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The morphological response of *Emiliania huxleyi* to seawater carbonate chemistry changes: an inter-strain comparison

Gerald Langer
ICTA, Autonomous University of Barcelona (UAB), 08193 Bellaterra, Spain: Gerald.Langer@awi.de

Ian Probert
CNRS / UPMC, Station Biologique de Roscoff, 29682 Roscoff, France

Gernot Nehrke
Alfred Wegener Institute for Polar and Marine Research, 27570 Bremerhaven, Germany

Patrizia Ziveri
FALW, Vrije Universiteit Amsterdam, 1081HV Amsterdam, The Netherlands

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Abstract Four strains of the coccolithophore *Emiliania huxleyi* (RCC1212, RCC1216, RCC1238, RCC1256) were grown in dilute batch culture at four CO_2 levels ranging from ~200 μatm to ~1200 μatm. Coccolith morphology was analyzed based on scanning electron micrographs. Three of the four strains did not exhibit a change in morphology over the CO_2 range tested. One strain (RCC1256) displayed an increase in the percentage of malformed coccoliths with increasing CO_2 concentration. We conclude that the sensitivity of the coccolith-shaping machinery to carbonate chemistry changes is strain-specific. Although it has been shown before that carbonate chemistry related changes in growth- and calcification rate are strain-specific, there seems to be no consistent correlation between coccolith morphology and growth or calcification rate. We did not observe an increase in the percentage of incomplete coccoliths in RCC1256, indicating that the coccolith-shaping machinery *per se* is affected by acidification and not the signalling pathway that produces the stop-signal for coccolith growth.

Keywords *Emiliania huxleyi*, morphology, carbonate chemistry

1. Introduction

In the context of ocean acidification, i.e. the decrease of surface seawater pH due to anthropogenic CO_2 emissions (Royal Society, 2005), the response of marine calcifiers to altered seawater carbonate chemistry has received particular attention. Coccolithophores, unicellular haptophyte algae that cover the cell surface with intracellularly-produced calcite platelets (coccoliths), have been shown to change growth rate and calcite- and organic carbon production in response to seawater carbonate chemistry changes (Riebesell et al., 2000; Langer et al., 2006; Feng et al., 2008). It is generally assumed that a decrease in calcite production, the most commonly observed response to seawater acidification, is detrimental for a coccolithophore. However, the function of calcification is unknown (Young, 1994) and so far there is no evidence that calcification plays a physiological role (Trimborn et al., 2007). Nevertheless, the products of calcification, i.e. the coccoliths and the resulting coccosphere, are most likely advantageous for the organism. In an Atomic Force Microscopy study of coccoliths, the authors concluded that "clearly, millions of years of natural selection have perfected the tailoring of coccolith biocrystals so that the mineral structure of the material is used to the greatest advantage" (Henriksen et al., 2003). To better understand the potential consequences of ocean acidification for coccolithophores, it is therefore important to study the effect of changing seawater carbonate chemistry not only on calcite production but also on coccolith morphology. The latter has been shown to be influenced by salinity (Green et al., 1998; Bollmann and Herrle, 2007) and temperature (Watabe and Wilbur, 1966; Langer et al., 2010). The carbonate chemistry effect on morphology was shown to be species specific (Langer et al., 2006). Moreover, conflicting results have been published on the response of a single species, namely *Emiliania huxleyi*. In the seminal study by Riebesell et al. (2000) an increase of the percentage of malformed coccoliths with increasing CO_2 concentration was reported. In contrast, no differences in coccolith morphology were found in a later study (Feng et al., 2008). However, coccolith morphology was not quantified in either of these studies, and in all of the studies cited above only one culture strain of *E. huxleyi* (and in every study a different strain) was used. Since the effect of carbonate chemistry changes on growth rate and calcite production in *E. huxleyi* is strain specific (Langer et al., 2009), it is probable that the effect on coccolith morphology is likewise. In order to test this hypothesis, we analyzed the coccolith morphology of the four *E. huxleyi* strains used by Langer et al. (2009).

2. Material and Methods

Clonal cultures of *Emiliania huxleyi* (strains RCC1212, RCC1216, RCC1238, and RCC1256) were grown in aged, sterile-filtered (0.2μm 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). The strains were obtained from the Roscoff Culture Collection (www.sb-roscoff.fr/Phyto/RCC). 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. Cells were pre-adapted to experimental conditions for approximately 12 generations and grown in dilute batch cultures (Langer et al., 2009). 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. For further experimental details see Langer et al. (2009).

Samples for scanning electron microscope analysis were filtered onto polycarbonate filters (0.8 µm pore size), dried in a drying cabinet at 60°C for 24 hours, then sputter-coated with gold-palladium. Imaging was performed with a Philips XL-30 digital scanning field-emission electron microscope. Four categories were used to describe the morphology of *Emiliania huxleyi*: 'normal', 'malformed', 'incomplete', and 'incomplete and malformed' coccoliths (for reference images for the categories, see Figures 1-4). An average of approximately 350 coccoliths was analyzed per sample (Langer and Benner, 2009).

3. Results and Discussion

3.1. Response patterns

Strain RCC1238 displayed more than 90% normally grown coccoliths under all CO_2 concentrations tested (Figure 5, Table 1). The same held for strain RCC1216 (Figure 6, Table 1). Both RCC1238 and RCC1216 are therefore considered as insensitive over the CO_2 ranges tested. We draw the same conclusion for strain RCC1212 (Figure 7, Table 1), although the result was less obvious. In RCC1212 a trend was observed towards a higher percentage of normal coccoliths under higher CO_2 concentration. This has to be interpreted with caution, however, because the differences were relatively small and the standard deviation relatively high (Table 1). The decrease in the percentage of normal coccoliths with increasing CO_2 in RCC1256 (Figure 8, Table 1) is, however, meaningful because the standard deviation was much smaller and the difference in question, i.e. the morphology at the highest CO_2 level compared to the other levels, was considerably larger. Hence, coccolith morphogenesis of this strain was impaired by acidification of seawater.

The absolute level of aberrant coccoliths in RCC1212 was much higher than in the other strains (Table 1). The reason for this is unknown. In general, the base level of malformation in cultured coccolithophores is highly variable (Langer and Benner, 2009), and as a rule cultured specimens display a higher percentage of malformed coccoliths than specimens

experiment	CO_2 [μatm]	normal	malformed	incomplete	malformed + incomplete
RCC1238 1	206	94.8	2.2	2.4	0.6
SD		2.1	0.9	1.0	0.8
RCC1238 2	395	93.9	1.7	3.3	1.1
SD		0.7	0.4	1.3	0.8
RCC1238 3	681	94.8	1.4	2.5	1.4
SD		2.0	0.6	0.9	0.6
RCC1238 4	929	93.3	0.5	2.8	3.4
SD		1.5	0.5	1.1	1.0
RCC1216 1	218	96.8	1.4	1.6	0.2
SD		1.7	0.7	1.1	0.3
RCC1216 2	422	97.6	0.6	1.5	0.2
SD		0.1	0.2	0.3	0.2
RCC1216 3	729	97.8	0.6	1.5	0.1
SD		0.1	0.4	0.2	0.2
RCC1216 4	1201	92.2	3.1	1.8	2.9
SD		4.2	1.5	1.0	1.7
RCC1212 1	194	51.0	42.8	4.0	2.3
SD		5.0	5.2	0.8	2.3
RCC1212 2	409	58.5	35.7	4.0	1.8
SD		2.9	1.6	1.9	0.5
RCC1212 3	752	66.0	27.5	4.0	2.6
SD		3.5	4.3	0.6	0.3
RCC1212 4	1096	65.2	28.2	2.2	4.4
SD		5.6	4.4	0.5	1.6
RCC1256 1	193	97.0	1.2	1.5	0.2
SD		1.1	0.2	0.9	0.4
RCC1256 2	399	97.3	1.0	1.6	0.2
SD		0.6	0.4	0.7	0.2
RCC1256 3	587	91.3	7.0	0.7	1.0
SD		0.8	1.2	0.2	0.3
RCC1256 4	915	61.0	29.4	1.4	8.2
SD		2.5	1.5	1.1	1.8

Table 1 Coccolith morphology (percentage of counted coccoliths) at different CO_2 levels. CO_2 partial pressures were taken from Langer et al. (2009). SD = standard deviation.

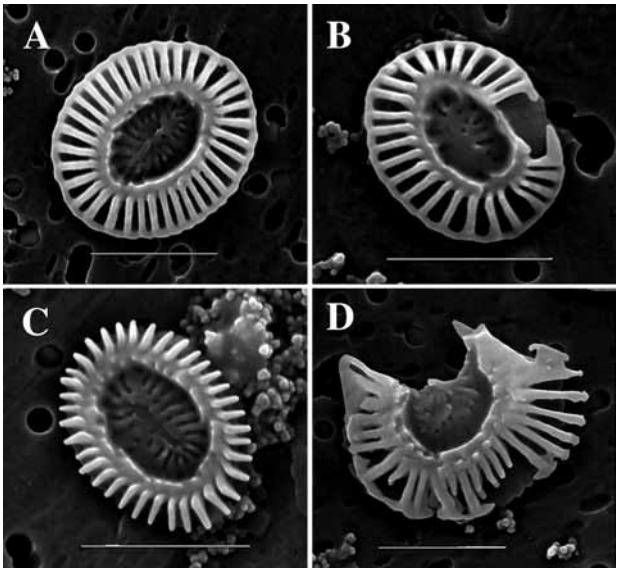

Figure 1 SEM images of Emiliania huxleyi (RCC1238) coccoliths. A) Normal B) Malformed C) Incomplete D) Incomplete and malformed. All coccoliths in distal view. All scalebars 2 μm.

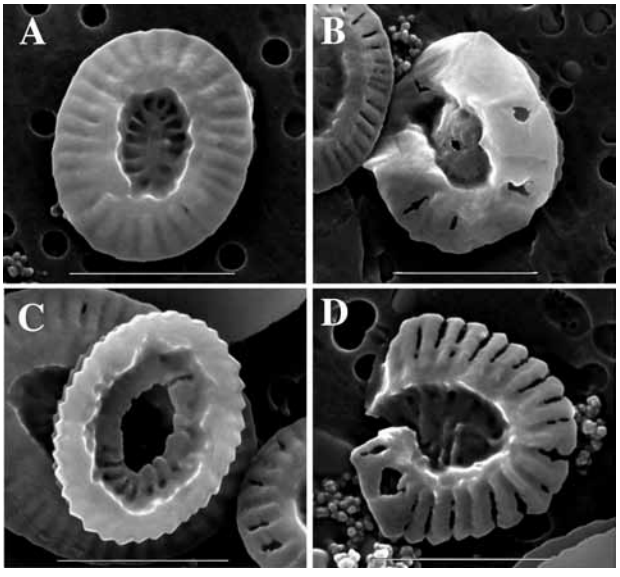

Figure 2 SEM images of Emiliania huxleyi (RCC1216) coccoliths. A) Normal B) Malformed C) Incomplete D) Incomplete and malformed. All coccoliths in distal view. All scalebars 2 μm.

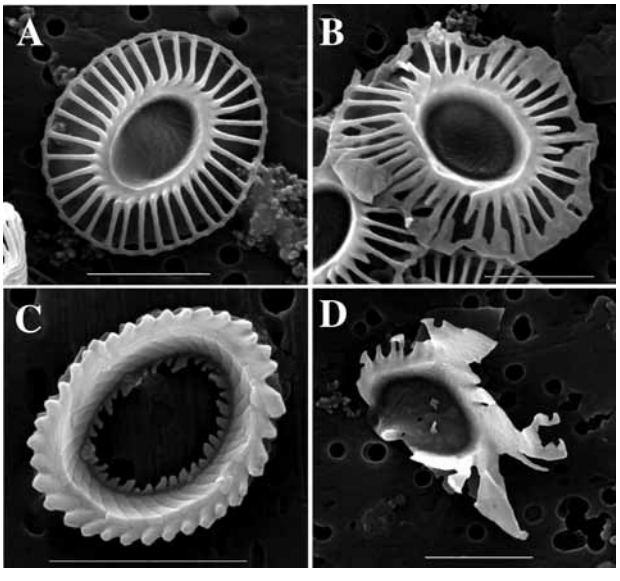

Figure 3 SEM images of Emiliania huxleyi (RCC1212) coccoliths. A) Normal B) Malformed C) Incomplete D) Incomplete and malformed. All coccoliths in distal view. All scalebars 2 μm.

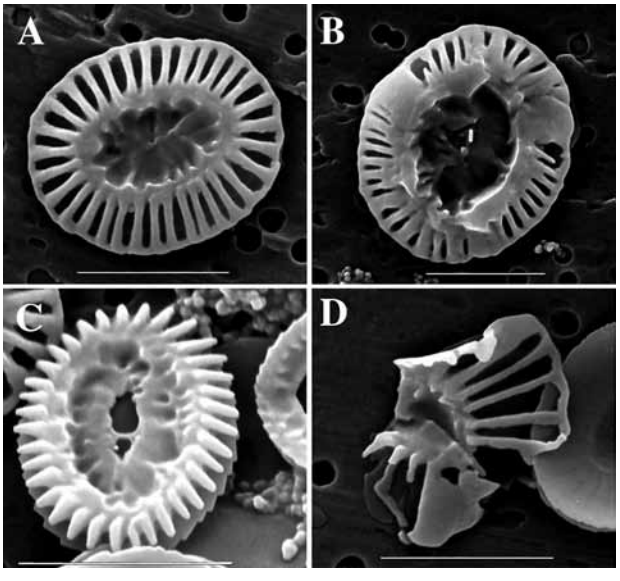

Figure 4 SEM images of Emiliania huxleyi (RCC1256) coccoliths. A) Normal B) Malformed C) Incomplete D) Incomplete and malformed. All coccoliths in distal view. All scalebars 2 μm.

from oceanic samples (Langer et al., 2006). The cause of the latter observation, often termed "culture artefacts", is also unknown (Langer and Benner, 2009). However, for the interpretation of our results it is important to note that the absolute level of malformation has been shown to have no influence on the response to carbonate chemistry changes in *Calcidiscus leptoporus* (Langer et al., 2006). Given that this also holds for *E. huxleyi*, the response of RCC1212 can be compared to the responses of the other strains.

It is therefore concluded that the four strains tested displayed two types of responses: either no change in morphology (RCC1238, RCC1216, and RCC1212), or a higher percentage of malformed coccoliths at a CO_2 partial pressure of ca. 900 μatm (RCC1256).

3.2. Strain specific responses

Since the initial study of Riebesell et al. (2000), the latter response pattern (i.e. higher malformation at higher CO_2) has been regarded as typical for *E. huxleyi* and indeed for coccolithophores. In our study, however, this response appears to be the exception rather than the rule. The origin of the differences in response remains to be discovered. Several environmental factors, which could presumably influence the response pattern, were discussed elsewhere (Langer et al., 2009). Distance from the shore of the site of strain isolation and morphotype of the strain can be ruled out (Langer et al., 2009). For details on the sites of strain isolation see also Table 2 and Figure 9. It may seem logical to assume that surface seawater CO_2 concentration at the site of strain isolation is a main factor influencing

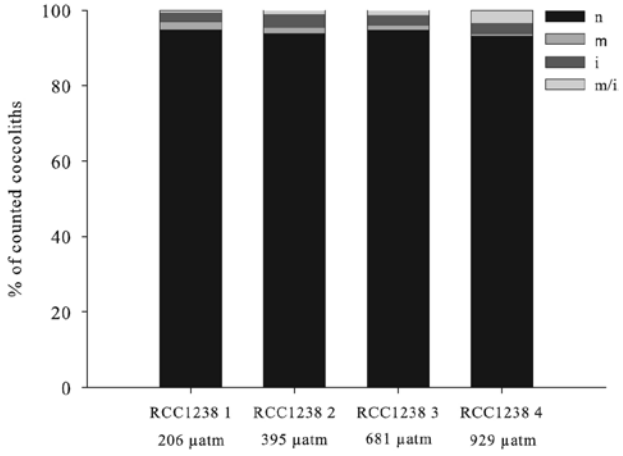

Figure 5 Percentage of normal (n), malformed (m), incomplete (i), malformed and incomplete (m/i) coccoliths (strain RCC1238) vs. CO_2 partial pressure. Values represent an average of triplicate experiments. CO_2 partial pressures were taken from Langer et al., (2009).

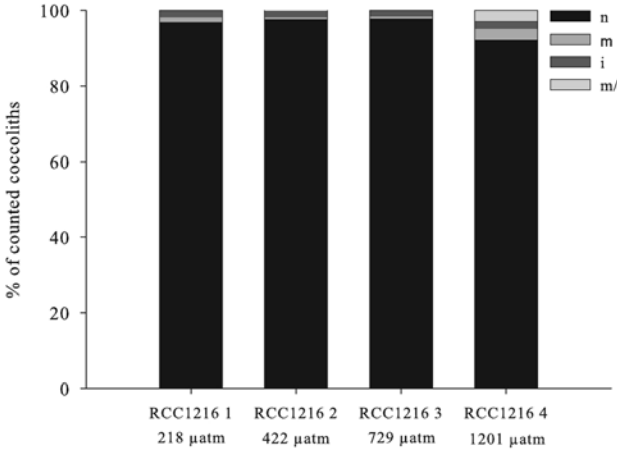

Figure 6 Percentage of normal (n), malformed (m), incomplete (i), malformed and incomplete (m/i) coccoliths (strain RCC1216) vs. CO_2 partial pressure. Values represent an average of triplicate experiments. CO_2 partial pressures were taken from (Langer et al., 2009).

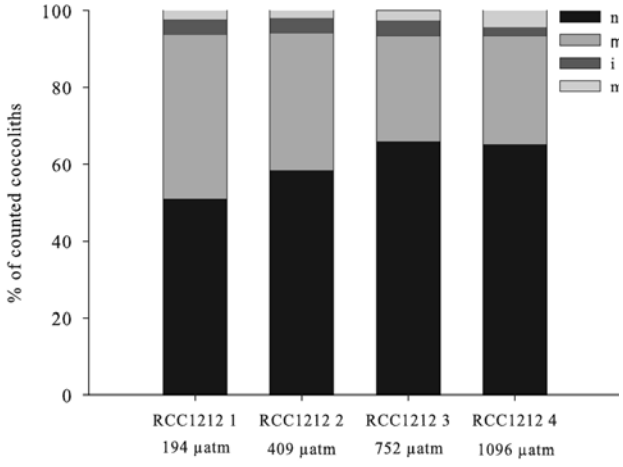

Figure 7 Percentage of normal (n), malformed (m), incomplete (i), malformed and incomplete (m/i) coccoliths (strain RCC1212) vs. CO_2 partial pressure. Values represent an average of triplicate experiments. CO_2 partial pressures were taken from (Langer et al., 2009).

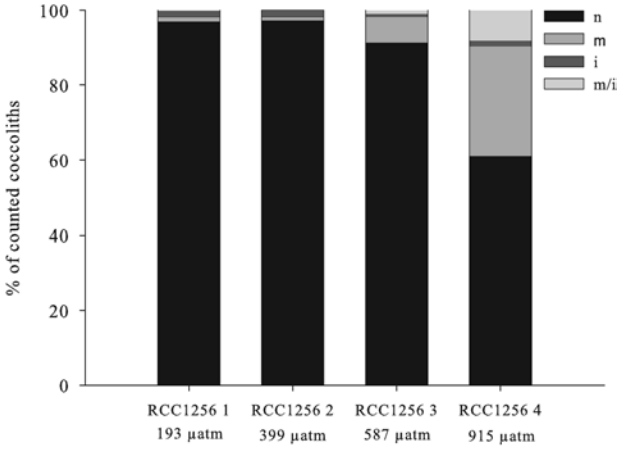

Figure 8 Percentage of normal (n), malformed (m), incomplete (i), malformed and incomplete (m/i) coccoliths (strain RCC1256) vs. CO_2 partial pressure. Values represent an average of triplicate experiments. CO_2 partial pressures were taken from (Langer et al., 2009).

the response pattern, see also Langer et al. (2009). South of Iceland, where RCC1256 was isolated, the surface water CO_2 partial pressure ranges from ca. 270 μatm in August to ca. 360 μatm in February (Takahashi et al., 2002). The CO_2 partial pressure at the three other sites of isolation is comparatively constant 320 μatm, Takahashi et al., (2002). However, it appears unlikely that the strain that has historically experienced varying CO_2 levels over the course of the year is particularly sensitive to acidification, whereas strains that have experienced quasi-constant carbonate chemistry are insensitive. This notion is strengthened when taking into account not only morphology but also growth rate, calcite- and organic carbon production (Langer et al., 2009). At any rate, all four strains may be regarded as belonging to different populations with specific genetic features (Langer et al., 2009), rendering it reasonable to attribute the different response patterns to these genetic features.

3.3. Comparison with literature data

Considering all available data on coccolithophores, there seems to be no consistent correlation between coccolith morphology and growth or calcification rate in carbonate chemistry manipulation experiments. In RCC1212, RCC1216 (Table 1, Langer et al., 2009), and CCMP371 (Feng et al., 2008) changes in growth and calcification rate, but no change in morphology, were observed. In RCC1256 (Table 1, Langer et al., 2009) changes in growth and calcification rate and morphology were observed, whereas B92/11 (Riebesell et al., 2000) and RCC1135 (Langer et al., 2006) changed calcification rate and morphology, but not growth rate. Finally, RCC1238 (Table 1, Langer et al., 2009) and RCC1200 (Langer et al., 2006) did not show a change in any of these parameters. These response patterns are difficult to interpret because the adverse effects of seawater acidification on coccolithophore calcification are not understood on a process level. It is not even known which

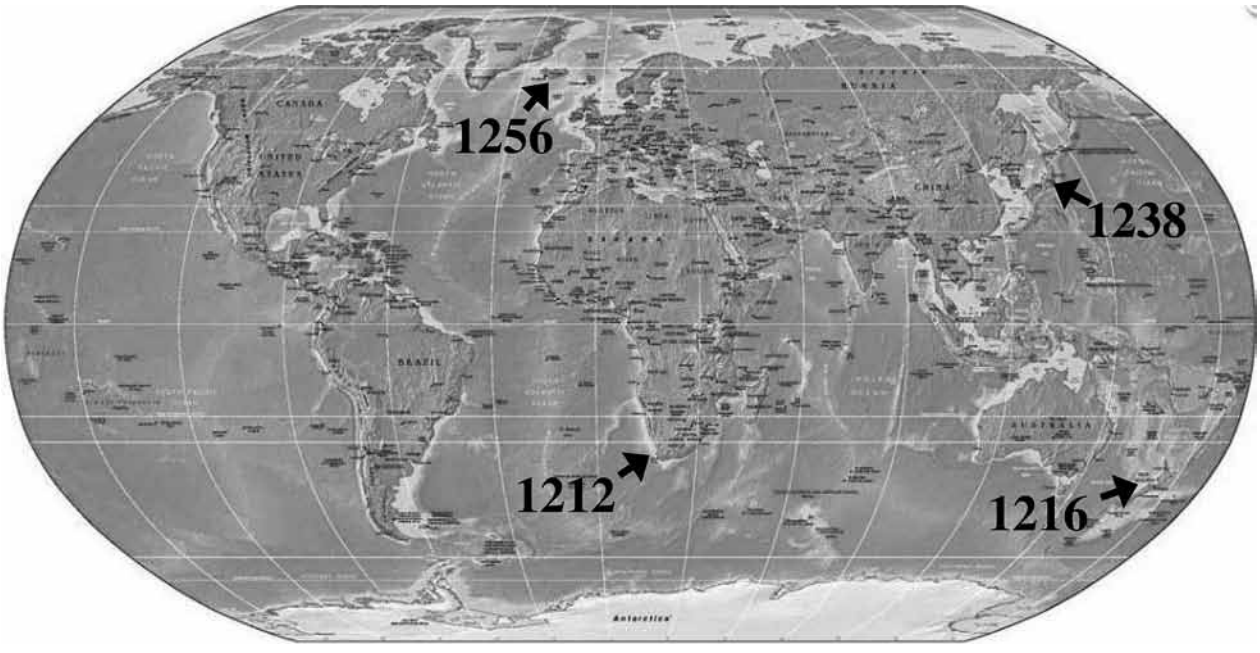

Figure 9 Map showing the sites of strain isolation. Map modified from http://www.justmaps.org/maps/thematics/physical.asp

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	Isolated by
RCC1212	9/2000	34°28'S 17°18'E (South Atlantic, off South Africa)	15°C	21°C	20°C	B	Ian Probert
RCC1216	9/1998	42°18'S 169°50'E (Tasman Sea, off New Zealand)	11°C	18°C	17°C	R	Ian Probert
RCC1238	11/2005	34°01'N 139°50' E (North Pacific, off Japan)	18°C	25°C	20°C	A	Ian Probert
RCC1256	7/1999	63°24'N 20°20W (North Atlantic, off Iceland)	9°C	14°C	17°C	A	Ian Probert

Table 2 Information on culture strains used in this study. Max. annual SST was taken from the World Ocean Atlas (www.nodc.noaa.gov).

parameter of the carbonate system causes these effects.

It was hypothesised that seawater pH changes upset the otherwise strictly controlled ion balance at the plasma membrane and thereby disturb intracellular ionic composition (Langer et al., 2006). This, in turn, would disrupt the specific function of polyelectrolytes involved in coccolithogenesis. Two types of polyelectrolytes have been shown to be involved in coccolithogenesis, namely coccolith-associated polysaccharides (Fichtinger-Schepman et al., 1981; Borman et al., 1982; Marsh et al., 2002; Henriksen et al., 2004; Henriksen and Stipp, 2009) and two elements of the cytoskeleton (Langer et al., 2010). In the latter study it was also shown that a malfunction of the cytoskeleton leads to a decreased growth rate as well as an increased percentage of malformed coccoliths. The lack of a correlation between morphology and growth rate in carbonate chemistry manipulation experiments (see above) suggests that the cytoskeleton is not the polyelectrolyte which is affected by acidification. It may, therefore, be worthwhile focusing research regarding this question on coccolith-associated polysaccharides.

It is also noteworthy that the percentage of incomplete coccoliths did not change in any of the tested strains (Table 1). In the context of developing a process-based understanding it is significant that the increase in malformations in higher than ambient CO_2 concentrations in RCC1256 (Table 1) was not accompanied by an increase in incomplete coccoliths, matching the observation for *C. leptoporus* RCC1135 (Langer et al., 2006). These observations clearly suggest that when acidification does have an effect, it is on morphogenesis per se and not the signalling pathway that produces the stop-signal for coccolith growth. This conclusion renders the overall lack of correlation between coccolith morphology and cellular calcite content / calcification rate (see above) plausible.

4. Conclusion

On the whole, the data presented here show firstly that the morphological response of *E. huxleyi* to short term acidification of seawater does not always correlate with the calcification or growth rate response. This finding is particularly relevant if it should indeed turn out that the product of calcification, i.e. the coccoliths, and not the process of calcification itself is of key importance for a coccolithophore. Secondly, it is shown that the morphological response is strain specific, once more highlighting the need to consider the potential for adaptation of coccolithophores (see also Langer et al., 2006; Langer et al., 2009).

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