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# The influence of lithogenic material on particulate inorganic carbon measurements of coccolithophores in the Bay of Biscay

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*Abstract*

Following earlier data suggesting a decoupling between coccolithophore abundance and its suggested proxy, particulate inorganic carbon (PIC), we investigated this relationship in the Bay of Biscay (northwest European shelf), between December 2009 and July 2010. Coccolithophore abundance, coccolith calcite, and PIC were determined in surface waters (5-m depth) along a transect crossing the Bay. *Emiliania huxleyi* was the most abundant species of coccolithophore and the main contributor of coccolith calcite (55–64%). PIC ranged from 0.07 to 11.7 mmol C $m^{-3}$, and coccolith calcite from 0.002 to 0.27 mmol C $m^{-3}$. Total PIC exceeded coccolith calcite in all samples, with only ~ 11% of the PIC attributed to coccoliths. Coccolithophores alone could not account for the PIC concentrations measured. Lithogenic particulate matter, with calcite and dolomite components, was observed in samples across the route and decoupled the relationship between PIC and coccolithophore abundance. Presence of lithogenic material and Mesozoic fossil coccoliths in the samples implies sediment resuspension. These findings question the suitability of PIC as a proxy for coccolithophore abundance and dynamics, particularly on or near continental shelves, where the resuspension and lateral transport of lithogenic calcite may decouple the potential relationship between PIC and coccolithophores.

Coccolithophores are the most abundant calcifying phytoplankton in the ocean, constituting around 1–20% of phytoplankton biomass (Poulton et al. 2006; Poulton et al. 2007; Poulton et al. 2010) and responsible for around half of oceanic carbonate production (Broecker and Clark 2009). Coccolithophores form layers of extracellular plates of calcium carbonate (calcite) known as coccoliths, although the function of these coccoliths is not fully understood (Paasche 2002). *Emiliania huxleyi* is the most abundant and well-documented species of coccolithophore, widespread at subpolar latitudes and forming intensive blooms that turn surface waters a milky white (Holligan et al. 1983).

Coccolithophores are of considerable interest as the production of coccolith calcite during blooms is a potentially large source of $CO_2$ (Paasche 2002), while export of coccoliths may have a significant role in the export of organic matter—a "ballast effect" (Klaas and Archer 2002). The balance of these two roles has considerable ramifications for the global carbon cycle. The expected response of calcifying plankton to ocean acidification is presently unclear, with culture studies producing conflicting responses of coccolithophores to increasing levels of $CO_2$ (Riebesell et al. 2000; Iglesias-Rodriguez et al. 2008; Langer et al. 2009). An ability to accurately characterize naturally occurring coccolithophore populations is essential for assessing their response to ocean acidification over time.

In the context of this work, the terms calcium carbonate concentration, calcite concentration, and particulate inorganic carbon (PIC) concentration are interchangeable in that they are all used to describe the same pool of particulate calcium carbonate. The concentration of PIC is often used as a proxy for coccolithophore abundance, with the PIC concentration deriving from coccolithophores referred to as coccolith calcite. Chemical techniques for measuring PIC are considered the most accurate methods of determining PIC (Balch and Fabry 2008). Despite widespread use (Fernandez et al. 1993; Balch et al. 2000; Poulton et al. 2010), PIC as a proxy for coccolithophore abundance has the serious drawback that coccolithophores are not the only oceanic source of PIC. Other pelagic calcifiers (foraminifera and pteropods) and detrital matter are known to contribute significantly to PIC (Broecker and Clark 2009; Poulton et al. 2010)—a salient point that is often forgotten.

The Southampton FerryBox project ran almost continuously from April 2002 to September 2010, on the MS *Pride of Bilbao*, a ferry operated by P&O European Ferries (Hydes et al. 2003). The route taken by the *Pride of Bilbao* between Portsmouth (50°81′N, 1°11′W) and Bilbao (43°34′N, 3°03′W) covered ~ 1000 km and a number of distinct oceanographic regions (Bargeron et al. 2006). These oceanographic regions can be broadly divided into three sections: the English Channel, the continental shelf from the entrance of the English Channel to the shelf break, and the deep waters of the Bay of Biscay (Fig. 1).

The central English Channel is shallow (< 50 m) and well mixed throughout the year, while to the west the channel deepens, with summer stratification (Borges and Frankignoulle 2003). In the Ushant region, to the west of Brittany, the mixed shallow water of the English Channel meets the deeper stratified water of the North Atlantic Ocean, forming the Ushant tidal front system (Bargeron et al. 2006). From Ushant, the transect crossed the shelf break

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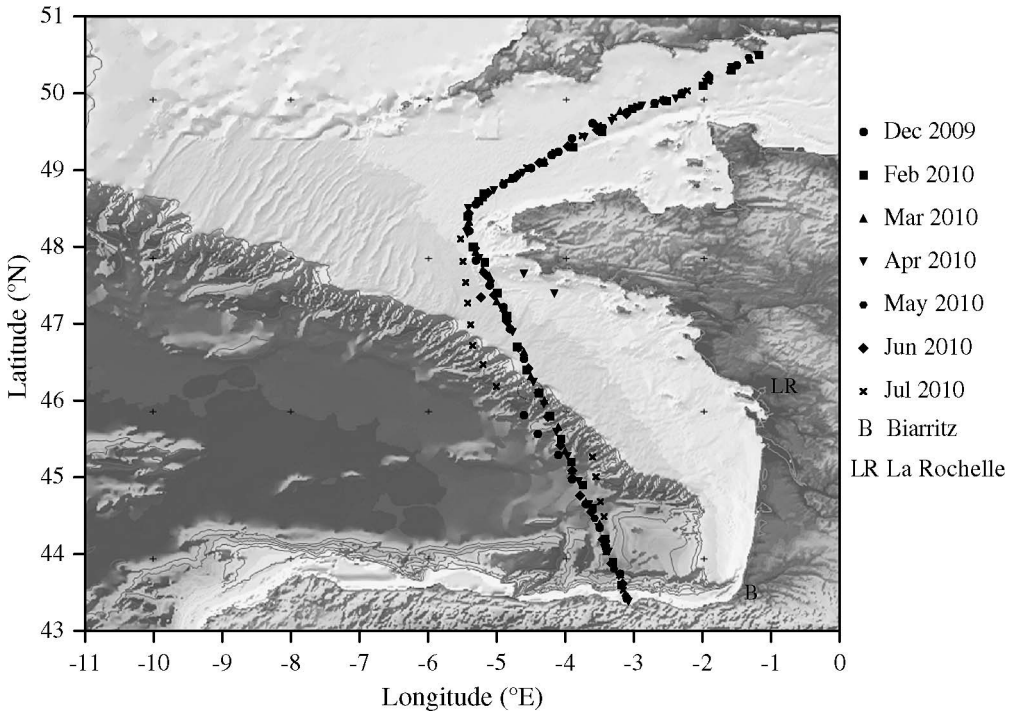


Fig. 1. Sampling stations from the Bay of Biscay.

with a large transition in depth from shallow shelf waters at 200 m to the deep waters (> 4000 m) of the Bay of Biscay (Fig. 1). After this, the route reached the Iberian shelf, a complex environment of underwater canyons, prior to entering Bilbao Harbour (Bargeron et al. 2006).

From 2006 to 2010, as part of the Southampton FerryBox project, the population of coccolithophores and their relationship with the environment were intensively studied: *E. huxleyi* abundance was determined using scanning electron microscopy (SEM), and PIC was measured using inductively coupled plasma optical emission spectroscopy (ICP-OES). The motivation for the present study came from earlier work on the same program; for samples collected between March and July 2009, estimates of the calcite contribution of coccospheres of *E. huxleyi* (assuming 15 coccoliths per cell [Paasche 2002], 0.035 pmol C per coccolith [Poulton et al. 2010]) found that *E. huxleyi* coccosphere calcite accounted for an average of only 8% of chemically determined PIC. Furthermore, PIC was higher than concurrent estimates of coccolith calcite in all samples (Fig. 2). It is hypothesized that this discrepancy could be due to one or more of the following candidate factors: (1) inaccurate measurements of PIC or coccolith abundance; (2) ignoring the calcite contribution from detached coccoliths of *E. huxleyi*; (3) other calcite-containing organisms; (4) the presence of species of coccolithophore other than *E. huxleyi*; and/or (5) the presence of suspended sediment (lithogenic calcite sources). Observed discrepancies between PIC and coccolithophore abundance are not limited to the Bay of Biscay, having previously been noted, but unexplained, in the equatorial Pacific, the Arabian Sea, and the Gulf of Maine (Balch and Kilpatrick 1996; Balch et al. 2000; Balch et al. 2008).

Despite being the most numerically abundant species of coccolithophore (Paasche 2002), the individual coccolith calcite content of *E. huxleyi* is relatively small, and other species may play a more significant role in calcite export (Ziveri et al. 2007). Furthermore, coccolithophores are not the only planktonic calcifying organisms. The tests of

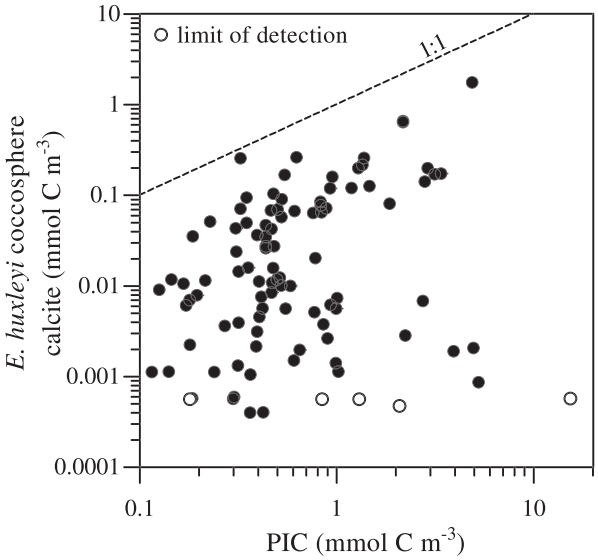


Fig. 2. Relationship between *Emiliania huxleyi* coccosphere calcite and particulate inorganic carbon (PIC) from samples taken between March and July 2009. Dashed line indicates a 1 : 1 ratio. In samples ($n = 5$) where PIC was measured and no coccospheres were present, the limit of detection for coccospheres was calculated from the volume considered in the 225 fields of view and is included in the plot.

pelagic foraminifera are formed of extremely pure calcite (Lea 1999) and have shell weights four to five orders of magnitude larger than coccosphere weights (Young and Ziveri 2000; Bijma et al. 2002), although foraminifera are relatively rare compared with coccolithophores (Baumann 2004). The purpose of this study was to determine the factors contributing to the observed lack of relationship between *E. huxleyi* abundance and PIC in the waters of the Bay of Biscay.

## Methods

*Sampling*—Sampling was performed approximately once a month from December 2009 to July 2010, with the exception of January, aboard the MS *Pride of Bilbao*. All crossings followed approximately the same route (Fig. 1). Only four samples were collected in May, and these have not been considered when determining coccolith calcite as a proportion of total PIC. Water samples were collected from the seawater intake for the ship's engine water cooling system (intake 5 m) through a stainless steel tap fitted with Tygon® tubing. Samples were collected on an hourly basis and prefiltered through a 200-$\mu$m mesh filter to remove zooplankton and adult foraminifera. Size-fractionated samples for PIC determination were collected for eight samples; two samples were collected in parallel with one sample prefiltered through a 50-$\mu$m mesh filter.

*Coccolithophore enumeration and identification*—Samples were collected for the determination of coccolithophore and coccolith abundance and species identification. Water samples (0.15–1.0 L) were filtered under low pressure ($\sim$ 400 mm Hg) using a two-stage vacuum filtration unit through polycarbonate membrane filters (25-mm diameter, 1.2-$\mu$m or 0.8-$\mu$m pore size) or cellulose nitrate filters (25-mm diameter, 0.45-$\mu$m or 0.8-$\mu$m pore size), with backing circles of nylon mesh (25-mm diameter, 50-$\mu$m pore size). Filters were rinsed with trace ammonium solution (pH $\sim$ 10) and air dried for 3–4 h before being stored in Millipore PetriSlides. Radial subsections of the polycarbonate membrane filters were fixed to aluminum stubs, sputter-coated with gold using a Hummer VI-A gold coater, and examined using a Leo 1450VP scanning electron microscope ($\times$5000).

Cells and coccoliths were identified to species level following Young et al. (2003). For each species, either 225 fields of view (FOVs) or 500 detached coccoliths (whichever came first) were counted per filter; cells were counted in the same FOVs in which the coccoliths were counted. The limit of detection was estimated to be 0.44–2.42 cells mL$^{-1}$ based on no cells being observed in the 225 FOVs. The concentration of non–*E. huxleyi* coccospheres was generally very low ($<$ 20 cells mL$^{-1}$) and was not considered separately from detached coccoliths of the same species, with the number of coccoliths per coccosphere estimated for each coccosphere by visual inspection (cf. Boeckel and Baumann 2008).

Coccolith calcite was estimated following Young and Ziveri (2000), measuring the distal shield lengths (maximum linear dimension of the coccolith) of up to 50 coccoliths per species using ImageJ (http://rsbweb.nih.gov/ij/). For those species whose calcite content was undefined in Young and Ziveri (2000), we calculated estimates using the author's values for "typical coccoliths," or coccoliths of a species with a similar shape. This optical technique for estimating coccolith calcite was compared with PIC measurements from a cultured strain of *E. huxleyi*. Coccolith calcite for *E. huxleyi* was estimated as 0.030–0.035 pmol C cocccolith$^{-1}$ using SEM measurements of distal shield length and 0.031–0.075 pmol C coccolith$^{-1}$ from the regression of PIC measurements and coccolith abundance. Generally coccolith calcite estimates from SEM measurements averaged $\sim$ 69% of measured PIC (44–111%) in the cultures, supporting the validity of the technique. The main sources of error in estimating coccolith calcite are from uncertainty in chosen coccolith shape (ks, $\sim$ 20%, Young and Ziveri 2000), error in SEM measurement of distal shield lengths (we estimate $\sim$10%), and error associated with the natural variation in coccolith distal shield for each species (e.g., *E. huxleyi* coccoliths varied from 1.9 to 5.5 $\mu$m in diameter in this study).

The SEM stubs were further examined for calcium using an SEM energy dispersive spectra (EDS) system; elemental point analysis and x-ray elemental mapping were undertaken using a Princeton Gamma-Tech interactive multimodal information extraction (PGT IMIX) light element detector.

Cellulose nitrate filters were mounted on slides using No. 75 Norland Optical Adhesive (Poulton et al. 2010) and examined under cross-polarized light ($\times$400 and $\times$1000, oil immersion) using either an Olympus BH2 microscope or a Brunel SP-300-XP Polarizing microscope with a Nikon Coolpix 6000 digital camera. Cells and coccoliths were identified but not enumerated using polarizing light microscopy.

*Particulate inorganic carbon*—Measurements of PIC using an ICP-OES were made on seawater samples (0.15–0.50 L) filtered onto polycarbonate membrane filters (25-mm diameter, 0.2-$\mu$m or 0.8-$\mu$m pore size), rinsed with trace ammonium solution, and extracted using 0.5 mL of 0.4 mol L$^{-1}$ nitric acid. During the course of the study, filter pore sizes were standardized from 0.2 $\mu$m to 0.8 $\mu$m. The effect of differing filter pore size was not significant (ANOVA, $F_{2,3} = 0.56$, $p = 0.62$). $Ca^{2+}$ concentrations were determined using a Perkin Elmer Optima 4300CV ICP-OES with the average relative standard deviation (RSD) of replicate $Ca^{2+}$ measurements $\sim$ 7% (range 5–9%).

## Results

*Particulate inorganic carbon*—During the course of the sampling, surface-water concentrations of PIC, as measured by ICP-OES, demonstrated a large temporal and spatial variability, ranging from 0.07 mmol C m$^{-3}$ (April, Bay of Biscay) to 11.7 mmol C m$^{-3}$ (March, central English Channel). The concentration of PIC was generally highest at the beginning and end of the route, with the highest concentrations observed in the central English Channel ($>$ 49°N; Fig. 3). A marked decrease in average PIC concen-

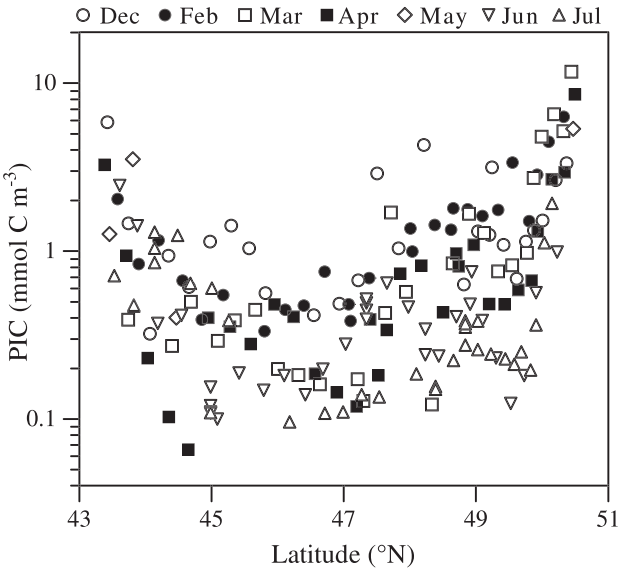


Fig. 3. Particulate inorganic carbon (PIC) concentration along the sampling route.

tration was observed from March to June (Table 1), coinciding with reduced PIC concentrations in the English Channel; the maximum PIC concentration in the English Channel in June (0.99 mmol C $m^{-3}$) was an order of magnitude smaller than that in March (11.7 mmol C $m^{-3}$). PIC minimums were generally observed in the oceanic waters of the Bay of Biscay (45–46.5°N). The difference between the PIC measurements of the size-fractionated samples was not statistically significant (Student's *t*-test, $t = 0.56$, df = 7, $p = 0.60$).

*Coccolithophore abundance—E. huxleyi* was the most abundant species of coccolithophore; detached coccoliths of *E. huxleyi* were present in all samples (64–35,925 coccoliths $mL^{-1}$; Fig. 4B) and coccospheres were present in almost all samples (Fig. 4A). The highest concentration of coccoliths and coccospheres was collected in an *E. huxleyi* bloom (1020 cells $mL^{-1}$) in May 2010. This study focused on non-bloom concentrations of coccolithophores, and, where appropriate, the bloom data were discarded or analyzed separately. The average ratio of detached coccoliths to cells for *E. huxleyi* was 152 (range from 13 to 542), exceeding the ratio observed in many field and

Table 1. Monthly averages of total PIC and the fraction of PIC accounted for by *Emiliania huxleyi* and all coccolithophore species (including *E. huxleyi*).

| Month | Total PIC (mmol C $m^{-3}$) | | Coccolith calcite fraction (%) | | | |
|---|---|---|---|---|---|---|
| | | | *E. huxleyi* | | All | |
| Dec | 1.59 | (0.32–5.84) | 4 | (0–12) | 6 | (1–14) |
| Feb | 1.53 | (0.33–6.30) | 6 | (1–18) | 10 | (1–36) |
| Mar | 1.66 | (0.12–11.7) | 7 | (0–23) | 11 | (1–31) |
| Apr | 1.02 | (0.07–8.56) | 12 | (0–31) | 19 | (0–56) |
| Jun | 0.48 | (0.10–2.45) | 7 | (0–37) | 10 | (0–38) |
| Jul | 0.47 | (0.10–1.92) | 5 | (1–27) | 8 | (1–31) |
| Mean | 1.08 | (0.07–11.7) | 7 | (0–37) | 11 | (0–56) |

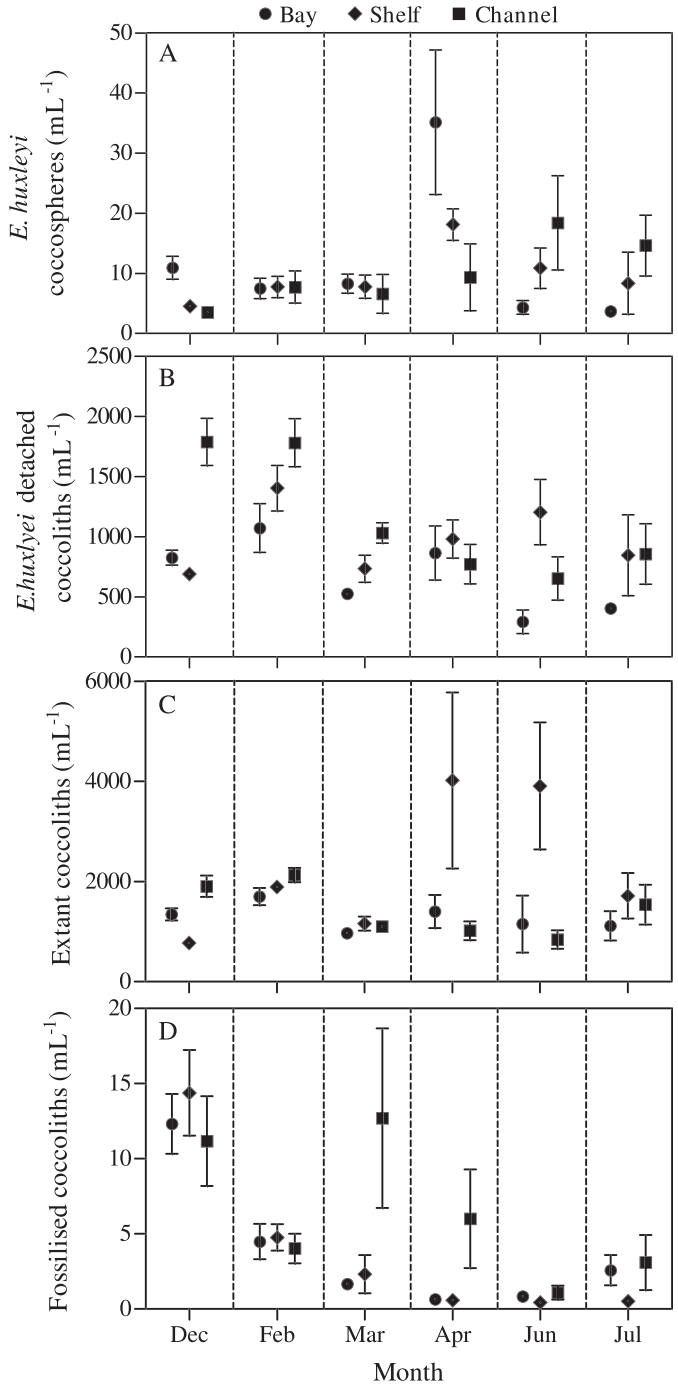


Fig. 4. Concentrations of (A) *Emiliania huxleyi* coccospheres, (B) *E. huxleyi* detached coccoliths, (C) detached extant coccoliths plus extant coccosphere coccoliths (excluding *E. huxleyi*), and (D) fossil coccoliths, averaged over the three oceanographic regions. Error bars are ± 1 standard error.

culture studies (Paasche 2002; Harlay et al. 2010; Poulton et al. 2010).

In addition to *E. huxleyi*, other species of coccolithophore were also present. Species observed in significant concentrations (> 10% total coccolith calcite) were: *Coccolithus pelagicus* ssp. *braarudii*, *Calcidiscus leptoporus*, *Helicosphaera carteri*, *Gephyrocapsa muellerae*, *Syracos-*

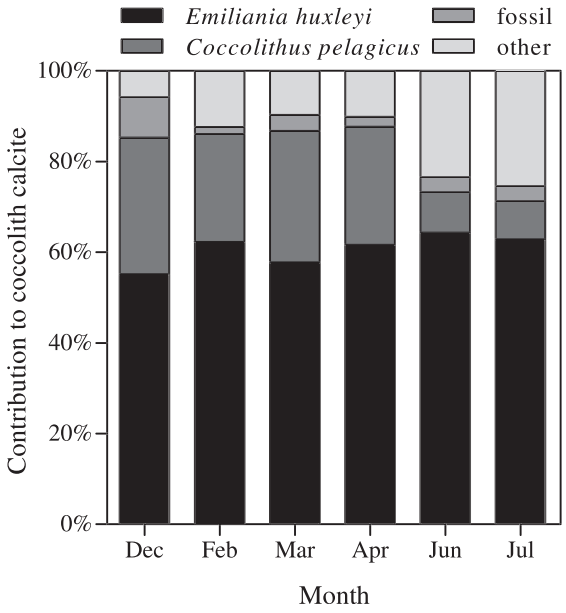


Fig. 5. Contribution of the major coccolithophore species to total coccolith calcite.

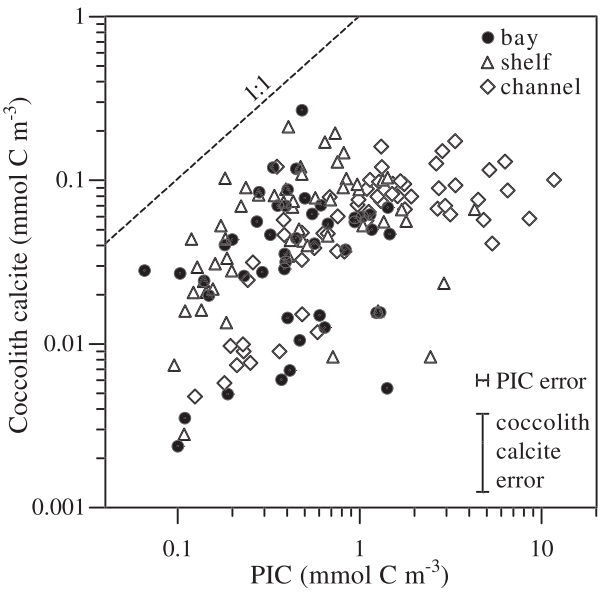


Fig. 6. Relationship between total coccolith calcite and particulate inorganic carbon (PIC). Dashed line indicates a 1 : 1 ratio. Coccolith calcite error is estimated as 50% and PIC error is the relative standard deviation (RSD, ~ 7%).

*phaera ossa*, *Syracosphaera pulchra* and its holococcolith (HOL) phase *S. pulchra* HOL *oblonga*, and another holococcolithophore *Coronosphaera mediterranea* HOL *wettsteinei*. Small (< 2 μm) species of *Syracosphaera* were in abundance (0–1200 coccoliths $mL^{-1}$) but could not be identified. The largest concentration of a coccolithophorid species other than *E. huxleyi* was found on the continental shelf in April, when *Syracosphaera bannockii* coccospheres (J. Young pers. comm.) peaked at 432 cells $mL^{-1}$. These concentrations far exceeded the highest concentration of other non–*E. huxleyi* species throughout the study.

The concentration of detached coccoliths plus coccosphere coccoliths (excluding *E. huxleyi* coccospheres) in non-bloom conditions ranged from 110 to 19,690 $mL^{-1}$ (Fig. 4C). The high number of extant coccoliths on the shelf in April is due to the *S. bannockii* bloom, while the high coccolith concentrations on the shelf in June derived mainly from holococcoliths. The extant coccolith calcite concentration ranged from 0.002 to 0.27 mmol C $m^{-3}$, averaging 0.057 ± 0.003 mmol C $m^{-3}$ over the study period. *E. huxleyi* was the main contributor of extant coccolith calcite over the study period (60–67%, Fig. 5), although it did not dominate every individual sample (range 10–99%).

*Fossil coccoliths*—Fossilized coccoliths of Mesozoic age (250–67 Myr), predominantly *Watznaueria* spp. (J. Young pers. comm.), were found in significant numbers across the route, with the highest concentrations observed in the English Channel (Fig. 4D). December was the only month in which fossilized coccoliths were observed in every sample (range 0.9–38 coccoliths $mL^{-1}$), and it had the highest concentration of fossils averaged across the route (12 coccoliths $mL^{-1}$). The decrease in fossil coccolith concentrations from December to February may be due to a decline in winter mixing, although we have no in situ data to support this. The highest individual concentration of fossils occurred in the English Channel in March (55 coccoliths $mL^{-1}$). The concentration of fossil coccoliths was much lower than that of extant coccolithophores. However, the estimated coccolith calcite contribution from fossil coccoliths was significant, reaching a maximum of 39% of total coccolith calcite, with the highest monthly average contribution in December (9%, Fig. 5). Accounting for both extant and fossil coccolithophores increased the average coccolith calcite concentration from 0.057 to 0.059 ± 0.003 mmol C $m^{-3}$ over the study period, while *E. huxleyi* remained the main contributor of coccolith calcite (55–64%, Fig. 5).

*Coccolith calcite as a proportion of total PIC*—If coccolith calcite was the principal component of PIC, a strong autocorrelation should exist between PIC and coccolith calcite. However, this was not observed; PIC was greater than coccolith calcite in all samples (Fig. 6), which implies that coccolith calcite was not the main driver of PIC. Coccoliths of *E. huxleyi* accounted for an average of only 7% (range 0.1–37%; Table 1) of total PIC. Accounting for all species of coccolithophore present increased the fraction of total PIC deriving from coccoliths to an average of 11% (range 0.3–56%; Table 1), which left ~ 89% of PIC unaccounted for. The smallest average discrepancy over one cruise was observed in April (19% of total PIC). Both the largest and smallest discrepancies between total PIC and total coccolith calcite, averaged over each region, were observed in the Bay of Biscay (26.8% in April and 2.8% in July).

*Particulate matter*—Under cross-polarized light, coccolithophore cells and detached coccoliths were identified by their distinctive birefringent patterns. Additional birefrin-

gence apparently not deriving from coccoliths was also observed in all samples (Fig. 7A). For samples with similar coccolith calcite estimates (0.101 and 0.103 mmol C $m^{-3}$) but differing PIC concentrations (0.18 and 11.7 mmol C $m^{-3}$), an increased level of birefringence was observed in those samples with higher PIC. High levels of non-coccolith particulate matter were identified in samples with the highest PIC concentrations (Fig. 7B). Elemental analysis identified calcium as a major constituent mineral in this particulate matter (Fig. 7C), whereas coccoliths did not dominate the total calcium signal in the image map.

## Discussion

As observed previously (Fig. 2), PIC exceeded coccolith calcite in all samples (Fig. 6). Despite dominance by *E. huxleyi* of coccolith calcite, accounting for only its contribution to PIC resulted in a significant underestimation of total coccolith calcite. Other coccolithophores contributed significantly to coccolith calcite in this study, and it is therefore essential to account for all species of coccolithophore present, particularly as *E. huxleyi* is only a minor contributor to the calcite flux out of the surface ocean (Ziveri et al. 2007). In this study, however, the discrepancy from considering only *E. huxleyi* is overshadowed, as, despite attempting to account for all species of coccolithophore present, the discrepancy between PIC and coccolith calcite remains significant (Fig. 6). The lack of a significant difference between the size-fractionated samples suggests no significant contribution from the $>$ 50-$\mu$m fraction to PIC. These findings are generally consistent with sediment trap records from the Bay of Biscay, where the coccolith contribution was estimated to be only 20% of the total PIC flux and contribution from other calcareous plankton was negligible (Beaufort and Heussner 1999).

*Role of lithogenic PIC*—Birefringence levels not attributable to coccoliths (Fig. 7A) suggested the presence of a non-coccolith source of calcite. In samples with elevated birefringence, high levels of particulate matter, not identifiable as coccoliths, were identified in SEM images (Fig. 7B), suggesting that the source of the increased birefringence was unidentified particulate matter (Fig. 7C). This was confirmed using SEM-EDS x-ray elemental mapping, demonstrating that the sources of particulate calcium were extensive across the FOV. The irregular structure and shape of the particulate matter suggested a lithogenic rather than biogenic origin. Dolomite (calcium magnesium carbonate—a lithogenic mineral, Gaines et al. 1997), was identified using detailed point analysis of a sample (R. Pearce pers. comm.), confirming a lithogenic origin for some of the particulate matter. The calcium signal detected from the particulate matter can be broadly partitioned into two categories: large pieces of calcium carbonate ($>$ 10-$\mu$m diameter) and small pieces with a much weaker calcium signal. Despite a much weaker calcium signal, the abundance of the small particles across the image suggests that their contribution is as important as the larger particles.

The presence of significant amounts of lithogenic PIC throughout the study area explains the discrepancy between coccolith calcite and PIC and is consistent with sediment trap records in the Bay of Biscay, which found that the bulk of the carbonate flux was detrital with a large lithogenic mineral fraction (Beaufort and Heussner 1999). The general trend in PIC is driven by the distribution of the lithogenic material. The highest levels of lithogenic PIC were observed in the central English Channel, as were the highest concentrations of PIC and some of the highest discrepancies between coccolith calcite and PIC.

The concentration of lithogenic PIC cannot be quantified as it can only be measured chemically, and such techniques cannot discriminate between biogenic and lithogenic sources of PIC. This fundamental problem in chemical measurements results in an overestimation of the coccolithophore contribution if other PIC contributors are present; in this study, the coccolith contribution has been overwhelmed by the lithogenic contribution, such that PIC cannot be used to infer the abundance of coccospheres and coccoliths.

*Sources of lithogenic material to the Bay of Biscay*—The presence of lithogenic material in surface-water samples implies resuspension of sediment. Further evidence for this comes from the presence of fossilized coccoliths and an abundance of reworked biogenic material, including diatoms and coccoliths. Increased levels of PIC, attributed to suspended lithogenic sediments, have previously been observed on the eastern side of the Gulf of Maine (Balch et al. 2008). The Gulf of Maine is a semi-enclosed shelf sea (Graziano et al. 2000) with several calcium carbonate beaches identified as the apparent source of lithogenic PIC (Balch et al. 2008). Our study area, however, covers a wide range of oceanographic regions with no obvious single source of lithogenic PIC.

Much of the coast of the Bay of Biscay is formed of rocks Cenozoic in age, with Mesozoic areas found between the Gironde and La Rochelle, between Biarritz and northeast Spain (Fig. 1), and within the English Channel (Choubert and Faure-Muret 1976). The presence of Mesozoic fossilized coccoliths throughout the route suggests sediment transport from these sources. Off-shelf transport of sediment within the Bay of Biscay has been intensively studied (Durrieu De Madron et al. 1999). Rivers and coastal runoff discharge large quantities of suspended particulate matter into the surface layer of the continental shelf region; the Gironde estuary supplies $\sim$ 70% of this lithogenic input (Durrieu De Madron et al. 1999). Lateral transport of lithogenic particulate matter off of the continental shelf has also been observed in sediment traps in the Bay of Biscay (Beaufort and Heussner 1999) and is considered an important component of the global oceanic carbon cycle through export of sediment to depth (Hwang et al. 2010). Elsewhere on the transect, apart from the Bay of Biscay, lateral transport is likely to be driven by the outflow from the well-mixed central English Channel (Borges and Frankignoulle 2003). Where the English Channel outflow meets the waters of the North Atlantic,

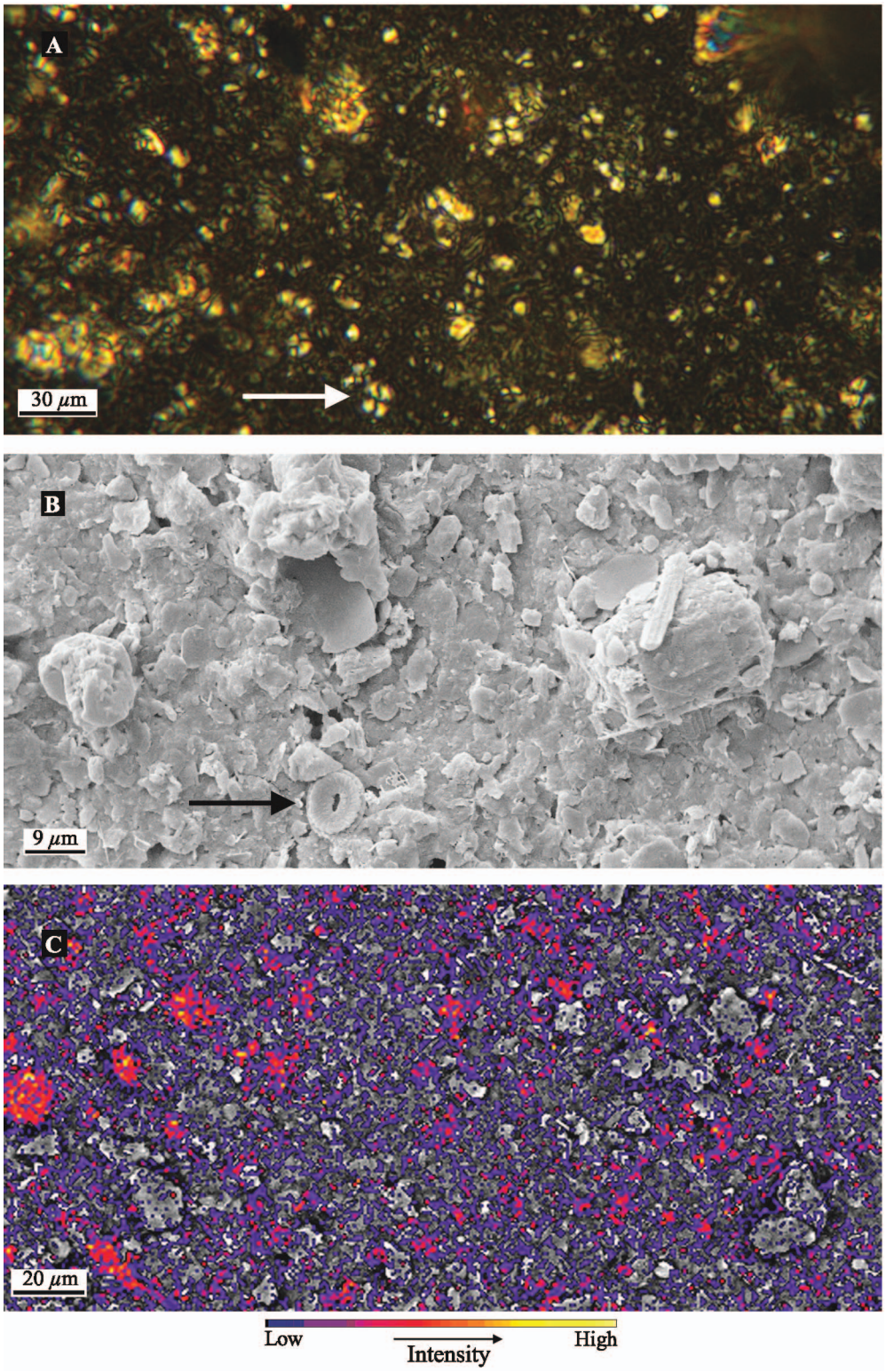


Fig. 7. Images of the particulate material in one sample collected during this study using different microscopic techniques: (A) birefringent image (×400) with the arrow highlighting a distinctive coccolith pattern, (B) SEM image of a Mesozoic fossil coccolith (*see* arrow) and an abundance of particulate matter (×5000), and (C) x-ray elemental map of calcium overlaid on an SEM image (×1500).

PIC (Fig. 3) and fossilized coccolith concentrations (Fig. 4D) generally decreased.

Whereas Durrieu De Madron et al. (1999) identified lateral transport to deep waters, our study has unexpectedly observed lateral transport to surface waters distant from the shelf. The increased concentration of fossilized coccoliths in the Bay of Biscay in the winter may be due to winter intensification of resuspension through reduced stratification and increased wind strength. It should be noted that the study area remains relatively close to the continental shelf margin (< 150 km); resuspended matter does not have to be transported far off the shelf to be observed in our study.

*Implications for PIC measurements*—The effect of the presence of lithogenic PIC in samples, elevating total PIC and decoupling PIC concentrations from coccolith calcite concentrations, questions the suitability of chemically determined PIC measurements as a robust proxy for observing the abundance of living coccolithophores. The decoupling we found may result in erroneous conclusions being made about the density of coccolithophores and their coccoliths. Many in situ studies on coccolithophores have concentrated on *E. huxleyi* and their blooms. In bloom conditions, coccolithophores dominate the PIC signal, with strong correlations found between PIC and the number of coccoliths in blooms in the northeast Atlantic (Fernandez et al. 1993), the western English Channel (Garcia-Soto et al. 1995), and the North Sea (Van Der Wal et al. 1995). Within coccolithophore blooms, PIC is dominated by detached coccoliths and aggregates (Fernandez et al. 1993; Van Der Wal et al. 1995) such that PIC can be a robust proxy for detached coccolith abundance.

Our study, however, was in non-bloom conditions; thus, the implications for PIC measurements in non-bloom conditions must be examined. In the Gulf of Maine, suspended lithogenic calcite was identified as the probable cause of the high PIC concentrations in the far eastern side of the Gulf, overwhelming the coccolithophore contribution (Balch et al. 2008). However, in the remainder of the Gulf, the source of PIC was attributed to coccolithophores (Balch et al. 2008); and a significant correlation between PIC and coccoliths has also been observed in non-bloom conditions in the central Iceland Basin ($r = 0.78$, Poulton et al. 2010). Very few studies have been made of coccolithophore dynamics in non-bloom conditions, and as such it is not possible to extrapolate globally the influence of lithogenic calcite on the validity of PIC as a proxy. However, the results from our study and the Gulf of Maine suggest that, particularly on or near continental shelves, PIC may be an unreliable method for characterizing coccolithophore populations and should not be used without examining the samples for the influence of lithogenic calcite.

A significant portion of the oceanic PIC standing stock is relatively refractory, whether formed of detached coccoliths and aggregates (Balch et al. 2005; Poulton et al. 2007) or resuspended lithogenic calcite (this study). Therefore, PIC concentrations give little information about coccolithophore community dynamics and are a poor proxy for oceanic coccolithophore abundance or calcite production (Poulton et al. 2007). Accurate measurements of calcification rates, such as those obtained with the micro-diffusion technique (Balch et al. 2000), combined with microscope counts of coccolithophores and detached coccoliths, offer the potential to distinguish between biogenic and lithogenic calcite signals and provide an insight into the dynamics of coccolithophore populations in the open ocean and coastal waters.

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## References

Balch, W. M., D. T. Drapeau, B. C. Bowler, E. S. Booth, L. A. Windecker, and A. Ashe. 2008. Space-time variability of carbon standing stocks and fixation rates in the Gulf of Maine, along the GNATS transect between Portland, ME, USA, and Yarmouth, Nova Scotia, Canada. J. Plankton Res. **30:** 119–139, doi:10.1093/plankt/fbm097

———, ———, and J. J. Fritz. 2000. Monsoonal forcing of calcification in the Arabian Sea. Deep-Sea Res. II **47:** 1301–1337, doi:10.1016/S0967-0645(99)00145-9

———, and V. J. Fabry. 2008. Ocean acidification: Documenting its impact on calcifying phytoplankton at basin scales. Mar. Ecol. Prog. Ser. **373:** 239–247, doi:10.3354/meps07801

———, H. R. Gordon, B. C. Bowler, D. T. Drapeau, and E. S. Booth. 2005. Calcium carbonate measurements in the surface global ocean based on Moderate-Resolution Imaging Spectroradiometer data. J. Geophys. Res. **110:** C07001, doi:10.1029/2004JC002560

———, and K. Kilpatrick. 1996. Calcification rates in the equatorial Pacific along 140 degrees W. Deep-Sea Res. II **43:** 971–993, doi:10.1016/0967-0645(96)00032-X

Bargeron, C. P., D. J. Hydes, D. K. Woolf, B. A. Kelly-Gerreyn, and M. A. Qurban. 2006. A regional analysis of new production on the northwest European shelf using oxygen fluxes and a ship-of-opportunity. Estuar. Coast. Shelf Sci. **69:** 478–490, doi:10.1016/j.ecss.2006.05.015

Baumann, K.-H. 2004. Importance of size measurements for coccolith carbonate flux estimates. Micropaleontology **50:** 35–43, doi:10.2113/50.Suppl_1.35

Beaufort, L., and S. Heussner. 1999. Coccolithophorids on the continental slope of the Bay of Biscay—production, transport and contribution to mass fluxes. Deep-Sea Res. II **46:** 2147–2174, doi:10.1016/S0967-0645(99)00058-2

Bijma, J., B. Honisch, and R. E. Zeebe. 2002. Impact of the ocean carbonate chemistry on living foraminiferal shell weight: Comment on "Carbonate ion concentration in glacial-age deepwaters of the Caribbean Sea" by W. S. Broecker and E. Clark. Geochem. Geophys. Geosyst. **3:** 1064, doi:10.1029/2002GC000388

Boeckel, B., and K.-H. Baumann. 2008. Vertical and lateral variations in coccolithophore community structure across the subtropical frontal zone in the South Atlantic Ocean. Mar. Micropaleontol. **67:** 255–273, doi:10.1016/j.marmicro.2008.01.014

Borges, A. V., and M. Frankignoulle. 2003. Distribution of surface carbon dioxide and air-sea exchange in the English Channel and adjacent areas. J. Geophys. Res. **108:** 3140, doi:10.1029/2000JC000571

Broecker, W., and E. Clark. 2009. Ratio of coccolith $CaCO_3$ to foraminifera $CaCO_3$ in late Holocene deep sea sediments. Paleoceanography **24:** PA3205, doi:10.1029/2009PA001731

Choubert, G., and A. Faure-Muret. 1976. Atlas géologique du monde. UNESCO, [Geological world atlas.]

Durrieu De Madron, X., P. Castaing, F. Nyffeler, and T. Courp. 1999. Slope transport of suspended particulate matter on the Aquitanian margin of the Bay of Biscay. Deep-Sea Res. II **46:** 2003–2027, doi:10.1016/S0967-0645(99)00053-3

Fernandez, E., P. Boyd, P. M. Holligan, and D. S. Harbour. 1993. Production of organic and inorganic carbon within a large-scale coccolithophore bloom in the northeast Atlantic Ocean. Mar. Ecol. Prog. Ser. **97:** 271–285, doi:10.3354/meps097271

Gaines, R. V., H. C. Skinner, E. E. Foord, B. Mason, and A. Rosenzweig. 1997. Dana's new mineralogy, 8th ed. Wiley.

Garcia-Soto, C., E. Fernandez, R. D. Pingree, and D. S. Harbour. 1995. Evolution and structure of a shelf coccolithophore bloom in the western English Channel. J. Plankton Res. **17:** 2011, doi:10.1093/plankt/17.11.2011

Graziano, L. M., W. M. Balch, D. Drapeau, B. C. Bowler, R. Vaillancourt, and S. Dunford. 2000. Organic and inorganic carbon production in the Gulf of Maine. Cont. Shelf Res. **20:** 685–705, doi:10.1016/S0278-4343(99)00091-6

Harlay, J., and others. 2010. Biogeochemical study of a coccolithophore bloom in the northern Bay of Biscay (NE Atlantic Ocean) in June 2004. Prog. Oceanogr. **86:** 317–336, doi:10.1016/j.pocean.2010.04.029

Holligan, P. M., M. Viollier, D. S. Harbour, P. Camus, and M. Champagne-Philippe. 1983. Satellite and ship studies of coccolithophore production along a continental shelf edge. Nature **304:** 339–342, doi:10.1038/304339a0

Hwang, J., E. R. M. Druffel, and T. I. Eglinton. 2010. Widespread influence of resuspended sediments on oceanic particulate organic carbon: Insights from radiocarbon and aluminum contents in sinking particles. Glob. Biogeochem. Cycles **24:** GB4016, doi:10.1029/2010GB003802

Hydes, D. J., and others. 2003. Use of a Ferry-Box system to look at shelf sea and ocean margin processes. Elsevier Oceanogr. Ser. **69:** 297–303, doi:10.1016/S0422-9894(03)80048-X

Iglesias-Rodriguez, M. D., and others. 2008. Phytoplankton calcification in a high-$CO_2$ world. Science **320:** 336, doi:10.1126/science.1154122

Klaas, C., and D. E. Archer. 2002. Association of sinking organic matter with various types of mineral ballast in the deep sea: Implications for the rain ratio. Glob. Biogeochem. Cycles **16:** 1116, doi:10.1029/2001GB001765

Langer, G., G. Nehrke, I. Probert, J. Ly, and P. Ziveri. 2009. Strain-specific responses of *Emiliania huxleyi* to changing seawater carbonate chemistry. Biogeosciences **6:** 2637–2646, doi:10.5194/bg-6-2637-2009

Lea, D. W. 1999. Trace elements in formainiferal calcite, p. 259–277. *In* B. K. S. Gupta [ed.], Modern foraminifera, 1st ed. Kluwer.

Paasche, E. 2002. A review of the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae), with particular reference to growth, coccolith formation, and calcification-photosynthesis interactions. Phycologia **40:** 503–529, doi:10.2216/i0031-8884-40-6-503.1

Poulton, A. J., T. R. Adey, W. M. Balch, and P. M. Holligan. 2007. Relating coccolithophore calcification rates to phytoplankton community dynamics: Regional differences and implications for carbon export. Deep-Sea Res. II **54:** 538–557, doi:10.1016/j.dsr2.2006.12.003

———, A. Charalampopoulou, J. R. Young, G. A. Tarran, M. I. Lucas, and G. D. Quartly. 2010. Coccolithophore dynamics in non-bloom conditions during late summer in the central Iceland Basin (July–August 2007). Limnol. Oceanogr. **55:** 1601–1613, doi:10.4319/lo.2010.55.4.1601

———, R. Sanders, P. M. Holligan, M. C. Stinchcombe, T. R. Adey, L. Brown, and K. Chamberlain. 2006. Phytoplankton mineralization in the tropical and subtropical Atlantic Ocean. Glob. Biogeochem. Cycles **20:** GB4002, doi:10.1029/2006GB002712

Riebesell, U., I. Zondervan, B. Rost, P. D. Tortell, R. E. Zeebe, and F. M. M. Morel. 2000. Reduced calcification of marine plankton in response to increased atmospheric $CO_2$. Nature **407:** 364–367, doi:10.1038/35030078

Van Der Wal, P., R. S. Kempers, and M. J. W. Veldhuis. 1995. Production and downward flux of organic-matter and calcite in a North-Sea bloom of the coccolithophore *Emiliania huxleyi*. Mar. Ecol. Prog. Ser. **126:** 247–265, doi:10.3354/meps126247

Young, J. R., M. Geisen, L. Cros, A. Kleijne, C. Sprengel, I. Probert, and J. Ostergaard. 2003. A guide to extant coccolithophore taxonomy. J. Nannoplankton Res., Special **1:** 1–125.

———, and P. Ziveri. 2000. Calculation of coccolith volume and its use in calibration of carbonate flux estimates. Deep-Sea Res. II **47:** 1679–1700, doi:10.1016/S0967-0645(00)00003-5

Ziveri, P., B. de Bernardi, K. H. Baumann, H. M. Stoll, and P. G. Mortyn. 2007. Sinking of coccolith carbonate and potential contribution to organic carbon ballasting in the deep ocean. Deep-Sea Res. II **54:** 659–675, doi:10.1016/j.dsr2.2007.01.006

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