Biogeosciences

Strain-specific responses of *Emiliania huxleyi* to changing seawater carbonate chemistry

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Abstract. Four strains of the coccolithophore *E. huxleyi* (RCC1212, RCC1216, RCC1238, RCC1256) were grown in dilute batch culture at four CO_2 levels ranging from ~200 μatm to ~1200 μatm. Growth rate, particulate organic carbon content, and particulate inorganic carbon content were measured, and organic and inorganic carbon production calculated. The four strains did not show a uniform response to carbonate chemistry changes in any of the analysed parameters and none of the four strains displayed a response pattern previously described for this species. We conclude that the sensitivity of different strains of *E. huxleyi* to acidification differs substantially and that this likely has a genetic basis. We propose that this can explain apparently contradictory results reported in the literature.

1 Introduction

Anthropogenic CO_2 emissions cause a decrease of surface seawater pH, a process termed ocean acidification (Royal Society, 2005). Among the adverse effects of ocean acidification on marine organisms, reduction in the capacity of calcifiers to build shells has received special attention because calcium carbonate precipitation in surface waters and its subsequent export to the sediments play important roles in the global carbon cycle (Van Cappellen, 2003). In terms of calcite export to sediments, the coccolithophores, unicellular haptophyte algae that cover the cell surface with minute intracellularly-produced calcite platelets (the coccoliths), are one of the most important groups of calcifiers in today's oceans (Baumann et al., 2004).

The question of how coccolithophores will respond to ocean acidification has attracted increasing attention over the last decade. To date, the majority of evidence stems from laboratory culture experiments. Following the seminal study by Riebesell et al. (2000), the first widely adopted notion was that coccolithophores decrease their calcification rate with increasing CO_2 concentration (decreasing pH) in a linear fashion (see also Zondervan et al., 2001). These studies were conducted on one culture strain of each of two closely related species, *E. huxleyi* and *Gephyrocapsa oceanica*, both gephyrocapsid coccolithophores that are relatively small ($\leq 8\,\mu$m), but numerically dominant in coccolithophore assemblages in modern oceans. A subsequent study showed that one culture strain of each of the two larger, heavily calcifying coccolithophores *Calcidiscus leptoporus* and *Coccolithus braarudii*, did not follow the response pattern previously reported for *E. huxleyi* (Langer et al., 2006). While *C. leptoporus* displayed an optimum curve, *C. braarudii* was insensitive over the CO_2(pH) range tested. This clearly demonstrated that there is no uniform response of coccolithophores to acidification.

For *E. huxleyi*, apparently conflicting results have been reported. In direct contrast to the results of Riebesell et al. (2000), a recent study reported that *E. huxleyi* increases its calcification rate in response to increasing CO_2 concentration under light saturation (Iglesias-Rodriguez et al., 2008a). Despite an active debate on the subject (Riebesell et al., 2008; Iglesias-Rodriguez et al., 2008b), it is presently unknown why apparently contradictory results have been obtained in different studies on *E. huxleyi*. One striking fact is that in every study a different culture strain of this species has been used. Bearing in mind that different species of coccolithophore have been shown to exhibit different responses to carbonate chemistry changes, it can be hypothesized that intra-specific responses also exist. To test this, we cultured four strains of *E. huxleyi* under light saturation and four different CO_2 concentrations, and measured, inter alia, calcification rate.

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Table 1. Information on culture strains used in this and previous studies. Max. annual SST was taken from the World Ocean Atlas (www.nodc.noaa.gov).

Strain Code	Isolation Date	Location of sample from which culture isolated	Seawater temperature at time of sampling	Max. annual SST at sampling location	Experimental temperature	Morphotype	Study
RCC1212	9/2000	34°28′ S 17°18′ E (South Atlantic, off South Africa)	15°C	21°C	20°C	B	This study
RCC1216	9/1998	42°18′ S 169°50′ E (Tasman Sea, off New Zealand)	11°C	18°C	17°C	R	This study
RCC1238	11/2005	34°01′ N 139°50′ E (North Pacific, off Japan)	18°C	25°C	20°C	A	This study
RCC1256	7/1999	63°24′ N 20°20′ W (North Atlantic, off Iceland)	9°C	14°C	17°C	A	This study
PLY B92/11A	4/1992	60°16′ N 5°14′ E (Bergen, Norway; mesocosm bag)	10°C	16°C	15°C	A	Riebesell et al., 2000
NZEH (COWPO6)		South Pacific, off New Zealand		~18°C	19°C	R	Iglesias-Rodriguez et al., 2008a
CCMP371	6/1987	32° N 62° W (Sargasso Sea; 50 m depth)	?	25°C	20 and 24°C		Feng et al., 2008

2 Material and methods

Clonal cultures of *E. huxleyi* (strains RCC1212, RCC1216, RCC1238, and RCC1256) were grown in aged, sterile-filtered (0.2 μm pore-size cellulose-acetate filters) North Sea seawater enriched with 100 μmol L^{-1} nitrate, 6.25 μmol L^{-1} phosphate, and trace metals and vitamins as in f/2 medium (Guillard and Ryther, 1962). Information on the strains, which were obtained from the Roscoff Culture Collection (www.sb-roscoff.fr/Phyto/RCC), is given in Table 1. Cultures were grown under a 16/8 hour light/dark cycle. Experiments were carried out at a light intensity of 400 μmol photons m^{-2} s^{-1} in an adjustable incubator (Rubarth Apparate GmbH, Germany). The temperature used was 17°C for RCC1216 and RCC1256, and 20°C for RCC1212 and RCC1238. We chose different temperatures as opposed to a standard temperature in order to grow each strain near its optimum temperature for growth. Salinity, measured with a conductivity meter (WTW Multi 340i) combined with a TetraCon 325 sensor, was 32. Cells were pre-adapted to experimental conditions for approximately 12 generations and grown in dilute batch cultures (Langer et al., 2007). Each data point presented in the tables and figures is the mean value of triplicate culture experiments. CO$_2$ levels were adjusted by adding calculated amounts of HCl or NaOH to the medium. Low cell densities ($<$50 000 cells ml^{-1}) even at the

termination of the experiments resulted in the consumption of less than 5% dissolved inorganic carbon (DIC), ensuring a quasi-constant carbonate system over the course of the experiment.

Samples for alkalinity measurements were filtered through 0.6 μm nominal pore-size glass fibre filters (Whatman GF/F), poisoned with 1 ml 35g L^{-1} HgCl$_2$, and stored in acid-washed 300 ml borosilicate flasks at 0°C. DIC samples were sterile-filtered through 0.2 μm pore-size cellulose-acetate syringe filters and stored in acid-washed 13 ml borosilicate flasks free of air bubbles at 0°C. Total alkalinity (TA) was calculated from linear Gran plots (Gran, 1952) after potentiometric titration (in duplicate) (Bradshaw et al., 1981; Brewer et al., 1986). DIC was measured photometrically (Stoll et al., 2001) in triplicate. Precision of the TA measurements was ~3 μmol L^{-1} and accuracy ~4 μmol L^{-1}. For DIC measurements, precision was ~4 μmol L^{-1} and accuracy ~5 μmol L^{-1}. The carbonate system was calculated from temperature, salinity, and the concentrations of DIC, TA, and phosphate using the DOS program CO$_2$ sys (Lewis and Wallace, 1998). The equilibrium constants of Mehrbach et al. (1973) refitted by Dickson and Millero (1987) were used. Samples for determination of total particulate carbon (TPC) and particulate organic carbon (POC) were filtered onto pre-combusted (12 h, 500°C) 0.6 μm nominal pore-size glass fibre filters (Whatman GF/F) and stored at -20°C.

Prior to analysis, 230 μL of an HCl solution (5 mol/L) was added on top of the POC filters in order to remove all inorganic carbon. TPC and POC were subsequently measured on a Euro EA Analyser (Euro Vector). Particulate inorganic carbon (PIC) was calculated as the difference between TPC and POC. For determination of cell density, samples were taken at the beginning and the end of experiment and counted immediately after sampling using a Coulter Multisizer III. Growth rate (μ) was calculated as :

$$\mu = (\ln c_1 - \ln c_0)\Delta t^{-1} \tag{1}$$

where c_0 and c_1 are the cell concentrations at the beginning and the end of experiment, respectively, and Δt is the duration of incubation in days.

Particulate inorganic carbon production, i.e. calcification rate (P_{PIC}, pg PIC cell^{-1} d^{-1}) was calculated according to:

$$P_{PIC} = \mu * (\text{cellular inorganic carbon content}) \tag{2}$$

with cellular inorganic carbon content = pg PIC per cell.

Particulate organic carbon production (P_{POC}, pg POC cell^{-1} d^{-1}) was calculated according to:

$$P_{POC} = \mu * (\text{cellular organic carbon content}) \tag{3}$$

with cellular organic carbon content = pg POC per cell.

3 Results and discussion

3.1 Summary of results

E. huxleyi RCC1212 and RCC1216 both slightly decreased growth rate in response to increasing CO_2 concentration (decreasing pH), while RCC1238 slightly increased growth rate (Fig. 1, Tables 2 and 3). RCC1256 displayed a marked decrease in growth rate with increasing CO_2 concentration (Fig. 1, Tables 2 and 3). Cellular PIC content decreased with increasing CO_2 concentration in RCC1212 and RCC1216, while it increased in RCC1256 (Fig. 2, Tables 2 and 3). RCC1238 exhibited no change in cellular PIC content over the range of CO_2 concentrations tested (Fig. 2, Tables 2 and 3). Cellular POC content increased slightly in RCC1216 with increasing CO_2 concentration, while cellular POC content of RCC1212 did not change (Fig. 2, Tables 2 and 3). In RCC1256 cellular POC content increased markedly with increasing CO_2 concentration and in RCC1238 cellular POC content remained unaltered up to $\sim$680 μatm CO_2 and decreased at $\sim$930 μatm CO_2 (Fig. 2, Tables 2 and 3). RCC1212 and RCC1216 both slightly decreased their PIC production, i.e. calcification rate, in response to increasing CO_2 (Fig. 3, Tables 2 and 3). RCC1256 showed an optimum curve with highest calcification rate at $\sim$600 μatm CO_2 and a pronounced decrease of calcification rate at $\sim$900 μatm CO_2 (Fig. 3, Tables 2 and 3). Calcification rate of RCC1238 did not change over the range of CO_2 concentrations tested (Fig. 3, Tables 2 and 3). POC production of RCC1212 and
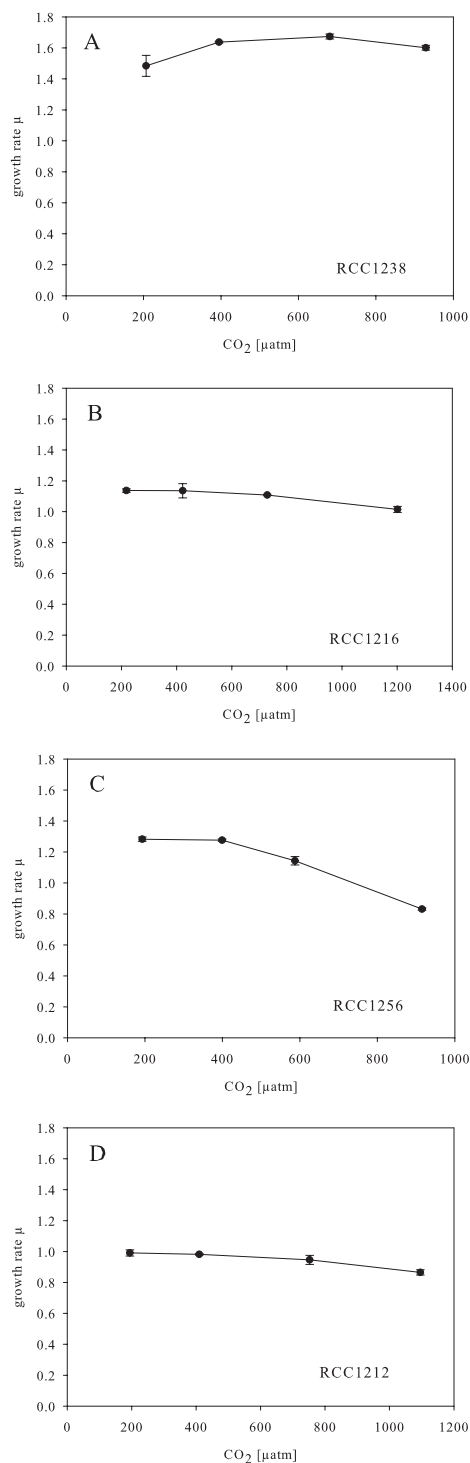

Fig. 1. Growth rates of the four *E. huxleyi* strains versus CO_2 concentration. **(A)** RCC1238, **(B)** RCC1216, **(C)** RCC1256, and **(D)** RCC1212. Errorbars represent standard deviation of three culture experiments.

Table 2. The carbonate system.

experiment	TA [μmol/kg]	TC [μmol/kg]	pH (NBS)	CO_2 [μatm]	HCO_3^- [μmol/kg]	CO_3^{2-} [μmol/kg]	omega calcite
RCC1238 1	2522	2086	8.45	206	1768	311	7.6
RCC1238 2	2302	2050	8.19	395	1858	179	4.4
RCC1238 3	2184	2039	7.96	681	1907	110	2.7
RCC1238 4	2107	2013	7.83	929	1902	80	2.0
RCC1216 1	2487	2102	8.42	218	1821	273	6.7
RCC1216 2	2281	2067	8.16	422	1898	153	3.7
RCC1216 3	2177	2060	7.93	729	1941	93	2.3
RCC1216 4	2075	2029	7.71	1201	1930	56	1.4
RCC1256 1	2456	2049	8.46	193	1756	286	7.0
RCC1256 2	2240	2022	8.17	399	1853	155	3.8
RCC1256 3	2136	1993	8.00	587	1865	106	2.6
RCC1256 4	2051	1974	7.81	915	1872	69	1.7
RCC1212 1	2517	2067	8.47	194	1741	319	7.8
RCC1212 2	2313	2066	8.18	409	1877	176	4.3
RCC1212 3	2203	2071	7.93	752	1944	103	2.5
RCC1212 4	2128	2053	7.77	1096	1947	71	1.7

Table 3. Growth rate, cellular POC and PIC content, POC and PIC production of the four *E. huxleyi* strains used SD = standard deviation. Experiment codes are the same as in Table 2.

experiment no.	growth rate μ	SD	cellular PIC content [pg PIC/cell]	SD	cellular POC content [pg POC/cell]	SD	PIC production [pg PIC/cell *day]	SD	POC production [pg POC/cell *day]	SD	PIC/ POC	SD
RCC1238 1	1.48	0.07	8.81	0.77	10.40	0.74	13.10	1.74	15.47	1.80	0.85	0.01
RCC1238 2	1.64	0.01	8.29	0.31	10.41	0.60	13.57	0.52	17.04	1.00	0.80	0.02
RCC1238 3	1.67	0.02	7.70	0.58	11.58	0.81	12.89	1.10	19.39	1.52	0.66	0.02
RCC1238 4	1.60	0.02	8.42	0.26	9.59	0.80	13.48	0.52	15.34	1.23	0.88	0.08
RCC1216 1	1.14	0.01	10.67	0.76	10.50	0.94	12.13	0.86	11.94	1.08	1.02	0.02
RCC1216 2	1.14	0.05	10.68	0.78	11.47	0.88	12.05	1.18	12.94	1.33	0.93	0.01
RCC1216 3	1.11	0.00	9.99	0.97	12.33	0.57	11.07	1.11	13.67	0.66	0.81	0.04
RCC1216 4	1.01	0.02	8.56	1.23	12.90	0.11	8.67	1.15	13.09	0.15	0.66	0.09
RCC1256 1	1.28	0.01	9.65	1.08	12.38	1.04	12.39	1.47	15.88	1.24	0.78	0.10
RCC1256 2	1.28	0.01	10.92	0.63	13.34	1.00	13.95	0.83	17.03	1.31	0.82	0.01
RCC1256 3	1.14	0.03	12.07	1.15	16.57	1.53	14.35	0.90	18.86	1.66	0.73	0.00
RCC1256 4	0.83	0.01	12.23	0.01	16.98	0.58	10.16	0.09	14.12	0.45	0.72	0.03
RCC1212 1	0.99	0.02	9.61	0.22	10.88	0.85	9.52	0.03	10.78	0.70	0.89	0.06
RCC1212 2	0.98	0.01	9.35	0.51	11.41	1.12	9.18	0.55	11.21	1.14	0.82	0.04
RCC1212 3	0.95	0.03	8.86	0.46	12.69	1.07	8.39	0.69	12.02	1.39	0.70	0.02
RCC1212 4	0.87	0.02	6.85	1.44	11.97	0.85	5.92	1.15	10.37	0.93	0.58	0.14

RCC1216 remained unaltered over the CO_2 range tested, while RCC1256 and RCC1238 both displayed an optimum curve with the highest POC production at $\sim$590 μatm CO_2 and $\sim$680 μatm CO_2, respectively (Fig. 3, Tables 2 and 3). Both strains (RCC1256 and RCC1238) showed a decrease in POC production at approx. 920 μatm CO_2 (Fig. 3, Tables 2 and 3). The PIC/POC ratio of RCC1216 and RCC1212 decreased by ca. 30% with increasing CO_2, in a linear fashion (Fig. 4). The PIC/POC ratio of RCC1256 varied little over the CO_2 range tested. Apart from a minimum at ca. 700 μatm CO_2, the same trend was observed in RCC1238 (Fig. 4).

3.2 Varying responses of *E. huxleyi* to changing carbonate chemistry

Under our experimental conditions, differences were found in every measured variable (Tables 2 and 3) for the four tested strains of *E. huxleyi* (RCC1212, RCC1216, RCC1238, RCC1256). Based on our results (Table 3, Figs. 1–4), we distinguish three types of responses represented by the following clones: (1) RCC1212 and RCC1216 (2) RCC1238 (3) RCC1256. Apart from the culture strain, the only other variable in this set of experiments was temperature, with two strains (RCC1216 and RCC1256) grown at 17°C and
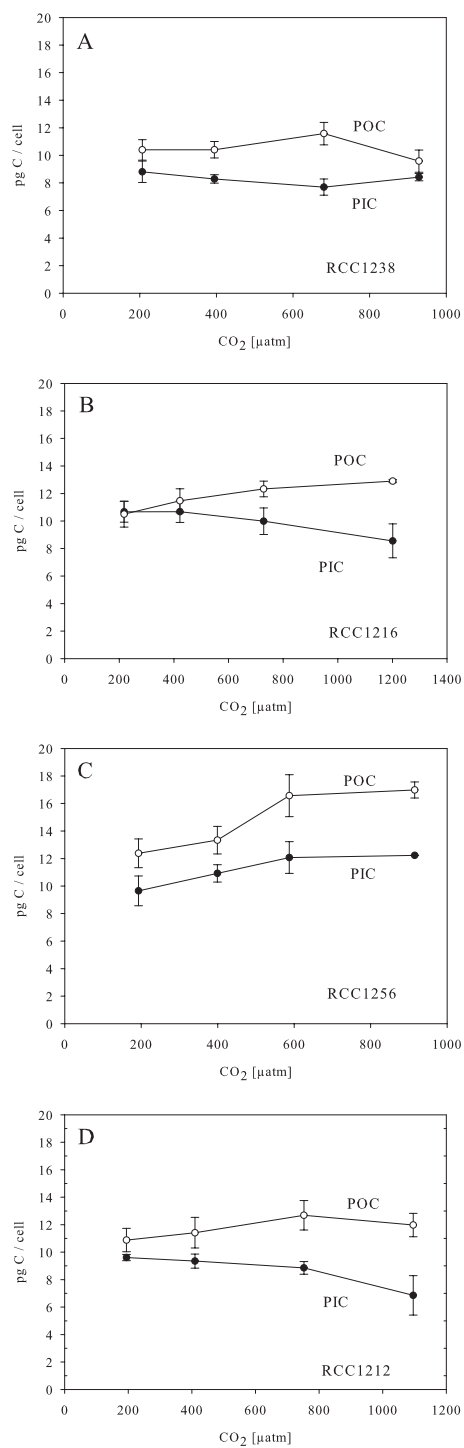
Fig. 2. Cellular PIC and POC content of the four *E. huxleyi* strains versus CO_2 concentration. **(A)** RCC1238, **(B)** RCC1216, **(C)** RCC1256, and **(D)** RCC1212. Open circles represent cellular POC content and closed circles represent cellular PIC content. Errorbars represent standard deviation of three culture experiments.
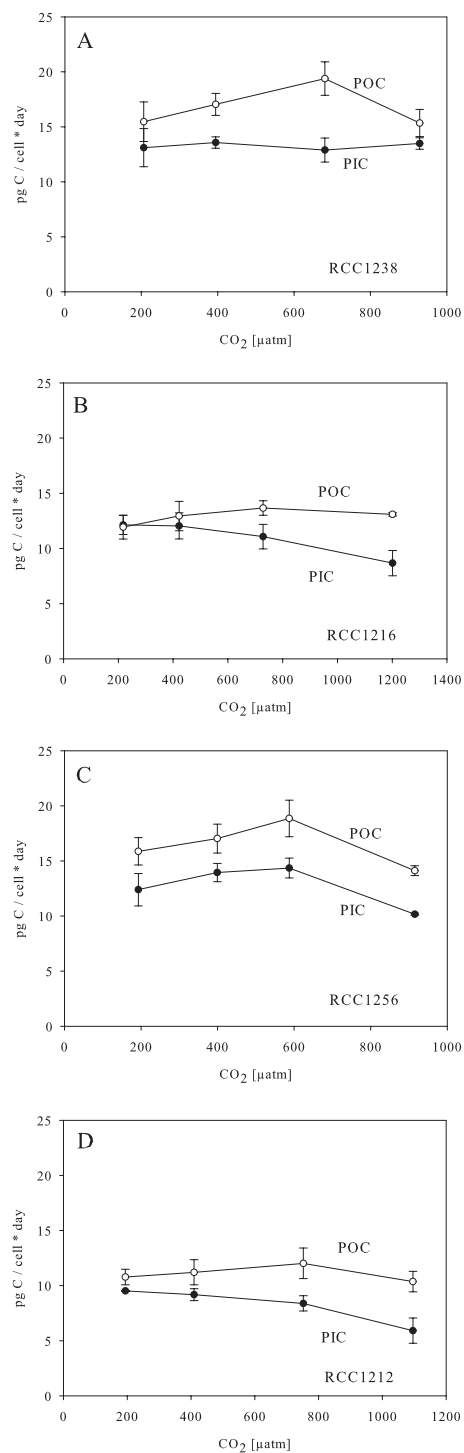
Fig. 3. PIC and POC production of the four *E. huxleyi* strains versus CO_2 concentration. **(A)** RCC1238, **(B)** RCC1216, **(C)** RCC1256, and **(D)** RCC1212. Open circles represent POC production and closed circles represent PIC production. Errorbars represent standard deviation of three culture experiments.
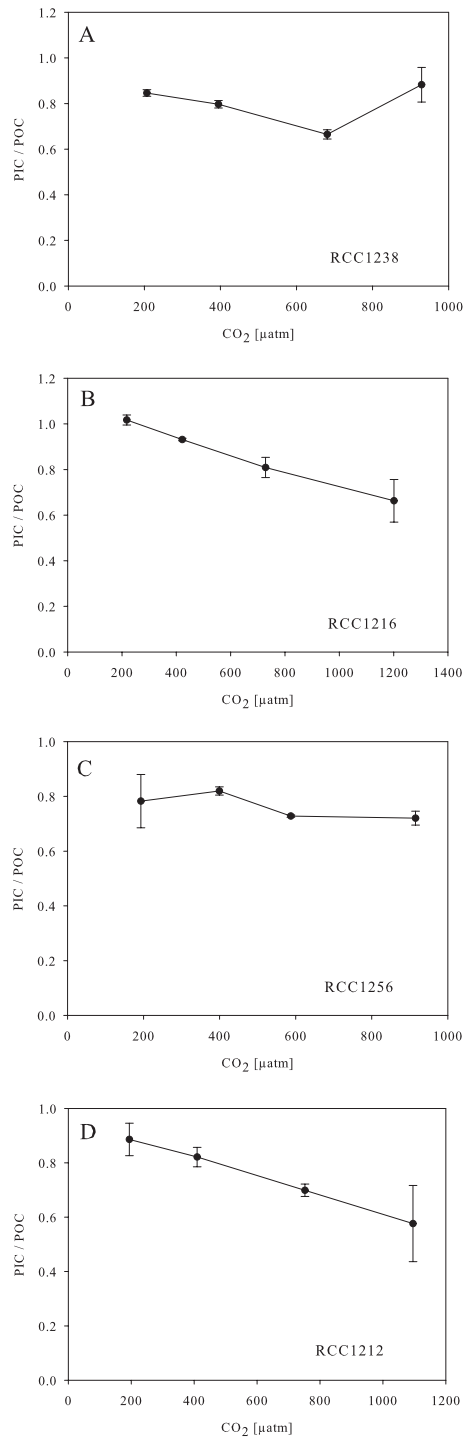
Fig. 4. PIC/POC ratio of the four *E. huxleyi* strains versus CO_2 concentration. **(A)** RCC1238, **(B)** RCC1216, **(C)** RCC1256, and **(D)** RCC1212. Errorbars represent standard deviation of three culture experiments.

two (RCC 1212 and RCC1238) at 20°C. The rationale for this was to compare responses between strains that were in a physiologically similar state in relation to their optimum growth rate. Temperature can influence metabolic processes at different rates and had a single temperature been used, comparison of responses could potentially have been biased by differing relative physiological condition of cells having differing temperature optima for growth. Therefore we grew every strain near its optimum temperature (Fig. 5). In fact, for *E. huxleyi* grown in light-saturating conditions, temperature does not appear to strongly influence PIC and POC production rates, at least over the range used in our experiment: Feng et al. (2008) showed that for a given combination of CO_2 and light conditions, a temperature shift of 4°C (from 20°C to 24°C) did not cause a significant quantitative or relative difference in PIC and POC responses in *E. huxleyi* strain CCMP371 (originating from the Sargasso Sea). The observation that at each temperature in our experiment the responses of the two strains differed leads us to conclude that temperature did not dictate the response pattern observed.

Presently, the scientific debate on the issue of differing responses of *E. huxleyi* in previous studies centres on experimental protocols in general, and on the method of carbonate chemistry manipulation in particular (Iglesias-Rodriguez et al., 2008a; Iglesias-Rodriguez et al., 2008b; Riebesell et al., 2008). In experimental studies on coccolithophores, the carbonate system has been manipulated either by changing TA or by changing DIC. The former is achieved by adding acid or base to the seawater, whereas the latter is achieved by bubbling a batch of seawater with CO_2-enriched/CO_2-depleted air. In the case of a DIC change, every parameter of the carbonate system apart from TA is changed; in the case of a TA change, every parameter of the carbonate system apart from DIC is changed (in a closed system). In the first study on *E. huxleyi*, the TA-manipulation method was employed and a decrease of calcification rate with increasing CO_2 concentration reported (Riebesell et al., 2000). In the most recent study, the DIC-manipulation method was employed and an increase of calcification rate with increasing CO_2 concentration was observed (Iglesias-Rodriguez et al., 2008a). The latter authors argued that their results were unlikely to be due to physiological traits of a particular strain of *E. huxleyi*, implying that the difference in response was due to the difference in the method of carbonate chemistry manipulation. If this were so, the results of Feng et al. (2008), who found a decrease of calcification rate with increasing CO_2 concentration even though they used the DIC-manipulation method, could not be explained. Likewise, the response of strains RCC1238 and RCC1256 in our study (Fig. 3, Table 3) would be aberrant. A literature review, which can be found in a paper by Ridgwell et al. (2009), provides more facts adding to the point. It is therefore highly unlikely that the method used to manipulate the carbonate system causes differences in response patterns.
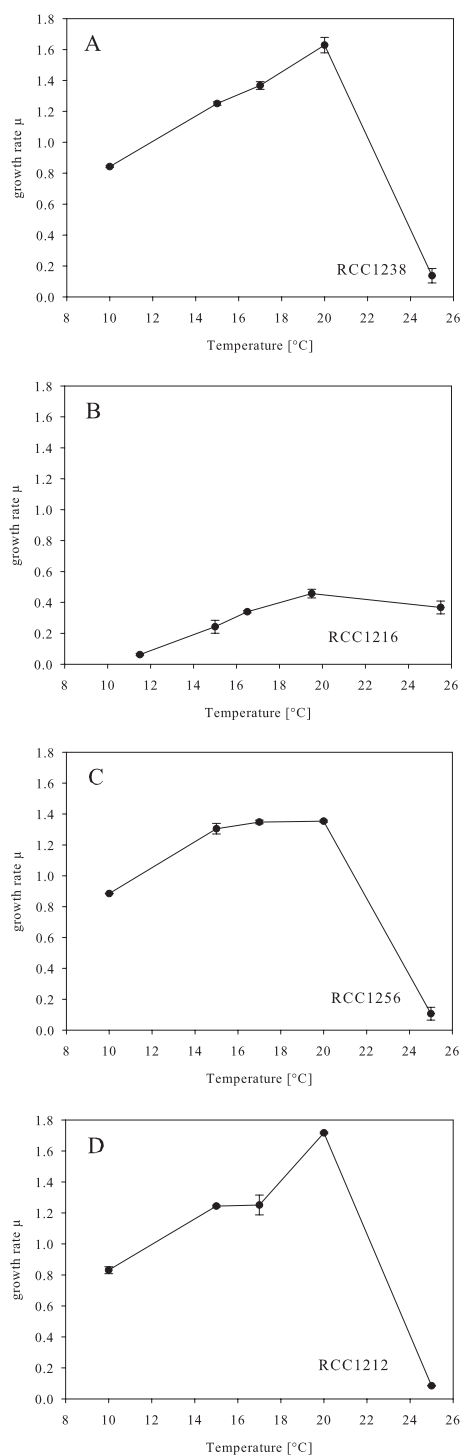
Fig. 5. Growth rates of the four *Emiliania huxleyi* strains versus temperature. **(A)** RCC1238, **(B)** RCC1216, **(C)** RCC1256, and **(D)** RCC1212. Errorbars represent standard deviation of three culture experiments.

In fact, it has recently been shown that the method of carbonate chemistry manipulation does not affect the response pattern of another *E. huxleyi* clone (Shi et al., 2009).

We therefore conclude that the sensitivity of different strains of *E. huxleyi* to changes in carbonate chemistry differs substantially. This becomes especially obvious when comparing the response patterns in detail, i.e. looking not only at calcification rate. In addition to calcification rate, the POC production, PIC and POC content, and growth rates of the different strains also showed different trends over the range of carbonate chemistry tested. We propose that these strain-specific differences can explain the apparent contradictions in the literature, because in every previous study a different strain was used (Feng et al., 2008; Iglesias-Rodriguez et al., 2008a; Riebesell et al., 2000, this study; see Table 1).

3.3 The origin of strain-specific responses

Considerable phenotypic variation is known between isolates classified as *E. huxleyi*. In this respect, focus has centred on morphological variation of coccoliths, reflecting the morphological tradition in coccolithophore taxonomy. *E. huxleyi* is currently separated into five morphotypes, where types A and B are the best characterized and most widely recognized (Young and Westbroek, 1991; Young et al., 2003). In the search for a genetic basis for this phenotypic variation, studies involving multiple *E. huxleyi* culture clones using molecular techniques such as RAPD and microsatellites have shown ample evidence of interclonal genotypic variation within the *E. huxleyi* morpho-species (Medlin et al., 1996, Iglesias-Rodriguez et al., 2006). One study has correlated phenotypic and genotypic variation, identifying a putative genetic marker for distinguishing *E. huxleyi* morphotypes (Schroeder et al., 2005). Pseudo-cryptic species have been documented in several coccolithophore taxa, with molecular data supporting earlier morphological and geological evidence (Sáez et al., 2003), but for *E. huxleyi* the question of whether different phenotypes or genotypes represent reproductively isolated species or interbreeding populations within a species complex remains to be resolved.

In contrast to studies on morphology and genetics, the majority of information on the physiology of *E. huxleyi* results from studies on single culture clones (see review of Paasche, 2002), in some cases demonstrating phenotypic plasticity, i.e. a phenotypic change in response to change in an environmental parameter. Multi-clone surveys of, for example, alkenone content (Conte et al., 1998) in *E. huxleyi* have been conducted, demonstrating variation between clones, which presumably has a genetic basis. Studies demonstrating phenotypic plasticity within clones, phenotypic variation between clones, and variability in phenotypic plasticity between clones (i.e. variable response patterns), as revealed in our study, are rarer.

Strain-specific responses in growth rate of *E. huxleyi* to temperature and salinity changes were described more than two decades ago, when it was found that strains isolated from geographically separated water masses within the western North Atlantic exhibited different responses (Brand, 1982; Brand, 1984). Coastal strains generally differed physiologically from oceanic strains (Brand, 1982, 1984). On the one hand, the two "coastal" (i.e. from within 10 km of the shore) strains used here (RCC1256 and RCC1238) indeed differed from the two 'oceanic' strains (RCC1212 and RCC1216). On the other hand, the two coastal strains (RCC1256 and RCC1238) did not behave similarly at all. Therefore, the distinction coastal / oceanic is not sufficient to explain strain-specific responses.

Surface seawater CO_2 concentrations at the locations of sampling for strain isolation do not provide a hint as to a reason for the differences in response pattern. The CO_2 concentration in the Tasman Sea (RCC1216), off Japan (RCC1238), and off South Africa (RCC1212) is approximately 320 μatm and does not vary greatly over the course of the year (Takahashi et al., 2002). South of Iceland (RCC1256), CO_2 concentration ranges from ca. 270 μatm in August to ca. 360 μatm in February (Takahashi et al., 2002). If ambient CO_2 concentration was the parameter determining the response pattern, all strains except RCC1256 would have been expected to have responded similarly, which was not the case.

The morphotype seems to have no influence on the response pattern. The two strains that exhibited similar responses belong to two different morphotypes, RCC1216 being a type R and RCC1212 being a type B, and the two coastal strains (RCC1238 and RCC1256), which differed in response from each other as well as from the oceanic strains, belong to the same morphotype, namely type A. Interestingly the two type A strains (RCC1238 and RCC1256) exhibit the weakest (RCC1238) and the strongest (RCC1256) responses. The intermediate responses are displayed by the type R (RCC1216) and type B (RCC1212). The type B morphotype is described as the more delicate form (compared to type A and type R) which is often assumed to be more vulnerable to acidification. Our data show that this is not the case. We think it more plausible to assume that the susceptibility to acidification related effects is connected with some physiological process, for instance a transmembrane transport of ions.

Since the strains used in the present study were isolated from samples from locations separated by at least a few thousand kilometers (Table 1), it appears reasonable to regard them at least as different populations with specific genetic features and potentially as genetically isolated cryptic or pseudo-cryptic species. RCC1256 (Iceland) and RCC1238 (Japan) showed two types of responses, neither of which resembled the response of RCC1212 (South Africa) and RCC1216 (New Zealand). The two southern-hemisphere strains, RCC1212 (South Africa) and RCC1216

(New Zealand) displayed very similar responses (see Table 3 for overview) and they might be regarded as being connected by the Antarctic circumpolar current, which could possibly allow for genetic exchange between the populations. As a general caveat, we note that experiments on many more strains would be needed to consolidate the conclusion that variability in the response of *E. huxleyi* to changing carbonate chemistry does not clearly correlate with biogeographic origin or morphotype of the strain.

We propose that the observed differences in response of *E. huxleyi* isolates to changing carbonate chemistry have a genetic basis. At present, it is impossible to speculate as to whether this could be due to, for example, gene differentiation, allelic sensitivity, or gene regulation effects (see Via et al., 1995). Relatively little is known about the physiological mechanisms, let alone the genetic basis, of carbon acquisition and processes involved in calcification in coccolithophores. A better understanding of these aspects would undoubtedly shed light on the variability in the response of coccolithophores (and other calcifiers) to ocean acidification.

3.4 Implications for model studies and the fate of coccolithophores

Calcification shifts the carbonate system towards higher CO_2 concentrations. Diminished calcification, and a decreased PIC/POC ratio, in the future would, therefore, lead to a negative feedback on atmospheric CO_2 concentration (Zondervan et al., 2001). Prediction of the strength of the CO_2-calcification feedback is impaired by species-specific responses to acidification in combination with the unresolved question of which calcifier is the most prolific (Ridgwell et al., 2007). If our results for *E. huxleyi* are representative for coccolithophores in general, or even for other calcifying groups such as the foraminifera, it would be unrealistic to define a representative response as an input for an Earth system model. Since this is an important and widely discussed issue, further studies addressing strain-specific effects are clearly warranted.

Such studies are also desirable for shedding light on the questions of if and how coccolithophores could cope with a future acidified ocean. Judging from culture studies only, it can be inferred that some strains will suffer from reduced calcification, e.g. *Gephyrocapsa oceanica* PC71 (Riebesell et al., 2000), some *E. huxleyi* strains (Table 3), and *Calcidiscus leptoporus* AC365 (=RCC1135) (Langer et al., 2006), while some will be unaffected, e.g. *E. huxleyi* RCC1238 (Table 3) and *Coccolithus braarudii* AC400 (=RCC1200) (Langer et al., 2006). Given that calcification actually is beneficial for coccolithophores (the function of calcification is still unknown), shifts in dominance between species and/or between clones within a species might therefore be expected.

However, short-term culture studies alone are not a sufficient basis from which conclusions regarding the fate of coccolithophores in the future can be drawn, because the

possibility of adaptation is not taken into account. Combining results from culture experiments with data from the sedimentary archive, it was hypothesized that *C. leptoporus* is in principle able to adapt to changing carbonate chemistry of seawater (Langer et al., 2006). The authors speculated that the population might be genetically diverse and that strain-specific responses to carbonate chemistry changes would lead to a high degree of adaptive potential of the species. The results for *E. huxleyi* presented here point to the possibility that this morphospecies is heterogeneous with regard to responses to changing carbonate chemistry. This highlights the need to consider adaptation when trying to predict the performance of coccolithophores in the future.

Edited by: S. Pantoja

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