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Interactive comment on "Ocean acidification reduces growth and grazing of Antarctic heterotrophic nanoflagellates" *by* Stacy Deppeler et al.

Anonymous Referee #2

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General Comments

Heterotrophic nanoflagellates play and important role in pelagic marine ecosystems as grazers of picoplankton and as prey for microheterotrophs (ciliates and heterotrophic dinoflagellates). Polar marine ecosytems are especially vulnerable to ocean acidification and a several studies have investigated the effects of Ocean acidification on Antarctic and Arctic pelagic microbial communities. This paper reports the results of a study which employed experimental minicosms to examine the effects of ocean acidification on pelagic microbial communities in Antarctica coastal waters. It contributes new observations on predator-prey interactions in response to acidification and sup-

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ports observations derived from a previous study undertaken the same location using a similar experimental approach. The novelty of the study, as compared to the previous study, lies in the incorporation of an initial acclimation period within the experimental design. It is also useful and somewhat novel to encounter a paper which repeats and reinforce the insights gained from earlier work. The paper is well presented with a well-referenced introduction and discussion. The methods and data interpretation are sound, and the conclusions are supported by the results. However, there are some weaknesses in data interpretation and conclusion, outlined below, which should be addressed. In summary, the paper should make a valuable contribution to the literature addressing a timely and relevant topic which should be of interest to readers of Biogeochemistry.

Specific Comments

Section 2.4 (Page 5, line 19): State volume of the single sample removed from each minicosm on each day for flow cytometry analysis (from which different sets of pseudoreplicates were subsequently removed for analysis of different microbial groups).

Section 2.4 (Page 5, line 22): Flow cytometry is the main method used to generate microbial data in the study so the authors should describe in full how flow cytometer sample flow rates were calibrated. This is important as flow cytometer flow rates are highly variable, and the exact volume analysed from each sample must be assessed independently. Poor calibration technique is therefore a significant source of error in some studies. Describing how flow rates were calibrated gives confidence that the microbial abundance data are accurate. It also reminds readers that rigorous procedures are required to generate accurate data from flow cytometers.

Section 2.4.3 (Page 6, line 18): The prokaryote abundance measurements (undertaken by flow cytometry using SYBR Green I stain and FL1 versus SSC plots) will include phototrophic prokaryotes (i.e. picophytoplankton) as well as heterotrophic prokaryotes, unless the picophytoplankton data (derived from analyses in Section 2.4.1) were

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subtracted from the prokaryote counts. This should be stated in this section of the methods. Heterotrophic prokaryotes will dominate these data, especially in Antarctic waters, so it is acceptable to treat the prokaryote results as representing mainly heterotrophic bacteria in subsequent discussions.

Section 2.5 (Page 7, Line 3): The authors correctly state that "...statistical differences among treatments should be interpreted conservatively..." due to lack of true replication. Clear trends between treatments can be clearly identified over the duration of incubation. However, conclusions based on the analysis of statistically significant differences between treatments (based on pseudoreplicates) at any one time point (page 7, line 1) are unconvincing. These include the subsequent statements (page 8, line 7; page 9, line 5) based on the picophytoplankton and prokaryote peak abundance analyses shown in Figure 7. Also the comparison of picophytoplankton and prokaryote growth rates with heterotrophic nanoflagellate abundance (page 9, line 20) shown in Figure 8. The respective conclusions from these analyses (that picoplankton and prokaryote abundance differ between treatments, and that reduced heterotrophic nanoflagellate abundance reduced grazing on picoplankton) are well-supported by the other analyses using data from several time points. I therefore question whether the authors should include the analyses presented in Figures 7 and 8.

Sections 3.4 (Page 8, line 15) and Section 4.2 (page 13, lines 12 and 23): I see no evidence in Fig 6b or Fig S3b that nanophytoplankton abundance was higher than the control in the 954 uatm treatment. The modelling data (shown in Table 2) may have revealed this but the modelling result is a simulation of the underlying data which, in turn, is based on pseudoreplicates. The figures clearly show that nanophytoplankton abundance was higher than the control in the 634 treatment, but the case for the 954 uatm treatment resulting in higher abundance is unconvincing.

Section 4.1 (Page 11, line 10): I am not convinced of the utility of the conclusion that heterotrophic nanoflagellate communities may change by 2050 due to ocean acidification. The abundance and composition of Antarctic heterotrophic nanoflagellate

communities may well change by 2050 for many reasons, and microcosm experiments undertaken over 18 days cannot simulate real environmental changes to entire ecosystems over decades. I suggest this conclusion is removed.

Section 4.1 (Page 11, line 13): The discussion on top-down control of heterotrophic nanoflagellates by the microheterotrophic community (heterotrophic dinoflagellates and ciliates) could include some additional considerations, as follows:

First, the study by Hancock et al. (2018) assessed microheterotroph abundance in Lugol's fixed samples of 2 to 10ml volume. It is not possible to derive meaningful microheterotroph data from such small sample volumes, so the statement (page 11, line 14) that treatments had no effect on the heterotrophic dinoflagellates and ciliates may not be valid.

Second, it would be useful to discuss the evidence for any switching in grazing pressure by microheterotrophs between nanophytoplankton and heterotrophic nanoflagellates. The fact that nanophytoplankton abundance was similar between treatments (except for 634 uatm – Figure 6b) suggests that heterotrophic dinoflagellates and ciliates were not exhibiting differential grazing pressure on heterotrophic nanoflagellates between treatments. This, in turn, lends support to the conclusion that the lower heterotrophic nanoflagellate abundances in high CO2 treatments were not a result of top-down pressure (assuming microheterotroph numbers were not affected by acidification and similar in each treatment). On the other hand, the observed shift in the composition of the nanophytoplankton community from Phaeocystis to Fragilariopsis in high CO2 treatments (page 13, line 21), as reported by Hancock et al. (2018), suggests that one would expect some differential microheterotrophic grazing between treatments and possible switching between nanophytoplankton and heterotrophic nanoflagellate prey.

Third, the consequences of screening the seawater used to fill the minicosm tanks through a 200 micron filter should be discussed. This action will have reduced top-down grazing pressure on microheterotrophs, possibly creating a differential trophic

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Discussion paper

cascade effect between treatments over the 18 days incubation. Any such effects may well have been minimal and equal across treatments. However, the potential effect of initial seawater screening should be discussed, especially with respect to the limits to which minicosm experiments can simulate the dynamics of in situ communities.

Section 4.1 (Page 12, line 5): Mixotrophic nanoflagellates will have been included within the nanophytoplankton counts due to the presence of chlorophyll (albeit possibly at low levels) within the cells. This should be mentioned in the methods or discussion text.

Section 4.3 (Page 13, line 32) and Section 4.4 (page 14, line 34): The results of Westwood et al. (2018) should be discussed as they are derived from the same location and draw similar conclusions to the present study (i.e. enhanced bacterial production and abundance in high CO2 treatments coinciding with reduced heterotrophic nanoflagellate abundance).

Technical Corrections

Page 1, Title: The title could be misinterpreted as reporting the effects of ocean acidification on the "...grazing of heterotrophic nanoflagellates." by their microzooplankton predators. A more accurate but unwieldly wording would be "...reduces growth of and grazing on heterotrophic nanoflagellates.". Perhaps rephrase as "...reduces growth and grazing impact of heterotrophic nanoflagellates".

Page 2, line 6: Correct spelling "whish" to "which".

Page 2, line 27: Use of the phrase "...in the present study..." implies that the observations referred to are part of the submitted manuscript rather than a different publication. Perhaps use the phrase "...concurrently observed amongst choanoflagellates in the present minicosm experiment..."?

Page 6, line12: The text refers to Figure 2a which shows a plot of FL3 versus FSC, rather than FL3 versus FL2 as stated in text.

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Page 9, Line 5: “Fig. 7” should read “Fig. 7b”.

Page 11, line 27: Add hyphen to change text to “. . .bloom-causing. . .”

Page 13, line 17: Refer to Fig S3b rather than Fig 6b as the treatment-specific dynamics of nanophytoplankton observed during the early stage of the experiment (days 1-9) are visible in Fig S3b but cannot be clearly resolved in Fig 6b.

Page 34, Table 2: Why are table columns the p-value data labelled “Day:”?

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