

1 **Population-specific responses in physiological rates of *Emiliana huxleyi* to a**
2 **broad CO₂ range**

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19 Running head: *population response of Emiliana huxleyi to CO₂*

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23 **Abstract**

24 Although coccolithophore physiological responses to CO₂-induced changes in
25 seawater carbonate chemistry have been widely studied in the past, there is limited
26 knowledge on the variability of physiological responses between populations from
27 different areas. In the present study, we investigated the specific responses of growth,
28 particulate organic (POC) and inorganic carbon (PIC) production rates of 3
29 populations of the coccolithophore *Emiliana huxleyi* from three regions in the North
30 Atlantic Ocean (Azores: 6 strains, Canary Islands: 5 strains, and Norwegian coast near
31 Bergen: 6 strains) to a CO₂ partial pressure ($p\text{CO}_2$) range from 120 μatm to 2630
32 μatm . Physiological rates of each population and individual strain increased with
33 rising $p\text{CO}_2$ levels, reached maximum and declined thereafter. Optimal $p\text{CO}_2$ for
34 growth and POC production rates and tolerance to low pH (i.e. high proton
35 concentration) was significantly higher in an *E. huxleyi* population isolated from the
36 Norwegian coast than in those isolated near the Azores and Canary Islands. This may
37 be due to the large environmental variability including large $p\text{CO}_2$ and pH
38 fluctuations in coastal waters off Bergen compared to the rather stable oceanic
39 conditions at the other two sites. Maximum growth and POC production rates of the
40 Azores and Bergen populations were similar and significantly higher than that of the
41 Canary Islands population. This pattern could be driven by temperature-CO₂-
42 interactions where the chosen incubation temperature (16 °C) was slightly below what
43 strains isolated near the Canary Islands normally experience. Our results indicate
44 adaptation of *E. huxleyi* to their local environmental conditions and the existence of

45 distinct *E. huxleyi* populations. Within each population, different growth, POC and
46 PIC production rates at different $p\text{CO}_2$ levels indicated strain-specific phenotypic
47 plasticity. Accounting for this variability is important to understand how or whether *E.*
48 *huxleyi* might adapt to rising CO_2 levels.

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67 **1 Introduction**

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69 Coccolithophores form a layer of calcium carbonate (CaCO₃) platelets (coccoliths)
70 around their cells. Coccoliths are of biogeochemical importance due to ballasting of
71 organic matter with CaCO₃, a phenomenon which is thought to promote the transport
72 of organic carbon to the deep ocean (Klaas and Archer, 2002; Rost and Riebesell,
73 2004). The coccolithophore *Emiliana huxleyi* forms extensive blooms under
74 favourable light intensity, temperature and nutrient conditions, with different
75 morphotypes in certain regions (Cook et al., 2011; Henderiks et al., 2012; Smith et al.,
76 2012; Balch et al., 2014; Krumhardt et al., 2017).

77 Variable responses of growth, photosynthetic carbon fixation and calcification rates
78 of different *E. huxleyi* strains to rising CO₂ levels have been reported (Langer et al.,
79 2009; Hoppe et al., 2011; Müller et al., 2015; Hattich et al, 2017) and are likely a
80 result of intra-specific variability of genotypes (Langer et al., 2009). Several recent
81 studies observed optimum curve responses in physiological rates of a single *E. huxleyi*
82 strain to a broad *p*CO₂ range from about 20 µatm to 5000 µatm, and linked them to
83 inorganic carbon substrate limitation at low *p*CO₂ and inhibiting H⁺ concentrations at
84 high *p*CO₂ (Bach et al., 2011, 2015; Kottmeier et al., 2016). Until now, studies on the
85 physiological responses of *E. huxleyi* to rising CO₂ are mostly based on a few
86 genotypes and little is known about the potential variability in CO₂ and H⁺ sensitivity
87 between and within populations. Recently, several studies found substantial variations
88 in CO₂ responses for N₂ 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 CO₂ 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 *p*CO₂ range from 120 μatm to 2630 μatm.

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112 **2 Materials and methods**

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114 **2.1 Cell isolation sites and experimental setup**

115 *Emiliana huxleyi* strains EHGKL B95, B63, B62, B51, B41 and B17 originated from
116 Raunefjord (Norway 60°18'N, 05°15'E) and were isolated by K. T. Lohbeck in May,
117 2009 (Lohbeck et al., 2012) at ~ 10 °C in-situ water temperature. *E. huxleyi* strains
118 EHGLE A23, A22, A21, A19, A13 and A10 originated from coastal waters near the
119 Azores (38°34'N, 28°42'W) and were isolated by S. L. Eggers in May or June, 2010
120 at ~ 17 °C in-situ water temperature. *E. huxleyi* strains EHGKL C98, C91, C90, C41
121 and C35 originated from coastal waters near Gran Canaria (27°58'N, 15°36'W) and
122 were isolated by K. T. Lohbeck in February, 2014 at ~ 18 °C in-situ water temperature.
123 Seasonal CO₂ concentration in the surface seawater ranges from 240 µatm to 400
124 µatm near Bergen, from 320 µatm to 400 µatm around the Azores and from 320 µatm
125 to 400 µatm around the Canary Islands (Table 1). Monthly surface seawater
126 temperature ranges from 6.0 to 16.0 °C near Bergen, 15.6 to 22.3 °C around the
127 Azores and from 18.0 to 23.5 °C around the Canary Islands (Table S1).

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

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

136 The six or five (in case of Canary Islands) strains of each region were used to test
137 the physiological response to varying CO₂ concentrations at constant total alkalinity
138 (TA). The experiment was performed in six consecutive incubations, with one strain
139 from each population (Azores, Bergen, Canary Islands) being cultured at a time (Fig.
140 S1). Monoclonal populations were always grown in sterile-filtered (0.2 µm diameter,
141 Sartobran[®] P 300, Sartorius) artificial seawater medium (ASW) as dilute batch
142 cultures at 200 µmol photons m⁻² s⁻¹ light intensity under a 16/8 h light/dark cycle
143 (light period: 5:00 a.m to 9:00 p.m.) at 16 °C which we consider to be a compromise
144 for the three different origins of the strains. Nutrients were added in excess (with
145 nitrate and phosphate concentrations of 64 µmol kg⁻¹ and 4 µmol kg⁻¹, respectively).
146 For the preparation of ASW and nutrient additions see Zhang et al. (2014). Calculated
147 volumes of Na₂CO₃ and hydrochloric acid were added to the ASW to achieve target
148 CO₂ levels at an average total alkalinity (TA) of 2319 ± 23 µmol kg⁻¹ (Pierrot et al.,
149 2006; Bach et al., 2011). Each strain was grown under 11 CO₂ levels ranging from
150 115 µatm to 3070 µatm without replicate. Mean response variables of all strains with
151 a population were calculated and mean CO₂ levels of all strains within a population
152 ranged from 120 µatm to 2630 µatm. Cells grew in the experimental conditions for at
153 least 7 generations, which corresponded to 4–7 days depending on cell division rates.
154 Cells were cultured for 4 days in 120–925 µatm CO₂, for 5 days in 1080–1380 µatm

155 CO₂, and for 6 or 7 days in 1550–2630 μatm CO₂. 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 $p\text{CO}_2$ levels changed less than 7% and 11%,
159 respectively, during the experimental growth phase.

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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 CO₂
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 $\mu\text{mol kg}^{-1}$ of phosphate and 0 $\mu\text{mol kg}^{-1}$ 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**

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

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

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

183

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

185 At 3:00 p.m. on the last day of incubation, cells for total particulate (TPC) and total
186 organic (TOC) carbon were filtered onto GF/F filters which were pre-combusted at
187 500 °C for 8 h. Samples of background particulate carbon (BPC) were determined in a
188 similar way but using filtered ASW without algae, which was previously adjusted to
189 target $p\text{CO}_2$ levels, and allowed to age for about 7 days under incubation conditions
190 (*see above*). All samples were placed at -20°C. BPC filters were used as blanks to
191 correct for organic carbon in the medium. TOC and BPC filters were acid fumed.
192 Afterwards, all filters were dried for 8 h at 60 °C. TPC, TOC and BPC were measured
193 using an Elemental Analyzer (EuroEA, Hekatech GmbH). The percentages of BPC in
194 TPC were about 20% at cell densities < 10,000 cells ml⁻¹ and about 10% at cell
195 densities > 40,000 cells ml⁻¹. POC was calculated as the difference between TOC and
196 BPC. PIC was calculated as the difference between TPC and TOC. POC and PIC
197 production rates were calculated as:

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

199 PIC production rate = μ (d^{-1}) \times (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

221 strains from different populations. A Shapiro–Wilk’s analysis was tested to analyze
222 residual normality. Statistical calculations were carried out using *R* and significance
223 was shown by $p < 0.05$.

224

225 **3 Results**

226

227 **3.1 Carbonate chemistry parameters**

228 Carbonate system parameters are shown in Table 2. Average $p\text{CO}_2$ levels of the ASW
229 ranged from 125 μatm to 2490 μatm for the Azores population, from 120 μatm to
230 2280 μatm for the Bergen population, and from 130 μatm to 2630 μatm for the
231 Canary Islands population. Corresponding pH_T values of the ASW ranged from 8.46
232 to 7.33 for the Azores population, from 8.47 to 7.37 for the Bergen population, and
233 from 8.45 to 7.31 for the Canary Islands population.

234

235 **3.2 Measured growth, POC and PIC production rates of each population**

236 As expected, growth rates, POC and PIC production rates of the three *E. huxleyi*
237 populations increased with rising $p\text{CO}_2$, reached a maximum, and then declined with
238 further $p\text{CO}_2$ increase (Fig. 1). Growth rates of the Azores and Bergen populations
239 were larger than those of the Canary Islands population at all investigated $p\text{CO}_2$ levels
240 (Fig. 1a). With rising $p\text{CO}_2$ levels beyond the $p\text{CO}_2$ optimum, decline in growth rates
241 was more pronounced in the Azores and Canary Islands populations than in the
242 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 $p\text{CO}_2$ levels (Fig. 1c) and decline in
245 POC production rates with increasing $p\text{CO}_2$ levels beyond the $p\text{CO}_2$ 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 $p\text{CO}_2$ 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 $p\text{CO}_2$**

254 Calculated optimum $p\text{CO}_2$ 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 $p\text{CO}_2$ 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).

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

271

272 **3.4 Physiological responses of individual strains to $p\text{CO}_2$**

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

277 For all strains within each population, optimum $p\text{CO}_2$ of POC production rates
278 were larger than optimum $p\text{CO}_2$ of growth rates or PIC production rates with the
279 exception of optimum $p\text{CO}_2$ of POC and PIC production rates of *E. huxleyi* strain
280 EHGLE A22 (Table 3). Compared to the Azores and Bergen populations, strains
281 isolated near the Canary Islands showed larger variation in optimum $p\text{CO}_2$ of PIC
282 production rates. Within the Azores population, variations in maximum values (V_{max})
283 and relative sensitivity constants (rs) of growth, POC and PIC production rates of all
284 strains were larger than those within the Bergen and Canary Islands populations (Fig.
285 3).

286

287 4 Discussion

288

289 We investigated growth, POC and PIC production rates of 17 *E. huxleyi* strains from
290 three populations to a broad $p\text{CO}_2$ range (120–2630 μatm). The three populations
291 differed significantly in growth and POC production rates at the investigated $p\text{CO}_2$
292 levels. The reaction norms of the individual strains and populations equaled an
293 optimum curve for all physiological rates (Figs. 1 and 3). However, we detected
294 distinct $p\text{CO}_2$ optima for growth, POC and PIC production rates, and different H^+
295 sensitivities for growth and POC production rates among them (Fig. 2). These results
296 indicate the existence of distinct populations in the cosmopolitan coccolithophore *E.*
297 *huxleyi*.

298 In comparison to the Azores and Canary Islands populations, variability in growth
299 rates between strains of the Bergen population was smaller even though they had
300 higher growth rates at all $p\text{CO}_2$ levels (Fig. 3). Furthermore, the Bergen population
301 showed significantly higher $p\text{CO}_2$ optima and lower H^+ sensitivity for growth and
302 POC production rates (Fig. 2). These findings indicate that the Bergen population may
303 be more tolerant to changing carbonate chemistry in terms of its growth and
304 photosynthetic carbon fixation rates. The Bergen strains were isolated from coastal
305 waters, while the Azores and Canary Islands strains were isolated from a more
306 oceanic environment. Seawater carbonate chemistry of coastal waters is usually more
307 dynamic than in the open ocean (Cai, 2011). In fact, previous studies have reported
308 that CO_2 and pH variability of the seawater off Bergen was larger than off the Azores

309 and Canary Islands (Table 1). In addition, due to riverine input, seawater upwelling
310 and metabolic activity of plankton communities, environmental variability in coastal
311 waters are larger than in open-ocean ecosystems (Duarte and Cerbrian, 1996). Doblin
312 and van Sebille (2016) suggested that phytoplankton populations should be constantly
313 under selection when experiencing changing environmental conditions. In this case,
314 the Bergen population, exposed to larger CO₂ or pH fluctuations, may have acquired a
315 higher capacity to acclimate to changing carbonate chemistry resulting in a higher
316 tolerance (or lower sensitivity) to rising CO₂ levels. In contrast, the Azores and
317 Canary Islands populations experience similar, less variable seawater carbonate
318 chemistry conditions in their natural environment, which could explain why they also
319 show similar *p*CO₂ optima and H⁺ sensitivity for physiological rates (Fig. 2).

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

331 reduced in comparison to the other populations (Fig. 2d).

332 Furthermore, compared to the Canary Islands population, the Azores population
333 had higher maximum growth and POC production rates, and similar optimum CO₂ for
334 these physiological rates. Again, this might be related to sub-optimal incubation
335 conditions as temperature has been found to significantly modulate CO₂ responses in
336 coccolithophores in terms of maximum rates, CO₂ optima and half-saturation, and H⁺
337 sensitivity (Sett et al., 2014; Gafar et al., 2018; Gafar and Schulz, 2018). In a similar
338 fashion, light can also modulate CO₂ responses, hence different requirements by
339 strains adapted to different light availabilities could also explain our observations
340 (Zhang et al., 2015; Gafar et al., 2018; Gafar and Schulz, 2018). Thus, with rising
341 CO₂, growth, photosynthetic carbon fixation and calcification rates of the Canary
342 Islands population cannot increase as much as in the Azores and Bergen populations.
343 In addition, the Canary Islands population showed smallest variability in optimum
344 *p*CO₂ and maximum values for growth and POC production rates (Fig. 2). The reason
345 may be that low incubation temperature predominantly limited growth and POC
346 production rates of the Canary Islands population, and decreased the sensitivities of
347 these physiological rates to rising *p*CO₂.

348 Before we started this experiment, strains isolated from the Azores, Bergen and
349 Canary Islands grew as stock cultures at 15 °C and 400 µatm for 4 years, 5 years and
350 3 months, respectively. Schaum et al. (2015) provide evidence that long-term
351 laboratory incubation affects responses of phytoplankton to different *p*CO₂ levels.
352 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 $p\text{CO}_2$ levels (Fig. 1).

355 Our results indicate that *E. huxleyi* populations are adapted to the specific
356 environmental conditions of their origin, resulting in different responses to increasing
357 $p\text{CO}_2$ 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 °C. In addition, these results will improve our
360 understanding on variation in physiological responses of different *E. huxleyi*
361 populations to climate change, and variation in production of different areas in future
362 oceans. The optimum temperature for growth of the Bergen population was about 22
363 °C and was 5 °C higher than the maximum SST in Bergen waters (Zhang et al. 2014).
364 Furthermore, in comparison to the Azores and Canary Islands populations, larger
365 optimum $p\text{CO}_2$ of growth rate indicates that the Bergen population may benefit more
366 from the rising CO_2 levels. PIC : POC ratios of the Azores and Bergen populations
367 declined with rising $p\text{CO}_2$, whereas PIC : POC ratios of the Canary Islands population
368 were rather constant (Figs. S6, S7). As changes in PIC : POC ratios of
369 coccolithophore blooms were suggested to impact on biological carbon pump (Rost
370 and Riebesell, 2004), variation in PIC : POC ratios of different populations indicates
371 that different regions might have different changes in marine carbon cycle in the
372 future ocean. In natural seawater, due to ocean currents and gene flow, populations at
373 any given location may get replaced by immigrant genotypes transported there from
374 other locations (Doblin and van Sebille, 2016). In addition, *E. huxleyi* is thought to

375 utilize HCO_3^- for calcification which generates protons, and increase in proton
376 concentration may mitigate the potential of the ocean to absorb atmospheric CO_2 and
377 then give a positive feedback to rising atmosphere CO_2 levels (Paasche, 2002).

378 Within a population, individual strains showed different growth, POC and PIC
379 production rates at different $p\text{CO}_2$ levels, indicating phenotypic plasticity of
380 individual strains (Reusch, 2014). Phenotypic plasticity constitutes an advantage for
381 individual strains to acclimate and adapt to elevated $p\text{CO}_2$ by changing fitness-
382 relevant traits and potentially to attenuate the short-term effects of changing
383 environments on fitness-relevant traits (Schaum et al., 2013).

384 The strain-specific CO_2 -response curves revealed considerable physiological
385 diversity in co-occurring strains (Fig. 3). Physiological variability makes a population
386 more resilient, increases its ability to persist in variable environments and potentially
387 forms the basis for selection (Gsell et al., 2012; Hattich et al., 2017). It is clear that
388 other environmental factors such as light intensity, temperature and nutrient
389 concentration affect the responses of physiological rates of individual *E. huxleyi*
390 strains to changing carbonate chemistry, and thus change the physiological variability
391 within populations (Zhang et al., 2015; Feng et al., 2017). However, different
392 sensitivities and requirements of each strain to the variable environments can allow
393 strains to co-exist within a population in the natural environment (Hutchinson, 1961;
394 Reed et al., 2010; Krueger-Hadfield et al., 2014). In a changing ocean, strain
395 succession is likely to occur and shift the population composition (Blanco-Ameijeiras
396 et al., 2016; Hattich et al., 2017). Strains with higher growth rates or other

397 competitive abilities may outcompete others (Schaum et al., 2013). Further, a
398 significant positive correlation between growth and POC production rate or POC
399 quota (Fig. S5) indicates that higher growth rate means larger populations and then
400 greater production.

401

402 **5 Conclusions**

403 In the present study, we found population-specific responses in physiological rates of
404 *E. huxleyi* to a broad $p\text{CO}_2$ range, which may have arisen from local adaptation to
405 environmental conditions at their origins. The existence of distinct *E. huxleyi*
406 populations and phenotypic plasticity of individual strains may both be important for
407 *E. huxleyi* when adapting to natural environmental variability and to ongoing climate
408 changes. Our results suggest that when assessing phytoplankton responses to
409 changing environments on a global scale, variability in population and strain
410 responses need to be considered. In this study, we only studied the effects of rising
411 CO_2 but future studies should take into account simultaneous effects from other
412 interacting factors such as light and temperature variability.

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

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

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441 **References**

- 442 Bach, L. T., Riebesell, U., Gutowska, M. A., Federwisch, L., and Schulz, K. G.: A
443 unifying concept of coccolithophore sensitivity to changing carbonate chemistry
444 embedded in an ecological framework, *Prog. Oceanogr.*, 135, 125–138, doi:
445 10.1016/j.pocean.2015.04.012, 2015.
- 446 Bach, L. T., Riebesell, U., and Schulz, K. G.: Distinguishing between the effects of
447 ocean acidification and ocean carbonation in the coccolithophore *Emiliana*
448 *huxleyi*, *Limnol. Oceanogr.*, 56, 2040–2050, doi: 10.4319/lo.2011.56.6.2040, 2011.
- 449 Balch, W. M., Drapeau, D. T., Bowler, B. C., Lyczkowski, E. R., Lubelczyk, L. C.,
450 Painter, S. C., and Poulton, A. J.: Surface biological, chemical, and optical
451 properties of the Patagonian Shelf coccolithophore bloom, the brightest waters of
452 the Great Calcite Belt, *Limnol. Oceanogr.*, 59, 1715–1732, doi:
453 10.4319/lo.2014.59.5.1715, 2014.
- 454 Blanco-Ameijeiras, S., Lebrato, M., Stoll, H. M., Iglesias-Rodriguez, D., Müller, M.
455 N., Méndez-Vicente, A., and Oschlies, A: Phenotypic variability in the
456 coccolithophore *Emiliana huxleyi*, *PLoS ONE*, 11, e0157697, doi:
457 10.1371/journal.pone.0157697, 2016.
- 458 Bradshaw, A. D.: Evolutionary significance of phenotypic plasticity in plants, *Adv.*
459 *Genet.*, 13, 115–155, doi: 10.1016/S0065-2660(08)60048-6, 1965.
- 460 Brand, L. E.: Genetic variability and spatial patterns of genetic differentiation in the
461 reproductive rates of the marine coccolithophores *Emiliana huxleyi* and
462 *Gephyrocapsa oceanica*, *Limnol. Oceanogr.*, 27, 236–245, doi:

463 10.4319/lo.1982.27.2.0236, 1982.

464 Cai W. J.: Estuarine and coastal ocean carbon paradox: CO₂ sinks or sites of terrestrial
465 carbon incineration?, *Ann. Rev. Mar. Sci.*, 3, 123–145, doi: 10.1146/annurev-
466 marine-120709-142723, 2011.

467 Carter, B. R., Radich, J. A., Doyle, H. L., and Dickson, A. G.: An automated system
468 for spectrophotometric seawater pH measurements, *Limnol. Oceanogr.: Methods*,
469 11, 16–27, doi: 10.4319/lom.2013.11.16, 2013.

470 Clayton, T. D., and Byrne, R. H.: Spectrophotometric seawater pH measurements–
471 Total hydrogen-ion concentration scale calibration of m-cresol purple and at-sea
472 results, *Deep Sea Res. I*, 40, 2115–2129, doi: 10.1016/0967-0637(93)90048-8,
473 1993.

474 Cook, S. S., Whittock, L., Wright S. W., and Hallegraeff, G. M.: Photosynthetic
475 pigment and genetic differences between two southern ocean morphotypes of
476 *Emiliana huxleyi* (Haptophyta), *J. Phycol.*, 47, 615–626, doi: 10.1111/j.1529-
477 8817.2001.00992.x, 2011.

478 Dickson, A. G., Afghan, J. D., and Anderson, G. C.: Reference materials for oceanic
479 CO₂ analysis: a method for the certification of total alkalinity, *Mar. Chem.*, 80,
480 185–197, doi: 10.1016/S0304-4203(02)00133-0, 2003.

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

484 Duarte, C. M., and Cerbrian, J.: The fate of marine autotrophic production, *Limnol.*

485 Oceanogr., 41, 1758–1766, 1996.

486 Feng Y. Y., Roleda, M. Y., Armstrong, E., Boyd, P. W., and Hurd, C. L.:
487 Environmental controls on the growth, photosynthetic and calcification rates of a
488 Southern Hemisphere strain of the coccolithophore *Emiliana huxleyi*, Limnol.
489 Oceanogr., 62, 519–540, doi: 10.1002/lno.10364, 2017.

490 Gafar, N. A., Eyre, B. D., and Schulz, K. G.: A conceptual model for projecting
491 coccolithophorid growth, calcification and photosynthetic carbon fixation rates
492 in response to global ocean change, Front. Mar. Sci., 4, 433, doi:
493 10.3389/fmars.2017.00433, 2018.

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

497 González-Dávila, M., and Santana-Casiano, M.: Seasonal and interannual variability
498 of sea-surface carbon dioxide species at the European Station for Time Series in
499 the Ocean at the Canary Islands (ESTOC) between 1996 and 2000, Glob.
500 Biogeochem. Cycles, 17, 1076, doi: 10.1029/2002GB001993, 2003.

501 Gsell, A. S., de Senerpont-Domis, L. N., Przytulska-Bartosiewicz, A., Mooij, W. M.,
502 van Donk, E, and Ibelings, B. W.: Genotype-by-temperature interactions may help
503 to maintain clonal diversity in *Asterionella formosa* (Bacillariophyceae), J.
504 Phycol., 48, 1197–1208, doi: 10.1111/j.1529-8817.2012.01205.x, 2012.

505 Hattich, G. S. I., Listmann, L., Raab, J., Ozod-Seradj, D., Reusch, T. B. H., and
506 Matthiessen, B.: Inter- and intraspecific phenotypic plasticity of three

507 phytoplankton species in response to ocean acidification, *Biol. Lett.*, 13,
508 20160774, doi: 10.1098/rsbl.2016.0774, 2017.

509 Henderiks, J., Winter, A., Elbrächter, M., Feistel, R., van der Plas, A., Nausch, G.,
510 and Barlow, R.: Environmental controls on *Emiliana huxleyi* morphotypes in the
511 Benguela coastal upwelling system (SE Atlantic), *Mar. Ecol. Prog. Ser.*, 448, 51–
512 66, doi:10.3354/meps09535, 2012.

513 Hoppe, C. J. M., Langer, G., and Rost, B.: *Emiliana huxleyi* shows identical
514 responses to elevated pCO₂ in TA and DIC manipulations, *J. Exp. Mar. Biol.*
515 *Ecol.*, 406, 54–62, doi: 10.1016/j.jembe.2011.06.008, 2011.

516 Hutchins, D. A., Fu, F. X., Webb, E. A., Walworth, N., and Tagliabue, A.: Taxon-
517 specific response of marine nitrogen fixers to elevated carbon dioxide
518 concentrations, *Nat. Geosci.*, 6, 790–795, doi: 10.1038/ngeo1858, 2013.

519 Hutchinson, G. E.: The paradox of the plankton, *Am. Nat.*, 95, 137–145, 1961.

520 Klaas, C., and Archer, D. E.: Association of sinking organic matter with various types
521 of mineral ballast in the deep sea: Implications for the rain ratio, *Glob.*
522 *Biogeochem. Cycles*, 16, 1116, doi: 10.1029/2001GB001765, 2002.

523 Kottmeier, D. M., Rokitta, S. D., and Rost, B.: H⁺-driven increase in CO₂ uptake and
524 decrease in HCO₃⁻ uptake explain coccolithophores' acclimation responses to
525 ocean acidification, *Limnol. Oceanogr.*, 61, 2045–2057, doi: 10.1002/lno.10352,
526 2016.

527 Kremp, A., Godhe, A., Egardt, J., Dupont, S., Suikkanen, S., Casabianca, S., and
528 Penna, A.: Intraspecific variability in the response of bloom-forming marine

529 microalgae to changed climate conditions, *Ecol. Evol.*, 2, 1195–1207, doi:
530 10.1002/ece.3.245, 2012.

531 Krueger-Hadfield, S. A., Balestreri, C., Schroeder, J., Highfield, A., Helaou ä, P.,
532 Allum, J., Moate, R., Lohbeck, K. T., Miller, P. I., Riebesell, U., Reusch, T. B. H.,
533 Rickaby, R. E. M., Young, J., Hallegraeff, G., Brownlee, C., and Schroeder, D. C.:
534 Genotyping an *Emiliania huxleyi* (prymnesiophyceae) bloom event in the North
535 Sea reveals evidence of asexual reproduction, *Biogeosciences*, 11, 5215–5234, doi:
536 10.5194/bg-11-5215-2014, 2014.

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

540 Krumhardt, K. M., Lovenduski, N. S., Debora Iglesias-Rodriguez, M., and Kleypas, J.
541 A.: Coccolithophore growth and calcification in a changing ocean, *Prog.*
542 *Oceanogr.*, 159, 276–295, doi: 10.1016/j.pocean.2017.10.007, 2017.

543 Langer, G., Nehrke, G., Probert, I., Ly, J., and Ziveri, P.: Strain-specific responses of
544 *Emiliania huxleyi* to changing seawater carbonate chemistry, *Biogeosciences*, 6,
545 2637–2646, doi: 10.5194/bg-6-2637-2009, 2009.

546 Levis, N. A., and Pfennig, D. W.: Evaluating ‘plasticity-first’ evolution in nature: key
547 criteria and empirical approaches, *Trends Eco. Evol.*, 31, 563–574, doi:
548 10.1016/j.tree.2016.03.012, 2016.

549 Lohbeck, K. T., Riebesell, U., and Reusch, T. B. H.: Adaptive evolution of a key
550 phytoplankton species to ocean acidification, *Nat. Geosci.*, 5, 346–351, doi:

551 10.1038/ngeo1441, 2012.

552 Müller, M. N., Trull, T. W., and Hallegraeff, G. M.: Differing responses of three
553 Southern Ocean *Emiliana huxleyi* ecotypes to changing seawater carbonate
554 chemistry, *Mar. Ecol. Prog. Ser.*, 531, 81–90, doi: 10.3354/meps11309, 2015.

555 Omar, A. M., Olsen, A., Johannessen, T., Hoppema, M., Thomas, H., and Borges, A.
556 V.: Spatiotemporal variations of $f\text{CO}_2$ in the North Sea, *Ocean Sci.*, 6, 77–89,
557 doi:10.5194/os-6-77-2010, 2010.

558 Paasche, E.: A review of the coccolithophorid *Emiliana huxleyi* (Prymnesiophyceae),
559 with particular reference to growth, coccolith formation, and calcification-
560 photosynthesis interactions, *Phycologia*, 40, 503–529, doi: 10.2216/i0031-8884-
561 40-6-503.1, 2002.

562 Palumbi, S. R.: Genetic divergence, reproductive isolation, and marine speciation.
563 *Ann. Rev. Ecol. Evol. Syst.*, 25, 547–572, doi:
564 10.1146/annurev.es.25.110194.002555, 1994.

565 Pancic, M., Hansen, P. J., Tammilehto, A., and Lundholm, N.: Resilience to
566 temperature and pH changes in a future climate change scenario in six strains of
567 the polar diatom *Fragilariopsis cylindrus*, *Biogeosciences*, 12, 4235–4244, doi: 10.
568 5194/bg-12-4235-2015, 2015.

569 Pierrot, D., Lewis, E., and Wallace, D. W. R.: MS Excel program developed for CO₂
570 system calculations, ORNL/CDIAC-105, Carbon Dioxide Information Analysis
571 Centre, Oak Ridge National Laboratory, U.S., Department of Energy, 2006.

572 Reed, T. E., Waples, R. S., Schindler, D. E., Hard, J. J., and Kinnison, M. T.:

573 Phenotypic plasticity and population viability: the importance of environmental
574 predictability, *Roc. R. Soc. B*, 277, 3391, doi: 10.1098/rspb.2010.0771, 2010.

575 Reusch, T. B. H.: Climate change in the oceans: Evolutionary versus phenotypically
576 plastic responses of marine animals and plants, *Evol. Appl.*, 7, 104–122, doi:
577 10.1111/eva.12109, 2014.

578 R ós, A. F., Pérez, F. F., Álvarez, M., Mintrop, L., González-Dávila, M., Santana-
579 Casiano, J. M., Lefèvre, L., and Watson, A. J.: Seasonal sea-surface carbon
580 dioxide in the Azores area, *Mar. Chem.*, 96, 35–51, doi:
581 10.1016/j.marchem.2004.11.001, 2005.

582 Rost, B., and Riebesell, U.: Coccolithophores and the biological pump: responses to
583 environmental changes, in: *Coccolithophores – From Molecular Biology to*
584 *Global Impact*, edited by: Thierstein, H. R. and Young, J. R., Springer, Berlin, 99–
585 125, 2004.

586 Roy, R. N., Roy, L. N., Lawson, M., Vogel, K. M., Moore, C. P., Davis W., and
587 Millero, F. J.: Thermodynamics of the dissociation of boric acid in seawater at S 5
588 35 from 0 degrees C to 55 degrees C, *Mar. Chem.*, 44, 243–248,
589 doi:10.1016/0304-4203(93)90206-4, 1993.

590 Rynearson, T. A., and Armbrust, E. V.: Genetic differentiation among populations of
591 the planktonic marine diatom *Ditylum Brightwellii* (Bacillariophyceae), *J. Phycol.*,
592 40, 34–43, doi: 10.1046/j.1529-8817.2004.03089.x, 2004.

593 Schaum, E., Rost, B., Millar, A. J., and Collins, S.: Variation in plastic responses of a
594 globally distributed picoplankton species to ocean acidification, *Nat. Clim.*

595 Change, 3, 298–302, doi: 10.1038/nclimate1774, 2013.

596 Schaum, E., Rost, B., Collins, S.: Environmental stability affects phenotypic evolution
597 in a globally distributed marine picoplankton, *The ISME Journal*, 10, 75–84, doi:
598 10.1038/ismej.2015.102, 2015.

599 Sett, S., Bach, L. T., Schulz, K. G., Koch-Klavsen, S., Lebrato, M., and Riebesell, U.:
600 Temperature modulates coccolithophorid sensitivity of growth, photosynthesis
601 and calcification to increasing seawater $p\text{CO}_2$, *PLoS ONE*, 9, e88308, doi:
602 10.1371/journal.pone.0088308, 2014.

603 Smith, H. E. K., Tyrrell, T., Charalampopoulou, A., Dumousseaud, C., Legge, O. J.,
604 Birchenough, S., Pettit, L. R., Garley, R., Hartman, S. E., Hartman, M. C., Sagoo,
605 N., Daniels, C. J., Achterberg, E. P., and Hydes, D. J.: Prodominance of heavily
606 calcified coccolithophores at low CaCO_3 saturation during winter in the Bay of
607 Biscay, *Proc. Natl. Acad. Sci. USA*, 109, 8845–8849, doi:
608 10.1073/pnas.1117508109, 2012.

609 Wisshak, M., Form, A., Jakobsen, J., and Freiwald, A.: Temperate carbonate cycling
610 and water mass properties from intertidal to bathyal depths (Azores),
611 *Biogeosciences*, 7, 2379–2396, doi:10.5194/bg-7-2379-2010, 2010.

612 Zhang, Y., Klapper, R., Lohbeck, K. T., Bach, L. T., Schulz, K. G., Reusch, T. B. H.,
613 and Riebesell, U.: Between- and within-population variations in thermal reaction
614 norms of the coccolithophore *Emiliana huxleyi*, *Limnol. Oceanogr.*, 59, 1570–
615 1580, doi: 10.4319/lo.2014.59.5.1570, 2014.

616 Zhang, Y., Bach, L. T., Schulz, K. G., and Riebesell, U.: The modulating effect of

617 light intensity on the response of the coccolithophore *Gephyrocapsa oceanica* to
618 ocean acidification, *Limnol. Oceanogr.*, 60, 2145–2157, doi:10.1002/lno.10161,
619 2015.

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640 **Table 1.** Surface seawater CO₂ levels and pH at the Azores, Bergen and Canary
 641 Islands.

	Location	Mean seasonal CO₂ (µatm)	Mean seasonal pH (total scale)	CO₂ variability (µatm)	References
Azores	38°34'N, 28°42'W	320 – 400	8.005 – 8.05	80	R ós 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	Gonz ález-D ávila 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 *Emiliana 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.

	$p\text{CO}_2$ (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 \pm 3	8.46 \pm 0.01	2358 \pm 12	1844 \pm 11	1485 \pm 13	355 \pm 5	5 \pm 0	8.5 \pm 0.1
	300 \pm 20	8.16 \pm 0.03	2339 \pm 27	2031 \pm 17	1803 \pm 18	218 \pm 13	11 \pm 1	5.2 \pm 0.3
	360 \pm 19	8.09 \pm 0.02	2322 \pm 30	2052 \pm 14	1849 \pm 9	190 \pm 10	13 \pm 1	4.5 \pm 0.3
	500 \pm 26	7.97 \pm 0.02	2301 \pm 23	2100 \pm 16	1933 \pm 14	149 \pm 8	18 \pm 1	3.5 \pm 0.2
	695 \pm 20	7.85 \pm 0.01	2317 \pm 11	2167 \pm 13	2023 \pm 14	118 \pm 2	25 \pm 1	2.8 \pm 0.1
	875 \pm 40	7.76 \pm 0.02	2320 \pm 19	2206 \pm 13	2076 \pm 10	99 \pm 5	32 \pm 1	2.4 \pm 0.1
	1110 \pm 119	7.66 \pm 0.05	2303 \pm 19	2222 \pm 23	2101 \pm 25	80 \pm 8	40 \pm 4	1.9 \pm 0.2
	1315 \pm 104	7.59 \pm 0.03	2308 \pm 18	2251 \pm 26	2133 \pm 26	70 \pm 4	48 \pm 4	1.7 \pm 0.1
	1665 \pm 107	7.50 \pm 0.03	2311 \pm 11	2286 \pm 15	2169 \pm 14	57 \pm 3	60 \pm 4	1.4 \pm 0.1
	1935 \pm 175	7.44 \pm 0.04	2308 \pm 15	2302 \pm 24	2183 \pm 21	50 \pm 4	70 \pm 6	1.2 \pm 0.1
2490 \pm 132	7.33 \pm 0.02	2320 \pm 12	2350 \pm 15	2220 \pm 13	40 \pm 2	90 \pm 5	0.9 \pm 0.1	
Bergen	120 \pm 3	8.47 \pm 0.01	2354 \pm 18	1834 \pm 18	1470 \pm 17	359 \pm 2	4 \pm 0	8.6 \pm 0.1
	290 \pm 16	8.17 \pm 0.02	2337 \pm 21	2024 \pm 12	1793 \pm 14	220 \pm 10	11 \pm 1	5.3 \pm 0.2
	355 \pm 18	8.10 \pm 0.02	2315 \pm 23	2045 \pm 11	1840 \pm 7	192 \pm 10	13 \pm 1	4.6 \pm 0.2
	490 \pm 18	7.98 \pm 0.02	2302 \pm 19	2096 \pm 14	1926 \pm 12	152 \pm 6	18 \pm 1	3.6 \pm 0.1
	670 \pm 22	7.86 \pm 0.01	2317 \pm 11	2162 \pm 10	2016 \pm 10	121 \pm 3	24 \pm 1	2.9 \pm 0.1
	855 \pm 52	7.77 \pm 0.03	2326 \pm 19	2206 \pm 15	2074 \pm 14	101 \pm 6	30 \pm 2	2.4 \pm 0.1
	1080 \pm 53	7.67 \pm 0.02	2316 \pm 26	2232 \pm 20	2110 \pm 18	83 \pm 5	39 \pm 2	2.0 \pm 0.1
	1280 \pm 71	7.60 \pm 0.02	2318 \pm 15	2257 \pm 17	2138 \pm 17	72 \pm 4	46 \pm 3	1.7 \pm 0.1
	1550 \pm 122	7.52 \pm 0.03	2300 \pm 19	2266 \pm 28	2150 \pm 27	60 \pm 4	56 \pm 4	1.4 \pm 0.1
	1800 \pm 235	7.47 \pm 0.05	2301 \pm 19	2286 \pm 33	2168 \pm 30	53 \pm 6	65 \pm 9	1.3 \pm 0.1
2280 \pm 147	7.37 \pm 0.02	2309 \pm 20	2326 \pm 27	2201 \pm 24	42 \pm 2	82 \pm 5	1.0 \pm 0.1	
Canary Islands	130 \pm 3	8.45 \pm 0.01	2344 \pm 38	1842 \pm 32	1491 \pm 26	347 \pm 7	5 \pm 0	8.3 \pm 0.2
	310 \pm 11	8.15 \pm 0.01	2317 \pm 24	2020 \pm 25	1798 \pm 25	210 \pm 4	11 \pm 1	5.0 \pm 0.1
	375 \pm 14	8.07 \pm 0.01	2295 \pm 14	2040 \pm 12	1846 \pm 13	182 \pm 5	14 \pm 1	4.3 \pm 0.1
	505 \pm 32	7.96 \pm 0.02	2297 \pm 19	2097 \pm 20	1930 \pm 23	148 \pm 7	18 \pm 1	3.5 \pm 0.2
	695 \pm 18	7.85 \pm 0.01	2312 \pm 20	2163 \pm 17	2020 \pm 15	118 \pm 3	25 \pm 1	2.8 \pm 0.1
	925 \pm 73	7.74 \pm 0.04	2319 \pm 26	2211 \pm 15	2083 \pm 12	95 \pm 8	33 \pm 3	2.3 \pm 0.1
	1180 \pm 53	7.64 \pm 0.02	2310 \pm 25	2239 \pm 20	2120 \pm 19	76 \pm 4	43 \pm 2	1.8 \pm 0.1
	1380 \pm 104	7.58 \pm 0.03	2323 \pm 5	2271 \pm 10	2154 \pm 11	68 \pm 5	50 \pm 4	1.6 \pm 0.1
	1740 \pm 98	7.48 \pm 0.02	2319 \pm 16	2298 \pm 16	2180 \pm 15	55 \pm 3	63 \pm 4	1.3 \pm 0.1
	2140 \pm 258	7.40 \pm 0.05	2312 \pm 9	2320 \pm 16	2197 \pm 13	46 \pm 5	78 \pm 10	1.1 \pm 0.1
2630 \pm 284	7.31 \pm 0.04	2317 \pm 13	2363 \pm 20	2225 \pm 14	37 \pm 3	98 \pm 8	0.8 \pm 0.1	

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665 **Table 3.** Calculated optimum $p\text{CO}_2$, 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.

strain	Growth rate			POC production rate			PIC production rate		
	optimum $p\text{CO}_2$ (μatm)	V_{max} (d^{-1})	rs	optimum $p\text{CO}_2$ (μatm)	V_{max} ($\text{pg C cell}^{-1} \text{d}^{-1}$)	rs	optimum $p\text{CO}_2$ (μatm)	V_{max} ($\text{pg C cell}^{-1} \text{d}^{-1}$)	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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677 **Figure Captions**

678 **Figure 1.** Optimum curve responses of measured and relative growth, particulate
679 organic (POC) and inorganic carbon (PIC) production rates of three *Emiliana huxleyi*
680 populations to a $p\text{CO}_2$ range from 120 μatm to 2630 μatm . Responses of measured (a)
681 and relative (b) growth rates to $p\text{CO}_2$. Responses of measured (c) and relative (d)
682 POC production rates to $p\text{CO}_2$. Responses of measured (e) and relative (f) PIC
683 production rates to $p\text{CO}_2$. Using the nonlinear regression model derived by Bach et al.
684 (2011), the curves were fitted based on average growth, POC and PIC production
685 rates of six strains from the Azores and Bergen, and of five strains from the Canary
686 Islands. Vertical error bars represent standard deviations of six growth, POC and PIC
687 production rates for the Azores and Bergen populations, and five growth, POC and
688 PIC production rates for the Canary Islands population. Horizontal error bars
689 represent standard deviations of six $p\text{CO}_2$ levels for the Azores and Bergen
690 populations and five $p\text{CO}_2$ levels for the Canary Islands populations. At the
691 population levels, 120 μatm and 2630 μatm was the lowest and highest $p\text{CO}_2$ level,
692 respectively.

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694 **Figure 2.** Calculated optimum $p\text{CO}_2$, calculated maximum value and fitted relative
695 sensitivity constant of growth, POC and PIC production rates of each population. (a)
696 optimum $p\text{CO}_2$ of growth rate; (b) optimum $p\text{CO}_2$ of POC production rates; (c)
697 optimum $p\text{CO}_2$ of PIC production rates; (d) maximum growth rate, (e) maximum
698 POC production rate, (f) maximum PIC production rate; (g) relative sensitivity

699 constant of growth rate; **(h)** relative sensitivity constant of POC production rate; **(i)**
700 relative sensitivity constant of PIC production rate. The line in the middle of each box
701 indicates the mean of 6 or 5 optimum $p\text{CO}_2$, 6 or 5 maximum values, and 6 or 5
702 relative sensitivity constants for growth, POC and PIC production rates in each
703 population. Bars indicate the 99% confidence interval. The maximum or minimum
704 data is shown as the small line on the top or bottom of the bar, respectively. Letters in
705 each panel represent statistically significant differences (Tukey HSD, $p < 0.05$).

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707 **Figure 3.** Optimum curve responses of growth, POC and PIC production rates of
708 individual *E. huxleyi* strains in the Azores (left), Bergen (medium) and Canary Islands
709 (right) populations to a CO_2 range from 115 μatm to 3070 μatm . Growth rates of each
710 strain as a function of $p\text{CO}_2$ within the Azores **(a)**, Bergen **(b)** and Canary Islands **(c)**
711 populations. POC production rates of each strain as a function of $p\text{CO}_2$ within the
712 Azores **(d)**, Bergen **(e)** and Canary Islands **(f)** populations. PIC production rates of
713 each strain as a function of $p\text{CO}_2$ within the Azores **(g)**, Bergen **(h)** and Canary
714 Islands **(i)** populations. At the strain levels, 115 μatm and 3070 μatm was the lowest
715 and highest $p\text{CO}_2$ level, respectively.

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