Marine Biology Research, 2005; 1: 68–78

ORIGINAL ARTICLE

The chemical microenvironment of the symbiotic planktonic foraminifer *Orbulina universa*

STEPHANIE KÖHLER-RINK[1] & MICHAEL KÜHL[2]

[1]*Microsensor Research Group, Max-Planck-Institute for Marine Microbiology, Bremen, Germany,* [2]*Marine Biological Laboratory, Institute of Biology, University of Copenhagen, Helsingør, Denmark*

Abstract

Microsensor measurements of CO_2, O_2, pH and Ca^{2+} in the vicinity of the symbiont-bearing planktonic foraminifer *Orbulina universa* showed major light-modulated changes in the chemical microenvironment due to symbiont photosynthesis, respiration of the holobiont, and precipitation of the calcite shell. Under saturating light conditions, microprofiles measured towards the shell surface showed an O_2 increase of up to 220% air saturation, a decrease in CO_2 concentration to 4.9 μM, and a pH increase to 8.8 due to symbiont photosynthesis. The Ca^{2+} concentration decreased to ~9.6 mM in two specimens, while it increased to 10.2–10.8 mM in three other specimens kept in light. In darkness, the respiration of the community decreased the O_2 concentration to 82% of air saturation, CO_2 increased up to 15 μM, the pH decreased to 8.0, and the Ca^{2+} concentration increased up to 10.4 mM. These data, and derived calculations of the distribution of HCO_3^- and CO_3^{2-} near the shell, showed that the carbonate system in the vicinity of *O. universa* was significantly different from conditions in the surrounding seawater, both in light and darkness, due to the metabolism of the foraminifer and its associated algae. Experimental light–dark cycles indicated a sufficient CO_2 supply sustaining high carbon fixation rates of the symbiotic algae via conversion of HCO_3^- or via CO_2 release from calcification and host respiration. Our findings on irradiance-dependent CO_2 and pH changes in the vicinity of symbiont-bearing planktonic foraminifera give direct experimental evidence for the predictions of isotope fractionation models used in palaeoclimatology stating that metabolic processes affect the isotopic carbon signal ($\delta^{13}C$) in foraminifera.

Key words: *Calcification, carbon isotope fractionation, carbonate system, CO_2, O_2, pH, and Ca^{2+} microenvironment, DBL, inorganic carbon sources*

Introduction

Symbiont-bearing planktonic foraminifera are widespread calcifying protozoa harbouring phototrophic microalgae (e.g. Lee et al. 1965; Bé 1977; Murray 1991; Hemleben & Spindler 1983). They are abundant in the euphotic zone of subtropical and tropical oceans, with highest densities of 10–100 individuals m^{-3} found at 10–50 m depths (Bradshaw 1959; Bé 1977). Planktonic foraminifera show diurnal and ontogenetic vertical migration patterns in the water column, and sink into deeper waters during reproduction (Spindler et al. 1978; Hemleben & Bijma 1994). The spinose foraminifer *Orbulina universa* is a ubiquitous species in subtropical and tropical waters and hosts high numbers of the endosymbiotic dinoflagellate *Gymnodinium béii* (Spero 1987). During daytime, the symbionts are light exposed within the host cytoplasm, which is stretched out along the calcite spines. High photosynthetic activities have been measured (Jørgensen et al. 1985; Rink et al. 1998) and symbiont-bearing planktonic foraminifera can be described as "hot spots" of primary productivity in oligotrophic seas (Spero & Parker 1985; Caron et al. 1995). However, the source of inorganic carbon sustaining symbiont photosynthesis has not been identified. The mechanisms involved in inorganic carbon uptake from the ambient seawater into the foraminiferal cytoplasm and the subsequent carbon fixation remain unknown.

The current knowledge of $CaCO_3$ precipitation mechanisms in symbiont-bearing planktonic forami-

Correspondence: S. Köhler-Rink, Microsensor Research Group, Max-Planck-Institute for Marine Microbiology, Celsiusstr. 1, D-28359 Bremen, Germany. E-mail: srink@mpi-bremen.de

Published in collaboration with the University of Bergen and the Institute of Marine Research, Norway, and the Marine Biological Laboratory, University of Copenhagen, Denmark

(Accepted 16 December 2004; Printed 30 March 2005)

ISSN 1745-1000 print/ISSN 1745-1019 online
DOI: 10.1080/17451000510019015

nifera is limited. In planktonic foraminifera, the nucleation of $CaCO_3$ crystals is facilitated by the existence of surfaces where calcite is deposited upon an organic matrix. The tertiary structure of this so-called primary organic lining is involved in the initiation and direction of calcification (e.g. Hemleben et al. 1977, 1986; Anderson & Bé 1978; Weiner & Erez 1984). The uptake and concentrating mechanisms of calcium and carbonate are energy dependent, and it has been suggested that the ions are stored in an inorganic carbon pool within the foraminiferal cytoplasm (ter Kuile et al. 1989a). Inorganic carbon is initially taken up in the form of bicarbonate (HCO_3^-), which is concentrated as carbonate (CO_3^{2-}) in special vesicles (Anderson & Faber 1984; ter Kuile & Erez 1988b; ter Kuile et al. 1989a; Erez et al. 1994).

Lea et al. (1995) postulated that symbiont photosynthesis can stimulate calcification in planktonic foraminifera. They measured up to three-fold higher calcification rates in *O. universa* under high light conditions as compared with individuals grown in the dark. Inhibition of symbiont photosynthesis with the inhibitor DCMU resulted in smaller shell growth rates of the foraminifer *Globigerinoides sacculifer* (Bé et al. 1982). However, the significance of symbiotic algae in the regulation of calcification and the potential influence of other physiological and biochemical processes await further studies.

Previous microsensor studies (Jørgensen et al. 1985; Rink et al. 1998) investigated the physicochemical microenvironment of symbiont-bearing planktonic foraminifera and demonstrated that photosynthesis and respiration caused significant changes in O_2 and pH levels in the vicinity of *O. universa* and *Globigerinoides sacculifer*. Based on these measurements, a diffusion-reaction model of the seawater chemistry in proximity to the shell of planktonic foraminifera was developed (Wolf-Gladrow et al. 1999) that simulates pH and concentration profiles of the carbonate species HCO_3^-, CO_3^{2-} and CO_2 during light and dark situations.

In micropalaeontological studies, stable isotope signatures of fossil foraminiferal shells are used to estimate the physical and chemical conditions of the water mass, wherein the foraminifera precipitated their shell carbonate. Based on the $^{18}O/^{16}O$ and $^{13}C/^{12}C$ isotope ratios conserved in the calcite shells, palaeoclimatic events such as changes in oceanic productivity and temperature changes can be reconstructed (Epstein et al. 1953; Duplessy 1978; Berger et al. 1981; Erez & Luz 1983; Spero & Deniro 1987; Wefer et al. 1999). This technique is based on the assumption that shell calcium carbonate is deposited in equilibrium with the ambient seawater (Emiliani 1954; Anderson & Arthur 1983). However, experimental measurements in benthic and planktonic foraminifera contradict this assumption (Erez 1978; Kahn 1979; Honjo & Erez 1981; Kahn & Williams 1981; Spero & Deniro 1987; Köhler-Rink & Kühl 2001) and it has been suggested that metabolic processes, so-called vital effects, change the isotopic composition of inorganic carbon ($\delta^{13}C$) during calcite precipitation in foraminifera (Erez & Honjo 1981; Spero & Williams 1988).

In this study, we present the first direct measurements of the CO_2 and Ca^{2+} microenvironment of symbiotic planktonic foraminifera. Combined measurements of CO_2, O_2, pH, and Ca^{2+} within the symbiont swarm demonstrated a dynamic response of the community to changing light conditions and a close interaction of photosynthesis, respiration, and calcification. We discuss the importance of foraminiferal metabolism on the chemical microenvironment, the isotopic carbon fractionation, and $CaCO_3$ precipitation.

Material and methods

Experimental organisms and sampling site

Adult specimens of *O. universa* with spherical shell diameters of 570–1000 μm were collected from surface waters near Curaçao, Netherlands Antilles in the Caribbean Sea during 3 weeks in March and April 1999. A Scuba diver captured individual organisms in glass jars at 5–10 m depths in the morning hours (08.00–10.00 h) (see also Rink et al. 1998). Individual specimens were maintained in glass jars with filtered seawater at room temperature (26°C). Measurements were performed on the day of sampling, starting around 11.00 h. Measured *O. universa* were in the pre-gametogenic state (Spero 1988) with symbiotic algae attached to the calcite spines. The number of investigated specimens was restricted to one to three per day due to the time-consuming microsensor preparations and measurements.

At the sampling site, surface waters were characterized by calcium supersaturation and high temperatures up to 28°C. In situ salinity was S = 38 and pH was 8.2. Experiments were conducted in the laboratory of the Caribbean Marine Research Station (CARMABI, Netherlands Institute of Sea Research).

Experimental set-up

Freshly collected foraminifera were placed on a Nylon mesh in a measuring chamber with filtered seawater, as described by Rink et al. (1998). The chamber was mounted in a water bath to reduce

temperature changes. Irradiance (0–1000 μmol photons m^{-2} s^{-1}) was provided by a fiber optic halogen lamp (Schott KL-1500, Germany) and was varied by neutral density filters (Oriel Inc., USA) inserted in the light path. Quantum scalar irradiance in the set-up was calibrated with a quantum scalar irradiance meter (QSL 101, Biospherical Instruments Inc., USA) at the position of the foraminifer. Experimental light–dark cycles were controlled by an electromechanical shutter (Vincent Assoc., USA) installed between the halogen lamp and the measuring chamber. Signal changes during light–dark cycles were recorded at a time resolution of 1 s. Microsensors were fixed to and positioned with a motorized micromanipulator (Märtzhäuser and LOT-ORIEL, Germany). The position of the microsensor tips relative to the foraminiferal shell surface was observed through a dissection microscope (Zeiss, SV 11, Germany). A custom-made program written in LabVIEW (National Instruments, USA) controlled the micromanipulator, shutter, and data acquisition.

Radial microprofiles were measured with a spatial resolution of 50–100 μm from the ambient seawater towards the shell surface of *O. universa*. For simultaneous measurements of two chemical compounds within the symbiont swarm, the two microsensors were placed in close proximity (<100 μm) at the shell surface of the foraminifer during experimental light–dark cycles. The size and position of the symbiont swarm was determined by the graded ocular scale of the dissection microscope. The foraminifera were allowed to acclimate to chamber conditions for 0.5–1 h prior to the experiments.

Microsensors

Microsensors for O_2, CO_2, pH, and Ca^{2+} were used. Clark-type O_2 microsensors (Revsbech 1989) were constructed with an outer tip diameter of ~7 μm, a t_{90} response time of 0.3 s, and a stirring sensitivity <1%. A linear calibration was performed from readings in aerated and N_2-flushed seawater, respectively. The O_2 detection limit was ~0.1 μM. CO_2 microsensors with a detection limit down to 1 μM and a response time of ~10 s were composed of an outer glass casing with an internal pH liquid ion-exchange (LIX) microelectrode (de Beer et al. 1997). The CO_2 microsensor was calibrated in a degassed phosphate buffer (50 mM, pH 8.0) by adding aliquot volumes of 200 mM carbonate solution. pH and Ca^{2+} were measured with LIX microelectrodes with tip diameters of 2–5 μm and a response time of <1 s (de Beer et al. 2000). The LIX microelectrodes were shielded with an outer casing containing 3 M KCl to reduce electrical noise (Kühl & Revsbech 2001). The detection limit for Ca^{2+} was 10 μM, and calibration was performed in 1, 10, and 20 mM Ca^{2+} solutions with added background ions (Mg^{2+}, Na^+, and K^+) at seawater concentration. pH microelectrodes were calibrated in standard pH buffers (Mettler Toledo, Germany).

O_2 signals were measured with a fast responding custom-made picoammeter. pH, CO_2, and Ca^{2+} were measured with high impedance mV meters (Mascom, Germany; Keithley, USA). Signals were recorded with a strip chart recorder (GOERZ, Austria) and a custom-built computerized data acquisition system (National Instruments, USA).

Net O_2, CO_2, and Ca^{2+} fluxes

The area-integrated fluxes, Q_t, of the measured solutes were calculated (in units of nmol h^{-1} $foraminifer^{-1}$) from the radial concentration gradient dC/dr, the surface area of the sphere $4\pi r^2$, and the molecular diffusion coefficient (D_0) (Jørgensen et al. 1985; Ploug et al. 1997; Rink et al. 1998). The CO_2 and O_2 gradients started outside the symbiont swarm towards the ambient seawater. Ca^{2+} uptake rates were calculated from gradients directly at the shell surface.

$$Q_t = 4\pi r^2 D_0 \frac{dC}{dr} \quad (1)$$

D_0 was 2.30×10^{-5} cm^2 s^{-1} for O_2, 1.83×10^{-5} cm^2 s^{-1} for CO_2, and 0.79×10^{-5} cm^2 s^{-1} for Ca^{2+} (25°C; S = 35) according to Broecker & Peng (1974) and Li & Gregory (1974).

Results

Microenvironment of CO_2, O_2, pH, and Ca^{2+}

The chemical microenvironment was influenced by the presence of a diffusive boundary layer (DBL) surrounding the shell of *O. universa* impeding the solute exchange between the ambient seawater and the foraminifer. The effective DBL thickness was 250–800 μm, as determined by the extrapolation of radial concentration gradients at the shell–seawater interface to the ambient seawater concentration (Jørgensen & Revsbech 1985; Ploug et al. 1997; Rink et al. 1998) (Figures 1 and 2). In the light, O_2 and pH increased and CO_2 decreased towards the shell surface of *O. universa* due to the photosynthesis by symbiotic microalgae (Figure 1A). The O_2 concentration and pH increased to 394 μM (218% air saturation) and pH 8.8, respectively, while the CO_2 concentration decreased to 4.9 μM at the shell surface. In the dark, the respiration of the holobiont caused O_2 and pH to decrease to 147 μM (82% air

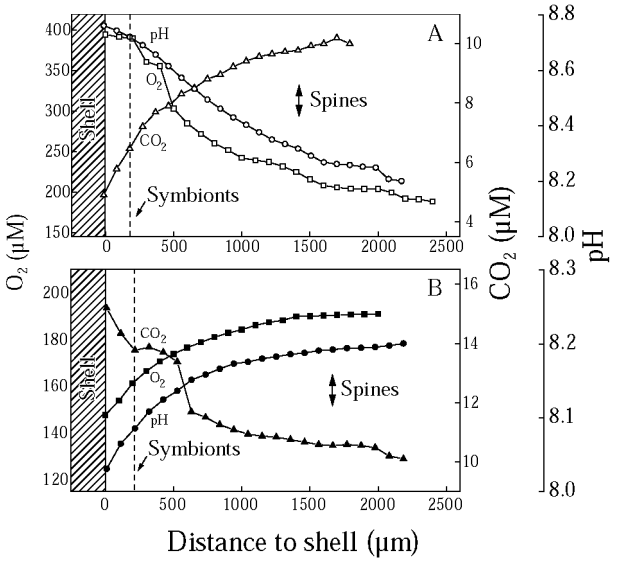

Figure 1. Light (A) and dark (B) concentration profiles of O_2, CO_2, and pH measured from the ambient seawater towards the shell surface of a single *Orbulina universa* specimen (scalar irradiance = 130 µmol photons m^{-2} s^{-1}). The vertical dashed line indicates the start of the symbiont swarm. The arrows indicate the outer extension of the calcite spines.

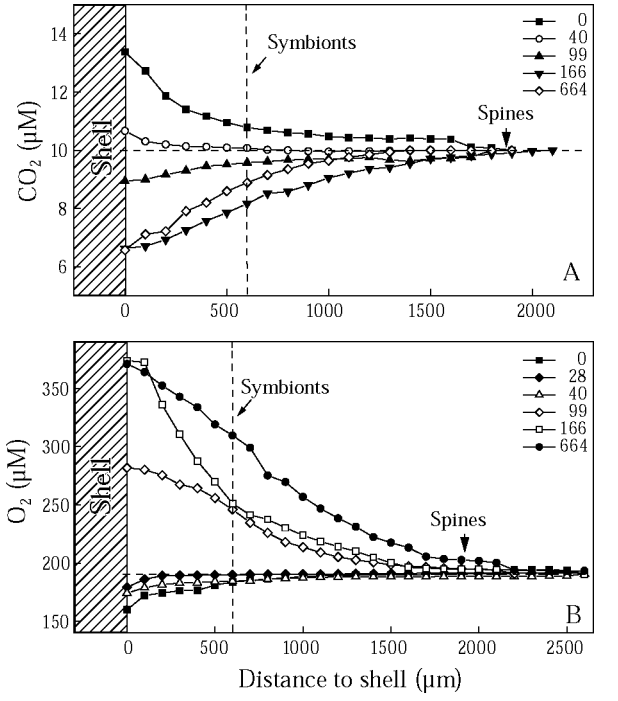

Figure 2. Radial concentration profiles of CO_2 (A) and O_2 (B) measured towards the shell surface of a single *Orbulina universa* specimen as a function of increasing irradiance. The numbers indicate incident irradiance in µmol photons m^{-2} s^{-1}. The horizontal dashed lines indicate ambient seawater concentrations. The vertical dashed lines indicate the start of the symbiont swarm. The arrows indicate the outer extension of the calcite spines.

saturation) and pH 8.03, respectively, while the CO_2 concentration increased up to 15 µM due to respiration and calcification (Figure 1B). Ambient seawater concentrations of O_2, CO_2, and pH were 190 µM, 10 µM, and pH 8.2, respectively. With increasing irradiance, the CO_2 concentration in the vicinity of the shell surface decreased, whereas the O_2 concentration increased (Figure 2). At an irradiance of 166 µmol photons m^{-2} s^{-1}, symbiont photosynthesis approached its saturation level, as indicated by both CO_2 and O_2 profiles (Figure 2).

Microprofiles of Ca^{2+} measured under light and dark conditions showed a significant concentration change between the ambient seawater and the shell surface of *O. universa* (Figure 3). In some specimens we measured a Ca^{2+} decrease towards the shell surface and thus a Ca^{2+} uptake of the foraminifer in the light (Figure 3A). Here the Ca^{2+} concentration at the shell surface decreased to 9.6 mM, but other specimens showed a Ca^{2+} concentration increase in the light of up to 10.8 mM (Figure 3A, B). All dark profiles showed a concentration increase of Ca^{2+} at the shell surface.

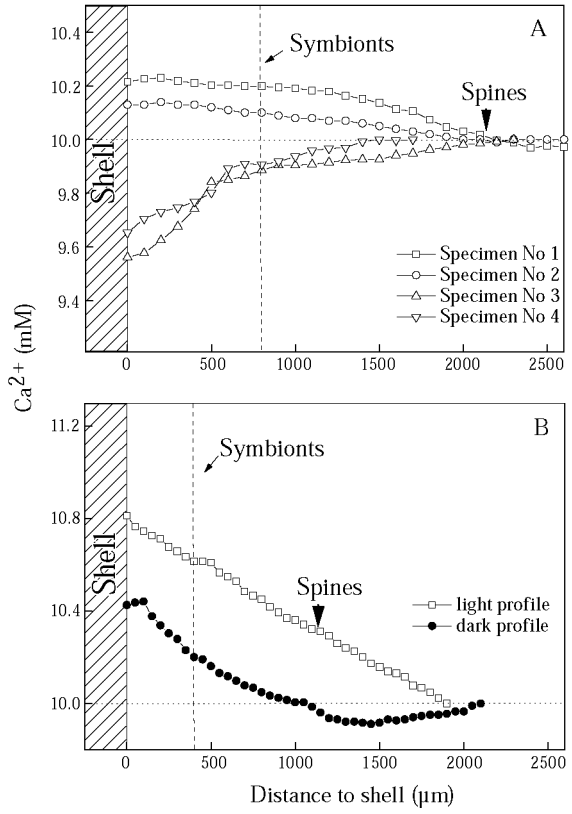

Figure 3. (A) Concentration profiles of Ca^{2+} measured in the light (specimen no 1+2: 560 µmol photons m^{-2} s^{-1}, specimen no 3+4: 260 µmol photons m^{-2} s^{-1}) towards the shell surface of four different specimens of *Orbulina universa*. (B) Light (600 µmol photons m^{-2} s^{-1}) and dark Ca^{2+} profiles measured in the same specimen. The horizontal dotted lines indicate ambient seawater concentrations. The vertical dashed lines indicate the start of the symbiont swarm. The arrows indicate the outer extension of the calcite spines.

Net O_2 and CO_2 fluxes

Rates of total net O_2 release and CO_2 uptake were calculated from the radial concentration gradients measured from the ambient seawater towards the shell surface as a function of irradiance (Figure 2). The photosynthetic light compensation point of the symbiont–host association, i.e. the irradiance where autotrophic and heterotrophic processes balanced each other and no net O_2 or CO_2 exchange was observed, E_c, was reached at ~40 µmol photons m^{-2} s^{-1} (Figure 4). Above 40 µmol photons m^{-2} s^{-1}, a net O_2 release and a net CO_2 uptake due to photosynthesis were measured. At all irradiance levels, the concentration gradients showed a much higher flux of O_2 as compared with CO_2. At high irradiance (664 µmol photons m^{-2} s^{-1}), net O_2 production was 15.6 nmol O_2 h^{-1} foraminifer^{-1} and net CO_2 uptake was 0.15 nmol O_2 h^{-1} foraminifer^{-1}. Thus, a high molar O_2/CO_2 conversion ratio of about 100 was calculated.

Dynamic microenvironmental changes at the shell surface

We measured pronounced variations in CO_2, O_2, and pH at the shell surface of *O. universa* during experimental light–dark cycles (Figures 5 and 6). In the light, symbiont photosynthesis rapidly increased the O_2 and pH concentrations and decreased the CO_2 concentration at the shell surface. When the light was switched off, the respiration of the association resulted in increasing CO_2 levels, and rapidly decreasing levels of O_2 and pH.

The total concentration change at the shell surface between light saturation and darkness was ~22 µM CO_2 and 339 µM O_2. pH varied about 1.2 units. The concentration changes generally increased with increasing irradiance (Figure 6). The O_2 responded immediately to changing light conditions. pH changed with a short delay, whereas the CO_2 response

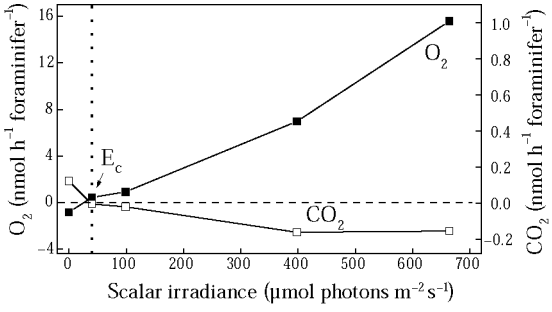

Figure 4. Area-integrated fluxes of O_2 and CO_2 (nmol h^{-1} foraminifer^{-1}) calculated from concentration gradients at the shell surface of *Orbulina universa* measured at increasing scalar irradiance (Figure 2). Note the different scales for O_2 and CO_2 fluxes. The vertical dotted line indicates the compensation irradiance, E_c, of ~40 µmol photons m^{-2} s^{-1}.

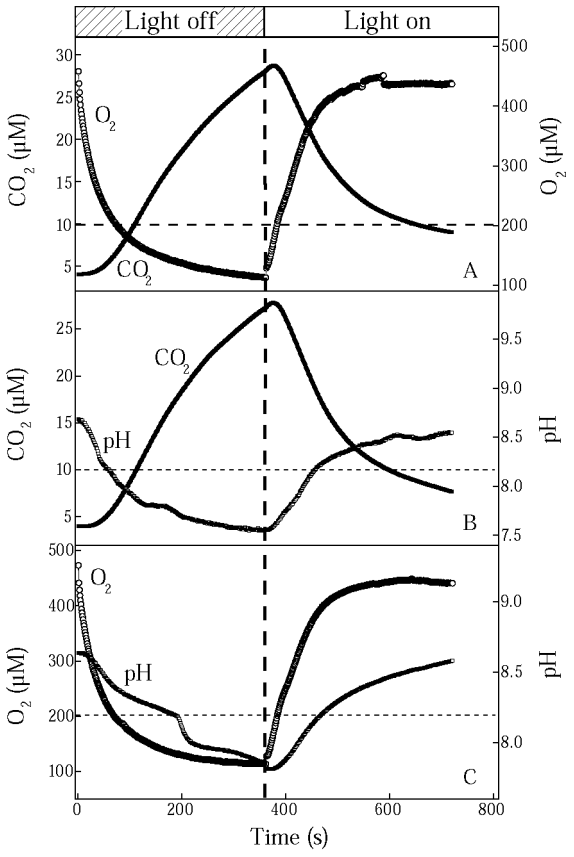

Figure 5. Concentrations of CO_2 and O_2 (A), CO_2 and pH (B), and O_2 and pH (C) measured during experimental light–dark cycles at the shell surface within the symbiont swarm of *Orbulina universa*. The vertical lines indicate the change from dark to light conditions (730 µmol photons m^{-2} s^{-1}). The horizontal lines indicate ambient seawater concentrations.

showed a delay of 17–100 s after the light–dark switch, i.e. significantly longer than the response time of the CO_2 microsensors. The CO_2 delay decreased from 50 s at 99 µmol photons m^{-2} s^{-1} to 35 s at 365 µmol photons m^{-2} s^{-1} (Figure 6).

Discussion

Irradiance effects on the chemical microenvironment

The presented data demonstrate the combined effect of symbiont photosynthesis, community respiration, and host calcification on the CO_2, O_2, pH, and Ca^{2+} levels around the shell of *O. universa*. Our measurements showed a strong influence of the prevailing light conditions on the chemical microenvironment (Figures 1–3). The dynamic CO_2 uptake rates of symbiont photosynthesis with increasing irradiance demonstrated that the CO_2 concentration changes determine the pH surrounding the foraminifer (Figures 1 and 5).

Planktonic symbiont-bearing foraminifera experience different irradiance regimes during their

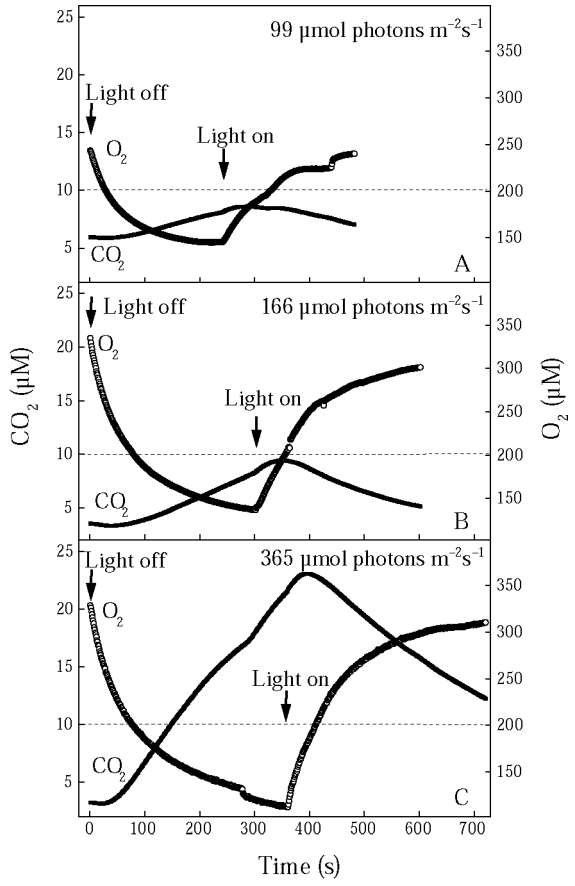

Figure 6. Dynamics of CO_2 and O_2 measured simultaneously during experimental light–dark cycles at the shell surface of *Orbulina universa* as a function of increasing irradiance [99 (A), 166 (B), and 365 (C) µmol photons m^{-2} s^{-1}]. The arrows indicate the change from dark to light conditions. The horizontal lines indicate ambient seawater concentrations.

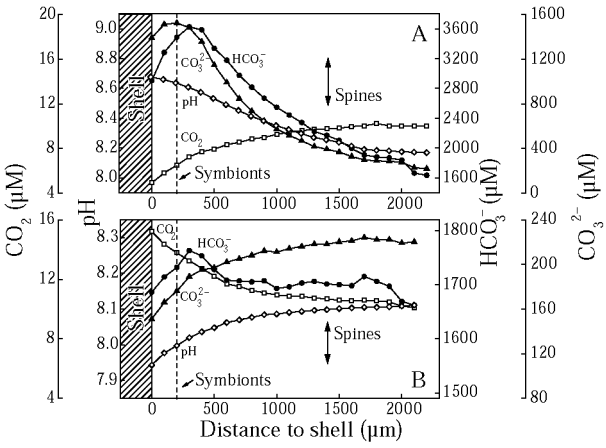

Figure 7. Calculated HCO_3^- and CO_3^{2-} profiles and measured CO_2 and pH profiles under light (A) and dark (B) conditions in *Orbulina universa*. Note the different scales in (A) and (B). The vertical dashed line indicates the start of the symbiont swarm. The arrows indicate the outer extension of the calcite spines.

lifecycle as they migrate vertically in the water column (Bradshaw 1959; Boltovsky 1973). Such migration patterns affect symbiont photosynthesis and, consequently, the local characteristics of the carbonate system due to changing microenvironmental conditions, as indicated by our results. In addition, foraminifera are exposed to diurnal variations in irradiance causing a continuously changing chemical microenvironment over a daily light cycle. Our experimental light–dark cycles do not reflect the effects of slowy varying light conditions, but demonstrate the solute dynamics due to short-term manipulations of the light field.

To evaluate local carbonate system changes in the vicinity of *O. universa* under light and dark conditions, we used the freely available program CO2SYS (v. 1.05) by E. Lewis and D. W. R. Wallace (http://cdiac.esd.ornl.gov/oceans/co2rprt.html). The program requires two CO_2 system parameters to determine the other carbonate system parameters. Assuming steady-state conditions, we calculated bicarbonate and carbonate profiles under light and dark conditions using our measured pH and CO_2 profiles (Figures 1 and 7). The model calculations included carbonate system parameters according to Mehrbach et al. (1973), Dickson & Millero (1987), and Dickson (1990) on the NBS pH scale. The program predicted unrealistic high concentrations of HCO_3^- and CO_3^{2-} in light, which have not been described in the literature so far. Our calculations thus demonstrated that the carbonate system close to the foraminiferal shell cannot be assumed to be in equilibrium and also indicated that light-enhanced calcification is caused by the CO_2 uptake of endosymbiotic algae.

The calculated HCO_3^- and CO_3^{2-} profiles showed a significant concentration increase towards the shell due to the increased pH by photosynthetic CO_2 fixation (Figure 7A). In the darkness, less pronounced gradients of HCO_3^- and CO_3^{2-} were predicted. A small HCO_3^- and CO_3^{2-} decrease towards the shell surface was calculated due to the measured pH decrease. This drop in pH could be explained by respiratory CO_2 release and/or HCO_3^- and CO_3^{2-} uptake due to calcification. The relative proportions of CO_2, HCO_3^-, and CO_3^{2-} in the vicinity of *O. universa* are thus regulated by the combined action of metabolic processes, the equilibrium reactions and the diffusional exchange of the carbonate species between the foraminiferal shell and the ambient seawater (Spero 1991; de Beer et al. 1997; Zeebe & Wolf-Gladrow 2001).

The irradiance-dependent variations in O_2, CO_2, and pH in the vicinity of *O. universa* give direct experimental evidence for the suggestion that symbiont photosynthesis and community respiration can influence the isotopic carbon composition of the

shell calcite in symbiont-bearing planktonic foraminifera (Spero & Deniro 1987; Spero & Williams 1988; Spero 1991). In laboratory culture experiments, Spero & Deniro (1987) showed that $\delta^{13}C$ values of *O. universa* ranged from $+2.77‰$ at highest irradiance to $+0.45‰$ in darkness. Several models were presented to elucidate these so-called "vital effects" on the inorganic carbon signal due to the metabolic processes of symbiont-bearing foraminifera (Spero 1991; Hemleben & Bijma 1994; Zeebe et al. 1999). Zeebe et al. (1999) published a numerical model that calculates the $\delta^{13}C$ values of *O. universa* in response to the metabolic processes of the foraminifer and its symbionts. The model predicted significant changes in the seawater $\delta^{13}C$ close to the shell surface due to symbiont photosynthesis and holobiont respiration. Generally, a $\delta^{13}C$ increase in all inorganic carbon species towards the shell surface under light conditions and depletion of $\delta^{13}C$ in the dark was predicted. Our experimental data support these predictions for *O. universa*.

Our study demonstrated that *O. universa* is surrounded by a layer of lower CO_2 concentration as compared with the ambient seawater during daytime when the symbiotic algae spread out along the calcite spines (Figure 1, 2A). The preferential use of $^{12}CO_2$ in photosynthetic carbon fixation (e.g. Wong & Sackett 1978; Spero & Deniro 1987; Goericke et al. 1994) means, however, that close to the shell there is a layer depleted in ^{12}C and enriched in ^{13}C relative to the ambient seawater (Zeebe et al. 1999). The light-dependent photosynthetic activity of the symbiotic algae (Figure 2A) will thus influence the $^{13}CO_2/^{12}CO_2$ ratio close to the shell surface and create a gradient in the $\delta^{13}C$ (Zeebe et al. 1999). Although photosynthetic CO_2 uptake is the process dominating the CO_2 variations close to the shell in the light (Figure 2A), concentration changes of inorganic carbon species affecting the inorganic carbon pools and the exchange with the ambient seawater may also influence the $^{13}CO_2/^{12}CO_2$ ratio (O'Leary 1981; Swart 1983; Zeebe et al. 1999).

In darkness, when the symbionts stay within the foraminiferal calcite chambers, *O. universa* is enclosed in a layer of higher CO_2 concentration as compared with the ambient seawater (Figures 1B, 2A). This layer would be depleted in ^{13}C relative to the ambient seawater because the foraminifer and its endosymbionts release respiratory CO_2 depleted in ^{13}C (DeNiro & Epstein 1978; Spero & Williams 1988). However, the respiratory effects on carbon fractionation in symbiont-bearing foraminifera might be compensated by the ^{13}C enrichment due to symbiont photosynthesis at irradiances above the light compensation point (>50 µmol photons m^{-2} s^{-1}), when there is a net CO_2 uptake (Rink et al. 1998). Reduced calcification rates of *O. universa* under dark conditions (Lea et al. 1995) indicate that incorporation of depleted ^{13}C during the night can play a minor role in total ^{13}C incorporation. In conclusion, the prevailing light conditions during pre-gametogenic calcification might strongly affect the isotopic ratio of the shell calcite in *O. universa*. We only present data on foraminifera in the pre-gametogenic state, but we speculate that similar microenvironmental controls can affect the isotopic ratio during gametogenic calcification.

Calcium microenvironment of planktonic foraminifera

The measured Ca^{2+} profiles describe the Ca^{2+} microenvironment of *O. universa* over a short time period during the growth of the adult spherical chamber. Our data indicate that Ca^{2+} uptake does not occur continuously (Figure 3) and that Ca^{2+} uptake can vary among individual specimens according to their specific status of calcification within their growth cycle. The Ca^{2+} microenvironment in symbiont-bearing foraminifera might also be affected by the daytime (Anderson & Faber 1984) and the feeding frequency (Caron et al. 1987). In addition, prevailing light conditions (Spero & Deniro 1987; Lea et al. 1995) and the ontogenetic state of the organism (Spero 1986; Hemleben & Bijma 1994) could play an important role. Thus, future studies should focus on Ca^{2+} measurements in specimens at a defined growth stage to get additional information about growth-specific Ca^{2+} dynamics.

Solute exchange processes between the ambient seawater, the intracellular Ca^{2+} pool (Anderson & Farber 1984; ter Kuile et al. 1989a) and the site of calcification affect the Ca^{2+} microenvironment. Diffusional transport of Ca^{2+} ions to the site of calcification was described for perforate and imperforate foraminifera (ter Kuile & Erez 1988b; ter Kuile et al. 1989a; Lea et al. 1995). An enzyme-mediated active Ca^{2+} transport was found in imperforate species (ter Kuile et al. 1989a).

The DBL surrounding the shell of *O. universa* is regulating the exchange of Ca^{2+} ions between the foraminifer and the surrounding seawater (Jørgensen et al. 1985; Rink et al. 1998). The mass transfer of solutes in the surrounding seawater and in between the network of spines covered by foraminiferal cytoplasm is thus fundamentally different. The calcite spines and the attached cytoplasm limit the local flow of seawater and the DBL impedes the transport of Ca^{2+} and CO_3^{2-} ions (Wieland et al. 2001; Ploug et al. 2002). It is possible to estimate the mean diffusion time of Ca^{2+} ions across the DBL (Jørgensen & Revsbech 1985; Mann & Lazier 1991). By using the diffusion coefficient (D) for

Ca^{2+} ions in seawater ($0.79*10^{-5}$ cm^2 s^{-1} at 25°C) (Li & Gregory 1974) and assuming an average thickness of the DBL of 400 μm (z), we calculated a diffusion time of $t = z^2/(0.9*D) \approx 3.5$ min. The transport of ions through a thinner DBL, caused e.g. by faster settling rates to the sea floor or by smaller foraminiferal sizes, would be much faster as the diffusion time is proportional to the square of the diffusion path length.

From the Ca^{2+} gradients showing net calcium uptake, we estimated total Ca^{2+} uptake rates in *O. universa* of about 1.4 nmol Ca^{2+} h^{-1} foraminifer^{-1}, assuming radial symmetry of the solute distribution. This calculated uptake rate is comparable with the calcification rates measured by Lea et al. (1995), who found an average Ca^{2+} uptake of 1.8 nmol Ca^{2+} h^{-1} in *O. universa* using a stable isotope technique that allowed the precise determination of $^{48}Ca/^{44}Ca$ ratios in single shells.

All Ca^{2+} profiles measured in darkness showed a Ca^{2+} increase towards the shell. In addition, during experimental light–dark cycles, we locally measured a concentration decrease of Ca^{2+} at the shell surface under light conditions and a Ca^{2+} increase in darkness relative to the ambient seawater level (data not shown). One explanation for the release of Ca^{2+} under dark conditions could be that elevated CO_2 concentrations and conditions of low pH create unfavourable conditions for the initiation of calcite precipitation. We measured a pH decrease to pH 7.5–7.9 and a CO_2 increase to 15–29 μM at the shell surface at a temperature of 26°C (S = 38). Respiratory CO_2 release by microbial metabolic activity is the dominating process controlling the calcium carbonate dissolution kinetics in deep-sea surface sediments (Archer et al. 1989; Schneider et al. 2000). In addition, reduced calcification rates at elevated CO_2 concentrations were found in coccolithophorids (Riebesell et al. 2000), in coralline macroalgae (Langdon et al. 2003), as well as in hermatypic corals (Marubini et al. 2001). Similar light-dependent Ca^{2+} and pH changes were measured with microsensors at the shell surface of a symbiont-bearing benthic foraminifer (Köhler-Rink & Kühl 2000) and in hermatypic corals (de Beer et al. 2000; Al-Horani et al. 2003). However, the mechanisms behind the calcium dynamics in foraminifera and especially the apparent high calcium release in darkness remain obscure and need further investigation. Recently, the application of microsensors and a range of specific inhibitors in a study of coral calcification pointed to a central role of host Ca-ATPase in the regulation of the calcium dynamics (Al-Horani et al. 2003), and similar studies on foraminifera now seem a natural next step.

Inorganic carbon sources for symbiont photosynthesis and calcification

Oceanic surface waters represent a large reservoir for dissolved inorganic carbon (DIC). It has been shown that DIC concentrations of seawater can saturate the photosynthesis of symbionts in benthic foraminifera and hermatypic corals (ter Kuile et al. 1989b; Goiran et al. 1996). In planktonic foraminifera, mechanisms of inorganic carbon fixation and DIC supply to the symbiotic algae have not yet been studied.

Inorganic carbon for photosynthetic assimilation and calcite precipitation is supplied from the large seawater reservoir via diffusion across the DBL. CO_2 can also be supplied from internal sources due to respiration and calcification. In benthic symbiotic foraminifera it was suggested that diffusion is the rate-limiting step for DIC uptake at lower DIC levels (ter Kuile et al. 1989a). Saturation of DIC uptake at higher DIC levels indicated the presence of a rate-limiting enzymatic step. The highest DIC uptake rates were found at pH 8–9 and the authors therefore suggested that HCO_3^- is the main inorganic carbon species taken up from the environment, which is then undergoing intracellular conversion to CO_2.

Our data show that CO_2 is not fully depleted at the shell surface of *O. universa*, even under saturating irradiance levels (Figures 1, 2, 5 and 6). This indicates that the symbionts are not CO_2 limited. Changing light conditions from light to darkness and vice versa showed a time lag in the CO_2 concentration changes measured at the shell surface (Figures 5 and 6). These changes were apparently not tightly coupled to the fast O_2 concentration changes due to photosynthesis. Furthermore, much higher total rates of O_2 release than CO_2 uptake per individual foraminifer were measured under saturating irradiance. The estimated molar O_2/CO_2 conversion ratio per foraminifer of ~100 at higher irradiance (660 μmol photons m^{-2} s^{-1}) showed that the CO_2 flux from the ambient seawater is by far not sufficient to support high rates of photosynthetic O_2 evolution. We therefore speculate that an efficient internal CO_2 supply mechanism must exist (Rink et al. 1998).

One possibility for a sufficient internal CO_2 supply, besides respiration and calcification, could be an enzymatic dehydration of HCO_3^- to CO_2. The symbionts or the host may actively regulate the CO_2 uptake via the enzyme carbonic anhydrase (CA) (e.g. Tsuzuki & Miyachi 1989; Raven 1994; Nimer et al. 1999). This would require an active uptake of HCO_3^- by the symbiont–host association. The fast pH increase of ~0.65 units within 100 s in

the light suggests the presence of an active HCO_3^- uptake mechanism, which is accompanied by an OH^- efflux or H^+ influx into the cytoplasm of the foraminifer (Badger 1987). Such co-transport of ions during the active transport of HCO_3^- has been described for calcifying algae (Borowitzka 1989) and for symbiont-bearing anthozoa (Allemand et al. 1998).

Conclusion

A close interaction of symbiont photosynthesis, community respiration, and calcification affects the microenvironment of *O. universa* during experimental light–dark cycles. A time delay in CO_2 changes and an immediate pH change during experimental light–dark cycles indicate an internal CO_2 supply sustaining the high photosynthetic activity of the symbionts. Conversion of HCO_3^- to CO_2 via CA seems an important internal CO_2 source for photosynthetic assimilation within planktonic foraminifera, in addition to the CO_2 supply from host respiration and calcification. Under saturating light conditions, the measured concentration profiles of CO_2, O_2, and pH indicate a microenvironment of decreased inorganic carbon due to symbiont photosynthesis in the vicinity of the foraminiferal shell. In darkness, CO_2 released by respiration and calcification influences the inter-conversion of inorganic carbon species in the vicinity of *O. universa*. The concentration dynamics of CO_2 and Ca^{2+} under light and dark conditions can thus change the calcite saturation state close to the shell. The local pH rise in the vicinity of planktonic foraminifera due to photosynthetic CO_2 uptake and the subsequent rise in CO_3^{2-} increases the degree of $CaCO_3$ saturation and can thereby enhance the calcium precipitation at the calcification site of *O. universa*.

The present study strongly suggests that the $^{13}CO_2/^{12}CO_2$ ratio ($\delta^{13}C$) in close proximity to the shell of *O. universa* is affected by (i) the preferential use of $^{12}CO_2$ for carbon fixation by symbiont photosynthesis and (ii) the enhanced ^{13}C depletion in respiratory CO_2 by community respiration. Metabolic processes change the CO_2 microenvironment and thus have an influence on the isotopic signature of the shell calcite in symbiont-bearing foraminifera.

Acknowledgements

We thank the staff of the Caribbean Marine Research Station (CARMABI) for providing laboratory facilities, and especially Brian Leysner for his help and support. Gaby Eickert is acknowledged for excellent technical assistance. We thank Hans-Peter Grossart for diving assistance and sampling of foraminifera. Brad Bebout, Volker Meyer and Roland Thar are thanked for their development of the microsensor data acquisition software and hardware used in the experiments. This study was financed by the Max-Planck-Gesellschaft (Germany) (SKR) and the Danish Natural Science Research Council (MK). Christoph Hemleben and an anonymous reviewer are thanked for constructive criticism.

References

Al-Horani FA, Al-Moghrabi SM, De Beer D. 2003. The mechanism of calcification and its relation to photosynthesis and respiration in the scleractinian coral *Galaxea fascicularis*. Mar-
ine Biology 142:419–26.

Allemand D, Furla P, Bénazet-Tambutté S. 1998. Mechanisms of carbon acquisition for endosymbiont photosynthesis in Anthozoa. Canadian Journal of Botany 76:925–41.

Anderson OR, Bé AWH. 1978. Recent advances in foraminifera fine structural research. In: Hedley RH, Adams CG, editors. Foraminifera. Vol. 3. London: Academic Press. p 122–202.

Anderson OR, Faber WW. 1984. An estimation of calcium carbonate deposition rate in the planktonic foraminifera *Globigerinoides sacculifer* using ^{45}Ca as a tracer; a recommended procedure for improved accuracy. Journal of Foraminifera Research 14:303–8.

Anderson TF, Arthur MA. 1983. Stable isotopes of oxygen and carbon and their application to sedimentologic and paleoenvironmental problems. In: Arthur MA, Anderson TF, Kaplan IR, Veizer J, Land LS, editors. Stable Isotopes in Sedimentary Geology. Tulsa: SEPM Short Course No. 10. p 1–151.

Archer D, Emerson S, Reimer C. 1989. Dissolution of calcite in deep-sea sediments: pH and oxygen microelectrode results. Geochimica Cosmochimica Acta 53:2831–45.

Badger MR. 1987. The CO_2-concentrating mechanism in aquatic phototrophs. The Biochemistry of Plants: a Comprehensive Treatise. New York: Academic Press. p 219–74.

Bé AWH. 1977. An ecological, zoogeographic and taxonomic review of recent planktonic foraminifera. In: Romsey ATS, editor. Oceanic Micropaleontology. London: Academic Press. p 1–100.

Bé AWH, Spero HJ, Anderson OR. 1982. Effects of symbiont elimination and reinfection on the life processes of the planktonic foraminifer *Globigerinoides sacculifer* in laboratory culture. Journal of the Marine Biological Association of the UK 62:435–51.

Berger WH, Bé AWH, Vincent E. 1981. Oxygen and carbon isotopes in foraminifera. Paleography Paleoclimatology and Paleoecology 33:1–278.

Boltovskoy E. 1973. Daily vertical migration and absolute abundance of living planktonic foraminifera. Journal of Foraminifera Research 3:89–94.

Borowitzka MA. 1989. Carbonate calcification in algae – initiation and control. In: Mann S, Webb J, Williams RJP, editors. Biomineralization. New York: VCH Publishers. p 63–94.

Bradshaw JE. 1959. Ecology of living planktonic foraminifera in the north and equatorial pacific ocean. The Cushman Foundation for Foraminiferal Research 10:25–64.

Broecker WS, Peng TH. 1974. Gas exchange rates between air and sea. Tellus 26:21–35.

Caron DA, Faber WW, Bé AWH. 1987. Growth of the spinose planktonic foraminifer *Orbulina universa* in laboratory culture and the effect of temperature on life processes. Journal of the Marine Biological Association of the UK 67:343–58.

Caron DA, Michaels AF, Swanberg NR, Howse FA. 1995. Primary productivity by symbiont-bearing planktonic sarcodines (Acantharia, Radiolaria, Foraminifera) in surface waters near Bermuda. Journal of Foraminifera Research 17:103–29.

De Beer D, Glud A, Epping E, Kühl M. 1997. A fast responding CO_2 microelectrode for profiling in sediments, microbial mats and biofilms. Limnology and Oceanography 42:1590–600.

De Beer D, Kühl M, Stambler N, Vaki L. 2000. A microsensor study of light enhanced Ca^{2+} uptake and photosynthesis in the reef-building hermatypic coral *Favia sp*. Marine Ecology Progress Series 194:75–85.

DeNiro MJ, Epstein S. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica Cosmochimica Acta 42:495–506.

Dickson AG. 1990. Standard potential of the reaction $AgCl(s) + 1/2H_2(g) = Ag(s) + HCl(aq)$ and the standard acidity constant of the ion HSO_4^- in synthetic sea-water from 273.15°K to 318.15°K. Journal of Chemical Thermodynamics 22:113–27.

Dickson AG, Millero FJ. 1987. A comparison of the equilibrium-constants for the dissociation of carbonic-acid in seawater media. Deep-Sea Research 34:1733–43.

Duplessy JC. 1978. Isotope studies. In: Gribbin J, editor. Climatic Change. Cambridge: Cambridge University Press. p 46–67.

Emiliani C. 1954. Depth habitats of some species of pelagic Foraminifera as indicated by oxygen isotope ratios. American Journal of Science 252:149–58.

Epstein S, Buchbaum R, Lowenstam HA, Urey HC. 1953. Revised carbonate–water isotopic temperature scale. Geological Society of America Bulletin 64:1315.

Erez J. 1978. Vital effect on stable-isotope composition seen in foraminifera and coral skeletons. Nature 273:199–202.

Erez J, Benthov S, Tishler C, Szafranek D. 1994. Intracellular calcium storage and the calcification mechanism of perforate foraminifera. Paleobios 16:30.

Erez J, Honjo S. 1981. Comparison of isotopic composition of planktonic foraminifera in plankton tows, sediment traps and sediments. Paleogeography Paleoclimatology and Paleoecology 33:129–56.

Erez J, Luz B. 1983. Experimental paleotemperature equation for planktonic foraminifera. Geochimica Cosmochimica Acta 47:1025–31.

Goericke R, Montoya JP, Fry B. 1994. Physiology of isotopic fractionation in algae and cyanobacteria. In: Lajtha K, Michener RH, editors. Stable Isotopes in Ecology and Environmental Science. Oxford: Blackwell Scientific. p 197–221.

Goiran C, Al-Moghrabi S, Allemand D, Jaubert J. 1996. Inorganic carbon uptake for photosynthesis by the symbiotic coral/dinoflagellate association. I. Photosynthetic performance of symbionts and dependence on sea water bicarbonate. Journal of Experimental Marine Biology and Ecology 199:207–25.

Hemleben Ch, Anderson OR, Berthold W, Spindler M. 1986. Calcification and chamber formation in foraminifera – a brief overview. In: Leadbeater BSC, Riding R, editors. Biomineralization in Lower Plants and Animals. Syst Ass Spec (30). London: The Systematics Association. p 237–49.

Hemleben Ch, Bé AWH, Anderson OR, Tuntivate-Choy S. 1977. Test morphology, organic layers and chamber formation of the planktonic foraminifer *Globorotalia menardii* (d Órbigny). Journal of Foraminifera Research 7:1–25.

Hemleben Ch, Bijma J. 1994. Foraminiferal population dynamics and stable carbon isotopes. In: Zahn R et al., editors. Carbon Cycling in the Glacial Ocean: Constraints on the Oceans Role in Global Change. NATO ASI Series G, Vol. I (17). Berlin: Springer.

Hemleben Ch, Spindler M. 1983. Recent advances in research on living planktonic foraminifera. Utrecht Micropaleontology Bulletin 30:141–70.

Honjo S, Erez J. 1981. Dissolution rates of calcium carbonate in the deep ocean: an in situ experiment in the North Atlantic. Earth Planet Science Letters 40:287–300.

Jørgensen BB, Revsbech NP. 1985. Diffusive boundary layers and the oxygen uptake of sediments and detritus. Limnology and Oceanography 30:111–22.

Jørgensen BB, Erez J, Revsbech NP, Cohen Y. 1985. Symbiotic photosynthesis in a planktonic foraminiferan, *Globigerinoides sacculifer* (Brady), studied with microelectrodes. Limnology and Oceanography 30:1253–67.

Kahn MI. 1979. Non-equilibrium oxygen and carbon isotope fractionation in tests of living planktonic foraminifera. Oceanologica Acta 2:195–208.

Kahn MI, Williams DF. 1981. Oxygen and carbon isotopic composition of living planktonic foraminifera from the northeast Pacific Ocean. Paleogeography Paleoclimatology and Paleoecology 33:47–76.

Köhler-Rink S, Kühl M. 2000. Microsensor studies of photosynthesis and respiration in larger foraminifera. I. The physicochemical microenvironment of *Marginopora vertebralis*, *Amphistegina lobifera*, and *Amphisorus hemprichii*. Marine Biology 137:473–86.

Köhler-Rink S, Kühl M. 2001. Microsensor studies of photosynthesis and respiration in the larger foraminifera *Amphistegina lobifera* and *Amphisorus hemprichii*. Ophelia 55:111–22.

Kühl M, Revsbech NP. 2001. Biogeochemical microsensors for boundary layer studies. In: Boudreau BP, Jørgensen BB, editors. The Benthic Boundary Layer. Oxford: Oxford University Press. p 180–210.

Langdon C, Broecker WS, Hammond DE, Glenn E, Fitzsimmons K, Nelson SG, Peng TH, Hajdas I, Bonani G. 2003. Effects of elevated CO_2 on the community metabolism of an experimental coral reef. Global Biogeochemical Cycles 17:1011.

Lea D, Martin P, Chan DA, Spero HJ. 1995. Calcium uptake and calcification rate in the planktonic foraminifer *Orbulina universa*. Journal of Foraminifera Research 25:14–23.

Lee JJ, Freudenthal HD, Kossoy V, Bè AWH. 1965. Cytological observations on two planktonic foraminifera, *Globigerina bulloides* (d'Orbigny, 1826) and *Globigerinoides ruber* (d'Orbigny, 1839) Cushman, 1972. Journal of Protozoology 12:531–42.

Li YH, Gregory S. 1974. Diffusion of ions in sea water and in deep-sea sediments. Geochimica Cosmochimica Acta 38:703–14.

Mann KH, Lazier JRN. 1991. Dynamics of Marine Ecosystems. Oxford: Blackwell Scientific.

Marubini F, Barnett H, Langdon C, Atkinson MJ. 2001. Dependence of calcification on light and carbonate ion concentration for the hermatypic coral *Porites compressa*. Marine Ecology Progress Series 220:153–62.

Mehrbach C, Culberso Ch, Hawley JE. 1973. Measurement of apparent dissociation-constants of carbonic-acid in seawater at atmospheric-pressure. Limnology and Oceanography 18:897–907.

Murray JW. 1991. Ecology and distribution of planktonic foraminifera. In: Lee JJ, Anderson R, editors. Biology of Foraminifera. London: Academic Press. p 255–84.

Nimer NA, Brownlee C, Merrett MJ. 1999. Extracellular carbonic anhydrase facilitates carbon dioxide availability for photosynthesis in the marine dinoflagellate *Prorocentrum micans*. Plant Physiology 120:105–11.

O'Leary MH. 1981. Carbon isotope fractionation in plants. Phytochemistry 20(4):553–67.

Ploug H, Hietanen S, Kuparinen J. 2002. Diffusion and advection within and around sinking, porous aggregates. Limnology and Oceanography 47:1129–36.

Ploug H, Kühl M, Buchholz B, Jørgensen BB. 1997. Anoxic aggregates – an ephemeral phenomenon in the pelagic environment. Aquatic Microbial Ecology 13:285–94.

Raven JA. 1994. Carbon fixation and carbon availability in marine phytoplankton. Photosynthesis Research 39:259–73.

Revsbech NP. 1989. An oxygen microelectrode with a guard cathode. Limnology and Oceanography 34:474–8.

Riebesell U, Zondervan I, Rost B, Tortell PD, Zeebe RE, Korel FMM. 2000. Reduced calcification of marine plankton in response to increased atmospheric CO_2. Nature 407:364–7.

Rink S, Kühl M, Bijma J, Spero HJ. 1998. Microsensor studies of photosynthesis and respiration in the symbiotic foraminifer *Orbulina universa*. Marine Biology 131:583–95.

Schneider RR, Schulz HD, Hensen C. 2000. Marine carbonates: their formation and destruction. In: Schulz HD, Zabel M, editors. Marine Geochemistry. Berlin: Springer.

Spero HJ. 1986. Symbiosis, chamber formation and stable isotope incorporation in the planktonic foraminifer, *Orbulina universa*. PhD dissertation, University of California at Santa Barbara.

Spero HJ. 1987. Symbiosis in the planktonic foraminifer *Orbulina universa* and the isolation of its symbiotic dinoflagellate *Gymnodinium béii* sp. nov. Journal of Phycology 23:307–17.

Spero HJ. 1988. Ultrastructural examination of chamber morphogenesis and biomineralization in the planktonic foraminifer *Orbulina universa*. Marine Biology 99:9–20.

Spero. 1991. Opening the carbon isotope "vital effect" black box, 2. Quantitative model for interpreting foraminiferal carbon isotope data. Paleoceanography 6(6):639–55.

Spero HJ, Deniro MJ. 1987. The influence of symbiont photosynthesis on the $\delta^{18}O$ values and $\delta^{13}C$ values of planktonic foraminiferal shell calcite. Symbiosis 4:213–28.

Spero HJ, Parker SL. 1985. Photosynthesis in the symbiotic planktonic foraminifer *Orbulina universa*, and its potential contribution to oceanic primary productivity. Journal of Foraminifera Research 15:273–81.

Spero HJ, Williams DF. 1988. Extracting environmental information from planktonic foraminiferal $\delta^{13}C$ data. Nature 335:717–9.

Spindler M, Anderson OR, Hemleben Ch, Bé AWH. 1978. Light and electron microscopic observations of gametogenesis in *Hastegerina pelagica* (Foraminifera). Journal of Protozoology 25:427–43.

Swart PK. 1983. Carbon and oxygen isotope fractionation in scleractinian corals: a review. Earth-Science Review 19:51–80.

Ter Kuile B, Erez J. 1988a. In situ growth rate experiments on the symbiont-bearing foraminifera *Amphistegina lobifera* and *Amphisorus hemprichii*. Journal of Foraminifera Research 14:262–76.

Ter Kuile B, Erez J. 1988b. The size and function of the internal inorganic carbon pool of the foraminifer *Amphistegina lobifera*. Marine Biology 99:481–8.

Ter Kuile B, Erez J, Padan E. 1989a. Mechanisms for the uptake of inorganic carbon by two species of symbiont-bearing foraminifera. Marine Biology 103:241–51.

Ter Kuile B, Erez J, Padan E. 1989b. Competition for inorganic carbon between photosynthesis and calcification in the symbiont-bearing foraminifer *Amphistegina lobifera*. Marine Biology 103:253–9.

Tsuzuki PD, Miyachi S. 1989. The function of carbonic anhydrase in aquatic photosynthesis. Aquatic Botany 34:85–104.

Wefer G, Berger WH, Bijma J, Fischer G. 1999. Clues to ocean history: a brief overview of proxies. In: Fischer G, Wefer G, editors. Use of Proxies in Paleoceanography: Examples from the South Atlantic. Berlin: Springer. p 1–68.

Weiner S, Erez J. 1984. Organic matrix of the shell of the foraminifer *Heterostegina depressa*. Journal of Foraminifera Research 14:206–12.

Wieland A, deBeer D, Damgard LR, Kühl M, Dusschoten D, Van As H. 2001. Fine-scale measurements of diffusivity in a microbial mat with nuclear magnetic resonance imaging Limnology and Oceanography 46:248–59.

Wolf-Gladrow DA, Bijma J, Zeebe RE. 1999. Model simulation of the carbonate system in the microenvironment of symbiont bearing foraminifera. Marine Chemistry 64:181–98.

Wong WW, Sackett WM. 1978. Fractionation of stable isotopes by marine phytoplankton. Geochimica Cosmochimica Acta 42:1809–15.

Zeebe RE, Bijma J, Wolf-Gladrow DA. 1999. A diffusion–reaction model of carbon isotope fractionation in foraminifera. Marine Chemistry 64:199–227.

Zeebe RE, Wolf-Gladrow DA. 2001. CO_2 in Seawater: Equilibrium, Kinetics, Isotopes. Elsevier Oceanography Series, Vol. 65. Amsterdam: Elsevier.

Editorial responsibility: Tom Fenchel