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S. Köhler-Rink · M. Kühl

Microsensor studies of photosynthesis and respiration in larger symbiotic foraminifera. I The physico-chemical microenvironment of *Marginopora vertebralis, Amphistegina lobifera* and *Amphisorus hemprichii*

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Abstract The physico-chemical microenvironment of larger benthic foraminifera was studied with microsensors for O_2, CO_2, pH, Ca^{2+} and scalar irradiance. Under saturating light conditions, the photosynthetic activity of the endosymbiotic algae increased the O_2 up to 183% air saturation and a pH of up to 8.6 was measured at the foraminiferal shell surface. The photosynthetic CO_2 fixation decreased the CO_2 at the shell down to 4.7 μM. In the dark, the respiration of host and symbionts decreased the O_2 level to 91% air saturation and the CO_2 concentration reached up to 12 μM. pH was lowered relative to the ambient seawater pH of 8.2. The endosymbionts responded immediately to changing light conditions, resulting in dynamic changes of O_2, CO_2 and pH at the foraminiferal shell surface during experimentally imposed light–dark cycles. The dynamic concentration changes demonstrated for the first time a fast exchange of metabolic gases through the perforate, hyaline shell of *Amphistegina lobifera*. A diffusive boundary layer (DBL) limited the solute exchange between the foraminifera and the surrounding water. The DBL reached a thickness of 400–700 μm in stagnant water and was reduced to 100–300 μm under flow conditions. Gross photosynthesis rates were significantly higher under flow conditions (4.7 nmol O_2 cm^{-3} s^{-1}) than in stagnant water (1.6 nmol O_2 cm^{-3} s^{-1}), whereas net photosynthesis rates were unaffected by flow conditions. The Ca^{2+} microprofiles demonstrated a spatial variation in sites of calcium uptake over the foraminiferal shells. Ca^{2+} gradients at the shell surface showed total Ca^{2+} uptake rates of 0.6 to 4.2 nmol cm^{-2} h^{-1} in *A. lobifera* and 1.7 to 3.6 nmol cm^{-2} h^{-1} in *Marginopora vertebralis*. The scattering and reflection of the foraminiferal calcite shell increased the scalar irradiance at the surface up to 205% of the incident irradiance. Transmittance measurements across the calcite shell suggest that the symbionts are shielded from higher light levels, receiving approximately 30% of the incident light for photosynthesis.

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S. Köhler-Rink (✉)
Max-Planck-Institute for Marine Microbiology,
Microsensor Research Group, Celsiusstr. 1,
28359 Bremen, Germany
Fax: +49-421-2028690
e-mail: srink@mpi-bremen.de

M. Kühl
Marine Biological Laboratory, University of Copenhagen,
Strandpromenaden 5, 3000 Helsingør, Denmark

Introduction

Larger, symbiont-bearing foraminifera occur in shallow regions of tropical and subtropical seas, where they contribute significantly to primary production, respiration and carbonate budgets of benthic communities (Lee and Bock 1976; Sournia 1976; Hansen and Buchardt 1977; Röttger et al. 1980; ter Kuile and Erez 1984; Lee and Hallock 1987; Langer et al. 1997). In their natural habitat, larger foraminifera are exposed to different hydrodynamic regimes, ranging from almost stagnant conditions to wave action. They live epibenthic on various substrates, such as sediments, rock surfaces, coral rubble and macroalgae. Standing stocks of benthic foraminifera can reach up to several thousand specimens per 10 cm^2 (Murray 1991). The oligotrophic environment of tropical seas was probably a major driving force in the development of symbiosis in foraminifera (Hallock 1981; Leutenegger 1984), which allowed the evolution of these giant protists with shell sizes of >10 cm in diameter (Smith and Wiebe 1977; Koba 1978; Lee and Hallock 1987; Krüger et al. 1996/97). Microfossils of benthic foraminiferal $CaCO_3$ shells are important biotracers for stratigraphical and paleoecological research. Therefore, studies of the biology of recent foraminifera are important for the interpretation of fossil foraminiferal assemblages (Murray 1976; ter Kuile and Erez 1984).

Larger foraminifera can host many different types of microalgal symbionts, belonging to the Bacillariophy-

ceae, Dinophyceae, Chlorophyceae and Rhodophyceae. The formation of these associations is still poorly understood because they form strongly restrictive host–symbiont relationships (Lee et al. 1980). Endosymbiotic diatoms are, e.g., extremely rare in the foraminiferal feeding habitat (Lee et al. 1989). The imperforate soritids *Marginopora vertebralis* and *Amphisorus hemprichii* live in symbiosis with dinoflagellates belonging to the genera *Symbiodinium* and *Amphidinium* (Leutenegger 1977; Lee and Lawrence 1990; Lee et al. 1997). The perforate species *Amphistegina lobifera* hosts small pennate diatoms, e.g. *Nitzschia frustulum*, *Fragilaria shiloi* and *N. panduriformis*.

The transparency of the wall and the compressed test, with its high surface area to volume ratio, were suggested to provide a good morphological basis for this symbiosis (Hallock 1979). The endosymbionts live in high numbers (hundreds to thousands) in the chamber endoplasm and in the ectoplasm that is distributed near the test openings and in the canal system. The symbionts are concentrated immediately below the lateral shell walls, where they are exposed to optimal light conditions. It has been suggested that they are well supplied with gases, ions and nutrients from the ambient seawater (Hansen and Dalberg 1979; Leutenegger and Hansen 1979; ter Kuile et al. 1989a). In *Amphistegina lobifera* the shell pores are associated with pore cups, where the symbionts are concentrated (Hansen and Buchardt 1977; Lee and Anderson 1991).

The amphistegenids are a very abundant foraminiferal group in shallow waters of tropical and subtropical seas (Hansen and Buchardt 1977; Hohenegger 1994; Hohenegger et al. 1999). *Amphistegina* spp. were found on illuminated surfaces of algae, macrophytes and sediments to 40 m depth in the Gulf of Aquaba, with maximum densities down to 10 m (Hansen and Buchardt 1977). The growth and reproduction of *Amphistegina* spp. are dependent on incident light (Hallock 1981). Furthermore, light intensity and spectral composition are suggested to influence the depth-related distribution pattern of symbiont-bearing species (Leutenegger 1977b; Hansen and Buchardt 1977; Lee et al. 1980).

Despite their importance in subtropical and tropical benthic communities, neither the metabolic activity of larger foraminifera nor its regulation by environmental variables has been intensively studied. Rates of carbon fixation of *Amphistegina lobifera* and *Marginapora vertebralis* were measured by Muller (1978) and Smith and Wiebe (1977), respectively. The primary production and respiration of *A. lobifera* and *Amphisorus hemprichii* were investigated with a manometer system by Lee et al. (1980). Effects of light and food on the growth of *Amphistegina lessonii*, *Heterostegina depressa* and *Peneroplis planatus* were measured by Röttger et al. (1980) and Faber and Lee (1991). Different roles of feeding in the metabolism of *A. lobifera* and *A. hemprichii* have been studied by ter Kuile et al. (1987) with radioisotope tracers of C and P. Ter Kuile et al. (1989a, b) found a competition for inorganic carbon between photosynthesis and calcification in *A. lobifera* and described the mechanisms for inorganic carbon uptake in perforate and imperforate species.

Microsensors have been used previously to study symbiotic systems like the planktonic foraminifera *Globigerinoides sacculifer* and *Orbulina universa*, and the hermatypic corals *Favia* sp. and *Acropora* sp. (Jørgensen et al. 1985; Kühl et al. 1995; Rink et al. 1998). In the present study we characterized for the first time the physico-chemical microenvironment of benthic foraminifera (*Marginopora vertebralis*, *Amphistegina lobifera* and *Amphisorus hemprichii*) with O_2, CO_2, pH and Ca^{2+} microsensors and a scalar irradiance microprobe. We investigated the influence of irradiance and flow velocity on photosynthesis and respiration of the foraminiferal–algal association.

Materials and methods

Sample collection

Larger foraminifera (*Amphistegina lobifera* Larsen and *Amphisorus hemprichii* Ehrenberg) (Fig. 1) growing on small biofilm-coated stones were hand collected in June 1998 from a depth of ca. 5 m in the Gulf of Aquaba, Red Sea, by snorkeling. In situ salinity was 40‰ and water temperature was 22 °C at the sampling site. Within a few days, samples were transported on the natural substrate from the field to the laboratory in Bremen, Germany, where they were kept in an aquarium with aerated artificial seawater (hw sea salt *professional*, DIN EN 45001; 40‰, pH 8). Cultures were maintained at room temperature (20 to 22 °C) under a natural light–dark cycle with a maximal irradiance of ca. 400 µmol photons m^{-2} s^{-1}.

Specimens of *Marginopora vertebralis* Quoy and Gaimard (Fig. 1) were collected in December 1998 at low tide, from macroalgae (*Halimeda macroloba*, *Chnoospora implexa*) growing in shallow pools of a reef flat surrounding Heron Island, Great Barrier Reef, Queensland, Australia. The water had a temperature of 26 °C and a salinity of 36‰. Laboratory measurements were performed on the day of sampling at the Heron Island Research Station (University of Queensland).

Experimental setup

For the microsensor measurements, a single benthic foraminifer was placed on the bottom of a small flow chamber, constructed of Plexiglas (Fig. 2A). The water flow was maintained with a submersible aquarium pump (Askoll, Italy). Flow was adjusted by a tubing system in a glass aquarium. Experimental flow velocities (n = 10) were estimated by timing the lateral displacement of small, freely suspended particles under a dissection microscope. In the experiments with *Amphisorus hemprichii* (*Amphistegina lobifera*) high flow was 4.0 cm s^{-1} (1.5 cm s^{-1}) and moderate flow was 2.2 cm s^{-1} (0.6 cm s^{-1}) (see Fig. 3; Table 1). The flow chamber was illuminated with a fiber optic halogen lamp (Schott KL-1500, Germany) equipped with a collimating lens and a heat filter. Scalar irradiance was measured at the bottom of the flow chamber with a quantum scalar irradiance meter (Biospherical Instruments Inc., QSL 101, USA) equipped with a small diffusing sphere (1.3 cm diam.). The scalar irradiance (0 to 1000 µmol photons m^{-2} s^{-1}) in the setup was adjusted by inserting neutral density filters (Oriel Inc., USA) into the light path. All light measurements refer to visible light (400 to 700 nm), i.e. photosynthetically available

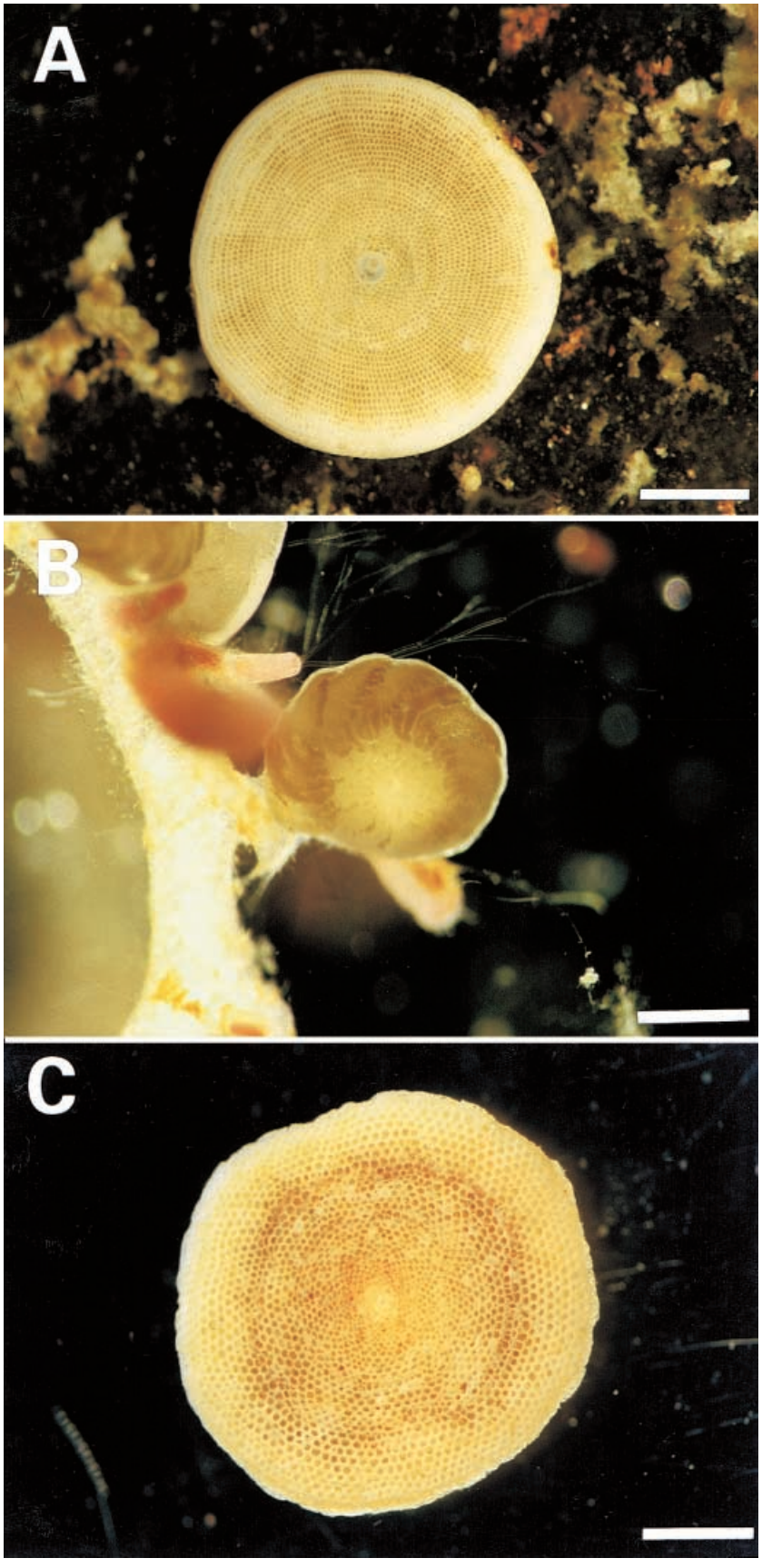


Fig. 1 Dorsal view of investigated foraminifera showing their yellow to brownish shells colored by dinoflagellate and diatom endosymbionts. Shell sizes of **A** *Marginopora vertebralis* with imperforate, disc-shaped test (diam. = 1.7–3.4 mm); **B** *Amphistegina lobifera* with thick-shelled, low-trochospiral test (diam. = 1.5–3.5 mm); **C** *Amphisorus hemprichii* with porcelaneous discoidal test (diam. = 3–5 mm) (scale bars = 1mm)

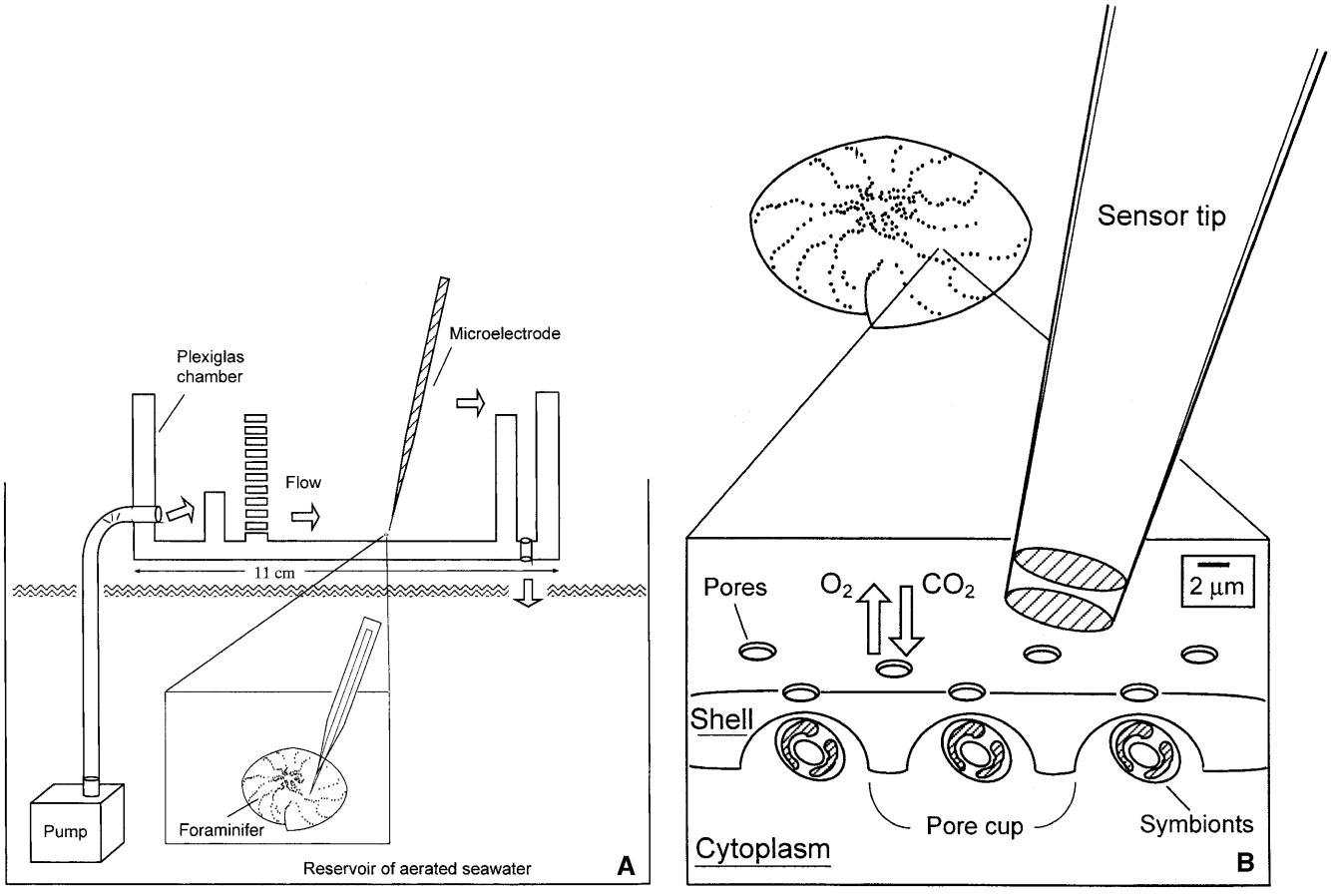


Fig. 2 **A** Schematic drawing of the experimental flow chamber. Seawater was pumped into the chamber and a lateral flow was created above the foraminifer. **B** Schematic drawing of a microsensor tip above the shell of *Amphistegina lobifera*. Influx and efflux of metabolic gases (O_2, CO_2) occurs through the perforate shell. Symbionts are associated with pore cups (Hansen and Buchardt 1977; Lee and Anderson 1991)

radiation (PAR). For photosynthesis experiments darkening was regulated by an electro-mechanical shutter (Vincent Association, USA), installed in the light path of the halogen lamp. The microsensors were mounted on a motorized micromanipulator (Märtzhäuser & LOT-ORIEL, Germany). The shutter control, data acquisition and the microsensor positioning were regulated by a custom-made, data-acquisition software programmed in LabVIEW (National Instruments, USA). Positioning of the microsensor tip relative to the foraminiferal shell surface was adjusted under a dissection microscope (Fig. 2B). Measurements were performed at ambient room temperature (26 and 20 °C, Australia and Bremen, respectively) under defined light conditions. The foraminifera were allowed to adapt to the flow chamber conditions for 0.5 to 1.0 h prior to the experiments.

Oxygen microsensors

Photosynthetic rates at the shell surface of the benthic foraminifera and O_2 profiles from the shell to the surrounding seawater were measured by Clark-type O_2 microsensors equipped with a guard cathode (Revsbech 1989) and connected to a picoammeter and a strip chart recorder (Servogor 124, Goerz, Austria). The microelectrodes had an outer tip diameter of 5 to 10 µm, a 90% response time of < 0.6 s, and a stirring sensitivity $< 1\%$. A linear calibration of the electrode signal was done at experimental temperatures in air-saturated seawater and in O_2-free seawater, degassed with N_2.

pH LIX microelectrodes

pH profiles and dynamics were measured with pH liquid ion exchange (LIX) microelectrodes (Lee and de Beer 1995; de Beer et al. 1997) in combination with a calomel reference electrode (Radiometer 401, Denmark). Both were connected to a high-impedance mV meter (Mascom, Germany). The tip diameter of the pH electrodes was ca. 5 µm, their dynamic range was pH 3 to 11, and their response time was ca. 10 s. The pH microelectrodes were calibrated in pH buffer solutions (Mettler Toledo, pH 4.01, 7.0 and 9.21, DIN 19266) at room temperature.

CO_2 microsensors

We constructed fast-responding CO_2 microsensors according to de Beer et al. (1997). The CO_2 microsensors were calibrated in a degased phosphate buffer (50 m*M*, pH 8.2) by adding aliquots of a 200 m*M* carbonate solution. The CO_2 microsensors had tip diameters of ca. 10 µm, a detection limit of ca. 0.5 µ*M* CO_2, and a response time of ca. 10 s.

Ca^{2+} microelectrodes

Ca^{2+} profiles from the shell surface towards the ambient seawater were measured with Ca^{2+} LIX microelectrodes in combination with a calomel reference electrode, both connected to a high-impedance mV meter (Keithley 617, USA) (Tsien and Rink 1980; Amman et al. 1987). The tip diameter was < 10 µm. Calibration was done in Ca^{2+} buffer solutions (1, 10 and 20 m*M*) with added background ions, i.e. seawater concentrations of Mg^{2+}, Na^{2+} and K^{+}.

Fiber optic microprobe

Profiles of quantum scalar irradiance (400 to 700 nm) from the shell surface to the ambient seawater were measured with a fiber optic scalar irradiance microprobe (Lassen et al. 1992) connected to a PAR meter (Kühl et al. 1997). Calibration procedures and more technical details were described by Kühl et al. (1997).

Gross photosynthesis

Oxygen microsensors with a fast response time were used for measurements of gross photosynthesis (in nmol O_2 cm^{-3} s^{-1}) at the shell surface of benthic foraminifera. Gross photosynthesis was estimated with the light–dark shift technique (Revsbech et al. 1981; Revsbech and Jørgensen 1983; Glud et al. 1992; Kühl et al. 1996), by measuring the rate of O_2 depletion over the first seconds after darkening. The O_2 depletion is equal to the photosynthetic O_2 production during the previous light period, assuming a steady state O_2 distribution before darkening, identical O_2 consumption before and during the dark period, and identical diffusive fluxes at the shell surface during the measurement.

Net photosynthesis and dark respiration

Net photosynthesis and dark respiration rates were calculated from measured steady-state O_2 profiles in the light and dark, respectively. Assuming a one-dimensional diffusion geometry, the rates were calculated as the diffusive O_2 flux, J, in nmol O_2 cm^{-2} s^{-1}, by Fick's first law:

$$J = -D_0 \frac{dC}{dz} \quad , \tag{1}$$

with the linear concentration gradient, dC/dz, over the diffusive boundary layer (DBL) (Jørgensen and Revsbech 1985), and the molecular O_2 diffusion coefficient in seawater, D_0. D_0 for O_2 is 2.32×10^{-5} cm^2 s^{-1} in seawater (36‰) at 26 °C and 1.96×10^{-5} cm^2 s^{-1} in seawater (40‰) at 20 °C, according to Broecker and Peng (1974) and Li and Gregory (1974).

Results

Physico-chemical microenvironment and DBL

The chemical microenvironment around the foraminiferal shells was affected by endosymbiont photosynthesis, calcification, and the combined respiration of host and microalgal symbionts. The exchange of photosynthetic and respiratory substrates/products between the foraminifer and the ambient seawater occurred over a DBL surrounding the foraminiferal shell. In an experiment with *Amphisorus hemprichii*, the DBL thickness decreased with flow velocity. Under stagnant conditions the DBL thickness reached 400 to 700 µm, and decreased to 100–175 µm under flow conditions (Fig. 3). The effective DBL thickness was measured by extrapolation of the O_2, CO_2 and pH gradients at the shell–seawater interface to the ambient seawater concentration according to Jørgensen and Revsbech (1985) and Jørgensen and Des Marais (1990).

The net photosynthesis rates calculated from the O_2 efflux from the shell of *Amphisorus hemprichii* were, however, not affected by the flow regime and reached 0.03 to 0.06 nmol O_2 cm^{-2} s^{-1} (Table 1). The dark respiration rates of *A. hemprichii* seemed to be influenced by the water flow. At higher flow velocity the dark respiration rate was two times higher than at moderate flow rate. Gross photosynthesis rates measured at the shell surface of *Amphistegina lobifera* were flow dependent (Table 1); the average gross rates under flow conditions (4.55 and 4.89 nmol O_2 cm^{-3} s^{-1}) were significantly higher than gross rates measured under stagnant conditions (1.62 nmol O_2 cm^{-3} s^{-1}). A thick DBL thus imposes limitations on gross photosynthesis of the

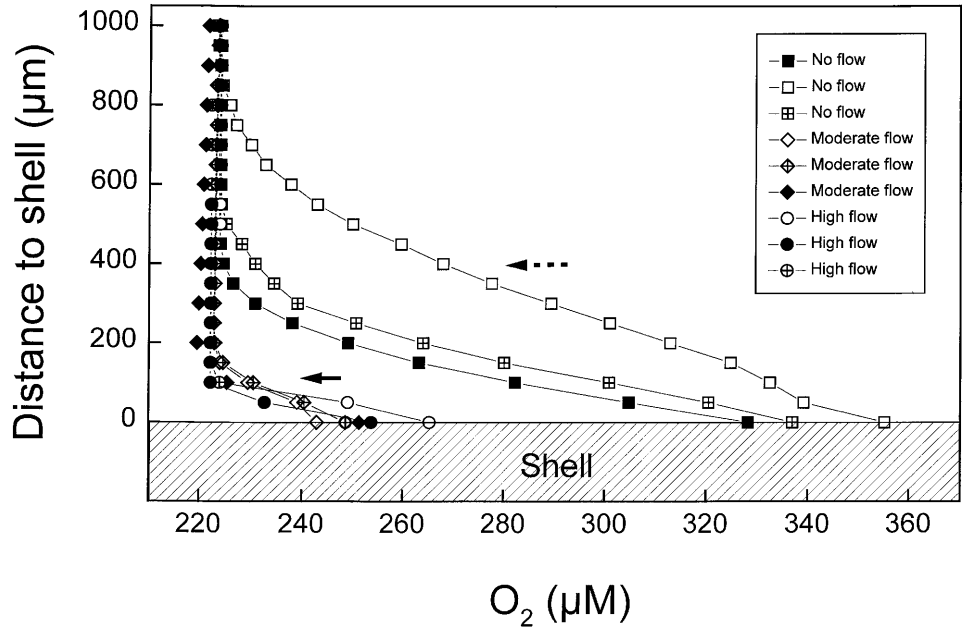


Fig. 3 *Amphisorus hemprichii*. O_2 concentration profiles measured under changing flow conditions towards the shell (*dashed arrow* DBL thickness under stagnant conditions; *solid arrow* DBL thickness under flow conditions; irradiance = 166 µmol photons m^{-2} s^{-1})

Table 1 *Amphisorus hemprichii*, *Amphistegina lobifera*. Gross photosynthesis (mean ± SD, nmol O_2 cm^{-3} s^{-1}) net photosynthesis and respiration rates (mean ± SD, nmol O_2 cm^{-2} s^{-1}) measured under changing flow velocities

	High flow	Moderate flow	No flow
A. hemprichii	4.0 cm s^{-1}	2.2 cm s^{-1}	
Net photosynthesis rate	0.06 ± 0.02	0.03 ± 0.01	0.06 ± 0.02
Dark respiration rate	0.05 ± 0.02	0.02 ± 0.01	–
A. lobifera	1.5 cm s^{-1}	0.6 cm s^{-1}	
Net photosynthesis rate	0.19 ± 0.07	0.19 ± 0.08	0.22 ± 0.01
Gross photosynthesis rate	4.89 ± 0.91	4.55 ± 1.81	1.62 ± 0.88

endosymbionts and on dark respiration rates of the symbiotic association.

Oxygen, CO_2 and pH profiles were measured in short intervals above one *Marginopora vertebralis* specimen (Fig. 4). All profiles demonstrated limited solute exchange between the foraminifer and the surrounding water due to the DBL above the shell surface. In light, the ambient O_2 concentration of 205 μ*M* started to increase ca. 400 μm above the shell of *M. vertebralis* and reached a concentration of 376 μ*M* (=183% air saturation) at the shell surface (Fig. 4A). Photosynthetic CO_2 fixation reduced the CO_2 concentration to 4.6 μ*M* and increased the pH to 8.6 at the shell surface, as compared to a CO_2 concentration of 10 μ*M* and a pH of 8.2 in the ambient seawater. Under dark conditions, the respiration of *Marginopora vertebralis* and its symbionts decreased the O_2 level down to 183 μ*M* (=91% air saturation) and increased the CO_2 up to 11.6 μ*M* at the shell surface (Fig. 4B). The pH was 8.2 at the shell surface. The other two foraminiferal species investigated in this study established similar O_2, CO_2 and pH environments in light and darkness.

The Ca^{2+} microenvironment near the shell surfaces of *Amphistegina lobifera* and *Marginopora vertebralis* exhibited significant changes in Ca^{2+} concentration compared to the surrounding seawater (Fig. 5). The Ca^{2+} profiles demonstrated a spatial heterogeneity of the Ca^{2+} concentration above the shells. In light, the Ca^{2+} concentration at the shell surface of *A. lobifera* decreased down to 9.9 m*M*, indicating a net uptake or consumption of Ca^{2+} (Fig. 5A). However, the Ca^{2+} dark profile showed a concentration increase up to 10.1 m*M* Ca^{2+} at the shell surface of *A. lobifera*. Most Ca^{2+} profiles measured in *A. lobifera* showed an uptake of Ca^{2+} ions from the surrounding seawater at the shell surface (Fig. 5B). Ca^{2+} profiles measured at different irradiances (500 and 1500 μmol photons m^{-2} s^{-1}, respectively) showed no significant effect of light (Fig. 5B). In *A. lobifera* average Ca^{2+} uptake rates reached 0.6 to 4.2 nmol Ca^{2+} cm^{-2} h^{-1} (Table 2). In *M. vertebralis* the Ca^{2+} environment changed over time between net uptake and net release of the Ca^{2+} ions (Fig. 5C); uptake rates varied between 1.7 and 3.6 nmol Ca^{2+} cm^{-2} h^{-1} (Table 2).

Profiles of quantum scalar irradiance, E_0 (PAR), measured above the shell of *Marginopora vertebralis* demonstrated an increase of E_0 (PAR) towards the foraminiferal shell (Fig. 6). The profiles were influenced by the presence of endosymbionts under the shell surface. The E_0 (PAR) profile measured in the center above the brownish area with dinoflagellates showed a smaller increase of scalar irradiance at the shell (160% of incident irradiance) as compared to the E_0 profile in the outer shell region where no symbionts were located (205% of incident irradiance). Measurements of light

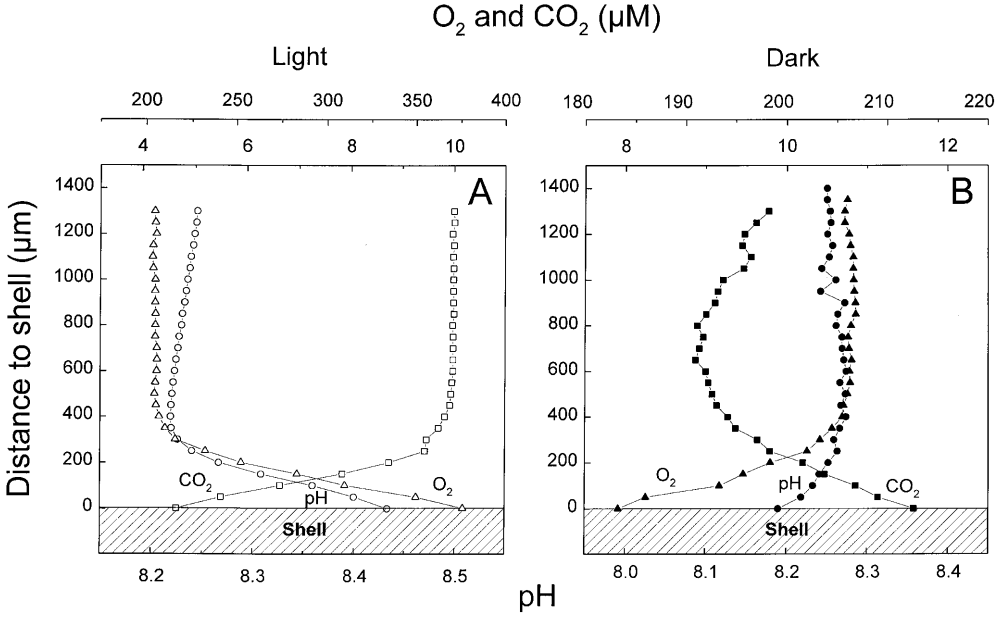
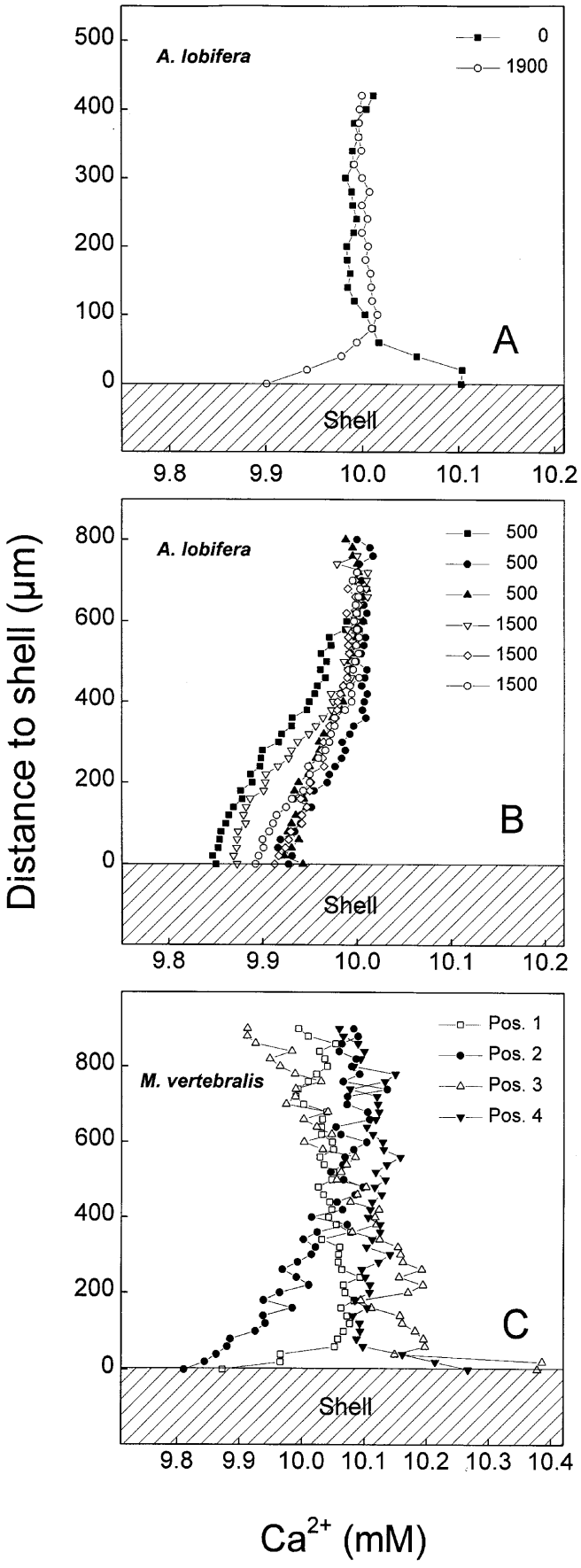


Fig. 4 *Marginopora vertebralis*. Concentration profiles of O_2 (△), CO_2 (□) and pH (○) under light (**A**) and dark (**B**) conditions (irradiance = 359 μmol photons m^{-2} s^{-1}). Profiles were measured from the shell surface towards the well-mixed surrounding seawater



◀

Fig. 5 Ca^{2+} concentration profiles measured **A** under light and dark conditions and **B** with varying incident irradiances in *Amphistegina lobifera* (numbers indicate incident irradiance in µmol photons m^{-2} s^{-1}). **C** Ca^{2+} concentration profiles measured at different positions on the shell surface of *Marginopora vertebralis*

transmission through the upper calcite layer of *M. vertebralis* showed an average transmittance of 0.31 ± 0.02 (n = 3). Thus the symbionts experience ca. 30% of the light incident on top of the foraminiferal shell.

O_2, CO_2 and pH dynamics

Dynamic variations of O_2, pH and CO_2 levels were measured at the shell surface of *Amphistegina lobifera* during experimental light–dark cycles (Fig. 7). After steady-state conditions of O_2, pH and CO_2 were recorded, the light was turned off. The O_2 concentration decreased rapidly from 147% (303 µ*M*) down to 88% air saturation (181 µ*M*) in <3 min. After the light was switched on again, O_2 increased to 100% air saturation (206 µ*M*) within 5 s. In the 3 min dark period, CO_2 increased from 7.2 up to 15.1 µ*M*. The pH variation with the light–dark shifts was less significant (~0.1 units) than that of O_2 and CO_2. pH at the shell surface decreased down to pH 8.02 in the darkness. O_2 and CO_2 at the shell surface changed immediately with the change in light conditions, whereas the pH signals showed a short delay (Fig. 7). The rapid concentration changes demonstrated for the first time a fast metabolic gas transport through the perforate shell of *A. lobifera*.

Discussion

Microenvironment of benthic foraminifera

The microenvironment around the shells of benthic symbiont-bearing foraminifera was largely controlled by

Table 2 *Amphistegina lobifera*, *Marginopora vertebralis*. Calcium uptake rates for *A. lobifera* (2.0 mm diam.) and *M. vertebralis* (1.7 to 3.4 mm diam.) calculated from locally measured Ca^{2+} uptake

Specimen No.	Average Ca^{2+} uptake (nmol cm^{-2} h^{-1})	Ca^{2+} uptake rate (nmol $foraminifer^{-1}$ h^{-1})
A. lobifera		
I	1.43	0.16
II	1.88	0.21
III	1.78	0.19
IV	4.21	0.46
V	0.60	0.07
Mean ± SD	1.98 ± 1.34	0.22 ± 0.15
M. vertebralis		
I	1.73	0.35
II	2.84	0.16
III	3.58	0.74
Mean ± SD	2.72 ± 0.93	0.42 ± 0.29

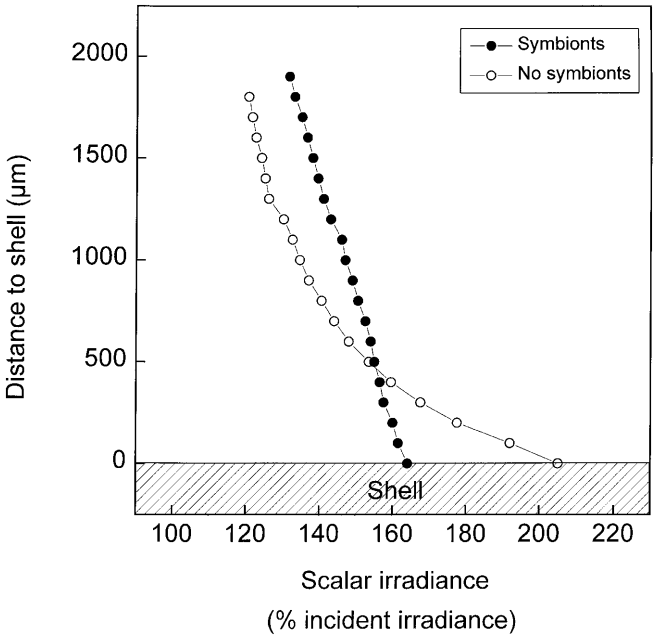


Fig. 6 *Marginopora vertebralis*. Scalar irradiance microprofiles measured towards the shell surface at a position with (●) and without (○) symbionts

the prevailing light and flow conditions (Figs. 3, 4, 7). Conditions of water flow caused steeper O_2 gradients across the DBL than stagnant water conditions (Fig. 3). Most profiles demonstrated a DBL thickness of around 400 µm (Figs. 3, 4). O_2, CO_2, pH and Ca^{2+} near the shell surface changed significantly compared to the concentrations in the surrounding seawater.

Under saturating irradiances, the endosymbiont photosynthesis resulted in an O_2 and pH increase towards the shell surface of *Marginopora vertebralis*. The CO_2 concentration above the shell of *M. vertebralis* and *Amphistegina lobifera* was not fully depleted by photosynthetic CO_2 fixation, and reached values between 4.6 and 7.3 µ*M*. Thus we suggest that the supply of dissolved inorganic carbon (DIC) was sufficient for the primary production of the endosymbionts under saturating irradiances. This is in agreement with previous DIC experiments in *A. lobifera* (ter Kuile et al. 1989a), which showed that photosynthesis of the endosymbiotic diatoms, both associated with the host and isolated in culture, was saturated at the inorganic carbon concentration of seawater. Beside the inorganic carbon reservoir of seawater, possible internal CO_2 sources available for photosynthesis could be due to a respiratory CO_2 release by the host or a conversion of HCO_3^- to CO_2 by the enzyme carbonic anhydrase (CA). Carbonic anhydrase activity was determined in the symbiotic microalgae of corals by Al-Moghrabi et al. (1996). A further supply of CO_2 could be due to the precipitation of $CaCO_3$ (McConnaughey 1989).

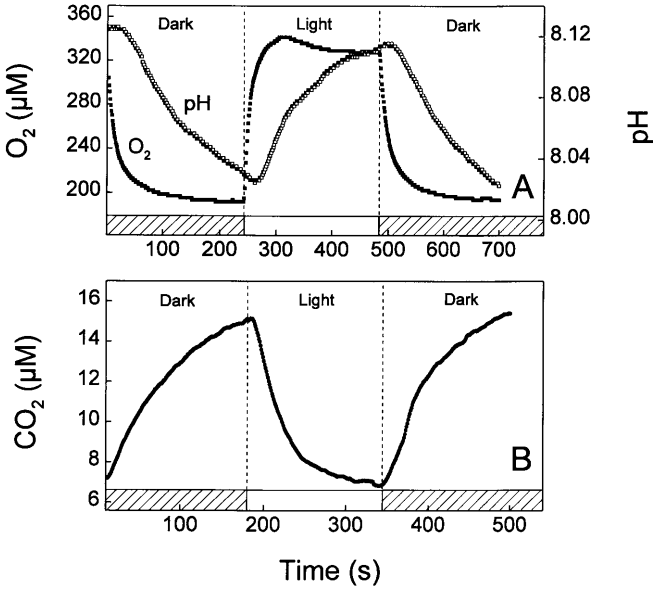


Fig. 7 *Amphistegina lobifera*. **A** O_2, pH and **B** CO_2 dynamics measured at the shell surface (irradiance = 697 µmol photons m^{-2} s^{-1})

The CO_2 gradients under light conditions demonstrated a net CO_2 uptake towards the shell surface. Due to the CO_2 fixation by symbiont photosynthesis, larger foraminifera represent a CO_2 sink during the daytime at saturating irradiances. In addition, the dark respiration rates measured in *Amphistegina lobifera* were 0.5 to 0.8 times smaller than the net O_2 production rates in light (Köhler-Rink and Kühl, unpublished). These observations contradict the suggestion that larger foraminifera contribute as a CO_2 source in reef communities (Langer et al. 1997).

The fast response of the endosymbionts to the changing light conditions resulted in dynamic changes in the chemical microenvironment at the foraminiferal shell (Fig. 7). Our data demonstrated a rapid influx/efflux of O_2 and CO_2 through the hyaline shell of *Amphistegina lobifera*. The ultrastructure of the perforate shell, therefore, allows a fast exchange of metabolic gases between the foraminiferal cytoplasm and the ambient seawater (Debenay et al. 1996). The passage of CO_2 through the pores of *Amphistegina* across the inner organic lining has already been studied by Leutenegger and Hansen (1979). Similar changes in O_2 and pH conditions due to symbiont photosynthesis were measured in the planktonic symbiotic foraminifera *Globigerinoides sacculifer* and *Orbulina universa* (Jørgensen et al. 1985; Rink et al. 1998). In comparison to the benthic species the symbionts of planktonic foraminifera, living within the cytoplasm, spread outside the shell, between the calcified spines, during daytime. In the symbiont swarm of *O. universa* O_2 reached up to 206% air saturation and pH was 8.8 at saturating irradiances. Endosymbionts living inside the tissue of hermatypic corals changed the O_2 concentration and pH of the tissue and its surroundings in the same way. Microsensor measurements in the tissue of *Favia* sp. and *Acropora* sp. detected a pH increase up to 8.5 and O_2 concentrations up to 250% of air saturation (Kühl et al. 1995).

The scalar irradiance profiles demonstrated an increase towards the shell surface of *Marginopora verte-*

bralis due to scattering of the incident light by the calcite crystals of the complex, porcelaneous shell texture (Debenay et al. 1996, 1999). Profiles measured in areas filled with symbionts showed a smaller increase, indicating reduced light reflection due to light absorption by the yellow-brownish microalgae (Fig. 6). Locally increased scalar irradiances were also found at the coral tissue surface of *Favia* sp. (Kühl et al. 1995), near the shell surface of the planktonic foraminifer *Orbulina universa* (Rink et al. 1998), and in the upper test of symbiont-containing didemnid ascidians (M. Kühl, unpublished data).

The thin foraminiferal calcite shell transmitted only 30% of the incident light and can thus protect the symbionts inside the cytoplasm against the damaging levels of high solar radiation often found in shallow waters, e.g. of lagoons or coral reefs. Whether the high light attenuation of the upper shell also includes some spectral filtering of light, e.g. by removal of UV light, remains to be investigated.

DBL and flow effects on photosynthesis and respiration

Larger foraminifera are surrounded by an environment of changing flow conditions that may affect the DBL around the foraminiferal shells (Jørgensen and Des Marais 1990; Jørgensen 2000). The DBL constitutes a barrier for ion and gas exchange between the seawater and the symbiotic association (Jørgensen et al. 1985; Kühl et al. 1995). Its thickness depends on the size and shape of the organism as well as on the water flow (Pasciak and Gavis 1974; Lazier and Mann 1989; Vogel 1994). A decrease in the DBL thickness will increase the solvent flux by increasing the concentration gradient and decreasing the time needed to equilibrate solvent concentrations (Patterson et al. 1991). Increasing water flow may therefore result in a better supply of O_2, DIC and nutrients like N and P to benthic foraminifera. Furthermore, foraminiferal feeding on suspended particulate matter by use of their pseudopodial network is strongly dependent on the rate of the ambient flow (Murray 1991; Vogel 1994).

The characteristic roughness of a surface is important for the boundary layer thickness, which increases with increasing roughness (Jørgensen and Revsbech 1985; Denny 1988; Jørgensen and Des Marais 1990; Vogel 1994). We speculate that the irregular surface textures of larger foraminiferal shells change the thickness and geometry of the DBL as was shown in microbial mats by Jørgensen and Des Marais (1990). In turn, the concentration gradients and thus the calculated diffusive influx/efflux of O_2 and Ca^{2+} measured at different shell positions may be influenced by the DBL changes. *Amphistegina lobifera* for example has a biconcave-shaped shell with a smooth surface, whereas *Marginopora vertebralis* and *Amphisorus hemprichii* have more irregular disc-shaped morphologies. Irregular surface textures (e.g. wave-like structures or depressions in the shell center) are typically found in relatively large shells of soritids, such as *M. vertebralis* and *A. hemprichii*.

We speculate that a decrease in thickness of the surrounding DBL may contribute to the enhanced growth rates reported for larger, benthic foraminiferal tests measured under conditions of water motion (Hallock and Hansen 1979; ter Kuile and Erez 1984; Hallock et al. 1986; Wetmore 1987). The Ca^{2+} influx from the ambient seawater could, e.g., increase under flow conditions and influence the direct calcium uptake for $CaCO_3$ precipitation or, alternatively, the formation of an internal Ca^{2+} pool (Hemleben et al. 1986; ter Kuile and Erez 1988; Erez et al. 1994). Wetmore and Plotnick (1992) proved that the test strength of larger benthic foraminifera (e.g. *Amphistegina gibbosa*) collected from a high-energy exposed reef was greater than that of individuals from a low-energy sheltered seagrass flat. In addition, individuals of *A. lobifera* increased in diameter and in mass more quickly in moving water than in stagnant water (Hallock et al. 1986).

The effect of flow on physiological processes of marine organisms has been the subject of numerous studies. Flow effects on diffusion-limited processes, such as photosynthesis, respiration and nutrient uptake, have been demonstrated in marine algae (Koehl and Alberte 1988; Pahlow et al. 1997), corals (Dennison and Barnes 1988; Patterson et al. 1991; Kühl et al. 1995) and sea anemones (Patterson and Sebens 1989). Changing flow conditions around the shell of *Amphistegina lobifera* affected symbiont photosynthesis (Table 1). Gross photosynthesis rates were significantly lower under stagnant conditions. We speculate that the enhanced gross photosynthesis rates could be due to a greater CO_2 release through increased respiration of the foraminifer under higher flow conditions. The endosymbionts, living inside the cytoplasm, may benefit from the respired CO_2. Haynes (1965) suggested that the host shell acts as a natural "greenhouse" offering a favorable habitat for the endosymbionts. The results of our flow experiments agree with investigations of water motion effects on corals. Increasing primary production and respiration rates with flow were measured in the coral *Montastrea annularis* (Patterson et al. 1991). Dennison and Barnes (1988) investigated water motion effects on the reef-building coral *Acropora formosa*, and found significantly reduced net photosynthesis and respiration in unstirred conditions. Lesser et al. (1994) detected a decrease in the enzymatic activity of CA when corals were exposed to increased water velocity. Their results indicate an effect of the surrounding flow conditions on the CO_2 supply for symbiont photosynthesis.

It is of interest to note here that some species of larger benthic foraminifera are motile and probably migrate within their habitats to positions where they find optimal growth conditions (Travis and Bowser 1991). The photoresponse of larger foraminifera was studied by Zmiri et al. (1974) and Lee et al. (1980). Lee et al. (1980) found a stronger phototaxic response than feeding response in *Amphisorus hemprichii*. In our study we

observed that *Amphistegina lobifera* tends to lift its shell from the substratum such that both shell sides are exposed to the water flow or the incident light (see also Hansen and Buchardt 1977). Furthermore, it was found clinging to exposed points, such as algal branches or stones. This motile activity could indicate the importance of water motion for the feeding strategy of *A. lobifera*. Future combined studies of foraminiferal behavior, their physico-chemical microenvironment and ecophysiology will be able to elucidate the mechanisms that control the different behavioral strategies of larger foraminifera in their natural environment.

Calcium microenvironment and calcification

In order to compare our Ca^{2+} uptake rates with published calcification rates of foraminifera we extrapolated the locally measured Ca^{2+} uptake to the total surface area of the foraminifera by using the formulas of Lee et al. (1988) for the biconcave-shaped *Amphistegina lobifera* (Eq. 2) and the disc-shaped *Marginopora vertebralis* (Eq. 3):

$$2\pi\left(\frac{D}{2}\right)\sqrt{\left(\frac{1}{4}D\right)^2+\left(\frac{1}{2}D\right)^2} \quad (2)$$

$$2\pi\left(\frac{1}{2}D\right)^2 + 2\pi\frac{1}{2}D * \text{height} \ . \quad (3)$$

Thereby, we estimated Ca^{2+} uptake rates of 0.22 ± 0.15 nmol Ca^{2+} foraminifer^{-1} h^{-1} in *A. lobifera* (individuals of 2 mm diameter, $n = 5$) and 0.42 ± 0.29 nmol in *M. vertebralis* (individuals of 1.7 to 3.4 mm diameter, $n = 3$) (Table 2).

Our calculated Ca^{2+} uptake rates are significantly lower than the calcification rates of benthic foraminifera reported by Duguay (1983), who found Ca^{2+} uptake rates of 8 nmol Ca^{2+} mg^{-1} dry wt h^{-1} in *Archais angulatus* (at 840 µmol photons m^{-2} s^{-1}) and ca. 13 nmol Ca^{2+} mg^{-1} dry wt h^{-1} in *Sorites marginalis* (240 µmol photons m^{-2} s^{-1}) by measuring the uptake of $^{45}CaCl_2$ as an indicator for calcification. The uptake rates of *Amphistegina lobifera* will be slightly higher when they are expressed per milligram dry weight. We found a fresh weight to dry weight ratio of 1.27 for *A. lobifera*. For comparison of Ca^{2+} uptake rates on a dry weight basis, however, it is important to point out the weight variations in benthic foraminifera (Duguay and Taylor 1978; Duguay 1983); the dry weight of benthic foraminifera changes during the ontogenetic cycle, due to an increase of cytoplasm and endosymbiont numbers, and the addition of calcium carbonate. Furthermore, growth of benthic foraminifera is influenced by the availability of food, temperature and salinity (Murray 1963), as well as light intensity and nutrient supply (Röttger et al. 1980; Hallock 1981; Hallock et al. 1986). Variations in calcium incorporation during the foraminiferal growth cycle and species-specific variations have been reported for the soritids *A. angulatus* and *S. marginalis* (Lee and Bock 1976; Duguay 1983). *S. marginalis* showed a two times higher calcium incorporation than did *A. angulatus*. Duguay (1983) suggested that this is caused by differences in the frequency and rate of chamber formation between the two species. Size variations were determined by Lee and Bock (1976), who measured 1.8-fold higher calcification rates in small *A. angulatus* than in larger specimens.

Our data do not show a correlation between the calcium uptake rates and the different magnesium contents of the foraminiferal shells. The high Mg^{2+} content (>20 mol% $MgCO_3$) in the porcelaneous shells of *Marginopora vertebralis* (Debenay et al. 1999) point to lower Ca^{2+} uptake rates in this species as compared to the low Mg^{2+} content (<6 mol% $MgCO_3$) of the hyaline shell of *Amphistegina lobifera* (Chave 1954). However, our microsensor measurements do not prove the precipitation of Ca^{2+} ions transported towards the shell surface. The measured Ca^{2+} gradients could also indicate a transport and subsequent immobilization of Ca^{2+}, e.g. into vesicles. Erez et al. (1994) described membrane-bound granules within the endoplasm of *A. lobifera*, which could serve as internal pools of Ca^{2+} for the calcification process. Furthermore, the regulation mechanisms of magnesium and calcium uptake and storage prior to calcite deposition are still unknown (Hemleben et al. 1986). It was reported that environmental parameters, such as water temperature, salinity and depth, affect the magnesium content of the calcite shells (Chave 1954; Delanay et al. 1985). Due to the fact that species with low and high Mg^{2+} calcite shells, such as *A. lobifera* and *A. hemprichii*, live in close association within the Gulf of Aquaba, we suggest that other factors may also influence the Mg^{2+} content. Bender et al. (1975) hypothesized that the precipitation of low Mg^{2+} calcite in planktonic foraminifera is affected by organic complexing agents produced by the foraminifera. Such agents could reduce the solution activity of Mg^{2+} by selectively complexing Mg^{2+} ions. However, the mechanisms that induce low Mg^{2+}/Ca^{2+} ratios in the shell calcite are still unknown, and variations in precipitation rates had no significant effect on the incorporation of Mg^{2+} into calcites (Burton and Walter 1987).

Some authors have used the $^{45}CaCl_2$ uptake technique to measure the precipitation of ^{45}Ca by a foraminiferal pool (Duguay and Taylor 1978; Duguay 1983) or by single foraminiferal shells (Anderson and Faber 1984). Ca^{2+} uptake rates of single planktonic foraminifera have been estimated from measured $^{48}Ca/^{44}Ca$ ratios (Lea et al. 1995). With the Ca^{2+} microsensor we measured Ca^{2+} gradients at the shell surface of a single foraminifer, but we were restricted to point measurements. The microsensor technique, therefore, determines the short-term Ca^{2+} situation at specific shell positions. We measured fluctuations in the Ca^{2+} microenvironment over time, but our data give no information on the time sequence of chamber calcification. Lea et al. (1995) did not find a general trend of changing calcification

rates over the growth cycle (60 h) of the planktonic foraminifer *Orbulina universa*. In the same species, Spero (1986) measured slower calcite addition during most of the shell-thickening period and faster addition of calcite several hours prior to gametogenesis. Anderson and Faber (1984) reported that chamber addition in *Globigerinoides sacculifer* is an incremental event and not a continuous process. The benthic species *Heterostegina depressa* showed chamber building activity every second or third day (Röttger 1972a, b). The frequency of chamber-forming periods was reduced at low temperatures or during extended dark periods.

Different aspects of the calcification process in foraminifera were investigated, but the basic mechanisms are still poorly understood (Erez 1978, 1983; Duguay 1983; ter Kuile and Erez 1988; Lea et al. 1995). A number of authors have discussed possible calcification theories (Hemleben et al. 1986; ter Kuile 1991; Debenay et al. 1996). One theory, biologically induced $CaCO_3$ fixation, is explained as a pH-driven process, in which CO_2 fixation by the host symbionts raises the pH, which, in turn, induces the precipitation of $CaCO_3$. This was suggested by Lea et al. (1995) for light-enhanced calcification in *Orbulina universa*. The planktonic species calcified two to three times more under high irradiance conditions (500 μmol photons m^{-2} s^{-1}) than individuals grown in less light (5 μmol photons m^{-2} s^{-1}) or in the dark. A symbiont-dependent stimulation of calcium carbonate production was also pointed out in the work of Duguay (1983). The benthic foraminifera *Sorites marginalis*, *Cyclorbiculina compressa* and *Archais angulatus* showed enhanced calcification under high light levels. Higher calcification rates under high light conditions were also reported by Lee and Zucker (1969) and Erez (1978). The Ca^{2+} increase in the dark profile of *Amphistegina lobifera*, which we measured directly after a light period (Fig. 5A), may demonstrate an influence of the light situation on the Ca^{2+} uptake.

The effect of ambient seawater pH on inorganic carbon uptake (C_i) was studied by ter Kuile et al. (1989a). In their study, the optimum pH for calcification ranged between 8.2 and 8.9 for *Amphistegina lobifera* and *Amphisorus hemprichii*, respectively. In *A. hemprichii* the C_i uptake into the shell skeleton was stimulated above pH 8.0, whereas the C_i uptake in *A. lobifera* did not show a significant change from pH 8.0 to 8.9. Our data demonstrate, however, that the shell surface pH changed significantly compared to the ambient pH of seawater. We measured a pH increase towards the foraminiferal shells of *A. hemprichii* (data not shown) and *Marginopora vertebralis* at high irradiance due to symbiont photosynthesis (Fig. 4). Our data could support the theory of biologically induced $CaCO_3$ precipitation, as alkaline conditions may have favored the chemical processes leading to calcification.

Alkaline conditions in the foraminiferal environment can also be induced by the surrounding substratum. Benthic foraminifera often live on or imbedded in microalgal biofilms or attached to macroalgae. *Marginopora vertebralis*, for example, lives on the calcareous green alga *Halimeda* sp. (Borowitzka and Larkum 1976). These phototrophic communities increase the surrounding seawater pH when exposed to light (Axelsson and Uusitalo 1988; Israel and Beer 1992).

Further calcification theories that have been proposed are: (1) an organic matrix, where a primary organic lining is controlling the calcification (Weiner and Erez 1984; Hemleben et al. 1986); and (2) an energy-dependent transport of carbonate into an inorganic carbon pool coupled with an active Ca^{2+}-concentrating mechanism (Anderson and Faber 1984; ter Kuile and Erez 1988; ter Kuile et al. 1989a). The "poison removal theory" (3) suggests that the presence of inhibiting ions, like ammonium, phosphate or magnesium, prevents the precipitation of calcite (Hemleben et al. 1986; ter Kuile 1991), which can, however, be induced spontaneously subsequent to the removal of these ions by the foraminifera.

Compared to *Amphistegina lobifera*, most profiles above the shell surface of *Marginopora vertebralis* demonstrated a release of Ca^{2+}. Possible explanations for the different Ca^{2+} profiles of the two species could be the texture of their calcite shells or the process of $CaCO_3$ precipitation. The transport of Ca^{2+} ions through the porous shell of *A. lobifera* is probably faster than through the imperforate shell of *M. vertebralis*. The Ca^{2+} gradient measured at the shell surface, thus, might demonstrate a transfer of Ca^{2+} into a Ca^{2+} pool as described by Anderson and Faber (1984). The very heterogeneous Ca^{2+} dynamics on the shell surface of *M. vertebralis* could indicate a different uptake mechanism through the porcelaneous, imperforate shell. During the biomineralization process of porcelaneous tests, the $CaCO_3$ nucleation occurs in Golgi vesicles, where secondary needles are constructed (Hemleben et al. 1986; ter Kuile and Erez 1988). The pre-formed needles are transported to the site of deposition, where they are released by exocytosis (Hemleben et al. 1986). In hyaline tests, the nucleation occurs on an organic membrane (Towe and Cifelli 1967; Hottinger 1986). This membrane provides a solid surface, where efficient nucleators can be absorbed and ions can be bound (Towe and Cifelli 1967; Addadi and Weiner 1985; Debenay et al. 1996).

Conclusions

The application of microsensors provided the first description of the physico-chemical microenvironment surrounding larger foraminifera. Based on these measurements we estimated rates of respiration, photosynthesis and calcification at high spatio-temporal resolutions and as a function of environmental variables like irradiance and water flow. The physico-chemical microenvironment around benthic foraminifera shells was largely controlled by the prevailing light and flow conditions. Due to the combined action of endosymbiont photosynthesis, host calcification and the respiration of host and microalgal symbionts, a dynamic microen-

vironment with respect to O_2, CO_2, pH and Ca^{2+} was found at the shell surfaces of larger foraminifera. The DBL thickness influenced the mass tranfer and solute exchange between the foraminifer and the surrounding seawater. Both respiration rates of the foraminiferal–algal association and the photosynthesis rates of the endosymbionts increased under flow conditions. Although the symbionts live inside the host cytoplasm, they showed a dynamic response to experimental light–dark cycles. The calcite shell provides the symbionts with protection against high levels of solar radiation.

Calcium microgradients demonstrated a net calcium uptake under light conditions in most cases. However, the heterogeneous Ca^{2+} microenvironment of the benthic foraminifera needs to be studied in more detail. To investigate the interaction between symbiont photosynthesis and host calcification, microsensor studies of O_2, CO_2, pH and Ca^{2+} dynamics combined with inhibitor experiments would be neccesary.

With the techniques presented here, the regulatory mechanisms of respiration, photosynthesis and calcification, and their interactions in benthic foraminifera and other symbioses can be investigated. Besides detailed ecophysiological studies, further investigations should focus on the study of benthic foraminifera in their natural environment, i.e. microsensor measurements of the foraminiferal physico-chemical microenvironment combined with behavioral studies of foraminifera within their natural habitat (e.g. attached to biofilm-coated stones).

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