Responses to comments

Dear referees,

We thank you for your supportive comments on our manuscript. Our detailed response in blue text to your comments is attached. Changes to the manuscript text are underlined.

Responses to comments of referee 1

Summary

Zhang et al. conducted a series of experiments with multiple strains of Emiliania huxleyi isolated from 3 different North Atlantic populations. Each strain was incubated under a broad range of pCO2 concentrations (about 120-2600uatm) but with constant total alkalinity to discern between effects due to changes in the carbonate systems and changes in CO2 levels. The physiological responses that Zhang et al measured were growth rates, PIC and POC production rates. They conclude that there were differences among strains and among populations but those differences depended on the physiological rate.

General comments

The manuscript is very well written. The ideas, methods and discussion are also clear and well structured, making the manuscript flow very well. This is high quality and thorough work and it deserves to be published. However, my main comment is perhaps related to the novelty of the work and I will make some suggestions as to how this could be addressed. Zhang et al. do a good job citing some of the previous relevant studies but their work would be better served by emphasizing how their work is significantly different and why this is important. We already know from studies like Iglesias-Rodriguez, Bach, Langer, etc., that there are CO2 effects in coccolithophore's physiological rates and we also know from Langer et al.'s work that these are species-specific and strain-specific responses, so (in my humble opinion) there is not much surprise in finding that there are population-specific differences. Throughout the manuscript the authors hint at the ideas of phenotypic plasticity and environmental variability. This, on the other hand is not so common, and I suggest that the authors elaborate more on this. They already show the pCO2 and temperature ranges in those 3 sites and it is used to explain the results. Fully accounting for this variability at the original field site is important and they should emphasize that. Acknowledging this variability is usually not done。

Response: We thank this referee for the positive comments. We summarized responses of growth, POC and PIC production rates of different *Emiliania huxleyi* strains to CO_2 and found that most of these studies focused on a few strains or a narrow range of CO_2 level (Table R1). In this study, we used 17 strains and measured growth, POC and PIC production rates at 120 µatm to 2630 µatm, which are different from previous studies. **These contents were shown in lines 84–87.**

For lines 333–336: When exposed to 16 °C, growth rate of the Canary Islands population might have been already below their optimum and hence significantly reduced in comparison to the other populations ~~thus it grew slower than the other populations~~ (Fig. 2d).

For lines 388–391: Phenotypic plasticity constitutes an advantage for individual strains to acclimate and adapt to elevated pCO_2 by changing ~~their~~ fitness-relevant traits and potentially to attenuate the effects of changing environments on fitness-relevant traits (Schaum et al., 2013).

For lines 395–397: Physiological variability makes a population more resilient, ~~and~~ increases its ability to persist in variable environments and potentially forms the basis for selection (Gsell et al., 2012; Hattich et al., 2017).

Table R1. Summary of the physiological responses of different *E. huxleyi* genotypes to various pCO_2 ranges at constant alkalinity condition. Symbols indicate: ↑ increased response, — no response, ↓ decreased response, ∩ optimum response.

E. huxleyi genotype	Isolated site	pCO_2 range (µatm)	Growth rate	POC pro.	PIC pro.	Incubation temp. (°C)	Reference
AC472	South Pacific, New Zealand	400 to 760	↑	—	↑	19	Fiorini et al., (2011)
EHTB 11.15	Trumpeter Bay, Tasmania	375 to 1650	—	∩	↓	14	Müller et al., (2015)
EHSO 5.14	Southern Ocean	300 to 1680	↓	∩	∩	14	Müller et al., (2015)
EHSO 5.11	Southern Ocean	259 to 1255	∩	∩	∩	14	Müller et al., (2015)
NIWA1108	Chatham Rise, New Zealand	80 to 1080	↑	↑	∩	4-25	Feng et al., (2017)
PLY M219 (NZEH)	New Zealand	380 to 750	↓	↓	↓	20	Shi et al., (2009)
PLY M219 (NZEH)	New Zealand	404 to 1066	↓	↑	↓	15	Hoppe et al., (2011)
PML B92/11A	Bergen, Norway	152 to 885	—	↑	↓	15	Riebesell et al., (2000)
PML B92/11A	Bergen, Norway	20 to 6000	∩	∩	∩	15	Bach et al., (2011)
RCC1212	South Atlantic, off South Africa	194 to 1096	↓	∩	↓	20	Langer et al., (2009)
RCC1216	Tasman Sea, off New Zealand	218 to 1201	↓	↑	↓	17	Langer et al., (2009)
RCC1238	North Atlantic, off Japan	206 to 929	↑	∩	—	20	Langer et al., (2009)
RCC1256	North Atlantic, off Iceland	193 to 915	↓	∩	∩	17	Langer et al., (2009)
RCC1256	Iceland	191 to 846	↓	↓	↓	15	Hoppe et al., (2011)
NZEH	New Zealand	280 to 750	↓	↑	↑	19	Iglesias-Rodriguez et al., (2008)

NZEH	New Zealand	395 to 1340	↓	↑	↑	19	Jones et al., (2013)

Bach, L. T., Riebesell, U., and Schulz, K.G.: Distinguishing between the effects of ocean acidification and ocean carbonation in the coccolithophore *Emiliania huxleyi*, Limnol. Oceanogr., 56, 2040–2050, 2011.

Beardall, J., and Raven, J. A.: Potential effects of global change on microalgal photosynthesis, growth and ecology, Phycologia, 43, 26–40, 2004.

Feng, Y. Y., Roleda, M. Y., Armstrong, E., Boyd, P. W., and Hurd, C. L.: Environmental controls on the growth, photosynthetic and calcification rates of a Southern Hemisphere strain of the coccolithophore *Emiliania huxleyi*, Limnol. Oceanogr., 62, 519–540.

Fiorini, S., Middelburg, J. J., and Gattuso, J. P.: Testing the effects of elevated pCO_2 on coccolithophores (prymnesiophyceae): comparison between haploid and diploid life stages, J. Phycol., 47, 1281–1291, 2011.

Hoppe, C. J. M., Langer, G., and Rost, B.: *Emiliania huxleyi* shows identical responses to elevated pCO_2 in TA and DIC manipulations, J. Exp. Mar. Biol. Ecol., 406, 54–62, 2011.

Iglesias-Rodriguez, M. D., Halloran, P. R., Rickaby, R. E. M., Hall, I. R., Colmenero-Hidalgo, E., Gittins, J. R., Green, D. R. H., Tyrrell, T., Gibbs, S. J., von Dassow, P., Rehm, E., Armbrust, E.V., and Boessenkool, K.P.: Phytoplankton calcification in a high-CO_2 world, Science, 320, 336–340, 2008.

Jones, B. M., Iglesias-Rodriguez, M. D., Skipp, P. J., Edwards, R. J., Greaves, M. J., Young, J. R., Elderfield, H., and O'Connor, C. D.: Responses of the *Emiliania huxleyi* proteome to ocean acidification, PLoS One 8(4), e61868, doi: 10.1371/journal.pone.0061868, 2013.

Langer, G., Nehrke, G., Probert, I., Ly, J., and Ziveri, P.: Strain-specific responses of *Emiliania huxleyi* to changing seawater carbonate chemistry, Biogeosciences, 6, 2637–2646, 2009.

Müller, M. N., Trull, T. W., and Hallegraeff, G. M.: Differing responses of three Southern Ocean *Emiliania huxleyi* ecotypes to changing seawater carbonate chemistry, Mar. Ecol. Prog. Ser., 531, 81–90, 2015.

Riebesell, U., Zondervan, I., Rost, B., Tortell, P. D., Zeebe, R. E., and Morel, F. M. M.: Reduced calcification of marine plankton in response to increased atmospheric CO_2, Nature, 407, 364–367, 2000.

Shi, D., Xu, Y., and Morel, F. M. M.: Effects of the pH/pCO_2 control method on medium chemistry and phytoplankton growth, Biogeosciences, 6, 1199–1207, 2009.

Specific comments

While isolating the effect of CO2 from changes in TA is a great idea, it also poses the question of whether the same experiment should have been repeated letting the TA change with CO2 concentration. It begs the question of "how would the results look like if TA could change?". After all, this is a more realistic situation and it would contribute to our understanding of E hux responses to a changing World. While I acknowledge that this would be an entire new project, I think it is my role to bring it up. Perhaps acknowledging the caveat would be enough.

Response: We did not 'isolate the effect of CO_2 from changes in TA', and our CO_2 manipulations are mimicking ongoing ocean acidification where CO_2/pH and DIC changes at constant TA.

As shown in Tables R2 and R3, rising pCO_2 level dominantly decreased pH at increasing TA conditions. According to studies of Bach et al. (2011), after optimum CO_2 levels, low pH inhibited growth, POC and PIC production. Thus, we expected that growth, POC and PIC production rates should show optimal curve responses to a broad CO_2 range at changing TA.

Table R2. Carbonate chemistry parameter at constant pCO_2 levels.

TA (µmol L^{-1})	**DIC** (µmol kg^{-1})	**pH** (total scale)	***p*CO$_2$** (µatm)	**HCO_3^-** (µmol kg^{-1})	**CO_3^{2-}** (µmol kg^{-1})	**CO_2** (µmol kg^{-1})	**Ω**
1500	1351.6	7.887	400	1245.0	93.7	12.9	2.24
1600	1436.9	7.912	400	1318.8	105.1	12.9	2.51
1700	1521.8	7.935	400	1391.8	117.1	12.9	2.80
1800	1606.3	7.957	400	1463.9	129.5	12.9	3.10
1900	1690.4	7.978	400	1535.1	142.4	12.9	3.41
2000	1774.2	7.997	400	1605.4	155.8	12.9	3.73
2100	1857.5	8.016	400	1675.0	169.6	12.9	4.06
2200	1940.6	8.033	400	1743.8	183.8	12.9	4.40
2300	2023.3	8.050	400	1811.9	198.4	12.9	4.75
2400	2105.6	8.066	400	1879.2	213.5	12.9	5.11
2500	2187.7	8.081	400	1945.9	228.9	12.9	5.47
2600	2269.4	8.095	400	2011.8	244.7	12.9	5.85
2700	2350.8	8.109	400	2077.1	260.8	12.9	6.24
2800	2432.0	8.122	400	2141.8	277.3	12.9	6.63

Table R3. Carbonate chemistry parameter at changing pCO_2 levels and changing TA conditions.

TA (µmol L^{-1})	**DIC** (µmol kg^{-1})	**pH** (total scale)	***p*CO$_2$** (µatm)	**HCO_3^-** (µmol kg^{-1})	**CO_3^{2-}** (µmol kg^{-1})	**CO_2** (µmol kg^{-1})	**Ω**
1500	1254.0	8.134	200	1101.0	146.5	6.5	3.51
1600	1436.9	7.912	400	1318.8	105.1	12.9	2.51
1700	1576.7	7.783	600	1470.2	87.1	19.4	2.08
1800	1701.7	7.694	800	1598.6	77.2	25.8	1.85
1900	1819.7	7.628	1000	1716.2	71.2	32.3	1.70
2000	1934.0	7.576	1200	1827.9	67.3	38.8	1.61
2100	2046.0	7.534	1400	1936.0	64.7	45.2	1.55
2200	2156.4	7.500	1600	2041.7	63.0	51.7	1.51
2300	2265.8	7.470	1800	2145.8	61.8	58.1	1.48
2400	2374.4	7.445	2000	2248.7	61.1	64.6	1.46
2500	2482.4	7.422	2200	2350.7	60.7	71.1	1.45
2600	2590.1	7.403	2400	2452.0	60.6	77.5	1.45
2700	2697.4	7.386	2600	2552.8	60.6	84.0	1.45
2800	2804.4	7.370	2800	2653.2	60.8	90.4	1.45

I am a bit confused about how the incubations were done (not saying it is wrong) but perhaps a diagram or flow chart would be helpful. I mention this in the technical comments section as well.

Response: We agree with this referee and present a flow chart which shows the experimental protocol. This flow chart was added in the supplement information as Figure S1.

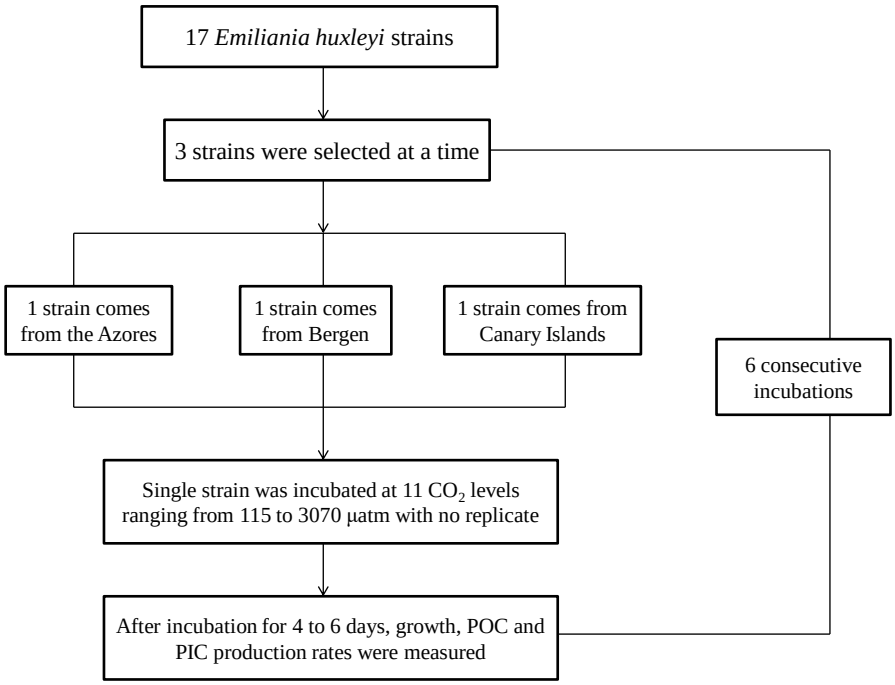

Figure R1 (S1). A flow chart of the experimental protocol.

Also, how realistic are CO_2 levels greater than 1500 uatm?

Response: According to business-as-usual CO_2 emissions (RCP8.5), atmospheric CO_2 level are projected higher than 1500 ppmv after 2200 (Meinshausen et al. 2011).

Meinshausen, M., Smith, S. J., Calvin, K., Daniel, J. S., Kainuma, M. L. T., Lamarque, J., Matsumoto, K., Montzka, S. A., Raper, S. C. B., Riahi, K., Thomson, A., Velders, G. J. M., and van Vuuren, D. P. P.: The RCP greenhouse gas concentrations and their extensions from 1765 to 2300, Climatic Change, 109, 213–241, 2011.

It is very interesting that they found almost no differences in PIC production rates among populations, yet growth and POC production rates did show differences at the population level. Why do you think this is? One factor that the authors mention briefly is temperature, I think that temperature-adaptation and temperature-CO2 interactions might have a greater role in explaining the differences than what the authors attribute to it. In some ways the 3 populations sit along a gradient of temperature and CO2 and depending on which physiological rate is studied, one parameter might be more important than the other. Zhang et al do mention that growing certain cultures under suboptimal temperatures may have set that

strain or population at a disadvantage from the beginning. Interactions between temperature and CO2 effects should not be discarded.

Response: We thank the referee for this suggestion.

For lines 337–348: These contents 'One of the reasons may be that compared to the Azores and Bergen populations, 16 ^{o}C likely causes lower carbon uptake and carbon-use efficiency of the Canary Islands population (Sett et al., 2014).' were replaced by 'Furthermore, compared to the Canary Islands population, the Azores population had higher maximum growth and POC production rates, and similar optimum CO_2 for these physiological rates. Again, this might be related to sub-optimal incubation conditions as temperature has been found to significantly modulate CO_2 responses in coccolithophores in terms of maximum rates, CO_2 optima and half-saturation, and H^+ sensitivity (De Bodt et al., 2010; Sett et al., 2014; Gafar et al., 2018; Gafar and Schulz, 2018). In a similar fashion light can also modulate CO_2 responses, hence different requirements by strains adapted to different light availabilities could also explain our observations (Zhang et al., 2015; Gafar et al., 2018; Gafar and Schulz, 2018).'

For lines 350–355: In addition, the Canary Islands population showed smallest variability in optimum pCO_2 and maximum values for growth and POC production rates (Fig. 2). The reason may be that low incubation temperature predominantly limited growth and POC production rates of the Canary Islands population, and decreased the sensitivities of these physiological rates to rising pCO_2.

De Bodt, C., Van Oostende, N., Harlay, J., Sabbe, K., and Chou, L: Individual and interacting effects of pCO_2 and temperature on *Emiliania huxleyi* calcification: study of the calcite production, the coccolith morphology and the coccosphere size, Biogeosciences, 1401–1412, 2010.

Gafar, N. A., Eyre, B. D., and Schulz, K. G. : A conceptual model for projecting coccolithophorid growth, calcification and photosynthetic carbon fixation rates in response to global ocean change, Front. Mar. Sci., 4, 433, doi: 10.3389/fmars.2017.00433, 2018.

Gafar, N. A., and Schulz, K. G. : A niche comparison of *Emiliania huxleyi* and *Gephyrocapsa oceanica* and potential effects of climate change, Biogeosci. Discuss., doi: 10.5194/bg-2018-88.

Another consideration is that Zhang et al do a great job by showing that there are different ranges of variability in the places where they were isolated from and they use this argument to explain the differences. However, their cultures are maintained at a constant CO2 concentration (and light pattern and temperature). As the authors suggest in this manuscript, the next generation of experiments should account for variability at its origin and hence variable environmental parameters (within a given range) in experimental designs. Plasticity and adaptation are key parameters to consider in the future.

Response: we agree with this referee.

For lines 388–391: Phenotypic plasticity constitutes an advantage for individual strains to acclimate and adapt to elevated pCO_2 by changing fitness-relevant traits and potentially to attenuate the short-term effects of changing environments on fitness-relevant traits (Schaum et al.,

2013).

Finally, Zhang et al found some very interesting results, some of which were not fully explored. For example, the optimum pCO2 is higher for Bergen than the other 2 regions, but the temperature optimum in Bergen is lower, what are the implications for future projections? Similarly, all strains but one showed that the pCO2 optimum for POC is greater than the optimum for PIC and growth rates, how do you think this might affect future PIC: POC ratios? What about the sensitivity constant results? OR Bergen populations experiencing the higher CO2 optimum and smallest variability between strains vs. Canary islands showing lowest optimums but highest variabilities in CO2 optimums….. These are just some examples of other interesting avenues to explore in the discussion.

Response: Agreed. The optimum temperature for growth of the Bergen population was about 22 ^{o}C and was 5 ^{o}C higher than the maximum SST in Bergen waters (Zhang et al. 2014). Furthermore, in comparison to the Azores and Canary Islands populations, larger optimum pCO_2 of growth rate indicates that the Bergen population may benefit more from the rising CO_2 levels at increasing temperatures. **These contents were added in lines 367–372.**

As shown in Fig. R2 (or S6 in the supplement), PIC : POC ratios of the Azores and Bergen populations declined with rising pCO_2, whereas PIC : POC ratios of the Canary Islands population were rather constant (Fig. S6). As changes in PIC ： POC ratios of coccolithophore blooms may impact on the biological carbon pump, different regions might see different changes in the future ocean. **These contents underlined were added in lines 372–376.**

As shown **in lines 304–306** in the manuscript or in Fig. 2, low sensitivity constant of growth rate of the Bergen population corresponded to high optimum CO_2 level.

For lines 350–355: In addition, the Canary Islands population showed smallest variability in optimum pCO_2 and maximum values for growth and POC production rates (Fig. 2). The reason may be that low incubation temperature predominantly limited growth and POC production rates of Canary Islands population, and decreased the sensitivities of these physiological rates to rising pCO_2.

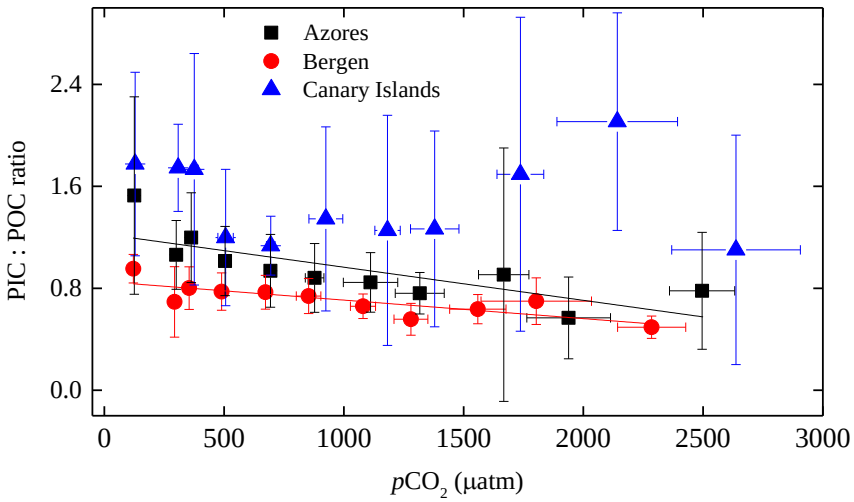

Figure R2 (S6). Responses of PIC : POC ratio of the Azores (square), Bergen (circular) and Canary Islands (diamond) populations to a CO_2 range from 120 µatm to 2630 µatm.

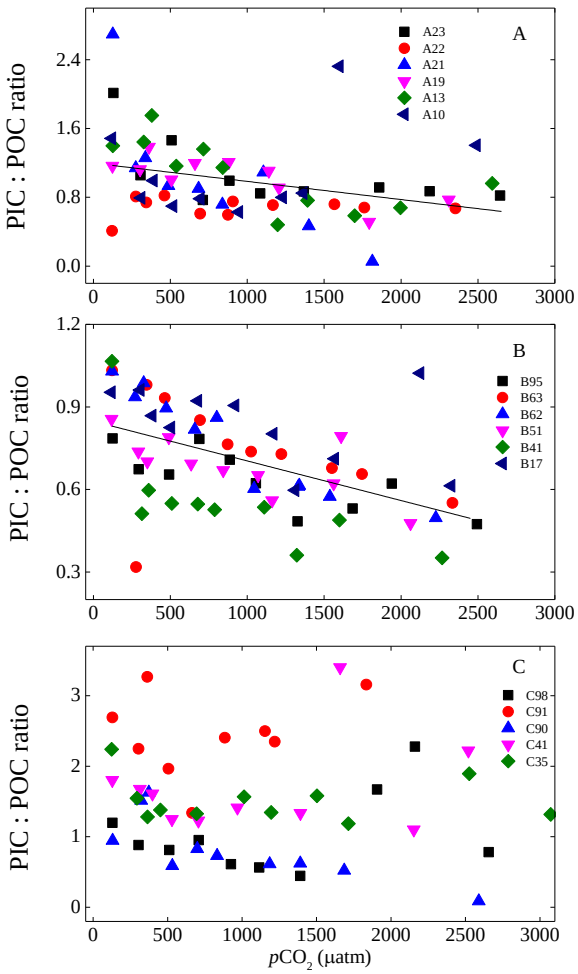

Figure R3 (S7). Response of PIC : POC ratio of individual *E. huxleyi* strain in the Azores (A), Bergen (B) and Canary Islands (C) populations to a CO_2 range from 115 µatm to 3070 µatm.

Technical comments Line 39: than that of

Response: **For lines 40–42:** Maximum growth and POC production rates of the Azores and Bergen populations were similar and significantly higher than that of the Canary Islands population.

Line 44-45: carbonate chemistry responses? Should it say instead "responses to changes in carbonate chemistry changes"?

Response: **For lines 48–51:** The existence of distinct ~~carbonate chemistry~~ responses to changes in carbonate chemistry between and within populations will likely benefit *E. huxleyi* to acclimate and adapt to rising CO_2 levels in the oceans.

Line 76: I recommend checking this new publication: Krumhardt et al. 2017. Coccolithophore growth and calcification in a changing ocean https://doi.org/10.1016/j.pocean.2017.10.007

Response: Krumhardt et al. (2017) developed an empirical coccolithophore model to investigate responses of growth and calcification of coccolithophores to changing environments (temperature,

CO_2, nutrient concentrations). This paper is now cited on line 76.
For lines 73–76: The coccolithophore *Emiliania huxleyi* forms extensive blooms under favourable light intensity, temperature and nutrient conditions, with different morphotypes in certain regions (Cook et al., 2011; Henderiks et al., 2012; Smith et al., 2012; Balch et al., 2014; Krumhardt et al., 2017).

Krumhardt, K. M., Lovenduski, N. S., Debora Iglesias-Rodriguez, M., and Kleypas, J. A.: Coccolithophore growth and calcification in a changing ocean, Prog. Oceanogr., 159, 276–295.

Line 135: "consecutive incubations" and then in Line 146 "each strain was grown under 11 CO2 levels: : :" then in line 150 and 158 "at least 7 generations: : :4-7 days depending on CO2 concentration : : :". can you explain the method in more detail, I am bit confused. Perhaps a supplementary diagram or flow chart figure would help.
Response: As mentioned above, a flow chart showing the experimental protocol was added to the supplement information (Figure S1).
For lines 138–140: The experiment was performed in six consecutive incubations, with one strain from each population (Azores, Bergen, Canary Islands) being cultured at a time (Fig. S1).

Line 202: For Eq 4 and 5, you cited Bach et al 2011, but could you please elaborate on this method. Can you also explain the sensitivity constant a bit more?
Response: **For lines 202–209:** In a broad pCO_2 range, physiological rates are expected to initially increase quickly until reaching an optimum and then decline towards further increasing CO_2 levels (e.g. Krug et al. 2011). Hence we used the following modified Michaelis-Menten equation (Bach et al. 2011) which was fitted to measured cellular growth, POC and PIC production rates and yielding theoretical optimum pCO_2 and maximum values for each of the three populations (combining the data of five or six strains) (Bach et al., 2011).

For lines 211–212: s, the sensitivity constant, depicts the slope of the decline after optimum CO_2 levels in response to rising H^+.

Line 207: Do these refer to figure S3?
Response: (**Lines 218–220)** Relative growth, POC and PIC production rates of each population are shown in Fig. 1b,d,f. Relative POC and PIC quotas of each population were shown in Fig. S2.

Line 295: "These findings indicate that the Bergen population may be more tolerant….." This is a great result! Environmental variability can tell us something about phenotypic plasticity.
Response: (**Lines 315–317**) Large environmental variability usually results in high tolerance of phytoplankton (Doblin and van Sebille, 2016). In this study, we cannot say that large environmental variability result in large or low phenotypic plasticity.

Doblin, M. A., and van Sebille, E.: Drift in ocean currents impacts intergenerational microbial exposure to temperature, Proc. Natl. Acad. Sci. USA., 113, 5700–5705, doi: 10.1073/pnas.1521093113, 2016.

Line 323 "likely causes the lower the carbon: : :" consider moving "the"

Response: **For lines 345–348:** we delete '*One of the reasons may be that compared to the Azores and Bergen populations, 16 ^{o}C likely causes lower the carbon uptake and carbon use efficiency of the Canary Islands population*'

Line 343: add and "s" to proton

Response: For lines 379–382: In addition, *E. huxleyi* is thought to utilize HCO_3^- for calcification which generates protons, and increase in proton concentration may mitigate the potential of the ocean to absorb atmospheric CO_2 (Paasche, 2002).

Line 345: consider adding "and" before "corresponding"

Response: For lines 382–385: we deleted this sentence '*Thus, due to population-specific growth and PIC production rates or quotas, changes in species composition, corresponding changes in PIC productions, may affect the ability of the ocean to take up CO_2.*'

Line 352: this conclusion seems to be out of place and not well justified

Response: For lines 391–393: we deleted this sentence '*Additionally, our results also suggest that strain-specific PIC quota may be the basis of variation in coccoliths of E. huxleyi within the morphotype A (Fig. S4) (Young, 1994; Paasche, 2002).*'

Lines 334-372: some very interesting ideas here but these paragraphs need some tightening.

Response: According to suggestions of this referee, we added and deleted some contents **in lines 365–382:** The ability to adapt to diverse environmental conditions is supposed to be one reason for the global distribution of *E. huxleyi* (Paasche, 2002), spanning a temperature range of about 30 ^{o}C. The optimum temperature for growth of the Bergen population was about 22 ^{o}C and was 5 ^{o}C higher than the maximum SST in Bergen waters (Zhang et al. 2014). Furthermore, in comparison to the Azores and Canary Islands populations, larger optimum pCO_2 of growth rate indicates that the Bergen population may benefit more from the rising CO_2 levels at increasing temperatures. PIC : POC ratios of the Azores and Bergen populations declined with rising pCO_2, whereas PIC : POC ratios of the Canary Islands population were rather constant (Fig. S6). As changes in PIC : POC ratios of coccolithophore blooms may impact on the biological carbon pump, different regions might see different changes in the future ocean. In natural seawater, due to ocean currents and gene flow, populations at any given location may get replaced by immigrant genotypes transported there from other locations (Doblin and van Sebille, 2016). In addition, *E. huxleyi* is thought to utilize HCO_3^- for calcification which generates protons, and increase in proton concentration may mitigate the potential of the ocean to absorb atmospheric CO_2 (Paasche, 2002).

Line 367-369: do you mean "dominated" or "dominating"? not sure I follow this argument.

Response: For lines 408–411: Further, a significant positive correlation between growth and POC

production rate or POC quota (Fig. S5) indicates that the dominating strains will also take up or fix dissolved inorganic carbon faster.

Responses to comments of referee 2 are shown as following:

GENERAL COMMENTS

The paper by Zhang et al. presents results from a large number of experiments on multiple geographically distinct strains of the coccolithophore Emiliania huxleyi. Each strain was exposed to a wide range of pCO2 concentrations and the authors examined differences in growth rates, photosynthetic rates (POC production) and calcification rates (PIC production). The authors conclude that significant variability exists in population-level sensitivity of physiological rates (most clearly growth and POC production) to pCO2. The paper is well written, with the data supporting the conclusions and the authors make some important and insightful conclusions. I have only two minor comments.

The first comment relates to a lack of any discussion or presentation of the variability in PIC:POC ratios and POC (or PIC) production between the different strains. Further information on the level of inter-strain variability in these parameters would strengthen and support the wider implications and conclusions made in the discussion. The second comment relates to the authors consideration of variability and stability in the different environmental conditions of the strain isolation locations – a large factor in these differences is likely to relate to different seasonal cycles and environmental drivers (ice-melt, riverine input, upwelling, etc). However, the authors only hint at the different factors influencing the relative stability of the different locations. Large-scale environmental differences will directly relate to the stability of the environment, as well as differing potential future perturbations for each

of them. Again, making these differences more explicit would support the wider implications of the study.

Response: We cultured 17 *Emiliania huxleyi* strains at 11 pCO_2 levels with no replicate. At each pCO_2 level, there is no replicate and this is the main reason that we did not discuss variability in physiological rates between strains within the population.

Regarding the variability in the PIC : POC ratio between the populations, we added these contents 'PIC : POC ratios of the Azores and Bergen populations declined with rising pCO_2, whereas PIC : POC ratios of the Canary Islands population were rather constant (Fig. S6). As changes in PIC : POC ratios of coccolithophore blooms may impact on the biological carbon pump, different regions might see different changes in the future ocean.' **in lines 372–376.**

For lines 313–315: we added these contents: 'In addition, due to riverine input, seawater upwelling and metabolic activity of plankton communities, environmental variability in coastal water are larger than in open-ocean ecosystems (Duarte and Cerbrian, 1996).'

Duarte, C. M., and Cerbrian, J.: The fate of marine autotrophic production, Limnol. Oceanogr., 41, 1758–1766, 1996.

SPECIFIC COMMENTS

Ln 27: Clarity is needed in the abstract on what the authors mean in terms of population-specific responses.

Response: In this study, 'population-specific responses' mean that growth, POC and PIC production rates of three *Emiliania huxleyi* populations were different at the same incubation conditions.

For lines 27–32: In the present study, we investigated the ~~population~~ specific responses of growth, particulate organic (POC) and inorganic carbon (PIC) production rates of ~~17 strains~~ 3 populations of the coccolithophore *Emiliania huxleyi* from three regions in the North Atlantic Ocean (Azores: 6 strains, Canary Islands: 5 strains, and Norwegian coast near Bergen: 6 strains) to a CO_2 partial pressure (pCO_2) range from 120 µatm to 2630 µatm.

Ln 28: More information on number of strains per environment would be good in the abstract.

Response: **For lines 27–32: see above**.

Ln 32: 'expected optimum curve responses' – may be expected by authors but not clear in the abstract. Some further background would be good.

Response: **For lines 32–34:** Physiological rates of each population and individual strain increased with rising pCO_2 levels, reached maximum and declined thereafter.

Ln 37: Could the authors elaborate more in terms of the role of seasonality (or lack thereof) in the stability of oceanic conditions.

Response: **For lines 37 to 39:** This may be due to the large environmental variability including large pCO_2 and pH fluctuations in coastal waters off Bergen compared to the rather stable oceanic

conditions at the other two sites.

In the discussion section, **for lines 313–315:** we added this sentence 'In addition, due to riverine input, seawater upwelling and metabolic activity of plankton communities, environmental variability in coastal water are larger than in open-ocean ecosystems (Duarte and Cerbrian, 1996).'

Lns 91-92: Would the authors consider adding 'geographically-distinct' strains to this line to emphasize both the importance of their own insights and the more general need to consider different strains of other widespread species.

Response: **For lines 91–94:** Hence, multiple strains, ideally from geographically distinct regions should be considered for investigating phytoplankton responses to climate change (Zhang et al., 2014; Blanco-Ameijeiras et al., 2016; Krumhardt et al., 2017).

Krumhardt, K. M., Lovenduski, N. S., Debora Iglesias-Rodriguez, M., and Kleypas, J. A.: Coccolithophore growth and calcification in a changing ocean, Prog. Oceanogr., 159, 276–295.

Lns 103-104: A plastic response also allows a strain to acclimate across an environmental gradient and widen its bio-geographical distribution. Rather than focus on just environmental change, what about environmental variability.

Response: **For lines 103–106:** Plasticity can be assessed by analyzing the reaction norm of one trait and a plastic response may allow a strain to acclimate across an environmental gradient and widen its bio-geographical distribution (Reusch, 2014; Levis and Pfennig, 2016).

Ln 126: How were all strains characterized and confirmed to be morphotype A (i.e. Distal shield length? Central area characteristics?)?

Response: Morphotype A was confirmed by scanning electron microscope.

For Lines 128–129: All 17 strains belong to morphotype A (determined by scanning electron microscopy) and have been deposited in the Roscoff culture collection (RCC)

Ln 140-141: Is this statement ('the best compromise') appropriate based on the authors end conclusion that the low experiment temperature relative to optimum growth conditions for the Canary Islands strains led to their low growth (and POC production)? It seems to be a compromise that had a definitive influence on the end outcome of the experiments. Is it not simpler to just delete this section (from the point of 'which ..' to the end) and come back to this in the discussion?

Reponse: **For lines 140–144:** Monoclonal populations were always grown in sterile-filtered (0.2 µm diameter, Sartobran® P 300, Sartorius) artificial seawater medium (ASW) as dilute batch cultures at 200 µmol photons m^{-2} s^{-1} light intensity under a 16/8 h light/dark cycle (light period: 5:00 a.m to 9:00 p.m.) at 16 °C which we consider to be ~~the best~~ a compromise for the three different origins of the strains.

Our results showed that low incubation temperature led to low growth and POC production rates of the Canary Islands population. In the discussion section, we compared influence of

temperature on physiological rate of three populations. **For lines 324–336:** In an earlier study (Zhang et al., 2014), growth rates of the same Azores and Bergen strains as used here were measured at 8–28 °C. While at 26–28 ℃ the Bergen strains grew slower than the Azores strains, at 8 ℃ the Azores strains grew slower than the Bergen strains. This illustrates nicely that local temperature adaptation can significantly affect growth of *E. huxleyi* strains in laboratory experiments. Considering these findings and the temperature ranges of the three isolation locations (Table S1), the incubation temperature of 16 ℃ used in the present study was lower than the minimum sea surface temperature (SST) commonly recorded at the Canary Islands. In contrast, SSTs of 16 ℃ and lower have been reported for Azores and Bergen waters (Table S1). When exposed to 16 °C, growth rate of the Canary Islands population might have been already below their optimum and hence significantly reduced in comparison to the other populations (Fig. 2d).

Lns 152-153 (cf Lns 174-175): How were initial cell densities measured/estimated?

Response: (**In line 156**) There was 590 ml seawater in the 500 ml glass bottles. Before cells were inoculated to new seawater, finial cell concentrations (C_0) were measured, and we calculated the inoculated volumes (V) according to V = (200 cell/ml x 590 ml)/C_0. By using this method, we think that the initial cell concentration was 200 cell/ml.

For lines **155–157:** Initial cell concentration was 200 cells ml^{-1} (estimated from measured pre-culture concentrations and known dilution) and final cell concentration was lower than 100,000 cells ml^{-1}.

Lns 289-290: An important result that should be emphasized in the abstract and conclusions.

Response: In the abstract, we added this content **in lines 45–46:** Our results indicate adaptation of *E. huxleyi* to their local environmental conditions and the existence of distinct *E. huxleyi* populations.

In the conclusion: we added this sentence **in lines 420–423:** The existence of distinct *E. huxleyi* populations and phenotypic plasticity of individual strains may both be important for *E. huxleyi* when adapting to natural environmental variability and to ongoing climate changes.

Lns 322-324: Suggest deleting ‘causes’ from this sentence.

Response: **For lines 345–348:** we delete these contents ‘*One of the reasons may be that compared to the Azores and Bergen populations, 16 °C likely causes lower the carbon uptake and carbon-use efficiency of the Canary Islands population (Sett et al., 2014).*’

Ln 351-352: Another potentially important conclusion, especially given the emphasis on determining time-dependent (or space-dependent) variations in coccolith-specific PIC quotas. However, the current paper lacks any details of the strain-specific variability in PIC quota and to what extent the different trends in pCO2-sensitivity (e.g. Fig. 3e) are driven by changes in growth rate and/or cellular (or coccolith) specific PIC quota. Can strain-specific information on PIC quota be added to the supplementary material to support this point with experimental data?

Response: PIC quota of population is shown in figure S2, and PIC quota of individual strain is shown in Figure S4. We measured PIC quota of individual strains at 11 pCO_2 levels **with no replicate.** This is the reason that we did not discuss PIC quota of individual strains.

We deleted this sentence **in lines 391–393:** '*Additionally, our results also suggest that strain-specific PIC quota may be the basis of variation in coccoliths of E. huxleyi within the morphotype A (Fig. S3) (Young, 1994; Paasche, 2002).*'

Ln 374: A two line conclusion seems relatively short based on the significant statements made in the conclusions. Either expand or delete?

Response: We added main result **in lines 420–423:** The existence of distinct *E. huxleyi* populations and phenotypic plasticity of individual strains may both be important for *E. huxleyi* when adapting to natural environmental variability and to ongoing climate changes.

For lines 425–426: we added ', and CO_2 response was modulated by other environmental factors such as temperature and light intensity.'

List of changes

Abstract

1 Lines 26–27: add ‘from different areas’

2 Line 27: delete ‘population’

3 Lines 28–29: add ‘3 populations’

4 Line 29: delete ‘17 strains’

5 Line 30: add ‘: 6 strains’, add ‘: 5 strains’

6 Line 31: add ‘: 6 strains’

7 Lines 32–33: change ‘displayed the expected optimum curve responses to the pCO_2 gradient’ to ‘increased with rising pCO_2 levels, reached maximum and declined thereafter’

8 Line 36: change ‘a’ to ‘the’

9 Line 37: change ‘fjord’ to ‘coast’

10 Line 38: add ‘environmental variability including large’, and delete ‘variability’

11 Line 39: add ‘fluctuations’

12 Line 41: add ‘that’

13 Lines 42–43: change ‘One of the reasons may be that the’ to ‘This pattern could be driven by temperature-CO_2-interactions where the’

14 Line 44: change ‘is’ to ‘was’

15 Line 46: add ‘and the existence of distinct *E. huxleyi* populations’

16 Lines 48–49: delete ‘carbonate chemistry’

17 Line 49: add ‘to changes in carbonate chemistry’

18 Line 50: add ‘and adapt’

Introduction

1 Line 76: add ‘; Krumhardt et al., 2017’

2 Line 91: change ‘These indicate that’ to ‘Hence,’

3 Lines 91–92: add ‘, ideally from geographically distinct regions’

4 Line 93: add ‘;’

5 Line 94: add ‘Krumhardt et al., 2017’

6 Line 105: change ‘to environmental change’ to ‘across an environmental gradient and widen its bio-geographical distribution’

Materials and methods

1 Lines 128–129: add ‘(determined by scanning electron microscopy)’

2 Line 129: change ‘at’ to ‘in’

3 Lines 139–140: add ‘(Fig. S1)’

4 Line 143: change ‘the best’ to ‘a’

5 Lines 156–157: add ‘(estimated from measured pre-culture concentrations and known dilution)’

6 Lines 202–206: add ‘In a broad pCO_2 range, physiological rates are expected to initially increase quickly until reaching an optimum and then decline towards further increasing CO_2 levels

(e.g. Krug et al. 2011). Hence we used the following modified Michaelis-Menten equation (Bach et al. 2011) which was fitted to measured cellular growth, POC and PIC production rates'

7 Lines 206–207: delete 'The nonlinear regression model (4) was used to fit growth, POC and PIC production rates'

8 Line 207: add 'and'

9 Line 211: delete 'is', add ',' and delete 'which indicates'

10 Line 212: delete 'the effect of', and add 'depicts the slope of the decline after optimum CO_2 levels in response to'

11 Line 213: delete 'the'

12 Line 214: add '(equation 5)', delete 'for physiological rates according to equation (5)', add 'and', and change 'M' to 'm'

13 Line 215: delete 'were calculated by using equation (4) based on K_m.'

14 Line 216: add 'following Bach et al., (2011).'

Discussion

1 Lines 313–315: add 'In addition, due to riverine input, seawater upwelling and metabolic activity of plankton communities, environmental variability in coastal waters are larger than in open-ocean ecosystems (Duarte and Cerbrian, 1996).'

2 Line 317: change 'ed with' to 'ing'

3 Line 329: add 'the', and change 'ed' to 'ion'

4 Lines 334–336: change 'thus it grew slower than the other populations' to 'hence significantly reduced in comparison to the other populations'

5 Lines 337–345: add 'Furthermore, compared to the Canary Islands population, the Azores population had higher maximum growth and POC production rates, and similar optimum CO_2 for these physiological rates. Again, this might be related to sub-optimal incubation conditions as temperature has been found to significantly modulate CO_2 responses in coccolithophores in terms of maximum rates, CO_2 optima and half-saturation, and H^+ sensitivity (De Bodt et al., 2010; Sett et al., 2014; Gafar et al., 2018; Gafar and Schulz, 2018). In a similar fashion light can also modulate CO_2 responses, hence different requirements by strains adapted to different light availabilities could also explain our observations (Zhang et al., 2015; Gafar et al., 2018; Gafar and Schulz, 2018).'

6 Lines 345–348: delete 'One of the reasons may be that compared to the Azores and Bergen populations, 16 ^oC likely causes lower the carbon uptake and carbon-use efficiency of the Canary Islands population (Sett et al., 2014).'

7 Lines 350–355: add 'In addition, the Canary Islands population showed smallest variability in optimum pCO_2 and maximum values for growth and POC production rates (Fig. 2). The reason may be that low incubation temperature predominantly limited growth and POC production rates of the Canary Islands population, and decreased the sensitivities of these physiological rates to rising pCO_2.'

8 Line 365: delete 'reflected in'

9 Line 366: add 'supposed to be one reason for'

10 Lines 367–376: add 'The optimum temperature for growth of the Bergen population was about

22 ^{o}C and was 5 ^{o}C higher than the maximum SST in Bergen waters (Zhang et al. 2014). Furthermore, in comparison to the Azores and Canary Islands populations, larger optimum pCO_2 of growth rate indicates that the Bergen population may benefit more from the rising CO_2 levels at increasing temperatures. PIC : POC ratios of the Azores and Bergen populations declined with rising pCO_2, whereas PIC : POC ratios of the Canary Islands population were rather constant (Fig. S6). As changes in PIC : POC ratios of coccolithophore blooms may impact on the biological carbon pump, different regions might see different changes in the future ocean.'

11 Line 377: change 'populations' to 'immigrant'

12 Line 378: add 'genotypes'

13 Lines 378–379: delete 'when having a higher potential to adapt to a changing environment'

14 Line 380: change 'take up' to 'is thought to utilize', and change 'to calcify and' to 'for calcification which'

15 Line 381: add 's'

16 Lines 382–385: delete 'Thus, due to population-specific growth and PIC production rates or quotas, changes in species composition, corresponding changes in PIC productions, may affect the ability of the ocean to take up CO_2.'

17 Line 389: add 'acclimate and', and delete 'their'

18 Lines 390–391: add 'and potentially to attenuate the short-term effects of changing environments on fitness-relevant traits'

19 Lines 391–393: delete 'Additionally, our results also suggest that strain-specific PIC quota may be the basis of variation in coccoliths of *E. huxleyi* within the morphotype A (Fig. S3) (Young, 1994; Paasche, 2002).'

20 Line 396: add ',', delete 'and', and add 'and'

21 Line 397: add 'potentially forms the basis for selection'

22 Line 404: add 'a', and delete 's'

23 Line 406: add 'er', and add 'or other'

24 Line 407: add 'competitive abilities', add 's', and delete 'strains in the oceans'

25 Line 408: change 'S' to 'Further, a s'

26 Line 409: change '4' to '5', change 'suggests' to 'indicates', and change 'ed' to 'ing'

27 Line 410: change 'can' to 'will', add 'or fix', and delete 'from the oceans or'

28 Line 411: delete 'fix carbon faster', and change 'this' to 'When extrapolated to the ocean, *E. huxleyi* blooms'

29 Line 412: change 'or the' to 'and its'

30 Line 413: delete 'of the oceans when large *E. huxleyi* blooms occur'

31 Line 414: change 'will' to 'has the potential to'

Conclusions

1 Lines 420–423: add 'The existence of distinct *E. huxleyi* populations and phenotypic plasticity of individual strains may both be important for *E. huxleyi* when adapting to natural environmental variability and to ongoing climate changes.'

2 Line 424: change 'or' to 'and'

3 Lines 425–426: add ', and CO_2 response was modulated by other environmental factors such as

temperature and light intensity.'

References

1 Lines 506–507: add 'Duarte, C. M., and Cerbrian, J.: The fate of marine autotrophic production, Limnol. Oceanogr., 41, 1758–1766, 1996.'

2 Lines 512–515: add 'Gafar, N. A., Eyre, B. D., and Schulz, K. G. : A conceptual model for projecting coccolithophorid growth, calcification and photosynthetic carbon fixation rates in response to global ocean change, Front. Mar. Sci., 4, 433, doi: 10.3389/fmars.2017.00433, 2018.'

3 Lines 516–518: add 'Gafar, N. A., and Schulz, K. G. : A niche comparison of *Emiliania huxleyi* and *Gephyrocapsa oceanica* and potential effects of climate change, Biogeosci. Discuss., doi: 10.5194/bg-2018-88.'

4 Lines 559–561: add 'Krug, S. A., Schulz, K. G., and Riebesell, U. : Effects of changes in carbonate chemistry speciation on *Coccolithus braarudii*: a discussion of coccolithophorid sensitivities, Biogeosciences, 8, 771–777, doi: 10.5194/bg-8-771-2011, 2011.'

5 Lines 562–564: add 'Krumhardt, K. M., Lovenduski, N. S., Debora Iglesias-Rodriguez, M., and Kleypas, J. A.: Coccolithophore growth and calcification in a changing ocean, Prog. Oceanogr., 159, 276–295.'

6 Lines 634–635: delete 'Young, J. R.: Variation in *Emiliania huxleyi* coccolith morphology in samples from the Norwegian EHUX experiment, 1992, Sarsia, 79, 417–425, 1994.'

Population-specific responses in physiological rates of *Emiliania huxleyi* to a broad CO_2 range

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Running head: *population response of* Emiliania huxleyi *to* CO_2

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Keywords: CO_2; coccolithophore; physiological rate; population; strain

Abstract

Although coccolithophore physiological responses to CO_2-induced changes in seawater carbonate chemistry have been widely studied in the past, there is limited knowledge on the variability of physiological responses between populations from different areas. In the present study, we investigated the ~~population~~ specific responses of growth, particulate organic (POC) and inorganic carbon (PIC) production rates of 3 populations ~~17 strains~~ of the coccolithophore *Emiliania huxleyi* from three regions in the North Atlantic Ocean (Azores: 6 strains, Canary Islands: 5 strains, and Norwegian coast near Bergen: 6 strains) to a CO_2 partial pressure (pCO_2) range from 120 µatm to 2630 µatm. Physiological rates of each population and individual strain ~~displayed the expected optimum curve responses to the pCO_2 gradient~~ increased with rising pCO_2 levels, reached maximum and declined thereafter. Optimal pCO_2 for growth and POC production rates and tolerance to low pH (i.e. high proton concentration) was significantly higher in an *E. huxleyi* population isolated from ~~a~~the Norwegian ~~fjord~~coast than in those isolated near the Azores and Canary Islands. This may be due to the large environmental variability including large pCO_2 and pH ~~variability~~ fluctuations in coastal waters off Bergen compared to the rather stable oceanic conditions at the other two sites. Maximum growth and POC production rates of the Azores and Bergen populations were similar and significantly higher than that of the Canary Islands population. ~~One of the reasons may be that the~~ This pattern could be driven by temperature-CO_2-interactions where the chosen incubation temperature (16 ^{o}C) ~~is~~was slightly below what strains isolated near the Canary Islands normally

experience. Our results indicate adaptation of *E. huxleyi* to their local environmental conditions and the existence of distinct *E. huxleyi* populations. Within each population, different growth, POC and PIC production rates at different pCO_2 levels indicated strain-specific phenotypic plasticity. The existence of distinct ~~carbonate chemistry~~ responses to changes in carbonate chemistry between and within populations will likely benefit *E. huxleyi* to acclimate and adapt to rising CO_2 levels in the oceans.

1 Introduction

Coccolithophores form a layer of calcium carbonate ($CaCO_3$) platelets (coccoliths) around their cells. Coccoliths are of biogeochemical importance due to ballasting of organic matter with $CaCO_3$, a phenomenon which is thought to promote the transport of organic carbon to the deep ocean (Klaas and Archer, 2002; Rost and Riebesell, 2004). The coccolithophore *Emiliania huxleyi* forms extensive blooms under favourable light intensity, temperature and nutrient conditions, with different morphotypes in certain regions (Cook et al., 2011; Henderiks et al., 2012; Smith et al., 2012; Balch et al., 2014; Krumhardt et al., 2017).

Variable responses of growth, photosynthetic carbon fixation and calcification rates of different *E. huxleyi* strains to rising CO_2 levels have been reported (Langer et al., 2009; Hoppe et al., 2011; Müller et al., 2015; Hattich et al, 2017) and are likely a result of intra-specific variability of genotypes (Langer et al., 2009). Several recent studies observed optimum curve responses in physiological rates of a single *E. huxleyi* strain to a broad pCO_2 range from about 20 µatm to 5000 µatm, and linked them to inorganic carbon substrate limitation at low pCO_2 and inhibiting H^+ concentrations at high pCO_2 (Bach et al., 2011; 2015; Kottmeier et al., 2016). Until now, studies on the physiological responses of *E. huxleyi* to rising CO_2 are mostly based on a few genotypes and little is known about the potential variability in CO_2 and H^+ sensitivity between and within populations. Recently, several studies found substantial variations in CO_2 responses for N_2 fixation rates between *Trichodesmium* strains, as well as for

growth rates between strains of *Gephyrocapsa oceanica*, *Ostreococcus tauri* and *Fragilariopsis cylindrus* (Hutchins et al., 2013; Schaum et al., 2013; Pancic et al., 2015; Hattich et al., 2017). ~~These indicate that~~ Hence, multiple strains, ideally from geographically distinct regions should be considered for investigating phytoplankton responses to climate change (Zhang et al., 2014; Blanco-Ameijeiras et al., 2016; Krumhardt et al., 2017).

Oceanographic boundaries formed by both ocean currents and environmental factors such as temperature, can limit dispersal of marine phytoplankton, reduce gene flow between geographic populations, and give rise to differentiated populations (Palumbi, 1994). Different populations were found to show different growth rates for *E. huxleyi*, *G. oceanica*, and *Skeletonema marinoi* at the same temperatures, and for *Ditylum brightwellii* at the same light intensities (Brand, 1982; Rynearson and Armbrust, 2004; Kremp et al., 2012; Zhang et al., 2014). Phenotypic plasticity describes the ability of a strain to change its morphology or physiology in response to changing environmental conditions (Bradshaw, 1965). Plasticity can be assessed by analyzing the reaction norm of one trait and a plastic response may allow a strain to acclimate ~~to environmental change~~ across an environmental gradient and widen its bio-geographical distribution (Reusch, 2014; Levis and Pfennig, 2016).

In order to better understand how local adaptation affects the physiological response of *E. huxleyi* to rising CO_2 conditions, we isolated 17 strains from three regions in the Atlantic Ocean, and assessed growth, carbon fixation and calcification responses of the population over a pCO_2 range from 120 µatm to 2630 µatm.

2 Materials and methods

2.1 Cell isolation sites and experimental setup

Emiliania huxleyi strains EHGKL B95, B63, B62, B51, B41 and B17 originated from Raunefjord (Norway 60°18'N, 05°15'E) and were isolated by K. T. Lohbeck in May, 2009 (Lohbeck et al., 2012) at ~ 10 °C in-situ water temperature. *E. huxleyi* strains EHGLE A23, A22, A21, A19, A13 and A10 originated from coastal waters near the Azores (38°34'N, 28°42'W) and were isolated by S. L. Eggers in May or June, 2010 at ~ 17 °C in-situ water temperature. *E. huxleyi* strains EHGKL C98, C91, C90, C41 and C35 originated from coastal waters near Gran Canaria (27°58'N, 15°36'W) and were isolated by K. T. Lohbeck in February, 2014 at ~ 18 °C in-situ water temperature. Seasonal CO_2 concentration in the surface seawater ranges from 240 µatm to 400 µatm near Bergen, from 320 µatm to 400 µatm around the Azores and from 320 µatm to 400 µatm around the Canary Islands (Table 1). Monthly surface seawater temperature ranges from 6.0 to 16.0 °C near Bergen, 15.6 to 22.3 °C around the Azores and from 18.0 to 23.5 °C around the Canary Islands (Table S1).

All 17 strains belong to morphotype A (determined by scanning electron microscopy) and have been deposited ~~at~~in the Roscoff culture collection (RCC) under the official names as shown above. Genetically different isolates, here called strains, were identified by 5 microsatellite markers (P02E09, P02B12, P02F11, EHMS37, EHMS15) (Table S2). For a description of primer testing, deoxyribonucleic acid

(DNA) extraction, DNA concentration measurements, and polymerase chain reaction (PCR) protocols see Zhang et al. (2014). The Azores and Bergen strains had been used earlier by Zhang et al. (2014).

The six or five (in case of Canary Islands) strains of each region were used to test the physiological response to varying CO_2 concentrations at constant total alkalinity (TA). The experiment was performed in six consecutive incubations, with one strain from each population (Azores, Bergen, Canary Islands) being cultured at a time (Fig. S1). Monoclonal populations were always grown in sterile-filtered (0.2 µm diameter, Sartobran® P 300, Sartorius) artificial seawater medium (ASW) as dilute batch cultures at 200 µmol photons m^{-2} s^{-1} light intensity under a 16/8 h light/dark cycle (light period: 5:00 a.m to 9:00 p.m.) at 16 °C which we consider to be ~~the best~~ a compromise for the three different origins of the strains. Nutrients were added in excess (with nitrate and phosphate concentrations of 64 µmol kg^{-1} and 4 µmol kg^{-1}, respectively). For the preparation of ASW and nutrient additions see Zhang et al. (2014). Calculated volumes of Na_2CO_3 and hydrochloric acid were added to the ASW to achieve target CO_2 levels at an average total alkalinity (TA) of 2319 ± 23 µmol kg^{-1} (Pierrot et al., 2006; Bach et al., 2011). Each strain was grown under 11 CO_2 levels ranging from 115 µatm to 3070 µatm without replicate. Mean response variables of all strains with a population were calculated and mean CO_2 levels of all strains within a population ranged from 120 µatm to 2630 µatm. Cells grew in the experimental conditions for at least 7 generations, which corresponded to 4–7 days depending on cell division rates. Cells were cultured for 4 days in 120–925 µatm CO_2, for 5 days in

1080–1380 µatm CO_2, and for 6 or 7 days in 1550–2630 µatm CO_2. Initial cell concentration was 200 cells ml^{-1} (estimated from measured pre-culture concentrations and known dilution) and final cell concentration was lower than 100,000 cells ml^{-1}. Dissolved inorganic carbon (DIC) concentrations and pCO_2 levels changed less than 7% and 11%, respectively, during the experimental growth phase.

2.2 pH_T and total alkalinity measurements

At 10:00 a.m. on the last day of incubations (at day 4–7 depending on CO_2 concentration), pH_T and TA samples were filtered (0.2 µm diameter, Filtropur S 0.2, Sarstedt) by gentle pressure and stored at 4°C for a maximum of 14 days. The entire sampling lasted less than 2 h. The pH_T sample bottles were filled with considerable overflow and closed tightly with no space. pH_T was measured spectrophotometrically (Cary 100, Agilent) using the indicator dye *m*-cresol purple (Sigma-Aldrich) similar to Carter et al. (2013) with constants of acid dissociation for the protonated and un-protonated forms reported in Clayton and Byrne (1993). TA was measured by open-cell potentiometric titration (862 Compact Titrosampler, Metrohm) according to Dickson et al. (2003). The carbonate system was calculated from measured TA, pH_T, (assuming 4 µmol kg^{-1} of phosphate and 0 µmol kg^{-1} of silicate) using the CO2 System Calculations in MS Excel software (Pierrot et al., 2006) with carbonic acid constants K_1 and K_2 as determined by Roy et al. (1993).

2.3 Growth rate measurements

177 At 1:00 p.m. on the last day of incubation, 25 ml samples were used to measure cell
178 concentration. Cell concentration was determined within two hours using a Z2 Coulter
179 Particle Counter (Beckman). Growth rate (μ) was calculated according to:

180 $$\mu = (\ln N_1 - \ln N_0) / d \qquad (1)$$

181 where N_1 is cell concentration on the last day of incubation, N_0 is 200 cells mL^{-1}, and
182 d is the time period for growth of algae in days.

183

184 **2.4 Particulate organic (POC) and inorganic (PIC) carbon measurements**

185 At 3:00 p.m. on the last day of incubation, cells for total particulate (TPC) and total
186 organic (TOC) carbon were filtered onto GF/F filters which were pre-combusted at
187 500 ^{o}C for 8 h. Samples of background particulate carbon (BPC) were determined in a
188 similar way but using filtered ASW without algae, which was previously adjusted to
189 target pCO_2 levels, and allowed to age for about 7 days under incubation conditions
190 (*see* above). All samples were placed at –20^{o}C. BPC filters were used as blanks to
191 correct for organic carbon in the medium. TOC and BPC filters were acid fumed.
192 Afterwards, all filters were dried for 8 h at 60^{o}C. TPC, TOC and BPC were measured
193 using an Elemental Analyzer (EuroEA, Hekatech GmbH). The percentages of BPC in
194 TPC were about 20% at cell densities < 10,000 cells ml^{-1} and about 10% at cell
195 densities > 40,000 cells ml^{-1}. POC was calculated as the difference between TOC and
196 BPC. PIC was calculated as the difference between TPC and TOC. POC and PIC
197 production rates were calculated as:

198 $$\text{POC production rate} = \mu\ (d^{-1}) \times (\text{TOC} - \text{BPC})\ (\text{pg C cell}^{-1}) \qquad (2)$$

199 PIC production rate = μ (d^{-1}) × (TPC – TOC) (pg C $cell^{-1}$) (3)

200

201 **2.5 Data analysis**

202 In a broad pCO_2 range, physiological rates are expected to initially increase quickly
203 until reaching an optimum and then decline towards further increasing CO_2 levels (e.g.
204 Krug et al. 2011). Hence we used the following modified Michaelis-Menten equation
205 (Bach et al. 2011) which was fitted to measured cellular growth, POC and PIC
206 production rates ~~The nonlinear regression model (4) was used to fit growth, POC and~~
207 ~~PIC production rates~~ and yielding theoretical optimum pCO_2 and maximum values
208 for each of the three populations (combining the data of five or six strains) (Bach et
209 al., 2011).

210 $$y = \frac{X \times pCO_2}{Y + pCO_2} - s \times pCO_2 \quad (4)$$

211 where X and Y are fitted parameters, and s ~~is~~, the sensitivity constant, ~~which indicates~~
212 ~~the effect of~~ depicts the slope of the decline after optimum CO_2 levels in response to
213 rising H^+. Based on the fitted X, Y and s, we calculated ~~the~~ pCO_2 optima (K_m)
214 (equation 5) ~~for physiological rates according to equation (5).~~ and ~~M~~maximum growth,
215 POC and PIC production rates ~~were calculated by using equation (4) based on K_m.~~
216 following Bach et al., (2011).

217 $$K_m = \sqrt{\frac{X \times Y}{s}} - Y \quad (5)$$

218 The relative values for growth, POC and PIC production rates were calculated as
219 ratios of growth, POC and PIC production rates at each pCO_2 level to the maximum
220 (highest) rates. We obtained the relative sensitivity constant by fitting function (4)

based on relative growth, POC and PIC production rates.

A one-way ANOVA was then used to test for statistically significant differences in theoretical optimum pCO_2, maximum value and relative sensitivity constant between populations. A Tukey HSD test was conducted to determine the differences between strains from different populations. A Shapiro–Wilk's analysis was tested to analyze residual normality. Statistical calculations were carried out using *R* and significance was shown by $p < 0.05$.

3 Results

3.1 Carbonate chemistry parameters

Carbonate system parameters are shown in Table 2. Average pCO_2 levels of the ASW ranged from 125 µatm to 2490 µatm for the Azores population, from 120 µatm to 2280 µatm for the Bergen population, and from 130 µatm to 2630 µatm for the Canary Islands population. Corresponding pH_T values of the ASW ranged from 8.46 to 7.33 for the Azores population, from 8.47 to 7.37 for the Bergen population, and from 8.45 to 7.31 for the Canary Islands population.

3.2 Measured growth, POC and PIC production rates of each population

Growth rates, POC and PIC production rates of the three *E. huxleyi* populations increased with rising pCO_2, reached a maximum, and then declined with further pCO_2 increase (Fig. 1). Growth rates of the Azores and Bergen populations were larger than

243 those of the Canary Islands population at all investigated pCO_2 levels (Fig. 1a). With
244 rising pCO_2 levels beyond the pCO_2 optimum, decline in growth rates was more
245 pronounced in the Azores and Canary Islands populations than in the Bergen
246 population (Fig. 1b).

247 Measured POC production rates of the Azores and Bergen populations were larger
248 than those of the Canary Islands population at all pCO_2 levels (Fig. 1c) and decline in
249 POC production rates with increasing pCO_2 levels beyond the pCO_2 optimum was
250 larger in the Azores and Canary Islands populations than in the Bergen population
251 (Fig. 1d).

252 Measured PIC production rates at investigated pCO_2 levels did not show significant
253 differences among the Azores, Bergen and Canary Islands populations (Fig. 1e).
254 Exceptions were that at 365–695 μatm, PIC production rates of the Azores population
255 were larger than those of the Canary Islands population (all $p < 0.05$).

256

257 **3.3 Physiological responses of populations to pCO_2**

258 Calculated optimum pCO_2 for growth, POC and PIC production rates of the Bergen
259 population were significantly larger than those of the Azores and Canary Islands
260 populations (all $p < 0.05$) (Fig. 2a–c). Optimum pCO_2 for these physiological rates
261 between the Azores and Canary Islands population were not different (all $p > 0.1$).

262 Calculated maximum growth rates, POC and PIC production rates were not
263 significantly different between the Azores and the Bergen populations (all $p > 0.1$)
264 (Fig. 2d–f). Maximum growth rate and POC production rate of the Canary Islands

population were significantly lower than those of the Azores and Bergen populations (both $p < 0.01$) (Fig. 2d,e). Maximum PIC production rates of the Canary Islands population were significantly lower than that of the Azores population ($p < 0.05$), while there was no difference to the Bergen population ($p > 0.1$) (Fig. 2f).

Fitted relative sensitivity constants for growth and POC production rates of the Bergen population were significantly lower than those of the Azores and Canary Islands populations ($p < 0.01$) (Fig. 2g, h). Fitted relative sensitivity constants for growth and POC production rates between the Azores and Canary Islands populations were not significantly different ($p > 0.1$). Fitted relative sensitivity constants for PIC production rates did not show difference among three populations ($p = 0.13$) (Fig. 2i).

3.4 Physiological responses of individual strains to pCO_2

Measured growth rates, POC and PIC production rates of 17 *E. huxleyi* strains showed optimum curve response patterns to the broad pCO_2 gradient (Fig. 3). Variations in calculated pCO_2 optima, maximum values and relative sensitivity constants of physiological rates were found between the strains (Table 3).

For all strains within each population, optimum pCO_2 of POC production rates were larger than optimum pCO_2 of growth rates or PIC production rates with the exception of optimum pCO_2 of POC and PIC production rates of *E. huxleyi* strain EHGLE A22 (Table 3). Compared to the Azores and Bergen populations, strains isolated near the Canary Islands showed larger variation in optimum pCO_2 of PIC production rates. Within the Azores population, variations in maximum values (V_{max})

and relative sensitivity constants (*rs*) of growth, POC and PIC production rates of all strains were larger than those within the Bergen and Canary Islands populations (Fig. 3).

4 Discussion

We investigated growth, POC and PIC production rates of 17 *E. huxleyi* strains from three populations to a broad pCO_2 range (120–2630 µatm). The three populations differed significantly in growth and POC production rates at the investigated pCO_2 levels. The reaction norms of the individual strains and populations equaled an optimum curve for all physiological rates (Figs. 1 and 3). However, we detected distinct pCO_2 optima for growth, POC and PIC production rates, and different H^+ sensitivities for growth and POC production rates among them (Fig. 2). These results indicate the existence of distinct populations in the cosmopolitan coccolithophore *E. huxleyi*.

In comparison to the Azores and Canary Islands populations, variability in growth rates between strains of the Bergen population was smaller even though they had higher growth rates at all pCO_2 levels (Fig. 3). Furthermore, the Bergen population showed significantly higher pCO_2 optima and lower H^+ sensitivity for growth and POC production rates (Fig. 2). These findings indicate that the Bergen population may be more tolerant to changing carbonate chemistry in terms of its growth and photosynthetic carbon fixation rates. The Bergen strains were isolated from coastal

waters, while the Azores and Canary Islands strains were isolated from a more oceanic environment. Seawater carbonate chemistry of coastal waters is usually more dynamic than in the open ocean (Cai, 2011). In fact, previous studies have reported that CO_2 and pH variability of the seawater off Bergen was larger than off the Azores and Canary Islands (Table 1). In addition, due to riverine input, seawater upwelling and metabolic activity of plankton communities, environmental variability in coastal waters are larger than in open-ocean ecosystems (Duarte and Cerbrian, 1996). Doblin and van Sebille (2016) suggested that phytoplankton populations should be constantly under selection when experiencinged with changing environmental conditions. In this case, the Bergen population, exposed to larger CO_2 or pH fluctuations, may have acquired a higher capacity to acclimate to changing carbonate chemistry resulting in a higher tolerance (or lower sensitivity) to rising CO_2 levels. In contrast, the Azores and Canary Islands populations experience similar, less variable seawater carbonate chemistry conditions in their natural environment, which could explain why they also show similar pCO_2 optima and H^+ sensitivity for physiological rates (Fig. 2).

In an earlier study (Zhang et al., 2014), growth rates of the same Azores and Bergen strains as used here were measured at 8–28 ^{o}C. While at 26–28 ℃ the Bergen strains grew slower than the Azores strains, at 8 ℃ the Azores strains grew slower than the Bergen strains. This illustrates nicely that local temperature adaptation can significantly affect growth of *E. huxleyi* strains in laboratory experiments. Considering these findings and the temperature ranges of the three isolationed locations (Table S1), the incubation temperature of 16 ℃ used in the present study

was lower than the minimum sea surface temperature (SST) commonly recorded at the Canary Islands. In contrast, SSTs of 16 ℃ and lower have been reported for Azores and Bergen waters (Table S1). When exposed to 16 ^{o}C, growth rate of the Canary Islands population might have been already below their optimum and hence significantly reduced in comparison to the other populations ~~thus it grew slower than the other populations~~ (Fig. 2d).

Furthermore, compared to the Canary Islands population, the Azores population had higher maximum growth and POC production rates, and similar optimum CO_2 for these physiological rates. Again, this might be related to sub-optimal incubation conditions as temperature has been found to significantly modulate CO_2 responses in coccolithophores in terms of maximum rates, CO_2 optima and half-saturation, and H^+ sensitivity (De Bodt et al., 2010; Sett et al., 2014; Gafar et al., 2018; Gafar and Schulz, 2018). In a similar fashion light can also modulate CO_2 responses, hence different requirements by strains adapted to different light availabilities could also explain our observations (Zhang et al., 2015; Gafar et al., 2018; Gafar and Schulz, 2018). ~~One of the reasons may be that compared to the Azores and Bergen populations, 16 ^{o}C likely causes lower the carbon uptake and carbon-use efficiency of the Canary Islands population (Sett et al., 2014).~~ Thus, with rising CO_2, growth, photosynthetic carbon fixation and calcification rates of the Canary Islands population cannot increase as much as in the Azores and Bergen populations. In addition, the Canary Islands population showed smallest variability in optimum pCO_2 and maximum values for growth and POC production rates (Fig. 2). The reason may be that low incubation

353 temperature predominantly limited growth and POC production rates of the Canary
354 Islands population, and decreased the sensitivities of these physiological rates to
355 rising pCO_2.

356 Before we started this experiment, strains isolated from the Azores, Bergen and
357 Canary Islands grew as stock cultures at 15 ^{o}C and 400 µatm for 4 years, 5 years and
358 3 months, respectively. Schaum et al. (2015) provide evidence that long-term
359 laboratory incubation affects responses of phytoplankton to different pCO_2 levels.
360 Thus, it is conceivable that the same selection history in the laboratory incubation
361 may contribute to a more similar response of growth, POC and PIC production rates
362 between the Azores and Bergen populations at low pCO_2 levels (Fig. 1).

363 Our results indicate that *E. hulxyei* populations are adapted to the specific
364 environmental conditions of their origin, resulting in different responses to increasing
365 pCO_2 levels. The ability to adapt to diverse environmental conditions is ~~reflected in~~
366 supposed to be one reason for the global distribution of *E. huxleyi* (Paasche, 2002),
367 spanning a temperature range of about 30 ^{o}C. The optimum temperature for growth of
368 the Bergen population was about 22 ^{o}C and was 5 ^{o}C higher than the maximum SST
369 in Bergen waters (Zhang et al. 2014). Furthermore, in comparison to the Azores and
370 Canary Islands populations, larger optimum pCO_2 of growth rate indicates that the
371 Bergen population may benefit more from the rising CO_2 levels at increasing
372 temperatures. PIC : POC ratios of the Azores and Bergen populations declined with
373 rising pCO_2, whereas PIC : POC ratios of the Canary Islands population were rather
374 constant (Fig. S6). As changes in PIC : POC ratios of coccolithophore blooms may

impact on the biological carbon pump, different regions might see different changes in the future ocean. In natural seawater, due to ocean currents and gene flow, populations at any given location may get replaced by ~~populations~~ immigrant genotypes transported there from other locations ~~when having a higher potential to adapt to a changing environment~~ (Doblin and van Sebille, 2016). In addition, *E. huxleyi* ~~take up~~ is thought to utilize HCO_3^- ~~to calcify and~~for calcification which generates protons, and increase in proton concentration may mitigate the potential of the ocean to absorb atmospheric CO_2 (Paasche, 2002). ~~Thus, due to population-specific growth and PIC production rates or quotas, changes in species composition, corresponding changes in PIC productions, may affect the ability of the ocean to take up CO_2.~~

Within a population, individual strains showed different growth, POC and PIC production rates at different pCO_2 levels, indicating phenotypic plasticity of individual strains (Reusch, 2014). Phenotypic plasticity constitutes an advantage for individual strains to acclimate and adapt to elevated pCO_2 by changing ~~their~~ fitness-relevant traits and potentially to attenuate the short-term effects of changing environments on fitness-relevant traits (Schaum et al., 2013). ~~Additionally, our results also suggest that strain-specific PIC quota may be the basis of variation in coccoliths of *E. huxleyi* within the morphotype A (Fig. S3) (Young, 1994; Paasche, 2002).~~

The strain-specific CO_2-response curves revealed considerable physiological diversity in co-occurring strains (Fig. 3). Physiological variability makes a population more resilient, ~~and~~ increases its ability to persist in variable environments and

potentially forms the basis for selection (Gsell et al., 2012; Hattich et al., 2017). It is clear that other environmental factors such as light intensity, temperature and nutrient concentration affect the responses of physiological rates of individual *E. huxleyi* strains to changing carbonate chemistry, and thus change the physiological variability within populations (Zhang et al., 2015; Feng et al., 2017). However, different sensitivities and requirements of each strain to the variable environments can allow strains to co-exist within a population in the natural environment (Hutchinson, 1961; Reed et al., 2010; Krueger-Hadfield et al., 2014). In a changing oceans, strain succession is likely to occur and shift the population composition (Blanco-Ameijeiras et al., 2016; Hattich et al., 2017). Strains with higher growth rates or other competitive abilities may outcompete others strains in the oceans (Schaum et al., 2013). Further, a Ssignificant positive correlation between growth and POC production rate or POC quota (Fig. 4S5) suggestsindicates that the dominateding strains canwill also take up or fix dissolved inorganic carbon faster from the oceans or fix carbon faster. When extrapolated to the ocean, *E. huxleyi* blooms This may increase the potential of the oceans to absorb CO_2 from the atmosphere or the and its carbon storage capacity of the oceans when large *E. huxleyi* blooms occur (Blanco-Ameijeiras et al., 2016), which willhas the potential to mitigate rising CO_2 levels in the atmosphere.

5 Conclusions

In the present study, we found population-specific responses in physiological rates of

E. huxleyi to a broad pCO_2 range, which may have arisen from local adaptation to environmental conditions at their origins. The existence of distinct *E. huxleyi* populations and phenotypic plasticity of individual strains may both be important for *E. huxleyi* when adapting to natural environmental variability and to ongoing climate changes. Our results suggest that when assessing phytoplankton responses to changing environments on a global scale, variability in population ~~or~~and strain responses need to be considered, and CO_2 response was modulated by other environmental factors such as temperature and light intensity.

Author contributions. YZ, LTB, UR designed the experiment. YZ, LL, RK performed the experiment. YZ prepare the manuscript and all authors analysed the data, reviewed and improved the manuscript.

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

Acknowledgements. The authors thank Jana Meyer for particulate organic and inorganic carbon measurements. This work was supported by the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung) in the framework of the collaborative project Biological Impacts of Ocean Acidification (BIOACID). Kai G. Schulz is the recipient of an Australian Research Council Future Fellowship (FT120100384). We also thank the China Postdoctoral Science Foundation (2017M612129) and Outstanding Postdoctoral Scholarship in State Key Laboratory of Marine Environmental Science at Xiamen University for their supports of Yong Zhang.

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662 **Figure Legends**

663 **Figure 1.** Optimum curve responses of measured and relative growth, particulate
664 organic (POC) and inorganic carbon (PIC) production rates of three *Emiliania huxleyi*
665 populations to a pCO_2 range from 120 µatm to 2630 µatm. Responses of measured (**a**)
666 and relative (**b**) growth rates to pCO_2. Responses of measured (**c**) and relative (**d**)
667 POC production rates to pCO_2. Responses of measured (**e**) and relative (**f**) PIC
668 production rates to pCO_2. Using the nonlinear regression model derived by Bach et al.
669 (2011), the curves were fitted based on average growth, POC and PIC production
670 rates of six strains from the Azores and Bergen, and of five strains from the Canary
671 Islands. Vertical error bars represent standard deviations of six growth, POC and PIC
672 production rates for the Azores and Bergen populations, and five growth, POC and
673 PIC production rates for the Canary Islands population. Horizontal error bars
674 represent standard deviations of six pCO_2 levels for the Azores and Bergen
675 populations and five pCO_2 levels for the Canary Islands populations. At the
676 population levels, 120 µatm and 2630 µatm was the lowest and highest pCO_2 level,
677 respectively.

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679 **Figure 2.** Calculated optimum pCO_2, calculated maximum value and fitted relative
680 sensitivity constant of growth, POC and PIC production rates of each population. **(a)**
681 optimum pCO_2 of growth rate; **(b)** optimum pCO_2 of POC production rates; **(c)**
682 optimum pCO_2 of PIC production rates; **(d)** maximum growth rate, **(e)** maximum
683 POC production rate, **(f)** maximum PIC production rate; **(g)** relative sensitivity

684 constant of growth rate; **(h)** relative sensitivity constant of POC production rate; **(i)**
685 relative sensitivity constant of PIC production rate. The line in the middle of each box
686 indicates the mean of 6 or 5 optimum pCO_2, 6 or 5 maximum values, and 6 or 5
687 relative sensitivity constants for growth, POC and PIC production rates in each
688 population. Bars indicate the 99% confidence interval. The maximum or minimum
689 data is shown as the small line on the top or bottom of the bar, respectively. Letters in
690 each panel represent statistically significant differences (Tukey HSD, $p < 0.05$).
691
692 **Figure 3.** Optimum curve responses of growth, POC and PIC production rates of
693 individual *E. huxleyi* strains in the Azores (left), Bergen (medium) and Canary Islands
694 (right) populations to a CO_2 range from 115 µatm to 3070 µatm. Growth rates of each
695 strain as a function of pCO_2 within the Azores (**a**), Bergen (**b**) and Canary Islands (**c**)
696 populations. POC production rates of each strain as a function of pCO_2 within the
697 Azores (**d**), Bergen (**e**) and Canary Islands (**f**) populations. PIC production rates of
698 each strain as a function of pCO_2 within the Azores (**g**), Bergen (**h**) and Canary
699 Islands (**i**) populations. At the strain levels, 115 µatm and 3070 µatm was the lowest
700 and highest pCO_2 level, respectively.
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706 **Table 1.** Surface seawater CO_2 levels and pH at the Azores, Bergen and Canary

707 Islands.

	Location	Mean seasonal CO_2 (µatm)	Mean seasonal pH (total scale)	CO_2 variability (µatm)	References
Azores	38°34'N, 28°42'W	320 – 400	8.005 – 8.05	80	Ríos et al., 2005 Wisshak et al., 2010
Bergen	60°18'N, 05°15'E	240 – 400	7.98 – 8.22	200	Omar et al., 2010
Canary Islands	27°58'N, 15°36'W	320 – 400	8.005 – 8.05	80	González-Dávila et al., 2003

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725 **Table 2.** Carbonate chemistry parameters (mean values for the beginning and end of
726 the incubations) of the artificial seawater for each *Emiliania huxleyi* population. pH
727 and TA samples were collected and measured before and at the end of incubation.
728 Data are expressed as mean values of six strains in the Azores and Bergen population,
729 and five strains in the Canary Islands population.

	pCO_2 (µatm)	pH (total scale)	TA (µmol kg^{-1})	DIC (µmol kg^{-1})	HCO_3^- (µmol kg^{-1})	CO_3^{2-} (µmol kg^{-1})	CO_2 (µmol kg^{-1})	Ω
Azores	125 ±3	8.46 ±0.01	2358 ±12	1844 ±11	1485 ±13	355 ±5	5 ±0	8.5 ±0.1
	300 ±20	8.16 ±0.03	2339 ±27	2031 ±17	1803 ±18	218 ±13	11 ±1	5.2 ±0.3
	360 ±19	8.09 ±0.02	2322 ±30	2052 ±14	1849 ±9	190 ±10	13 ±1	4.5 ±0.3
	500 ±26	7.97 ±0.02	2301 ±23	2100 ±16	1933 ±14	149 ±8	18 ±1	3.5 ±0.2
	695 ±20	7.85 ±0.01	2317 ±11	2167 ±13	2023 ±14	118 ±2	25 ±1	2.8 ±0.1
	875 ±40	7.76 ±0.02	2320 ±19	2206 ±13	2076 ±10	99 ±5	32 ±1	2.4 ±0.1
	1110 ±119	7.66 ±0.05	2303 ±19	2222 ±23	2101 ±25	80 ±8	40 ±4	1.9 ±0.2
	1315 ±104	7.59 ±0.03	2308 ±18	2251 ±26	2133 ±26	70 ±4	48 ±4	1.7 ±0.1
	1665 ±107	7.50 ±0.03	2311 ±11	2286 ±15	2169 ±14	57 ±3	60 ±4	1.4 ±0.1
	1935 ±175	7.44 ±0.04	2308 ±15	2302 ±24	2183 ±21	50 ±4	70 ±6	1.2 ±0.1
	2490 ±132	7.33 ±0.02	2320 ±12	2350 ±15	2220 ±13	40 ±2	90 ±5	0.9 ±0.1
Bergen	120 ±3	8.47 ±0.01	2354 ±18	1834 ±18	1470 ±17	359 ±2	4 ±0	8.6 ±0.1
	290 ±16	8.17 ±0.02	2337 ±21	2024 ±12	1793 ±14	220 ±10	11 ±1	5.3 ±0.2
	355 ±18	8.10 ±0.02	2315 ±23	2045 ±11	1840 ±7	192 ±10	13 ±1	4.6 ±0.2
	490 ±18	7.98 ±0.02	2302 ±19	2096 ±14	1926 ±12	152 ±6	18 ±1	3.6 ±0.1
	670 ±22	7.86 ±0.01	2317 ±11	2162 ±10	2016 ±10	121 ±3	24 ±1	2.9 ±0.1
	855 ±52	7.77 ±0.03	2326 ±19	2206 ±15	2074 ±14	101 ±6	30 ±2	2.4 ±0.1
	1080 ±53	7.67 ±0.02	2316 ±26	2232 ±20	2110 ±18	83 ±5	39 ±2	2.0 ±0.1
	1280 ±71	7.60 ±0.02	2318 ±15	2257 ±17	2138 ±17	72 ±4	46 ±3	1.7 ±0.1
	1550 ±122	7.52 ±0.03	2300 ±19	2266 ±28	2150 ±27	60 ±4	56 ±4	1.4 ±0.1
	1800 ±235	7.47 ±0.05	2301 ±19	2286 ±33	2168 ±30	53 ±6	65 ±9	1.3 ±0.1
	2280 ±147	7.37 ±0.02	2309 ±20	2326 ±27	2201 ±24	42 ±2	82 ±5	1.0 ±0.1
Canary Islands	130 ±3	8.45 ±0.01	2344 ±38	1842 ±32	1491 ±26	347 ±7	5 ±0	8.3 ±0.2
	310 ±11	8.15 ±0.01	2317 ±24	2020 ±25	1798 ±25	210 ±4	11 ±1	5.0 ±0.1
	375 ±14	8.07 ±0.01	2295 ±14	2040 ±12	1846 ±13	182 ±5	14 ±1	4.3 ±0.1
	505 ±32	7.96 ±0.02	2297 ±19	2097 ±20	1930 ±23	148 ±7	18 ±1	3.5 ±0.2
	695 ±18	7.85 ±0.01	2312 ±20	2163 ±17	2020 ±15	118 ±3	25 ±1	2.8 ±0.1
	925 ±73	7.74 ±0.04	2319 ±26	2211 ±15	2083 ±12	95 ±8	33 ±3	2.3 ±0.1
	1180 ±53	7.64 ±0.02	2310 ±25	2239 ±20	2120 ±19	76 ±4	43 ±2	1.8 ±0.1
	1380 ±104	7.58 ±0.03	2323 ±5	2271 ±10	2154 ±11	68 ±5	50 ±4	1.6 ±0.1
	1740 ±98	7.48 ±0.02	2319 ±16	2298 ±16	2180 ±15	55 ±3	63 ±4	1.3 ±0.1
	2140 ±258	7.40 ±0.05	2312 ±9	2320 ±16	2197 ±13	46 ±5	78 ±10	1.1 ±0.1
	2630 ±284	7.31 ±0.04	2317 ±13	2363 ±20	2225 ±14	37 ±3	98 ±8	0.8 ±0.1

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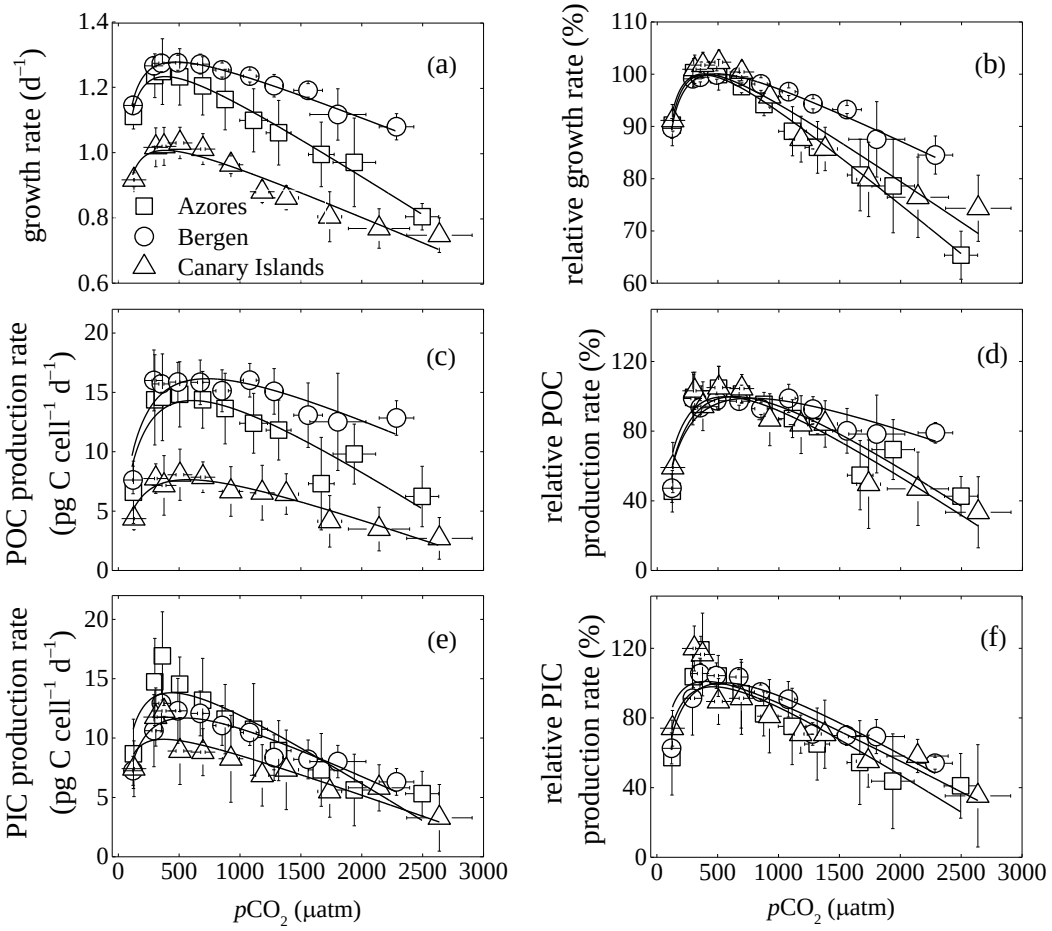
Table 3. Calculated optimum pCO_2, calculated maximum value (V_{max}) and fitted relative sensitivity constant (*rs*, ‰) of growth, POC and PIC production rates of each *E. huxleyi* strain.

	Growth rate			POC production rate			PIC production rate		
strain	**optimum pCO_2 (µatm)**	**V_{max} (d^{-1})**	***rs***	**optimum pCO_2 (µatm)**	**V_{max} (pg C $cell^{-1}$ d^{-1})**	***rs***	**optimum pCO_2 (µatm)**	**V_{max} (pg C $cell^{-1}$ d^{-1})**	***rs***
A23	392	1.21	0.22	673	12.47	0.50	323	13.45	0.38
A22	436	1.27	0.16	591	17.33	0.33	635	12.28	0.40
A21	392	1.25	0.22	707	15.45	0.50	396	16.73	1.11
A19	371	1.26	0.24	512	16.17	0.56	480	18.92	0.67
A13	244	1.08	0.13	756	9.84	0.63	471	11.72	0.57
A10	432	1.32	0.20	549	14.42	0.48	385	11.69	0.24
B95	534	1.26	0.10	762	13.46	0.20	562	9.13	0.33
B63	436	1.26	0.11	633	16.66	0.27	615	12.93	0.45
B62	456	1.29	0.11	945	17.27	0.18	488	14.00	0.43
B51	499	1.29	0.11	660	16.77	0.35	492	11.87	0.48
B41	542	1.25	0.09	984	18.34	0.38	553	9.46	0.37
B17	490	1.32	0.14	761	15.19	0.30	625	12.77	0.47
C98	400	1.03	0.16	644	8.44	0.54	440	6.40	0.31
C91	393	0.97	0.21	413	4.83	0.60	195	10.87	0.33
C90	384	0.97	0.12	546	8.28	0.34	284	8.52	0.50
C41	393	1.01	0.14	609	7.64	0.45	545	11.15	0.30
C35	378	1.05	0.17	596	8.87	0.44	464	12.68	0.34


(a)
(b)
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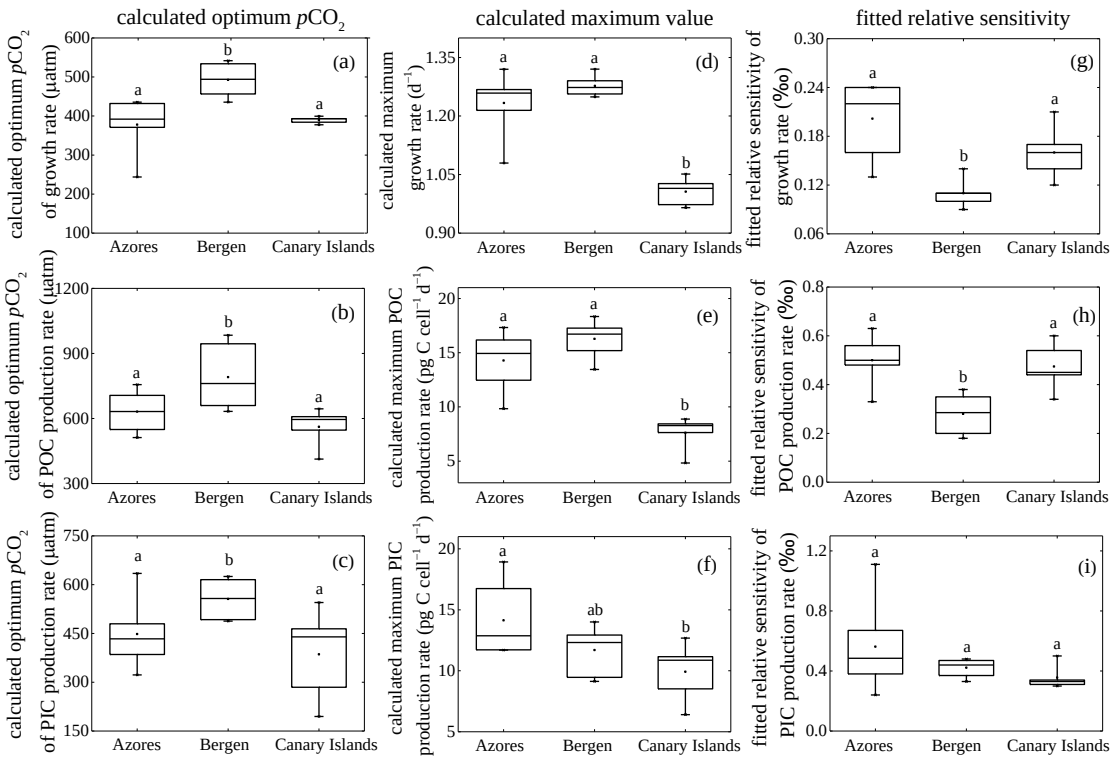
(e)
(f)
growth rate (d^{-1})
relative growth rate (%)
POC production rate (pg C cell^{-1} d^{-1})
relative POC production rate (%)
PIC production rate (pg C cell^{-1} d^{-1})
relative PIC production rate (%)
Azores
Bergen
Canary Islands
pCO$_2$ (µatm)
pCO$_2$ (µatm)

Figure 1


calculated optimum pCO_2
calculated maximum value
fitted relative sensitivity
calculated optimum pCO_2 of growth rate (µatm)
calculated maximum growth rate (d^{-1})
fitted relative sensitivity of growth rate (‰)
calculated optimum pCO_2 of POC production rate (µatm)
calculated maximum POC production rate (pg C $cell^{-1}$ d^{-1})
fitted relative sensitivity of POC production rate (‰)
calculated optimum pCO_2 of PIC production rate (µatm)
calculated maximum PIC production rate (pg C $cell^{-1}$ d^{-1})
fitted relative sensitivity of PIC production rate (‰)
(a)
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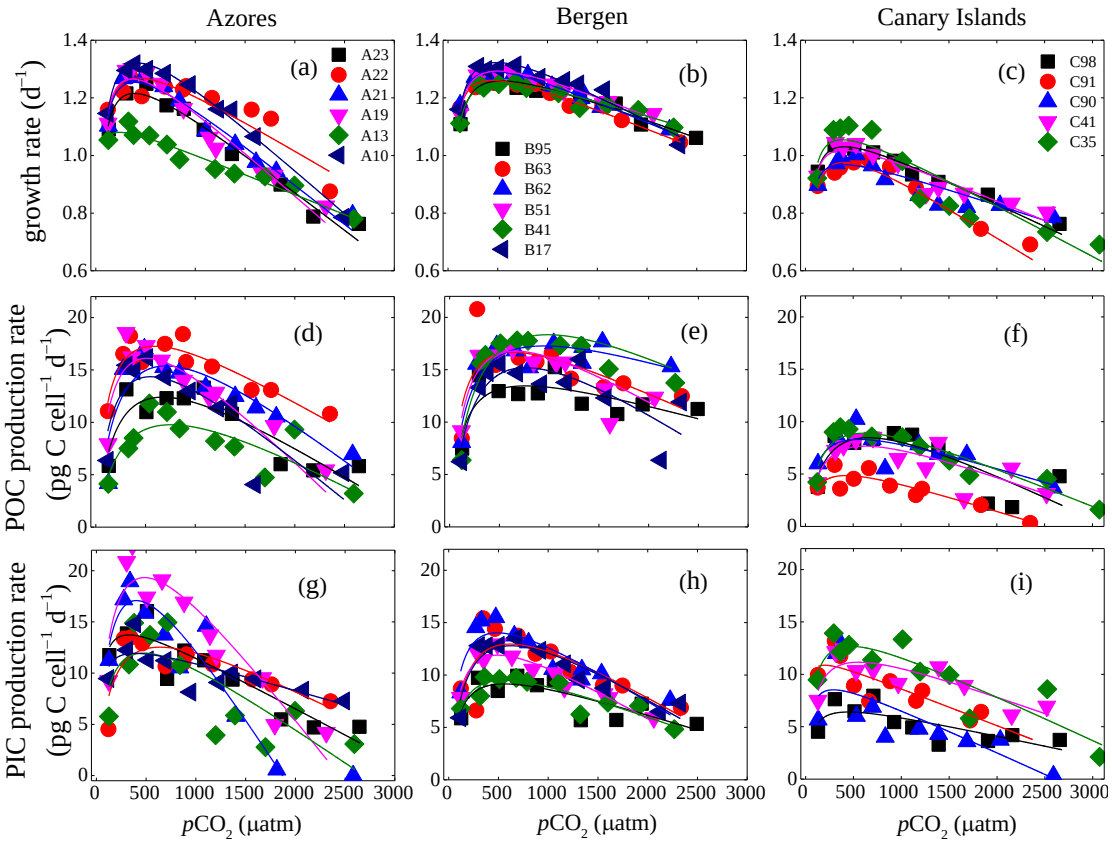
Azores
Bergen
Canary Islands

Figure 2


Azores
Bergen
Canary Islands
growth rate (d^{-1})
POC production rate (pg C cell^{-1} d^{-1})
PIC production rate (pg C cell^{-1} d^{-1})
(a)
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C90
C41
C35
pCO$_2$ (µatm)

Figure 3