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Microsensor studies of photosynthesis and respiration in the larger symbiont bearing foraminifera Amphistegina lobifera, and Amphisorus hemprichii

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OPHELIA 55 (2): 111-122 (December 2001)

MICROSENSOR STUDIES OF PHOTOSYNTHESIS AND RESPIRATION IN THE LARGER SYMBIONT BEARING FORAMINIFERA *AMPHISTEGINA LOBIFERA*, AND *AMPHISORUS HEMPRICHII*

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ABSTRACT

The photosynthesis and respiration of the larger foraminifera *Amphistegina lobifera* and *Amphisorus hemprichii* was studied with O_2, CO_2, and pH microsensors, and with a miniature gas exchange chamber. The diffusive transport of O_2 and CO_2 through both perforate (*A. lobifera*) and imperforate (*A. hemprichii*) calcite shells of the foraminifera was fast and allowed investigations of endosymbiont photosynthesis by microsensor measurements at the shell surface. Gross photosynthesis versus scalar irradiance (P vs. E_0) curves showed onset of light saturation (E_k) at 95-198 µmol photons m^{-2} s^{-1}. No photoinhibition was observed up to an irradiance of 2000 µmol photons m^{-2} s^{-1}. Net photosynthesis (at saturating irradiance) and dark respiration rates were 3.7-22.7 and 5.6-14.3 nmol O_2 foraminifer^{-1} h^{-1}, respectively. Simultaneous CO_2, pH and O_2 measurements at the shell surface of *A. hemprichii* during experimental light-dark cycles showed rapid concentration changes of all three variables upon light-dark or dark-light shifts. The dynamics of O_2 and CO_2 at the shell surface of *A. hemprichii* showed unequal net conversion rates of O_2 and CO_2 during experimental light-dark cycles. The molar O_2/CO_2 conversion ratio at the shell surface of the foraminifera was ~2 in darkness and ~6 at saturating irradiance, pointing to a large internal supply of CO_2 in the host-symbiont association and the use of bicarbonate as source for inorganic carbon. The carbonate chemistry in the vicinity of symbiont-bearing larger foraminifera is thus strongly affected by the combined action of photosynthesis, respiration and calcification, and cannot be considered in equilibrium with the surrounding sea water. This has important implications for paleoenvironmental analysis and interpretation of the stable isotope composition of foraminiferal calcite shells and the derived models used for reconstructing climate and productivity in the past.

Keywords: photosynthesis, inorganic carbon sources, respiration, larger foraminifera, symbiosis, P vs. E_0 curves, *Amphestigina lobifera, Amphisorus hemprichii.*

INTRODUCTION

Larger foraminifera live in oligotrophic tropical reef environments and harbor symbiotic diatoms or dinoflagellates (Lee et al. 1980, Lee et al. 1997). An efficient carbon and nutrient recycling within the foraminiferal-algal association has been suggested (Lee et al. 1974, Caron et al. 1995). The microalgae release O_2 and photosynthates like polyglucan, glucose, and lipids to the cytoplasm of the foraminiferal host (Kremer et al. 1980) and can, thereby, provide a major part of the organic carbon required for host metabolism (Battey 1992). Furthermore, the endosymbionts contribute to the calcification of foraminiferal shells, as calcium carbonate precipitation appears to be metabolically coupled to the photosynthetic reactions in foraminifera, corals, and calcifying algae (Goreau 1959, Hallock 1981, Duguay 1983, Lea et al. 1995, Falkowski and Raven 1997, de Beer et al. 2000).

Only a few studies have investigated the use and uptake mechanisms of inorganic carbon (C_i) for endosymbiont photosynthesis in larger foraminifera. Carbon dioxide has to permeate several membranes from the ambient seawater to the site of CO_2 fixation. The direct uptake of $^{14}CO_2$ through the thin lateral test walls of the imperforate species *Amphisorus hemprichii* was measured by Hansen and Dalberg (1979), and in an earlier study we found a fast exchange of O_2 and CO_2 through the perforate shell of *Amphistegina lobifera* (Köhler-Rink and Kühl 2000). Ter Kuile et al. (1989a) assumed different C_i transport mechanisms in perforate and

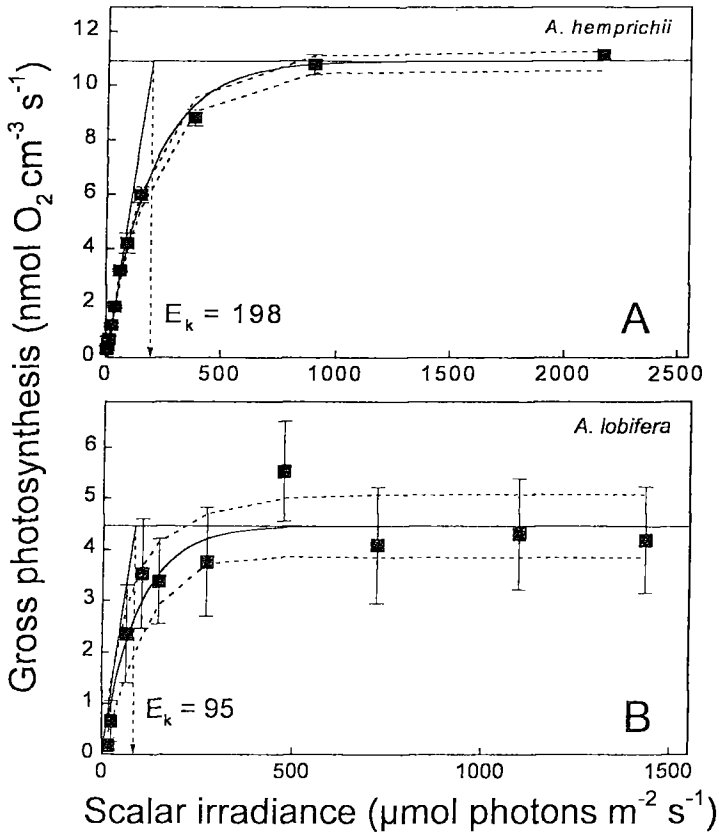

Fig. 1. P vs. E_0 curves obtained from individual specimens of *Amphisorus hemprichii* (A) and *Amphistegina lobifera* (B). Gross photosynthesis rates as a function of increasing scalar irradiance were measured at different positions on the shell surface. Symbols and error bars indicate mean ± standard deviation (n = 3-7). Solid lines indicate an exponential function fitted to the data (Webb et al. 1974). Dashed lines indicate 95% confidence intervals. E_k denotes the irradiance at onset of photosynthesis saturation.

imperforate larger foraminifera due to different calcification mechanisms. In a second study, ter Kuile et al. (1989b) concluded that photosynthesis and calcification compete for inorganic carbon in perforate species, while no competition was present in the imperforate species. It was hypothesized that the presence of carbonic anhydrase (CA), which catalyses the conversion of HCO_3^- to CO_2, shifts the balance between photosynthesis and calcification in favour of photosynthesis.

Few studies of irradiance effects on photosynthesis in larger foraminifera have been published. Stimulation of growth and ^{14}C uptake as a function of irradiance has been demonstrated in laboratory and field studies of *Amphistegina* spp., *Heterostegina depressa*, and *Sorites marginalis* (Röttger 1976, Hallock 1981, Duguay 1983). Lee et al. (1980) found photosynthesis inhibition in *A. lobifera* and *A. hemprichii* at very high irradiances (>3300 µmol photons m^{-2} s^{-1}). The species *Amphistegina lessonii* and *H. depressa* were photoinhibited at lower irradiances (~330 µmol photons m^{-2} s^{-1}).

The first data on the chemical microenvironment of larger foraminifera were published by Köhler-Rink and Kühl (2000). In the present study we used O_2, pH, and CO_2 microsensors as well as a miniature gas exchange chamber system to further investigate the photosynthesis and respiration of the symbiont-bearing larger foraminifera *A. lobifera* and *A. hemprichii*. We focus on gas exchange and on local dynamics of water chemistry on the shell surface of the foraminifera. Our data add to the small database of published photosynthesis and respiration rates in larger foraminifera and present new information on the local dynamics of

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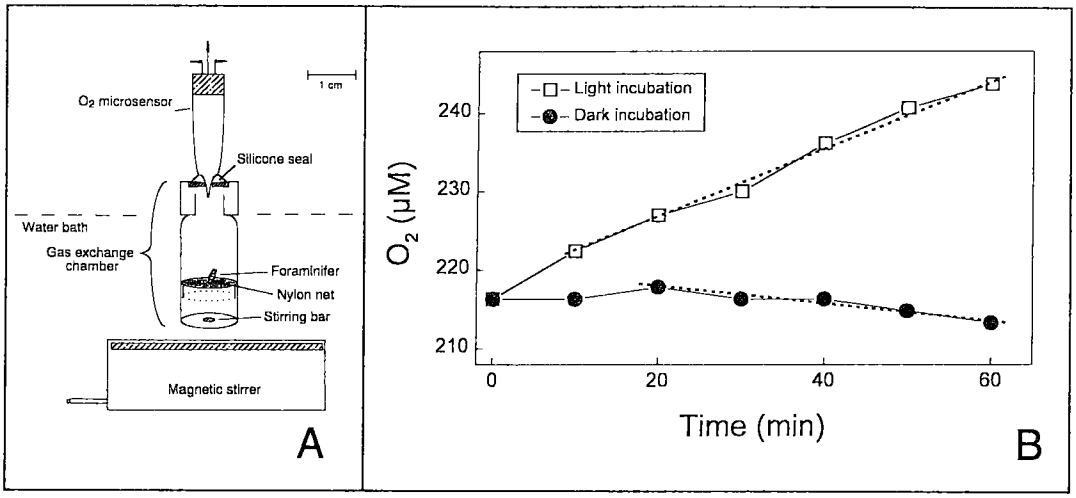

Fig. 2. **A** Schematic drawing of a flux chamber (V=1.6 ml) for gas exchange measurements on single foraminifera. An O_2 microsensor was inserted through the airtight lid into the glas chamber. **B** Net O_2 production and consumption of *Amphistegina lobifera* measured over a time of 1 h (scalar irradiance=598 µmol photons m^{-2} s^{-1}). Dashed lines indicate calculated slopes.

the carbonate system in the vicinity of foraminifera.

RESULTS

Gross photosynthesis

Gross photosynthesis rates at the shell surface versus scalar irradiance (P vs. E_0) showed no photoinhibition in *A. hemprichii* and *A. lobifera* up to 2000 µmol photons m^{-2} s^{-1} (Fig. 1). The P vs. E_0 curves exhibited a P_{max} of 4.5 nmol O_2 cm^{-3} s^{-1} for *A. lobifera* (one specimen investigated) and 10.9-16.6 nmol O_2 cm^{-3} s^{-1} for *A. hemprichii* (three specimens investigated). The scalar irradiance at the onset of light saturation, E_k, was 95 µmol photons m^{-2} s^{-1} for *A. lobifera* and 164-198 µmol photons m^{-2} s^{-1} for *A. hemprichii.*

Net O_2 production and consumption

Net O_2 production (= gross photosyntheis - symbiont respiration - host respiration) at saturating irradiance (598 µmol photons m^{-2} s^{-1}) and net O_2 consumption under dark conditions (= the sum of symbiont and host respiration) were measured for single foraminifera during short-term incubations in a miniature gas exchange chamber (Fig. 2). Net O_2 production rates of *A. lobifera* ranged between 3.7 and 22.7 nmol O_2 $foraminifer^{-1}$ h^{-1} (n=5) and their dark respiration rates varied between 5.6 and 14.3 nmol O_2 $foraminifer^{-1}$ h^{-1} (n=5) (Table 1). One specimen of *A. hemprichii* showed a net O_2 production of 13 nmol O_2 $foraminifer^{-1}$ h^{-1} and a dark respiration of 9.9 nmol O_2 $foraminifer^{-1}$ h^{-1}. We calculated an average ratio of net photosynthesis to respiration (P_{net}/R_{dark}) of 1.5 ± 0.6 (mean ± SD, n=5) for *A. lobifera*, and of 1.3 (n=1) for *A. hemprichii.*

Dynamics of O_2, CO_2, and pH at the shell surface

Measurements of O_2, CO_2, and pH dynamics were performed at the shell surface of the imperforate species *A. hemprichii* during experimental light-dark cycles (Fig. 3). Due to the symbiont photosynthesis, O_2 and pH increased while CO_2 decreased during the light period. In darkness the combined host and symbiont respiration resulted in an O_2 and pH decrease and a CO_2 increase at the shell surface. Oxygen, CO_2 , and pH at the shell of *A. hemprichii* changed simultaneously and at a fast rate during experimental light-dark cycles (Fig. 3). The CO_2 concentration at the shell surface varied from ~4-6 µM in darkness to ~20-24 µM in the light. The CO_2 level in the surrounding waters was ~10 µM. The pH at the shell surface varied from ~pH 8.10 in darkness to ~pH 8.20 in the light. The pH in the surrounding waters was ~ pH 8.15. The O_2 concentration at the shell surface of *A. hemprichii* varied from ~150 µM (~70% of the air saturated surrounding water) in darkness to ~240 µM (~110% of air saturation) in the light.

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Table 1. Net oxygen production, dark respiration and estimated gross photosynthesis of *Amphistegina lobifera* and *Amphisorus hemprichii*.

Foraminifer Diameter (mm)	Net O_2 production	Dark respiration (nmol O_2 foraminifer^{-1} h^{-1})	Gross photosynthesis
Amphistegina lobifera			
2.0	22.69	11.34	34.03
1.9	8.37	5.58	13.95
1.8	3.72	5.58	9.30
1.8	16.08	14.29	30.37
1.7	14.74	7.15	21.89
Mean ± SD	13.12 ± 7.31	8.79 ± 3.87	21.91 ± 10.51
Amphisorus hemprichii			
3.9	13.00	9.90	22.90

Average rates (n=2) of O_2 release/uptake (dO_2/dt) and of CO_2 uptake/release (dCO_2/dt) were calculated over the first seconds of the experimental light-dark and dark-light shifts. In the dark *A. hemprichii* showed an O_2 uptake rate of 1.03 and CO_2 release of 0.53 µM s^{-1}. In

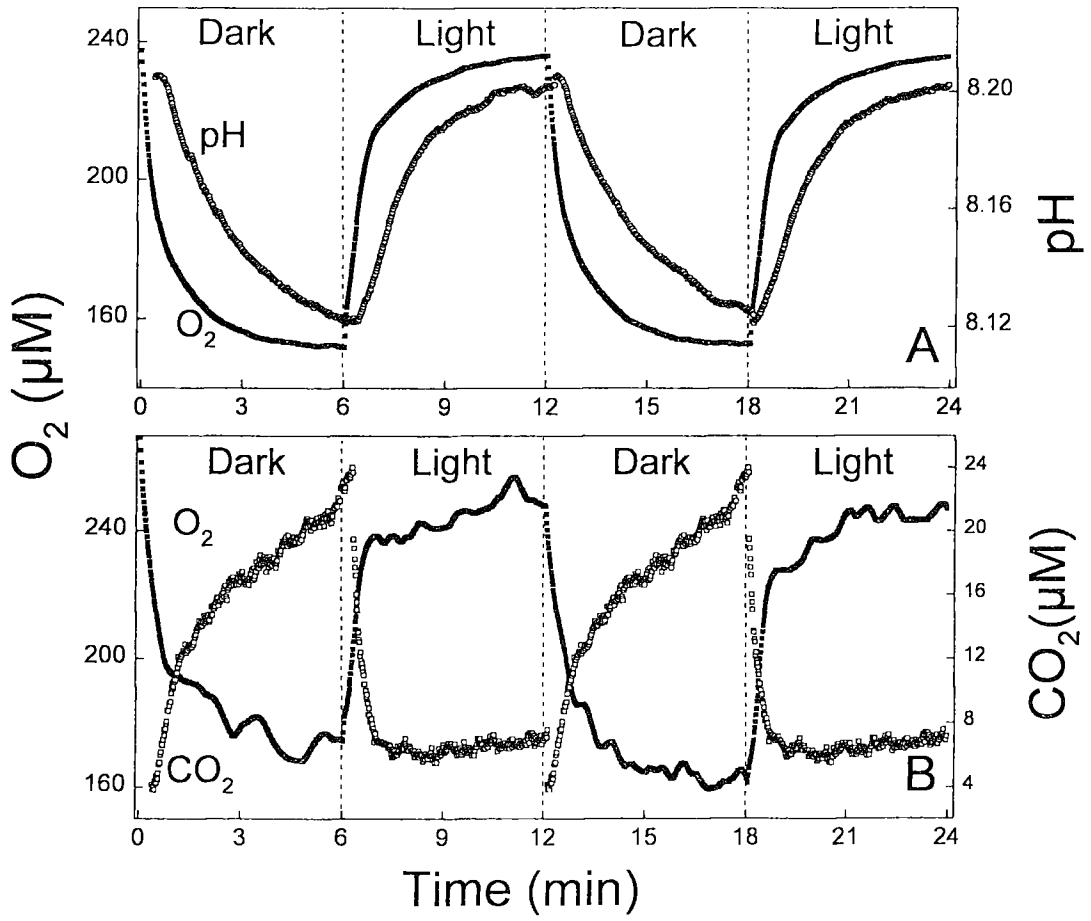

Fig. 3. Combined measurements of O_2 and pH (A), and O_2 and CO_2 (B) dynamics at the shell surface of *Amphisorus hemprichii* during experimental light-dark cycles (scalar irradiance = 332 µmol photons m^{-2} s^{-1}). Dashed lines indicate experimental light switches.

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the light periods we measured a CO_2 uptake of 0.31 μM s^{-1} compared to an O_2 release of 1.98 μM s^{-1}. The O_2/CO_2 molar conversion ratio at the shell surface of *A. hemprichii* thus was ~2 in darkness and ~6 under saturating irradiances.

DISCUSSION

Irradiance effects on symbiont photosynthesis

The photosynthesis vs. scalar irradiance (P vs. E_0) characteristics of the benthic foraminifera (Fig. 1) were comparable to those reported for isolated high-irradiance adapted symbiotic algae (Alberte et al. 1986, Iglesias-Prieto and Trench 1994). The maximum gross photosynthesis rates (P_{max}) of the benthic foraminifera *A. hemprichii* and *A. lobifera* were of the same magnitude as photosynthesis rates measured in planktonic foraminifera and corals (Kühl et al. 1995, Rink et al. 1998). *A. hemprichii*, hosting symbiotic dinoflagellates, showed higher E_k values (164-198 μmol photons m^{-2} s^{-1}) as compared to the investigated *A. lobifera* harboring symbiotic diatoms (95 μmol photons m^{-2} s^{-1}). Our E_k values fall within the range reported for endosymbionts of planktonic foraminifera and hermatypic corals, i.e. 75-275 μmol photons m^{-2} s^{-1} (Jørgensen et al. 1985, Kühl et al. 1995, Rink et al. 1998). It is important to note, however, that photosynthesis vs. irradiance curves are prone to change due to photoacclimation, and more comprehensive studies of the photosynthetic performance of larger formainifera under *in-situ* light conditions are needed. Our data were obtained on a rather limited number of specimens and we did not attempt to normalize our data to body-size or biomass (e.g. chlorophyll content or dry weight) in order to investigate the intra-species variation of total gross photosynthesis. Our light-dark shift measurements relate to the gross photosynthesis of symbionts found close to the shell surface, and it is difficult to extrapolate from such data to the total gross photosynthesis per specimen.

Most studies of the photosynthesis response curves of symbiotic dinoflagellates do not show a decreasing photosynthesis, i.e. photoinhibition, at high irradiance (Harland and Davies 1995, Goiran et al. 1996). In our study of *A. hemprichii* and *A. lobifera* no photoinhibition was observed up to 2000 μmol photons m^{-2} s^{-1}. Symbiotic algae living in high-irradiance environments have developed short-term photoprotective mechanisms to suppress the oxidative damage to the photosynthetic apparatus e.g. the interconversion of carotenoids via the xanthophyll cycle or via enhanced thermal dissipation in the Photosystem II reaction centers (Falkowski and Raven 1997). Long-term photoacclimatory processes can involve changes in the abundance and composition of photosynthetic pigments (Iglesias-Prieto and Trench 1994). We speculate that the symbionts of benthic foraminifera are able to adapt their pigment content to environmental light stress and probably possess protective mechanisms against intense radiation and UV-damage, but the presence of such adaptation mechanisms is yet to be demonstrated. A light shielding function of the calcite shells of benthic foraminifera also avoids photoinhibition at high irradiances. In a previous study (Köhler-Rink and Kühl 2000), we estimated that ~30% of the incident irradiance (400-700 nm) is transmitted through the calcite shell to the symbionts within the host cytoplasm. Lee et al. (1980) measured photoinhibition in *A. hemprichii* and *A. lobifera* at very high incident irradiances (>3300 μmol photons m^{-2} s^{-1}). With our data on light transmission, the data of Lee et al. (1980) would thus indicate photoinhibition at an irradiance >1000 μmol photons m^{-2} s^{-1} below the calcite shell. Pulses of high irradiance can reach the sea floor in clear shallow waters due to focusing effects of surface waves (Dera and Gordon 1968, Falkowski et al. 1990), but to our knowledge the importance of such high irradiance pulses for the photosynthetic performance of aquatic phototrophs has not been investigated.

Primary production of benthic foraminifera

The gross photosynthesis rates and net O_2 production rates measured on whole specimens in the present study are in the same order of magnitude as previously reported primary production rates of benthic foraminifera as measured with 14C incorporation and respirometry (Smith and Wiebe 1977, Muller 1978, Lee et al. 1980, Röttger et al. 1980, Hallock 1981, Duguay 1983, ter Kuile et al. 1989a). *Sorites marginalis* showed maximum carbon uptake rates of 100 ng C (mg dw)$^{-1}$ h^{-1} and *Archais angulatus* reached up to 50 ng C (mg dw)$^{-1}$ h^{-1} (Duguay 1983). Assuming a O_2/CO_2 conversion ratio of 1 and a dry weight of 0.6 mg per *A. angulatus* specimen (Duguay 1983) this rate would

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amount to ~2.6 nmol O_2 foraminifer^{-1} h^{-1}. Hallock (1981) reported comparable rates of primary production in *A. lobifera* (2.9 x 10^{-5} mg 14C h^{-1} foraminifer^{-1} =~2.4 nmol O_2 foraminifer^{-1} h^{-1}) and in *Amphistegina lessonii* (1.95 x 10^{-5} mg 14C h^{-1} foraminifer^{-1} =~1.6 nmol O_2 foraminifer^{-1} h^{-1}). Ter Kuile et al. (1989a) estimated a maximum Ci uptake of 0.2 µg C mg^{-1} foraminifer^{-1} h^{-1} assuming an average weight of 298 µg and a surface area of 1.76 mm^2 for a 1 mm large *A. lobifera*. Calculating the C uptake on a per foraminifer basis results in ~5 nmol C foraminifer^{-1} h^{-1}, which is comparable to our measurements. In this comparison we assumed a photosynthetic quotient, PQ, of 1. However, PQ values can depend on prevailing O_2 and CO_2 concentrations, nutrients used by photosynthesis, light conditions, photosynthetic products as well as the foraminiferal calcification (Burris 1981, Gattuso and Jaubert 1988, Geider and Osborne 1992).

We measured total net O_2 production and consumption of the foraminiferal-algal association (nmol O_2 foraminifer^{-1} h^{-1}). Assuming similar respiration rates in darkness and light, we estimated an average gross photosynthesis rate of 22 nmol O_2 h^{-1} for *A. lobifera* (n=5) and 23 nmol O_2 h^{-1} for *A. hemprichii* (n=1) (Table 1). Gross photosynthesis rates could, however, be even higher due to higher respiration rates in light as compared to dark respiration (Kühl et al. 1995, Rink et al. 1998). In addition, the comparison of net photosynthesis and dark respiration rates in *A. lobifera* and *A. hemprichii* point to an efficient carbon and nutrient cycling in the foraminiferal-algal association. P_{net}/R_{dark} ratios of 1.3-1.5 indicate that the symbiotic carbon release can account for a major part of the foraminiferal carbon assimilation. This is in line with earlier studies showing that diatom symbionts in the benthic foraminifer *Archais angulatus* release ~60% of the non-respired fixed carbon to their host (Lee et al. 1974). In addition, the oxygen production of the symbionts in light alleviates the oxygen limitation of host respiration in larger foraminifera. Diffusion limitation is evident from oxygen profiles measured in darkness towards the shell surface of *A. hemprichii* (Köhler-Rink and Kühl 2000) and *A. lobifera* (data not shown), and the observation that dark respiration of the foraminifera increases with increasing flow velocity (Köhler-Rink and Kühl 2000).

Importance of foraminifera for coral reef productivity

The total annual production on coral reefs ranges between 300 and 5500 g C m^{-2} yr^{-1}, which is significantly higher than the open ocean productivity of 21-183 g C m^{-2} yr^{-1} (Muscatine 1990, Gattuso et al. 1996). Symbiont bearing foraminifera live in dense populations on sediments and as epifauna on macroalgae, seaweeds, and corals. Duguay (1983) reported maximum population densities of *Archais angulatus* of >15.000 foraminifera m^{-2}. Hansen and Buchardt (1977) found densities of 3 individuals cm^{-2} of living *Amphistegina* sp. Muller (1974) found 1.41- 41.8 x 10^4 foraminifera m^{-2} of the species *Amphistegina madagascariensis*. The potential contribution of symbiotic foraminifera to the primary production within a reef system was first described by Sournia (1976) who measured the primary production of sand communities that were dominated by symbiotic foraminifera and estimated daily rates of 0.43 - 1.33 g C m^{-2} d^{-1}. Boucher et al. (1998) found a net production of ~29 g C m^{-2} yr^{-1} in a barrier reef sediment.

To give a first approximation of the daily rate of net primary production of benthic larger foraminifera based on our data of *A. lobifera* we assumed a net production of 13 nmol O_2 h^{-1} foraminifer^{-1} (Table 1), a 12:12 h light: dark period, and a population density of 15.000 individuals m^{-2}. In accordance with Muscatine (1980) the photosynthetic O_2 evolution was converted into carbon units using the empirical relationship of g C = 0.375 * g O_2 (Alberte et al. 1986). Thereby, we calculated a potential daily primary productivity of 14 mg C m^{-2} d^{-1} amounting to a yearly rate of about 5.1 g C m^{-2} yr^{-1}. However, this value probably overestimates the production rate due to carbon losses by growth, excretion, or symbiont photorespiration. In addition, foraminifera densities vary and the incident irradiance changes in a daily pattern and with depth (Muller 1974, Lee and Bock 1976, Falkowski et al. 1984, Muscatine 1990). However, benthic foraminifera can reach maximum densities up to 40.000 individuals m^{-2} after a reproduction period and this would, therefore, lead to higher production rates.

Dynamic microenvironmental changes

Larger foraminifera live in a dynamic environment of variable light and flow conditions, which modulate the chemical microenviron-

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ment in the vicinity of the foraminifera (Köhler-Rink and Kühl 2000). In the light, photosynthetic uptake of inorganic carbon (CO_2, HCO_3^-) increases the pH, which in turn increases the CO_3^{2-} concentration according to the seawater carbonate system:

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \Leftrightarrow 2\,H^+ + CO_3^{2-}$$

(Barnes and Chalker 1990, Stumm and Morgan 1996). In the dark, opposite concentration changes are to be expected due to CO_2 release by respiration and calcification. The level of CO_2 is thus a key variable for inorganic carbon transfer between these processes.

We found rapid concentration changes of O_2 and CO_2 at the shell surface of *A. hemprichii* (Fig. 3) demonstrating for the first time a fast exchange of metabolic gases through imperforate shells of benthic foraminifera. Thus the porcelaneous shell does not seem to limit the diffusive exchange of dissolved gases across the calcite wall significantly. Fast gas exchange of O_2 and CO_2 were also measured with microsensors at the hyaline perforate shell of *A. lobifera* (Köhler-Rink and Kühl, 2000). The fast response of the chemical microenvironment to changes in irradiance underlines the close coupling of autotrophic and heterotrophic processes within the foraminiferal-algal association, where phototrophic O_2 release and CO_2 uptake of the symbiotic algae occurs in close proximity to the respiratory O_2 consumption and CO_2 production of the host.

Combined recordings of the O_2 and CO_2 concentrations at the shell surface of *A. hemprichii* (this study) and of *A. lobifera* (Köhler-Rink and Kühl 2000) showed simultaneous changes of both. The immediate CO_2 response indicates a rapid reaction of the CO_2 fixation process of the endosymbionts that, subsequently, resulted in a pH increase at the shell surface (Fig. 3). The dynamics of the chemical microenvironment has also been measured at the tissue surface of the large foraminifera *Marginopora vertebralis* (Köhler-Rink and Kühl unpublished data) and of a *Favia* sp. coral (de Beer et al. 2000) , but here a time delay in the CO_2 and pH dynamics was recorded during experimental light-dark shifts, and the external CO_2 concentration changes were apparently not tightly coupled to microalgal photosynthesis. A fast O_2 dynamics at the tissue surface in these species indicated, however, that symbiont photosynthesis was supplied with sufficient CO_2, apparently not originating directly from the surrounding seawater.

In addition to photosynthesis and respiration, the extracellular pH changes can be affected by several metabolic processes including nitrogen assimilation, organic acid assimilation/excretion, as well as cation and anion fluxes (Smith and Raven 1976, Stumm and Morgan 1996). However, it still remains unknown if these processes influence the fast pH variations at the shell surface during the light-dark cycles. The importance of photosynthetic products in altering pH during photosynthesis experiments is considered of minor importance (Geider and Osborne 1992).

Inorganic carbon sources

To sustain the high photosynthesis rates measured in symbiotic foraminifera there is a need for sufficient supply of inorganic carbon (C_i). In larger foraminifera inorganic carbon can be supplied by diffusive transport of external CO_2 and HCO_3^- and/or by internal sources like respiration and calcification (Fig. 4). Transport of external C_i occurs from the ambient seawater through the foraminiferal shell into the cytoplasm of the foraminifer, where the symbionts are located (Hansen and Dalberg 1979, ter Kuile et al. 1989a, Köhler-Rink and Kühl 2000). The rate of C_i diffusion to the microalgae is an important factor regulating the inorganic carbon supply for photosynthesis. The molecules have to permeate several membranes to reach the site of CO_2 fixation. In foraminifera the diffusional C_i uptake is additionally limited by a diffusive boundary layer (DBL) that surrounds the calcite shell (Köhler-Rink and Kühl 2000). The thickness of the prevailing DBL is affected by the flow conditions, the characteristic roughness of the surface and the size of the organism (Jørgensen and Revsbech 1985, Vogel 1994). In previous experiments with benthic foraminifera we measured a flow dependent DBL thickness of 100-700 µm and we found enhanced gross photosynthesis and respiration rates under increased flow velocities (Köhler-Rink and Kühl 2000), while net photosynthesis rates were indifferent.

The large HCO_3^- pool in the surrounding sea water constitutes a major Ci source for photosynthesis. However, the uncatalyzed conversion of HCO_3^- to CO_2 in seawater is slower than the rate at which CO_2 can be assimilated by marine algae. Therefore maximum rates of carbon fixation can only be maintained by

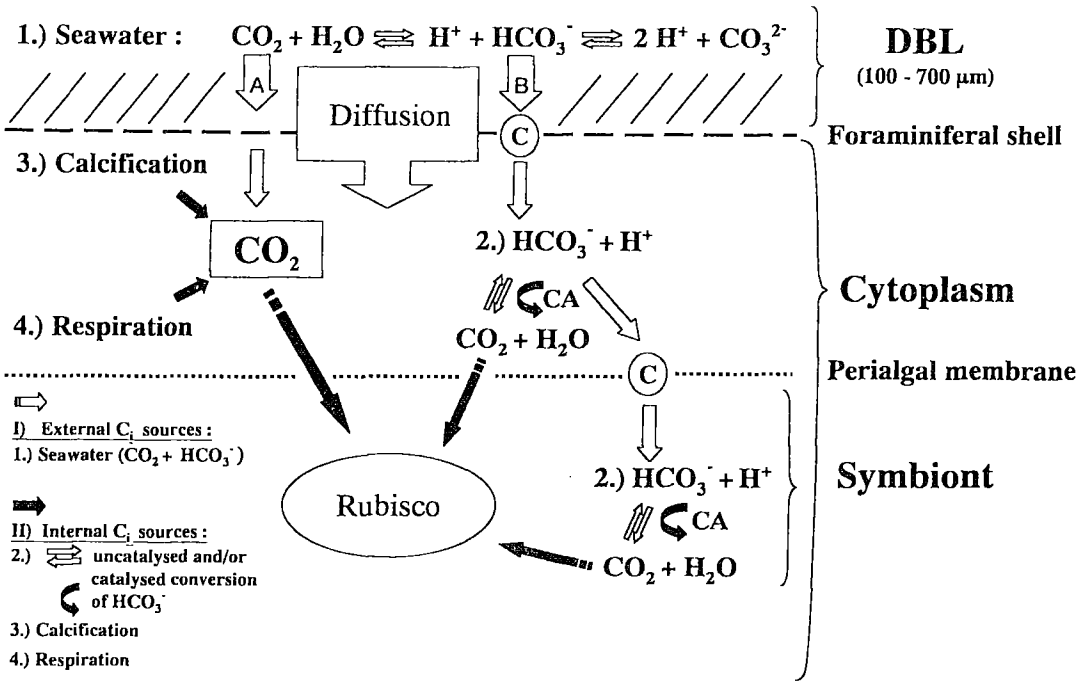

Fig. 4. Schematic diagram of potential external and internal sources of inorganic carbon (C_i) and related processes for photosynthetic carbon assimilation of the symbionts in larger foraminifera (A = CO_2 uptake; B = HCO_3^- uptake; DBL = diffusive boundary layer; CA = carbonic anhydrase; Rubisco = Ribulose-1,5-bisphoshat carboxylase/oxygenase; C = carrier for HCO_3^- transport) (based on Al-Moghrabi et al. 1996, Falkowski and Raven 1997).

HCO_3^- if dehydration is catalyzed enzymatically by carbonic anhydrase (CA) (Raven 1994, Falkowski and Raven 1997). Two active inorganic carbon transport processes have been reported for symbiotic cnidarian species (Weis et al. 1988, Al-Moghrabi et al. 1996): i) a CA catalyzed dehydration of extracellular HCO_3^- and subsequent diffusive CO_2 transport to the Rubisco and ii) an active HCO_3^- transport across the plasma membrane via a carrier protein and subsequent internal conversion to CO_2 by CA. Under conditions of external CO_2 limitation extracellular activity of carbonic anhydrase could be demonstrated (Tsuzuki and Miyachi 1989, Badger and Price 1992). Carbonic anhydrase activity was also found intracellularly on the plasmalemma (Falkowski and Raven 1997, Nimer et al. 1999), and in the symbiotic dinoflagellate *Symbiodinium* spp. light-activated intracellular CA activity was demonstrated (Leggat et al. 1999).

Results of net O_2 and CO_2 flux calculations at the shell surface of larger foraminifera showed higher rates of O_2 release as compared to the CO_2 uptake due to symbiont photosynthesis in the light. Molar O_2/ CO_2 conversion ratios at the shell surface of the perforate species *A. lobifera* were higher in the light as compared to darkness. Similar results have also been found in the imperforate species *Marginopora vertebralis* (Köhler-Rink and Kühl 2000). This indicates a major internal CO_2 supply resulting from respiratory CO_2 release and/or the conversion of HCO_3^- to CO_2 catalyzed by carbon anhydrase. Mechanisms for inorganic carbon uptake in *A. lobifera* and *A. hemprichii* were previously studied by ter Kuile et al. (1989a). They measured photosynthesis saturation at Ci levels of seawater in the cultured symbionts of *A. lobifera*, i.e. the diatom *Fragilaria shiloi*, and suggested that Ci is transported into the foraminifer in the form of HCO_3^- and, subsequently, is converted intracellularly to CO_2, which is fixed by the symbionts. In *A. hemprichii* a parallel uptake of CO_2 and HCO_3^- was assumed. Supplemental CO_2 can also be produced as a result of carbonate deposition at the foraminiferal shell. If HCO_3^- is used as a substrate for calcification the reaction: $2\,HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + CO_2 + H_2O$ results in a net CO_2 release (Stumm and Morgan 1996).

The use of microsensor techniques now

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allows detailed studies of symbiont photosynthesis and the CO_2 and pH microenvironment. Detailed information on the carbonate system in the vicinity of the foraminiferal shell can now be gained and incorporated in model calculations, as it has already been done in planktonic foraminifera (Wolf-Gladrow et al. 1999). Furthermore, future microsensor experiments in combination with specific inhibitors of e.g. external and internal CA could reveal more details of the mechanisms involved in inorganic carbon dynamics in foraminifera. Our data show that the carbonate system close to the shell of larger foraminifera cannot simply be considered in equilibrium with the carbonate system in the ambient seawater; an assumption that often has been used for the interpretation of foraminiferal isotope data (Epstein et al. 1953, Duplessy et al. 1970). Our results support other observations indicating that vital effects, i.e. physiological processes that cause an isotopic disequilibrium, play a major role in variations of oxygen (^{18}O) and carbon (^{13}C) isotopic compositions of foraminiferal calcite shells (Williams et al. 1981, Wefer and Berger 1991).

MATERIALS AND METHODS

Sampling of foraminifera

The perforate species *Amphistegina lobifera* and the imperforate *Amphisorus hemprichii* were hand collected from ~ 5m depth by snorkeling in the clear oligotrophic water of the Gulf of Aqaba, Red Sea in June 1998. The *in-situ* salinity was S=40 and the water temperature was 22 °C. The foraminifera, living attached to small biofilm-coated stones, were transported to the laboratory in Bremen, Germany within a few days. They were maintained in an aquarium with aerated artificial seawater (Sel Marine, HW, Sea Salt Professional; S=40; pH 8) at room temperature (20–22 °C) under a natural light-dark cycle. The light source was a halogen lamp (Osram Dulux 11W/21, Germany) and maximal irradiance was ~400 µmol photons m^{-2} s^{-1}. *A. lobifera* specimens used in this study had a diameter of 1.1-3.5 mm and *A. hemprichii* specimens had a diameter of 1.6-7.4 mm. Prior to experiments, the foraminifera were carefully cleaned off adhered algae with an artist's brush and rinsed several times in seawater.

Experimental setup

A foraminifer was placed on the bottom of a small flow chamber (Köhler-Rink and Kühl 2000) that was illuminated with a fiber optic halogen lamp (Schott KL-1500, Germany). Different irradiance levels (0-2000 µmol photons m^{-2} s^{-1}) were obtained with varying combinations of neutral density filters (Oriel Inc., USA) inserted in the light path. In the setup, quantum scalar irradiance (E_0) was measured at the foraminiferal position with a quantum scalar irradiance meter (Biospherical Instruments Inc., QSL 101, USA). Experimental light-dark shifts were performed with an electro-mechanical shutter (Vincent Assoc., USA), installed in the light path of the halogen lamp. The shutter, data acquisition, and microsensor positioning were controlled via custom made software (LabVIEW; National Instruments, USA). The microsensors were fixed to a motor driven micromanipulator (Märtzhäuser & LOT-ORIEL, Germany) and the surface positioning was controlled with the aid of a dissection microscope. Measurements were performed at ambient room temperature (20-22°C) under a defined light and flow regime. The latter was created with an underwater aquarium pump circulating aerated water (S=40) through the flow chamber. The foraminifera were allowed to adapt to the flow chamber conditions for 0.5-1.0 h prior to the experiments.

Microsensor measurements

Clark-type O_2 microsensors (Revsbech 1989) with a t_{90} response time of <0.4 s were used for measurements of gross photosynthesis rates and O_2 dynamics at the shell surface of foraminifera. Carbon dioxide and pH dynamics at the foraminiferal shell were measured according to de Beer et al. (1997) with a LIX-type pH microelectrode in combination with a calomel reference electrode (Radiometer 401, Denmark) and with a CO_2 microsensor. More detailed accounts of microsensor characteristics, calibration methods, and data acquisition are presented elsewhere (Köhler-Rink and Kühl 2000).

Photosynthesis and respiration measurements

Symbiont gross photosynthesis rates (nmol O_2 cm^{-3} s^{-1}) were performed as point measurements with the light-dark shift technique (Revsbech and Jørgensen 1983). Fast responding O_2 microsensors were positioned at the shell surface, and the initial rate of O_2 depletion within the first seconds after darkening is

identical with the rate of gross photosynthesis during the preceding light period (Revsbech and Jørgensen 1983, Glud et al. 1992, Kühl et al. 1996). The photosynthesis is measured just around the electrode tip at a spatial resolution of ~0.1 mm, which allowed for monitoring the activity of symbionts just below the shell surface.

Photosynthesis vs. irradiance curves

Gross photosynthesis rates at the shell surface of the foraminifera were measured at increasing irradiance from 0 to 2000 µmol photons m^{-2} s^{-1}. For each investigated specimen 3-7 different positions at the shell surface were investigated. The P vs. E_0 data were fitted by non-linear regression (Origin 3.0, MicroCal Software, Inc.) with the exponential function of Webb et al. (1974)

$$P = P_m [1 - \exp(-\alpha E_0 / P_m)] \quad (2)$$

where P_m is the maximal photosynthetic rate at light saturation and a the initial slope of the P vs. E_0 curve. The irradiance for onset of photosynthesis saturation, E_k, was calculated as $E_k = P_m/\alpha$.

Net O_2 production and consumption

Total rates of oxygen exchange (nmol O_2 foraminifer^{-1} h^{-1}) of single specimens were measured with an O_2 microsensor in a small flux chamber (V=1.6 ml) (Fig. 2A). Robust Clark-type O_2 microsensors were made with a short shaft and an outer tip diameter of ~1 mm (Glud et al. 1994). The electrode was inserted through a silicone/Teflon seal of the glass chamber lid and fixed with additional silicone (Fig. 2A). For the measurements, artificial sterile filtered seawater (Sel Marine, Germany) was used to exclude contamination and background O_2 consumption in the chamber. The foraminifera were cleaned carefully with an artist's brush and washed several times in seawater, before they were transferred to the chamber. Continuous mixing in the chamber was maintained with a miniature magnetic stirrer bar controlled by an underwater stirrer (Variomag, H+P Labortechnik, Germany). The setup was installed in a thermostated water bath. Light was provided from the side by a halogen lamp (Schott, KL1500, Germany) and was measured at the chamber position with a quantum scalar irradiance meter (Biospherical Instruments Inc., QSL 101, USA). Linear calibration of the O_2 electrodes was done from readings in aerated and N_2 flushed seawater of known temperature and salinity, respectively. Total rates of oxygen production or consumption were calculated from the increase/decrease in O_2 concentration measured over a time period of 1-2 hours.

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