# *Interactive comment on* "Impact of ocean acidification on Arctic phytoplankton blooms and dimethylsulfide production under simulated ice-free and under-ice conditions" *by* Rachel Hussherr et al.

**Anonymous Referee #2**

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The manuscript provides a good account of the potential effects on OA of Baffin Bay seawater in the Arctic Ocean and it's affect on various variables such as Chl a, pH, nutrients, DMSPt and DMS etc., The manuscript is well presented and figures and tables are very clearly produced. Significant changes have been highlighted in the 10 day incubation experiment. Whilst the authors state that the rapid change in pH investigated over 10 days is not representative of the gradual OA that is taking place their study does reflect potential extreme responses. However, some further acknowledgement of this should be made in the discussion and in particular acknowledge that organisms do adapt to changes which may well affect the validity of some the discussion and conclusions.

We wish to thank the referee for his positive remarks.

In response to the comment regarding the capacity of the organisms to acclimate to changes in pH, we added the following sentence:

L 628 – 634, new sentence: "However, it is important to keep in mind that our short-term experiment precludes any acclimation of the algae to their new environment, something that is likely to take place in nature with a more gradual change in pH. In that regard, two studies have highlighted the acclimation capacity/evolutionary adaptation of the strong DMS(P) producer *Emiliana Huxleyi* to decreases in pH (Lohbeck et al., 2012; 2014). More studies are needed to fully assess how the acclimation capacity of phytoplankton will combine with short-term physiological responses to environmental stressors to shape future DMS emissions and climate."

L 730 – 731, the following passage was added in the conclusion: "...although our results do not account for the acclimation/evolutionary adaptation potential of natural microbial communities."

The abstract should contain more of the important findings mentioned in the text. Go through and highlight these changes in discussion and make sure they are included in the abstract.

This is a good point. The following sentences were added to the abstract:

- L 32 – 35: “During our experiment, a sharp increase in $DMSP_T$ and DMS concentrations coincided with the exhaustion of $NO_3^-$ in most of the microcosms, suggesting that the nutrient stress stimulated DMS(P) synthesis by the diatom community.”

- L 36 – 37: “The pH-induced decreases in Chl *a* concentration suggest a decrease in net carbon fixation by diatoms under low pH conditions.”

The introduction is well stated although there should be some attempt perhaps in the discussion to state why different authors find different affects of OA on phytoplankton response.

L 575 - 581, in part 4.2 “Phytoplankton community and nutrient uptake response to the pH gradient”, a new sentence is discussing the contrasting results found by our study and the very similar study of Richier et al. We give there some hypotheses that could explain the contrasting responses of phytoplankton to OA observed between these studies. These hypotheses could also be valid for other OA experiments that reported contrasting results (Thoisen et al., 2015; Villafane et al., 2015)

L 575 - 581: “In contrast to our study, Richier et al. (2014) reported a negative impact of ocean acidification not only on nanophytoplankton but on picophytoplankton as well during a microcosm experiment using a similar methodology. In this study conducted with water from the northwest European shelf, lowering the pH resulted in a decrease in the abundance (cell number) and biomass (Chl *a*) of phytoplankton < 10 $\mu$m. These contrasting results could reflect differences in the initial picophytoplankton community composition and possible species-specific physiological response to OA. By contrast, (...)”

Methods. Are the expts 9 days or 10 days-it is not clear. As the authors removed the large grazers could microzooplankton affected the results? Why was alkalinity kept constant? Surely in the natural environment and in particular a bloom event alkalinity would change as well as the concentration and ion activities of some of the constituents measured?

The whole experiment lasted 10 days, but the incubation lasted 9 days: T0 was the day when we filled the bags and did the initial acidification. We then started sampling the bags at T1 for the incubation experiment. This inconsistency was corrected in the revised version of the manuscript. To avoid confusion, we are now stating that the experiment lasted 9 days.

The point about removing the large grazers is valid, although the practice is common for this type of experiment. Unfortunately, we can only speculate about how removing the large grazers may have affected our results. Nonetheless, the absence of a relationship between the abundance of heterotrophic protists and the $H^+$ and DMS concentrations suggest that grazing by microzooplankton did not have a significant influence on the observed results. For sake of clarity, a sentence was added so the readers are made aware that removing the large grazers may have affected the relative importance of microzooplankton grazing on small autotrophic and heterotrophic cells (see L 667 – 671, later in the document).

The alkalinity in our samples was kept constant only during the initial process of acidification, and we did not control alkalinity during the following 9 days. However the alkalinity varied only slightly (< 2% variation) between day 1 and day 9 in all bags. The variations could be attributed mostly to biological phosphate and silicate uptake or $CaCO_3$ precipitation/dissolution (Richier et al., 2014), $CaCO_3$ reactions being the main process responsible for non-conservative TA variation (Cross et al., 2013). However, calcareous species are believed to be absent from our experiment (see Poulin et al. 2011), so we suggest that this process would have been minimal and negligible.

We also note that the majority of the salts in seawater are non-reactive ions (see Riebesell et al., 2010), and their ion activities would have remained essentially constant during the incubation experiments, since salinity did not vary. Changes in minor/trace seawater constituent concentrations (e.g., nitrate, phosphate, silicate, iron, organic ligands, which would be biologically taken up or produced) could surely affect trace element complexation in solution, but these would not affect the pH or total alkalinity within the precision of our measurements.

Results: See the sticky notes added to the manuscript and please attend to them. Can you say what species were mainly reflected in the nanoplankton. Were any calcareous?

At the peak of the bloom, the nanoplankton were mostly composed (74% of total cells) of small (< 20 $\mu$m) centric diatoms *Chaetoceros* species. Unfortunately, we could not identify the *Chaetoceros* to the species level. We found no calcareous species in the samples, but since we preserved the samples with acidic Lugol, calcareous species may not have been preserved. Irrespective, no calcareous species have yet been identified in these waters (see Poulin et al. 2011).

Discussion and Conclusion: see the sticky notes. These parts need to be carefully gone over and some sentences modified.

The suggested modifications and re-wording have been carefully applied. Here are more specific responses to some of your comments:

- L 105: Have any of these studies considered speciation differences in CO2 in seawater? Have any of these studies measured or calculated CO2 throughout the experiment and compared with the speciation of CO2 in controls?

The concentrations of the individual species of the carbon dioxide system in solution cannot be measured directly (Dickson et al., 2007), but are derived from pH and total alkalinity measurements using the MS Excel macro $CO_2$SYS (Pierrot et al., 2006).

None of the cited studies reported the speciation between the different species in water ($CO_2$, $HCO_3^-$ and $CO_3^{2-}$). As these studies were focused mostly on the biological impact of OA on the microbial and planktonic communities and their influence on DMS dynamics, the authors may had chosen to focus their sampling and analysis efforts on the biological community.

We calculated the speciation of $CO_2$ for all microcosms at T1, T4 and T9. As expected, $HCO_3^-$ represented around 94% of the carbonate species in all microcosms at T1, T4 and T9. The

proportion between free $CO_2$ and $CO_3^{2-}$ varied between 7- 0.6% depending of the pH, with the lowest $CO_3^{2-}$ / highest $CO_2$ values observed at the lowest pH, which is concordant with the information found in the literature (see the diagram of $CO_2$, $HCO_3^-$ and $CO_3^{2-}$ concentrations in function of pH for example). Despite these natural changes due to pH modification in the microcosms, theses percentages ($HCO_3^-$/ $CO_3^{2-}$/ $CO_2$) did not change during the incubation and remain relatively constant between T1, T4 and T9. Moreover, measuring the impact of each individual species on the monitored biological processes would have required many buffered experiments. This would have been logistically very difficult to conduct on board of the Amundsen.

- L 131: You state above seawater temperature is -1.35oC why difference?

Since our deck-incubator was cooled with circulating surface water, we had no control over the temperature. However, all HL and LL bags were in the same incubator and were therefore held at the same temperature.

L 142 – 145, new sentence added: "Since our deck incubator was cooled with circulating surface water, we had no control over the incubation temperature (mean temperature of 4.3 ± 1.6°C over the 9-day experiment). However, all bags were in the same incubator and, therefore, held to the same temperature."

- L150-152: Can you be more specific?

When $CO_2$ dissolves in the ocean, it combines with water ($H_2O$) to form carbonic acid ($H_2CO_3$). This acid then dissociates to form one proton ($H^+$) ion and one bicarbonate ($HCO_3^-$), and thus, the absorption of $CO_2$ does not impact alkalinity (e.g., Riebesell et al., 2010).

- L160-163: What about changes in ion activities and complexation effects that also affect pH? Did you test for these effects? As alkalinity was kept constant this is not what would happen in the natural environment? Could discuss these issues in discussion

Please, see our response to your general comment about the method at the beginning of this document.

- L 203-204: How much seawater was sampled for analysis and how did this loss of volume affect the results?

To minimise potential low-volume effects, we removed at most half the initial volume of each bag over the course of the 9-day experiment (ca.5 out of 10 L). Since large grazers had been removed prior to the incubation, we believe that the gradual decrease in volume had no impact on the relative abundance of the protists assemblage.

L 217: "At least half of the initial volume of the microcosms was still..." has been changed to "At least half of the initial volume of the microcosms (5L) remained ..."

- L262: How much seawater was taken?

20 mL samples were taken directly from each bag with a syringe connected to the luer-lock port of each bag.

L 276: "Water for FRRF measurements was taken" has been changed to "For FRRF measurements, 20 mL of seawater were taken (...)".

- L330: Did you calculate concentrations of H+ ions or activities?

Proton ($H^+$) concentrations were measured spectrophotometrically on the total proton concentration scale under the constant ionic medium convention (e.g. Dickson et al., 2007; section SOP 6b)

- L 338: But could it not also increase due to decreasing volume of seawater in the bags as you take more sample out for analysis? Also could plankton growth affect Ca and Mg ions? which could affect activities of H+? In natural ocean these complexation affects would be diluted but perhaps not in incubation experiments?

Plankton growth will affect macronutrient concentrations (nitrate, phosphate, silicate), but it is the uptake of $CO_2$ for organic carbon production that will affect pH most. Calcium and magnesium are major constituents of seawater and their concentrations in seawater are not significantly (< 0.5%) affected by plankton growth, except in areas of massive biogenic $CaCO_3$ precipitation such as on the Bahamas Bank and Persian Gulf. Given the presumed absence of calcareous species and the low concentration of these micronutrients in seawater, their uptake should not affect pH values within the precision our measurements. However, due to the limitation of our experiment, we could neither substantiate nor reject the possible impact of these ions complexation on the results.

- L 389: This statement seems at variance with the above?

The 1-2 days lag between the peak in chlorophyll *a* and the peak in nanoplankton abundance did not affect much the overall correlation between the two variables. The lag probably reflects a decrease in chlorophyll *a* synthesis (and thus chlorophyll *a* cell quota), as the dividing cells were becoming nitrogen limited.

L 397 – 398, new sentence added: "The lag probably reflects a decrease in chlorophyll *a* synthesis (and thus chlorophyll *a* cell quota) as the dividing cells were becoming nitrogen limited."

- L 542: Don't you mean Si concentrations? If not how did you measure Si consumption?

L 561: "$Si(OH)_4$: $NO_3^-$ consumption" has been changed to "$Si(OH)_4$: $NO_3^-$ concentration".

- L 565: Did you measure any production of free CO2 in your expts? If not can you calculate it for the length of your expt? Is it significant?

We did not measure $CO_2$ production *per se* during the incubation. If there was any $CO_2$ production (this includes all bacterial respiration processes), this would have been implicitly captured by the change in pH and DIC over time. However, our measurements only show the net effect of $CO_2$ production and consumption. To unravel any individual processes, we would have need labelled incubations.

- L 591: But this assumes DMS is mainly produced from DMSPt? Could it not be produced directly into seawater as a stress response?

Both algae and bacteria can produce DMS from DMSP. In algae, DMS can be produced as a stress response. This stress response is, however, only present in species with DMSP lyase capacity such as the prymnesiophytes *Phaeocystis spp*. and *Emiliana hyxleyi*. So far, DMSP lyases were never found in diatoms, the dominant group during our experiment. In the open ocean, bacteria are though to be responsible for most of the transformation of dissolved DMSP (released by the algae in the medium) into DMS. For this reason, we believe that most of the DMS produced during our incubation resulted from bacterial activity. Since non-diatom species present in the assemblage may have contributed to this production, our statement refers to both direct exudation of DMS by algae as well as bacterial DMSP cleavage. To make this point clear, the sentence was modified as follow:

L 617 – 619: "Together, these results suggest that ongoing OA will have a stronger impact on the algal and bacterial DMSP transformation into DMS than on the synthesis of DMSP by algae (...)"

- L 602: This is a long bow from a 10 days experiment. It suggests that organisms don't adapt to change.

L 628: Deleted passage: "...during the next centuries, with OA potentially counteracting the predicted stimulation of DMS production due to sea-ice retreat and the consequent increase in primary production (Six et al., 2013)."

Passage added to the discussion: please, see our response to your general comment at the beginning of the document.

- L 611: You can get a measure of DMSPt and DMS production rates or consumption rates by taking the concentration measurements on the different days to see if production or consumption varied much as a function of the elapsed time between measurements. You are comparing concentrations of DMSPt /DMS with changes in ion activities of H+ not an ideal comparison. As DMSPd did not change much it seems unlikely that bacterial activity had much effect being swamped by the increases in Chl a?

Since both DMSP and DMS are produced and consumed by distinct pathways, day-to-day changes in the size of their pools only allow a calculation of net changes in DMSP and DMS, not their gross changes.

Because pH is simply equal to the negative logarithm of $[H^+]$, the linear relationship between different dependant variables and pH, as often indicated in the literature, is not appropriate. This

is the reason why we choose to compare DMSPt and DMS with $H^+$ concentration ($[H^+]$). A similar type of representation was also adopted by Archer et al. (2013) and Hopkins and Archer (2014) during their mesocosm/microcosm experiments.

- L 617: Some reference should be made to changes in these ratios also observed in polar waters by Jones et al. (1998)

L 654 – 656, new sentence added: "Although the decrease of this ratio could also be due to an increased grazing of diatoms by microzooplankton (Jones et al., 1998), we found no significant relationship between the micrograzers and $H^+$ or DMS."

- L 631: Whilst you removed large grazers you presumably still had microzooplankton present?

This is right. The abundance of heterotrophic protists (mostly ciliates and choanoflagellates) was measured at T0, T5 and T9 in 6 pH treatments during the study (8.07, 7.61 and 7.22 at both high and low light). Their abundance varied from 786,008 to 15,254,481 cells $L^{-1}$. However, we found no significant relationship between their abundance and $H^+$ or DMS concentrations.

To clarify our argument, some details were added to the discussion:

L 667 – 671: "Heterotrophic protists were present in the microcosms, with abundances varying between ca. 786,008 to 15,254,481 cells $L^{-1}$ (data not shown). Although removing large grazers before the incubation may have affected the relative importance of the microzooplankton grazing on phytoplankton, no relationship between protists abundance and $H^+$ or DMS could be found."

- L 638: a large biomass of centric diatoms could have increased DMS and affected DMSPt. Why not use the % of each species found and apply this % to DMS and DMSPt production (concentrations measured) and see if any trends.?

The large diatom bloom that took place during our experiment certainly contributed to the increase in DMSPt, but since DMSP lyases have never yet been identified in diatoms, they are likely not directly responsible for the associated increases in DMS concentrations.

- L 639: Jones et al (1998) suggest an inverse correlation occurs in polar waters of the Southern Ocean for diatoms and dinoflagellates. Could something like this occur in your incubation bags?

We cannot respond to this question since the few dinoflagellates present in the water at the beginning of the experiment quickly disappeared. The dinoflagellates seem to be too fragile to survive the initial filling of the bags.

- L 656: Yes this is an important point and makes sense in a melting sea ice environment (see Vance et al. 2013?)

L 697 – 699, new sentence added: “These results suggest that phytoplankton exiting the ice pack would not necessarily experience a light shock as previously noted by others (Vance et al., 2013, Galindo et al., 2016).”

- L 680: Too much speculation. This last sentence should be removed. As Chl a increased and is a function of SRD.

L 719: The following sentence was deleted: “These results further suggest that SRD may not be the main factor driving net DMS production in Arctic waters, similar to results from the northeast Atlantic, where Belviso and Caniaux (2009) found that the SRD accounted for only 19% to 24% of DMS variations during the summer.”

- L 682: very rapid OA!

L 721: “During this study, we demonstrated that OA could negatively impact” has been changed to “During this study, we demonstrated that a rapid decrease in surface water pH could negatively impact”

- L 688: but Chl *a* and DMSPt increased as Chaetocerous increased?

It is true that Chl *a* and DMSPt concentrations increased in parallel with the bloom of *Chaetoceros spp*. during the experiment, but the amplitude of the peak in Chl *a* and DMSPt decreased as the pH decreased.

- L 712: This last bit seems to be at variance with what you have stated earlier?

We agree with the reviewer.

L 750 – 753, new sentence added: “(...), our results show that Arctic diatoms may bloom under light conditions much lower than the one tested here. This apparent capacity of Arctic diatoms to growth under extremely low light conditions should be explored in future studies.”

Overall I would recommend publication with attention paid to the minor comments. Also the authors should end their discussion with what future studies should concentrate on wrt. Baffin Bay to extend the field and make these expts more relevant to actual conditions in the field.

L 753 - 755, new sentence added: “As short-term impacts of OA on the DMS cycle become clearer, future studies should focus on assessing the potential adaptation and tolerance mechanisms of microbial DMS(P) producers, mechanisms that likely develop on a time scale closer to the natural OA rate.”

Please also note the supplement to this comment: http://www.biogeosciences-discuss.net/bg-2016-501/bg-2016-501-RC2- supplement.pdf

Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-501, 2016. C2