Below, the response and implemented changes to the referees' comments are presented as 1) referee's comment, 2) author's response, 3) changes made in the manuscript. Different referees are indicated by their name.

Douglas Campbell

1) Table 1: The left-most column is not labeled; it is pCO2, I think.

2) We agree the left-most column should be labeled (it displays 'pH treatments').

3) The column in question is now labeled 'pH treatments'.

1) Figure 1: I think the legend is wrong. It says the 3 panels are a 1C, b 5C and 8C, but each panel has curves at each of these temperatures, and is labeled with a strain name. Then in the legend is says 6 strains were analyzed, but only 3 strains are labeled on the panels.

2) We agree Figure 1 caption is wrong.

3) The previous caption: '*The mean maximum growth rates (d^{-1}) of strains D5A4, D10A12, D4D11, D8G3, D8F4 and D3G1 cultivated at (a) 1 °C, (b) 5 °C and (c) 8 °C, and all four pH treatments. Error bars represent ± one standard deviation.*' has been replaced with:

'The mean maximum growth rates (d^{-1}) for strains (a) D10A12, (b) D4D11, and (c) D3G1 cultured at temperatures of 1 °C, 5 °C and 8 °C, and four different pH treatments. Error bars represent ± SD. Note that the data are categorical, and the dotted lines serve to show the trend.'

1) Figure 3: 'pH dilutions' is not a good term. Media dilutions to control pH, perhaps? In panel B, the dotted line is labeled 'Expected pH', but the Y axis shows temperature. Pasting error, again?

2) We agree that '*pH dilutions*' is not a good term, and that in panel B, the labels '*Expected pH*' and '*Measured pH*' are wrong. We agree that Figure 3 caption should be modified.

3) In Figure 3: The term '*pH dilutions*' has been changed to 'pH adjustments'.

In panel B, the labels have been changed to: 'Expected temperature' and 'Measured temperature'. The modified Figure 3 can be found at the end of the document.

In Figure 3 caption, the previous caption: *'(a) An example of pH dilutions in the pH treatment 7.4 at 5 °C, shown as a function of time. (b) An example of temperature fluctuations in the treatment with pH 7.4, shown as a function of time. The first three days represent the acclimation period and are not included in the results. Error bars represent ± one SD.*' has been modified to:

'(a) An example of pH adjustments in the pH treatment 7.4 at 5 °C, shown as a function of time. (b) An example of temperature fluctuations in the treatment with pH 7.4, shown as a function of time. The first three days represent the acclimation period and are not included in the results. Error bars represent ± SD.'

1) Figure 2: has the same legend as Figure 1, but I think it applies only to Figure 2. Pasting error for Figure 1 legend?

2) Yes, Figure 2 caption does apply only to Figure 2.

3) Figure 2 caption: '*The mean maximum growth rates (d^{-1}) of strains D5A4, D10A12, D4D11, D8G3, D8F4 and D3G1 cultivated at (a) 1 °C, (b) 5 °C and (c) 8 °C, and all four pH treatments. Error bars represent ± one standard deviation.*' has been modified to:

'The mean maximum growth rates (d^{-1}) of strains D5A4, D10A12, D4D11, D8G3, D8F4 and D3G1 cultivated at (a) 1 °C, (b) 5 °C and (c) 8 °C, and all four pH treatments. Error bars represent ± SD. Note that the data are categorical, and the dotted lines serve to show the trend.'

1) Materials & Methods: How long/how many cellular generations were cells grown before the growth rate estimates? Were they fully acclimated to the conditions?

2) The total acclimation period to different treatments was 8 days (as described in Materials and methods, section Experimental setup). All the strains were treated the same way: 48 hours at the specific temperature and pH 8.0, followed by dividing the culture into two sub-cultures and adjusting pH to 7.7 (temperature remained the same). After 24 hours, the later sub-culture was divided into two sub-cultures again, and pH of one of them adjusted to 7.4. Again, after 24 hours the 7.4 sub-culture was divided, and pH of one of them adjusted to 7.1. From here on, all the sub-cultures at different pH treatments were grown for three days, and on the third day (day 0), the experimental flasks were inoculated with a cell concentration of 1000 cells mL^{-1} and L1 medium of the pH-specific value. Sampling was initiated on day 3, and thus the

time period from day 0 to day 3 was considered as part of the acclimation period (in total 8 days).

3) pg 4632 line 11: The sentence '*After two days of acclimation, pH of the cultures was lowered to pH 7.7, 7.4 and 7.1 in steps of 0.3 pH units every 24 h by addition of strongly acidified L1 medium (pH 0.49±0.02).*' has been modified to:

'After two days of acclimation at final temperatures, pH of the cultures was lowered to pH 7.7, 7.4 and 7.1 in steps of 0.3 pH units every 24 h by addition of strongly acidified L1 medium (pH 0.49±0.02; 5 days of acclimation period).'

pg 4633 line 8: The sentence '*Thus, the time period from day 0 to day 3 was considered as part of the acclimation period and not included in the results*' has been modified to:

'Thus, the time period from day 0 to day 3 was considered as part of the acclimation period and not included in the results (in total 8 days of acclimation period).'

1) Discussion: "…the growth rates of all three strains increased with alkalinity (from pH 7.1 to 8.0)" pH is not the same thing as total alkalinity; given the complexities of marine carbonate systems, it is important to be terminologically and conceptually precise. Did alkalinity increase with increasing pH? Or not? Usually in marine media total alkalinity is fixed.

2) We agree that the terms '*alkalinisation*' and '*alkalinity*' were misused here (we did not measure alkalinity).

3) pg 4639 line 22 and pg 4640 line 7: The terms have been replaced with 'increased pH' and 'decreasing acidification', respectively.

Penelope Ajani

1) Abstract: I think it is important to state that DIC, nutrients and molecular characterization (ITS) was included in this study.

2) The measurements of DIC and nutrients were taken in order to exclude them from the list of parameters, which could potentially interfere with the growth of the species. The molecular characterization was performed to confirm the identity of the strains as well as the similarity

among them. With this in mind, we believe that stating the measurements in question in the abstract will not improve the overall understanding of the topic.

1) Materials and Methods: what depth were the water samples collected and what was the collection method?

2) We agree that the depth and collection method should be added.

3) pg 4631 line 14: The sentence '*Water samples were collected from Disko Bay (69°11 N, 53°31 W) on the west coast of Greenland.*' has been modified and changed to:

'Water samples were collected from Disko Bay (69°11 N, 53°31 W), Western Greenland from the upper 20 m surface layer with a 20 μm mesh plankton net.'

1) It is not obvious why the strains were reduced in number beyond the first set of experiments. This could be made clearer.

2) We agree that the reason why the strains were reduced in number could be made clearer.

3) pg 4632 line 3: The paragraph '*Based on these results, the second set of experiments was carried out with a reduced number of strains (D3G1, D4D11 and D10A12) at 8 °C and with all the pH treatments, and the last set with the same reduced number of strains at 1 °C.*' has been modified and changed to:

'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).'

Katherina Petrou

1) The title is not quite appropriate for the content. It should reflect more the phenotypic plasticity and intra-specific variability rather than resilience. Also, there were only 3 strains used across temperature manipulations not 6.

2) 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).

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.

1) The authors have analyzed their data using a two factor ANOVA, yet present their data both in the text and Figure 2 as 3-factorial; temperature, pH and strain. Three-way ANOVA would be more appropriate, as testing the significance of differences between the strains is fundamental to the hypothesis and therefore equally as important than the environmental factors (pH and temperature) being tested. Statistics need to reflect the hypothesis being tested, the data presentation and the discussion.

2) 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.

3) pg 4635 line 2: The paragraph '*All analyses were performed using IBM SPSS Statistics (version 22). Differences between the treatments were tested using two-way ANOVA, with Bonferroni's correction and Student's t-test. The normal distribution of data was tested using a Shapiro-Wilk test. Levene's test verified the equality of variances in the data (homogeneity of variances). The level of significance used was 0.05.*' has been modified and changed to:

'All analyses were performed using IBM SPSS Statistics (version 22). Differences between the treatments were tested using three-way ANOVA with temperature and pH as fixed factors, and strain as a random factor. A statistically significant three-way interaction was followed up

with simple two-way interactions at all levels, applying a Bonferroni adjustment, and simple main effects for fixed factors pH and temperature. The normal distribution of data was tested using a Shapiro-Wilk test and homogeneity of variances using Levene's test. The level of significance used was 0.05.'

pg 4635 line 11: The paragraph '*The differences in growth rates within and among the strains were tested using two-way ANOVA, and a significant interaction between temperature and pH on growth rate was found (all P values < 0.05). This means that the effect of temperature on growth rates depends on pH, and vice versa.*' has been modified to:

'The differences in growth rates within and among the strains were tested using three-way ANOVA, and a significant interaction among temperature, pH and strain on growth rate was found ($P < 0.05$). This means that the effect of each of the three parameters on growth rates depends on the set of the other two (e.g. the effect of temperature on growth rates depends on pH and strain).'

pg 4639 line 19: The paragraph '*This study showed a statistically significant interaction between pH and temperature on the growth of all F. cylindrus strains cultivated at four pH treatments and three temperatures (two-way ANOVA, P < 0.05).*' has been modified to:

'This study showed a statistically significant interaction among pH, temperature and strain on the growth of all *F. cylindrus* strains cultivated at four pH treatments and three temperatures (three-way ANOVA, $P < 0.05$).'

pg 4640 line 9: The sentence '*These two observed patterns illustrate that the combination of both parameters counterbalances each other.*' has been modified to:

'These two observed patterns illustrate that the combination of pH and temperature counterbalances each other, with the response also being dependent on a strain.'

pg 4640 line 20: The sentence '*However, one has to take into consideration that the largest variability was found among the strains.*' has been modified to:

'However, one has to take into consideration that the largest variability was found among the strains (the random factor).'

1) Data on strains D10A12, D4D11 and D3G1 is doubly represented in Fig 1 and 2, where the exact same data is re-plotted separated by temperature instead of strain and with the 3 additional strains added at 5 degrees only. One of the figures should be removed.

2) Fig. 1 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 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.

1) Also, I agree with reviewer Dr Ajani, that there is no rationale provided as to why the authors only analyzed a subset of the 6 strains for 1 and 8 degrees. This needs to be clarified.

2) We agree that the reason why the strains were reduced in number should be clarified. See the response to Penelope Ajani.

1) The authors have put lines connecting the growth rates across the pH measurements. This is misleading and incorrect, as the data is categorical. The lines need to be removed. The data would be better replotted as bar graphs, which I feel would be more fitting, similar to the data in Fig 2 of the supplementary. In general, I find the data presentation could be improved to assist the reader in interpreting the findings.

2) 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.

3) The modified Figure 1 and Figure 2 can be found at the end of the document. For the modified captions, see the response to Douglas Campbell.

1) The supplementary figures more clearly and accurately represent the data. They also include the statistical significance information, which is missing from the main figures. However, again, the same data is plotted 4 times in the supplementary and also a table provided with the same values. This should be reduced.

2) 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 Q_{10} 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.

1) Figure 3 is not data and is therefore non-essential. I recommend making it supplementary or removing it altogether.

2) 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.

1) With the removal of 2 figures, there is now only one figure and two small tables, therefore, I strongly encourage the authors to consider making this study a note paper, as I don't feel that there is sufficient significant data to warrant an entire discussion paper.

2) 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; most studies use only one parameter) 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.

1) The authors discuss the data in both combined effects and individual effects. However, they detected a significant interaction, which means that the data only need be discussed in terms of the combined effect. This may of course change once a 3rd factor (strain) is included.

2) 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.

1) The results section needs refining and could be reduced substantially. In general, figures should be sufficient to describe the patterns to the reader. Therefore, the text should report on the statistically significant aspects of the data and main trends.

2) 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 letters above the bar plots in the supplement.

3) Removed:

pg 4636 line 16: '*Within the pH 7.1 treatment, significant differences were observed among the growth rates of all the strains ($P < 0.05$), except between D8G3 and D4D11, and D5A4 and D10A12 ($P > 0.05$; Fig. S3-IIa). Within the pH 7.4 and 7.7 treatments, significant differences between the growth rates of each pair of the strains were observed ($P < 0.05$), except between D8G3 and D4D11 ($P > 0.05$; Fig. S3-IIb-c). Within the 8.0 pH treatment, significant differences were observed among the growth rates of all the strains ($P < 0.05$; Fig. S3-IId).*'

pg 4637 line 15: '*Within all four pH treatments, significant differences between the growth rates of each pair of strains were observed (P < 0.05).*'

pg 4637 line 26: '*In all four pH treatments, significant differences were observed among the growth rates of each pair of the strains (P < 0.05).*'

pg 4636 line 7: '*pairwise comparison*'

pg 4637 line 10: '*pairwise comparison*'

pg 4637 line 22: '*pairwise comparison*'

pg 4638 line 4: '*pairwise comparison*'

1) Please be specific in your subheadings. At 4.1.1 please change 'multiple' to 3 F. cylindrus strains.

2) We agree the subheading should be more specific.

3) pg 4639 line 14: The subheading '*Combined effects of temperature and pH on growth of the multiple F. cylindrus strains*' has been changed to:

'Combined effects of temperature, pH and strain on growth of *F. cylindrus*'

1) Take care when using the term alkalinity instead of higher pH, as they aren't the same thing.

2) We agree that the term 'alkalinity' was misused. See the response to Douglass Campbell.

1) The authors should try to avoid ambiguous terms such as 'greatest impact' or 'slightly'.

2) 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.

3) pg 4637 line 20: The term '*slightly*' has been removed.

1) The section in which the authors mention the natural pH range of Arctic phytoplankton (7.5-8.3) should be disclosed much earlier on, as it shows the inherent plasticity the strains would be expected to have, due to the seasonal or diel fluctuations that occur in their habitat. This provides a framework in which to discuss the results,

particularly with respect to the minimal effect on pH changes from 7.4-8.0 in their experiments.

2) 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.

1) With respect to the data presentation; the growth data could be shown in a way as to be more informative when interpreting the data in terms of phenotypic plasticity, for example it could be a figure that shows the relative change in growth rates across strains, pH and temperature, or it could simply plot the range in growth rates (0.2-0.7 d-1) across the six strains. Alternatively, the authors could consider using the supplementary figure 2 (temperature and pH in 3 strains) and the supplementary table (with all the strains at 5 degrees) to represent their data set in the main manuscript. The only supplementary figures would then be Sup Fig 1, table on carbonate chemistry and the Figure 3 from the main paper.

2) 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.

Anonymous referee

1) Page 4628 line16-18, is there any field observation which can support the author's prediction?

2) Field studies recording the presence of the species in question at different temperatures do exist. The species has most often been found widespread in polar and sub-polar regions of the Arctic and the Antarctic (Lundholm and Hasle, 2008; Hasle, 1965; Poulin et al., 1990). Moreover, the records from more southern areas also exist, e.g. the Baltic Sea, North Atlantic (Hasle, 1965), although it has been hypothesized that the cells were carried to those areas by the southward currents (Hasle, 1965; Von Quillfeldt, 2001). As pH levels are rarely recorded

and almost not-existing in the Arctic (Thoisen et al., 2015) we do not have an access to field data supporting the prediction regarding pH.

1) Page 4269 line7, the paper of Feng et al. 2008 is based on experimental researches other than modeling study.

2) The citation of the paper will be removed since the paper is not a modeling study.

3) pg 4629 line 7: The reference 'Feng et al., 2008' has been removed.

1) Page 4631 line15, In Fig2, the growth rates of the 6 strains showed very big differences. Did these strains also showed big differences on morphology and molecular sequences? If not, why?

2) As mentioned in the Results, the molecular analyses showed that the ITS1. 5.8S and ITS2 sequences of all six strains were identical, showing that they belong to the same species. We did not characterize genetic differences any further, as this was not within the scope of the paper. With regard to morphology, we examined three of the strains (D10A12, D4D11 and D3G1). Their morphometric data were overlapping except for a difference in valve length (expected among strains of pennate diatoms) - all in agreement with *F. cylindrus sensu* in Lundholm and Hasle (2008).

1) Page 4632 line 17, References need to be included to support the selection of light level. In general, the light level in polar regimes is very low because of ice cover, deeper mixed layer and half year darkness.

2) The light intensity level in this study (90-100 μmol photons m^{-2} s^{-1}) was selected based on the measurements of the surface irradiation in late summer in the Arctic regions, which is approximately 1000 μmol photons m^{-2} s^{-1} (Platt et al., 1982), and according to Nielsen and Hansen (1999), approximately 5-10 % of the surface irradiance penetrates down to the depth of 20 m. Based on that, we assumed that the light level of 90-100 μmol photons m^{-2} s^{-1} could be found in Disko Bay in April-May in the upper 20 m surface layer, when the day length reaches 17-18 hours.

3) pg 4632 line 6: The sentence '*The cells were exposed to 90-100 μmol photons* m^{-2} s^{-1}*, following a light:dark cycle of 16:8 h.*' has been modified to:

'The cells were exposed to 90-100 μmol photons m^{-2} s^{-1} (Nielsen and Hansen, 1999; Platt et al., 1982), following a light:dark cycle of 16:8 h.'

Nielsen, T. G. and Hansen, B. W.: Plankton community structure and carbon cycling on the western coast of Greenland during the stratified summer situation. I. Hydrography, phytoplankton and bacterioplankton, Aquat Microb Ecol, 16, 205-216, 1999.

Platt, T., Harrison, W. G., Irwin, B., Horne, E. P., and Gallegos, C. L.: Photosynthesis and photoadaptation of marine phytoplankton in the Arctic, Deep Sea Res A, 29, 1159-1170, 1982.

1) Page 4638 line7, Page 4639 line10, Please double check the data. The SD values of temperature and pH could up to 0.6C and 0.03 units, respectively. The daily fluctuations of temperature and pH in Fig 3 are at least 3 times of 0.05C and 0.03 units, respectively. In addition, the quality of experimental operation actually reflects on daily fluctuation.

2) We agree that the SD value of temperature in the text is wrong (± 0.05 °C). The typo will be corrected to ± 0.6 °C, as presented in Table 2.

3) pg 4638 line 7: The sentence '*Temperature and pH in the experimental treatments fluctuated minimally around the designated values (< 0.05 °C and < 0.03 units, respectively) (Fig. 3, Table 2).*' has been modified to:

'Temperature and pH in the experimental treatments fluctuated minimally around the designated values (< 0.6 °C and < 0.03 units, respectively) (Fig. 3, Table 2).'

pg 4639 line 9: The sentence '*Throughout the experiment, the temperature and pH of the experimental treatments fluctuated minimally (< 0.05 °C and < 0.03 units, respectively; Table 2), making the treatments clearly separate from each other, and thus enabling an evaluation of the combined effects of ocean acidification and temperature on several strains of a microalgal species.*' has been modified to:

'Throughout the experiment, the temperature and pH of the experimental treatments fluctuated minimally (< 0.6 °C and < 0.03 units, respectively; Table 2), making the treatments clearly separate from each other, and thus enabling an evaluation of the combined effects of ocean acidification and temperature on several strains of a microalgal species.'

1) Page 4628, line16-18, Page 4640 line 1-2, Page 4644 line 16-19, Due to the complexity of nature, laboratory experimental design is incomplete to mimic real environmental change. So the laboratory data could not simply used to predict what will happen in nature even prediction is under limited conditions. However, it helps if field observations could support author's point. In natural conditions, many marine

phytoplankton can be found across a very big range of temperature, pH, light etc. A lot of F. cylindrus can be found at California coastal water with an annual temperature range of ~12-20C. This is a real proof which strongly supports that diatom F. cylindrus could adapt to high temperature.

2) We agree with the reviewers point in as much as only temperature and pH as well as multiple strains were considered, whereas in the field, the situation is more complex compared to the experimental setup. Incubations of natural populations also have some their shortcomings (grazers, pathogens, etc.), which is why the following sentence will be added in the end of the discussion:

3) pg 4644 line 19: 'It should be noted that controlled laboratory experiments cannot mimic real environmental changes (Schlüter et al., 2014), yet they are at present the only direct way for simulating plausible future climate changes and examining their effects on marine phytoplankton.'

Other changes:

pg 4630 line 15: The sentence '*Similarly, Nielsen et al. (2011) reported that the investigated coastal plankton communities from temperate regions were unaffected by projected 21 century changes in pH and free* CO_2.' has been modified to

'Similarly, Nielsen et al. (2011) reported that the investigated coastal plankton communities from temperate regions were unaffected by projected 21st century changes in pH and free CO_2.'

pg 4640 line 21: The sentence '*Some strains showed better performance than others when cultivated in the same conditions, indicating that these strains may display high resilience to the changes in pH and temperature predicted for the 21 century (e.g. present study; Kremp et al., 2012; Langer et al., 2009).*' has been modified to

'Some strains showed better performance than others when cultivated in the same conditions, indicating that these strains may display high resilience to the changes in pH and temperature predicted for the 21st century (e.g. present study; Kremp et al., 2012; Langer et al., 2009).'

1

Resilience to temperature and pH changes in a future climate change scenario in six strains of the polar diatom *Fragilariopsis cylindrus*

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Abstract

The effects of ocean acidification and increased temperature on physiology of six strains of the polar diatom *Fragilariopsis cylindrus* from Greenland were investigated. Experiments were performed under manipulated pH levels (8.0, 7.7, 7.4, and 7.1) and different temperatures (1, 5 and 8 °C) to simulate changes from present to plausible future levels. Each of the 12 scenarios was run for 7 days, and a significant interaction between temperature and pH on growth was detected. By combining increased temperature and acidification, the two factors counterbalanced each other, and therefore no effect on the growth rates was found. However, the growth rates increased with elevated temperatures by ~20–50 % depending on the strain. In addition, a general negative effect of increasing acidification on growth was observed. At pH 7.7 and 7.4, the growth response varied considerably among strains. However, a more uniform response was detected at pH 7.1 with most of the strains exhibiting reduced growth rates by 20–37 % compared to pH 8.0. It should be emphasized that a significant interaction between temperature and pH was found, meaning that the combination of the two parameters affected growth differently than when considering one at a time. Based on these results, we anticipate that the polar diatom *F. cylindrus* will be unaffected by changes in temperature and pH within the range expected by the end of the century. In each simulated scenario, the variation in growth rates among the strains was larger than the variation observed due to the whole range of changes in either pH or temperature. Climate change may therefore not affect the species as such, but may lead to changes in the population structure of the species, with the strains exhibiting high phenotypic plasticity, in terms of temperature and pH tolerance towards future conditions, dominating the population.

1 Introduction

The Arctic Ocean is currently experiencing fast environmental changes, such as warming and sea ice loss, as well as sea ice and ecosystem structure changes due to natural and anthropogenic factors (Arrigo, 2014; Nicolaus et al., 2012; Turner and Overland, 2009). According to some models, the average sea surface temperature (SST) in some areas of the global ocean will increase by 1–4 °C over the next 100 years (Alley et al., 2007~~; Feng et al., 2008~~), with the largest changes happening in the Arctic (Gradinger, 1995; Hansen et al., 2010). At high latitudes above the Arctic Circle, the average surface air warming rate was found to be about 0.7 °C per decade (~6 °C by the end of the 21st century), which will have a

strong impact on the SST of the Arctic Ocean (Comiso, 2010). These changes may impact algal communities via changes in physical forcing, biogeochemical cycling, and food web interactions due to loss of habitat (Boras et al., 2010; Fountain et al., 2012; Johannessen and Miles, 2011; Melnikov, 2005). Higher temperatures may intensify heterotrophic processes in sea ice, via increased grazing rates and nutrient regeneration (Melnikov, 2009). Earlier melting of snow cover may accelerate the timing of ice algal blooms, but it is difficult to predict their impact; and mismatching in timing between the phytoplankton production and the reproductive cycle of key Arctic secondary producers could have negative consequences for the entire lipid-driven Arctic marine ecosystem (Søreide et al., 2010). Recent studies on ocean surface warming suggest increased phytoplankton productivity as a consequence of increased temperatures (Feng et al., 2009; Mock and Hoch, 2005; Torstensson et al., 2012). Mock and Hoch (2005) reported that given enough time, the polar diatom *Fragilariopsis cylindrus* could efficiently adjust its photosynthesis to diverse temperatures. Similarly, Torstensson et al. (2012) showed that an elevated temperature (from 0.5 to 4.5 °C) increased the growth rate of the benthic/sea ice diatom *Navicula directa*.

Next to rapid changes in the ocean surface temperature and their consequences on the marine ecosystem, ocean acidification is expected to occur relatively fast in the Arctic environment. The major reasons are its unique features, such as cold and relatively fresh surface waters which promote high CO_2 solubility (Yamamoto et al., 2012). According to Alley et al. (2007), the atmospheric partial pressure of CO_2 (pCO_2) is likely to exceed 700 parts per million (ppm) by the year 2100. In the open oceans, where phytoplankton biomass and primary productivity is usually low, this will be accompanied by a seawater pH decline from a global preindustrial level of ~8.2 to about 7.8 (Alley et al., 2007; Orr et al., 2005; Yamamoto et al., 2012), with low seasonal variability (Feely et al., 2009). However, in coastal ecosystems pH displays large seasonal and diurnal fluctuations due to high primary production, respiration, upwelling and water residence time (Duarte et al., 2013; Thoisen et al., 2015).

To date, experimental data on phytoplankton tolerance to decreasing pH and rising SST are scarce and mostly only available for phytoplankton from temperate coastal waters. Berge et al. (2010) investigated the tolerance of eight temperate phytoplankton species from four groups (dinoflagellates, cryptophytes, diatoms, prymnesiophytes) to lowered pH, and showed that marine phytoplankton was, in general, resistant to climate change in terms of ocean acidification. Similarly, Nielsen et al. (2011) reported that the investigated coastal plankton

communities from temperate regions were unaffected by projected ~~21st~~ 21st century changes in pH and free CO_2. Iglesias-Rodriguez et al. (2008) reported increased calcification and primary production of the coccolithophore haptophyte *Emiliania huxleyi* at elevated CO_2 concentrations. On the other hand, Riebesell et al. (2000) and Feng et al. (2008) showed decreasing calcification rates and malformed coccoliths of the same species at increasing acidification. A recent study on ocean acidification in the polar areas showed negative effects on growth rates of the brine algal community, when exposed to pH below 7.6 (McMinn et al., 2014). Likewise, Torstensson et al. (2012) reported somewhat reduced growth rates of the polar diatom *Navicula directa* at increased pCO_2 levels (960 ppm; pH ~7.7).

Experimental data on combined effects of elevated temperatures and decreased pH on the growth of phytoplankton from polar waters remain limited and poorly understood (Slagstad et al., 2011). Most studies investigating climate effects on phytoplankton use only one strain as representative of a species despite it being well documented that species are genetically and physiologically diverse. Therefore, conclusions based on single strains could potentially be misguiding. The aim of the present study was to simulate pH and temperature changes from present to probable future levels, to be able to evaluate their potential impact on the growth of the polar diatom species *Fragilariopsis cylindrus* (Grunow) Krieger, based on six strains of the species. *F. cylindrus* is one of the most widespread and common diatoms in polar and sub-polar regions (Kang and Fryxell, 1992; Lundholm and Hasle, 2008), and an important species in terms of biomass and primary production during spring blooms in the Arctic Sea (von Quillfeldt, 2000). It is common in pack ice as well as in the water column throughout the year (Kang et al., 1993; Kang and Fryxell, 1992), although its relative abundance considerably decreases after late spring (von Quillfeldt, 2000).

2 Materials and methods

2.1 Cultures

Water samples were collected from Disko Bay (69°11 N, 53°31 W), Western Greenland from the upper 20 m surface layer with a 20 µm mesh plankton net.~~Water samples were collected from Disko Bay (69°11N, 53°31W) on the west coast of Greenland.~~ Six different clonal strains (D3G1, D4D11, D10A12, D5A4, D8F4 and D8G3) of *Fragilariopsis cylindrus* were isolated into clonal cultures in April (D3G1 – 23.4.2011, D4D11 and D10A12 – 26.4.2011,

D5A4 – 29.4.2011) and May (D8F4 and D8G3 – 7.5.2011) 2011 by isolating single cells or single chains. The strains were cultured at 4 °C at 20-30 µmol photons $m^{-2}s^{-1}$ following a light : dark cycle of 16 : 8 h, and the medium used was L1 (Guillard and Hargraves, 1993) based on autoclaved 0.2 µm filtered seawater with a salinity of 33.

2.1.1 Experimental setup

The experiments were carried out at three different temperatures, 1 °C, 5 °C, and 8 °C, and four different pH treatments, pH 8.0, 7.7, 7.4, and 7.1. The experiments were designed to ensure that the cells were kept in the exponential growth phase; hence, for the total duration of the experiments, maximum growth rates were observed. The first set of experiments with six strains (D3G1, D4D11, D10A12, D5A4, D8F4 and D8G3) was carried out at 5 °C and all four pH treatments. 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). ~~Based on these results, the second set of experiments was carried out with a reduced number of strains (D3G1, D4D11 and D10A12) at 8 °C and with all the pH treatments, and the last set with the same reduced number of strains at 1 °C.~~ The cells were exposed to 90–100 µmol photons $m^{-2}s^{-1}$ (Nielsen and Hansen, 1999; Platt et al., 1982) following a light : dark cycle of 16 : 8 h.

For acclimation, each of the six strains was grown in L1 medium, based on 0.2 µm filtered seawater, with a pH value of 8.0, at a temperature of 5 °C, and at a light intensity of 90–100 µmol photons $m^{-2}s^{-1}$ for two days. Three of the strains were acclimated to 8 °C and 1°C, in steps of ~2 °C per day. After two days of acclimation at final temperatures, pH of the cultures was lowered to pH 7.7, 7.4 and 7.1 in steps of 0.3 pH units every 24 hours by addition of strongly acidified L1 medium (pH 0.49 ± 0.02; 5 days of acclimation period). The pH level of the acidified L1 medium was lowered by using gaseous CO_2 (Air Liquid Denmark A/S. UN 1013 Carbon Dioxide, Class 2, 2A, ADR). During the days of acclimation to different temperatures and pH treatments, the strains were grown in 65 mL flasks with L1 medium and exposed to the same light intensity (90–100 µmol photons $m^{-2}s^{-1}$) following a light : dark cycle of 16 : 8 h. The L1 growth medium was selected to ensure nutrient-replete conditions for the algal cultures during the experiment (Lundholm et al., 2004).

The experimental flasks (65 mL) were inoculated with a cell concentration of 1000 cells mL^{-1} and L1 medium of the pH-specific value. All experiments were performed in triplicates. For enumeration of cells, 2 mL were withdrawn and fixed with 30 µL of acidic Lugol's solution (2 % final concentration). Before sub-sampling, each flask was gently rotated vertically at least 15 times to ensure that the cells were equally distributed. Sub-sampling was carried out at approximately 10 a.m. every day, starting with those grown at pH 7.1 and followed by those at 7.4, 7.7 and 8.0. Volumes removed for sub-sampling were replaced with equal volumes of adjusted L1 medium. To avoid large fluctuations of pH, the cultures were diluted on a daily basis with pH-specific media. If desired pH was not obtained after dilution, a few drops of acidified L1 medium were added to lower pH of the samples. The pH level was measured before and after dilution. For cell counting, an inverted light microscope (OLYMPUS CKX31, 100 × magnification) and a Sedgewick-Rafter chamber were used, and a minimum of 400 cells from each sample was counted, corresponding to a deviation of ± 10 % using 95 % confidence limits (Utermöhl, 1958). Sampling was initiated on day 3 to allow the experimental cultures to acclimate to the experimental conditions and to overcome the initial lag phase (day 0–day 2). Thus, the time period from day 0 to day 3 was considered as part of the acclimation period and not included in the results (in total 8 days of acclimation period).

Temperature and pH were measured using a WTW pH 340i pH-meter with a SenTix 41 electrode, with a sensor detection limit of 0.01. The pH electrode was calibrated weekly (2 point calibration) using Sentron buffers of pH 7.0 and 10.0 dilutions.

2.1.2 Dissolved inorganic carbon and nutrients

The concentration of dissolved inorganic carbon (DIC) in fresh media (all four pH treatments) was measured in triplicate. Measurements were done using an infrared gas analyzer (IRGA) and a bicarbonate standard solution (2 mmol L^{-1}), as described in Nielsen et al. (2007).The concentration of carbon species (bicarbonate ion HCO_3^-, carbonate ion CO_3^{2-}, and dissolved carbon dioxide CO_2 (aq) and carbonic acid H_2CO_3) in the media was calculated from pH, salinity, temperature and DIC, using the CO2SYS.XLS program *(set of constants*: K1, K2 from Mehrbach et al. (1973) refit by Dickson and Millero (1987); *$KHSO_4$* from Dickson (1990); *pH scale* as seawater scale (mol/kg-SW) (Lewis and Wallace, 2014).

Samples (3 × 50 ml) for measurements of inorganic nutrients (nitrate NO_3^-, phosphate PO_4^{3-} and silicate $Si(OH)_4$) were taken from L1 medium (pH 8.0) and frozen immediately. The samples were analyzed at the Institute for Bioscience, University of Aarhus, following procedures of Hansen and Koroleff (2007).

2.1.3 Maximum growth rates

Assuming exponential growth of the cells, the maximum growth rates were calculated from the logarithmic curves of cell growths (logarithmic cumulative cell concentrations versus days) using the equation

$$\ln N_1 = \ln N_0 + \mu(t_1 - t_0), \quad (1)$$

where N_0 and N_1 are the number of cells at time t_0 and t_1.

The maximum growth rate for a given strain and pH treatment at a specific temperature was calculated employing linear regression for the steepest part of the growth curve. Linear regression was carried out for each replica of the strain at a given treatment, and the mean of maximum growth rates of the three replicates at a given treatment was taken as the maximum growth rate for that combination of strain and treatment. The temperature coefficients, Q_{10} for the growth rates, as a consequence of increased temperature by 10 °C, were calculated according to the equation

$$Q_{10} = \left(\frac{\mu_{T_2}}{\mu_{T_1}}\right)^{\left(\frac{10}{T_2 - T_1}\right)}, \quad (2)$$

where μ_{T1} and μ_{T2} are the maximum growth rates at temperatures T_1 = 1 °C and T_2 = 8 °C.

2.2 Molecular characterization

All six *F. cylindrus* strains (D3G1, D4D11, D10A12, D5A4, D8F4 and D8G3) were used for molecular characterization of ITS1, 5.8S and ITS2 (ITS – Internal transcribed spacer) of the nuclear rDNA. Cells of each of the six strains were concentrated and frozen. DNA extractions, sequencing and alignment followed Lundholm and Hasle (2008).

2.3 Statistical analyses

All analyses were performed using IBM SPSS Statistics (version 22). Differences between the treatments were tested using three-way ANOVA with temperature and pH as fixed factors, and strain as a random factor. A statistically significant three-way interaction was followed up with simple two-way interactions at all levels, applying a Bonferroni adjustment, and simple main effects for fixed factors pH and temperature. The normal distribution of data was tested using a Shapiro-Wilk test and homogeneity of variances using Levene's test. The level of significance used was 0.05.~~All analyses were performed using IBM SPSS Statistics (version 22). Differences between the treatments were tested using two-way ANOVA, with Bonferroni's correction and Student's t-test. The normal distribution of data was tested using a Shapiro-Wilk test. Levene's test verified the equality of variances in the data (homogeneity of variances). The level of significance used was 0.05.~~

3 Results

3.1 Growth of *Fragilariopsis cylindrus* strains

All strains, cultivated at all combinations of three different temperatures and four different pH treatments, grew exponentially as a function of time, with an acclimation period of three days (Fig. S1 in the Supplement). The differences in growth rates within and among the strains were tested using ~~two~~three-way ANOVA, and a significant interaction among temperature, pH and strain on growth rate was found ($P < 0.05$). This means that the effect of each of the three parameters on growth rates depends on the set of the other two (e.g. the effect of temperature on growth rates depends on pH and strain).~~and a significant interaction between temperature and pH on growth rate was found (all P-values < 0.05). This means that the effect of temperature on growth rates depends on pH, and vice versa.~~

3.1.1 Growth vs. temperature - at four different pH treatments

A general positive effect of increased temperature on the growth rates was observed at all four different pH treatments (Fig. 1). Comparisons of the maximum growth rates among the three different temperatures showed highest growth rates at 8 °C in all four pH treatments, followed by those at 5 °C and 1 °C (Fig. 1). The trend was the same for each of the three strains, D10A12, D4D11 and D3G1. The resulting Q_{10} values according to Eq. (2) are shown in Table 1. The Q_{10} values illustrate that the growth rates, as a consequence of increased temperature

by 10 °C are a strain-specific feature, e.g. growth rate of strain D3G1 increased rapidly with increasing temperature by Q_{10} value of 3.35 (pH 7.1), whereas growth rate of strain D10A12 increased by 1.29 (pH 7.1).

Strain D10A12 showed the overall highest growth rates at the highest temperature (8 °C). At pH 7.1, significant differences were found between the growth rates at 1 °C and the two higher temperatures (5 °C and 8 °C). At pH 7.4 and 8.0, significant differences were observed among all temperatures, whereas at pH 7.7, significant differences were found between 8 °C and the two lower temperatures ($P < 0.05$; Fig. 1a, Fig. S2a). In strains D4D11 and D3G1, significant differences were found between the growth rates for all combinations of treatment (~~pairwise comparisons,~~ $P < 0.05$; Fig. 1b and c, Fig. S2b and c).

3.1.2 Growth vs. pH - at three different temperatures

A general negative effect of increased acidification at three different temperatures on the growth rates was observed (Fig. 2).

At 5 °C, the maximum growth rates were highest in strains D5A4 and D10A12, irrespective of the pH treatment. The maximum growth rates of the three other strains (D4D11, D8F4 and D8G3) were approximately 50–% smaller than those of D5A4 and D10A12 for every pH value, but approximately twice as high as the lowest growth rates observed in strain D3G1 (Fig. 2b, Table S1 in the Supplement). ~~Within the pH 7.1 treatment, significant differences were observed among the growth rates of all the strains (P < 0.05), except between D8G3 and D4D11, and D5A4 and D10A12 (P > 0.05; Fig. S3-IIa). Within the pH 7.4 and 7.7 treatments, significant differences between the growth rates of each pair of the strains were observed (P < 0.05), except between D8G3 and D4D11 (P > 0.05; Fig. S3-IIb-c). Within the 8.0 pH treatment, significant differences were observed among the growth rates of all the strains (P < 0.05; Fig. S3-IId).~~

Overall we found a decrease in growth rates from pH 8.0 to pH 7.1 at 5 °C (Fig. 2b, Table S1), yet with variation among strains. In strain D8F4, the highest maximum growth rates were observed at pH 8.0, and gradually lower growth rates were observed with increasing acidification. The maximum growth rates in strains D4D11 and D5A4 overall decreased with increasing acidification, although a slight increase at pH 7.4 was observed. In strains D8G3 and D3G1, the maximum growth rates increased from pH 8.0 to pH 7.4, and then decreased at pH 7.1. The maximum growth rates in strain D10A12 were approximately the same in all four

pH treatments. In D4D11 and D3G1 strains, significant differences between the growth rates were observed for all combinations of pH treatment ($P < 0.05$), and no significant differences for any pH combination in strain D10A12 ($P > 0.05$). Within strains D8G3 and D5A4, significant differences between the growth rates were observed for all combinations of pH treatment ($P < 0.05$), except for the pH combination 7.7 – 8.0 ($P > 0.05$). Similarly, significant differences between the growth rates for all combinations of the pH treatment ($P < 0.05$) apart from the pH combination 7.4 – 7.7 were observed in strain D8F4 (~~pairwise comparisons,~~ $P > 0.05$; Fig. S3-II).

At 1 °C, strain D10A12 exhibited the highest maximum growth rates, irrespective of the pH treatment (Fig. 2a). The maximum growth rates of strains D4D11 and D3G1 were approximately 50 % and 70 % smaller than those of D10A12 for every pH value (Fig. 2a, Table S2). ~~Within all four pH treatments, significant differences between the growth rates of each pair of strains were observed ($P < 0.05$).~~

For all three strains grown at 1 °C, the maximum growth rates at different pH treatments were highest at pH 8.0, and gradually decreased with increased acidification in strains D10A12 and D3G1. In strain D4D11, the maximum growth rates first decreased from pH 8.0 to pH 7.7, then ~~slightly~~ increased at pH 7.4, and again decreased at pH 7.1 (Fig. 2a, Table S2). Within all three strains, significant differences among the growth rates were observed for all combinations of pH treatment (~~pairwise comparisons,~~ $P < 0.05$; Fig. S3-I).

At 8 °C, the maximum growth rates were highest in strain D10A12, irrespective of the pH treatment, followed by strain D4D11 (~30-% lower) and strain D3G1 (~60-% lower) (Fig. 2c, Table S3). ~~In all four pH treatments, significant differences were observed among the growth rates of each pair of the strains ($P < 0.05$).~~

In the three strains grown at 8 °C, the maximum growth rates were highest at pH 8.0, and gradually lowered with increasing acidification (Fig. 2c, Table S3). In D3G1 strain, significant differences were observed among the growth rates for all combinations of pH treatments ($P < 0.05$). Significant differences among the growth rates for all combinations of the pH treatments except for the pH combination 7.4 – 7.7 ($P > 0.05$) were observed in strains D10A12 and D4D1 (~~pairwise comparisons,~~ $P < 0.05$; Fig. S3-III).

3.2 Experimental temperature, pH, DIC and nutrients

Temperature and pH in the experimental treatments fluctuated minimally around the designated values (< ~~0.05~~0.6 °C and < 0.03 units, respectively) (Fig. 3, Table 2). The DIC concentrations increased with decreasing pH of the medium. In all treatments, the concentration of HCO_3^- exceeded 90 % of the total inorganic carbon, with the highest share being observed at pH 7.1, and the lowest at pH 8.0. The concentration of CO_2 (aq) and H_2CO_3 decreased with increasing pH, from 199.8 ± 4.3 µmol L^{-1} at pH 7.1 to 24.3 ± 1.4 µmol L^{-1} at pH 8.0, whereas the concentration of CO_3^{2-} increased from 16.9 ± 0.4 µmol L^{-1} at pH 7.1 to 96.7 ± 4.9 µmol L^{-1} at pH 8.0 (Table S4). The concentrations of nutrients NO_3^-, PO_4^{3-} and $Si(OH)_4$ from L1 medium (pH 8.0) were 523.04 ± 5.70 µM, 30.06 ± 0.85 µM and 47.44 ± 4.03 µM, respectively, which fitted the Si:N:P = 16:16:1 ratio of marine diatoms (Justić et al., 1995).

3.3 Molecular identification

The sequences of ITS1, 5.8S and ITS2 of all six strains were identical to each other and also identical to strain Real9 of *F. cylindrus* in Genbank with accession number EF660056, confirming their identity and similarity.

4 Discussion and conclusions

4.1 Growth of multiple strains of the Arctic diatom *Fragilariopsis cylindrus* at different temperatures and pH

Fragilariopsis cylindrus is an ecologically important polar sea-ice and phytoplankton species, and as a model organism it may help us improve the understanding of the consequences resulting from changes in the atmospheric CO_2 concentration and concurrent SST rise in high-latitude environments. By manipulating temperature and pH levels in laboratory experiments, plausible future climate change scenarios were simulated. Throughout the experiment, the temperature and pH of the experimental treatments fluctuated minimally (< ~~0.05~~0.6 °C and < 0.03 units, respectively; Table 2), making the treatments clearly separate from each other, and thus enabling an evaluation of the combined effects of ocean acidification and temperature on several strains of a microalgal species.

4.1.1 Combined effects of temperature ~~and~~, pH and strain on growth of ~~the multiple~~ *F. cylindrus* ~~strains~~

Future marine phytoplankton will not be exposed solely to a decrease in pH but also to other concurrent changes such as increased SST, which is why it is important to consider cumulative effects of multiple climate stressors (e.g. present study; Schlüter et al., 2014; Xu et al., 2014). This study showed a statistically significant interaction among pH, temperature and strain on the growth of all *F. cylindrus* strains cultivated at four pH treatments and three temperatures (three-way ANOVA, $P < 0.05$).~~This study showed a statistically significant interaction between pH and temperature on the growth of all *F. cylindrus* strains cultivated at four pH treatments and three temperatures (two-way ANOVA, P < 0.05).~~ An overall positive effect of increased temperature and ~~alkalinisation~~ increased pH on the growth rates at the same time was detected for *F. cylindrus*, despite the variability in strain-specific responses (Figs. 1 and 2).

The variation in the growth rates within a single species suggests variation in evolutionary potential within species (Beaufort et al., 2011; Langer et al., 2009), which is why it is important to take intra-specific diversity into account when trying to understand the physiology and evolution of natural populations (Collins et al., 2014). This study showed that different strains of *F. cylindrus* can be affected by climate change in different ways. At a temperature of 5 °C and different pH treatments, some strains experienced positive, negative or no effects when treated with specific pH treatment. In contrast, a more general pattern in growth rates was observed at 1 °C and 8 °C at specific pH treatments – (1) the growth rates of all three strains increased with ~~alkalinity~~ decreasing acidification (from pH 7.1 to 8.0), and at the same time (2) all three strains exhibited highest growth rates at pH 8.0 which further increased with elevated temperature (from 1 °C to 8 °C). These two observed patterns illustrate that the combination of pH and temperature counterbalances each other, with the response also being dependent on a strain.~~These two observed patterns illustrate that the combination of both parameters counterbalances each other.~~ Here is why: if the growth rates of the three strains observed at pH 8.0 and temperature 1 °C, which represent the present conditions in the Arctic environments, are compared with the growth rates obtained at pH 7.7 and temperature 5 °C, which are the conditions expected by the year 2100, no effect of the elevated temperature and acidification can be found (e.g. D3G1 $\mu = 0.22 \pm 0.00$ and $\mu = 0.23$

± 0.00, respectively). Similar results were observed for *F. cylindrus* strain from the Antarctic when exposed to pH 7.8 and temperature of 6 °C (Xu et al., 2014).

In contrast, if one parameter is examined at a time, a general positive effect of increased temperature (see Sect. 4.1.2), and a general negative effect of increased acidification (see Sect. 4.1.3) is found. However, one has to take into consideration that the largest variability was found among the strains (the random factor). Some strains showed better performance than others when cultivated in the same conditions, indicating that these strains may display high resilience to the changes in pH and temperature predicted for the 21st~~21~~st century (e.g. present study; Kremp et al., 2012; Langer et al., 2009). Climate change may therefore lead to alterations in strain composition, with the strains exhibiting high phenotypic plasticity, in terms of temperature and pH tolerance, dominating the population. To our knowledge, this is the first study reporting the intra-specific variability of a phytoplankton species from the polar environments in response to elevated temperatures and ocean acidification.

4.1.2 Effects of temperature on growth of the multiple *F. cylindrus* strains

A change of temperature had significant effects on *F. cylindrus* growth rates (Fig. 2). The increasing growth rates correlated to elevated temperatures despite the variability in strain-specific responses. One of the fastest growing strains (D10A12) generally exhibited 50 % higher growth rates than the other strains, irrespective of the treatment. The growth rates were always highest at 8 °C regardless of pH. The slowest growing strain (D3G1) displayed the biggest differences in growth rates when cultured at elevated temperatures, with ~50 % higher growth rates at 8 °C compared to those at 1 °C. The fastest growing strain (D10A12) exhibited ~20 % higher growth rates at 8 °C compared to 1 °C. The resulting Q_{10} values were found to be strain-specific, ranging from 1.29 to 3.35 (1–8 °C; Table 1). These results are in agreement with findings on *F. cylindrus* exhibiting increased growth rates at elevated temperatures by approximately 45 % (Q_{10} = 2.28) in the temperature range of -1–7 °C (Mock and Hoch, 2005). Likewise, Torstensson et al. (2012) reported that at elevated temperatures (from 0.5 to 4.5 °C) the growth rates of the benthic/sea ice diatom *Navicula directa* increased by approximately 43 %. As similar data on polar phytoplankton are restricted, the present study provides important information for evaluating the effects of temperature increase in polar areas. *Fragilariopsis cylindrus*, a polar microalgal representative, is well-adapted to a wide range of temperatures due to the phenotypic variation among strains and temperature

adaptation of individual strains. We found that increased temperatures had greatest impact on the slowest growing strain (Table 1, Fig. 2).

The noteworthy variability in strain-specific responses, with growth rates varying up to ~65 % suggests that some strains perform better when exposed to perturbations in the environment than others (Fig. 2, Tables S1-3). This high phenotypic plasticity in terms of temperature tolerance could be explained by the large temperature fluctuations that occur concomitantly with changes in solar irradiance, which phytoplankton experiences on daily and seasonal bases in polar environments. In Disko Bay, the spring SST vary from -1.1 ± 0.3 °C to 2.3 ± 2.0 °C (based on 4-year temperature data provided by DiskoBasis/Arctic Station, Faculty of Science, University of Copenhagen), and from -1.8 °C to 6.7 °C during the year (Hansen et al., 2012) with an average SST of 1.8 ± 1.2 °C (March–December; DiskoBasis/Arctic Station). Similar intra-specific variation has also been observed among strains of the diatom *Skeletonema marinoi* from two geographic areas. Kremp et al. (2012) reported that *S. marinoi* strains from the North Sea, which also experience large temperature fluctuations in their natural environment, uniformly exhibited higher growth rates at elevated temperatures (at 24 °C compared to 20 °C), indicating that the ability to adjust to varying temperature is advantageous for species. In contrast, *S. marinoi* strains from the temperate Adriatic Sea, where the temperature is known to be more stable, responded to elevated temperatures in different ways, with some strains being unaffected and others being positively or negatively affected by temperature changes (Kremp et al., 2012). The present study confirms previous notion of high intra-specific variability within species, and emphasizes, that this variation might be even larger than the variation observed due to changing environmental factors, stressing the need for several strains when exploring the environmental effects on species.

4.1.3 Effects of pH on growth of the multiple *F. cylindrus* strains

Acidification results in both decreasing pH and increasing CO_2 concentration. Generally, rising CO_2 is considered to facilitate photosynthetic carbon fixation by some phytoplankton groups (Riebesell, 2004). The direct effect of changes in environmental pH is less clear but recent studies have shown that it can affect intracellular pH and membrane potential, as well as enzyme activity (McMinn et al., 2014). In the present study, the concentration of DIC and the carbon species at pH 8.0 corresponded well to the concentrations found in the ocean surface; ~2 mmol L^{-1} DIC, with ~90 % HCO_3^-, ~9 % CO_3^{2-}, and ~1 % CO_2 (aq) and H_2CO_3 (Feely et al., 2009; Riebesell, 2004). DIC increased with increased acidification from ~2.15

mmol L^{-1} at pH 8.0 to ~2.65 mmol L^{-1} at pH 7.1 (Table S4), which is in agreement with the predictions by Feely et al. (2009).

The present study found a general negative effect of increasing acidification on *F. cylindrus* growth, and similarly to what was observed for temperature, a significant variability among the strains was observed (Fig. 1). A decrease in pH to 7.7, which is the expected global change in pH by the end of this century, and further decrease to 7.4, negatively affected growth of most of the strains. Reduced growth rates by 2–23 % for a pH of 7.7 and 4–29 % for 7.4, were observed (compared to pH 8.0). On the other hand, some strains were unaffected by the increased seawater acidity, and some of them were even positively affected (the growth rates increased up to 15 % for a pH of 7.7 and 25 % for a pH of 7.4 compared to pH 8.0). A further decrease in pH to 7.1 reduced the growth rates of most of the strains by 20–37 %, as compared to those at pH 8.0. Similarly, McMinn et al. (2014) reported that the brine algal community experienced significantly reduced growth rates at pH 7.6, and when treated at pH 7.2, the growth decreased by 50 % compared to a pH of 8.0. Reduced growth rates due to increased acidification were also observed in *Navicula directa*. At a pCO_2 level of 960 ppm (corresponding to pH ~7.7), the diatom experienced reduced growth by 5 % as compared to 380 ppm (corresponding to pH ~8.1) (Torstensson et al., 2012). Similar to our results, a recent study on ocean acidification in the Arctic marine ecosystem found decreased growth rates of the polar phytoplankton community when exposed to increased seawater acidity; community growth rates gradually decreased with increasing acidification (from 8.0 to 7.4) with a greater reduction at pH 7.1, where the community experienced reduced growth rates by 55 % as compared to pH 8.0 (Thoisen et al., 2015).

To date, studies on phytoplankton responses to ocean acidification have mainly been focused on temperate or tropical regions, and only a few studies have been carried out in polar regions (e.g. present study; McMinn et al., 2014; Thoisen et al., 2015; Torstensson et al., 2012). However, increased pCO_2 (~1000 ppm, which corresponds to pH ~7.7) affected tropical phytoplankton communities, which were found to respond with decreased primary production by 7–36 % (Gao et al., 2012). In contrast, Berge et al. (2010) and Nielsen et al. (2010) showed that the growth of coastal marine phytoplankton was, in general, unaffected by ocean acidification (pH ~7.0–8.5 and 7.6–8.0, respectively). They speculated that common natural pH fluctuations in coastal regions made phytoplankton more pH-tolerant in these areas and therefore growth was not affected. Similar pH fluctuations were observed in the Arctic coastal

waters (Disko Bay) during the spring bloom in 2012, with a pH gradient of 7.5–8.3. The pH fluctuation was found to be caused by the transition from the polar night period and the dominating respiration processes (pH ~7.5) to the polar day period with the increasing phytoplankton biomass and concomitant photosynthesis (Thoisen et al., 2015). The present study showed that *F. cylindrus* is generally well-adapted to acidification down to ~pH 7.4, although with notable strain-specific response variability. Some strains (e.g. D10A12, D4D11, D5A4, and D8G3) were slightly affected by the lower pH (-10 % < μ < +10 %), whereas others (e.g. D3G1, D8F4) responded with greater reduction in the growth rates (< 29 %). Similar observations on the strain-specific response were also reported among other phytoplankton species (Kremp et al., 2012; Langer et al., 2009). Thus, these observations suggest that shifts in dominance among strains due to ocean acidification might be expected.

Long-term adaptation to environmental parameters of *F. cylindrus* strains was not considered in this study. However, the adaptation is expected to increase phenotypic plasticity (Schlüter et al., 2014) of the strains, and therefore the ability of the species to adapt to future climate conditions should increase even more. It should be noted that controlled laboratory experiments cannot mimic real environmental changes (Schlüter et al., 2014), yet they are at present the only direct way for simulating plausible future climate changes and examining their effects on marine phytoplankton.

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Table 1. The Q_{10} values (1–8 °C) for D10A12, D4D11 and D3G1 strains were calculated according to Eq. (2), based on the mean maximum growth rates displayed in Table S2 and Table S3.

pH treatments	D10A12	D4D11	D3G1
7.1	1.29	2.42	3.35
7.4	1.54	2.06	2.34
7.7	1.48	2.45	2.81
8.0	1.46	2.36	2.43

Table 2. The average temperatures ± SD (°C) and pH values ± SD in the experimental treatments from day 0 to day 7; $n^a = 12$; $n^b = 24$.

	Average temperatures (°C)		
	1.4 ± 0.6 °C[a]	5.4 ± 0.1 °C[b]	7.7 ± 0.2 °C[a]
Average pH values	7.10 ± 0.01[a]	7.09 ± 0.02[b]	7.11 ± 0.01[a]
	7.37 ± 0.01[a]	7.38 ± 0.02[b]	7.40 ± 0.01[a]
	7.64 ± 0.01[a]	7.67 ± 0.02[b]	7.68 ± 0.02[a]
	7.91 ± 0.01[a]	7.95 ± 0.01[b]	7.95 ± 0.03[a]

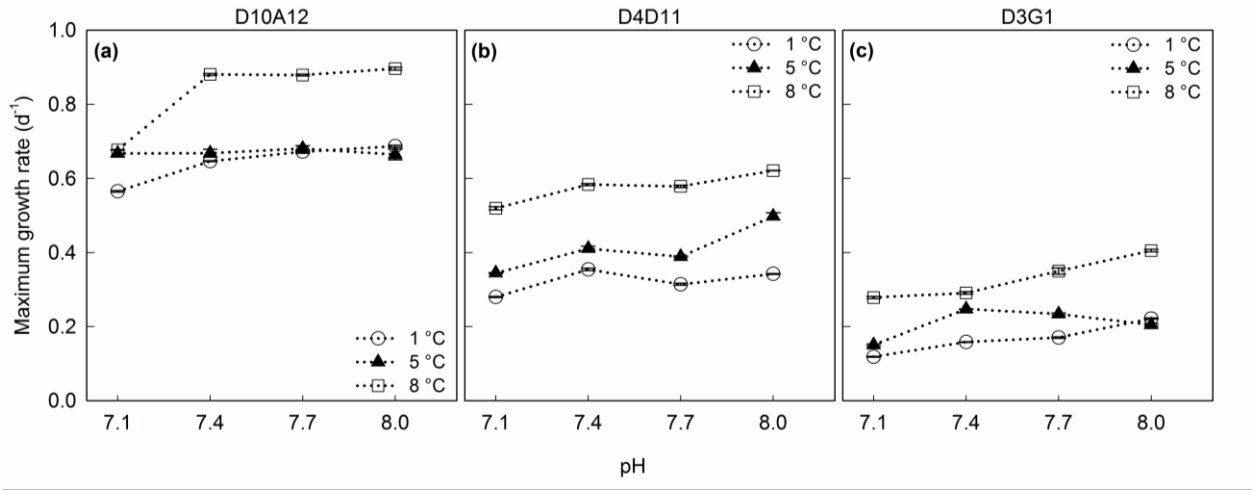

Figure 1. The mean maximum growth rates (d^{-1}) for strains (a) D10A12, (b) D4D11, and (c) D3G1 cultured at temperatures of 1 °C, 5 °C and 8 °C, and four different pH treatments. Error bars represent ± ~~one~~ SD. Note that the data are categorical, and the dotted lines serve to show the trend.

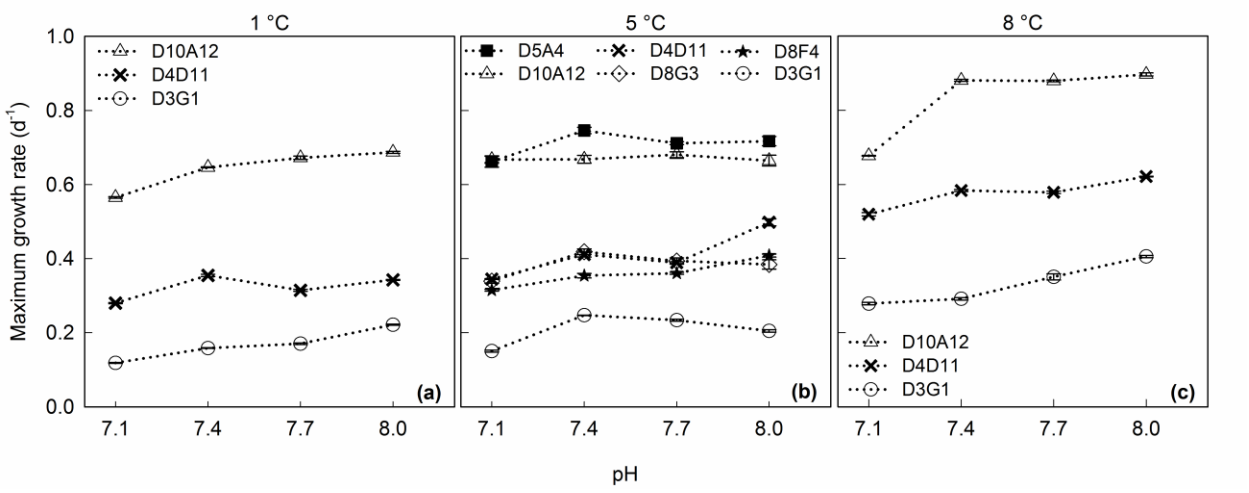

Figure 2. The mean maximum growth rates (d^{-1}) of strains D5A4, D10A12, D4D11, D8G3, D8F4 and D3G1 cultivated at (a) 1 °C, (b) 5 °C and (c) 8 °C, and all four pH treatments. Error bars represent ± ~~one~~ SD. Note that the data are categorical, and the dotted lines serve to show the trend.

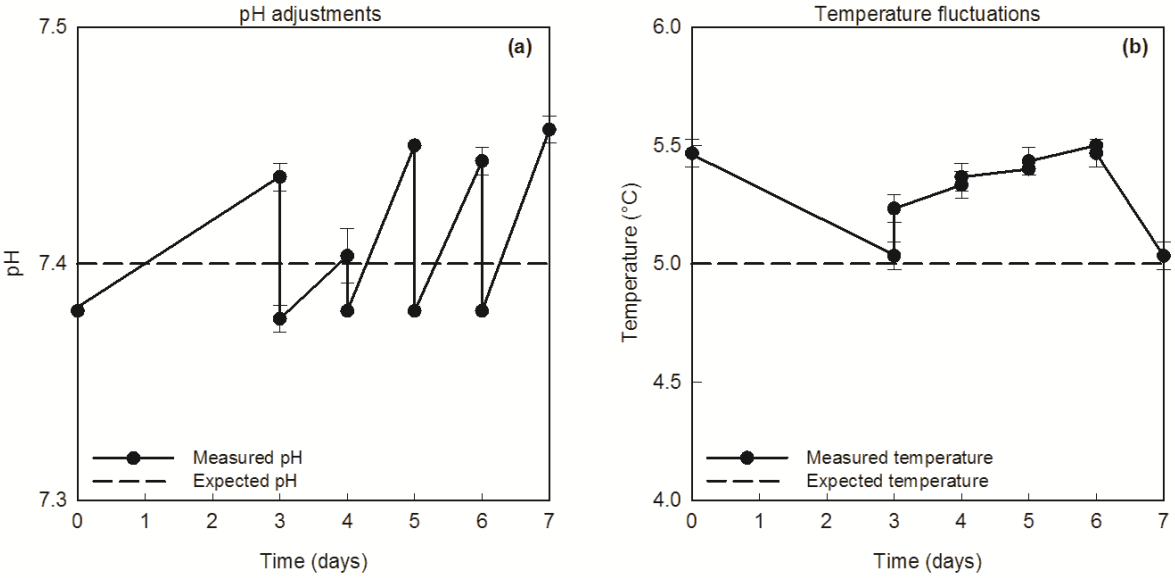

Figure 3. (a) An example of pH ~~dilutions~~ adjustments in the pH treatment 7.4 at 5 °C, shown as a function of time. (b) An example of temperature fluctuations in the treatment with pH 7.4, shown as a function of time. The first three days represent the acclimation period and are not included in the results. Error bars represent ± ~~one~~ SD.