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Discussions

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Interactive comment on "Resilience to temperature and pH changes in a future climate change scenario in six strains of the polar diatom *Fragilariopsis cylindrus*" by M. Pančić et al.

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Dear Katherina Petrou,

We would like to thank you for your comments on the content. They are very helpful for us to improve the manuscript. Below you can find our response to your comments.

Re 1 and 4: The title is not appropriate for the content, and why did the authors analyze a subset of 6 strains for 1 and 8 °C? The aim of this study was to investigate the response of different strains of a common polar diatom species to various temperature and pH changes. According to the IPCC assessment reports, the air temperature in the Arctic will increase by ~6 °C by the end of the century, which will have a strong impact

on the SST of the Arctic Ocean. Assuming that the SST will reach 5 °C by the end of the 21st century, the first set of experiments was conducted at the said temperature and 4 different pH treatments. It has been observed that the six investigated strains clustered into three groups as shown in Fig. 2b, and based on that the second and the third set of experiments were carried out at the same pH conditions and changed temperatures, and with the reduced number of strains (taking one strain from each of the three groups). The number of strains was reduced assuming that similar clustering will also be observed at changed temperatures.

This study reports that the investigated species shows resilience to the changes in temperature and pH predicted for the 21st century, when looking at the impact of combining temperature and pH changes. Furthermore, the study shows that some strains display better performance than others when cultivated under the same conditions, highlighting the importance of investigating multiple strains of a species to avoid misguiding conclusions based on one strain as representative of an entire species. With this in mind, we believe that the title is appropriate and it fits the results obtained in this study.

Regarding the comment 4, the following sentence was added in the section "Materials and methods": 'Based on the observation from the first set of experiments, which showed clustering of six strains into three groups (Fig. 2b), further experiments at 1 °C and 8 °C with all pH treatments were carried out with reduced number of strains (taking one strain from each group – D3G1, D4D11 and D10A12). The subset of the six strains was analyzed assuming that the similar clustering will also be observed at changed temperatures.

Re 2: Data were analyzed using two-way ANOVA, why not three-way ANOVA. We agree that three-way ANOVA is more appropriate. We now have also run three-way ANOVA with temperature and pH as fixed factors, and strain as a random factor. We found significant three-way interaction ($P < 0.05$). We followed up a statistically significant three-way interaction with simple two-way interactions at all levels (T*pH, T*strain,

pH*T, all significant interaction) and simple main effects for fixed factors pH and temperature. The type of ANOVA used for the statistical analysis will be changed in the manuscript (section 2.3) accordingly.

Re 9: The data are discussed in both combined effects and individual effects, although a significant interaction was detected, and the data only need to be discussed in terms of the combined effect. We agree that after a significant interaction is found one does usually not report individual effects. However, in our study we found it important to also discuss the simple main effects of the fixed factors, because the effect of pH changes alone was found to be very different compared to the effect of temperature changes, and again very different compared to the combined effect of both the factors, which is also discussed in the section 4. With this in mind, we believe that reporting the effects of single factors and then combined factors on the species were necessary in order to show the importance of taking more stressors into account when trying to predict the plausible response to future changes.

Re 3: The same data is doubly represented in Fig. 1 and Fig. 2. Fig. 1 (the latter choice of the two within-subject factors in Re 2) compares the growth rates within specific strain cultivated at 12 different simulated climate change scenarios and also visually compares the performance among three strains, whereas Fig. 2 (the former choice of the two within-subject factors in Re 2) compares the growth rates among all investigated strains at different temperatures. We believe that plotting this complex data set the way we did, can help considerably with understating the investigated relations, and we simply see no other way of presenting it without losing the readers if we do it any other way.

Re 5: Lines connecting the growth rates across the pH measurements are misleading and incorrect, as the data are categorical. The reason why we used lines was to highlight the trend that we observed. However, we do agree that using solid lines is wrong since the data are categorical, and they will be replaced with dotted lines as we want to keep the message. We will add a short explanation in the captions, explaining

that the data are categorical and the dotted lines serve to show the trend.

Re 6: Supplementary figures are clearer; the same data are plotted 4 times. We decided to put the bar plots with statistics together with the tables in the supplementary file since the former include the statistical information as you mentioned, and the latter the summary of the growth rates we measured and were used for the Q10 values we calculated (data not presented in the manuscript at any point). However, we believe that the plots we used in the manuscript more clearly illustrate the message we want to give (the trend), and the bar plots give more detailed information of what is significant, however the trend cannot be seen, which is why we put them in the supplement.

Re 7: Figure 3 is not data and is therefore non-essential. We agree that Figure 3 is not data, but we believe that it graphically explains the methodology we used in our experiments. It shows the minimal fluctuations around the designated pH and temperature values we examined in this study, and so it reflects the quality of the experimental operation.

Re 8: Make this study a note paper. The data we present in this paper are substantial (in terms of interaction we found – environmental factors and multiple strains) and report the plausible effects of global change on phytoplankton in polar areas. Experimental data on combined effects of environmental factors on a phytoplankton growth performance in the context of global change remain limited and poorly understood, and most studies use only a single strain as representative of an entire species despite documented physiological variability among the strains of the species. This study finds that the effects of single parameters (temperature and pH) on growth performance of the model species counterbalance each other, and due to the high variation among strains that we found, the global change may not affect the species as such, but rather the population structure of the species. The paper thus presents results that are new to science, adds important input to the debate on global change and the basal part of the marine ecosystem. The study also represents a substantial amount of data, and thus we believe that they would be better presented in a discussion paper than in a

note paper.

Re 10: The results section needs refining and could be reduced substantially. In the section 3.1.2 the following will be removed: Page 4636 lines 16-23, Page 4637 lines 15-16 and lines 26-27. The rest reports only significant findings which are also supported with the p-values in the brackets and also with the bar plots in the supplement (letters above the bars).

Re 11: Be specific in your subheadings, change 'multiple' to 'three' at 4.1.1. The term 'multiple' will be removed accordingly ('Combined effects of temperature and pH on growth of F. cylindrus strains').

Re 12: Take care when using the term alkalinity. The term 'alkalinity' will be replaced with 'increased pH' as also reported in our response to Dr Campbell (Page 4639 line 22, Page 4640 line 7).

Re 13: Try to avoid ambiguous terms such as 'greatest' and 'slightly'. The term 'slightly' on Page 4637 line 20 will be removed. On Page 4644 line 10, the term 'slightly' is explained with the specific change in growth rates. Similarly, the term 'greatest' on Page 4641 line 21 is further explained with Q_{10} values displayed in Table 1.

Re 14: The authors mention the natural pH range of Arctic phytoplankton should be disclosed much earlier on, as it shows the inherent plasticity the strains would be expected to have. To our knowledge there are no studies supporting the inherent plasticity which you said the strains would be expected to have (due to the wide pH range the Arctic phytoplankton communities experience during the spring blooms) since the dynamics of pH during the spring blooms in the Arctic coastal regions has never been measured before. The pH range found in the Arctic coastal region (Thoisen et al., 2015) was the first study that measured in situ pH levels during the spring bloom in the area.

Re 15: The growth data could be shown in a different way. We plotted the data in a way we believed they would best be presented. We decided to put the line (dotted line)

plots in the manuscript to present the trends. To support and additionally explain our findings, we presented the data with the bar plots (including the statistical significant information) and tables in the supplement.

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