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

Stacy Deppeler et al.

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Reviewer 1:

General comments

This work is part of a minicosm investigation of the effects of increasing fCO2 levels on a natural planktonic microbial community of Prydz Bay, East Antarctica, and deals with the response of heterotrophic flagellates (HNF), nano- and picophytoplankton, and prokaryotes. The design of the experiments was similar to that of previous studies in East Antarctica, but with an initial CO2 acclimation period. The publications (one of

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them, at least, in Biogeosciences) on the same minicosm experiment, and will have benefitted from the reviews of the previous works.

Overall, the manipulations appear to have been competently carried out and the text is well written. Concerning the discussion, I appreciated, in particular, the consideration given to potential community shifts, in addition to physiological changes. Some comments on aspects that could be improved are given below.

Specific comments

The main results of these accompanying works tend to appear late in the text; they should rather be presented up front in the introduction, so that the reader can better appreciate what is the context for and the contribution of the present study.

Response: We agree that presenting the previously published results of this minicosm study in the Introduction will provide greater context for the results presented. We will update the Introduction to include a summary of the previously published findings of this minicosm study.

Some conclusions go further than supported by the presented results. For example, the statement (whether correct or not): “Therefore, it is likely that increasing CO2 will cause the phytoplankton community to shift from a summer community that is currently dominated by large diatoms to one composed of smaller species or morphotypes of nano- and picophytoplankton.” (lines 27-29 of page 13) does not derive from the work shown in the present manuscript (or if the authors believe so, it should be much better discussed).

Response: This is true, we did not analyse the phytoplankton community >50 μm

in size so cannot solely base this conclusion on the results of this manuscript. This conclusion took into account the additional data provided by microscopic analysis of the microphytoplankton community in Hancock et. al (2018). We will reconsider this conclusion in its current location and will update this section of text to be more specific to the work in the present manuscript. We will provide further discussion of the combined published results of the greater minicosm study in the Conclusion.

Other comments

It would be helpful for the readers to give more details on the statistical analyses (for example, explain "I" in tables S2-S5, number of time points and of pseudoreplicates).

Response: We regret not having provided sufficient information regarding the statistical analysis. A number of changes will be made to the presentation and interpretation of statistical analyses (see also other referee comments below) and more clarification will be provided regarding pseudoreplicate numbers and abbreviations displayed in statistical tables.

It would be helpful to repeat somewhere that the prokaryote group here is supposed to include few or no cyanobacteria.

Response: The referee is entirely correct, the prokaryote analysis is of the heterotrophic prokaryote community only. This is because autotrophic prokaryotes (ie, cyanobacteria) were not detected in our study. We did mention in the Introduction that cyanobacteria are very rare in coastal Antarctic waters but we will reiterate this information in the Methods to make it abundantly clear that they were not detected in our flow cytometry results and were not part of the prokaryote analysis.

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Line 3 of page 9. Eliminate “treatments”.

Response: We will fix this sentence to remove the extra word.

Lines 6-7 of page 10. “acclimating cells over the years to decades . . . is unachievable in most experimental designs”. It is also doubtful to expect that the same cells/taxa would be acclimating for years or decades in natural settings.

Response: This is true and we will amend the wording of this sentence to acknowledge this.

Lines 7-8 of page 13. “dominated by large diatoms and ...” Which were the main “large diatom taxa”?

Response: Previous observational studies of East Antarctic waters, of which the study site is located, identified a diverse range of large diatom taxa (e.g. Davidson et. al, 2010). The most abundant during summer were generally Fragilariopsis sp., Chaetoceros sp., Thalassiosira sp., Navicula sp., and Pseudo-nitzschia sp. In our minicosm study, the dominant species were large centric and pennate diatoms such as Thalassiosira sp. and Fragilariopsis sp. (see Hancock et. al, 2018). We will update this sentence to specify that we are discussing East Antarctic phytoplankton communities and provide examples of the dominant large diatom genera in early summer in this region.

Lines 28-29 of page 13. “a summer community that is currently dominated by large

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diatoms". This would not apply to many Antarctic areas.

Response: We disagree with this statement. Summer blooms of large diatoms have been observed frequently across East Antarctic coastal regions and the Antarctic Peninsula (e.g. Ducklow et. al, 2007, Davidson et. al, 2010). The Ross Sea is one region where this is not necessarily the case and where large blooms of Phaeocystis antarctica are observed during the summer months (Arrigo et. al, 2000). That said, we do acknowledge that the driving factors for community composition differ around Antarctica. Our experiment was performed in East Antarctic waters and we did not intend our statement to encompass all Antarctic waters. We will update our conclusions to make this clearer.

Line 31 of page 13. "Increases of prokaryote .."

Response: We will fix this.

Explanation of Fig. 7: "prokaryotes" instead of "prokryotes".

Response: We will fix this.

Explanation of Fig. 9: Add indication that the abscisssa shows the picophytoplankton and prokaryote abundances on the day before decline. For example: "Heterotrophic nanoflagellate abundance (y axis) on the day before (a) picophytoplankton and (b) prokaryote abundance (shown in x axis) declined in each ... "

Response: We will revise the figure explanation to clarify the identity of the axes.

References:

Arrigo, K.R., DiTullio, G.R., Dunbar, R.B., Robinson, D.H., VanWoert, M., Worthen, D.L., Lizotte, M.P., 2000. Phytoplankton taxonomic variability in nutrient utilization and primary production in the Ross Sea. J. Geophys. Res. Ocean. 105, 8827–8846. https://doi.org/10.1029/1998JC000289

Davidson, A.T., Scott, F.J., Nash, G. V., Wright, S.W., Raymond, B., 2010. Physical and biological control of protistan community composition, distribution and abundance in the seasonal ice zone of the Southern Ocean between 30 and 80°E. Deep Sea Res. Part II Top. Stud. Oceanogr. 57, 828–848. https://doi.org/10.1016/j.dsr2.2009.02.011

Ducklow, H.W., Baker, K., Martinson, D.G., Quetin, L.B., Ross, R.M., Smith, R.C., Stammerjohn, S.E., Vernet, M., Fraser, W., 2007. Marine pelagic ecosystems: the West Antarctic Peninsula. Philos. Trans. R. Soc. B Biol. Sci. 362, 67–94. https://doi.org/10.1098/rstb.2006.1955

Hancock, A.M., Davidson, A.T., McKinlay, J., McMinn, A., Schulz, K.G., van den Enden, R.L., 2018. Ocean acidification changes the structure of an Antarctic coastal protistan community. Biogeosciences 15, 2393–2410. https://doi.org/10.5194/bg-15-2393-2018

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Reviewer 2:

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

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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 supports 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 methods and data interpretation are 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).

Response: We will update the flow cytometry methods to include the volume information.

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

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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.

Response: We agree that understanding the flow cytometry methods is important. The "high" and "low" flow rates for each flow cytometer was calibrated by performing a linear regression of sample volume analysed by increasing time increments (in mins). This allowed us to determine the volume analysed for each different assay, based on the run time and flow speed setting. We appreciate the reviewer drawing our attention to the calibration data, as in doing so we found an error in the calculation of the pico- and nanophytoplankton abundance after changing to the FACSCalibur instrument on Day 16. This error was applied across all treatments so did not affect the overall CO2 treatment trends, but it did reduce the abundance observed on days 16-18. We have taken this error very seriously and have re-analysed all of our data to ensure that our results and conclusions have not changed. In the updated manuscript we will include more detail in the Methods to explain how the flow rate and volume calibrations were performed and put the data into a table in the Supplement. We will also update the individual flow cytometry methods for each group to specify the exact flow rates and volumes for each assay so that the calculations are clearer. Lastly, we will include an explanation that we had to use a different flow cytometer on day 16 because the FACScan broke down. All figures and tables will be remade to ensure they display the corrected data.

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

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.

Response: Autotrophic prokaryotes (ie, cyanobacteria) are rare in the region where we performed our experiment (see Wright et. al, 2009) and we did not observe any in our flow cytometry analysis. We have mentioned this in the Introduction but we will include this information in the Methods as well to make it clearer that the prokaryote data included only heterotrophic species.

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.

Response: Upon reviewing the results, we agree there is not a good case for higher abundance of the 953 μatm treatment in the nanophytoplankton community. This result was based on a model that we accept was not well-fitted to the abundance of the treatments. Based on the above feedback of the statistical analysis in Sections 2.5 & 3.4 and our correction of pico- and nanophytoplankton abundance on days 16-18, we are reassessing our statistical methods. Examples of this are providing more robust modelling of growth curves through the use of generalized additive models (GAMs) to assess temporal changes in the abundance of the various microbial groups and removal of single time point analyses.

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.

Response: We understand and have acknowledged in the Discussion that simulating real environmental changes to ecosystems over decades is a limitation of our experimental design. We also acknowledge that changes in CO2 are one of a number of environmental factors that will influence these communities with climate change (see Deppeler and Davidson, 2017). We will reconsider the wording of this conclusion and supply caveats around the onset and magnitude of additional stressors that may affect the HNF community response to ocean acidification.

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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.

Response: The reviewer has misinterpreted the methods for Lugol's-fixed microheterotroph analysis in Hancock et. al (2018). The 2-10 ml samples were sedimented concentrates of seawater that were derived from 960 ml of sea water. Hancock et. al (2018) did, however, acknowledge that microheterotroph abundance was low (1% of all cells) and that a lack of CO2 response may have been related to these low counts. We shall therefore add that the response may not have been apparent due to the low abundance of these species in the experiment.

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

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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.

Response: This is an interesting consideration and we will re-evaluate our results and include discussion on possible changes in grazing pressure by microheterotrophs and heterotrophic nanoflagellates. Low abundances of heterotrophic dinoflagellates and ciliates in all treatments does suggest that grazing pressure on HNF was low and that reductions in heterotrophic nanoflagellate abundance at higher CO2 levels were not likely caused by increased grazing from larger taxa. We will also consider in the Discussion how a shift in the dominant nanophytoplankton taxa might affect grazing dynamics.

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 topdown grazing pressure on microheterotrophs, possibly creating a differential trophic 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.

Response: This is also an interesting consideration and we will include discussion on how a reduction in top-down pressure of larger zooplankton species may have affected the results. We routinely pre-screen the microbial community by 200 μm in these experiments because small differences in the abundance of large grazers among tanks could greatly affect the trajectory and composition of the succession in the tanks, thereby masking any CO2-induced effect. We do appreciate that grazing of

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>100% of daily production is observed in waters in this region (see Pearce et al. 2010). For this study, pre-screening by <200 μm allowed us greater control by only varying one environmental factor so we could focus on the effect of CO2 on the microbial community dynamics. We will include some consideration around how this may have affected our results in the Discussion.

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.

Response: This is true, we still lack a thorough understanding of the mixotrophic community so we cannot comment on what proportion of nanophytoplankton cells are mixotrophic species. We will provide acknowledgement that mixotrophs will be part of the nanophytoplankton data. In addition to this, we will clarify that chlorophyll-containing mixotrophic cells will only be present among the nanophytoplankton but are not included in the heterotrophic nanoflagellate counts due to our removal of chlorophyll-containing cells as a first step to identifying heterotrophic cells.

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).

Response: A comprehensive analysis of the results of Westwood et. al (2018) in relation to bacterial production in this minicosm study has been provided in Deppeler

et. al (2018). However, we appreciate that the findings of Westwood et. al (2018) provide valuable support to the current paper and will include further analysis and comparison with the results reported by them in our Discussion.

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.

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:"?

Response: We agree with all the above technical corrections kindly provided by the

reviewer and will amend the text accordingly. In response to the final comment about the column label "Day:", the "Day:506" specifies the interaction term for the variables "Day" and "506" (i.e., CO2 treatment) in the statistical model. So, this is the result of the response of the CO2 treatment over time. We will make a number of changes to the presentation and interpretation of the statistical analyses (see above comments), so the information provided in text and in the tables is clear to the reader.

References:

Deppeler, S.L., Davidson, A.T., 2017. Southern Ocean Phytoplankton in a Changing Climate. Front. Mar. Sci. 4, 1–18. https://doi.org/10.3389/fmars.2017.00040

Deppeler, S., Petrou, K., Schulz, K.G., Westwood, K., Pearce, I., McKinlay, J., Davidson, A., 2018. Ocean acidification of a coastal Antarctic marine microbial community reveals a critical threshold for CO2 tolerance in phytoplankton productivity. Biogeosciences 15, 209–231. https://doi.org/10.5194/bg-15-209-2018

Hancock, A.M., Davidson, A.T., McKinlay, J., McMinn, A., Schulz, K.G., van den Enden, R.L., 2018. Ocean acidification changes the structure of an Antarctic coastal protistan community. Biogeosciences 15, 2393–2410. https://doi.org/10.5194/bg-15-2393-2018

Pearce, I., Davidson, A.T., Thomson, P.G., Wright, S., van den Enden, R., 2010. Marine microbial ecology off East Antarctica (30 - 80°E): Rates of bacterial and phytoplankton growth and grazing by heterotrophic protists. Deep Sea Res. Part II Top. Stud. Oceanogr. 57, 849–862. https://doi.org/10.1016/j.dsr2.2008.04.039

Westwood, K.J.J., Thomson, P.G.G., van den Enden, R.L.L., Maher, L.E.E., Wright, S.W.W., Davidson, A.T.T., 2018. Ocean acidification impacts primary and bacterial production in Antarctic coastal waters during austral summer. J. Exp. Mar. Bio. Ecol. 498, 46–60. https://doi.org/10.1016/j.jembe.2017.11.003

Wright, S.W., Ishikawa, A., Marchant, H.J., Davidson, A.T., van den Enden, R.L., Nash, G. V., 2009. Composition and significance of picophytoplankton in Antarctic waters. Polar Biol. 32, 797–808. https://doi.org/10.1007/s00300-009-0582-9

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Changes made to manuscript for resubmission:

- All changes discussed in responses to Reviewers 1 and 2 (above).
- General tidy up of language and improvement of writing through entire manuscript.
- Moved discussion of mixotrophy from Discussion section 4.1, Heterotrophic nanoflagellates to more suitable position in section 4.2, Nano- and picophytoplankton.
- Update of Fig. 6 to GAM model fits of the data, in line with new statistical analysis methods.
- Removal of Fig. 7 and 8 and corresponding results from text, following reviewer comments on single-point statistical analyses.
- Update of Fig. 9 (now Fig. 7) to larger font size.
- Update of Table 2 and 3 to focus only on growth rate statistical analysis and remove modelled curve analysis. Sequence of tables switched due to updated position in text.
- Update of Supplement:
    - Removed model fits and ANOVA tables for modelled curve analysis.
    - Removed ANOVA tables for single-point abundance statistics.
    - Added ANOVA tables for growth rate trend statistical analysis.
    - Added flow cytometry flow rates and equations for counts.

Ocean acidification reduces growth and grazing impact of Antarctic heterotrophic nanoflagellates

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Abstract. High-latitude oceans have been identified as particularly vulnerable to ocean acidification if anthropogenic CO_2 emissions continue. Marine microbes are an essential part of the marine food web and are a critical link in biogeochemical processes in the ocean, such as the cycling of nutrients and carbon. Despite this, the response of Antarctic marine microbial communities to ocean acidification is poorly understood. We investigated the effect of increasing fCO_2 on the growth of heterotrophic nanoflagellates (HNF), nano- and picophytoplankton, and prokaryotes (heterotrophic bacteria and Archaea) in a natural coastal Antarctic marine microbial community from Prydz Bay, East Antarctica. At CO_2 levels $\geq$634 µatm, HNF abundance was reduced, coinciding with ~~significantly~~ increased abundance of picophytoplankton and prokaryotes. This increase in picophytoplankton and prokaryote abundance was likely due to a reduction in top-down control of grazing HNF. Nanophytoplankton abundance was ~~significantly~~ elevated in the 634 ~~and 953~~ µatm ~~treatments~~treatment, suggesting that moderate increases in CO_2 may stimulate growth. ~~Changes in~~ The taxonomic and morphological differences in CO_2-tolerance we observed are likely to favour dominance of microbial communities by prokaryotes, nano- and picophytoplankton. Such changes in predator-prey interactions with ocean acidification could have a significant effect on the food web and biogeochemistry in the Southern Ocean~~. Based on these results, it is likely that the phytoplankton community composition in these waters will shift to communities dominated by prokaryotes, nano- and picophytoplankton. This may intensify~~, intensifying organic matter recycling in surface waters, ~~leading to a decline in~~ reducing vertical carbon flux, ~~as well as a~~ and reducing the quality~~and quantity of food available to higher trophic organisms~~, quantity and availability of food for higher trophic levels.

1 Introduction

Oceanic uptake of anthropogenic CO_2 has ~~resulted in~~ caused a ~0.1 unit decline in ~~pH in the oceans~~ oceanic pH since pre-industrial times (Sabine, 2004; Raven et al., 2005), with ~40% of this uptake occurring in the Southern Ocean (Takahashi et al., 2012; Frölicher et al., 2015). In addition, the low overall water temperature and naturally low $CaCO_3$ saturation state ~~make~~ of the Southern Ocean makes it particularly vulnerable to ocean acidification (Orr et al., 2005; McNeil and Matear,

2008). ~~Coastal Antarctic~~ Close to the Antarctic continent, Southern Ocean waters are regions of high productivity, that provide an essential food source for the abundance of life in Antarctica (Arrigo et al., 2008). In recent decades, these waters have seen reductions in pH (Roden et al., 2013) and it is crucial that we understand the impact of ocean acidification projections on the base of this essential food web. While large phytoplankton, such as diatoms and dinoflagellates, are often believed to be responsible for most of the energy transfer to higher trophic levels in this region, picophytoplankton, prokaryotes, mixotrophic phytoflagellates, microheterotrophs, and heterotrophic nanoflagellates (HNF) also play important roles in grazing and ~~the carbon cycle~~ biogeochemical element cycling (Azam et al., 1991; Sherr and Sherr, 2002; Smetacek et al., 2004).

Marine microbes are ~~an essential~~ a fundamental part of the marine food web and are a critical link in biogeochemical processes, such as the cycling of nutrients and carbon (Azam and Malfatti, 2007). Globally, it is estimated that ~80-100% of daily primary production is either consumed by grazers or lost via processes such as cell lysis and sinking (Behrenfeld, 2014). Grazing can profoundly affect phytoplankton abundance in marine ecosystems, with microzooplankton consuming on average 60-75% of daily primary production (Landry and Calbet, 2004) and HNF grazing between 20-100% of daily bacterial production ~~(Pearce et al., 2010; Safi et al., 2007)~~(Safi et al., 2007; Pearce et al., 2010). Prokaryotes salvage dissolved organic matter released from phytoplankton primary production, ~~whish~~ which is returned to the food web upon grazing by HNF (Pearce et al., 2010; Buchan et al., 2014). Prokaryotes also produce essential micronutrients and vitamins required for phytoplankton growth (Azam and Malfatti, 2007; Buchan et al., 2014; Bertrand et al., 2015) and are important in the supply of nutrients to microzooplankton in Antarctic waters over winter, when primary productivity is low (Azam et al., 1991). This transfer of organic matter between primary producers, prokaryotes~~(bacteria and Archaea)~~, and protozoa forms the microbial loop, upon which all life in the ocean relies (Azam et al., 1983; Fenchel, 2008).

In Antarctic waters, heterotrophic flagellates make a significant contribution to the top-down control of phytoplankton and prokaryote productivity. ~~They can achieve growth rates that~~ Their growth rates can exceed that of their ~~phytoplanktonic~~ prey and their grazing can significantly alter the microbial community composition (Bjørnsen and Kuparinen, 1991; Archer et al., 1996; Pearce et al., 2010). ~~Heterotrophic flagellates, microzooplankton, and ciliates of all sizes (2->200) have been observed grazing on picophytoplankton (0.2-2) and prokaryotes (0.1-5) (Safi et al., 2007).~~ Despite their importance in marine ecosystems, ~~they remain relatively~~ their response to ocean acidification remains largely unstudied (Caron and Hutchins, 2013). ~~Difficulties in identification of HNF in natural seawater samples has no doubt contributed to the scarcity of published studies (Rose et al., 2004).~~ Of the few studies that have included heterotrophic flagellates, most ~~studies~~ have focused on the larger microzooplankton community (20-200 μm), reporting no changes in abundance or grazing rates with elevated CO_2 (Suffrian et al., 2008; Aberle et al., 2013; Davidson et al., 2016). However, ~~ocean acidification effects on microzooplankton grazers may also be indirect, due to~~ indirect effects of ocean acidification on microzooplankton have been observed, through changes in the abundance and composition of their prey (Rose et al., 2009b). ~~Thomson et al. (2016), in their Antarctic minicosm study, reported a~~ Difficulties in identification of HNF in natural seawater samples has no doubt contributed to the scarcity of published studies on this group (Rose et al., 2004). A negative effect of ~~ocean acidification~~ increased CO_2 on HNF abundance ~~when concentrations were $\geq$750 . Species-specific~~ was observed in a previous Antarctic mesocosm study, which the authors suggest led to a reduction in grazing mortality of picophytoplankton and prokaryotes (Thomson et al., 2016). In the present

mesocosm study, Hancock et al. (2018) reported species-specific responses to ocean acidification ~~have also been observed amongst choanoflagellates in the present study (Hancock et al., 2018)~~amongst choanoflagellate species (bacterivorous HNF), exposing a hitherto unrecognised layer of complexity to predicting the effects of ocean acidification on microbial communities.

~~When assessing~~ A global assessment of ocean acidification studies ~~globally,~~ by Schulz et al. (2017) reported a general trend toward increased abundance of picophytoplankton with declining ocean pH. The cyanobacterium *Synechococcus* and picoeukaryotes in the prasinophyte class were identified as the key beneficiaries of increased CO_2 ~~levels, potentially through increased~~ , possibly through down regulation of energetically costly CO_2 ~~concentration~~ and HCO_3^- transporters (carbon concentrating mechanisms, CCMs) as CO_2 concentration increased in the relatively small diffusive boundary layer of these small cells ~~, allowing for down regulation of energetically costly and transporters into the cell~~ (Beardall and Giordano, 2002). Unlike temperate oligotrophic ecosystems, cyanobacteria are very rare in Antarctic waters~~(Wright et al., 2009; Lin et al., 2012; Flombaum~~ ~~meaning the picophytoplankton in waters~~ , so the picophytoplankton community south of the Polar Front are composed largely of eukaryotes ~~. This group~~ (Wright et al., 2009; Lin et al., 2012; Flombaum et al., 2013; Liang et al., 2016). In this region, picophytoplankt can comprise up to 33% of total phytoplankton biomass (Wright et al., 2009; Lin et al., 2012). ~~A minicosm study on natural communities of coastal Antarctic marine microbes observed an increase in picoeukaryote abundance at~~ In coastal Antarctic waters, the abundance of picoeukaryotes was found to increase with elevated CO_2 ~~levels above 750 , although their results~~ concentration (Thomson et al., 2016). However, the authors suggested that this ~~may have been~~ was likely due to a reduction in top-down control of the HNF community, ~~as opposed to a~~ rather than direct promotion of ~~picoeukaryote growth(Thomson et al., 2016)~~ ~~.~~

~~In~~ their growth. In a companion paper to the present study, Deppeler et al. (2018) reported a down-regulation of extracellular CCM activity in phytoplankton cells <10 µm in suze at high CO_2 but it is not known whether this resulted in a concurrent increase in productivity for this size group as primary productivity measurements were performed on the whole community. Overall, primary productivity rates were significantly reduced in high CO_2 treatments ($\geq$1140 µatm), suggesting CO_2 was not beneficial for phytoplankton growth at these levels.

Studies investigating natural marine microbial communities ~~, prokaryotes have been shown to have a high tolerance to ocean acidification , with little effect on~~ have shown prokaryotes are tolerant to ocean acidification and show little CO_2-induced effect on their abundance or productivity (Grossart et al., 2006; Allgaier et al., 2008; Paulino et al., 2008; Wang et al., 2016). Prokaryote abundance and production is generally linked to increased primary production, with peaks in abundance often occurring immediately after the peak of a phytoplankton bloom (Pearce et al., 2007; Buchan et al., 2014). This is likely due to ~~increased~~ an increase in availability of dissolved organic matter, released by phytoplankton during growth, viral lysis, or bacterial degradation of dead cells (Azam and Malfatti, 2007). A CO_2-induced increase in the production of organic matter and the formation of transparent exopolymer particles ~~has been~~ by phytoplankton was reported in a ~~natural community Endres et al. (2014)~~ mesocosm study in a Norwegian fjord (Endres et al., 2014). This promoted bacterial abundance and stimulated enzyme production for organic matter degradation, suggesting that ocean acidification may increase the flow of carbon through the microbial loop in surface waters~~Endres et al. (2014)~~. Shifts in prokaryote community composition have also been reported, although with no significant ~~effect on~~ change in total prokaryote abundance ~~(Roy et al., 2013; Bergen et al., 2016; Zhang et al., 2013).~~

Instead, the composition and abundance of prokaryote communities appear to (Roy et al., 2013; Zhang et al., 2013; Bergen et al., 2016). Despite the apparent resilience of prokaryotes to ocean acidification, several authors suggest they may be indirectly affected by ocean acidification by altering biotic factors that influence their growth and mortalitychanges in substrate availability due to changes in phytoplankton composition and abundance (e.g. Piontek et al., 2010; Celussi et al., 2017). Given the critical role of heterotrophic prokaryotes in remineralisation and carbon flux, it is vital to better understand the direct and indirect effects of ocean acidification on their communities.

In our study, a A natural community of marine microbes from Prydz Bay, East Antarctica was exposed to increasing levels of fCO_2 , levels up to 1641 µatm , in 650 L minicosms. The l minicosms, during the 2014/15 austral summer. In the present study, the abundance of HNF, nano- and picophytoplankton , and prokaryotes was measured and the results used to assess whether nanophytoplankton (2-20 µm), picophytoplankton (0.2-2 µm), and heterotrophic prokaryotes were measured by flow cytometry to determine whether CO_2 had an effect on the growth rate and abundance of each of these microbial groups; and whether predator-prey interactions between these communities could be inferred. A previous community-level studyin the Antarctic reported a decline in HNF abundance and an increase in picophytoplankton and prokaryotic abundance when range of additional measurements were also taken during this 18 day study, of which many findings have been published elsewhere (Deppeler et al., 2018; Hancock et al., 2018; Petrou et al., 2019). These studies reported that high CO_2 concentrations were ≥750 (Davidson et al., 2016; Thomson et al., 2016; Westwood et al., 2018). We used levels caused: reductions in photosynthetic performance, primary productivity, and particulate organic matter production (Deppeler et al., 2018); decreased abundance of microplanktonic diatoms (>20 µm) and *Phaeocystis antarctica* (Hancock et al., 2018); and reduced diatom silicification (Petrou et al., 2019). In contrast, there was no CO_2-induced effect on bacterial productivity (Deppeler et al., 2018) or the abundance of nano-sized diatoms, which were dominated by *Fragilariopsis* sp. (≤20 µm, Hancock et al., 2018). Henceforth, these studies will be referred to as "coincident studies". A previous minicosm study took place at the same location over the 2008/09 austral summer (henceforth referred to as "complementary studies"; Davidson et al., 2016; Thomson et al., 2016; Westwood et al. . We utilised a similar experimental design to Thomson et al. (2016) this previous minicosm study, but added an initial CO_2 acclimation period at low light to determine whether this acclimation would alter the response previously reported.

2 Methods

2.1 Minicosm

A natural microbial assemblage from Prydz Bay, Antarctica was incubated in six 650 l polythene tanks (minicosms) and exposed to six CO_2 treatments; ambient (343 µatm), 506, 634, 953, 1140, and 1641 µatm. Before commencement of the experiment, all minicosms were acid washed with 10% vol:vol AR HCl, rinsed thoroughly with MilliQ water, and finally rinsed with seawater from the sampling site. Seawater to fill the minicosms was collected from amongst the decomposing fast ice in Prydz Bay at Davis Station, Antarctica (68° 35' S 77° 58' E) on 19th November, 2014. A 7000 l polypropylene reservoir tank was filled by helicopter, using multiple collections in a thoroughly rinsed 720 l Bambi bucket. The seawater was then gravity fed from the reservoir to the minicosms through Teflon-lined hose, fitted with a 200 µm pore size Arkal

filter to exclude metazooplankton that would significantly graze the microbial community. Microscopic analysis showed that very few metazooplankton and nauplii passed through the pre-filter and they were seldom observed throughout the experiment (see Hancock et al., 2018). Thus, it is unlikely that their grazing ~~effected~~ affected the CO_2-induced trends in community composition in our study. All minicosms were filled simultaneously to ensure uniform distribution of microbes.

The six minicosms were housed in a temperature-controlled shipping container, with the water temperature in each minicosm maintained at 0.0 ± 0.5 ~~°C~~°C. The temperature in each minicosm was maintained by offsetting the cooling of the shipping container against warming of the tank water with two 300 W Fluval aquarium heaters connected via Carel temperature controllers and a temperature control program. Each minicosm was sealed with an acrylic lid and the water was gently mixed by a shielded high-density polyethylene auger, rotating at 15 rpm.

Minicosms were illuminated by two 150 W HQI-TS (Osram) metal halide lamps on a 19:5 h light:dark cycle. Low intensity light (0.9 ± 0.22 µmol photons m^{-2} s^{-1}) was provided for the first 5 d to slow phytoplankton growth while the CO_2 levels were gradually raised to the target concentration for each minicosm (see below). Following this 5 d CO_2 acclimation period, light was progressively increased over 2 d to a final light intensity of 90.5 ± 21.5 µmol photons m^{-2} s^{-1}. The microbial assemblages were then incubated for 10 d with samples taken at regular intervals (see below) and no further addition of ~~seawater or nutrients~~ nutrients or seawater (except for the small volume required for carbonate chemistry modification, see below). For further details on minicosm setup see Deppeler et al. (2018).

2.2 Carbonate chemistry calculation and manipulation

Carbonate chemistry was measured throughout the experiment, allowing the fugacity of CO_2 (fCO_2) to be manipulated to the desired values over the first 5 d of acclimation and then maintained for the remainder of the experiment. Samples were taken daily from each minicosm in 500 ml glass-stoppered bottles (Schott Duran) following the guidelines of Dickson et al. (2007), with sub-samples for dissolved inorganic carbon (DIC, 50 ml glass-stoppered bottles) and pH on the total scale (pH_T, 100 ml glass stoppered bottles) gently pressure filtered (0.2 µm) following Bockmon and Dickson (2014). For each minicosm, DIC was measured in triplicate by infrared absorption on an Apollo SciTech AS-C3 analyser equipped with a Li-cor LI-7000 detector calibrated with five prepared sodium carbonate standards (Merck Suprapur) and daily measurements of a certified reference material batch CRM127 (Dickson, 2010). DIC measurements were converted to µmol kg^{-1} using calculated density from known sample temperature and salinity.

Measurements of pH_T were performed using the pH indicator dye m-cresol purple (Acros Organics) following Dickson et al. (2007) and measured by a GBC UV-vis 916 spectrophotometer at 25 °C in a 10 cm thermostated cuvette. A syringe pump (Tecan Cavro XLP 6000) was used for sample delivery, dye addition, and mixing to minimise contact with air. An offset for dye impurities and instrument performance (+0.003 pH units) was determined through measurement of pH_T of CRM127 and comparison with the calculated pH_T from known DIC and total alkalinity (TA), including silicate and phosphate. Salinity was measured in situ using a WTW197 conductivity meter and used with measured DIC and pH_T to calculate practical alkalinity (PA) at 25 °C, using the dissociation constants for carbonic acid determined by Mehrbach et al. (1973) and Lueker et al. (2000).

Total carbonate chemistry speciation was then calculated for in situ temperature conditions from measured DIC and calculated PA.

During the acclimation period, the fCO_2 in each minicosm was adjusted daily in increments until the target level was reached, after which fCO_2 was kept as constant as possible for the remainder of the experiment. Twice-daily measurements of pH were performed in the morning (before sampling) and the afternoon using a portable, NBS-calibrated probe (Mettler Toledo) to determine the amount of DIC to be added to the minicosm. Adjustment of the fCO_2 in each minicosm was performed by addition of a calculated volume of 0.2 µm filtered CO_2-saturated natural seawater to 1000 ml infusion bags and drip-fed into the minicosms at ~50 ml min^{-1}. One minicosm was maintained close to the fCO_2 of the initial (ambient) sea water (343 µatm) and was used as the control treatment, against which the effects of elevated fCO_2 were measured. The mean fCO_2 levels in the other five minicosms were 506, 634, 953, 1140, and 1641 µatm. For further details of the carbonate chemistry sampling methods, calculations, and manipulation see Deppeler et al. (2018).

2.3 Nutrient analysis

Concentrations of the macronutrients nitrate plus nitrite (NOx), soluble reactive phosphorus (SRP), and molybdate reactive silica (silicate) were measured in each minicosm during the experiment. Samples were taken on days 1, 3, and 5 during the CO_2 acclimation period and every 2 days for the remainder of the experiment (days 8-18). Samples were obtained following the protocol of Davidson et al. (2016). Briefly, seawater samples were filtered through 0.45 µm Sartorius filters into 50 ml Falcon tubes and frozen at -80 ~~°C~~ °C for analysis in Australia. Determination of the concentration of NOx, SRP, and silicate were performed by Analytical Services Tasmania, using flow injection analysis.

2.4 Flow Cytometry

Flow cytometric analyses were performed daily to determine the abundance of small protists (HNF, pico- and nanophytoplankton, and prokaryotes) in each minicosm during the experiment. Samples were pre-filtered through a 50 µm mesh (Nitex), stored in the dark at 4 ~~°C~~°C, and analysed within 6 h of collection, following Thomson et al. (2016). Samples were analysed using a Becton Dickinson FACScan ~~or FACSCalibur flow cytometer~~ flow cytometer until day 15 after which, the instrument broke down and analysis was performed on a Becton Dickinson FACSCalibur. Both instruments were fitted with a 488 nm laser ~~.~~ and MilliQ water was used as sheath fluid for all ~~analysis.~~ analyses. PeakFlow Green 2.5 µm beads (Invitrogen) were added to samples as an internal fluorescence and size standard. Final cell numbers were calculated from event counts on bivariate scatter plots divided by the analysed volume.

The analysed volume for each flow cytometer was calibrated ~~to the sample run time and flow rate and was used to calculate final cell concentrations from event counts on bivariate scatter plots. PeakFlow Green 2.5 beads (Invitrogen)were added to samples as an internal fluorescence and size standard~~ by measuring the weight change of 1 ml seawater run for 1, 2, 3, 4, 5, and 10 min at high and low flow settings on each instrument. This weight change was converted to ml by dividing by 1.027, the density of seawater at 4 °C with salinity 34.3 PSU (Table S1). A linear regression was fitted to each data set and the analysed sample volume was determined by entering the sample run time (x) into the equations (Table S2). Average flow rates

in ml min^{-1} for each instrument at both flow settings was determined by dividing each analysed volume by the run time. The standard deviation for all mean flow rates on both instruments was <0.004. Details of instrument flow rates and equations for flow cytometry counts can be found in Table S2.

2.4.1 Pico- and nanophytoplankton abundance

Three pseudoreplicate 1 ml samples for pico- and nanophytoplankton abundance were prepared from each minicosm seawater sample. Each sample was placed in a beaker of ice and run for 3 min at a high flow rate of ~~~40 for FACScan and ~70 for~~ 36.5 µl min^{-1} for the FACScan and 67.2 µl min^{-1} for the FACSCalibur, resulting in an analysed volume of ~~0.1172 and 0.2093~~ 0.1140 and 0.2036 ml, respectively. Phytoplankton populations were separated into regions based on their chlorophyll autofluorescence in bivariate scatter plots of red (FL3) versus orange fluorescence (FL2~~) (~~; Fig. 1a). The pico- and nanophytoplankton communities were determined from relative cell size in side scatter (SSC) versus FL3 fluorescence bivariate scatter plots (Fig. 1b). Cyanobacteria, which have high orange and low red fluorescence due to possessing phycoerythrin, were not evident in FL3 versus FL2 scatter plots and were deemed absent in this study. Final cell counts in cells l^{-1} were calculated from event counts in the phytoplankton regions and analysed volume.

2.4.2 Heterotrophic nanoflagellate abundance

Heterotrophic nanoflagellate (HNF) abundance was determined using LysoTracker Green (Invitrogen) staining following the protocol of Thomson et al. (2016). A 1:10 working solution of LysoTracker Green was prepared daily by diluting the commercial stock into 0.22 µm filtered seawater. For each minicosm sample, 10 ml of seawater was stained with 7.5 µl of working solution to a final stain concentration of 75 nM. Stained samples were then incubated in the dark on ice for 10 min. Triplicate 1 ml sub-samples were taken from the stained sample and run for 10 min at a high flow rate of ~~~40 for FACScan and ~70 for~~ 36.5 µl min^{-1} for the FACScan and 67.2 µl min^{-1} for the FACSCalibur, resulting in an analysed volume of ~~0.4153 and 0.7203~~ 0.4043 and 0.7006 ml, respectively.

LysoTracker Green stained HNF abundances were determined in green fluorescence (FL1) versus forward scatter (FSC) plots after removal of phytoplankton and detritus particles following Rose et al. (2004) and Thomson et al. (2016) and shown in Fig. 2. Phytoplankton were identified by high chlorophyll autofluorescence in bivariate scatter plots of FL3 versus ~~FL2~~ FSC fluorescence (Fig. 2a) and detritus was identified by high SSC in FL1 fluorescence versus SSC plots (Fig. 2b). HNF abundance was then determined in a bivariate plot of FL1 fluorescence versus FSC with phytoplankton and detritus particles removed. Mixotrophic species would have been excluded from HNF counts, due to their chlorophyll fluorescence in FL3 versus FSC plots. Remaining particles larger than the 2.5 µm PeakFlow Green beads were counted as HNF (Fig. 2~~c~~d). Final cell counts in cells l^{-1} were calculated from event counts and analysed volume.

2.4.3 Prokaryote abundance

Prokaryote abundance measurements relate to heterotrophic prokayotes only, as autotrophic prokaryotes were not present in the minicosms (see above). Samples for prokaryote abundance were stained for 20 min with 1:10,000 dilution SYBR Green I (Invitrogen) following Marie et al. (2005). Three pseudoreplicate 1 ml samples were prepared from each minicosm seawater sample and were run for 3 min at a low flow rate ~~(~12)~~of 7.5 µl min^{-1} for the FACScan and 15.6 µl min^{-1} for the FACSCalibur, resulting in an analysed volume of ~~0.0260 and 0.0491 on the FACScan and FACSCalibur~~0.0254 and 0.0478 ml, respectively. Prokaryote abundance was determined from SSC versus FL1 fluorescence bivariate scatter plots (Fig. 3). Final cell counts in cells l^{-1} were calculated from event counts and analysed volume. High background noise was observed on the flow cytometer on day 1, likely due to suspended detritus from when the minicosm tanks were filled, which obscured cell counts at this time. Background interference had cleared on day 2, allowing analysis to start from this day.

2.5 Statistical analysis

Microbial community growth in the minicosms was measured in six unreplicated ~~f~~CO_2 treatments and thus, triplicate subsamples from individual minicosms represent within-treatment pseudoreplicates. Therefore, means and standard error of these pseudoreplicate samples only provide the within-treatment sampling variability for each procedure. For the purpose of analysis, we treated pseudoreplicates as independent to provide an informal assessment of the difference among treatments. ~~A curved (quadratic) regression model~~

A generalized additive model (GAM) was fitted to each CO_2 treatment over time ~~for all analyses using the~~ to visually assess temporal changes in the abundance of each microbial group using the *~~Stats~~mgcv* ~~package in R (R Core Team, 2016), with an omnibus test of differences between the trends in~~ and *ggplot2* packages in R (Wood, 2011; R Core Team, 2016; Wickham, 2016). Taking into account the pseudo-replicated sampling method, further statistical analysis of these curves was not performed. For growth rate analysis, a linear regression model was fitted on natural log-transformed data for each CO_2 ~~treatments over time assessed by ANOVA. Growth rates were calculated from linear regression on the region that marked~~ treatment over the incubation period during which each microbial group sustained steady-state logarithmic growth~~and the~~. Growth rates for each treatment were determined from the slope estimate of the linear model. An omnibus test of differences between the ~~trends in~~ linear models for each CO_2 ~~treatments over time~~ treatment was assessed by ANOVA ~~. For peak abundance measurements, differences between treatments were tested by one-way ANOVA, followed by a post-hoc Tukey test to determine which treatments differed~~to determine significant differences between the growth trends for each microbial group. The lack of replication in our study and limited number of time points at which each minicosm was sampled means that the trends within treatments are indicative and the statistical differences among treatments should be interpreted conservatively. The significance level for all tests was set at <0.05.

3 Results

3.1 Carbonate chemistry

The carbonate chemistry of the initial seawater was measured as a pH_T and DIC of 8.08 and 2187 µmol kg^{-1}, respectively, resulting in a calculated fCO_2 of 356 µatm and a PA of 2317 µmol kg^{-1} (Fig. 4, S1; Table S1). Measurements of carbonate chemistry during the acclimation period showed a stepwise increase in fCO_2, after which the CO_2 level remained largely constant, with treatments ranging from 343 to 1641 µatm and a pH_T range from ~~8.1~~ 8.10 to 7.45 (Fig. 4; Table 1). Some decline in fCO_2 was observed in the high CO_2 treatments towards to the end of the experiment indicating that the addition of CO_2-saturated seawater was insufficient to fully compensate for its out-gassing into the headspace and drawdown by phytoplankton photosynthesis.

3.2 Nutrients

There was little variance in nutrient concentrations among all treatments at the start of the experiment (Table S1). Concentrations of NOx fell from 26.2 ± 0.74 uM on day 8 to below detection limits on day 18 (Fig. 5a), with the 1641 µatm treatment being drawn down the slowest. SRP concentrations were drawn down in a similar manner as NOx, falling from 1.74 ± 0.02 uM to 0.13 ± 0.03 uM on day 18 in all treatments (Fig. 5b). Silicate was replete throughout the experiment in all treatments, with initial concentrations of 60.0 ± 0.91 uM falling to 43.6 ± 2.45 uM (Fig. 5c). Silicate draw-down was highest in the 634 µatm and lowest in the 1641 µatm treatment.

3.3 Picophytoplankton abundance

Picophytoplankton abundance did not change during the CO_2 acclimation period~~and remained~~, remaining at ~~2.0~~ 2.04 ± 0.02 x10^6 ~~. Cell abundance~~ cells l^{-1}. Cell numbers increased in all treatments from day 8, with ~~a significantly enhanced growth rate in the 953~~ treatments ≤506 µatm ~~treatment when compared with the control (Table 2, 3). Abundance peaked~~ peaking on day 12 ~~in treatments ≤506 at 5.5 ± 0.61 x10^6 but continued to rise in treatments ≥634~~ and all higher CO_2 treatments continuing to grow until day 13 (Fig. 6a). ~~Despite a faster growth rate in the~~ Steady-state logarithmic growth rates were calculated between days 8 to 12 (Fig. S2) and are presented in Table 2. The omnibus test of trends in fCO_2 treatment over time for picophytoplankton steady-state growth indicated there was a significant difference between treatments ($F_{5,78}$ = 2.85, p < 0.01; Table S3). Examining the significance of the individual linear model terms indicated that only the 953 µatm ~~treatment, peak abundance in this treatment was similar to the~~ growth rate was significantly different to the control (p < 0.01; Table 3), with a higher growth rate of 0.32 (Table 2). Despite the similarity in growth rates among treatments, there was a difference between peak abundances. The highest were observed in the 953 and 1641 µatm ~~treatment (7.8~~ treatments, which reached 8.11 ± 0.05 x10^6 ~~), while the~~ cells l^{-1} (Fig. 6a). The 634 and 1140 µatm treatments peaked ~~at a slightly lower abundance of 6.9~~ slightly lower at 7.06 ± ~~0.02~~ 0.03 x10^6 ~~(Fig. 6a)~~ cells l^{-1} and following this, the control (343 µatm) and 506 µatm treatments peaked at 5.28 ± 0.17 x10^6 cells l^{-1} and 4.47 ± 0.13 x10^6 cells l^{-1}, respectively. After reaching their peak, cell numbers

rapidly declined in all treatments until day 18, falling to ~~0.8~~ 0.50 ± ~~0.03~~ 0.01 x10^6 cells l^{-1}. The 506 µatm treatment was excluded from analysis on day 18 due to ~~very~~ high background noise on the flow cytometer, ~~resulting in artificially elevated event~~ which caused artificially elevated counts.

~~Abundance curves for each treatment were modelled from days 8 to 18, excluding the acclimation period when no growth occurred. The omnibus test of trends in picophytoplankton abundance among treatments over time indicated there was no significant difference among treatments (Table 2, S2). However, examination of the model fits showed that whilst there was a reasonable fit to the data set (Adjusted R^2 = 0.82; Table 2), the constraints of limited data meant that the high abundance values between days 12-14 in the treatments ≥634 were not well fitted (Fig. S2). Despite this, the models did show the general trend of increased abundance in treatments ≥634 . Analysis of the differences between peak abundances revealed that treatments ≥634 reached significantly higher maximum abundances than the control, while the 506 treatment was significantly lower (Fig. 7a).~~

3.4 Nanophytoplankton abundance

Nanophytoplankton abundance declined during the CO_2 acclimation period in all treatments, falling from a mean initial abundance of ~~1.2~~ 1.19 ± 0.03 x10^6 ~~to 0.9~~ cells l^{-1} to 0.96 ± 0.02 x10^6 cells l^{-1} on day 7. Following acclimation, nanophytoplankton abundance increased in ~~treatments ≤953 until day 18, while treatments ≥1140 remained low through to day 9 before increasing~~ all treatments until day 15, after which growth plateaued (Fig. 6b~~, S3). Analysis of steady-state~~). Steady-state logarithmic growth rates ~~revealed that growth rates in the 634, 1140, and 1641 treatments were significantly higher than the control (Table 2, 3). In spite of this, comparison of the trends between modelled abundance curves for each~~ were calculated between days 9 to 15 (Fig. S2) and are presented in Table 2. There was a significant difference between growth trends among CO_2 ~~treatment indicated that the~~ treatments ($F_{5,113}$ = 5.92, $p < 0.01$; Table S4), with significance due to enhanced growth rates in treatments ≥634 ~~and 953 treatments were significantly enhanced compared to the control (Table 2, S3~~µatm (Table 3). In the 634 µatm ~~treatment, elevated nanophytoplankton abundance was observed~~ treatment, cell numbers were substantially higher than all other treatments from day 12 through to day 18, reaching a final abundance of ~~15~~ 8.83 ± ~~0.4~~ 0.24 x10^6 cells l^{-1} (Fig. 6b). ~~Despite lower abundance on days 8-9, enhanced~~ Enhanced growth rates in treatments ≥~~1140 led to final abundances similar to the~~ 953 µatm ~~treatment on day~~ also led to cell numbers exceeding the control by day 15, averaging 5.61 ± 0.12 x10^6 cells l^{-1}. Between days 15 and 18, ~~reaching 12~~ abundance in treatments ≥953 µatm dipped and then recovered to a final abundance of 6.64 ± ~~0.5~~ 0.06 x10^6 ~~(Fig. 6b, S3). The lowest nanophytoplankton~~ cells l^{-1}. It is uncertain whether the large dip in abundance on day ~~18 was in the treatments ≤~~16 was due to a reduction in cell numbers in the tanks or associated with the change in flow cytometer on this day. Growth rates in the control and 506 µatm ~~, which were 10~~ treatments were the slowest (0.22-0.23, Table 2), displaying less of a plateau in growth between days 15-18 and reaching a final abundance of 5.96 ± ~~0.3~~ 0.15 x10^6 cells l^{-1}, only slightly less than the ≥953 µatm treatments.

3.5 Heterotrophic nanoflagellate abundance

HNF abundance was initially low (~~0.9~~ 0.94 ± 0.04 x10^5 cells l^{-1}) and remained ~~at a similar abundance~~ steady throughout the CO_2 acclimation period ~~. Abundance increased from day 8 in all treatments, but by day 9 was lower in~~ (Fig. 6c), with a

small dip in cell numbers observed in the treatments ≥~~634~~ 953 µatm ~~than ≤506 treatments, at 1.9 ± 0.08 x10^5 and 2.9 ± 0.18 x10^5 , respectively and remained lower until day 15~~ on day 7 (Fig. ~~6c). Growth rate analysis between days~~ S2). From day 8, HNF abundance increased in all treatments until day 15, with cell numbers in the control and ~~15 revealed that growth rates were significantly slower in the~~ 506 µatm ~~treatment and significantly faster in the 1641~~ treatments consistently higher than all other treatments (≥634 µatm~~treatment, when compared with the control treatment (Table 2, 3~~; Fig. 6c). From day 15 to 18, the control, 634, ~~and~~ 953, and 1641 µatm treatments continued to rise, ~~reaching 3.2 ± 0.07 x10^6 ,~~ while abundance in the 506 and 1140 µatm ~~treatment stabilisedbetween days 16 and 18, reaching 2.6~~ treatments stabilised. Steady-state logarithmic growth rates were calculated between days 8 to 15 (Fig. S2) and are presented in Table 2. The omnibus test of trends in fCO_2 treatment over time showed there was a significant difference between the treatments ($F_{5,131}$ = 5.40, $p < 0.01$; Table S5), due to significant differences in growth trends of the 506 and 1641 µatm treatments (Table 3). Examining the growth rates of each of these treatments revealed that the 506 µatm treatment was slower than the control (0.32, p = 0.02), while the 1641 µatm treatment was faster (0.40, p = 0.02; Table 2, 3). The slower growth rate of the 506 µatm treatment appears to be due to a higher initial abundance on day 8 (2.42 ± ~~0.95~~ 0.35 x10^6 ~~. HNF abundance remained lower~~ ^{5} cells l^{-1}) than the control ~~in the~~ (1.86 ± 0.05 x10^5 cells l^{-1}). Despite a higher growth rate in the 1641 µatm treatment, cell numbers in the highest CO_2 treatments, 1140 and 1641 µatm, remained consistently lower than the control throughout the entire growth period (days 8 and 18), reaching abundances on day 18 of ~~2.1~~ only 2.12 ± 0.02 x10^6 and ~~2.5~~ 2.62 ± 0.11 x10^6 cells l^{-1}, respectively (Fig. 6c). The ~~omnibus test among modelled abundance curves for each treatment over time indicated that HNF abundance in at least one treatment differed significantly from the control (Table 2, S4). Examination of the significance of individual curve terms revealed that this reflected the significantly lower abundance of HNF in these two highest treatments (1140 and~~ 506 µatm treatment plateaued after day 16, with a final abundance similar to the 1641 µatm ~~; Table 2).~~ treatment, at 2.66 ± 0.02 cells l^{-1}. In contrast, the 634 and 953 µatm treatments continued to rise, exceeding the control after day 16 and reaching 3.42 ± 0.08 x10^6 cells l^{-1} on day 18, with the control treatment slightly lower at 3.13 ± 0.04 x10^6 cells l^{-1}.

3.6 Prokaryote abundance

~~Prokaryote abundance increased in~~ Prokaryote abundance was similar in all fCO_2 treatments at the start of the acclimation period (2.10 ± 0.04 x10^8 cells l^{-1}) and increased after day 4 in treatments ≥634 µatm~~during the acclimation period, with growth rates in treatments~~ , while abundance in treatments ≤506 µatm remained unchanged (Fig. 6d). Due to the large fluctuation in cell numbers between days 4 to 7 in treatments ≥634 µatm, steady-state logarithmic growth was not observed (Fig. S2). However, prokaryote growth rates were calculated from linear regression between days 4 to 8 to assess differences in prokaryote growth among treatments during the CO_2 acclimation period (Table 2). There was a significant difference between growth trends among CO_2 treatments ($F_{5,77}$ = 3.59, $p < 0.01$; Table S6). Treatments ≥953 µatm ~~significantly higher~~ all displayed significant differences in growth trends and were faster than the control ~~between days 4 and 8~~ (Table 2, 3). ~~In contrast, abundance in treatments treatments ≤506 remained unchanged (Fig. 6d).~~ Between days 7 and 11, prokaryote ~~abundance~~ cell numbers remained steady in all treatments, with ~~abundances~~ abundance higher than the control in treatments ≥634 µatm ~~significantly higher than the control~~ (Fig. ~~7~~6d). During this time, ~~the mean abundance was 3.09 ± 0.02 x10^8 for~~ abundance

was highest in treatments ≥953 µatm, ~~2.47~~ with an average abundance of 3.17 ± ~~0.02~~ 0.03 x10^8 ~~in~~ cells l^{-1}, followed by the 634 µatm treatment, ~~and 2.07~~ at 2.53 ± ~~0.03~~ 0.05 x10^8 ~~in treatments ≤~~cells l^{-1}. The control and 506 µatm ~~(Fig. 6d). After~~ had similar abundances, averaging 2.12 ± 0.03 x10^8 cells l^{-1}. From day 12, prokaryote ~~abundance declined~~ cell numbers declined rapidly in all treatments, falling to ~~0.6~~ 0.58 ± ~~0.06~~ 0.05 x10^7 ~~by day 17.~~ cells l^{-1} by day 18.

~~Prokaryote abundance curves were modelled for each treatment from days 4 to 18, excluding days 2 and 3 when no growth occurred. There was no significant difference between treatments in the omnibus test among modelled abundance curves (Table S5) but curves for the 953 and 1140 treatments differed significantly from the control (Table 2). In a similar manner to the picophytoplankton data, the models did not well represent the high values in the treatments ≥953 (Fig. S2). Whilst no significant differences were reported for the~~

3.7 Microbial community interaction

Although grazing experiments were not performed, interactions between HNF and their phytoplankton and prokaryote prey were assessed visually. There appeared to be no correlation between HNF and nanophytoplankton abundance, as nanophytoplankton only displayed higher cell numbers than the control in the 634 ~~and 1641~~ µatm ~~treatments, the general trend in the modelled curves did follow that of the analysis, with increased abundance in all treatments~~ treatment, which showed no relationship to the CO_2-induced reduction in HNF abundance at levels ≥634 µatm.

3.8 ~~Microbial community interaction~~

~~Although grazing experiments were not performed~~In contrast, the co-occurrence of slowed HNF growth with increased picophytoplankton ~~and prokaryote abundance~~ abundance between days 8 and 13 in CO_2 treatments ≥634 µatm ~~suggests~~ suggested that the picophytoplankton ~~and prokaryote communities were~~ communities may have been released from grazing pressure. ~~Growth rates of prokaryotes and picophytoplankton were compared with HNF abundance on day 8 and 13, respectively, to examine whether trophic interactions could be inferred. Picophytoplankton had a negative but non-significant trend (Fig. ??a; Table S6), while prokaryotes displayed a significant negative trend with HNF abundance (Fig. ??b; Table S7). This suggests that reduced HNF abundance reduced grazing mortality of the picoplankton community. This hypothesis~~ This hypothesis of a reduction in grazing pressure by HNF at increased CO_2 was further supported by the observation that above a threshold HNF abundance there was a rapid decline in both the picophytoplankton and prokaryote abundance, irrespective of treatment and the duration of incubation. For picophytoplankton, ~~this decline occurred~~ cell numbers rapidly declined when HNF abundance reached ~~0.84~~ 0.87 ± 0.02 x10^6 cells l^{-1} (Fig. ~~??~~7a) and for prokaryotes it occurred ~~after HNF abundance reached 0.31~~ once HNF abundance had reached 0.32 ± 0.02 x10^6 cells l^{-1} (Fig. ~~??~~7b). Interestingly, the rate of decline in picophytoplankton and prokaryote abundances in the fCO_2 treatments ≥634 µatm was greater than the control and 506 µatm treatments. ~~However, thisprovided no benefit to HNF abundance in these treatments , which never surpassed that of the control~~ (Despite this, only HNF in the 634 and 953 µatm treatments reached abundances as high as the control at the conclusion of the experiment, suggesting that high CO_2 (≥1140 µatm) continued to have a negative effect on HNF growth (Fig. 6c).

4 Discussion

Mesocosm experiments are useful in assessing the effects of environmental perturbations on multiple trophic levels of a marine ecosystem (Riebesell et al., 2008). Our results suggest that there are both direct effects of elevated CO_2 on nanophytoplankton and indirect effects of trophic interactions occurring between HNF and their prokaryotic and picoplanktonic prey that can significantly alter the composition and abundance of organisms at the base of the food web.

Exposing cells to a gradual change in CO_2 during an acclimation period allows cells an opportunity to adjust their physiology to environmental change and may alleviate some of the stress experienced when changes are imposed rapidly (Dason and Colman, 2004). However, little is known about the time scales required for the changes in physiology necessary to optimise cellular tolerance of CO_2-induced stress. In addition, acclimating ~~cells~~ and adapting microbial communities over the years to decades anticipated for anthropogenic ocean acidification, whilst retaining similar taxonomic composition to natural communities, is unachievable in ~~most~~ current experimental designs. Acknowledging these limitations, a gradual increase in fCO_2 over 5 days was included in this study to assess whether acclimation would moderate the previously observed response of Antarctic microbial communities that were exposed to rapid changes in CO_2 (Davidson et al., 2016; Thomson et al., 2016; Westwood et al., 2018).

The results of the current study were similar to those ~~reported previously (Davidson et al., 2016; Thomson et al., 2016; Westwood et al., 2~~ in the complementary studies that lacked acclimation (Davidson et al., 2016; Thomson et al., 2016; Westwood et al., 2018). Thus, it appears that an acclimation period had no discernible effect on the response of the community to enhanced CO_2. Hancock et al. (2018), in their coincident study on microbial community composition, did observe a significant change in ~~microbial community composition in all treatments~~ the community composition between days 1 and 3 but no ~~further change in community composition~~ difference was found between ~~any of the treatmentsduring the acclimation~~individual treatments. Therefore, ~~they attributed~~ this initial change was attributed to acclimation of the community to the minicosm tanks and not a response to increasing CO_2. This lack of ~~acclimation~~ community-level acclimation, through selection of CO_2-tolerant species in high-CO_2 treatments, may be due ~~to ineffectiveness of the acclimation we used or~~ an ineffective acclimation or alternatively, indicative of a community already adapted to the highly variable CO_2 ~~experienced by the marine microbial community~~ at the study site. Here, CO_2 levels have been measured to vary by $\sim$450 μatm throughout the year, with highest CO_2 levels experienced at the end of winter and ~~strong~~ low CO_2 levels during the austral summer when there is strong CO_2 draw-down ~~occurring in the Austral summer~~ due to high primary productivity (Gibson and Trull, 1999; Roden et al., 2013). Marine organisms exposed to highly variable environments such as this have been shown to be more tolerant of changes in CO_2 (Boyd et al., 2016)~~and have also been demonstrated in this region (e.g Thomson et al., 2016; Deppeler et al., 2018)~~.

It is also possible that the acclimation under low light conditions did not allow the cells to adjust their physiology effectively and that much of the acclimation occurred after the light levels were increased. Indeed, ~~phytoplankton~~ in our coincident study measuring phytoplankton productivity and photophysiology, phytoplankton cell health (measured by photochemical quantum yield; F_v/F_m) was high during the low light acclimation period and a CO_2-induced decline in health was ~~only~~ observed when light intensity was increased between days 5 and 8 ~~(see Deppeler et al., 2018)~~(Deppeler et al., 2018). Synergistic effects of

CO_2 and light stress have been observed in a number of phytoplankton studies, with declines in growth, productivity, and ~~cell health (~~F_v/F_m ~~) reported under a~~ reported under combined high CO_2 and light intensity ~~(Trimborn et al., 2017; Gao et al., 2012a, b; Li et al. . In our study,~~ conditions (e.g. Gao et al., 2012a, b; Li et al., 2015; Trimborn et al., 2017). Deppeler et al. (2018) did note that the phytoplankton community ~~did appear~~ appeared to acclimate to this light and CO_2 stress over time, with F_v/F_m increasing in all treatments after day ~~12 (Deppeler et al., 2018).~~ 12. HNF and prokaryotes are not reliant on light for growth but displayed similar growth patterns to Thomson et al. (2016)'s complementary study with no acclimation. With increasing CO_2 levels, prokaryote abundance increased and HNF growth was limited. Consequently, it is likely that ~~the acclimation was either incomplete or ineffective~~cellular physiology did not change in any microbial group during the acclimation period. Despite this, the similarity of our results with those previously reported does allow us to gain a more comprehensive understanding of the seasonal and temporal effects of ocean acidification on the marine microbial community in this region.

Top-down grazing pressure on the microbial community is an important dynamic in the growth and composition of the microbial community in the Southern Ocean (Smetacek et al., 2004). Our experimental design included pre-screening of the natural seawater community through a 200 µm mesh, which may have modified trophic dynamics by removing macrozooplankton grazers and thus, reduce top-down grazing pressure on microheterotrophs. We chose to exclude macrozooplankton in order to remove an environmental factor that could differentially alter the mortality of microbes among CO_2 treatments. Small differences in the abundance of large grazers among the tanks could have greatly affected the growth and composition of the microbial community, to the point of removing all protists from the tanks, masking any CO_2-induced effects. We recognise that removing higher trophic levels is a limitation of minicosm experiments to simulate the full dynamics of in situ communities. However, pre-screening by $<$200 µm allowed for greater control of our experiment by allowing us to vary only one environmental factor so we could focus of the effect of CO_2 on microbial community dynamics.

4.1 Heterotrophic nanoflagellates

Our study indicates that HNF abundance is negatively affected by elevated CO_2. ~~This contrasts with the study by Moustaka-Gouni et al. (20~~ HNF abundance was reduced when CO_2 levels were $\geq$634 µatm and remained lower than the ambient treatment at levels $\geq$1140 µatm. These observations are consistent with complementary studies in Prydz Bay, Antarctica, that reported a reduction in HNF abundance when CO_2 was $\geq$750 µatm in both high and low nutrient conditions (Davidson et al., 2016; Thomson et al., 2016) . These results contrast with those reported by Moustaka-Gouni et al. (2016) from a Baltic Sea mesocosm study, who found ~~no effect of~~ high CO_2 concentration (1040 ppm) had little effect on the HNF community~~when exposed to levels up to 1040 ppm. As HNF cells are difficult to identify by microscopy in fixed samples (Sherr et al., 1993; Sherr and Sherr, 1993), we~~ . Interestingly, they also demonstrated that HNF communities form complex food webs and trophic interactions between species can change with environmental conditions and prey availability. We were unable to determine whether species-specific sensitivities led to the reduction in HNF abundance ~~and differences in growth rates among treatments were due to~~ with high CO_2 ~~-induced effects on the entire HNF community or if species-specific sensitivities changed the community composition.~~ . However, Hancock et al. (2018) reported a CO_2-related change in the relative abundances of ~~two~~ choanoflagellate species at CO_2 levels $\geq$634 µatm (see 4.4 below)~~and thus~~. Therefore, it is possible that other CO_2-induced changes to the HNF

community composition may have occurred. ~~Previous experiments in Prydz Bay, Antarctica also reported a reduction in HNF abundance when was ≥750 in both high and low nutrient conditions (Thomson et al., 2016).~~ The consistency of ~~these results over the Austral~~ reduced abundance of HNF with increased CO_2 over the austral summer and between years ~~suggests that if emissions continue to increase at rates similar to the IPCC~~ indicate that ocean acidification alone may significantly alter the HNF growth and community structure by 2050 (following RCP8.5 projections, ~~the abundance and composition of HNF communities may change around 2050 (IPCC, 2013)~~IPCC, 2013). However, it must be acknowledged that a number of environmental factors will influence microbial communities with the onset of climate change (see Deppeler and Davidson, 2017) and the sequence and severity of these additional stressors will be important in determining the nature and magnitude of the effect of ocean acidification on this community.

Increased top-down control by heterotrophic dinoflagellates and ciliates on the HNF community may have led to the lower abundance of HNF in the high CO_2 treatments. ~~However, this was unlikely as~~ Hancock et al. (2018) saw no effect of CO_2 on the composition or abundance of the microheterotrophic community in ~~our study.~~ their coincident study. Although, they did acknowledge that microheterotroph abundance was low in all treatments (~1% of all cells) and therefore, any CO_2 response may not have been apparent. Low abundances of heterotrophic dinoflagellates and ciliates in all treatments would suggest that grazing pressure on HNF was low and thus, any reduction in HNF abundance at higher CO_2 levels were not likely caused by increased grazing from larger taxa. Few other studies have investigated the effect of ocean acidification on heterotrophic protists and as yet there are no reports of direct effects of elevated CO_2 on microheterotrophic grazing rates, abundance, or taxonomic composition (Suffrian et al., 2008; Aberle et al., 2013). One study by Rose et al. (2009a) did report an increase in microzooplankton abundance when a natural North Atlantic microbial community was exposed to high CO_2 (690 ppm). However, this increased abundance was ~~thought~~ believed to be an indirect effect of CO_2-induced promotion of phytoplankton abundance and a change in the phytoplankton community composition, as opposed to a direct effect of ocean acidification on microzooplankton physiology. A shift in the dominant nanophytoplankton taxa was reported by Hancock et al. (2018), with a threshold in this change appearing between 634-953 µatm (see 4.2 below). The prymnesiophyte *Phaeocystis antarctica* was dominant in treatments ≤634 µatm, whilst in higher CO_2 treatments, *P. antarctica* was considerably reduced, resulting in a shift in dominance to the diatom *Fragilariopsis* sp. (<20 µm size). Low microzooplankton grazing rates have been reported in Antarctic waters dominated by colonial *P. antarctica* (Safi et al., 2007; Caron et al., 2000; Pearce et al., 2010), suggesting that a shift in dominance to more palatable small diatom species with increasing CO_2 may lead to a concurrent increase in microzooplankton and subsequent increase in HNF grazing.

It is difficult to evaluate the potential reasons for reduced abundance ~~in~~ of the HNF community in high CO_2 treatments as the mechanism(s) responsible for CO_2 sensitivity in HNFs are unstudied (Caron and Hutchins, 2013). Heterotrophs do not require CO_2 for growth, thus increased [H+] from lowered pH is likely the dominant driver of the effects observed (Sommer et al., 2015). The CO_2 sensitivity of heterotrophic flagellates may be governed by the effectiveness of the mechanism(s) they possesses to regulate intracellular pH (Pörtner, 2008). However, little is known about the pH sensitivities of heterotrophic flagellates. Among the few studies on flagellates, a decline in pH influenced the swimming behaviour of a harmful algal bloom causing raphidophyte (Kim et al., 2013) and an inability to control intracellular pH disrupted the growth of the autotrophic

dinoflagellates *Amphidinium carterae* and *Heterocapsa oceanica* (Dason and Colman, 2004). Disruption of flagella motility has also been observed in marine invertebrate sperm, due to inhibition of the internal pH gradients required to activate signalling pathways (Nakajima, 2005; Morita et al., 2010; Nakamura and Morita, 2012). Whilst these examples do not provide evidence for direct inhibition of HNF growth, they do highlight the diverse sensitivities of flagellates to changes in pH that require further investigation. Size may also play a part in CO_2 sensitivity, with size-related declines in the external pH boundary layer meaning small cells are likely to be more affected by lower ocean pH (Flynn et al., 2012). As heterotrophs respire CO_2 and do not photosynthesise, it is likely that pH would be even lower at the cell surface than for autotrophs. This may explain why HNFs showed reduced growth rates in our study while the larger microheterotrophs ~~were unaffected (see Hancock et al., 2018) .~~ may have been unaffected (see Hancock et al., 2018).

~~This study highlights the need for additional research on the nanoflagellate community. There is an increasing understanding of the prevalence of mixotrophy in the marine microbial community (Gast et al., 2018; Mitra et al., 2014; Stoecker et al., 2017) . Mixotrophs are able to utilise both autotrophic and heterotrophic methods of energy production and consumption, although the methods employed can be diverse (Stoecker et al., 2017). It is currently unknown how mixotrophic phytoflagellates will respond to ocean acidification. Caron and Hutchins (2013) speculated that with an increasing concentration of DIC at increasing levels of , autotrophic energy production may be more efficient. However, the simultaneous increase in may have negative effects on both heterotrophic and autotrophic cellular mechanisms, causing multiple stresses to mixotrophic physiology. As molecular methods are allowing for better identification of mixotrophic species (Gast et al., 2018), further research into how these species respond to increasing may now be possible. Whilst iron was not a limiting factor for phytoplankton in the coastal region studied (Davidson et al., 2016), it is a significant driver on the ecology of the marine microbial community in a majority of the Southern Ocean (Martin et al., 1990). Iron limitation has been found to lessen the impact of on some diatom species, especially in combination with other stressors (Hoppe et al., 2013). No studies to date have investigated the effect of ocean acidification on HNF in the iron-limited Southern Ocean, despite their dominance in the microbial community this region (Safi et al., 2007). Thus, it is imperative that further study be done.~~

4.2 Nano- and picophytoplankton

~~A significant~~ An increase in picophytoplankton abundance was observed in our study when CO_2 levels were $\geq$634 μatm (Fig. 6a)~~. Increased abundance of picophytoplankton has been reported in~~ , agreeing with other ocean acidification studies ~~on natural communities around the world (e.g. Brussaard et al., 2013; Schulz et al., 2013; Biswas et al., 2015; Crawfurd et al., 2017). In contrast, Antarctic community studies report varying responses to elevated~~ globally that have reported an increase in abundance of picophytoplankton at elevated CO_2 ~~. Shifts toward~~ levels (e.g. Brussaard et al., 2013; Schulz et al., 2013; Biswas et al., 2015; Crawfurd et al., 2017). However, studies on phytoplankton communities in other Antarctic regions have reported shifts towards larger diatom species ~~have been reported in coastal waters of the Ross Sea(Feng et al., 2010; Tortell et al., 2008), while there was no -induced change to growth or community composition at a site on the Antarctic Peninsula(Young et al., 2015)~~(Ross Sea; Feng et al., 2010; Tortell et al., 2008) or no change (Antarctic Peninsula; Young et al., 2015). This variability in response among sites in Antarctic waters may be due to factors such as differences in microbial ~~composition~~ community seasonal succession or study methods ~~. Picophytoplankton~~

were either not counted (Feng et al., 2010; Tortell et al., 2008) or were considered negligible (Young et al., 2015) in these studies. The significant that excluded picophytoplankton analysis. The increase in picophytoplankton abundance at CO_2 levels $\geq$634 µatm that we report reported here is similar to the findings of Thomson et al. (2016) in their complementary study at the same siteand using similar methods, indicating that this response is consistent across different seasonal and temporal environmentsseasons and between years. It has been suggested that increased abundance of picophytoplankton may be due to increases in productivity derived from more readily-available CO_2 at the cell surface, allowing more passive diffusion of CO_2 into the cell, and thus, reduced requirements for energy-intensive carbon concentration mechanisms (CCMs) (Riebesell et al., 1993; Paulino . CCMs were down-regulated concentrating mechanisms (CCMs; Riebesell et al., 1993; Paulino et al., 2008; Schulz et al., 2013; Calbet et . Down-regulation of CCMs in the high CO_2 treatment (1641 µatm) treatment in both small in small cells (<10 µm) and large (≥10) cells in our was reported in our coincident study (Deppeler et al., 2018). We did not observe any increase in However, it is uncertain whether this resulted in increased primary productivity for this size group, as primary productivity from CCM down-regulation in this treatment (Deppeler et al., 2018) although, small changes in exponential growth get amplified over time and are difficult to pick up in primary productivity measurements , which are representative for the entire community. were performed on the whole community. Instead, primary productivity was significantly reduced when CO_2 levels were ≥1140 µatm, suggesting CCM down-regulation did not have a significant positive effect on growth.

Larger cell surface to volume ratios The larger cell surface area to volume ratio in small cells, allowing increased nutrient utilisation in nutrient-limited environments, has also been invoked to explain the increased abundance of picophytoplankton with elevated CO_2 (Schulz et al., 2013). Size-related differences in growth rates may allow picophytoplankton to establish a bloom faster than larger phytoplankton species (e.g. Newbold et al., 2012). However, this is not seen in nutrient-replete East Antarctic waters, where early summer blooms are dominated by large diatomsand , such as *Thalassiosira* sp. and *Phaeocystis antarctica**Fragilariopsis* sp. (>20 µm), and the prymnesiophyte *P. antarctica* in its colonial life-stage (Davidson et al., 2010). It was also not observed in this study, where only the 953 µatm treatment displayed a significantly enhanced picophytoplankton growth rate (Table 23). Increased rates of nutrient draw-down were observed in the 634-953 µatm CO_2 treatments (Fig. 5), suggesting that moderate increases in CO_2 may stimulate phytoplankton growth, but further increases in CO_2 (≥1140 µatm) led to significant reductions in primary productivity (Deppeler et al., 2018).

Nanophytoplankton abundance was significantly higher highest in the 643 and 953 µatm treatmentstreatment, with significantly increased growth rates in the treatments ≥634 , 1140, and 1641 µatm treatments (Fig. 6b; Table 23). This was likely due to favourable conditions, including the inhibition of growth of larger phytoplankton species, that allowed nano-sized phytoplankton to thrive at higher CO_2 levels (Hancock et al., 2018). The initial decline in nanophytoplankton abundance in all treatments between days 1 and 7 may have been due to acclimation of the community to the mesocosms minicosms or grazing by microzooplankton. Increasing light intensity had a temporary inhibitory effect on growth at CO_2 levels ≥1140 µatm between days 8 and 9 (Fig. 6bS2), suggesting that the significantly enhanced growth rates in these treatments between days 9 and 15 may have been caused by an increase in relative abundance of more tolerant species. The Interestingly, whilst no negative effect of CO_2 was observed on the overall nanophytoplankton abundance, there were very strong species-specific responses to increasing CO_2, resulting in a significant change in community structure. In their coincident study, Hancock et al. (2018) identified

the most abundant nanophytoplankton species present in the minicosms ~~were~~ as *Fragilariopsis* ~~spp. and~~ sp. (<20 µm) and ~~*Phaeocystis*~~ *P. antarctica* in it's colonial form~~(Hancock et al., 2018)~~. These species displayed a CO_2-related threshold in dominance around 634 µatm, with a shift from *P. antarctica* to *Fragilariopsis* ~~spp~~sp. in the high CO_2 treatments~~(Hancock et al., 2018)~~. Thus, it is likely that the relative fitness of both of these species ~~is~~ was increased with a moderate increase in CO_2 level, explaining the higher abundance observed at ~~643 and 953~~ 634 µatm CO_2. ~~Interestingly, whilst no negative effect of was observed on the overall nanophytoplankton abundance, there were very strong species-specific responses to increasing , resulting in a significant change in community structure (Hancock et al., 2018).~~ Increased abundance of *Fragilariopsis* ~~spp~~sp. with elevated CO_2 has also been observed in other ocean acidification studies on natural Antarctic microbial communities (Hoppe et al., 2013; Davidson et al., 2016). Therefore, ~~it is likely that~~ increasing CO_2 ~~will cause the phytoplankton community to shift from a summer community that is currently dominated by large diatoms to one composed of smaller species or morphotypes of nano- and picophytoplankton.~~ may not result in a change in total nanophytoplankton abundance but may instead result in a shift in the summer nanophytoplankton community composition, with increased abundance of small diatoms over *P. antarctica* colonies.

There is an increased understanding of the prevalence of mixotrophy in the marine microbial community (Mitra et al., 2014; Stoecker et a. Therefore, it is possible that mixotrophic nanoflagellates were included in our nanophytoplankton counts, due to the presence of chlorophyll in their cells. Mixotrophs are able to utilise both autotrophic and heterotrophic methods of energy production and consumption, although the range methods employed can be diverse (Stoecker et al., 2017). It is currently unknown how mixotrophic phytoflagellates will respond to ocean acidification. Caron and Hutchins (2013) speculated that autotrophic energy production may be more efficient with increasing levels of CO_2, owing to increased availability of dissolved inorganic carbon species, an essential substrate for photosynthesis, with lower pH. However, the simultaneous increase in $[H^+]$ may have negative effects on both heterotrophic and autotrophic cellular mechanisms, causing multiple stresses to mixotrophic physiology. As molecular methods are allowing for better identification of mixotrophic species, further research into how these species respond to increasing CO_2 may now be possible (Gast et al., 2018).

4.3 Prokaryotes

~~There was a significant increase in abundance of prokaryotes at~~ The prokaryote community responded favourably to increasing CO_2, displaying increased abundance when CO_2 levels were ≥634 µatm (Fig. 6d~~; Table 2). Increases~~). This increase in prokaryote abundance with elevated CO_2 was also observed in ~~previous~~ complementary studies at Prydz Bay~~(Thomson et al., 2016) , as well as in~~ , who reported consistent increases in prokaryote abundance and production with CO_2 levels ≥780 µatm, in high and low nutrient conditions spanning early to late-summer (Thomson et al., 2016; Westwood et al., 2018). An increase in prokaryote abundance with increasing CO_2 has also been reported in Arctic mesocosms (Endres et al., 2014; Engel et al., 2014). ~~Other studieshave reported no influence of~~ Although in other studies, CO_2 had no influence on the prokaryote community (Grossart et al., 2006; Allgaier et al., 2008; Paulino et al., 2008; Newbold et al., 2012)~~, suggesting that the prokaryote community~~ . Thus, it is anticipated that heterotrophic prokaryotes will tolerate increasing CO_2 levels ~~(Reviewed in Hutchins and Fu, 2017)~~ and in some instances, may thrive (reviewed in Hutchins and Fu, 2017). Like HNF, prokaryotes do not require CO_2 for growth,

although it appears they ~~are~~ may be more resistant to large variations in pH. ~~However~~Despite this, there is evidence that CO_2 may ~~affect prokaryotes by inducing~~ induce changes in community composition, selecting for more tolerant ~~species or allowing rare species to emerge~~ or rare species (Krause et al., 2012; Roy et al., 2013; Zhang et al., 2013; Bergen et al., 2016). This may be related to differential responses of phylogenetic groups to maintaining pH homeostasis in either acid and alkaline conditions (Padan et al., 2005; Bunse et al., 2016). The mechanisms for transporting ~~hydrogen ions (H^+)~~ out of the cell are energetically demanding and may reduce the energy available for growth. Whether these energy demands are increased or decreased with ocean acidification depends upon the different strategies for pH homeostasis employed by individual prokaryote species (Teira et al., 2012). In ~~their study, Teira et al. (2012) observed a significant increase~~ addition to this, significant increases in growth efficiency with elevated CO_2 ~~in one bacterial strain, although no~~ may not result in an increase in productivity or abundance ~~resulted~~(Teira et al., 2012). Instead, these changes may affect dissolved organic carbon consumption~~(Endres et al., 2014)~~, with potential impacts on organic matter cycles (Endres et al., 2014).

4.4 Community interactions

The coincidence of the increase in picophytoplankton and prokaryote abundances with reduced ~~abundance of~~ HNF suggests that these communities were being released from grazing pressure at CO_2 levels $\geq$634 µatm. Grazing rates in East Antarctica are on average, 62% of primary production per day, up to a maximum of 220% (Pearce et al., 2010). In addition, >100% of prokaryote production can be removed by micro- and nanoheterotrophs when ~~Chl~~ chlorophyll *a* concentration and prokaryote abundance is high (Pearce et al., 2010). The rapid decline in abundance we observed in picophytoplankton and prokaryotes after 12 days incubation is entirely consistent with the rapid rates of grazing observed in other Antarctic marine microbial communities in this region. In relation to fCO_2, it is reasonable to hypothesise that the lower abundances of these prey ~~sizes~~ in the control and 506 µatm treatments may have been due to stronger top-down control on the community as opposed to a reduction in growth rate. Grazing control of the picophytoplankton community has been proposed in other mesocosm studies to explain both positive (Paulino et al., 2008; Rose et al., 2009a) and negative (Meakin and Wyman, 2011; Newbold et al., 2012) changes in picophytoplankton abundance, although they were not confirmed by HNF counts. In our minicosm study, the rapid decline in prokaryote abundance coincided with a dramatic increase in choanoflagellate abundance, ~~bactivorous~~ bacterivorous eukaryotes, between days 14 and 16 (Hancock et al., 2018). Furthermore, picophytoplankton and prokaryotes in all CO_2 treatments both declined after HNF abundance ~~reached~~ appeared to reach a critical threshold (Fig. ~~??~~7), suggesting that at this point their growth was unable to exceed the top-down control of grazing. Thomson et al. (2016) and Westwood et al. (2018), in their complementary studies, also noted that higher numbers of prokaryotes coincided with reduced HNF abundance across differing microbial community compositions and nutrient availabilities in Prydz Bay, suggesting that this response is likely to be consistent on both seasonal and temporal scales.

Species-specific differences in the sensitivity of HNF to CO_2 may lead to significant changes in the composition of the picophytoplankton and prokaryote communities. HNF food webs are complex and successional changes in taxa occur during phytoplankton blooms (Moustaka-Gouni et al., 2016). In ~~our~~ their coincident study, Hancock et al. (2018) observed species-specific differences in the CO_2 tolerances of choanoflagellate species, where *Bicosta antennigera* displayed significant CO_2

sensitivity at levels ≥634 µatm while other choanoflagellate species (principally *Diaphanoeca multiannulata*) were unaffected. This change in HNF community composition with increased CO_2 did not affect the total prokaryote abundance but may have implications for the prokaryotic community composition through selective grazing. Changes in prokaryote community composition have been observed in other mesocosm studies (Roy et al., 2013; Zhang et al., 2013; Bergen et al., 2016). There is also evidence that different prokaryote phylogenetic groups have preferences for organic substrates produced by different phytoplankton taxa (Sarmento and Gasol, 2012), leading to the possibility that future changes in prokaryote community composition could impact organic matter recycling.

As viral abundance was not determined in our study, we cannot exclude viral lysis as an explanation for the rapid decline in picophytoplankton and prokaryote abundance. Viral lysis can account to up to 25% of daily production, although grazing by micro- and nanoheterotrophs can be twice as high (Evans et al., 2003; Pearce et al., 2010). In an Arctic mesocosm study, the decline of a picophytoplankton bloom coincided with a large increase in viral abundance (Brussaard et al., 2013). However, later in the study, picophytoplankton were heavily also grazed by microzooplankton. Bacteriophages are the dominant viruses in the Prydz Bay area (Pearce et al., 2007; Thomson et al., 2010; Liang et al., 2016), with viral abundance displaying no correlation to picophytoplankton (Liang et al., 2016). This suggests that viral lysis was unlikely to be the main cause of the decline in picophytoplankton numbers but may have affected the prokaryotes.

5 Conclusions

The results of this study show how ocean acidification can exert both direct and indirect influences on the interactions among trophic levels within the microbial loop. Our study reinforces findings in near shore waters off East Antarctica ~~(Davidson et al., 2016; Thom~~ that HNF abundance is reduced when CO_2 is ≥634 µatm, irrespective of temporal changes in the physical and biological environment among seasons and years (Davidson et al., 2016; Thomson et al., 2016; Westwood et al., 2018). This likely resulted in a decline in grazing mortality of picophytoplankton and prokaryotes, allowing these communities to increase in abundance. ~~Such changes in predator-prey interactions with ocean acidification could have significant effects on the food web and biogeochemistry in the Southern Ocean.~~ HNF are an important link in carbon transfer to higher trophic levels as they are grazed upon by microzooplankton and thereafter by higher trophic organisms (Azam et al., 1991; Sherr and Sherr, 2002). Grazing is also a critical determinant of phytoplankton community composition and standing stocks (Sherr and Sherr, 2002). Therefore, the changes in predator-prey interactions with ocean acidification we observed in this study could have significant effects on the food web and biogeochemistry in coastal Antarctic waters.

Our results, together with those of the coincident studies by Deppeler et al. (2018) and Hancock et al. (2018), indicate it is likely that increasing CO_2 will cause a shift away from blooms dominated by large diatoms and *P. antarctica* towards communities increasingly dominated by prokaryotes, ~~nano-~~ nano-sized diatoms, and picophytoplankton. Large phytoplankton cells contribute significantly to deep ocean carbon sequestration (Tréguer et al., 2018). They are also the preferred food source for higher trophic organisms, especially the Antarctic krill *Euphausia superba* (Haberman et al., 2003; Meyer et al., 2003; Schmidt et al., 2006). *E. superba* have been found to graze less efficiently on phytoplankton cells <10 µm (Quetin and Ross,

1985; Kawaguchi et al., 1999; Haberman et al., 2003). ~~Therefore~~Thus, a shift to smaller-celled communities will likely alter the structure of the Antarctic food web. Furthermore, increases in prokaryote abundance will likely intensify the breakdown of organic matter in surface waters, further contributing in a decline in the sequestration of carbon from summer phytoplankton blooms into the deep ocean.

Data availability. Experimental data used for analysis are available via the Australian Antarctic Data Centre.

Environmental data: Deppeler, S.L., Davidson, A.T., Schulz, K.: Environmental data for Davis 14/15 ocean acidification minicosm experiment, Australian Antarctic Data Centre, http://dx.doi.org/10.4225/15/599a7dfe9470a, 2017, (updated 2017).

Flow cytometry data: Deppeler, S.L., Schulz, K.G., Hancock, A., Pascoe, P., Mckinlay, J., Davidson, A.T. (2018, updated 2018) Data for manuscript 'Ocean acidification reduces growth and grazing of Antarctic heterotrophic nanoflagellates' Australian Antarctic Data Centre, http://dx.doi.org/10.4225/15/5b234e4bb9313, 2018 (updated 2018)

Micoscopy data: Hancock, A.M., Davidson, A.T., Mckinlay, J., Mcminn, A., Schulz, K., Van Den Enden, D. (2017, updated 2018) Ocean acidification changes the structure of an Antarctic coastal protistan community Australian Antarctic Data Centre, http://dx.doi.org/10.4225/15/592b83a5c7506, 2018 (updated 2018)

Author contributions. AD conceived and designed the experiments. AD led and oversaw the minicosm experiment and PP, SD, and AH performed the experiments. SD and AD performed the data analysis. KS performed the carbonate system measurements and manipulation. JM provided statistical guidance. SD wrote the manuscript with all other authors providing contributions and critical review of the manuscript.

Competing interests. The authors declare that they have no conflict of interest.

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References

Aberle, N., Schulz, K. G., Stuhr, A., Malzahn, A. M., Ludwig, A., and Riebesell, U.: High tolerance of microzooplankton to ocean acidification in an Arctic coastal plankton community, Biogeosciences, 10, 1471–1481, https://doi.org/10.5194/bg-10-1471-2013, 2013.

Allgaier, M., Riebesell, U., Vogt, M., Thyrhaug, R., and Grossart, H.-P.: Coupling of heterotrophic bacteria to phytoplankton bloom development at different pCO_2 levels: a mesocosm study, Biogeosciences, 5, 1007–1022, https://doi.org/10.5194/bg-5-1007-2008, 2008.

Archer, S. D., Leakey, R. J. G., Burkill, P. H., and Sleigh, M. A.: Microbial dynamics in coastal waters of East Antarctica: Herbivory by heterotrophic dinoflagellates, Marine Ecology Progress Series, 139, 239–255, https://doi.org/10.3354/meps139239, 1996.

Arrigo, K. R., van Dijken, G. L., and Bushinsky, S.: Primary production in the Southern Ocean, 1997-2006, Journal of Geophysical Research: Oceans, 113, C08 004, https://doi.org/10.1029/2007JC004551, 2008.

Azam, F. and Malfatti, F.: Microbial structuring of marine ecosystems, Nature Reviews Microbiology, 5, 782–791, https://doi.org/10.1038/nrmicro1747, 2007.

Azam, F., Fenchel, T., Field, J. G., Gray, J. C., Meyer-Reil, L. A., and Thingstad, F.: The ecological role of water-column microbes in the sea, Marine Ecology Progress Series, 10, 257–264, https://doi.org/10.3354/meps010257, 1983.

Azam, F., Smith, D. C., and Hollibaugh, J. T.: The role of the microbial loop in Antarctic pelagic ecosystems, Polar Research, 10, 239–243, https://doi.org/10.1111/j.1751-8369.1991.tb00649.x, 1991.

Beardall, J. and Giordano, M.: Ecological implications of microalgal and cyanobacterial CO_2 concentrating mechanisms, and their regulation, Functional Plant Biology, 29, 335, 2002.

Behrenfeld, M. J.: Climate-mediated dance of the plankton, Nature Climate Change, 4, 880–887, https://doi.org/10.1038/nclimate2349, 2014.

Bergen, B., Endres, S., Engel, A., Zark, M., Dittmar, T., Sommer, U., and Jürgens, K.: Acidification and warming affect prominent bacteria in two seasonal phytoplankton bloom mesocosms, Environmental Microbiology, 18, 4579–4595, https://doi.org/10.1111/1462-2920.13549, 2016.

Bertrand, E. M., McCrow, J. P., Moustafa, A., Zheng, H., McQuaid, J. B., Delmont, T. O., Post, A. F., Sipler, R. E., Spackeen, J. L., Xu, K., Bronk, D. a., Hutchins, D. a., and Allen, A. E.: Phytoplankton–bacterial interactions mediate micronutrient colimitation at the coastal Antarctic sea ice edge, Proceedings of the National Academy of Sciences, 112, 9938–9943, https://doi.org/10.1073/pnas.1501615112, 2015.

Biswas, H., Jie, J., Li, Y., Zhang, G., Zhu, Z. Y., Wu, Y., Zhang, G. L., Li, Y. W., Liu, S. M., and Zhang, J.: Response of a natural phytoplankton community from the Qingdao coast (Yellow Sea, China) to variable CO_2 levels over a short-term incubation experiment, Current Science, 108, 1901–1909, 2015.

Bjørnsen, P. K. and Kuparinen, J.: Growth and herbivory by heterotrophic dinoflagellates in the Southern Ocean, studied by microcosm experiments, Marine Biology, 109, 397–405, https://doi.org/10.1007/BF01313505, 1991.

Bockmon, E. E. and Dickson, A. G.: A seawater filtration method suitable for total dissolved inorganic carbon and pH analyses, Limnology and Oceanography: Methods, 12, 191–195, https://doi.org/10.4319/lom.2014.12.191, 2014.

Boyd, P. W., Cornwall, C. E., Davison, A., Doney, S. C., Fourquez, M., Hurd, C. L., Lima, I. D., and McMinn, A.: Biological responses to environmental heterogeneity under future ocean conditions, Global Change Biology, 22, 2633–2650, https://doi.org/10.1111/gcb.13287, 2016.

Brussaard, C. P. D., Noordeloos, A. A. M., Witte, H., Collenteur, M. C. J., Schulz, K., Ludwig, A., and Riebesell, U.: Arctic microbial community dynamics influenced by elevated CO_2 levels, Biogeosciences, 10, 719–731, https://doi.org/10.5194/bg-10-719-2013, 2013.

Buchan, A., LeCleir, G. R., Gulvik, C. A., and González, J. M.: Master recyclers: features and functions of bacteria associated with phytoplankton blooms, Nature Reviews Microbiology, 12, 686–698, https://doi.org/10.1038/nrmicro3326, 2014.

Bunse, C., Lundin, D., Karlsson, C. M. G., Vila-Costa, M., Palovaara, J., Akram, N., Svensson, L., Holmfeldt, K., González, J. M., Calvo, E., Pelejero, C., Marrasé, C., Dopson, M., Gasol, J. M., and Pinhassi, J.: Response of marine bacterioplankton pH homeostasis gene expression to elevated CO_2, Nature Climate Change, 1, 1–7, https://doi.org/10.1038/nclimate2914, 2016.

Calbet, A., Sazhin, A. F., Nejstgaard, J. C., Berger, S. A., Tait, Z. S., Olmos, L., Sousoni, D., Isari, S., Martínez, R. A., Bouquet, J.-M., Thompson, E. M., Båmstedt, U., and Jakobsen, H. H.: Future Climate Scenarios for a Coastal Productive Planktonic Food Web Resulting in Microplankton Phenology Changes and Decreased Trophic Transfer Efficiency, PLoS ONE, 9, e94 388, https://doi.org/10.1371/journal.pone.0094388, 2014.

Caron, D. A. and Hutchins, D. A.: The effects of changing climate on microzooplankton grazing and community structure: drivers, predictions and knowledge gaps, Journal of Plankton Research, 35, 235–252, https://doi.org/10.1093/plankt/fbs091, 2013.

Caron, D. A., Dennett, M. R., Lonsdale, D. J., Moran, D. M., and Shalapyonok, L.: Microzooplankton herbivory in the Ross Sea, Antarctica, Deep-Sea Research Part II: Topical Studies in Oceanography, 47, 3249–3272, https://doi.org/10.1016/S0967-0645(00)00067-9, 2000.

Celussi, M., Malfatti, F., Annalisa, F., Gazeau, F., Giannakourou, A., Pitta, P., Tsiola, A., and Del Negro, P.: Ocean acidification effect on prokaryotic metabolism tested in two diverse trophic regimes in the Mediterranean Sea, Estuarine, Coastal and Shelf Science, 186, 125–138, https://doi.org/10.1016/j.ecss.2015.08.015, 2017.

Crawfurd, K. J., Alvarez-Fernandez, S., Mojica, K. D. A., Riebesell, U., and Brussaard, C. P. D.: Alterations in microbial community composition with increasing fCO_2: a mesocosm study in the eastern Baltic Sea, Biogeosciences, 14, 3831–3849, https://doi.org/10.5194/bg-14-3831-2017, 2017.

Dason, J. S. and Colman, B.: Inhibition of growth in two dinoflagellates by rapid changes in external pH, Canadian Journal of Botany, 82, 515–520, https://doi.org/10.1139/b04-023, 2004.

Davidson, A., McKinlay, J., Westwood, K., Thomson, P., van den Enden, R., de Salas, M., Wright, S., Johnson, R., and Berry, K.: Enhanced CO_2 concentrations change the structure of Antarctic marine microbial communities, Marine Ecology Progress Series, 552, 93–113, https://doi.org/10.3354/meps11742, 2016.

Davidson, A. T., Scott, F. J., Nash, G. V., Wright, S. W., and Raymond, B.: Physical and biological control of protistan community composition, distribution and abundance in the seasonal ice zone of the Southern Ocean between 30 and 80°E, Deep Sea Research Part II: Topical Studies in Oceanography, 57, 828–848, https://doi.org/10.1016/j.dsr2.2009.02.011, 2010.

Deppeler, S., Petrou, K., Schulz, K. G., Westwood, K., Pearce, I., McKinlay, J., and Davidson, A.: Ocean acidification of a coastal Antarctic marine microbial community reveals a critical threshold for CO_2 tolerance in phytoplankton productivity, Biogeosciences, 15, 209–231, https://doi.org/10.5194/bg-15-209-2018, 2018.

Deppeler, S. L. and Davidson, A. T.: Southern Ocean Phytoplankton in a Changing Climate, Frontiers in Marine Science, 4, 1–18, https://doi.org/10.3389/fmars.2017.00040, http://journal.frontiersin.org/article/10.3389/fmars.2017.00040/full, 2017.

Dickson, A.: Standards for Ocean Measurements, Oceanography, 23, 34–47, https://doi.org/10.5670/oceanog.2010.22, 2010.

Dickson, A., Sabine, C., and Christian, J., eds.: Guide to Best Practices for Ocean CO_2 Measurements, North Pacific Marine Science Organization, Sidney, British Columbia, 2007.

Endres, S., Galgani, L., Riebesell, U., Schulz, K.-G., and Engel, A.: Stimulated Bacterial Growth under Elevated pCO_2: Results from an Off-Shore Mesocosm Study, PLoS ONE, 9, e99 228, https://doi.org/10.1371/journal.pone.0099228, 2014.

Engel, A., Piontek, J., Grossart, H.-P., Riebesell, U., Schulz, K. G., and Sperling, M.: Impact of CO_2 enrichment on organic matter dynamics during nutrient induced coastal phytoplankton blooms, Journal of Plankton Research, 36, 641–657, https://doi.org/10.1093/plankt/fbt125, 2014.

Evans, C., Archer, S. D., Jacquet, S., and Wilson, W. H.: Direct estimates of the contribution of viral lysis and microzooplankton grazing to the decline of a *Micromonas* spp. population, Aquatic Microbial Ecology, 30, 207–219, https://doi.org/10.3354/ame030207, 2003.

Fenchel, T.: The microbial loop - 25 years later, Journal of Experimental Marine Biology and Ecology, 366, 99–103, https://doi.org/10.1016/j.jembe.2008.07.013, 2008.

Feng, Y., Hare, C., Rose, J., Handy, S., DiTullio, G., Lee, P., Smith, W., Peloquin, J., Tozzi, S., Sun, J., Zhang, Y., Dunbar, R., Long, M., Sohst, B., Lohan, M., and Hutchins, D.: Interactive effects of iron, irradiance and CO_2 on Ross Sea phytoplankton, Deep Sea Research Part I: Oceanographic Research Papers, 57, 368–383, https://doi.org/10.1016/j.dsr.2009.10.013, 2010.

Flombaum, P., Gallegos, J. L., Gordillo, R. a., Rincon, J., Zabala, L. L., Jiao, N., Karl, D. M., Li, W. K. W., Lomas, M. W., Veneziano, D., Vera, C. S., Vrugt, J. A., and Martiny, A. C.: Present and future global distributions of the marine Cyanobacteria *Prochlorococcus* and *Synechococcus*, Proceedings of the National Academy of Sciences, 110, 9824–9829, https://doi.org/10.1073/pnas.1307701110, 2013.

Flynn, K. J., Blackford, J. C., Baird, M. E., Raven, J. A., Clark, D. R., Beardall, J., Brownlee, C., Fabian, H., and Wheeler, G. L.: Changes in pH at the exterior surface of plankton with ocean acidification, Nature Climate Change, 2, 510–513, https://doi.org/10.1038/nclimate1489, 2012.

Frölicher, T. L., Sarmiento, J. L., Paynter, D. J., Dunne, J. P., Krasting, J. P., and Winton, M.: Dominance of the Southern Ocean in Anthropogenic Carbon and Heat Uptake in CMIP5 Models, Journal of Climate, 28, 862–886, https://doi.org/10.1175/JCLI-D-14-00117.1, 2015.

Gao, K., Helbling, E. W., Häder, D. P., and Hutchins, D. A.: Responses of marine primary producers to interactions between ocean acidification, solar radiation, and warming, Marine Ecology Progress Series, 470, 167–189, https://doi.org/10.3354/meps10043, 2012a.

Gao, K., Xu, J., Gao, G., Li, Y., Hutchins, D. A., Huang, B., Wang, L., Zheng, Y., Jin, P., Cai, X., Häder, D.-p., Li, W., Xu, K., Liu, N., and Riebesell, U.: Rising CO_2 and increased light exposure synergistically reduce marine primary productivity, Nature Climate Change, 2, 519–523, https://doi.org/10.1038/nclimate1507, 2012b.

Gast, R. J., Fay, S. A., and Sanders, R. W.: Mixotrophic Activity and Diversity of Antarctic Marine Protists in Austral Summer, Frontiers in Marine Science, 5, 1–12, https://doi.org/10.3389/fmars.2018.00013, 2018.

Gibson, J. A. E. and Trull, T. W.: Annual cycle of fCO_2 under sea-ice and in open water in Prydz Bay, East Antarctica, Marine Chemistry, 66, 187–200, https://doi.org/10.1016/S0304-4203(99)00040-7, 1999.

Grossart, H.-p., Allgaier, M., Passow, U., and Riebesell, U.: Testing the effect of CO_2 concentration on the dynamics of marine heterotrophic bacterioplankton, Limnology and Oceanography, 51, 1–11, https://doi.org/10.4319/lo.2006.51.1.0001, 2006.

Haberman, K. L., Quetin, L. B., and Ross, R. M.: Diet of the Antarctic krill (*Euphausia superba* Dana), Journal of Experimental Marine Biology and Ecology, 283, 79–95, https://doi.org/10.1016/S0022-0981(02)00466-5, 2003.

Hancock, A. M., Davidson, A. T., McKinlay, J., McMinn, A., Schulz, K. G., and van den Enden, R. L.: Ocean acidification changes the structure of an Antarctic coastal protistan community, Biogeosciences, 15, 2393–2410, https://doi.org/10.5194/bg-15-2393-2018, 2018.

Hoppe, C. J. M., Hassler, C. S., Payne, C. D., Tortell, P. D., Rost, B., and Trimborn, S.: Iron Limitation Modulates Ocean Acidification Effects on Southern Ocean Phytoplankton Communities, PLoS ONE, 8, e79 890, https://doi.org/10.1371/journal.pone.0079890, 2013.

Hutchins, D. A. and Fu, F.: Microorganisms and ocean global change, Nature Microbiology, 2, 1–11, https://doi.org/10.1038/nmicrobiol.2017.58, 2017.

IPCC: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, https://doi.org/10.1017/CBO9781107415324, 2013.

Kawaguchi, S., Ichii, T., and Naganobu, M.: Green krill, the indicator of micro- and nano-size phytoplankton availability to krill, Polar Biology, 22, 133–136, https://doi.org/10.1007/s003000050400, 1999.

Kim, H., Spivack, A. J., and Menden-Deuer, S.: pH alters the swimming behaviors of the raphidophyte *Heterosigma akashiwo*: Implications for bloom formation in an acidified ocean, Harmful Algae, 26, 1–11, https://doi.org/10.1016/j.hal.2013.03.004, 2013.

Krause, E., Wichels, A., Giménez, L., Lunau, M., Schilhabel, M. B., and Gerdts, G.: Small Changes in pH Have Direct Effects on Marine Bacterial Community Composition: A Microcosm Approach, PLoS ONE, 7, e47 035, https://doi.org/10.1371/journal.pone.0047035, 2012.

Landry, M. R. and Calbet, A.: Microzooplankton production in the oceans, ICES Journal of Marine Science, 61, 501–507, https://doi.org/10.1016/j.icesjms.2004.03.011, 2004.

Li, G., Brown, C. M., Jeans, J. A., Donaher, N. A., McCarthy, A., and Campbell, D. A.: The nitrogen costs of photosynthesis in a diatom under current and future pCO_2, New Phytologist, 205, 533–543, https://doi.org/10.1111/nph.13037, 2015.

Liang, Y., Bai, X., Jiang, Y., Wang, M., He, J., and McMinn, A.: Distribution of marine viruses and their potential hosts in Prydz Bay and adjacent Southern Ocean, Antarctic, Polar Biology, 39, 365–378, https://doi.org/10.1007/s00300-015-1787-8, 2016.

Lin, L., He, J., Zhao, Y., Zhang, F., and Cai, M.: Flow cytometry investigation of picoplankton across latitudes and along the circum Antarctic Ocean, Acta Oceanologica Sinica, 31, 134–142, https://doi.org/10.1007/s13131-012-0185-0, 2012.

Lueker, T. J., Dickson, A. G., and Keeling, C. D.: Ocean pCO_2 calculated from dissolved inorganic carbon, alkalinity, and equations for K_1 and K_2: validation based on laboratory measurements of CO_2 in gas and seawater at equilibrium, Marine Chemistry, 70, 105–119, https://doi.org/10.1016/S0304-4203(00)00022-0, 2000.

Marie, D., Simon, N., and Vaulot, D.: Phytoplankton Cell Counting by Flow Cytometry, in: Algal Culturing Techniques, edited by Anderson, R. A., chap. 17, pp. 253–267, Academic Press, San Diego, CA, USA, https://doi.org/10.1016/B978-012088426-1/50018-4, 2005.

Martin, J. H., Gordon, R. M., and Fitzwater, S. E.: Iron in Antarctic waters, Nature, 345, 156–158, https://doi.org/10.1038/345156a0, 1990.

McNeil, B. I. and Matear, R. J.: Southern Ocean acidification: A tipping point at 450-ppm atmospheric CO_2, Proceedings of the National Academy of Sciences, 105, 18 860–18 864, https://doi.org/10.1073/pnas.0806318105, 2008.

Meakin, N. G. and Wyman, M.: Rapid shifts in picoeukaryote community structure in response to ocean acidification, The ISME Journal, 5, 1397–1405, https://doi.org/10.1038/ismej.2011.18, 2011.

Mehrbach, C., Culberson, C. H., Hawley, J. E., and Pytkowicx, R. M.: Measurement of the Apparent Dissociation Constants of Carbonic Acid in Seawater At Atmospheric Pressure, Limnology and Oceanography, 18, 897–907, https://doi.org/10.4319/lo.1973.18.6.0897, 1973.

Meyer, B., Atkinson, A., Blume, B., and Bathmann, U. V.: Feeding and energy budgets of larval antarctic krill *Euphausia superba* in summer, Marine Ecology Progress Series, 257, 167–177, https://doi.org/10.3354/meps257167, 2003.

Mitra, A., Flynn, K. J., Burkholder, J. M., Berge, T., Calbet, A., Raven, J. A., Granéli, E., Glibert, P. M., Hansen, P. J., Stoecker, D. K., Thingstad, F., Tillmann, U., Väge, S., Wilken, S., and Zubkov, M. V.: The role of mixotrophic protists in the biological carbon pump, Biogeosciences, 11, 995–1005, https://doi.org/10.5194/bg-11-995-2014, 2014.

Morita, M., Suwa, R., Iguchi, A., Nakamura, M., Shimada, K., Sakai, K., and Suzuki, A.: Ocean acidification reduces sperm flagellar motility in broadcast spawning reef invertebrates, Zygote, 18, 103–107, https://doi.org/10.1017/S0967199409990177, 2010.

Moustaka-Gouni, M., Kormas, K. A., Scotti, M., Vardaka, E., and Sommer, U.: Warming and Acidification Effects on Planktonic Heterotrophic Pico- and Nanoflagellates in a Mesocosm Experiment, Protist, 167, 389–410, https://doi.org/10.1016/j.protis.2016.06.004, 2016.

Nakajima, A.: Increase in intracellular pH induces phosphorylation of axonemal proteins for activation of flagellar motility in starfish sperm, Journal of Experimental Biology, 208, 4411–4418, https://doi.org/10.1242/jeb.01906, 2005.

Nakamura, M. and Morita, M.: Sperm motility of the scleractinian coral *Acropora digitifera* under preindustrial, current, and predicted ocean acidification regimes, Aquatic Biology, 15, 299–302, https://doi.org/10.3354/ab00436, 2012.

Newbold, L. K., Oliver, A. E., Booth, T., Tiwari, B., DeSantis, T., Maguire, M., Andersen, G., van der Gast, C. J., and Whiteley, A. S.: The response of marine picoplankton to ocean acidification, Environmental Microbiology, 14, 2293–2307, https://doi.org/10.1111/j.1462-2920.2012.02762.x, 2012.

Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R. M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R. G., Plattner, G.-K., Rodgers, K. B., Sabine, C. L., Sarmiento, J. L., Schlitzer, R., Slater, R. D., Totterdell, I. J., Weirig, M.-F., Yamanaka, Y., and Yool, A.: Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms, Nature, 437, 681–6, https://doi.org/10.1038/nature04095, 2005.

Padan, E., Bibi, E., Ito, M., and Krulwich, T. A.: Alkaline pH homeostasis in bacteria: New insights, Biochimica et Biophysica Acta - Biomembranes, 1717, 67–88, https://doi.org/10.1016/j.bbamem.2005.09.010, 2005.

Paulino, A. I., Egge, J. K., and Larsen, A.: Effects of increased atmospheric CO_2 on small and intermediate sized osmotrophs during a nutrient induced phytoplankton bloom, Biogeosciences, 5, 739–748, https://doi.org/10.5194/bg-5-739-2008, 2008.

Pearce, I., Davidson, A., Bell, E., and Wright, S.: Seasonal changes in the concentration and metabolic activity of bacteria and viruses at an Antarctic coastal site, Aquatic Microbial Ecology, 47, 11–23, https://doi.org/10.3354/ame047011, 2007.

Pearce, I., Davidson, A. T., Thomson, P. G., Wright, S., and van den Enden, R.: Marine microbial ecology off East Antarctica (30 - 80°E): Rates of bacterial and phytoplankton growth and grazing by heterotrophic protists, Deep Sea Research Part II: Topical Studies in Oceanography, 57, 849–862, https://doi.org/10.1016/j.dsr2.2008.04.039, 2010.

Petrou, K., Baker, K. G., Nielsen, D. A., Hancock, A. M., Schulz, K. G., and Davidson, A. T.: Acidification diminishes diatom silica production in the Southern Ocean, Nature Climate Change, 9, 781–786, https://doi.org/10.1038/s41558-019-0557-y, 2019.

Piontek, J., Lunau, M., Händel, N., Borchard, C., Wurst, M., and Engel, A.: Acidification increases microbial polysaccharide degradation in the ocean, Biogeosciences, 7, 1615–1624, https://doi.org/10.5194/bg-7-1615-2010, 2010.

Pörtner, H. O.: Ecosystem effects of ocean acidification in times of ocean warming: A physiologist's view, Marine Ecology Progress Series, 373, 203–217, https://doi.org/10.3354/meps07768, 2008.

Quetin, L. B. and Ross, R. M.: Feeding by Antarctic Krill, Euphausia superba: Does Size Matter?, in: Antarctic Nutrient Cycles and Food Webs, edited by Siegfried, W., Condy, P., and Laws, R., pp. 372–377, Springer Berlin Heidelberg, Berlin, Heidelberg, https://doi.org/10.1007/978-3-642-82275-9_52, 1985.

R Core Team: R: A Language and Environment for Statistical Computing, 2016.

Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P., Riebesell, U., Shepherd, J., Turley, C., and Watson, A.: Ocean acidification due to increasing atmospheric carbon dioxide, Tech. Rep. June, The Royal Society, 2005.

Riebesell, U., Wolf-Gladrow, D. A., and Smetacek, V.: Carbon dioxide limitation of marine phytoplankton growth rates, Nature, 361, 249–251, https://doi.org/10.1038/361249a0, 1993.

Riebesell, U., Bellerby, R. G. J., Grossart, H.-P., and Thingstad, F.: Mesocosm CO_2 perturbation studies: from organism to community level, Biogeosciences, 5, 1157–1164, https://doi.org/10.5194/bg-5-1157-2008, 2008.

Roden, N. P., Shadwick, E. H., Tilbrook, B., and Trull, T. W.: Annual cycle of carbonate chemistry and decadal change in coastal Prydz Bay, East Antarctica, Marine Chemistry, 155, 135–147, https://doi.org/10.1016/j.marchem.2013.06.006, 2013.

Rose, J., Feng, Y., Gobler, C., Gutierrez, R., Hare, C., Leblanc, K., and Hutchins, D.: Effects of increased pCO_2 and temperature on the North Atlantic spring bloom. II. Microzooplankton abundance and grazing, Marine Ecology Progress Series, 388, 27–40, https://doi.org/10.3354/meps08134, 2009a.

Rose, J. M., Caron, D. A., Sieracki, M. E., and Poulton, N.: Counting heterotrophic nanoplanktonic protists in cultures and aquatic communities by flow cytometry, Aquatic Microbial Ecology, 34, 263–277, https://doi.org/10.3354/ame034263, 2004.

Rose, J. M., Feng, Y., DiTullio, G. R., Dunbar, R. B., Hare, C. E., Lee, P. a., Lohan, M., Long, M., Smith, W. O. J., Sohst, B., Tozzi, S., Zhang, Y., and Hutchins, D. a.: Synergistic effects of iron and temperature on Antarctic phytoplankton and microzooplankton assemblages, Biogeosciences, 6, 3131–3147, https://doi.org/10.5194/bg-6-3131-2009, 2009b.

Roy, A.-S., Gibbons, S. M., Schunck, H., Owens, S., Caporaso, J. G., Sperling, M., Nissimov, J. I., Romac, S., Bittner, L., Mühling, M., Riebesell, U., LaRoche, J., and Gilbert, J. A.: Ocean acidification shows negligible impacts on high-latitude bacterial community structure in coastal pelagic mesocosms, Biogeosciences, 10, 555–566, https://doi.org/10.5194/bg-10-555-2013, 2013.

Sabine, C. L.: The Oceanic Sink for Anthropogenic CO_2, Science, 305, 367–371, https://doi.org/10.1126/science.1097403, 2004.

Safi, K. A., Griffiths, F. B., and Hall, J. A.: Microzooplankton composition, biomass and grazing rates along the WOCE SR3 line between Tasmania and Antarctica, Deep Sea Research Part I: Oceanographic Research Papers, 54, 1025–1041, https://doi.org/10.1016/j.dsr.2007.05.003, 2007.

Sarmento, H. and Gasol, J. M.: Use of phytoplankton-derived dissolved organic carbon by different types of bacterioplankton, Environmental Microbiology, 14, 2348–2360, https://doi.org/10.1111/j.1462-2920.2012.02787.x, 2012.

Schmidt, K., Atkinson, A., Petzke, K.-J., Voss, M., and Pond, D. W.: Protozoans as a food source for Antarctic krill, *Euphausia superba*: Complementary insights from stomach content, fatty acids, and stable isotopes, Limnology and Oceanography, 51, 2409–2427, https://doi.org/10.4319/lo.2006.51.5.2409, 2006.

Schulz, K. G., Bellerby, R. G. J., Brussaard, C. P. D., Büdenbender, J., Czerny, J., Engel, A., Fischer, M., Koch-Klavsen, S., Krug, S. A., Lischka, S., Ludwig, A., Meyerhöfer, M., Nondal, G., Silyakova, A., Stuhr, A., and Riebesell, U.: Temporal biomass dynamics of an Arctic plankton bloom in response to increasing levels of atmospheric carbon dioxide, Biogeosciences, 10, 161–180, https://doi.org/10.5194/bg-10-161-2013, 2013.

Schulz, K. G., Bach, L. T., Bellerby, R. G. J., Bermúdez, R., Büdenbender, J., Boxhammer, T., Czerny, J., Engel, A., Ludwig, A., Meyerhöfer, M., Larsen, A., Paul, A. J., Sswat, M., and Riebesell, U.: Phytoplankton Blooms at Increasing Levels of Atmospheric Carbon Dioxide: Experimental Evidence for Negative Effects on Prymnesiophytes and Positive on Small Picoeukaryotes, Frontiers in Marine Science, 4, 1–18, https://doi.org/10.3389/fmars.2017.00064, 2017.

Sherr, E. B. and Sherr, B. F.: Preservation and Storage of Samples for Enumeration of Heterotrophic Protists, in: Handbook of Methods in Aquatic Microbial Ecology, edited by Kemp, P. F., Sherr, B. F., Sherr, E. B., and Cole, J. J., chap. 25, pp. 207–212, CRC Press, Boca Raton, FL, 1993.

Sherr, E. B. and Sherr, B. F.: Significance of predation by protists, Antonie van Leeuwenhoek, 81, 293–308, https://doi.org/10.1023/A:1020591307260, 2002.

Sherr, E. B., Caron, D. A., and Sherr, B. F.: Staining of Heterotrophic Protists for Visualization via Epifluorescence Microscopy, in: Handbook of Methods in Aquatic Microbial Ecology, edited by Kemp, P. F., Sherr, B. F., Sherr, E. B., and Cole, J. J., chap. 26, pp. 213–227, CRC Press, Boca Raton, FL, 1993.

Smetacek, V., Assmy, P., and Henjes, J.: The role of grazing in structuring Southern Ocean pelagic ecosystems and biogeochemical cycles, Antarctic Science, 16, 541–558, https://doi.org/10.1017/S0954102004002317, 2004.

Sommer, U., Paul, C., and Moustaka-Gouni, M.: Warming and Ocean Acidification Effects on Phytoplankton—From Species Shifts to Size Shifts within Species in a Mesocosm Experiment, PLOS ONE, 10, e0125 239, https://doi.org/10.1371/journal.pone.0125239, 2015.

Stoecker, D. K., Hansen, P. J., Caron, D. A., and Mitra, A.: Mixotrophy in the Marine Plankton, Annual Review of Marine Science, 9, 311–335, https://doi.org/10.1146/annurev-marine-010816-060617, 2017.

Suffrian, K., Simonelli, P., Nejstgaard, J. C., Putzeys, S., Carotenuto, Y., and Antia, A. N.: Microzooplankton grazing and phytoplankton growth in marine mesocosms with increased CO_2 levels, Biogeosciences, 5, 1145–1156, https://doi.org/10.5194/bg-5-1145-2008, 2008.

Takahashi, T., Sweeney, C., Hales, B., Chipman, D., Newberger, T., Goddard, J., Iannuzzi, R., and Sutherland, S.: The Changing Carbon Cycle in the Southern Ocean, Oceanography, 25, 26–37, https://doi.org/10.5670/oceanog.2012.71, 2012.

Teira, E., Fernández, A., Álvarez-Salgado, X. A., García-Martín, E. E., Serret, P., and Sobrino, C.: Response of two marine bacterial isolates to high CO_2 concentration, Marine Ecology Progress Series, 453, 27–36, https://doi.org/10.3354/meps09644, 2012.

Thomson, P., Davidson, A., and Maher, L.: Increasing CO_2 changes community composition of pico- and nano-sized protists and prokaryotes at a coastal Antarctic site, Marine Ecology Progress Series, 554, 51–69, https://doi.org/10.3354/meps11803, 2016.

Thomson, P. G., Davidson, A. T., van den Enden, R., Pearce, I., Seuront, L., Paterson, J. S., and Williams, G. D.: Distribution and abundance of marine microbes in the Southern Ocean between 30 and 80°E, Deep Sea Research Part II: Topical Studies in Oceanography, 57, 815–827, https://doi.org/10.1016/j.dsr2.2008.10.040, 2010.

Tortell, P. D., Payne, C. D., Li, Y., Trimborn, S., Rost, B., Smith, W. O., Riesselman, C., Dunbar, R. B., Sedwick, P., and DiTullio, G. R.: CO_2 sensitivity of Southern Ocean phytoplankton, Geophysical Research Letters, 35, L04 605, https://doi.org/10.1029/2007GL032583, 2008.

Tréguer, P., Bowler, C., Moriceau, B., Dutkiewicz, S., Gehlen, M., Aumont, O., Bittner, L., Dugdale, R., Finkel, Z., Iudicone, D., Jahn, O., Guidi, L., Lasbleiz, M., Leblanc, K., Levy, M., and Pondaven, P.: Influence of diatom diversity on the ocean biological carbon pump, Nature Geoscience, 11, 27–37, https://doi.org/10.1038/s41561-017-0028-x, 2018.

Trimborn, S., Thoms, S., Brenneis, T., Heiden, J. P., Beszteri, S., and Bischof, K.: Two Southern Ocean diatoms are more sensitive to ocean acidification and changes in irradiance than the prymnesiophyte *Phaeocystis antarctica*, Physiologia Plantarum, 160, 155–170, https://doi.org/10.1111/ppl.12539, 2017.

Wang, Y., Zhang, R., Zheng, Q., Deng, Y., Van Nostrand, J. D., Zhou, J., and Jiao, N.: Bacterioplankton community resilience to ocean acidification: evidence from microbial network analysis, ICES Journal of Marine Science: Journal du Conseil, 73, 865–875, https://doi.org/10.1093/icesjms/fsv187, 2016.

Westwood, K. J., Thomson, P. G., van den Enden, R. L., Maher, L. E., Wright, S. W., and Davidson, A. T.: Ocean acidification impacts primary and bacterial production in Antarctic coastal waters during austral summer, Journal of Experimental Marine Biology and Ecology, 498, 46–60, https://doi.org/10.1016/j.jembe.2017.11.003, 2018.

Wickham, H.: ggplot2: Elegant Graphics for Data Analysis, Springer-Verlag New York, https://ggplot2.tidyverse.org, 2016.

Wood, S. N.: Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models, Journal of the Royal Statistical Society (B), 73, 3–36, 2011.

Wright, S. W., Ishikawa, A., Marchant, H. J., Davidson, A. T., van den Enden, R. L., and Nash, G. V.: Composition and significance of picophytoplankton in Antarctic waters, Polar Biology, 32, 797–808, https://doi.org/10.1007/s00300-009-0582-9, 2009.

Young, J., Kranz, S., Goldman, J., Tortell, P., and Morel, F.: Antarctic phytoplankton down-regulate their carbon-concentrating mechanisms under high CO_2 with no change in growth rates, Marine Ecology Progress Series, 532, 13–28, https://doi.org/10.3354/meps11336, 2015.

Zhang, R., Xia, X., Lau, S. C. K., Motegi, C., Weinbauer, M. G., and Jiao, N.: Response of bacterioplankton community structure to an artificial gradient of pCO_2 in the Arctic Ocean, Biogeosciences, 10, 3679–3689, https://doi.org/10.5194/bg-10-3679-2013, 2013.

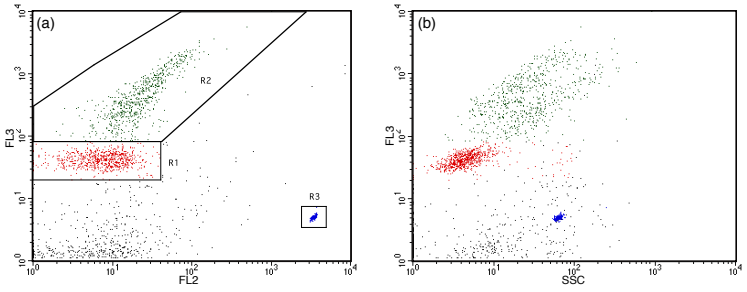

Figure 1. Nano- and picophytoplankton regions identified by flow cytometry. (a) Two separate regions identified based on red (FL3) versus orange (FL2) fluorescence scatter plot. (b) Picophytoplankton (R1) and nanophytoplankton (R2) communities determined from side scatter (SSC) versus FL3 fluorescence scatter plot. PeakFlow Green 2.5 μm beads (R3) used as fluorescence and size standard.

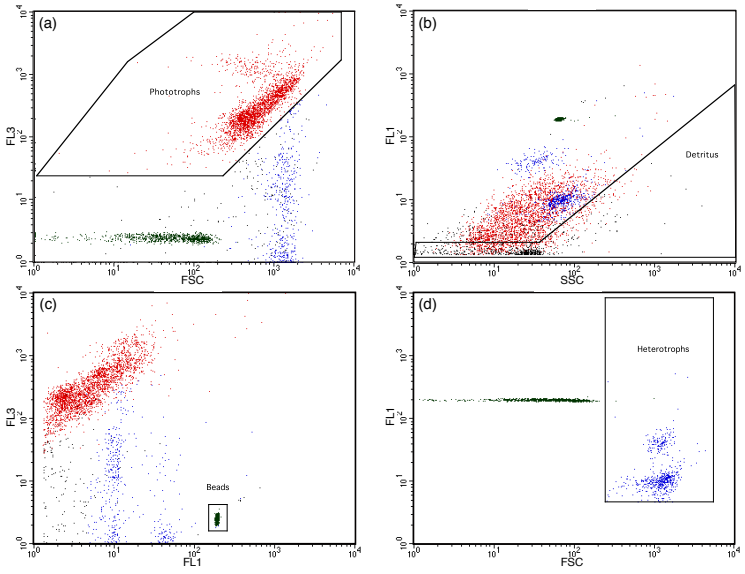

Figure 2. LysoTracker Green-stained heterotrophic nanoflagellates identified by flow cytometry. (a) Phytoplankton identified based on red (FL3) versus ~~orange~~ forward scatter (~~FL2~~FSC) fluorescence scatter plots. (b) Detritus particles identified from high side scatter (SSC) versus LysoTracker Green fluorescence (FL1). (c) PeakFlow Green 2.5 µm beads identified from high FL1 versus ~~low red (~~FL3 ~~)~~ fluorescence. (d) Phytoplankton and detritus from (a) and (b) removed from FL1 and ~~forward scatter (~~FSC ~~)~~ plot and remaining LysoTracker Green-stained particles >2.5 µm were counted as heterotrophic nanoflagellates.

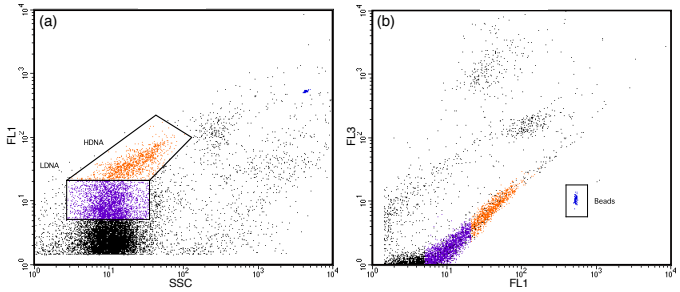

Figure 3. Prokaryote regions identified by flow cytometry. (a) SYBR-Green I-stained high DNA (HDNA) and low DNA (LDNA) prokaryote regions identified from side scatter (SSC) versus green fluorescence (FL1) scatter plots. (b) Prokaryote cells determined from high FL1 versus low red (FL3) fluorescence. PeakFlow Green 2.5 μm beads used as fluorescence and size standard.

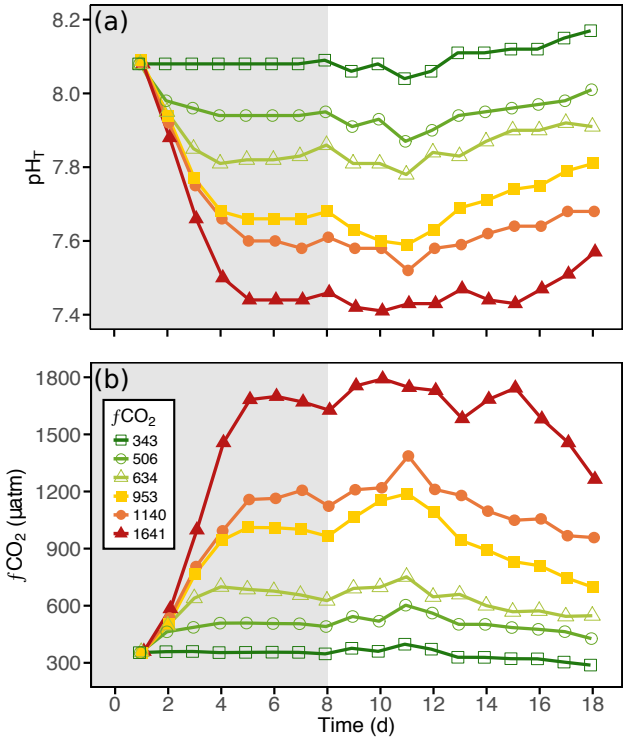

Figure 4. The (a) pH on the total scale (pH_T) and (b) fugacity of CO_2 (fCO_2) carbonate chemistry conditions in each of the minicosm treatments over time. Grey shading indicates CO_2 and light acclimation period.

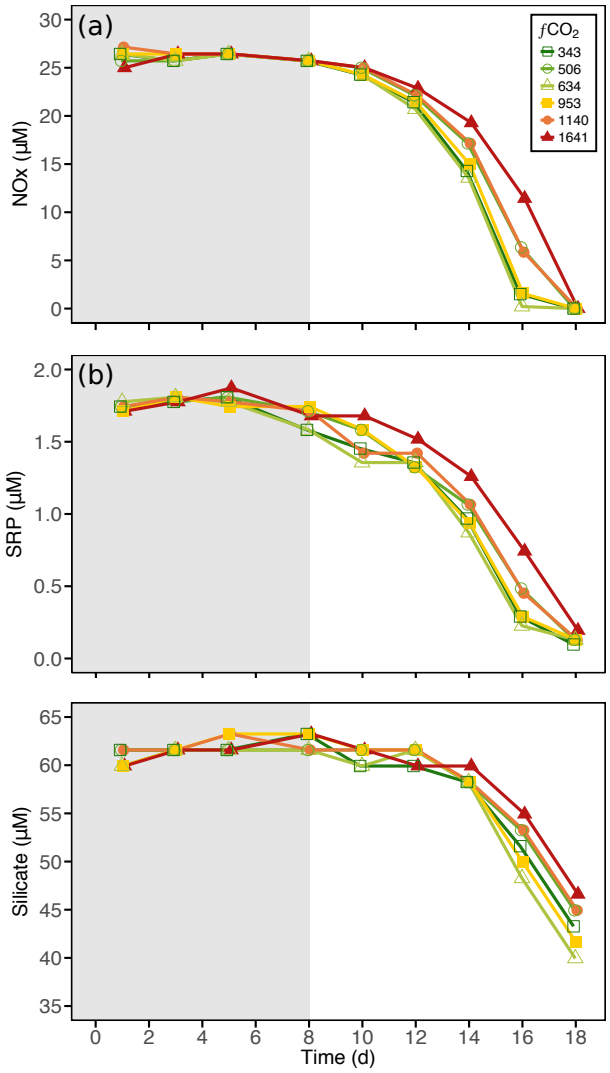

Figure 5. Nutrient concentration in each of the minicosm treatments over time. (a) Nitrate + nitrite (NOx), (b) soluble reactive phosphorus (SRP), and (c) molybdate reactive silica (Silicate). Grey shading indicates CO_2 and light acclimation period.

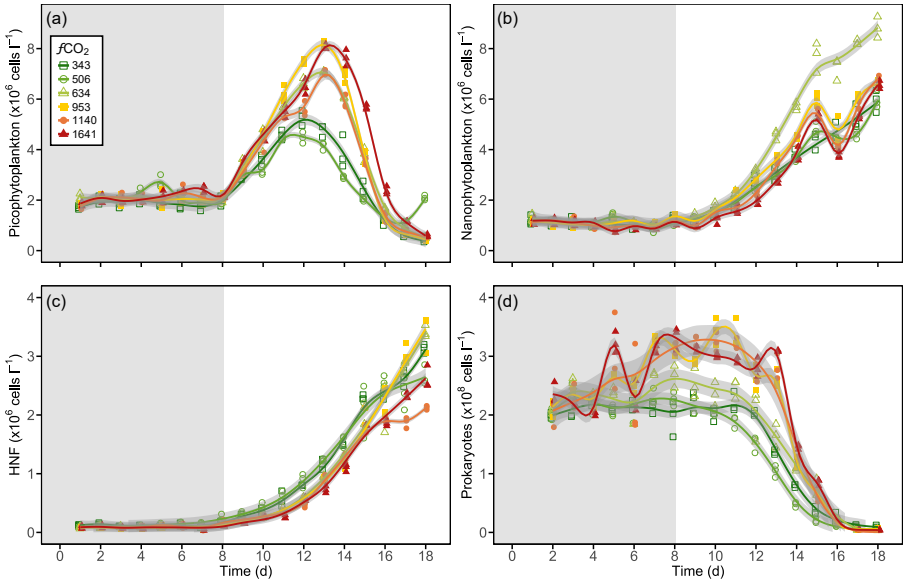

Figure 6. ~~Abundance of~~ Generalized additive model fits for (a) picophytoplankton, (b) nanophytoplankton, (c) heterotrophic nanoflagellates (HNF), and (d) prokaryotes in each of the minicosm treatments over time. ~~Error bars display standard error of pseudoreplicate samples~~Shading above and below fitted lines (grey) displays 95% confidence interval for model predictions. Grey shading on plot background indicates CO_2 and light acclimation period.

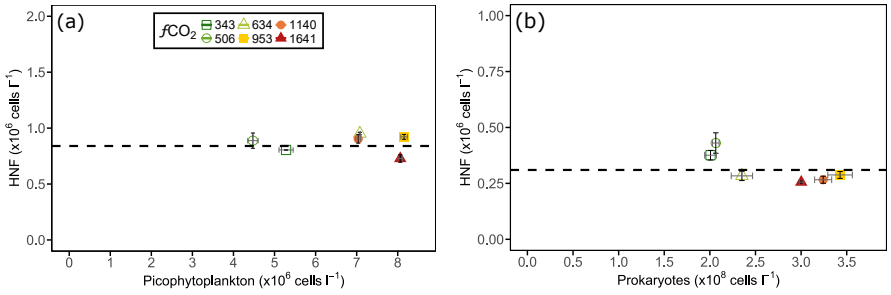

Figure 7. ~~Peak abundances of~~ Heterotrophic nanoflagellate (HNF) abundance (y-axis) on the day before (a) picophytoplankton and (b) ~~prokryotes~~ prokaryote abundance declined (shown on x-axis) in each of the minicosm treatments. ~~Letters indicate significantly different groupings assigned by post-hoc Tukey test.~~ Error bars display standard error of pseudoreplicate samples of HNFs (grey) and picophytoplankton/prokaryotes (black). Dotted line indicates threshold heterotrophic nanoflagellate abundance of (a) 0.87 $\pm$ 0.02 x10^6 cells l^{-1} and (b) 0.32 $\pm$ 0.02 x10^6 cells l^{-1}.

Table 1. Mean carbonate chemistry conditions in minicosms

Tank	fCO_2 (µatm)	pH_T	DIC (µmol kg^{-1})	PA (µmol kg^{-1})
1	343 ± 30	8.10 ± 0.04	2188 ± 6	2324 ± 11
2	506 ± 43	7.94 ± 0.03	2243 ± 8	2325 ± 10
3	634 ± 63	7.85 ± 0.04	2270 ± 5	2325 ± 12
4	953 ± 148	7.69 ± 0.07	2314 ± 11	2321 ± 11
5	1140 ± 112	7.61 ± 0.04	2337 ± 5	2320 ± 10
6	1641 ± 140	7.45 ± 0.04	2377 ± 8	2312 ± 10

Data are mean ± one standard deviation of triplicate pseudoreplicate measurements, fCO_2; fugacity of CO_2, pH_T; pH on the total scale, DIC; dissolved inorganic carbon, PA; practical alkalinity

~~Comparison of (a) picophytoplankton (day 13) and (b) prokaryote (day 8) steady-state growth rates against heterotrophic nanoflagellate abundance. Error bars display standard error of pseudoreplicate samples of heterotrophic nanoflagellates. Dotted line indicates linear regression trend (Data in Table S6, S7).~~

~~Heterotrophic nanoflagellate abundance on the day before (a) picophytoplankton and (b) prokaryote abundance declined in each of the minicosm treatments. Error bars display standard error of pseudoreplicate samples of heterotrophic nanoflagellates (grey) and picophytoplankton/prokaryotes (black). Dotted line indicates threshold of heterotrophic nanoflagellate abundance of (a) 0.84 ± 0.02 x10^6 and (b) 0.31 ± 0.02 x10^6 .~~

Table 2. ~~ANOVA results comparing trends~~ Steady-state logarithmic growth rates in ~~each~~ fCO_2 ~~treatment over time against the control~~treatments

	Days	~~Adjusted~~ R^2 343 µatm	~~Day:~~506 ~~p-value~~ µatm	~~Day:~~634 ~~p-value~~ µatm	~~Day:~~953 ~~p-value~~ µatm	~~Day:~~1140 ~~p-value~~ µatm	~~Day:~~1641 ~~p-value~~ µatm
Pico	~~$F_{12,182}$ = 74.6~~ 8-12	~~0.82~~ 0.25	~~0.38~~ 0.21	~~0.80~~ 0.29	~~0.57~~ **0.32**	~~0.76~~ 0.25	~~0.08~~ 0.26
Nano	~~$F_{12,311}$ = 478.8~~ 9-15	~~0.95~~ 0.23	~~0.47~~ 0.22	**~~<0.01~~0.30**	**~~0.01~~0.27**	~~0.10~~ **0.28**	~~0.78~~ **0.29**
HNF	~~$F_{12,307}$ = 634.3~~ 8-15	~~0.96~~ 0.36	~~0.15~~ **0.32**	~~0.88~~ 0.37	~~0.99~~ 0.37	**~~<0.01~~** 0.34	**~~<0.01~~0.40**
Prok	~~$F_{12,256}$ = 131.5~~ 4-8	~~0.85~~ 0.02	~~0.39~~ 0.02	~~0.49~~ 0.04	**~~<0.05~~0.09**	**~~0.04~~0.08**	~~0.08~~ **0.09**

Bold text denotes treatments with trends in steady-state logarithmic growth significantly different to the control (343 µatm, $p < 0.05$), shown in Table 3. Days; days from which the linear regression for growth rates was modelled, shown in Fig. S2, Pico; picophytoplankton, Nano; nanophytoplankton, HNF; heterotrophic nanoflagellates, Prok; prokaryotes.

Table 3. ANOVA results comparing trends in steady-state logarithmic growth for each fCO_2 treatment over time against the control

	F	Adjusted R^2	506 p-value	634 p-value	953 p-value	1140 p-value	1641 p-value
Pico	$F_{11,81}$ $_{11,78}$ = 144.7 113.8	0.95 0.94	0.71 0.17	0.12 0.13	**<0.01**	0.48 0.87	0.98 0.47
Nano	$F_{11,132}$ $_{11,114}$ = 611.1 552.6	0.98	0.34 0.45	**<0.01**	0.29 **<0.01**	**<0.05 0.01**	**< 0.01**
HNF	$F_{11,131}$ = 518.6	0.98	**0.02**	0.30	0.32	0.39	**0.02**
Prok	$F_{11,113}$ $_{11,77}$ = 12.94 9.334	0.51	0.52 0.21	0.17 0.06	**<0.01**	**<0.01**	**<0.01**

Bold text denotes significant p-values (<0.05). Pico; picophytoplankton, Nano; nanophytoplankton, HNF; heterotrophic nanoflagellates, Prok; prokaryotes.

Steady-state logarithmic growth rates in treatments 343 506 634 953 1140 1641 Pico 0.25 0.26 0.29 **0.32** 0.23 0.25 Nano 0.26 0.25 **0.32** 0.27 **0.28** **0.29** HNF 0.36 **0.32** 0.38 0.37 0.34 **0.40** Prok 0.00 0.01 0.02 **0.07** **0.07** **0.07**