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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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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 (pCO_2) range from 120 µatm to 2630 µatm. Physiological rates of each population and individual strain displayed the expected optimum curve responses to the pCO_2 gradient. Optimal pCO_2 for growth and POC production rates and tolerance to low pH (i.e. high proton concentration) was significantly higher in an *E. huxleyi* population isolated from a Norwegian fjord than in those isolated near the Azores and Canary Islands. This may be due to the large pCO_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 ^{o}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 pCO_2 levels indicated strain-specific phenotypic plasticity. The existence of distinct carbonate

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chemistry responses between and within populations will likely benefit *E. huxleyi* to acclimate to rising CO_2 levels in the oceans.

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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 pCO_2 range from about 20 µatm to 5000 µatm, and linked them to inorganic carbon substrate limitation at low pCO_2 and inhibiting H^+ concentrations at high pCO_2 (Bach et al., 2011; 2015; Kottmeier et al., 2016). Until now, studies on the physiological responses of *E. huxleyi* to rising CO_2 are mostly based on a few genotypes and little is known about the potential variability in CO_2 and H^+ sensitivity between and within populations. Recently, several studies found substantial variations in CO_2 responses for N_2 fixation rates between *Trichodesmium* strains, as well as for

growth rates between strains of *Gephyrocapsa oceanica*, *Ostreococcus tauri* and *Fragilariopsis cylindrus* (Hutchins et al., 2013; Schaum et al., 2013; Pancic et al., 2015; Hattich et al., 2017). These indicate that 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 pCO_2 range from 120 µatm to 2630 µatm.

2 Materials and methods

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112 **2.1 Cell isolation sites and experimental setup**

113 *Emiliania huxleyi* strains EHGKL B95, B63, B62, B51, B41 and B17 originated from
114 Raunefjord (Norway 60°18'N, 05°15'E) and were isolated by K. T. Lohbeck in May,
115 2009 (Lohbeck et al., 2012) at ~ 10 °C in-situ water temperature. *E. huxleyi* strains
116 EHGLE A23, A22, A21, A19, A13 and A10 originated from coastal waters near the
117 Azores (38°34'N, 28°42'W) and were isolated by S. L. Eggers in May or June, 2010
118 at ~ 17 °C in-situ water temperature. *E. huxleyi* strains EHGKL C98, C91, C90, C41
119 and C35 originated from coastal waters near Gran Canaria (27°58'N, 15°36'W) and
120 were isolated by K. T. Lohbeck in February, 2014 at ~ 18 °C in-situ water temperature.
121 Seasonal CO_2 concentration in the surface seawater ranges from 240 µatm to 400
122 µatm near Bergen, from 320 µatm to 400 µatm around the Azores and from 320 µatm
123 to 400 µatm around the Canary Islands (Table 1). Monthly surface seawater
124 temperature ranges from 6.0 to 16.0 °C near Bergen, 15.6 to 22.3 °C around the
125 Azores and from 18.0 to 23.5 °C around the Canary Islands (Table S1).

126 All 17 strains belong to morphotype A and have been deposited at the Roscoff
127 culture collection (RCC) under the official names as shown above. Genetically
128 different isolates, here called strains, were identified by 5 microsatellite markers
129 (P02E09, P02B12, P02F11, EHMS37, EHMS15) (Table S2). For a description of
130 primer testing, deoxyribonucleic acid (DNA) extraction, DNA concentration
131 measurements, and polymerase chain reaction (PCR) protocols see Zhang et al.
132 (2014). The Azores and Bergen strains had been used earlier by Zhang et al. (2014).

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133 The six or five (in case of Canary Islands) strains of each region were used to test
134 the physiological response to varying CO_2 concentrations at constant total alkalinity
135 (TA). The experiment was performed in six consecutive incubations, with one strain
136 from each population (Azores, Bergen, Canary Islands) being cultured at a time.
137 Monoclonal populations were always grown in sterile-filtered (0.2 µm diameter,
138 Sartobran® P 300, Sartorius) artificial seawater medium (ASW) as dilute batch
139 cultures at 200 µmol photons m^{-2} s^{-1} light intensity under a 16/8 h light/dark cycle
140 (light period: 5:00 a.m to 9:00 p.m.) at 16 ^{o}C which we consider to be the best
141 compromise for the three different origins of the strains. Nutrients were added in
142 excess (with nitrate and phosphate concentrations of 64 µmol kg^{-1} and 4 µmol kg^{-1},
143 respectively). For the preparation of ASW and nutrient additions see Zhang et al.
144 (2014). Calculated volumes of Na_2CO_3 and hydrochloric acid were added to the ASW
145 to achieve target CO_2 levels at an average total alkalinity (TA) of 2319 ± 23 µmol kg^{-1}
146 (Pierrot et al., 2006; Bach et al., 2011). Each strain was grown under 11 CO_2 levels
147 ranging from 115 µatm to 3070 µatm without replicate. Mean response variables of all
148 strains with a population were calculated and mean CO_2 levels of all strains within a
149 population ranged from 120 µatm to 2630 µatm. Cells grew in the experimental
150 conditions for at least 7 generations, which corresponded to 4–7 days depending on
151 cell division rates. Cells were cultured for 4 days in 120–925 µatm CO_2, for 5 days in
152 1080–1380 µatm CO_2, and for 6 or 7 days in 1550–2630 µatm CO_2. Initial cell
153 concentration was 200 cells ml^{-1} and final cell concentration was lower than 100,000
154 cells ml^{-1}. Dissolved inorganic carbon (DIC) concentrations and pCO_2 levels changed

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less than 7% and 11%, respectively, during the experimental growth phase.

2.2 pH_T and total alkalinity measurements

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

2.3 Growth rate measurements

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:

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

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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 pCO_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:

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

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

2.5 Data analysis

The nonlinear regression model (4) was used to fit growth, POC and PIC production

rates 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 rising H^+. Based on the fitted X, Y and s, we calculated the pCO_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.

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

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

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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).

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243 Exceptions were that at 365–695 µatm, PIC production rates of the Azores population
244 were larger than those of the Canary Islands population (all $p < 0.05$).
245
246 **3.3 Physiological responses of populations to pCO_2**
247 Calculated optimum pCO_2 for growth, POC and PIC production rates of the Bergen
248 population were significantly larger than those of the Azores and Canary Islands
249 populations (all $p < 0.05$) (Fig. 2a–c). Optimum pCO_2 for these physiological rates
250 between the Azores and Canary Islands population were not different (all $p > 0.1$).
251 Calculated maximum growth rates, POC and PIC production rates were not
252 significantly different between the Azores and the Bergen populations (all $p > 0.1$)
253 (Fig. 2d–f). Maximum growth rate and POC production rate of the Canary Islands
254 population were significantly lower than those of the Azores and Bergen populations
255 (both $p < 0.01$) (Fig. 2d,e). Maximum PIC production rates of the Canary Islands
256 population were significantly lower than that of the Azores population ($p < 0.05$),
257 while there was no difference to the Bergen population ($p > 0.1$) (Fig. 2f).
258 Fitted relative sensitivity constants for growth and POC production rates of the
259 Bergen population were significantly lower than those of the Azores and Canary
260 Islands populations ($p < 0.01$) (Fig. 2g, h). Fitted relative sensitivity constants for
261 growth and POC production rates between the Azores and Canary Islands populations
262 were not significantly different ($p > 0.1$). Fitted relative sensitivity constants for PIC
263 production rates did not show difference among three populations ($p = 0.13$) (Fig. 2i).
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265 **3.4 Physiological responses of individual strains to pCO_2**

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

270 For all strains within each population, optimum pCO_2 of POC production rates
271 were larger than optimum pCO_2 of growth rates or PIC production rates with the
272 exception of optimum pCO_2 of POC and PIC production rates of *E. huxleyi* strain
273 EHGLE A22 (Table 3). Compared to the Azores and Bergen populations, strains
274 isolated near the Canary Islands showed larger variation in optimum pCO_2 of PIC
275 production rates. Within the Azores population, variations in maximum values (V_{max})
276 and relative sensitivity constants (*rs*) of growth, POC and PIC production rates of all
277 strains were larger than those within the Bergen and Canary Islands populations (Fig.
278 3).

279

280 **4 Discussion**

281

282 We investigated growth, POC and PIC production rates of 17 *E. huxleyi* strains from
283 three populations to a broad pCO_2 range (120–2630 µatm). The three populations
284 differed significantly in growth and POC production rates at the investigated pCO_2
285 levels. The reaction norms of the individual strains and populations equaled an
286 optimum curve for all physiological rates (Figs. 1 and 3). However, we detected

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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). 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 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 °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 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 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 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. hulxyei* 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 the global distribution of *E. huxleyi* (Paasche, 2002), spanning a temperature range of about 30 ^{o}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 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 pCO_2 levels, indicating phenotypic plasticity of individual strains (Reusch, 2014). Phenotypic plasticity constitutes an advantage for individual strains to adapt to elevated pCO_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

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375 In the present study, we found population-specific responses in physiological rates of
376 *E. huxleyi* to a broad pCO_2 range, which may have arisen from local adaptation to
377 environmental conditions at their origins. Our results suggest that when assessing
378 phytoplankton responses to changing environments on a global scale, variability in
379 population or strain responses need to be considered.
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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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596 **Figure Legends**

597 **Figure 1.** Optimum curve responses of measured and relative growth, particulate
598 organic (POC) and inorganic carbon (PIC) production rates of three *Emiliania huxleyi*
599 populations to a pCO_2 range from 120 µatm to 2630 µatm. Responses of measured (**a**)
600 and relative (**b**) growth rates to pCO_2. Responses of measured (**c**) and relative (**d**)
601 POC production rates to pCO_2. Responses of measured (**e**) and relative (**f**) PIC
602 production rates to pCO_2. Using the nonlinear regression model derived by Bach et al.
603 (2011), the curves were fitted based on average growth, POC and PIC production
604 rates of six strains from the Azores and Bergen, and of five strains from the Canary
605 Islands. Vertical error bars represent standard deviations of six growth, POC and PIC
606 production rates for the Azores and Bergen populations, and five growth, POC and
607 PIC production rates for the Canary Islands population. Horizontal error bars
608 represent standard deviations of six pCO_2 levels for the Azores and Bergen
609 populations and five pCO_2 levels for the Canary Islands populations. At the
610 population levels, 120 µatm and 2630 µatm was the lowest and highest pCO_2 level,
611 respectively.

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613 **Figure 2.** Calculated optimum pCO_2, calculated maximum value and fitted relative
614 sensitivity constant of growth, POC and PIC production rates of each population. **(a)**
615 optimum pCO_2 of growth rate; **(b)** optimum pCO_2 of POC production rates; **(c)**
616 optimum pCO_2 of PIC production rates; **(d)** maximum growth rate, **(e)** maximum
617 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.

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640 **Table 1.** Surface seawater CO_2 levels and pH at the Azores, Bergen and Canary

641 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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659 **Table 2.** Carbonate chemistry parameters (mean values for the beginning and end of
660 the incubations) of the artificial seawater for each *Emiliania huxleyi* population. pH
661 and TA samples were collected and measured before and at the end of incubation.
662 Data are expressed as mean values of six strains in the Azores and Bergen population,
663 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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665 **Table 3.** Calculated optimum pCO_2, calculated maximum value (V_{max}) and fitted
666 relative sensitivity constant (*rs*, ‰) of growth, POC and PIC production rates of each
667 *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

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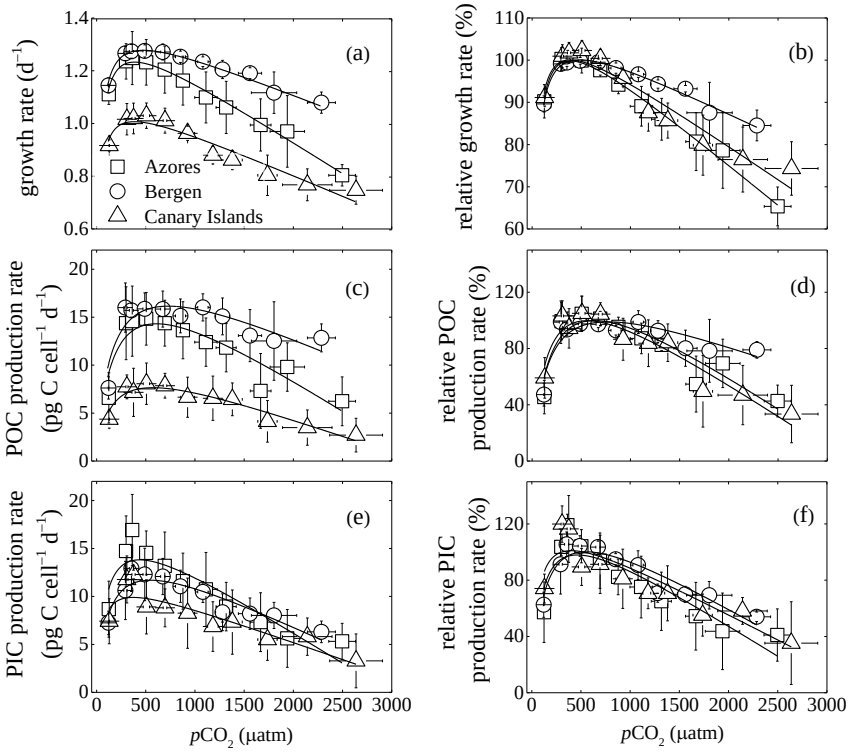

Figure 1

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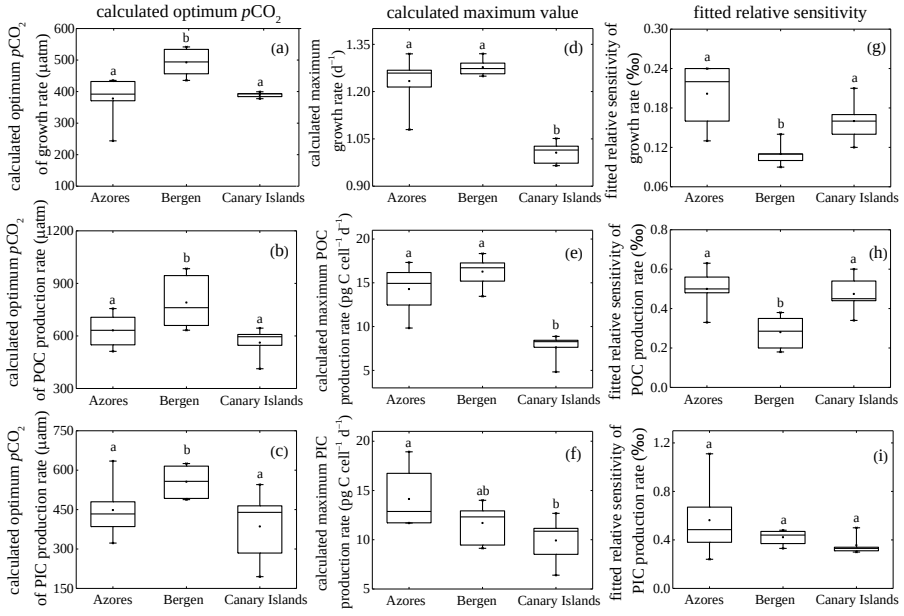

Figure 2

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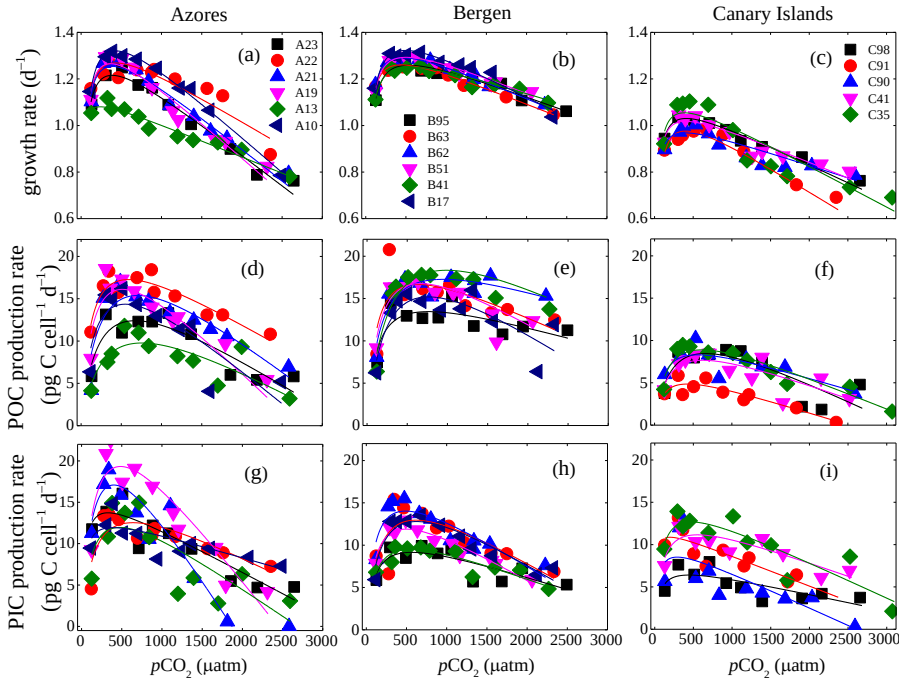

Figure 3