

Determinants of the PIC:POC response in the coccolithophore *Emiliana huxleyi* under future ocean acidification scenarios

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Abstract

We use a meta-analysis to quantify the response of *Emiliana huxleyi* particulate inorganic carbon (PIC) to particulate organic carbon (POC) ratio under different laboratory conditions and changes in carbonate chemistry. There is an overarching trend of decreasing PIC:POC across all ecotypes irrespective of the strain, isolation date, isolation location, and method of acidification. The variability about this overall trend is explained by the different nutrient and light regimes used in each experiment, but there is no evidence for a strain-specific response that might be expected if strains had adapted to the average environmental conditions at the locations from which the strain was isolated; indeed, each strain shows a comparably broad physiological window. We propose that *E. huxleyi* PIC:POC exhibits a plastic response to carbonate conditions that can be predicted by the seawater concentrations of aqueous CO₂, total alkalinity, and phosphate conditions. This relationship now requires field validation as well as longer-term studies of *E. huxleyi* response to variable environmental conditions.

The increasing carbon dioxide (CO₂) concentration in the atmosphere is leading to large-scale changes in the terrestrial and marine environments. In the oceans, sea surface temperatures are increasing (Ramanathan and Feng 2008), and changes are occurring to the ocean chemistry. The oceans are a natural sink for CO₂, absorbing about 25% of CO₂ emissions (Sabine et al. 2004); however, CO₂ reacts with water to form carbonic acid, which changes the natural balance of carbon in the oceans and leads to a decrease in pH and carbonate ions in a process recently termed ocean acidification (OA; Caldeira and Wickett 2003). The predicted CO₂ emissions for the year 2100 using a business-as-usual scenario will result in a decrease in pH from the current global average of 8.1 to a global average of 7.7 (IPCC 2007), whereas the global average preindustrial pH level was about 8.2.

A variety of responses have been characterized in marine organisms exposed to changing pH, even when the same function (e.g., calcification) was investigated (Ries et al. 2009; Hendriks et al. 2010). Although meta-analyses of the effects of OA on invertebrates have been conducted, the high level of variability prevents general trends from being formulated even with respect to one species or one process. Calcification has been the focus of much research into the effects of OA on marine organisms because of the resulting decrease in carbonate ion concentration ([CO₃²⁻]). Some marine organisms produce calcium carbonate (CaCO₃) shells and liths (armor plates), which will become more costly for organisms to maintain as [CO₃²⁻] decreases (Pörtner 2008; Findlay et al. in press). Although calcification has been studied within several phyla (including

Cnidaria, Mollusca, and Crustacea), only in the coccolithophore *Emiliana huxleyi* has there been a relatively extensive account of the calcification response in geographically separated strains of a single species (Langer et al. 2009; Ridgwell et al. 2009), thus making it an amenable biological model for the investigation of OA effects on a large geographical scale, an approach that can help to verify whether general response trends can be found and help to refine models.

E. huxleyi is one of the most ubiquitous calcareous phytoplankton species (Berge 1962; Holligan et al. 1993), and can tolerate a broad range of temperatures (1–41°C) and salinities (15–45) (Brown and Yoder 1994), making it an easy species to culture, and for this reason it is arguably the most well studied of all the phytoplankton (Iglesias-Rodríguez et al. 2006). Over recent decades, researchers have examined the responses of perhaps 10 different strains of *E. huxleyi* living at different geographic locations characterized by different environmental conditions. Indeed, Iglesias-Rodríguez et al. (2006) suggested that a high level of genetic diversity exists in populations of *E. huxleyi*. In addition, Ridgwell et al. (2009) argued that *E. huxleyi* should be regarded as a diverse assemblage of genotypes with highly variable calcification characteristics and ecological adaptations, and Langer et al. (2009) reported that the four *E. huxleyi* strains they analyzed differed substantially in their level of sensitivity to OA, this likely having a genetic basis. This high variability makes future projections of the response of *E. huxleyi* across the global ocean complicated but nevertheless important.

There are several reasons to attempt to make sense of the response of coccolithophores to OA, as they play a major role in biogeochemical cycles. They contribute significantly to the carbon cycle, both through photosynthesis (taking up CO₂ to form organic carbon compounds) and calcification of their liths (removing carbon from seawater to form inorganic carbon compounds) (Sikes and Fabry

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1994). The sinking of their liths once they have died contributes to the biological pump of capturing carbon from the surface ocean and sinking down to the deep ocean (Westbroek et al. 1993). However, calcification is a net source of CO₂, with deposition of 1 mol of calcium carbonate releasing 0.6 mol CO₂ into seawater (Gattuso et al. 1995). The particulate organic carbon (POC) content, or production, of coccolithophores is often used as a measure of net autotrophic productivity, whereas particulate inorganic carbon (PIC) content or production is used as a measure of net calcification. PIC and POC are therefore consequential quantities, or traits, that are produced as a result of physiological processes (photosynthesis, respiration, and calcification). Environmental factors can influence the physiological functioning of coccolithophores, resulting in a change in production of PIC and POC. The ratio between PIC and POC gives a measure of the export ratio and is an important term for modeling carbon cycling in the oceans (Ridgwell et al. 2009).

The environmental conditions thought to allow *E. huxleyi* to form large blooms include high light, as they bloom only in highly stratified upper surface waters, and low nitrate conditions, with phosphate levels being more important (Tyrrell and Taylor 1996); and, more recently, [CO₃²⁻] has been suggested as a contributing factor: Merico et al. (2006) demonstrated that coccolithophore blooms followed the spring bloom in the Barents Sea when the calcite saturation state (Ω_{calcite}) was high, Findlay et al. (2008) showed agreement with data and modeling from the Norwegian Sea, and Tyrrell et al. (2008) argued that *E. huxleyi* was absent from the Baltic Sea but present in the Black Sea because of low wintertime Ω_{calcite} . There is also evidence from the natural environment that less heavily calcified ecotypes are dominant at lower saturation states (Cubillos et al. 2007), and Beaufort et al. (2008) inferred that size and weight of liths of coccolithophores in the Pacific Ocean correlated with the carbonate system parameters, especially alkalinity. Triantaphyllou et al. (2010) additionally showed a seasonal variation in size and calcification of coccoliths in the Aegean Sea, which correlated with temperature and productivity but also potentially with changes in the carbonate system, although exactly which parameters ([HCO₃⁻], [CO₃²⁻], alkalinity, dissolved inorganic carbon [DIC], etc.) affected the growth of the coccolithophores obviously could not be resolved from these field studies. Tyrrell and Taylor (1995) showed using data and modeling of the North Atlantic that *E. huxleyi* was not associated with low CO₂ conditions, as might be expected if it had evolved to be a successful competitor in a low CO₂ environment.

Variation in laboratory methodology or experimental design might explain some of the variability observed in *E. huxleyi* response to a change in carbonate chemistry. However, the environment from which the organism was originally collected is also likely to shape an organism's ability to respond to changes in environmental conditions. Many taxa and populations show local adaptation and or acclimatization to a suite of environmental conditions that another taxon, population, or strain from a different location would be unable to tolerate or would react

differently to (Stillman 2003; Calosi et al. 2008). Hence, the existing level of adaptation may lead to taxa and strain-specific responses to OA: *see*, for example, differences in the responses to OA in genetically distinctive clonal lines of the bryozoan *Celleporella hyalina* (Pistevos et al. 2011). Thus, the level of physiological adaptation of different functions (i.e., photosynthesis, calcification) in different strains of *E. huxleyi* could contribute to the changes observed in the PIC:POC ratio. It is important in attempting to constrain the consequences of OA to consider strain- and or population-level adaptation to specific environmental conditions (Dobzhansky 1973).

In this study, using a meta-analysis approach, we quantitatively characterize for the first time the relationship between the response in PIC:POC of genetically different strains of the coccolithophore *E. huxleyi* collected at different locations and the pH and carbonate system parameters (and other environmental variables), while controlling for experimental conditions. Further, we provide a test for the hypothesis proposed by Ridgwell et al. (2009) of the possible existence of an analogy between Eppley curve (Eppley 1972) encapsulation of temperature-growth behavior and the CO₂-calcification system. Finally, on the basis of our results we propose a model to predict *E. huxleyi* response (PIC:POC) to different environmental conditions, and calculate the expected PIC:POC value in current and future environmental scenarios over the locations where the strains were collected.

Methods

Meta-analysis—We chose to study the ratio between PIC and POC (PIC:POC), a measure often used as a carbon export ratio, of the coccolithophore *E. huxleyi* exposed to different pH conditions. Although PIC and POC are routinely measured in CO₂ experiments, they are sometimes provided as quota values and other times reported as production rates. This makes it difficult to compare these values across studies. PIC:POC, however, provides a standard unit-less value that can be compared across treatments and experiments. CO₂ experiments conducted on *E. huxleyi* were chosen from a literature search of all published data. Studies were excluded if there were no data on PIC or POC. Studies that used either acid-base or CO₂ bubbling as a mechanism for altering the carbonate system were included in the analysis, and mechanism was considered as a factor. Data were gathered on the experimental conditions (nitrate, phosphate, light level, temperature, and salinity) as well as strain name, date and location of isolation (using latitudinal and longitudinal coordinates), and morphotype, if the information was available (Table 1). To provide an understanding of ambient environmental conditions experienced at the location of isolation of each strain, data were obtained from the interpolated Carbon Dioxide Information Analysis Centre (CDIAC) database developed by Goyet et al. (2000). The data gathered were DIC, total alkalinity (TA), temperature, and salinity, as well as calculated Ω_{calcite} , pH, pCO₂, and [CO₃²⁻] at the nearest latitude and longitude using Ocean Data View (version 4) (Table 2). In total, nine

Table 1. Conditions in each experiment, including the strain of *E. huxleyi*, the experimental conditions (Lab., laboratory; Meso., mesocosm), the method of acidification (CO₂, CO₂ bubbling; Acid-base, acid-base manipulation), the isolation date of the strain, the location that the strain was isolated (given to nearest latitude [Lat.] and longitude [Long.]), the light levels, the phosphate and nitrate concentrations, the temperature (Temp), the salinity, and the pH total scale (pH_T) range. Expt., experiment; nr, not reported.

Expt. No.	Strain No.	Strain name	Expt.	Method	Isolation date	Lat.	Long.	Light ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Phosphate ($\mu\text{mol L}^{-1}$)	Nitrate ($\mu\text{mol L}^{-1}$)	Temp (°C)	Salinity	pH _T range	Reference
1	1	RCC1256	Lab.	Acid-base	1999	63.5°N	20.5°W	400	6.25	100	17	32	8.33–7.69	Langer et al. 2009
2	2	Natural population	Meso.	CO ₂	2001	60.5°N	4.5°E	150	0.5	17	10	31.3	8.32–7.83	Delille et al. 2005
3	3	PML B92/11A	Lab.	Acid-base	1992	60.5°N	5.5°W	150	6.25	100	15	31	8.45–7.80	Riebesell et al. 2000
4	3	PML B92/11A	Lab.	Acid-base	1992	60.5°N	5.5°W	150	6.25	100	15	31	8.39–7.81	Zondervan et al. 2001
5	3	PML B92/11A	Lab.	Acid-base	1992	60.5°N	5.5°W	150	6.25	100	15	31	8.16–7.77	Zondervan et al. 2001
6	4	TW1	Lab.	CO ₂	2001	39.5°N	1.5°E	570	5	15	17	nr	8.02–7.84	Sciandra et al. 2003
7	5	RCC1212	Lab.	Acid-base	2000	34.5°S	17.5°E	400	6.25	100	20	32	8.34–7.64	Langer et al. 2009
8	6	NZEH	Lab.	CO ₂	1992	47.5°S	168.5°E	150	6.25	100	19	34	8.15–7.79	Iglesias-Rodríguez et al. 2006
9	6	NZEH	Lab.	CO ₂	1992	47.5°S	168.5°E	150	6.25	100	20	35	8.10–7.80	Shi et al. 2009
10	6	NZEH	Lab.	Acid-base	1992	47.5°S	168.5°E	150	6.25	100	20	35	8.10–7.80	Shi et al. 2009
11	7	RCC1216	Lab.	Acid-base	1998	41.5°S	170.5°E	400	6.25	100	17	32	8.30–7.58	Langer et al. 2009
12	8	RCC1238	Lab.	Acid-base	2005	34.5°N	140.5°E	400	6.25	100	20	32	8.32–7.69	Langer et al. 2009
13	2	Bergen	Lab.	Acid-base	2005	60.5°N	5.5°W	140	3.6	88	16	33	8.21–7.60	Müller et al. 2010
14	9	AC481	Lab.	CO ₂	2003	49.5°N	2.5°W	150	1	32	13	35.6	8.25–7.80	De Bodt et al. 2010
15	9	AC481	Lab.	CO ₂	2003	49.5°N	2.5°W	150	1	32	18	35.6	7.95–7.80	De Bodt et al. 2010

Table 2. Conditions at each location (latitude and longitude) of the carbonate parameters taken from the CDIAC interpolated dataset available at http://odv.awi.de/en/data/ocean/global_alkamity_tco2/ (Goyet et al. 2000).

Expt. No.	Strain No.	Lat.	Long.	Temp (°C)	Salinity	pH _T	pCO ₂	Ω _{calcite}	CO _{2(aq)} (μmol kg ⁻¹)	CO _{3²⁻} (μmol kg ⁻¹)	HCO _{3⁻} (μmol kg ⁻¹)	DIC (μmol kg ⁻¹)	TA (μmol kg ⁻¹)
1	1	63.5°N	20.5°W	8.05	35.13	8.171	284.5	3.87	13.3	162.6	1916.0	2091.9	2322.3
2, 13	2	60.5°N	4.5°E	8.46	33.27	7.901	562.2	2.12	26.2	88.1	2002.7	2117.0	2223.6
3, 4, 5	3	60.5°N	5.5°W	9.81	35.29	8.090	360.4	3.47	15.8	145.9	1956.6	2118.3	2320.8
6	4	39.5°N	1.5°E	18.40	37.42	8.129	323.3	5.31	10.1	226.5	1857.3	2093.9	2416.1
7	5	34.5°S	17.5°E	18.94	35.40	8.115	343.4	4.74	11.3	199.1	1812.1	2022.5	2305.1
8, 9, 10	6	47.5°S	168.5°E	11.86	34.46	8.122	336.1	3.71	29.8	155.3	1861.6	2046.7	2265.5
11	7	41.5°S	170.5°E	15.99	34.99	8.152	313.0	4.52	11.2	189.8	1812.3	2013.3	2284.0
12	8	34.5°S	140.5°E	21.38	34.33	7.983	422.6	3.82	13.4	158.6	1715.0	1887.0	2111.3
14, 15	9	49.5°N	2.5°W	12.97	34.89	8.023	414.0	3.33	16.5	139.8	1914.2	2070.5	2262.9

studies were included (totaling 15 experiments), comprising nine strains collected in nine localities (see Table 1).

As much information as possible of the carbonate system was gathered for each experiment; where data were missing from the literature, values were calculated using CO2sys (Pierrot et al. 2006) using the dissociation constants of Mehrbach et al. (1973) as refitted by Dickson and Millero (1987). Initial conditions were taken so as to represent the conditions at the start of the growth phase of the experiments, which could therefore be expected to represent conditions experienced at the beginning of a coccolithophore bloom in the real world. Additionally, pH was converted, where necessary, so that all pH values reported here are on the total scale.

An assessment was carried out to investigate if there were any overall correlations between PIC:POC and each of the experimental conditions (correlation coefficient rho is provided together with the degrees of freedom and probability statistic). Further analysis was carried out using PRIMER6 (Anderson et al. 2008) to correlate the pattern of PIC:POC across all the experiments with their associated experimental conditions. The Biological-Environmental Stepwise (BEST) routine in PRIMER6 was used on normalized experimental data with Euclidian distance as the resemblance measure. PRIMER6 uses nonparametric measures and permutations to produce test statistics based on distance matrices (resemblance measures; Clarke 1993). Here we use Euclidian distance as an appropriate measure for environmental data to produce the distance matrix. The experimental data were normalized to remove bias in the distance matrix, as the variables are not on comparable measurement scales (Clarke and Gorley 2006). The rationale for the BEST procedure is to find the “best” match between the multivariate among-sample patterns of an assemblage (in this case the PIC:POC values at each pH condition for each strain of *E. huxleyi*) and that from environment variables associated with those samples (in this case the experimental conditions). The output from this routine gives the best correlation (rho) from all the combinations of the experimental variables. A global test statistic was generated by carrying out permutation tests with 999 permutations (Clarke 1993).

To assess if there were differences in the vulnerability of strains from different locations, and therefore different field environment conditions, to changes in pH, an effect size was calculated using effect size correlation, phi (Φ), for each experiment (Sheskin 1997). Φ is related to the correlation coefficient and Cohen’s *d* (Cohen 1988) and estimates the extent of the relationship between two variables. We used effect size correlation to measure the correlation of PIC:POC response to changing pH rather than selecting PIC:POC values at two specific pH levels and using an effect size measure of two independent groups. Φ is calculated by finding the square root of the chi-square probability value (with 1 df), divided by the sample size *N*:

$$\Phi = \sqrt{\frac{\chi^2}{N}} \quad (1)$$

Table 3. Analysis of the five best linear models with different number of predictor variables.

Predictor variable	Residual sum of squares	AIC_c	ΔAIC_c	R^2 (%)	R^2_{adj} (%)
$CO_{2aq}^*+TA^*$	0.57199	-211.539	0.00	45.5	43.1
$CO_{2aq}^*+TA^*+phosphate$	0.54722	-211.332	0.21	47.8	44.3
$CO_{2aq}^{**}+TA^*+phosphate+pH_T$	0.54064	-209.439	2.10	48.4	43.8
$CO_{2aq}^*+TA+phosphate+CO_3^{2-} +\Omega_{calcite}$	0.52552	-208.224	3.31	49.9	44.1
CO_{2aq}^*	0.64511	-207.917	3.62	38.5	37.2

* $p < 0.05$; ** $p = 0.0685$.

The PIC:POC value (experimental result) at each pH level was compared to the average PIC:POC value in each experiment, based on the null hypothesis that there is no change in PIC:POC with changing pH. A meta-analysis was used to assess whether the strain, absolute latitudinal position, environmental conditions (data obtained from Goyet et al. 2000: temperature, salinity, and carbonate system parameters), morphotype, collection date, and method of acidification correlated with the effect size.

Model development—The results from the BEST analysis provided combinations of experimental variables (carbonate parameters, nutrients, temperature, salinity, light, etc.) that could be used to generate a general linear model, which could then be used to predict PIC:POC. In Minitab (version 15) a Best subsets regression was run using the chosen variables and regression analysis was carried out on models using sequentially more predictor variables. The models were assessed using the R^2_{adj} , which gives the percentage of response variable variation that is explained by its relationship with one or more predictor variables (R^2), adjusted for the number of predictors in the model, and the ΔAIC_c , which is the difference in Akaike Information Criterion (AIC), corrected for sample size (AIC_c), between models when compared to the PIC:POC data. The preferred model has the lowest AIC_c , and the ΔAIC_c thus allows a comparison between this preferred model and other models. The model with best predictive capability was then used to predict PIC:POC for real conditions taken from the localities of where the strains were collected.

Results

PIC:POC response to experimental conditions—There was no significant relationship between PIC:POC and any of experimental temperature, salinity, nitrate, phosphate, light conditions, acidification method, absolute latitude of original strain location, morphotype, isolation date, DIC concentration, or bicarbonate ion concentration. There was a significant positive correlation between PIC:POC and experimental TA (df = 50, $r = 0.5704$, $p = 0.001$), $[CO_3^{2-}]$ (df = 50, $r = 0.6435$, $p = 0.001$), $\Omega_{calcite}$ (df = 50, $r = 0.6545$, $p = 0.001$), and pH total scale (pH_T) (df = 50, $r = 0.6052$, $p = 0.001$) (Table 3; Fig. 1). Further, there was a significant negative correlation between PIC:POC and pCO_2 (df = 50, $r = 0.5962$, $p = 0.001$) and between PIC:POC and aqueous CO_2 concentration (CO_{2aq}) (df = 50, $r = 0.6203$, $p = 0.001$) (Fig. 1). All these significant

correlations were found to be linear except for the correlations of PIC:POC with $\Omega_{calcite}$ and PIC:POC with $[CO_3^{2-}]$, which both had logarithmic relationships (Fig. 1).

The PRIMER analysis confirmed these individual correlations, demonstrating that the best combination of factors that correlates most strongly with the pattern of PIC:POC was TA, CO_{2aq} , and phosphate (best correlation $\rho = 0.345$, permutations = 999, $p = 0.001$). The formation of a distance matrix in PRIMER highlights the outliers with respect to PIC:POC response and can be illustrated by plotting the points on a multidimensional scaling (MDS) plot (Fig. 2). The outliers correspond to experiments 2 and 12. The most distant point (Fig. 2), corresponding to experiment 12, has the lowest PIC:POC value correlating with the highest CO_2 conditions ($42.1 \mu mol kg^{-1}$), and although it lies further away from other points, it falls along the CO_2 trajectory. The other outlying point (experiment 2) lies to the left and slightly above the other points, demonstrating that, although the main response was due to the CO_2 effect on PIC:POC, there was also some influence of temperature (experiment 2 had the lowest temperature, conducted at $10^\circ C$).

Overall there was no significant difference in the response of PIC:POC with the different strains or methods of acidification (Fig. 2).

PIC:POC effect size—There was no significant correlation between the PIC:POC effect size and any of the environmental conditions (environmental temperature, salinity, or carbonate system parameters), strain, isolation date, or isolation location (absolute latitude) (global test statistic: best correlation $\rho = 0.295$, permutations = 999, $p = 0.176$). Indeed, the only significant correlation between experimental conditions and environmental conditions was between the experimental temperature and the environmental temperature (excluding any studies that purposely elevated the experimental temperatures in order to test the temperature response of coccolithophores), this relationship being positive (df = 10, $r = 0.591$, $p = 0.0430$).

Model results—The seven variables that together produced the BEST PRIMER correlation were TA, phosphate, CO_{2aq} , pCO_2 , CO_3^{2-} , $\Omega_{calcite}$, and pH_T . In Minitab (version 15) analysis was conducted using these seven variables and regression best subsets analysis was carried out on five models using sequentially more predictor variables. The models were assessed using the R^2_{adj} and the AIC_c (Table 3). Based on the AIC_c , the best model was

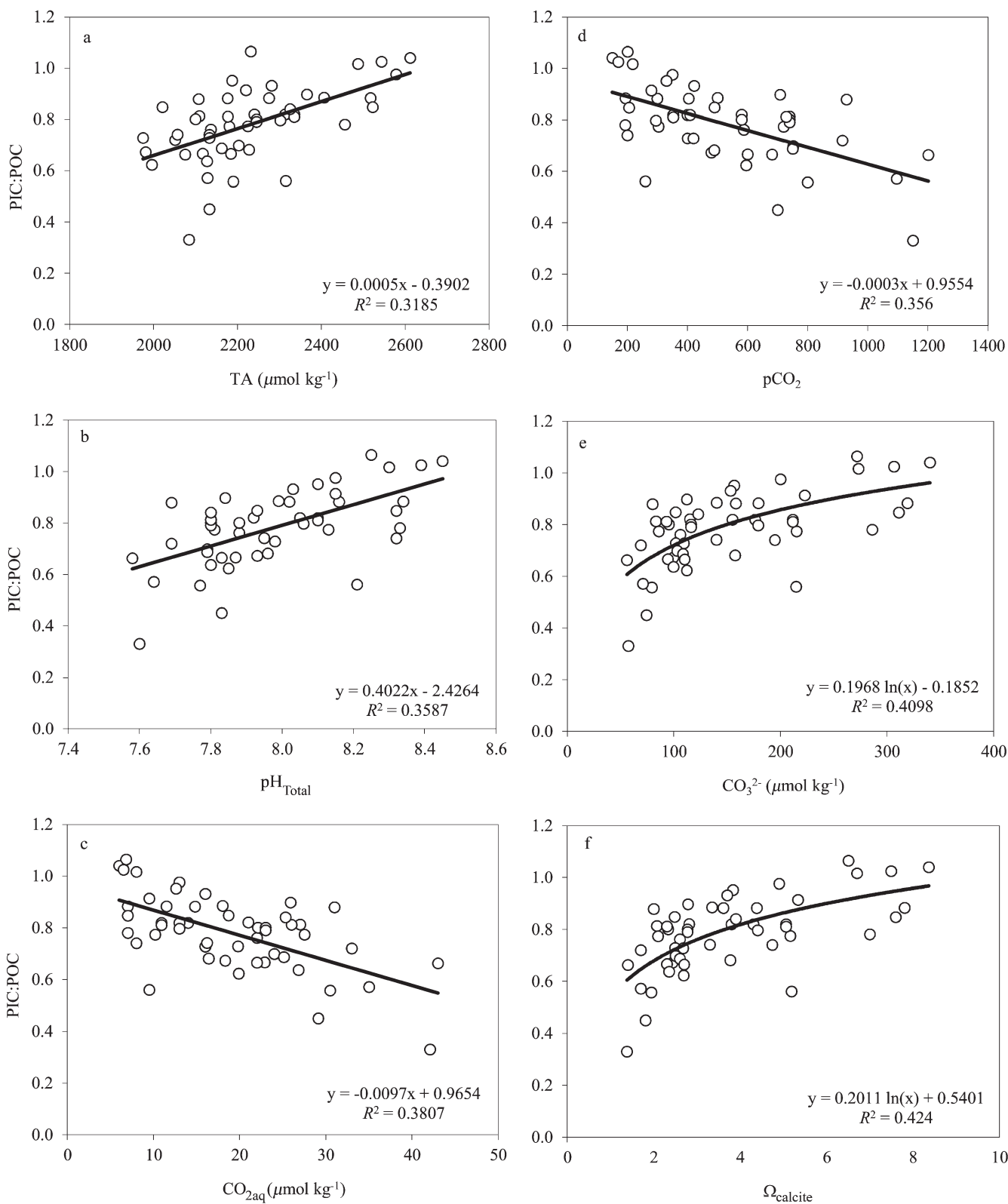


Fig. 1. PIC:POC of all experiments correlated with (a) TA, (b) pH_T , (c) $\text{CO}_{2\text{aq}}$, (d) pCO_2 , (e) $[\text{CO}_3^{2-}]$, and (f) Ω_{calcite} . Open circles represent the data point, and the line is the best fit (R^2 and equation are provided for each figure).

Resemblance: S17 Bray Curtis similarity 2D Stress: 0.01

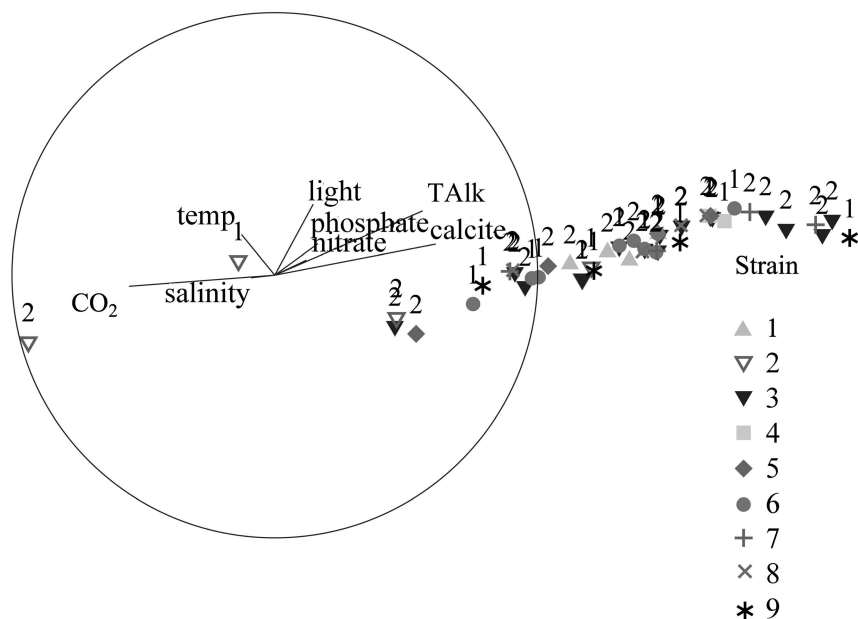


Fig. 2. MDS ordination of PIC:POC from all experiments overlaid with the experimental variables. Symbols (defined in the legend) represent the different strains (strain numbers are given in Table 1), and the numbers above each symbol represent the method of acidification: method 1 = bubbling with CO₂, method 2 = acid or base adjustment. The MDS is overlaid with trajectories representing the influence of the experimental conditions. The majority of the PIC:POC response follows the CO_{2aq} (CO₂) and TA (TALK) lines.

with TA and CO_{2aq}; however, the model using TA, CO_{2aq}, and phosphate had an improved R^2_{adj} by 1.2 and only slightly higher AIC_c ($\Delta AIC_c = 0.21$). By ranking and scoring the models by their respective values of ΔAIC_c and r^2_{adj} and averaging such scores, we show that although there is much similarity between the model AIC_c and r^2_{adj} values, the best model, taking into account both statistics, is the model using TA, CO_{2aq}, and phosphate. Analysis of each variable component within the model shows, however, that only TA and CO_{2aq} are statistically significant contributors to the prediction (Table 3).

The predictive model is therefore

$$\text{PIC : POC} = 0.266 - 0.00178 \times [\text{CO}_{2\text{aq}}] + 0.000264 \times [\text{TA}] + 0.0118 \times [\text{phosphate}] \quad (2)$$

This model underestimates PIC:POC at higher values and overestimates PIC:POC at lower values, where there were fewer data points to constrain the model (Fig. 3a).

The model was then used to predict the PIC:POC value for each geographic location of each strain using the environmental values gathered from the dataset of Goyet et al. (2000). The average PIC:POC under current conditions was 0.803 ($\sigma^2 = 0.0588$), whereas under a future OA scenario, where pH decreases by 0.3 units from ambient conditions (while maintaining constant TA and constant phosphate), the average PIC:POC decreased to 0.717 ($\sigma^2 = 0.0326$) (Fig. 3b). The main predictor is the CO₂ concentration, which has a distributional gradient over the world's oceans determined by a number of factors involving the

water mass properties but also the anthropogenic input (highest in the western Atlantic) as well as upwelling locations and areas of high riverine input.

Discussion

Overarching trends in laboratory experiments—In order to understand and predict future responses of coccolithophores to increased pCO₂, a large number of experiments have recently been performed that have yielded a surprising diversity of results. Explanations for apparently contradictory results have included differences in experimental conditions, strain (genetic adaptation), and CO₂ manipulation techniques. The ability to extrapolate from cultures that have been in laboratory conditions for several decades to natural populations is also often discussed. Ridgwell et al. (2009) summarized qualitatively the different responses and the relationship between acidification method and experimental conditions, concluding that methodological differences in acidification have negligible influence on the results, whereas strain difference may be notable, particularly where strains have been in culture for many years. Ridgwell et al. (2009) showed that no study had yet reported an increased PIC:POC in response to increasing OA, yet PIC:POC is the most useful value for helping estimate the effects of OA on global carbonate production, and for use in models. Here we have shown that although there are variable responses in PIC and POC in the literature across *E. huxleyi* experiments, the response to OA in terms of PIC:POC ratio is surprisingly uniform across experiments.

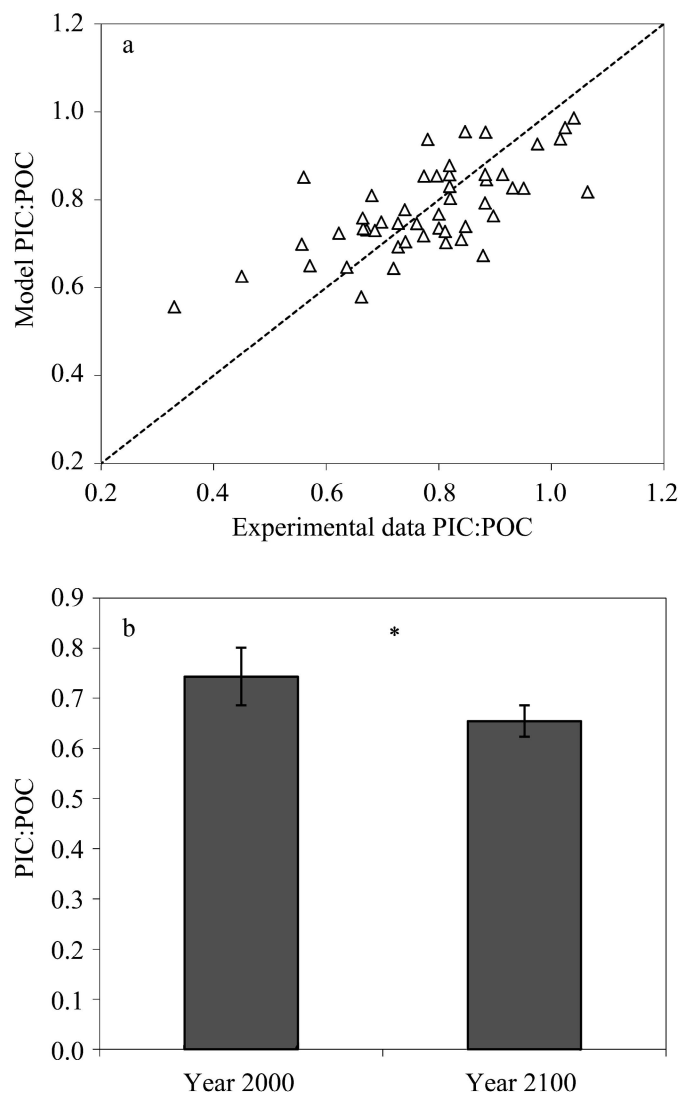


Fig. 3. (a) Experimental values for PIC:POC for each study compared with the predicted PIC:POC values using the model. (b) Model results of PIC:POC in response to changing atmospheric CO_2 concentration from year 2000 value of 0.038 kPa (380 ppm) to year 2100 value of 0.074 kPa (740 ppm). The model is $\text{PIC:POC} = 0.266 - 0.00178 \times [\text{CO}_{2\text{aq}}] + 0.000264 \times [\text{TA}] + 0.0118 \times [\text{phosphate}]$. The asterisk indicates a significant difference ($p < 0.001$).

Out of all the experimental conditions the main drivers for PIC:POC, explaining nearly half the variation, appear to be TA, $\text{CO}_{2\text{aq}}$, and phosphate (Eq. 2). The remaining variability can be accounted for by the differences in light and nutrient conditions used in the experiments. We found that CO_3^{2-} and calcite had logarithmic relationships with PIC:POC, whereas pH, TA, and $\text{CO}_{2\text{aq}}$ had linear relationships, but HCO_3^- did not have any correlation with PIC:POC. This may be because HCO_3^- does not vary significantly over the pH range investigated here. Previous modeling attempts, as highlighted by Ridgwell et al. (2009), have included export ratio (PIC:POC) calculated from $\text{CO}_{2\text{aq}}$ (Heinze et al. 2004), Ω_{calcite} (Gehlen et al. 2007; Ridgwell et al. 2007), or $[\text{CO}_3^{2-}]$ (Hofmann and Schelln-

huber 2009) although these were parameterized by only one or two individual experiments.

Additionally, when looking across all the experimental results, we found no evidence to suggest that the method of acidification and experimental conditions affected the overall trend of declining PIC:POC with increasing CO_2 and decreasing pH. Our analysis thus agrees with previous evaluations that show that method of acidification has little influence on the response in PIC:POC (Ridgwell et al. 2009; Shi et al. 2009). The overall PIC:POC relationship with changes in the carbonate system therefore appears to hold despite differing experimental conditions. This provides a relationship with the carbonate system that can be used in modeling studies, as suggested by Ridgwell et al. (2009) with regards to the idea of building a pH or carbonate system parallel to the Eppley curve of encapsulation for temperature-growth behavior (Eppley 1972). This model will be useful to fine-tune our ability to predict the likely effect of OA on coccolithophore export ratio, assuming that these organisms respond to OA in the time scale of the experiments used in this meta-analysis through plastic responses rather than adaptive responses, which cannot be assessed by these studies. However, more data would help develop this idea, particularly as there is a lack of information from the polar and the tropical regions; despite coccolithophores' having been observed in the high Nordic seas and into the Barents Sea (Smyth et al. 2004) and through the Bering Strait (Merico et al. 2003), PIC:POC values have not yet been obtained from these locations.

We also found that the only environmental condition (temperature, salinity, and carbonate parameters) that correlated between the laboratory and the expected environmental conditions was temperature. This suggests that conditions in the laboratory experiments may not always have been representative of the environmental conditions, particularly in relation to the carbonate system. In addition, as the correlation between laboratory and field temperatures was positive, our results stress the fact that often in the laboratory conditions are maintained to produce maximum growth rates rather than natural levels experienced in the field. This analysis highlights the need to understand the microscale and local dynamics as well as studying global averages. The annual average pH, for example, is unlikely to represent the conditions prevalent during a coccolithophore bloom, which will vary from start to end of the bloom as carbon levels change through photosynthesis, respiration, and calcification (Findlay et al. 2008). Yet all OA experiments to date use global averages for current and future levels of pH or CO_2 . However, there must be some caution with this interpretation, because the environmental data have come from an interpolated global map, whereas the coccolithophores used in experiments may be coastal or even estuarine, and hence salinity and the carbonate parameters will be even more variable in these locations. Additionally, the Goyet et al. (2000) map provides data only for the maximum mixed layer and so misses the seasonality of the carbonate system that could influence coccolithophores on much shorter timescales. This dataset was, however, the most extensive available in terms of geographic coverage for the carbonate system.

It is important to consider the caveats associated with meta-analysis. Meta-analysis provide information only of the data available in the literature; if there are publication biases (i.e., significant results are more likely to be published) then this will skew the overall trends. There are also problems associated with meta-analysis when differences in experimental conditions are not considered. This study uses the experimental conditions where possible as correlative factors to determine if they do or do not influence the PIC:POC response. However, not all studies in the literature provide all the information required, e.g., information on salinity in some cases was not available. This again could lead to some bias in our interpretations.

Response sensitivity across strains—By quantitatively evaluating results presented in the literature, we found no difference in the effect size (i.e., the response in PIC:POC to OA) between strains, locations, and environmental conditions. This assessment provides some indication of whether the different responses observed between experiments and strains (in terms of PIC and/or POC) results in strain-specific sensitivity to OA in terms of export ratio (PIC:POC). Although *E. huxleyi* populations appear highly genetically diverse (Iglesias-Rodríguez et al. 2006), our results suggest that such a degree of intraspecific genetic diversity (demonstrated by a large variability in PIC responses and in POC responses) does not translate into functional variability (e.g., export ratio), as suggested by Iglesias-Rodríguez et al. (2006), and demonstrated by the fact that the PIC:POC response to changes in the carbonate system appears highly conserved within an environmentally relevant pH.

The similar response in PIC:POC found in all ecotypes (strains) across the gradients in carbonate system parameters (CO_2 , pH, etc.) suggests that the coccolithophore strains do not show any specific level of adaptation to regional or local carbonate conditions. However, there is still limited understanding from these short-timescale experiments of whether a change from present (early 21st century) to future (end of 21st century) average carbonate conditions would result in genetic adaptation, or whether the range of variability in carbonate conditions that these organisms currently experience would allow the extant populations to respond to these shifts in the carbonate system. Further assessment of natural assemblages and the conditions in which they thrive would help to develop whether there can be shifts from one ecotype dominance to another. Our analysis furthers the work of Ridgwell et al. (2009) by assessing the conditions within the experiments, as well as the differences between ecotypes (strains) and the environmental conditions in which they were originally located. A next step would be to understand the extent of variability to which these organisms are exposed in the natural environment, as well as the plastic responses of strains and scope for further adaptation to low pH–high CO_2 conditions.

Our analysis also provides the first quantitative assessment that time spent in culture does not appear to affect the response of PIC:POC to OA. However, it is important to note that estimated culture times are of several years, timescales during which a high number of generations can occur, and together with a variety of uncontrolled selective

processes, this may prevent us from appreciating the real potential for adaptation of *E. huxleyi*, especially as the rates of calcification, photosynthesis, and cell growth in coccolithophores are dynamic, alter on short timescales, show strong diel periodicity, and are affected by cell division rate (Paasche 2002). A recent long-term study by Müller et al. (2010) suggested that changing the carbonate system more slowly and over several generations causes similar responses in PIC:POC as seen in shorter-term experiments. Examples from the field have shown that coccolithophores are seasonally dynamic in their growth, coccolith size, and thickness (a measure of calcification), increasing in size and becoming more heavily calcified in the winter and early spring (Triantaphyllou et al. 2010).

Environmental conditions and wider implications—Interestingly, although not a significant contributor, experimental phosphate was a dominant predictor of PIC:POC. The phosphate levels were not limiting in any of the experiments (see Table 1); indeed, *E. huxleyi* is believed to have enhanced growth in low-phosphate conditions, which allows it to dominate over more phosphate-limited phytoplankton species (Eppley et al. 1969; Iglesias-Rodríguez et al. 2002; Satoh et al. 2009). Previous models for export ratio have not included any nutrient parameters, yet under analysis of a wide range of results, as shown here, phosphate concentration does appear important.

The water–mass properties and physical dynamics experienced by these organisms ultimately determine what conditions they are exposed to. As $\text{CO}_{2\text{aq}}$ and TA appear to be the dominant predictors for PIC:POC, we can assess the response in relation to the current levels of $\text{CO}_{2\text{aq}}$ and TA found in the oceans. For example, the northern Atlantic has high levels of anthropogenic CO_2 compared to the North Pacific (Sabine et al. 2004); deep water rich in CO_2 have been shown to encroach on the surface water, particularly in upwelling areas on the eastern Pacific (Feely et al. 2008; E. S. Mayol et al. unpubl.). We might therefore predict, then, that these areas of high CO_2 conditions already have lower PIC:POC levels, which could be further reduced by OA, as it appears there is no local adaptation that would make these organisms equally vulnerable to environmental change despite their location.

The reduction in PIC:POC appears, in the majority of experiments, to result from a decrease in PIC (Riebesell et al. 2000; Langer et al. 2009). A decrease in PIC with stable POC and specific growth rate, as in these cases (Riebesell et al. 2000; Langer et al. 2009), would reduce the weight of cells, reducing sinking rates (Armstrong et al. 2002; Klaas and Archer 2002), but would also reduce the potential CO_2 released by calcification (Ridgwell et al. 2007). In the cases of Iglesias-Rodríguez et al. (2008) and Shi et al. (2009), PIC:POC decreased with increasing CO_2 because although both PIC and POC increased, there was a slight mismatch in the magnitude of increase, i.e., POC increased more. In other studies, an increase in POC is seen alongside a decrease in PIC (Zondervan et al. 2001), which results in even greater decreases in the PIC:POC ratio with increasing CO_2 .

The PIC:POC response to OA does not appear to be strain specific but can be predicted from TA, $\text{CO}_{2\text{aq}}$, and phosphate

conditions as a phenotypically plastic response to the environment. PIC:POC can be characterized by an overall relationship with the carbonate system, such that there is a linear decrease in PIC:POC with increasing TA, increasing $\text{CO}_{2\text{aq}}$, and decreasing pH, and a logarithmic decrease in PIC:POC with decreasing $[\text{CO}_3^{2-}]$ and decreasing Ω_{calcite} . Furthermore, PIC:POC variation with environmental conditions can be characterized to specific water masses, although this relationship now needs validation from field results. There appears to be no complication that local populations or different strains have become biologically isolated to respond differently to the changes in chemistry, most likely because they experience a wide range of conditions. This means that a general increase in CO_2 or decrease in pH over the global oceans will ultimately produce comparable responses in genetically distinctive strains of *E. huxleyi*, yet there will be regional differences in the rate of change and therefore the relative contribution to the export ratio.

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