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Interactive comment on "Acidification counteracts negative effects of warming on diatom silicification" *by* Alexandra Coello-Camba and Susana Agustí

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This paper deals with physiological response of Arctic diatoms to increasing of temperature and pCO2. Cell volume, valve thickness and silicon incorporation rate of diatoms were examined by using the natural diatom community. The authors showed that cell volume and valve thickness of diatoms were decreased as increase of temperature and pCO2, while silicon incorporation rates were increased. The authors described that increase CO2 and water temperature affect negative effect of diatom silicification. It has taken great efforts to incubate many large bottles in this study. Also, there is new information on silicon incorporation rates of diatoms using a novel fluorescence bye PDMPO. However, this manuscript contains significant problems in experimental

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designs and methods they used. Therefore, data the authors analyzed do not support their conclusion and are inadequate to lead their conclusion. I suggest the current manuscript doesn't merit to be published. Please clarify my questions listed below.

- Introduction P2 line 14-23. My main concern is that the reason why they used the natural phytoplankton community is unclear. In the introduction section the authors describes that they analyzed the effects of temperature and pCO2 on cell and valve dimensions and silicification, and possible interactions. For such purpose incubation experiments using unialgal strains in laboratory under severely controlled condition are appropriate to demonstrate physiological response of diatoms to environmental changes. Advantages using natural plankton community are to evaluate ecosystem responses, such as competition among the other phytoplankton, species succession among diatoms and effect of grazing by microzooplankton. - Authors' response: The main reason why we use natural phytoplankton communities here is to test the actual responses of Arctic communities. Laboratory data cannot reflect the complexity of the biotic and abiotic interactions that take place in nature and in particular in Arctic waters. Silicification processes have been demonstrated to be influenced by the concentration of silica present in the medium, influencing the amount of silica eventually incorporated to the cell (Finkel et al. 2010). Moreover, different environmental factors acting simultaneously are difficult to reproduce in the laboratory. Here our interest was focused on the process of silicification in arctic communities, as affected by increased levels of temperature and pCO2. This study focuses more on the biogeochemical consequences than on the diatom physiology alone. The use of cultures will imply a strong simplification of the environment.

- Methods: P2 line 33-P3 line 9. The authors should describe when, where and how they obtained water samples for general readers. - Authors' response: We improved the description of the sampling with this information included now in the new Table 1. In lines 25-29, page 2 of the new manuscript we refer the reader to the previous publications where these experiments were described in more detail, " Three incubation

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experiments were conducted in the Arctic Ocean using natural phytoplankton communities sampled at the surface waters. These experiments were run at the University Centre in Svalbard (UNIS) in Longyearbyen during the summers of 2009 and 2010 (see Coello-Camba et al., 2014 and 2015). Information on sampling location, dates and conditions of temperature and pCO2 treatments are also described in new Table 1".

- P2 line 31-32. While setup conditions of pCO2 were described to be 380 ppm and 1000 ppm in the materials and method section, the results at 217.7 ppm, 780.8 ppm and 1652 ppm were shown. Which is right? - Authors' response: In agreement with the reviewer's observation we realize that this information was not well described. We referred to the planned treatments, although we should instead refer to the actual treatments. The methods were well described in Coello-Camba et al. (2014). According to this, we improved the description in the Methods section (lines 33-34, page 2): "Seven temperature treatments were set for the 2009 experiments and three temperature treatments in 2010; in this last experiment temperatures were combined with three pCO2 treatments (Table 1)", and (lines 13-14, page 6): "The average CO2 values actually measured along the experiment resulted in 217.7 (37), 780.8 (46) and 1652(72) ppm respectively".

- P3 line 8. Was the setup condition of light intensity (200mol, continuous light) appropriate? At least the authors should show daily PAR at the same latitude for reference. - Authors' response: We chose this value based on the PAR measurements performed at noon in 22 Arctic stations on a previous cruise (July 2007). Using a PUV 2500 Biospherical radiometer, the average PAR value at 5 m deep was 146 μmol photons m-2 s-1, reaching a maximum value of 470 μmol photons m-2 s-1 on July 14th, and a mimimum of 45 μmol photons m-2 s-1on July 22th. We added this information to the revised manuscript on lines 13-16, page 3.

- P3 line 13. Why were the concentrated water samples frozen? This procedure would damage diatom frustules. - Authors' response: The samples were frozen to preserve

them until microscopic analysis on land. This procedure would not affect cell size or thickness, the main features to be measured here.

- P3 line 14-15. Were the density of dead (empty) diatom cells checked before cleaning procedure? - Authors' response: Yes, the abundance of empty diatoms was low. We checked the viability of some of the groups found, showing high viability along the experiment.

- P3 line 27-28. How did the authors measure height of diatom frustules to the pervalvar axis to calculate cell volume? - Authors' response: As indicated by the reviewer, the referred information is missing. We have added the following information on the revised version of the manuscript: " Centric diatoms are more likely to appear in the microscope slide on a valve view, so the measurement of cell heights was more difficult to get. This way, we used an estimation of the average cell heights for each group based on the measurements performed in Olenina et al. (2006)" in lines 2-5, page 4. Valve height is more conservative between species than their diameter. Olenina et al. (2006) showed that the range of Coscinodiscus spp. and Thalassiosira spp. diameters was larger than the range of their valve heights, that were very conservative.

- P3 line 38. The authors used 250 mL subsamples from 20L incubation bottles for measuring silicon incorporation rates. Did the authors conduct CO2 bubbling or monitor pH value in the subsamples? - Authors' response: No, the CO2 levels were monitored along all the experiments, but for the PDMPO subsamples this was not feasible. We, as other authors (i.e. Sugie et al., 2013), followed a standard procedure to perform these measurements in pCO2 experiments. It is not necessary to bubble CO2 into the PDMPO bottles as the relatively low biomass present in these subsamples together with the short incubation time allows the assumption that the change in the carbonate chemistry of the bottles during this 24h incubation period was small (Sugie et al., 2013).

- Results: P6 line 10-15. I can't understand this sentence. Were the cell volumes of the centric diatoms 21.4m decreased, although their diameters did not change? This

means that height of diatoms to the pervalvar axis. Please clarify this more detail. - Authors' response: The referred paragraph has been modified (lines 31-32, page 6) to make more clear that the diameter decreased with temperature. We removed the quotation of cell diameter i.e. 21.4 μm to avoid misunderstandings. Now we refer to the different cell groups as Thalassiosira sp. populations 1 and 2 or Coscinodicus sp.

- Discussion: P7 line 2-10. I disagree this conclusion. It is unclear whether size of the same species was decreased or the dominant diatoms successed from large species to small species because diatoms were not identified to species. - Authors' response: We have added to the revised manuscript the identification to genus level of the diatom groups studied here (lines 23-26, page 6), as Coscinodiscus sp. (21.4 0.38 μm initial cell diameter) from the 2009 open sea community experiment, and Thalassiosira sp. population 1 (7.4 0.04 μm initial cell diameter) from the 2009 fjord community experiment), and population 2 (6.6 0.04 μm initial cell diameter) from the 2010 experiment. These groups were clearly differentiated from other less abundant groups and we can say that it is the size of these groups that decreased with temperature, instead of species succession from bigger to smaller species.

- P7 line 24-26. I disagree this conclusion. It is possible that a decrease in silica incorporation rates was due to lower abundance of total diatoms at higher temperature. The authors should show the initial concentrations of biogenic silica or total biomass of diatoms in 250 mL subsamples before incubation with PDMPO. - Authors' response: To determine the silica incorporation rate we followed the standard procedure described in Leblanc and Hutchins (2005) and Shimizu et al. (2011). This measurement is an incorporation rate, a time-related parameter. We estimated the silica incorporation rates per unit of diatom biomass in the revised manuscript (values shown in lines 5-6, page 8), and observed that this ratio did not show any significant relationship with increased temperature or pCO2. Silicification is performed by active cells, although the measurement of biomass is not related to the state of the cells and includes no actively growing cells and a component of detritical biomass. Probably, the presence of non

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active cell biomass influenced the incorporation rate vs. biomass ratio and prevented us for finding clear responses of the ratio with increased temperature or pCO2. The incorporation rates showed here reflecting the silicification process help us to identify the overall silicification responses of the communities and thus the consequences for the biogeochemical cycles.

- P7 line 27-32. I disagree this conclusion. It is possible that an increase in silica incorporation rates was due to higher abundance of total diatoms at higher pCO2. The initial concentrations of biogenic silica or total biomass of diatoms in 250 mL subsamples should be shown. - Authors' response: See above.

- Table 1 Please show the longitudes and latitudes at sampling locations. - Authors' response: The suggested information has been added to Table 1.

- Fig. 2A. Is this figure the result at 380 ppm or 1000 ppm? Please describe more details in the figure caption. - Authors' response: In this figure we show the effect of temperature considering all pCO2 treatments.

- Fig. 2 B. Is this figure the result at 1.8C, 6.7C or 10.3C? Please describe more details in the caption. - Authors' response: In this figure we show the effect of temperature considering all temperature treatments.

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Table 1

	Experiment		
	ATP 2009		ATP 2010
	Open sea	Fjord	
Sampling location	SE of Svalbard	Isfjorden	Isfjorden
Latitude/Longitude	77°N / 28°E	78°N / 14°E	78°N / 13°E
Experiment dates	1-10 July	10-19 July	24 June-8 July
Sampling water T (°C)	-1.19	6.2	1.4
	1.6	1.2	1.8
	2.6	3	
Mean incubation	4.5	4.1	
T measured	5.5	5.5	
(°C, ±0.1)	7.6	-	6.7
	8.5	8.3	
	10.5	10	10.3
Mean pCO_2			217.7 (±37)
values measured	-	-	780.8 (±46)
(ppm, ±SE)			1652 (±72)

Fig. 1. New Table 1