Responses to reviewers' comments

Anonymous Referee #2

The present paper adds to the growing body of literature that explores the effects on changing environmental drivers on marine phytoplankton.

I appreciate the effort that was taken in the experimental design, particularly the prolonged acclimation phases for each treatment and the gradient design to retrieve functional response curves to each driver. Obviously, the experimental design was flawless and all analysis have been conducted appropriately. Generally, I also appreciate the usefulness of culture experiments, despite their limitations, as I think a lot can be learned about physiological mechanisms that are relevant for the "bigger picture".

However, regarding the present paper, I am not quite sure what the main conclusions and the "new lessons learned" actually are, and what the paper should be highly cited for. Certainly, the study provides some nice physiological information on two species, which might certainly be of some use e.g. to compare to other culture experiments.

The main message seems to be that the diatom *Pseudo-nitzschia subcurvata* might be favored under global warming relative to *Phaeocystis antarctica*, due to its higher optimum temperature and thermal tolerance. However, the fact that different species and/or functional groups have different thermal response curves is not new. It is quite obvious that this might lead to some degree of reorganization of community structure or spatial shifts of species distributions. However, I don't see such new insights from the present study. I would have loved to read about possible physiological mechanisms behind the observed responses, for instance, the interactive effects of temperature and CO2 on *P. subcurvata*. There are several recent studies that discussed responses to multiple stressors in much more detail (e.g. Brennan & Collins 2015).

Response to Anonymous Referee #2

Thanks for the comments. We agree that the effects of temperature and CO_2 have been examined on certain phytoplankton. Our research aimed to add to this body of literature by testing how interactions between these two global change factors may affect the composition of two dominant phytoplankton groups in the Ross Sea, Antarctica, with the intention of shedding light on potential climate change-driven changes in this pristine polar food web and the biogeochemical cycles of C.N. P and Si in the Ross Sea. Thus, while we agree that determining mechanistic physiological drivers for these observed responses is something that should be done,

for this Biogeosciences paper our emphasis is on documenting net effects on ecology biogeochemistry.

We do appreciate the suggestion to consider the possible mechanisms of interactive effects of these two variables on our two model species. New discussion and references on general interactive effects of temperature and CO_2 on the enzyme efficiencies, protein translation, and growth of phytoplankton have been added on lines 438-446.

Furthermore, the authors state that CO2 effects on elemental composition were negligible, and mention several other studies that observed differential effects. However, no possible explanations for these contrasting results, e.g. based on physiology or differences in experimental setup, is provided.

We cite literature and discuss this subject on lines 447 – 477. As the reviewer noted, there have been contrasting results observed in various studies of CO_2 effects on phytoplankton elemental composition. We added some explanation on lines 459– 477 that the effects of CO_2 on elemental ratios might be related to changes in biochemical composition, or to species-specific and experimental design effects.

One critical aspect that I'm missing in particular is the role of nutrient status. Responses to temperature and CO2 have been tested in semi-continuous cultures with permanent nutrient replete conditions in this study. The authors state that CO2 effects were negligible, which is indeed in agreement with earlier studies that were conducted under nutrient-saturated conditions. However, a number of recent studies have demonstrated, that physiological responses to CO2, as well as temperature, tend to be much stronger under nutrient-limited conditions (e.g. *Sala et al., 2015*) or in the transition from exponential to stationary growth (e.g. *Taucher et al., 2015*). This is particularly true for elemental ratios, which are also prominently discussed in the present paper.

Therefore, I wonder how relevant and representative the results of the present study might be? What do the authors think, how might the response have looked like under more realistic nutrient conditions, e.g. a transition to nutrient depletion? Furthermore, how relevant is *P. subcurvata* in the study region at all (in terms of biomass)? And what about other important diatom species in the study region? Without discussing such aspects, I find it hard to justify larger- scale

extrapolations to phytoplankton community structure or even biogeochemical cycles, as done by the authors.

We intentionally did the experiment with replete nutrients, because the Ross Sea is typically an HNLC area, with plenty of macronutrients throughout much of the growing season. This HNLC situation occurs throughout all of the spring and most of the summer in McMurdo Sound where these cultures were isolated, however by late fall nutrients have usually been depleted to fairly low levels. The culture medium was based on real Ross Sea water with additional nutrients added, so we think that our experiments are relevant relative to the dominant nutrient-replete condition. Our experiments should thus be representative of most of the growing season in this area, and we have added a new paragraph and a reference to support this on lines 363-366. We agree experiments with nutrient-limited phytoplankton would also be interesting from a physiological point of view, but they may arguably be less ecologically relevant for most of the season in this particular regime. We added text to say this, as well as the Sala et al and Taucher et al references on lines 366-371.

Although we don't have an estimate of the exact relative abundance of P. subcurvata in Ross Sea phytoplankton communities, it is one of the commonly encountered species there. For instance, it was found to be dominant in Ross Sea incubations published by Tortell et al. (2008), and in our own experimental warming incubations conducted in the Ross Sea (currently being prepared for publication).

In fact, the tendency to extrapolate the findings from the culture experiments to large-scale biogeochemical cycling (e.g. export flux) seems rather far-fetched. What about possible food-web effects resulting from a transition from *Phaeocystis* to diatoms? Particularly with regard to predictions on future export, it seems odd that the discussion goes straight from physiological responses (under artificial constant exponential growth conditions) to predictions on future export, without mentioning possible shifts in food web structure. For instance, how might the grazer community respond to a shift from *Phaeocystis* to diatoms? And how might this in turn influence export patterns?

If our warming experiments prove to be predictive, we think there may indeed be large biogeochemical changes as diatoms like this one replace the less warm-adapted Phaeocystis. As we note in the text, the influences of these two phytoplankton groups on biogeochemical cycling of carbon and nutrients are quite different. However, we do agree with the reviewer that we should have done a better job of considering food web effects as well. New discussion about this point has been added on lines 398-401.

Besides, I generally agree with the other reviewers that the writing style of the paper is rather tiresome. The results section reads very generic and large parts of the discussion are somewhat repetitive as they just state the same as already said in the results. I think a more focused and in-depth discussion combined with a more appealing writing style would make the paper a lot better.

Thanks for the comments, we have tried to edit the manuscript to make it easier to read, and have added next text on points of interest raised by the reviewers (see below).

Responses to the specific comments:

- The 8°C treatment is a rather unrealistic scenario. Of course, it is desirable to observe an effect in such experiments, but I wonder about the environmental relevance of this treatment, as such temperature cannot be expected for the near future.

Just as the reviewer commented, we aimed to observe an effect in this experiment, and we agree that the temperature of the Ross Sea won't increase to 8°C any time soon. However, the large temperature increase may provide information about the responses of these two groups of phytoplankton to warming that cannot be easily detected over smaller scales of temperature increase. The use of this high temperature also led us to an interesting observation, which is one of our major points in the Discussion: the fact that the growth rates of P. subcurvata increased as temperature increased up until 8°C means that this species is currently growing well below its optimum temperature. In contrast, growth rates of P. antarctica decreased quickly above 8C, and are instead closer to optimal at current (and near-future) Ross Sea temperatures. The diatom thus seems to be 'pre-adapted' to outcompete the prymnesiophyte in a warmer Ross Sea. We re-emphasized this with new text on p. 336-343.

- Q10 values for growth of 2.11 and 3.17 seem rather high. Usually, values of 1-2 have been reported for autotrophic processes. It might we worthwhile to embed the presented findings with earlier studies on temperature responses.

It has been known for some time that Q10 values for polar marine organisms are typically much higher than the canonical value of 2, with rates often doubling with a temperature increase of 4C or less (Clarke, A. 1983. Life in cold water: the physiological ecology of polar marine ectotherms. Oceanogr. Mar Biol. Annu. Rev. 21, 341–453). We added this reference and some

discussion on lines 328-330. Even in temperate environments, there are higher reported Q10 values for phytoplankton, For instance, Zhu et al. (2017) observed that the Q10 value of Pseudo-nitzschia australis was 3.3, and Tadonléké et al. (2010) observed that the Q10 value of photosynthetic rates of phytoplankton in a lake could be 2.41.

- Elemental ratios at different temperatures might be difficult to interpret, as the cultures experienced differences in length of growth period (i.e. number of cell divisions) and nutrient uptake, with differences in left- over nutrients at the end of the incubations. Thus, they might not be in the same physiological state

We agree that the two phytoplankton species would have a different number of cell divisions at different temperatures. However, we used semi-continuous culture techniques, which means that all the phytoplankton were maintained in continual exponential growth stage, and so all treatments were sampled at the same growth stage. Thus, the physiological state of the phytoplankton in the different temperatures was relatively similar. We added some text to make this point more clearly in the Methods (lines 115-177).

- Competition: Why does P. subcurvata outcompete Phaeocystis at 0°C ? According to the thermal response curves, Phaecystis should have a higher growth rate. Was there any difference in experimental conditions compared to the thermal response experiments?

It is true that P. subcurvata slightly increased its abundance relative to Phaeocystis even at 0C, although obviously this shift was much more pronounced at 6C. The experimental conditions were as identical as we could make them for the thermal response experiment and co-incubation experiment. The growth rates of the P. subcurvata and Phaeocystis cultures were not significantly different at 0C in unialgal cultures (Fig. 1), but it is certainly possible that there were other types of competitive interactions not related to temperature when these two phytoplankton were grown together. For instance, the nutrient uptake and utilization strategy of P. subcurvata could have provided it with an advantage in the co-incubation, which may be worth further research. We added some new text to discuss this on lines 336-343.

- Fig 5: It would be helpful if the scale of the y-axis would be identical in all panels.

Thanks for this helpful comment. All the panels have been changed to the same y-axis scale.

References:

Tortell, P. D., Payne, C. D., Li, Y., Trimborn, S., Rost, B., Smith, W. O., ... & DiTullio, G. R. (2008). CO2 sensitivity of Southern Ocean phytoplankton. *Geophysical Research Letters*, *35*(4).

Zhu, Z., Qu, P., Fu, F., Tennenbaum, N., Tatters, A. O., & Hutchins, D. A. (2017). Understanding the blob bloom: Warming increases toxicity and abundance of the harmful bloom diatom Pseudo-nitzschia in California coastal waters. Harmful Algae, 67, 36-43.

Tadonléké, R. D. (2010). Evidence of warming effects on phytoplankton productivity rates and their dependence on eutrophication status. Limnology and Oceanography, 55(3), 973-982.

Individual and interactive effects of warming and CO_2 on *Pseudo-nitzschia subcurvata* an *Phaeocystis antarctica*, two dominant phytoplankton from the Ross Sea, Antarctica

Zhi Zhu[1], Pingping Qu[1], Jasmine Gale[1], Feixue Fu[1], David A. Hutchins[1]

1. Department of Biological Science, University of Southern California, Los Angeles, CA 90089, USA.

Correspondence to: David A. Hutchins (dahutch@usc.edu)

Abstract: We investigated the effects of temperature and CO_2 variation on the growth and elemental composition of cultures of the diatom *Pseudo-nitzschia subcurvata* and the prymnesiophyte *Phaeocystis antarctica*, two ecologically dominant phytoplankton species isolated from the Ross Sea, Antarctica. To obtain thermal functional response curves, cultures were grown across a range of temperatures from 0°C to 14°C. In addition, a co-culturing experiment examined the relative abundance of both species at 0°C and 6°C. CO_2 functional response curves were conducted from 100 to 1730 ppm at 2°C and 8°C to test for interactive effects between the two variables. The growth of both phytoplankton was significantly affecte by temperature increase, but with different trends. Growth rates of *P. subcurvata* increased wit temperature from 0°C to maximum levels at 8°C, while the growth rates of *P. antarctica* only increased from 0°C to 2°C. The maximum thermal limits of *P. subcurvata* and *P. antarctica* where growth stopped completely were 14°C and 10°C, respectively. Although *P. subcurvata* outgrew *P. antarctica* at both temperatures in the co-incubation experiment, this happened mu faster at 6°C than at 0°C. For *P. subcurvata*, there was a significant interactive effect in which the warmer temperature decreased the CO_2 half saturation constant for growth, but this was no the case for *P. antarctica*. The growth rates of both species increased with CO_2 increases up to 425 ppm, and in contrast to significant effects of temperature, the effects of CO_2 increase on th

1 Introduction

Global temperature is predicted to increase 2.6°C to 4.8°C by 2100 with increasing anthropogenic CO_2 emissions (IPCC, 2014). The temperature of the Southern Ocean has increased even faster than global average temperature (Meredith and King, 2005), and predicte future climate warming may profoundly change the ocean carbon cycle in this region (Sarmien et al., 1998). The Ross Sea, Antarctica, is one of the most productive area in the ocean, and features annual austral spring and summer algal blooms dominated by *Phaeocystis* and diatom that contribute as much as 30% of total primary production in the Southern Ocean (Arrigo et al 1999, 2008; Smith et al., 2000, 2014a). The responses of phytoplankton in the Ross Sea to futu temperature change (Rose et al., 2009; Xu et al., 2014; Zhu et al., 2016) in combination with intensified stratification (Sarmiento et al., 1998) could lead to intensified future diatom blooms (Smith et al. 2014b), and the physiological effects of warming may partially compensate for a lack of iron throughout much of this region (Hutchins and Boyd, 2016).

In the Ross Sea, the colonial prymnesiophyte *Phaeocystis antarctica* typically blooms i austral spring and early summer, and diatoms including *Pseudo-nitzschia subcurvata* and *Chaetoceros* spp. bloom later in the austral summer (Arrigo et al., 1999, 2000; DiTullio and Smith, 1996; Goffart et al., 2000; Rose et al., 2009). Both diatoms and *P. antarctica* play an important role in anthropogenic CO_2 drawdown and the global carbon cycle; additionally, they contribute significantly to the global silicon and sulfur cycles, respectively (Arrigo et al., 1999 Tréguer et al., 1995; Schoemann et al., 2005). Furthermore, the N: P and C: P ratios of *P. antarctica* are higher than those of diatoms, and thus they contribute unequally to the carbon, nitrogen, and phosphorus cycles (Arrigo et al., 1999, 2000). Diatoms are preferred by many planktonic herbivores over *P. antarctica*, and so the two groups also differentially influence th

dominant groups of phytoplankton. Smith and Jones (2015) presented evidence for the importance of deep mixing and the critical depth for the timing of transitions from *P. antarctic* to diatom blooms. Zhu et al. (2016) observed that a 4ºC temperature increase promoted the growth rates of several dominant diatoms isolated from Ross Sea, including *P. subcurvata*, *Chaetoceros* sp., and *Fragilariopsis cylindrus*, but not the growth rates of *P. antarctica*. In addition, both field and laboratory research has suggested that temperature increase and iron addition can synergistically promote the growth of Ross Sea diatoms (Rose et al., 2009; Zhu et al., 2016; Hutchins and Boyd, 2016). Thus, it is possible that phytoplankton community structu in this region may change in the future under a global warming scenario.

In addition to temperature increases, ocean uptake of 30% of total emitted anthropogen CO_2 has led to a 0.1 pH unit decrease in surface water, corresponding to a 26% increase in acidity (IPCC, 2014). The global CO_2 concentration is predicted to increase to around 800 ppm by 2100, which will lead to a further decrease in surface seawater pH of 0.3–0.4 units (Orr et a 2005; IPCC, 2014). CO_2 increases have been found to promote the growth and affect the physiology of many but not all phytoplankton species tested (Fu et al., 2007, 2008; King et al., 2011; Xu et al., 2014; Hutchins and Fu 2017).

Research on the effects of CO_2 increases on *Phaeocystis antarctica* and Antarctic diato is still scarce. Xu et al. (2014) suggested that future conditions (higher temperature, CO_2, and irradiance) may shift phytoplankton community structure towards diatoms and away from *P. antarctica* in the Ross Sea. Trimborn et al. (2013) discovered that the growth rates of *P. antarctica* and *P. subcurvata* were not significantly promoted by high CO_2 relative to ambient CO_2 at 3°C. In contrast, Wang et al. (2010) observed that the growth rates of the closely relate temperate colonial species *Phaeocystis globosa* increased significantly at 750 ppm CO_2 relativ to 380 ppm CO_2.

and warming on the growth of phytoplankton from the Ross Sea (Rose et al., 2009; Zhu et al., 2016; Hutchins and Boyd 2016). Thus, an important goal of phytoplankton research is to also gain an understanding of how global warming together with ocean acidification may shift the phytoplankton community in the Ross Sea (Arrigo et al., 1999; DiTullio et al., 2000). This stud aimed to explore the effects of increases in temperature and CO_2 availability, both individually and in combination, on *P. antarctica* and *P. subcurvata* isolated from the Ross Sea, Antarctica. These results may shed light on the potential effects of global change on the marine ecosystem and the cycles of carbon and nutrients in the highly productive coastal polynyas of Antarctica.

2 Materials and Methods

2.1 Strains and growth conditions

P. subcurvata and *P. antarctica* were isolated from the ice edge in McMurdo Sound (77.62° S, 165.47° E) in the Ross Sea, Antarctica during January 2015; *P. antarctica* cultures grew as small colonies (~4-12 cells) in all the experiments. All stock cultures were grown in Aquil* medium (100 µmol L^{-1} NO_3^-, 100 µmol L^{-1} SiO_4^{4-},10 µmol L^{-1} PO_4^{3-}) made with 0.2 µM filtered seawater that was collected from the same Ross Sea locale as the culture isolates (Sund et al., 2005). Stock and experimental cultures were grown in Fe-replete Aquil medium (0.5 µM Although phytoplankton in the open Ross Sea polynya are generally proximately iron-limited (Ryan-Keogh et al. 2017), these culture conditions are relevant to the coastal McMurdo Sound ice edge environment in the early spring when Fe is relatively abundant, and typically not limiting. This 'winter reserve' iron is then drawn down in this nearshore environment over the course of the seasonal algal bloom to eventually reach limiting levels (Sedwick et al., 2011; Bertrand et al., 2015). Our experiments address warming and acidification responses in *P. subcurvata* and *P. antarctica* in the absence of any differential effects of Fe availability;

For thermal functional response curves, experimental cultures of both phytoplankton were grown in triplicate 500 ml acid washed polycarbonate bottles and gradually acclimated by series of step-wise transfers to a range of temperatures, including 0°C, 2°C, 4°C, 6°C, 8°C, and 10°C (*P. antarctica* died at 10°C) under the same light cycle as stock cultures. Cultures were diluted semi-continuously following Zhu et al. (2016), allowing them to be maintained in continuous exponential growth and so facilitating comparisons between treatments in the same physiological growth stage. All of the cultures were acclimated to their respective temperatures for 8 weeks before the commencement of the experiment. At this point, after the growth rates were verified to be stable for at least three to five consecutive transfers, the cultures were sampled 48 h after dilution (Zhu et al., 2016).

For CO_2 functional response curves, *P. antarctica* and *P. subcurvata* were also grown i triplicate in a series of six CO_2 concentrations from ~100 ppm to ~1730 ppm in triplicate 500 m acid washed polycarbonate bottles at both 2°C and 8°C using same dilution technique as above The CO_2 concentration was achieved by gently bubbling with 0.2 μm filtered air/CO_2 mixture (Gilmore, CA) and carbonate system equilibration was ensured by pH and dissolved inorganic carbon (DIC) measurements (King et al., 2015, see below).

An additional experiment tested whether temperature-related trends in growth rates observed in monocultures were maintained when both species were grown together in a simple model community. For this examination of thermal effects on the growth of *P. antarctica* and *subcurvata* in co-culture (pre-acclimated to respective temperatures), the isolates were mixed a equal Chl *a* (chlorophyll *a*) concentrations and grown together for 6 days in triplicate bottles at both 0°C and 6°C. These temperatures chosen to span the optimum growth ranges of both species (see Results, below). The relative abundance of each phytoplankton was then calculate

immediately were preserved with Lugol's (final concentration 2%) and stored at 4°C until counting. Specific growth rates (d^{-1}) were calculated following Eq. (1):

$$\mu = (\ln N_1 - \ln N_0)/t, \quad (1)$$

where N_0 and N_1 are the cell density at the beginning and end of a dilution period, respectively, and t is the duration of the dilution period (Zhu et al. 2016). The Q_{10} of growth rates was calculated following Chaui-Berlinck et al. (2002) as Eq. (2):

$$Q_{10} = (\mu_2/ \mu_1)^{10/(T_2 - T_1)}, \quad (2)$$

where μ_1 and μ_2 are the specific growth rates of the phytoplankton at temperatures T_1 and T_2, respectively. The growth rates were fitted to Eq. (3) to estimate the thermal reaction norms of each species:

$$f(T) = ae^{bT}(1- ((T-z)/(w/2))^2), \quad (3)$$

where specific growth rate *f* depends on temperature (T), temperature niche width (*w*), and oth empirical parameters *z*, *a*, and *b* were estimated by maximum likelihood (Thomas et al., 2012; Boyd et al., 2013). Afterwards, the optimum temperature for growth and maximum growth rat were estimated by numerically maximizing the equation (Boyd et al., 2013). The growth rates all the species at all the CO_2 levels were fitted to Michaelis-Menten equation as Eq. (4):

$$\mu = \mu_{max} S/(K_m + S), \quad (4)$$

to estimate maximum growth rates (μ_{max}) and half saturation constants (K_m) for CO_2 concentration (S). In the CO_2 curve experiments growth rates for both these autotrophic specie were assumed to be zero at 0 ppm CO_2, and in the thermal curve experiments growth rates wer assumed to be zero at -2°C, approximately the freezing point of seawater.

2.4 Elemental and Chl *a* analysis

Culture samples for particulate organic carbon/nitrogen (POC/PON) and particulate organic phosphorus (POP) analyses were filtered onto pre-combusted (500°C for 2 h) GF/F

followed Fu et al. (2007), and BSi analysis followed Paasche et al. (1973). An aliquot of 30 to ml from each treatment replicate was filtered onto GF/F filters and extracted with 90% acetone -20°C for 24 h for Chl *a* analysis. The Chl *a* concentration was then determined using the non- acidification method on a 10-AU^{TM} fluorometer (Turner Design, CA) (Fu et al., 2007).

2.5 pH and dissolved inorganic carbon (DIC) measurements

pH was measured using a pH meter (Thermo Scientific, MA), calibrated with pH 7 and 10 buffer solutions. For DIC analyses, an aliquot of 25 mL was preserved with 200 µL 5% HgC and stored in the dark at 4^{o}C until analysis. Total DIC was measured using a CM140 Total Inorganic Carbon Analyzer (UIC Inc., IL). An aliquot of 5 mL sample was injected into the sparging column of Acidification Unit CM5230 (UIC Inc., IL) followed by 2 ml 10% phospho acid. By using flow rates controlled pure nitrogen as carrier gas, and the CO_2 released from the DIC pool in the sample was quantified with a CM5015 CO_2 Coulometer (UIC Inc., IL) using absolute coulometric titration. The carbonate buffer system was sampled for each of the triplic bottles in each treatment at the beginning and end of the experiments; reported values are final ones. The pCO_2 in growth media was calculated using CO2SYS (Pierrot et al., 2006). These carbonate system measurements are shown in Table 1, along with the corresponding calculated pCO_2 values calculated. Kinetic parameters were calculated using the individual calculated pC values for each replicate (see above), but for convenience, the CO_2 treatments are referred to in the text using the mean value of all experimental bottles, rounded to the nearest 5 ppm: these values are 100 ppm, 205 ppm, 260 ppm, 425 ppm, 755 ppm, and 1730 ppm.

2.6 Statistical analysis

All statistical analyses and model fitting, including student t-tests, ANOVA, Tukey's HSD test, two-way ANOVA, and thermal reaction norms estimation were conducted using the open source statistical software R version 2.1.2 (R Foundation)

Temperature increase significantly affected the growth rates of both *P. antarctica* and *P. subcurvata*, but with different trends ($p < 0.05$) (Fig. 1). The specific growth rates of *P. subcurvata* increased from 0°C to 8°C ($p < 0.05$), and then significantly decreased at 10°C ($p < 0.05$) (Fig. 1). The growth rates of *P. antarctica* significantly increased from 0°C to 2°C, and plateaued at 4°C and 6°C, and then significantly decreased from 6°C to 8°C ($p < 0.05$) (Fig. 1). *P. antarctica* and *P. subcurvata* stopped growing at 10°C and 14°C, respectively (Fig. 1A). The specific growth rates of *P. subcurvata* were not significantly different from those of *P. antarctica* at 0°C, 2°C and 4°C, but became significantly higher than *P. antarctica* at 6°C, and remained significantly higher than *P. antarctica* through 8°C and 10°C ($p < 0.05$) (Fig. 1A). The optimum temperatures for growth of *P. antarctica* and *P. subcurvata* were 4.85°C and 7.36°C, respectively, both well above the current temperature in the Ross Sea, Antarctica (Table 2). In addition, the estimated temperature niche width of *P. subcurvata* (-2°C – 12.19°C) is wider than that of *P. antarctica* (-2.0°C to 9.52°C) (Table 2); calculated minimum temperatures estimated from the thermal niche width equation were less than -2.0°, the freezing point of seawater, and growth is assumed to terminate at -2.0°. The Q10 value of the growth rate of *P. antarctica* from 0°C to 4°C is 2.11, which is lower than the Q10 values 3.17 for *P. subcurvata* over the same temperature interval ($p < 0.05$) (Table 2).

3.2 Temperature effects on elemental composition

The C: N and N: P ratios of *P. subcurvata* were unaffected by changing temperature (Fig. 2A, B), but the C: P, C: Si, and C: Chl *a* ratios of this species were significantly affected ($p < 0.05$) (Fig. 2C, D, Fig. 3). The C: P ratios of *P. subcurvata* were slightly but significantly lower in the middle of the tested temperature range. They were higher at 8°C and 10°C than at 2°C, 4°C, and 6°C ($p < 0.05$) (Fig. 2C), and also significantly higher at 10°C than at 0°C (Fig. 2C). The C: Si ratios of *P. subcurvata* showed a similar pattern of slightly lower values at mid-range

of the thermal gradient. At 0°C, 8°C and 10°C, C: Chl *a* ratios were significantly higher than a 2°C, 4°C, and 6°C ($p < 0.05$), and also significantly higher at 10°C than at 0°C and 8°C (Fig. 3

The C: N, N: P, C: P, and C: Chl *a* ratios of *P. antarctica* were not significantly differe across the temperature range (Fig. 2A, B, C, Fig. 3). The N: P ratios of *P. antarctica* were significantly higher than those of *P. subcurvata* at 2°C, 6°C, and 8°C ($p < 0.05$) (Fig. 2B). Additionally, the C: P ratios of *P. antarctica* were significantly higher than those of *P. subcurvata* at 6°C and 8°C ($p < 0.05$) (Fig. 2C), and the C: Chl *a* ratios of *P. antarctica* were significantly higher than values of *P. subcurvata* at all the temperatures tested ($p < 0.05$) (Fig.

Temperature change significantly affected the cellular carbon (C) quotas, cellular nitrogen (N) quotas, cellular phosphorus (P) quotas, cellular silica (Si) quotas, and cellular Chl quotas of *P. subcurvata* ($p < 0.05$) (Table 3). The cellular C and N quotas of *P. subcurvata* wer significantly higher at 8°C than at 0°C ($p < 0.05$) (Table 3), the cellular P quotas of *P. subcurvata* were significantly higher at 4°C than at 0°C, 2°C, and 10°C ($p < 0.05$) (Table 3), a the cellular Si quotas of *P. subcurvata* were significantly higher at 8°C than at 0°C and 2°C. S quotas were also significantly higher at 4°C and 6°C than at 0°C ($p < 0.05$) (Table 3). The extreme temperatures significantly decreased the cellular Chl *a* quotas of *P. subcurvata*, as the cellular Chl *a* quotas of this species were significantly higher at 4°C, 6°C, and 8°C than at 0°C and 10°C ($p < 0.05$) (Table 3).

Temperature change significantly affected the cellular P quotas and cellular Chl *a* quot of *P. antarctica* ($p < 0.05$), but not the cellular C and N quotas ($p > 0.05$) (Table 3). The cellul P quotas of *P. antarctica* were significantly higher at 0°C than at 8°C ($p < 0.05$) (Table 3), and the Chl *a* quotas of the prymnesiophyte were significantly lower at 8°C than at 0°C, 2°C, and 6°C ($p < 0.05$) (Table 3).

3.3 Co-incubation at two temperatures

prymnesiophyte at both temperatures by day 6, this increase was larger and happened much faster at 6°C (from 31% to 72%) relative to 0°C (from 31% to 38%) ($p < 0.05$) (Fig. 4).

3.4 CO_2 effects on specific growth rates at two temperatures

The carbonate system was relatively stable across the range of CO_2 levels during the course of the experiment (Table 1). CO_2 concentration significantly affected the growth rates o *P. subcurvata* at both temperatures (Fig. 5). The growth rates of the diatom at 2°C increased steadily with CO_2 concentration increase from 205 ppm to 425 ppm ($p < 0.05$), but were saturated at 755 ppm and 1730 ppm (Fig. 5A). Similarly, the growth rates of *P. subcurvata* at 8°C increased with CO_2 concentration increase from 205 ppm to 260 ppm ($p < 0.05$), and were saturated at 425 ppm, 755 ppm and 1730 ppm (Fig. 5B). The growth rates of the diatom at all CO_2 concentrations tested at 8°C were significantly higher than at 2°C ($p < 0.05$); for instance, the maximum growth rate of *P. subcurvata* at 8°C was 0.88 d^{-1}, significantly higher than the value of 0.60 d^{-1} at 2°C ($p < 0.0.5$) (Table 4). In addition, the pCO_2 half saturation constant (K_r of *P. subcurvata* at 8°C was 10.7 ppm, significantly lower than 66.0 ppm at 2°C ($p < 0.0.5$) (Table 4). Thus, temperature and CO_2 concentration increase interactively increased the growtl rates of *P. subcurvata* ($p < 0.05$).

CO_2 concentration also significantly affected the growth rates of *P. antarctica* at both 2°C and 8°C. The growth rates of the prymnesiophyte at both 2°C and 8°C increased w CO_2 concentration increase from 100 ppm to 260 ppm ($p < 0.05$), and were saturated at 425 pp and 755 ppm (Fig. 5C, D). The growth rates of *P. antarctica* at 2°C decreased slightly at 1730 ppm relative to 425 ppm and 755 ppm ($p < 0.05$) (Fig. 5C). The maximum growth rate of *P. antarctica* at 8°C was 0.43 d^{-1}, significantly lower than the value of 0.61 d^{-1} at 2°C ($p < 0.05$) (Table 4). The pCO_2 half saturation constants of *P. antarctica* at 2°C and 8°C were not

CO_2 concentration variation didn't affect the C: N, N: P, or C: P ratios of *P. subcurvata* either 2°C or 8°C. The C: Si ratios of *P. subcurvata* were significantly higher at 1730 ppm relative to lower pCO_2 levels, except at 755 ppm at 8°C ($p < 0.05$) (Table 5). The N: P ratios of *P. subcurvata* at 8°C were significantly higher than at 2°C at all the CO_2 levels tested except 10 ppm ($p < 0.05$) (Table 5). The C: P ratios of *P. subcurvata* at 8°C were significantly higher tha at 2°C at all the CO_2 levels tested ($p < 0.05$) (Table 5). The C: Si ratios of *P. subcurvata* at CO_2 levels lower than 755 ppm at 8°C were significantly lower than at 2°C ($p < 0.05$) (Table 5). Th higher temperature also significantly increased the C: Chl *a* ratios of *P. subcurvata* at all the C levels tested ($p < 0.05$) (Table 5). Additionally, the temperature increase and CO_2 concentratio increase interactively decreased the C: Chl *a* ratios of *P. subcurvata* ($p < 0.05$) (Table 5).

The CO_2 concentration increase did not affect the C: N, N: P, and C: P ratios of *P. antarctica* at either 2°C or 8°C. The carbon to Chl *a* ratios of *P. antarctica* were significantly higher at 1730 ppm than at all lower CO_2 concentrations at 2°C. Similarly, at 8°C the carbon to Chl *a* ratios of this species also were significantly higher at 425 ppm, 755 ppm, and 1730 ppm than at lower CO_2 concentrations ($p < 0.05$) (Table 5), and significantly higher at 1730 ppm tha at 425 ppm and 755 ppm ($p < 0.05$) (Table 5).

The warmer temperature significantly decreased the C: N ratios of *P. antarctica* at 260 ppm and 755 ppm CO_2 ($p < 0.05$) (Table 5), and C: P ratios also decreased at 100 ppm and 205 ppm($p < 0.05$) (Table 5). The C: Chl *a* ratios of *P. antarctica* at CO_2 levels higher than 205 ppm were significantly higher at 8°C relative to 2°C ($p < 0.05$) (Table 5). Temperature and CO_2 concentration increase interactively increased the C: Chl *a* ratios of *P. antarctica* ($p < 0.05$) (Table 5).

The CO_2 concentration increase didn't affect the cellular C, N, P, or Si quotas of *P. subcurvata* at 2°C, or the C quotas and N quotas at 8°C. The Si quotas of *P. subcurvata* vara

297 cellular Si quota of *P. subcurvata* at all the CO_2 levels tested except 1730 ppm ($p < 0.05$) (Tab
298 6). Additionally, warming and CO_2 concentration interactively decreased the cellular Si quotas
299 *P. subcurvata* ($p < 0.05$) (Table 6).

300 The C, N, and P quotas of *P. antarctica* were not affected by CO_2 increase at 2°C, and
301 and P quotas were not affected by CO_2 increase at 8°C, either. However, the C quota of *P.*
302 *antarctica* at 1730 ppm CO_2 was significantly higher than CO_2 levels lower than 755 ppm at 8°
303 ($p < 0.05$) (Table 6). The Chl *a* per cell of *P. antarctica* at 1730 ppm CO_2 was significantly less
304 than at lower CO_2 levels at both 2°C and 8°C ($p < 0.05$) (Table 6). For *P. antarctica*, the Chl *a*
305 per cell values at 100 ppm, 205 ppm, and 755 ppm CO_2 at 8°C were significantly lower relativ
306 to 2°C ($p < 0.05$) (Table 6). Temperature increase and CO_2 concentration increase interactively
307 increased the C and N quotas of *P. antarctica* ($p < 0.05$) (Table 6).

308 **4 Discussion**

309 As has been documented in previous work, the diatom *P. subcurvata* and the
310 prymnesiophyte *P. antarctica* responded differently to warming (Xu et al., 2014; Zhu et al.
311 2016). In the Ross Sea as elsewhere, temperature determines both phytoplankton maximum
312 growth rates (Bissinger et al., 2008) and the upper limit of growth (Smith, 1990) in a species-
313 specific manner. Thermal functional responses curves of phytoplankton typically increase in a
314 normally distributed pattern, with growth rates increasing up to the optimum temperature range
315 and then declining when temperature reaches inhibitory levels (Boyd et al., 2013; Fu et al., 201
316 Xu et al., 2014; Hutchins and Fu, 2017). Specific growth rates of *P. subcurvata* reached optim
317 levels at 8°C, demonstrating that this species grows fastest at temperatures substantially above
318 any temperatures found in the present-day Ross Sea. In contrast, growth rates of *P. antarctica*
319 saturated at 2°C. This suggests that *P. subcurvata* may be a superior competitor over *P.*
320 *antarctica* in any realistically foreseeable warming scenario.

Pa CO_2, 150 µmol photons m^{-2} s^{-1}) relative to the "current condition" (2°C, 39 Pa CO_2, and 50 µmol photons m^{-2} s^{-1}) and the "year 2060 condition" (4°C, 61 Pa CO_2, and 100 µmol photons m^{-2} s^{-1}). In our study, the Q10 value of *P. subcurvata* from 0°C to 4°C was 3.11, nearly 50% higher than the Q10 value of *P. antarctica* across the same temperature range (2.17), and simil to the Q10 values observed for different strains of these two species in Zhu et al. (2016). Such Q10 values that substantially exceed the canonical value of 2 are often observed in polar marin organisms (Clarke et al. 1983, Hutchins and Boyd 2016). Our results showed that the maximal thermal limit of *P. antarctica* was reached at 10°C, as was also observed by Buma et al. (1991) while *P. subcurvata* did not cease to grow until 14°C. Clearly, *P. subcurvata* has a superior tolerance to higher temperature compared to *P. antarctica*.

The co-incubation experiment with *P. subcurvata* and *P. antarctica* at 0°C and 6°C confirmed that the diatom retained its growth advantage at the higher temperature when growi together with *P. antarctica*. Although the growth rates of the *P. subcurvata* and *Phaeocystis* cultures were not significantly different at 0°C in unialgal cultures (Fig. 1), the diatom slightly outcompeted the prymnesiophyte even at this temperature. It is possible that there were other types of competitive interactions not related to temperature when these two phytoplankton wer grown together. For instance, the nutrient uptake and utilization strategy of *P. subcurvata* coul have provided it with an advantage in the co-incubation. However, the competitive advantage enjoyed by the diatom was clearly largest at the higher temperatures, so thermal effects on competition are still evident from this experiment. Although we do not know what role (if any) competition for resources like nutrients may have played in determining the outcome of this experiment, it did demonstrate clearly that thermal growth response trends in simple model communities are in general consistent with those seen in unialgal cultures. Xu el al. (2014)

often dominant in cooler waters in the springtime, while diatoms often dominate in summer (DiTullio and Smith, 1996; Arrigo et al., 1999; DiTullio et al., 2000; Liu and Smith, 2012).

Besides temperature, mixed layer depth and irradiance also likely play a role in the competition between diatoms and *P. antarctica* (Arrigo et al., 1999; Arrigo et al., 2010, Smith and Jones 2015). Arrigo et al. (1999) observed that *P. antarctica* dominated the southern Ross Sea region with deeper mixed layers, while diatom dominated the regions with shallower mixe layer depths. The niches of these two groups of phytoplankton are difficult to define by either light or by temperature, since shallow surface stratification tends to promote both solar heating and high irradiance, while deep mixing often lowers both light and temperatures. It is worth considering whether these two phytoplankton groups are each best adapted to a different environmental matrix of both variables. This concept of different light/temperature niches for Ross Sea diatoms and *P. antarctica* is worthy of further investigation.

Our experiments used nutrient-replete conditions, which are relevant to most of the Ros Sea HNLC region throughout most of the growing season. However, major nutrients sometim become depleted late in the season on McMurdo Sound, the origin of our culture isolates, as Fe inputs are somewhat higher in these nearshore waters (Bertrand et al. 2015). Experiments usin nutrient-limited phytoplankton frequently find differing responses to CO_2 and temperature compared to those of nutrient-replete cells, including sometimes enhanced effects of these glob change factors on elemental ratios (Taucher et al. 2015, Sala et al. 2016). Our experiments und high nutrient, Fe-replete conditions thus are likely to best predict possible biological effects of future high CO_2 and temperature during the first half or more of the Ross Sea growing season.

Temperature change affected the C: P, N: P and C: Si ratios of *P. subcurvata*, due to th combined effects of the different responses of cellular C, P, and Si quotas. The C: P and N:P

temperatures have been documented in other studies as well (Xu et al., 2014; Boyd et al., 2015 Hutchins and Boyd, 2016). This result suggests that the amount of carbon exported per unit phosphorus by *P. subcurvata* (and perhaps other diatoms) in the Ross Sea may increase as temperature increases in the future (Toseland et al., 2013).

In contrast, the decreasing trend of C: Si ratios in *P. subcurvata* appears to be largely d to higher cellular Si quotas at temperatures at and above 4°C. Although the physiological reason(s) for increased silicification with warming are currently not understood, this trend also may have biogeochemical consequences. This decrease of cellular C: Si ratios at higher temperature may tend to enhance Si export, with the qualification that biogenic Si remineralization rates also increase with temperature (Ragueneau et al. 2000), and thus could potentially offset this trend.

Previous studies have shown that nutrient drawdown by diatoms and *P. antarctica* are different, due to differing elemental ratios of these two groups (Arrigo et al., 1999; Smith et al. 2014a; Xu et al., 2014). Our results generally corresponded to this trend, as the N: P ratios of *P antarctica* were higher than *P. subcurvata* at 2°C, 6°C and 8°C and C: P ratios of *P. antarctica* were higher than *P. subcurvata* at 6°C and 8°C ($p < 0.05$) (Fig. 2). Although elemental ratios o the prymnesiophyte were largely unaffected by temperature, a predicted increase of diatom and decrease of *P. antarctica* contributions to phytoplankton production caused by warming will likely change nutrient export ratios (Smith et al., 2014a, b). It is possible that N and C export p unit P may decrease with a phytoplankton community shift from *P. antarctica* dominance to diatom dominance (Arrigo et al., 1999; Smith et al., 2014a, b; Xu et al., 2014). However, food web effects may compensate for the effects of temperature on ~~the~~ biogeochemical cycles, as diatoms ~~is~~are a preferred food ~~to~~source for zooplankton grazers, compared~~ing~~ to *Phaeocystis*

405 pCO_2 can sometimes drop to very low levels (Tagliabue and Arrigo, 2016). However, at CO_2
406 concentrations beyond current atmospheric levels of ~400 ppm, growth rates of *P. subcurvata*
407 *P. antarctica* were CO_2-saturated. Although a general model prediction suggests that an
408 atmospheric CO_2 increase from current levels to 700 ppm could increase the growth of marine
409 phytoplankton by 40% (Schippers et al., 2004), our results instead correspond to previous studi
410 which showed negligible effects of elevated CO_2 on various groups of phytoplankton (Goldma
411 1999; Fu et al., 2007; Hutchins and Fu 2017). In particular, Trimborn et al. (2013) found that
412 increasing CO_2 had no effect on growth rates of Southern Ocean isolates of *P. subcurvata* and
413 *antarctica*. The minimal effects of changing CO_2 levels on many phytoplankton groups have
414 been suggested to be due to efficient carbon concentrating mechanisms (CCMs) that allow ther
415 to avoid CO_2 limitation at low pCO_2 levels (Burkhardt et al., 2001; Fu et al., 2007; Tortell et al
416 2008). For instance, both *P. subcurvata* and *P. antarctica* have been shown to strongly
417 downregulate activity of the important CCM enzyme carbonic anhydrase as CO_2 increases
418 (Trimborn et al. 2013). Clearly, though, for our two species their CCM activity was not sufficie
419 to completely compensate for carbon limitation at low pCO_2 levels. Although speculative, it is
420 possible that *P. antarctica* could have an ability to subsidize growth at very low CO_2 levels
421 through oxidation of organic carbon from the colony mucilage. Our results also showed that
422 very high CO_2 (1730 ppm) significantly reduced the growth rate of *P. antarctica* relative to 42
423 ppm and 755 ppm at 2°C; negative effects of high CO_2 on an Antarctic microbial community
424 were also observed by Davidson et al. (2016). This inhibitory effect might be due to the
425 significantly lower pH at 1730 ppm (~7.4), which could entail expenditures of additional energ
426 to maintain pH homeostasis within cells.

427 Warming from 2°C to 8°C had a significant interactive effect with CO_2 concentration i

future additional competitive advantage over *P. antarctica* in the late growing season when pC
can be low (Tagliabue and Arrigo, 2016) and temperatures higher, although temperatures are
generally never as high as 8°C in the current Ross Sea (Liu and Smith, 2012). The interactive
effects of temperature and CO_2 on *P. subcurvata* might be due to ~~the~~ elevated enzyme and
protein translation efficiencies~~y~~ ~~and translation efficiency~~ at higher temperature, which may
decrease the CO_2 requirement ~~in~~of the Calvin cycle and facilitate allocation of fixed carbon to
growth (Toseland et al., 2013, Hutchins and Boyd 2016). On the other ~~side~~hand, 8°C ~~might be~~
clearly close to the upper thermal limit of *P. antarctica*, ~~thus the growth rates of *P. antarctica*~~
~~decreased at 8°C not increased~~suggesting that it's biochemical efficiencies decline rapidly abo
this temperature. The ~~CO_2~~ $K_{1/2}$ of *P. antarctica* for CO_2 ~~at 2°C~~ was however significantly lowe
than that of *P. subcurvata* at 2°C~~at this temperature~~, which may be advantageous to the
prymnesiophyte when water temperatures are low in the spring.

The effects of pCO_2 variation on the elemental ratios of *P. subcurvata* and *P. antarctic*
were minimal relative to those of temperature increase. Previous research on the effects of CO_2
on the elemental ratios of phytoplankton has shown that the elemental composition of
phytoplankton may change with CO_2 availability (Burkhardt et al., 1999; Fu et al., 2007, 2008;
Tew et al., 2014; reviewed in Hutchins et al., 2009). Hoogstraten et al. (2012) found that CO_2
concentration change didn't change the cellular POC, PON, C: N ratios, or POC to Chl *a* ratios
of the temperate species *Phaeocystis globosa*. In contrast, Reinfelder (2014) observed that the
and P quotas of several diatoms decreased with increasing CO_2 and led to increased C: N, N: P
and C: P ratios. King et al. (2015) found that high CO_2 could increase, decrease or not affect th
C: P and N: P ratios of several different phytoplankton species. Our results resemble those of
studies with other phytoplankton that found that the effects of CO_2 concentration can be

other cellular components), or to differences in experimental design, which can make inter-comparisons problematic (Hutchins and Fu 2017)~~species specific~~.

In contrast to C: N: P ratios, we observed that the C: Si ratios of *P. subcurvata* were significantly higher at 1730 ppm compared to almost all of the lower CO_2 levels. This increase C: Si ratios was due to a decrease in cellular Si quotas at 1730 ppm CO_2. Milligan et al. (2004) observed that the silica dissolution rates of a temperate diatom increased significantly in high CO_2 relative to in low CO_2 cultures. Tatters et al. (2012) found a similar trend in the temperate toxic diatom *Pseudo-nitzschia fraudulenta*, in which cellular C: Si ratios were higher at 765 pp than at 200 ppm CO_2. This suggests that future increases in diatom silicification at elevated pCO_2 could partially or wholly offset the decreased silicification and higher dissolution rates o silica observed at warmer temperatures (above); to fully predict net trends, further interactive experiments focusing on silicification as a function across a range of both temperature and pC are needed.

In conclusion, our results indicate that *P. subcurvata* from the Ross Sea are better adap to higher temperature than is *P. antarctica*. Diatoms are a diverse group, but if their general thermal response is similar to that of this *Pseudo-nitzschia* species, they may thrive under futu global warming scenarios while the relative dominance of *P. antarctica* in this region may wan In contrast, another recent study has suggested that warming might indirectly favor *P. antarcti* springtime dominance by leading to large areas of open water at a time when incident light penetration is low and mixed layers are still relatively deep (Ryan-Keogh et al. 2017). Because of the differences in elemental ratios in the two groups, ecological shifts that favor diatoms ma significantly increase the export of phosphorus and silicon relative to carbon and nitrogen, whi increased *P. antarctica* dominance will increase carbon export relative to nutrient fluxes, as we

consider in both the present and future in this region (Smith and Jones, 2015). Thus, in addition to warming and CO_2 increases, the interactive effects of light and Fe with these two factors should also be considered (Xu et al., 2014; Boyd et al., 2015; Hutchins and Boyd 2016; Hutchi and Fu 2017). Considering the differences between the responses of the diatom and *P. antarcti* to warming and ocean acidification seen here, as well to warming and Fe in previous work (Zh et al., 2016), models attempting to predict future changes in community structure and primary production in the Ross Sea polynya may need to realistically incorporate a complex network of interacting global change variables.

Author contribution

Z. Zhu, F. X. Fu, D. A. Hutchins designed the experiments, Z. Zhu, P. Qu, and J. Gale carried them out, and Z. Zhu and D. A. Hutchins wrote the manuscripts.

Competing interests

The authors declare that they have no conflict of interest.

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706

707 Table 1. The measured pH and dissolved inorganic carbon (DIC), and calculated pCO_2 of *P. subcurvat*

708 and *P. antarctica* at 2°C and 8°C in each treatment. Values represent the means and errors are the

709 standard deviations of triplicate bottles.

710

P. subcurvata		*P. antarctica*	
2°C	8°C	2°C	8°C
pH			
8.36±0.04	8.51±0.04	8.40±0.03	8.45±0.03
8.25±0.04	8.36±0.01	8.22±0.04	8.29±0.01
8.07±0.01	8.17±0.01	8.09±0.02	8.14±0.00
7.86±0.02	7.99±0.01	7.85±0.01	7.94±0.00
7.68±0.01	7.79±0.02	7.65±0.01	7.75±0.00
7.35±0.01	7.46±0.02	7.34±0.01	7.45±0.00
DIC (µmol/kg)			
1890.1±26.6	1846.5±15.8	1847.1±30.0	1831.1±22.7
2049.1±10.8	1985.7±2.1	2033.9±15.0	2014.2±19.9
2131.3±9.4	2067.5±4.7	2136.6±5.6	2085.3±15.3
2190.4±2.8	2156.1±13.9	2168.1±12.4	2167.4±21.5
2260.0±22.2	2234.8±10.3	2252.1±11.5	2238.7±12.0
2340.1±19.4	2334.5±18.8	2338.2±12.1	2323.7±11.5
pCO_2 (ppm)			
109.1±9.3	94.4±10.1	96.6±9.5	108.8±8.8
158.6±15.5	150.3±3.6	171.2±14.4	183.6±4.2
263.1±5.9	254.2±9.9	246.4±9.9	280.3±0.6
450.2±17.3	414.9±12.0	462.2±12.1	480.9±4.7
740.9±10.6	708.8±23.5	786.9±10.3	784.1±4.8
1751.2±35.9	1675.3±49.4	1769.9±59.5	1720.3±18.3

711
712
713

714 Table 2. Statistical comparison of the results for each of the three thermal traits: Optimum temperature

715 (ºC), Maximum growth rate (d^{-1}) and temperature niche width (W)* of *P. subcurvata* and *P. antarctica*.

716

Species	Optimum temperature (ºC)	Maximum growth rates (d^{-1})	W upper CI	W lower CI	Q_{10}
P. subcurvata	7.36	0.86	12.19	< -2.0	3.17
P. antarctica	4.85	0.66	9.52	< -2.0	2.11

717

718 * The statistical results for the lower bound of temperate niche width in both species were lower
719 than -2.0ºC, the freezing point of seawater

720

721 Table 3. The effects of temperature on the C quota (pmol cell^{-1}), N quota (pmol cell^{-1}), P quota (pmol
722 cell^{-1}), Si quota (pmol cell^{-1}), and chl *a* per cell (pg cell^{-1}) of *P. subcurvata* and *P. antarctica.* Values
723 represent the means and errors are the standard deviations of triplicate bottles.
724

	P. subcurvata	*P. antarctica*
C quota		
0°C	1.91±0.14	2.64±0.34
2°C	2.11±0.19	2.49±0.41
4°C	2.15±0.12	2.50±0.23
6°C	2.07±0.13	2.26±0.18
8°C	2.33±0.14	2.17±0.22
10°C	2.17±0.13	
N quota		
0°C	0.27±0.03	0.39±0.03
2°C	0.29±0.03	0.36±0.02
4°C	0.33±0.02	0.40±0.01
6°C	0.31±0.01	0.35±0.02
8°C	0.36±0.05	0.34±0.03
10°C	0.33±0.04	
P quota		
0°C	0.02±0.00	0.03±0.00
2°C	0.02±0.00	0.02±0.00
4°C	0.03±0.00	0.03±0.01
6°C	0.03±0.00	0.02±0.00
8°C	0.03±0.00	0.02±0.00
10°C	0.02±0.00	
Si quota		
0°C	0.23±0.02	
2°C	0.23±0.06	
4°C	0.30±0.01	
6°C	0.30±0.03	
8°C	0.34±0.01	
10°C	0.28±0.04	
Chl a per cell (pg/cell)		
0°C	0.48±0.01	0.23±0.03
2°C	0.57±0.07	0.22±0.02
4°C	0.64±0.01	0.20±0.01
6°C	0.68±0.05	0.21±0.00
8°C	0.58±0.03	0.17±0.02
10°C	0.46±0.03	

725
726

728 Table 4. Comparison of the curve fitting results for maximum growth rate (d^{-1}) and half saturation

729 constants (K_m), calculated from the CO_2 functional response curves of *P. subcurvata* and *P. antarctica*

730 2°C and 8°C. Values represent the means and errors are the standard errors from fitting.

731 .

Species	Maximum growth rates (d^{-1})	K_m
P. subcurvata		
2°C	0.60±0.18	66.4±10.39
8°C	0.88±0.02	9.8±5.34
P. antarctica		
2°C	0.61±0.02	26.4±8.23
8°C	0.41±0.02	22.1±11.15

732

733 Table 5 The effects of CO_2 on the C: N, N: P, C: P, C: Si, and C: Chl *a* ratios of *P. subcurvata* and *P.*

734 *antarctica* at 2°C and 8°C. Values represent the means and errors are the standard deviations of triplica

735 bottles.

	P. subcurvata		*P. antarctica*	
	2°C	8°C	2°C	8°C
C: N				
100 ppm	6.6±0.26	7.1±0.68	7.22±0.50	6.95±0.35
205 ppm	6.7±0.24	7.5±0.32	7.74±0.21	6.56±1.15
260 ppm	6.7±0.32	7.3±0.18	8.07±0.52	6.99±0.27
425 ppm	6.7±0.05	6.6±0.05	7.21±0.81	6.19±0.13
755 ppm	6.8±0.20	7.1±0.68	7.98±0.44	6.79±0.22
1730 ppm	7.1±0.82	7.4±1.07	8.15±0.48	7.05±0.91
N: P				
100 ppm	10.4±0.85	14.5±2.28	16.4±1.24	13.9±0.20
205 ppm	10.8±1.01	13.3±0.42	16.6±1.12	15.7±2.77
260 ppm	10.3±1.28	14.0±0.56	14.3±1.24	14.5±2.38
425 ppm	11.3±0.84	16.5±0.28	17.1±1.83	17.2±1.98
755 ppm	9.9±0.28	14.3±1.34	14.2±2.60	11.6±4.11
1730 ppm	10.4±1.02	15.5±1.84	15.5±0.56	15.1±1.85
C: P				
100 ppm	68.6±3.10	101.0±6.43	117.7±4.08	96.7±4.86
205 ppm	72.7±4.82	99.3±7.05	128.2±5.98	101.0±1.91
260 ppm	69.1±7.68	103.0±4.88	115.5±7.25	101.0±13.04
425 ppm	76.3±5.19	109.0±2.20	122.3±4.85	106.0±11.14
755 ppm	67.2±1.38	101.0±5.80	113.5±22.50	78.6±27.09
1730 ppm	73.4±1.22	114.0±5.99	126.2±12.10	105.0±6.26
C: Si				
100 ppm	7.8±0.80	5.6±0.32		
205 ppm	7.4±0.30	5.6±0.24		
260 ppm	7.3±0.23	6.1±0.38		
425 ppm	7.5±0.23	6.1±0.06		
755 ppm	7.4±0.66	6.3±0.36		
1730 ppm	8.0±0.88	7.1±0.47		
C: Chl *a* (μg/μg)				
100 ppm	43.6±1.14	70.7±5.01	160.4±6.68	197.4±29.35
205 ppm	45.2±2.91	67.3±4.42	157.5±4.95	194.0±17.14
260 ppm	41.6±3.31	60.1±9.45	138.3±15.19	169.8±9.20
425 ppm	37.2±2.58	72.5±2.35	180.2±20.10	232.4±20.47
755 ppm	42.2±3.62	68.7±6.29	167.5±5.06	282.5±15.30
1730 ppm	46.3±2.23	85.3±15.70	276.5±36.57	460.3±15.21

736

737

743 Table 6 The effects of CO_2 on the C quota (pmol cell^{-1}), N quota (pmol cell^{-1}), P quota (pmol cell^{-1}), S

744 quota (pmol cell^{-1}), and chl *a* per cell (pg cell^{-1}) of *P. subcurvata* and *P. antarctica* at 2°C and 8°C.

745 Values represent the means and errors are the standard deviations of triplicate bottles.

	P. subcurvata		*P. antarctica*	
	2°C	8°C	2°C	8°C
C quota				
100 ppm	2.0±0.15	2.64±0.06	2.57±0.03	2.15±0.22
205 ppm	2.1±0.12	2.67±0.31	2.72±0.28	2.35±0.19
260 ppm	1.9±0.04	2.28±0.18	2.51±0.36	2.21±0.04
425 ppm	1.8±0.04	2.43±0.15	2.31±0.05	2.28±0.46
755 ppm	2.1±0.09	2.26±0.05	2.47±0.17	2.81±0.15
1730 ppm	2.1±0.30	2.47±0.18	2.43±0.10	2.96±0.30
N quota				
100 ppm	0.30±0.03	0.38±0.04	0.36±0.03	0.31±0.03
205 ppm	0.30±0.03	0.36±0.03	0.35±0.03	0.36±0.06
260 ppm	0.29±0.01	0.31±0.02	0.31±0.06	0.32±0.02
425 ppm	0.27±0.01	0.37±0.06	0.32±0.03	0.37±0.05
755 ppm	0.30±0.02	0.32±0.03	0.31±0.03	0.41±0.01
1730 ppm	0.29±0.05	0.34±0.06	0.30±0.03	0.43±0.10
P quota				
100 ppm	0.03±0.00	0.03±0.00	0.02±0.00	0.02±0.00
205 ppm	0.03±0.00	0.03±0.00	0.02±0.00	0.02±0.00
260 ppm	0.03±0.00	0.02±0.00	0.02±0.00	0.02±0.00
425 ppm	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.01
755 ppm	0.03±0.00	0.02±0.00	0.02±0.00	0.04±0.02
1730 ppm	0.03±0.00	0.02±0.00	0.02±0.00	0.03±0.00
Si quota				
100 ppm	0.26±0.02	0.47±0.04		
205 ppm	0.28±0.02	0.48±0.07		
260 ppm	0.27±0.01	0.37±0.03		
425 ppm	0.25±0.01	0.40±0.04		
755 ppm	0.28±0.03	0.36±0.03		
1730 ppm	0.26±0.01	0.35±0.05		
Chl a per cell (pg/cell)				
100 ppm	0.54±0.05	0.45±0.04	0.19±0.01	0.13±0.02
205 ppm	0.54±0.04	0.48±0.05	0.21±0.02	0.15±0.02
260 ppm	0.56±0.03	0.46±0.04	0.22±0.04	0.16±0.01
425 ppm	0.60±0.04	0.40±0.04	0.16±0.02	0.12±0.01
755 ppm	0.59±0.06	0.40±0.03	0.18±0.01	0.12±0.00
1730 ppm	0.53±0.06	0.35±0.05	0.11±0.02	0.08±0.01

746

747

749
750 **Figure legends**

751 Fig. 1. Thermal functional response curves showing specific growth rates (and fitted curves) o
752 *Pseudo-nitzschia subcurvata* and *Phaeocystis antarctica* across a range of temperatures from 0
753 to 14^{o}C. Values represent the means and error bars represents the standard deviations of triplic
754 samples.

755

756 Fig. 2. The C: N ratios (A), N: P ratios (B), and C: P ratios (C) of *Pseudo-nitzschia subcurvata*
757 and *Phaeocystis antarctica* and (D) the C: Si ratios of *Pseudo-nitzschia subcurvata* from the
758 thermal response curves shown in Fig. 1 for a range of temperatures from 0^{o}C to 10^{o}C. Values
759 represent the means and error bars represents the standard deviations of triplicate samples.

760

761 Fig. 3. The C: Chl *a* ratios of *Pseudo-nitzschia subcurvata* and *Phaeocystis antarctica* from th
762 thermal response curves shown in Fig. 1 for a range of temperatures from 0^{o}C to 10^{o}C. Values
763 represent the means and error bars represents the standard deviations of triplicate samples.
764 .

765 Fig. 4. The relative abundance of *Pseudo-nitzschia subcurvata* in a 6 day competition
766 experiment with *Phaeocystis antarctica* at 0°C and 6°C. The competition experiments were
767 started with equal Chl *a* concentrations for both species, and the relative abundance was
768 calculated based on cell counts. Values represent the means and error bars represents the
769 standard deviations of triplicate samples.

770

771 Fig. 5. CO_2 functional response curves showing specific growth rates (and fitted curves) acros
772 range of CO_2 concentrations from ~100 ppm to ~1730 ppm at 2°C and at 8°C. *Pseudo-nitzsch*

Fig. 1

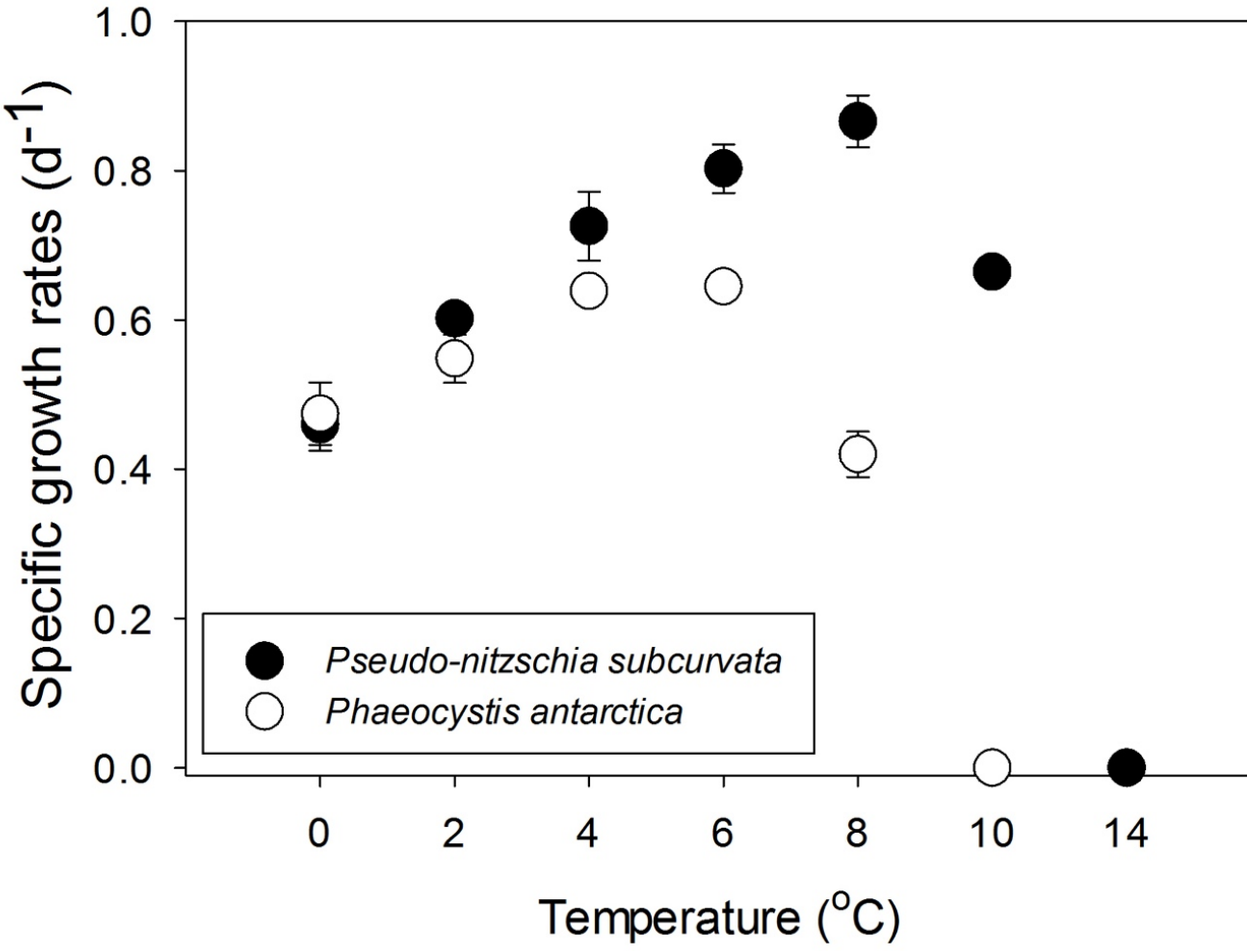
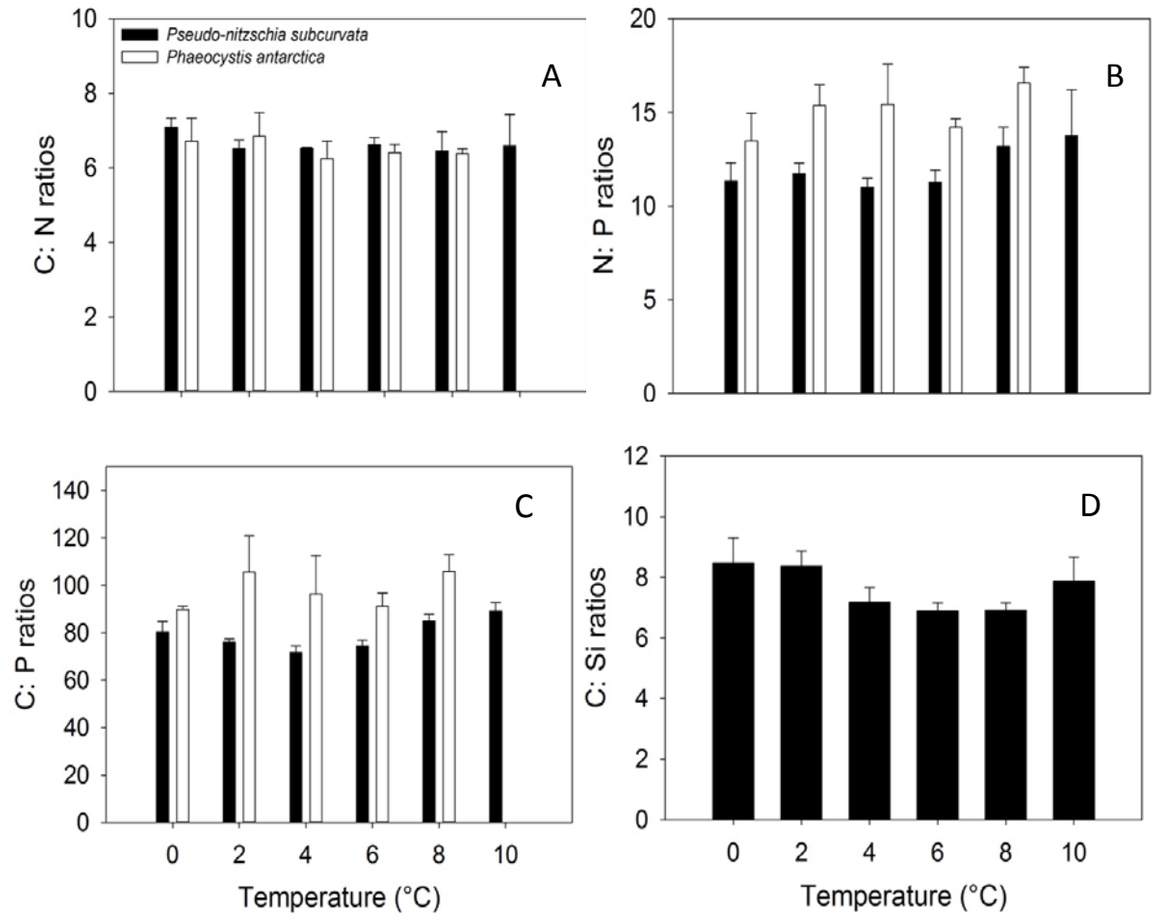
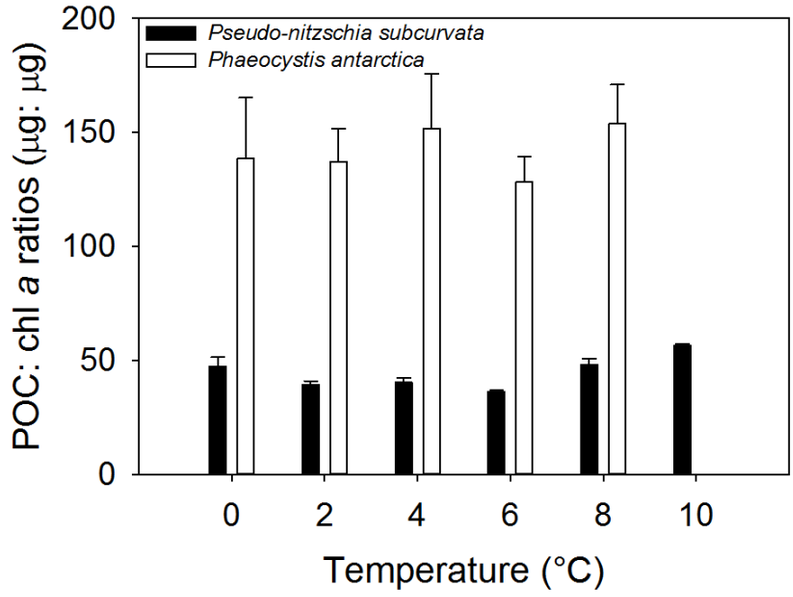

Specific growth rates (d-1)
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
14
Temperature (°C)
Pseudo-nitzschia subcurvata
Phaeocystis antarctica

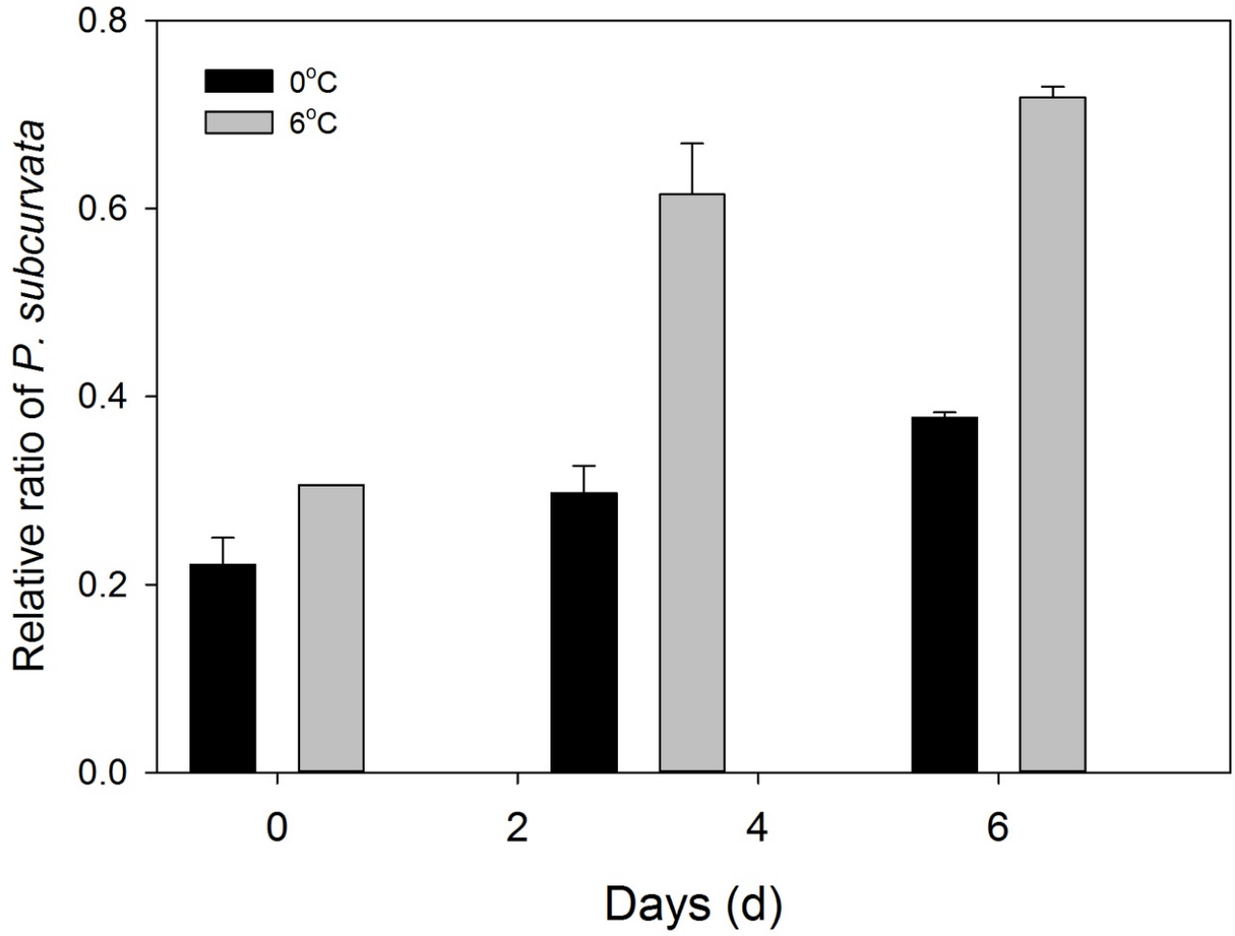
Fig. 2


Pseudo-nitzschia subcurvata
Phaeocystis antarctica
A
B
C
D
C: N ratios
N: P ratios
C: P ratios
C: Si ratios
Temperature (°C)
Temperature (°C)

825 Fig. 3


Pseudo-nitzschia subcurvata
Phaeocystis antarctica
POC: chl a ratios (µg: µg)
200
150
100
50
0
0
2
4
6
8
10
Temperature (°C)

826 Fig. 4



827
828

829 Fig. 5