Assessing the applicability of *Emiliania huxleyi* coccolith morphology as a sea-surface salinity proxy

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Abstract

Culture experiments were used to assess the applicability of *Emiliania huxleyi* coccolith morphology as a palaeo–sea-surface salinity (SSS) proxy. Coccolith morphology was dependent on salinity over a range reflecting present day marine conditions; both coccolith size and the number of coccolith elements increased linearly with increasing salinity. Using regression analysis, the effect of salinity on coccolith morphology was compared to those previously observed in sediment core-top and plankton data. No significant differences were found between the slopes of these data, suggesting that salinity is the primary control on *E. huxleyi* coccolith size and element number in the ocean. However, the intercepts of the culture data were significantly higher. A combination of experimental and literature analysis indicated that temperature and nutrients were unlikely to be the causes of this discrepancy. Literature analysis also highlighted that coccolith size data from marginal environments displayed different intercepts to those from the open-ocean data. This suggests that discrete morphotypes exist in these marginal locations. We, therefore, recommend that the original *E. huxleyi* coccolith morphology palaeo-SSS transfer function requires further evaluation before being routinely applied.

The ability to reconstruct historical sea-surface salinity (SSS) patterns is essential to understanding past ocean circulation and climatic change (Hay et al. 2006; Hay 2008). However, unlike temperature, palaeo-SSS still cannot be reconstructed with reliability (Schmidt 1999; Rohling 2000, 2007). As a step towards addressing this problem, recent studies (Bollmann and Herrle 2007) have indicated that the morphology of external calcite plates (coccoliths) of the ubiquitous and abundant coccolithophorid *Emiliania huxleyi* may provide a useful proxy with which to reconstruct SSS. Analysis of sediment core-top samples (~Holocene in age) has revealed that *E. huxleyi* coccolith size is linearly related to present annual mean SSS between 33 and 39, though the relationship deviated from linear outside this range (Bollmann and Herrle 2007). Further, a linear relationship between coccolith size and in situ SSS (32–39) has been demonstrated for *E. huxleyi* plankton samples (Bollmann et al. In press). Correlations between ocean SSS and coccolith morphology are encouraging for the use of coccoliths as a proxy for palaeo-SSS reconstructions. However, a direct relationship needs to be established between salinity and *E. huxleyi* coccolith morphology under controlled laboratory conditions to assess the robustness of such a proxy. To date, culture studies have indicated that reduced salinity (14–34) affects coccolith morphology in some strains of *E. huxleyi* (Paasche et al. 1996; Green et al. 1998). However, the limited number of salinity treatments makes the quantification of this relationship difficult. Further, the limited overlap between previous experimental measurements and the present SSS range (mostly between 33 and 38) precludes comparison of this effect with data from environmental samples.

In this study we assess coccolith morphology from *E. huxleyi* cultures grown in the laboratory over a broad range of salinities (26–41). Because temperature may also affect coccolith morphology (Watabe and Wilbur 1966), we also test the effect of temperatures between 10°C and 20°C on coccolith size. Finally, we explicitly compare these laboratory-based measurements of the coccolith morphology vs. salinity relationship with those made from sediment core-top and plankton samples to establish whether a consistent trend between morphology and salinity occurs. In this way we assess the general applicability of *E. huxleyi* coccolith morphology as a proxy for palaeo-SSS. Our analysis reveals a direct relationship between coccolith morphology and salinity consistent with observations from sediment core-top and plankton samples. However, there were differences between the intercepts of the experimentally derived coccolith morphology vs. salinity relationships and those for the environmental samples, and we discuss potential environmental and geographic reasons for these inconsistencies.

Methods

*Culturing and morphological analysis—Emiliania huxleyi* strain PLY B92/11 (isolated from Bergen Fjord, W. Norway) was grown in semi-continuous batch culture (Brand and Guillard 1981) in borosilicate glass tubes (13-mm diam.) each filled with 15 mL of artificial sea water (ASW) at ~30 μmol photons m⁻² s⁻¹ (broadly represent-
tative for natural *E. huxleyi* growth conditions; Kirk 1994; Cortés et al. 2001) continuous light (Philips TL-D/865 Super 80). The use of continuous light ensured that cultures did not undergo synchronized division, which could bias results (Müller et al. 2008). ASW was made using deionized water, 0.5 g Tricine L\(^{-1}\) (Sigma, T-0377) to prevent precipitation of salts during autoclaving, and variable concentrations of synthetic sea salt (Ultramarine, Waterlife Research Industries). After autoclaving, f/2 enrichment media was added (Sigma, G0154; Guillard 1975). Salinities were determined using an Autosal 8400 (Guildline Instruments).

Cultures were grown at 10 salinities (between 18 and 41) and at four temperatures (between 10°C and 20°C). Cultures were acclimated to each treatment for 
.10 generations. Throughout acclimation and subsequent experimentation, cultures were mixed twice daily and maintained in exponential growth phase at cell concentrations below \(3.0 \times 10^4\) cells mL\(^{-1}\). Acclimated specific growth rates were between 0.05 d\(^{-1}\) and 0.7 d\(^{-1}\) (salinity gradient) and 0.2 d\(^{-1}\) and 0.95 d\(^{-1}\) (temperature gradient). In mid-exponential phase, one sample from each treatment was taken for coccolith morphological analysis using a scanning electron microscope (SEM). Samples were filtered onto polycarbonate filters (0.4-\(\mu\)m pore size), rinsed in \(\text{NH}_4\)OH-buffered \(\text{H}_2\)O (pH 8.5), mounted on stubs, and sputter-coated with \(\sim 15\) nm of gold-palladium. Cultures grown below a salinity of 26 failed to produce sufficient coccoliths and were excluded from analysis.

Images were captured using a Philips XL30 SEM on 30 detached, flat-lying coccoliths per sample. Length and width of both the coccolith distal shield (DL and DW, respectively) and the central area (CAL and CAW, respectively) were measured and the number of distal shield elements (NE) was counted (Fig. 1) using ImageJ 1.38 (http://rsb.info.nih.gov/ij/) at a resolution of 0.008 \(\mu\)m. Measurements were calibrated using 2-\(\mu\)m microsphere standards (Duke Scientific). The mean values of each morphological variable were calculated for each treatment.

**Statistical analysis**—Least squares linear regressions of each morphological variable as a function of salinity were calculated from data collected at 15°C, and slopes were tested against the null hypothesis that they were not significantly different from zero (\(t\)-test, \(z = 0.05\); Zar 1999). Data collected at 10°C, 17°C, and 20°C were tested against the predicted value from the 15°C regression equation to determine if temperature had a significant effect on coccolith morphology (\(t\)-test, \(z = 0.01\) after Bonferroni correction; Sokal and Rohlf 1995; Zar 1999).

**Fig. 1.** Coccolith of *Emiliania huxleyi*, showing distal shield length (DL) and width (DW), central area length (CAL) and width (CAW), and distal shield elements.

**Fig. 2.** Locations of sediment core-top (circles; Bollmann and Herrle 2007), plankton (stars; Bollmann et al. In press), culture (triangles; this study; Paasche et al. 1996; Green et al. 1998; Beaufort et al. 2007), and mesocosm (squares; Batvik et al. 1997) samples. See Table 2 for details.
Table 1. $r^2$, intercept, and slope for linear regressions of *E. huxleyi* coccolith morphological variables (distal shield length [DL], distal shield width [DW], central area length [CAL], central area width [CAW], and number of elements [NE]) for this culture study, plankton samples (Bollmann et al. In press), and sediment core-top samples between salinities of 33 and 39 (Bollmann and Herrle 2007).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Culture</th>
<th>Plankton</th>
<th>Sediment core-top</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL</td>
<td>0.95</td>
<td>0.65</td>
<td>-0.84*</td>
</tr>
<tr>
<td>DW</td>
<td>0.94</td>
<td>0.48</td>
<td>0.05*</td>
</tr>
<tr>
<td>CAL</td>
<td>0.87</td>
<td>0.81</td>
<td>-3.05*</td>
</tr>
<tr>
<td>CAW</td>
<td>0.93</td>
<td>0.74</td>
<td>-2.30*</td>
</tr>
<tr>
<td>NE</td>
<td>0.75</td>
<td>26.3</td>
<td>0.47</td>
</tr>
</tbody>
</table>

* Denotes a significant difference ($\alpha = 0.05$) for comparisons of both intercept and slope of plankton and sediment core-top data sets with those of this culture study.

**Literature analysis**—Coccolith DL, DW, CAL, and CAW data from sediment core-top and plankton samples with salinities between 32 and 39 (Bollmann and Herrle 2007; Bollmann et al. In press) were used to determine the slope and intercept of each morphology–salinity relationship. The slopes and intercepts from this study were then compared to the field data ($t$-test, $\alpha = 0.05$; Zar 1999). Due to significant differences between intercepts (see Results), number-of-element data were also obtained from 22 sediment core-tops used in previous analyses (Bollmann and Herrle 2007) and compared to the culture results. A significant difference between the intercepts of the two number-of-element data sets prompted further analysis of the literature, which is dealt with in the Discussion. The locations of all sediment core-top, plankton, and culture samples used in this analysis are shown in Fig. 2 (see Table 2 for details).

**Results**

**Culture coccolith morphology vs. salinity relationships**—All measured coccolith morphological variables of *E. huxleyi* grown in culture were significantly related to salinity in a positive linear fashion (Table 1). The variable best related to salinity was DL (Fig. 3A), although DW, CAL, and CAW all gave high $r^2$ values compared to the NE (Table 1). Coccoliths grown at 10°C, 15°C, 17°C, and 20°C were not significantly different in size.

**Culture comparison with plankton and sediment core-top data**—There were no significant differences between the slopes of the salinity vs. morphology relationships derived from plankton and sediment core-top samples and those derived from our laboratory measurements (Fig. 3B; Table 1). However, the intercepts of the culture data were significantly higher than those of both plankton and sediment core-top data sets (Fig. 3B; Table 1).

**Discussion**

**Culture coccolith morphology vs. salinity relationships**—The linear increase of *E. huxleyi* coccolith morphological variables with increasing salinity between 26 and 41 observed in this study supports the trends observed in previous culture work. Using salinities between 14 and 34, three out of four *E. huxleyi* strains, including the strain used here, produced smaller coccoliths at lower salinities (Paasche et al. 1996; Green et al. 1998). Our culture results build on these studies and show that morphological variables also respond to salinity over the range encountered in the present ocean (mostly between 33 and 38). Therefore, comparisons can be made of the relationships between coccolith morphology and salinity observed in the laboratory and those observed in the field.

**Culture comparison with plankton and sediment core-top data**—Both sediment core-top and plankton analyses (Bollmann and Herrle 2007; Bollmann et al. In press) found *E. huxleyi* coccoliths to increase in size linearly with increasing salinities from 32 to 39 (sediment core-top data outside this salinity range are discussed later). The degree of high similarity in slopes between the coccolith morphology vs. salinity regressions for culture and for plankton and sediment core-top data (Fig. 3B; Table 1) supports the hypothesis that salinity increase causes the linear change in *E. huxleyi* coccolith size observed in the ocean (Bollmann and Herrle 2007). However, the higher intercept of the morphological data presented here indicates that the absolute sizes of coccoliths at any given salinity may be larger for culture than for environmental samples (Fig. 3B; Table 1). Therefore, we investigated whether culture conditions or other factors might be responsible for this size difference. For this we used coccolith DL data from this study and from the literature (where exact locations and salinities were known).

**Environmental effects on coccolith size**—First, temperature has been shown to affect coccolith size in one *E. huxleyi* strain (Watabe and Wilbur 1966). For our strain, however, coccolith size remained stable at different temperatures (Fig. 3A). Further, the temperature range used in this study (10–20°C) encompasses ~35% of the data used in both plankton and sediment core-top studies (1–30°C; Bollmann and Herrle 2007; Bollmann et al. In press). Second, the nutrient-replete conditions used in cultures differ from those experienced in the ocean and could be hypothesized to have caused the difference in intercepts. However, *E. huxleyi* coccolith size from other cultures grown in nutrient-replete media (Table 2) correspond with both sediment core-top and plankton data and culture data from this study (Fig. 3C). Finally, the low light levels and
Biogeographic effects on coccolith size—Previous work suggests that coccoliths from Norwegian coastal waters belong to a larger morphotype than those in other regions (Batvik et al. 1997). Indeed, the strain used in this study (PLY B92/11) was isolated off the western coast of Norway in 1992. Further, data collected in 1991 at the start of mesocosm experiments (Batvik et al. 1997) from the same location (as the PLY B92/11 isolation site) correspond well with our culture data when analyzed as a function of their in situ salinity (Fig. 3C). Finally, data from cultured strains of *E. huxleyi* and sediment core-top samples (not corresponding with the linear salinity vs. morphology relationship) from the Oslofjord and the Skagerrak (Table 2) correspond well with other data from Norwegian coastal waters (Fig. 3D). Therefore, environmental data support the high intercept of our culture results. This provides strong evidence in favor of the suggestion (Young 1994; Batvik et al. 1997) that *E. huxleyi* populations from southwest Norwegian coastal waters belong to a local subtype (Fig. 3D).

Coccolith sizes from sediment core-top samples in two further geographic regions—the northern Red Sea, and the Black Sea—appear to deviate from the open-ocean plankton and sediment core-top data (Fig. 3D). This may also indicate separate morphotypes in these marginal environments, although these data are still sparse. Nevertheless, these data, combined with those from Norwegian 15°C experiments. Standard deviation for each data point is ~0.4 μm. (B) Culture results from this study (open circles) and data from plankton and sediment core-top samples between salinities of 32 and 39 (open triangles; Bollmann and Herrle 2007; Bollmann et al. In press). Linear regression is through data for all temperatures. (C) All literature data separated by sample type: culture results from this study (open circles), data from plankton and sediment core-top samples between salinities of 18 and 40 (open triangles; Bollmann and Herrle 2007; Bollmann et al. In press), other nutrient-replete cultures (filled circles; Paasche et al. 1996; Green et al. 1998; Beaufort et al. 2007), and data from the start of mesocosm experiments off the coast of western Norway (filled diamonds; Batvik et al. 1997). (D) All literature data separated by sample location: plankton and sediment core-top samples from open-ocean environments (open inverted triangles), cultures from open-ocean environments (filled inverted triangles), all data from Norwegian coastal waters (open circles), and sediment core-top samples from the northern Red Sea (filled triangles) and the Black Sea (filled squares). See Table 1 for more information about sample locations.

Fig. 3. *E. huxleyi* coccolith DL measurements from this study and from the literature as a function of salinity. Solid lines represent linear regressions, dotted lines represent the 95% confidence interval. (A) Culture results from this study grown at 10°C (filled circles), 15°C (open circles), 17°C (filled triangles), and 20°C (open triangles). Linear regression is only through data from growth rates (see Methods) experienced by cells in this study could also be hypothesized to have affected coccolith size. However, corresponding with our results are coccolith size data (Green et al. 1998) from the same strain as we use here, which were cultured at high light intensities (200 μmol photons m⁻² s⁻¹) and experienced higher growth rates (1.0 d⁻¹ and 1.6 d⁻¹; Paasche et al. 1996). Therefore, differences in temperature, nutrient and light levels, and growth rate do not appear to provide reasonable explanations of the observed size discrepancy.
coastal waters, suggest that *E. huxleyi* in marginal environments are morphologically different to those in the open ocean (Fig. 3D). The existence of genetic divides between populations from marginal waters and from the open ocean has been noted on the grounds of physiological and biochemical evidence (Conte et al. 1995; Paasche 2002). This recognition of biogeographic constraints on *E. huxleyi* morphotypes is an important step in the development of the original palaeo-SSS transfer function (Bollmann and Herrle 2007). The proxy proposed by Bollmann and Herrle (2007) is potentially still applicable to the open ocean where its calibration data set displayed a linear salinity vs. coccolith morphology response. However, this study has shown that the original proxy is not universally applicable. Analyses of *E. huxleyi* strains from both marginal and open-ocean settings are now required to ascertain the generality of the morphological divide.

In conclusion, our culture data support a direct relationship between *E. huxleyi* coccolith morphology and salinity. This is consistent with observations from sediment core-top and plankton samples and reaffirms the potential of *E. huxleyi* coccolith morphology as a palaeo-SSS proxy. However, coccoliths from Norwegian coastal waters, the Black Sea, and the Northern Red Sea, deviate from the linear response observed in the open ocean, likely indicating different morphotypes in non-open-ocean settings. We, therefore, recommend that the coccolith morphology palaeo-SSS proxy proposed by Bollmann and Herrle (2007) requires further evaluation before its routine application.

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**References**


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