

## ***Interactive comment on “Individual and interactive effects of warming and CO<sub>2</sub> on *Pseudo-nitzschia subcurvata* and *Phaeocystis antarctica*, two dominant phytoplankton from the Ross Sea, Antarctica” by Zhi Zhu et al.***

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Response to Anonymous Referee #1

We agree with the reviewer that there are many factors that may affect the growth and competitive success of Ross Sea phytoplankton, and we also agree that iron is certainly among the most important. In fact, as the reviewer notes we have worked extensively on this topic in the past, including a recent paper that used these same two Antarctic species to examine the interactions between iron limitation and warming (Zhu et al. 2016, Marine Ecology Progress Series 550: 39–51). We have also compared the

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responses of *Phaeocystis* and another Ross Sea diatom (*Fragilariopsis*) to changing iron availability under a ‘clustered’ matrix of present or future temperature, CO<sub>2</sub>, and light (Xu et al. 2014, Limnol. Oceanogr., 59, 1919–1931). In terms of experimental logistics, though, it is not practical to simulate all potential future changes in one set of factorial experiments, so we decided to focus particularly on temperature and CO<sub>2</sub> and their interactions in this one.

These iron-replete experiments are arguably more realistic for our isolates than they would be for many other Southern Ocean phytoplankton, since our cultures came from coastal McMurdo Sound. This is an area that often experiences extended periods of springtime iron-replete conditions, before eventually transitioning to late summer iron-limited conditions as the annual bloom progresses and depletes the iron (Bertrand et al. 2015, PNAS 112). We mentioned this rationale for doing the experiments with added iron on lines 93-96 in the Methods, but we can add more justification and detail during revisions. It has also been suggested by some research that as iron addition stimulates the growth of both diatoms and *Phaeocystis*, iron availability may not be a major distinguishing factor in the competition between these two groups. As it is, we agree with the reviewer that our experiments address warming and acidification responses in the absence of any differential effects of iron limitation, and we will plan to add text explicitly stating this. We disagree with this reviewer that there is a lack of community interest in understanding the interactions between warming and acidification in Ross Sea phytoplankton, regardless of other important factors like iron limitation, stratification, UV, etc, and the other two reviewers seem to agree with us on this point.

Responses to the specific comments:

1. We will update the citations to include the recent related papers and reviews in our manuscript, as we are aware that new studies have come out since we wrote and submitted this manuscript. For the comments on the literature now cited in our paper, line 37: Arrigo et al. (1999) and Smith et al. (2000) reported the distribution and seasonal cycle of diatom and *Phaeocystis* in the Ross Sea, the same coastal Southern

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Ocean polynya that our isolates came from. It seems a matter of semantics, but we can certainly refer strictly to the 'Ross Sea' in the text rather than generally to the coastal portion of the Southern Ocean. Arrigo et al. (2008) studied the production of all the Southern Ocean, and found that the Ross Sea is one of most productive regions in the entire area. line 39: We can add some of the numerous other available references to warming in the region other than Sarmiento et al. (1998). We can also cite Rose et al. (2009), Xu et al. (2014) and Zhu et al. (2016) here to mention that temperature increases may promote the growth of phytoplankton in the Southern Ocean, and so may compensate for the decrease of carbon export predicted by Sarmiento et al. (1998). line 52: we will change zooplankton to microzooplankton in our manuscript; line 32: Instead of Gille (2002), we will cite references more appropriate to surface warming such as Meredith and King (2005).

2. We agree that the full range of temperatures we used exceeds likely maximum warming levels in the region, at least for the next few centuries. However, there is considerable value in examining the full range from 0C to 10C in order to generate complete thermal functional response curves for these two phytoplankton, allowing us to calculate key quantitative parameters including maximum growth temperatures, maximum growth rates, optimum temperatures, etc, and so thoroughly understand their overall thermal physiology. In fact, using a broad range of temperatures enabled the interesting and significant observation that both species (but especially the pennate diatom) are in fact typically growing well below their optimum growth temperatures in the current Ross Sea. One of our most important results is that all degrees of foreseeable future warming, far from being deleterious, will in fact increase the potential maximum growth rates of the diatom relative to the prymnesiophyte. At any rate, the (relatively) near future two degree temperature increase mentioned by the reviewer is already included in our thermal response curves, which thus offer both the near-term environmentally relevant information this reviewer requests, as well as considerably more information on physiology at higher temperatures. The fact that we performed full thermal response curves rather than just examining two temperatures in a simple

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dichotomous 'current' and 'future' scenario was commented on by Reviewer 3 as being one of the best features of our experimental design. For these same reasons, we also deliberately extended our full CO<sub>2</sub> response curves out to very elevated pCO<sub>2</sub> values that are not likely to occur in the Ross Sea in the near future, but that still offer useful information about physiological responses to acidification. The competition experiment (which was really a co-growth experiment, as was pointed out by Reviewer 3) was run at 6C because this temperature lies within the optimum growth range for both species (see Fig. 1). The experiment was simply intended to test if our thermal response curves for both species individually were indeed predictive of growth rates in simple co-cultured model communities. It was not intended to accurately simulate near-future warming levels, but rather see if warmer conditions favor the diatom even when grown together with *Phaeocystis*. We will add text to better explain this in the revised manuscript.

3. We will mention in our revised manuscript that our *Phaeocystis* cultures mostly consisted of small colonies.

4. Yes, certainly growth rates increase with temperature, but the surprise as noted above is that optimum temperatures especially for the diatom are well above any currently relevant temperatures for the Ross Sea. We will make this key point more clearly in the revised manuscript.

5. Similar C:chl ratios ranges have been observed by Smith et al. (2000) in situ in the Southern Ocean, and by Xu et al. (2014) and Zhu et al. (2016) in lab cultures. It seems likely that the amount of organic C associated with colonial *Phaeocystis* may be quite variable, as it depends not only on cell biomass but also especially on the amount of mucilage produced by the cells under any particular growth condition.

6. We'll update our manuscript to mention that zero growth rate at zero CO<sub>2</sub> was assumed. As the reviewer notes, it is reasonable to assume that an obligate photoautotroph cannot grow at zero CO<sub>2</sub> concentrations, and in fact there would be no practical

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way to grow cells at 0 pCO<sub>2</sub> to obtain a real data point here. Likewise, it might be difficult to grow them even at 25 or 50 uatm, as from our curves it looks like growth rates would be very low here. The possibility of mucilage oxidation serving as a supplementary source of CO<sub>2</sub> for *Phaeocystis* at low ambient pCO<sub>2</sub> is an interesting if speculative idea, and we can add text to mention this during revisions.

7. We'll update the wording to get rid of light intensity and use irradiance instead, and can delete the term "co-variables" for temperature and light. As the reviewer notes there are some situations where deeper water can be slightly warmer than the surface mixed layer, but in general shallow mixed layers will have often have a tendency to warm up from solar heating relative to underlying water, depending of course on the amount of ice melting occurring. We can rewrite this text to make this discussion more realistic and relevant.

8. We agree that Si remineralization rates are temperature dependent, and will add this point into our discussion. 9. We agree that we cannot extrapolate one diatom to all diatoms; we focused on *Pseudo-nitzschia subcurvata* as our previous work in both the field and the lab suggests it will be a particular beneficiary of warming in the Ross Sea, but we will carefully qualify our conclusions appropriately in the revisions. Likewise, we also cannot be 100% sure how the phytoplankton community composition and elemental fluxes will be shifted by global change, and so we will broaden our discussion to include the alternative possibility in the new JGR paper mentioned here.

#### Response to Anonymous Referee #2

1. We will try to rewrite our manuscript to better highlight the interesting aspects of the study for readers.

2. We agree that comparisons with the results of the related study by Trimborn et al. (2013) are needed, but we have already cited this paper and made some of these comparisons in our manuscript on lines 360-365. We can add further text to compare the two studies in more depth, though.

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3. The typo will be corrected in our revised manuscript

#### Response to Referee #3, Andrew McMinn.

We appreciate the reviewer's positive comments about our experimental methods, the use of recently isolated cultures, and the importance of our study.

1. We mentioned that *Phaeocystis* and diatoms contribute in significantly different ways to biogeochemical cycles of nutrients in the introduction due to their different stoichiometry, but can re-emphasize this in our revisions. The elemental ratio analyses helped to us to confirm these differences, and reveal potential effects of warming and acidification on these elemental ratios both in living phytoplankton and potentially in exported particles. We can make our reasons for including these data more evident in the revised paper.

2. We agree iron is an important factor for the growth of phytoplankton in the Southern Ocean. As noted in our response to Reviewer 1, we addressed iron limitation and its interactions with warming in Zhu et al. (2016) and interactions of iron with combined treatments of warming, CO<sub>2</sub> and irradiance in Xu et al. (2014), and will modify the text to point readers to these papers and recognize that iron is clearly an important factor that can't be neglected in the Ross Sea.

3. We agree the competition experiment might be better described as a co-incubation or co-growth experiment to show whether the growth rates observed in unialgal cultures are repeatable in mixed cultures. 6C was chosen because the growth rates of these phytoplankton started to differentiate significantly at this temperature, and this temperature also lies within the optimal range for growth for both isolates (see Fig. 1). As noted in our response to Reviewer 1, we did not intend this experiment to simulate any specific future scenario. We agree with the reviewer's point that the experiment tests the relative maximum growth rates of both species at this temperature, rather than being a true 'competition' experiment. We would like to retain these results for the reasons mentioned above, but will not refer to it as a competition experiment (and will

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explain why this is so), but rather we will refer to it as a co-culture experiment.

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