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B/Ca in coccoliths and relationship to calcification vesicle pH and dissolved inorganic carbon concentrations

Heather Stoll [a,b,*], Gerald Langer [c], Nobumichi Shimizu [d], Kinuyo Kanamaru [b]

[a] Dept. Geología, Universidad de Oviedo, Arias de Velasco s/n, 33005 Oviedo, Asturias, Spain
[b] Geoscience Dept., University of Massachusetts at Amherst, Amherst, MA 01003, USA
[c] Institute of Environmental Science and Technology (ICTA), Universitat Autònoma de Barcelona (UAB), Bellaterra 08193, Spain
[d] Geology and Geophysics Dept., Woods Hole Oceanographic Institute, Woods Hole, MA, USA

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Abstract

Coccolithophorid algae are microscopic but prolific calcifiers in modern and ancient oceans. When the pH of seawater is modified, as may occur in the future due to ocean acidification, different species and strains of coccolithophorids have exhibited diverse calcification responses in laboratory culture. Since their biomineralization is a completely intracellular process, it is unclear why their response should be affected by extracellular seawater pH. Variations in the B/Ca in coccoliths are potential indicators of pH shifts in the intracellular coccolith vesicle where calcification occurs, because B/Ca in abiogenic calcites increases at higher pH due to the greater abundance of borate ions, the only B species incorporated into calcite. We used a SIMS ion probe to measure B/Ca of coccoliths from three different strains of *Emiliania huxleyi* and one strain of *Coccolithus braarudii braarudii* cultured under different seawater pH conditions to ascertain if the B/Ca can be used to elucidate how coccolithophorids respond to changing ocean pH. These data are interpreted with the aid of a conceptual model of cellular boron acquisition by coccolithophorids. Based on uptake in other plants, we infer that boron uptake by coccolithophorid cells is dominated by passive uptake of boric acid across the lipid bilayer. Subsequently, in the alkaline coccolith vesicle (C.V.), boron speciates according to the C.V. pH, and borate is incorporated into the coccolith. At increasing seawater pH, the relative abundance of the neutral boric acid in seawater decreases, lowering the potential B flux into the cell. Homeostasis or constant pH of the coccolith vesicle results in a decrease of the B/Ca in the coccolith with increasing seawater pH. In contrast, if coccolith vesicle pH increases with increasing seawater pH, then the B/Ca will increase as the fraction of borate in the coccolith vesicle increases. The coccolith B/Ca is also expected to depend inversely on the dissolved inorganic carbon (DIC) concentration in the coccolith vesicle. The B/Ca in cultured coccoliths is much lower than that of foraminifera or corals and limits precision in the analysis. Modest variations in DIC or pH of the coccolith vesicle can account for the observed trends in B/Ca in cultured coccoliths. The model shows that paired measurements of B/Ca and B isotopic composition of the calcite could distinguish between regulation of pH or DIC in the coccolith vesicle.

1. INTRODUCTION

B/Ca has been proposed as a paleo-carbonate ion or paleo-pH proxy due to the preferential incorporation of the borate ion relative to boric acid into the calcite lattice (Yu et al., 2007; Foster, 2008). Boron in seawater exists in two species, $B(OH)_3$ and $B(OH)_4^-$ with a pK^* of 8.6 at 25 °C and salinity of 35 (Dickson et al., 2007). In abiogenic carbonate precipitated directly from seawater at different

* Corresponding author at: Dept. Geología, Universidad de Oviedo, Arias de Velasco s/n, 33005 Oviedo, Asturias, Spain. Tel.: +34 657 972 624.
E-mail addresses: hstoll@geol.uniovi.es, hstoll@geo.umass.edu (H. Stoll).

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pH values, the incorporation of B in the carbonate covaries with the proportion of B present as $B(OH)_4^-$ in the parent solution (Sanyal et al., 2000).

In biogenic carbonates, the relative importance of cellular regulation vs. external pH on the solid carbonate B/Ca remains to be characterized for most organisms. If biogenic carbonate were a passive recorder of extracellular pH or carbonate chemistry, their fossil B/Ca could be a useful tool for reconstructing past variations of pH in these systems. Conversely, if cellular processes regulate the organism's calcifying environment, the B/Ca may help elucidate the mechanisms by which cells respond to changing pH or carbonate chemistry of their environment. This latter insight would be very useful since many marine calcifying organisms may be disrupted by future ocean acidification (Orr et al., 2005), and some coccolithophorids appear to have calcified more heavily during past periods of higher ocean pH and saturation state (Beaufort et al., 2011).

Here, we focus on the relationship of solid carbonate B/Ca to calcification in the coccolithophorids. Coccolithophorids have shown sensitivity of calcification to ocean pH or carbonate mineral saturation state (Riebesell et al., 2000; Langer et al., 2006a, 2009; Iglesias-Rodriguez et al., 2008). A significant disruption of calcification could have important consequences for global biogeochemical cycles since coccolithophorid algae are commonly regarded as a major producer of calcium carbonate in open ocean settings, and changes in their calcite production may alter the efficient ballasting of organic carbon to the deep ocean (Zondervan et al., 2001; Klaas and Archer, 2002; Barker et al., 2003b). Nevertheless, the sign and magnitude of such feedbacks is uncertain because coccolithophorids have revealed a complex response of calcification to ocean acidification. First experiments with one strain of *Gephyrocapsa oceanica* and *Emiliania huxleyi* showed strong disruption of calcification with decreased pH (Riebesell et al., 2000). Subsequent experiments with *Calcidiscus leptoporus* showed development of an optimum calcification at modern CO_2, whereas those with *Coccolithus braarudii* showed no change in calcification as a function of seawater pH (Langer et al., 2006a). Recently, strain-specific responses of calcification to pH have been diagnosed in *E. huxleyi* (Langer et al., 2009) which appear to reflect substantial genotypic variability in this taxa (Medlin et al., 1996; Schroeder et al., 2005; Iglesias-Rodriguez et al., 2006). In those strains that are strongly affected by external pH, the mechanism of disruption of intracellular calcification remains uncertain. One hypothesis is that coccolithophorids may be sensitive to external pH due to the operation of a proton channel which exports protons from the cytosol and which is hindered by reduced proton gradient from cytosol to seawater when the seawater is more acidic (Brownlee and Taylor, 2004).

Coccolithophorids appear to be unique among marine calcifying organisms in not using a seawater reservoir for calcification (Brownlee and Taylor, 2004). In addition, they regulate the pH of the calcification space (Anning et al., 1996). Consequently, we develop a model that includes the most likely mechanisms of B uptake into the cell and simulates the response of coccolith B/Ca and B isotopic composition to the cellular regulation of pH and dissolved inorganic carbon concentrations in the calcifying space.

We analyze B/Ca in several strains of two calcifying species which have been the subject of the largest numbers of studies, *E. huxleyi* and *C. braarudii*. Our original objective was the measurement of both the B/Ca and B isotopic composition of coccoliths, since the boron isotopic composition has been widely used as a pH proxy (Sanyal et al., 1997; Pearson and Palmer, 2000). However, we found a low boron concentration in coccoliths which precludes precise isotopic measurements in the quantity of culture sample available to us.

2. METHODS

We measured B/Ca using secondary ion mass spectrometry because we aimed to develop a method that will also be applicable to sediments containing fossil coccoliths. No method exists to efficiently remove B-containing, similarly-sized detrital and authigenic minerals from coccolith-size fractions of sediments, and picking coccoliths (Stoll and Shimizu, 2009) is viable for ion probe but not for wet-chemistry ICP-MS methods given that 10^6 individuals are required to achieve the 100 μg carbonate needed for ICP-MS (Yu et al., 2005). Due to the low B concentrations and precision of the ion probe for B isotopes, measurements of B isotopes are currently not feasible in coccolith samples picked from sediments.

2.1. 1Culture

Cultures were grown in aged, sterile-filtered (0.2 μm pore-size cellulose-acetate filters) natural seawater. Nutrients were enriched to 100 μmol nitrate, 6.25 μmol phosphate, and f/2 levels of trace elements and vitamins according to standard nutrient recipes (Keller et al., 1987). Clonal cultures of *E. huxleyi* (strains RCC1212, RCC1238, and RCC1256) were obtained from the Roscoff Culture Collection (http://www.sb-roscoff.fr/Phyto/RCC) and were grown at the optimal temperatures for each strain, temperatures of 17 °C for RCC1256, and 20 °C for RCC1212 and RCC1238. Strains 1256 and 1238 are A morphotypes, and strain 1212 is a B morphotype (Young et al., 2003). *Coccolithus braarudii braarudii* strain AC400 was used in the experiments. Cultures were grown in 16/8 h light/dark cycle at a photon intensity of 400 μmol photons $m^{-2}s^{-1}$. Salinity, measured with a conductivity meter (WTW Multi 340i) and TetraCon 325 sensor, was 32. Cells were pre-adapted to experimental conditions for approximately 12 generations and grown in dilute batch cultures (cell density <50,000 cells/ml for *E. huxleyi* and <5000 cells/ml for *C. braarudii*). For all experiments, with the exception of a batch of *C. braarudii* grown in 2007, culture pH and carbonate system parameters were initially modified via adjustment of the total alkalinity (TA) by addition of acid (HCl) or base (NaOH). In the 2007 set of experiments with *C. braarudii*, culture pH and the carbonate system parameters were initially modified by adjustments of the total dissolved inorganic carbon (DIC) by bubbling of air with different CO_2 partial pressures. In previous comparisons, manipulation of TA vs. DIC has not

resulted in different responses of the growth of coccolithophorids and diatoms (Shi et al., 2009) so it is not clear that the observed lower growth rates in the *C. braarudii* experiments from 2007 are due to the way in which media was prepared. Measurements of growth rate, calcification rate, and organic carbon fixation rate are detailed in Langer et al. (2009). Concentrations of DIC, TA, and phosphate were measured at the end of the experiments, using methods detailed previously for the *E. huxleyi* cultures (Langer et al., 2009). The carbonate system parameters were calculated from temperature, salinity, and the DIC, TA, and phosphate concentrations using the DOS program CO2sys (Lewis and Wallace, 1998). Calculated pH is reported throughout on the total pH scale. Over the course of the experiments, media chemistry evolves due to extraction of inorganic carbon and nitrate for organic cellular components and $CaCO_3$ shells. We used standard equilibrium constants (Mehrbach et al., 1973) as subsequently refitted (Dickson and Millero, 1987). Culture carbonate system parameters and growth rates are given in the Appendix A.

For B/Ca analysis, culture samples were collected on 0.45 μm polycarbonate filters, dried and stored in petri dishes. All subsequent containers used to process the sample were cleaned by sonication three times in high purity (Milli-Q) de-ionized distilled water.

2.2. Cleaning of culture samples

Culture samples, which by nature contain a much higher proportion of organic matter than typical marine sediments, must be cleaned to remove organic phases potentially containing B or Ca and to prevent combustion under the ion beam during analysis.

To reduce Fe-oxyhydroxides that may form on the outside of cells (Tang and Morel, 2006) while retaining the integrity of the calcite (Apitz, 1991), the filter was placed on a plastic filter funnel and 10 mL of freshly prepared reducing solution (prepared using 5 g hydroxylamine hydrochloride (Alfa Aesar ACS) in 100 mL 12% NH_4OH (Alfa Aesar ACS)) were added and reacted 20 min before the solution was vacuum filtered. Next, 100 mL of ultrapure distilled water was pumped through the filter to remove the reducing solution. The filter was placed in a 15 mL centrifuge tube with 5 mL of an alkaline oxidizing solution consisting of 0.3% H_2O_2 (Alfa Aesar ACS) in 0.1 M u NaOH (Fluka Analytical TraceSelect), a solution that is frequently used in the cleaning of foraminifera (Barker et al., 2003a). The tube was sonicated briefly to resuspend particles off the filter and the filter was removed. Subsequently, the solution was placed in a boiling water bath for 10 min. The supernatant solution was removed by centrifugation, and the oxidation process was repeated four times. The sample was rinsed once in high purity distilled water, transferred to a new clean centrifuge tube, and rinsed again at least three times. The sample was dried for several days in an oven at 80 °C, forming a dry pellet at the base of the tube.

We evaluated this cleaning method and a similar method which used an oxidizing solution composed of 50% v/v 5% NaOCl (VWR CPR Rectapur) and 30% H_2O_2 (Alfa Aesar ACS) (Bairbakhish et al., 2001) at room temperature. To assess whether both or either method were effective at removing B associated with organic phases, we took a pure carbonate (Cambrian Marble powder from Stockbridge Formation, North Adams, MA), crushed it with an agate mortar and pestle, and sieved it at 20 μm yielding particles in the range of 1–5 μm, similar to coccoliths. We contaminated a split of this powdered carbonate with organic matter from cultures of *Synechococcus* (photosynthetic bacteria) and with mixed algal populations growing in seawater carboys. We then applied the two cleaning methods described above and evaluated if, following cleaning, the contaminated aliquots had the same B/Ca as the original powdered marble.

The comparison of cleaning methods showed that the peroxide oxidation yielded B/Ca indistinguishable from that of the original pure carbonate and therefore was an effective cleaning method (Fig. 1). This comparison also confirms that cleaning steps do not introduce B enrichment or depletion artifacts in the original carbonate. In contrast, the NaOCl oxidation yielded highly variable B/Ca ratios. In some of the analyzed ion probe spots, the ratio was comparable to the original carbonate but in others it was 10-fold higher. It was impossible to measure the contaminated and uncleaned aliquots to determine their end-member B concentrations because the organic phases in these samples combusted under the ion beam and led to negligible sample current.

2.3. Mounting for ion probe

Prior to mounting for ion probe analysis, samples were placed in a 110 °C oven for at least 12 h. Samples were mounted in aluminum holders which contained a central area of 8 mm diameter by 3 mm thick polished indium, which is a conductive but soft (melting temperature = 157 °C) substrate. Cleaned carbonate pellets were split and a portion of the powder was placed in the center of the indium. A polished aluminum disk was placed against the aluminum/indium mount and the two were pressed together

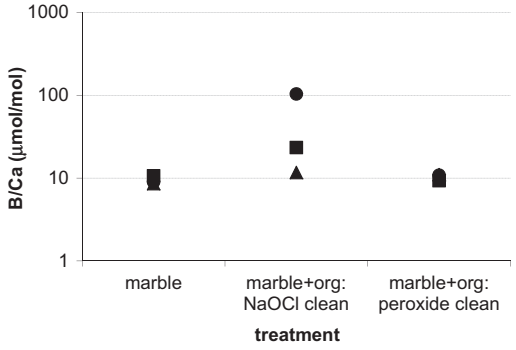

Fig. 1. Comparison of B/Ca in a natural marble sample (Stockbridge Formation, Massachusetts), and in a marble sample contaminated with marine organic matter and subsequently cleaned using NaOCl method (Bairbakhish et al., 2001) or using hot H_2O_2 method (Barker et al., 2003a). Different symbols represent different spots analyzed on the sample.

in a bench vise for 30 min to reduce sample porosity and improve ion yield. Following manufacture, the samples were stored in a vacuum dessicator. Just prior to analysis, samples were coated with 30 nm of gold. Samples which had been insufficiently compacted gave highly variable $^{11}B/^{40}Ca$ during measurement and/or very low sample current. In this case, the samples were repressed in the vise for 30 min and a new gold coat applied. This treatment typically reduces the variability of the measurement, but does not change the mean of the replicate sample points.

2.4. Ion probe analysis

Samples were analyzed on the Cameca IMS 1280 at the Woods Hole Oceanographic Institution. A beam of $^{133}Cs+$ ions with 12.7 keV energy and a current of 5–6 nA was focused to a ~20-micron diameter spot. Negatively-charged secondary ions of B were accelerated into a double-focusing mass spectrometer with 10 keV energy. During the course of this study, it was found that B in carbonates ionized much better as B^- upon Cs^+ bombardment (>20 cps/ppm/nA) than as B^+ upon conventional O^- bombardment (~4 cps/ppm/nA), which made it possible to determine B/Ca at sub-ppm B concentrations in coccoliths. A mass resolving power (MRP) of 3100 was used to separate $^{24}Mg^{16}O$ (required MRP = 2300) and $^{12}C2^{16}O$ (MRP = 1240) from ^{40}Ca. We measured counts at mass 10.7 (background), 11 (B), and 40 (Ca) in an ascending mass order, and repeated the cycle 20 times (two blocks of 10 cycles) with wait times of 4, 2, and 3 s, and count times of 5, 10, and 5 s, respectively. With a ^{11}B count rate of $\sim 2 \times 10^3$, we got a total of 4×10^5 ^{11}B counts per second (cps) with a counting uncertainty of 0.16%. The ^{40}Ca count rate (cps) was $\sim 2.5 \times 10^4$ with total counts of $2.5 \times 10^6$and a counting uncertainty of 0.06%. Data were collected at 4–10 locations on the pressed sample pellet. ^{40}Ca intensities during sample measurements ranged from 8500 to 50,000 cps. This count rate is lower than would be attained by measuring Ca in positive ion mode (e.g. with O^- bombardment) but this does not jeopardize measurement precision which is limited by the count rate on B. No systematic correlation is observed between $^{11}B/^{40}Ca$ and the ^{40}Ca intensity.

A calibration curve was established with four carbonate standards of varying B content from 0.3 ppm (calcite, Carrara) to 29.2 ppm (calcite, 0875), as determined by TIMS isotope dilution at the Institute for the Study of Earth's Interior, Okayama University, Misasa, Japan. Representative calibration data are shown in Table 1. All sample analyses lie between the middle two standards (OKA at 6.15 μmol/mol and ODP209 at 145 μmol/mol) and the slope of the regression in this range is indistinguishable from the full regression of all four standards. The sample data collected at 4–10 locations on the sample pellet have a precision which varies from sample to sample: the average two standard error ranges from 5% to 52% of the sample mean, averaging 14% of the sample mean (all data and standard errors appear in Appendix A). Average precision may differ from session to session, resulting in an average relative standard error (r.s.e.) of 9% for the RCC1256 series but 19% for the EH1G series.

The reproducibility of the biological responses as well as the reproducibility of cleaning methods and ion probe analysis was assessed by analyzing pellets from replicate cultures bottles of *E. huxleyi*. Replicates were available from both the lowest and highest pH conditions for strains RCC1238 and RCC1256, a total of four experimental conditions. The average B/Ca of each pellet, derived from multiple ion probe determinations on each pellet, was compared to its replicate culture. The relative standard deviation of each replicate pair ranges from 2% to 25% and averages 16% (Appendix A). The relative standard deviation of each replicate pair is highly correlated ($r^2 = 0.92$, p value 0.043) with the maximum analytical r.s.e. among the two replicates, estimated from multiple ion probe determinations on the same pellet, as described in the preceding paragraph. This correlation suggests that the overall uncertainty of the B/Ca measurements is not represented best by a uniform error bar but rather one scaling with the analytical uncertainty for that measurement. Hence, in figures, we employ an error bar equal to 2 standard errors of the analytical mean for each pellet.

3. RESULTS

3.1. *Coccolithus braarudii braarudii*

B/Ca in *C. braarudii* range from5 to 22 μmol/mol in the 2009 experiment (Fig. 2a) and from 5 to 8 μmol/mol in the slow growing 2007 experiment (Fig. 2b). In the former experiment, the linear regression suggests increasing B/Ca with increasing pH ($r^2 = 0.78$, $p = 0.11$). Whereas both culture experiments featured an identical range in pH and pCO_2, coccoliths grew at a much slower rate in the 2009 experiment (0.42 vs. 0.86/day in the DIC experiment). The difference in B/Ca between the two experiments is most pronounced at the low-CO_2, high pH conditions.

3.2. *E.* huxleyi

B/Ca in *E. huxleyi* vary significantly among the strains examined. Ratios are much higher in the A morphotypes RCC1256and RCC1238, 55–25 μmol/mol, compared with

Table 1
Calibration curve for ion probe measurements, showing measured ^{11}B:^{40}Ca intensity ratios and their associated standard error, along with the established concentrations for the standards as described in the text.

Standard	Mean $^{11}B/^{40}Ca$	s.e. of mean	ppm B	μmol/mol B/Ca
nsbc0709-carr	4.5E-05	9.7E-06	0.306	2.78
nsbc0709-OKA	8.1E-04	1.7E-05	0.676	6.15
nsbc0709-odp209	2.8E-02	5.3E-03	15.6	142
nsbc0709-0875	7.3E-02	8.6E-04	29.2	265

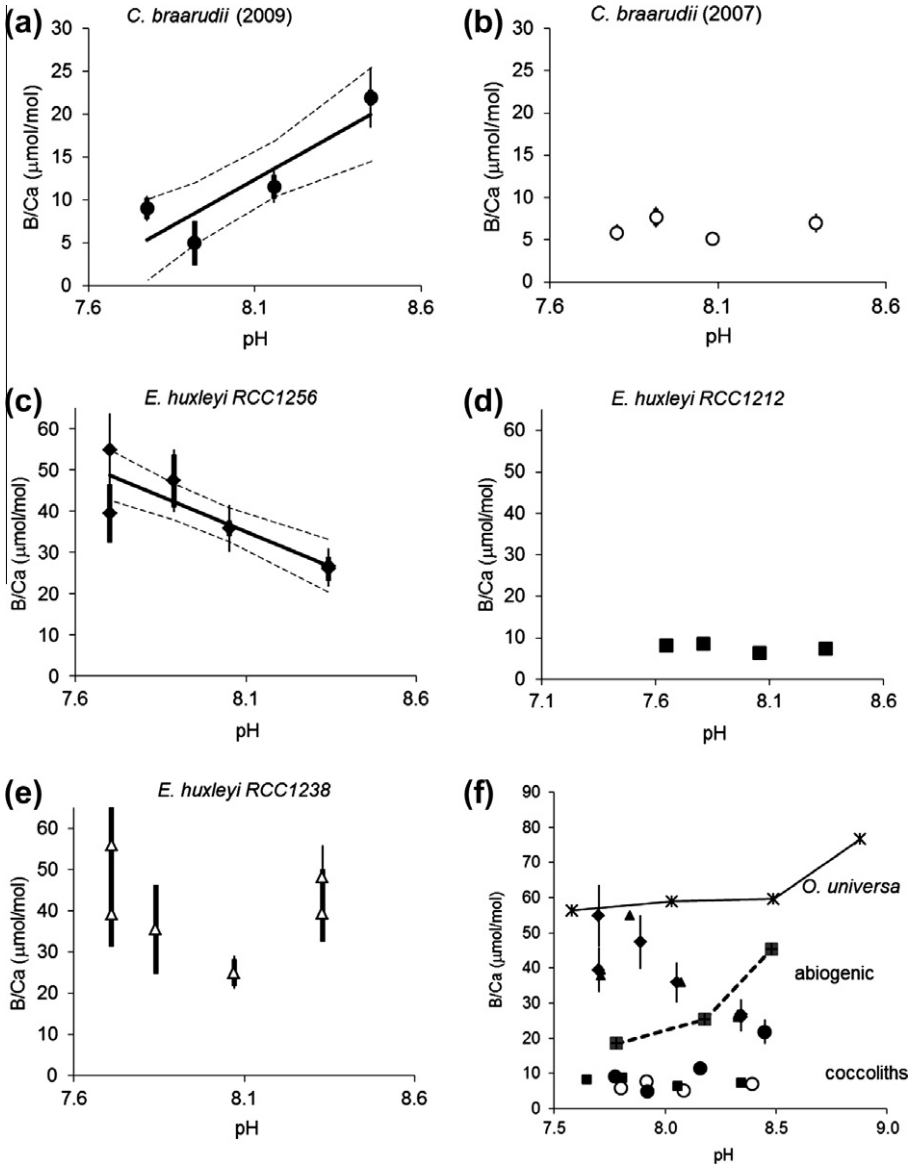

Fig. 2. (a) through (e) Measured B/Ca in coccoliths as a function of seawater pH. Where error is larger than symbol size, heavy error bars on coccolith determinations represent the 2 s.e. analytical error of the ion probe determinations as reported in Section 2.4; thin error bar indicates 1 r.s.d of 16%, the average r.s.d. of replicates, visible in the cases where this is significantly larger than the 2 s.e. analytical standard error. Where B/Ca shows systematic change with seawater pH, a trendline and 90% confidence interval for the trendline is indicated on the figure. In (a) the linear regression has r^2 0.78 and p value 0.11. In (c) the linear regression has r^2 0.77 and p value 0.022. (f) B/Ca for all measured coccoliths compared to estimates from abiogenic calcite experiments (large gray square with dashed bold line) and measurements from cultures of foraminifera *Orbulina universa* (×*symbol*)(Sanyal et al., 1996, 2000). For abiogenic calcites, plotted values are calculated for B content of natural seawater, which is 17-fold lower than the B content used in the precipitation experiments (Sanyal et al., 2000). In order to represent the experiments of foraminifera and abiogenic calcites on the total pH scale, these have been adjusted from NBS to total scale by subtracting 0.12. In all experiments except 2b, pH was modified directly by addition of acid or base as described in the text.

the B morphotype RCC1212, 6–9 μmol/mol (Fig. 2c–e). The differences between RCC1212 and the other two *E. huxleyi* strains are highly significant (two-tailed t-test, $p = 1.3 \times 10^{-7}$). The RCC1212 strain has a significantly lower average growth rate (0.94/day) than the other two strains (1.34/day) as well as lower average calcification rate (Appendix A).

In RCC1256, the six data points define a significant linear decrease in B/Ca with increasing seawater pH ($r^2 = 0.77$, $p = 0.022$). Across the range of pH conditions for cultures of RCC1212, the ratios are constant within analytical uncertainty. Within the large analytical uncertainty in RCC1238, no significant trend with seawater pH is evident.

The highest B/Ca values in the two *E. huxleyi* strains are comparable to those observed at comparable pHs in foraminifera *Orbulina universa* and the overall range of coccoliths is comparable to those measured in abiogenic calcites, when the abiogenic calcites are corrected to seawater B concentrations (Fig. 2f). Nevertheless, with increasing pH, abiogenic calcites and foraminifera *O. universa* show increasing B/Ca, unlike any of the *E. huxleyi* strains. The ranges in B/Ca of *C. braarudii* are lower than those of foraminifera or abiogenic calcites grown at comparable seawater pH.

4. DISCUSSION

In analogy to abiogenic and other biogenic calcites, we assume that borate is likely to be the principal boron species incorporated into coccolith calcite, substituting for the carbonate anion (Hemming and Hanson, 1992; Hemming et al., 1995, 1998; Sanyal et al., 2000; Rae et al., 2011). Yet, coccolithophorid calcification is fundamentally different from that of coral and foraminifera which use endocytosis of seawater for Ca and DIC supply (de Nooijer et al., 2009a). Coccoliths form intracellularly in a specialized vesicle which does not contain advected seawater (Brownlee and Taylor, 2004). Coccoliths are produced one at a time in the coccolith vesicle and subsequently extruded through the cell membrane to form an interlocking layer of plates covering the cell exterior. The coccolith vesicle has a small volume and behaves as an open system during calcification, refilled by continuous flux of Ca in the course of production of a single coccolith (Langer et al., 2006b). The B concentration of the calcifying fluid in this vesicle may differ substantially from that of seawater because B species must either be able to diffuse (in the case of uncharged species) or be transported across the cell membrane.

Limited information on the composition of the calcifying fluid and ion uptake/transport in the coccolithophorid cell hampers our ability to establish an absolute pH estimate or DIC concentration for the calcifying vesicle. Thus, we aim to assess the direction of trends and the approximate amplitudes of changes that might occur in the B/Ca of coccoliths as a result of variations in the seawater pH, the pH of the calcifying vesicle, and the DIC of the calcifying vesicle. Since this model differs significantly from models used for organisms like foraminifera or corals which calcify from a seawater reservoir, model predictions of the B isotopic composition of the coccoliths may offer a fundamental test of the relevance of the model and are described in Electronic annex EA-1.

4.1. A simple model of cellular boron uptake and incorporation in coccoliths

A broad base of studies on boron acquisition by plant and animal cells provides important constraints on the likely mechanisms of B uptake by coccolithophorids. In combination with studies on coccolith biomineralization (Brownlee and Taylor, 2004) and pH determinations in the coccolithophorid cytoplasm and calcifying vesicles (Anning et al., 1996), this framework enables us to develop a model for B uptake and incorporation into coccoliths. The fundamentals of the model and their justification are described successively. This model is the simplest scenario for B uptake by cells, speciation in the coccolithophorid cell and coccolith vesicle, and incorporation into calcite which meets current observational constraints, although future improvements in our understanding of the physiology of calcification in coccolithophorids may enable further refinements of the model.

4.1.1. Boron enters the cell exclusively as boric acid

B uptake occursby boric acid diffusion across the lipid bilayer of the cell membrane (Tanaka and Fujiwara, 2008), a process that is universal in higher plants, and also occurs in animal cells as well as artificial lipids (Dordas and Brown, 2000, 2001). This uptake is the major source of B in higher plants, under non-limiting (>100 μM) B concentrations (Tanaka and Fujiwara, 2008). Given the relatively high B concentration of seawater (>400 μM) relative to soil waters, we suggest this is also the principal uptake mechanism for coccolithophorids (Fig. 3a). Boron is known to be an essential micro-nutrient for diatoms and marine algal flagellates (Loomis and Durst, 1992) and, assuming B quotas of coccolithophorids are similar to those of higher plants, the seawater boric acid concentration is expected to be non-limiting. Alternative sources such as uptake of the charged borate species with a specialized transporter such as BOR1 (Takano et al., 2002) or active uptake of boric acid with a specialized transporter such as NIP (Tanaka and Fujiwara, 2008) would both entail a higher energetic cost to the cell and are therefore expected to be much less significant.

4.1.2. Cytoplasm total boron concentrations are equal to seawater boric acid concentrations

Because of effective membrane permeability to boric acid, cellular boron concentrations reach equilibrium with extracellular concentrations as boric acid concentrations increase (Dordas and Brown, 2000). The extracellular seawater concentration of boric acid, in turn, will vary with seawater pH, as illustrated in Fig. 3b. For example, over the pH range of the cultures (7.8–8.6), we might expect a ~40% decrease in cellular boric acid concentrations as extracellular pH increases and boric acid concentrations decrease from ~350 to 210 μmol kg^{-1} .

Once boric acid crosses the cell membrane, it will speciate into borate and boric acid according to the cell pH (Fig. 3a and b). Previous fluorescent probe studies of cytosol pH for both *E. huxleyi* and *C. braarudii* reveal that pH is always significantly lower (pH 6.3–7.6) than the seawater culture media (pH 8.3) (Anning et al., 1996). At this low pH, nearly all (93–97%) of the boric acid taken up across the cell membrane remains as boric acid in the cytoplasm. Hence, we model cytoplasm B as boric acid and equivalent to seawater boric acid concentrations.

4.1.3. Boric acid exclusively enters the coccolith vesicle

Uptake of B into the coccolith vesicle may occur through passive uptake of the boric acid across the vesicle

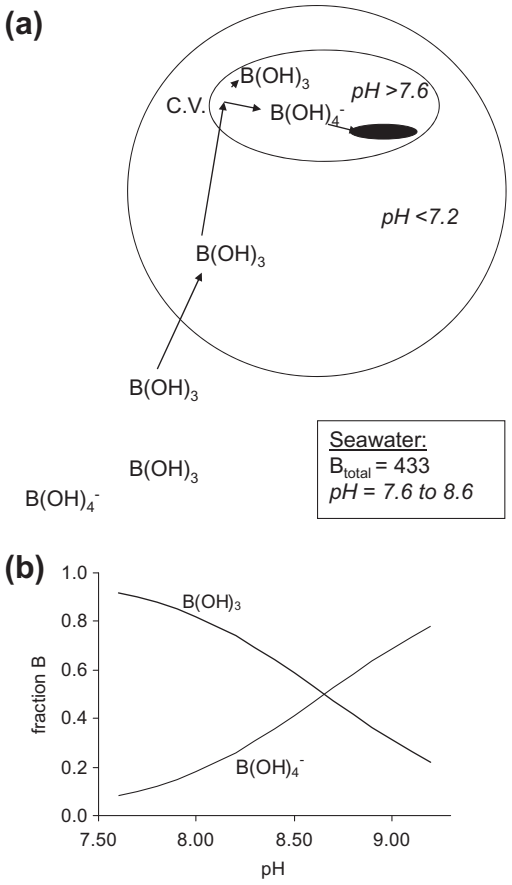

Fig. 3. (a) Schematic of cell model described in Section 4.1. The scale of the coccolith vesicle relative to the cell is exaggerated to illustrate the B speciation. B concentration in μmol kg^{-1} based on B/Salinity relationship (Lee et al., 2010) and salinity of 35. (b) Speciation of B in seawater as a function of pH (total scale), assuming temperature of 20 °C and salinity of 35(Dickson et al., 2007).

membrane or via transport in the acidic Golgi body, an organelle linked to ion transport for calcification (Seksek et al., 1995). Because intracellular as well as extracellular membranes should be permeable only to boric acid, in the model, the coccolith vesicle has a boric acid concentration identical to that of the cytosol and seawater. Within the coccolith vesicle, B will speciate to borate and boric acid depending on coccolith vesicle pH. B speciation is inferred to have a p*K* identical to that in seawater, although the exact p*K* in the coccolith vesicle may be slightly different depending on the ionic composition of the calcifying fluid. A different p*K* could change the absolute borate concentrations in the coccolith vesicles but not the trends. Because the partitioning of B in calcite is very small, the fraction of borate removed to the growing coccolith is negligible compared to the B in the coccolith vesicle, and its replenishment in the coccolith vesicle by diffusion from the cytosol. For this reason, we are able to model the system as a steady state rather than transport-limited system.

4.1.4. Coccolith vesicle pH sets the speciation of borate and boric acid

The pH in the coccolith vesicle determines the relative amount of borate, which is the only form assumed to incorporate into the calcite lattice. Coccolithophorids are likely to regulate the pH in the coccolith vesicle because a higher pH reduces the need for Ca and DIC transport and hence the energetic requirements of calcification. Published fluorescent probe studies in *C. braarudii* indicate one mode in which coccolith vesicle pH is low and covaries with cytosol pH over the range of 6.4–7.2, and another in which coccolith vesicle pH is elevated (7.6–8.3) while cytosol pH remains constant (Anning et al., 1996). We infer therefore that the calcification vesicle pH, at the time of coccolith production, is likely to be in the higher pH range of >7.6 and may extend even beyond 8.3 as the small coccolith vesicle may preclude determination of pH at the moment of calcification. The calcification vesicle may adopt the lower range of pH (6.4–7.2) when calcite is not being produced to avoid diverting cytoplasm CO_2 to an alkaline vesicle which could compete with CO_2 demand from photosynthesis. Cells may have an optimum range of coccolith vesicle pH required for calcification, and may seek to maintain this optimum pH (homeostasis). Nevertheless, changes in the external seawater pH could affect the efficiency of cellular pH regulation, if, for example, a proton channel which exports protons from the cytosol is hindered by a reduced proton gradient between the cytosol and seawater when the seawater is more acidic (Brownlee and Taylor, 2004).

4.1.5. Coccolith vesicle DIC concentrations are actively regulated by the cell and DIC speciation is controlled by coccolith vesicle pH

Bicarbonate transported into the cell is the major source of DIC for calcification (Brownlee and Taylor, 2004) and also a significant source of C for photosynthesis in *E. huxleyi* (Rost et al., 2006), because, in modern seawater, the concentration of dissolved inorganic carbon (DIC) in the form of uncharged carbon dioxide ($CO_{2(aq)}$) in equilibrium with the atmosphere is about 10 μmol L^{-1},insufficient to obtain maximum photosynthetic growth rates of microalgae.Active uptake of inorganic carbon leads to cellular DIC enrichment up to 20-fold (Badger et al., 1998) and, perhaps significantly greater localized enrichment if the intracellular DICpool is concentrated in small volume compartments like the chloroplast or coccolith vesicle. The total DIC concentration in the coccolith vesicle is poorly constrained: some studies suggest concentrations comparable to seawater whereas others imply up to a 10-fold enrichment in DIC (Anning et al., 1996). It may be beneficial for the cell to regulate the DIC concentration in the coccolith vesicle since the solubility product of CO_3^{2-} and Ca^{2+} must match or exceed saturation for calcification to proceed. If coccolith vesicle pH were variable over the range of 7.6–8.6, then a constant concentration of DIC and Ca^{2+} would result in large (8-fold) variation in CO_3^{2-} and of the Ca^{2+}/CO_3^{2-} at saturation. Conversely, to maintain a constant molar Ca^{2+}/CO_3^{2-} in the coccolith vesicle would require a comparable variation in DIC or Ca^{2+} concentration over the pH range of 7.6–8.6. During abiogenic

crystallization, large (2–3-fold) variations in the stoichiometry of the calcifying fluid can cause changes in isotropy of crystal step growth (Davis et al., 2005) which could interfere with the close cellular control over the crystallography of the growing coccolith and may induce cells to regulate the pH of the calcification vesicle. Malformation is observed in *E. huxleyi* coccoliths grown in media with Ca concentrations half of those of normal seawater (Herfort et al., 2004). Defining a particular cellular mechanism for regulation of DIC in the coccolith vesicle is beyond the scope of this contribution, nevertheless, in the model, we explore the consequences of cells maintaining a constant DIC in the coccolith vesicle, and of cells varying the DIC in the coccolith vesicle to maintain a constant degree of oversaturation. The range of values we explore in the model is given in the relevant figure caption for each model experiment.

To calculate HCO_3^- and CO_3^{2-} based on the modeled DIC and pH of the coccolith vesicle, we employ standard carbonic acid dissociation constants for seawater(Dickson et al., 2007). If the major ionic composition of calcifying fluid differs significantly from seawater, the absolute value of dissociation constants may differ, but, as noted above, this would slightly affect the absolute calculated B/Ca for the coccoliths but not the direction or magnitude of changes in B/Ca.

4.1.6. Boron partitioning in the coccolith is a function of the borate–bicarbonate ratio in the coccolith vesicle

Based on inorganic precipitation experiments, the mechanism of B incorporation in calcite has been inferred to be an exchange reaction dependent on the ratio of borate to HCO_3^- in the solution (Hemming and Hanson, 1992):

$$CaCO_3 + B(OH)_4^- \leftrightarrow Ca(HBO_3) + HCO_3^- + H_2O \quad (1)$$

The borate uptake has previously been described (Zeebe and Wolf-Gladrow, 2001; Yu et al., 2007):

$$K_D = [B/Ca]_{calcite}/[B(OH)_4^-/HCO_3^-]_{aq.} \quad (2)$$

The most relevant estimates of partitioning coefficient come from a study of abiogenic carbonate precipitation in Mg-free seawater (Sanyal et al., 2000), but the estimates of K_D are subject to two uncertainties. First of all, in that study, the pH was characterized but the solution chemistry, temperature, and salinity are not fully documented leading to uncertainty in DIC and therefore HCO_3^- concentration estimates. For our purpose and subsequent calculations, we assume modest seawater DIC, salinity and temperature values (DIC = 2000 μmol, $S = 35$, and temperature of 25 °C) and seawater boric acid and carbonic acid dissociation constants (Dickson et al., 2007). Differences in solution chemistry and dissociation constants may yield different absolute partitioning coefficients but will not broaden the relative range of K_D values for the suite of pH conditions considered in our model. Secondly, in that study, calcite was precipitated with the aid of seed crystals whose final mass contribution to the analyzed calcite is not well constrained. A lower estimate of K_D (0.0004–0.0005) results from the assumption that seed crystals, through dissolution-reprecipitation, completely re-equilibrated with the experimental solution. An upper estimate of K_D (0.0012–0.0018) is obtained if the seed crystals did not re-equilibrate with the experimental solutions and B was strictly incorporated in the overgrowth. The extent of re-equilibration in the experiment depends on a variety of factors such as the purity and size of the seed crystals as well as the experiment duration, which are not documented in that study. Irrespective of the scenario, B partitioning in calcite is much less variable (±25% of the mean) than common divalent cations in calcite which can exhibit 5-fold variations in partitioning coefficients as a function of saturation state or precipitation rate (Lorens, 1981; Tesoriero and Pankow, 1996).

In our model simulations, we employ a constant abiogenic K_D of 0.0005. The value chosen is near the lower end of K_D estimated from abiogenic studies (i.e. assuming substantial re-equilibration of seed crystals) and similar to the value of 0.00063 estimated for planktonic foraminifera grown in seawater pH of 8.6, for which there should be minimal difference between seawater pH and calcification vacuole pH (de Nooijer et al., 2009b; Rollion-Bard and Erez, 2010). Given a partitioning coefficient of 0.0005 and a coccolith vesicle DIC comparable to seawater (2200 μM) yields a range of B/Ca of coccoliths which is comparable to that observed in our culture experiments. Nonetheless, if the actual partitioning coefficient were threefold higher (e.g. K_D of 0.0015 consistent with the no seed crystal re-equilibration scenario described above) then the model fits would require that the DIC concentration in the coccolith vesicle be threefold higher to produce the same coccolith B/Ca value. A higher DIC is compatible with evidence for strong inorganic carbon accumulation in coccolithophorid cells (Badger et al., 1998).

In sensitivity tests,we examine the effect of K_D varying by ±35% of the chosen value of 0.0005. This is implemented as a linearly increasing K_D from 0.000325 at coccolith vesicle pH 7.6–0.000675 at coccolith vesicle pH 8.6. This positive correlation is most consistent with the results of Sanyal et al. (2000), but contrasts with the suggestion that decreasing foraminiferal K_D with higher pH could result from competition with carbonate ion (Allen et al., 2011).

4.2. Model predictions for B in coccoliths

In the first series of model experiments, we maintain a constant pH and DIC in the coccolith vesicle while varying seawater pH. We illustrate the coccolith B/Ca response to three potential pH values for the coccolith vesicle: 8.3, 8.0, and 7.8. In each case, as seawater pH increases, there is reduced cellular uptake of boric acid resulting in reduced borate in the coccolith vesicle and a lower B/Ca of the coccolith (Fig. 4a). Consequences for B isotopes for this and the subsequent model experiments are described in EA-1.

In the second series of model experiments, the pH in the coccolith vesicle positively covaries with seawater pH while the coccolith vesicle DIC remains constant. We illustrate four potential ranges of variation in CV pH: from 7.6–8.6 (identical to seawater pH variation), from 7.6 to 7.9, from

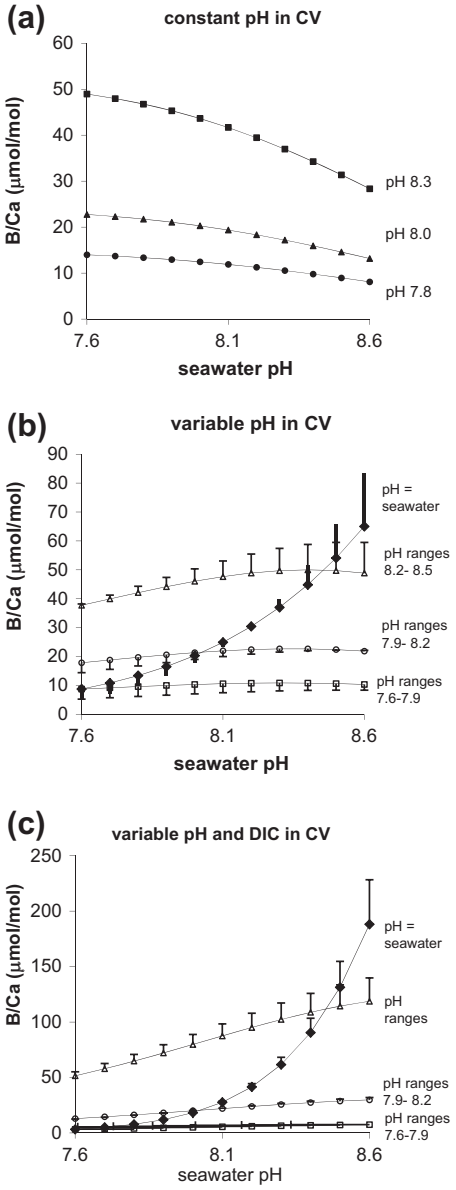

Fig. 4. Modeled variations in coccolith B/Ca ratio. (a) Case for constant pH of coccolith vesicle, assuming coccolith vesicle DIC of 2200 μmol kg^{-1}. Three pH values are indicated: pH 8.3 (filled squares), pH 8.0 (open triangles) and pH 7.8 (solid circles). (b) Case for variable pH of coccolith vesicle, and constant coccolith vesicle DIC of 2200 μmolkg^{-1}. Solid diamond indicates coccolith vesicle pH identical to seawater pH. Cases of attenuated covariation of C.V. pH with seawater pH are shown for C.V. pH 8.2–8.5 (open triangles), pH 7.9–8.2 (open circles) and pH 7.6–7.9 (open squares). (c) Case for variable pH of coccolith vesicle, and DIC varying to maintain a constant carbonate ion concentration in the CV of 200 μmol kg^{-1}.For pH 8.2–8.5 this entails DIC decrease from 1600 to 900 μmol kg^{-1}, for pH 7.9–8.2 this entails DIC decrease from 3000 to 1600 μmol kg^{-1} and for pH 7.6 to 7.9 this entails DIC decrease from 5900 to 3000 μmol kg^{-1} . Symbols for pH variation as in panels (b). In (b) and (c) the vertical error bars show the difference between B/Ca expected for K_D of 0.0005 and the B/Ca that results if the $K_D$varied by ±35% over the coccolith vesicle pH range from 7.6 to 8.6, as described in the Section 4.1.6 of the text. The model calculations were done assuming seawater speciation constants for boron and carbonate system given by (Dickson et al., 2007), for salinity of 35 and temperature of 20 °C and pH on total scale.

7.9 to 8.2, and from 8.2 to 8.5. If the pH in the coccolith vesicle is identical to seawater pH, then borate in the coccolith vesicle will increase because the proportional change in vesicular borate with increasing pH is greater than the proportional decrease in ambient boric acid. This leads to a steep rise in B/Ca of coccoliths with increasing seawater pH (Fig. 4b).

In this second series of model experiments, we also evaluate the effect of small decreases in K_D at higher coccolith vesicle pH, using vertical error bars in Fig. 4b to indicate the extent of additional variation. These small variations in K_D have a minor impact on the trends in coccolith B/Ca. Therefore, unless the existing abiogenic experiments dramatically underestimate the variability in boron partitioning in calcite, the principal sources of variation in coccolith B/Ca remain the pH of the seawater and the calcifying vesicle, and, as shown subsequently, the DIC concentration of the coccolith vesicle.

In the final set of model experiments, we use the same variations in CV pH as in the second set of model experiments, but also evaluate the effect of decreasing DIC concentration with increasing coccolith vesicle pH in order to maintain a constant $[CO^3_{2-}]$ in the coccolith vesicle (Fig. 4c). If the coccolith vesicle pH increases from 7.6 to 8.6, this entails an 8-fold reduction in DIC; for lesser changes in coccolith vesicle pH, the DIC change is proportionally smaller as indicated in the figure legend. In all cases, this variation in DIC steepens the increase in B/Ca with increasing seawater pH (Fig. 4c) with respect to the case of constant coccolith vesicle DIC (Fig. 4b).

4.3. Interpreting processes responsible for B/Ca variations in coccoliths from culture experiments

4.3.1. Relationship to calcification and growth rate

According to our model, the cellular B content is set uniquely by the seawater pH and, for any given seawater pH, all species should have the same cellular B content. If our model captures the primary processes regulating B/Ca in coccoliths, then variations in B/Ca at constant pH might be useful for discerning differences in vesicle pH or DIC among different strains and species (Fig. 5a).For each of the four seawater pH conditions used in our experiments, at least two populations of B/Ca are distinguished; the low ratios of *E. huxleyi* RC1212 and *C. braarudii* 2007 experiments, and the high ratios characteristic of *E. huxleyi* strains 1256 and 1238. Although we cannot infer the absolute pH or DIC variations involved, the model can be used to estimate the magnitude of variation in either coccolith vesicle pH (assuming constant DIC) or DIC (assuming constant pH) that could explain the observed range between the low B/Ca and high B/Ca populations at a given seawater pH. For example, at the seawater pH of 7.9, the variation in B/Ca among the different strains could be explained by pH of the calcification vesicle ranging from 7.7 to 8.3 (assuming constant DIC of 2200 μmol kg^{-1}), or by DIC of the calcification vesicle ranging from 9000 to 1800 μmol kg^{-1} (assuming constant pH of 8.3; Fig. 5b). The B isotopic composition of coccoliths would differ significantly in these two scenarios, as indicated in EA-1.

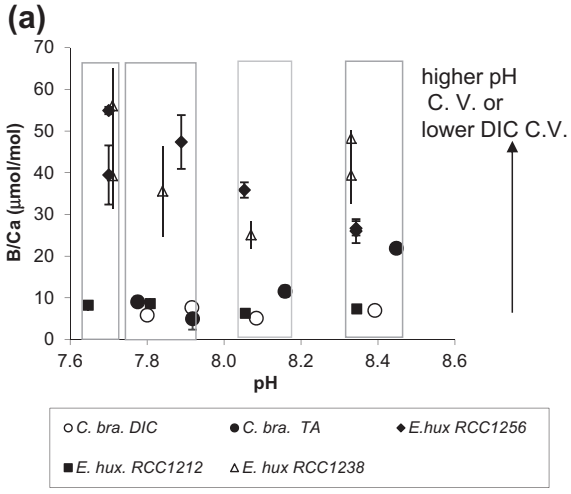

Fig. 5. (a) Comparison of B/Ca in all cultured coccoliths as a function of culture pH (adjusted to total pH scale). Rectangles group experiments in a similar seawater pH range, within which most variation is inferred to arise from different coccolith vesicle pH and/or DIC. Error bars in measurements as in Fig. 2. (b) For an example case of constant seawater pH of 7.9, estimate of the degree of variation in coccolith vesicle pH or DIC (in μmol kg^{-1}) which would be required to produce the range from low B/Ca (zone A, coinciding with values found in *E. huxleyi* RCC1212 and *C. braarudii*) to high B/Ca (zone B, coinciding with values found in *E. huxleyi* RCC1256 and RCC1238). Filled circles exhibit modeled variation for variable pH at a constant coccolith vesicle DIC of 2200 μmol kg^{-1}, open squares exhibit modeled variation for DIC (in μmol kg^{-1}) assuming a constant coccolith vesicle pH of 8.3. pH on total proton scale.

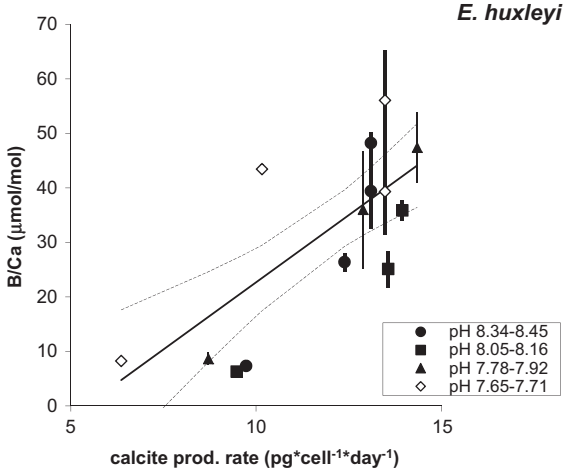

Fig. 6. Coccolith B/Ca vs. calcification rate in *E. huxleyi* experiments. Linear regression through entire dataset is shown along with 90% confidence intervals on the regression ($r^2 = 0.48$, $p = 0.0028$). For simplicity a single error estimate from the 2 r.s.e. analytical uncertainty is shown. pH ranges shown are on total proton scale.

In the *E. huxleyi* experiments, calcification rates also define two clearly distinct populations of samples (Fig. 6). The population with low calcification rate coincides with low B/Ca values. The population B/Ca broadens at the lowest seawater pH, as the absolute calcification rates decrease. A linear regression through the data is significant ($r^2 = 0.48$, $p = 0.0028$). The association between calcification rate and B/Ca may reflect differing regulation of calcification in different strains. A higher pH in the calcification vesicle reduces the energetic cost of calcification (Anning et al., 1996) by reducing the concentration of dissolved inorganic carbon required to attain saturation for any given Ca concentration. In this way, a higher coccolith vesicle pH might enable a greater calcite production rate. Because calcification rate is positively correlated with growth rate in these experiments (Langer et al., 2009), it is also possible that higher growth rates deplete DIC concentrations in the coccolith vesicle. Alternatively, if higher calcification rates reflect greater crystal growth rates of individual coccoliths, rather than shorter time spans between the production of successive coccoliths, and the scope of kinetic effects on boron partitioning is underestimated in existing abiogenic experiments, a higher B partitioning coefficient at faster growth rates could, in theory, increase B/Ca at higher calcification rate. However, the scope of variation in K_D is not documented in abiogenic systems and, if foraminiferal empirical K_D were taken as evidence of greater variability, such variation is in the wrong direction: higher seawater pH conditions which favor higher calcification rates in foraminifera(Spero et al., 1997) are accompanied by decreases in empirical K_D (Allen et al., 2011).The association between calcification rate and B incorporation in coccoliths merits further study as it may provide a new technique to trace the cellular processes of calcification and its regulation.

In the case of *C. braarudii*, no data exist for calcification rate but the two experiments define populations of samples of two different growth rates. B/Ca exhibit variability only for experiments at high pH (>8.17). At the highest pH, the B/Ca is higher in the rapidly growing population (Fig. 7). In this strain, when seawater pH is high and $CO_{2\ aq}$ is more limited, then faster growth rates coincide with either higher coccolith vesicle pH or lower coccolith vesicle DIC. It has been shown that several classes of phytoplankton shift the ratio of CO_2 to HCO_3^- uptake, and upregulate certain carbon acquisition systems under low $CO_{2\ aq}$ conditions such as occur in the high pH conditions of these cultures (Kranz

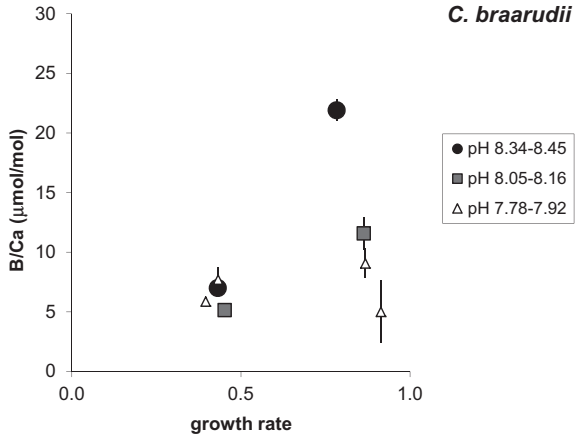

Fig. 7. Coccolith B/Ca vs. growth rate in *C. braarudii* experiments. For simplicity, a single error estimate from the 2 r.s.e. analytical uncertainty is shown. pH ranges shown are on total proton scale.

et al., 2009; Trimborn et al., 2009) . Hence, it is possible that under CO_2 limitation, maintenance of high growth rates requires shifts in the mechanisms of calcification which are not induced with fast growth rates. Additional experiments are needed to see if this trend is general among other strains and if it is also related to calcification rate.

4.3.2. Relationship to calcification and growth rate

The model can constrain the magnitude of coccolith vesicle DIC or pH variations in response to seawater pH excursion and reflected by B/Ca of each strain. Because of the uncertainties in DIC concentration and K_D, we cannot estimate absolute coccolith vesicle pH by this method, but we can estimate the magnitude of variation within the likely pH range of the coccolith vesicle (Anning et al., 1996). The current modest analytical precision in B/Ca determinations also contributes to ambiguity in interpretations and,as this is improved, B/Ca may provide tighter constraints on coccolith vesicle processes.

Constant pH in the coccolith vesicle yields a pattern of decreasing B/Ca with increasing seawater pH (Fig. 4a). The scope of this resulting decrease in B/Ca could explain, within analytical uncertainties, the range of variation in B/Ca in the rapidly growing *E. huxleyi* strain RCC1256 (Fig. 8a). If this process were dominant, it would imply pH homeostasis in the coccolith vesicle over most of the seawater pH range, in particular the maintenance of alkaline coccolith vesicle pH as seawater pH becomes more acidic. This strain exhibited an optimum calcification rate with increasing acidification (Langer et al., 2009), which may be consistent with homeostasis of pH during ocean acidification. Alternatively, the B/Ca data could be explained by sympathetic variation in pH of the coccolith vesicle (pH 8.3–8.6) and seawater, coupled with an increase in DIC in the coccolith vesicle from 1800 to 5000 μmol kg^{-1} as seawater pH increases (Fig. 8a). This latter scenario would imply a fourfold increase in carbonate ion concentration in the coccolith vesicle over the range of seawater pH from 7.9 to 8.6.

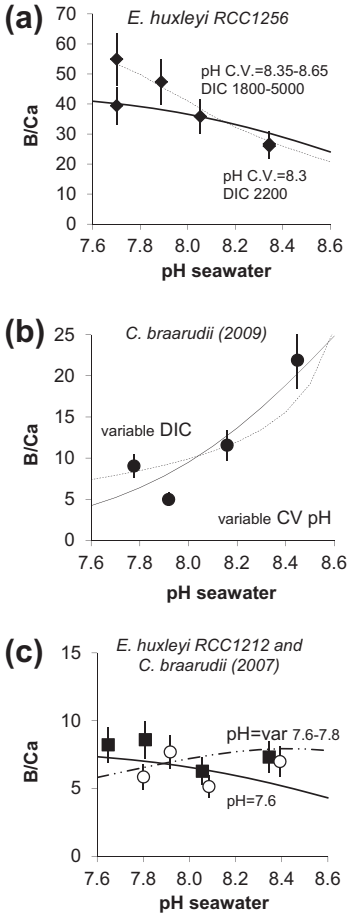

Fig. 8. Comparison of magnitude of B/Ca changes in models with B/Ca measurements in coccoliths grown at different seawater pH. All B/Ca in μmol/mol and DIC in μmol kg^{-1}; for simplicity a single error estimate from the 2 r.s.e. analytical uncertainty is shown. Culture pH converted to total pH scale and model simulations run for culture salinity value of 32. (a) Measured B/Ca of *E. huxleyi* strains RCC1256. Solid curve shows model for constant coccolith vesicle pH of 8.3 and DIC 2200 μmol kg^{-1}; dashed line shows variable coccolith vesicle pH (increasing linearly from 8.35 to 8.65 with increasing seawater pH) and variable coccolith vesicle DIC (increasing linearly from 1800 to 5000 μmol kg^{-1} with increasing seawater pH). (b) Measured B/Ca of *C. braarudii* AC400 grown in 2009. Solid line shows model for variable coccolith vesicle pH and DIC (pH of coccolith vesicle increasing from 7.7 to 8.2 as seawater pH increases, and coccolith vesicle DIC varying to maintain constant carbonate ion concentration of 200 μmol kg^{-1}). Dashed line shows model for constant coccolith vesicle pH of 8.35 and DIC decreasing with increasing seawater pH from 11,000 to 1800 μmol/L. (c) Variations in coccolith B/Ca in *E. huxleyi* RCC1212 (filled square) and *C braarudii* grown in 2007 (open square) compared with models of constant coccolith vesicle pH of 7.6 (solid line) and models of variable coccolith vesicle pH (pH variations 7.6–7.8). In both cases, constant coccolith vesicle DIC of 2200 μmol kg^{-1} is assumed. B/Ca all reported in μmol/mol.

Sympathetic variation in pH between seawater and the coccolith vesicle yields increasing B/Ca with increasing seawater pH (Fig. 4b and c). The B/Ca trends from the

rapidly growing 2009 *C. braarudii* experiments could be accounted for by coccolith vesicle pH changes comparable in magnitude to those of the seawater (Fig. 8b).Alternatively, the B/Ca data could be accounted for by a constant pH in the coccolith vesicle and DIC concentrations which decrease from 10,000 to 2000 μmol kg^{-1} as seawater pH increases (Fig. 8b). The B isotopic composition of coccoliths would differ significantly in these two scenarios, as indicated in EA-1.

The overall lower B/Ca of the 2007 *C. braarudii* experiments and slower growing *E. huxleyi*strain RCC1212 suggest overall lower coccolith vesicle pH and/or higher DIC, as illustrated in Fig. 6. The constancy of B/Ca ratios, despite changes in seawater pH, is consistent with either constant or only slightly varying coccolith vesicle pH (Fig. 8c). The RCC1212was the only *E. huxleyi* strain exhibiting appreciable calcification rate decrease with seawater acidification (Langer et al., 2009). The disruption of calcification in this species at low seawater pH cannot be unambiguously ascribed to variation in pH in the coccolith vesicle based on the B/Ca data. Yet, if coccolith vesicle DIC values are comparable to those of other *E. huxleyi* strains, then lower overall B/Ca indicate that average pH in the coccolith vesicle in this strain is lower than for the other strains, perhaps making it more susceptible to disruption of calcification by acidification of seawater. A further comparison across a larger number of strains would be required to establish if there is a relationship between sensitivity to acidification and the pH or DIC of the coccolith vesicle at normal seawater pH.

5. CONCLUSIONS

We have constructed a simple model for boron uptake and speciation in coccolithophorids which accommodates all existing constraints from coccolithophorid biomineralization and uptake of boron across cell membranes. Whereas uncertainties in the absolute values of a number of parameters preclude using the model to solve for absolute pH in the coccolith vesicle, the model can assess the degree and direction of changes in coccolith vesicle pH or DIC which would be required to produce trends in B/Ca observed in coccolithophorids. Because the pH and DIC concentration are key parameters in determining the saturation state of vesicle fluid and the onset of calcite precipitation, constraints in these parameters from measurement of coccolith B/Ca and boron isotopes may reveal the mechanisms behind calcification responses of coccolithophorids to changes in the ocean pH and carbonate mineral saturation state.

The first analyses of B/Ca in coccoliths reveal an intriguing trend of higher B/Ca at higher calcification rates of *E. huxleyi*. If the higher B/Ca reflect higher coccolith vesicle pH, it would indicate that coccolithophorids may regulate calcification vesicle pH to promote calcification or reduce the energetic cost of DIC transport. This association merits further study as it may provide a new technique to trace the cellular processes of calcification and its regulation. Our model reveals that the combination of B/Ca and B isotopic measurements in coccoliths (shown in EA-1) could distinguish the dual pH and DIC control of saturation state in the coccolithophorid vesicle and the regulation of calcification. Therefore, developing measurements of boron isotopes in coccolithophorids from culture should be a priority to ascertain the mechanism of calcification response to changes in the ocean's carbonate saturation state.

The model provides a quantitative basis to evaluate the role of changing DIC and pH in the calcifying vesicle. While tailored to coccolithophorids, several aspects of the conceptual model may be applicable to other marine calcifiers such as foraminifera and corals for which B concentration and isotopes are studied. Whereas most interpretations of the B system in these organisms are based on the assumption of precipitation from seawater, it is possible that there is additional cross-membrane transport of boric acid into the calcification space. It is also known that many organisms elevate the pH and may also modify the DIC of the calcifying space (Bentov et al., 2009; de Nooijer et al., 2009b). Explicit calculation of these modifications may also improve interpretation of B concentrations and isotopic ratios in these other marine calcifiers.

AUTHOR CONTRIBUTIONS

G.L. conducted the culture experiments, N.S. developed the SIMS measurement protocol and sample mounting procedure, H.M.S. conceived of the B indicator for calcification rate and developed and evaluated the cleaning procedure; N.S., H.M.S. and K.K. conducted the SIMS measurements; H.M.S. developed the cell model and wrote the paper.

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APPENDIX A

Culture parameters (Langer et al., 2009) including media chemistry measured at time of harvest, and B/Ca measurements. Measured pH on NBS scale was converted to total scale using CO2sys.

APPENDIX B. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gca.2011.12.003.

Species	Strain	Exper.	Replicate	Growth rate μ	Temp. °C	TA (μmol kg^{-1})	TC (μmol kg^{-1})	pH (NBS)	CO2 (μatm)	HCO_3^- (μmol kg^{-1})	CO_3^{2-} (μmol kg^{-1})	Omega calcite	pH (total)	Calcification rate (pg calcite $*$ $cell^{-1}$ $*$ day^{-1})	B/Ca (μmol/mol)	2 s.e. analytical uncertainty in B/Ca (μmol/mol)	Std. dev. culture replicate
C. braarudii	AC 400	2009		0.78	17	2521	2028	8.57	144.3	1675	348.3	8.48	8.45		22	0.9	
C. braarudii	AC 400	2009		0.86	17	2239	1967	8.28	297.6	1768	188.2	4.58	8.16		12	1.4	
C. braarudii	AC 400	2009		0.91	17	2056	1905	8.03	523	1778	108.3	2.64	7.92		5.0	2.6	
C. braarudii	AC 400	2009		0.87	17	2022	1922	7.89	743.8	1816	79.4	1.93	7.78		9.1	1.3	
C. braarudii	AC 400	2007		0.43	17	2124	1726	8.51	143	1456	265.5	6.47	8.39		7.0	0.4	
C. braarudii	AC 400	2007		0.45	17	2059	1839	8.20	335.9	1677	150	3.65	8.08		5.2	0.3	
C. braarudii	AC 400	2007		0.43	17	2087	1936	8.03	533.7	1807	109.6	2.67	7.92		7.7	1.0	
C. braarudii	AC 400	2007		0.40	17	2145	2033	7.91	741	1918	88.9	2.16	7.80		5.9	0.4	
E. huxleyi	RCC 1256		A	1.28	17	2456	2049.0	8.46	193	1756	286	7	8.34	12.4	27	1.7	0.47
E. huxleyi	RCC 1256		B	1.28	17	2456	2049.0	8.46	193	1756	286	7	8.34	12.4	26	2.9	
E. huxleyi	RCC 1256			1.28	17	2240	2022.0	8.17	399	1853	155	3.8	8.05	14.0	36	1.8	
E. huxleyi	RCC 1256			1.14	17	2136	1993.0	8	587	1865	106	2.6	7.89	14.4	47	6.5	
E. huxleyi	RCC 1256		A	0.83	17	2051	1974.0	7.81	915	1872	69	1.7	7.70	10.2	40	0.9	10.96
E. huxleyi	RCC 1256		B	0.83	17	2051	1974.0	7.81	915	1872	69	1.7	7.70	10.2	55	8.6	
E. huxleyi	RCC 1212			1.00	20	2517	2067.0	8.47	194	1741	319	7.8	8.35	9.7	7.3	0.3	
E. huxleyi	RCC 1212			0.99	20	2313	2066.0	8.18	409	1877	176	4.3	8.06	9.5	6.3	0.1	
E. huxleyi	RCC 1212			0.93	20	2203	2071.0	7.93	752	1944	103	2.5	7.81	8.7	8.6	1.2	
E. huxleyi	RCC 1212			0.85	20	2128	2053.0	7.77	1096	1947	71	1.7	7.65	6.4	8.2	0.5	
E. huxleyi	RCC 1238		A	1.48	20	2522	2086.0	8.45	206	1768	311	7.6	8.33	13.1	39	6.9	6.24
E. huxleyi	RCC 1238		B	1.48	20	2522	2086.0	8.45	206	1768	311	7.6	8.33	13.1	48	1.9	
E. huxleyi	RCC 1238			1.64	20	2302	2050.0	8.19	395	1858	179	4.4	8.07	13.57	25	3.3	
E. huxleyi	RCC 1238			1.67	20	2184	2039.0	7.96	681	1907	110	2.7	7.84	12.89	36	10.8	
E. huxleyi	RCC 1238		A	1.6	20	2107	2013.0	7.83	929	1902	80	2	7.71	13.48	56	9.1	11.84
E. huxleyi	RCC 1238		B	1.6	20	2107	2013.0	7.83	929	1902	80	2	7.71	13.48	39	7.8	

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