Population-specific responses in physiological rates of *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 from different areas. In the present study, we investigated the specific responses of growth, particulate organic (POC) and inorganic carbon (PIC) production rates of 3 populations of the coccolithophore *Emiliania huxleyi* from three regions in the North Atlantic Ocean (Azores: 6 strains, Canary Islands: 5 strains, and Norwegian coast near Bergen: 6 strains) to a CO_2 partial pressure (pCO_2) range from 120 µatm to 2630 µatm. Physiological rates of each population and individual strain increased with rising pCO_2 levels, reached maximum and declined thereafter. Optimal pCO_2 for growth and POC production rates and tolerance to low pH (i.e. high proton concentration) was significantly higher in an *E. huxleyi* population isolated from the Norwegian coast than in those isolated near the Azores and Canary Islands. This may be due to the large environmental variability including large pCO_2 and pH 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. This pattern could be driven by temperature-CO_2-interactions where the chosen incubation temperature (16 ^{o}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 pCO_2 levels indicated strain-specific phenotypic plasticity. The existence of distinct responses to changes in carbonate chemistry between and within populations will likely benefit *E. huxleyi* to acclimate and adapt to rising CO_2 levels in the oceans.

1 Introduction

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

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

89 growth rates between strains of *Gephyrocapsa oceanica*, *Ostreococcus tauri* and
90 *Fragilariopsis cylindrus* (Hutchins et al., 2013; Schaum et al., 2013; Pancic et al.,
91 2015; Hattich et al., 2017). Hence, multiple strains, ideally from geographically
92 distinct regions should be considered for investigating phytoplankton responses to
93 climate change (Zhang et al., 2014; Blanco-Ameijeiras et al., 2016; Krumhardt et al.,
94 2017).

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

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

2 Materials and methods

2.1 Cell isolation sites and experimental setup

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

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

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

The six or five (in case of Canary Islands) strains of each region were used to test the physiological response to varying CO_2 concentrations at constant total alkalinity (TA). The experiment was performed in six consecutive incubations, with one strain from each population (Azores, Bergen, Canary Islands) being cultured at a time (Fig. S1). Monoclonal populations were always grown in sterile-filtered (0.2 µm diameter, Sartobran® P 300, Sartorius) artificial seawater medium (ASW) as dilute batch cultures at 200 µmol photons m^{-2} s^{-1} light intensity under a 16/8 h light/dark cycle (light period: 5:00 a.m to 9:00 p.m.) at 16 ^{o}C which we consider to be a compromise for the three different origins of the strains. Nutrients were added in excess (with nitrate and phosphate concentrations of 64 µmol kg^{-1} and 4 µmol kg^{-1}, respectively). For the preparation of ASW and nutrient additions see Zhang et al. (2014). Calculated volumes of Na_2CO_3 and hydrochloric acid were added to the ASW to achieve target CO_2 levels at an average total alkalinity (TA) of 2319 ± 23 µmol kg^{-1} (Pierrot et al., 2006; Bach et al., 2011). Each strain was grown under 11 CO_2 levels ranging from 115 µatm to 3070 µatm without replicate. Mean response variables of all strains with a population were calculated and mean CO_2 levels of all strains within a population ranged from 120 µatm to 2630 µatm. Cells grew in the experimental conditions for at least 7 generations, which corresponded to 4–7 days depending on cell division rates. Cells were cultured for 4 days in 120–925 µatm CO_2, for 5 days in 1080–1380 µatm

155 CO_2, and for 6 or 7 days in 1550–2630 µatm CO_2. Initial cell concentration was 200
156 cells ml^{-1} (estimated from measured pre-culture concentrations and known dilution)
157 and final cell concentration was lower than 100,000 cells ml^{-1}. Dissolved inorganic
158 carbon (DIC) concentrations and pCO_2 levels changed less than 7% and 11%,
159 respectively, during the experimental growth phase.
160

161 **2.2 pH_T and total alkalinity measurements**

162 At 10:00 a.m. on the last day of incubations (at day 4–7 depending on CO_2
163 concentration), pH_T and TA samples were filtered (0.2 µm diameter, Filtropur S 0.2,
164 Sarstedt) by gentle pressure and stored at 4°C for a maximum of 14 days. The entire
165 sampling lasted less than 2 h. The pH_T sample bottles were filled with considerable
166 overflow and closed tightly with no space. pH_T was measured spectrophotometrically
167 (Cary 100, Agilent) using the indicator dye *m*-cresol purple (Sigma-Aldrich) similar
168 to Carter et al. (2013) with constants of acid dissociation for the protonated and un-
169 protonated forms reported in Clayton and Byrne (1993). TA was measured by open-
170 cell potentiometric titration (862 Compact Titrosampler, Metrohm) according to
171 Dickson et al. (2003). The carbonate system was calculated from measured TA, pH_T,
172 (assuming 4 µmol kg^{-1} of phosphate and 0 µmol kg^{-1} of silicate) using the CO2
173 System Calculations in MS Excel software (Pierrot et al., 2006) with carbonic acid
174 constants K_1 and K_2 as determined by Roy et al. (1993).
175

176 **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 (μ) was calculated according to:

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

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 ^{o}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 pCO_2 levels, and allowed to age for about 7 days under incubation conditions (*see* above). All samples were placed at –20^{o}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 ^{o}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:

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

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

200

201 **2.5 Data analysis**

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

208 $$y = \frac{X \times pCO_2}{Y + pCO_2} - s \times pCO_2 \quad (4)$$

209 where X and Y are fitted parameters, and s, the sensitivity constant, depicts the slope
210 of the decline after optimum CO_2 levels in response to rising H^+. Based on the fitted X,
211 Y and s, we calculated pCO_2 optima (K_m) (equation 5) and maximum growth, POC
212 and PIC production rates following Bach et al., (2011).

213 $$K_m = \sqrt{\frac{X \times Y}{s}} - Y \quad (5)$$

214 The relative values for growth, POC and PIC production rates were calculated as
215 ratios of growth, POC and PIC production rates at each pCO_2 level to the maximum
216 (highest) rates. We obtained the relative sensitivity constant by fitting function (4)
217 based on relative growth, POC and PIC production rates.

218 A one-way ANOVA was then used to test for statistically significant differences in
219 theoretical optimum pCO_2, maximum value and relative sensitivity constant between
220 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).

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

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

252

253 **3.3 Physiological responses of populations to pCO_2**

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

258 Calculated maximum growth rates, POC and PIC production rates were not
259 significantly different between the Azores and the Bergen populations (all $p > 0.1$)
260 (Fig. 2d–f). Maximum growth rate and POC production rate of the Canary Islands
261 population were significantly lower than those of the Azores and Bergen populations
262 (both $p < 0.01$) (Fig. 2d,e). Maximum PIC production rates of the Canary Islands
263 population were significantly lower than that of the Azores population ($p < 0.05$),
264 while there was no difference to the Bergen population ($p > 0.1$) (Fig. 2f).

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

3.4 Physiological responses of individual strains to pCO_2

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

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

4 Discussion

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

In comparison to the Azores and Canary Islands populations, variability in growth rates between strains of the Bergen population was smaller even though they had higher growth rates at all pCO_2 levels (Fig. 3). Furthermore, the Bergen population showed significantly higher pCO_2 optima and lower H^+ sensitivity for growth and POC production rates (Fig. 2). These findings indicate that the Bergen population may be more tolerant to changing carbonate chemistry in terms of its growth and photosynthetic carbon fixation rates. The Bergen strains were isolated from coastal waters, while the Azores and Canary Islands strains were isolated from a more oceanic environment. Seawater carbonate chemistry of coastal waters is usually more dynamic than in the open ocean (Cai, 2011). In fact, previous studies have reported that CO_2 and pH variability of the seawater off Bergen was larger than off the Azores

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

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

reduced in comparison to the other populations (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). Thus, with rising CO_2, growth, photosynthetic carbon fixation and calcification rates of the Canary Islands population cannot increase as much as in the Azores and Bergen populations. In addition, the Canary Islands population showed smallest variability in optimum pCO_2 and maximum values for growth and POC production rates (Fig. 2). The reason may be that low incubation 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 ^{o}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 pCO_2 levels. Thus, it is conceivable that the same selection history in the laboratory incubation

353 may contribute to a more similar response of growth, POC and PIC production rates
354 between the Azores and Bergen populations at low pCO_2 levels (Fig. 1).
355 Our results indicate that *E. hulxyei* populations are adapted to the specific
356 environmental conditions of their origin, resulting in different responses to increasing
357 pCO_2 levels. The ability to adapt to diverse environmental conditions is supposed to
358 be one reason for the global distribution of *E. huxleyi* (Paasche, 2002), spanning a
359 temperature range of about 30 ^{o}C. The optimum temperature for growth of the Bergen
360 population was about 22 ^{o}C and was 5 ^{o}C higher than the maximum SST in Bergen
361 waters (Zhang et al. 2014). Furthermore, in comparison to the Azores and Canary
362 Islands populations, larger optimum pCO_2 of growth rate indicates that the Bergen
363 population may benefit more from the rising CO_2 levels at increasing temperatures.
364 PIC : POC ratios of the Azores and Bergen populations declined with rising pCO_2,
365 whereas PIC : POC ratios of the Canary Islands population were rather constant (Fig.
366 S6). As changes in PIC ： POC ratios of coccolithophore blooms may impact on the
367 biological carbon pump, different regions might see different changes in the future
368 ocean. In natural seawater, due to ocean currents and gene flow, populations at any
369 given location may get replaced by immigrant genotypes transported there from other
370 locations (Doblin and van Sebille, 2016). In addition, *E. huxleyi* is thought to utilize
371 HCO_3^- for calcification which generates protons, and increase in proton concentration
372 may mitigate the potential of the ocean to absorb atmospheric CO_2 (Paasche, 2002).
373 Within a population, individual strains showed different growth, POC and PIC
374 production rates at different pCO_2 levels, indicating phenotypic plasticity of

individual strains (Reusch, 2014). Phenotypic plasticity constitutes an advantage for individual strains to acclimate and adapt to elevated pCO_2 by changing fitness-relevant traits and potentially to attenuate the short-term effects of changing environments on fitness-relevant traits (Schaum et al., 2013).

The strain-specific CO_2-response curves revealed considerable physiological diversity in co-occurring strains (Fig. 3). Physiological variability makes a population more resilient, 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 ocean, 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 (Schaum et al., 2013). 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. When extrapolated to the ocean, *E. huxleyi* blooms may increase the potential of the oceans to absorb CO_2 from the atmosphere and its carbon

storage capacity (Blanco-Ameijeiras et al., 2016), which 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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640 **Figure Legends**

641 **Figure 1.** Optimum curve responses of measured and relative growth, particulate
642 organic (POC) and inorganic carbon (PIC) production rates of three *Emiliania huxleyi*
643 populations to a pCO_2 range from 120 µatm to 2630 µatm. Responses of measured (**a**)
644 and relative (**b**) growth rates to pCO_2. Responses of measured (**c**) and relative (**d**)
645 POC production rates to pCO_2. Responses of measured (**e**) and relative (**f**) PIC
646 production rates to pCO_2. Using the nonlinear regression model derived by Bach et al.
647 (2011), the curves were fitted based on average growth, POC and PIC production
648 rates of six strains from the Azores and Bergen, and of five strains from the Canary
649 Islands. Vertical error bars represent standard deviations of six growth, POC and PIC
650 production rates for the Azores and Bergen populations, and five growth, POC and
651 PIC production rates for the Canary Islands population. Horizontal error bars
652 represent standard deviations of six pCO_2 levels for the Azores and Bergen
653 populations and five pCO_2 levels for the Canary Islands populations. At the
654 population levels, 120 µatm and 2630 µatm was the lowest and highest pCO_2 level,
655 respectively.

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657 **Figure 2.** Calculated optimum pCO_2, calculated maximum value and fitted relative
658 sensitivity constant of growth, POC and PIC production rates of each population. **(a)**
659 optimum pCO_2 of growth rate; **(b)** optimum pCO_2 of POC production rates; **(c)**
660 optimum pCO_2 of PIC production rates; **(d)** maximum growth rate, **(e)** maximum
661 POC production rate, **(f)** maximum PIC production rate; **(g)** relative sensitivity

662 constant of growth rate; **(h)** relative sensitivity constant of POC production rate; **(i)**
663 relative sensitivity constant of PIC production rate. The line in the middle of each box
664 indicates the mean of 6 or 5 optimum pCO_2, 6 or 5 maximum values, and 6 or 5
665 relative sensitivity constants for growth, POC and PIC production rates in each
666 population. Bars indicate the 99% confidence interval. The maximum or minimum
667 data is shown as the small line on the top or bottom of the bar, respectively. Letters in
668 each panel represent statistically significant differences (Tukey HSD, $p < 0.05$).
669
670 **Figure 3.** Optimum curve responses of growth, POC and PIC production rates of
671 individual *E. huxleyi* strains in the Azores (left), Bergen (medium) and Canary Islands
672 (right) populations to a CO_2 range from 115 µatm to 3070 µatm. Growth rates of each
673 strain as a function of pCO_2 within the Azores (**a**), Bergen (**b**) and Canary Islands (**c**)
674 populations. POC production rates of each strain as a function of pCO_2 within the
675 Azores (**d**), Bergen (**e**) and Canary Islands (**f**) populations. PIC production rates of
676 each strain as a function of pCO_2 within the Azores (**g**), Bergen (**h**) and Canary
677 Islands (**i**) populations. At the strain levels, 115 µatm and 3070 µatm was the lowest
678 and highest pCO_2 level, respectively.
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684 **Table 1.** Surface seawater CO_2 levels and pH at the Azores, Bergen and Canary

685 Islands.

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

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

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

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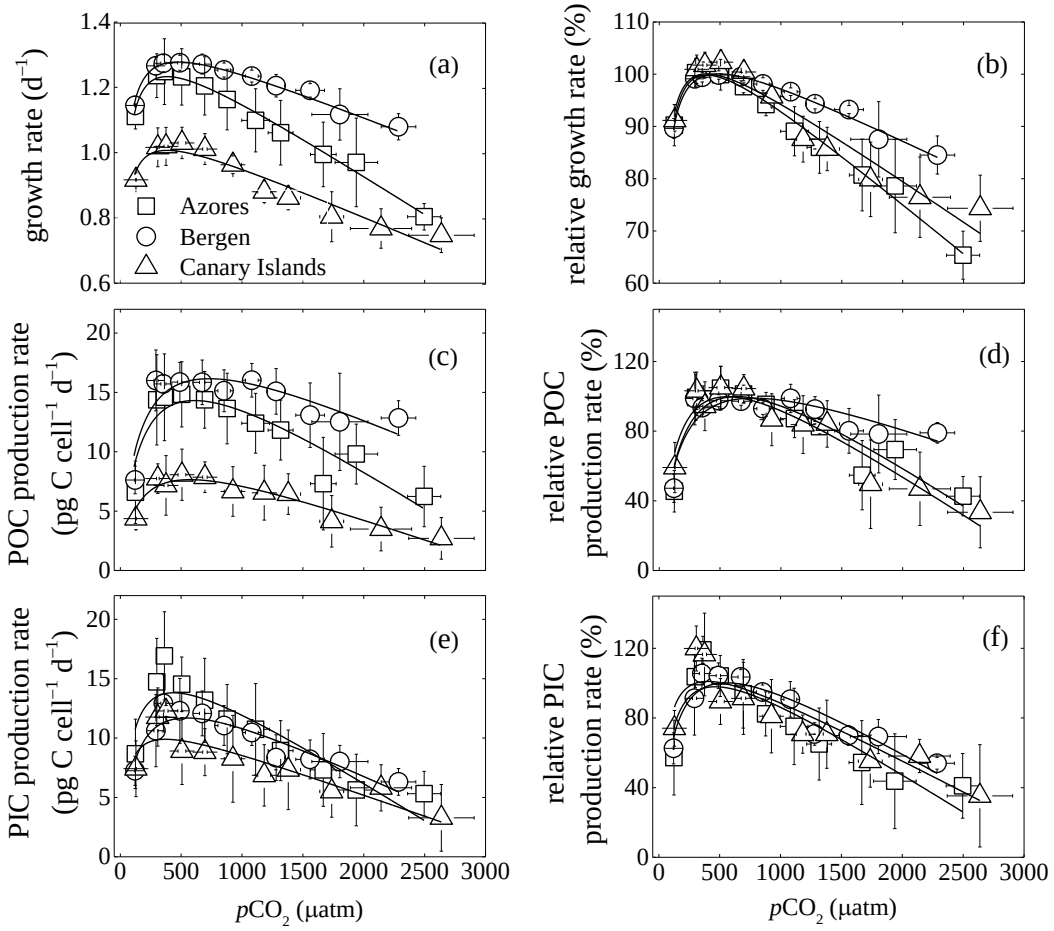
Table 3. Calculated optimum pCO_2, calculated maximum value (V_{max}) and fitted relative sensitivity constant (*rs*, ‰) of growth, POC and PIC production rates of each *E. huxleyi* strain.

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


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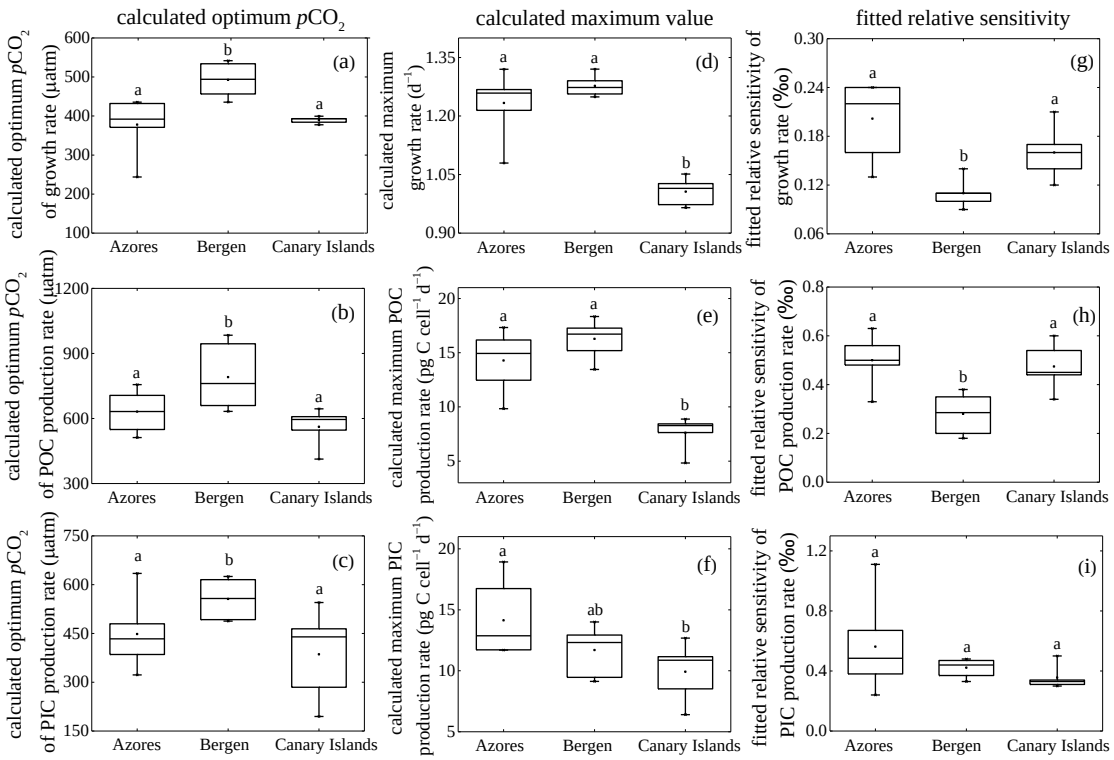
growth rate (d^{-1})
relative growth rate (%)
POC production rate (pg C cell^{-1} d^{-1})
relative POC production rate (%)
PIC production rate (pg C cell^{-1} d^{-1})
relative PIC production rate (%)
Azores
Bergen
Canary Islands
pCO$_2$ (µatm)
pCO$_2$ (µatm)

Figure 1


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

Figure 2

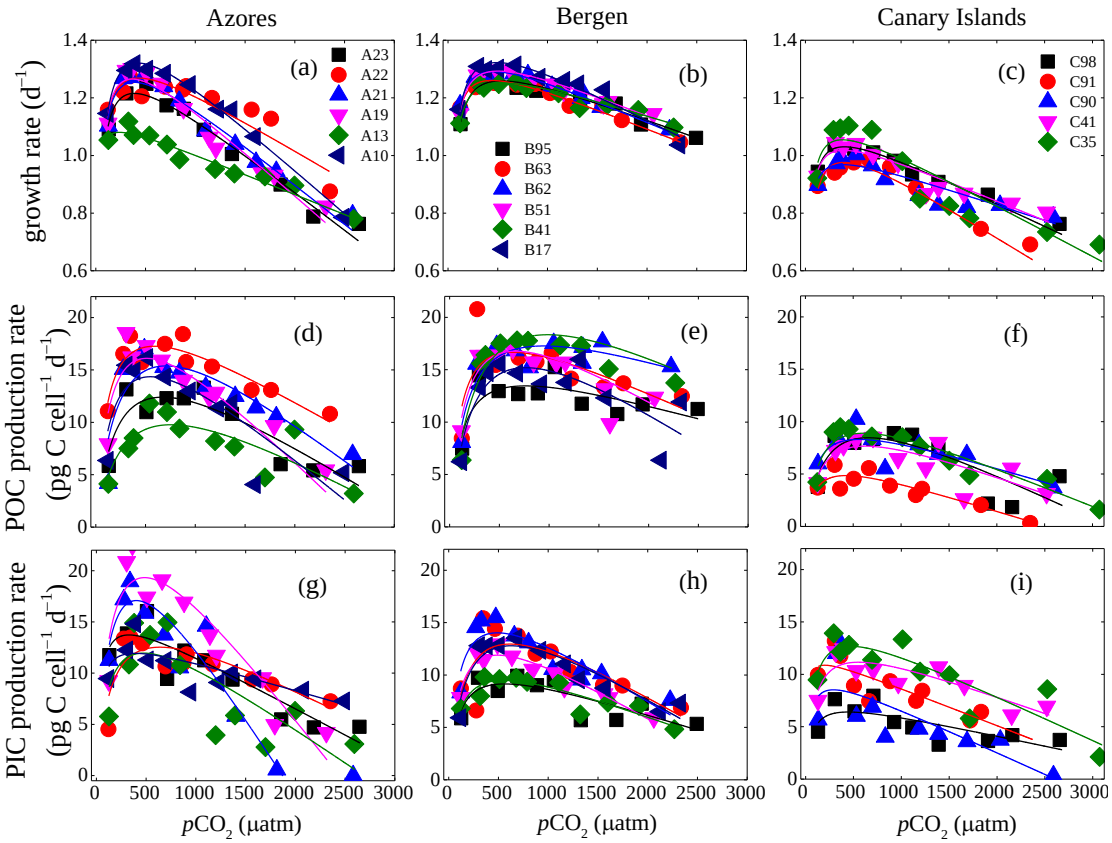
Azores
Bergen
Canary Islands
growth rate (d^{-1})
POC production rate (pg C cell^{-1} d^{-1})
PIC production rate (pg C cell^{-1} d^{-1})
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
A23
A22
A21
A19
A13
A10
B95
B63
B62
B51
B41
B17
C98
C91
C90
C41
C35
pCO$_2$ (µatm)

Figure 3