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Coccolithophore calcification is independent of carbonate chemistry in the tropical ocean

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Abstract

Short-term experiments indicate that seawater acidification can cause a decrease in the rate of calcification by coccolithophores, but the relationship between carbonate chemistry and coccolithophore calcification rate in natural assemblages is still unclear. During the Malaspina 2010 circumnavigation, we measured primary production, calcification, coccolithophore abundance, particulate inorganic carbon (PIC) concentration, and the parameters of the carbonate system, along basin-scale transects in the tropical Atlantic, Indian and Pacific oceans. Euphotic layer-integrated calcification and mean cell-specific calcification in the euphotic layer ranged between 2–10 mgC m^{-2} d^{-1} and 5–20 pgC $cell^{-1}$ d^{-1}, respectively. We found a significant relationship between primary production and calcification, such that the calcification to primary production (CP/PP) ratio was relatively invariant among ocean basins, with an overall mean value of 0.05 ± 0.04. Extrapolating this value to the entire ocean would result in a global pelagic calcification rate of 2.4 PtC yr^{-1}. The mean PIC concentration in surface waters was 1.8 ± 1.6 mgC m^{-3} and its turnover time averaged 20 d. We combined our data of calcification, primary production, and carbonate chemistry from Malaspina 2010 with those obtained during two previous cruises in the northern Arabian Sea. Both the CP/PP ratio and cell-specific calcification were largely constant across a wide range of calcite saturation state (1.5–6.5), $[HCO_3^-]/[H^+]$ (0.08–0.24; mol: μmol), and pH (7.6–8.1), which indicates that calcification by natural coccolithophore assemblages was independent of carbonate chemistry. Our results suggest that coccolithophore calcification, at least in tropical regions, may not be decreasing in the currently acidifying ocean.

Anthropogenic ocean acidification involves a decrease in the pH, the concentration of carbonate ions $[CO_3^{2-}]$, and the saturation state for calcium carbonate (Ω) of seawater (Orr et al. 2005), which has been identified as a major threat for different groups of calcifying organisms including mollusks, corals and coccolithophores (Doney et al. 2009). Coccolithophores are single-celled, photosynthetic algae characterized by producing an exoskeleton of calcium carbonate (calcite) plates called coccoliths. These organisms constitute a key plankton functional group (Iglesias-Rodríguez et al. 2002) and play major ecological and biogeochemical roles as they have been estimated to contribute 20% of the total marine primary production (Rousseaux and Gregg 2013) and $>50\%$ of total marine calcium carbonate export (Broecker and Clark 2009). Whereas net primary production converts CO_2 into organic matter, some of which is exported to the deep ocean (the organic carbon pump), calcification releases CO_2 and leads to a sinking flux of calcium carbonate and a decrease in surface ocean's alkalinity (the carbonate carbon

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pump). The ratio between calcification and primary production in the upper ocean is closely linked to the ratio between the sinking fluxes of calcium carbonate and organic carbon (the rain ratio), which is a primary determinant of CO_2 exchange between the ocean and the atmosphere (Archer and Maier-Reimer 1994; Rost and Riebesell 2004; Tyrrell 2008).

Although there are discrepancies among individual studies, meta-analyses of laboratory and shipboard experiments with cultures and natural populations indicate a significant, negative effect of acidification on coccolithophore calcification (Kroeker et al. 2013; Meyer and Riebesell 2015). However, extrapolation of these experimental results to natural conditions at sea is not straightforward. One concern is that many algal strains used in laboratory experiments have been maintained in dense cultures at high pH values, which may have unwittingly selected for genotypes that are less able to adapt to low and/or changing pH conditions (Joint et al. 2011). Furthermore, the short duration of most experiments imply that phytoplankton populations are not given time to adapt to the new conditions and, perhaps, not even to attain full physiological acclimation. In this regard, recent long-term, multi-generation laboratory experiments with nutrient-replete cultures have found that coccolithophores can adapt to high-CO_2 conditions and sustain higher calcification rates than control populations (Lohbeck et al. 2012; Benner et al. 2013; Schluter et al. 2014). An additional aspect is that growth conditions during experiments are usually nutrient-replete and constant over time, whereas natural populations often experience nutrient limitation (Moore et al. 2013) and large changes in carbonate chemistry over relatively small temporal and spatial scales (Joint et al. 2011). In this context, a valuable approach is to assess the relationship between carbonate chemistry and pelagic calcification using observations across natural gradients in the ocean.

After analyzing the coccolith mass of dominant coccolithophore species from several oceanic regions, mostly with sediment samples, Beaufort et al. (2011) found a consistent pattern of decreasing coccolith mass with decreasing $[CO_3^{2-}]$ and Ω, hence supporting a negative effect of acidification on calcification. In contrast, Smith et al. (2012), studying the seasonal patterns in the Bay of Biscay, observed that the relative abundance of the most heavily calcified *Emiliania huxleyi* morphotype was highest in winter, when Ω was lowest. It must be noted that coccolith mass and calcium carbonate quotas, per se, cannot be readily interpreted as calcification rates if the turnover time of cell calcite is unknown. For instance, high calcite quotas can result from reduced rates of cell division (Paasche and Brubak 1994; Paasche 2002; Zondervan et al. 2002) or changes in species composition (Charalampopoulou et al. 2011) rather than increased calcification rate. A recent study that used both coccolith mass data and estimated cell division rates to determine calcite production rates across the Paleocene-Eocene thermal maximum concluded that other factors in addition to ocean acidification are more likely to explain the variability in coccolithophore calcification (O'Dea et al. 2014).

Since the ^{14}C-uptake technique was adapted to measure coccolithophore calcification rates over short-time scales, numerous field measurements have been obtained (e.g., Balch et al. 2007), but efforts have concentrated on *E. huxleyi* blooms in temperate and high latitudes (e.g., Balch et al. 1992; Fernández et al. 1993; Van Der Wal et al. 1995; Marañón and González 1997). Comparatively, calcification in tropical and subtropical regions has received less attention (Balch and Kilpatrick 1996; Balch et al. 2000; Poulton et al. 2006), despite the fact that coccolithophores show enhanced contributions to total phytoplankton biomass (Cermeño et al. 2008) and productivity (Rousseaux and Gregg 2013) in these regions, and that calcification during non-bloom conditions in the oligotrophic ocean may dominate global calcium carbonate budgets (Sarmiento et al. 2002). To the best of our knowledge, the relationship between coccolithophore calcification rates and seawater carbonate chemistry has not been investigated yet in tropical and subtropical oceanic regions.

Here, we report on concurrent measurements of calcification and photosynthesis carried out in the euphotic layer of the Atlantic, Indian, and Pacific oceans during the Malaspina 2010 circumnavigation. We also make use of calcification and carbonate chemistry data obtained during two U.S. JGOFS cruises in the Arabian Sea (Balch et al. 2000). Our main goals are (1) to provide basin-scale estimates of calcification rate and the calcification to primary production ratio in the tropical ocean and (2) to test the hypothesis that the variability in coccolithophore calcification is affected by seawater carbonate chemistry.

Materials and methods

Sampling

The Malaspina 2010 circumnavigation expedition took place onboard R/V *Hespérides* during the period 14 December 2010–14 July 2011 and consisted of seven transects that crossed tropical and subtropical waters of the Atlantic, Indian and Pacific oceans (Fig. 1). The transects were: Cádiz to Rio de Janeiro from 14 December 2010 to 13 January 2011; Rio de Janeiro to Cape Town from 17 January 2011 to 06 February 2011, Cape Town to Perth from 11 February 2011 to 13 March 2011; Perth to Sydney from 17 March 2011 to 30 March 2011; Auckland to Hawaii from 16 April 2011 to 08 May 2011; Hawaii to Panama from 13 May 2011 to 10 June 2011; and Cartagena de Indias to Cartagena, Spain from 19 June 2011 to 14 July 2011.

Hydrography, irradiance, and carbonate chemistry

Vertical profiles of temperature and salinity were obtained with a SBE911 Plus Conductivity-Temperature-Depth (CTD) probe attached to a rosette equipped with Niskin bottles. The vertical attenuation of photosynthetically active radiation (PAR) was measured with a Satlantic OCP-100FF radiometer. Seawater pH (at the total scale) was measured

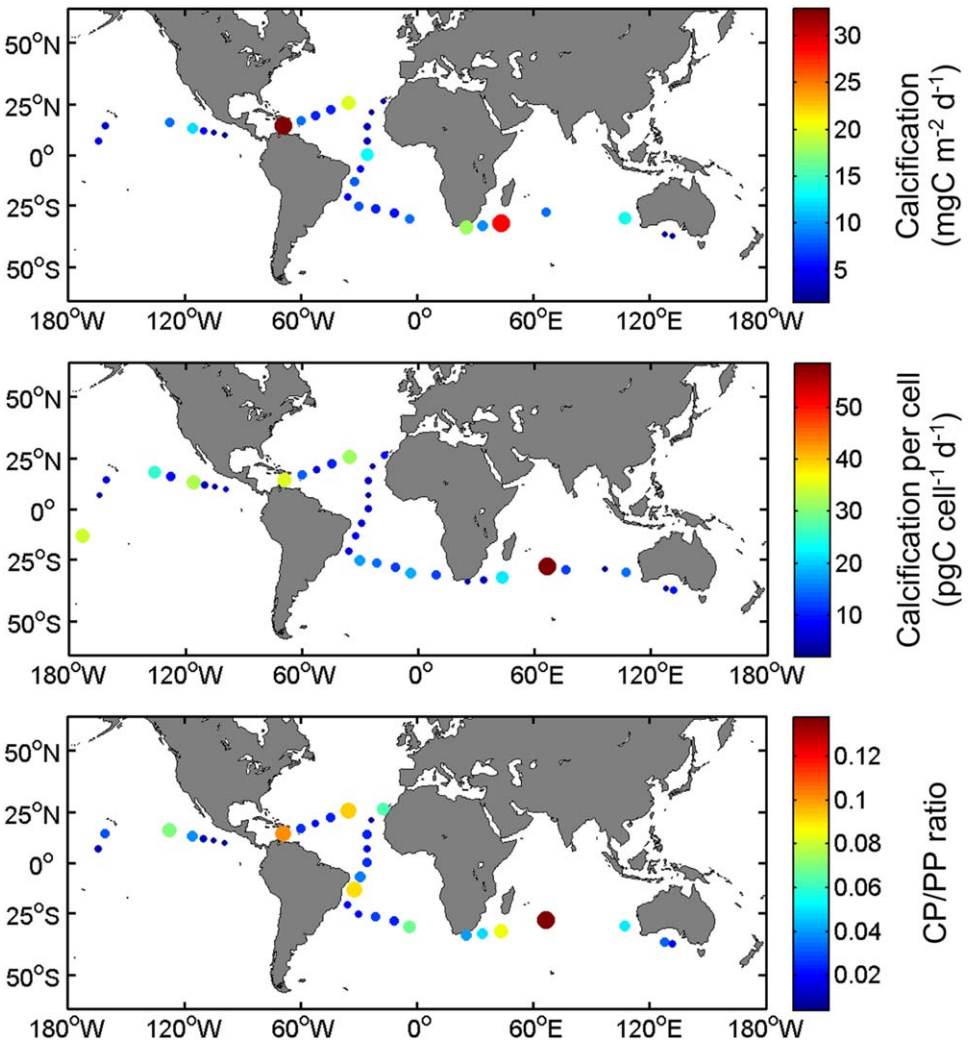

Fig. 1. Calcification during the Malaspina 2010 circumnavigation. For each sampling station, the color scale indicates (**A**) euphotic layer-integrated calcification rate (mgC m^{-2} d^{-1}), (**B**) mean cell-specific calcification (pgC $cell^{-1}$ d^{-1}) in the euphotic layer, and (**C**) mean calcification to primary production (CP/PP) ratio in the euphotic layer. Larger circles indicate higher values. Integrated calcification rates were not calculated when less than 3 depth values were available.

spectrophotometrically (Clayton and Byrne 1993) and alkalinity was determined potentiometrically by titration at endpoint detection (Mintrop et al. 2000). Additional pH and alkalinity data for the present analysis were obtained from the U.S. Biological and Chemical Oceanography Data Management Office database. Specifically, we used pH and alkalinity data from cruises TT049 and TT053, carried out onboard R/V *Thomas G. Thomson* in the Arabian Sea as part of the U.S. JGOFS program (http://www.bco-dmo.org/dataset/2536; Principal Investigators: Catherine Goyet and Frank Millero). During these cruises, pH and alkalinity were measured following the procedures described by Millero et al. (1998). For the ensemble of the Malaspina and Arabian Sea cruises, dissolved inorganic carbon (DIC), carbonate and bicarbonate ion concentrations, saturation states, and all parameters of the CO_2 system in seawater (always reported at in situ conditions) were computed from pH, alkalinity, temperature, and salinity with the dissociation constants given by Dickson and Millero (1987), using the CO2SYS program, version 2.1.

Coccolithophore abundance and diversity

We used the Utermöhl technique to examine coccolithophore abundance and diversity under the inverted microscope. Samples (250 mL) were collected from surface, 20% PAR depth and 1% PAR depth and stored in glass bottles with hexamine-buffered formaldehyde (4% final formalin concentration) as a fixative. A 100-mL aliquot of sample was allowed to settle in a composite chamber. After 48 h, the bottom was removed and one transect of the chamber was examined at 312X magnification to count the smaller, more frequent cells.

Table 1. Mean (± standard deviation) surface temperature and salinity, surface concentration of nitrate, phosphate and Chl *a*, and depth of the deep chlorophyll maximum (DCM), in the different basins studied during the Malaspina 2010 circumnavigation. Data correspond only to stations where calcification was measured. *n* is the number of stations visited in each region.

Region	Surf. temp. °C	Surf. salin. PSU	NO_3 μmol L^{-1}	PO_4 μmol L^{-1}	Chl *a* μg L^{-1}	DCM depth (m)	*n*
North Atlantic	26.6 ± 2.5	36.4 ± 0.8	0.40 ± 0.13	0.05 ± 0.02	0.15 ± 0.09	96 ± 35	10
South Atlantic	25.3 ± 3.3	36.3 ± 0.5	0.23 ± 0.12	0.11 ± 0.03	0.06 ± 0.03	113 ± 28	8
Indian	23.0 ± 2.4	35.5 ± 0.2	0.53 ± 0.62	0.07 ± 0.08	0.10 ± 0.04	97 ± 36	9
North Pacific	26.9 ± 2.5	34.3 ± 0.5	0.34 ± 0.29	0.12 ± 0.03	0.23 ± 0.08	73 ± 45	8

Table 2. Calcification in the tropical ocean. Mean (± standard deviation) calcification (CP, mgC m^{-2} d^{-1}) and calcification to primary production (CP/PP) ratio in the euphotic layer of the tropical and subtropical (29°N-39°S) Atlantic, Indian, and Pacific oceans. *n* is the number of stations visited in each region.

Region	CP	CP/PP	*n*	References
Atlantic Ocean (29°N-32°S)	8 ± 7	0.04 ± 0.03	18	This study
Atlantic Ocean (29°N-12°S)	16 ± 10	0.06 ± 0.05	5	Poulton et al. (2006)
Indian Ocean (28°S-39°S)	12 ± 9	0.05 ± 0.03	7	This study
Arabian Sea (25°N-10°N)	41 ± 47	0.03 ± 0.04	52	Balch et al. (2000)
Pacific Ocean (17°N-7°N)	5 ± 4	0.06 ± 0.04	7	This study
Equatorial Pacific (12°N-12°S)	28 ± 25	0.05 ± 0.06	40	Balch and Kilpatrick (1996), Balch et al. (2011)

Subsequently, the whole chamber bottom was scanned at 125X magnification to record larger, less frequent forms.

Particulate inorganic carbon

The concentration of particulate inorganic carbon (PIC) was determined by inductively coupled plasma (ICP) atomic emission spectrometry. 500-mL samples from the 100%, 20%, and 1% PAR levels were filtered through 0.4-μm pore-size polycarbonate filters, which were repeatedly rinsed with 0.02 M $K_2B_4O_7$ to eliminate seawater. Filters were wrapped in aluminum foil and stored at −20°C until analysis. After the cruise, filters were extracted in 2% nitric acid in the laboratory, and calcium concentration was determined on a Perkin Elmer Optima 3200 RL ICP atomic emission spectrometer, monitoring also sodium as a proxy of seawater contamination. PIC concentration was calculated on the basis of the measured calcium content, assuming all the particulate calcium was in the form of calcium carbonate (Fernández et al. 1993). PIC data are not available for the stations sampled in the Pacific ocean.

Photosynthesis and calcification

We used the ^{14}C-uptake and microdiffusion technique (Paasche and Brubak 1994; Balch and Kilpatrick 1996; Poulton et al. 2006) to determine simultaneously, on the same samples, the incorporation of dissolved inorganic carbon into particulate organic carbon (primary production) and particulate inorganic carbon (calcification). Samples were collected from surface (3 m) and the 20% and 1% PAR attenuation depths into acid-washed, 250-mL polystyrene bottles. For each depth, four bottles (three light replicates and one blank killed with hexamine-buffered formalin at 4% final concentration) were spiked with 1.85–3.7 MBq (50–100 μCi) of ^{14}C-labelled sodium bicarbonate ($NaH^{14}CO_3$) and placed inside on-deck incubators during 24 h. The incubators were cooled with running seawater pumped from the surface (for surface samples) or with water circulating through a refrigerator (for deeper samples) so that all samples were incubated within ±2°C of their in situ temperature. Irradiance levels inside the incubator were controlled with a combination of neutral density and blue (Mist Blue, Lee filters) filters.

Incubation was ended by filtration under low-vacuum pressure through 0.4-μm pore size polycarbonate filters, which were then thoroughly rinsed with 0.2-μm filtered seawater to remove the non-incorporated, ^{14}C-labelled DIC. After processing each filter with the micro-diffusion procedure (Balch and Kilpatrick 1996), sample radioactivity was measured with a Wallac scintillation counter. To calculate the rates of primary production and calcification, the disintegrations per minute (DPM) counts measured in the formalin-killed blank bottle were subtracted from the DPM counts measured in the light bottles. The mean value of the coefficient of variation for triplicate samples was 18% for primary production and 33% for calcification. Primary production rates measured with the micro-diffusion technique showed good agreement (Spearman's $r = 0.85$, $p < 0.01$, $n = 95$)

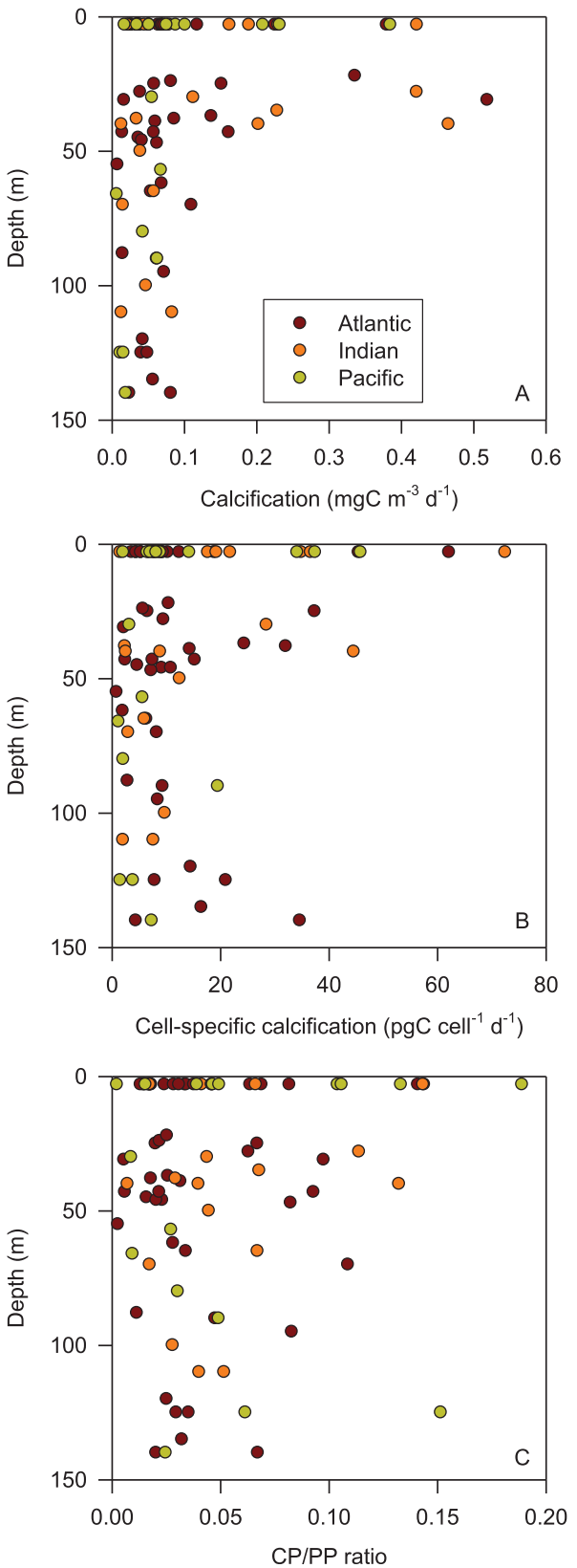

Fig. 2. Vertical distribution of (**A**) calcification rate (mgC m^{-3} d^{-1}), (**B**) cell-specific calcification (pgC $cell^{-1}$ d^{-1}), and (**C**) the calcification to primary production ratio for all data combined.

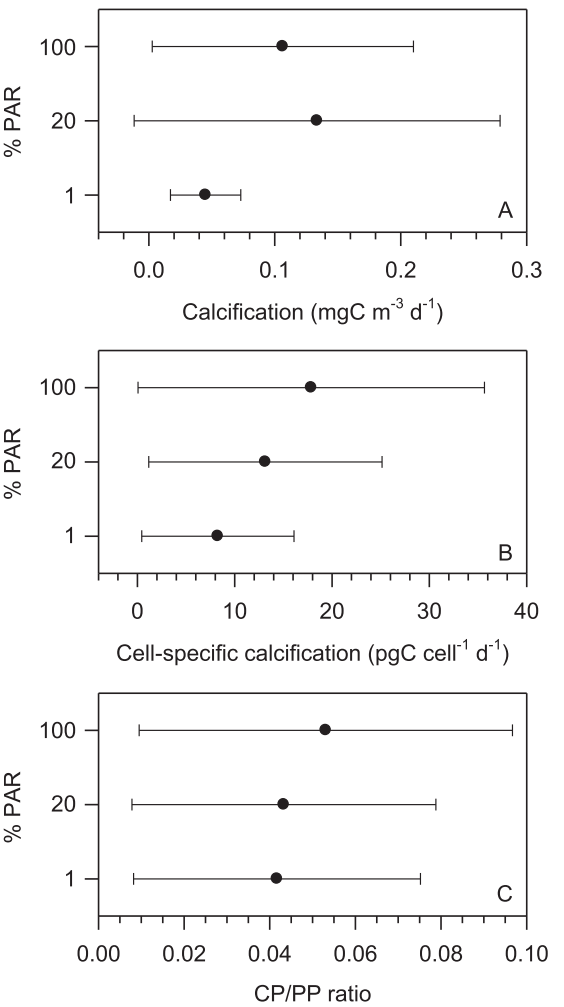

Fig. 3. Calcification across the euphotic zone. Mean and standard deviation for (**A**) calcification rate (mgC m^{-3} d^{-1}), (**B**) cell-specific calcification (pgC $cell^{-1}$ d^{-1}), and (**C**) the calcification to primary production ratio at each level of photosynthetically active radiation (PAR) for all data combined.

with those measured with the conventional ^{14}C-uptake technique during parallel, independent experiments (Pinedo-González et al. 2015). Areal rates of primary production and calcification (mgC m^{-2} d^{-1}) were calculated by trapezoidal integration over the depth of the euphotic zone.

Additional calcification data used in the present analysis were obtained during U.S. JGOFS cruises TT049 and TT053 in the Arabian Sea and have been reported before (Balch et al. 2000). The methods used in these cruises for phytoplankton sampling, on-deck ^{14}C-incubation, and sample processing with the micro-diffusion technique, were the same as those used during the Malaspina 2010 expedition.

All data used in this work are accessible at the Malaspina 2010 Expedition Geodatabase (http://metamalaspina.imedea.uib-csic.es) and the U.S. Biological and Chemical

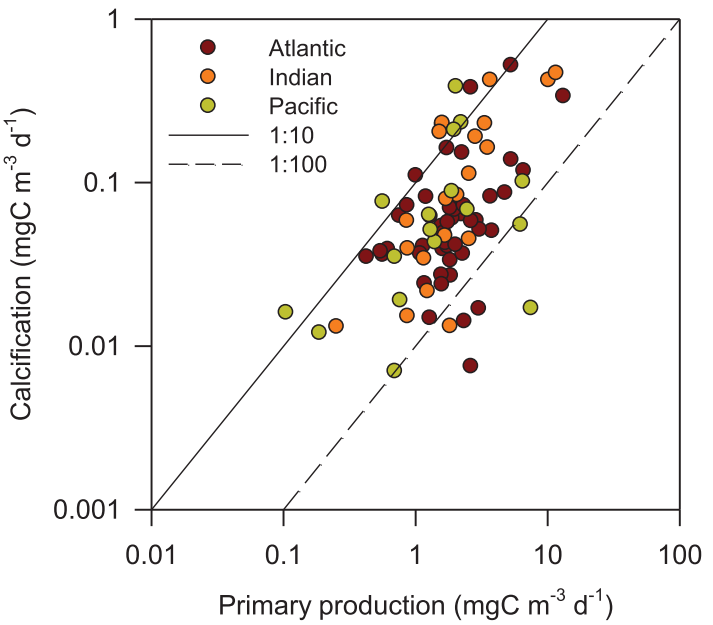

Fig. 4. Relationship between primary production and calcification rate. Lines indicate the 0.1 and 0.01 calcification to primary production ratio. The linear fit (reduced major axis regression) between the two variables was $y = 0.048\ x - 0.020$ ($R^2 = 0.32$, $n = 85$, $p < 0.01$).

Oceanography Data Management Office database (http://www.bco-dmo.org), and can also be obtained from the authors on request.

Results

General oceanographic context

Previous reports have already described the geographical variability of temperature, salinity, nutrient concentration and chlorophyll *a* (Chl *a*) concentration (Pinedo-González et al. 2015), as well as that of the nutrient vertical diffusive fluxes (Fernández-Castro et al. 2015), during the Malaspina 2010 expedition. Excluding two stations located to the south of Australia, all the stations where calcification was measured (Fig. 1) were characterized by warm (> 22°C) surface temperatures and strong thermal stratification, typical of open-ocean tropical regions. Considering the different basins sampled, the mean surface temperature ranged between 23°C in the Indian Ocean and 27°C in the North Pacific Ocean (Table 1). Mean surface nutrient concentrations were in the range 0.2–0.5 μmol L^{-1} for nitrate and 0.05–0.12 μmol L^{-1} for phosphate. Surface Chl *a* concentration showed mean, basin-scale values ranging between 0.06 μg L^{-1} in the South Atlantic and 0.23 μg L^{-1} in the North Pacific. In most stations, the deep chlorophyll maximum (DCM) was located at a depth greater than 60 m (Table 1).

Variability of calcification rates

During most of the circumnavigation, euphotic layer-integrated calcification rates were generally within the range 2–10 mgC m^{-2} d^{-1} (Fig. 1). Although higher values (15–30 mgC m^{-2} d^{-1}) were measured in some stations of the North Atlantic and the Indian oceans, no significant differences were found between oceans in their mean calcification rates (Table 2). The mean cell-specific calcification rate in the euphotic layer typically ranged between 5 pgC $cell^{-1}$ d^{-1} and 20 pgC $cell^{-1}$ d^{-1}, with a few higher values (> 30 pgC $cell^{-1}$ d^{-1}) in the North Atlantic and Indian oceans (Fig. 1). The integrated calcification to primary production (CP/PP) ratio, which generally ranged between 0.02 and 0.08, was (mean ± 1 standard deviation) 0.04 ± 0.03, 0.05 ± 0.03 and 0.06 ± 0.04 in the Atlantic, Indian, and Pacific oceans, respectively (Table 2). The overall, mean CP/PP ratio for the entire Malaspina 2010 expedition was 0.05 ± 0.04. Calcification rates tended to decrease with depth

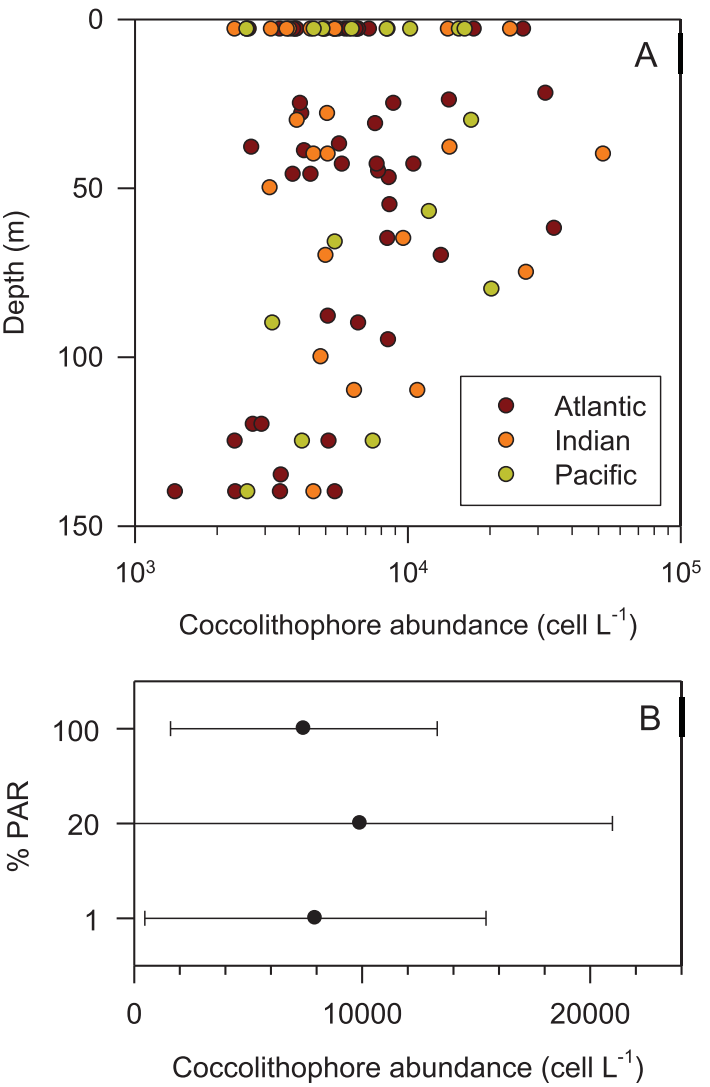

Fig. 5. Coccolithophore abundance (cell L^{-1}) over depth for each ocean and mean values for each PAR level with all data combined. Bars represent the standard deviation.

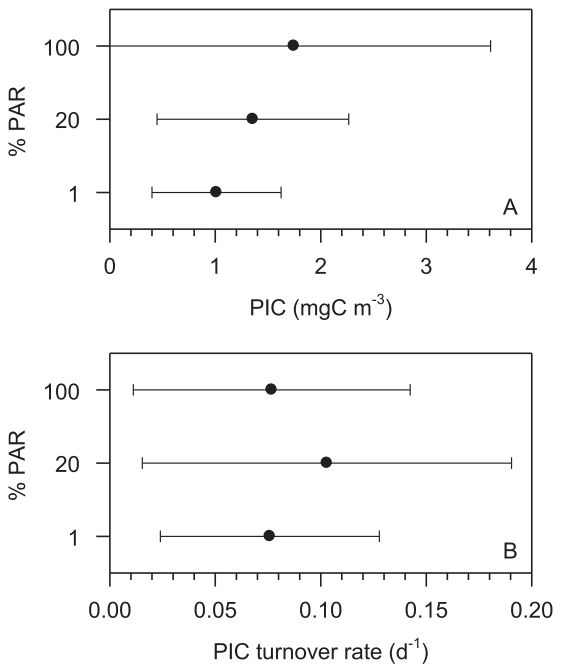

Fig. 6. Mean concentration (mgC m^{-3}) and turnover rate (d^{-1}) of particulate inorganic carbon (PIC) at each PAR level with data from the Atlantic and Indian oceans.

in all oceans (Figs. 2, 3). By irradiance level, the lowest calcification rates, both absolute and cell-specific, were measured at the 1% PAR depth (Fig. 3A,B). A significant, negative correlation with depth was found for both absolute calcification rate (Pearson's $r = -0.27$, $p = 0.014$, $n = 85$) and primary production ($r = -0.28$, $p = 0.006$, $n = 95$). In contrast, the CP/PP ratio was not correlated with depth ($r = -0.08$, $p = 0.485$, $n = 85$) and showed similar mean values at the three irradiance levels considered (Fig. 2C). There was a significant relationship between primary production and calcification, such that in most samples of all basins the CP/PP ratio was within the range 0.01–0.1 (Fig. 4). The linear regression model (reduced major axis regression) between primary production (independent variable) and calcification (dependent variable) was $y = 0.048\ x - 0.020$ ($R^2 = 0.32$, $n = 85$, $p < 0.01$).

Coccolithophore abundance

Coccolithophore abundances in most samples were in the range 2000–20,000 cell L^{-1} (Fig. 5). Abundances tended to decrease toward the base of the euphotic layer. No marked inter-ocean differences in coccolithophore abundance and vertical distribution were observed. In most stations, *E. huxleyi* and *Gephyrocapsa* spp. contributed 60–80% of total coccolithophore abundance (data not shown). Among other species, those with the highest abundance and occurrence

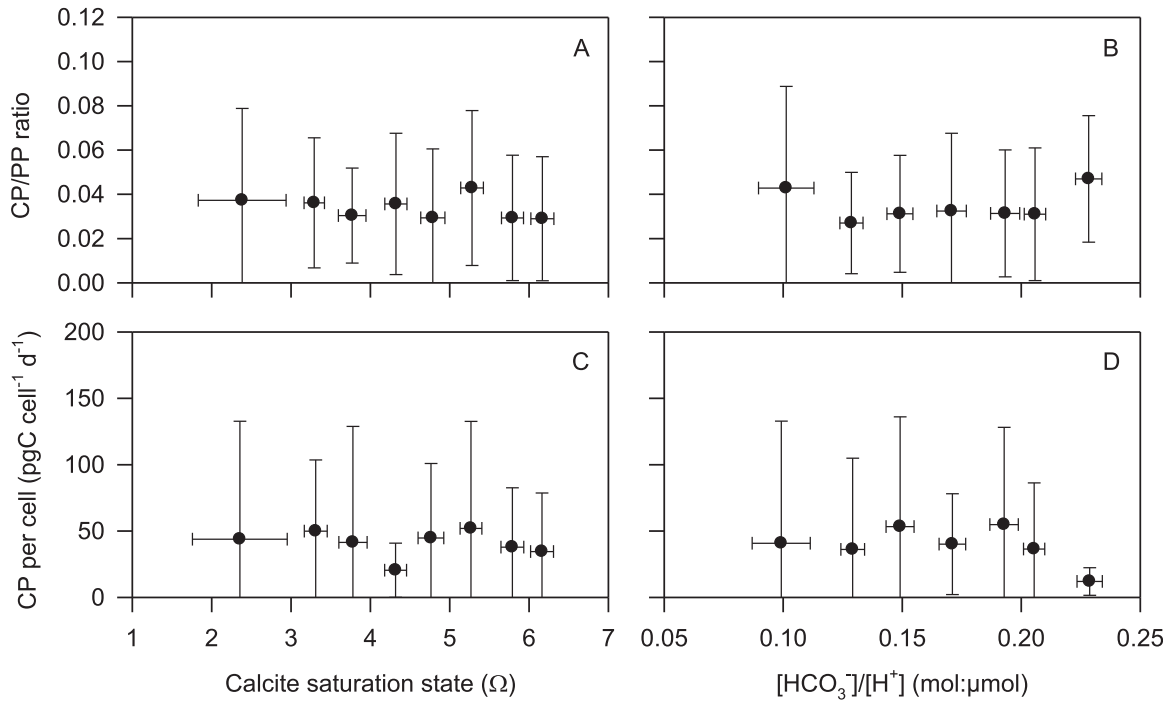

Fig. 7. Calcification and carbonate chemistry. Calcification to primary production (CP/PP) ratio (**A**,**B**) and cell-specific calcification (**C**,**D**) as a function of calcite saturation (Ω) (**A**,**C**) and $[HCO_3^-]/[H^+]$ (**B**,**D**) for the Malaspina 2010 and Arabian Sea data combined. Plotted are the mean and standard deviation bars of binned data. Data bin limits were 1-3, 3-3.5, 3.5-4, 4-4.5, 4.5-5, 5-5.5, 5.5-6, and 6-7 for Ω, and 0.08-0.12, 0.12-0.14, 0.16-0.18, 0.18-0.20, 0.20-0.22, and 0.22-0.24 for $[HCO_3^-]/[H^+]$ (mol: µmol).

rates were *Discosphaera tubifera*, *Syracosphaera pulchra*, *Umbellosphaera irregularis*, *U. sibogae*, and *Rhabdosphaera clavigera*.

Particulate inorganic carbon

The concentration of particulate inorganic carbon (PIC) in surface waters of the Atlantic and Indian oceans, which generally ranged between 0.5 mgC m^{-3} and 2 mgC m^{-3}, took a mean value of 1.8 mgC m^{-3} (Fig. 6A). PIC concentration decreased with depth, reaching values around 1 mgC m^{-3} at the base of the euphotic layer. We calculated the PIC turnover rate (d^{-1}) by dividing the daily calcification rate by PIC concentration. Most PIC turnover rates were within the range 0.02–0.2 d^{-1}. The mean PIC turnover rates at the three different PAR levels assayed were similar and took values around 0.08–0.1 d^{-1} (Fig. 6B).

Carbonate chemistry

In the Malaspina 2010 expedition, calcite saturation state (Ω) and $[HCO_3^-]/[H^+]$ (mol: μmol) typically ranged between 4–6 and 0.15–0.25, respectively (Supporting Information Fig. S1), with a corresponding pH range of approximately 7.9–8.1 (pH data not shown). In four stations located between 10 and 14°N in the eastern North Pacific, Ω, $[HCO_3^-]/[H^+]$ and pH at the base of the euphotic layer were in the range 1–2, 0.05–0.10, and 7.6–7.7, respectively. Ω tended to decrease with depth and took lower values in the Indian than in the Atlantic Ocean. In the Arabian Sea cruises, samples near the base of the euphotic layer often had Ω values in the range 2–3. Considering together the Malaspina and the Arabian Sea cruises, the entire data set covered a relatively large range of Ω (1.5–6.5), $[HCO_3^-]/[H^+]$ (0.08–0.24), and pH (7.6–8.1).

Calcification and carbonate chemistry

Taking together all data from the Malaspina 2010 and the Arabian Sea cruises, we found that the CP/PP ratio was not correlated either to Ω ($r = -0.085$, $p = 0.12$, $n = 342$) or to $[HCO_3^-]/[H^+]$ ($r = 0.006$, $p = 0.91$, $n = 342$) (Supporting Information Fig. S2A,B). Similarly, cell-specific calcification was not correlated either to Ω ($r = -0.035$, $p = 0.54$, $n = 318$) or to $[HCO_3^-]/[H^+]$ ($r = -0.078$, $p = 0.18$, $n = 318$) (Supporting Information Fig. S2C,D). Accordingly, the binned CP/PP and cell-specific calcification data (Fig. 7) showed an invariant distribution across the entire range of Ω and $[HCO_3^-]/[H^+]$, suggesting that calcification rate, when normalized either to primary production or to coccolithophore cell abundance, is independent from carbonate chemistry in the tropical ocean.

Discussion

Large-scale variability in calcification

Our measurements provide a broad view of basin-scale calcification rates in the oligotrophic ocean and suggest that the calcification to primary production (CP/PP) ratio, which took a mean value of 0.05 ± 0.04 during the Malaspina 2010 expedition, is relatively invariant across large spatial scales in tropical and subtropical latitudes. This value of the CP/PP ratio supports the results of Sarmiento et al. (2002) who, using a biogeochemical-transport box model, calculated a global value of 0.06 for the calcium carbonate to organic carbon shallow export ratio. Given that in temperate and high-latitude regions, and particularly during coccolithophore blooms, the CP/PP ratio is often higher (Fernández et al. 1993; Poulton et al. 2010), and assuming a global primary production of 48.5 PgC yr^{-1} (Field et al. 1998), one could use the value of 0.05 to calculate an estimate of global calcification of 2.4 PgC yr^{-1}. This figure is higher than the value of 1.6 PgC yr^{-1} obtained by Balch et al. (2007) using satellite measurements, although uncertainty regarding the variability of the CP/PP ratio, particularly in high-latitude regions, prevents us from concluding that these estimates are significantly different. Our estimate is also higher than current geochemical estimates of global community calcification in the upper ocean (0.8–1.4 PgC yr^{-1}) (Feely et al. 2004), but it must be born in mind that, while our measurements represent short-term, near-to-gross calcification rates, calcite dissolution in the upper ocean over seasonal time scales will result in a lower value of net annual (exportable) calcification.

The absolute calcification rates we measured are lower than those reported before for tropical waters of the Atlantic (Poulton et al. 2006, 2007), Pacific (Balch and Kilpatrick 1996; Balch et al. 2011) and Indian (Balch et al. 2000) oceans (Table 2). This difference is likely due to the fact that earlier studies had a better coverage of areas with enhanced biological productivity, such as the Pacific equatorial upwelling and the northern Arabian Sea, whereas our observations were conducted mostly in strongly oligotrophic conditions (Table 1). However, our estimates of the CP/PP ratio are close to those obtained in previous studies (Table 2), which suggests that when conditions in the tropical ocean become less oligotrophic and more favorable for primary productivity (e.g., due to increased nutrient supply from deep waters) coccolithophore calcification is also enhanced to a similar extent. The relative constancy of the CP/PP ratio, as determined with the ^{14}C-uptake technique, is also consistent with the results of geochemical studies that show small differences in net community production and calcification among the subtropical gyres of all oceans (Louanchi and Najjar 2000; Lee 2001). The contrast between the subtropical Atlantic and Pacific oceans in their deep-sea sediment calcium carbonate content, particularly in the northern hemisphere (Heinze et al. 1999), would thus result mostly from differences in preservation in the ocean's interior rather than differences in production within the euphotic zone.

It has been noted that coccolithophore abundance tends to covary with that of other phytoplankton during non-bloom conditions (Balch et al. 2000). Global maps of estimated coccolithophore biomass (Rousseaux and Gregg 2015) and calcification (Balch et al. 2007) describe large-scale geographical patterns that resemble those of surface

Chl *a* concentration and primary production climatologies. Accordingly, Poulton et al. (2007) found that calcification rate was strongly related to Chl *a* concentration and primary production in a large set of observations collected around the world's oceans. One could therefore expect calcification rates during the Malaspina expedition to be related to nitrogen supply, which is the main factor limiting phytoplankton growth in the subtropical gyres (Moore et al. 2013). However, when we explored the relationship between nitrate diffusive fluxes (Fernández-Castro et al. 2015) and euphotic layer-integrated calcification, mean cell-specific calcification, and the mean CP/PP ratio, no significant correlations were found ($r = -0.28$, $p = 0.19$, $n = 23$ for integrated calcification; $r = -0.12$, $p = 0.54$, $n = 28$ for cell-specific calcification; $r = -0.16$, $p = 0.43$, $n = 28$ for the CP/PP ratio). This may have been due to the relatively narrow range of variability in phytoplankton biomass and productivity encountered in our cruises, and also to the fact that mesoscale variability, which can be important in determining coccolithophore abundance and calcification (Charalampopoulou et al. 2011), was not resolved during the circumnavigation.

Coccolithophore abundance, cell-specific calcification, and PIC turnover

The coccolithophore abundances determined during the Malaspina 2010 cruises, which ranged between 2000 cell L^{-1} and 20,000 cell L^{-1} in most samples, were comparable to those reported by previous studies in tropical, open-ocean regions. Balch and Kilpatrick (1996), during a transect from 12°N to 12°S across the Equatorial Pacific, found abundances below 5000 cell L^{-1} in the less productive waters at latitudes greater than 9°, and above > 20,000 cell L^{-1} near the Equator. During two cruises in the northern Arabian Sea, Balch et al. (2000) measured average coccolithophore abundances of ca. 12,000 and 17,000 cell L^{-1} in July/August and October/November 1995, respectively. More recently, Dandonneau et al. (2006) reported surface abundances in the range 20,000–60,000 and 8,000–20,000 cell L^{-1} during repeated cruises in the Pacific Equatorial Divergence and the South Pacific Subtropical Gyre provinces, respectively. Regarding cell-specific calcification, the rates measured during the Malaspina cruises (range: 1–70 pgC cell^{-1} d^{-1}) were broadly similar to those reported by Balch et al. (2000) for the Arabian Sea and Poulton et al. (2010) for the North Atlantic.

The PIC turnover rates we calculated imply turnover times that varied widely between 1 d and 100 d, similar to the ranges reported before (Balch and Kilpatrick 1996; Balch et al. 2000; Poulton et al. 2007). The average turnover time for PIC was around 20 d, considerably higher than the turnover time estimated for both bulk phytoplankton (Marañón 2005; Huete-Ortega et al. 2011) and prymnesiophytes (Gutiérrez-Rodríguez et al. 2011) in oligotrophic waters (2–6 d). This discrepancy could be interpreted as an indication that coccolithophores sustain slower growth rates than the ensemble of the phytoplankton assemblage in the tropical ocean. Coccolithophores, on account of their larger cell size, may experience a stronger degree of nutrient limitation than picophytoplankton, which are better adapted to live in nutrient-impoverished waters and dominate the phytoplankton community in the oligotrophic ocean (Marañón 2015). Another, non-exclusive explanation, is that a substantial fraction of the suspended PIC is in the form of free coccoliths (Balch and Kilpatrick 1996; Balch et al. 2000), and therefore the calculated PIC turnover time must be longer than the turnover time of cellular (attached) calcite. Also in contrast to phytoplankton growth rates, which decrease markedly with depth (Marañón et al. 2000; Pérez et al. 2006), PIC turnover rates were rather similar in the different PAR levels analyzed. The relative constancy of PIC turnover across the euphotic zone is consistent with the common observation that calcification is a less light-dependent process than photosynthesis (Balch et al. 1992; Zondervan 2007).

Carbonate chemistry and calcification

The study of the CP/PP ratio is receiving increased attention in the context of global oceanic change because both calcification and photosynthesis can be regulated by the same environmental variables, such as irradiance, dissolved inorganic carbon chemistry, and nutrient availability (Rost and Riebesell 2004; Zondervan 2007; Mackey et al. 2015), and also because this ratio is closely linked to the rain ratio and can mediate a negative feedback of ocean acidification on atmospheric CO_2 (Zondervan et al. 2001). The comparative paucity of field studies investigating the CP/PP ratio makes our measurements, obtained over a wide range of carbonate chemistry conditions, particularly relevant.

It has been recently argued that the ratio between $[HCO_3^-]$ and $[H^+]$ may be a more influential parameter than carbonate saturation (Ω) for the regulation of calcium carbonate biotic precipitation, which would be stimulated by HCO_3^- and inhibited by H^+ (Bach et al. 2015). Therefore, we have considered the $[HCO_3^-]/[H^+]$ ratio as a descriptor of carbonate chemistry, in addition to calcite Ω.

We found that the CP/PP ratio does not decrease with decreasing Ω and $[HCO_3^-]/[H^+]$. Given that photosynthesis in phytoplankton tends to increase or remain unchanged with increasing acidification (Kroeker et al. 2013; Mackey et al. 2015), the constancy of the CP/PP ratio over a wide range Ω and $[HCO_3^-]/[H^+]$ strongly suggests that community calcification is not reduced under high-DIC conditions. We also found no correlation between cell-specific calcification and Ω or $[HCO_3^-]/[H^+]$. These patterns do not support the hypothesis that the variability in pelagic calcification, at the community level, is affected by carbonate chemistry, but are consistent with reports of a lack of correlation between the

carbonate system and coccolith morphology and calcite content in both fossil and extant coccolithophore assemblages (Langer et al. 2006; Berger et al. 2014; Young et al. 2014). Similarly, no clear relationship between calcite saturation and coccolithophore calcification has been found in the central Iceland Basin (Poulton et al. 2010) or in the north-west European shelf (Poulton et al. 2014).

Our results need to be reconciled with the observation that coccolith mass decreases with decreasing carbonate saturation across multiple spatio-temporal gradients (Beaufort et al. 2011). An important issue is that other critical environmental factors, such as nutrient supply, covary with carbonate chemistry, because the concentration of dissolved inorganic nutrients is stoichiometrically linked to that of DIC through the synthesis and remineralization of organic matter, and also because temperature affects both CO_2 solubility and nutrient vertical transport across multiple spatial and temporal scales. Calcification is a less regulated process than photosynthesis, at least in *E. huxleyi* (Paasche 1998, 2002), and therefore under strong nutrient limitation (which in the ocean is often associated with low DIC concentration and hence high pH and Ω values) calcite precipitation tends to continue while the synthesis of organic matter, and thus cellular growth, slows down, giving way to high calcite quotas (Paasche 2002; Rost and Riebesell 2004; Zondervan 2007). In addition, variations in coccolith mass in multispecific assemblages are mostly due to changes in the abundance of different species having distinct morphologies and calcite contents (Beaufort et al. 2011; Charalampopoulou et al. 2011), but also distinct ecological niches. For instance, a small, lightly calcified, fast-growing species such as *E. huxleyi* shows increased abundances in productive environments, whereas a larger, heavily calcifying species such as *Gephyrocapsa oceanica*, which has slower maximum growth rates (Buitenhuis et al. 2008), is associated with warm, oligotrophic conditions (Winter et al. 1994; Beaufort et al. 2011). Thus, biogeographic patterns in the cellular calcite content of coccolithophores may result from environmental selection of functional traits, such as cell size, resource acquisition abilities, and maximum growth rates, that are not necessarily related to the impacts of carbonate chemistry on calcification.

Calcification and ocean change

Assuming that coccoliths confer a net benefit to cells (Raven and Crawfurd 2012), a negative correlation between seawater calcite saturation and calcification rate would imply a prospect of decreasing biological fitness for coccolithophores in an acidifying ocean. Our results, however, suggest that natural coccolithophore assemblages are able to sustain similar rates of calcification across a wide range of carbonate chemistry conditions, which highlights the ability of cells to acclimate to changing concentrations of H^+ and dissolved inorganic carbon species (Joint et al. 2011). We suggest that other oceanographic conditions, which are related directly to the ecological niche of coccolithophores (such as water column stratification, vertical nutrient supply, and mean irradiance in the upper mixed layer, among others) may be more critical in determining their fate in a changing ocean. Indeed, a poleward expansion in the distribution of coccolithophore blooms during the last few decades has been observed (Winter et al. 2014), in spite of the low calcite saturation typical of high-latitude waters. Similarly, an increase in coccolithophore occurrence from 1965 through to 2010 has been reported recently for the temperate North Atlantic (Rivero-Calle et al. 2015). The linkage between thermal stratification, nutrient supply, and the relative abundance of coccolithophores and diatoms indicates that the former group is likely to become more prevalent in a warming ocean, due to their higher nutrient affinity and lower resource requirements (Cermeño et al. 2008).

Conclusions

We have shown that the calcification to primary production (CP/PP) ratio, despite having a considerable degree of variability at the local scale, takes a remarkably similar mean value (0.05) throughout the various basins of the tropical ocean. Extrapolating this value to the entire ocean would result in an estimate for global pelagic calcification of 2.4 PtC yr^{-1}. We have also found that both the CP/PP ratio and cell-specific calcification are invariant over a wide range of pH, $[HCO_3^-]/[H^+]$ and Ω, which leads us to reject the hypothesis that the variability of coccolithophore calcification in tropical regions is affected by carbonate chemistry. Although anthropogenic ocean acidification will undoubtedly cause enhanced calcium carbonate dissolution in the sediments, with important biogeochemical consequences (Feely et al. 2004; Tyrrell 2008), our results suggest that pelagic calcification rate may not be decreasing in the currently acidifying ocean.

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