The physico-chemical microenvironment of symbiont-bearing foraminifera and their photosynthetic and respiratory characteristics studied with microsensors

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Stephanie Köhler-Rink

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1. Gutachter: Prof. Dr. Friedrich Widdel
2. Gutachter: Assistant Professor Dr. Michael Kühl

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Preface

The aim of this thesis was the investigation of photosynthesis, respiration, and calcification of symbiont-bearing planktonic and benthic foraminifera. Microsensors were used to study the combined effect of these major metabolic processes to the microenvironment around the foraminiferal shells. A general introduction to this thesis is given in chapter 1. The results are presented and discussed in chapter 2 - 5 (chapter 2 was published in Marine Biology 131: 583-595; chapter 4 is in press for publication in Marine Biology.)

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Chapter 1

General Introduction

1. General Introduction

Foraminifera are small marine, eukaryotic organisms of the phylum Sarcomastigophora in the sub-kingdom Protozoa (Fig. 1). They are structurally single-celled species, showing no tissue-level organization. Foraminifera protrude a network of pseudopodia for entrapping food, which are mainly algae, diatoms, protozoa, and microcrustaceans. The nutrient material is digested within the cytoplasm or drawn into the test, where digestion occurs in the endoplasm. Furthermore, pseudopodia are used for locomotion, attachment, and expulsion of waste material. The majority of foraminifera lives on the sea bottom, where they are either free-living or attached to the substrate. Most foraminifera are equipped with substantial shells of one or several chambers of mineral material (commonly calcium carbonate) according to a specific morphology (Bé 1977, Farmer 1980, Caron and Swanberg 1990, Murray 1991, Lee and Anderson 1991, Mather and Bennet 1994).

Kingdom : Protista (or Protoctista)
Subkingdom : Protozoa
Phylum : Sarcomastigophora
Subphylum : Sarcodina
Superclass : Rhizopodea
Class: Lobosea (amoebae)
Class : Granuloreticulosea (**foraminifera**)
Superclass : Actinopodea
Class : Acantharea (acantharia)
Class : Polycystinea (radiolaria)
Class : Phaeodaria (radiolaria)
Class : Heliozoa (heliozoa)

Fig. 1 Taxonomic position of foraminifera (based on Lee et al. 1985, Caron and Swanberg 1990).

1. 1. Ecology of symbiotic foraminifera

1. 1. 1. Planktonic foraminifera

Planktonic foraminifera are worldwide distributed marine protists that inhabit all oceans from the tropics to the polar seas (Bé 1977, Boltovskoy and Wright 1976, Murray 1991). They are floating microplanktonic organisms that are transported by the oceanic current system (Boltovskoy and Wright 1976). The productivity of planktonic foraminifera depends on several biological factors including their life span, growth rate, reproduction cycle, mortality, and turnover rate of each species (Bé 1977). Five major faunal provinces (polar, subpolar, transition, subtropical, tropical) of living planktonic foraminifera were described that show a clear correlation with the surface circulation pattern of the ocean (Sverdrup et al. 1942). Furthermore, the distribution of planktonic foraminifera is influenced by abiotic parameters like temperature, salinity, light, oxygen, and nutrient concentrations. The cold regions are marked by a low diversity and only a few indigenous species, whereas the warm regions have a high diversity and many indigenous species (Bé 1977). Highest densities were reported for plankton-rich areas of the major currents, boundary currents, divergence zones, and upwelling areas. Oligotrophic central water masses and continental shelves showed low densities. The abundance of living foraminifera in surface waters ranged from < 1 up to 100.000 specimens per 1 m^3 (Berger 1969). The concentration is greatest in surface waters and decreases rapidly with depth. In coastal waters with relatively steep bottom slope and no significant input from freshwater run off, as found e.g. along the Californian coast, numerous planktonic foraminifera have been observed. At greater distances from the shore the number of planktonic species increases. Symbiont-bearing species are restricted to the euphotic zone, where the photoautotrophic microalgae are exposed to sufficient light for photosynthesis. The highest densities of symbiont-bearing planktonic foraminifera are generally found in a depth of 10-50 m below the surface (Bradshaw 1959, Bé 1960a, Boltovskoy 1964). Furthermore, the majority of spinose species (i.e. with attached calcite spines) are surface dwellers, whereas the non-spinose species live preferentially below 50 m.

Planktonic foraminifera show diurnal migration patterns and a vertical migration during their ontogeny. During their growth they migrate between the reproductive depth (30 - >200 m) and the uppermost part of the photic zone (Berger 1969, Spindler and Hemleben 1983). In addition, it was recognized that the reproductive cycle of symbiont-bearing spinose foraminifera is controlled by the lunar cycle. The population of spinose species is strongly reduced during the period of the full moon. After this period a steady increase in individual numbers was found in the surface waters (Spindler et al. 1978, Hemleben and Bijma 1994).

1. 1. 1. 1. Experimental organism

The spinose symbiont-bearing species ***Orbulina universa***, investigated in the present study, is described as an ubiquitous species in subtropical, tropical, and transitional waters. Highest population densities were found in the surface layers of strong current systems and upwelling regions near the continental margins (Bradshaw 1959). *O. universa* is a widely distributed species in the Equatorial and Southeast Pacific (Parker 1960) and in the subtropical zones (20 - 40° latitude) of the Atlantic and Indian ocean (Bé and Tolderlund 1971). In the North and Equatorial Pacific highest densities were observed in the cool California Current and near Hawaii (Bradshaw 1959). The dinoflagellate endosymbionts of *O. universa* were described as the small coccoid species *Gymnodinium béii* (Spero 1987).

O. universa is characterized as „intermediate water" species living predominantly in a depth of 50 - 100 m. The species tolerates salinities of 23 - 46‰ and temperatures of 12 - 31° C (Bradshaw 1959, Bé and Tolderlund 1971). Observations at Bermuda and Barbados showed a reproduction depth of *O. universa* between 100 - 200 m (Hemleben and Spindler 1983).

The sampling sites where we collected *O. universa* for this study were (1) the California Current near the Island Santa Catalina, California (Fig. 2A), and (2) the surface waters near Curaçao, Netherland Antilles, Caribbean Sea (Fig. 2B).

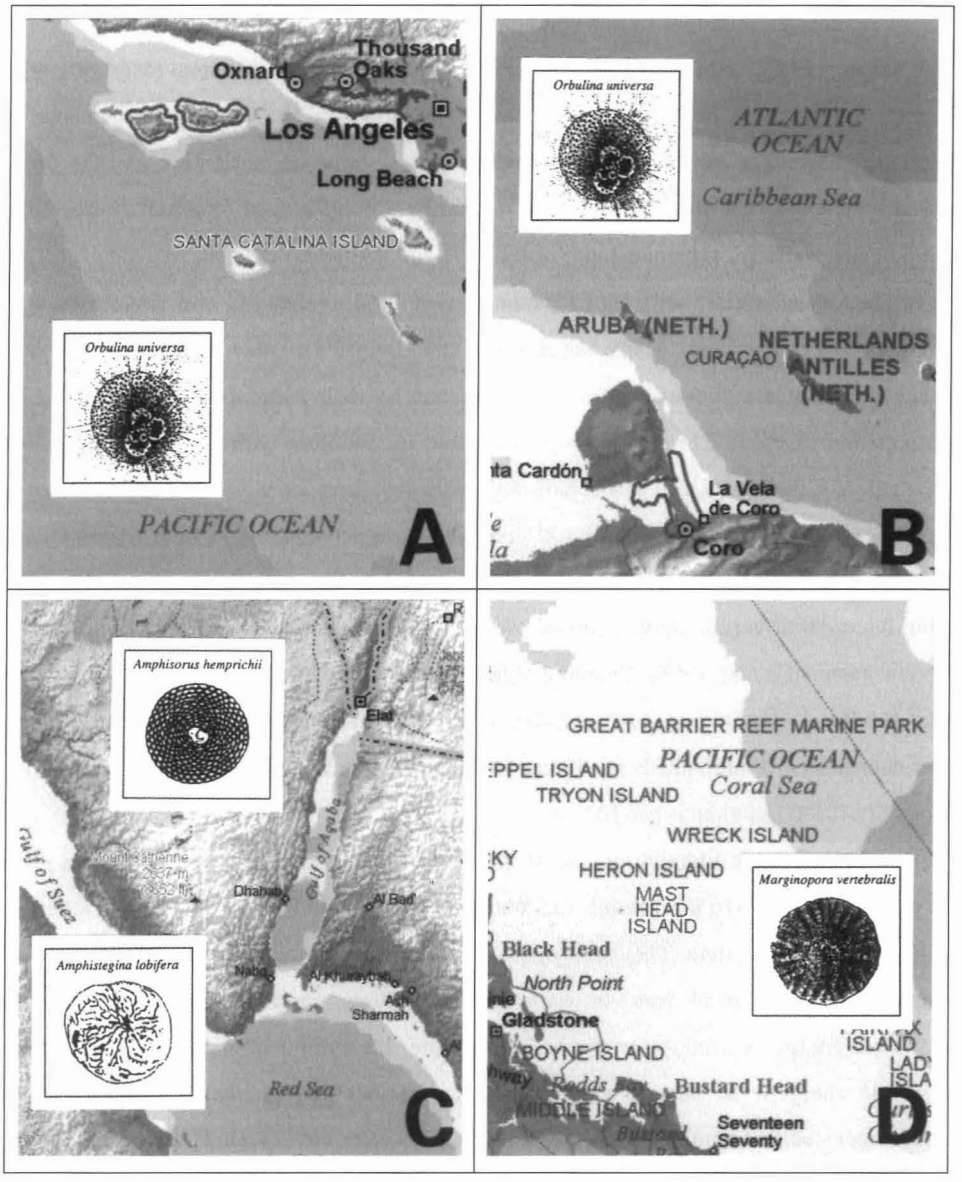

Fig. 2 Sampling sites of the present study. *Orbulina universa* was collected near Santa Catalina Island (**A**) and Curaçao, Netherland Antilles (**B**). *Amphisorus hemprichii* and *Amphistegina lobifera* were colleted in the Gulf of Aqaba (**C**) and at Heron Island, Great Barrier Reef (**D**).

1. 1. 2. Benthic foraminifera

Benthic foraminifera live as epifauna on various substrates such as rocks, seaweeds, macroalgae, corals, and the sediment, others as infauna within the sediment (Murray 1991). Benthic foraminifera inhabit diverse types of ecosystems. The major environments of benthic foraminifera are marshes, mangroves, lagoons, estuaries, shelf seas, and the deep-sea sediment. The distribution of benthic foraminifera is influenced by abiotic factors like temperature, salinity, substrate type, turbidity, light, nutrients, oxygen, and tidal energy. These factors interrelate with biotic parameters of food availability and abundance, and interspecific competition. Many species are free living, i.e. able to move with their pseudopodia, others live attached to the substratum by their pseudopodia (clinging) or by cementation (sessile). The relationship of benthic foraminifera with their substratum, the orientation of their tests, and to a certain degree the test form are closly linked with feeding strategies and the exposure to physical energy (e.g. water motion) in their environments (Murray 1991). Benthic foraminifera exhibit a typical patchy distribution. Several species from intertidal or very shallow waters show patchy distribution patterns on a scale of < 1 to several meters (Buzas 1968). Standing stocks can reach up to several hundred specimens cm^{-2} (Muller 1974, Duguay 1983, Murray 1991). Oligotrophic areas of low fertility show low densities (< 10 individuals per 10 cm^2) whereas high fertile areas have a higher standing stock of > 1000 individuals per 10 cm^2.

Most benthic foraminifera are of meiofaunal size (< 2 mm) but some species grow to giant protists of >16 mm (Smith and Wiebe 1977, Lee and Hallock 1987). The latter are called larger foraminifera. They are symbiont-bearing species that occur in shallow areas of tropical and subtropical seas. In warm shallow waters, metabolic rates are high and sufficient energy is needed for survival. Therefore, the symbiosis with autotrophic algae gives an energetic advantage, especially in nutrient-poor environments. Several authors have suggested that benthic foraminifera in shallow-water environments are adapted to the environmental conditions of their local habitat (Hallock et al. 1986, Murray 1991, Wetmore and Plotnick 1992). For example, the test strength of larger foraminifera that inhabit a high-energy exposed reef (i.e. high wave action) was greater as compared to species collected from a low-energy, sheltered seagrass flat. Moreover, the growth of some larger foraminifera mainly depends on photosynthesis of their endosymbionts and the

concentrations of nitrate and micronutrients in the surrounding seawater (Röttger et al. 1980). The different groups of endosymbionts seem to have varying light requirements. The study of Leutenegger (1984) investigated the depth distribution of several benthic foraminiferal groups. Foraminifera that host chlorophyceans were restricted to shallow waters (0 - 15 m). Dinophycean and rhodophycean symbionts were found in foraminifera living in a depth of 0 - 70 m, and diatom bearing species lived in depths of 0 - 130 m.

1. 1. 2. 1. Experimental organisms

For the present study, three different species of larger benthic foraminifera were collected from two different sites.

1. ***Marginopora vertebralis*** that belongs to the family Soritidae of the suborder Miliolina (= imperforated species), is characterized by a discoidal porcelaneous, calcerous shell. *M. vertebralis* that lives in symbiosis with dinoflagellates is a conspicious form in beach sediments, reaching shell diameters up to 2 cm. We collected *M. vertebralis* on the reef flat of Heron Island, Great Barrier Reef (Fig. 2D), where we found *M. vertebralis* attached to the calcerous algae *Halimeda macroloba*. *M. vertebralis* was described as major contributor to reef sedimentation (Smith and Wiebe 1977).

2. ***Amphisorus hemprichii,*** also a member of the family Soritidae (suborder Miliolina), consists of an imperforate porcelaneous shell (Hansen and Dalberg 1979). The dinoflagellate endosymbionts are usually distributed in parts of the shell away from digestive activities of the host. Like many other imperforate species, *A. hemprichii* acquires a significant part of its energy from ingestion, mainly of unicellular algae (ter Kuile et al. 1987).

3. ***Amphistegina lobifera*** is a member of the family Amphisteginidae (suborder Rotaliina = perforate species). As compared to *M. vertebralis* and *A. hemprichii*, this species hosts diatom endosymbionts (e.g. *Nitzschia* sp., *Fragilaria* sp.). Furthermore, it consists of a perforate calcerous shell. Amphistegenids are a very abundant group in shallow waters of tropical and subtropical seas where they live on surfaces of algae, macrophytes, and sediments (Hansen and Buchardt 1977, Hottinger 1993, Hohenegger 1994). Growth of *A. lobifera* was stimulated by the addition of nutrients to the medium (ter Kuile et al 1997). The ingestion of food seemed to be a less important energy source in this species.

For this study, *A. lobifera* and *A. hemprichii* were collected in the Gulf of Aqaba, Northern Red Sea (Fig. 2C), where they grow on biofilm coated stones in the sandy intertidal zone. For microsensor studies they were transported to the laboratory of the Max-Planck-Institute for Marine Microbiology, Bremen, Germany.

1. 1. 3. Foraminifer-symbiont interactions

The term „**symbiosis**" describes an association between different species of organisms with mutual benefit (Smith and Douglas 1987, Murray 1991, Lee and Anderson 1991) (Table 1). The association of foraminifera with unicellular algae is very succesful and a widespread phenomenon in the photic zone of nutrient-poor tropic and subtropic waters.

Table 1 Interactions between foraminifera and their symbiotic algae (based on Hausmann and Hülsmann 1996)

Symbionts benefit from:	Foraminifer benefits from:
- Optimal conditions for photosynthesis (light, CO_2)	- Release of symbiont photosynthates
- Habitat, constant environment, transportation	- Symbiont assimilation of metabolic waste products (nitrogen and phosphorus compounds)
- N- and P-containing host metabolites	
- Protection against predation	

It is suggested that foraminifera provide a good cellular habitat for the establishment and maintenance of algal symbionts due to the fact that they can host a diversity of algal types (Lee and Anderson 1991). Within the host cytoplasm, the symbionts are non-motile and lack cell envelopes such as diatom frustules, dinophycean thecae, or cysts. The symbionts are seperated from the host by a perialgal vacuole membrane, and it is still not known how they are protected against digestion by the host.

The endosymbionts probably provide a major part of organic carbon required for the host metabolism by releasing photosynthates to the foraminiferal cytoplasm. Photo-assimilatory products including polyglucan, glucose, lipids, and glycerol have been

identified in several larger foraminifera (Kremer et al. 1980, Battey 1992). The contribution of diatom symbionts to the nutrition of the benthic species *Archais angulatus* has been investigated by Lee et al. (1974). They found that 60% of the non-respired fixed carbon was released to the host. In addition, isolated symbionts of the benthic foraminifer *Amphistegina* spp. showed a stimulated **release of photosynthates** in the presence of host homogenate (Smith and Douglas 1987, Lee et al. 1994). This activating factor has not been identified in foraminifera but its presence suggested that the host tissue affects the **translocation of nutrients** within the symbiotic association. Recently, Gates et al. (1999) identified the `host factor´ in a symbiont-bearing sea anemone as a mixture of free amino acids. Its presence caused enhanced carbon fixation rates and photosynthetic O_2 production in the endosymbiotic algae. Other possible pathways of carbon transfer from the symbionts to the host are the processes of symbiont autolysis or digestion. Furthermore, it is suggested that the symbionts **convert metabolic waste products** (ammonia, urea, glycerophosphate) of the host into vitamins, amino acids, and enzymes, which could otherwise inhibit the host metabolism (Murray 1991). Thus, an efficient cycling of nutrients between both partners takes place, which supports the survival in oligotrophic waters where external supply with N and P is limited.

The importance of feeding for the growth of some larger foraminifera was found by Faber and Lee (1991). Feeding experiments with the benthic species *Peneroplis planatus* showed no growth of starved specimens, that were maintained in the light. The imperforate species *Amphisorus hemprichii*, *Archais angulatus,* and *Sorites marginalis* did not grow in experiments with no food supply. These species gain most of their carbon by feeding on unicellular algae from the substrate (Farber and Lee 1991). In other larger foraminifera feeding supplies only a minor part of energy and organic carbon. Nutrition experiments with *Amphistegina lessonii* demonstrated that the species is mainly photoautotrophic and growth was possible with light as the only energy source (Röttger et al. 1980).

In planktonic foraminifera on the other hand, digestion of plankton organisms is a major source of organic compounds and digestion of zooplankton prey results in a large **supply of N and P** (Bé et al. 1981, Spindler et al. 1984, Lee and Anderson 1991). The symbionts of planktonic species probably benefit from the food debris of caught prey within the pseudopodial network of the host, where it is digested. The study of Jørgensen et al. (1985), however, demonstrated that the symbiotic association of the planktonic

Globigerinoides sacculifer could be highly autotrophic at light saturation when the dinoflagellates produced approximately the tenfold amount of the organic matter needed for host respiration. Jørgensen et al. (1985) suggested that this enormous organic production could theoretically cover the carbon requirement over the whole diurnal light-dark cycle. Surface dwelling spinose foraminifera commonly have symbionts and in oligotrophic areas such as the central water masses the environment is poor in nutrients. Thus, the presence of symbionts might assist planktonic foraminifera to survive in areas of low food supply.

The close interaction of symbiotic algae and foraminifera was proven with **isotope fractionation experiments** of benthic and planktonic species (Honjo and Erez 1981, Spero and Deniro 1987, Uhle et al. 1998). The shells of the benthic foraminifer *Heterostegina depressa* e.g. became depleted in ^{18}O and ^{13}C with increasing irradiance (Zimmerman et al. 1983). Enhanced uptake of $^{12}CO_2$ by symbiont photosynthesis resulted in an enrichment of ^{13}C in the inorganic carbon pool. Subsequently, photosynthesis results in a depletion of the ^{13}C of the foraminiferal shell, whereas the respiration of the foraminiferal-algal association increases the ^{13}C value.

Uhle et al. (1998) used $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope measurements to elucidate the various metabolic and biosynthetic pathways e.g. of the nitrogen and carbon flow within planktonic foraminifera. They suggested that the changes in isotope data in *Orbulina universa* indicate a translocation of C and N from the symbionts to the host. Furthermore, they assumed that certain amino acids such as glutamic acid, valine, and isoleucine might be directly supplied from the fed *Artemia* nauplii to the foraminiferal host.

Radio tracer studies of carbon translocation from the host to its symbionts were performed with ^{14}C-labelled food. After 24 h the symbionts became heavily labelled by ^{14}C (Lee et al. 1988b). Additionally, the foraminifer-algal symbiosis may benefit from the **translocation of O_2 and CO_2**. It was hypothezised that O_2 produced by photosynthesis is utilized by the respiration of the host, whereas respired CO_2 may supply inorganic carbon for photosynthetic fixation in the symbiotic algae (Lee 1977, Jørgensen et al. 1985).

1. 2. Processes affecting the chemical microenvironment of symbiont-bearing foraminifera

1. 2. 1. Photosynthesis

The autotrophic symbiotic algae living in the foraminiferal cytoplasm remove CO_2 and release O_2 due to their photosynthetic activity (Fig. 3). All oxygenic photoautotrophs incorporate CO_2 to form carbohydrates by adding four electrons and four protons to the carbon atom. The net equation of oxygenic photosynthesis can be described by:

$$CO_2 + 2\,H_2O \Rightarrow CO_2 + 4[H] + O_2 \Rightarrow CH_2O + H_2O + O_2 \qquad (1).$$

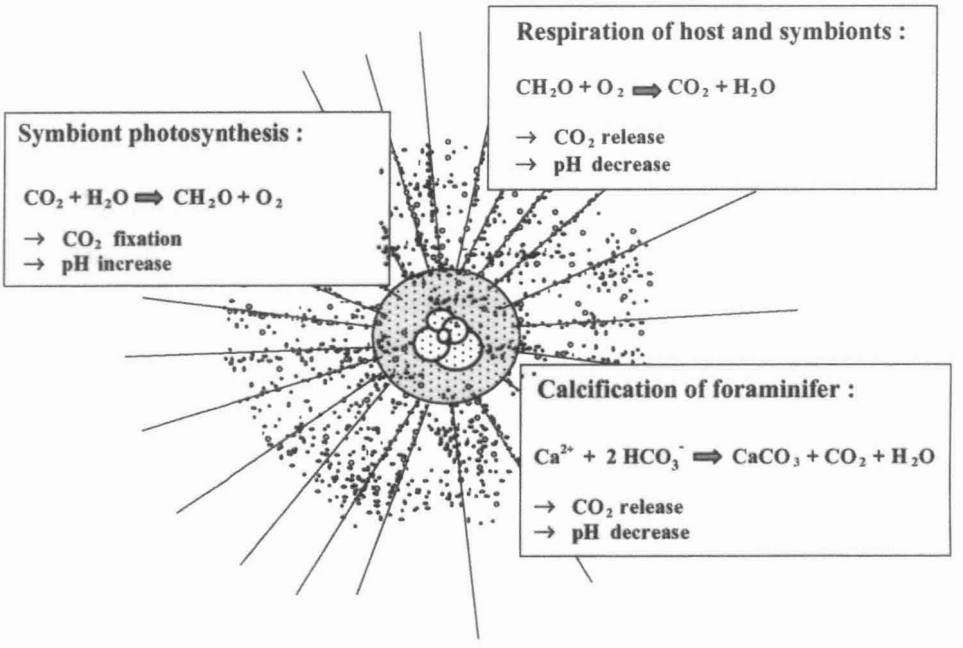

Fig. 3 Processes affecting the chemical microenvironment of symbiotic foraminifera.

Photosynthesis is a multistep process comprised of two independent series of reactions (Badger 1985, Beardall 1989, Jones 1992, Raven 1994, Falkowski and Raven 1997, Foyer and Quick 1997). In the following a brief description of the light and dark reactions will be given. The **„light" reactions** depend on the capture of photons by the

photosynthetic pigments. The two systems of light reactions are called photosystem I (PSI) and photosystem II (PSII). The concept of the two light reactions connected by an electron transport chain is called the Z-scheme. The first step in oxygenic electron flow is the splitting of water into oxygen and hydrogen atoms. An electron from water is donated to the oxidized P680 molecule, the reaction center of PSII. Light energy converts P680 into a strong reductant capable of reducing the intermediate acceptor phaeophytin a. From phaeophytin a the electron travels through several membrane carriers including quinones, cytochromes, and plastocyanin, that donate electrons to photosystem I. The electron is accepted by the reaction center chlorophyll of PSI, P700. The oxidation of P700 is followed by the transfer of the electron to a phylloquinone (A_1). From latter the electron is passed to an iron-sulfur complex (F_X) which is then oxidized by a second iron-sulfur complex (F_B/F_A). F_B/F_A transfers the electron to a nonheme iron protein, ferredoxin, which reduces $NADP^+$ to NADPH.

During the transfer of an electron from the acceptor in PSII to P700, electron transport occurs in a thermodynamic favorable direction. This generates a proton motive force from which ATP can be produced. The NADPH and ATP generated by the electron transport chain in the thylakoid membranes couple the light reaction to carbon fixation. In eukaryotic algae both the electron carrier as well as the enzymes which use the NADH and the ATP to convert CO_2 and H_2O to carbohydrates, are localized in the chloroplast. In symbiotic algae the enzymes for CO_2 fixation (e.g. ribulose-1,5-bisphosphate carboxylase/oxygenase) are located in the pyrenoid within the chloroplast (Leutenegger 1984, Raven 1994, Al-Moghrabi 1996). Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is involved in the photosynthetic carbon reduction cycle (PCRC), the globally most significant metabolic pathway for carbon reduction (Geider and Osborne 1992, Jones 1992, Raven 1997).

ATP and NADPH provided by the „light" reactions are used to reduce CO_2 into complex organic molecules. The reaction that lead to carbon reduction can proceed in the dark as well as in the light. There are two characteristic pathways of carbon fixation during the dark reactions (Raven 1994, Badger 1995, Falkowski and Raven 1997). First a carboxylation reaction incorporates inorganic CO_2 into ribulose-1,5-bisphosphate. This is catalyzed by the enzyme ribulose bisphophate carboxylase/oxygenase (Rubisco). The enzyme catalyzes both a carboxylase and oxygenase reaction. Latter results in oxygenation

of ribulose-1,5-bisphosphate and the production of 2-phosphoglycolate and 3-phosphoglycerate (PGA). Carboxylation results in the production of two molecules 3-phosphoglycerate. The rate of each reaction is dependent on the concentrations of O_2 and CO_2 in the chloroplast. Plants where primary carboxylation of atmospheric CO_2 resulted in the production of PGA were denoted as **„C3" plants**. In the „C_3" pathway the first product of carbon fixation is a 3-carbon compound called 3-phosphoglyceric acid (PGA) which is then converted to triose phosphate using ATP and NADPH. In **„C4" plants** CO_2 is initially incorporated into phosphoenolpyruvate by the action of phosphoenolpyruvate carboxylase producing oxaloacetate (OAA) as the first product of fixation, with subsequent formation of other 4-carbon compounds (malate and aspartate). The 4-carbon compounds are decarboxylated, and the CO_2 thus released is fixed by the enzymes of the PCR cycle. The initial fixation by PEP carboxylase acts as a `CO_2 concentrating´ mechanism because PEP carboxylase has a much higher affinity for CO_2 than does ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco).

The inorganic carbon fixation by marine algae involves carboxylation of Rubisco (Geider and Osborne 1992). Experiments of Burns and Beardall (1987) indicate that microalgae do not have a C_4 mechanism as originally suggested from $\delta^{13}C$ experiments. In marine diatoms, enzymatic studies indicate that ribulose bisphosphate carboxylase was the predominant active enzyme (Descolas and Fontugne 1985). Zimba et al. (1990) detected 3-phosphoglycerate as the first product of photosynthetic carbon fixation in marine benthic diatoms. They assumed that the predominant carbon fixation pathway is similar to that in C_3 plants. Symbiotic algae are suggested to belong to the group of C_3 plants (Streamer et al. 1993). The process of carbon fixation of the diverse types of endosymbiotic algae associated with foraminifera remains to be identified.

CO_2 is the major substrate for photosynthesis in marine environments (Raven 1994, Raven 1997). In seawater of pH 8.2, the CO_2 concentration reaches ~1% of the total inorganic carbon (DIC) and > 95% of the inorganic carbon is present as HCO_3^-. Rates of inorganic carbon fixation in marine algae can only be accelerated by the transport of C_i (CO_2, HCO_3^-) from the surrounding seawater into the organism. CO_2 can cross the plasmalemma or chloroplast envelope membrane to the site of carbon fixation (Raven 1997). CO_2 is dissolved in the membrane lipid phases, diffuses across the membrane, and dissolves back to the aqueous phase intracellularly.

An active transport of HCO_3^- in marine phytoplankton as well as in several symbiotic algae has been proven (Badger et al. 1980, Weiss et al. 1988, Al-Moghrabi et al. 1996). This C_i pump was described as the **carbon-concentrating mechanism** (CCM) (Burns and Beardall 1987, Badger and Price 1992, Falkowski and Raven 1997). The CCM elevates CO_2 around the active site of the primary photosynthetic carboxylating enzyme **Rubisco** (ribulose-1,5-bisphosphat carboxylase).

The **photosynthetic quotient** (PQ) is the ratio of O_2 evolved to CO_2 consumed. This metabolic quotient provides information on the physiology and metabolism of plants. Variations in the PQ have been attributed to the effects of nitrate assimilation and photorespiration in marine algae (Geider and Osborne 1992). The PQ value depends further on O_2 and CO_2 concentrations, nutrients used by photosynthesis, light conditions, and on the photosynthetic products (Burris 1981, Gattuso and Jaubert 1988). In addition, the process of calcification which leads to a reduction of TCO_2 without affecting O_2 could influence the PQ value. The stoichiometry of inorganic carbon assimilation of carbohydrates approximates the release of one O_2 molecule for one fixed CO_2 molecule. Organic carbon in lipids and proteins is more reduced increasing the PQ. Beside the different reductants which increase the PQ value, the process of photorespiration reduces the value (Geider and Osborne 1992). Conditions of O_2 supersaturation that enhance photorespiration reduced the PQ value in microalgae (Burris 1981). In unicellular algae PQ values ranged between 0.7 - 4.2 (Arantza 1999). The values increased with increasing experimental irradiance and were higher in algae grown at higher irradiances. Downton et al. (1976) also found a wide range of PQ values for symbiotic microalgae ranging between 1.3 - 5. Gattuso and Jaubert (1990) investigated the PQ of endosymbionts associated with their host coral, which reached values between 1.14 - 1.57. The authors suggested that light controls the quality of products photosynthesized.

1. 2. 2. Respiration

The combined respiration of the foraminifer and its symbionts results in O_2 uptake and CO_2 release of the community (Fig. 3). Respiration is generally known as the oxidation of organic compounds to CO_2 and H_2O by a series of oxidative reactions:

$$CH_2O + O_2 \Rightarrow CO_2 + H_2O \qquad (2).$$

Respiration is the sum of metabolic processes that consume O_2 and evolve CO_2. Two main types of respiration are described, dark respiration and photorespiration (Burris 1977, Geider and Osborne 1989, Beardall and Raven 1990, Krömer 1995, Falkowski and Raven 1997). The **dark respiration** includes various pathways of substrate oxidation such as glycolysis, the oxidative pentose phosphate pathway, and the tricarboxylic acid (TCA or Krebs) cycle. Dark respiration can occur in all cells under dark and light conditions. In most primary producers it provides the sole source of energy during periods when photosynthesis is inactive. In algae dark respiration varies widely in dependence of photosynthesis. On the average the dark respiration of microalgae is in the order of ~10% of the gross production (Beardall and Raven 1990).

In **glycolysis**, hexose is oxidized to pyruvate, and the oxidation is coupled to the reduction of NAD^+. In the absence of O_2, pyruvate can be anaerobically oxidized to lactate or, via pyruvate decarboxylase, to acetaldehyde and ethanol. The major role of glycolysis is to provide substrates for further respiratory oxidative processes.

The **oxidative pentose phosphate pathway** phosphorylates and subsequently oxidizes hexose to produce NADPH; CO_2, and pentose phosphate. The latter is converted via heptulose and tetrose phosphate to hexose phosphate and triose phosphate. Hexose and triose phosphates can be recycled or fed into glycolytic reactions.

In the **Krebs cycle**, the pyruvate produced in glycolysis is aerobically oxidized to CO_2 by a sequence of electron transfer reactions. These oxidations are mediated by NAD^+ and FAD. Intermediates in the Krebs cycle are withdrawn to form carbon skeletons for amino acids, lipids, tetrapyroles, and other biosynthetic processes.

The fourth component of dark respiration is the **respiratory electron transport** in the inner mitochondrial membrane. This process oxidizes most of the NADH generated in

the Krebs cycle. It leads to the phosphorylation of ADP via a proton flux through the ATP synthase complex and is the largest source of ATP in respiratory processes.

The second type of respiration described for microalgae that consumes O_2 and produces CO_2 is called **photorespiration** (Burris 1977, Ogren and Badger 1985, Beardall 1989, Beardall and Raven 1990, Falskowski and Raven 1997). Photorespiration is enhanced under conditions of high light and/ or reduced CO_2 supply or increased O_2 concentrations. In this pathway CO_2 is produced via the photorespiratory carbon oxidation (PCO) cycle (also known as the glycolytic pathway). Photorespiration is obligatorily coupled to the operation of the PCR cycle and therefore occurs only in the light. Moreover, the process is affected by the concentration of both O_2 and CO_2 due to the competitive nature of oxygenation versus carboxylation of the enzyme Rubisco. Increases in CO_2 concentration increase the proportion of ribulose-1,5-bisphosphate (RuBP) that is carboxylated. Under high partial pressure of O_2 the oxygenase activity and thus the carbon lost in photorespiration increases. In contrast to terrestrial „C3" plants photorespiration in aquatic plants is usually low (Raven 1984). Aquatic inorganic carbon suppress the oxygenase and stimulate the carboxylase activities of Rubisco.

The **respiratory quotient** (RQ) of algae is measured as the molar ratio of CO_2 released to O_2 absorbed during respiration. Respiration of glucose and other hexoses results in a respiratory quotient of 1. The oxidation of reduced compounds such as fats or proteins yields a quotient of less than 1 (0.7 for many lipids, 0.8 for some proteins). Oxidized compounds including organic acids yield a RQ greater than 1 (about 1.33 for citric acid) (Jones 1992).

1. 2. 3. Calcification

Calcification contributes to the formation of CO_2, leading to a higher pCO_2 while simultaneously reducing the concentration of total dissolved inorganic carbon:

$$\mathbf{Ca^{2+} + 2HCO_3^- \Rightarrow CaCO_3 + CO_2 + H_2O} \qquad (3).$$

In the literature a number of hypothesis about biogenic calcification in foraminifera exists. The **„Theory of biological induced $CaCO_3$ precipitation"** is explained as a pH driven

process in symbiotic foraminifera that displaces the carbonate equilibrium of the seawater (Borowitzka 1982a, Duguay 1983, Lea et al. 1995). The principal processes controlling the pH in the vicinity of the foraminifer are the photosynthetic removal and respiratory addition of CO_2 (Fig. 3). Photosynthetic fixation of CO_2 raises the pH and increases the carbonate concentration, largely at the expense of bicarbonate, according to the following equilibrium reaction :

$$\mathbf{H_2O + CO_2 \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+ \Leftrightarrow CO_3^{2-} + 2H^+} \qquad (4).$$

At high pH the equilibrium reactions are shifted towards the right and at low pH to the left. For example a pH shift from 8 to 9 will cause a fivefold increase in carbonate concentration (Barnes and Chalker 1990, Falkowski and Raven 1997).

The precipitation of $CaCO_3$ occurs when the solubility product of the ions in solution is exeeded (Barnes and Chalker 1990). Furthermore, the nucleation of $CaCO_3$ crystals is facilitated by the existence of surfaces. The tendency of surfaces to order the ions decreases the time or degree of supersaturation that is required for nucleation. Surfaces of existing crystals provide ideal substrates for nucleation of new crystal growth. This process is suggested in foraminifera by the **„Theory of an organic matrix“** (Hemleben et al. 1977, Anderson and Bé 1978, Röttger et al. 1984, Weiner and Erez 1984). The tertiary structure of a primary organic lining is involved in the initiation and direction of calcification by the spatial ordering of calcium and carbonate ions. The orientation in which a crystal is nucleated can be controlled by the pre-existing orientation of favouring substances.

The inhibition of $CaCO_3$ precipitation in foraminifera by a number of ions like ammonium, phosphate, and magnesium is suggested by the **„Poison removal theory“** (Borowitzka 1977, Chalker 1983, Swart 1983). This theory proposes that the organism removes ions and thereby induces a spontaneous precipitation. Simkiss (1964b) supposed that photosynthesis in corals removes phosphate from the internal environment and thus enhances calcification.

Calcification is a process requiring metabolic energy. The uptake and concentrating mechanisms of calcium and carbonate are energy-dependent. In benthic and planktonic foraminifera the existence of an inorganic carbon pool was suggested by studies of Anderson and Faber (1984), ter Kuile and Erez (1988), and ter Kuile et al. (1989a). The

energy- dependent carbonate uptake was formulated as the **„Theory of energy-dependent concentration of reactants".** Moreover, it was hypothesized that photosynthesis could enhance calcification. Photoautotrophic endosymbionts supply energy via the photosynthate release to the host and/ or remove of interferring substances due to the nutrient uptake from the sites of calcification (Barnes and Chalker 1990). The calcification in symbiotic foraminifera is probably the result of combined physico-chemical processes as well as of biologically induced reactions as mentioned in the previous hypothesis. Despite the investigation of different aspects of foraminiferal calcification (carbon supply, light enhancement) our knowledge about the basic mechanisms is still quite limited.

1. 3. The geological importance of planktonic foraminifera

Based on the global distribution of planktonic foraminifera through passive transport by ocean currents, on their enormous productivity, and on their sensitivity to environmental variations, isotope measurements of foraminiferal shell calcite have been used as a standard tool for the interpretation of ancient marine conditions (Bé and Tolderlund 1971). Their skeletons are abundantly preserved in the oceanic sediments in the so-called `**Globigerina ooze**´, which is the most widespread sediment type over greater parts of the deep Atlantic and much of the Indian and Pacific Oceans, covering nearly 50% of the deep-sea floor. It contains up to 95% calcium carbonate, mainly in the form of foraminiferal shells (Sverdrup et al. 1942, Bradshaw 1959). The enormous productivity of planktonic foraminifera is reflected by the fact e.g. that several thousand specimens (> 200 μm size) have been found in one gram of `Globigerina ooze sediment´ (Correns 1939).

Planktonic foraminiferal shells play a major role in paleoclimatology and paleo-geography, e.g. to reconstruct the paleotemperature, paleosalinity, and productivity of past oceans (Epstein et al. 1953, Emiliani 1954, Berger et al. 1971, Erez 1978, Berger et al. 1981, Erez and Luz 1983, Broecker and Peng 1986). Oxygen isotope data contain important information about the physical and chemical environment where the organisms precipitated their shell carbonate. Based on the assumption that the foraminiferal shells precipitated in equilibrium with the ambient seawater the $^{18}O/^{16}O$ ratio is used e.g. to reconstruct the paleotemperature.

^{12}C is the most common carbon form, which constitutes 98.9% of the natural carbon in the world. 1.1% is present in the form of ^{13}C (Falkowski and Raven 1997). In chemical processes involving carbon, the lighter isotope ^{12}C undergoes a higher rate of collision as a consequence of its smaller mass as compared to ^{13}C. This tendency is called isotopic fractionation and leads to different $^{13}C/^{12}C$ ratios in different natural C pools. Disequilibrium precipitation of $CaCO_3$ shells can be explained in terms of ontogenetic migration and biological fractionation in foraminifera that modifies the $\delta^{13}C$ signal of foraminiferal shells. The seawater pool of total CO_2 ($\sum CO_2 = HCO_3^-$, CO_3^{2-}, CO_2) that surrounds the symbiont-bearing foraminifer is enriched with ^{13}C due to the preferentially uptake of ^{12}C by symbiont photosynthesis. Therefore, enhanced photosynthetic activity of the endosymbionts increases the ^{13}C value of inorganic carbon in the surrounding seawater. Both the symbiont density, which increases with foraminiferal size, as well as their light-dependent photosynthesis that is regulated due to the foraminiferal position in the euphotic zone, affect the ^{13}C signal of the foraminiferal shells (Hemleben and Bijma 1994). Other metabolic processes such as the respiration of the community counteracts this enrichment (Spero 1992). Respiration processes, that are mainly affected by temperature, organism size, and feeding rate decrease the ^{13}C value.

1. 4. Microsensors

Microsensors can measure chemical and physical parameters with **high spatial resolution** due to their small tip diameters (1 - 20 μm). In previous studies they were mainly used to measure environmental conditions and metabolic processes in complex microbial communities (e.g. microbial mats, biofilms and aggregates) (Jørgensen and Revsbech 1985, Revsbech and Jørgensen 1986, Kühl and Jørgensen 1992, Ploug et al. 1997, de Beer et al. 1997). But they also found increasing applications in studies of symbiotic associations like the symbiotic planktonic foraminifer *Globigerinoides sacculifer* (Jørgensen et al. 1985), the hermatypic corals *Favia* spp. and *Acropora* spp. (Kühl et al. 1995, de Beer et al. 2000), and the didemnid ascidian *Diplosoma virens* (Kühl et al., unpublished). Recently, the O_2 and pH microenvironment of two symbiont bearing radiolaria, *Sphaerozoum punctatum* and *Physematium mulleri*, was studied (Köhler-Rink et al., unpublished). The **small dimensions** of microsensors allow the investigation of the physico-chemical microenvironment and rates of metabolic processes at high spatial and temporal resolution in such small, sensitive organisms. Several chemical parameters can be measured simultaneously without any destruction of the organisms. The combined use of O_2, CO_2, and pH microsensors can therefore provide important information about basic regulatory mechanisms of the major metabolic processes like photosynthesis and respiration in such symbiotic communities.

This thesis presents for the first time the application of CO_2 and Ca^{2+} microsensors and a fiber optic scalar irradiance microprobe (PAR) in studies of symbiont-bearing foraminifera. In combination with O_2 and pH microsensors the physico-chemical microenvironment and the regulation of photosynthesis, respiration, and calcification has been investigated in symbiotic planktonic and benthic foraminifera. The following table and graph summarize the different types of microsensors used in this study (Table 2, Fig. 4).

Table 2 Microsensors used in the present study

Sensor type	Principle	Tip size (µm)	Detection limit	References
1. Clark-type oxygen microsensor	Reduction of O_2 on a Au-cathode behind a silicone membrane	5 - 15	0.1 µM O_2	Revsbech (1989)
2. Glass pH microelectrode	Build-up of a potential across a special pH glass	20 - 200	pH 1 -14	Thomas (1978)
3. LIX microelectrode	Build-up of a potential across a liquid ion-exchanger membrane (LIX)	1 - 10		
a) pH			a) pH 3 - 11	de Beer et al. (1997)
b) Ca^{2+}			b) 10 µM Ca^{2+} (seawater)	Tsien and Rink (1980) Amman et al. (1987)
4. CO_2 microsensor	CO_2 induced pH change of a bicarbonate solution behind a silicone membrane	10 - 20	< 1 µM CO_2	de Beer et al. (1997)
5. Fiber optic microprobe	Measurement of quantum scalar irradiance (400 - 700 nm) with a small diffusing sphere at the tapered end of an optical fiber	70 - 200	< 1 µmol photons m^{-2} s^{-1}	Lassen et al. (1992a) Kühl et al. (1994b, 1997)

* modified from Kühl and Revsbech (2000)

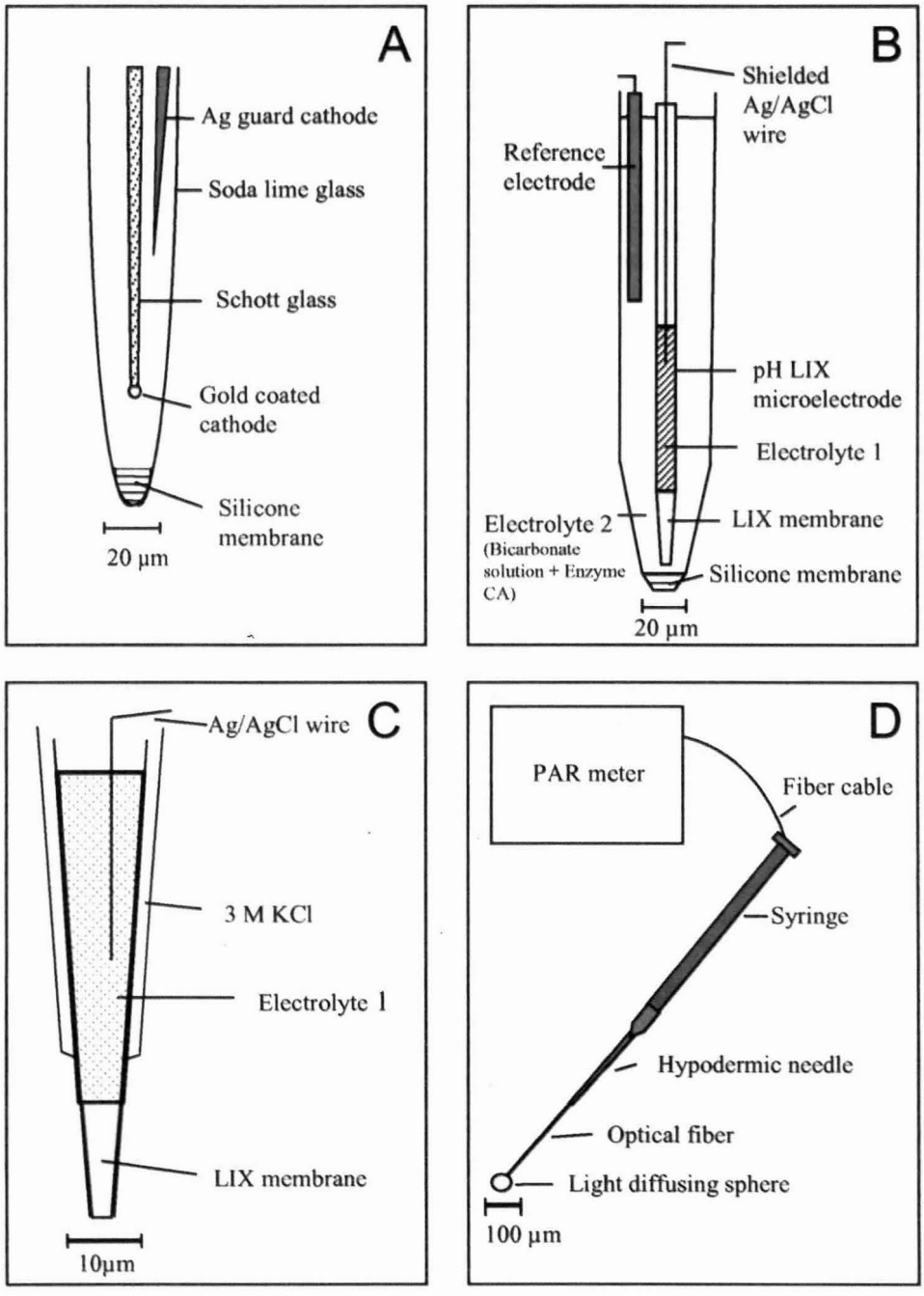

Fig. 4 Microsensors. Clark-type oxygen microsensor (**A**), CO_2 microsensor (**B**), LIX microelectrode (pH, Ca^{2+}) (**C**), fiber optic microprobe (PAR) (**D**).

1. 5. Outline of this thesis

This thesis investigates the physico-chemical microenvironment of symbiont-bearing planktonic and benthic foraminifera with microsensors. The aim of this thesis was to study the effect of metabolic processes including photosynthesis, respiration, and calcification on the chemical microenvironment of the foraminiferal-algal association.

In chapter 2 microsensors for O_2, pH, and light (PAR) were used to investigate the physico-chemical microenvironment of the planktonic species *Orbulina universa*. The effect of light on algal photosynthesis and on the respiration of the symbiotic system was studied. Chapter 3 complemented the investigation of the chemical microenvironment of *O. universa*. The aim of this study was to investigate the influence of symbiont photosynthesis on the CO_2 and Ca^{2+} microenvironment of this planktonic species. Furthermore, the potential inorganic carbon sources for symbiont photosynthesis were studied. For this purpose we did combined measurements of O_2 and CO_2 fluxes and studied simultaneous concentration changes of O_2, CO_2, and pH within the symbiont swarm.

In addition to the planktonic foraminifera chapter 4 and 5 describe the investigation of symbiont-bearing benthic foraminifera. The combined effect of photosynthesis, respiration, and calcification on the chemical microenvironment of the larger foraminifera *Marginopora vertebralis*, *Amphisorus hemprichii*, and *Amphistegina lobifera* was studied with O_2, CO_2, pH, and Ca^{2+} microsensors (chapter 4). Additionally, irradiance effects on photosynthesis of the symbiotic microalgae that live inside the foraminiferal cytoplasm and on the foraminiferal microenvironment were investigated (chapter 5). Potential inorganic carbon sources for photosynthetic assimilation were studied. Furthermore, a small measuring chamber was developed to estimate the total O_2 production and consumption rates of single larger foraminifera. The combination of this method with the point measurements of gross and net photosynthesis allowed a detailed study of the primary production of symbiont-bearing foraminifera with O_2 and CO_2 microsensors.

1. 6. References

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Chapter 2

Microsensor studies of photosynthesis and respiration in the symbiotic foraminifer *Orbulina universa*

Stephanie Rink[1], *Michael Kühl*[1], *Jelle Bijma*[2], *Howard J. Spero*[3]

[1]Max-Planck-Institute for Marine Microbiology, Microsensor Research Group, Celsiusstr. 1, D-28359 Bremen, Germany.

[2]Alfred-Wegener-Institute for Polar and Marine Research, Columbusstr., D-27568 Bremerhaven

[3]Department of Geology, University of California, Davis, CA 95616

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ABSTRACT

Oxygen and pH microelectrodes were used to investigate the microenvironment of the planktonic foraminifer *Orbulina universa* and its dinoflagellate endosymbionts. A diffusive boundary layer (DBL) surrounds the foraminiferal shell and limits the O_2 and proton transport from the shell to the ambient seawater and *vice versa*. Due to symbiont photosynthesis, high O_2 concentrations of up to 206% air saturation and a pH of up to 8.8, i.e. 0.5 pH units above ambient seawater, were measured at the shell surface of the foraminifer at saturating irradiances. The respiration of the host-symbiont system in darkness decreased the O_2 concentration at the shell surface to < 70% of the oxygen content in the surrounding air saturated water. The pH at the shell surface dropped to 7.8 in darkness. We measured a mean gross photosynthetic rate of 8.5 ± 4.0 nmol O_2 h^{-1} $foraminifer^{-1}$. The net photosynthesis averaged 5.3 ± 2.7 nmol O_2 h^{-1}. In the light, the calculated respiration rates reached 3.9 ± 1.9 nmol O_2 h^{-1}, whereas the dark respiration rates were significantly lower (1.7 ± 0.7 nmol O_2 h^{-1}). Experimental light-dark cycles demonstrated a very dynamic response of the symbionts to changing light conditions. Gross photosynthesis versus scalar irradiance curves (P vs. E_o curves) showed light saturation irradiances (E_k) of 75 and 137 µmol photons m^{-2} s^{-1} in two *O. universa* specimens, respectively. No inhibition of photosynthesis was observed at irradiance levels up to 700 µmol photons m^{-2} s^{-1}. The light compensation point of the symbiotic association was 50 µmol photons m^{-2} s^{-1}. Radial profile measurements of scalar irradiance (E_o) inside the foraminifera showed a slight increase at the shell surface up to 105% of the incident irradiance (E_d).

INTRODUCTION

Planktonic symbiont-bearing foraminifera often occur in oligotrophic ocean waters. Probably due to their close relationship with autotrophic dinoflagellates, they can survive in nutrient limited environments. Symbiont-bearing foraminifera populate the euphotic zone, where the symbionts are exposed to light levels sufficient for photosynthesis. It has been

suggested that the zooxanthellae live well protected in the cytoplasm of the host where they benefit from the respired CO_2 as well as from nitrogen and phosphorus from prey digested by the foraminifer (Bé 1977, Jørgensen et al. 1985, Gastrich and Bartha 1988). The density of endosymbionts can reach a mean of 3.300 cells per foraminifer and specific photosynthetic rates of 1.72 pmol C symbiont^{-1} h^{-1} were measured at saturating irradiances (Spero and Parker 1985). The importance of the endosymbionts for the host was demonstrated in experiments, where the symbionts were treated with the photosynthetic inhibitor DCMU. Bé et al. (1982) thus found significantly shorter survival times, reduced shell growth rates, and a smaller final shell size after inhibition of zooxanthellae photosynthesis.

Spinose planktonic foraminifera have a perforate calcareous shell with thin spines. The spines can reach a length of several mm and enlarge the effective surface area of the foraminifera thereby increasing the chance of capturing prey with its sticky rhizopodial network (Bé 1977). Due to the enormous productivity of foraminiferal shells large parts of the ocean floor are covered with them and constitute the so called „globigerina ooze". Because the geochemical composition, i.e. the stable carbon and oxygen isotope composition, planktonic foraminiferal shells can be used for paleo-environmental reconstructions of the world´s oceans for the last 120 * 10^6 years, these organisms have become a major tool in geology to reconstruct the productivity of past oceans. However, photosynthesis of endosymbionts can affect the isotopic composition of the foraminiferal shells due to the higher affinity of the CO_2 fixing enzyme for $^{12}CO_2$ (see e.g. Spero and de Niro 1987).

Symbiotic associations of planktonic spinose foraminifera with microalgae have been reported for at least seven species. The predominant algal symbionts are coccoid dinoflagellates (Hemleben et al. 1989). They are found in the species *Orbulina universa*, *Globigerinoides sacculifer*, *G. ruber*, and *G. conglobatus* (Spindler and Hemleben 1980, Hemleben and Spindler 1983, Spero 1987). The endosymbiont of *O. universa*, an opportunistic species from the temperate to tropical provinces (Bé 1977), is the dinoflagellate *Gymnodinium béii*. The species *Globigerinella aequilateralis*, *Globigerina cristata*, and *G. falconensis* host symbiotic chrysophycophytes (Spindler and Hemleben 1980, Gastrich 1987, Faber et al. 1988). Oxygen and pH microelectrodes have already been used to study symbiotic associations, such as the foraminifer *G. sacculifer* and the

hermatypic corals, *Favia* sp. and *Acropora* sp. (Jørgensen et al. 1985, Kühl et al. 1995). Microsensor techniques proved to be useful tools for measuring the processes of photosynthesis and respiration with a high spatial and temporal resolution in these symbiotic associations (Revsbech and Jørgensen 1986). The light-dark shift technique (Revsbech et al. 1981, Revsbech and Jørgensen 1983) measures gross photosynthetic rates independent of the respiration process, and light and dark respiration rates in symbiont-host systems can be assessed independently. Due to their small tip diameter, microsensors can be used without any destruction of the organism and several measurements in one specimen, e.g. under changing light or temperature conditions, are possible.

Photosynthesis in planktonic symbiotic foraminifera has previously been investigated with two different techniques. Jørgensen et al. (1985) used O_2 microsensors to measure the gross and net photosynthetic rates of *G. sacculifer* (Jørgensen et al. 1985). Radio tracer ^{14}C methods have been used to estimate the cell specific carbon uptake of symbionts of two different species (Spero and Parker 1985, Gastrich and Bartha 1988). It was estimated that a single *O. universa* specimen would contribute approximately 0.2% of the fixed carbon in 1 m^3 of seawater (Spero and Parker 1985). The foraminifer-algal association has been characterized as a „hot spot" of productivity in oligotrophic seawater.

Symbiont-bearing planktonic foraminifera are cosmopolitan calcifying organisms, but there are still a lot of open questions about their biology and the physiological and biochemical interactions of the host-symbiont association. Several hypotheses about their mutual benefit, e.g. the nutritional relationship, the transport of metabolic gases, and the calcification process are discussed in the literature (e.g. Erez 1983, Jørgensen et al. 1985, Hemleben et al. 1989, Lea et al. 1995). Although the photosynthetic rates of the symbionts of *O. universa* have been studied (Spero and Parker 1985), the microenvironment of this foraminifer and its importance for host-symbiont interaction is still unknown. We used O_2 and pH microsensors and a fiber-optic scalar irradiance microprobe to investigate the physico-chemical microenvironment of this symbiotic system. Our study demonstrates the influence of changing light conditions on the foraminifer-algal symbiosis and a close coupling of photosynthesis and respiration in *O. universa*.

MATERIALS AND METHODS

Collection Adult *O. universa* with sphere diameters ranging between 290 to 550 μm (Fig. 1A) were hand-collected by SCUBA divers in the surface waters of the California Bight, near Santa Catalina Island, California between July and August 1995.

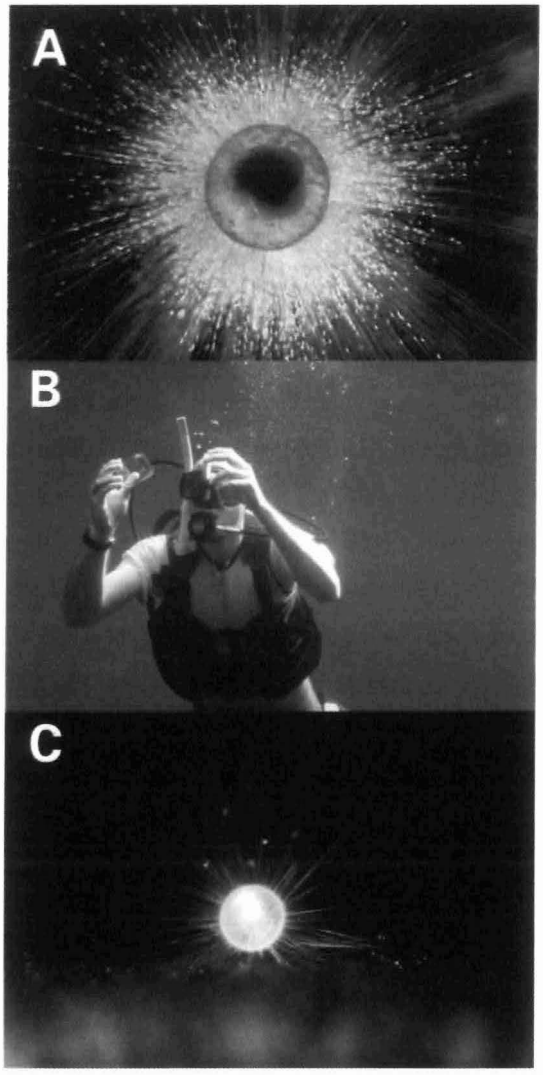
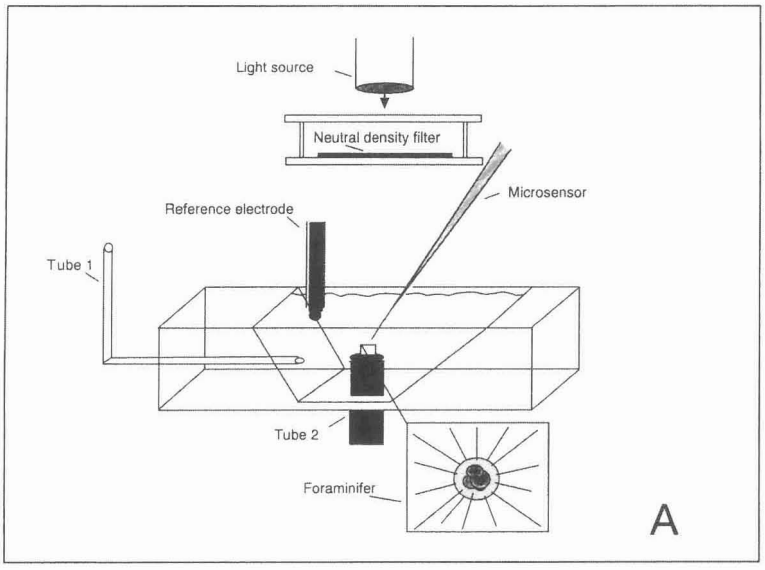


Fig. 1A Adult *Orbulina universa* with dinoflagellate symbionts surrounding the shell. Juvenile trochospiral shells are visible in the center of the transparent spherical chamber (diameter of the spherical shell was ~ 500 μm) (photo: T. Mashiotta). **B** Collection of planktonic foraminifera by SCUBA diving. Individual specimens are sampled in glass jars (photo: E. Meesters). **C** *Orbulina universa* sticking to the nylon mesh inside the measuring chamber.

Individual specimens were sampled in glass jars at a depth of 5 – 10 m (Fig. 1B). During the collection period the mean water temperature was 19.2° C. Light measurements at the collection site showed an average downwelling irradiance of 2100 µmol photons m^{-2} s^{-1} at the water surface at full sunlight (S. Anderson, pers. comm.). After sampling in the morning hours (9 – 11 a.m.), individual foraminifera were kept in separate glass vessels at 22° C and ~80 µmol photons m^{-2} s^{-1} without feeding. Experiments were conducted within less than 24 h after collection in the laboratory of the Wrigley Institute for Environmental Studies (WIES).

Experimental setup For microsensor measurements, a specimen was placed on a nylon mesh in a small Plexiglas chamber (V = 10 ml) with filtered seawater (Figs. 1C, 2A). The microsensors were manually positioned with a micromanipulator (Märtzhäuser, Germany). The angle of inclination of the microsensor was 30° relative to the vertically incident light. Positioning of the microsensor tip relative to the foraminiferal shell surface was adjusted under a dissection microscope. Measurements were performed at room temperature (20 - 22° C) in a dark room under defined light conditions. The light source was a fiber optic halogen lamp (Schott KL-1500) equipped with a collimating lens, and incident irradiance (0 - 1000 µmol photons m^{-2} s^{-1}) was adjusted by neutral density filters (Oriel). Downwelling quantum irradiance (400 - 700 nm) was measured with a quantum irradiance meter (LiCor, LI 189). The light was controlled by a mechanical shutter, installed in the light path of the halogen lamp, without influencing the light quality. The specimens were allowed to adapt to conditions in the measuring chamber for 0.5 - 1 h and the experiments were started when the symbionts were distributed in a concentrical halo around the shell (Figs. 1A, 2B).



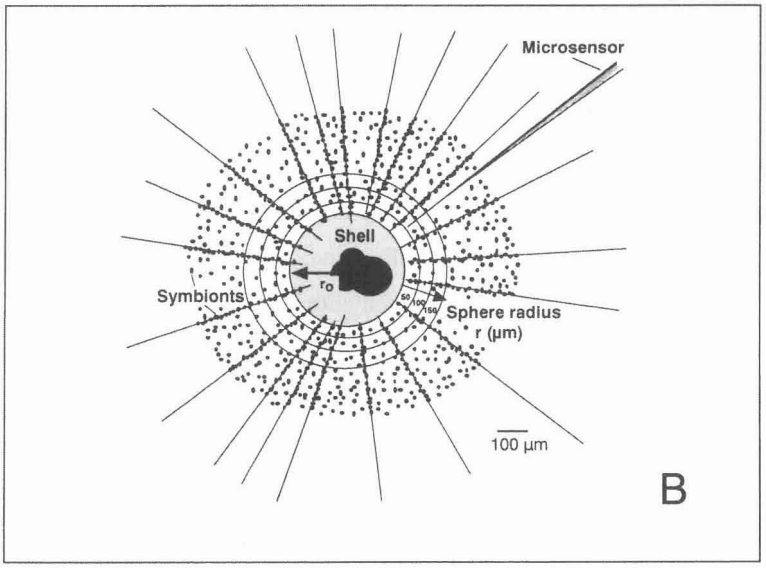


Fig. 2 A Schematic drawing of the measuring chamber (V = 10 ml) with a single foraminifer placed on a nylon mesh. Microsensor positioning was done with a micromanipulator and the incident light was adjusted by neutral density filters. **B** Schematic drawing of an adult *Orbulina universa*. Concentric spheres of 50 µm thickness indicate the microsensor positioning for the photosynthesis measurements (r_o = radius of the spherical shell; r = distance to the shell).

Oxygen microelectrodes Photosynthetic rates and radial concentration profiles of O_2 from the ambient seawater to the shell surface were measured by a Clark-type O_2 microelectrode with a guard cathode connected to a picoammeter and a strip chart recorder (Revsbech 1989). The microelectrodes had an outer tip diameter of 5 - 12 µm, a 90% response time of < 0.4 - 1.8 s and a stirring sensitivity of 0 - 2%. Linear calibration of the electrode signal was done at room temperature in air saturated seawater and in O_2 free seawater (reduced with sodium dithionite). The O_2 concentration of the air saturated seawater was determined by Winkler-titration (Grasshoff et al. 1983).

pH microelectrodes pH was measured with glass pH microelectrodes (Revsbech et al. 1983) in combination with a calomel reference electrode (Radiometer, Denmark) both connected to a high impedance mV meter. The pH electrodes had a pH sensitive tip of 12 - 25 µm diameter and of 80 - 150 µm length. They were calibrated in NBS buffers (Mettler Toledo, pH 4, 7, and 9) at room temperature.

Scalar irradiance measurements A fiber optic microprobe (Lassen et al. 1992a) connected to a PAR meter (Kühl et al. 1997) was used for measuring radial profiles of quantum scalar irradiance (400 - 700 nm) from the surroundings towards the shell of *O. universa*. The diameter of the scalar irradiance microprobe tip was < 100 µm. Linear calibration of the fiber optic scalar irradiance microprobe was done in darkness and in a collimating light field at a known downwelling irradiance over a black light trap (Kühl et al. 1997). Downwelling irradiance was measured with a quantum irradiance meter (LiCor, LI 189). All light measurements in this paper refer to visible light (400 - 700 nm), i.e. the photosynthetic available radiation for oxygenic photosynthesis.

Photosynthesis measurements Oxygen microelectrodes with a fast response time (< 0.5 s) were used for measurements of gross and net photosynthesis. Gross photosynthesis was estimated with the light-dark shift technique (Revsbech et al. 1981, Revsbech and Jørgensen 1983) by measuring the initial decrease of O_2 in the first seconds after darkening. The O_2 depletion is equal to the photosynthetic O_2 production during the previous light period (more details in Revsbech and Jørgensen 1983, Glud et al. 1992, Kühl et al. 1996). Gross photosynthetic rates, P (r), were measured inside the symbiotic swarm at 50 µm intervals starting at the shell surface. Radial profiles of photosynthetic activity were used to calculate the total gross photosynthetic production assuming that the symbionts surround the shell in a spherical symmetry (Fig. 2B). The total gross photosynthetic rate, P_{total}, in

nmol O_2 h^{-1} foraminifer^{-1} was calculated as the sum of the photosynthetic rates, measured per volume of each concentric segment in the symbiotic halo (Jørgensen et al. 1985) :

$$\sum_i P(r_i)\left(\frac{4}{3}\pi(r_i + r_{i-1})^3 - (r_{i-1})^3\right) \qquad \text{(I)}$$

where i = 0, 50, 100...μm.

Net photosynthesis and respiration rates were calculated from the measured steady state O_2 profiles in light and in darkness, respectively. The area integrated O_2 flux, Q_t, in nmol O_2 h^{-1} foraminifer^{-1}, was calculated by the radial gradient dC/dr, the molecular O_2 diffusion coefficient in seawater, D, and the surface area of the sphere $4\pi r^2$ (Jørgensen et al. 1985, Ploug et al. 1997):

$$Q_t = 4\pi r^2 D \frac{dC}{dr} \qquad \text{(II).}$$

Respiration measurements The respiration of the symbiont-host system in the light was calculated as the difference between total gross photosynthesis and net photo-synthesis (Jørgensen et al. 1985). In the dark, the O_2 flux to the sphere is determined by the combined respiration rate of the foraminifer and the symbionts, and dark respiration was calculated from the O_2 profiles measured in the dark by using equation II.

P vs. $E_o$curves Gross photosynthetic rates (nmol O_2 cm^{-3} s^{-1}) were measured with the light-dark shift method at the shell surface inside the symbiont swarm as a function of increasing scalar irradiance. *Orbulina universa* was exposed to each irradiance level for 15 - 30 min before the measurements started. Light intensities (0 - 700 μmol photons m^{-2} s^{-1}) were adjusted with neutral density filters (Oriel). An exponential function: $P = P_m (1 - \exp(-\alpha E_o / P_m))$ (Webb et al. 1974) was fitted to the P vs. E_o data measured at the shell surface, where P_m is the light-saturated photosynthetic rate and α the initial slope of the P vs. E_o curve at subsaturating scalar irradiance (Geider and Osborne 1992).

RESULTS

Microenvironment of the symbiotic *O. universa*

The zooxanthellae of *O. universa* showed a diurnal migration pattern. During the day, the dinoflagellates spread out on the rhizopodial network between the spines, while at night they were located inside the shells. During our experiments the symbionts formed a 200 - 400 µm thick concentric halo surrounding the spherical shell of the foraminifer (Figs. 1A, 2B).

Around the shell, a diffusive boundary layer (DBL) was established, that limited the solute transport between the surrounding seawater and the foraminifer. In the light, the O_2 concentration started to increase in the distal part of the spines and very high concentrations were measured towards the shell (Figs. 3A, 7A). Profiles of gross photosynthesis inside the symbiont swarm showed highest rates at the foraminiferal shell, where a maximum gross photosynthesis up to 13.7 nmol O_2 cm^{-3} s^{-1} was measured (Fig. 3C). The photosynthetic activity of the symbionts and the presence of a DBL thus created a microenvironment of high pH and high O_2 concentrations around the shell of *O. universa* as compared to the ambient seawater (Figs. 3A, B). At the shell surface, we measured O_2 supersaturation up to 206% of air saturation at high irradiances (Fig. 3A). During measurements of the dark profiles the symbionts moved into the shell. In darkness, the respiration of the foraminifer and the symbionts decreased the O_2 concentration to

< 80% air saturation at the shell surface of this specimen (Fig. 3A). Due to photosynthetic CO_2 fixation in the light, pH increased to up to 8.8 at the shell surface under saturating light conditions. In darkness, pH was lowered down to pH 7.9 at the shell surface as a result of CO_2 release during respiration of the host and its symbionts (Fig. 3B). The average rate of gross photosynthesis per adult *O. universa* was 8.9 nmol O_2 h^{-1} $foraminifer^{-1}$ (Table 1), but rates of 13.9 nmol O_2 h^{-1} $foraminiferat^{-1}$ saturating irradiance (782 µmol photons m^{-1} s^{-1}) were found in one specimen (No I). The net photosynthetic rate of the same specimen reached 8.7 nmol O_2 h^{-1}.

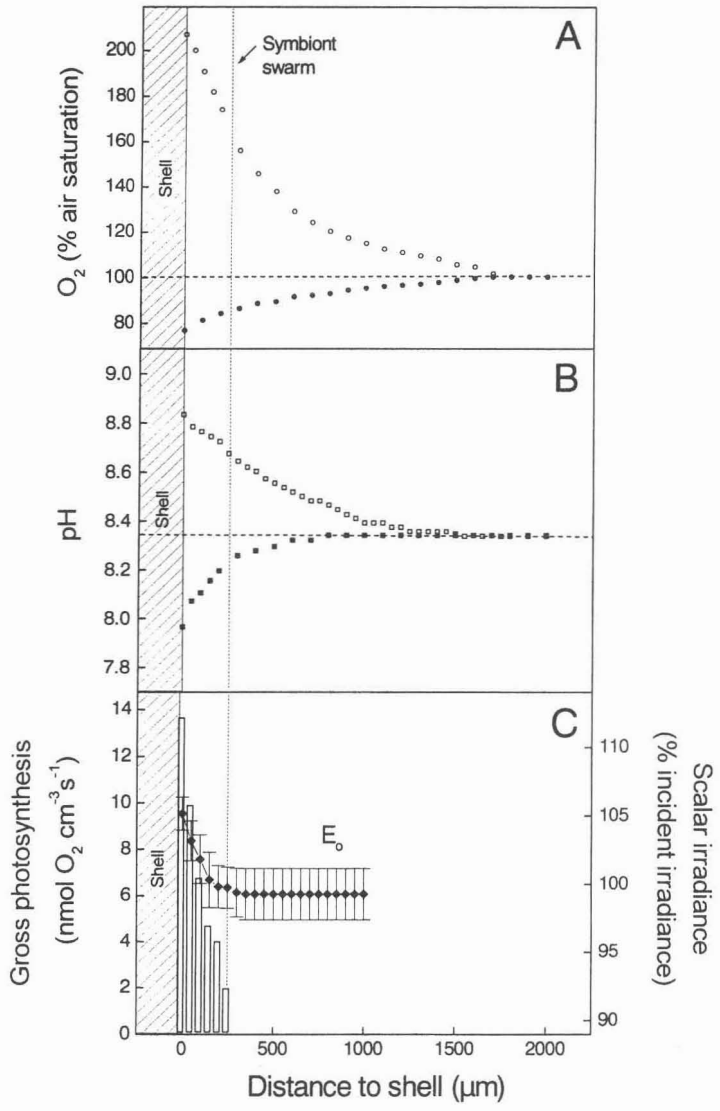


Fig. 3 Light profiles (O) and dark profiles (●) of O_2 (**A**) and pH light (□) and dark(■) profiles (**B**) measured from the ambient seawater to the spherical shell (E_o = ~700 µmol photons m^{-2} s^{-1}). Profiles of scalar irradiance (♦) and gross photosynthesis (bars) measured in steps of 50 µm towards the shell surface of *Orbulina universa* (**C**). The arrow indicates the outer periphery of the symbiont swarm.

Radial O_2 and pH profiles measured at different positions in the foraminifer showed similar concentration gradients (data not shown) supporting our assumption of a radial symmetry of solute concentration and diffusion around the foraminiferal shell under stagnant conditions. Radial profiles of scalar irradiance from the ambient seawater to the shell showed values up to 105% of the incident irradiance (Fig. 3C). This increase probably resulted from light scattering and reflection within the spines and the calcite shell.

Table 1 *Orbulina universa*. Photosynthesis and respiration measured in several individuals of different sizes at saturating irradiances

Foraminifer	Shell diameter	Incident irradiance	Photosynthesis		Respiration	Percentage of gross photosynthesis
			Gross	Net		
No.	(μm)	(μmol photons m^{-2} s^{-1})	(nmol O_2 h^{-1} foraminifer^{-1})			
I	554	782	13.89	8.72	5.17	37%
II	554	782	11.00	5.06	5.94	54%
III	463	288	9.26	4.57	4.69	51%
IV	473	446	8.16	6.45	1.71	21%
V	297	750	2.29	0.57	1.72	75%
Mean ± SD	468 ± 105	609 ± 228	8.92 ± 4.29	5.07 ± 2.99	3.85 ± 1.99	47.6 ± 20.14

Oxygen, pH, and photosynthesis at the shell surface

Experimental light-dark cycles resulted in very dynamic changes in the O_2 production at the shell surface (Fig. 4). After a steady state O_2 level was reached, light was turned off and the O_2 level decreased from 190% to 80% air saturation within 5 min. When the light was turned on again, the O_2 concentration increased immediately and reached 100% air saturation within 15 s. A steady state supersaturation of 190% was reached again after 3 - 4 min.

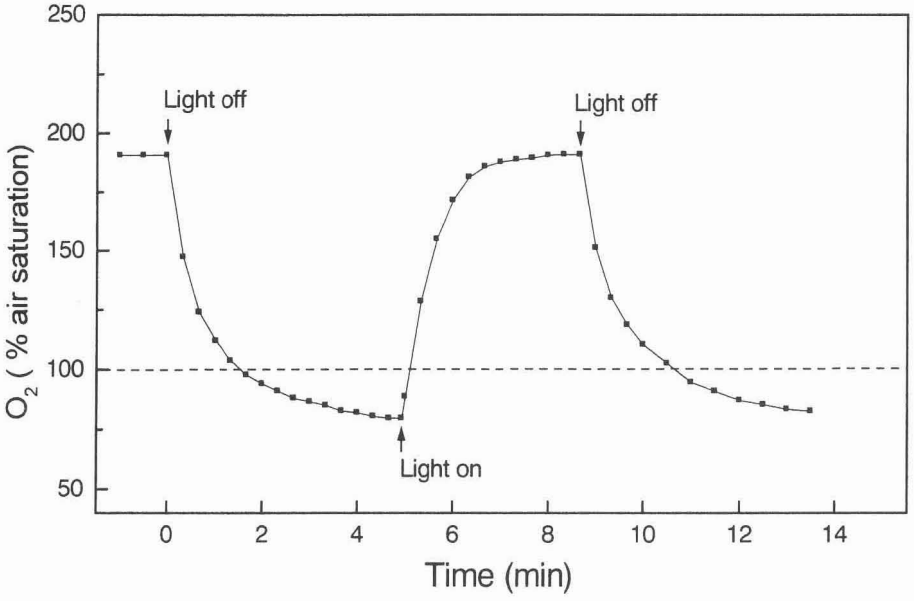


Fig. 4 O_2 dynamics at the shell surface of *Orbulina universa* during experimental light-dark cycles. Incident irradiance was 683 µmol photons m^{-2} s^{-1}. Dashed line indicates the O_2 concentration of the ambient seawater.

Oxygen and pH conditions at the surface of the foraminiferal shell were investigated as a function of scalar irradiance (Fig. 5). The O_2 and pH versus scalar irradiance curves demonstrated the saturation of photosynthesis with increasing incident light. Both pH and the O_2 level at the shell surface saturated at approximately 250 µmol photons m^{-2} s^{-1}.

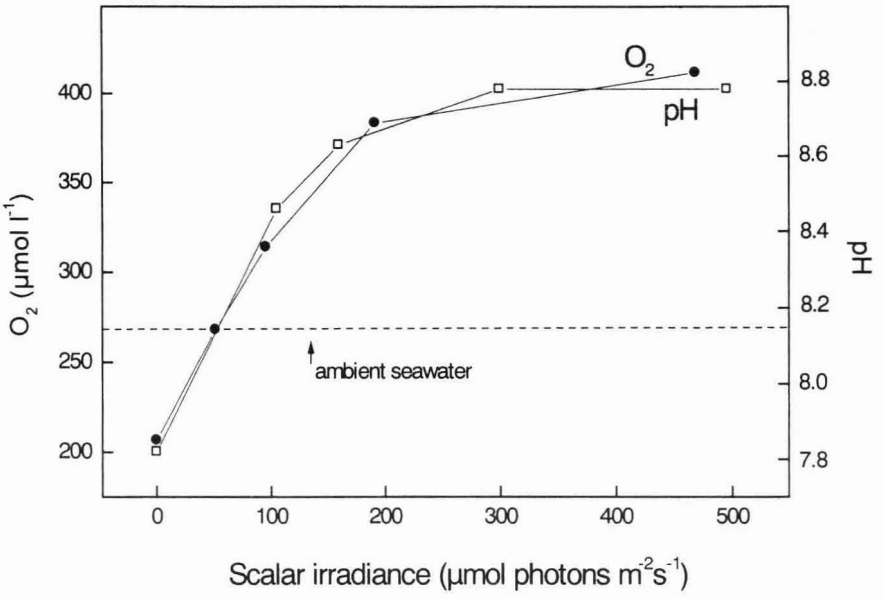


Fig. 5 O_2 (●) and pH (□) measured as a function of scalar irradiance (µmol photons m^{-2} s^{-1}) at the shell surface of *Orbulina universa*. Dashed line: ambient seawater level of O_2 and pH.

Gross photosynthetic rates at the shell surface increased with increasing scalar irradiance (Figs. 6A, B). The exponential function of Webb et al. (1974) was fitted to the P vs. E_0 measurements and estimated a maximum photosynthetic rate of 9.3 nmol O_2 cm^{-3} s^{-1} in one specimen. The initial slope α in the linear part of this P vs. E_0 curve was 0.07 (Fig. 6A). The onset of light saturation of photosynthesis expressed by the light saturation irradiance, E_k, was P_{max}/α = 137 µmol photons m^{-2} s^{-1}. In a second specimen, we found a lower E_k of 75 µmol photons m^{-2} s^{-1} caused by a lower P_{max} of 5.6 nmol O_2 cm^{-3} s^{-1} and the same initial slope (α = 0.07) (Fig. 6B). Up to 700 µmol photons m^{-2} s^{-1} no photoinhibition was observed in *O. universa*.

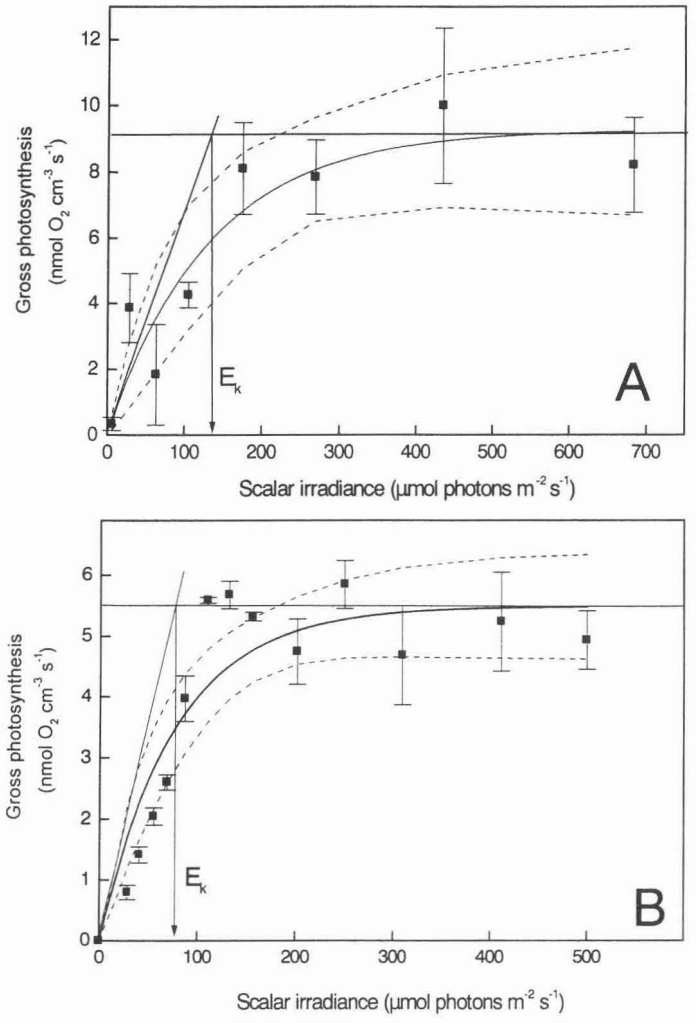


Fig. 6 Gross photosynthetic rates vs. scalar irradiance (400-700 nm) measured at the shell surface of two *Orbulina universa* specimens (**A** + **B**). An exponential function (Webb et al. 1974) (solid line) was fitted to the data by a nonlinear least-square Levenberg-Marquardt algorithm (Origin 4.1, MicroCal Software, Inc.) (A: $r^2 = 0.86$, $\chi^2 = 1.95$; B: $r^2 = 0.85$, $\chi^2 = 0.63$). Dashed lines: 95% confidence intervals. E_k = onset of light saturation.

Radial distribution of O_2 and pH

Radial O_2 and pH profiles in dependence of the incident irradiance were measured from the ambient seawater towards the shell surface (Fig. 7). The O_2 concentration started to increase outside the spines and reached the highest values at the shell surface due to the presence of the DBL. The O_2 profiles varied as a function of the light level (Fig. 7A).

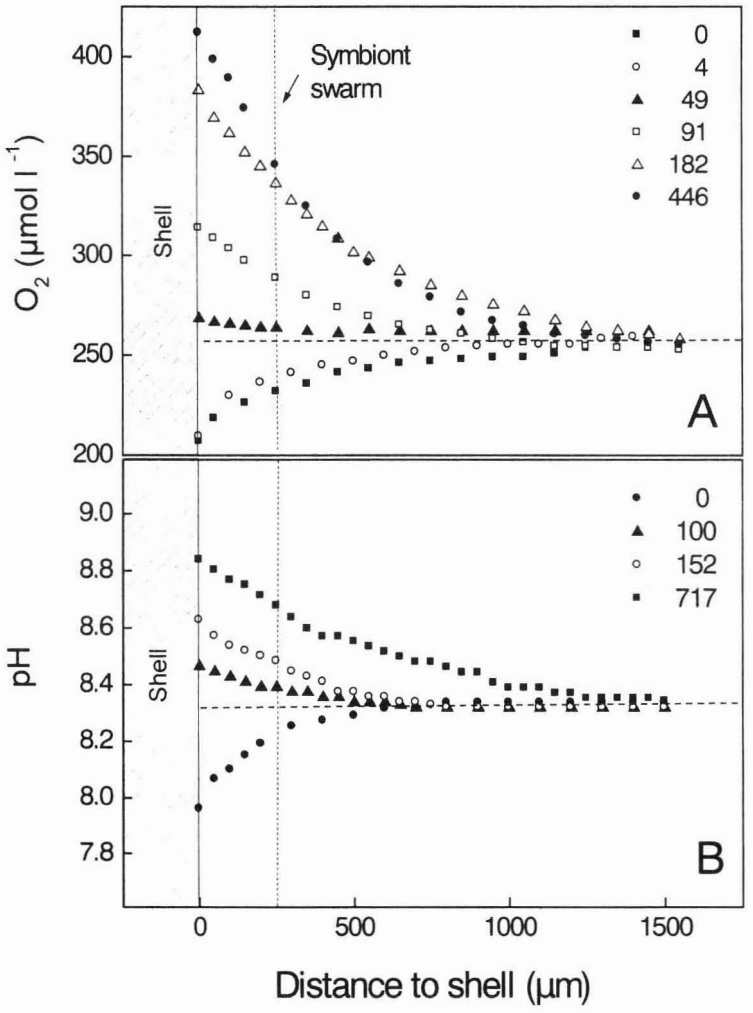


Fig. 7 Radial steady state O_2 (**A**) and pH (**B**) profiles as a function of increasing irradiance. The arrow indicates the outer periphery of the symbiont swarm. Numbers indicate incident irradiance (µmol photons m^{-2} s^{-1}). Dotted lines: O_2 concentration and pH of the bulk seawater.

The pH increased towards the surface of the shell from the ambient seawater level at the end of the spines. Due to increasing photosynthetic CO_2 fixation with irradiance and the presence of a DBL we measured increasing pH values at the shell surface (Fig. 7B). The highest pH of 8.8 was found at 717 µmol photons m^{-2} s^{-1}. In the darkness the surface pH of this specimen decreased to 7.9.

At 50 µmol photons m^{-2} s^{-1} the compensation irradiance, E_c, where the respiratory O_2 consumption of the system balanced the zooxanthellae O_2 production, was reached (Fig. 8). With increasing incident irradiance (> 50 µmol photons m^{-2} s^{-1}) the photosynthetic O_2 production exceeded the O_2 uptake and net photosynthesis approached saturation at > 450 µmol photons m^{-2} s^{-1} (Fig. 8).

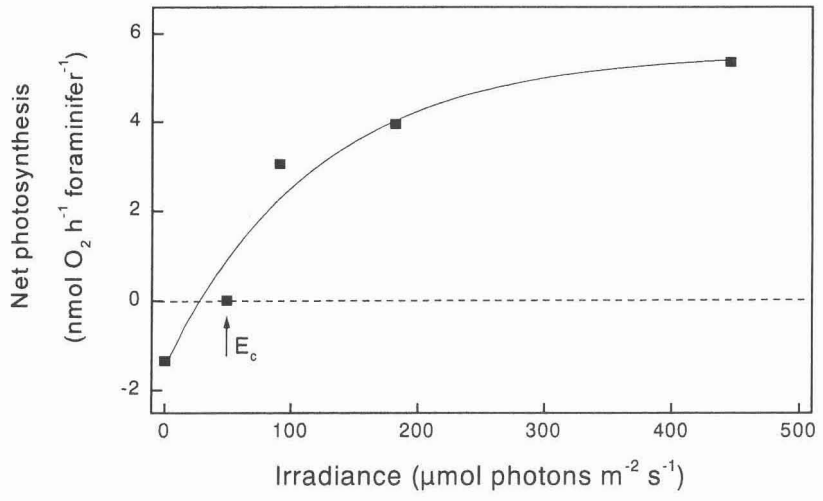


Fig. 8 Net photosynthesis of *Orbulina universa* (nmol O_2 h^{-1}) as a function of incident irradiance. The compensation light intensity, E_c, was found at 50 µmol photons m^{-2} s^{-1}.

Respiration rates in light and darkness

In the light we observed a high variability of respiration rates in different specimens (Table1). When light respiration was calculated as % of gross photosynthesis we found an average of 47.6 ± 20.1% (n=5) (Table 1, Fig. 9). *Orbulina universa* and its zooxanthellae showed a lower average O_2 consumption in darkness (1.7 ± 0.7 nmol O_2 h^{-1}; n=24) (data not shown) compared to the respiration at light saturation (3.9 ± 1.9 nmol O_2 h^{-1}; n=5, see Table 1). Thus, respiration was stimulated in the light by a factor of 2.

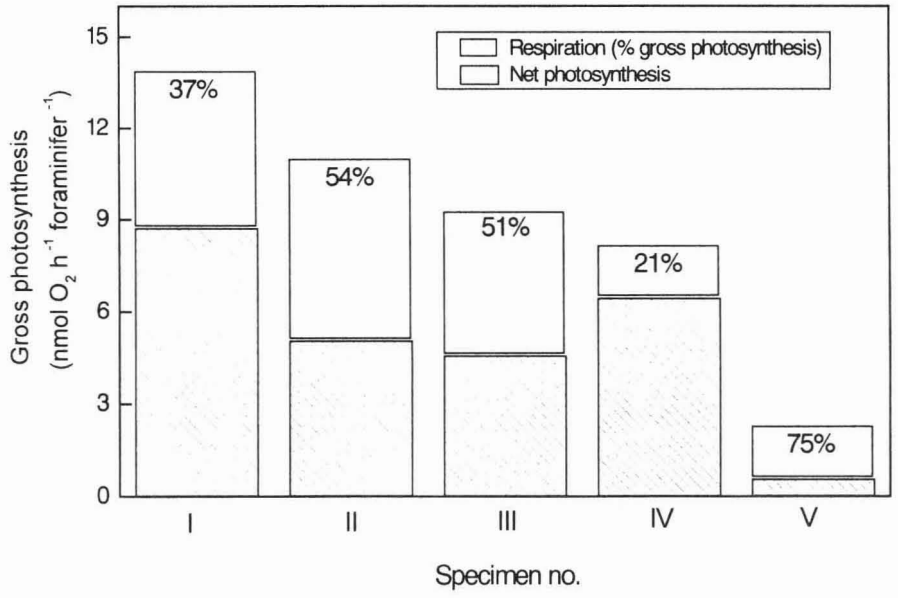


Fig. 9 Photosynthesis and respiration rates of different *Orbulina universa* specimens (No I-V) calculated in % of gross photosynthesis.

DISCUSSION

Foraminiferal microenvironment

The O_2 and pH of the microenvironment around the foraminiferal shell differs from the ambient seawater, depending on the rates of photosynthesis and respiration of the host-symbiont association. The pH varied approximately one unit between saturating irradiances and dark conditions, and the O_2 level ranged between < 70 - 206% of air saturation. The foraminifer and its endosymbionts thus live in a dynamic microenvironment of constantly shifting physico-chemical conditions.

The steep O_2 and pH gradients from the shell to the bulk medium at higher irradiances (> 150 µmol photons m^{-2} s^{-1}) (Figs. 7A, B) are caused by the high photosynthetic activity of the endosymbionts and the existence of a diffusive boundary layer (DBL) that surrounds the shell of the foraminifer (Jørgensen et al. 1985). The DBL constitutes a barrier for the mass transfer of gases, ions, and other solutes between the foraminifer and the ambient seawater. The thickness of the DBL around a sphere is generally measured by extrapolating the gradient of O_2 at the sphere-water interface to the ambient seawater concentration (Jørgensen and Revsbech 1985, Ploug et al. 1997). While the DBL thickness around the shell of *O. universa* could be estimated in the dark (~200 µm) when the symbionts reside inside the shell, the DBL thickness in light could not be estimated by the extrapolation method due to the presence of the symbiont swarm around the shell. The steady state O_2 gradients towards the shell in the light are thus affected by diffusion as well as photosynthesis and respiration.

The relative importance of small scale physical processes around the shell and between the spines (eddy and molecular diffusion) are still unknown and should be investigated to characterize the DBL in more detail. Due to the presence of the calcite spines, the DBL probably shows different characteristics than a sublayer over a sphere with a smooth surface (e.g. turbulent wakes) (Mann and Lazier 1991).

To understand zooxanthellae photosynthesis the scalar irradiance is the most relevant light intensity parameter (Kirk 1994, Kühl et al. 1995). In hermatypic corals, Kühl et al. (1995) measured scalar irradiance profiles with a fiber optic microprobe and demonstrated that the scalar irradiance could reach up to 180% of the downwelling irradiance at the tissue surface. This increase was explained by multiple scattering and

diffuse reflection of light within the coral tissue-skeleton matrix. Our measurements showed a slight increase of scalar irradiance towards the spherical shell of *O. universa* that is probably caused by the combined scattering of the calcite spines and the reflection of light by the spherical shell (Fig. 3C). The light measurements thus demonstrated no significant self shading of the dinoflagellate cells inside the swarm.

Photosynthetic rates

The photosynthetic rates determined for *O. universa* are similar to published data. The photosynthetic productivity of *O. universa*, when measured with the ^{14}C method (Spero and Parker 1985), showed a photosynthetic rate per symbiotic dinoflagellate of 1.72 pmol C h^{-1}. Assuming an average symbiont density of about 3.3 * 10^3 algae per adult *O. universa* (Spero and Parker 1985), the total photosynthetic rate of a single foraminifer would amount to a rate of 5.7 nmol C h^{-1} *Globigerinoides sacculifer* showed a mean gross photosynthetic rate of 18 nmol O_2 foraminifer^{-1} h^{-1} and a net photosynthesis of 15 nmol O_2 foraminifer^{-1} h^{-1} (Jørgensen et al. 1985). The carbon fixation rates of symbiotic planktonic foraminifera collected in the surface waters near Bermuda ranged between 1.2 - 4.2 nmol C h^{-1} foraminifer^{-1} (Caron et al. 1995). Assuming an O_2/ CO_2 conversion ratio of unity, these numbers compare well with the rates measured in this study.

During our experiments some zooxanthellae remained in the calcite shell. Because we only measured the gross photosynthesis towards the shell surface, we did not record the O_2 production inside the shell, which may not be negligible. Earlier measurements of the photosynthetic rates inside the shell of the symbiotic *G. sacculifer* showed a high O_2 production. Jørgensen et al. (1985) estimated an O_2 production of 3.8 nmol O_2 h^{-1} inside the shell. Thus the total gross photosynthetic rates per *O. universa* specimen we report here could be underestimated.

Due to the close coupling of photoautotrophic and heterotrophic processes in the symbiont-bearing foraminifera, the photosynthesis measurements with the ^{14}C method shows some disadvantages. Geider and Osborne (1992) pointed out methodological and interpretative problems of the ^{14}C method, e.g. the impossibility to measure the light respiration as well as the transport of carbon between the intracellular carbon pools. In symbiotic associations, the ^{14}C method probably understimates the production rates due to

the production of unlabeled CO_2 by respiration (Michaels 1991). Here we estimated the photosynthesis and respiration rates of *O. universa* from O_2 gradients and discrete measurements inside the symbiont swarm. Because we did not determine the chlorophyll *a* content of the endosymbionts and the number of endosymbionts, we present the rates on a per foraminifer basis.

The radial profiles of gross photosynthesis inside the symbiont swarm of *O. universa* showed a significant increase towards the shell surface. This is due to the fact that the symbiont density increased towards the shell. When measurements of gross photosynthetic rates were done by the light-dark shift technique, the zooxanthellae tended to move back into the shell of *O. universa* after a while. Our measurements of total gross photosynthesis are based on point measurements with a spatial resolution of 50 - 100 µm. This means that the O_2 production within the symbiont swarm was measured for a small volume around the electrode tip (Jørgensen et al. 1985). Consequently, a change of the spatial distribution of the zooxanthellae will affect the photosynthetic rates.

The variability of the gross photosynthetic rates is probably due to several reasons. First, the symbiont photosynthetic activity is affected by the available light and the nutrient supply. Second, the number of symbionts and their distribution may play an important role. Spero and Parker (1985) observed a positive correlation between the shell diameter and the symbiont number of juvenile *O. universa*. The symbiont density depends on the rate of cell division of the endosymbionts and on the age of *O. universa*. The dinoflagellate *Gymnodinium béii* shows division rates of 0.65 per day (25° C) in culture (Spero 1987). Although Spero and Parker (1985) could not determine a correlation between the size of the adult chamber and the number of symbionts, we observed a positive correlation between the size of the spherical shell and the total gross photosynthetic rate (Table 1). The specimen with the largest shell diameter showed the highest total gross photosynthesis. Lea et al. (1995) found that the shell diameter of *O. universa* specimens is independent of age. Therefore, the diameter of the spherical shell can not serve as an estimate for age. The correlation between the total gross photosynthetic rate and the foraminiferal shell size as well as the number of symbionts should be proved in further studies, e.g. by detailed pigment analysis.

The total photosynthetic rates of the symbiotic foraminifera can also be influenced by the pigment content of the symbiotic dinoflagellates. For example Bijma (1986) studied

the pigment composition of symbionts of *Globigerinoides ruber* and *G. sacculifer* and found a ~1.5 times higher chlorophyll *a*/carotenoid ratio in the symbionts of *G. ruber*. The type of endosymbionts is a further important parameter affecting the total photosynthesis. In some planktonic foraminifera smaller chrysophyte symbionts occur in higher abundances than the bigger dinoflagellate symbionts (Caron et al. 1995). In addition, daily variations of the photosynthetic rates were demonstrated in ^{14}C experiments with *O. universa* (Spero and Parker 1985). The photosynthetic rates of the symbiotic dino-flagellates started to increase in the late morning and highest rates were found in the late afternoon.

Light regulation of photosynthesis

Measurements of O_2 profiles and pH profiles (Figs. 7A, B) showed a very dynamic response to the incident light intensity and experimental light-dark cycles demonstrated a rapid reaction of the symbionts to changing irradiances (Fig. 4). Light-dark cycle experiments in *G. sacculifer* showed similar O_2 dynamics at the shell surface (Jørgensen et al. 1985).

The onset of light saturation of the symbiont photosynthesis (E_k) was estimated in two specimens of *O. universa* of different sizes. The E_k values were found at irradiances of 75 and 137 µmol photons m^{-2} s^{-1}, respectively. The difference is due to the different maximum photosynthetic rates (P_{max}) of the two specimens because both P-I curves showed nearly identical slopes (α) of 0.067 and 0.07 (Figs. 6A, B). The specimen with the higher E_k value also had a larger diameter (483 µm compared to 297 µm). One explanation for the higher E_k is thus a higher number of endosymbionts. However, higher P_{max} values can also indicate high growth irradiances (Herzig and Dubinsky 1992).

The study of photosynthesis versus irradiance curves in several symbiotic systems reported E_k values between 160 - 390 µmol photons m^{-2} s^{-1} (Jørgensen et al. 1985, Spero and Parker 1985, Kühl et al. 1995). *Globigerinoides sacculifer* collected in the Gulf of Aqaba showed higher E_k values of 160 - 170 µmol photons m^{-2} s^{-1} (Jørgensen et al. 1985) as compared to *O. universa*. ^{14}C measurements of photosynthetic rates of *O. universa* showed a much higher E_k value of 386 µmol photons m^{-2} s^{-1} (Spero and Parker 1985). The onset of light saturation at higher light levels demonstrate the adaptation of the symbionts to high irradiances in the surface waters. An adaptation to high light exposure is also indicated by

the fact that no photoinhibition was observed in our study even at high irradiances (Figs. 6A, B).

The calculation of the onset of light saturation (E_k) is also affected by the definition of the light field parameter (Kühl et al. 1995). Photosynthesis versus irradiance curves plotted against the downwelling irradiance (P vs. E_d) result in a lower E_k compared to the photosynthesis versus scalar irradiance curves (P vs. E_o) (Kühl et al. 1995). In our study, the E_k values estimated from the P vs. E_d curves were only slightly lower due to the smaller difference between E_d and E_o at the shell surface. However, scalar irradiance is always the most relevant light field parameter when measuring light regulation of photosynthesis in microscale (Kühl et al. 1994, Kühl and Jørgensen 1994).

The light compensation point (E_c) is dependent on gross photosynthesis and respiration of the host-symbiont system. In addition, processes that change the symbiotic light respiration, e.g. the mitochondrial respiration or photorespiration, may influence the light compensation point. A change of the foraminiferal light respiration due to growth rate or prey digestion may also result in a change of the compensation light intensity. Respiration measurements of *O. universa* before and after feeding thus demonstrated an increase of the respiration rate within a few hours after feeding with one day old *Artemia* nauplii (Rink, unpublished). Falkowski and Owens (1980) found a dependence of the light compensation point on the irradiance level during growth. The compensation light intensity of the symbiotic *G. sacculifer* was 26 - 30 µmol photons m^{-2} s^{-1} (Jørgensen et al. 1985). Compared to *O. universa* this lower compensation point is probably caused by adaptation to lower irradiances (150 µmol photons m^{-2} s^{-1}) during the maintenance in the laboratory several days before measurements (Jørgensen et al. 1985).

Light measurements at full sunlight at the collecting site showed irradiances up to 2070 µmol photons m^{-2} s^{-1} at the surface and 556 µmol photons m^{-2} s^{-1} in 12 m depth (S. Anderson 1995, pers. comm.). Depth profiles of scalar irradiance (E_o) measured at the collection site showed a mean light attenuation coefficient (K_o) of 0.07 (SD ± 0.023)(Fig. 10). The light compensation point (E_c) of *O. universa* would thus be reached in a depth of ca. 50 m at the sampling site (Fig. 10). Theoretically, a net O_2 production of the symbiont-host system is possible down to this water depth at full sunlight. The O_2 production of *O. universa* exhibited a pronounced light dependency (Fig. 7A) and high net primary

production rates of the symbiotic *O. universa* are limited to the regions of photosynthesis saturating irradiances in the surface waters (0 - 35m).

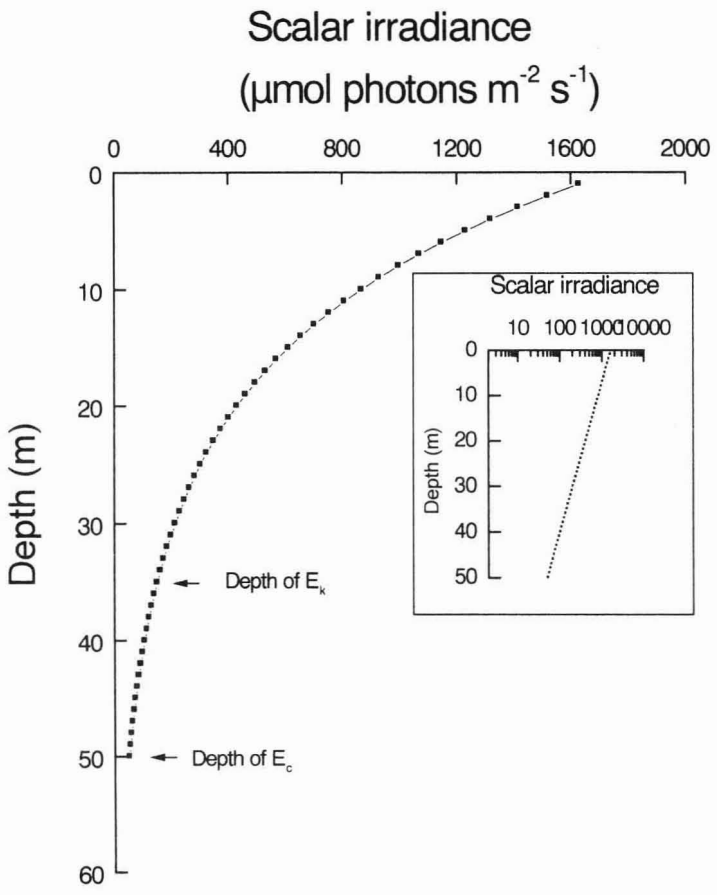


Fig. 10 Depth profile of scalar irradiance (E_o) calculated with the subsurface value $E_o(0)$ = 1747 µmol photons m^{-2} s^{-1} and a light attenuation coefficient K_o = 0.07. $E_o(0)$ was measured under sunny conditions in the California Current (Catalina Island) (E_k = 137 µmol photons m^{-2} s^{-1}, E_c = 50 µmol photons m^{-2} s^{-1}). Picture insert shows log-transformed data.

Primary production of planktonic foraminifera

The symbiont-bearing foraminifera constitute microenvironments of concentrated photosynthetic activity (Caron and Swanberg 1990) and were reported to have the highest rates of primary production in plankton communities because of the extremly high density of the endosymbiotic algae in their cytoplasm. Due to the high algal biomass, the amount of primary production occuring in the symbiont-host association is generally much higher than

in an equivalent volume of seawater (Jørgensen et al. 1985, Spero and Parker 1987). Jørgensen et al. (1985) estimated that a single foraminifer would increase the CO_2 fixation rate in a 125 ml productivity bottle 5-fold above the CO_2 fixation in ambient seawater. Spero and Parker (1985) made the assumption that a single large *O. universa* may represent a potential source of net primary production that would contribute approximately 0.2% of the fixed carbon in 1 m^3 of seawater.

Although the associations are packages of high productivity it is still difficult to estimate their total primary production. The foraminiferal part of the total phytoplankton primary production is dependent on their population density in the oceans (Bé 1977). Their productivity depends on the population dynamics and the patchiness of foraminifera. The distribution of most species shows a correlation with sea surface temperatures (Bradshaw 1959). Currents and mixing of surface waters may also cause a change of the foraminiferal distribution. Changes of the depth habitat due to the lunar periodicity of the reproductive cycle were reported by Hemleben and Spindler (1983).

Diurnal variations of the depth habitat, rising of the foraminifera during the daytime and falling in the night, are discussed by Berger (1969) and Boltovskoy (1973). Bradshaw (1959) suggested that the rapidly production of O_2 by the symbiotic algae in the protoplasm could form oxygen bubbles that increase the buoyancy of the foraminifera during the day and could cause a rising to the surface. Fairbanks and Wiebe (1980) observed a maximum abundance of planktonic foraminifera in the deep chlorophyll maximum layer (DCM) with changing seasonal depth levels. They suggested that the foraminifera exploit the DCM as a major source for food and nutrients. Population studies of Bé et al. (1985) showed seasonally changing abundances of planktonic foraminifera in the Panama Basin. Because of this distributional patchiness in horizontal and vertical direction the estimation of the primary productivity by planktonic foraminifera is difficult and only be possible for small oceanic areas that are well studied.

To calculate the net primary production of *O. universa* from our microsensor data we assumed a density of 5 specimens m^{-3} (Spero and Parker 1985). An average net photosynthetic rate of 5 nmol O_2 h^{-1} foraminifer^{-1} (Table 1) over a daily light exposure about 10 h would result in a production of 0.25 µmol O_2 m^{-3} day^{-1} at light saturation. For the same parameters Jørgensen et al. (1985) found a three times higher primary production of 0.75 µmol O_2 m^{-3} day^{-3} for *G. sacculifer* in the Gulf of Aqaba. The whole population of *G.*

sacculifer would contribute about 0.1% of the mean yearly primary production in the Gulf. Caron et al. (1995) reported that the total symbiont production of sarcodines (acantharia, radiolaria, foraminifera) in oligotrophic waters of the Sargasso Sea contribute only a small fraction (< 1 %) of the total primary production. They found production rates of acantharia and foraminifera to contribute with an average of ~5% to the total annual primary production in the surface waters. A vertical biomass distribution for foraminifera was given by Michaels (1991) who formulated a depth dependent relationship for symbiont productivity that is related to the exponential decline of the light field.

The percentage of the total primary production of planktonic foraminifera in 1 m^3 of seawater is probably overestimated and the production rates are more variable because several parameters are limiting the primary production rates as mentioned before. Symbiont densities and productivties as well as light exposure and nutrient supply are changing the maximum net O_2 production. If the planktonic foraminifera are changing their depth habitat due to vertical migration light will be a limiting factor.

There are still open questions about the nutritional relationship in the foraminifer-dinoflagellate symbiosis. For instance, which kind of photosynthates are released by the dinoflagellates and how much of the primary fixed carbon is translocated to the host. With regard to the predation on plankton the significance of the photosynthate supply for the energy budget of the host will be of great interest. Due to the vertical ontogenetic migration of the planktonic foraminifera a combination of two energy sources, planktonic prey, and photosynthates, is probably of importance. Detailed investigations of the migration patterns and changing abundances of *O. universa* and other species in the water column would help to provide more information about their total primary productivity (Hemleben and Bijma 1994).

Respiration in light and darkness

One advantage of the microsensor technique compared to other methods is the possibility to estimate the respiration rate of the symbiont-host system in the light. We were able to calculate the light respiration of a *O. universa* by measuring the total gross photosynthesis and the net photosynthesis of the same specimen. Direct comparison of dark and light respiration rates was therefore possible. In the light, we found higher respiration

rates of the symbiont-host association (3.9 nmol O_2 h^{-1} $foraminifer^{-1}$) compared to the dark respiration (1.7 nmol O_2 h^{-1} $foraminifer^{-1}$). This enhanced respiration in the light was described for several symbiotic systems (Edmunds and Davies 1988, Kühl et al. 1995, Harland and Davies 1995) and for microalgae (Falkowski et al. 1985, Grande et al. 1989). Different mechanisms are discussed to explain the enhanced respiration in the light (Falkowski et al. 1985, Weger et al. 1989).

The respiration of the host is enhanced in the light via the production of photosynthates by the dinoflagellate endosymbionts. Symbionts of larger benthic foraminifera have been shown to release soluble photosynthates like polyglucan, glycerol, glucose, and lipids (Kremer et al. 1980). The zooxanthellae probably increase the quantity of respiratory subtrates translocated to the host in the light. The tissue of larger foraminifera contains some activating factors that stimulate the release of the photosynthates. Lee et al. (1984) found that the level of the photosynthate release of isolated endosymbionts increased dramatically in the presence of host homogenates. Due to the supply of carbohydrates and lipids by the endosymbionts foraminiferal respiration can thus be stimulated in the light.

Photosynthesis results in O_2 supersaturation around the foraminiferal shell that could stimulate the respiration of the symbionts and the foraminifer. This internal O_2 supply alleviates the diffusion limitation due to the presence of the DBL. Experiments showed increased dark respiration when the symbiotic sea anemone *Anemonia viridis* was exposed to hyperoxic water (Harland and Davies 1995). They suggested, therefore, that the day time respiration is influenced by the O_2 release of the endosymbionts. Also, Jørgensen et al. (1985) suggested that the limited O_2 supply in the darkness due to the presence of the DBL caused reduced dark respiration rates. They measured a decrease of the O_2 at the shell of *G. sacculifer* down to 50% of air saturation in darkness. In *O. universa* we found an O_2 decrease to the shell surface down to 67% air saturation during darkness.

A higher O_2 consumption in the light can also be caused by photorespiration. Photorespiration is defined as a light dependent O_2 uptake and CO_2 release due to the bifunctional enzyme Rubisco (Falkowski et al. 1985, Beardall and Raven 1990). The high O_2/ CO_2 ratio produced by the photosynthesis of the zooxanthellae could promote the oxygenase activity of Rubisco. However, an efficient inorganic carbon uptake mechanism present in most microalgae seems to be able to decrease the importance of photo-respiration

(Beardall and Raven 1990). To our knowledge no investigation of photorespiration or inorganic carbon uptake by the foraminiferal symbionts has been reported in the literature.

In principle the pseudocyclic photophosphorylation (Mehler reaction) represents another light induced O_2 consuming process (Raven and Beardall 1981, Falkowski et al. 1985). However, Glud et al. (1992) suggested that the measurement of gross photosynthetic rates with the light-dark shift method probably does not include the O_2 consumed by the Mehler reaction.

Due to the limitation of the ^{14}C method to measure respiration in the light, some authors investigated the dark respiration after exposure to high irradiances. This process of post-illuminated O_2 consumption in the darkness was discussed for different microalgae (Burris 1977, Falkowski et al. 1985, Weger et al. 1989, Beardall et al. 1994) as well as for symbiotic sea anemones (Harland and Davies 1995) and corals (Edmunds and Davies 1988). Burris (1977) obtained a post-illumination burst of oxygen uptake in the dinoflagellate *Glenodinium* sp. and in the zooxanthellae of the coral *Pocillophora capitata* that lasted about 5 - 10 min. The dinoflagellates showed a longer post-illumination burst compared to other algae (1 - 2 min). Burris (1977) explained this increase by the possibility of a different photorespiratory pathway or by higher dark respiration rates. Beardall et al. (1994) demonstrated that low light adapted cells of *Thalassiosira weissflogii* were more susceptible to the enhanced post-illumination respiration (EPIR) compared to cells grown under high light conditions. Harland and Davies (1995) found a stimulation of dark respiration of 39% after 6 h exposure to saturating irradiance (300 µmol photons m^{-2} s^{-1}). The reef coral *Porites porites* showed a mean increased dark respiration rate of 39% relative to the pre-illumination dark respiration rate (Edmunds and Davies 1988).

The estimation of the light respiration with the microsensor technique showed much higher respiration rates during light conditions compared to the dark respiration rates (Kühl et al. 1995). Jørgensen et al. (1985) measured for *G. sacculifer* a similar respiration rate in the light (3.0 nmol O_2 h^{-1} foraminifer^{-1}) as we did for *O. universa* but they did not find a lower dark respiration (2.7 nmol O_2 h^{-1}). In *O. universa*, we found a two times lower dark respiration (1.7 ± 0.7 nmol O_2 h^{-1}, n=24). If we assume higher total photosynthetic rates per foraminifer due to additional O_2 production inside the shell, the difference between respiration rates in the light and darkness may be even larger. Generally, the dark respiration rates of microalgae are in the order of 10% of the gross photosynthesis (Beardall and Raven

1990). In our study we measured a mean total dark respiration of the symbiont host association of 1.7 nmol O_2 h^{-1} and an average total gross photosynthetic rate of 8.9 nmol O_2 h^{-1}. If we assume that 50% of the total O_2 uptake is due to the symbiont respiration (Jørgensen et al. 1985), the dark respiration rate of the zooxanthellae is nearly in the order of 10% of the gross photosynthesis.

The P/R ratio is used to estimate the physiological state of marine microalgae and to scale the relationship of consumption and production of organic material (Burris 1977). This ratio has been investigated for several algal species and the numbers for dinoflagellates varied between 1.3 and 5.7 (Humphrey 1975, Burris 1977, Daneri et al. 1992). The zooxanthellae of coelenterate hosts can supply most of the carbon required by the host, as was demonstrated in 70 species of corals with P/R ratios of 2.4 ± 1.5 (Battey 1992). In our study we measured a mean net photosynthesis of 5.0 nmol O_2 h^{-1} during light saturation and an average dark respiration of 1.7 nmol O_2 h^{-1} foraminifer^{-1}. Consequently, the P_{net}/R_{dark} ratio of the symbiont-host system is about 3, which indicates that the required carbon for the foraminifer can be supplied by its symbionts. However, to estimate, if the net primary production of the endosymbionts can provide the required organic carbon for growth and respiration of the symbiont-host association, the total net photosynthesis over the daily light period as well as the growth rates and the respiration rates of the host and the symbionts have to be calculated on a daily basis.

It has been suggested that foraminifera supply their endosymbionts with the respired CO_2 (Bé 1977). The respiration of *O. universa* in the light showed an average rate of 48% of the gross photosynthesis. This value demonstrates a much higher CO_2 availability for the symbionts as compared to free living dinoflagellates. Geider and Osborne (1989) reported, that a dark respiration vs. photosynthesis rate of 0.25 is generally found in dinoflagellates. *Orbulina universa* can, thus, supply its endosymbionts with additional CO_2, that may support the photosynthetic CO_2 fixation. However, recent model calculations (Wolf-Gladrow et al., subm) as well as laboratory experiments (Bijma et al., subm) demonstrate that *G. béii* in symbiosis with *O. universa* as well as isolated in culture also tap into the bicarbonate pool as a carbon source.

Conclusions

Microsensors are useful tools for studying photosynthetic processes in symbiotic systems and for comparing light and dark respiration rates. The respiration of *O. universa* in the light was significantly higher than dark respiration. Possible mechanism for this observation might be the increase of respiratory substrate (photosynthates) released by the symbionts and/ or photorespiration.

Varying incident irradiances caused dynamic changes of the symbiont photosynthetic activity that affected the chemical microenvironment around the foraminiferal shell. High photosynthetic rates in combination with a slow efflux of O_2 and protons due to the diffusive boundary layer created an O_2 oversaturation and a pH increase in the foraminiferal microenvironment as compared to the ambient seawater. The symbiotic associations of *O. universa* thus represent highly productive „hot spots" in the light saturated photic zone of oligotrophic pelagic environments.

To understand the complexity of interactions between photosynthesis, respiration, and calcification in symbiotic foraminifera, new methods have to be explored. A new CO_2 microsensor (de Beer et al. 1997) could provide more information about the CO_2 uptake and dynamics. Furthermore the CO_2 microsensors could be used in combination with Ca^{2+} microelectrodes (Tsien and Rink 1980, Amman et al. 1987) to investigate the process of calcification in symbiont-bearing foraminifera.

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Chapter 3

The CO_2, O_2, pH, and Ca^{2+} microenvironment of the symbiotic planktonic foraminifer *Orbulina universa* studied with microsensors

Stephanie Köhler-Rink[1], *Michael Kühl*[2]

[1]Max-Planck-Institute for Marine Microbiology, Microsensor Research Group, Celsiusstr. 1, D-28359 Bremen, Germany.

[2]Marine Biological Laboratory, University of Copenhagen, Strandpromenaden 5, DK-3000 Helsingør, Denmark

ABSTRACT

We used microsensors for O_2, pH, and photosynthesis rate measurements together with the first direct CO_2 and Ca^{2+} measurements in the vicinity of a symbiont-bearing foraminifer *Orbulina universa*. The chemical microenvironment was affected by the combined processes of symbiont photosynthesis, respiration of the community, and host calcification. Furthermore, the presence of a diffusive boundary layer (DBL) with a thickness of 250 - 800 µm limited the solute exchange between the foraminifer and the surrounding seawater. Under saturating light conditions, microprofiles measured towards the shell surface showed an O_2 increase up to 210% air saturation, a CO_2 decrease down to 4.9 µM, and a pH increase up to pH 8.8 due to symbiont photosynthesis. In darkness, the respiration of the community decreased the O_2 concentration down to 82% air saturation, CO_2 increased up to 15 µM, and pH decreased down to pH 8.0. Consequently, the carbonate system in the vicinity of the foraminifer was significantly different from conditions in the surrounding seawater both in light and darkness. Net fluxes of O_2 and CO_2 measured at the shell surface in the symbiont swarm demonstrated much higher rates of O_2 influx and efflux as compared to the CO_2 fluxes. At high irradiance (664 µmol photons m^{-2} s^{-1}) a molar O_2/CO_2 conversion ratio of about 38.5 was estimated. Combined concentration measurements of CO_2 and pH in the symbiont swarm during experimental light-dark cycles showed a time delay of the CO_2 response, whereas simultaneously measured O_2 and pH concentrations changed immediately when the light conditions changed. These observations indicate sufficient CO_2 supply for high carbon fixation rates of the symbiotic algae via conversion of HCO_3^- or via CO_2 release from calcification and host respiration.

Calcium concentration profiles as well as Ca^{2+} dynamics measured during experimental light-dark cycles demonstrated a light dependent Ca^{2+} microenvironment of *O. universa*. Microprofiles measured from the ambient seawater towards the shell surface showed a significant Ca^{2+} concentration change at the shell as compared to the seawater level (10 mM). In the light, Ca^{2+}concentration decreased down to 9.65 mM and under dark conditions Ca^{2+} increased up to 10.8 mM. Our findings on the carbonate system and calcification in the vicinity of foraminifera have important implications for paleoclimatic interpretations on foraminiferal analyses.

INTRODUCTION

Symbiotic planktonic foraminifera are widespread calcifying protozoa living in symbiosis with phototrophic microalgae (Lee et al. 1965, Bé 1977, Murray 1991, Hemleben and Spindler 1983). They are most abundant in the euphotic zone of subtropical and tropical oceans with highest densities of 10 - 100 individuals m^{-3} (Bradshaw 1959, Bé 1977). Symbiont-bearing planktonic foraminifera inhabit the euphotic zone and reach depth down to ca. 100 m. Highest numbers have been found at 10 - 50 m depth, where the endosymbionts are exposed to sufficient irradiance. The planktonic foraminifera show diurnal and ontogenetic vertical migration patterns in the water column, and sinking into deeper waters was observed during reproduction.

Orbulina universa was described as an ubiquitos species in subtropical, tropical, and transitional waters. Like most spinose species, it host high numbers of the endosymbiotic dinoflagellate *Gymnodinium béii* (Spero 1987). During daytime, when the symbionts are light-exposed in the host cytoplasm strechted out along the calcite spines, high photosynthetic activities have been measured (Jørgensen et al. 1985, Rink et al. 1998). Symbiont-bearing planktonic foraminifera can be described as "hot spots" of primary productivity in oligotrophic seas. However, the inorganic carbon sources that the endosymbionts use to maintain such high photosynthetic activities still remain to be identified.

It has been demonstrated that photosynthesis of the symbiotic microalgae can affect the calcification process in planktonic foraminifera. Lea et al. (1995) measured up to three times higher calcification rates in *O. universa* under high light conditions as compared to individuals grown in the dark. Thus, they hypothezised that symbiont photosynthesis enhances calcification and creates a microenvironment with optimal conditions for $CaCO_3$ precipitation. The role of endosymbionts in relation to the shell growth was studied in the species *Globigerinoides sacculifer* that builts up one chamber per day. Inhibition of symbiont photosynthesis with the inhibitor DCMU resulted in smaller shell growth rates of the foraminifera (Bé et al. 1982).

The physico-chemical microenvironment of several symbiotic systems including planktonic foraminifera (Jørgensen et al. 1985, Rink et al. 1998), benthic foraminifera (Köhler-Rink and Kühl 2000), corals (Kühl et al. 1995, de Beer et al. 2000), didemnid

ascidians (Kühl, unpublished), and radiolaria (Köhler-Rink et al. unpublished) has been studied with microsensors for O_2, pH, CO_2, Ca^{2+}, and scalar irradiance (PAR). In the planktonic foraminifera *O. universa* and *G. sacculifer* such experiments demonstrated significantly different O_2 and pH levels at the shell surface as compared to the ambient seawater conditions. In the light, O_2 supersaturation up to 200% air saturation and pH values up to 8.8 were measured (Jørgensen et al. 1985, Rink et al. 1998). Moreover, O_2 microsensores were used to study the effect of light on photosynthesis and respiration of the foraminifera demonstrating enhanced respiration rates in the light as compared to the dark respiration (Rink et al. 1998). Based on such microsensor measurements, a diffusion-reaction model of the seawater chemistry surrounding the shell of symbiont-bearing foraminifera has been applied (Wolf-Gladrow et al. 1999). With this model concentration profiles of the carbonate species HCO_3^-, CO_3^{2-}, and CO_2 including pH have been simulated during light and dark situations.

In the present study, we used microsensors for CO_2, O_2, pH, and Ca^{2+} to obtain additional information about the chemical microenvironment of the symbiotic species *O. universa* under light and dark conditions. 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 in planktonic foraminifera. Our experiments with the photosystem II inhibitor DCMU (3-(3-4 dichlorophenyl-1.1-dimethylurea) indicate a complex dependence of the Ca^{2+} micro-environment on the foraminiferal metabolism.

MATERIALS AND METHODS

Sampling site Adult specimen of *Orbulina universa* with spherical shell diameters of 570 - 1000 µm were collected from surface waters near Curaçao, Netherland Antilles in the Caribbean Sea in March and April 1999. Individual organisms were captured in glas jars by Scuba divers at 5 - 10 m depth. At the sampling site, surface waters were characterized by calcium supersaturation and high temperatures up to 28° C. In-situ salinity was 38‰ and pH was 8.2. Experiments were conducted in the laboratory of the Caribbean Marine Research Station (CARMABI, Netherlands Institute of Sea Research). Individual specimen were maintained in glas jars with filtered seawater at room temperature of 26 °C. Measurements were performed at the day of sampling.

Microsensor measurements and experimental setup For microsensor measurements, 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. Incident light was provided by a fiber optic halogen lamp (Schott KL-1500, Germany). Light intensity (0 - 1000 µmol photons m^{-2} s^{-1}) was varied by neutral density filters (Oriel Inc., USA) inserted in the light path and was calibrated with a quantum scalar irradiance meter (QSL 101, Biospherical Instruments Inc., USA). Experimental light-dark cycles were controlled by an electro-mechanical shutter (Vincent Association, USA) installed between the halogen lamp and the measuring chamber. The microelectrodes were fixed to a motor controlled micromanipulator (Märtzhäuser & LOT-ORIEL, Germany). Their positioning relative to the foraminiferal shell surface was observed through a dissection microscope (Zeiss, SV 11, Germany). Micromanipulator and shutter control as well as data acquisition were controlled by the program LabVIEW (National Instruments) that also collected the signals by the custom made data acquisition system (Insight, PyroImagination).

Microprofiles with a spatial resolution of 50 - 100 µm were measured from the ambient seawater towards the shell surface of *O. universa*. For simultaneous measurements of two chemical compounds the two microsensors were placed in close proximity (< 100 µm) at the shell surface of the foraminifer during experimental light-dark cycles.

Microsensors Microsensors for O_2, CO_2, pH, and Ca^{2+} were used. Clark-type O_2 microelectrodes (Revsbech 1989) were constructed with an outer tip diameter of 7 µm, a

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. The O_2 detection limit was 0.1 µM. CO_2 microelectrodes with a detection limit down to 1 µM and a response time of ca. 10 s were composed of an outer glas casing with an internal pH liquid ion-exchange (LIX) microelectrode. The CO_2 sensor was calibrated in a degassed phosphate buffer (50 mM, pH 8.0) by adding aliquots of 200 mM carbonate solution (de Beer et al. 1997). pH and Ca^{2+} were measured with liquid ion-exchange (LIX) microelectrodes with tip diameters of 2 - 5 µm and a response time of < 1 s. The LIX microelectrodes are shielded with an outer casing containing 1 M KCl to reduce electrical noise. The detection limit for Ca^{2+} was 10 µM, and calibration was performed in 1, 10, and 20 mM Ca^{2+} solutions with added back ground ions of seawater concentration (Mg, Na, and K). pH microelectrodes were calibrated in standard pH buffers (Mettler Toledo).

Oxygen 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 and Keithley, USA). Signals were recorded with a strip chart recorder (Servogor, SV 124) connected to the computer data acquisition system.

DCMU experiments The herbicide DCMU (3-(3,4-dichlorophenyl)-1,1-dimethyl urea), an inhibitor of photosystem II, was dissolved in ethanol and added to the seawater (10^{-5} M final concentration) to inhibit symbiont photosynthesis and foraminiferal calcification (Erez 1983). During light-dark cycles, O_2 and Ca^{2+} were simultaneously measured at the shell surface of *O universa* before and after the incubation time of 0.5 h with inhibitor.

RESULTS

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

Photosynthesis, respiration, and calcification in *O. universa* affected the chemical microenvironment in the vicinity of the foraminiferal shell (Fig. 1-3).

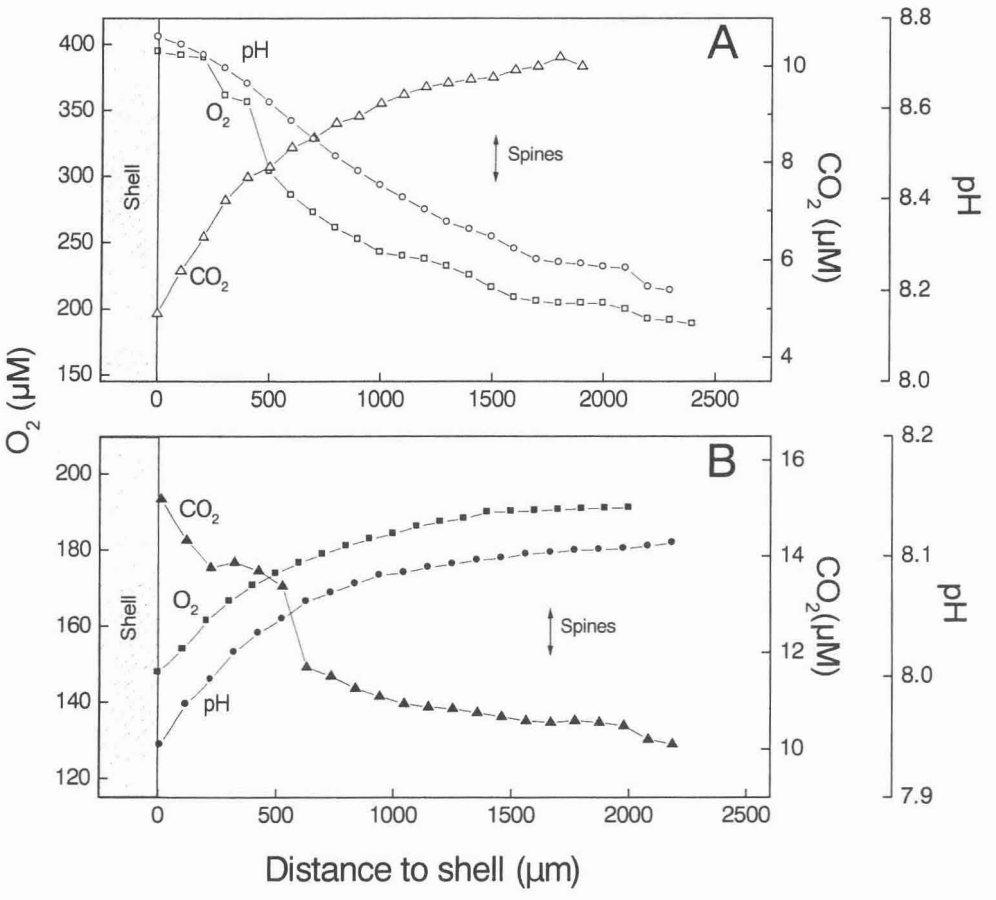

Fig. 1 Light (**A**) and dark (**B**) concentration profiles of CO_2 (Δ), O_2 (□), and pH (○) measured from the ambient seawater towards the shell surface of *Orbulina universa* (scalar irradiance = 130 µmol photons m^{-2} s^{-1}).

In light an O_2 and pH increase and a CO_2 decrease towards the shell surface was due to the photosynthesis of the symbiotic algae (Fig. 1A). Oxygen and pH increased up to 394 µM (218% air saturation) and pH 8.76, respectively. CO_2 was lowered down to 4.9 µM at the shell surface. Under dark conditions the respiration of the community caused an O_2 and pH decrease down to 147 µM (82% air saturation) and pH 8.03, respectively. Due to respiration and/or calcification CO_2 increased up to 15 µM (Fig. 1B). Ambient seawater concentrations of O_2, CO_2, and pH were 180 µM, 10 µM, and pH 8.2, respectively.

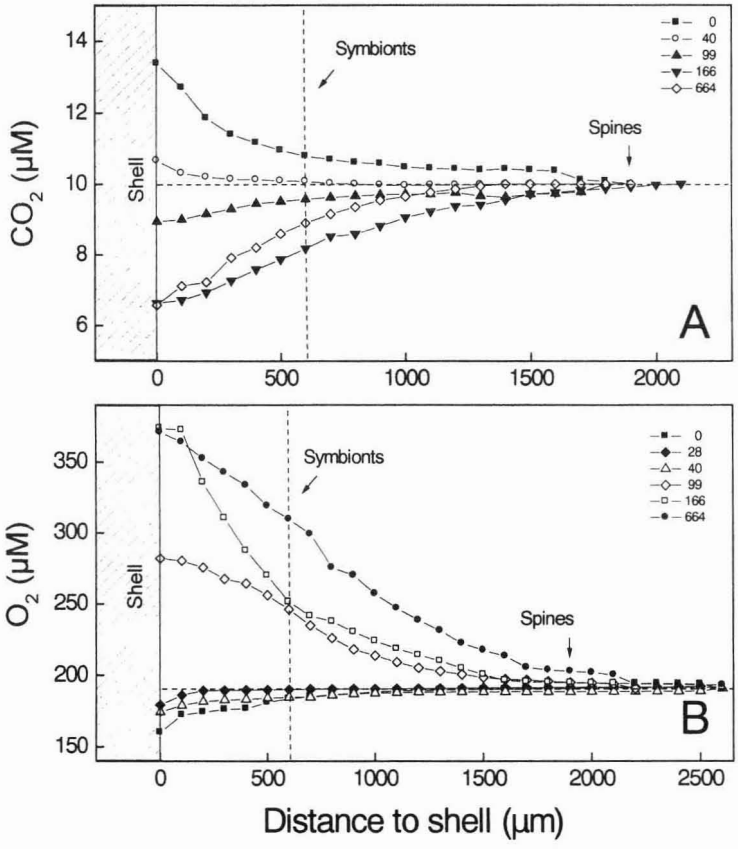

Fig. 2. Radial concentration profiles of CO_2 (**A**) and O_2 (**B**) were measured towards the shell surface of *Orbulina universa* as a function of increasing irradiance. Numbers indicate incident irradiance in µmol photons m^{-2} s^{-1}. Horizontal dashed lines indicate ambient seawater concentrations. Vertical dotted lines indicate the start of the symbiont swarm.

With increasing irradiance the CO_2 concentration towards the shell surface decreased, whereas O_2 increased (Fig. 2). At 166 µmol photons m^{-2} s^{-1} symbiont photosynthesis approached its saturation level as indicated by both, CO_2 and O_2 profiles (Fig. 2).

Ca^{2+} microprofiles measured under light and dark conditions showed a significant concentration change between the ambient seawater and the shell surface of *O. universa* (Fig. 3). Most profiles demonstrated a Ca^{2+} decrease towards the shell surface and thus a Ca^{2+} uptake of the foraminifer in light (Fig. 3A).

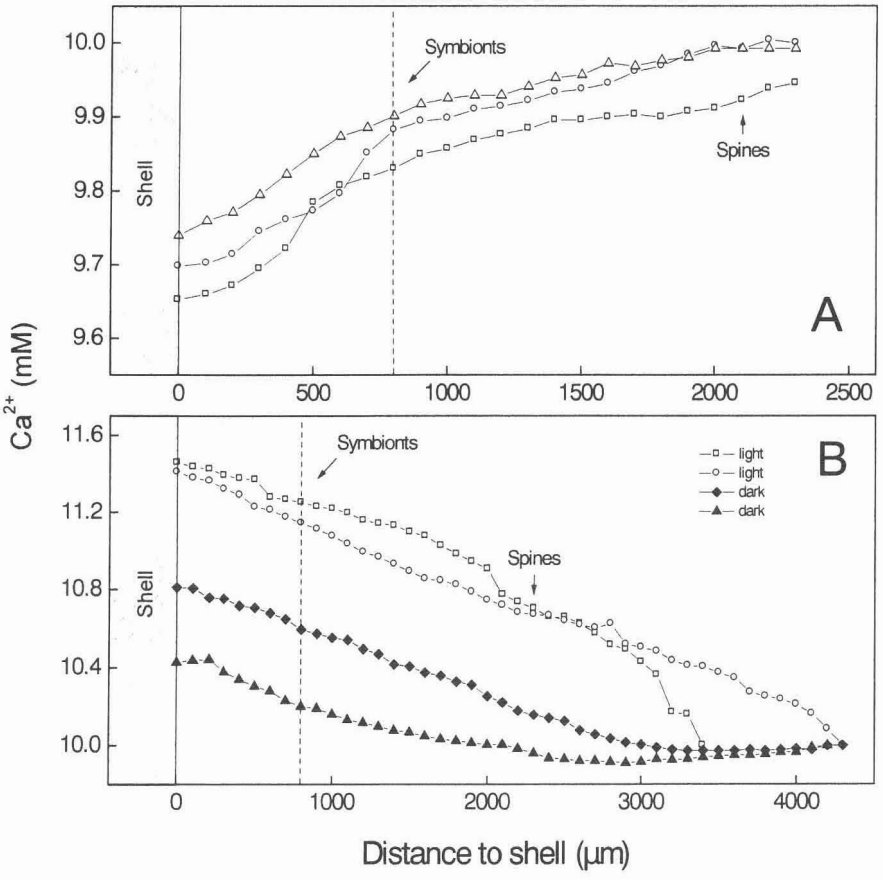

Fig. 3 Concentration profiles of Ca^{2+} measured in the light towards the shell surface of *Orbulina universa* (**A**). Light and dark Ca^{2+} profiles measured with a second specimen (**B**). Dotted lines indicate the start of the symbiont swarm.

The Ca^{2+} concentration at the shell surface decreased to 9.65 mM. However, in a few specimens the Ca^{2+} concentration increased in the light (Fig. 3B). All dark profiles showed a concentration increase of Ca^{2+} up to 10.8 mM at the shell surface.

In addition to the metabolic activity of the foraminifer the chemical microenvironment was influenced by the presence of a diffusive boundary layer (DBL) surrounding the shell of *O. universa*, that limited the solute exchange between the ambient seawater and the foraminifer. The DBL reached a thickness of 250 - 800 μm as determined by the extrapolation of the concentration gradient at the shell-seawater interface to the ambient seawater concentration (Jørgensen and Revsbech 1985, Ploug et al. 1997, Rink et al. 1998) (Figs. 1, 2).

Net O_2 and CO_2 fluxes

The CO_2 uptake and O_2 release rate was calculated from the concentration gradients measured at the shell as a function of irradiance (Fig. 2). The light compensation point of symbiont photosynthesis (E_c) was reached at ~75 μmol photons m^{-2} s^{-1} (Fig. 4), when no net O_2 or CO_2 exchange was observed. Above 75 μmol photons m^{-2} s^{-1} a net O_2 release and a net CO_2 uptake due to photosynthesis was measured. At all irradiance level, concentration gradients showed much higher fluxes of O_2 as compared to CO_2. At high irradiance (664 μmol photons m^{-2} s^{-1}) net O_2 production was 77 nmol O_2 cm^{-2} h^{-1} and CO_2 uptake 2 nmol O_2 cm^{-2} h^{-1}. Thus, a molar O_2/CO_2 conversion ratio of 38.5 was calculated.

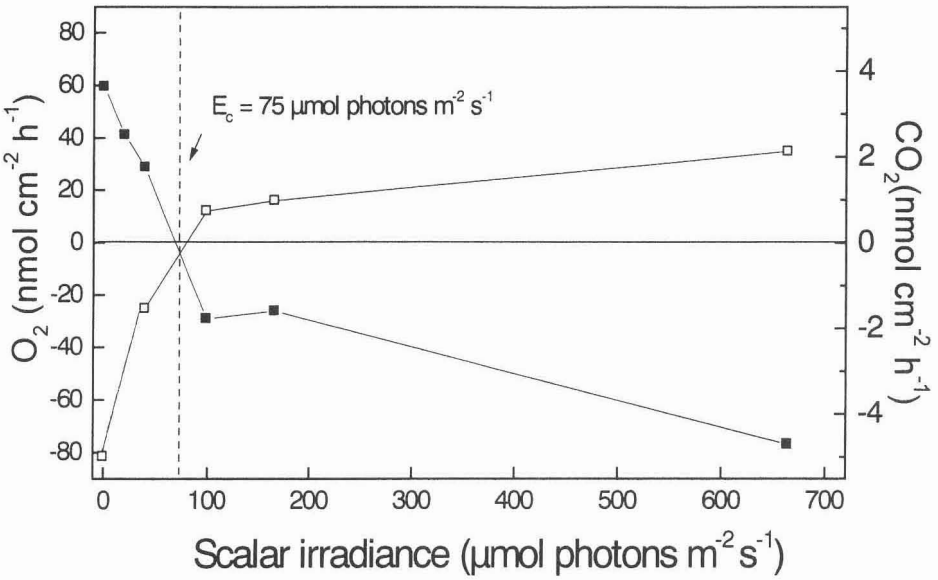

Fig. 4 Net fluxes of O_2 (□) and CO_2 (■) (nmol cm^{-2} h^{-1}) were calculated from measured concentration gradients at the shell surface of *Orbulina universa* with increasing scalar irradiance (Fig. 2). Note the different scales for O_2 and CO_2 fluxes. Vertical dashed line indicates the compensation irradiance, E_c = 75 µmol photons m^{-2} s^{-1}.

Dynamic shell surface conditions

We measured significant variations of the CO_2, O_2, pH, and Ca^{2+} concentration at the shell surface of *O. universa* during experimental light-dark cycles (Figs. 5, 6, 7). Combined measurements of CO_2, O_2, and pH demonstrated a dynamic response of the foraminiferal-algal symbiosis with changing irradiances. Under light conditions the symbiont photosynthesis increased the O_2 and pH concentrations and decreased the CO_2 level at the shell surface. When light was switched off the respiration of the association increased the CO_2 again and O_2 and pH values decreased.

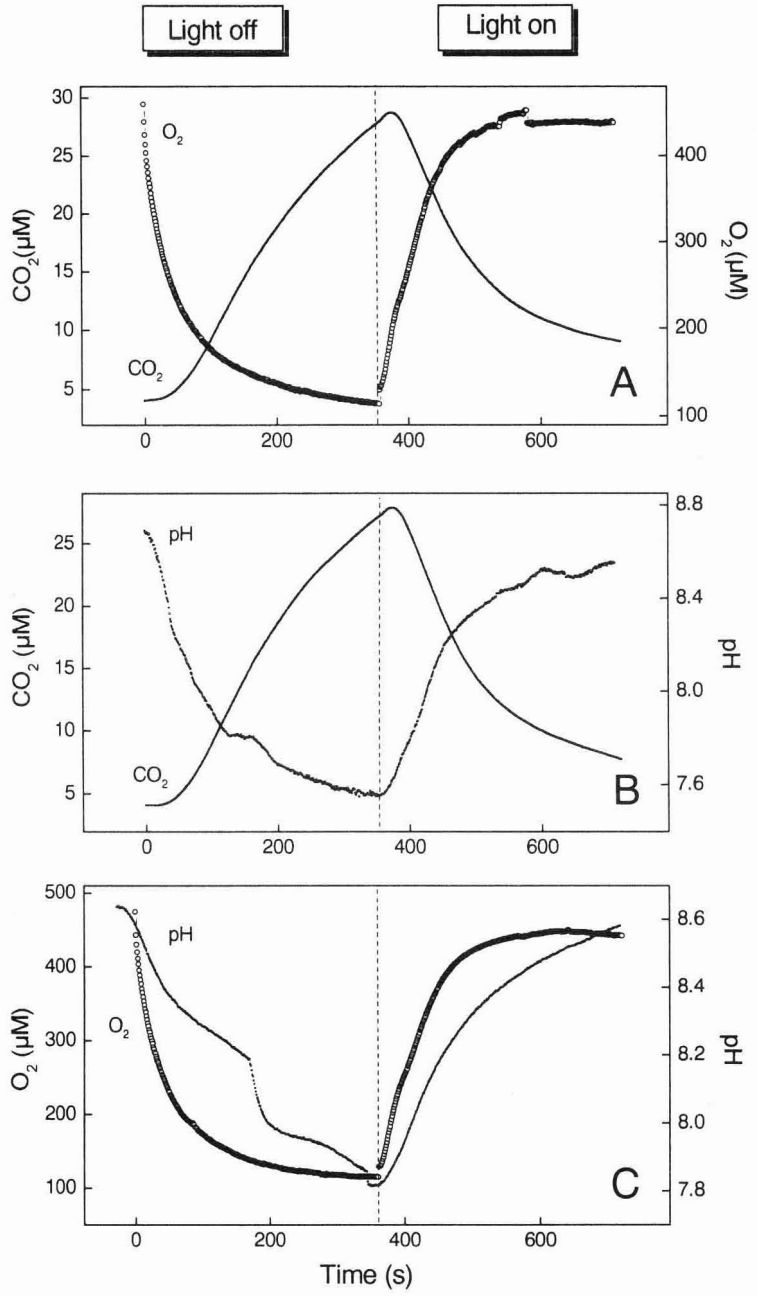

Fig. 5 CO_2 and O_2 (**A**), CO_2 and pH (**B**), and O_2 and pH (**C**) concentrations were measured simultaneously during experimental light-dark cycles at the shell surface of *Orbulina universa* within the symbiont swarm. Dotted lines indicate the change from dark to light conditions.

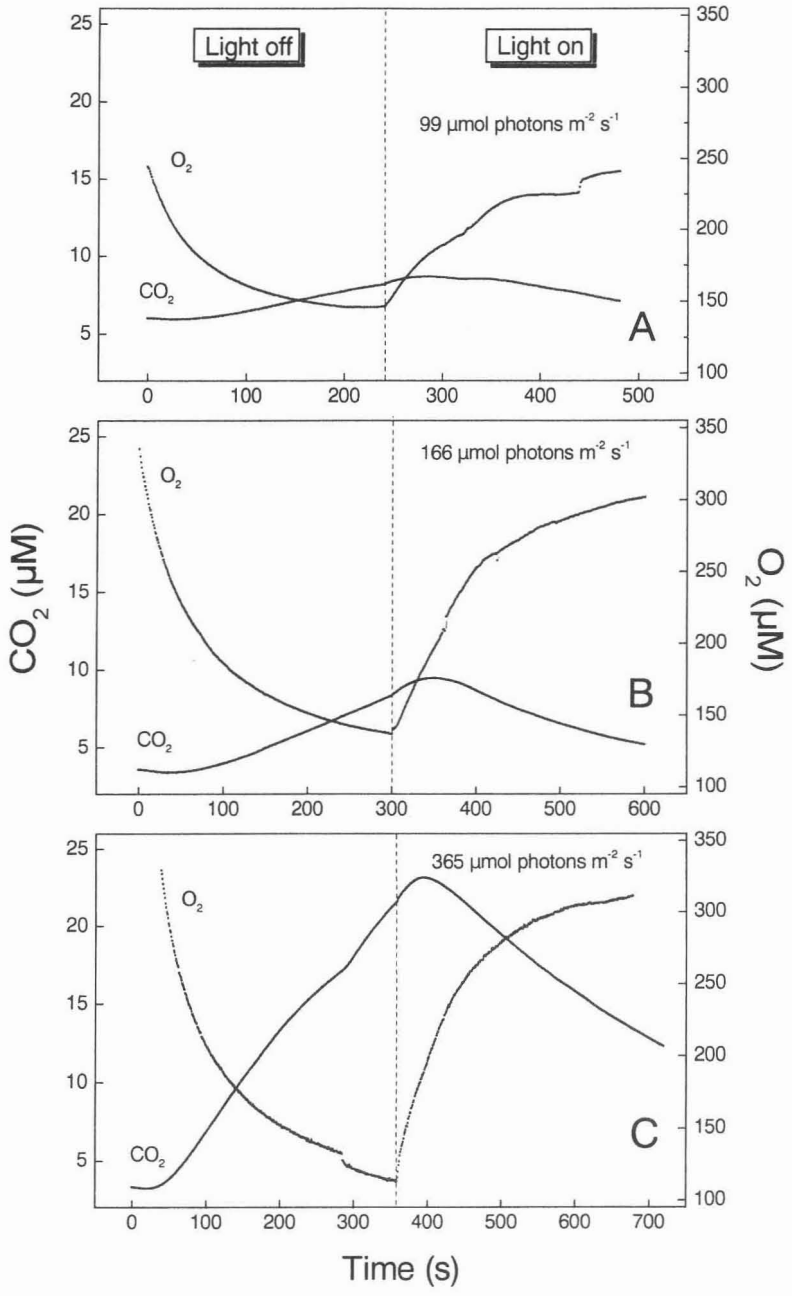

Fig. 6 CO_2 and O_2 dynamics were measured simultaneously during experimental light-dark cycles at the shell surface of *Orbulina universa* with increasing irradiances (99 (**A**), 166 (**B**), 365 (**C**) µmol photons m^{-2} s^{-1}). Dotted lines indicate the change from dark to light conditions.

The total concentration change at the shell surface between light saturation values and dark conditions was ~ 22 μM CO_2, 339 μM O_2, and a ΔpH of 1.2 units. The concentration changes depended on the irradiance and generally increased with increasing irradiance (Fig. 6). Both the O_2 and pH responded immediately to changing light conditions, while the CO_2 response showed a time delay of 17 - 100 s, which decreased with inceasing irradiance (Figs. 5, 6).

A light-dependend change of Ca^{2+} concentration was also shown (Fig. 7). Within a 3 min dark period, Ca^{2+} concentration increased 60 μM at the shell surface and decreased again about 110 μM in the following light period (Fig. 7A).

After DCMU treatment the O_2 and Ca^{2+} concentration at the shell surface changed significantly (7B). Due to the inhibition of symbiont photosynthesis O_2 decreased down to 122 μM and Ca^{2+} down to 6.9 μM. Despite the DCMU treatment small concentration changes of O_2 and Ca^{2+} during experimental light-dark shifts could be observed (Fig. 7B). During the dark period O_2 decreased about 27 μM. When light was switched on again no initial concentration change was measured until O_2 increased slightly after 90 s. Ca^{2+} decreased within the dark period about 120 μM and increased immediately when light turned on again. While the concentration change of Ca^{2+} in the presence of DCMU was in the same order of magnitude the dynamics showed a reverse behaviour as compared to measurements without inhibitor.

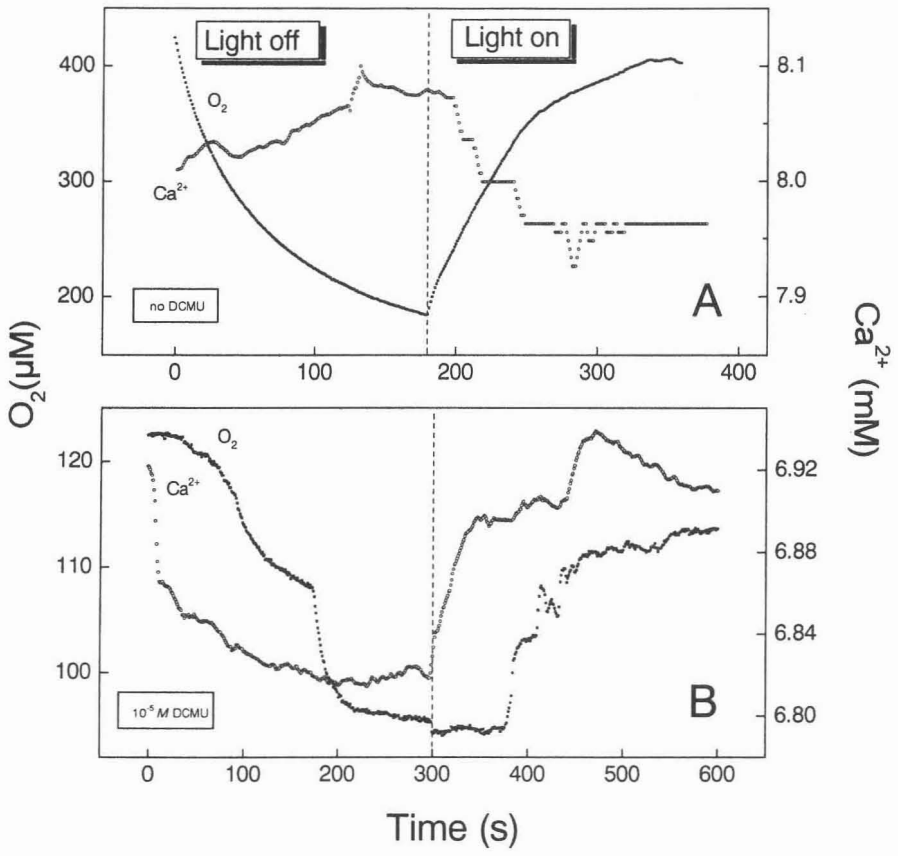

Fig. 7 Ca^{2+} and O_2 dynamics were measured simultaneously during experimental light-dark cycles at the foraminiferal shell surface before (**A**) and after (**B**) DCMU treatment with 10^{-5} M inhibitor concentration. Dotted lines indicate the change from dark to light conditions. Note the different O_2 and Ca^{2+}concentration scales in (A) and (B).

DISCUSSION

Chemical microenvironment of *O. universa*

With the present microsensor study we could 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 clearly show the influence of the prevailing light conditions on the chemical microenvironment (Figs. 1-3). Beside the incident light, ambient flow conditions can affect the concentration gradients towards the shell of planktonic foraminifera (Jørgensen et al. 1985). Exchange of O_2, DIC, and nutrients is limited due to the hydrodynamics of the seawater surrounding the organisms in their natural habitat (Pasciak and Gavis 1974, Mann and Lazier 1991, Vogel 1994). Microsensor studies of flow effects on benthic foraminifera demonstrated a decrease in the diffusive boundary layer (DBL) thickness with increasing flow (Köhler-Rink and Kühl 2000) leading to an enhanced gas exchange with the surrounding seawater. In the benthic species *Amphistegina lobifera* gross photosynthesis rates of the symbiotic algae were stimulated significantly by increasing flow velocities (Köhler-Rink and Kühl 2000).

The photosynthetic activity of the dinoflagellate symbionts changed the seawater chemistry in the surroundings of *O. universa* significantly (Figs. 1, 2). The pH changes towards the shell resulted from the combined effect of CO_2 fixation, respiration, and calcification of the host-symbiont association. Photosynthetic uptake of inorganic carbon (CO_2, HCO_3^-) under light conditions caused a pH increase at the shell surface that results in a CO_3^{2-} concentration increase (Barnes and Chalker 1990, Stumm and Morgan 1996, Falkowski and Raven 1997). Under dark conditions, CO_2 increased due to respiration and calcification (Fig. 1B, 2A) and opposite concentration changes of the carbonate system are to be expected. Thus, biologically induced CO_2 variations influenced the carbonate chemistry in the vicinity of the shell and probably affect the process of foraminiferal calcite precipitation (Spero 1992).

Planktonic foraminifera are exposed to changing incident irradiances during their life cycle due to vertical migrations in the water column (Bradshaw 1959, Boltovsky 1973). Such migration pattern would result in a change of the local characteristics of the carbonate system, as indicated by our results. In the surface waters with higher irradiance the chemical microenvironment of planktonic symbiotic foraminifera is characterized by higher pH

values due to lower CO_2 and HCO_3^- concentrations. When foraminifera reach deeper zones less photosynthetic activity will result in a pH decrease and CO_2 and HCO_3^- concentrations would be higher. In addition to lower irradiances, deeper dwelling organisms are further exposed to decreasing water temperatures. Temperature changes alter the pH and the solubility of CO_2, and thus affect the interconversion of inorganic carbon species and the degree of calcite saturation (Wollast and Vanderborght 1994, Stumm and Morgan 1996). In *O. universa* $\delta^{18}O$ values indicated an increasing wall thickening due to decreasing temperatures during migration to deeper water (Deuser et al. 1981).

In micropaleontological studies isotope data 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 foraminiferal calcite shells, paleoceanographic and paleoclimatic events have been reconstructed (Epstein 1953, Berger et al. 1971, Duplessy 1978, Erez and Luz 1983, Spero and Deniro 1987). This technique is based on the assumption that shell calcium carbonate is deposited in equilibrium with the ambient seawater (Emiliani 1954, Valentine 1973, Anderson and Arthur 1983). However, some authors demonstrated that this assumption is not valid for many benthic and planktonic species (Erez 1978, Honjo and Erez 1981, Spero and Deniro 1987) and it has been suggested that a nonequilibrium isotope fractionation in symbiont-bearing foraminifera is caused by changes in the isotopic composition of CO_2 available for calcification. Foraminiferal metabolism and symbiont photosynthesis affect the isotopic composition of the shell calcite. The symbiont photosynthesis increases the ^{13}C content of the ambient CO_2 pool, since $^{12}CO_2$ is preferantially used by photosynthetic fixation. The respiration of the foraminifer releases CO_2 that is depleted in ^{13}C relative to the ambient seawater CO_2 pool. Thus, at lower light intensities a trend towards more negative shell $\delta^{13}C$ values was found, and was explained by the increased influence of respired CO_2 on the $\delta^{13}C$ value of the CO_2 pool available for calcification (Goreau 1977, Spero and Deniro 1987). Honjo and Erez (1981) investigated several species that demonstrated pronounced and well defined deviations in $\delta^{18}O$ values and strong deviations among $\delta^{13}C$ values due to metabolic CO_2 effects.

The carbonate system of planktonic foraminifera was modeled with a diffusion-reaction model by Wolf-Gladrow et al. (1999) to estimate the influence of photosynthesis, respiration, and calcification processes on the uptake and production of the carbonate

species in the vicinity of the foraminiferal shell. Model results predict e.g. that the carbonate concentration decreases below 50% of the seawater value due to the foraminifer calcification. Furthermore, Zeebe et al. (1999) presented a numerical model that calculates the $\delta^{13}C$ values of foraminiferal shells in response to the seawater carbonate system and to the metabolic processes of the foraminifer and its symbionts, so-called `vital effects´. The model, that based on the inorganic carbonate chemistry, predicts that the $\delta^{13}C$ value of *O. universa* is, however, a result of calcite precipitation and such vital effects.

The present microelectrode study of the chemical microenvironment of *O. universa* demonstrates significant variations of the pH and CO_2 concentrations in the vicinity of the foraminifer due to the changing photosynthetic activity of the symbiotic algae. Therefore, our data give direct experimental evidence for the suggestion that symbiont photosynthesis may influence the precipitation of the shell calcite in symbiont-bearing foraminifera.

Calcification in planktonic foraminifera

Light enhanced calcification has been described in several symbiotic calcifying communities like hermatypic corals (Muscatine 1980, Barnes and Chalker 1990), benthic and planktonic foraminifera (Erez 1983, Duguay 1983, Anderson and Faber 1984, Gastrich and Bartha 1988, Lea et al. 1995). Our Ca^{2+} measurements with microsensors at the shell surface of *O. universa* demonstrated a light dependend Ca^{2+} microenvironment. However, the Ca^{2+} microprofiles showed intraspecific differences (Fig. 3). The measured profiles represent the Ca^{2+} environment over a short time period during the growth of the adult spherical chamber of *O. universa*. Our data suggest that Ca^{2+} uptake does not occur permanently and varies among individual specimens according to their specific status of calcification within their growth cycle. As described in the literature, planktonic foraminifera calcify during the daytime as well as in the night (Bé 1980, Spero 1986). The calcification of the spherical shell of *O. universa* occurs in two stages. The first pre-gametogenic calcification is characterized by slow calcite addition in the majority of the shell thickening period. This process occurs during the day as well as in the night. Several hours prior to gametogenesis a thick calcite layer is added to the shell surface. During this gametogenic calcification 13 - 28% of the shell mass is produced within several hours primarily in the late afternoon (Spero 1986). Further studies should focus on Ca^{2+}

measurements in specimens at a defined growth stage to get additional information about growth specific Ca^{2+} dynamics during the ontogeny of foraminifera.

From measured Ca^{2+} gradients we estimated total Ca^{2+} uptake rates in *O. universa* and found an average uptake of 1.4 nmol Ca^{2+} h^{-1} foraminifer^{-1}. We calculated the area integrated Ca^{2+} flux, Q_t, using the radial gradient dC/dr, the molecular diffusion coefficient, D_0, and the surface area of the spherical shell, $4\pi r^2$ (Jørgensen et al. 1985, Ploug et al. 1997, Rink et al. 1998). D_0 for Ca^{2+} ions in seawater is $0.793*10^{-5}$ cm^{-2} s^{-1} (Li et al. 1974). Our calculated uptake rate is quite comparable to 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 allows the precise determination of $^{48}Ca/^{44}Ca$ ratios in single shells. Lea et al. (1995) suggested that the Ca^{2+} is incorporated directly from the surrounding seawater into the shell calcite. They also showed that the calcification of the spherical shell in individuals grown under high light conditions was 2 - 3 times higher than in individuals grown under low light or in darkness. Anderson and Faber (1984) investigated the calcium carbonate deposited during new chamber addition in *Globigerinoides sacculifer*, which added chambers at the rate of one chamber per 24 h. The addition of a new chamber usually occured in the early morning, at rates of 3.9 nmol Ca^{2+} h^{-1} chamber^{-1}.

Our Ca^{2+} profiles measured in darkness demonstrated a Ca^{2+} increase towards the shell. Furthermore, experimental light-dark cycles at the shell surface showed a concentration decrease of Ca^{2+} under light conditions and a Ca^{2+} increase in the darkness relative to the ambient seawater level. The preliminary results of our first inhibitor experiments in *O. universa* demonstrated a Ca^{2+} concentration decrease in the dark period after DCMU treatment and an increase immediately when the light switched on. This observation indicates a complex interaction between the Ca^{2+} microenvironment and the photosynthetic activity of the endosymbionts.

Similar Ca^{2+} and O_2 concentration changes were measured with microsensors at the tissue surface of the coral *Favia* sp. (de Beer et al. 2000). During experimental light-dark cycles they also found an O_2 increase and Ca^{2+} decrease in the light and opposite concentration changes in the darkness. Their DCMU inhibitor experiments also demonstrated a Ca^{2+} increase when light switched on after a dark period. Compared to the Ca^{2+} variations of about 100 µM we measured in *O. universa*, the Ca^{2+} changes in the tissue of *Favia* sp. were much higher, and varied up to 600 µM between light and dark maxima.

Due to the presence of photosynthesizing algae in both systems conditions of high pH and higher Ca^{2+} concentrations as well as reduced inorganic carbon levels were measured during light conditions within the host-algal association. The comparable results of the microsensor studies in calcifying foraminifera and corals could therefore indicate similar mechanisms regulating the chemical processes within the symbiotic communities.

Carbon sources for symbiont photosynthesis

Oceanic surface waters represent a large reservoir for dissolved inorganic carbon (DIC). At seawater salinity and a pH of 8.2 about 10 µM of dissolved CO_2 and about 2000 µM HCO_3^- are available (Stumm and Morgan 1996). DIC concentrations of seawater can saturate the photosynthesis of symbiotic algae in benthic foraminifera and hermatypic corals (ter Kuile et al. 1989b, Goiran et al. 1996). Despite their importance as primary producers the mechanisms of DIC supply in symbiotic algae are poorly understood (Muscatine 1980). In coral-dinoflagellate associations it was suggested that HCO_3^- is the main species taken up for photosynthesis. Isolated symbiotic algae of corals can utilize both CO_2 and HCO_3^- (Goiran et al. 1996). In benthic symbiotic foraminifera ter Kuile et al. (1989a) studied the DIC uptake mechanisms for photosynthesis and calcification. They suggested that diffusion is the rate limiting step for DIC uptake in the perforate species *Amphistegina lobifera* at lower DIC levels. At higher DIC levels saturation occured and indicated the presence of a rate limiting enzymatic step. Highest DIC uptake rates were found at pH 8 - 9. The authors therefore suggested that HCO_3^- is the main inorganic carbon species taken up from the environment, which is then subsequently converted to CO_2 intracellularly.

In planktonic foraminifera mechanisms of carbon fixation and DIC supply to the symbiotic algae remain unknown. However, in the present study we demonstrate that CO_2 is not fully depleted due to symbiont photosynthesis as shown by CO_2 profiles measured under saturating light levels and by CO_2 light-dark cycles (Figs. 1, 2, 5, 6). Furthermore, combined measured O_2 and CO_2 cycles indicate a fast CO_2 supply fueling the immediate O_2 increase after the light was switched on (Figs. 5A, 6). Variations of CO_2 concentration during experimental light-dark cycles demonstrated a time lag after light-dark shifts before a concentration increase/decrease occured. Under saturating irradiance (664 µmol photons m^{-2} s^{-1}) much higher rates of O_2 release than CO_2 uptake were measured. The estimated molar

O_2/CO_2 conversion ratio of ~ 38 demonstrated that the CO_2 influx is not sufficient to support high rates of O_2 evolution. We therefore speculate that an efficient internal CO_2 supply mechanism exists (Rink et al. 1998).

Several `CO_2-concentrating mechanisms´ for algal photosynthesis have been described (Badger et al. 1980, Burns and Beardall 1987). In *O. universa*, sufficient CO_2 for the intense photosynthetic carbon fixation within the symbiont swarm could be supplied by an extracellular enzymatic dehydration of HCO_3^-. The enzyme carbonic anhydrase (CA) enhances the interconversion of HCO_3^- and CO_2 (Tsuzuki and Miyachi 1989, Raven 1994, Nimer et al. 1999). If active HCO_3^- uptake would be the main source for CO_2 assimilation we would not measure immediate pH responses when the light was switched on (Fig. 5 B, C), as HCO_3^- uptake changes the total alkalinity of the surrounding seawater but does not affect the external pH (Sikes et al. 1980). Moreover, the time scale for the uncatalysed conversion of CO_2 from HCO_3^- is in the order of 100 s (Stumm and Morgan 1996). Within 100 s we measured a pH increase of ca. 0.65 units. This fact further supports our assumption about an enhanced CO_2 supply that caused a fast pH increase at the shell surface.

Conclusions

The interaction of symbiont photosynthesis, community respiration, and host calcification could be demonstrated by measuring the shell surface concentrations of CO_2, O_2, pH, and Ca^{2+} during experimental light dark-cycles. The time delay of CO_2 changes and the immediate pH variations during experimental light-dark cycles indicate an internal CO_2 supply mechanisms sustaining the high photosynthetic activity of the symbionts. Conversion of HCO_3^- to CO_2 via external or internal CA could be a potential CO_2 source for photosynthetic assimilation within planktonic foraminifera. Furthermore, the processes of respiration and calcification could supply additional CO_2.

Under saturating light conditions concentration measurements of CO_2, O_2, and pH suggest a microenvironment of decreased inorganic carbon and increased alkalinity due to the symbiont photosynthesis around the foraminiferal shell. In the darkness, the CO_2 release by respiration and calcification may influence the interconversion of the inorganic carbon species in the surrounding seawater. Their concentration changes under light and dark conditions, however, affect the process of $CaCO_3$ precipitation.

With Ca^{2+} microelectrodes microenvironments of small and sensitive calcifying organisms such as planktonic foraminifera can be studied with high spatial and temporal resolution. Experimental light-dark cycles demonstrated a dynamic Ca^{2+} environment of *O. universa*. Future studies should focus on inhibitor experiments to obtain further information about the regulation of calcification in symbiont-bearing foraminifera. Furthermore, long term Ca^{2+} experiments with foraminifera at defined growth stages could detect possible ontogenetic changes of the Ca^{2+} microenvironment. Combined studies with microsensors and e.g. Ca tracer techniques might help to understand the fast $CaCO_3$ precipitation of foraminiferal shells and their attached calcite spines.

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Chapter 4

Microsensor studies of photosynthesis and respiration in larger symbiotic foraminifera

I. The physico-chemical microenvironment of *Marginopora vertebralis, Amphistegina lobifera,* and *Amphisorus hemprichii*

Stephanie Köhler-Rink[1], *Michael Kühl*[2]

[1] Max-Planck-Institute for Marine Microbiology, Microsensor Research Group, Celsiusstr. 1, D-28359 Bremen, Germany

[2] Marine Biological Laboratory, University of Copenhagen, Strandpromenaden 5, DK-3000 Helsingør, Denmark

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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 pH reached up to 8.6 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 CO_2 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}) as compared to 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 - 4.2 nmol cm^{-2} h^{-1} in *Amphistegina lobifera* and 1.7 – 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.

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 for evolution of these giant protists with shell sizes of more than 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 Bacillariophyceae, 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 genus *Symbiodinium* and *Amphidinium* (Leutenegger 1977b, Lee and Lawrence 1990, Lee et al. 1997). The perforate species *Amphistegina lobifera* host small pennate diatoms like *Nitzschia frustulum*, *Fragilaria shiloi*, and *N. panduriformis.*

The transparancy of the wall and the compressed test, with its high surface area to volume ratio, was suggested to provide a good morphological basis for this symbiosis (Hallock 1979). The endosymbionts live in high numbers of 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 get 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 *A. lobifera* the shell pores are associated to 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* sp. 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* sp. is dependent on the 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, the metabolic activity of larger foraminifera and its regulation by environmental variables, has not been intensively studied. Rates of carbon fixation of *A. lobifera* and *M. vertebralis* were measured by Muller (1978) and Smith and Wiebe (1977), respectively. The primary production and respiration of *A. lobifera* and *A. hemprichii* were investigated with a manometer system by Lee et al. (1980). Effects of light and food on the growth of *A. 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 were 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 this study we characterised for the first time the physico-chemical microenvironment of benthic foraminifera (*M. vertebralis*, *A. lobifera*, and *A. 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* and *Amphisorus hemprichii*) (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 - 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* (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 temperature was 26 °C and had a salinity of 36‰. Laboratory measurements were performed on the day of sampling at the Heron Island Research Station (University of Queensland).

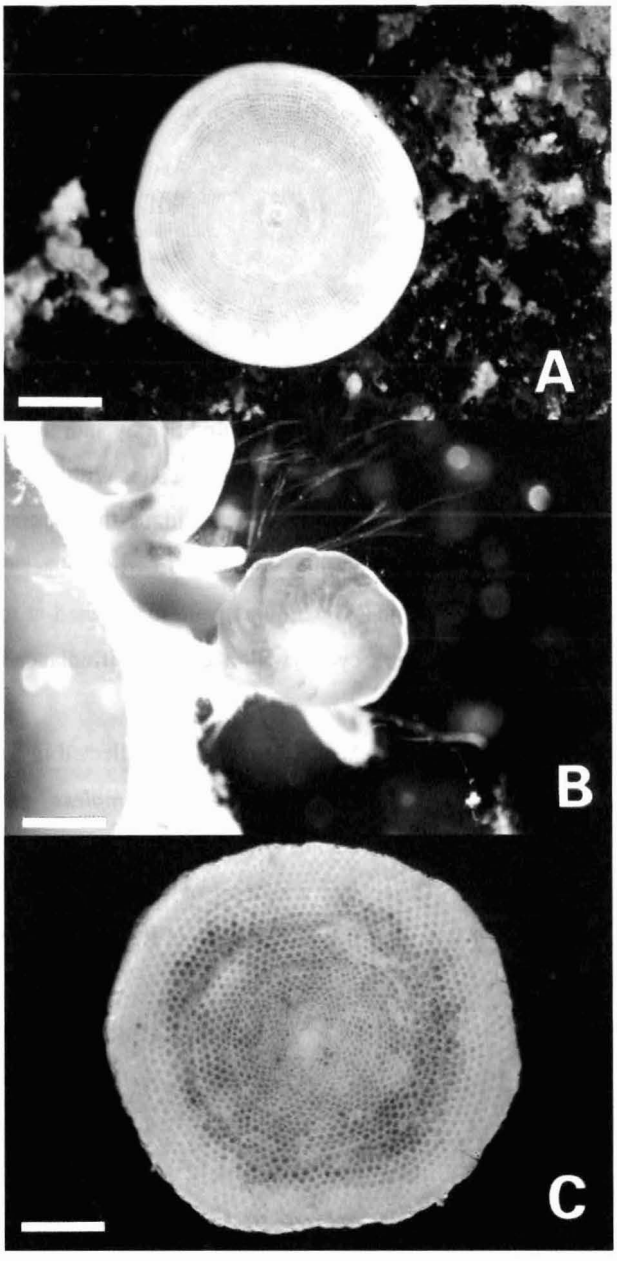

Fig. 1 The dorsal view to the foraminifera shows their yellow to brownish shells coloured by their dinoflagellate and diatom endosymbionts. The shell sizes of the imperforate disc-shaped *Marginopora vertebralis* QUOY & GAIMARD measured between 1.7 - 3.4 mm (**A**). The thick shelled low-trochospiral test of *Amphistegina lobifera* LARSEN had diameters between 1.5 - 3.5 mm (**B**). The porcelanous discoidal tests of *Amphisorus hemprichii* EHRENBERG sized about 3 - 5 mm (**C**) (size bars = 1 mm).

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 *A. hemprichii* (*A. 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}) (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 (diameter 1.3 cm). The scalar irradiance (0 -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 - 700 nm), i.e. photosynthetically available radiation. 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 °C 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 - 1.0 h prior to the experiments.

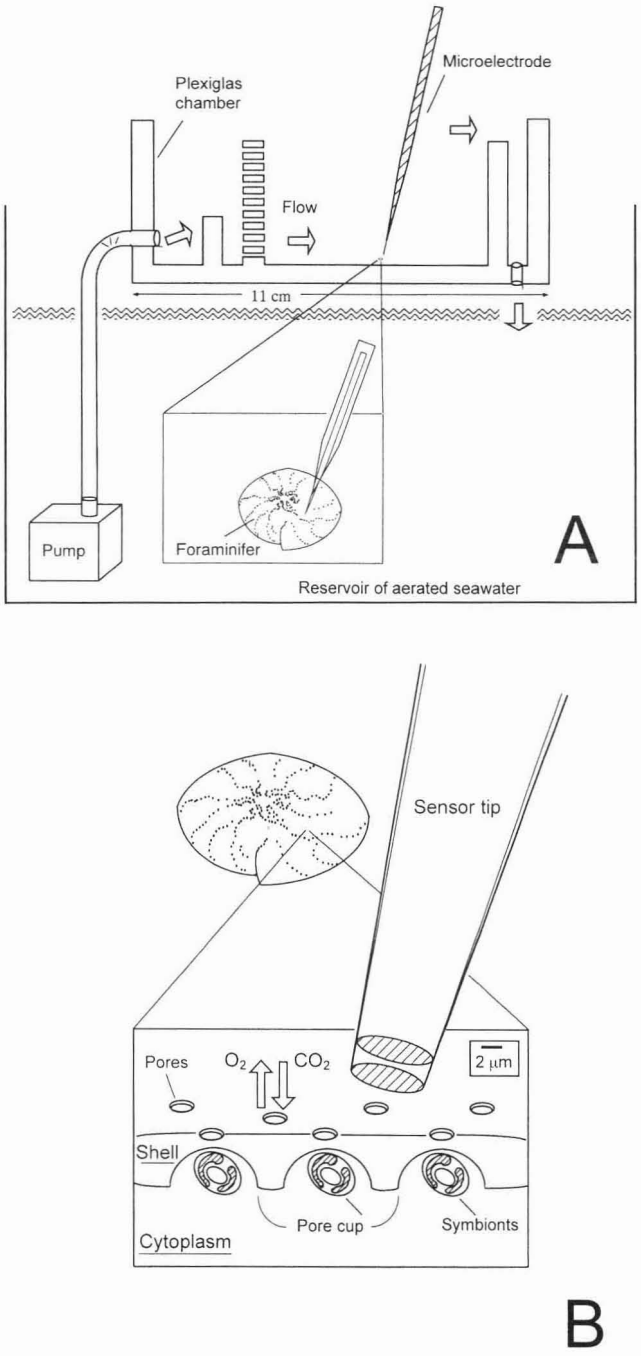

Fig. 2 **A**. Schematic drawing of the flow chamber. Seawater was pumped into the chamber and a lateral flow was created above the foraminifer. **B**. Microsensor tip above the shell of *Amphistegina lobifera*. Symbionts are associated to pore cups.

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 - 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 - 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 mM, pH 8.0) by adding aliquots of a 200 mM carbonate solution. The CO_2 microsensors had tip diameters of ca. 10 µm, a detection limit of ca. 0.5 µM 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 mM) with added background ions, i.e. seawater concentrations of Mg^{2+}, Na^{2+}, and K^+.

Fiber optic microprobe Profiles of quantum scalar irradiance (400 - 700 nm) from the shell surface to the ambient seawater were measured with a fiber optic scalar irradiance microprobe (Lassen et al. 1992a) 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} h^{-1}, by Fick´s first law:

$$J = -D_0 \frac{dC}{dz} \qquad \text{(I)}$$

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 diffusive boundary layers

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 occured over a diffusive boundary layer (DBL) surrounding the foraminiferal shell. In an experiment with *A. hemprichii* the DBL thickness decreased with flow velocity. Under stagnant conditions the DBL thickness reached up to 400 - 700 μm and decreased to 100 - 175 μm under moderate and high flow conditions, respectively (Fig. 3).

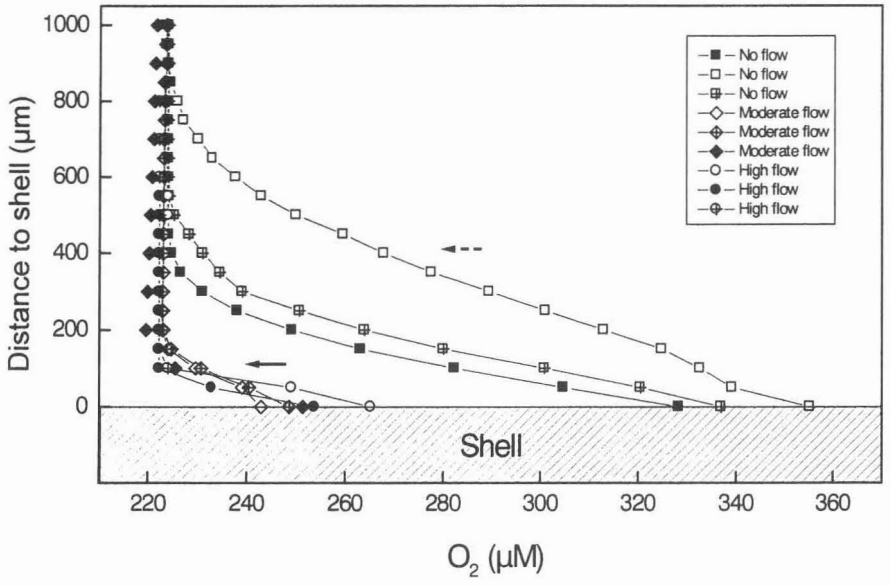

Fig. 3 O_2 concentration profiles measured under changing flow conditions towards the shell of *Amphisorus hemprichii* (dashe arrow = DBL thickness under stagnnant conditions; black arrow = DBL thickness under flow conditions; iradiance = 166 μmol photons m^{-2} s^{-1}).

The effective DBL thickness was measured by the 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 out of the shell of *A. hemprichii* were, however, not affected by the flow regime and reached 0.03 - 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 *A. lobifera* were flow dependent (Table 1).

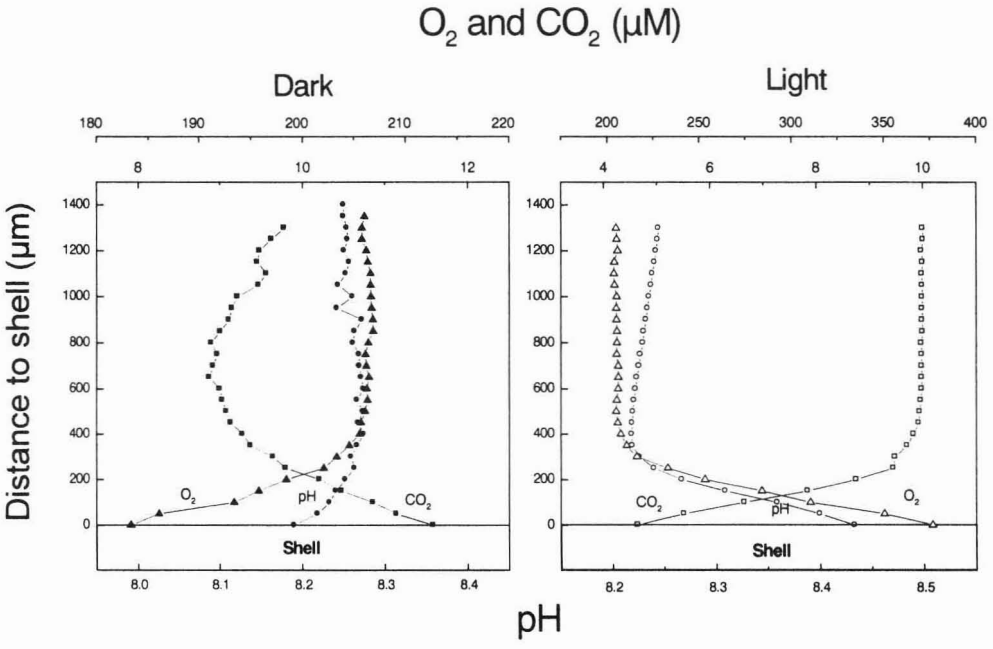

Fig. 4 *Marginopora vertebralis*. Concentration profiles of O_2, pH and CO_2 in dark (**A**) and light (**B**) conditions (irradiance = 359 µmol photons m^{-2} s^{-1}). Profiles were measured from the shell surface towards the well mixed surrounding seawater.

The average gross rates under flow conditions (4.55 and 4.89 nmol O_2 cm^{-3} s^{-1}) were significantly higher as compared to the 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 endosymbionts and on dark respiration rates of the symbiotic association.

Table 1 Gross photosynthesis, net photosynthesis, and respiration rates measured under changing flow conditions in *Amphisorus hemprichii* and *Amphistegina lobifera*

Rates	Species	Flow velocity (cm s^{-1})		
		High flow (1.5 - 4 cm s^{-1})	Moderate flow (0.14 - 2.2 cm s^{-1})	No flow
Net photosynthesis (Mean ± SD in nmol O_2 cm^{-2} s^{-1})	*A. hemprichii*	0.06 ± 0.02	0.03 ± 0.01	0.06 ± 0.02
Dark respiration (Mean ± SD in nmol O_2 cm^{-2} s^{-1})		0.05 ± 0.02	0.02 ± 0.01	
Net photosynthesis (Mean ± SD in nmol O_2 cm^{-2} s^{-1})	*A. lobifera*	0.19 ± 0.07	0.19 ± 0.08	0.22 ± 0.01
Gross photosynthesis (Mean ± SD in nmol O_2 cm^{-3} s^{-1})		4.89 ± 0.91	4.55 ± 1.81	1.62 ± 0.88

Oxygen, CO_2, and pH profiles were measured in short intervals above one *M. vertebralis* specimen (Fig. 4). All profiles demonstrated a limited solute exchange between the foraminifer and the surrounding water caused by the diffusive boundary layer (DBL) above the shell surface. In the light the ambient O_2 concentration of 205 µM started to increase ca. 400 µm above the shell of *M. vertebralis* and reached a concentration of 376 µM (= 183% air saturation) at the shell surface (Fig. 4A). Photosynthetic CO_2 fixation lowered the CO_2 concentration down to 4.6 µM and increased the pH up to 8.6 at the shell surface as compared to a CO_2 concentration of 10 µM and a pH of 8.2 in the ambient seawater.

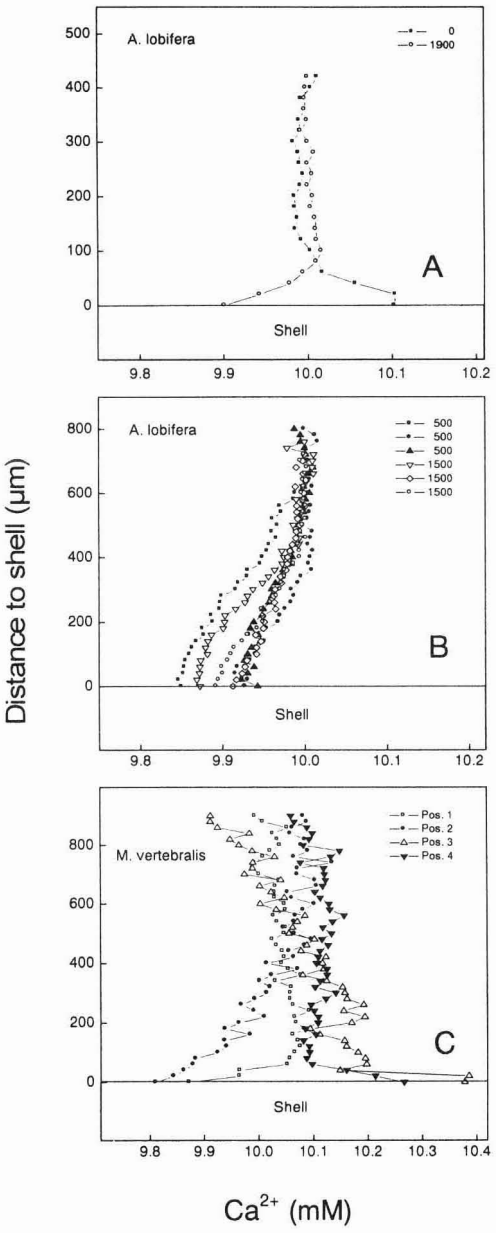

Fig. 5 Ca^{2+} concentration profiles measured under light and dark conditions (**A**) and with varying incident irradiances (**B**) in *Amphistegina lobifera*. Ca^{2+} concentration profiles measured at different positions at the shell surface of *Marginopora vertebralis* (**C**).

Under dark conditions, the respiration of *M. vertebralis* and its symbionts decreased the O_2 level down to 183 µM (= 91% air saturation) and increased the CO_2 up to 11.6 µM at the shell surface (Fig. 4B). The pH was 8.2 at the shell surface. The three different foraminiferal species investigated in this study established similar O_2, CO_2, and pH environments in light and darkness as shown here for *M. vertebralis*.

The Ca^{2+} microenvironment around *A. lobifera* and *M. vertebralis* exhibited significant changes of the Ca^{2+} concentration near the shell surfaces as compared to the surrounding seawater (Fig. 5). The Ca^{2+} profiles demonstrated a spatial heterogeneity of Ca^{2+} concentration above the shells. In the light, the Ca^{2+} concentration at the shell surface of *A. lobifera* decreased down to 9.9 mM, indicating a net uptake or consumption of Ca^{2+} (Fig. 5A). 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). However, the Ca^{2+} dark profile showed a concentration increase up to 10.1 mM Ca^{2+} at the shell surface of *A. lobifera*. 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 - 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 *M. 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 centre 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 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.

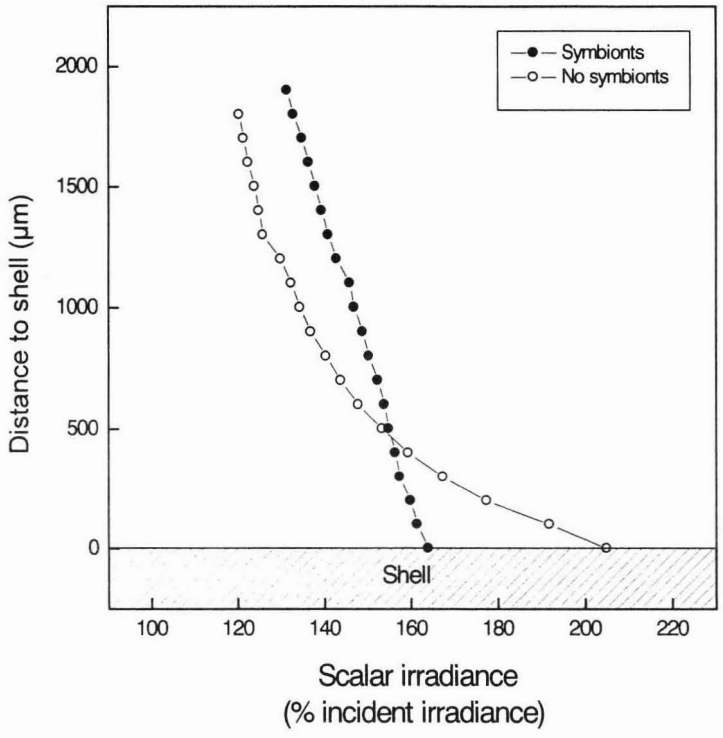

Fig. 6 Scalar irradiance microprofiles measured towards the shell surface of *Marginopora vertebralis* at a position with symbionts (●) and without (○).

O_2, CO_2, and pH dynamics

Dynamic variations of O_2, pH, and CO_2 levels were measured at the shell surface of *A. 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 µM) down to 88% air saturation (181 µM) in less than 3 min.

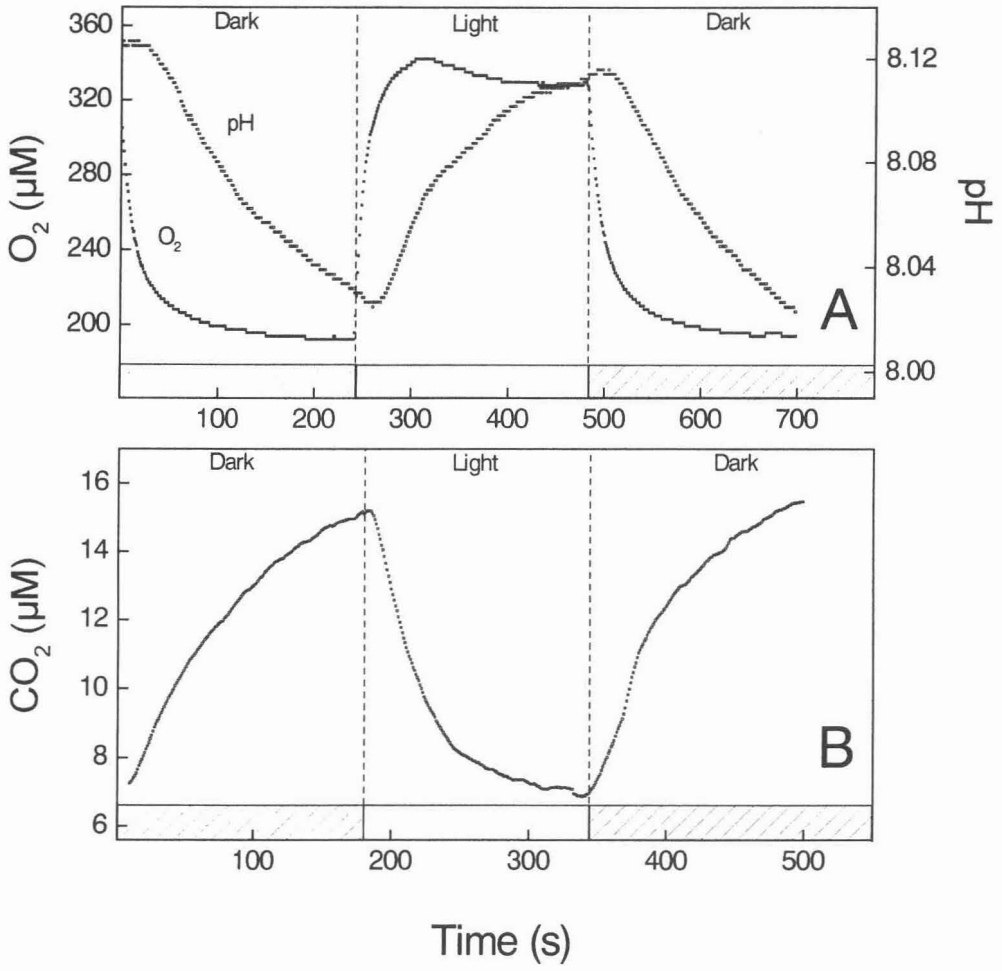

Fig. 7 O_2, pH (A) and CO_2 (B) dynamics at the shell surface of *Amphistegina lobifera* (irradiance = 697 µmol photons m^{-2} s^{-1}).

With returning light O_2 increased immediately up to 100% air saturation (206 µM) within 5 s. In the 3 min dark period, CO_2 increased from 7.2 µM up to 15.1 µM. The pH variation with the light-dark shifts was less significant (~ 0.1 units) as compared to 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 of light conditions whereas the pH signals showed a short time 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 the prevailing light and flow conditions (Figs. 3, 4, 7). Conditions of water flow caused steeper O_2 gradients across the diffusive boundary layer (DBL) as compared to stagnant water (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 *M. vertebralis*. The CO_2 concentration above the shell of *M. vertebralis* and *A. lobifera* was not fully depleted by the photosynthetic CO_2 fixation, and reached values between 4.6 and 7.3 μM. Thus we suggest a sufficient DIC supply 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 demonstrated 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 1989c).

The CO_2 gradients in the light 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 *A. lobifera* were 1.5 - 6 times smaller as compared to the net O_2 production rates in the 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 of the chemical microenvironment at the foraminiferal shell (Fig. 7). Our data demonstrated a rapid in/efflux of O_2 and CO_2 through the hyaline shell of *A. 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 was already studied by Leutenegger and Hansen (1979). Similar changes of O_2 and pH conditions due to symbiont photosynthesis were measured in the planktonic symbiotic foraminifera *G. sacculifer* and *O. 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 inbetween the calcified spines during day time. 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 surrounding 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 *M. vertebralis* due to scattering of the incident light by the calcite cristalls of the complex porcelaneous shell texture (Debenay et al. 1996, Debenay et al. 1999). Profiles measured in areas filled with symbionts showed a smaller increase, indicating a 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 *O. universa* (Rink et al. 1998), and in the upper test of symbiont containing didemnid ascidians (M. Kühl unpublished data).

The thin calcite test transmitted only 30% of the incident light and can thus protect the symbionts inside the foraminifera against 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.

Diffusive boundary layer and flow effects on photosynthesis and respiration

Larger foraminifera are surrounded by an environment of changing flow conditions that may affect the diffusive boundary layer (DBL) around the foraminiferal shell (Jørgensen and Des Marais 1990, Jørgensen in press). 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 the benthic foraminifera with O_2, DIC, and nutrients like N and P. Furthermore, foraminiferal feeding on suspended particulate matter by use of their pseudopodial network is strongly dependend 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 changes the thickness and geometry of the DBL (Jørgensen and Des Marais 1990). Subsequently, 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. *A. lobifera* for example has a biconcave shaped shell with a smooth surface, whereas *M. vertebralis* and *A. hemprichii* have a more irregular disc-shaped morphology. Irregular surface textures (e.g. wave-like structures or depressions in the shell centre) are typically found in bigger 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. *A. gibbosa*) collected from a high-energy exposed reef increased as compared to 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 as compared to stagnant conditions (Hallock et al. 1986).

The effect of flow on physiological processes of marine organisms has been 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 *A. lobifera* affected the symbiont photosynthesis (Table 1). Gross photosynthesis rates were significantly lower under stagnant conditions. We speculate that the enhanced gross photosynthesis rates could be caused due to a higher CO_2 release by an increased respiration of the foraminifer under higher flow conditions. The endosymbionts, living inside the cytoplasm, may probably benefit from the respired CO_2. Haynes (1965) suggested that the host shell acts as a natural „greenhouse„ that offers a favourable 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 enzymatic activity of carbonic anhydrase (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. (1980a). Lee et al. (1980a) found a stronger phototaxic response of *A. hemprichii* as compared to its feeding response. In our study we could observe that *A. 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 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 behaviour, their physico-chemical microenvironment, and ecophysiology will be able to elucidate the mechanisms that control the different behavioural 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 *A. lobifera* (eq. 1) and the disc-shaped *M. vertebralis* (eq. 2):

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

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

Table 2 Calcium uptake rates in *Amphistegina lobifera* and *Marginopora vertebralis*.

Foraminifer	Specimen No.	Average calcium uptake (nmol cm^{-2} h^{-1})	Uptake per specimen (nmol h^{-1})	
			size (1mm)	size (2mm)
A. lobifera	1	1.43	0.103	0.156
	2	1.88	0.135	0.205
	3	1.78	0.128	0.194
	4	4.21	0.303	0.458
	5	0.6	0.043	0.07
	average	1.98	0.14	0.22
	± SD	± 1.34	± 0.09	± 0.15
			size (1.7 - 3.4 mm)	
M. vertebralis	1	1.73	0.35	
	2	2.84	0.16	
	3	3.58	0.74	
	average	2.72	0.42	
	± SD	± 0.93	± 0.29	

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

Our calculated Ca^{2+} uptake rates are significantly lower than calcification rates of benthic foraminifera reported by Duguay (1983), who found Ca^{2+} uptake rates of 8 nmol Ca^{2+} mg dry weight^{-1} h^{-1} in *Archais angulatus* (at 840 µmol photons m^{-2} s^{-1}) and ca. 13 nmol Ca^{2+} mg dry weight^{-1} 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. Our uptake rates will be larger when expressed per mg 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 it is, however, important to point to the weight variations of the benthic foraminifera (Dugay and Taylor 1978, Dugay 1983). Dry weights of benthic foraminifera are changing during their 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 of calcium incorporation during the foraminiferal growth cycle and species-specific variations were reported for the soritids *A. angulatus* and *S. marginalis* (Lee and Bock 1976, Dugay 1983). *S. marginalis* showed two times higher calcium incorporation as compared to *A. angulatus*. Dugay (1983) suggested that this is caused by differences in frequency and rate of chamber formation of 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 *M. 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 *A. 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 for Ca^{2+} for the calcification process. Furthermore, the regulation of the magnesium and calcium uptake and storage prior to the

calcite deposition is 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 in the habitat of the Gulf of Aquaba we suggest that other factors 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 used the $^{45}CaCl_2$ uptake technique to measure the precipitation of ^{45}Ca by a foraminiferal pool (Duguay and Taylor 1978, Dugay 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 measure 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 of the Ca^{2+} microenvironment over time, but our data give no information about the time sequence of the chamber calcification. Lea et al. (1995) did not find a general trend of changing calcification rates over the growth cycle of 60 hours in the planktonic *O. universa*. In the same species Spero (1986) measured a slower calcite addition in the stage of pre-gametogenic calcification and a faster addition of calcite prior to gametogenesis. Anderson and Faber (1984) reported that chamber addition in *G. sacculifer* is an incremental event and not a continuous process. The benthic species *Heterostegina depressa* showed a chamber building activity every second or third day (Röttger 1972b, c). The frequency of chamber formation was reduced at low temperatures or extended dark periods.

Different aspects of the calcification process in foraminifera were investigated, but the basic mechanisms are still poorly understood (Erez 1978, Duguay 1983, ter Kuile and Erez 1988, Lea et al. 1995). A number of authors discussed possible calcification theories (Hemleben et al. 1986, ter Kuile 1991, Debenay et al. 1996). One theory, the biological induced $CaCO_3$ fixation is explained as a pH driven process, where CO_2 fixation by the host

symbionts rises the pH that, subsequently, induces the precipitation of $CaCO_3$. This was suggested by Lea et al. (1995) for light enhanced calcification in *O. universa*. The planktonic species calcified 2 - 3 times more under high light conditions (500 µmol photons m^{-2} s^{-1}) than individuals grown under low 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 *S. marginalis*, *C. compressa*, and *A. 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 *A. lobifera* that we measured directly after a light period (Fig. 4A) 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 *A. lobifera* and *A. 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 - 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 *M. vertebralis* at high irradiance due to the symbiont photosynthesis (Fig. 4). Our data could support the theory of biologically induced $CaCO_3$ precipitation as alkaline conditions could favour the chemical processes for 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. *M. vertebralis* for example lives on the calcarous green alga *Halimeda* sp. (Borowitzka and Larkum 1976). These phototrophic communities increase the surrounding seawater pH during light conditions (Axelsson 1988, Israel and Beer 1992).

Further calcification theories discussed *a)* an organic matrix, where a primary organic lining is controlling the calcification (Weiner and Erez 1984, Hemleben et al. 1986), or *b)* an energy dependent carbonate concentration 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„ 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 removal of these ions by the foraminifera.

Compared to *A. lobifera* most profiles above *M. 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 supply of Ca^{2+} into a Ca^{2+} pool as described by Anderson and Faber (1984). The very heterogeneous Ca^{2+} dynamic on the shell surface of *M. vertebralis* could indicate a different uptake mechanism through the porcelanous imperforate shell. During the biomineralisation 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 preformed 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 Cifelly 1967, Hottinger 1986). This membrane provides a solid surface where efficient nucleators can be absorbed and ions can be bound (Towe and Cifelly 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 resolution 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 microenvironment 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 were increasing with flow. Although the symbionts live inside the host cytoplasm they showed a dynamic response to experimental light-dark cycles. The calcite shell provides protection of the symbionts against high levels of solar radiation.

Calcium microgradients demonstrated a net calcium uptake in the light 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 the host calcification, microsensor studies of O_2, CO_2, pH, and Ca^{2+} dynamics combined with inhibitor experiments would be nessecary.

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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Chapter 5

Microsensor studies of photosynthesis and respiration in larger symbiotic foraminifera

II. Irradiance effects on photosynthesis and respiration of *Marginopora vertebralis*, *Amphistegina lobifera*, and *Amphisorus hemprichii*

Stephanie Köhler-Rink[1], *Michael Kühl*[2]

[1]Max-Planck-Institute for Marine Microbiology, Microsensor Research Group, Celsiusstr. 1, D-28359 Bremen, Germany.

[2] Marine Biological Laboratory, University of Copenhagen, Strandpromenaden 5, DK-3000 Helsingør, Denmark

ABSTRACT

Irradiance effects on symbiont photosynthesis of larger benthic foraminifera and their microenvironment were studied with O_2, CO_2, and pH microelectrodes. With increasing irradiance and under experimental light-dark cycles, a dynamic response of the foraminiferal-algal association was observed. Significant changes of the chemical micro-environment near the foraminiferal shell have been measured under light and dark conditions. In *Amphisorus hemprichii* and *Amphistegina lobifera* we could demonstrate that endosymbiont photosynthesis was not photoinhibited up to 2000 µmol photons m^{-2} s^{-1}. Photosynthesis versus irradiance curves showed light saturation levels (E_k) between 95 - 198 µmol photons m^{-2} s^{-1} indicating an adaptation of symbiont photosynthesis to high light conditions.

In addition to point measurements of gross and net photosynthesis, we used a new „mini-net" chamber to measure net O_2 production rates (= net photosynthesis) and net O_2 uptake rates (= dark respiration) of single foraminifera. Net photosynthesis and dark respiration of *A. lobifera* ranged between 3.7 - 25.5 and 5.6 - 14.6 nmol O_2 h^{-1}, respectively. These rates are comparable to rates found in other symbiotic associations such as planktonic foraminifera or radiolaria. Combined measurements of O_2 and CO_2 dynamics at the foraminiferal shell showed molar O_2/CO_2 conversion ratios > 6, i.e. a higher O_2 efflux than CO_2 influx under light conditions and a higher O_2 influx than CO_2 efflux in the darkness. These results and observations of relatively slow concentration changes of CO_2 and pH measured during experimental light-dark cycles may indicate that CO_2 is supplied by enzymatic conversion of HCO_3^-. In addition to the seawater reservoir of inorganic carbon the respiration of the community and the foraminiferal $CaCO_3$ precipitation are potential CO_2 sources for symbiont photosynthesis.

INTRODUCTION

Larger foraminifera of oligotrophic tropical reef communities live in close relationship with specific symbiotic diatoms or dinoflagellates (Lee et al. 1980, Lee et al. 1997). It was suggested that the symbionts assimilate metabolic waste products of the foraminifer containing nitrogen and phosphorus, e.g. ammonia, urea, and organic phosphate, which otherwise could inhibit the host metabolism (Murray 1991). The foraminifer additionally supplies respiratory CO_2 for photosynthesis driven carbon fixation of the symbiotic algae. The microalgae supply O_2 to the host respiration and release photosynthates like polyglucan, glucose, and lipids to the foraminiferal cytoplasm (Kremer et al. 1980). Thereby they provide a major part of the organic carbon required for the host metabolism (Battey 1992). Diatom symbionts in the benthic foraminifer *Archais angulatus* release e.g. 60% of the nonrespired fixed carbon to its host (Lee et al. 1974). In planktonic foraminifera the symbiotic primary production contributes 39% to the host carbon budgets as estimated by Caron et al. (1995). The dinoflagellates of the planktonic species *Orbulina universa* e.g. produced more O_2 than was consumed by the host-symbiont system resulting in a ratio of net photosynthesis to respiration (P_{net}/R_{dark}) of about 3 (Rink et al. 1998). Consequently, a large part of the microalgal production is cycled within the symbiotic association and an efficient carbon and nutrient recycling thus takes place (Murray 1976, Lee et al. 1980, Smith and Douglas 1987). Furthermore, the endosymbionts contribute to the calcification of foraminiferal shells and coral skeletons as calcium carbonate precipitation appears to be metabolically coupled to the photosynthetic reactions in foraminifera, corals, and calcifying algae (Goreau 1959, Hallock 1981a, Duguay 1983, Lea et al. 1995, Falkowski and Raven 1997).

High photosynthetic rates of endosymbiotic microalgae were reported for corals (Muscatine et al. 1981, Kühl et al. 1995, Goiran et al. 1996), planktonic foraminifera (Jørgensen et al. 1985, Spero and Parker 1985, Rink et al. 1998), benthic foraminifera (Hallock 1981a, Duguay 1983, Köhler-Rink and Kühl 2000), radiolaria (Caron et al. 1995, Köhler-Rink et al. unpublished) and didemnid ascidians (Alberte et al. 1986, Kühl et al., unpublished). The photosynthesizing endosymbionts contribute significantly to the primary production in reef communities. Global net photosynthetic production by symbiotic microalgae was estimated to $4.6 * 10^8$ metric tons of carbon yr^{-1} (Muscatine et al. 1981).

Endosymbiont photosynthesis has been investigated with a number of methods. Net photosynthesis was measured manometrically in the benthic foraminifera *Amphistegina lobifera* and *Amphisorus hemprichii* from the Gulf of Aqaba (Lee et al. 1980) and in symbiotic soft corals from the Great Barrier Reef (Fabricius and Klumpp 1995). $^{14}CO_2$ uptake of algal symbionts has been studied in *A. hemprichii* by Hansen and Dalberg (1979). They could demonstrate a direct uptake of $^{14}CO_2$ through the thin lateral test walls of this imperforate species. Primary production was measured as ^{14}C uptake in the benthic species *A. lobifera*, *A. lessonii*, and *Marginopora vertebralis* (Smith and Wiebe 1977, Muller 1978, Hallock 1981a). Recently, measurements of the photosynthetic activity of symbiotic algae in corals, clams, and sea anemone were performed in-situ with a new underwater pulse amplitude modulated (PAM) fluorometer that measures the effective quantum yields of photosystem II (Beer et al. 1998, Ralph et al. 1999). Oxygen microelectrodes have been used to investigate net and gross photosynthesis rates of symbiotic microalgae in planktonic foraminifera (Jørgensen et al. 1985, Rink et al. 1998), benthic larger foraminifera (Köhler-Rink and Kühl 2000), and hermatypic corals (Kühl et al. 1995, de Beer et al. 1999).

In an earlier paper we presented a microsensor study of the physico-chemical microenvironment of larger foraminifera and discussed the role of the diffusive boundary layer (DBL) that surrounds the foraminifera (Köhler-Rink and Kühl 2000). The present study investigates the light regulation of the endosymbiont photosynthesis. O_2, pH, and CO_2 microsensors were used to measure gross photosynthesis, net photosynthesis, and dark respiration of *Marginopora vertebralis*, *Amphistegina lobifera*, and *Amphisorus hemprichii*. Furthermore, we present a new approach to determine net O_2 evolution and uptake of larger foraminifera in a closed mini chamber to estimate the total net primary production and dark respiration of a single specimen.

MATERIALS AND METHODS

Sampling The perforate species *Amphistegina lobifera* and the imperforate *Amphisorus hemprichii* were hand collected by snorkeling in the clear oligotrophic water of the Gulf of Aqaba, Red Sea in June 1998. Samples were taken in a depth of ca. 5 m. The in-situ salinity was 40‰ and the water temperature was 22 °C. The foraminifera, 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, 40‰, pH 8) at room temperature (20 – 22 °C) with a natural light-dark cycle. Maximal irradiance was ca. 400 µmol photons m^{-2} s^{-1}. *A. lobifera* specimens used in this study ranged between 1.1 - 3.5 mm and *A. hemprichii* between 6.6 - 7.4 mm in diameter.

Marginopora vertebralis was collected at low tide from the macro algae *Halimeda macroloba* and *Chnoospora implexa* in warm shallow pools (26 °C/ 36‰ salinity) of the Heron Island reef flat, Great Barrier Reef, Queensland, Australia in December 1998. Experiments with 1.7 - 3.4 mm large individuals were performed on the day of sampling at the Heron Island Research Station (University of Queensland). Prior to experiments the foraminifera were carefully cleaned off adhered algae with an artist brush and rinsed several times in artifical seawater.

Experimental setup The 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 light intensities (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 with a quantum scalar irradiance meter (Biospherical Instruments Inc., QSL 101, USA). Experimental light-dark shifts were performed with an electro-mechanical shutter (Vincent Association, USA), installed in the light path of the halogen lamp. The shutter, the data acquisition, and the microsensor positioning were controlled via a custom made data acquisition 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 (26 °C and 20 °C, Australia and Bremen, respectively) under a

defined light and flow regime. The latter was created with an underwater aquarium pump. 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) were used for measurements of gross photosynthesis rates and O_2 dynamics at the foraminiferal shell surface, and of steady state O_2 microprofiles in light and darkness, respectively. CO_2 and pH dynamics at the foraminiferal shell and profiles from the shell surface to the ambient seawater were measured with a LIX-type pH microelectrode (Lee and de Beer 1995, de Beer et al. 1997) in combination with a calomel reference electrode (Radiometer 401, Denmark) and with a CO_2 microsensor according to de Beer et al. (1997). The electrode characteristics, calibration methods, and data acquisition are described in more detail by Köhler-Rink and Kühl (2000).

Photosynthesis and respiration measurements Measurements of symbiont gross photosynthesis rates were performed with the light-dark shift technique (Revsbech and Jørgensen 1983, Glud et al. 1992). Fast responding O_2 microsensors were positioned at the shell surface, and the rate of O_2 depletion within the first seconds after darkening was measured. Detailed accounts of the light-dark shift method are published elsewhere (Revsbech and Jørgensen 1983, Glud et al. 1992, Kühl et al. 1996).

Net photosynthesis rates were estimated as the net O_2 flux out of the foraminiferal shell as calculated from the linear concentration gradient of O_2 over the DBL by using Fick´s first law of diffusion

$$J = -D_0 \frac{dC}{dz} \quad (1)$$

where D_0 is the molecular diffusion coefficient of O_2 in seawater (Broecker and Peng 1974, Li and Gregory 1974) and dC/dz the concentration gradient over the diffusive boundary layer (DBL) (Jørgensen and Revsbech 1985). 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, respectively.

The dark respiration of the community was estimated from the O_2 flux towards the foraminiferal shell in darkness using eq. 1.

Photosynthesis vs. irradiance curves Gross photosynthesis rates at the shell surface of the foraminifer were measured with increasing irradiance from 0 - 2000 µmol photons m^{-2} s^{-1}. The P vs. E_0 curves were fitted by non-linear curve fitting (Origin 3.0, MicroCal Software, Inc.) with the exponential function of Webb et al. (1974)

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

where P_m is the maximal photosynthetic rate at light saturation and α the initial slope of the P vs. E_0 curve.

Net O_2 production and consumption Total rates of oxygen production or consumption of single specimens were measured with an O_2 microelectrode in a „mini-net" chamber (Vol. ~1.6 ml) (Fig. 1A). Robust Clark-type O_2 electrodes 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 glas chamber lid and fixed with additional silicone (Fig. 1A). For the measurements, artificial sterile filtered seawater (Sel marine, hw) was used to exclude contamination and background O_2 consumption in the chamber. The foraminifera were cleaned carefully with an artists brush and washed several times in seawater, before they were transferred in the chamber. Continuous mixing in the chamber was maintained with a mini 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 slopes of concentration increase and decrease (dO_2/dt) measured over a time period of 1 - 2 hours.

RESULTS

Net O_2 production and consumption

The total net O_2 production (= net photosynthesis) at saturating irradiance (598 µmol photons m^{-2} s^{-1}) and the total net O_2 consumption under dark conditions (= dark respiration) were measured for single foraminifera during short term incubations in a „mini-net" chamber (Fig. 1A). A 1 h time course of the net O_2 production and consumption of one *A. lobifera* specimen is shown in Fig. 1B. The net O_2 production rates of *A. lobifera* ranged between 3.7 - 25.5 nmol O_2 h^{-1} (n=5) and their dark respiration rates varied between 5.6 - 14.6 nmol O_2 h^{-1} (Table 1). One *A. hemprichii* specimen showed a net O_2 production rate of 13 nmol O_2 h^{-1} and a dark respiration rate of 9.9 nmol O_2 h^{-1}.

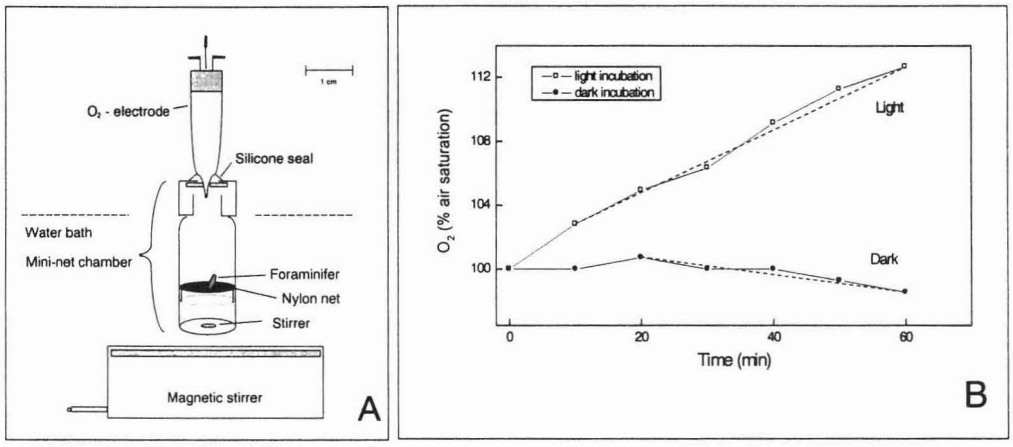

Fig. 1 **A** Schematic drawing of the closed „mini-net" chamber (Vol. 1.6 ml). The O_2 microelectrode was inserted through the air tight lid into the glas chamber. **B** *Amphistegina lobifera*. Net O_2 production and consumption measured over a time of 1 h (scalar irradiance = 598 µmol photons m^{-2} s^{-1}).

Table 1 *Amphistegina lobifera*. Net photosynthesis, dark respiration, and gross photosynthesis

Foraminifer Diameter (mm)	Net photosynthesis	Dark respiration (nmol O_2 h^{-1} foraminifer^{-1})	Gross photosynthesis
I. 2.0	25.53	14.58	40.11
II. 1.9	8.37	5.58	13.95
III. 1.8	3.72	5.58	9.30
IV. 1.8	16.08	14.29	30.88
V. 1.7	14.74	7.14	21.88
Mean ± SD	13.69 ± 7.41	9.43 ± 4.12	23.12 ± 11.11

Irradiance effects on the microenvironment

The foraminiferal microenvironment was investigated as a function of irradiance by measuring O_2, pH, and CO_2 microprofiles from the shell surface to the ambient seawater (Fig. 2). The profiles showed the presence of a diffusive boundary layer (DBL) of 100 - 300 μm thickness.

At the shell surface of *M. vertebralis* the O_2 concentration and pH changed significantly with increasing irradiance due to symbiont photosynthesis (Figs. 2A, B). The light dependence of photosynthesis was also demonstrated by the CO_2 increase at the shell surface of *A. lobifera* from 7.4 μM at light saturation up to 15.4 μM under dark conditions (Fig. 2C). Ambient levels of O_2, pH, and CO_2 in the surrounding seawater were 206 μmol O_2, pH 8.24, and 10 μmol CO_2, respectively. In the dark, the O_2, pH, and CO_2 profiles were determined by the combined respiration of the host-symbiont association. Lower O_2 and pH, and higher CO_2 levels at the shell surface as compared to the ambient seawater were measured (Fig. 2). Between darkness and light saturation O_2 increased about 50 μM and the pH changed about 0.4 units.

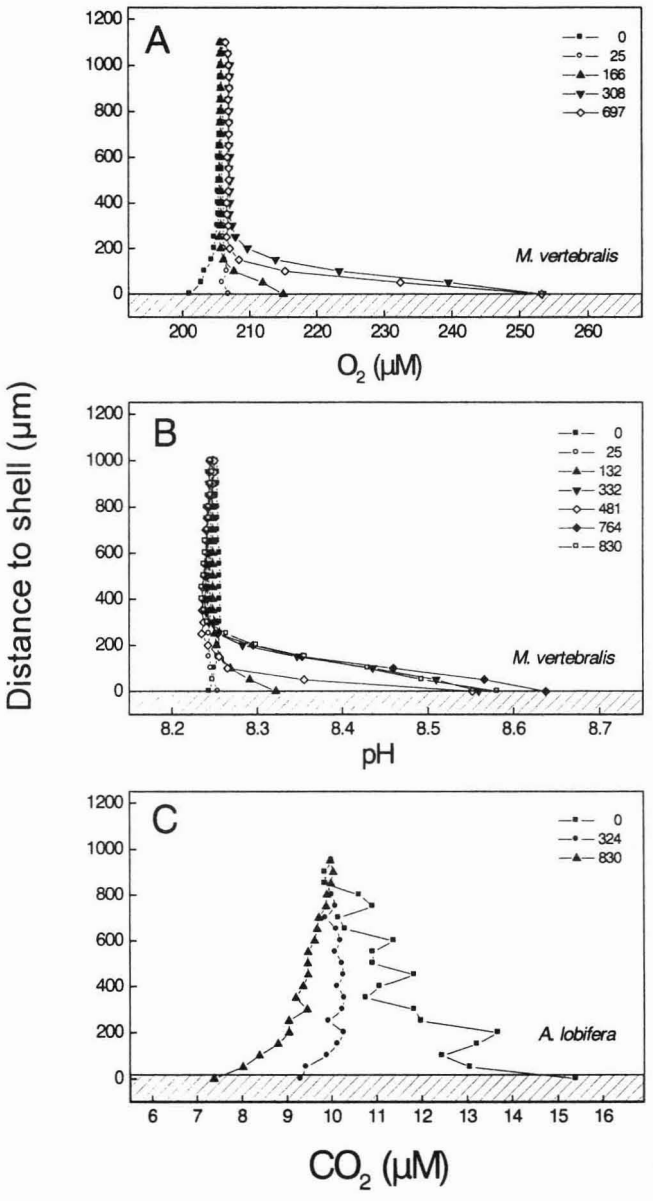

Fig. 2 O_2 and pH microprofiles in *Marginopora vertebralis* (**A**, **B**) and CO_2 microprofiles in *Amphistegina lobifera* (**C**) measured from the shell surface towards the surrounding seawater as a function of increasing irradiance. Numbers indicate scalar irradiance (µmol photons m^{-2} s^{-1}).

The O_2 and pH profiles demonstrated a compensation irradiance, E_c, of 25 µmol photons m^{-2} s^{-1} in *M. vertebralis*. At this irradiance the photosynthetic O_2 release balances the respiratory O_2 uptake.

O_2, CO_2, pH, and photosynthesis at the shell surface

The O_2 and pH at the shell surface of *M. vertebralis* were measured as a function of scalar irradiance. O_2 and pH levels increased with irradiance and approached a saturation level above 300 µmol photons m^{-2} s^{-1} (Fig. 3A).

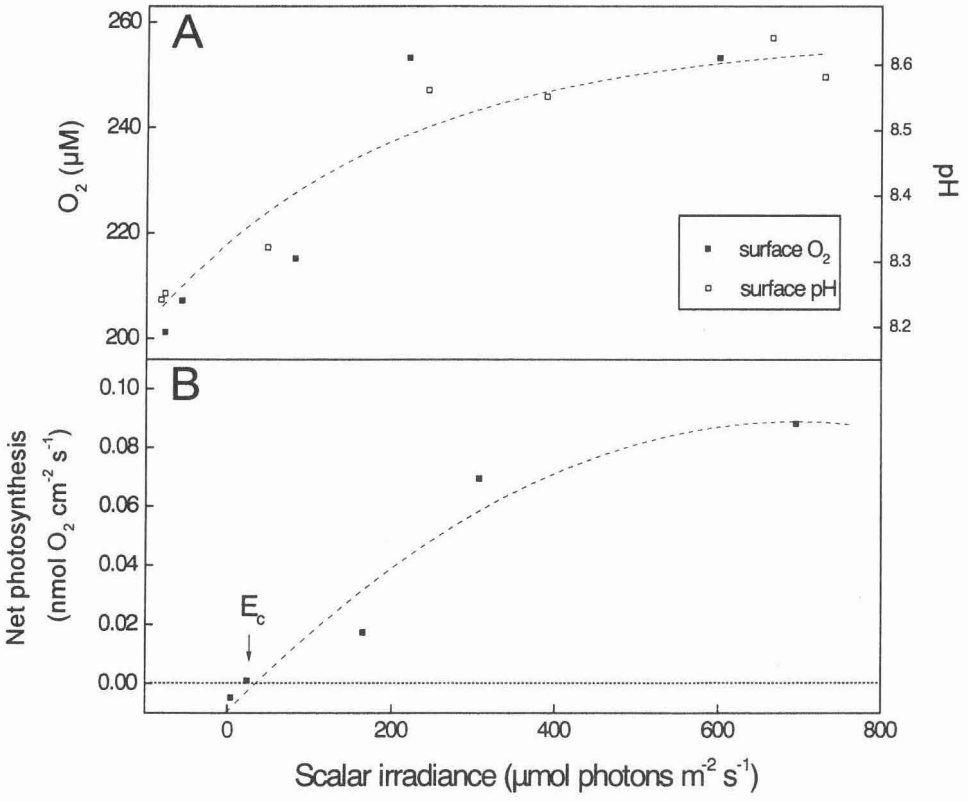

Fig. 3 *Marginopora vertebralis*. Shell surface values of O_2 (■) and pH (□) plotted against scalar irradiance (**A**). Net photosynthesis rates (nmol O_2 cm^{-2} s^{-1}) calculated from O_2 gradients near the shell surface as a function of irradiance (**B**). The compensation irradiance (E_c), indicated by the dashed lines, was 25 µmol photons m^{-2} s^{-1}.

Highest O_2 concentration of 253 µM and a surface pH value of 8.64 were found at 700 µmol photons m^{-2} s^{-1}. Net photosynthesis rates of *M. vertebralis* were calculated from measured O_2 microprofiles (Fig. 3B) and a maximum rate of 0.08 nmol O_2 cm^{-2} s^{-1} was found at 680 µmol photons m^{-2} s^{-1}. The gross photosynthetic rates of the endosymbionts, measured at the shell surface as a function of scalar irradiance, are shown in Fig. 4.

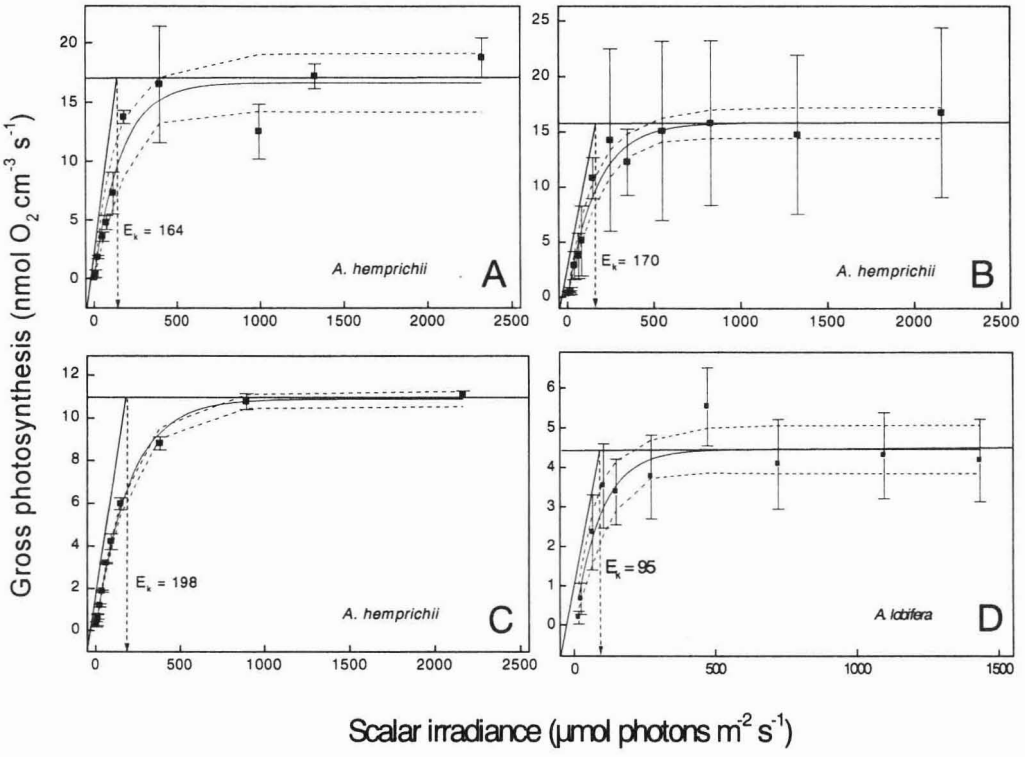

Fig. 4 P vs. E_o curves in *Amphisorus hemprichii* (**A-C**) and *Amphistegina lobifera* (**D**). Gross photosynthesis rates with increasing scalar irradiance were measured at the shell surface. Solid curves indicate the exponential function fitted to the data (Webb et al. 1974). Dashed lines indicate 95% confidence intervals (E_k = onset of light saturation).

Photosynthesis versus scalar irradiance measurements (P vs. E_0) demonstrated no photoinhibition in *A. hemprichii* and *A. lobifera* up to 2000 µmol photons m^{-2} s^{-1}. The exponential function of Webb et al. (1974) was fitted to the P vs. E_0 data. Thereby, we estimated a P_{max} ranging between 4 - 17 nmol O_2 cm^{-3} s^{-1} and an initial slope (α) that varied

between 0.06 - 0.10. The scalar irradiance at the onset of light saturation was determined as $E_k = P_{max}/\alpha$. E_k values of *A. hemprichii* ranged between 164 and 198 µmol photons m^{-2} s^{-1} while the E_k of *A. lobifera* was 95 µmol photons m^{-2} s^{-1}.

Dynamics of O_2, CO_2, and pH

Measurements of O_2, CO_2, and pH dynamics were performed at the shell surface of *M. vertebralis* and *A. hemprichii* during experimental light-dark cycles (Figs. 5, 6). Due to the symbiont photosynthesis, an O_2 and pH increase and a CO_2 decrease were measured during the light period.

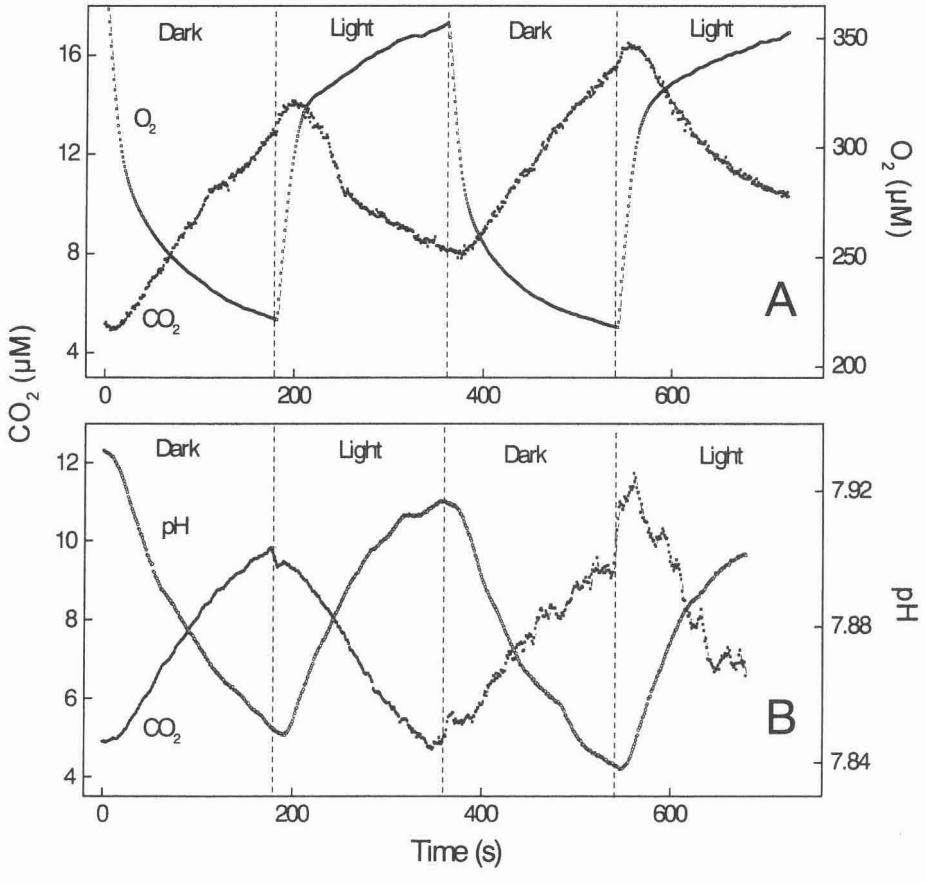

Fig. 5 Combined measurements of CO_2 and O_2 (**A**), and CO_2 and pH (**B**) dynamics at the shell surface of *Marginopora vertebralis* during experimental light-dark cycles (scalar irradiance = 359 - 398 µmol photons m^{-2} s^{-1}). Dashed lines indicate light switches.

In darkness, when photosynthesis was stopped, the combined host and symbiont respiration resulted in an O_2 and pH decrease and CO_2 increase at the shell surface. During a 3 min dark period the O_2 concentration at the shell surface of *M. vertebralis* decreased from 362 µM down to 221 µM (Fig. 5A) and increased again up to the light saturation level in the following 3 min light period. In parallel, the CO_2 concentration increased from 5.1 µM up to 12.8 µM in the dark and showed a time lag of ca. 20 - 30 s in the following light period before the concentration decreased again (Fig. 5A). The combined pH and CO_2 measurements in *M. vertebralis* showed a pH change of 0.06 - 0.08 units and a CO_2 change of 4.9 - 7.6 µM between the light and dark maxima (Fig. 5B).

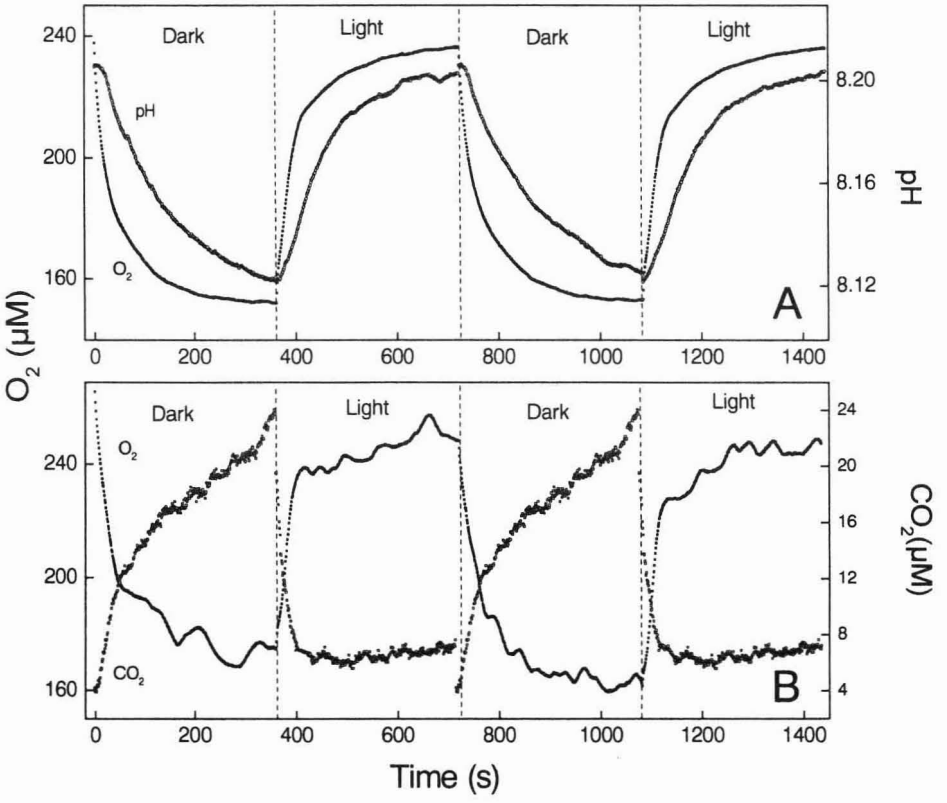

Fig. 6 Combined measurements of O_2 and pH (**A**), and O_2 and CO_2 (**B**) dynamics at the shell surface of *Amphisorus hemprichii* during experimental light-dark cycles (scalar irradiance = 332 µmol photons m^{-2} s^{-1}). Dashed lines indicate light switches.

The surface concentrations exhibited a time delay when the light situation changed. In comparison, the O_2, CO_2, and pH concentrations at the shell of *A. hemprichii* demonstrated simultaneous changes during the experimental light-dark cycles (Fig. 6).

In the first dark period O_2 decreased 86 µM and pH decreased 0.08 units. The CO_2 concentration increased from 4 µM up to 24 µM. Between the light and dark saturation values the O_2, CO_2, and pH at the shell surface of *A. hemprichii* varied between 73 - 112 µM, 11 – 19 µM, and 0.07 - 0.08 units, respectively. Whereas the CO_2 variations between dark maxima and light minima of *M. vertebralis* were smaller as compared to *A. hemprichii*, the concentration differences of O_2 were bigger for *M. vertebralis* between light and darkness (Figs. 5, 7). In addition, we found faster shell surface dynamics of O_2, CO_2, and pH in *A. hemprichii* as compared to *M. vertebralis* (Figs. 5, 6, 7, 8).

In the first seconds of the experimental light-dark and dark-light shifts, measured at the shell surface of *A. hemprichii*, average rates (n = 2) of O_2 release/uptake were compared to average rates of CO_2 uptake/release (Fig. 8A, B, Table 2). In the dark *A. hemprichii* showed an O_2 uptake rate of 1.03 and CO_2 release of 0.53 µM s^{-1} (Table 2). In the light periods we measured an CO_2 uptake of 0.31 µM s^{-1} compared to an O_2 release of 1.98 µM s^{-1}. Thus, we found much higher molar conversion rates of O_2 as compared to the rates of CO_2 conversion, both in light and darkness.

Table 2 *Amphisorus hemprichii*. Rates of O_2 and CO_2 uptake and release

	(µM s^{-1})
Light-dark shifts	
O_2 uptake	1.03
CO_2 release	0.53
Dark-light shifts	
O_2 release	1.98
CO_2 uptake	0.31

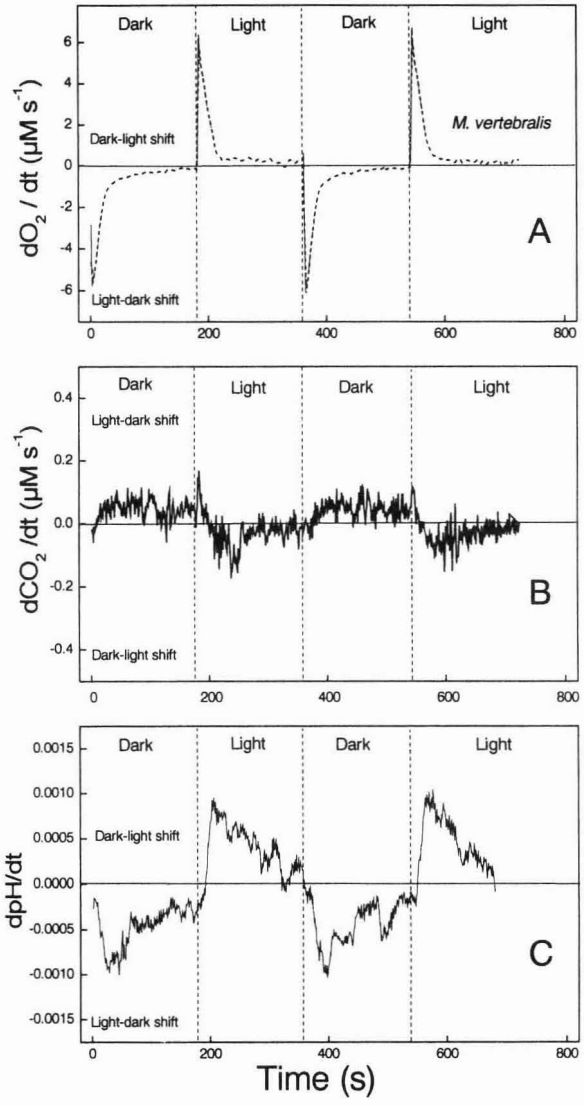

Fig. 7 O_2, CO_2, and pH dynamics (dC/dt in µM O_2/CO_2 s^{-1} or dpH/dt), as calculated from the light-dark and dark-light shifts in *Marginopora vertebralis* (Fig. 5). Dashed lines indicate light switches.

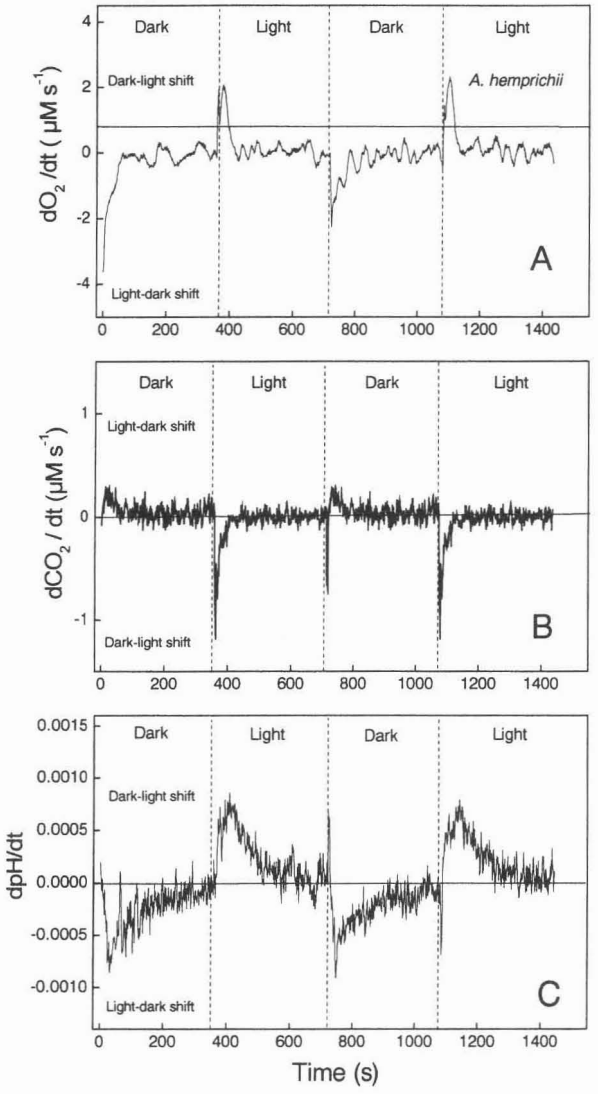

Fig. 8 O_2, CO_2, and pH dynamics (dC/dt in µM O_2/CO_2 s^{-1} or dpH/dt), as calculated from the light-dark and dark-light shifts in *Amphisorus hemprichii* (Fig. 6). Dashed lines indicate light switches.

DISCUSSION

In the following, we will first discuss the dynamic changes of the chemical microenvironment observed near the shell of larger foraminifera and continue with the effect of incident irradiance to the endosymbiont photosynthesis. Furthermore, we compare the primary production rates of benthic foraminifera measured with microsensors to previous studies and will discuss various inorganic carbon sources for endosymbiont photosynthesis in larger foraminifera.

Dynamic microenvironmental changes

Symbiotic larger foraminifera live in a dynamic environment largely determined by the prevailing light and flow conditions. Scalar irradiance affected the photosynthetic activity of the endosymbiotic algae and thus the chemical microenvironment adjacent to the foraminiferal shells as demonstrated by the dynamic response of the O_2, CO_2, and pH profiles with increasing incident light (Fig. 2). Enhanced photosynthesis with increasing light levels increased the O_2 and pH concentrations and decreased the CO_2 at the foraminiferal shell. Thereby, the pH dependent composition of the seawater carbonate system ($C_T = HCO_3^- : CO_3^{2-} : CO_2$) will be changed (Stumm and Morgan 1996). The ratio of the carbonate species and, subsequently, the composition of the chemical environment around the foraminiferal shell is regulated by the photosynthetic CO_2 fixation of the endosymbionts.

The fast response of the chemical microenvironment during experimental light-dark cycles indicated a close coupling of autotrophic and heterotrophic processes within the foraminiferal-algal association (Figs. 5, 6). The initial rates of O_2 release and uptake, measured at the shell surface of *M. vertebralis* and *A. hemprichii*, that were in the same order of magnitude support this assumption (Fig. 7, 8). Combined recordings of O_2 and CO_2 at the shell surface of *A. hemprichii* showed simultaneous changes of both concentrations. The immediate CO_2 response could indicate a rapid reaction of the CO_2 fixation process of the endosymbionts that, subsequently, resulted in an immediate pH increase at the shell surface (Fig. 6). The CO_2 response in *M. vertebralis* on the other hand showed a time lag up to 30 s until a concentration change was observed (Fig. 5). This time delay following the

light changes was also shown by the pH changes in *M. vertebralis* and demonstrates a close coupling between the pH and CO_2 signals. pH changes at the shell surface, however, were driven by the CO_2 responses due to the endosymbiont photosynthesis and the respiration of the community. Similar dynamics of the chemical environment of symbiotic organisms have been measured within the symbiont swarm of the planktonic foraminifer *O. universa* (Rink et al. 1998, Köhler-Rink and Kühl, unpublished) and in the tissue of the coral *Favia* sp. (Kühl et al. 1995, de Beer et al. 1999). De Beer et al. (1999) found a similar time delay in the CO_2 and pH dynamics at the coral tissue surface as compared to our findings with *M. vertebralis* during experimental light-dark shifts. In both symbiotic systems, the external CO_2 concentration changes were not immediately coupled to the start and end of the microalgal photosynthesis, i.e. the onset and eclipse of light. Within the symbiont swarm of *O. universa* the same phenomenon was observed. However, as indicated by the fast O_2 dynamics, symbiont photosynthesis of *M. vertebralis* was supplied with sufficient CO_2, that is not delivered immediately from the surrounding seawater.

The rapid concentration changes of O_2 and CO_2 at the shell surface also demonstrated for the first time a fast exchange of metabolic gases through the imperforate shells of *M. vertebralis* and *A. hemprichii*. Thus porcelaneous shells do not limit the gaseous transport across the calcite wall. Similar fluxes of O_2 and CO_2 were measured with microsensors at the hyaline shell of *A. lobifera* with its perforate structure (Köhler-Rink and Kühl, 2000).

Irradiance effects on symbiont photosynthesis

In the marine environment endosymbiotic microalgae are exposed to a wide range of light intensities of varying spectral composition. The relationship between photosynthesis and irradiance is described by photosynthesis vs. irradiance (P-E) curves, which contain important information on the functioning of various components of the photosynthetic apparatus and their response to environmental changes (Geider and Osborne 1992). Environmental variables such as light, nutrients, and temperature affect the light saturated and light-limited rates of photosynthesis. Light adaptation can affect the P-E parameters, α and P_{max}, depending on the strategy of photoadaptation (Richardson et al. 1983).

The photosynthesis vs. scalar irradiance ($P\text{-}E_0$) curves of the symbiotic benthic foraminifera were similar to $P\text{-}E_0$ curves obtained from isolated symbionts and other symbiotic organisms including planktonic foraminifera and corals. The initial photosynthesis rates increased proportionally to the increasing irradiance, became saturated, and approached a maximal gross photosynthesis at highest irradiance (Fig. 4). The $P\text{-}E_0$ characteristics of the benthic species were comparable to those reported for high light adapted symbiotic algae. High light adapted cells exhibit less steep initial slopes of the P-E curves (α), higher light saturation levels for photosynthesis (E_k), and higher compensation irradiance levels (E_c) as compared to cells adapted to low light conditions (Alberte et al. 1986, Iglesias-Prieto and Trench 1994). In addition, most studies of the photosynthesis response curves of symbiotic dinoflagellates do not show a decreasing photosynthesis, i.e. photoinhibition, at high irradiance (Falkowski and Dubinsky 1981, Chalker et al. 1988, Harland and Davies 1995, Goiran et al. 1996). In comparison, investigations of phytoplankton dinoflagellates demonstrated low E_c and E_k values and photoinhibition at low irradiance (200 µmol photons m^{-2} s^{-1}) (Prezelin 1976, Richardson et al. 1983).

The maximum gross photosynthesis rates of the benthic foraminifera *A. hemprichii* and *A. lobifera,* measured in nmol O_2 cm^{-3} s^{-1}, were in the same order of magnitude as compared to photosynthesis rates measured in planktonic foraminifera and corals (Kühl et al. 1995, Rink et al. 1998). The onset of light saturation (E_k) of *A. hemprichii* and *A. lobifera* varied between 95 and 198 µmol photons m^{-2} s^{-1}. *A. hemprichii* that hosts symbiotic dinoflagellates showed higher E_k values as compared to *A. lobifera* that lives in symbiosis with diatoms. E_k values reported for endosymbionts of planktonic foraminifera and hermatypic corals ranged between 75 and 275 µmol photons m^{-2} s^{-1} (Jørgensen et al. 1985, Kühl et al. 1995, Rink et al. 1998)(Table 3). High light adapted endosymbionts (*Symbiodinium* sp.) isolated from a jellyfish, corals, and zoanthides showed E_k values of 123 - 154 µmol photons m^{-2} s^{-1}, whereas low light adapted cells had E_k values of 67 - 109 µmol photons m^{-2} s^{-1} (Iglesias-Prieto and Trench 1994).

Microprofiles of O_2 and pH in *M. vertebralis* demonstrated a light compensation point, E_c, of 25 µmol photons m^{-2} s^{-1} (Fig. 2). At this irradiance the photosynthetic O_2 production is equal to the respiratory O_2 uptake of the foraminiferal-algal association.

Table 3 Photosynthesis and respiration rates and *P vs. E* parameters measured in symbiotic foraminifera

	Gross photosynthesis	Net photosynthesis	Respiration	E_k#	E_c#	Method	Reference
I. Benthic foraminifera							
I. *M. vertebralis*		0.25*				^{14}C	Smith and Wiebe 1977
II. a) *A. lobifera*		317*			165	^{14}C	Lee et al. 1980
b) *A. hemprichii*		166*					
III. a) *A. lobifera*		3.56*				^{14}C	Röttger et al. 1980
b) *A. lessonii*		4.64*					
IV a) *A. lobifera*		2.4 *				^{14}C	Hallock 1981a
b) *A. lessonii*		1.6*					
V. *A. angulatus*		2.6*				^{14}C	Duguay 1983
VI. *A. lobifera*		4.97*				^{14}C	ter Kuile et al. 1989a
VII. a) *A. lobifera*	23.1*	13.7*	9.4*	95		O_2 micro-sensors	Present study
b) *A. hemprichii*	24.9*	13*	9.9*	164-198			Present study
Isolated symbionts							
I. *Fragilaria shiloi* (of *A. lobifera*)		150+				^{14}C	ter Kuile et al. 1989a
II. Planktonic foraminifera							
I. *G. sacculifer*	18*	14.9*	3.0*	160-170	26-30	O_2 micro-sensors	Jørgensen et al. 1985
II. *G. ruber*		0.48*				^{14}C	Gastrich and Bartha 1988
III. *Different species*		1.0 - 4.0*				^{14}C	Caron et al. 1995
IV. *O. universa*	8.9*	5*	3*	75 - 137	50	O_2 micro-sensors	Rink et al. 1998
Isolated symbionts							
I. *G. béii* (of *O. universa*)		1.72 * 10^{-3}"		386		^{14}C	Spero and Parker 1985

* nmol O_2 h^{-1}
"nmol C $algae^{-1}$ h^{-1}
\+ µM C mg Chl^{-1} h^{-1}
# µmol photons m^{-2} s^{-1}

In the natural reef habitat, the benthic species would be exposed to this light compensation irradiance in a depth of ca. 90 m (Jerlov 1976, Kirk 1994) or in shallower shaded areas, e.g. under large coral heads. The E_c in *M. vertebralis* was smaller as compared to the reported light compensation points of 165 µmol photons m^{-2} s^{-1} for *A. hemprichii* and *A. lobifera* (Lee et al. 1980) or for planktonic foraminifera with 26 - 50 µmol photons m^{-2} s^{-1} (Jørgensen et al. 1985, Rink et al. 1998)(Table 3). In the coral *Stylophora pistillata* photosynthesis saturated above 600 µmol photons m^{-2} s^{-1} and E_c was reached at 350 µmol photons m^{-2} s^{-1} (Falkowski and Dubinsky 1981).

Species-specific variations of P-E characteristics could derive e.g. from different methological approaches and the application of different mathematical expressions to fit the P vs. E data (Chalker 1981). In addition, the photophysiological differences of the endosymbiotic algae could result from measurements of varying symbiont densities or algal adaptations to prevailing environmental parameters (light, temperature). The photoadaptation in symbiotic algae may in turn influence the distribution of their hosts (Hansen and Buchardt 1977, Hallock 1981a, Leutenegger 1984, Lee et al. 1989, Hollaus and Hottinger 1997). Leutenegger (1984) found a definite correlation between depth distribution of benthic foraminifera, the types of symbionts, and the prevailing light conditions. Foraminifera bearing chlorophyceans dwell in very shallow waters (0 - 15 m), those with dinophyceans and rhodophyceans between 0 - 70 m, and diatom-bearing species between 0 - 130 m water depth. Leutenegger (1984) concluded that light intensity, substrate type, and topography among other parameters determined the distribution of symbiont-bearing foraminifera. Distribution profiles measured in the Gulf of Aqaba showed highest densities of *A. lobifera* down to 10 m. At depth greater 40 m the species dissapears (Hansen and Buchardt 1977). This observation support the assumption that the diatom symbionts (e.g. *Nitzschia* sp., *Fragilaria* sp.) of *A. lobifera* are adapted to higher levels of solar radiation. At depth > 35m light intensity decreased to less than 70 µmol photons m^{-2} s^{-1} as measured by ter Kuile and Erez (1984). The photoacclimatory capabilities of the endosymbionts probably limit foraminiferal live to this water depth.

Growth experiments with isolated symbionts support this findings. Isolated diatoms of the benthic species *Amphistegina lessonii* (*Fragilaria shiloi* and *Nitzschia laevis*) grew fastest at irradiances of ca. 16 µmol photons m^{-2} s^{-1} whereas diatoms from *Heterostegina depressa* (*Nitzschia valdestriata* and *Nitzschia panduriformis*) grew best at very low light

intensities (ca. 1 µmol photons m^{-2} s^{-1}) (Lee et al. 1982b). Thus, the primary depth determing factor of larger foraminifera seems to be the light dependence of their photoautotrophic symbionts.

In the transparent nutrient poor waters of tropical seas microalgae are often exposed to high light levels. In particular in shallow areas damaging solar radiation levels prevail (Battey 1992). Jerlov (1950) postulated that UV must be a significant biological factor to depth of 20 m in clear oceanic waters. Especially in the tropics, high levels of UV (290 - 400 nm) reach the ocean surface (Baker et al. 1980). Exposure to UV radiation and/or high levels of PAR can result in cell damage and photoinhibition of photosynthesis and a subsequent decrease in cellular growth rate. Ekelund (1991) could demonstrate that growth and motility of marine dinoflagellates were inhibited by UV-B. Symbiotic algae developed adaptation mechanisms to high light exposure depending e.g. on changes of their cellular pigment contents, differing pigment ratios and/ or enzyme activity (Falkowski and Owens 1980). Furthermore, carotenoids play an essential role in the process of photoprotection of light-harvesting proteins (LHC) and core complexes (Iglesias-Prieto and Trench 1997, Larkum and Howe 1997).

Recently, physiological changes in the photosynthetic unit and the *Chl*-protein complexes during photo-acclimation of symbiotic dinoflagellates have been shown (Iglesias-Prieto and Trench 1994, Iglesias-Prieto and Trench 1997). Under super-saturating irradiances the symbiotic dinoflagellates of the jellyfish *Cassiopeia xamachana* e.g. showed a species-specific enrichment of photo-protective xanthophylls. Increased xanthophyll levels can provide an effective pathway for nonradiative dissipation of excessive excitation. These data further support the assumption that symbiotic microalgae living in high-light environments have developed different photo-adaptive strategies.

In our study photosynthesis in *A. hemprichii* and *A. lobifera* showed no photoinhibition up to 2000 µmol photons m^{-2} s^{-1}. We therefore suggest that the symbionts of benthic foraminfera are able to adapt their pigment content to environmental light stress and probably possess protective mechanisms against intense radiation and UV-damage. However, the presence of such adaptations in pigment composition are yet to be demonstrated. In hermatypic corals high levels of protective pigments, especially UV-absorbing compounds were measured (Falkowski et al. 1990, Kinzie 1993). One family of UV-blocking substances, the mycosporine like amino acids (MAAs), act as spectrally

specific UV sunscreens in several symbiotic and non-symbiotic invertebrates and microalgae e.g. in sea anemone, scleractinian corals, and dinoflagellates. By accumulating UV-absorbing MAAs the dinoflagellate *Gymnodinium sanguineum* showed an increased resistence to UV inhibition of photosynthesis (Neale et al. 1998). MAA-rich colonies of the coral *Acropora microphthalma* showed no UV inhibition in freshly isolated zooxanthellae (Shick et al. 1995). The presence of MAAs in symbiotic foraminifera has so far not been studied. However, due to the exposure to high irradiances UV-protective compounds could be expected. Future studies of the UV photophysiology of foraminifera would therefore be of great interest.

Another explanation for the apparent lack of photoinhibition could be a light shielding by the calcite shells of benthic foraminifera. In a previous study, we measured that only 30% of the incident irradiance was transmitted through the calcite shell to the symbionts that live in the host cytoplasm (Köhler-Rink and Kühl, 2000). 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 light levels > 1000 µmol photons m^{-2} s^{-1} below the calcite shell. Light absorbance of the tissue in didemnid ascidians was 60 - 80% of the incident light (Alberte et al. 1986). Therefore the authors concluded that the symbiont *Prochloron* never experience irradiances > 1000 µmol photons m^{-2} s^{-1}. In corals, the specialized environment of symbiotic dinoflagellates in the host tissue was suggested to act to reduce or eliminate photoinhibition (Long et al. 1994, Hoegh-Guldberg and Jones 1999).

Primary production of benthic foraminifera

Larger benthic foraminifera are major contributors to the primary production of tropical reef communities (Muller 1974, Hallock 1981a). They live in dense populations attached to benthic substrates as epifauna on macroalgae, seaweeds, and corals. Duguay (1983) reported maximum population densities of *Archais angulatus* of > 15.000 foraminifera m^{-2} collected at Largo Sound, Florida. Hansen and Buchardt (1972) found densities of 90 individuals 30 cm^{-2} of living *Amphistegina* sp. in a depth of 20 m at Whadi Thaba in the Gulf of Aqaba. In a field study at Oahu, Hawai Muller (1974) investigated the life cycle of *Amphistegina madagascariensis*. This species showed a tenfold increase in

density during spring and early summer. Large variations of productivity and a 6-month period of reproductive activity between February and August were measured. The total population varied between 1.41 - 41.8 10^4 foraminifera m^{-2} with a maximum density in July.

The high gross photosynthesis rates and high net O_2 production rates measured in the present study reflect the high primary productivity of the algal-foraminiferal associations. Our results are in the same order of magnitude as previously reported primary production rates measured in several larger foraminifera species by ^{14}C incorporation and respirometry (Smith and Wiebe 1977, Muller 1978, Lee et al. 1980, Röttger et al. 1980, Hallock 1981a, Duguay 1983, ter Kuile et al. 1989a) (Table 3). *Sorites marginalis* e.g. 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 dry weight of 0.6 mg *A. angulatus*$^{-1}$ (Duguay 1983) and a CO_2/O_2 conversion ratio of unity (Muscatine 1990, Sikes et al. 1980) this rate would amount to ca. 2.6 nmol O_2 foraminifer^{-1} h^{-1}. Hallock (1981a) reported comparable rates of primary production in *A. lobifera* (2.9 $*10^{-5}$ mg ^{14}C h^{-1} foraminifer^{-1} = ca. 2.4 nmol O_2 foraminifer^{-1} h^{-1}) and *in A. lessonii* (1.95$*10^{-5}$ mg ^{14}C h^{-1} foraminifer^{-1} = ca. 1.6 nmol O_2 foraminifer^{-1} h^{-1}). Respirometric studies by Lee et al. (1980) showed 1.9 times higher O_2 evolution rates of *A. hemprichii* as compared to *A. lobifera*. *A. hemprichii* evolved 46 nmol O_2 µg protein^{-1} h^{-1} and *A. lobifera* 34 nmol O_2 µg protein^{-1} h^{-1} (at 198 µmol photons m^{-2} s^{-1}). O_2 evolution on a per organism basis would result in 317 nmol O_2 foraminifer^{-1} h^{-1} in *A. lobifera* (average protein content 4.9 µg foram^{-1}, Lee et al. 1980) and 166 nmol O_2 foraminifer^{-1} h^{-1} in *A. hemprichii* (average protein content 6.9 µg foraminifer^{-1}). However, the expression of O_2 evolution rates on the basis of protein content probably overestimates the primary production rates. Ter Kuile et al. (1989a) estimated a maximum C_i 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 big *A. lobifera*. Calculating the C uptake on a per foraminifer basis results in ca. 4.97 nmol C foraminifer^{-1} h^{-1} which is comparable to our measurements. One explanation for the higher production rates in *A. hemprichii* could be the amount of photosynthetic pigments. Pigment analysis of *A. lobifera* and *A. hemprichii* demonstrated a much higher content of *Chl a*, *Chl b*, and *Chl c* in *A. hemprichii* (total content = 0.89 mg organism^{-1}) as compared to *A. lobifera* with 0.054 mg organism^{-1} (Lee et al. 1980).

The primary production rates measured in this study are similar to previously reported rates of other symbiotic protozoa such as planktonic foraminifera and radiolaria.

Net photosynthesis rates measured in planktonic foraminifera ranged between 1.0 and 15.0 nmol O_2 foraminifer^{-1} h^{-1} (Jørgensen et al. 1985, Caron et al. 1985, Spero et al. 1985, Rink et al. 1998) (Table 3). Solitary radiolaria showed higher average rates of ca. 43.0 nmol C organism^{-1} h^{-1} (Caron et al. 1985).

In this study, we used O_2 microsensors to measure i) net photosynthesis (nmol O_2 cm^{-2} s^{-1}) and gross photosynthesis (nmol O_2 cm^{-3} s^{-1}) rates at the shell surface of benthic foraminifera and ii) the total net O_2 production of the foraminiferal-algal association (nmol O_2 h^{-1}). To compare this two different methods we extrapolated the shell surface rates to total surface area rates of the biconcave shaped *A. lobifera* by using the formula of Lee et al. (1988):

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

Assuming a diameter (D) of 3.0 mm (± 0.42, n = 5) and an average net photosynthesis rate of 0.11 nmol O_2 cm^{-2} s^{-1} (± 0.079, n = 26) we estimated a total net O_2 production rate of 62.0 nmol O_2 foraminifer^{-1} h^{-1}. This approximation largely overestimates the average total net photosynthesis rate of 14 nmol O_2 h^{-1} that we measured in *A. lobifera* with the net chamber technique (Table 1). In addition, the use of this new chamber allows the estimation of the total gross photosynthesis rate of larger foraminifera (Table 1). We estimated an average gross rate of 23 nmol O_2 h^{-1} for *A. lobifera* and 25 nmol O_2 h^{-1} for *A. hemprichii* (data not shown) by the summation of the net photosynthesis and dark respiration rates. Total gross photosynthesis rates in planktonic foraminifera measured with O_2 microsensors are similar. Jørgensen et al. (1985) estimated 18 nmol O_2 h^{-1} in the species *G. sacculifer* and Rink et al. (1998) found a gross rate of 8.9 nmol O_2 h^{-1} in *O. universa* (Table 3).

In a coral reef community macroalgae, seagrass, as well as symbiotic microalgae that live in association with several metazoan hosts (e.g. corals, sea anemone, ascidiens) and larger foraminifera largely contribute to the primary production of this ecosystem. The total annual production on coral reefs ranges between 300 - 5500 g C m^{-2} yr^{-1} in comparison to an open ocean productivity of 21 - 183 g C m^{-2} yr^{-1} (Muscatine 1990, Gattuso et al. 1996). The gross community primary production of Younge Reef (Great Barrier Reef) ranged between 9 - 15 g C m^{-2} d^{-1}. Normalized to the surface area of individual colonies Falkowski and

Dubinsky (1981) estimated a mean productivity of 2.63 g C m^{-2} d^{-1} (961 g C m^{-2} yr^{-1}) for the coral *Stylophora pistillata*. Porter (1988) reported a gross production of 510 g C m^{-2} yr^{-1} for the coral *Montastrea annularis* living in a depth of 10 m. In a seagrass community, epibenthic algae showed a mean net productivity of 4.2 g C m^{-2} d^{-1} (Pollard and Kazuhiro 1993).

To give a rough estimation of the daily rate of net primary production due to benthic foraminifera in their natural habitat we assumed an average net production of 14 nmol O_2 h^{-1} foraminifer^{-1}, a 12:12 light dark period, and a population density of 15.000 individuals m^{-2}. According to 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 15 mg C m^{-2} d^{-1} amounting to a yearly rate of 5.5 g C m^{-2} yr^{-1}. This value probably overestimates the production rate due to the carbon loss by e.g. growth, soluble carbon secretion 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 1984, Muscatine 1990). On the other hand, one has to note that benthic foraminifera can reach maximum densities up to 40.000 individuals m^{-2}. Therefore, after a reproduction period, the increased population would amount even higher production rates. In comparison to the corals, however, the primary production of larger benthic foraminifera seems to contribute a small part to the total reef production on an annual basis.

The comparison with different approaches demonstrated that the microsenor technique is very useful to estimate the primary production of larger benthic foraminifera. Furthermore, O_2 microsensors allowed short term experiments to investigate e.g. irradiance effects in one foraminifer (P-E_o curves) or to estimate the total net O_2 production or consumption of a single organism under changing environmental conditions. Compared to traditional techniques, we could thereby calculate the total gross primary productivity of the foraminiferal-algal association.

Inorganic carbon sources

To sustain high primary production rates as measured in symbiotic foraminifera there is a need for sufficient supply of inorganic carbon (C_i). In larger foraminifera inorganic carbon could be supplied from internal (e.g. respiration, calcification) and/ or external sources (Fig. 9). The C_i transport 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). This diffusional C_i uptake of the foraminifer is limited by a diffusive boundary layer (DBL) that surrounds the foraminiferal shells. The thickness of the prevailing DBL is affected by the flow conditions and the characteristic roughness of the surface (Jørgensen and Revsbech al. 1985, Jørgensen and Des Marais 1990, Vogel 1994). In our previous flow experiments with benthic foraminifera we measured a DBL thickness of 100 - 700 μm and found enhanced gross photosynthesis and respiration rates under increased flow velocities (Köhler-Rink and Kühl, 2000).

Measurements of the CO_2 microenvironment around the shell of *A. lobifera* at saturating irradiances (830 μmol photons m^{-2} s^{-1}) (Fig. 2C) and experimental light-dark cycles in *A. hemprichii* and *M. vertebralis* (Figs. 5, 6) demonstrated that CO_2 is not totally depleted by photosynthesis at the foraminiferal shell surface. Furthermore, pH microsensor measurements showed a significant pH increase around the foraminiferal shells as compared to the ambient seawater values. The observation of high pH values at the foraminiferal shells up to 8.65 (Fig. 2B) indicate that the seawater CO_2 concentration must be low (Gavis and Furgeson 1975). Therefore we suggest that altenative CO_2 sources exist, that supply suffcient CO_2 for symbiont photosynthesis.

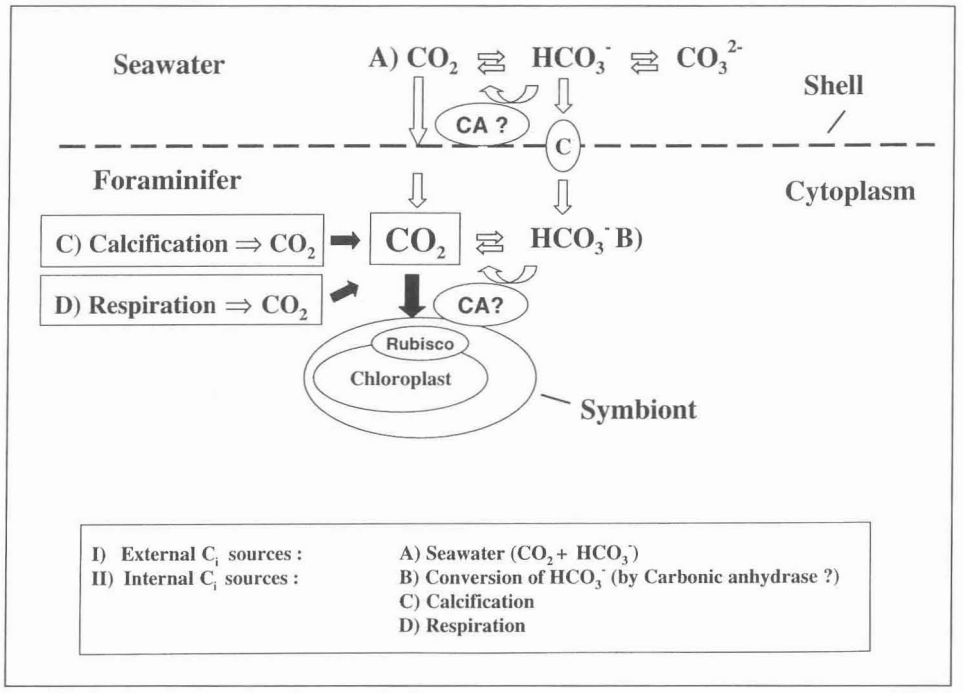

Fig. 9 Potential external and internal sources of inorganic carbon (C_i) and related processes for photosynthetic assimilation of the symbionts in larger foraminifera (CA = enzyme carbonic anhydrase, Rubisco = ribulose-1,5-bisphoshat carboxylase/oxygenase, C = carrier for HCO_3^- transport)(based on Al-Moghrabi et al. 1996, Falkowski and Raven 1997).

One possible C_i source for photosynthetic CO_2 assimilation is the ambient seawater reservoir due to the large concentration of HCO_3^- (Fig. 9). In seawater (35‰) of pH 8.21 > 95% of the inorganic carbon is present in form of HCO_3^-, while the CO_2 concentration only amounts to ~ 1% of the total inorganic carbon content (C_T). In addition, the uncatalysed conversion of HCO_3^- to CO_2 is frequently slower than the rate at which CO_2 can be assimilated by marine algae. Therefore maximum rates of carbon fixation can only be maintained if the reaction is catalyzed enzymatically (Raven 1994, Falkowski and Raven 1997).

CO_2 concentrating mechanisms (i.e. active inorganic C transport processes) have been reported for several symbiotic cnidarian species (Weis et al. 1988, Al Moghrabi et al. 1996) and for different types of marine phytoplankton (Badger et al. 1980, Burns and Beardall 1987). Two DIC uptake mechanisms have been described (Fig. 9): i) an enzymatic dehydration of extracellular HCO_3^-, or ii) an active HCO_3^- transport across the plasma membrane via a carrier protein (Al Moghrabi et al. 1996). The enzyme carbonic anhydrase (CA), that catalyses the reversible hydration-dehydration of CO_2 plays an important role in the dissolved inorganic carbon (DIC) uptake (Badger and Price 1992). CA activity was found on the plasmalemma and as an extracellular, soluble enzyme (Falkowski and Raven 1997).

In the coral *Galaxea fascicularis* e. g. Al-Moghrabi et al. (1996) detected a carrier mediated transport of HCO_3^- into the host cells. The pH measurements in *M. vertebralis* (Fig. 5B) could indicate that CO_2 and not HCO_3^- is entering the foraminiferal cytoplasm for photosynthetic fixation. The pH shifts measured at the shell surface during light-dark cycles followed the CO_2 response. The external pH would not be affected if HCO_3^- is entering the cells (Sikes et al. 1980). The different responses of the pH and CO_2 dynamics in *M. vertebralis* and *A. hemprichii* to experimental light-dark cycles could therefore indicate different CO_2 supply mechanisms for symbiont photosynthesis or point to different CO_2 fixation mechanisms.

Results of CO_2 and O_2 flux calculations at the shell surface of *M. vertebralis* (Köhler-Rink and Kühl, 2000) showed higher rates of O_2 release as compared to the CO_2 uptake due to symbiont photosynthesis in the light. In the darkness, respiratory O_2 uptake was ~ 18 x higher as compared to the CO_2 release of the community (Table 4). The smaller rates of CO_2 uptake could suggest slower chemical reactions of the HCO_3^- dehydroxylation in the seawater near the foraminiferal shell as compared to the fast photosynthetic CO_2 fixation rates of the endosymbionts (Falkowski and Raven 1997). The higher O_2 flux out of the foraminiferal shell could further indicate an internal CO_2 supply resulting from respiratory CO_2 release and/ or the conversion of HCO_3^- to CO_2 (Fig. 9).

Table 4 *Marginopora vertebralis*. Shell surface gradients of O_2 and CO_2 under light and dark conditions

	nmol cm^{-2} s^{-1}
Light :	
CO_2 uptake	0.004
O_2 release	0.15
Dark :	
CO_2 release	0.0013
O_2 uptake	0.023

In larger foraminifera respiratory CO_2 supply of the community is probably not sufficient for the photosynthetic CO_2 fixation. Based on the ratio of total net O_2 production vs. consumption (0.69) measured in *A. lobifera* (Table 1), we suggest that the endo-symbionts are probably dependent on further CO_2 sources. Supplemental CO_2 could also be produced as a result of carbonate deposition of the foraminiferal shell. If HCO_3^- is used as a substrate for calcification the reaction: $2\ HCO_3^- + Ca^{2+} \Leftrightarrow\ CaCO_3 + CO_2 + H_2O$ would result in a net CO_2 release (Stumm and Morgan 1996, Falkowski and Raven 1997). Further studies in this direction need to be done to answer these questions of CO_2 supply and CO_2 translocation within the foraminiferal cytoplasm.

Carbon uptake mechanisms in benthic foraminifera and the CO_2 sources for symbiont photosynthesis are rarely investigated. The use of microsensor techniques allows detailed studies about the symbiont photosynthesis and the CO_2 and pH microenvironment. Thus, a reconstruction of the carbonate system of the surrounding seawater in the vicinty of the foraminiferal shell is now possible. Mechanisms for inorganic carbon uptake in *A. lobifera* and *A. hemprichii* have been studied with the ^{14}C method by ter Kuile et al. (1989a). They measured photosynthesis saturation at C_i levels of seawater in the cultured symbionts of *A. lobifera* (*Fragilaria shiloi*). The photosynthetic C_i uptake of the symbionts within *A. lobifera* and *A. hemprichii* also showed no significant increase above C_i seawater concentrations at ca. 750 µmol photons m^{-2} s^{-1}. Therefore, the authors suggested an enzymatic step limiting the C_i uptake. They further discussed the transport from the host

cytoplasm into the symbiont cells and suggested that in *A. lobifera* C_i diffuses into the foraminifer in the form of HCO_3^-, that is converted intracellularly to CO_2 and subsequently fixed by the symbionts (Fig. 9). In *A. hemprichii* a parallel uptake of CO_2 and HCO_3^- was assumed. The increase of CO_2 concentration due to the addition of CA to the medium stimulated the symbiont photosynthesis of *A. lobifera* but not of *A. hemprichii*. We speculate that this result could also indicate photosynthesis saturation of *A. hemprichii* before the addition of CA, depending on the prevailing light situation and the photosynthetic characteristics of the endosymbionts within the measured foraminifera. Alternatively, CA was already present in *A. hemprichii*. Results of our P vs. E studies demonstrated that photosynthesis of *A. lobifera* and *A. hemprichii* reached light saturation between 100 - 200 µmol photons m^{-2} s^{-1}. We also showed that individual foraminifera of one species could have different light saturation values (E_k) (Fig. 5).

Conclusions

The photosynthetic activity of the symbiotic microalgae demonstrated a dynamic response to changing light conditions. Combined measurements of O_2, CO_2, and pH concentrations at the shell surfaces showed a close interaction of the autotrophic and heterotrophic processes resulting in rapid microenvironmental changes under varying light conditions.

The study of symbiont light requirements and their photophysiology support the assumption of high-light adaptation mechanisms in larger foraminifera as indicated by the lack of photoinhibition and high light saturation values (E_k). In addition, the photoacclimatory capabilities of the endosymbionts could influence the distribution pattern of larger foraminifera. Based on total O_2 production and consumption measurements, and point measurements of gross and net photosynthesis with O_2 microelectrodes it is now possible to estimate the primary production rates of benthic foraminifera much more accurately.

Our knowledge about inorganic carbon sources, the C_i pathways and translocation to the endosymbionts is still very limited. However, microsensor studies as presented here in combination with e.g. use of specific inhibitors have a large potential to elucidate the mechanisms of C_i uptake and CO_2 supply mechanisms in symbiotic foraminifera.

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Summary

Biological studies of symbiotic foraminifera focused i) on foraminiferal life cycles, ii) their cytology and fine structure, iii) growth and calcification under different light and nutrient regimes, and iiii) the identification of symbiotic algae. Still very little is known about physiological processes of foraminifera living in symbiosis with phototrophic microalgae, e.g. the interrelations of the symbiotic partners, including the transport of nutrients and gases within the association. In addition, it is of interest to understand the impact of changing physico-chemical parameters including light intensity and the hydrodynamics of the surrounding seawater on the physiology of the foraminifer-algal symbiosis. Furthermore, the basic mechanisms involved in the calcium carbonate precipitation of foraminiferal shells are poorly understood.

This thesis investigated the physico-chemical conditions in the vicinity of symbiont-bearing foraminifera and their metabolic activities with high spatial and temporal resolution by using different types of microsensors. Microsensors for O_2, pH, and scalar irradiance (PAR) have been used to study the physico-chemical microenvironment of the planktonic species *Orbulina universa* (chapter 2). For microsensor measurements a single foraminifer was placed on a nylon mesh in a small measuring chamber. Significant concentration changes of O_2 and pH as compared to the ambient seawater conditions were found in the vicinity of the shell due to photosynthesis of the symbiotic algae and respiration of the host-symbiont system. In addition, the presence of a diffusive boundary layer that surrounds the foraminiferal shell limited the solute exchange between *O. universa* and the ambient seawater. Under light conditions, O_2 supersaturation and a pH increase were measured. Dark conditions were characterized by an O_2 depletion and pH decrease towards the shell surface. Radial profiles of scalar irradiance showed a slight increase near the calcite shell due to light reflection and scattering by the shell and its attached calcite spines. Photosynthesis vs. irradiance ($P\text{-}E_o$) curves demonstrated an adaptation of the symbiotic microalgae to higher light levels as indicated by a high light saturation irradiance (E_k). In addition, no photoinhibition was observed up to 700 µmol photons m^{-2} s^{-1}. Furthermore, respiration rates of the foraminifer-algal association were significantly higher in the light as compared to the respiration under dark conditions. This observation could be explained by an increase in

respiratory substrates supplied by the release of photosynthates and/ or the process of photorespiration which is enhanced at higher O_2 concentrations.

The study of planktonic foraminifera was continued with the application of CO_2 and Ca^{2+} microsensors, two important parameters with respect to photosynthesis and calcification in symbiotic foraminifera (chapter 3). Combined studies of CO_2, O_2, pH, and Ca^{2+} concentrations in the surroundings of *O. universa* demonstrated dynamic concentration changes under changing light conditions. Symbiont photosynthesis and the respiration of both partners changed the carbonate chemistry in dependence of the incident irradiance. Under saturating light conditions combined CO_2 and pH measurements indicate an increase in seawater alkalinity and a decrease of inorganic carbon concentration due to the symbiont photosynthesis. The microenvironment changed completely under dark conditions when respiration dominated. A significant CO_2 increase and pH decrease were observed. O_2, CO_2, and pH dynamics measured within the symbiont swarm suggest a fast CO_2 supply mechanism that delivers sufficient CO_2 for the high photosynthetic rates of the endosymbionts. The conversion of HCO_3^- and the processes of respiration and calcification are potential sources for CO_2 supply in symbiont- bearing foraminifera.

The second part of this study (chapters 4 and 5) investigates the metabolic processes of symbiotic benthic foraminifera and their physico-chemical microenvironment with O_2, CO_2, pH, and Ca^{2+} microsensors. We found a dynamic microenvironment at the shell surface of larger foraminifera due to the combined processes of symbiont photosynthesis, respiration of the association, and host calcification (chapter 4). The symbionts that live intracellularly in the foraminiferal shells showed a dynamic response to changing incident irradiances and to experimental light-dark cycles. The dynamic concentration changes at the shell surface demonstrated for the first time a fast exchange of metabolic gases through the perforate hyaline shells and the imperforate porcelaneous shells of larger foraminifera. Microsensors were further used to investigate the diffusive boundary layer (DBL) adjacent to the foraminiferal shell surface. The DBL showed an increase in thickness, when the water flow was reduced. At higher flow velocity we could measure significantly higher gross photosynthesis and respiration rates as compared to stagnant conditions.

Calcium microprofiles measured towards the shell of larger foraminifera showed spatial variations in the sites of calcium uptake. We found different Ca^{2+} dynamics in the perforate *Amphistegina lobifera* as compared to the imperforate *Marginopora vertebralis*.

Based on Ca^{2+} flux measurements close to the shell surface we were able to estimate their rates of Ca^{2+} uptake. In addition, the application of the fiber optic microprobe to measure scalar irradiance (PAR) was important to estimate the light transmittance of the foraminiferal shell. Transmittance measurements showed that the symbionts in the foraminiferal cytoplasm receive approximately 30% of the incident light.

The effect of the prevailing light conditions on symbiont-bearing larger foraminifera was studied in chapter 5. Endosymbiont photosynthesis was largely affected by the incident irradiance. Subsequently, dynamic concentration changes of O_2, CO_2, and pH have been measured near the foraminiferal shell with increasing irradiances. Symbiont light requirements suggest an adaptation to high-light conditions as indicated by the lack of photoinhibition and the high light saturation values (E_k). The inorganic carbon (C_i) supply for photosynthetic fixation of symbiotic microalgae (e.g. diatoms and dinoflagellates) living associated with foraminifera is poorly understood. The results of our measurements showed that the microsensor technique gives a large potential to investigate the mechanisms of C_i supply and possible inorganic carbon sources for symbiont photosynthesis.

Furthermore, the O_2 microsensors are useful to measure the primary production of symbiont-bearing foraminifera. In short term experiments, point measurements of gross and net photosynthesis at the shell surface of foraminifera gave important information about the response of symbiont photosynthesis to environmental changes e.g. varying incident irradiance and water flow conditions. The total O_2 production and consumption of single larger foraminifera was measured with a new developed setup. This „mini-net" chamber allows the investigation of foraminiferal primary production rates under changing temperature and light conditions.

In summary, the present thesis demonstrates the useful application of microsensors to investigate important metabolic processes including photosynthesis, respiration, and calcification of symbiont-bearing calcifying organisms, such as planktonic and benthic foraminifera.

List of publications

Contributions of (co-) authors to the manuscripts presented in this thesis

1. **Microsensor studies of photosynthesis and respiration in the symbiotic foraminifer *Orbulina universa***

   S. Rink, M. Kühl, J. Bijma, H. Spero

   Concepted by M. Kühl, J. Bijma, H. Spero and S. Rink,
   Writing of the manuscript by S. Rink with editorial help from M. Kühl

2. **The CO_2, O_2, pH and Ca^{2+} microenvironment of the symbiotic planktonic foraminifer *Orbulina universa***

   S. Köhler-Rink, M. Kühl

   Concepted by S. Köhler-Rink and M. Kühl, writing of the manuscript by
   S. Köhler-Rink with editorial help from M. Kühl

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

   S. Köhler-Rink, M. Kühl

   Concepted by S. Köhler-Rink and M. Kühl, writing of the manuscript by
   S. Köhler-Rink with editorial help from M. Kühl

4. **Microsensor studies of photosynthesis and respiration in larger symbiotic foraminifera II. Irradiance effects on photosynthesis and respiration of *Marginopora vertebralis*, *Amphistegina lobifera*, and *Amphisorus hemprichii***

   S. Köhler-Rink, M. Kühl

   Concepted by S. Köhler-Rink and M. Kühl, writing of the manuscript by
   S. Köhler-Rink with editorial help from M. Kühl