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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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Coello-Camba and Agusti describe the results of three experiments, testing the effect of temperature and pCO2 on diatom cell volume and valve thickness. I do not believe that they have tested the actual effect on diatom silicification as stated in the title for reasons that I explain further down in this review. The topic of how climate change related environmental drivers affect phytoplankton physiology and thereby possibly their ability to protect themselves against grazers is a very important one. However, I feel that this manuscript lacks important information, especially in the methods section and I am not convinced that the experimental set up and some of the methods used and especially the amount of data analysed are appropriate to allow the authors to draw

the conclusions they did. I therefore do not recommend publication of this manuscript unless the authors can clarify all the issues addressed in detail below.

- Methods: Page 2, Line 32: You state that in the 2010 experiment you had two pCO2 treatments at 380 and 1000 ppm. However, in the figure 2 there are three pCO2 treatments at 217.7 (which would be pre-industrial), 780.0 and 1652 ppm which would both be future scenarios. In figure 3 you only show 780.8 and 1652 ppm. Which one is true? If you did not have and ambient control treatment at 380 ppm please explain why. - Authors'R 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". In figure 3, only the two higher CO2 values appear because, as indicated in the methods section (lines 14-15, page 5), we used the lowest pCO2 value as reference point in order to apply the IA method.

- Page 2, Line 35: How many 20l bottles were incubated? Only one per treatment without replication? - Authors' response: We added information on the number of replicate bottles in lines 37-38, page 2 of the revised manuscript: "For the 2009 experiments we used two replicate bottles for each treatment, and three replicates for each treatment in the 2010 experiment."

- Page 2, Line 38-Page 3 Line 5: Were the bottles constantly bubbled during incubation or was the target pCO2 just adjusted in the beginning? If yes, it will have changed during incubation, was pH monitored? - Authors' response: The bottles were incubated under a constant bubbling of air-CO2; this information has been added to the manuscript in lines 3-5, page 3: "Throughout the 2010 experiment the target pCO2

level was achieved by fitting each experimental 20 L bottle with a bubbling system connected to CO2 bottles and air mixture bottles. The gas mixture was continuously provided by mass flow controllers (model GFC17, Aalborg Instruments and Controls, Inc.) (...)". Besides this, total hydrogen ion concentrations (i.e., pH) and total alkalinity (TA) were measured daily along the experiment (information added in lines 10-11, page 3).

- Page 3, line 8: 200 μmol photons constant light seems very high. Can you show environmental data to show that these are average light intensities phytoplankton is exposed to in Arctic summer considering the deep mixing? - 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.

- Page 3 line 10 following: My major concern about the cell size and valve thickness measurements is that I don't know how you can be sure that you measured individual frustules from the same species. Following the cleaning procedure, the cells will be empty and broken. Were you able to identify the species or at least genus? If yes please report them. If not how can you be sure that you did not measure cells from different species in each treatment? Also how many cells did you measure from each treatment? In figure 1 it seems like you only took one measurement per treatment as there are no error bars. If this is true, I do not believe that your data show any temperature trend but simply the natural variability in cell size that you find in any diatom species. In line 27 and 28 you state that you determined cell volume from the closest geometric figure. Did you measure the frustule height for each measured cell or did you just use the same estimated height for all measurements throughout all treatments? If you do not have the exact cell height for each cell you measured

your cell volume estimations will be very inaccurate. Cell height in diatoms is much more variable than frustule diameter even within the same species. At least you would need to know which species you have measured in each treatment and use an average literature value for its cell height. - Authors' response: The measurements performed during our experiments were done on the most abundant groups, and they were clearly differentiated from other groups. The process of cell cleaning has been described as a necessary methodology in order to facilitate diatom identification and analysis using light microscopy (i. e. Identifying marine phytoplankton (Tomas, 1997); Phytoplankton identification manual (Verlecar & Desai, 2004)), so in fact this step facilitates diatom identification. Also, according to the reviewer's comment we have added to the revised manuscript the identification to genus level of the diatom groups studied here (lines 22-26, 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". - As the reviewer observed here, the error bars in Figure 1 were missing, so we have completed this figure by adding the error bars to the plots. - 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.

- Page 3 line 30 following: If I understand this correctly you used the PDMPO uptake over 24 hours I sub-incubations to determine the silicification in your treatments. But you do not describe any biomass measurements to relate to PDMPO uptake, do you just assume that growth rates and grazing were exactly the same in all treatments?

Total PDMPO uptake does not say anything about silicification if you don't know the diatom biomass. The lower Si uptake at high temperatures could also mean that less diatoms grew in total, or more of less silicified diatom species but it does not necessarily mean that each diatom species changed its silicification. - 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 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.

- Page 4, Line 23 following: I don't really understand why you are using this model if the assumption that temperature and pCO2 have additive effects is clearly wrong and does not fit your data. I don't see how this is helpful. - Authors' response: The simple and intuitive interpretation of joint effects is only appropriate under the condition of a linear relationship between the intensity of the single stress factors and their effects (Coors and De Meester, 2008). Thus, the summation of effects could not be applied here as increased temperature and pCO2 typically show non-linear dose-response curves. The use of a mathematical model will help on the identification of the degree of interaction. The independent action (IA) model used here assumes additivity, and denotes synergy and antagonism by positive and negative significant deviations of the observed relative to the predicted effect, respectively, as described in Payne et al. (2001). This model is widely used in toxicology and has been successfully applied to test the interaction be-

tween stressors as toxic chemicals in zooplankton (Coors & De Meester, 2008; Carreja et al., 2016). Moreover, this method allows a good graphic representation of the results. We have added in the revised manuscript a better explanation of the usefulness of the model applied (lines 1-6, page 5). "In order to determine mathematically the existence of synergy or antagonism in the effects of increased temperature and pCO2 in silica incorporation rates of diatoms, we used the independent action (IA) model described by Payne et al. (2001). This model has been recommended for the prediction of the joint effects of dissimilarly acting factors (stressors that influence independently the regulation of a life-history trait by different mechanisms). It assumes additivity and denotes non-additivity by deviations of the measured from the predicted (reference) responses (Coors and De Meester, 2008)".

- Page 5, Line 1 following: The estimation of sinking rates does only apply for dead cells as you assume constant cytoplasm density for all species and throughout all treatments. This should be made clear here and later in the results and discussion. - Authors' response: We improved the description in the revised manuscript. The lack of cytoplasm density measurements in the literature compels us to use a constant value for living cells (Miklasz and Denny (2010). We used the same assumption in the formula to estimate the potential sinking rates of living diatoms. In the revised version of the manuscript, in lines 36-37, page 5, we added the following information: " We have assumed here a constant value for cytoplasm and valve densities in living diatoms as there are very few literature data on this topic (Miklasz and Denny, 2010)" and it is not clear yet if the cytoplasm density could be species-specific (Miklasz and Denny, 2010). We used the equation described in Miklasz and Denny (2010) to estimate the changes in the potential sinking speed of a diatom due to variations in valve size and thickness.

- Page 5, Line 18-20: This should go in the results section - Authors' response: As suggested by the reviewer, the referred sentence has been relocated to the Results section (lines 17-18, page 6).

- Page 5, Line 27,28: I hope the pH values did not vary between 8.2 and 7.5 which

would be massive but that these are indeed the pH values for the three individual pCO2 treatments. Please state how and when pH was measured and if these are the start values or average over the course of the incubation. - Authors' response: These pCO2 values for each treatment have been obtained from daily measurements, as indicated on a previous response: " Total hydrogen ion concentrations (i.e., pH) and total alkalinity (TA) were measured daily along the experiment (see Coello-Camba et al. 2014)"(lines 10-11, page 3). This information has been clarified in lines 14-16, page 6: "The pH values obtained for each pCO2 treatment by averaging daily measurements of the last week of incubation were 8.2 (0.1), 7.9 (0.1) and 7.5 (0.0) pH units ".

- Tables 1 and 2: I think these two tables should be combined. Also in table 1 you state that the temperatures for the first two experiments were 1.5 – 10.5 and from 1 – 10 in experiment 3 but in table 2 temperature goes from 1.6 to 10.5 and from 1.2 to 10 in the first two experiments and from 1.8 to 10.3 in exp. 3. Also pCO2 in table 1 is once again described as 380 and 1000ppm. Please make sure you report the correct values throughout. - Authors' response: According to the reviewer's suggestion, both tables have been combined (new Table 1).

- Table 3: I cannot believe that all the cells you measured had the exact same size (e.g. 21.4 _m in exp.1). There is always a natural variance in cell size in every species and in this study it is absolutely crucial to know what the natural variability is in order to be able to estimate changes in cell size caused by the different treatments. So please report the number of individual cells you measured in each treatment and the actual cell size of each. - Authors' response: We added more information to indicate the natural variability in the cell size of the diatoms described here in the revised version of the manuscript. To do so, we added the standard errors in the measurements of cell valve diameters (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. We also

added in the methods section of the revised manuscript the minimum number of cells measured per treatment (lines 26-27, page 6).

- Figure 1: From this figure I am not convinced that you were able to perform the measurements of valve thickness and cell diameter with the precision that you report. In table 3 you have calculated a change in valve thickness of less than 1 nM per _C. As the temperature range you have tested is 10_C this would mean that you have actually measured a 10 nM difference in valve thickness between these two treatments using light microscopy! I find this hard to believe. - Authors' response: Although the theoretical maximum resolving power in optical microscopy is 0.2 μm at 1000x magnification, this parameter is quite dependent on the detection mode used. Digital imaging systems allow image enhancement and perform considerably better contrast than the nonlinear human eye, so the standard resolution criteria do not apply when these image analysis softwares are used (Hajjar et al., 1999). This way, the method we followed here used 1600x magnification allowing a maximum resolving power of 0.125 μm, plus image analysis system, had an adequate resolution (0.05 μm approx.) to perform the measurements of valve thicknesses. Besides this, as we indicated in the text, we did not observe significant variations in the valve thickness measurements with temperature or pCO2 (lines 34-35, page 6). We added the methodological information in the revised manuscript lines 36-39, page 3.

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Figure 1

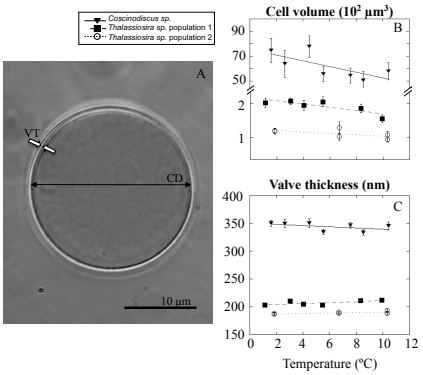

Fig. 1. New Figure 1

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 (ºC)	-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. 2. New Table 1