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

Received 19 June 2002; received in revised form 18 February 2003; accepted 22 February 2003

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 pH, the lower boron isotope values correspond to a reduction in pH 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: δ11B; planktonic foraminifera; vital effects; symbionts; paleo-pH

1. Introduction

Data from experiments with living foraminifera have confirmed the hypothesis that seawater pH is the dominant environmental control on the 11B/10B content (δ11B) of planktonic foraminifera shells (Hemming and Hanson, 1992; Sanyal et al., 2001, 1996, 2000; Spivack et al., 1993). Although measurements of foraminiferal δ11B are not yet a routine tool in paleoceanography, several studies have published paleo-pH reconstructions across different geological timescales with encouraging results (Palmer et al., 1998;
Whereas pH is the primary environmental control on shell $\delta^{11}$B, several physiological processes can modify the pH of the calcifying microenvironment, potentially complicating straightforward interpretation of $\delta^{11}$B data. For instance, microelectrode studies have revealed that pH 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 pH in the foraminiferal microenvironment, respiration releases CO$_2$ and decreases pH. 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. pH-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 pH 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 pH. 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; Masliotta 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$_3$BO$_3$) per l seawater. The drop in pH upon adding H$_3$BO$_3$ was readjusted to ambient pH 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 $pH$ values (on the NBS scale) were determined potentiometrically. Carbonate chemistry analyses were calibrated against certified reference materials 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 $\mu$m 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 $\mu$l 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$_3^+$ 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 (12C14N16O-ions), mass 26 (12C14N-ions) was monitored during each measurement. No interferences were detected. The $^{11}$B/$^{10}$B ratio was corrected for isotopic interferences on mass 43 ($^{10}$B16O17O-ions) by subtraction of 0.00078 from the 43/42 ratio (Spivack and Edmond, 1986).

The fractionation $\varepsilon$ between natural seawater (NS) and calcite (C) is usually calculated as: $\varepsilon_{\text{NS–C}} = \delta^{11}$B$_{\text{NS}} – \delta^{11}$B$_{\text{C}}$. 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-
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):

\[
\delta^{11}B_{\text{NC}} = \alpha_{\text{NS-MS}} \times \delta^{11}B_c + (\alpha_{\text{NS-MS}} - 1) \times 1000 \tag{1}
\]

where \(\alpha_{\text{NS-MS}}\) is a factor expressing the isotope difference between modified and natural seawater \(\left(\alpha_{\text{NS-MS}} = (\delta^{11}B_{\text{NS}} + 1000)/(\delta^{11}B_{\text{MS}} + 1000)\right)\). \(\delta^{11}B_{\text{NC}}\) 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 \(2\sigma_{\text{mean}}\) for multiple sample runs. \(\delta^{11}B\) (\%) \(= (R_s/R_{\text{std}} - 1) \times 1000\), \(R_s = ^{11}B/^ {10}B\) of sample, \(R_{\text{std}} = ^ {11}B/^ {10}B\) of NBS 951 boric acid standard. Seawater standard \(= 39.5 \pm 0.34\) %.

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 \(pH\) the \(\delta^{11}B\) of LL \(\text{Orbulina universa}\) shells is 1.5% lower than that of specimens grown under HL (Table 1; Fig. 1). Shifting 951 boric acid standard were measured at a filament temperature of 925 ± 10°C.

Table 1
Boron isotopic composition of cultured \(\text{Orbulina universa}\) and modified seawater chemistry

<table>
<thead>
<tr>
<th>Light (μmol photons m(^{-2}) s(^{-1}))</th>
<th>pH (culture water)</th>
<th>Alkalinity (μmol kg(^{-1}))</th>
<th>Seawater (\delta^{11}B_{\text{MS}}) (%)</th>
<th>(\text{Orbulina universa}) n</th>
<th>(\delta^{11}B_{\text{NC}}) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>321 ± 8</td>
<td>8.16 ± 0.02</td>
<td>3147 ± 13</td>
<td>−8.9 ± 0.1</td>
<td>5</td>
<td>−25.6 ± 0.6</td>
</tr>
<tr>
<td>19 ± 2</td>
<td>8.15 ± 0.03</td>
<td>3154 ± 8</td>
<td>−9.1 ± 0.4</td>
<td>6</td>
<td>−27.2 ± 0.3</td>
</tr>
</tbody>
</table>

Isotope results are based on 70 shells per sample. Errors are expressed as \(2\sigma_{\text{mean}}\) for multiple sample runs. \(\delta^{11}B\) (\%) \(= (R_s/R_{\text{std}} - 1) \times 1000\), \(R_s = ^{11}B/^ {10}B\) of sample, \(R_{\text{std}} = ^ {11}B/^ {10}B\) of NBS 951 boric acid standard. Seawater standard \(= 39.5 \pm 0.34\) %.

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 \(pH\) the \(\delta^{11}B\) of LL \(\text{Orbulina universa}\) shells is 1.5% lower than that of specimens grown under HL (Table 1; Fig. 1). Shifting 951 boric acid standard were measured at a filament temperature of 925 ± 10°C.

Table 2
Boron isotopic composition of plankton tow \(\text{Orbulina universa}\) and \(\text{Globigerina bulloides}\)

<table>
<thead>
<tr>
<th>Species</th>
<th>Ambient pH</th>
<th>(\delta^{11}B) (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Orbulina universa})</td>
<td>8.12 ± 0.02</td>
<td>20.5 ± 0.5</td>
<td>2</td>
</tr>
<tr>
<td>(\text{Globigerina bulloides})</td>
<td>8.12 ± 0.02</td>
<td>19.0 ± 0.9</td>
<td>2*</td>
</tr>
</tbody>
</table>

Results are based on approximately 300 shells per sample. Errors are expressed as \(2\sigma_{\text{mean}}\) for multiple sample runs. \(\delta^{11}B\) (\%) \(= (R_s/R_{\text{std}} - 1) \times 1000\), \(R_s = ^{11}B/^ {10}B\) of sample, \(R_{\text{std}} = ^ {11}B/^ {10}B\) of NBS 951 boric acid standard. Seawater standard \(= 39.5 \pm 0.34\) %.

* = 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. $^{11}$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 $\sim 2.7\%$. In order to determine the pH at the site of calcification under LL conditions, we moved the theoretical curve for $^{11}$B of B(OH)$_4^-$ vs. pH (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 pH (see arrows).

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

Our calculated, $^{11}$B-based, pH offset between HL and LL conditions is smaller than the HL-dark pH offset measured by Rink et al. (1998) using microelectrodes. Rink et al. (1998) measured the pH 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 $\mu$mol photons m$^{-2}$ s$^{-1}$, respectively (all on the NBS scale). Interpolating between the latter two values we estimate a pH of 8.7 for the HL conditions in our culture experiments ($\sim 320$ $\mu$mol photons m$^{-2}$ s$^{-1}$). The difference between our HL conditions and complete darkness should therefore be $\sim 0.75$ pH units. Although the pH 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 $\sim 59$ nmol C d$^{-1}$ instead of $\sim 87$ 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 pH measurements made by Rink et al. (1998). With regard to pH, 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 (at pH $\approx 7.95$) and during the day (at pH $\approx 8.46$) yields a weighted, time integrated pH of 8.29 for O. universa. Based on symbiont density and photosynthetic rates during the remaining illuminated period. Therefore, the integrated pH difference predicted for foraminifera grown under a HL-dark cycle compared to shells grown in complete darkness is therefore reduced to $\sim 0.34$ instead of $\sim 0.75$ units.

Finally, it should be kept in mind that we did not keep the LL-foraminifera in the dark but at $\sim 19$ $\mu$mol 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), symbiotic photosynthesis still removes CO$_2$. Therefore, the actual microenvironment pH under LL conditions is ca. 0.1 pH 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 pH difference of
\( \sim 0.24 \) units. Our experimental result of a \( \sim 0.2 \)
\( \Delta pH \) difference between LL and HL grown speci-
mens agrees well with these calculations. See,
however, the discussion in Section 3.4 and Fig.
3 for the potential effect of increased boron con-
centration on these data.

3.2. Plankton tows

The \( \delta^{11}B \) value of *Orbulina universa* collected in
plankton tows (20.5 \( \pm \) 0.5\%) is identical to that
of *O. universa* cultured under LL conditions (Ta-
ble 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 \( pH \) (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. How-
ever, light level measurements made at the collec-
tion site in August 1987 yielded irradiance levels
between 2188 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \) at the sur-
face and 361–123 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \) at 18–27
m water depth (H.J. Spero, unpublished data). These irradiiances would suggest all the tow-col-
clected 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 sym-
bionts expected from a similar sized sphere as it

![Fig. 3. Potential effect of higher boron concentration on ex-
perimental \( \delta^{11}B \) 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 nat-
ural seawater boron concentrations (1 \( \times \) B), whereas right
grey bar refers to tenfold enriched total boron concentrations
(10 \( \times \) B). HL conditions as indicated by open symbols are
320 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \) in both studies, closed symbols
refect 19 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \) (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 \( \mu \text{m} \), respectively. See Zeebe et al. (in press)
for model details. The model was run relative to the theoreti-
cal \( \delta^{11}B_{\text{B(OH)}} \)–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 \( \times \) B, the agree-
ment 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 \( \delta^{11}B–\delta^{11}B_{\text{B(OH)}} \) at pH 8.16.

![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 carbon-
ate was also precipitated in artiﬁcial seawater, the \( \delta^{11}B \) val-
ues of Sanyal et al. (2000) were converted to the natural sea-
water 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. \( pH \) (dashed line; Kakihana et al.,
1977).]
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 δ11B 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 δ11B 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 pH measurements have never been conducted on this species, it is well known that the addition of respiratory CO2 decreases pH in symbiont-bearing foraminifera by up to 0.3 units (Jorgensen et al., 1985; Rink et al., 1998; Wolfe-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 δ11B compared to *O. universa* and the inorganic precipitation results is reasonable under the assumption of a lower pH 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 δ11B 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 isotopic 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 ~ +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 δ11B 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 \( \sim 2.2\% \) between shells grown under HL (\( \delta^{11}B = 23.9\%_e, n=2 \)) and LL (\( \delta^{11}B = 21.7\%_e, n=1 \)) conditions. Although the \( \delta^{11}B \) of *Orbulina universa* was measured \( \sim 2\% \) 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 \( \delta^{11}B-pH \) 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 \( \sim 200 \) shells to 60–70 shells. However, the addition of boric acid also lowers the seawater \( pH \). We chose to titrate with NaOH to raise \( pH \) 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 \( \text{N}_2 \). This latter method has the disadvantage that upon reaching the original \( pH \), the DIC concentration in the culture solution would have been reduced by almost 700 \( \mu\text{mol kg}^{-1} \) to ca. 1290 \( \mu\text{mol kg}^{-1} \). 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 \( pH \) 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 \( pH \) 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 \( \sim 19 \mu\text{mol photons m}^{-2} \text{s}^{-1} \) 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 \( p_H \) and \( ^{11}B \) at the site of calcification: \( \Delta p_H \sim -0.34 \) (experimental data according to Spero and Parker, 1985 and Rink et al., 1998) and \( \Delta ^{11}B \sim -1.5 \) to \(-3\% \) (value depending on assumed thickness of the symbiont halo, Fig. 3 according to Zeebe et al., in press). At present, the database 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 \(^{11}B\), it is essential to repeat the experiment of Sanya et al. (2001) and to compare exclusively individuals grown in the laboratory at 1 \( \times \) B and 10 \( \times \) B, rather than laboratory (10 \( \times \) B) and field grown (1 \( \times \) B) foraminifera.

Regardless of the magnitude of the \(^{11}B\) 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 \(^{11}B\) of different foraminifera species can be brought into agreement. Despite interlaboratory calibration issues, this study demonstrates the importance of foraminifera physiology on shell \(^{11}B\) 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 \textit{Orbulina universa} \(^{11}B\) 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 \( p_H \), the empirical relationships established by Sanyal et al. (1996) and Sanyal et al. (2001) for \textit{O. universa} and \textit{Globigerinoides sacculifer} should deviate in shape from the theoretical \( B(OH)_4 \) curve by Kakihana et al. (1977). We suggest that the use of \(^{11}B\) as a proxy for \( p_H \) 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 \( p_H \), our results suggest that symbiont-bearing foraminifera like \textit{Orbulina universa} and \textit{Globigerinoides sacculifer} should generally record higher \(^{11}B\) values and symbiont-barren species such as \textit{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 \(^{11}B\) 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 \( p_H \) and \(^{11}B\) by Kakihana et al. (1977), the differences in \( p_H \) can be estimated.

5. Uncited references

Zeebe, 1999

Acknowledgements

We thank the staff of the Catalina Marine Science Center for providing facilities to make this work possible. We gratefully acknowledge field help by Laurie Juranek, M. Thomas, H. Iverson and the Catalina dive crew. Invaluable was A. Sanyal’s introduction into the secrets of \(^{11}B\) analysis and boron enriched culture experiments. Of great benefit were suggestions and comments by D. Lea, D. Wolf-Gladrow and H. Fischer and technical discussions with A. Deyhle and N. Gusson. Reviews by N.G. Hemming and an anonymous reviewer were very encouraging and the helpful suggestions are gratefully acknowledged. This research was supported by DAAD Grant D/00 20292 (B.H.), NEBROC (B.H., J.B.), NSF

MARMIC 928 8-4-03
References


