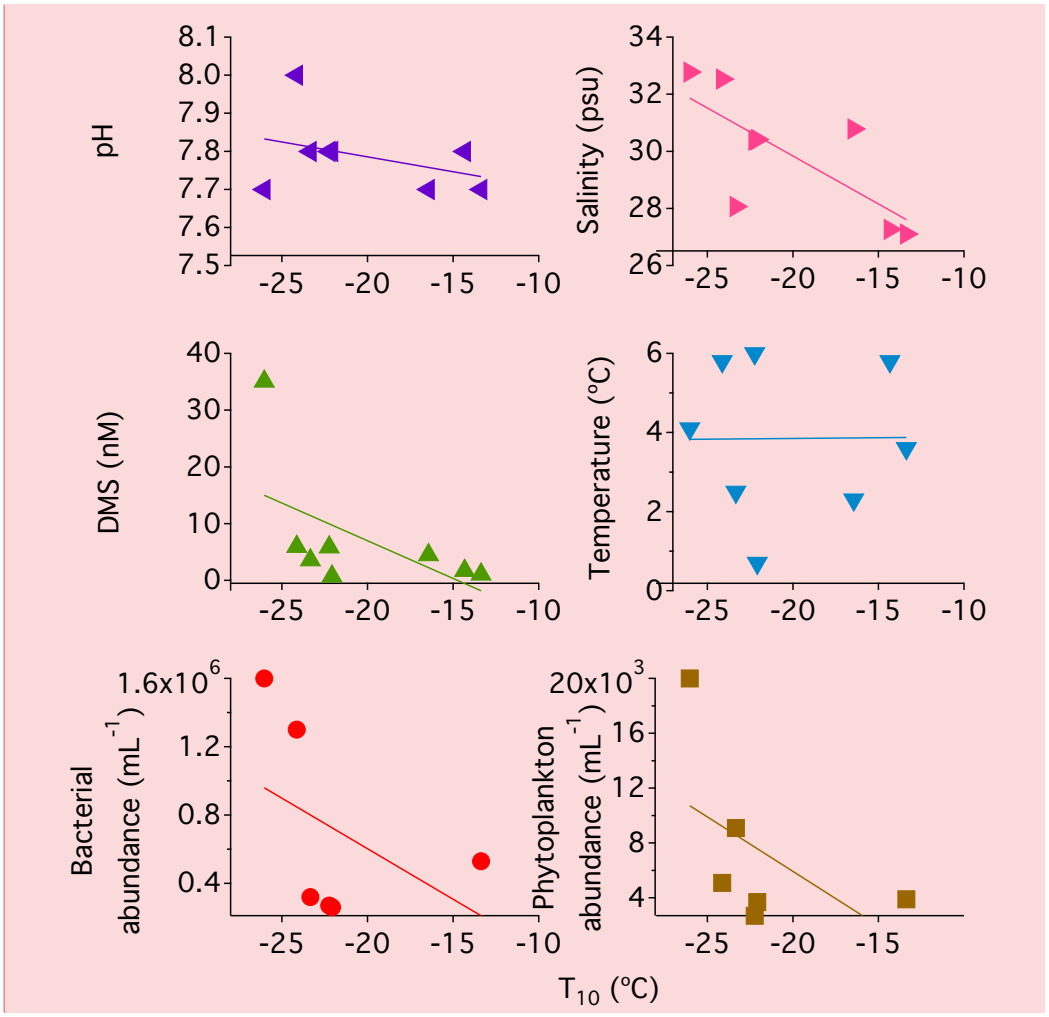

Figure S1 - Correlation plots between chemical and physical properties, and T_{10}-values in the bulk seawater. R and p values can be found in Table 2 in the main text.

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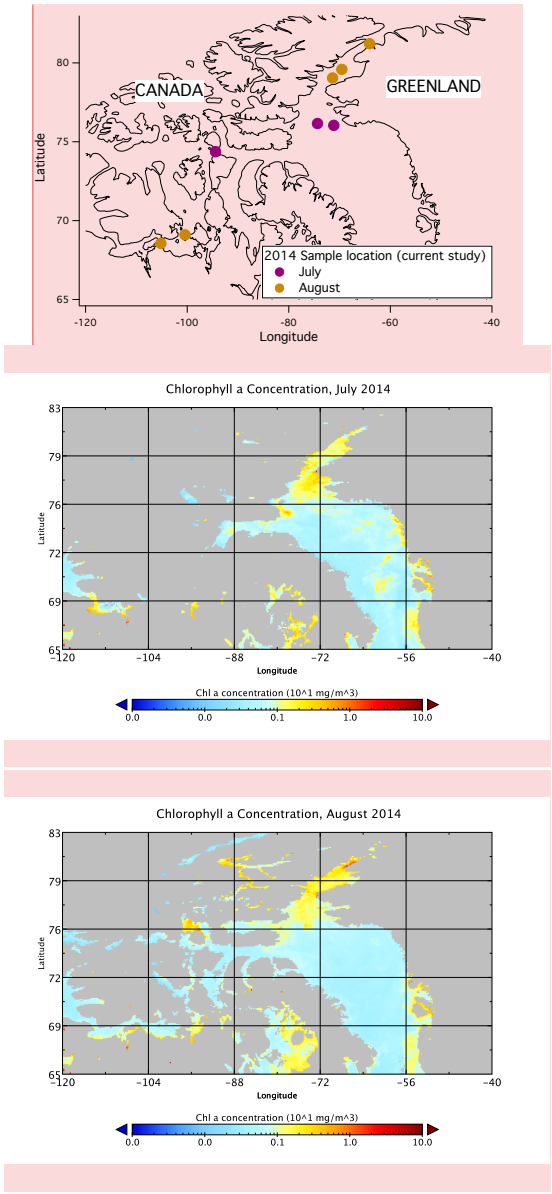

Figure S2 - Sample locations and monthly average chlorophyll *a* concentrations for sampling during the current study. Chlorophyll *a* concentrations were obtained from the NASA Ocean Biology Distributed Active Archive Centre (OB.DAAC).

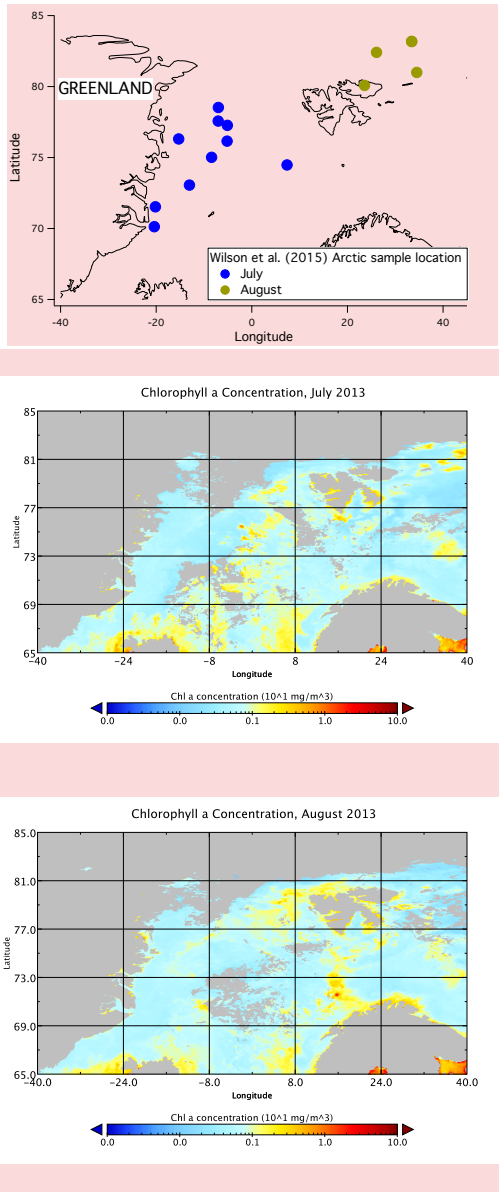

Figure S3 - Sample locations and monthly average chlorophyll *a* concentrations for sampling during the Wilson et al. (2015) study in the Arctic. Chlorophyll *a* concentrations were obtained from the NASA Ocean Biology Distributed Active Archive Centre (OB.DAAC).

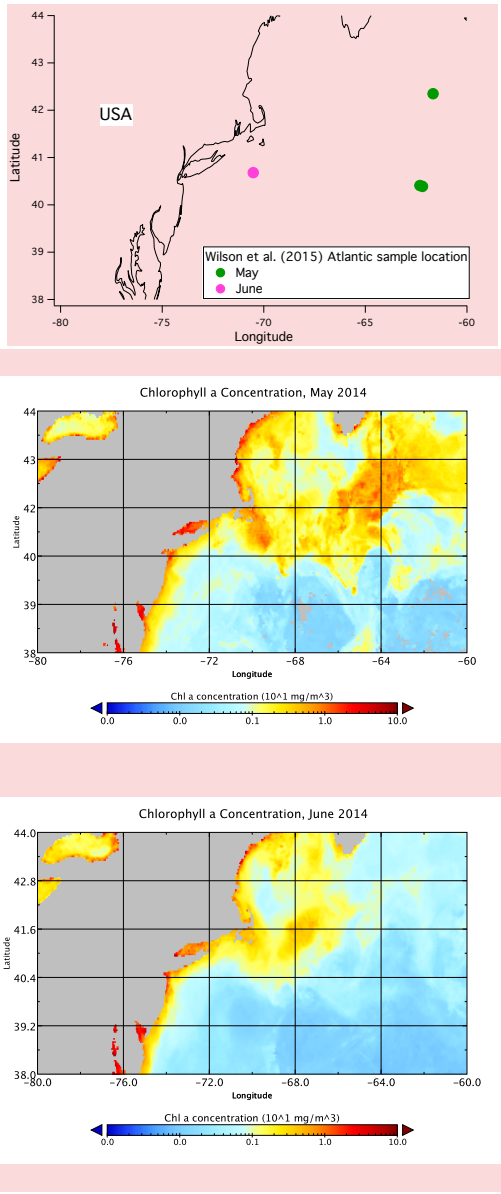

Figure S4 - Sample locations and monthly average chlorophyll *a* concentrations for sampling during the Wilson et al. (2015) study in the Atlantic. Chlorophyll *a* concentrations were obtained from the NASA Ocean Biology Distributed Active Archive Centre (OB.DAAC).

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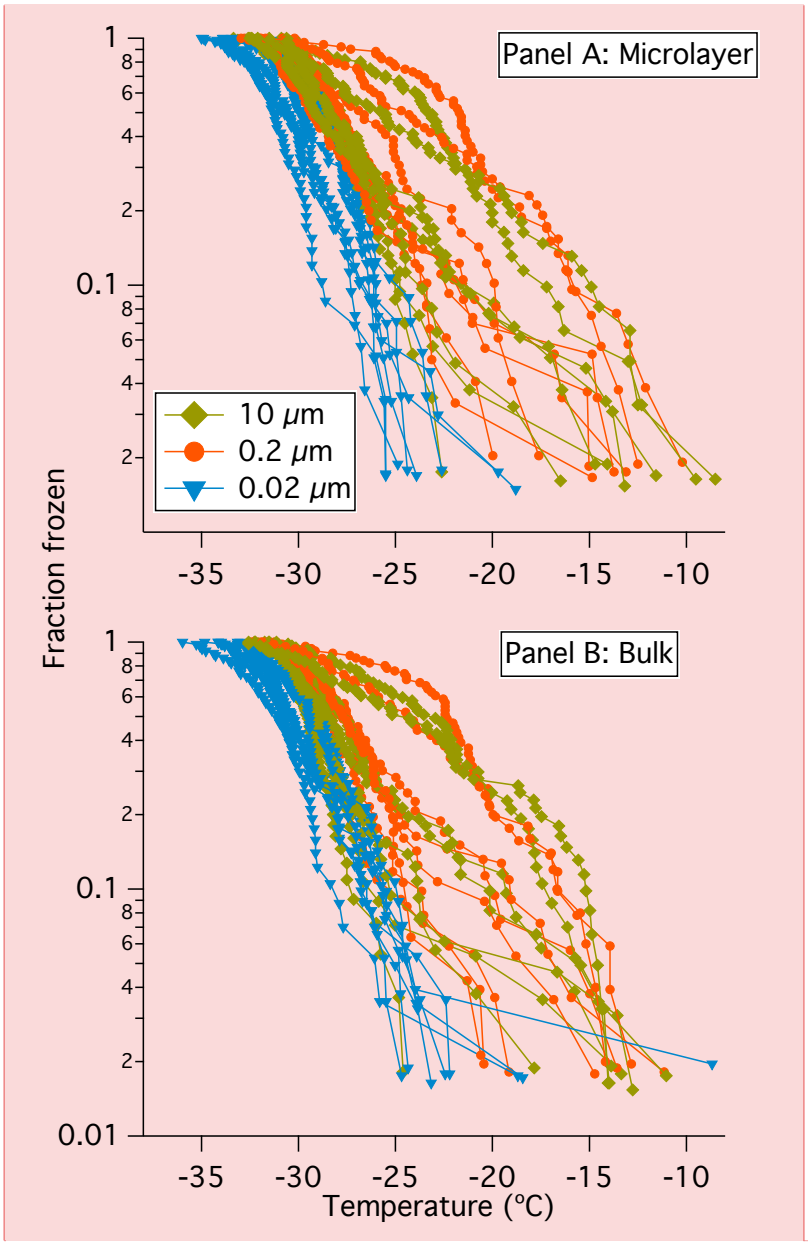

Figure S5 - Plots of the fraction of droplets frozen (in the immersion mode) versus temperature for samples filtered with 10 µm, 0.2 µm and 0.02 µm filters. Panel A and Panel B correspond to the microlayer and bulk seawater, respectively. Each set of line and markers represents results for 3 repeat experiments of each sample or "blank", adding up to a total of between 45 to 60 freezing events in each set. All microlayer and bulk seawater freezing points have been corrected for freezing point depression to account for dissolved salts in seawater (Section 2.2.4). The uncertainty in temperature is not shown but is ± 0.3 °C.

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