Production of aerosol containing ice nucleating particles (INPs) by fast growing phytoplankton

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Public justification (visible to the public if the article is accepted and published):
I would like to thank the authors for incorporating the suggestions made by both reviewers. The revised version looks pretty good and it is almost ready for publication; however, there are some minor comments that need to be properly addressed before I can accept the manuscript.

Reviewer #1:
The conclusion could benefit from a short introduction sentence e.g. "In this study we investigated … "., since the length of the manuscript increased within the review process. The authors could bring back the reader to the overall big picture and summarize and conclude their results better.

Following the reviewer's suggestion, we have added the following (Lines 702-703):

"In this study, we investigated whether the physiological status of phytoplankton, especially growth rates, can be linked to the properties of primary marine aerosol and ability to act as effective INPs."

Clearly, the high freezing temperature of the procedure blank is the main issue of the presented methodology, limiting the interpretation of the results as I already mentioned in my first comment. Although, the authors already improved the methodology between the two sampling campaigns, further improvements could be considered for future studies. I whish that the authors could include a (short) outlook in the conclusion that helps designing further campaigns. The authors can think of experimental improvements that allow to lower the procedure blank freezing temperatures and share it with the scientific community to help build upon their research.

As the reviewer noted, we have already improved the methodology in the previous revision. We agree that blanks are an issue for offline ice nucleation measurements in general. This important topic could be the topic of a future review paper, but we do not feel that the conclusion of this paper is the correct place for such a discussion.

Line 102: Change "The ASW was made with high purity analytical grade salts. Nevertheless, the large mass of salts in artificial seawater represents a source of potential contamination" to "The ASW was made with high purity analytical grade salts. Nevertheless, the large mass of salts in artificial seawater represents a source of potential contamination for ice nucleation experiments".
Changed as suggested.

Line 165: sometimes you write "dewpoint" and sometimes "dew point".
Changed to 'dew point' throughout the manuscript.

Line 253: “The image analysis method was based on Engel (2009), see Thornton and Chen (2017) for details.” Can you include an example image in the SI?
Figure S2 has been added to the Supplementary Information – this shows unprocessed light microscopy images (i.e. the ‘raw data’) for TEP and CSP samples collected from the MART during the growth of *Thalassiosira weissflogii.*

Line 541: Change “… GRH hypothesis … ” to “… GRH … ” since the acronym already implies the word hypothesis.
Corrected as suggested.

Line 684: Change “Different phytoplankton have different have significantly different …” to “Different phytoplankton have significantly different …”
Corrected as suggested.

Reviewer #2:
L331: “… per day and mean INP freezing temperatures…” – can this be added to the figure 2a caption? Right now it only refers to the temperatures as “INP freezing temperatures” rather than “mean INP freezing temperatures”. There are inconsistencies throughout and perhaps the same freezing temperature is referred to throughout the text, in which case it may be simplest to state in the methods “mean freezing temperatures are used to described INP spectra”.
Changes have been made to the Results text and figure legends (Figures 2 and 3) to make it clear when temperatures represent ‘mean freezing temperatures’.

L 338: “freezing temperatures” should be “mean freezing temperatures”?
Changed as suggested.

Figure 3a: are these mean freezing temperatures, or onset freezing temperatures (L385-386)? Can this also be added to the figure caption (specify if the plotted temperatures in 3a are mean, median, or onset)?
Changes have been made to the Results text and figure legends (Figures 2 and 3) to make it clear when temperatures represent ‘mean freezing temperatures’.

L388: “Fraction frozen curves showed that freezing occurred at temperatures significantly warmer than the procedural blanks at the beginning of the experiment (days 2 and 3) and at the end of the experiment (days 16 and 29).” – What about Day 7, which is above the ASW+L1 data for many temperatures? I also think it’s important to point out that days 2 and 3 are >7? degrees higher than the procedural blanks and days 7, 16, and 29 are only higher by ~2 degrees (or whatever these specific values are).

The explanation of what's happening in this graph is already included in the previous line of the existing text (lines 391 to 392):

“The onset of ice nucleation on days 2 and 3 were -19.1 ºC and -22.3 ºC, respectively (Fig. 3c). On these days, the fraction of INPs frozen reached 100% at -24 ºC (Fig. 3c). In contrast, the

onset of nucleation was < -27 ºC for sampling days at slower growth rates later in the experiment (Fig. 3c). Fraction frozen curves showed that freezing occurred at temperatures significantly warmer than the procedural blanks at the beginning of the experiment (days 2 and 3) and at the end of the experiment (days 16 and 29)."

We have not added any text describing Day 7 as we hope that the reader can discern sufficient information from Figure 3C itself. It would extend the results section significantly if we were to describe all the individual plots.

L705: "Significantly, our results are the first to show that fast growing phytoplankton are a source of INPs that catalyse freezing at relatively warm temperatures" – What temperatures, specifically?

The following text has been added (lines 714 to 716):

"Mean freezing temperatures during the early growth phase of the MART cultures were > -24 ºC, which was warmer than the mean freezing temperature of the procedural blanks (-34.9 for ASW and -31.3 ºC for ASW+L1 nutrients)."

Editor:
L44 and L61: There is a missing space after the "period".
Corrected as suggested.

L73: I think "limitation, stressors that are" should be "limitation and stressors that are"
Changed to the following (lines 72 to 73):
"The amount of DOM released by phytoplankton increases when cells are stressed by environmental factors associated with bloom collapse, such as nutrient limitation (Thornton, 2002; 2014).

L98: Here and a long the text "h" and "hour" are used. I suggest using "h" along the text.
'hour' has been replaced with 'h' in several places, particularly in the methods.

L100: "L1": please define it, especially because a similar term is used for the PIXE cascade impactor. Actually, I suggest changing the nomenclature of the PIXE stages to avoid confusions.
Lines 99 to 100 have been changed to the following:
"Prior to introduction to the MART, phytoplankton were grown in artificial seawater (ASW) (Harrison et al., 1980; Berges et al., 2001) supplemented with trace metals and vitamins from the L1 medium recipe of Guillard and Hargraves (1993)."

L109: Here "Thalassiosira weissflogii" is called "T. weissflogii"; however, in the following text the authors jump back and forth between both names. Please after L109 call "Thalassiosira weissflogii" as "T. weissflogii" (including figures and tables).

T. weissflogii has been used throughout the text after its initial introduction by its full scientific name.

L136: “Samples collected on L1 impaction stage (0.06-1 µm aerodynamic diameter)”. Based on the previous line, it seems that L1 contains the larger particles. Please double check this.

We thank the Editor for catching this. The stages were listed out of order (6, 3, and L1). This has been corrected.

The text now reads: “… PIXE cascade impactor with the following stages: 6, 3, and 01, corresponding to 8, 1 and 0.06 µm diameter, respectively (Fig. 1). Samples collected on the L1 impaction stage (0.06-1 µm aerodynamic diameter) were analysed. Aerosol were collected for 2 h at an air flow of 1 L min^{-1} through the sampler. Samples were stored at -80 °C.”

L199 and the following text: “ml” should be “mL”.
Changed as requested.

L258: “(EEm)” is not necessary as it was not used in the following text.
The acronym ‘EEM’ has been deleted.

L331 and the following text: “Synechococcus elongatus” is called “Synechococcus”. Please use the entire name (or S. elongatus if appropriate).
S. elongatus has been used throughout the text to refer to the organism grown in the MART. It is listed as *S. elongatus* in the culture collection it was obtained from. The term *Synechococcus* is used to refer to organisms counted in the field by flow cytometry as we could not determine which ‘species’ or strains were encountered in the North Atlantic. The use of *Synechococcus* is consistent with Wilbourn et al. (2020), which originally presented the field data that was used to calculate growth rates in this manuscript. Further, it is common in the biological oceanography literature to use the genus name *Synechococcus* without a species name as the genetic diversity of this organism does not fit into conventional definitions of species.

L403: DOM was defined in L70
Corrected.

L442: TEP and CSP were defined in L236
Corrected.

L442: FDOM was defined in L256
Corrected.

Figure 1: What is the meaning of PIXE A and PIXE B? in L135, only 1 cascade impactor with 3 stages is mentioned.
This has been explained in the revised text on sample collection with the PIXE impactors (lines 135-140):

“For offline ice nucleation measurements, size-sorted aerosol samples were collected on combusted aluminium foil substrates inside a PIXE cascade impactor with the following stages: 6, 3, and 01, corresponding to 8, 1 and 0.06 µm diameter, respectively (Fig. 1). Samples

collected on the L1 impaction stage (0.06-1 µm aerodynamic diameter) were analysed. Aerosol were collected for 2 h at an air flow of 1 L min^{-1} through the sampler. Samples were stored at -80 °C. There were two PIXE cascade impactors in the system (Fig. 1), but only samples from PIXE A were used in the analysis. PIXE B served as a backup system to ensure that back-up samples were available if there were issues such as instrument failure."

Figure 2: Please include the meaning of the open circles and green squares as in Figure 3.
The Figure 2 legend has been corrected and is consistent with Figure 3.

Figure 4. I think the y-axis in both panels can go in logarithmic scale.
We agree that it is convention to plot these type of data on a log scale. This convention arises from the wide range of concentrations of INP observed in the atmosphere at different temperatures. Data collected during one field campaign can cover several orders of magnitude in terms of INP number concentration. However, in our MART experiments we observed a narrow range of INP concentrations, both with temperature and across different days of the experiment. Therefore, there was no advantage to plotting our data on a log scale.

Figure 5: I think "Fraction of ice nucleating particles (INPs)" should be "Fraction of droplets"
Yes – this has been changed to 'fraction of droplets' in the figure legend.

Table S1: "bank" should be "blank"
Changed to 'blank'.

Kianna McFadden (co-author) wanted to associate her ORCID digital identifier (0000-0003-1383-2503) with her name, but I could not work out how to add it at this stage of the submission.