



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

Yong Zhang^{1,5}, Lennart T. Bach¹, Kai T. Lohbeck^{1,2,6}, Kai G. Schulz³, Luisa Listmann², Regina Klapper⁴, and Ulf Riebesell¹

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

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

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

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

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

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

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

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Abstract. Although coccolithophore physiological responses to CO₂-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 three populations of the coccolithophore *Emiliana huxleyi* from three regions in the North Atlantic Ocean (Azores: six strains, Canary Islands: five strains, and Norwegian coast near Bergen: six strains) to a CO₂ partial pressure ($p\text{CO}_2$) range from 120 to 2630 μatm . Physiological rates of each population and individual strain increased with rising $p\text{CO}_2$ levels, reached a maximum and declined thereafter. Optimal $p\text{CO}_2$ for growth, 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 $p\text{CO}_2$ 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–CO₂ interactions where the chosen incubation temperature (16 °C) was slightly below what strains isolated near the Canary Islands normally experience. Our results indicate adaptation of *E. huxleyi* to their local environmental conditions and the existence of distinct *E. huxleyi* populations. Within each population, different growth, POC, and PIC production rates at different $p\text{CO}_2$ levels indicated strain-specific phenotypic plasticity. Accounting for this variability is important to understand how or whether *E. huxleyi* might adapt to rising CO₂ levels.

1 Introduction

Coccolithophores form a layer of calcium carbonate (CaCO₃) platelets (coccoliths) around their cells. Coccoliths are of biogeochemical importance due to ballasting of organic matter with CaCO₃, 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 *Emiliana huxleyi* forms extensive blooms under favorable 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 CO₂ levels have been reported (Langer et al., 2009; Hoppe et al., 2011; Müller et al., 2015; Hattich et al., 2017) and are likely a result of intra-specific variability of genotypes (Langer et al., 2009). Several recent studies observed optimum curve responses in physiological rates of a single *E. huxleyi* strain to a broad *p*CO₂ range from about 20 to 5000 µatm, and linked them to inorganic carbon substrate limitation at low *p*CO₂ and inhibiting H⁺ concentrations at high *p*CO₂ (Bach et al., 2011, 2015; Kottmeier et al., 2016). Until now, studies on the physiological responses of *E. huxleyi* to rising CO₂ are mostly based on a few genotypes and little is known about the potential variability in CO₂ and H⁺ sensitivity between and within populations. Recently, several studies found substantial variations in CO₂ responses for N₂ 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; Pančić et al., 2015; Hattich et al., 2017). Hence, multiple strains, ideally from geographically distinct regions, should be considered for investigating phytoplankton responses to climate change (Zhang et al., 2014; Blanco-Ameijeiras et al., 2016; Krumhardt et al., 2017).

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

In order to better understand how local adaptation affects the physiological response of *E. huxleyi* to rising CO₂ conditions, we isolated 17 strains from three regions in the Atlantic Ocean, and assessed growth, carbon fixation, and calcification responses of the population over a *p*CO₂ range from 120 to 2630 µatm.

2 Materials and methods

2.1 Cell isolation sites and experimental setup

Emiliana huxleyi strains EHGKL B95, B63, B62, B51, B41, and B17 originated from Raunefjord (Norway, 60°18' N, 05°15' E) and were isolated by Kai 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 Sarah 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 Kai T. Lohbeck in February 2014 at ~18 °C in situ water temperature. Seasonal CO₂ concentration in the surface seawater ranges from 240 to 400 µatm near Bergen, from 320 to 400 µatm around the Azores, and from 320 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 in the Supplement).

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 five 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 CO₂ 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 in the Supplement). 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: 05:00 to 21:00) at 16 °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 and 4 µmol kg⁻¹, respectively). For the preparation of ASW and nutrient additions see Zhang et al. (2014). Calculated volumes of Na₂CO₃ and hydrochloric acid were added to the ASW to achieve target CO₂ levels at an average TA of 2319 ± 23 µmol kg⁻¹ (Pierrot et al., 2006; Bach et al., 2011). Each strain was grown under 11 CO₂ levels ranging from 115 to 3070 µatm without replicate. Mean response variables of

Table 1. Surface seawater CO₂ levels and pH at the Azores, Bergen, and Canary 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)

all strains with a population were calculated and mean CO₂ levels of all strains within a population ranged from 120 to 2630 µatm. Cells grew in the experimental conditions for at least seven generations, which corresponded to 4–7 days depending on cell division rates. Cells were cultured for 4 days in 120–925 µatm CO₂, for 5 days in 1080–1380 µatm CO₂, and for 6 or 7 days in 1550–2630 µatm CO₂. Initial cell concentration was 200 cells mL⁻¹ (estimated from measured pre-culture concentrations and known dilution) and final cell concentration was lower than 100 000 cells mL⁻¹. Dissolved inorganic carbon (DIC) concentrations and *p*CO₂ levels changed less than 7 and 11 %, respectively, during the experimental growth phase.

2.2 pH_T and total alkalinity measurements

At 10:00 on the last day of incubations (at day 4–7 depending on CO₂ 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. The 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 13:00 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 the following equation:

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

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 15:00 on the last day of incubation, cells for total particulate (TPC) and total organic (TOC) carbon were filtered onto GF/F filters which were pre-combusted at 500 °C for 8 h. Samples of background particulate carbon (BPC) were determined in a similar way but using filtered ASW without algae, which was previously adjusted to target *p*CO₂ 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 follows:

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

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

2.5 Data analysis

In a broad *p*CO₂ range, physiological rates are expected to initially increase quickly until reaching an optimum and then decline towards further increasing CO₂ levels (e.g., Krug et al., 2011). Hence we used the following modified Michaelis–Menten equation (Bach et al. 2011), which was fitted to measured cellular growth, POC, and PIC production rates, and yield theoretical optimum *p*CO₂ and maximum values for each of the three populations (combining the data of five or six strains) (Bach et al., 2011).

$$y = \frac{X \times p\text{CO}_2}{Y + p\text{CO}_2} - s \times p\text{CO}_2, \quad (4)$$

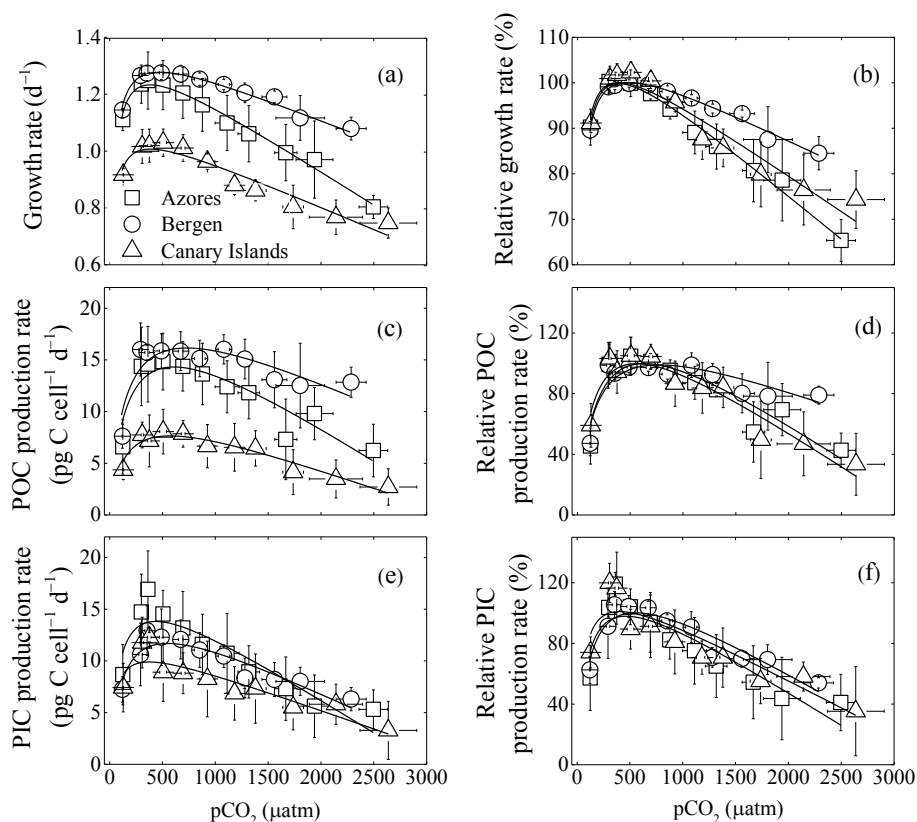


Figure 1. Optimum curve responses of measured and relative growth, particulate organic (POC) and inorganic carbon (PIC) production rates of three *Emiliana huxleyi* populations to a $p\text{CO}_2$ range from 120 to 2630 μatm . Responses of measured (a) and relative (b) growth rates to $p\text{CO}_2$.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

where X and Y are fitted parameters, and s , the sensitivity constant, depicts the slope of the decline after optimum CO₂ levels in response to rising H⁺. Based on the fitted X , Y and s , we calculated $p\text{CO}_2$ optima (K_m) (Eq. 5) and maximum growth, POC, and PIC production rates following Bach et al. (2011).

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

The relative values for growth, POC, and PIC production rates were calculated as ratios of growth, POC, and PIC production rates at each $p\text{CO}_2$ level to the maximum (highest) rates. We obtained the relative sensitivity constant by fitting function (4) based on relative growth, POC, and PIC production rates.

A one-way ANOVA was then used to test for statistically significant differences in theoretical optimum $p\text{CO}_2$, maximum value and relative sensitivity constant between pop-

ulations. A Tukey HSD test was conducted to determine the differences between strains from different populations. A Shapiro–Wilk’s analysis was tested to analyze residual normality. Statistical calculations were carried out using *R* and significance was shown by $p < 0.05$.

3 Results

3.1 Carbonate chemistry parameters

Carbonate system parameters are shown in Table 2. Average $p\text{CO}_2$ levels of the ASW ranged from 125 to 2490 μatm for the Azores population, from 120 to 2280 μatm for the Bergen population, and from 130 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.

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

	$p\text{CO}_2$ (μatm)	pH (total scale)	TA ($\mu\text{mol kg}^{-1}$)	DIC ($\mu\text{mol kg}^{-1}$)	HCO_3^- ($\mu\text{mol kg}^{-1}$)	CO_3^{2-} ($\mu\text{mol kg}^{-1}$)	CO_2 ($\mu\text{mol kg}^{-1}$)	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	

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

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

Measured POC production rates of the Azores and Bergen populations were larger than those of the Canary Islands population at all $p\text{CO}_2$ levels (Fig. 1c) and decline in POC pro-

duction rates with increasing $p\text{CO}_2$ levels beyond the $p\text{CO}_2$ optimum was larger in the Azores and Canary Islands populations than in the Bergen population (Fig. 1d).

Measured PIC production rates at investigated $p\text{CO}_2$ levels did not show significant differences among the Azores, Bergen and Canary Islands populations (Fig. 1e). Exceptions were that at 365–695 μatm , PIC production rates of the Azores population were larger than those of the Canary Islands population (all $p < 0.05$).

3.3 Physiological responses of populations to $p\text{CO}_2$

Calculated optimum $p\text{CO}_2$ for growth, POC, and PIC production rates of the Bergen population were significantly larger than those of the Azores and Canary Islands popula-

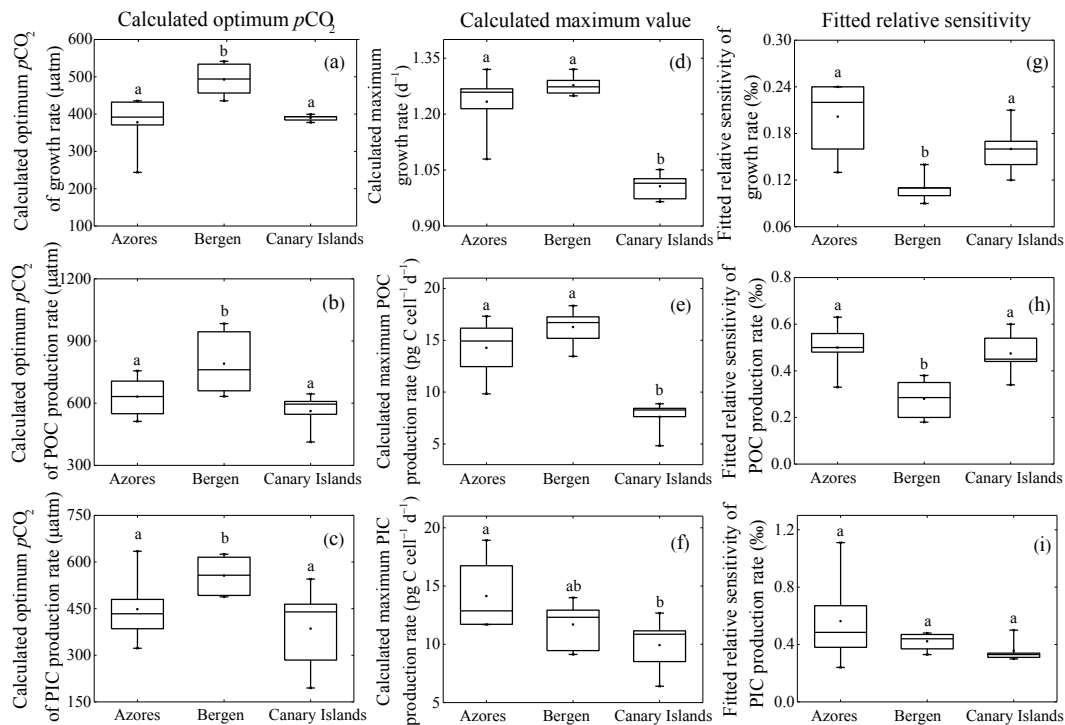


Figure 2. Calculated optimum $p\text{CO}_2$, calculated maximum value and fitted relative sensitivity constant of growth, POC, and PIC production rates of each population. (a) optimum $p\text{CO}_2$ of growth rate; (b) optimum $p\text{CO}_2$ of POC production rates; (c) optimum $p\text{CO}_2$ of PIC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

tions (all $p < 0.05$) (Fig. 2a–c). Optimum $p\text{CO}_2$ for these physiological rates between the Azores and Canary Islands population were not different (all $p > 0.1$).

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

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

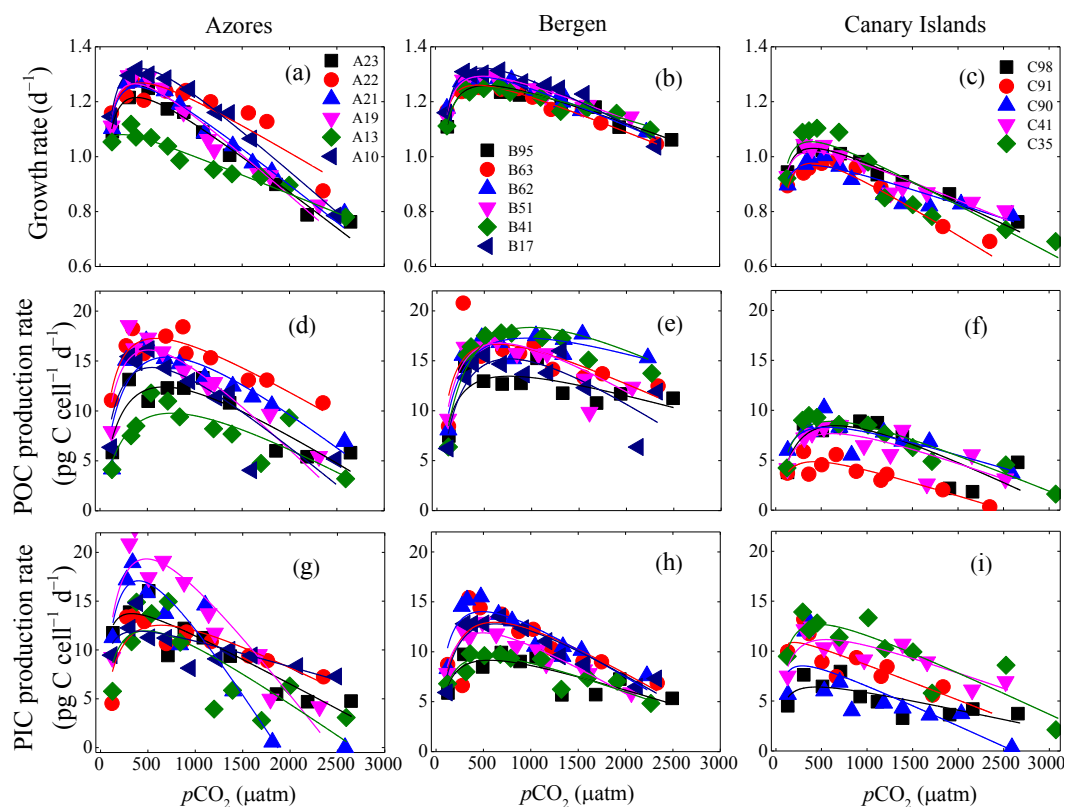
3.4 Physiological responses of individual strains to $p\text{CO}_2$

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

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

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

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

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

4 Discussion

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

In comparison to the Azores and Canary Islands populations, variability in growth rates between strains of the Bergen population was smaller even though they had higher growth rates at all $p\text{CO}_2$ levels (Fig. 3). Furthermore, the Bergen population showed significantly higher $p\text{CO}_2$ optima and lower H^+ sensitivity for growth and POC production rates (Fig. 2). These findings indicate that the Bergen population may be more tolerant to changing carbonate chemistry in terms of its growth and photosynthetic carbon fixation rates. The Bergen strains were isolated from coastal waters, while the Azores and Canary Islands strains were isolated from a more oceanic environment. Seawater carbonate chemistry of coastal waters is usually more dynamic than in the open ocean (Cai, 2011). In fact, previous studies have reported that CO₂ and pH variability of the seawater off Bergen was larger than off the Azores and Canary Islands (Table 1). In addition, due to riverine input, seawater upwelling, and metabolic activity of plankton communities, environmental variability in coastal waters are larger than in open-ocean ecosystems (Duarte and Cerbrian, 1996). Doblin and van Sebille (2016) suggested that phytoplankton populations should be constantly under selection when experiencing changing environmental conditions. In this case, the Bergen population, exposed to larger CO₂ or pH fluctuations, may have acquired a higher capacity to acclimate to changing carbonate chemistry resulting in a higher tolerance (or lower sensitivity) to rising CO₂ levels. In contrast, the Azores and Canary Islands populations experience similar, less variable seawater carbonate chemistry conditions in their natural environment, which could explain why they also show similar $p\text{CO}_2$ 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 how adaptation to local temperature can significantly affect growth of *E. huxleyi* strains in laboratory experiments. Considering these findings and the temperature ranges of the three isolation locations (Table S1), the incubation temperature of 16 °C used in the present study was lower than the

minimum sea surface temperature (SST) commonly recorded at the Canary Islands. In contrast, SSTs of 16 °C and lower have been reported for Azores and Bergen waters (Table S1). When exposed to 16 °C, growth rate of the Canary Islands population might have already been 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 CO₂ for these physiological rates. Again, this might be related to sub-optimal incubation conditions as temperature has been found to significantly modulate CO₂ responses in coccolithophores in terms of maximum rates, CO₂ optima and half-saturation, and H^+ sensitivity (Sett et al., 2014; Gafar et al., 2018; Gafar and Schulz, 2018). In a similar fashion, light can also modulate CO₂ responses, hence different requirements by strains adapted to different light availabilities could also explain our observations (Zhang et al., 2015; Gafar et al., 2018; Gafar and Schulz, 2018). Thus, with rising CO₂, growth, photosynthetic carbon fixation and calcification rates of the Canary Islands population cannot increase as much as in the Azores and Bergen populations. In addition, the Canary Islands population showed smallest variability in optimum $p\text{CO}_2$ and maximum values for growth and POC production rates (Fig. 2). The reason may be that low incubation temperature predominantly limited growth and POC production rates of the Canary Islands population, and decreased the sensitivities of these physiological rates to rising $p\text{CO}_2$.

Before we started this experiment, strains isolated from the Azores, Bergen, and Canary Islands grew as stock cultures at 15 °C and 400 μatm for 4 years, 5 years and 3 months, respectively. Schaum et al. (2015) provide evidence that long-term laboratory incubation affects responses of phytoplankton to different $p\text{CO}_2$ levels. Thus, it is conceivable that the same selection history in the laboratory incubation may contribute to a more similar response of growth, POC, and PIC production rates between the Azores and Bergen populations at low $p\text{CO}_2$ levels (Fig. 1).

Our results indicate that *E. huxleyi* populations are adapted to the specific environmental conditions of their origin, resulting in different responses to increasing $p\text{CO}_2$ levels. The ability to adapt to diverse environmental conditions is supposed to be one reason for the global distribution of *E. huxleyi* (Paasche, 2002), spanning a temperature range of about 30 °C. In addition, these results will improve our understanding on variation in physiological responses of different *E. huxleyi* populations to climate change, and variation in production of different areas in future oceans. The optimum temperature for growth of the Bergen population was about 22 °C and was 5 °C higher than the maximum SST in Bergen waters (Zhang et al., 2014). Furthermore, in comparison to the Azores and Canary Islands populations, larger optimum $p\text{CO}_2$ of growth rate indicates that the Bergen population may benefit more from the rising CO₂ levels. PIC : POC ra-

tios of the Azores and Bergen populations declined with rising $p\text{CO}_2$, whereas PIC:POC ratios of the Canary Islands population were rather constant (Figs. S6, S7). As changes in PIC:POC ratios of coccolithophore blooms were suggested to impact on biological carbon pump (Rost and Riebesell, 2004), variation in PIC:POC ratios of different populations indicates that different regions might have different changes in marine carbon cycle in the future ocean. In natural seawater, due to ocean currents and gene flow, populations at any given location may get replaced by immigrant genotypes transported there from other locations (Doblin and van Sebille, 2016). In addition, *E. huxleyi* is thought to utilize HCO_3^- for calcification which generates protons, and increase in proton concentration may mitigate the potential of the ocean to absorb atmospheric CO₂ and then give a positive feedback to rising atmosphere CO₂ levels (Paasche, 2002).

Within a population, individual strains showed different growth, POC, and PIC production rates at different $p\text{CO}_2$ levels, indicating phenotypic plasticity of individual strains (Reusch, 2014). Phenotypic plasticity constitutes an advantage for individual strains to acclimate and adapt to elevated $p\text{CO}_2$ 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 CO₂-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 out compete others (Schaum et al., 2013). Further, a significant positive correlation between growth and POC production rate or POC quota (Fig. S5) indicates that higher growth rate means larger populations and then greater production.

5 Conclusions

In the present study, we found population-specific responses in physiological rates of *E. huxleyi* to a broad $p\text{CO}_2$ 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. In this study, we only studied the effects of rising CO₂ but future studies should take into account simultaneous effects from other interacting factors such as light and temperature variability.

Data availability. The manuscript and all data can be found in following link: <https://issues.pangaea.de/projects/PDI/issues/PDI-17737?filter=allopenissues>, last access: 15 June 2018.

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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 analyzed the data, reviewed and improved the manuscript.

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

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