Population-specific responses in physiological rates of \textit{Emiliania huxleyi} to a broad CO$_2$ range

Yong Zhang,$^{1,5,*}$ Lennart T. Bach,$^1$ Kai T. Lohbeck,$^{1,2,6}$ Kai G. Schulz,$^3$ Luisa Listmann,$^2$ Regina Klapper,$^4$ Ulf Riebesell$^1$

$^1$Biological Oceanography, GEOMAR Helmholtz-Centre for Ocean Research Kiel, Kiel, Germany

$^2$Evolutionary Ecology of Marine Fishes, GEOMAR Helmholtz-Centre for Ocean Research Kiel, Kiel, Germany

$^3$Centre for Coastal Biogeochemistry, School of Science, Environment and Engineering, Southern Cross University, Lismore, NSW, Australia

$^4$Goethe-University, Institute for Ecology, Evolution and Diversity; Senckenberg Gesellschaft für Naturforschung, Senckenberg Biodiversity and Climate Research Centre, Frankfurt am Main, Germany

$^5$State Key Laboratory of Marine Environmental Science, College of Ocean and Earth Sciences, Xiamen University (Xiang-An Campus), Xiamen 361102, China

$^6$Department of Marine Sciences, University of Gothenburg, Gothenburg, Sweden

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*Correspondence to: Yong Zhang (zhangyong1983@xmu.edu.cn)

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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. 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, Canary Islands, and Norwegian coast near Bergen) 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. 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 a Norwegian fjord than in those isolated near the Azores and Canary Islands. This may be due to the large $p$CO$_2$ and pH variability 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 of the Canary Islands population. One of the reasons may be that the chosen incubation temperature (16 °C) is slightly below what strains isolated near the Canary Islands normally experience. Our results indicate adaptation of *E. huxleyi* to their local environmental conditions. Within each population, different growth, POC and PIC production rates at different $p$CO$_2$ levels indicated strain-specific phenotypic plasticity. The existence of distinct carbonate
chemistry responses between and within populations will likely benefit *E. huxleyi* to acclimate 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).

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 multiple strains should be considered for investigating phytoplankton responses to climate change (Zhang et al., 2014; Blanco-Ameijeiras et al., 2016).

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 (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 $p$CO$_2$ 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 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\textsubscript{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. Monoclonal populations were always grown in sterile-filtered (0.2 μm diameter, Sartobran\textsuperscript{®} P 300, Sartorius) artificial seawater medium (ASW) as dilute batch cultures at 200 μmol photons m\textsuperscript{-2} s\textsuperscript{-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\textsuperscript{-1} and 4 μmol kg\textsuperscript{-1}, respectively). For the preparation of ASW and nutrient additions see Zhang et al. (2014). Calculated volumes of Na\textsubscript{2}CO\textsubscript{3} and hydrochloric acid were added to the ASW to achieve target CO\textsubscript{2} levels at an average total alkalinity (TA) of 2319 ± 23 μmol kg\textsuperscript{-1} (Pierrot et al., 2006; Bach et al., 2011). Each strain was grown under 11 CO\textsubscript{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\textsubscript{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\textsubscript{2}, for 5 days in 1080–1380 μatm CO\textsubscript{2}, and for 6 or 7 days in 1550–2630 μatm CO\textsubscript{2}. Initial cell concentration was 200 cells ml\textsuperscript{-1} and final cell concentration was lower than 100,000 cells ml\textsuperscript{-1}. Dissolved inorganic carbon (DIC) concentrations and pCO\textsubscript{2} levels changed...
less than 7% and 11%, respectively, during the experimental growth phase.

2.2 pH\textsubscript{T} and total alkalinity measurements

At 10:00 a.m. on the last day of incubations (at day 4–7 depending on CO\textsubscript{2} concentration), pH\textsubscript{T} and TA samples were filtered (0.2 \textmu m 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\textsubscript{T} sample bottles were filled with considerable overflow and closed tightly with no space. pH\textsubscript{T} was measured spectrophotometrically (Cary 100, Agilent) using the indicator dye \textit{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, pH\textsubscript{T}, (assuming 4 \textmu mol kg\textsuperscript{-1} of phosphate and 0 \textmu mol kg\textsuperscript{-1} of silicate) using the CO\textsubscript{2} System Calculations in MS Excel software (Pierrot et al., 2006) with carbonic acid constants \textit{K}_1 and \textit{K}_2 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 $^\circ$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 $-20^\circ$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$^\circ$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:

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

\[
\text{PIC production rate} = \mu \times (d^{-1}) \times (\text{TPC} - \text{TOC}) \text{ (pg C cell}^{-1})
\]

### 2.5 Data analysis

The nonlinear regression model (4) was used to fit growth, POC and PIC production
rates yielding theoretical optimum \( p\text{CO}_2 \) and maximum values for each of the three populations (combining the data of five or six strains) (Bach et al., 2011).

\[
y = \frac{X \times p\text{CO}_2}{Y + p\text{CO}_2} - s \times p\text{CO}_2 \tag{4}
\]

where \( X \) and \( Y \) are fitted parameters, and \( s \) is the sensitivity constant which indicates the effect of rising \( H^+ \). Based on the fitted \( X, Y \) and \( s \), we calculated the \( p\text{CO}_2 \) optima \((K_m)\) for physiological rates according to equation (5). Maximum growth, POC and PIC production rates were calculated by using equation (4) based on \( K_m \).

\[
K_m = \sqrt{\frac{X \times Y}{s} - Y} \tag{5}
\]

The relative values for growth, POC and PIC production rates were calculated as ratios of growth, POC and PIC production rates at each \( p\text{CO}_2 \) 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 \( p\text{CO}_2 \), 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 $p\text{CO}_2$ 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 $p\text{CO}_2$, reached a maximum, and then declined with further $p\text{CO}_2$ increase (Fig. 1). Growth rates of the Azores and Bergen populations were larger than those of the Canary Islands population at all investigated $p\text{CO}_2$ levels (Fig. 1a). With rising $p\text{CO}_2$ levels beyond the $p\text{CO}_2$ 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 $p\text{CO}_2$ levels (Fig. 1c) and decline in POC production rates with increasing $p\text{CO}_2$ levels beyond the $p\text{CO}_2$ optimum was larger in the Azores and Canary Islands populations than in the Bergen population (Fig. 1d).

Measured PIC production rates at investigated $p\text{CO}_2$ 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 $p$CO$_2$

Calculated optimum $p$CO$_2$ 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 $p$CO$_2$ 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 $p\text{CO}_2$

Measured growth rates, POC and PIC production rates of 17 *E. huxleyi* strains showed optimum curve response patterns to the broad $p\text{CO}_2$ gradient (Fig. 3). Variations in calculated $p\text{CO}_2$ optima, maximum values and relative sensitivity constants of physiological rates were found between the strains (Table 3).

For all strains within each population, optimum $p\text{CO}_2$ of POC production rates were larger than optimum $p\text{CO}_2$ of growth rates or PIC production rates with the exception of optimum $p\text{CO}_2$ 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 $p\text{CO}_2$ 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 $p\text{CO}_2$ range (120–2630 μatm). The three populations differed significantly in growth and POC production rates at the investigated $p\text{CO}_2$ 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 $p$CO$_2$ 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 $p$CO$_2$ levels (Fig. 3). Furthermore, the Bergen population showed significantly higher $p$CO$_2$ 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). Doblin and van Sebille (2016) suggested that phytoplankton populations should be constantly under selection when experienced 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 \( p\text{CO}_2 \) optima and \( \text{H}^+ \) 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 \( E. \text{huxleyi} \) strains in laboratory experiments.

Considering these findings and the temperature ranges of 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 thus it grew slower than the other populations (Fig. 2d). 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 \( \text{CO}_2 \), growth, photosynthetic carbon fixation and calcification rates of the Canary Islands population cannot increase as much as in the Azores and Bergen populations.

Before we started this experiment, strains isolated from the Azores, Bergen and Canary Islands grew as stock cultures at 15 °C and 400 µatm 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 $p$CO$_2$ 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 $p$CO$_2$ 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 $p$CO$_2$ levels. The ability to adapt to diverse environmental conditions is reflected in the global distribution of *E. huxleyi* (Paasche, 2002), spanning a temperature range of about 30 °C. In natural seawater, due to ocean currents and gene flow, populations at any given location may get replaced by populations 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 $\text{HCO}_3^-$ to calcify and generate proton, 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 $p$CO$_2$ levels, indicating phenotypic plasticity of individual strains (Reusch, 2014). Phenotypic plasticity constitutes an advantage for individual strains to adapt to elevated $p$CO$_2$ by changing their 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 (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 changing oceans, strain succession is likely to occur and shift the population composition (Blanco-Ameijeiras et al., 2016; Hattich et al., 2017). Strains with high growth rates may outcompete other strains in the oceans (Schaum et al., 2013). Significant positive correlation between growth and POC production rate or POC quota (Fig. 4S) suggests that the dominated strains can also take up dissolved inorganic carbon faster from the oceans or fix carbon faster. This may increase the potential of the oceans to absorb CO$_2$ from the atmosphere or the carbon storage capacity of the oceans when large *E. huxleyi* blooms occur (Blanco-Ameijeiras et al., 2016), which will 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. Our results suggest that when assessing
phytoplankton responses to changing environments on a global scale, variability in
population or strain responses need to be considered.
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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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 $\mu$atm to 2630 $\mu$atm. 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 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 $\mu$atm and 2630 $\mu$atm 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 $pCO_2$, 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 $pCO_2$ within the Azores (a), Bergen (b) and Canary Islands (c) populations. POC production rates of each strain as a function of $pCO_2$ within the Azores (d), Bergen (e) and Canary Islands (f) populations. PIC production rates of each strain as a function of $pCO_2$ 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 $pCO_2$ level, respectively.
**Table 1.** Surface seawater CO₂ levels and pH at the Azores, Bergen and Canary Islands.

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<th>Location</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.

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

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Figure 1

(a) Growth rate (d⁻¹) vs. $pCO_2$ (µatm) for Azores, Bergen, and Canary Islands.
(b) Relative growth rate (%).
(c) POC production rate (pg C cell⁻¹ d⁻¹) vs. $pCO_2$ (µatm).
(d) Relative POC production rate (%).
(e) PIC production rate (pg C cell⁻¹ d⁻¹) vs. $pCO_2$ (µatm).
(f) Relative PIC production rate (%).
Figure 2
Figure 3