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 120-2600µatm) 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 CO2 and found that most of these studies focused on a few strains or a narrow range of CO2 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 pCO₂ 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 pCO₂ ranges at constant alkalinity condition. Symbols indicate: ↑ increased response, — no response, ↓ decreased response, ∩ optimum response.

<table>
<thead>
<tr>
<th><em>E. huxleyi</em> genotype</th>
<th>Isolated site</th>
<th>pCO₂ range (µatm)</th>
<th>Growth rate</th>
<th>POC pro.</th>
<th>PIC pro.</th>
<th>Incubation temp. (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC472</td>
<td>South Pacific, New Zealand</td>
<td>400 to 760</td>
<td>↑</td>
<td>—</td>
<td>↑</td>
<td>19</td>
<td>Fiorini et al., (2011)</td>
</tr>
<tr>
<td>EHSO 5.14</td>
<td>Southern Ocean</td>
<td>300 to 1680</td>
<td>↓</td>
<td>∩</td>
<td>∩</td>
<td>14</td>
<td>Müller et al., (2015)</td>
</tr>
<tr>
<td>EHSO 5.11</td>
<td>Southern Ocean</td>
<td>259 to 1255</td>
<td>∩</td>
<td>∩</td>
<td>∩</td>
<td>14</td>
<td>Müller et al., (2015)</td>
</tr>
<tr>
<td>NIWA1108</td>
<td>Chatham Rise, New Zealand</td>
<td>80 to 1080</td>
<td>↑</td>
<td>↑</td>
<td>∩</td>
<td>4-25</td>
<td>Feng et al., (2017)</td>
</tr>
<tr>
<td>PLY M219 (NZEH)</td>
<td>New Zealand</td>
<td>380 to 750</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>20</td>
<td>Shi et al., (2009)</td>
</tr>
<tr>
<td>PLY M219 (NZEH)</td>
<td>New Zealand</td>
<td>404 to 1066</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>15</td>
<td>Hoppe et al., (2011)</td>
</tr>
<tr>
<td>B92/11A</td>
<td>Bergen, Norway</td>
<td>20 to 6000</td>
<td>∩</td>
<td>∩</td>
<td>∩</td>
<td>15</td>
<td>Bach et al., (2011)</td>
</tr>
<tr>
<td>RCC1212</td>
<td>South Atlantic, off South Africa</td>
<td>194 to 1096</td>
<td>↓</td>
<td>∩</td>
<td>↓</td>
<td>20</td>
<td>Langer et al., (2009)</td>
</tr>
<tr>
<td>RCC1216</td>
<td>Tasman Sea, off New Zealand</td>
<td>218 to 1201</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>17</td>
<td>Langer et al., (2009)</td>
</tr>
<tr>
<td>RCC1238</td>
<td>North Atlantic, off Japan</td>
<td>206 to 929</td>
<td>↑</td>
<td>∩</td>
<td>—</td>
<td>20</td>
<td>Langer et al., (2009)</td>
</tr>
<tr>
<td>RCC1256</td>
<td>North Atlantic, off Iceland</td>
<td>193 to 915</td>
<td>↓</td>
<td>∩</td>
<td>∩</td>
<td>17</td>
<td>Langer et al., (2009)</td>
</tr>
<tr>
<td>RCC1256</td>
<td>Iceland</td>
<td>191 to 846</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>15</td>
<td>Hoppe et al., (2011)</td>
</tr>
<tr>
<td>NZEH</td>
<td>New Zealand</td>
<td>280 to 750</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>19</td>
<td>Iglesias-Rodriguez et al., (2008)</td>
</tr>
</tbody>
</table>


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 $p$CO$_2$ 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 $p$CO$_2$ levels.

<table>
<thead>
<tr>
<th>TA (μmol L$^{-1}$)</th>
<th>DIC (μmol kg$^{-1}$)</th>
<th>pH (total scale)</th>
<th>$p$CO$_2$ (μatm)</th>
<th>HCO$_3^-$ (μmol kg$^{-1}$)</th>
<th>CO$_3^{2-}$ (μmol kg$^{-1}$)</th>
<th>CO$_2$ (μmol kg$^{-1}$)</th>
<th>Ω</th>
</tr>
</thead>
<tbody>
<tr>
<td>1500</td>
<td>1351.6</td>
<td>7.887</td>
<td>400</td>
<td>1245.0</td>
<td>93.7</td>
<td>12.9</td>
<td>2.24</td>
</tr>
<tr>
<td>1600</td>
<td>1436.9</td>
<td>7.912</td>
<td>400</td>
<td>1318.8</td>
<td>105.1</td>
<td>12.9</td>
<td>2.51</td>
</tr>
<tr>
<td>1700</td>
<td>1521.8</td>
<td>7.935</td>
<td>400</td>
<td>1391.8</td>
<td>117.1</td>
<td>12.9</td>
<td>2.80</td>
</tr>
<tr>
<td>1800</td>
<td>1606.3</td>
<td>7.957</td>
<td>400</td>
<td>1463.9</td>
<td>129.5</td>
<td>12.9</td>
<td>3.10</td>
</tr>
<tr>
<td>1900</td>
<td>1690.4</td>
<td>7.978</td>
<td>400</td>
<td>1535.1</td>
<td>142.4</td>
<td>12.9</td>
<td>3.41</td>
</tr>
<tr>
<td>2000</td>
<td>1774.2</td>
<td>7.997</td>
<td>400</td>
<td>1605.4</td>
<td>155.8</td>
<td>12.9</td>
<td>3.73</td>
</tr>
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<td>2100</td>
<td>1857.5</td>
<td>8.016</td>
<td>400</td>
<td>1675.0</td>
<td>169.6</td>
<td>12.9</td>
<td>4.06</td>
</tr>
<tr>
<td>2200</td>
<td>1940.6</td>
<td>8.033</td>
<td>400</td>
<td>1743.8</td>
<td>183.8</td>
<td>12.9</td>
<td>4.40</td>
</tr>
<tr>
<td>2300</td>
<td>2023.3</td>
<td>8.050</td>
<td>400</td>
<td>1811.9</td>
<td>198.4</td>
<td>12.9</td>
<td>4.75</td>
</tr>
<tr>
<td>2400</td>
<td>2105.6</td>
<td>8.066</td>
<td>400</td>
<td>1879.2</td>
<td>213.5</td>
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<td>8.081</td>
<td>400</td>
<td>1945.9</td>
<td>228.9</td>
<td>12.9</td>
<td>5.47</td>
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<td>2600</td>
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<td>400</td>
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<td>5.85</td>
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<td>2350.8</td>
<td>8.109</td>
<td>400</td>
<td>2077.1</td>
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<td>12.9</td>
<td>6.24</td>
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<tr>
<td>2800</td>
<td>2432.0</td>
<td>8.122</td>
<td>400</td>
<td>2141.8</td>
<td>277.3</td>
<td>12.9</td>
<td>6.63</td>
</tr>
</tbody>
</table>

Table R3. Carbonate chemistry parameter at changing $p$CO$_2$ levels and changing TA conditions.

<table>
<thead>
<tr>
<th>TA (μmol L$^{-1}$)</th>
<th>DIC (μmol kg$^{-1}$)</th>
<th>pH (total scale)</th>
<th>$p$CO$_2$ (μatm)</th>
<th>HCO$_3^-$ (μmol kg$^{-1}$)</th>
<th>CO$_3^{2-}$ (μmol kg$^{-1}$)</th>
<th>CO$_2$ (μmol kg$^{-1}$)</th>
<th>Ω</th>
</tr>
</thead>
<tbody>
<tr>
<td>1500</td>
<td>1254.0</td>
<td>8.134</td>
<td>200</td>
<td>1101.0</td>
<td>146.5</td>
<td>6.5</td>
<td>3.51</td>
</tr>
<tr>
<td>1600</td>
<td>1436.9</td>
<td>7.912</td>
<td>400</td>
<td>1318.8</td>
<td>105.1</td>
<td>12.9</td>
<td>2.51</td>
</tr>
<tr>
<td>1700</td>
<td>1576.7</td>
<td>7.783</td>
<td>600</td>
<td>1470.2</td>
<td>87.1</td>
<td>19.4</td>
<td>2.08</td>
</tr>
<tr>
<td>1800</td>
<td>1701.7</td>
<td>7.694</td>
<td>800</td>
<td>1598.6</td>
<td>77.2</td>
<td>25.8</td>
<td>1.85</td>
</tr>
<tr>
<td>1900</td>
<td>1819.7</td>
<td>7.628</td>
<td>1000</td>
<td>1716.2</td>
<td>71.2</td>
<td>32.3</td>
<td>1.70</td>
</tr>
<tr>
<td>2000</td>
<td>1934.0</td>
<td>7.576</td>
<td>1200</td>
<td>1827.9</td>
<td>67.3</td>
<td>38.8</td>
<td>1.61</td>
</tr>
<tr>
<td>2100</td>
<td>2046.0</td>
<td>7.534</td>
<td>1400</td>
<td>1936.0</td>
<td>64.7</td>
<td>45.2</td>
<td>1.55</td>
</tr>
<tr>
<td>2200</td>
<td>2156.4</td>
<td>7.500</td>
<td>1600</td>
<td>2041.7</td>
<td>63.0</td>
<td>51.7</td>
<td>1.51</td>
</tr>
<tr>
<td>2300</td>
<td>2265.8</td>
<td>7.470</td>
<td>1800</td>
<td>2145.8</td>
<td>61.8</td>
<td>58.1</td>
<td>1.48</td>
</tr>
<tr>
<td>2400</td>
<td>2374.4</td>
<td>7.445</td>
<td>2000</td>
<td>2248.7</td>
<td>61.1</td>
<td>64.6</td>
<td>1.46</td>
</tr>
<tr>
<td>2500</td>
<td>2482.4</td>
<td>7.422</td>
<td>2200</td>
<td>2350.7</td>
<td>60.7</td>
<td>71.1</td>
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</tr>
<tr>
<td>2600</td>
<td>2590.1</td>
<td>7.403</td>
<td>2400</td>
<td>2452.0</td>
<td>60.6</td>
<td>77.5</td>
<td>1.45</td>
</tr>
<tr>
<td>2700</td>
<td>2697.4</td>
<td>7.386</td>
<td>2600</td>
<td>2552.8</td>
<td>60.6</td>
<td>84.0</td>
<td>1.45</td>
</tr>
<tr>
<td>2800</td>
<td>2804.4</td>
<td>7.370</td>
<td>2800</td>
<td>2653.2</td>
<td>60.8</td>
<td>90.4</td>
<td>1.45</td>
</tr>
</tbody>
</table>
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.

![Flow Chart]

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

Also, how realistic are CO₂ levels greater than 1500 uatm?

Response: According to business-as-usual CO₂ emissions (RCP8.5), atmospheric CO₂ level are projected higher than 1500 ppmv after 2200 (Meinshausen et al. 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 °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 CO2 for these physiological rates. Again, this might be related to sub-optimal incubation conditions as temperature has been found to significantly modulate CO2 responses in coccolithophores in terms of maximum rates, CO2 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 CO2 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 pCO2 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 pCO2.


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 pCO2 by changing fitness-relevant traits and potentially to attenuate the short-term effects of changing environments on fitness-relevant traits (Schaum et al.,
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 °C and was 5 °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 pCO2 of growth rate indicates that the Bergen population may benefit more from the rising CO2 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 pCO2, 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 CO2 level.

For lines 350–355: In addition, the Canary Islands population showed smallest variability in optimum pCO2 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 pCO2.
**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,
CO₂, 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).


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:" and then in line 160 "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 pCO₂ range, physiological rates are expected to initially increase quickly until reaching an optimum and then decline towards further increasing CO₂ 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 pCO₂ 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 CO₂ 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.

"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 °C likely causes lower the carbon uptake and carbon use efficiency of the Canary Islands population’

Line 343: add "s" to proton

Response: For lines 379–382: In addition, *E. huxleyi* is thought to utilize $\text{HCO}_3^-$ 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 °C. The optimum temperature for growth of the Bergen population was about 22 °C and was 5 °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 $p$CO$_2$ 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 $p$CO$_2$, 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 $\text{HCO}_3^-$ 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 p\(\text{CO}_2\) levels with no replicate. At each p\(\text{CO}_2\) 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 p\(\text{CO}_2\), 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).’


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 (p\(\text{CO}_2\)) 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 p\(\text{CO}_2\) 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 p\(\text{CO}_2\) 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).


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?
Response: 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 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 °C the Bergen strains grew slower than the Azores strains, at 8 °C 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 °C 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 °C 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 (*C₀*) were measured, and we calculated the inoculated volumes (*V*) according to *V* = (200 cell/ml x 590 ml)/*C₀*. 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 pCO₂ 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 CO₂ 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 $p$CO$_2$ gradient’ to ‘increased with rising $p$CO$_2$ 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 $p$CO$_2$ 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 CO₂ levels in response to’

11 Line 213: delete ‘the’

12 Line 214: delete ‘(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 Kₘ.’

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 CO₂ for these physiological rates. Again, this might be related to sub-optimal incubation conditions as temperature has been found to significantly modulate CO₂ responses in coccolithophores in terms of maximum rates, CO₂ 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 CO₂ 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 °C 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 pCO₂ 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 pCO₂.’

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 °C and was 5 °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.

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
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 17 strains of the coccolithophore Emiliania huxleyi from three regions in the North Atlantic Ocean (Azores: 6 strains, Canary Islands: 5 strains, and Norwegian fjord coast near Bergen: 6 strains) to a CO$_2$ partial pressure ($p$CO$_2$) range from 120 µatm to 2630 µatm. Physiological rates of each population and individual strain displayed the expected optimum curve responses to the $p$CO$_2$-gradient increased with rising $p$CO$_2$ levels, reached maximum and declined thereafter. Optimal $p$CO$_2$ 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 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 $p$CO$_2$ 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°C) 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 $p\text{CO}_2$ 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 $p$CO$_2$ range from about 20 µatm to 5000 µatm, and linked them to inorganic carbon substrate limitation at low $p$CO$_2$ and inhibiting $H^+$ concentrations at high $p$CO$_2$ (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 CO₂ 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 CO₂ 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 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 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$_2$CO$_3$ 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 CO₂, and for 6 or 7 days in 1550–2630 μatm CO₂. 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 pCO₂ levels changed less than 7% and 11%, respectively, during the experimental growth phase.

2.2 pHₜ and total alkalinity measurements

At 10:00 a.m. on the last day of incubations (at day 4–7 depending on CO₂ concentration), pHₜ 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 pHₜ sample bottles were filled with considerable overflow and closed tightly with no space. pHₜ 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 unprotonated 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, pHₜ, (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 K₁ and K₂ as determined by Roy et al. (1993).

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

$$\mu = \frac{\ln N_1 - \ln N_0}{d}$$  

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

### 2.4 Particulate organic (POC) and inorganic (PIC) carbon measurements

At 3:00 p.m. on the last day of incubation, cells for total particulate (TPC) and total organic (TOC) carbon were filtered onto GF/F filters which were pre-combusted at 500 °C for 8 h. Samples of background particulate carbon (BPC) were determined in a similar way but using filtered ASW without algae, which was previously adjusted to target $p$CO$_2$ levels, and allowed to age for about 7 days under incubation conditions (see above). All samples were placed at −20°C. BPC filters were used as blanks to correct for organic carbon in the medium. TOC and BPC filters were acid fumed. Afterwards, all filters were dried for 8 h at 60°C. TPC, TOC and BPC were measured using an Elemental Analyzer (EuroEA, Hekatech GmbH). The percentages of BPC in TPC were about 20% at cell densities < 10,000 cells ml$^{-1}$ and about 10% at cell densities > 40,000 cells ml$^{-1}$. POC was calculated as the difference between TOC and BPC. PIC was calculated as the difference between TPC and TOC. POC and PIC production rates were calculated as:

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

(2)
PIC production rate = $\mu \, (d^{-1}) \times (TPC - TOC) \, (pg \, C \, cell^{-1}) \quad (3)$

2.5 Data analysis

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. The nonlinear regression model (4) was used to fit 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).

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

where $X$ and $Y$ are fitted parameters, and $s$ is the sensitivity constant, which indicates the effect of $pCO_2$ (H+) on physiological rates. Based on the fitted $X$, $Y$ and $s$, we calculated the $pCO_2$ optima ($K_m$) (equation 5) for physiological rates according to equation (5). Maximum growth, POC and PIC production rates were calculated by using equation (4) based on $K_m$ following Bach et al., (2011).

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

The relative values for growth, POC and PIC production rates were calculated as ratios of growth, POC and PIC production rates at each $pCO_2$ level to the maximum (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
those of the Canary Islands population at all investigated $pCO_2$ levels (Fig. 1a). With rising $pCO_2$ levels beyond the $pCO_2$ optimum, decline in growth rates was more pronounced in the Azores and Canary Islands populations than in the Bergen population (Fig. 1b).

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

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

### 3.3 Physiological responses of populations to $pCO_2$

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

Calculated maximum growth rates, POC and PIC production rates were not significantly different between the Azores and the Bergen populations (all $p > 0.1$) (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 CO₂ 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 experiencing changing environmental conditions. In this case, the Bergen population, exposed to larger CO₂ 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 CO₂ 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 pCO₂ 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 °C. While at 26–28 °C the Bergen strains grew slower than the Azores strains, at 8 °C 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 isolated locations (Table S1), the incubation temperature of 16 °C 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 °C 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 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 °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 $p$CO$_2$ 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 $pCO_2$.

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

Our results indicate that *E. huxleyi* populations are adapted to the specific environmental conditions of their origin, resulting in different responses to increasing $pCO_2$ levels. The ability to adapt to diverse environmental conditions is reflected in supposed to be one reason for the global distribution of *E. huxleyi* (Paasche, 2002), spanning a temperature range of about 30 °C. The optimum temperature for growth of the Bergen population was about 22 °C and was 5 °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 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 HCO₃⁻ to calcify and/or calcification which generates protons, and increase in proton concentration may mitigate the potential of the ocean to absorb atmospheric CO₂ (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 CO₂.

Within a population, individual strains showed different growth, POC and PIC production rates at different pCO₂ levels, indicating phenotypic plasticity of individual strains (Reusch, 2014). Phenotypic plasticity constitutes an advantage for individual strains to acclimate and adapt to elevated pCO₂ 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 CO₂-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 significant positive correlation between growth and POC production rate or POC quota (Fig. 4S) suggests that the dominating strains eat will 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 will has 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 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.

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References


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Figure Legends

**Figure 1.** Optimum curve responses of measured and relative growth, particulate organic (POC) and inorganic carbon (PIC) production rates of three *Emiliania huxleyi* populations to a pCO$_2$ range from 120 µatm to 2630 µatm. Responses of measured (a) and relative (b) growth rates to pCO$_2$. Responses of measured (c) and relative (d) POC production rates to pCO$_2$. Responses of measured (e) and relative (f) PIC production rates to pCO$_2$. Using the nonlinear regression model derived by Bach et al. (2011), the curves were fitted based on average growth, POC and PIC production rates of six strains from the Azores and Bergen, and of five strains from the Canary Islands. Vertical error bars represent standard deviations of six growth, POC and PIC production rates for the Azores and Bergen populations, and of five growth, POC and PIC production rates for the Canary Islands population. Horizontal error bars represent standard deviations of six pCO$_2$ levels for the Azores and Bergen populations and five pCO$_2$ levels for the Canary Islands populations. At the population levels, 120 µatm and 2630 µatm was the lowest and highest pCO$_2$ level, respectively.

**Figure 2.** Calculated optimum pCO$_2$, calculated maximum value and fitted relative sensitivity constant of growth, POC and PIC production rates of each population. (a) optimum pCO$_2$ of growth rate; (b) optimum pCO$_2$ of POC production rates; (c) optimum pCO$_2$ of PIC production rates; (d) maximum growth rate, (e) maximum POC production rate, (f) maximum PIC production rate; (g) relative sensitivity
constant of growth rate; (h) relative sensitivity constant of POC production rate; (i) relative sensitivity constant of PIC production rate. The line in the middle of each box indicates the mean of 6 or 5 optimum $p\text{CO}_2$, 6 or 5 maximum values, and 6 or 5 relative sensitivity constants for growth, POC and PIC production rates in each population. Bars indicate the 99% confidence interval. The maximum or minimum data is shown as the small line on the top or bottom of the bar, respectively. Letters in each panel represent statistically significant differences (Tukey HSD, $p < 0.05$).

**Figure 3.** Optimum curve responses of growth, POC and PIC production rates of individual *E. huxleyi* strains in the Azores (left), Bergen (medium) and Canary Islands (right) populations to a CO$_2$ range from 115 µatm to 3070 µatm. Growth rates of each strain as a function of $p\text{CO}_2$ within the Azores (a), Bergen (b) and Canary Islands (c) populations. POC production rates of each strain as a function of $p\text{CO}_2$ within the Azores (d), Bergen (e) and Canary Islands (f) populations. PIC production rates of each strain as a function of $p\text{CO}_2$ within the Azores (g), Bergen (h) and Canary Islands (i) populations. At the strain levels, 115 µatm and 3070 µatm was the lowest and highest $p\text{CO}_2$ level, respectively.
Table 1. Surface seawater CO₂ levels and pH at the Azores, Bergen and Canary Islands.

<table>
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<th>Location</th>
<th>Mean seasonal CO₂ (µatm)</th>
<th>Mean seasonal pH (total scale)</th>
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Table 2. Carbonate chemistry parameters (mean values for the beginning and end of the incubations) of the artificial seawater for each *Emiliania huxleyi* population. pH and TA samples were collected and measured before and at the end of incubation. Data are expressed as mean values of six strains in the Azores and Bergen population, and five strains in the Canary Islands population.

<table>
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Table 3. Calculated optimum $p$CO$_2$, calculated maximum value ($V_{\text{max}}$) and fitted relative sensitivity constant ($rs$, ‰) of growth, POC and PIC production rates of each *E. huxleyi* strain.

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Figure 1
Figure 2
Figure 3