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The influence of symbiont photosynthesis on the boron isotopic composition of foraminifera shells

Bärbel Hönisch [a,*], Jelle Bijma [a], Ann D. Russell [b], Howard J. Spero [b], Martin R. Palmer [c], Richard E. Zeebe [a], Anton Eisenhauer [d]

[a] *Alfred Wegener Institute for Polar and Marine Research, 27570 Bremerhaven, Germany*
[b] *Department of Geology, University of California, Davis, CA 95616, USA*
[c] *Southampton Oceanography Centre, Southampton, SO14 3ZH, UK*
[d] *GEOMAR, 24148 Kiel, Germany*

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Abstract

Culture experiments were carried out with the planktonic foraminifer *Orbulina universa* under high and low light levels in order to determine the influence of symbiont photosynthetic activity on the boron isotopic composition of shell calcite. Under low light (reduced photosynthetic rates) the boron isotopic composition of the tests is 1.5‰ lower compared to shells grown under high light (elevated photosynthetic rates). In terms of inferred *p*H, the lower boron isotope values correspond to a reduction in *p*H of approximately 0.2 units. The boron isotopic composition of *Orbulina universa* from plankton tows is similar to that of shells grown under low light conditions in the laboratory. These data are consistent with reduced symbiont concentrations in recently secreted shells. In addition to laboratory and field grown *O. universa*, we present the first data for a symbiont-barren foraminifer, *Globigerina bulloides*. Data obtained for *G. bulloides* fall ~1.4‰ below those of the field grown *O. universa*. Although the plankton tow results are preliminary, they support the hypothesis that respiration and photosynthesis are the key physiological parameters responsible for species-specific vital effects.

Keywords: $\delta^{11}B$; planktonic foraminifera; vital effects; symbionts; paleo-*p*H

1. Introduction

Data from experiments with living foraminifera have confirmed the hypothesis that seawater *p*H is the dominant environmental control on the $^{11}B/^{10}B$ content ($\delta^{11}B$) of planktonic foraminifera shells (Hemming and Hanson, 1992; Sanyal et al., 2001, 1996, 2000; Spivack et al., 1993). Although measurements of foraminiferal $\delta^{11}B$ are not yet a routine tool in paleoceanography, several studies have published paleo-*p*H reconstructions across different geological timescales with encouraging results (Palmer et al., 1998;

* Corresponding author. Present address: Lamont-Doherty Earth Observatory of Columbia University, Palisades, NY 10964-8000, USA. Tel.: +1-845-365-8828; Fax: +1-845-365-8155.
E-mail address: hoenisch@ldeo.columbia.edu (B. Hönisch).

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Pearson and Palmer, 2000; Sanyal and Bijma, 1999; Sanyal et al., 1997, 1995; Spivack et al., 1993).

Whereas *p*H is the primary environmental control on shell $\delta^{11}B$, several physiological processes can modify the *p*H of the calcifying microenvironment, potentially complicating straightforward interpretation of $\delta^{11}B$ data. For instance, microelectrode studies have revealed that *p*H in the calcifying microenvironment of symbiont-bearing foraminifera varies with light levels (Jørgensen et al., 1985; Rink et al., 1998). Although symbionts remove CO_2 during photosynthesis, thereby increasing *p*H in the foraminiferal microenvironment, respiration releases CO_2 and decreases *p*H. Results from diffusion–reaction model simulations support these microsensor studies (Wolf-Gladrow et al., 1999), showing that respiration and symbiont-photosynthesis, along with diffusion and chemical reactions, control the availability of CO_3^{2-} and HCO_3^- for the calcification process. The carbonate ion effect on shell $\delta^{13}C$ of planktonic foraminifera (Bijma et al., 1998; Spero et al., 1997) can also be partly explained by the influence of these physiological processes (Zeebe et al., 1999).

Comparison of empirical $\delta^{11}B$ vs. *p*H-relationships has revealed significant offsets between inorganic and biogenic calcification as well as among foraminifera species (Sanyal et al., 2001). It was speculated that species-specificity could be due to differences in microenvironment *p*H and/or due to differences in the relative proportion of calcite precipitated during day and night (Sanyal et al., 2001). Similarly, Hemming et al. (1998) attributed more positive boron isotope values in a coral during periods of high primary productivity to enhanced symbiont photosynthetic activity and a therefore higher *p*H. This study investigates the influence of symbiont photosynthetic activity on the boron isotopic composition of *Orbulina universa* grown in the laboratory. In order to estimate the effects on field grown foraminifera, we compare experimental data with plankton tow samples of *O. universa* and the symbiont-barren *Globigerina bulloides*.

2. Methods

2.1. Foraminifera collection and culturing

Foraminifera were cultured using previously established methods (Lea and Spero, 1992; Mashiotta et al., 1997; Spero et al., 1997). Juvenile (presphere) *Orbulina universa* were hand collected by scuba divers in July and August 2000 from surface waters of the San Pedro Basin, approximately 2 km NNE of the Wrigley Institute for Environmental Studies, Santa Catalina Island, CA. Surface seawater for culturing was collected at the dive site, filtered through a 0.8-μm membrane filter and its boron concentration was subsequently modified using the method of Sanyal et al. (2001). To reduce the large number of shells required for isotope analysis, the boron concentration in the culture solution was increased tenfold by adding 0.27 g of boric acid (H_3BO_3) per l seawater. The drop in *p*H upon adding H_3BO_3 was readjusted to ambient *p*H of 8.16 by titration with NaOH. Samples of the culture solution were taken at the beginning and end of the experiment, acidified with ultrapure HCl and archived for later determination of the boron isotope value.

After collection, individual foraminifera were examined under an inverted light microscope for identification of species and general condition and then transferred to 115-ml glass jars containing the experimental filtered seawater. Culture jars were closed to the atmosphere and maintained at a constant temperature in a 22 ± 0.3°C water bath, the approximate summer sea surface temperature at the collection site. For each experiment, seventy individuals were grown in the laboratory. Foraminifera were grown under the following conditions: (1) a 12-h high light (HL):12-h dark cycle where light levels were adjusted to above P_{max} (315–326 μmol photons m^{-2} s^{-1}), and (2) a 12-h low light (LL; 18–20 μmol photons m^{-2} s^{-1}):12-h dark cycle. Both experiments utilized high output, cool white, fluorescent bulbs. The former light levels exceed the saturating irradiances for symbionts in *Orbulina universa*, whereas the latter are lower than the light compensation point (Rink et al., 1998). During the 6–15-day culture period, *O. universa* secretes and

calcifies a spherical chamber. The foraminifera were fed a 1-day old *Artemia* sp. nauplius (brine shrimp) every third day. Upon termination of the experiment following foraminiferal gametogenesis, the empty shells were rinsed in ultrapure water and archived for later analysis.

Alkalinity was determined by Gran-titration at the start and termination of the experiment. At the same time, dissolved inorganic carbon (DIC) samples were collected, poisoned with saturated $HgCl_2$ solution and measured coulometrically at the Alfred Wegener Institute, Bremerhaven, Germany. Seawater *p*H values (on the NBS scale) were determined potentiometrically. Carbonate chemistry analyses were calibrated against certified reference material supplied by Dr. A.G. Dickson, University of California, San Diego, CA. The experimental carbonate chemistry data are reported in Table 1.

Plankton tow samples were collected at the dive site in order to determine the ambient boron isotopic composition of field *Orbulina universa* and the symbiont-barren *Globigerina bulloides*. Nets with a mesh size of 153 μm were towed at 0–20 m depth. Selected foraminifera shells were rinsed in distilled water, dried and archived. The samples were treated in a low temperature asher to remove organic matter and to better distinguish between juvenile *O. universa* and *G. bulloides*. Approximately 300 shells of each species were collected. Most *O. universa* had built their spherical chambers shortly before collection. Shells were very thin and none of the collected specimens of the two species showed signs of gametogenic calcification. Total sample weight before cleaning was no more than 1 mg for *O. universa* and 0.6 mg for *G. bulloides*.

2.2. Analytical techniques

With the exception of the plankton tow samples, only gametogenic individuals from the culture experiments were used for analysis. All specimens were rinsed in distilled water to remove sea salts, dried and weighed. The shells of each experiment were pooled, crushed and bleached with 4–6% sodium hypochlorite to remove organic matter and then rinsed, ultrasonicated and centrifuged repeatedly with distilled water to remove soluble salt and eventually adsorbed B. In a laminar flow bench, the cleaned carbonate was dissolved in 2N quartz distilled (i.e. boron free) HCl. The dissolved sample, containing approximately 5 ng of B, was loaded on a rhenium zone refined filament, and 1 μl of boron-free seawater was added to enhance ionization and suppress fractionation (Hemming and Hanson, 1994). Samples were dried at an initial ion current of 0.8 A, followed by a 1 min period at 1.2 A. Loaded filaments were kept under an infrared lamp until mounted into the mass spectrometer. Isotope data were collected on a Finnigan MAT 262 RPQ^+ Thermal Ionization Mass Spectrometer (TIMS) at GEOMAR in Kiel, Germany. The BO_2^- ion method was used following previously published procedures (Sanyal et al., 1996, 1997). For the culture experiments each sample was run at least four times. Cultured foraminifera samples were measured at a filament temperature of 915 ± 10°C. While we seldom observed time-dependent fractionation in these boron enriched samples, the small plankton tow samples started fractionating after 20–30 min of acquisition. We could therefore only complete two acceptable runs for *Orbulina universa* and a single acquisition for *Globigerina bulloides*. However, initial values of the fractionating runs were consistent with the results of acceptable analyses. Runs were accepted if the fractionation was less than 1 ‰ over 30 min of acquisition.

To rule out isobaric interferences on mass 42 with organic contamination ($^{12}C^{14}N^{16}O$-ions), mass 26 ($^{12}C^{14}N$-ions) was monitored during each measurement. No interferences were detected. The $^{11}B/^{10}B$ ratio was corrected for isotopic interferences on mass 43 ($^{10}B^{16}O^{17}O$-ions) by subtraction of 0.00078 from the 43/42 ratio (Spivack and Edmond, 1986).

The fractionation ε between natural seawater (NS) and calcite (C) is usually calculated as: $\varepsilon_{(NS-C)} = \delta^{11}B_{NS} - \delta^{11}B_C$. This equation gives a good approximation when the isotopic composition of NS and modified seawater (MS) are the same. Because the modified seawater used in the culture experiments had a significantly different isotopic composition from natural seawater (Ta-

Table 1
Boron isotopic composition of cultured *Orbulina universa* and modified seawater chemistry

Light (μmol photons m^{-2} s^{-1})	*p*H (culture water)	Alkalinity (μmol kg^{-1})	Seawater $\delta^{11}B_{MS}$ (‰)	n	*Orbulina universa* $\delta^{11}B_C$ (‰)	n	$\delta^{11}B_{NC}$ (‰)
321 ± 8	8.16 ± 0.02	3147 ± 13	−8.9 ± 0.1	5	−25.6 ± 0.6	4	22.0 ± 0.6
19 ± 2	8.15 ± 0.03	3154 ± 8	−9.1 ± 0.4	6	−27.2 ± 0.3	4	20.5 ± 0.3

Isotope results are based on 70 shells per sample. Errors are expressed as $2\sigma_{mean}$ for multiple sample runs. $\delta^{11}B$ (‰) = (R_s/R_{std}−1)*1000, $R_s = {}^{11}B/{}^{10}B$ of sample, $R_{std} = {}^{11}B/{}^{10}B$ of NBS 951 boric acid standard. Seawater standard = 39.5 ± 0.34‰. n = number of replicate analyses. $\delta^{11}B_{NC}$ is the $\delta^{11}B_C$ after conversion to the natural seawater scale ($\delta^{11}B_{NS}$ = 39.5‰), see text and Eq. 1 for details. While seawater modification left DIC unchanged at ambient values of 1987 ± 13 μmol kg^{-1}, the tenfold boron concentration increased total alkalinity above ambient values of 2257 ± 10 μmol kg^{-1}. Carbonate chemistry remained constant over the course of the experiments.

ble 1), all analyses were corrected for this difference in order to allow comparison to previously published data. To convert our data to the natural seawater scale we applied the following equation (Zeebe and Wolf-Gladrow, 2001):

$$\delta^{11}B_{NC} = \alpha_{NS-MS} * \delta^{11}B_c + (\alpha_{NS-MS} - 1) * 1000 \quad (1)$$

where α_{NS-MS} is a factor expressing the isotope difference between modified and natural seawater ($\alpha_{NS-MS} = (\delta^{11}B_{NS}+1000)/(\delta^{11}B_{MS}+1000)$). $\delta^{11}B_{NC}$ is the value of the calcite if it had been grown in natural seawater.

The boron isotopic compositions are listed in Table 1 and Table 2. Errors are expressed as $2\sigma_{mean}$. Repeated analyses of natural seawater used as a laboratory standard resulted in an average value of 39.58 ± 0.34‰ (n = 9; filament temperature: 900 ± 10°C).

For laboratory intercomparison, additional analyses of the culture samples were performed on a Micromass VG Sector 54 TIMS at the Southampton Oceanography Centre (SOC), Southampton, UK. Analysis followed the method outlined in Palmer et al. (1998). Samples and NBS 951 boric acid standard were measured at a filament temperature of 925 ± 10°C.

3. Results and discussion

Here we present the data obtained from our experiments. The data set is internally consistent and the results are reasonable with regard to theoretical considerations. However, we found systematic offsets from previously published calibration curves. Although the offsets do not affect the conclusions of this and most previous studies, the underlying problem will be discussed in more detail in Sections 3.3 and 3.4.

3.1. Laboratory experiments

The results of our experiments clearly show the influence of symbiont photosynthetic activity on the boron isotopic composition of the shell. At equal culture water *p*H the $\delta^{11}B$ of LL *Orbulina universa* shells is 1.5‰ lower than that of specimens grown under HL (Table 1; Fig. 1). Shifting

Table 2
Boron isotopic composition of plankton tow *Orbulina universa* and *Globigerina bulloides*

Species	Ambient *p*H	$\delta^{11}B$ (‰)	n
Orbulina universa	8.12 ± 0.02	20.5 ± 0.5	2
Globigerina bulloides	8.12 ± 0.02	19.0 ± 0.9	2*

Results are based on approximately 300 shells per sample. Errors are expressed as $2\sigma_{mean}$ for multiple sample runs. $\delta^{11}B$ (‰) = (R_s/R_{std}−1)*1000, $R_s = {}^{11}B/{}^{10}B$ of sample, $R_{std} = {}^{11}B/{}^{10}B$ of NBS 951 boric acid standard. Seawater standard = 39.5 ± 0.34‰. n = number of replicate analyses. * = runs incomplete according to criteria for acceptable runs, see text for details.

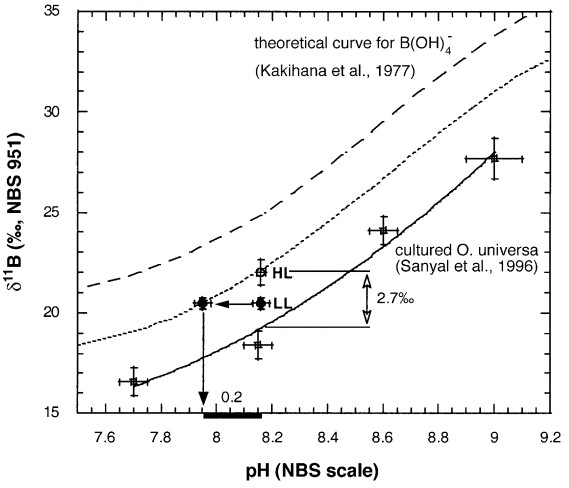

Fig. 1. Comparison of the boron isotopic composition in shells of *Orbulina universa* cultured under HL (open circle) and LL (filled circle). Shells were grown in modified seawater with tenfold increased boron concentrations. To account for the isotopic difference between culture medium and natural seawater, shell data were converted to the natural seawater scale (i.e. $\delta^{11}B_{NS} = 39.5‰$, equation 1, Zeebe and Wolf-Gladrow, 2001). Also shown (solid curve) is the empirical HL curve for *O. universa* established by Sanyal et al. (1996). Note that our data are offset to Sanyal's values by ~+2.7‰. In order to determine the *p*H at the site of calcification under LL conditions (arrow-pointed circle), we moved the theoretical curve for $\delta^{11}B$ of $B(OH)_4^-$ vs. *p*H (dashed line; Kakihana et al., 1977) onto our HL data point (dotted line). The reflection of the LL data at the shifted curve thus yields the *p*H (see arrows).

the theoretical curve for $\delta^{11}B$ of $B(OH)_4^-$ (Kakihana et al., 1977) so it passes through our HL data, the $\delta^{11}B$ for the LL group implies a decrease in *p*H of ~0.2 units.

Our calculated, $\delta^{11}B$-based, *p*H offset between HL and LL conditions is smaller than the HL-dark *p*H offset measured by Rink et al. (1998) using microelectrodes. Rink et al. (1998) measured the *p*H within the spine microenvironment of *Orbulina universa*, reporting values of 7.95 units in the dark, and 8.85 and 8.65 at 717 and 152 μmol photons m^{-2} s^{-1}, respectively (all on the NBS scale). Interpolating between the latter two values we estimate a *p*H of 8.7 for the HL conditions in our culture experiments (~320 μmol photons m^{-2} s^{-1}). The difference between our HL conditions and complete darkness should therefore be ~0.75 *p*H units. Although the *p*H offset is considerably larger than our boron isotope data predict, we note that the microsensor data are spot measurements under specific illumination conditions. In contrast, our shell data reflect an integrated signal over several diurnal light–dark cycles. Two studies support this argument. Firstly, Spero and Parker (1985) have shown that symbiont photosynthetic rates in *O. universa* display a daily periodicity. For any given 12-h illumination period, symbionts only photosynthesize at a maximum rate for 4–6 h with lower rates during the remaining illuminated period. Based on symbiont density and photosynthetic rates provided in that study, the integrated photosynthetic rate for one light period is calculated at ~59 nmol C d^{-1} instead of ~87 nmol C d^{-1} which would be calculated if the maximum photosynthetic rate had been maintained for the full 12-h illuminated period. Therefore, the integrated symbiont photosynthetic effect is only 68% of the spot *p*H measurements made by Rink et al. (1998). With regard to *p*H, the computed integrated value for a full light period is therefore only 8.46 instead of 8.70. Secondly, culture experiments by Lea et al. (1995) further showed that calcification in *O. universa* varies among specimens and is not strictly limited to the daylight hours. They calculated that on average, 33% of the spherical shell is precipitated during the night. Using a simple mass balance, the influence of combining calcite secreted during the night (@ *p*H = 7.95) and during the day (@ *p*H = 8.46) yields a weighted, time integrated *p*H of 8.29 for the HL group. The *p*H difference predicted for foraminifera grown under a HL-dark cycle compared to shells grown in complete darkness is therefore reduced to ~0.34 instead of ~0.75 units.

Finally, it should be kept in mind that we did not keep the LL-foraminifera in the dark but at ~19 μmol photons m^{-2} s^{-1}. Although this is below the light compensation point for the *Orbulina universa* symbiotic association (association respiration rate = symbiont photosynthetic rate) (Rink et al., 1998), symbiont photosynthesis still removes CO_2. Therefore, the actual microenvironment *p*H under LL conditions is ca. 0.1 *p*H unit higher than that in shells grown in the dark (cf.

Rink et al., 1998). Using this line of argument, we calculate an effective HL–LL *p*H difference of ~0.24 units. Our experimental result of a ~0.2 *p*H difference between LL and HL grown specimens agrees well with these calculations. See, however, the discussion in Section 3.4 and Fig. 3 for the potential effect of increased boron concentration on these data.

3.2. Plankton tows

The $\delta^{11}B$ value of *Orbulina universa* collected in plankton tows (20.5 ± 0.5‰) is identical to that of *O. universa* cultured under LL conditions (Table 2; Fig. 2). This observation is in contrast to sediment coretop data for this species, which were shown to be isotopically similar to $\delta^{11}B$ of shells grown in the laboratory at ambient *p*H (Sanyal et al., 1996). Because our plankton tow foraminifera were collected at depths down to 20 m, one could argue that lower irradiance levels at this depth may have reduced photosynthetic activity. However, light level measurements made at the collection site in August 1987 yielded irradiance levels between 2188 µmol photons m^{-2} s^{-1} at the surface and 361–123 µmol photons m^{-2} s^{-1} at 18–27 m water depth (H.J. Spero, unpublished data). These irradiances would suggest all the tow-collected shells were exposed to light levels that were higher than the HL levels in the laboratory. To explain the low isotopic value in the tows, we therefore hypothesize that the thinly calcified specimens collected in plankton tows are not fully calcified and may not contain the density of symbionts expected from a similar sized sphere as it

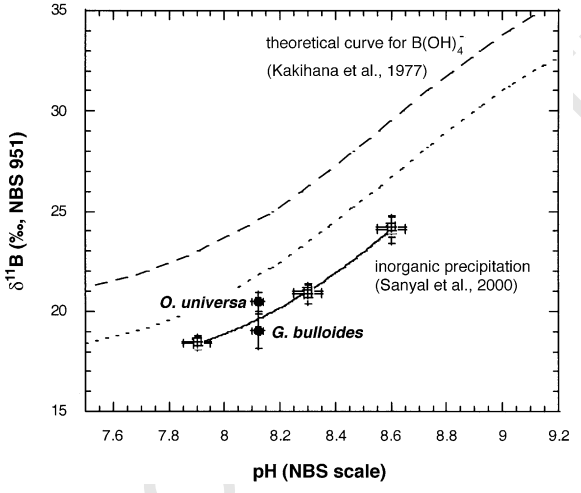

Fig. 2. Comparison of the boron isotopic composition of the symbiont-bearing foraminifera *Orbulina universa* and the symbiont-barren *Globigerina bulloides* (filled circles) taken from plankton tows and inorganic carbonates (open circles and solid line; Sanyal et al., 2000). As the inorganic carbonate was also precipitated in artificial seawater, the $\delta^{11}B$ values of Sanyal et al. (2000) were converted to the natural seawater scale according to Zeebe and Wolf-Gladrow (2001, equation 1). Also shown is the reference curve for our *O. universa* cultured under HL conditions (dotted line; see also Fig. 1 and discussion in the text) and the theoretical curve for $\delta^{11}B$ of $B(OH)_4^-$ vs. *p*H (dashed line; Kakihana et al., 1977).

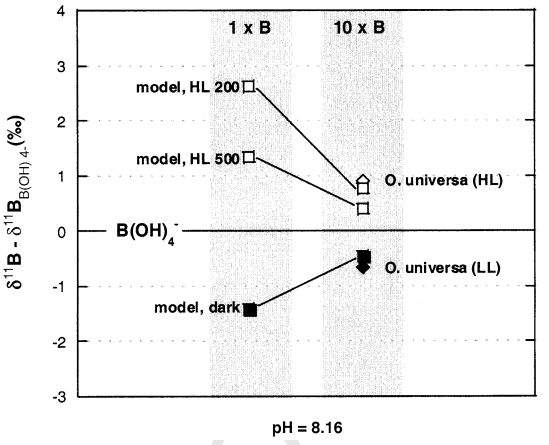

Fig. 3. Potential effect of higher boron concentration on experimental $\delta^{11}B$ results (diamonds; this study) as argued in a diffusion–reaction model study by Zeebe et al. (in press) (squares). Symbols on left gray bar are model results for natural seawater boron concentrations (1× B), whereas right gray bar refers to tenfold enriched total boron concentrations (10× B). HL conditions as indicated by open symbols are 320 µmol photons m^{-2} s^{-1} in both studies, closed symbols reflect 19 µmol photons m^{-2} s^{-1} (this study) and darkness (model). Model results labeled by HL 500 and HL 200 refer to HL conditions with an assumed symbiont halo thickness of 500 and 200 µm, respectively. See Zeebe et al. (in press) for model details. The model was run relative to the theoretical $\delta^{11}B_{B(OH)4^-}$-fractionation curve by Kakihana et al. (1977). For comparison between experimental and model results, the offset of the experimental data from the x-axis had to be chosen arbitrarily. If the model is run at 10× B, the agreement between both results is good. See text for differences in model assumptions and an alternative explanation for the smaller HL–LL offset in the experimental results. Note that all data reflect $\delta^{11}B - \delta^{11}B_{B(OH)4^-}$ at pH 8.16.

approaches gametogenesis. The photosynthetic impact on the boron isotopic composition is therefore assumed to be reduced at such an early stage suggesting plankton tow samples are not the ideal source of *O. universa* material for testing the boron isotope calibration.

The $\delta^{11}B$ of symbiont-barren *Globigerina bulloides* (19.0 ± 0.9‰) was 1.4‰ lower than that of the *Orbulina universa* shells collected from the same plankton tows. Because this is the first $\delta^{11}B$ datum ever measured on a symbiont-barren species, it cannot be compared to literature data. However, the dominant physiological process that affects the carbonate chemistry of *G. bulloides* at the site of calcification is respiration. Although *p*H measurements have never been conducted on this species, it is well known that the addition of respiratory CO_2 decreases *p*H in symbiont-bearing foraminifera by up to 0.3 units (Jørgensen et al., 1985; Rink et al., 1998; Wolf-Gladrow et al., 1999) and is therefore expected to influence *G. bulloides* similarly. Comparison of this datum point with data from inorganic precipitation experiments (Sanyal et al., 2000) demonstrates that *G. bulloides* falls slightly below the inorganic precipitation curve (Fig. 2). Given the uncertainty of absolute differences between studies and samples and the single datum presented here, the similarity between *G. bulloides* and the inorganic precipitation experiments is promising. The lower $\delta^{11}B$ compared to *O. universa* and the inorganic precipitation results is reasonable under the assumption of a lower *p*H at the site of calcification due to respiration.

3.3. Analytical offset

Our HL data are offset from the empirical equation of Sanyal et al. (1996), based on cultured *Orbulina universa*, by approximately +2.7‰ (Fig. 1). At this stage we cannot explain the offset although part of the explanation could be due to lower light intensities in Sanyal's experiments (no additional illumination was provided apart from the normal laboratory ceiling lighting), it is unlikely that irradiances were lower than the LL levels studied in our experiments. Besides probable differences in the light regime, major differences between the two experimental set-ups are the use of boron enriched seawater and the fact that specimens underwent gametogenesis in our experiments. While Sanyal et al. (2001) ruled out the possibility that higher boron alkalinity in artificial seawater affects experimental $\delta^{11}B$ values, comparison between pregametogenic experimental individuals and postgametogenic shells derived from sediments (Sanyal et al., 1996) supported the notion that gametogenesis does not influence the boron isotopic fractionation significantly. Since the experimental methods were equal apart from these differences, there is no explanation for the offset to be expected from the experimental point of view.

The only remaining difference is the laboratory and the mass spectrometer on which the samples were analyzed. Data for the previously published empirical relationships on foraminifera and inorganic calcite were all established in the same laboratory (Sanyal et al., 1996, 2000, 2001). However, offsets between laboratories have already been reported in the literature. For instance, Hemming et al. (1998) compared marine coral boron isotope data studied by Vengosh et al. (1991), Hemming and Hanson (1992) and Gaillardet and Allègre (1995). They found offsets up to 3‰ between studies, although measurements were conducted on the same modern coral species. Analyses on the coral *Porites* (Hönisch and Bijma, unpublished data) are similar to data published by Hemming and Hanson (1992) and Gaillardet and Allègre (1995), indicating our analytical techniques are sound and comparable among laboratories. Furthermore, data acquired on *Globigerinoides sacculifer* at SOC (M.R. Palmer) are offset by $\sim$+2‰ to similar samples analyzed by Sanyal et al. (2001). Our own repeated analyses on different samples of *G. sacculifer* revealed a much closer similarity in $\delta^{11}B$ between this species and *Orbulina universa* than the one reported by Sanyal et al. (2001).

We suggest that the origin for the observed differences must be within the analytical procedure. The offsets may be laboratory specific, maybe even specific for different (biogenic) carbonates. Two possible causes of interlaboratory offsets include procedural differences such as the filament

temperature at which the analysis is performed, and differences in standardization. For instance, the temperature at which the analysis is performed is species-specific and is adjusted to the amount of boron present in the carbonate. Furthermore, many laboratories use internal seawater standards to calibrate their data instead of the NBS 951 boric acid standard. Neither standard is a carbonate, and matrix differences may be more important than previously assumed. The difference between the 43/42 ratio of biogenic carbonates and seawater on the one hand, and the boric acid standard on the other, may be too large to make any of these non-carbonates a reasonable standard. There is a clear need to define an international carbonate standard for boron isotopic analysis.

Despite the possibility of specific laboratory offsets, relative differences between samples of the same species seem to be constant. Repeated analyses of our cultured samples at SOC revealed a difference of ~2.2‰ between shells grown under HL ($\delta^{11}B = 23.9$‰, $n = 2$) and LL ($\delta^{11}B = 21.7$‰, $n = 1$) conditions. Although the $\delta^{11}B$ of *Orbulina universa* was measured ~2‰ heavier at SOC compared to GEOMAR, the relative difference between the two cultured shell samples obtained in both laboratories is the same within error. Hence, using a known $\delta^{11}B$–*p*H relationship, comparison of relative differences between samples is therefore feasible. However, comparison of absolute values raised in different laboratories seems to be inappropriate until identification of the underlying issues.

3.4. The effect of increased boron concentration

The use of boron enriched seawater was a substantial improvement to the investigation of boron isotope systematics in the laboratory (Sanyal et al., 2001). Increasing the boron concentration in the culture water to tenfold the natural seawater concentration increases the boron concentration in the shells proportionately and allows us to reduce the large sample sizes required for $\delta^{11}B$ analyses from ~200 shells to 60–70 shells. However, the addition of boric acid also lowers the seawater *p*H. We chose to titrate with NaOH to raise *p*H back to ambient values. This increases total alkalinity but brings the concentrations of the carbonate species back to the initial values of the natural sea water. The alternative method, keeping alkalinity constant, would have required us to lower DIC by bubbling the solution with an inert gas such as N_2. This latter method has the disadvantage that upon reaching the original *p*H, the DIC concentration in the culture solution would have been reduced by almost 700 μmol kg^{-1} to ca. 1290 μmol kg^{-1}. The concentrations of all carbonate species would then decrease significantly in such a solution. This would not only affect the chemical gradients in the microenvironment of the foraminifera and therefore the impact of the life processes on the *p*H at the site of calcification, but also reduce the final shell weight significantly (e.g. Bijma et al., 1999), producing less material for $\delta^{11}B$ analysis.

Sanyal et al. (2001) provided evidence that the use of increased boron concentrations in laboratory experiments does not change the $\delta^{11}B$ relative to shells grown under natural boron concentrations in the field. However, the $\delta^{11}B$ offset between shells grown under HL and LL in this study is ca. 2.6‰ smaller than that predicted for foraminifera grown in natural sea water on the basis of a diffusion–reaction model (Zeebe et al., in press). In that paper it is argued that the difference could be due to the experimentally increased boron concentration which buffers the impact of photosynthesis and respiration on the *p*H at the site of calcification. Consequently, the isotopic difference between shells grown under HL and LL would be significantly smaller at tenfold boron concentration (10× B) compared to natural conditions. Fig. 3 demonstrates the good agreement between the numerical results at 10× B and the measured offset found in the experiments.

Although this agreement is good, there is a difference between the theoretical arguments applied to account for the small HL–LL offset: whereas Zeebe et al. (in press) find the solution in the increased boron concentration, the daily periodicity in the symbiont photosynthetic rate and the low photosynthetic activity at ~19 μmol photons m^{-2} s^{-1} are essential components of the line of

argument provided in Section 3.1 but are not included in the numerical approach by Zeebe et al. (in press). Both lines of argument appear equally admissible and yield a similar difference in effective pH and $\delta^{11}B$ at the site of calcification: $\Delta p\mathrm{H} \sim -0.34$ (experimental data according to Spero and Parker, 1985 and Rink et al., 1998) and $\Delta\delta^{11}B \sim -1.5$ to -3‰ (value depending on assumed thickness of the symbiont halo, Fig. 3 according to Zeebe et al., in press). At present, the data base is too small and the analytical errors are too large to resolve this discrepancy. To conclusively rule out a potential effect of increased boron concentration on $\delta^{11}B$, it is essential to repeat the experiment of Sanyal et al. (2001) and to compare exclusively individuals grown in the laboratory at 1× B and 10× B, rather than laboratory (10× B) and field grown (1× B) foraminifera.

Regardless of the magnitude of the $\delta^{11}B$ difference between species grown in HL and LL, a significant difference exists. The experiments presented here were not designed to define a correction factor by which the $\delta^{11}B$ of different foraminifera species can be brought into agreement. Despite interlaboratory calibration issues, this study demonstrates the importance of foraminifera physiology on shell $\delta^{11}B$ and shows the necessity to concentrate on monospecific foraminifera assemblages. Ironically, these are the same issues that had to be addressed in the early years of oxygen and carbon isotope analyses for paleoceanography.

4. Conclusions

The results presented here suggest a dependence of *Orbulina universa* $\delta^{11}B$ on symbiont photosynthetic activity similar to the observation by Hemming et al. (1998) on corals in periods of high symbiont productivity. Although the effect is significant, we suggest it is constant for monospecific foraminifera samples. If respiration and photosynthesis of the foraminifer–symbiont association changed significantly with varying seawater pH, the empirical relationships established by Sanyal et al. (1996) and Sanyal et al. (2001) for *O. universa* and *Globigerinoides sacculifer* should deviate in shape from the theoretical $B(OH)_4^-$ curve by Kakihana et al. (1977). We suggest that the use of $\delta^{11}B$ as a proxy for pH is not compromised by the vital effect presented here.

If photosynthesis and respiration are the major parameters affecting deviations of the shell isotopic signature from seawater pH, our results suggest that symbiont-bearing foraminifera like *Orbulina universa* and *Globigerinoides sacculifer* should generally record higher $\delta^{11}B$ values and symbiont-barren species such as *Globigerina bulloides* lower values compared to inorganic calcites. Culture and field data presented here are consistent with this hypothesis but deviate from earlier published data. In order to better understand the controls over $\delta^{11}B$ in foraminifera and to compare results from different laboratories, it is essential to resolve the interlaboratory analytical offsets discussed herein. Nevertheless, as long as modern samples of a certain species are available, they can be used as a reference for ancient samples of the same species. Using the shape of the theoretical relationship between pH and $\delta^{11}B$ by Kakihana et al. (1977), the differences in pH can be estimated.

5. Uncited references

Zeebe, 1999

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