Biogeosciences

Effects of ocean acidification on calcification of symbiont-bearing reef foraminifers

K. Fujita[1], **M. Hikami**[1,2], **A. Suzuki**[3], **A. Kuroyanagi**[2], **K. Sakai**[4], **H. Kawahata**[2], and **Y. Nojiri**[5]

[1]Department of Physics and Earth Sciences, University of the Ryukyus, Okinawa, Japan
[2]Atmosphere and Ocean Research Institute, The University of Tokyo, Kashiwa, Japan
[3]Geological Survey of Japan, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan
[4]Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan
[5]Center for Global Environmental Research, National Institute for Environmental Studies, Tsukuba, Japan

Abstract. Ocean acidification (decreases in carbonate ion concentration and pH) in response to rising atmospheric pCO_2 is generally expected to reduce rates of calcification by reef calcifying organisms, with potentially severe implications for coral reef ecosystems. Large, algal symbiont-bearing benthic foraminifers, which are important primary and carbonate producers in coral reefs, produce high-Mg calcite shells, whose solubility can exceed that of aragonite produced by corals, making them the "first responder" in coral reefs to the decreasing carbonate saturation state of seawater. Here we report results of culture experiments performed to assess the effects of ongoing ocean acidification on the calcification of symbiont-bearing reef foraminifers using a high-precision pCO_2 control system. Living clone individuals of three foraminiferal species (*Baculogypsina sphaerulata*, *Calcarina gaudichaudii*, and *Amphisorus hemprichii*) were subjected to seawater at five pCO_2 levels from 260 to 970 µatm. Cultured individuals were maintained for about 12 weeks in an indoor flow-through system under constant water temperature, light intensity, and photoperiod. After the experiments, the shell diameter and weight of each cultured specimen were measured. Net calcification of *B. sphaerulata* and *C. gaudichaudii*, which secrete a hyaline shell and host diatom symbionts, increased under intermediate levels of pCO_2 (580 and/or 770 µatm) and decreased at a higher pCO_2 level (970 µatm). Net calcification of *A. hemprichii*, which secretes a porcelaneous shell and hosts dinoflagellate symbionts, tended to decrease at elevated pCO_2. Observed different responses between hyaline and porcelaneous species are possibly caused by the relative importance of

elevated pCO_2, which induces CO_2 fertilization effects by algal symbionts, versus associated changes in seawater carbonate chemistry, which decreases a carbonate concentration. Our findings suggest that ongoing ocean acidification might favor symbiont-bearing reef foraminifers with hyaline shells at intermediate pCO_2 levels (580 to 770 µatm) but be unfavorable to those with either hyaline or porcelaneous shells at higher pCO_2 levels (near 1000 µatm).

1 Introduction

The oceans have taken up about one-third of the anthropogenic carbon dioxide released into the atmosphere over the past 200 years (Sabine et al., 2004). According to the Intergovernmental Panel on Climate Change (IPCC) Special Report on Emission Scenarios (SRES), atmospheric CO_2 will rise further, up to a partial pressure of 500–1000 µatm by the end of the century (Meehl et al., 2007). This absorption of CO_2 into the surface ocean changes seawater chemistry, resulting in decreases in pH and carbonate ion concentration, and increases in the concentrations of bicarbonate and hydrogen ions. It will also lead to a decrease in the saturation state of seawater with respect to calcite and aragonite, two common forms of calcium carbonate secreted by marine calcifying organisms (Orr et al., 2005). Calcification of marine calcareous organisms is strongly dependent on the carbonate saturation state of seawater, suggesting that ocean acidification will adversely affect marine calcifying taxa, with potentially severe implications for marine ecosystems such as coral reefs (Kleypas et al., 1999).

Culture experiments with reef calcifying organisms such as corals, coralline algae, molluscs, and foraminifers suggest that ocean acidification will adversely impact their calcification (e.g. Gattuso et al., 1998; Kuffner et al., 2008; Kuroyanagi et al., 2009; Ries et al., 2009), leading to a reduction in coral-reef biodiversity and the net accumulation of reef carbonates in the future (Hoegh-Guldberg et al., 2007). Large, reef-dwelling benthic foraminifers (defined as mature individuals > 1 mm in diameter) are shelled protists that are host to algal endosymbionts (Lee, 1998; Hallock, 1999). They are one of the primary producers and carbonate producers in coral reefs (Hallock, 1981; Langer et al., 1997; Hohenegger, 2006; Fujita and Fujimura, 2008), secreting high-magnesium calcite (HMC; Raja et al., 2005), the solubility of which can exceed that of aragonite at a similar seawater pCO_2 level (Morse et al., 2006). Therefore, reef foraminifers with high-magnesium calcite shells may be the "first responders" among reef calcifying organisms to the decreasing saturation state of seawater caused by ocean acidification.

During calcification, foraminifers are able to elevate the pH at the site of calcification (vesicles or seawater vacuoles) by one unit above seawater pH (Erez, 2003; Bentov et al., 2009; de Nooijer et al., 2009). In acidified seawater, foraminifers would require more energy to elevate the intracellular pH, leading to a decrease in calcification. Previous culturing results have indicated that shell weights of both planktonic and benthic foraminifers reduce with decreasing $[CO_3^{2-}]$ or pH (Bijma et al., 1999, 2002; Dissard et al., 2010; Lombard et al., 2010). Calcification (measured as increments of shell weight and diameter) of *Marginopora*, a large, dinoflagellate symbiont-bearing benthic foraminifer, is reduced as pH becomes lower (Kuroyanagi et al., 2009). Culture experiments using ^{14}C tracer techniques of reef foraminifers have also shown that the carbon uptake ratio is dependent on pH and concentrations of inorganic carbon and calcium, and decreases when the pH or the concentrations of these elements are lower than those of the present seawater (ter Kuile et al., 1989).

However, the effects of ocean acidification on calcification of diverse large, algal symbiont-bearing and reef-dwelling benthic foraminifers have been assessed in only one species (*Marginopora kudakajimensis*; Kuroyanagi et al., 2009). Recent studies have shown that the response of marine calcifying organisms to ocean acidification varies both within and among species (e.g. Fabry, 2008; Langer et al., 2009; Ries et al., 2009; Ridgwell et al., 2009). Moreover, in our previous study (Kuroyanagi et al., 2009), we controlled the pH by adding an acid or base, rather than by bubbling CO_2-enriched air through the seawater, though the latter would better reproduce the present anthropogenic changes and the ocean's response. Therefore, in this study, we conducted a culture experiment using a high-precision pCO_2 control system to assess the effect of ocean acidification on calcification of symbiont-bearing reef foraminifers.

2 Materials and methods

2.1 Target species

Three species of large, algal symbiont-bearing benthic foraminifers were selected for this study: *Baculogypsina sphaerulata* (Parker and Jones), *Calcarina gaudichaudii* d'Orbigny in Ehrenberg, and *Amphisorus hemprichii* Ehrenberg. These species are commonly found on macrophytes on coral-reef flats in the northwest Pacific (e.g. Hohenegger, 1994) and are important primary producers and carbonate producers in their environments (e.g. Sakai and Nishihira, 1981; Hohenegger, 2006; Fujita and Fujimura, 2008). Shells of all three species are composed of high-magnesium calcite (HMC; approx. 10 Mg mol %; Saraswati et al., 2004). Shell walls of *B. sphaerulata* and *C. gaudichaudii* are perforate and have a hyaline (i.e. clear, glassy) appearance, whereas those of *A. hemprichii* are imperforate and appear porcelaneous (i.e. shiny, white, and smooth like porcelain). *Baculogypsina sphaerulata* and *C. gaudichaudii* are host to diatom endosymbionts, whereas *A. hemprichii* is host to dinoflagellate endosymbionts (Lee, 1998). In addition, these three species have been observed to reproduce asexually during spring and summer (Sakai and Nishihira, 1981; Hohenegger, 2006). Thus, asexually reproduced clone individuals can be used for culture experiments to exclude the effect of genetic variability on the experimental results. For more detailed information on the taxonomy, biology, and ecology of these species, see Röttger and Krüger (1990) and Hohenegger (1994).

2.2 Maintenance until asexual reproduction

Adult individuals just before asexual reproduction (agamont or shizont) were collected during low tide on nearshore reef flats: *B. sphaerulata* and *C. gaudichaudii* northeast of Ikei Island, Okinawa, Japan (26°23′ N, 128°00′ E) on 26 April and 22 May 2009, and *A. hemprichii* on a reef moat along the Oudo coast, south of Okinawa Island (26°05′ N, 127°42′ E) on 28 May 2009. Collected individuals were maintained separately in small Petri dishes filled with natural seawater at room temperature (approximately 25 °C) under a natural light:dark cycle near a window (light intensity, ~100 µmol m^{-2} s^{-1}). Because *C. gaudichaudii* and *B. sphaerulata* are commonly found in high-energy reef-flat environments (Hohenegger, 1994), these species were maintained under continuous water motion produced by using a continuous-action shaker (approximately 30 rpm; in vitro Shaker, Wave-PR, TAITEC Inc., Saitama, Japan). In contrast, *A. hemprichii* individuals were maintained under stagnant conditions on a flat shelf, because they are commonly found in relatively calm reef-moat environments (Hohenegger, 1994). These individuals were not fed during maintenance, and the culture medium was changed weekly.
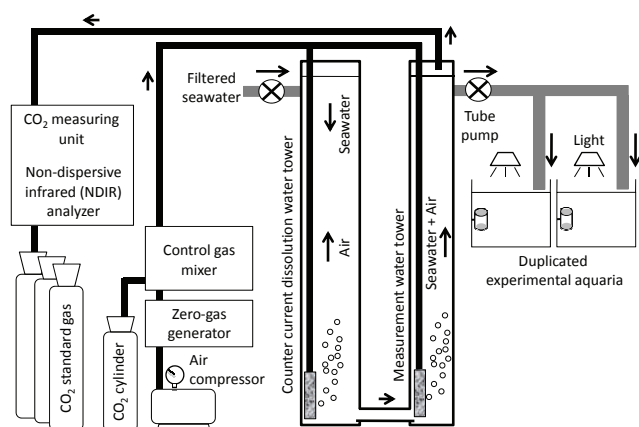
Fig. 1. The schematic diagram of culturing set-up. See the text for explanation.

After a few weeks, these adult individuals reproduced asexually. Juveniles of the clone populations were kept under the same conditions as the adults for 4 to 6 weeks until the experiments were started. To determine differences in characteristics within species under experimental conditions, two clone populations of each species were used, tagged as populations α (alpha) and β (beta).

2.3 High-precision pCO$_2$ control system

Clone individuals of the three foraminiferal species were placed into culture cages and subjected to seawater with different pCO$_2$ levels. pCO$_2$ in each culture cage was adjusted by bubbling CO$_2$ gas through the seawater, and a constant pCO$_2$ level was maintained by a high-precision pCO$_2$ control system developed with the cooperation of Kimoto Electric Co., Ltd. (Osaka, Japan). This system is composed of CO$_2$ dissolution and measurement towers (height, 1.78 m), into which gaseous CO$_2$ is dissolved in seawater by bubbling it from the bottom of the towers (Fig. 1). The seawater pCO$_2$ is continually monitored in the equilibrated air flowing out from the measurement tower surface and adjusted as needed by controlling the CO$_2$ concentration in the air by mixing CO$_2$ and dilution-air (dried air with a low CO$_2$ concentration) into the filtered (pore size, 1 µm) seawater.

The pCO$_2$ of the culture medium was adjusted to one of five pCO$_2$ levels (approximately 260, 360, 580, 770, and 970 µatm, Table 1) corresponding to the estimated preindustrial value, the present value, and future values predicted by IPCC SRES (Meehl et al., 2007), respectively. Seawater temperature and chemistry (salinity, pH, and total alkalinity) were measured twice a week, and the averaged values were used for calculating the saturation state of seawater with respect to calcite (Table 1).

2.4 Culture protocol

Each foraminifer culture cage consisted of a clear vinyl hose (5 cm long; inside diameter, 2.5 cm) with a cell strainer (100 µm mesh, BD Falcon, Inc) at each end. For each clone population, 40 individuals of each species (i.e. a total of 120 individuals per cage) were put into a cage. A set of five cages for the five pCO$_2$ levels were prepared for each of the two clone populations α and β. Before the start of experiment, another 40 clone individuals of each species were preserved in ethanol. Each cage was placed in a water bath and attached to the tank wall with a suction cup. Thus, there were a total of 10 water baths (five treatments × two clone populations). Seawater within the culture cages was circulated by fixing them in a path of a water jet within the water bath. Culture cages containing clone individuals were maintained for approximately 12 weeks (from 6 July to 30 September 2009) at a constant water temperature (27.5 ± 0.1 °C), light intensity (60 µmol m^{-2} s^{-1}), and photoperiod (12 h). During the culture experiments, filamentous algae attaching to the surfaces of the culture cages were removed biweekly with brushes. Small air bubbles inside the cages, probably produced by the photosynthesis of the algal symbionts, were removed with a pipette.

At the end of the experiment, the cages were removed from the water baths and kept cool until later analysis. Cultured individuals were picked from each cage, cleaned with a brush and distilled water, and air-dried. Of the 40 cultured individuals, 20 were selected randomly for measurements, excluding dead individuals (i.e. those that did not grow or had no protoplasm). Mortality during the experiments was very low, less than 2.5 % for each population. Twenty individuals from those preserved in ethanol at the start of the experiment (hereafter, "initial individuals") were also randomly selected for comparison with the cultured individuals.

2.5 Measurements

The shell diameter and shell weight of each initial and cultured individual was measured. The shell diameter (the average of the maximum diameter from the final chamber and that crossing the first at the center of the test and perpendicular to it) was measured to the nearest 1 µm on microscope photographs using image analysis software (Photomeasure, Kenis Inc., Osaka, Japan). Shell weight was measured to the nearest 0.1 µg using a microbalance (Thermo Cahn C-35 microbalance, Thermo Electron Corporation).

Growth (calcification) rates were estimated from the increment of shell diameter and weight during the experimental period. Although time-course measurements have not been conducted in this experiment to avoid the loss of cultured specimens and to minimize stress to them during manipulation, our previous culture experiments using clones of *Marginopora kudakajimensis* (Kuroyanagi et al., 2009) statistically confirmed that shell diameter increased with time

Table 1. Mean physical and chemical conditions in each pCO_2 treatment. Standard deviations are shown for pH_T and pCO_2. The mean salinity and total alkalinity, which were measured repeatedly during the experiments, were 34.4 and 2188 $\mu mol\,kg^{-1}$, respectively. The value of DIC (dissolved inorganic carbon), Ω_{cal} and Ω_{arg} (saturation state of seawater with respect to calcite and aragonite) was calculated from the total alkalinity and pCO_2.

Treatment	Temperature (°C)	pH_T at 25 °C	pCO_2 (μatm)	DIC (μmol kg^{-1})	Ω_{cal}	Ω_{arg}
Preindustrial	27.5	8.170 ± 0.028	261 ± 29	1796	6.39	4.24
Present	27.4	8.072 ± 0.033	360 ± 19	1865	5.32	3.53
Future 1	27.5	7.926 ± 0.039	580 ± 30	1958	3.91	2.60
Future 2	27.5	7.826 ± 0.035	774 ± 56	2007	3.18	2.11
Future 3	27.6	7.756 ± 0.038	972 ± 43	2043	2.69	1.78

over the range of seawater pH examined (from 7.7 to 8.3; NBS scale). On the other hand, culturing studies of other species of reef foraminifers (*Amphistegina* spp.) showed that growth rates of juvenile clones were faster than those of matured ones (Hallock et al., 1986). Thus, calcification rates obtained in this experiment are averaged or underestimated.

2.6 Statistical analysis

The shell diameter and weight data sets were analyzed by one-way analysis of variance (ANOVA) for differences among the five pCO_2 levels. When significant differences were observed within a data set, the difference between each pair of means was analyzed with a Tukey's Honestly Significant Difference (HSD) test. These statistical analyses were performed with JMP 7.0.2 (SAS Institute Inc.).

3 Results

All individuals cultured at different pCO_2 levels grew more than twice as large as the initial individuals during the experimental period (Fig. 2). SEM images of cultured specimens did not show any traces of shell dissolution. Shell weight was positively correlated with shell diameter, but the statistical analysis indicated that shell weight was more dependent on pCO_2 than shell diameter, as reported by Kuroyanagi et al. (2009) as well. This difference is partly because shell diameter does not exactly reflect three-dimensional shell growth, in particular in *B. sphaerulata* and *C. gaudichaudii*, which have subglobular to bilenticular shells with spines (Fig. 2). Thus, the following description of culturing results is focused on foraminiferal shell weight.

3.1 *Baculogypsina sphaerulata*

The mean shell weight of initial individuals of *B. sphaerulata* was about 4 μg for both populations (Fig. 3). At the end of experiment, the mean shell weight of the cultured individuals varied from 15 to 25 μg. The shell weight was relatively heavier at intermediate pCO_2 levels in both clone
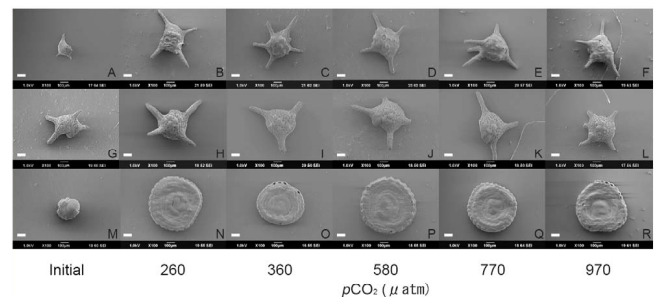

Fig. 2. SEM images of cultured individuals at various pCO_2 levels (scale bar = 100 μm). Photographs are shown for average-sized specimens from the population α of each species. (A to F) *Baculogypsina sphaerulata*, (G to L) *Calcarina gaudichaudii*, (M to R) *Amphisorus hemprichii*. (A, G, M) initial individuals, (B, H, N) specimens subjected to 260 μatm, (C, I, O) specimens subjected to 360 μatm, (D, J, P) specimens subjected to 580 μatm, (E, K, Q) specimens subjected to 770 μatm, (F, L, R) specimens subjected to 970 μatm.

populations. One-way ANOVA indicated significant differences in shell weight among the different pCO_2 levels in both populations ($F = 4$; $P < 0.001$ for population α, $P < 0.0001$ for population β). Tukey's HSD post hoc test of each population showed that individuals grown at a pCO_2 of 770 μatm had significantly heavier shell weights than those grown at 360 or 970 μatm ($P < 0.05$). Mean shell weights of individuals grown at a pCO_2 of 580 μatm were significantly heavier than those of individuals grown at 970 μatm ($P < 0.05$). In population β, individuals grown at 580 μatm were also significantly heavier than those grown at 260 or 360 μatm ($P < 0.05$). Moreover, in both populations, individuals grown at 260 μatm were found to be heavier than those grown at 360 μatm, although mean shell weight was not statistically significant between these two treatments.

3.2 *Calcarina gaudichaudii*

The mean shell weight of initial individuals of *C. gaudichaudii* was about 16 and 13 μg for population α, and β,
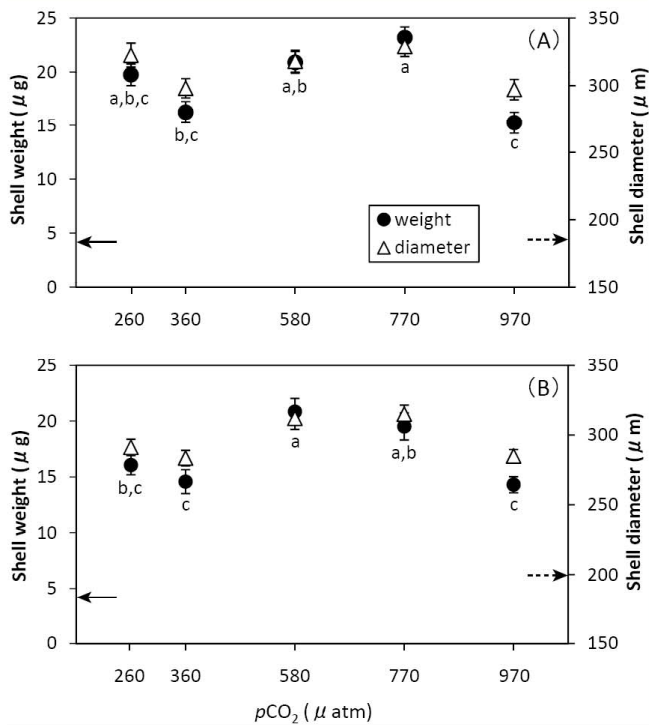
Fig. 3. Mean shell diameter and weight of *Baculogypsina sphaerulata* after culture for about 12 weeks at five pCO_2 levels. Values are means $\pm$ SE of the 20 clone individuals sampled in each treatment. Different letters above plotted treatment values indicate a significant difference ($\alpha = 0.05$) in shell weight according to Tukey's HSD post hoc test. Dashed and solid arrows show mean shell diameter and mean weight of clone individuals, respectively, at the start of the experiment. **(A)** Population α, **(B)** population β.
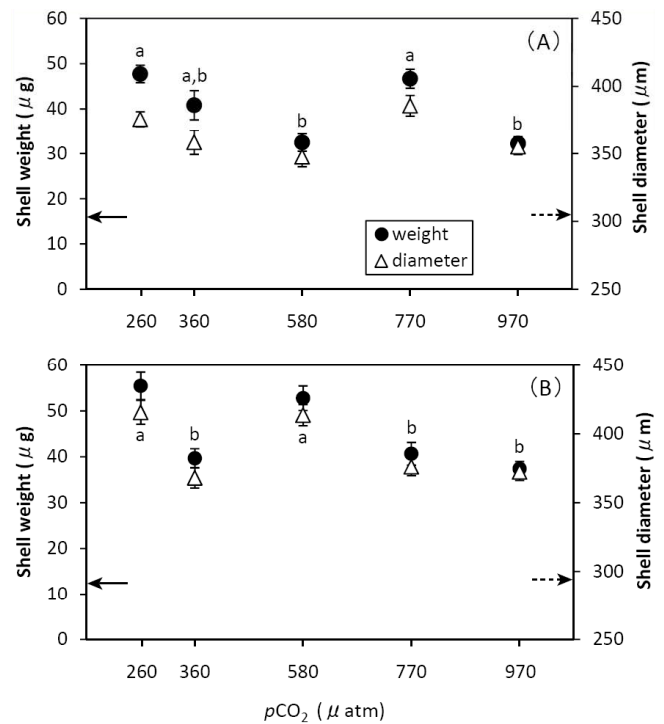

Fig. 4. Mean shell diameter and weight of *Calcarina gaudichaudii* after culture for about 12 weeks at five pCO_2 levels. Values are means $\pm$ SE of the 20 clone individuals sampled in each treatment. Different letters above plotted treatment values indicate a significant difference ($\alpha = 0.05$) in shell weight according to Tukey's HSD post hoc test. Dashed and solid arrows show mean shell diameter and weight of clone individuals, respectively, at the start of the experiment. **(A)** Population α, **(B)** population β.

respectively (Fig. 4). At the end of experiment, the mean shell weight of the cultured individuals varied from 30 to 50 μg in population α, and from 35 to 55 μg in population β. It was generally lower at elevated pCO_2, but higher at intermediate pCO_2 levels. One-way ANOVA indicated significant differences in shell weight among different pCO_2 levels in both populations ($F = 4$, $P < 0.0001$). Tukey's HSD post hoc test of each population showed that individuals grown at a pCO_2 of 260 μatm had significantly heavier shell weights than those grown at 970 μatm ($P < 0.05$). In population α, individuals grown at 260 and 770 μatm were significantly heavier than those grown at 580 or 970 μatm ($P < 0.05$). In population β, individuals grown at 260 and 580 μatm were significantly heavier than those grown at 360, 770, or 970 μatm ($P < 0.05$).

3.3 *Amphisorus hemprichii*

The mean shell weight of initial individuals of *A. hemprichii* was about 5 and 9 μg for population α and β, respectively (Fig. 5). At the end of experiment, the mean shell weight of the cultured individuals varied from 30 to 50 μg in population

α, and from 30 to 83 μg in population β. The mean shell weight tended to decrease at elevated pCO_2. One-way ANOVA indicated significant differences in shell weight among different pCO_2 levels in both populations ($F = 4$; $P < 0.01$ for population α, and $P < 0.0001$ for population β). Tukey's HSD post hoc test showed that in population α, individuals grown at a pCO_2 of 580 μatm had significantly heavier shell weights than those grown at 770 or 970 μatm ($P < 0.05$). In population β, individuals grown at a pCO_2 of 260 μatm had significantly heavier shell weights than those grown at pCO_2 levels of 580 μatm or more ($P < 0.05$). Mean shell weights of individuals grown at 770 μatm were significantly lighter than those grown at other pCO_2 levels in population β ($P < 0.05$).

4 Discussion

4.1 Scrutiny of culture experiments

Prior to discussing the effect of elevated seawater pCO_2 on the calcification of symbiont-bearing reef foraminifers, we
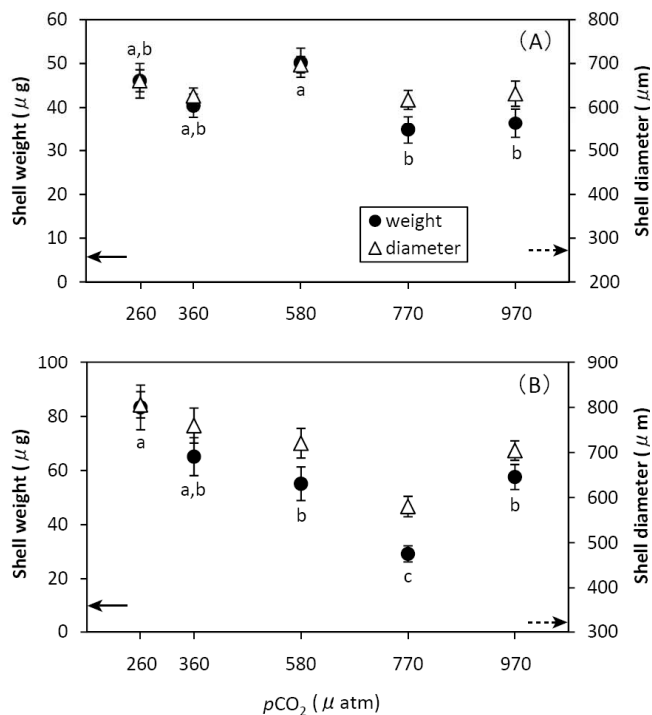
Fig. 5. Mean shell diameter and weight of *Amphisorus hemprichii* after culture for about 12 weeks at five pCO_2 levels. Values are means ± SE of the 20 clone individuals sampled in each treatment. Different letters above plotted treatment values indicate a significant difference ($\alpha = 0.05$) in shell weight according to Tukey's HSD post hoc test. Dashed and solid arrows show mean shell diameter and weight of clone individuals, respectively, at the start of experiment. **(A)** Population α, **(B)** population β.

need to scrutinize causes producing variability in the data, possible problems associated with culturing experiments, and other factors that might affect calcification rates.

Reef foraminifers are usually subject to a large diurnal variation in reef-water pCO_2, which ranges from 100 to 1000 µatm (e.g. Suzuki, 1994). Thus our studied ranges of carbonate chemistry are not beyond the tolerance limit of the physiology and growths of reef foraminifers. However, clone individuals were not pre-incubated in cultured conditions prior to experiments. The introduction to altered seawater carbonate chemistry may require time for foraminifers to acclimate and therefore cause them stressed particularly at the beginning of the experiment.

Environmental variables other than carbonate chemistry affect calcification rates. Light intensity in this experiment ($60 \, \mu\text{mol m}^{-2}\text{s}^{-1}$) was much darker than average daytime levels in their foraminiferal habitats as well as light saturation levels in a short-term light experiment (Fujita and Fujimura, 2008). Measurements of photosynthesis and respiration of the three foraminiferal species with algal symbionts in various temperatures (Fujita, unpublished data) indicate that net photosynthesis was enhanced in higher temperature

(30 °C) than that in the cultured condition (27.5 °C). However, long-term exposures of high light and temperature may cause stress (bleaching) on symbiont-bearing foraminifers. Water motion also influences the metabolic activity of these foraminifers. Because *B. sphaerulata* and *C. gaudichaudii* live in high-energy environments and *A. hemprichii* prefers relatively calm environments (Hohenegger, 1994), water motions inside the cages might have been weaker than those in the natural environment, in particular for the two hyaline species. Furthermore, cultured foraminifers were not fed during the experiments. Lee et al. (1991) showed that food availability did not influence growth rates of symbiont-bearing perforate (hyaline) foraminifer (*Amphistegina lobifera*), whereas imperforate (porcelaneous) foraminifers (*Amphisorus hemprichii* and *Marginopora kudakajimensis*) grew better in fed conditions than in unfed conditions. Therefore, all these variables might have not been optimum conditions for cultured foraminifers. Nevertheless, since cultured individuals were maintained in the homogeneous conditions except for pCO_2, observed differences in the calcification rates can be interpreted to be caused by different pCO_2 levels.

Experimental results indicate that responses of foraminiferal calcification (measured by the increment of shell weight and diameter) to different pCO_2 levels varied between two clone populations within a species. The variability between clone populations suggests the presence of diverse genotypes or ecotypes within a foraminiferal species and/or a symbiotic algal type, which have different physiological adaptations to pCO_2, as suggested for the cocolithophore *Emiliania huxleyi* (Langer et al., 2009; Ridgwell et al., 2009). Langer et al. (2009) demonstrated that four strains of *E. huxleyi* did not show a uniform response to carbonate chemistry changes, which likely has a genetic basis. Although the possibility of various genotypes or ecotypes has not yet been demonstrated in symbiont-bearing reef foraminifers, diverse genotypes have been found within a single morphospecies of planktonic foraminifers from geographically separated water masses (e.g. de Vargas et al., 2002).

The variability in average shell weight among five pCO_2 treatments within a clone population is mostly due to different growth rates of the cultured specimens (i.e. the number of chambers added during the experimental period). Kuroyanagi et al. (2009) also showed both shell weight and diameter of *Marginopora kudakajimensis* decreased with lowering seawater pH. Variability in shell wall thickness among individuals subjected to different pCO_2 levels may be partly attributed to differences in shell weight, as shown in planktonic foraminifers (Barker and Elderfield, 2002; de Moel et al., 2009; Moy et al., 2009). De Moel et al. (2009) showed that lighter shells were produced in planktonic foraminifers influenced by ocean acidification and seasonal upwelling. Our previous experiment (Kuroyanagi et al., 2009) also showed the shell weight of *Marginopora kudakajimensis* decreased

with lowering seawater pH for specimens with an identical shell diameter. Detailed SEM examinations are necessary to find differences in shell wall thickness of the cultured specimens among different pCO_2 levels.

4.2 Effects of pCO_2 on the calcification of symbiont-bearing reef foraminifers

As discussed above, experimental results in this study may contain some "noises" affecting calcification rates. Thus we discuss below mainly statistically significant results that were common to the two clone populations within a species.

Calcification was enhanced at intermediate pCO_2 levels in the two species with a hyaline shell (*B. sphaerulata* at 580 and 770 µatm and *C. gaudichaudii* at 580 or 770 µatm), compared with that at the present pCO_2 level. In contrast, calcification in *A. hemprichii* did not increase significantly at intermediate pCO_2 levels. Furthermore, calcification in all three species studied tended to be reduced at the highest pCO_2 level (970 µatm).

The result of the two hyaline species is similar to the findings of previous culture experiments by Iglesias-Rodrigues et al. (2008) and Ries et al. (2009). Iglesias-Rodrigues et al. (2008) reported increased calcification and net primary production in the coccolithophore *Emiliania huxleyi* at high pCO_2 (750 µatm). Ries et al. (2009) obtained parabolic calcification response patterns (i.e. positive at intermediate pCO_2 [600 to 900 µatm] and negative at the highest pCO_2 [2856 µatm]) in four of the 18 benthic marine taxa that they examined.

Enhanced calcification of the two species with a hyaline shell at intermediate pCO_2 levels may be related to increases in the concentrations of dissolved inorganic carbon (in particular, of bicarbonate ions; Table 1). However, ^{14}C tracer studies of inorganic carbon uptake by the perforate (hyaline) foraminifer *Amphistegina lobifera* demonstrated that only a very small increase of uptake was observed at inorganic carbon concentrations higher than those of present seawater (ter Kuile et al., 1989). This is possibly because regardless of external pH and carbonate chemistry, the intracellular pH and the ratio between dissolved carbon dioxide, bicarbonate, and carbonate ions can be modified by foraminiferal protoplasm (Bentov et al., 2009; de Nooijer et al., 2009).

Photosynthesis by algal symbionts may be enhanced under intermediate pCO_2 levels. The photosynthesis by algal symbionts provides host foraminifers with substantial photosynthates, which become a source of energy for metabolic activity (Hallock, 2000). In addition, photosynthates (carbohydrates) may be used for the organic matrix in foraminiferal shells, which is critical in initiating and directing chamber formation (Hallock, 1999). The removal of host metabolites such as NH_4^+ and PO_4^{3-} by symbionts might aid calcification of the host foraminifers because such metabolites can interfere with crystal formation (ter Kuile et al., 1989). Thus, enhanced photosynthesis by algal symbionts may promote

protoplasmic growth and chamber formation (i.e. CO_2 fertilization effects). Enhancement of calcification by photosynthesis has been also demonstrated by Iglesias-Rodrigues et al. (2008) in *E. huxleyi*, and suggested by Ries et al. (2009) in coralline red and calcareous green algae. Although other experimental results have suggested that photosynthesis by isolated in situ diatom symbionts is saturated at the inorganic carbon concentration of the present seawater (ter Kuile et al., 1989), further studies are needed to measure photosynthetic rates of algal symbionts at various pCO_2 levels.

In contrast to *B. sphaerulata* and *C. gaudichaudii*, calcification in *A. hemprichii* did not increase significantly at intermediate pCO_2 levels. A declining calcification trend of *A. hemprichii* at intermediate pCO_2 levels is possibly attributed to the decrease in carbonate concentration. Culture experiments using ^{14}C tracer techniques showed that uptake of dissolved inorganic carbon into the shell of *A. hemprichii* increased linearly as a function of the carbonate concentration (ter Kuile et al., 1989). Therefore, for this species, seawater carbonate chemistry may largely influence calcification, compared with CO_2 fertilization effects discussed above.

Calcification in all three species studied tended to be reduced at the highest pCO_2 level (970 µatm). These results suggest that pCO_2 levels of around 1000 µatm and higher (pH of 7.7 on the seawater scale) are a limiting factor for CO_2 fertilization effects for the two hyaline species. Le Cadre et al. (2003) showed that in low pH seawater, pseudopodial emission of a hyaline species (*Ammonia*) was reduced or stopped, implying a disturbance of the metabolic activity of the foraminifer. Further studies are needed to investigate metabolic activity in relation to chamber formation (calcification) of symbiont-bearing foraminifers in high pCO_2 seawater.

Although not all observations were statistically significant, calcification tended to decrease from pCO_2 of 260 to 360 µatm in the three species examined. Culture experiments using ^{14}C tracer techniques (ter Kuile et al., 1989) have demonstrated that in perforate species (*A. lobifera*) the optimum pH for calcification is between pH 8.2 and 8.9 (NBS scale), but in an imperforate species (*A. hemprichii*), they found no optimum pH for calcification, which increased very steeply above pH 8.4 (NBS scale). These results indicate that reef foraminifers with both hyaline and porcelaneous shells can calcify more effectively in seawater at a pH higher than the present level.

Our findings suggest that ongoing ocean acidification favor symbiont-bearing reef foraminifers with hyaline shells at intermediate pCO_2 levels (580 to 770 µatm) but be unfavorable to those with either hyaline or porcelaneous shells at higher pCO_2 levels. In future, relatively high calcification rates of symbiont-bearing hyaline foraminifers but low calcification rates of porcelaneous ones compared with previous rates may be attributed to ocean acidification. These contrasting differences in calcification rates may lead to the

dominance of hyaline foraminifers over porcelaneous ones in symbiont-bearing reef foraminiferal communities.

This possibility also explains the dominance of large benthic foraminifers with hyaline shells during the warm period of the Cenozoic. Atmospheric CO_2 concentrations were more than 1000 ppm for the Paleocene and early Eocene, then declined into the middle Eocene (Pearson and Palmer, 2000; Demicco et al., 2003). Although large benthic foraminifers diversified following the terminal Cretaceous extinctions, a reduction of the genetic diversity and a simultaneous diversification at species level and a considerable increase of their shell size and adult dimorphism, which are referred to as a larger foraminiferal turnover, appears to have been coeval with the Late Paleocene Thermal Maximum (Orue-Etxebarria et al., 2001), when atmospheric CO_2 exceeded over 2000 ppm (Pearson and Palmer, 2000; Demicco et al., 2003). During the middle Eocene, when atmospheric CO_2 levels were similar to or higher than present (Pearson and Palmer, 1999), large benthic foraminifers with hyaline shells such as *Nummulites* extremely diversified and dominated tropical, shallow-water carbonate environments (Hallock et al., 1991). Their modern descendants host diatom symbionts in tropical shelf environments (Lee, 1998). Thus, diversification and predominance of hyaline, large benthic foraminifers during the middle Eocene may partly be explained by relatively high pCO_2, which induced CO_2 fertilization effects by algal symbionts.

References

Barker, S. and Elderfield, H.: Foraminiferal calcification response to glacial-interglacial changes in atmospheric CO_2, Science, 297, 833–836, doi:10.1126/science.1072815, 2002.

Bentov, S., Brownlee, C., and Erez, J.: The role of seawater endocytosis in the biomineralization process in calcareous foraminifera, P. Natl. Acad. Sci. USA, 106, 21500–21504, doi:10.1073/pnas.0906636106, 2009.

Bijma, J., Spero, H., and Lea, D.: Reassessing foraminiferal stable isotope geochemistry: impact of the oceanic carbonate system (experimental results), in: Use of Proxies in Paleoceanography: Examples from the South Atlantic, edited by: Fischer, G. and Wefer, G., Springer-Verlag, Berlin, Heidelberg, Germany, 489–512, 1999.

Bijma, J., Honisch, B., and Zeebe, R.: Impact of the ocean carbonate chemistry on living foraminiferal shell weight: comment on "Carbonate ion concentration in glacial-age deepwaters of the Caribbean Sea", edited by: W. S. Broecker and E. Clark, Geochem. Geophys. Geosyst., 3, 1064, 2002.

de Moel, H., Ganssen, G. M., Peeters, F. J. C., Jung, S. J. A., Kroon, D., Brummer, G. J. A., and Zeebe, R. E.: Planktic foraminiferal shell thinning in the Arabian Sea due to anthropogenic ocean acidification?, Biogeosciences, 6, 1917–1925, doi:10.5194/bg-6-1917-2009, 2009.

de Nooijer, L. J., Toyofuku, T., and Kitazato, H.: Foraminifera promote calcification by elevating their intracellular pH, P. Natl. Acad. Sci. USA, 106, 15374–15378, doi:10.1073/pnas.0904306106, 2009.

de Vargas, C., Bonzon, M., Rees, N. W., Pawlowski, J., and Zaninetti, L.: A molecular approach to biodiversity and biogeography in the planktonic foraminifer *Globigerinella siphonifera* (d'Orbigny), Mar. Micropaleontol., 45, 101–116, doi:10.1016/s0377-8398(02)00037-3, 2002.

Dissard, D., Nehrke, G., Reichart, G. J., and Bijma, J.: Impact of seawater pCO_2 on calcification and Mg/Ca and Sr/Ca ratios in benthic foraminifera calcite: results from culturing experiments with *Ammonia tepida*, Biogeosciences, 7, 81–93, doi:10.5194/bg-7-81-2010, 2010.

Demicco, R. V., Lowenstein, T. K., and Hardie, L. A.: Atmospheric pCO_2 since 60 Ma from records of seawater pH, calcium, and primary carbonate mineralogy, Geology, 31, 793–796, doi:10.1130/g19727.1, 2003.

Erez, J.: The source of ions for biomineralization in foraminifera and their implications for paleoceanographic proxies, Rev. Mineral. Geochem., 54, 115–149, doi:10.2113/0540115, 2003.

Fabry, V. J.: Marine calcifiers in a high-CO_2 ocean, Science, 320, 1020–1022, doi:10.1126/science.1157130, 2008.

Fujita, K. and Fujimura, H.: Organic and inorganic carbon production by algal symbiont-bearing foraminifera on northwest pacific coral-reef flats, J. Foramin. Res., 38, 117–126, doi:10.2113/gsjfr.38.2.117, 2008.

Gattuso, J. P., Frankignoulle, M., Bourge, I., Romaine, S., and Buddemeier, R. W.: Effect of calcium carbonate saturation of seawater on coral calcification, Global Planet. Change, 18, 37–46, 1998.

Hallock, P.: Production of carbonate sediments by selected large benthic foraminifera on two Pacific coral reefs, J. Sediment. Petrol., 51, 467–474, 1981.

Hallock, P.: Symbiont-bearing foraminifera, in: Modern Foraminifera, edited by: Sen Gupta, B. K., Kluwer Academic Publishers, Dordrecht, Netherlands, 123–139, 1999.

Hallock, P.: Symbiont-bearing foraminifera: harbingers of global change?, Micropaleontology, 46, 95–104, 2000.

Hallock, P., Forward, L. B., and Hansen, H. J.: Influence of environment on the test shape of *Amphistegina*, J. Foramin. Res., 16, 224–231, 1986.

Hallock, P., Premoli Silva, I., and Boersma, A.: Similarities between planktonic and larger foraminiferal evolutionary trends through Paleogene paleoceanographic changes, Palaeogeogr., Palaeoclimatol., Palaeoecol., 83, 49–64, 1991.

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., Harvell, C. D., Sale, P. F., Edwards, A. J., Caldeira, K., Knowlton, N., Eakin, C. M., Iglesias-Prieto,

R., Muthiga, N., Bradbury, R. H., Dubi, A., and Hatziolos, M. E.: Coral reefs under rapid climate change and ocean acidification, Science, 318, 1737–1742, doi:10.1126/science.1152509, 2007.

Hohenegger, J.: Distribution of living larger foraminifera NW of Sesoko-jima, Okinawa, Japan, P.S.Z.N. I: Mar. Ecol., 15, 291–334, 1994.

Hohenegger, J.: The importance of symbiont-bearing benthic foraminifera for West Pacific carbonate beach environments, Mar. Micropaleontol., 61, 4–39, 2006.

Iglesias-Rodriguez, M. D., Halloran, P. R., Rickaby, R. E. M., Hall, I. R., Colmenero-Hidalgo, E., Gittins, J. R., Green, D. R. H., Tyrrell, T., Gibbs, S. J., von Dassow, P., Rehm, E., Armbrust, E. V., and Boessenkool, K. P.: Phytoplankton calcification in a high-CO_2 world, Science, 320, 336–340, doi:10.1126/science.1154122, 2008.

Kleypas, J. A., Buddemeier, R. W., Archer, D., Gattuso, J. P., Langdon, C., and Opdyke, B. N.: Geochemical consequences of increased atmospheric carbon dioxide on coral reefs, Science, 284, 118–120, 1999.

Kuffner, I. B., Andersson, A. J., Jokiel, P. L., Rodgers, K. S., and Mackenzie, F. T.: Decreased abundance of crustose coralline algae due to ocean acidification, Nat. Geosci., 1, 114–117, doi:10.1038/ngeo100, 2008.

Kuroyanagi, A., Kawahata, H., Suzuki, A., Fujita, K., and Irie, T.: Impacts of ocean acidification on large benthic foraminifers: results from laboratory experiments, Mar. Micropaleontol., 73, 190–195, doi:10.1016/j.marmicro.2009.09.003, 2009.

Langer, G., Nehrke, G., Probert, I., Ly, J., and Ziveri, P.: Strain-specific responses of *Emiliania huxleyi* to changing seawater carbonate chemistry, Biogeosciences, 6, 2637–2646, doi:10.5194/bg-6-2637-2009, 2009.

Langer, M. R., Silk, M. T., and Lipps, J. H.: Global ocean carbonate and carbon dioxide production: the role of reef foraminifera, J. Foramin. Res., 27, 271–277, 1997.

Le Cadre, V., Debenay, J. P., and Lesourd, M.: Low pH effects on *Ammonia beccarii* test deformation: implications for using test deformations as a pollution indicator, J. Foramin. Res., 33, 1–9, 2003.

Lee, J. J.: "Living sands"—larger foraminifera and their endosymbiotic algae, Symbiosis, 25, 71–100, 1998.

Lee, J. J., Sang, K., ter Kuile, B., Strauss, E., Lee, P. J., and Faber, W. W.: Nutritional and related experiments on laboratory maintenance of three species of symbiont-bearing, large foraminifera, Mar. Biol., 109, 417–425, doi:10.1007/bf01313507, 1991.

Lombard, F., da Rocha, R. E., Bijma, J., and Gattuso, J.-P.: Effect of carbonate ion concentration and irradiance on calcification in planktonic foraminifera, Biogeosciences, 7, 247–255, doi:10.5194/bg-7-247-2010, 2010.

Meehl, G. A., Stocker, T. F., Collins, W. D., Friedlingstein, P., Gaye, A. T., Gregory, J. M., Kitoh, A., Knutti, R., Murphy, J. M., Noda, A., Raper, S. C. B., Watterson, I. G., Weaver, A. J., and Zhao, Z.-C.: Global climate projections, in: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, edited by: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., and Miller, H. L., Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 747–845, 2007.

Morse, J. W., Andersson, A. J., and Mackenzie, F. T.: Initial responses of carbonate-rich shelf sediments to rising atmospheric pCO_2 and "ocean acidification": Role of high Mg-calcites, Geochim. Cosmochim. Acta, 70, 5814–5830, doi:10.1016/j.gca.2006.08.017, 2006.

Moy, A. D., Howard, W. R., Bray, S. G., and Trull, T. W.: Reduced calcification in modern Southern Ocean planktonic foraminifera, Nat. Geosci., 2, 276–280, doi:10.1038/Ngeo460, 2009.

Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R. M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R. G., Plattner, G.-K., Rodgers, K. B., Sabine, C. L., Sarmiento, J. L., Schlitzer, R., Slater, R. D., Totterdell, I. J., Weirig, M.-F., Yamanaka, Y., and Yool, A.: Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms, Nature, 437, 681–686, 2005.

Orue-Etxebarria, X., Pujalte, V., Bernaola, G., Apellaniz, E., Baceta, J. I., Payros, A., Nuñez-Betelu, K., Serra-Kiel, J., and Tosquella, J.: Did the Late Paleocene thermal maximum affect the evolution of larger foraminifers? Evidence from calcareous plankton of the Campo Section (Pyrenees, Spain), Mar. Micropaleontol., 41, 45–71, doi:10.1016/s0377-8398(00)00052-9, 2001.

Pearson, P. N. and Palmer, M. R.: Middle Eocene seawater pH and atmospheric carbon dioxide concentrations, Science, 284, 1824–1826, doi:10.1126/science.284.5421.1824, 1999.

Pearson, P. N. and Palmer, M. R.: Atmospheric carbon dioxide concentrations over the past 60 million years, Nature, 406, 695–699, 2000.

Raja, R., Saraswati, P. K., Rogers, K., and Iwao, K.: Magnesium and strontium compositions of recent symbiont-bearing benthic foraminifera, Mar. Micropaleontol., 58, 31–44, 2005.

Ridgwell, A., Schmidt, D. N., Turley, C., Brownlee, C., Maldonado, M. T., Tortell, P., and Young, J. R.: From laboratory manipulations to Earth system models: scaling calcification impacts of ocean acidification, Biogeosciences, 6, 2611–2623, doi:10.5194/bg-6-2611-2009, 2009.

Ries, J. B., Cohen, A. L., and McCorkle, D. C.: Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification, Geology, 37, 1131–1134, doi:1131-1134.10.1130/g30210a.1, 2009.

Röttger, R. and Krüger, R.: Observations on the biology of Calcarinidae (Foraminiferida), Mar. Biol., 106, 419–425, 1990.

Sabine, C. L., Feely, R. A., Gruber, N., Key, R. M., Lee, K., Bullister, J. L., Wanninkhof, R., Wong, C. S., Wallace, D. W. R., Tilbrook, B., Millero, F. J., Peng, T.-H., Kozyr, A., Ono, T., and Rios, A. F.: The Oceanic sink for anthropogenic CO_2, Science, 305, 367–371, doi:10.1126/science.1097403, 2004.

Sakai, K. and Nishihira, M.: Population study of the benthic foraminifer *Baculogypsina sphaerulata* on the Okinawan reef flat and preliminary estimation of its annual production, in: Proceedings of the 4th International Coral Reef Symposium, volume 2, Manila, 18–22 May 1981, 763–766, 1981.

Saraswati, P. K., Seto, K., and Nomura, R.: Oxygen and carbon isotopic variation in co-existing larger foraminifera from a reef flat at Akajima, Okinawa, Japan, Mar. Micropaleontol., 50, 339–349, doi:10.1016/S0377-8398(03)00099-9, 2004.

Suzuki, A.: Seawater CO_2 system and its transformation caused by photosynthesis and calcification in coral reefs. – theory and measurements of reef metabolisms –, Bull. Geol. Surv. Japan, 45, 573–623, 1994.

ter Kuile, B., Erez, J., and Padan, E.: Mechanisms for the uptake of inorganic carbon by two species of symbiont-bearing foraminifera, Mar. Biol., 103, 241–251, 1989.