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Calcification in the planktonic foraminifera *Globigerina bulloides* linked to phosphate concentrations in surface waters of the North Atlantic Ocean

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Abstract

Marine calcifiers, such as planktonic foraminifera, form a major component of the global carbon cycle, acting as both a source and sink of CO_2. Understanding factors that affect calcification in these organisms is therefore critical in predicting how the oceans will respond to increased CO_2 concentrations in the atmosphere. Here, size-normalised weights (SNWs) of the planktonic foraminifera *Globigerina bulloides*, collected from the surface waters of the North Atlantic, are compared with in situ carbonate ion concentrations ($[CO_3^{2-}]$), optimum growth conditions (implied from *G. bulloides* abundances), and nutrient concentrations. Significant positive relationships suggest that phosphate concentration ($[PO_4^{3-}]$) has the greatest effect on *G. bulloides* SNWs, with reduced test masses at higher concentrations (range: 0.04–0.31 µM). $[CO_3^{2-}]$ appears to have a minor effect over the range of values examined (148–181 µmol kg^{-1}), and no evidence was found for increased SNWs under apparent optimum growth conditions. These findings point to the potential importance of phosphate concentration in determining calcification rates in foraminifera, a factor which has been overlooked by previous studies on these organisms. The confirmation of these results via carefully controlled culture studies is recommended in the future.

1 Introduction

Marine calcifying organisms secrete shells of calcium carbonate ($CaCO_3$) and form a major component of the global carbon cycle, transferring approximately 3 billion tons of $CaCO_3$ to the sea-floor annually (Milliman, 1993). While $CaCO_3$ transferred to the seafloor represents a long-term sink of carbon dioxide (CO_2), the production of $CaCO_3$, releasing CO_2 into the surrounding water, represents a source over shorter timescales (Purdie and Finch, 1994).

The oceans are estimated to have absorbed between 30–40 % of anthropogenically released carbon dioxide (CO_2; Sabine et al., 2004; Zeebe et al., 2008), thereby

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mitigating some of the effects of climate change. This, however, has come at the cost of reduced oceanic pH values (Caldeira and Wickett, 2003), a phenomenon termed "ocean acidification". The ongoing "acidification" of the oceans is proposed to have an adverse effect on marine calcifiers via shifts in seawater carbonate chemistry and associated reductions in carbonate ion concentrations [(CO_3^{2-})] (e.g. Gattuso et al., 1998; Riebesell et al., 2000; Müller et al., 2010). However, a recent comparison of 18 calcifying organisms suggests that a range of responses to reduced [CO_3^{2-}] are likely (Reis et al., 2009). This is reinforced by studies on coccolithophores which have found mixed responses in calcification under increased pCO_2 conditions (Riebesell et al., 2000; Langer et al., 2006; Iglesias-Rodriguez et al., 2008).

Planktonic foraminifera are ubiquitous open ocean protozoans and comprise an estimated 23–56 % of the total open ocean marine $CaCO_3$ flux to the deep sea (Schiebel, 2002). Understanding the factors controlling calcification in these organisms is, therefore, critical in predicting how the oceanic carbon pump will respond to increased pCO_2 in the atmosphere. Additionally, size-normalised weights (SNWs) of these organisms, which are essentially a measure of test (shell) thickness and therefore calcification rate, are a potentially important proxy for enabling paleoatmospheric pCO_2 variations beyond ice-core records to be evaluated (Spero et al., 1997). This is based on the assumption that SNWs of these organisms are strongly linked to [CO_3^{2-}], which in turn is used as a proxy for pCO_2 in the atmosphere.

Planktonic foraminiferal SNWs are generally reduced under lower [CO_3^{2-}], although a large amount of inter and intra-specific diversity in response to [CO_3^{2-}] exists (see Table 1 for a summary). There is also a suggestion that SNWs may be greatest under optimum growth conditions (de Villiers, 2004), which are represented by the geographic location where each individual species is most abundant, either because favourable growth conditions result in higher rates of calcification, or a larger proportion of the population reaches maturity where calcite crust formation takes place. This hypothesis, however, is not supported in a recent comparison between SNWs and both the absolute and relative abundance of *Globigerina bulloides* and *Globigerinoides ruber* using

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samples from the Arabian Sea (Beer et al., 2010a). Therefore, it has been suggested that environmental controls in addition to [CO_3^{2-}] influence foraminiferal calcification rate and hence SNWs.

Nutrient concentrations (NO_3^- and PO_4^{3-}) are also likely to be important in determining foraminiferal calcification rates. In culture experiments, increased "water fertility" has been shown to result in larger test sizes via increases in prey availability (Bijma et al., 1992), but it is unknown whether concomitant increases in test thickness, and therefore SNWs, also occur. It may be that high [PO_4^{3-}] actually reduces SNWs as substantial evidence exists for the inhibition of calcification by phosphate (PO_4^{3-}) via the adsorption of calcium hydrogen phosphate ($CaHPO_4$) onto the calcite surface, blocking active crystal growth sites and slowing $CaCO_3$ precipitation (Lin and Singer, 2006). Reduced calcification rates have been observed at elevated phosphate concentrations in coral reefs (Kinsey and Davies, 1979), calcifying green algae (Demes et al., 2009), and coccolithophores (Paasche and Brubank, 1994). However, the influence of phosphate on foraminiferal calcification has yet to be investigated.

Here, SNWs of the planktic foraminifer *Globigerina bulloides*, from two size fractions (150–200 µm and 200–250 µm) collected from surface waters at 10 locations in the North Atlantic Ocean, are compared to in situ [CO_3^{2-}], optimum growth conditions (implied from *G. bulloides* abundances), and nutrient concentrations (NO_3^- and PO_4^{3-}) in order to investigate the environmental factor/s controlling calcification rates of this species in the natural environment.

2 Material and methods

2.1 Sample collection

G. bulloides samples and in situ environmental data were collected from 10 locations in the North Atlantic (Fig. 1) on board the RRS Discovery D340 Extended Ellett Line Cruise between 11 and 20 June 2009. Specimens of *G. bulloides* were obtained as

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recommended by Hemleben et al. (1989) using a plankton net towed at the surface with a 0.5 m diameter opening (area 0.196 m^2) and a mesh size of 120 µm; the use of a flowmeter allowed for the quantification of water passing through the net. Seawater samples were immediately preserved using formalin buffered with sodium borate (30 g per l^{-1}) to provide a final formalin concentration of 4 % and a pH of 8.1.

2.2 Isolation of *G. bulloides* specimens

A 1 ml sub-sample from each plankton trawl was transferred to a 1 ml glass Sedgewick-Rafter chamber using an automatic pipette. Individual specimens were removed under a dissecting microscope using a micro-pipette and transferred into de-ionised water (buffered with 7.8×10^{-4} M sodium tetraborate and 1.01×10^{-3} M sodium hydroxide). This buffer solution was chosen as it left minimal residue when foraminifera were dried (discussed below).

The above procedure was repeated until a minimum of 80 individuals had been isolated from each sample. Following this, hydrogen peroxide (H_2O_2) was added to the buffered de-ionised water (final concentration of 3 %) in order to dissolve any organic material adhering to the outside of the tests. After 45 min the foraminifera containing solution was decanted into a petri-dish. Foraminifera were transferred, using a micro-pipette, into another petri-dish and 5 ml of buffered de-ionised water added to dilute any remaining H_2O_2, thereby adhering to the recommendations of Moy et al. (2009) of not exceeding 1 h in H_2O_2. Specimens were finally transferred onto pre-marked areas of petri-slides using a micro-pipette and left to evaporate in air. Once dried, specimens of *G. bulloides* were isolated from other species, according to the defining characteristics described by Bé (1977), and then separated into 2 size fractions (150–200 µm and 200–250 µm), using a calibrated microscope graticule.

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2.3 Calculation of *G. bulloides* abundance

In order to quantify the number of *G. bulloides* at each sample site, average abundances were determined in a 1 ml aliquot of the preserved net sample transferred to a 1 ml glass Sedgewick-Rafter chamber under a dissecting microscope. This was repeated on average 75 times (range: 27–198). The mean numbers of *G. bulloides* per ml of sample were converted to numbers per m^3 using the flowmeter readings.

At 3 of the 10 sites, the flowmeter recorded very little water flow over the 4–5 min sample period (less than 50 m^3). This was unlikely to be a true reflection of the actual flow and is suspected to reflect a failure of the flowmeter. For these three net samples an estimate of the flow rate (AF) was calculated by dividing the total volume of water entering the net (TV) by the total time that the net was in the water (T), from the 7 samples where the flowmeter was deemed to have worked adequately (Eq. 1). This was considered the best course of action based on the fact that for these 7 samples, net deployment time was positively, and significantly, correlated with the volume of water sampled (Linear regression: $R^2 = 0.58$, $F_{1,6} = 6.89$, $P = 0.047$).

$$\mathrm{AF} = \frac{\mathrm{TV}\ (\mathrm{m}^3)}{T\ (s)}$$

$$\mathrm{AF} = 0.98\,\mathrm{m}^3\,\mathrm{s}^{-1} \quad (1)$$

This average flow rate (AF) was then multiplied by the number of seconds that the net was in the water (TW), for each of the 3 samples where the flowmeter failed, to provide an approximate volume of water passing through the net for these samples (AV) (Eq. 2).

$$\mathrm{AV} = \mathrm{AF} \cdot \mathrm{TW}$$

$$\mathrm{AV} = 0.98\,\mathrm{m}^3\,\mathrm{s}^{-1} \cdot \mathrm{TW} \quad (2)$$

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2.4 SNW analysis

SNWs are required to determine factors that affect test wall thickness and density, and therefore test weights of foraminifera. This works by removing the influence that test size has on weight: essential, as test-size has been shown to vary with ambient environmental conditions that occur during growth (Hecht, 1976; Schmidt et al., 2006). Two methods of determining SNWs are commonly used. The simplest method is to weigh specimens that have been picked from a narrow size fraction (typically 50 µm), with the data being termed the "sieve-based weight" (SBW; Broecker and Clark, 2001). The second method involves measuring the size (typically diameter or area) of each individual picked from a narrow size-fraction. Test weights are then normalised to the mean measured test size to obtain a "measurement-based weight" (MBW; e.g. Barker and Elderfield, 2002). Here the MBW method is used as SBWs are in part determined by test size (Beer et al., 2010b), implying that this is not an effective size-normalisation procedure.

Following the measurement of test diameters, using micrograph images taken at a known magnification with an integrated microscope (Leica MZ8) and camera system (Nikon D5000 Digital SLR), *G. bulloides* specimens were weighed, in aluminium capsules (5 × 9 mm), in groups of 10–25 individual tests, using a microbalance (Sartorius ME-5, precision = 1 µg). Weights were determined following transference to an environmentally controlled weighing room for 2 h, therefore allowing tests to equilibrate with the ambient atmospheric moisture content of the room.

During the measuring of test diameters, some foraminifera (57 out of 309 specimens) were found to be outside the desired size ranges (both size fractions; on average +17 µm). Specimens less than 25 µm outside the desired size range were included in the final analysis in order to maintain as large a sample size as possible; the size-normisation procedure is capable of removing any influence that these tests would have had on the overall results.

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Mean SBWs were calculated by dividing the average mass per sample (10–25 tests) by the number of *G. bulloides* in the sample. MBW_{diam} for each sample was calculated by normalizing SBW to the mean diameter for the corresponding size (Eq. 3).

$$MBW_{diameter} = \frac{\text{mean SBW}_{sample} \cdot \text{mean diameter}_{size\ fraction}}{\text{mean diameter}_{sample}} \quad (3)$$

2.5 $[CO_3^{2-}]$ measurements

Dissolved inorganic carbon (DIC) and alkalinity samples were taken from the non-toxic seawater supply (intake at ~5 m depth) and stored in borosilicate glass bottles (250 ml). A saturated solution of mercuric chloride (7 g/100 ml) was added to the samples in a 0.02 % volume ratio (50 µl) in order to eradicate any biological activity; samples were then stored in the dark prior to analysis. The DIC and total alkalinity (TA) values were derived from versatile instrument for the determination of titration alkalinity (VINDTA) analyses. These were inserted in the CO_2sys.exe program (Lewis and Wallace, 1998) together with input conditions (temperature, pressure, phosphate and silicate concentrations) and output conditions (temperature and pressure) in order to calculate $[CO_3^{2-}]$ for each sample site.

2.6 Dissolved inorganic nutrients

Water samples were collected directly from the CTD Niskin water bottles into 250 ml acid cleaned polythene bottles. Samples were stored at 4° C prior to analysis within 24 h of collection. Nitrate and phosphate measurements were made in triplicate using a Lachat *QuikChem 8500* flow injection autoanalyser according to the manufacturers recommended procedures. Nutrient standards were prepared in deionised water and the samples run in a carrier stream of dionised water. Salt correction of the result was performed by running a small number of Low Nutrient Sea Water samples (OSIL, http://www.osil.co.uk, Batch LNS 17, Salinity 35) during each sample batch run and

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the mean concentration subtracted from sample results. A standard reference solution prepared from nutrient standard solutions, containing 1 µM PO_4^{3-} and 10 µM NO_3^- was run at the start and end of each sample batch. As well as providing an independent check on analysis accuracy it also provided a correction of calibration drift during the course of each sample batch analysis.

2.7 Statistical analysis

Parametric linear regressions were performed using SigmaStat statistical software in order to determine if the size-normalised weights of *G. bulloides* varied significantly with variations in [CO_3^{2-}], abundance of *G. bulloides*, and nutrient concentrations (phosphate and nitrate). All data were found to have constant variance (Bartlett's test for equal variance) and to be normally distributed (Kolmogorov-Smirnov test for normality).

3 Results

$MBW_{diameter}$ values were obtained, by normalising SBW values to mean diameters, and used in this study in order to isolate the influence of test wall thickness and density, from that of size, on test weight. In order to determine if this method is effective, $MBW_{diameter}$ and SBW were compared with mean test diameters (Fig. 2). There was a statistically significant relationship, in both size fractions, between SBW(µg) and mean test diameters (Linear regression: 150–200 µm, $R^2 = 0.47$, $F_{1,9} = 6.98$, $P = 0.030$; 200–250 µm, $R^2 = 0.52$, $F_{1,9} = 8.77$, $P = 0.018$). No statistically significant relationships were observed when comparing MBWs with mean test diameters (Linear regression: 150–200 µm, $R^2 = 0.35$, $F_{1,9} = 4.27$, $P = 0.073$; 200–250 µm, $R^2 = 0.34$, $F_{1,9} = 4.04$, $P = 0.079$).

A weak positive relationship between SNW and [CO_3^{2-}] was observed for both size fractions over the sampled range of [CO_3^{2-}] (148.38–181.38 µmol kg^{-1}; Fig. 3a).

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The sign and gradients of change were +0.09 µg per 10 µmol kg^{-1} and +0.13 µg per 10 µmol kg^{-1} for the 150–200 µm and 200–250 µm size fractions, respectively. Neither of these slopes, however, were significantly different from zero (Linear regression: 150–200 µm, $R^2 = 0.35$, $F_{1,9} = 4.26$, $P = 0.073$; 200–250 µm, $R^2 = 0.32$, $F_{1,9} = 3.71$, $P = 0.090$).

In situ abundances of *G. bulloides* ranged between 4–53 individuals m^{-3}. No statistically significantly relationships between abundance and SNW (Fig. 3b) were found in either size fraction (Linear regression: 150–200 µm, $R^2 = 0.20$, $F_{1,9} = 2.06$, $P = 0.19$; 200–250 µm, $R^2 = 0.32$, $F_{1,9} = 3.90$, $P = 0.084$).

The relationship between concentrations of surface NO_3^- and PO_4^{3-} was statistically significant (Fig. 4; $R^2 = 0.93$, $F_{1,9} = 114.7$, $P < 0.001$). SNWs were inversely related to NO_3^- and PO_4^{3-} concentrations (Fig. 3c, d). [PO_4^{3-}] ranged between 0.038 and 0.32 µM. The gradients of change in test mass in response to phosphate concentrations were −1.60 µg (150–200 µm) and −2.11 µg (200–250 µm) per 1 µM. The slopes were significantly different from zero in both size fractions (Linear regression: 150–200 µm, $R^2 = 0.62$, $F_{1,9} = 12.90$, $P = 0.007$; 200–250 µm, $R^2 = 0.55$, $F_{1,9} = 9.75$, $P = 0.014$). NO_3^- (+NO_2^-) concentrations ranged between 0.019 and 12.35 µM. The gradients of change in SNWs in response to NO_3^- concentrations were −0.35 µg (150–200 µm) and −0.37 µg (200–250 µm) per 10 µM. The slope in the 150–200 µm size fraction was significantly different from zero (Linear regression: 150–200 µm, $R^2 = 0.65$, $F_{1,9} = 14.30$, $P = 0.005$), whereas the slope in the 200–250 µm size fraction was not (Linear regression: 200–250 µm, $R^2 = 0.38$, $F_{1,9} = 5.00$, $P = 0.06$).

The suggestion that SNWs decrease with increasing nutrient concentrations raises the question of how nutrient concentrations impact *G. bulloides* abundance. Comparing *G. bulloides* abundance to NO_3^- and PO_4^{3-} concentrations suggests that abundance increases with increasing concentrations of these two nutrients (Fig. 5a, b): the slopes observed were both significantly different from zero (Linear regression: PO_4^{3-}, $R^2 = 0.55$, $F_{1,9} = 10.05$, $P = 0.013$; NO_3^-, $R^2 = 0.48$, $F = 7.42$, $P = 0.026$).

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4 Discussion

4.1 Effectiveness of the size-normalisation procedure

While no statistically significant relationships were observed when comparing MBWs with mean test diameters for either size fraction, significant relationships were observed when comparing SBWs to test diameters. This suggests that the size-normalisation procedure employed as part of this study (i.e. using MBWs as opposed to SBWs) adequately isolates the influence of test wall thickness and density from that of size, on test weight. Therefore, we are confident that the SNWs used here are a good reflection of calcification rate, and not simply test size.

4.2 A consideration of potential factors controlling SNWs

4.2.1 $[CO_3^{2-}]$

The findings presented here suggest that at present day pCO_2 concentrations, $[CO_3^{2-}]$ is not a factor exerting significant control on calcification rates in *G. bulloides* in the North Atlantic Ocean. However, the range of $[CO_3^{2-}]$ (148–181 µmol kg^{-1}) is smaller than in previous studies, which have artificially imposed high $[CO_3^{2-}]$ in culture (Spero et al., 1997; Bijma et al., 1999; Lombard et al., 2010) or used samples from sediment cores (Barker and Elderfield, 2002; Gonzalez-Mora et al., 2008; de Moel et al., 2009; Moy et al., 2009). Despite this, the sign and gradient of change observed here are comparable to those derived from *G. bulloides* specimens collected from surface waters in the Arabian Sea (Beer et al., 2010a), and *Globigerinoides sacculifer* and *Orbulina universa* specimens in culture (Bijma et al., 1999; Lombard et al., 2010). As the majority of studies to date have found SNWs to be positively related to $[CO_3^{2-}]$, not only in *G. bulloides* but also in other species of foraminifera (Table 1), it is likely that reductions in $[CO_3^{2-}]$ in the future will adversely affect calcification in these organisms.

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It would appear that species specific responses to reduced [CO_3^{2-}] exist in planktonic foraminifera (Table 1), as has been observed in studies on coccolithophores (Riebesell et al., 2000; Langer et al., 2006; Iglesias-Rodriguez et al., 2008). It is, however, important to consider that [CO_3^{2-}] is a useful proxy but not necessarily a direct driver of calcification. Calcification tends not to occur at surfaces in direct contact with seawater, but in relatively isolated compartments within the cell (e.g. Erez, 2003; Bentov et al., 2009; de Nooijer et al., 2009). Additionally, ion transport channels tend to transport bicarbonate as opposed to carbonate ions (Carre et al., 2006): precipitating $CaCO_3$ from HCO_3^- and/or CO_2 via a series of reactions (Portner, 2008). Therefore, the ability to modify carbonate chemistry within microenvironments, convert HCO_3^- to CO_3^{2-}, and/or utilize HCO_3^- directly in calcification as is the case in coccolithophores (Paasche, 2002), may result in the range of responses exhibited by different species of foraminifera to increased pCO_2 in seawater.

G. bulloides is a non-symbiont bearing species. Foraminifera species which harbour algal symbionts may be better able to withstand changes in [CO_3^{2-}] due to the production of ATP from photosynthesis, providing energy for concentration of inorganic carbon into vesicles, removal of ions that inhibit calcification (e.g. ter Kuile, 1991), and/or the conversion of HCO_3^- to CO_3^{2-} via pH regulation at the site of calcification (Rink et al., 1998). Non-symbiont bearing species are likely to have a higher sensitivity to reduced [CO_3^{2-}]. This is a factor that may explain the larger decrease of test weights between the last glacial maximum to present day conditions for *G. bulloides* (Barker and Elderfield, 2002; Moy et al., 2009) compared to the symbiotic *G. ruber* (de Moel et al., 2009), and the differences in response to [CO_3^{2-}] exhibited by these two species in the surface waters of the Arabian Sea (Beer et al., 2010a). However, a recent study by Fujita et al. (2011) demonstrates that it is important to be careful when extrapolating these responses to future ocean acidification scenarios. The effects of increased pCO_2 on three species of symbiont-bearing reef foraminifers were examined, and although two of the species exhibited enhanced calcification at intermediate pCO_2, further increases beyond 970 µatm reduced calcification. Therefore, in the short term there may well be

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winners and losers in response to ocean acidification amongst foraminifera, but in the long term, in the absence of any adaptive strategies, these organisms may find their geographical range restricted to lower latitudes where [CO_3^{2-}] will be highest (Feely et al., 2004).

4.2.2 Optimum growth conditions

de Villiers (2004) suggested that SNWs are linked more closely to optimum growth conditions than [CO_3^{2-}]. de Villiers used relative abundance as a proxy for optimum growth conditions assuming that favourable environmental variables will result in greater abundances of foraminifera. Beer et al. (2010a) were unable to lend support to this hypothesis. Results from the current study also found no statistically significant relationship between SNWs and *G. bulloides* abundances in both size fractions examined. Although three out of the ten abundance values were calculated using average flow rates, the abundance counts presented here are arguably more reliable than those used by de Villiers (2004), which relied on generalised geographic trends. Confidence can also be placed in these findings based on the high volume of sample that these abundance counts were based on (average of 75 ml; range: 27–198 ml), and the fact that abundances found here are in strong agreement with expected abundances in the North Atlantic for this time of year (Schiebel and Hemleben, 2001). It is also likely that the size-normalisation procedure (the use of SBWs) employed by de Villiers (2004) was inadequate in isolating the effect that test size had on mass. It is therefore possible that SNWs correlated with factors which also influenced size. As planktonic foraminifera are known to reach their maximum size in their preferred water mass (e.g. Hecht, 1976; Schmidt et al., 2004), this may explain the trends observed by de Villiers (2004) and why no subsequent studies have been able to find support for the optimal growth conditions hypothesis.

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4.2.3 Nutrient concentrations

SNWs are inversely related to both NO_3^- and PO_4^{3-} concentrations. As concentrations of phosphate and nitrate were closely correlated to each other, it is difficult to infer whether this effect is due to the combination, or just one of these nutrients acting in isolation. There is, however, no evidence in the literature for reduced calcification in marine calcifying organisms under high NO_3^- concentrations.

Substantial evidence does exist for inhibition of calcification by high concentrations of phosphate. Phosphate has long been recognised as an inhibitor of calcite formation, adsorbing onto the calcite surface, blocking active crystal growth sites and impeding calcite precipitation (e.g. Simkiss, 1964; Pytkowicz, 1973; Reddy, 1977; Mucci, 1986; House, 1987). Lin and Singer (2006) identified the phosphate species responsible for this inhibition as calcium hydrogen phosphate ($CaHPO_4$), which alters the formation and subsequent growth of surface nuclei, resulting in reduced precipitation kinetics. Although there is no direct evidence for this phenomenon occurring in planktonic foraminifera, studies have been carried out on other marine calcifiers. For example, coccolithophores grown in phosphate replete mediums have been demonstrated to have lower calcification rates than cells grown under phosphate limited conditions (Paasche and Brubank, 1994), while phosphate concentrations of 20 µM have been shown to decrease biomineralisation in the calcifying green alga *Halimeda incrassata* by 15 % (Demes et al., 2009). Similarly, a >50 % reduction in coral calcification has been attributed to elevated phosphate concentrations (2 µM), maintained via discontinuous fertilisation over an 8 month period (Kinsey and Davies, 1979). Although the phosphate concentrations in these experiments are at least 6 times greater than the highest concentrations found in the present study (0.32 µM), there is indirect evidence for phosphate inhibition of calcification at concentrations much closer to these values: one of the highest phosphate concentrations reported for waters in direct proximity to a coral reef (0.6 µM), is associated with one of the lowest overall calcification rates (Smith and Kinsey, 1976). This suggests that phosphate may be the main factor influencing

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SNWs of foraminifera in the present study, and is re-enforced by the fact that relationships between [PO_4] and SNW are statistically significant in both size fractions. While this is by no means proof that phosphate is a major factor influencing calcification rates in planktonic foraminifera in the ocean, it would possibly explain the occurrence of heavier tests during non-upwelling periods (de Moel et al., 2009) and should at least be considered by future studies, especially as calcite inhibition by PO_4^{3-} is exacerbated at lower pH (Lin and Singer, 2006), therefore potentially becoming more of an issue in future "acidified" oceans.

A confounding factor is that nutrient concentrations (PO_4 and NO_3) and abundance of *G. bulloides* were positively and significantly correlated with each other, a finding that is consistent with observations of maximum frequencies of *G. bulloides* at high nutrient concentrations (e.g. Bé and Tolderlund, 1971; Hemleben et al., 1989; Schiebel et al., 2001). Taken together, these findings suggest that high nutrient concentrations lead to a larger number of organisms with thinner tests. This may be explained in two ways: higher nutrient concentrations favour increased growth and reproduction of *G. bulloides*, perhaps due to increased prey abundance (Bijma et al., 1992), but at the price of thinner tests resulting from phosphate inhibition of calcification. Alternatively, increased growth and reproductive output are energetically costly to individual *G. bulloides*, which subsequently invest less energy into calcification, resulting in thinner tests. These two explanations may not necessarily be mutually exclusive and, once again, separating out the effects of nitrate and phosphate is beyond the scope of the particular observational approach adopted here.

5 Summary and conclusions

These findings point to the potential importance of phosphate in determining foraminiferal test masses in the ocean via inhibition of calcification, a factor which has previously been overlooked by previous studies on these organisms. However, it is important to stress that the relationships observed here do not necessarily imply

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causality; culture studies are perhaps best suited for assessing those environmental factors that are simply correlated with SNWs of foraminifera and those which exert control. If phosphate is important in inhibiting calcification in planktonic foraminifera then it is likely that the effects of ocean acidification may be exacerbated in areas with high phosphate concentrations such as coastal upwelling regions. More broadly, these results, combined with the conflicting evidence regarding factors controlling calcification in planktonic foraminifera leads us to echo the sentiments of de Villiers (2004): the interpretation of SNW variations in direct response to $[CO_3^{2-}]$, or any other single factor, should be done so with caution. The intrinsically complex nature of ecosystems should not be overlooked in the search for simple correlative relationships, but should be embraced and incorporated into future studies on the response of marine calcifiers to global environmental change.

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Table 1. Inter and intra-specific diversity in the response of different foraminifera species to $[CO_3^{2-}]$. Studies that utilized data from: sediment cores (a), laboratory cultures (b), and plankton net samples (c) are shown.

Species	$[CO_3^{2-}]$ positively related to SNW	$[CO_3^{2-}]$ negatively related to SNW	No response to $[CO_3^{2-}]$
Orbulina universa	Spero et al. (1997)[b] Bijma et al. (1999)[b] Lombard et al. (2010)[b]		
Globigerina bulloides	Barker and Elderfield (2002)[a] Moy et al. (2009)[a] de villiers (2004)[a] Gonzalez-Mora et al. (2008)[a] Beer et al. (2010a) [c]		Bijma et al. (1999)[b]
Globorotalia truncatulinoides	de Villiers (2004)[a]		
Neogloboquadrina pachyderma			de Villiers (2004)[a] Gonzalez-Mora et al. (2008)[a]
Globigerinoides ruber	de Moel et al. (2009)[a] Gonzalez-Mora et al. (2008)[a]	Beer et al. (2010a)[c]	
Globigerinoides sacculifer	Lombard et al. (2010)[b]		

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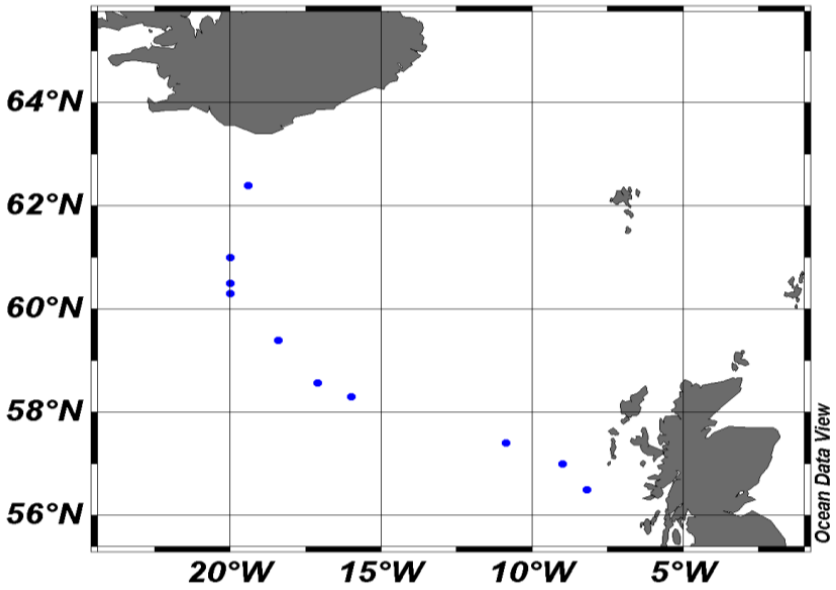

Fig. 1. Stations sampled during the D340 Extended Ellett Line Cruise to the North Atlantic in June 2009 (blue dots).

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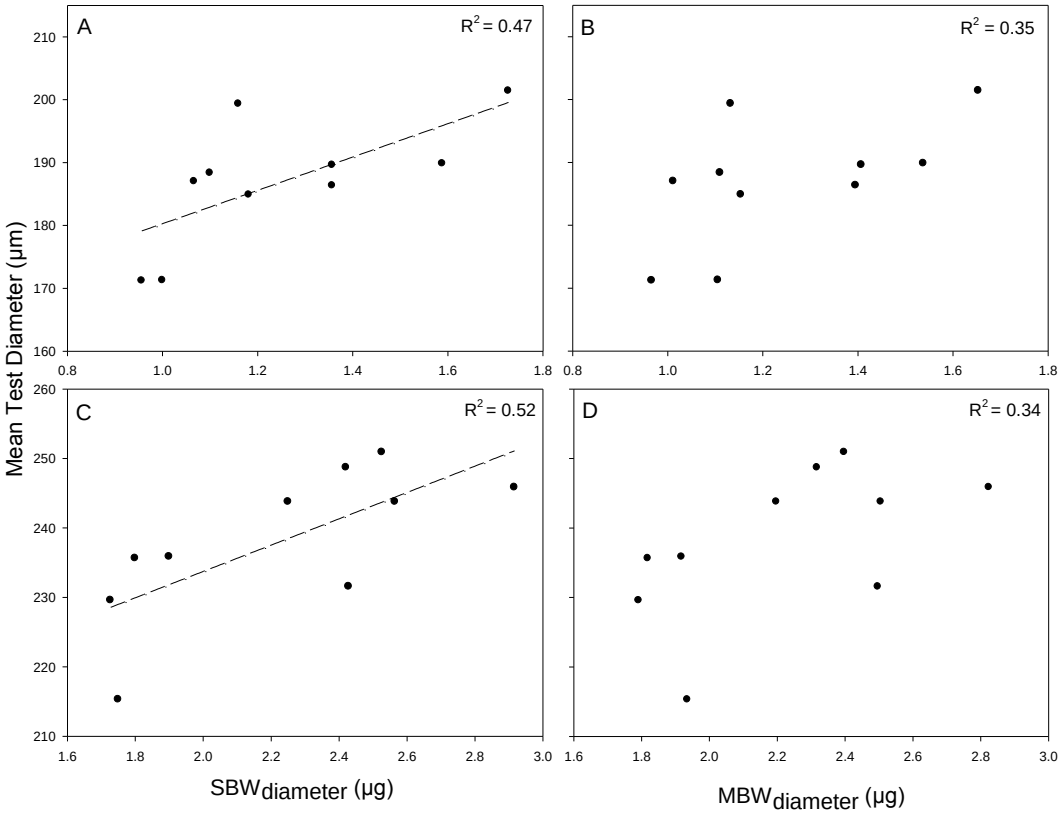

Fig. 2. The mean sieve-based weight (SBW; µg) and measurement-based weight ($MBW_{diameter}$; µg) versus the mean diameter (µm) for *G. bulloides* from 150–200 µm **(A, B)** and 200–250 µm **(B, C)** size-fractions. Dashed lines represent relationships that are statistically significant at the 95 % confidence level.

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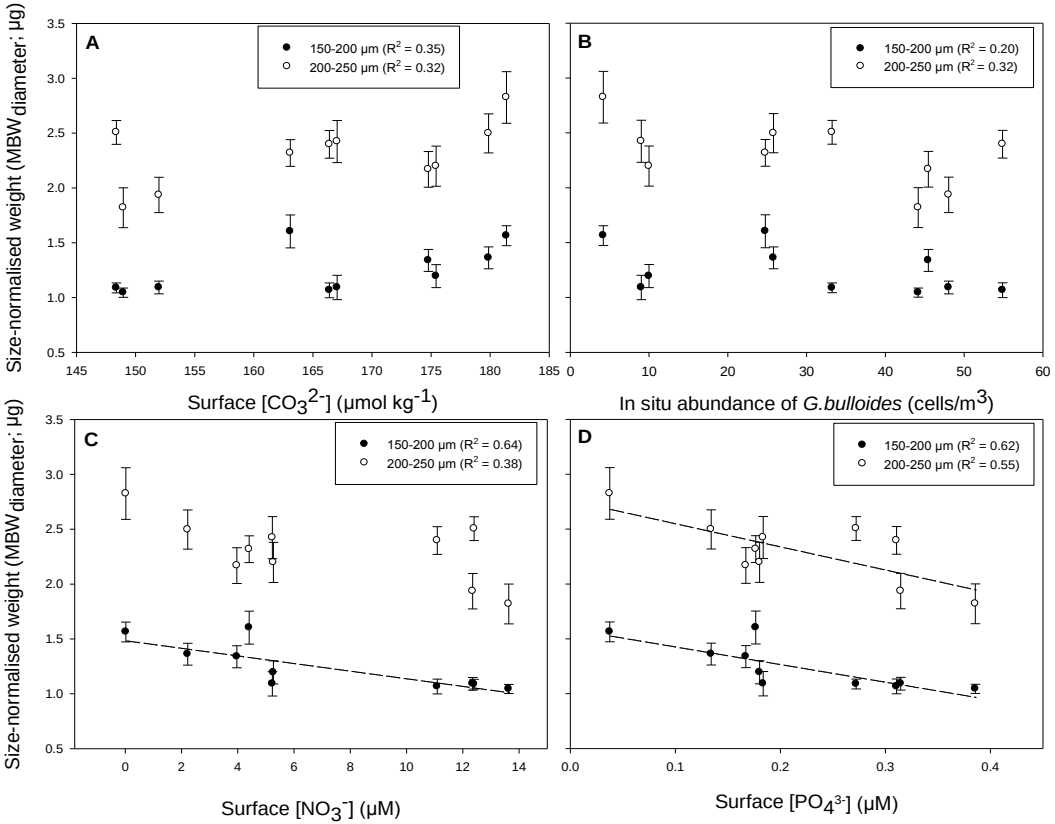

Fig. 3. SNWs of *G. bulloides* for 150–200 µg (filled symbols) and 200–250 µg (hollow symbols) size fractions compared to: **(A)** [CO_3^{2-}], **(B)** in situ abundance of *G. bulloides*, **(C)** [NO_3^-], and **(D)** [PO_4^{3-}]. Error bars represent the reciprocal of the number of specimens weighed per aliquot multiplied by mean specimen weight. Dashed lines represent relationships statistically significant at the 95 % confidence level.

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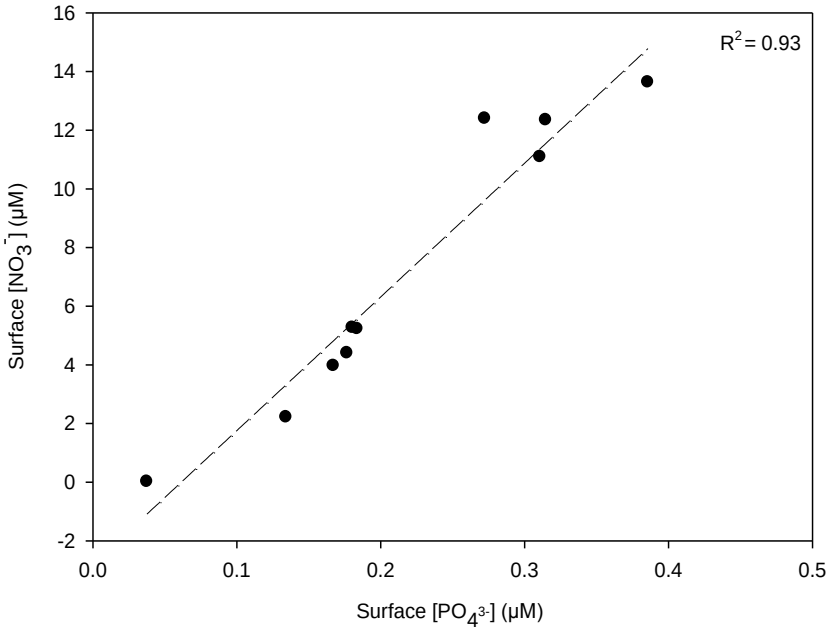

Fig. 4. Surface phosphate concentrations compared to surface nitrate concentrations (including NO_2^-). Dashed line represents statistical significance at the 95 % confidence level.

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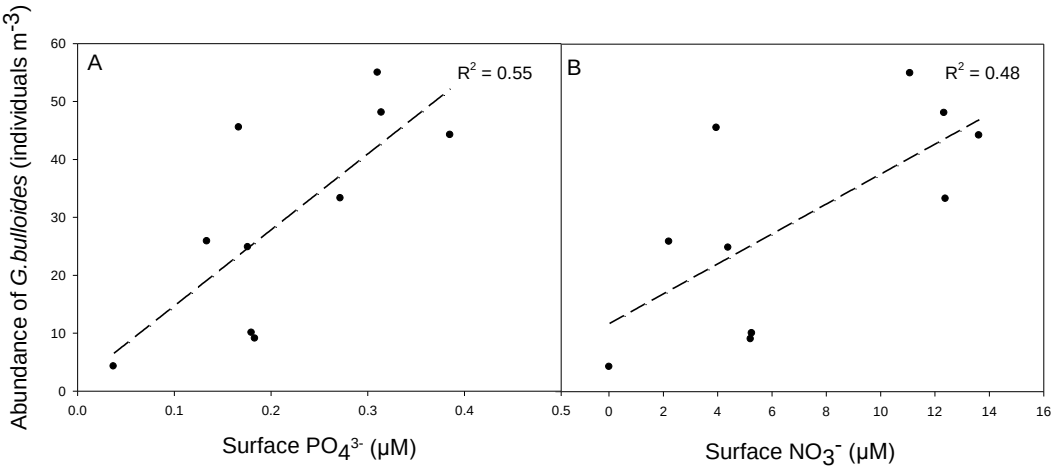

Fig. 5. Surface nitrate (**A**; including NO_2^-) and phosphate **(B)** concentrations compared to in situ abundance of *G. bulloides*. Dashed lines represent relationships that are statistically significant at the 95 % confidence level.

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