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PHOTOSYNTHESIS AND RESPIRATION IN FIVE SPECIES OF BENTHIC FORAMINIFERA THAT HOST ALGAL ENDOSYMBIONTS

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ABSTRACT

Oxygen production and consumption were measured in five species of benthic foraminifers using a "Clark-type" oxygen electrode. Net photosynthesis and respiration were calculated and normalized to µg chlorophyll *a* for the chlorophyte-bearing soritid foraminifers *Archaias* (*Ar.*) *angulatus* and *Cyclorbiculina compressa*, and the diatom-bearing amphisteginids *Amphistegina gibbosa*, *Am. lessonii*, and *Am. radiata*. Chlorophyll *a* concentrations were 40–50% lower in *C. compressa* than in the four other species. Photosynthesis/Irradiance (P/E) curves were generated by fitting data to the hyperbolic tangent equation, $P = P_{max} \tanh(\alpha E / P_{max})$, yielding the derived photosynthetic parameters, P_{max}, α, and E_k. Calculated maximum oxygen production (P_{max}), when normalized to chl *a*, was 3–4× higher in the soritids than in the amphisteginids. Photosynthetic efficiency (α) was approximately two-fold higher in *Am. gibbosa* and ~50% higher in *Am. lessonii* than in the soritids. Calculated P/E data for *Am. radiata* were too variable to estimate an α. Median E_k, which indicates approaching light saturation, was 13 µmol photon m^{-2} s^{-1} for *Am. gibbosa* and *Am. radiata*, 26 µmol photon m^{-2} sec^{-1} for *Am. lessonii*, and 86 and 122 µmol photon m^{-2} sec^{-1} respectively for *Ar. angulatus* and *C. compressa*. These values are consistent with the habitats occupied by these foraminifers and with results of previous studies that used other methods. Median factorial metabolic scope, which is the ratio of respiration rate under normal activity to resting metabolic rate, was 2–4 for the amphisteginids versus 9–10 for the soritids. *Archaias angulatus*, *C. compressa* and *Am. lessonii* appear to be net primary producers, whereas *Am. gibbosa* and *Am. radiata* are net consumers.

INTRODUCTION

Larger benthic foraminifers (LBF) are so abundant in many reef environments that they were called "living sands" by Lee (1998). Most free-living LBF host algal endosymbionts in a relationship analogous to that in zooxanthellate corals (Lee and Anderson, 1991). Unlike corals, which have exclusively dinoflagellate symbionts, LBF taxa host a variety of symbiont taxa including chlorophytes, rhodophytes, diatoms, and dinoflagellates (e.g., Lee and Anderson, 1991). Although fewer than 10% of extant families of the class Foraminifera host algal endosymbionts, these families locally account for substantial carbonate production (e.g., Hallock, 1981a). Globally, LBF account for roughly 0.5% of the total annual carbonate production (Langer, 2008).

Algal symbioses offer several possible advantages to foraminifers. Host foraminifers may utilize organic carbon produced by symbiont photosynthesis as energy sources (Muller, 1978; Hallock, 1981b). Within the cell, uptake of carbon dioxide during photosynthesis may enhance calcification rates (Duguay and Taylor, 1978; ter Kuile, 1991; Erez, 2003). In low-nutrient environments, algal symbionts may utilize nutrient wastes produced by the host foraminifer (Hallock, 1999). The benefits from symbiosis and the variety of endosymbionts hosted have apparently enabled different LBF taxa to adapt to environments over a wide range of light availability (Hallock, 1988, 1999).

Despite the widespread abundance and carbonate productivity of LBF, relatively few studies have examined basic metabolic processes such as photosynthesis and respiration (see Fujita and Fujimura, 2008, and references therein). Using a differential manometer system, Lee and others (1980) demonstrated net primary production in *Amphistegina* (*Am.*) *lobifera* Larsen, which is the shallowest-dwelling Indo-Pacific amphisteginid, at light levels of 10 klx (~180 µmol photon m^{-2} s^{-1}). Rink and others (1998) examined photosynthesis, respiration, and calcification rates using microsensors to measure O_2, CO_2, pH, and Ca^{2+} at the test surface of the planktonic foraminifer *Orbulina universa* d'Orbigny, which hosts dinoflagellate endosymbionts. Köhler-Rink and Kühl (2000, 2001) then studied the LBF species *Am. lobifera* and *Marginopora vertebralis* Quoy and Gaimard using similar technology.

Nobes and others (2008) used pulse-amplitude modulated fluorometry to examine physiological responses of *Amphistegina* spp., *Calcarina* spp., and *Heterostegina depressa* d'Orbigny. One shortcoming of their study with respect to *Amphistegina* and *Calcarina* was that their cultures mixed species from different subenvironments of the reef: the three amphisteginid species show distinct depth zonation (e.g., Hallock 1984; Hohenegger, 1994, 2004), so mixing the common reef-flat dwelling *Am. lobifera* with *Am. lessonii* d'Orbigny and the typically deeper fore-reef-dwelling *Am. radiata* (Fichtel and Moll) complicates comparisons with their results.

Most studies of photosynthesis and calcification have employed uptake of radioisotopes (Erez, 2003, and references therein). Duguay and Taylor (1978) recorded primary production and calcification in *Archaias* (*Ar.*) *angulatus* (Fichtel and Moll) using photosynthetic carbon fixation as a measure of photosynthesis. They reported that *A. angulatus* reached light saturation at light intensity of ~200 µmol m^{-2} s^{-1}. Duguay (1983) reported that calcium and carbon uptake approach saturation in *Ar. angulatus* between 250–500 µmol photon m^{-2} s^{-1} and found no photoinhibition at light intensities as high as 1000 µmol photon m^{-2} s^{-1}. Others that have used radioisotopic procedures to study calcification and productivity in benthic foraminifers include Lee and Bock (1976), Smith

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(1977), Smith and Wiebe (1977), Leutenegger and Hansen (1979), Lee and others (1980), and ter Kuile (1991).

The objective of our study was to utilize Clark-type oxygen electrode technology to estimate photosynthesis and respiration rates in five species of LBF. Resulting data enabled us to generate photosynthesis/irradiance curves for each species, and calculate other basic metabolic functions. The broader goal of this research is to determine if these basic functions provide insight into the habitats of the foraminifers.

Taxa Studied

Five common species of LBF, which were collected for other research projects, were used in this study. Three species are abundant in carbonate shelf and reef environments of the subtropical/tropical western Atlantic and Caribbean: *Ar. angulatus*, *Cyclorbiculina compressa* d'Orbigny and *Amphistegina gibbosa* d'Orbigny. *Amphistegina lessonii* occurs abundantly in the Indo-Pacific, while *Am. radiata* is most common in the western Pacific.

Archaias angulatus is the shallowest dwelling of the species studied, ranging from depths less than 1 m in relatively turbid coastal waters to 20 m or more in very clear open-shelf or reef-margin habitats. They are commonly abundant among seagrass beds of *Thalassia testudinum* König (Duguay, 1983). Densities of *Ar. angulatus* have been observed as high as 15×10^4 individuals m^{-2} in the Florida Keys, where they can produce roughly 60 g $CaCO_3$ m^{-2} yr^{-1} (Hallock and others, 1986a). *Archaias angulatus* harbor the chlorophyte endosymbiont *Chlamydomonas hedleyi* Lee, Crocket, Hagen and Stone (Lee and others, 1974), which provides the host with less than 10% of its organic carbon needs (Lee and Bock, 1976). A majority of its organic-carbon requirements come from grazing. Photosynthesis clearly enhances calcification in this species (Duguay and Taylor, 1978).

Cyclorbiculina compressa inhabits slightly deeper waters than *Ar. angulatus*, typically 3–40 m depth, depending upon water transparency. They can be found on seagrass and in filamentous algal mats in open reef environments (e.g., Hallock and Peebles, 1993). Lutze and Wefer (1980) reported densities of *C. compressa* up to 200/m^2 in Harrington Sound, Bermuda. *Cyclorbiculina compressa* hosts the chlorophyte endosymbiont *Chlamydomonas provasoli* Lee, McEnery and Kahn (Lee and others, 1979). As in *Ar. angulatus*, *C. compressa* will not calcify without its symbionts or in the dark (Duguay, 1983).

Amphistegina gibbosa is the smallest and deepest living of the western Atlantic species examined. They can be found from depths less than 1 m down to 100 m, but are commonly abundant at 5–40 m (Hallock, 1999; Baker and others, 2009). These foraminifers are found on a variety of reef substrates including coral rubble and macrophytes. *Amphistegina gibbosa* host diatom endosymbionts belonging to several genera, though *Nitzschia frustulum* var. *symbiotica* Lee and Reimer emend. was the dominant species isolated from specimens collected in the Florida Keys (Lee and others, 1995; Lee, 1998).

Amphistegina lessonii is the Pacific sibling of *Am. gibbosa,* although *Am. lessonii* populations have slightly shallower depth distributions and somewhat higher light tolerances (Hallock and others, 1986b). These foraminifers are most abundant at depths of 10–40 m (e.g., Hallock, 1984; Hohenegger, 1994, 2004). In shallow, well-lit environments, *Am. lessonii* avoids damage from intense light by cryptic behavior (Hallock, 1999). This species also hosts diatom endosymbionts belonging to several genera (e.g., Lee and others, 1993).

Amphistegina radiata is a somewhat deeper dwelling species (e.g., Hohenegger, 1994, 2004), typically found in greatest abundance at depths of 20–50 m. Like the other two amphisteginids, it hosts diatom endosymbionts (Lee and others, 1993).

In general, soritid foraminifers inhabit shallower, higher light environments than the amphisteginids examined (Hallock, 1999; Baker and others, 2009), yet paradoxically, symbiosis apparently is a more important source of energy for the amphisteginids (ter Kuile and others, 1987). These soritids are prevalent in environments with somewhat more abundant food resources (Hallock and Peebles, 1993). Therefore, the primary role of the chlorophyte symbionts may be enhancement of calcification (Duguay, 1983). In the low-nutrient environments inhabited by amphisteginids, diatom endosymbionts can provide organic carbon that is otherwise in limited supply (Hallock, 1999). The ability of diatoms to utilize light in the blue–green range allows their hosts to exploit deeper habitats (Leutenegger, 1984).

METHODS

Collection of Specimens

Specimens of *Cyclobiculina compressa* and *Am. gibbosa* were collected from 10–30 m depths using SCUBA at Conch and Tennessee reefs in the Florida Keys, USA (locations described in Hallock and others, 1995). *Amphistegina lessonii* and *Am. radiata* were collected from 20 m depth by SCUBA divers in Papua New Guinea at Ambitle Island (location described in Pichler and others, 2006). Pieces of coral rubble were collected under water, placed in zippered plastic bags and taken to a field laboratory or shipboard, where the rubble was brushed free of foraminifers, sediment, and other debris. Sequential rinsing and decanting from the samples removed the organic debris and fine sediment. The remaining sediment and foraminifers were then placed in plastic jars for transport. Upon arrival at the laboratory, the samples were again rinsed with seawater and transferred to 150×15 ml petri dishes and placed in an environmental chamber. They were kept under a 12-hour light/dark schedule at 25°C. Maintenance light intensity was 5–10 μmol photon m^{-2} s^{-1} under cool-white fluorescent lighting. After approximately 24 hours the organisms migrated to the surface of the sediment where they were easily located under a stereomicroscope and removed with forceps for use in experimental procedures.

Specimens of *Ar. angulatus* were collected from a shallow (1–2 m depth) seagrass bed in Florida Bay directly behind the Keys Marine Laboratory located in Layton, Florida, USA (for location and habitat description, see Fujita and Hallock, 1999). Clumps of seagrass blades and filamentous algae were shaken into zippered plastic bags to release

foraminifers and associated sediment. This concentrate was rinsed with seawater to remove most phytal debris, and then the plastic bags were topped off with oxygen for transport. A sample of *Thalassia testudinum* and filamentous algae was separately bagged. Upon arrival at the laboratory, the sediment and the phytal samples were reunited in a small aquarium and an air stone was added to provide the biota with adequate dissolved oxygen. The aquarium was exposed to indirect sunlight (up to tens of μmol photon m^{-2} s^{-1} depending on time of day and cloud cover).

Photosynthesis and Respiration Trials

The ''Clark-type'' oxygen electrode is an electrochemical cell that has its cathode and anode connected by an electrolyte ''bridge'' (Clark, 1956). When a polarizing voltage is applied to the cell, ionization of the electrolyte induces current flow through the electrode. The magnitude of the current flow is proportional to the concentration of dissolved oxygen in the electrolyte solution. The concentration of oxygen in the electrolyte is in turn proportional to the oxygen in the surrounding environment

Photosynthesis and respiration rates were measured as net oxygen evolution using an oxymeter coupled to a DW1 oxygen electrode unit illuminated by a LS2 light source (Hansatech Ltd., UK). The Hansatech® Oxygen Electrode Disk in the DW1 unit utilizes the Clark-type oxygen electrode developed by Delieu and Walker (1981). The theory, setup, challenges, precautions, and calculations associated with measurements using such electrodes, including discussion of the instrument and light source we used, can be found in Walker (1987).

The DW1 oxygen-electrode unit has a sleeve surrounding a 3-ml reaction chamber that allows water from a temperature-controlled water bath to circulate around the chamber in order to maintain constant temperature. For our setup, the chamber and water-sleeve unit rested on a magnetic stirrer that drove a magnetic stir bar inside the chamber to maintain a well-mixed water sample. The entire apparatus was placed in a dark container (a cardboard box lined with black paper). Two holes were cut in the box: one to allow for passage of the water hoses from the temperature-controlled bath to the sleeve and a second to provide a window for light to enter from the light source. For dark trials, the entire assembly was wrapped in opaque black plastic.

Before each trial, the chamber was flushed with nitrogen gas to calibrate to zero oxygen concentration. Then the chamber was flushed with aerated seawater to calibrate to oxygen saturation for the seawater used in the trials. All experiments were conducted at salinity of 36 and temperature of 25°C. The setup procedure included a control run to determine if the instrument signal exhibited drift. If drift was detected during the blank trial, the electrode was cleaned, reassembled, and retested prior to running trials with foraminifers.

Individual foraminifers used in experimental trials were picked from sample dishes under the microscope or hand picked from the aquarium. Each specimen was examined under a stereomicroscope to ascertain that it exhibited the appearance of a normal, healthy, adult-sized individual (e.g., Hallock and others, 1995; Baker and others, 2009), and then was cleaned by removing any debris or algae attached to the test or held by the reticulopodia. Fine-tipped paintbrushes and forceps were used to clean specimens (e.g., Duguay and Taylor, 1978; Lee and others, 1980).

For the larger species *C. compressa*, *Ar. angulatus*, and *Am. radiata*, single specimens were placed in small bags made from plankton netting (0.125 mm mesh) and suspended in the reaction chamber just above the stir bar. Because *Am. lessonii* and *Am. gibbosa* are relatively smaller, multiple specimens were required to produce measurable changes; therefore, three *Am. lessonii* specimens and five *Am. gibbosa* were put into the mesh bag for each of their respective trials.

For each trial, the specimen or group of specimens was held in the dark in the reaction chamber for one hour to ascertain that respiration, as indicated by a decline in oxygen concentration, was detectable. Change in oxygen concentration during the last ten minutes of the dark period was recorded as pre-trial respiration rate. As soon as the respiration trial was complete, photosynthesis trials began, proceeding from the lowest to the highest light intensity. Change in oxygen concentration was recorded at each light intensity after ten minutes.

The Hansatech® LS2 light source is a tungsten quartz halogen bulb with a typical spectrum of four 300–750 nm that provides a uniform field of illumination when positioned horizontal to the chamber (Walker, 1987). Photosynthetically active radiation (PAR), as determined using a LICOR® LI-250 light meter, was varied for the trials using neutral density filters and by moving the light source predetermined distances to attenuate the light. Light intensities were measured a) in air in front of the chamber, b) with the chamber removed and the light sensor at the distance of the middle of the inner chamber where specimens were located, and c) behind the water-filled chamber, in order to correct for light attenuation by the apparatus.

The goal was to test light intensities that would yield data points for dark respiration, light-limited photosynthesis, light-saturated photosynthesis, and photoinhibition. *Archaias angulatus* specimens were tested sequentially at 0 (dark trial), 0.96, 17, 37, 48, 92, 180, 230, 540, 660, 780, 860, 1300, and again at 0 μmol photon m^{-2} s^{-1}; *C. compressa* was tested in the same sequence excluding the trial at 860 μmol photon m^{-2} s^{-1}. The *Amphistegina* spp. were tested sequentially at 0, 0.96, 3.1, 11, 17, 37, 48, 92, 180, 230, 540, 780, 1300, and 0 μmol photon m^{-2} s^{-1}. Although measured to higher precision, we report PAR intensities at two significant digits because our interest is in the shape of the curves generated, not the precise intensities, which were determined by the realities of light source, filters, and distances.

After completion of a set of light trials, the light source was turned off and a second respiration trial was run in darkness. Then the specimen or group of specimens was removed from the chamber and examined under a stereomicroscope to measure maximum diameter of each individual and record if color changes had occurred during

the experiment. After the specimens were blotted dry and weighed, they were placed on a filter pad, wrapped in aluminum foil, and stored at −39°C until chlorophyll extractions were performed.

Chlorophyll Extraction

Chlorophyll (chl *a*) extraction and measurement was performed following the methanol procedure of Holm-Hansen and Rieman (1978) modified for larger foraminifers (Hallock and Talge, 1994). Specimens were removed from the freezer and placed in cuvettes containing 5 ml of methanol, and crushed with a sterile glass rod. The cuvettes were covered with parafilm to prevent evaporation, then wrapped in aluminum foil to keep them in the dark during extraction. Samples were placed on a shaker in a refrigerator (4°C) and agitated for 18 hours, after which they were removed and left to warm to 25°C. Chlorophyll *a* concentration in the methanol was then measured for each sample using a Turner® 10 AU fluorometer and standardized to the mass of the specimen from which the chlorophyll was extracted.

Data Analysis

To estimate pre- and post-light rates of dark respiration, as well as net photosynthesis rates, we recorded rates of change in oxygen concentrations in the dark and at up to 12 light intensities for individual specimens or groups of specimens of each foraminiferal species. Net oxygen production or consumption estimates represent the combination of symbiont photosynthesis and holobiont (symbiont + host) respiration. Metabolic factorial scope (MFS), which is the ratio of post-trial to pre-trial oxygen production rates (Hernández-León and Ikeda, 2005), was determined for each species.

Derived parameters of photosynthesis were calculated using standard procedures (Neale, 1987) from the measurements of the rate of photosynthesis at each light intensity. Photosynthesis to irradiance (P/E) curves (Fig. 1) were generated for each trial by fitting the raw data from each individual foraminifer or group of foraminifers to the equation described by Jassby and Platt (1976):

$$P = P_{max} \tanh(\alpha E / P_{max})$$

where P is rate of photosynthesis, P_{max} is the experimentally derived maximum rate of photosynthesis, tanh is the abbreviation for hyperbolic tangent, α represents photosynthetic efficiency, and E is irradiance. Irradiance at the maximum rate of photosynthesis (E_k) was calculated by dividing P_{max} by α.

When measuring oxygen production or consumption in phytoplankton, dark respiration is often minimal and quickly overtaken by oxygen production, so positive values are recorded at low light intensities. When measuring oxygen production or consumption in a host-algal unit (holobiont), the oxygen consumption of the host is added to the oxygen consumption of the symbiont, so oxygen production must be somewhat higher to overtake respiration. Thus, we typically recorded negative values at low light intensities in all species and at most light intensities in two species. However, a decrease in oxygen consumption, when compared to initial dark readings, was commonly observed at the lower light intensities. Thus, raw values of oxygen production were assumed to represent net photosynthesis. To generate gross photosynthesis rates for each trial, the pre-trial dark oxygen uptake rate was subtracted from each subsequent data point in the light trial series before fitting each measured data set to the above equation. The P/E curves (Fig. 1) and corresponding values for P_{max}, α, and E_k were thereby determined using gross photosynthesis rates.

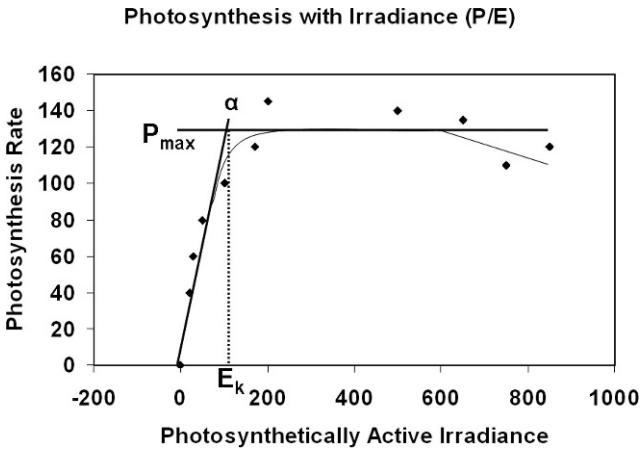

FIGURE 1. Sample photosynthesis/irradiance curve showing P_{max}, α, and E_k parameters (adapted from Neale, 1987).

Regressions were run on P/E data for each specimen normalized to μg chl *a*. To determine if differences existed between mean photosynthesis values at specific light intensities, two-tailed Student t-tests (Zar, 1984) were performed. At higher light intensities, when photosynthesis values declined, multiple one-tailed Students t-tests (Zar, 1984) were performed starting at the highest light intensity. T-tests were performed on data from successively lower light intensities until no significant difference was found. A significance level of 0.05 was used throughout.

Potential daily net oxygen production ($Prod_{net}$) was estimated as follows:

$$Prod_{net}\ \text{foram}^{-1} = [(P_{max} \times 10\ \text{hr}) - (R_I \times 24\text{hr})] \times \text{chl a mg}^{-1} \times \text{mg foram}^{-1}$$

where R_I is initial respiration. This calculation is based on the assumptions that saturation photosynthesis occurs 10 hr per day and respiration over the entire 24 hr is equivalent to that measured at the start of trials. Another possibility would be to use post-illumination respiration rates, but those are respiration rates under stress. Reality is likely somewhere between those endpoints.

RESULTS

For each specimen, diameter, mass, and chl a concentration were measured, then the ratio of chl a to mass was calculated (Tables 1, 2). Rates of oxygen consumption or production were measured in ten specimens each of *Ar.*

TABLE 1. Physical parameters, including maximum diameter (Diam), chl *a* extracted, mass, and chl *a* normalized to mass, for each soritid foraminifer used in the photosynthesis to irradiance observations shown in Figures 2 and 3 (AA = *Archaias angulatus*; CC = *Cyclorbiculina compressa*). Because not all data are normally distributed, data are summarized as median values.

Specimen ID	Diam (mm)	Chl a (μg)	Mass (mg)	μg Chl *a* /mg foram
AA01	3.00	0.50	2.20	0.23
AA02	2.90	0.24	1.62	0.15
AA03	2.95	0.25	1.72	0.14
AA04	2.40	0.19	1.06	0.18
AA05	2.15	0.42	1.08	0.39
AA06	2.65	0.63	1.75	0.36
AA07	2.55	0.39	1.75	0.22
AA08	4.25	1.18	3.85	0.31
AA09	2.95	0.55	1.79	0.31
AA10	2.90	0.54	2.08	0.26
AA median	2.90	0.46	1.75	0.25
CC01	3.85	0.24	2.90	0.08
CC02	3.95	0.25	3.10	0.08
CC03	5.30	0.69	6.30	0.11
CC04	3.50	0.19	2.10	0.09
CC05	3.60	0.18	2.30	0.08
CC06	5.60	0.80	7.70	0.10
CC07	5.00	0.65	5.20	0.13
CC08	3.80	0.23	2.70	0.08
CC09	4.15	0.45	3.30	0.14
CC10	3.60	0.20	2.10	0.10
CC median	3.90	0.25	3.00	0.10

angulatus, Cyclorbiculina compressa, and *Am. radiata.* Ten groups of *Am. gibbosa* were assessed using five specimens per group (i.e., a total of 50 specimens were utilized). Because available specimens of *Am. lessonii* were more limited, five groups with three individuals each were analyzed. Resulting data sets are presented in Figure 2A–E. Data summarized in Figures 2F and 3, and in Table 3, are presented as medians and ranges because not all data were normally distributed.

Median chl *a* concentrations for *Ar. angulatus* and the three amphisteginids were ~0.2 μg chl a/mg total weight, and approximately half that for the more massive *C. compressa*. Median pre-trial oxygen consumption in the dark ranged from a low of 9 nmoles O_2 hr^{-1} μg chl a^{-1} in *Ar. angulatus* to a high of 30 nmoles O_2 hr^{-1} μg chl a^{-1} in *Am. radiata*. The two soritids had post-trial respiration rates roughly 10 fold higher than their pre-trial rates, resulting in metabolic factorial scope (MFS) medians of 9–10. In contrast, median MFSs in the amphisteginids were only 2–4.

Photosynthesis versus irradiance (P/E) plots for the five species revealed that both soritids and *Am. lessonii* were net producers of oxygen under experimental conditions (Fig. 2A–C & F). All three species exhibited net photosynthesis at light intensities of 17 μmol photon m^{-2} s^{-1} and higher (Fig. 3). In contrast, *Am. gibbosa* and *Am. radiata* exhibited reductions in net oxygen consumption (Fig. 2D–F), indicating that photosynthesis was occurring but did not exceed respiration of the holobiont. Interestingly, at the lowest intensity light trials (0.96 μmol photon m^{-2} s^{-1}), *Am. lessonii* and *gibbosa* revealed an increase in oxygen consumption compared to initial dark respiration (Fig. 3)

Calculated maximum photosynthetic rates (P_{max}) were 120 and 140 nmoles O_2 hr^{-1} μg chl a^{-1} for the soritids, *Ar. angulatus* and *C. compressa* respectively, but only 42 nmoles O_2 hr^{-1} μg chl a^{-1} for *Am. lessonii* (Table 3). Those rates were reached at median calculated irradiances (E_k) of 86, 122, and 26 μmol photon m^{-2} s^{-1} respectively for those species. Calculated median P_{max} values for *Am. gibbosa* and *Am. radiata* were 34 and 17 nmoles O_2 hr^{-1} μg chl a^{-1} respectively, in both cases occurring at a median E_k of ~13 μmol photon m^{-2} s^{-1}.

Four of the five species exhibited declines in median oxygen production at light intensities >540 μmol photon m^{-2} s^{-1} (Fig. 2F), indicating photoinhibition; *C. compressa* exhibited photoinhibition only at 860 and 1300 μmol photon m^{-2} s^{-1}. In all species, visible changes in color could be seen using stereomicroscopy at the completion of light trials. The centers of the chlorophyte-bearing taxa had normal green coloration, but outer perimeters were white. The amphisteginids typically appeared slightly pale and uneven in color.

DISCUSSION

This study produced both expected results and some interesting surprises. An example of a predictable result was the difference in P/E responses between soritids and amphisteginids, which paralleled those of free-living relatives of their symbionts. Among the surprises were the differences in respiration rates and metabolic scope between the two groups, as well as their different potential to be primary producers.

Light requirements for growth and photosynthesis have long been known to differ significantly among the different algal classes (e.g., Richardson and others, 1983; Kirk, 1994). Diatoms can survive and grow at very low photon flux densities and can tolerate relatively high light intensities for limited periods of time, but they cannot increase their rate of photosynthesis to take advantage of the increase in radiant energy. In contrast, chlorophytes typically are unable to grow in very low light environments and they reach maximum photosynthesis rates in much higher light intensities (Richardson and others, 1983). In our study, the chlorophyte-bearing soritids reached P_{max} values, when normalized to chl *a*, that were 3–8× higher that those attained by the diatom-bearing amphisteginids (Table 3). Fujita and Fujimura (2008) similarly found that, at light saturation, dinoflagellate-bearing *Marginopora kudakajimensis* Gudmundson photosynthesized about 4–5× faster than diatom-bearing *Baculogypsina sphaerulata* (Parker and Jones) and *Calcarina gaudichaudii* d'Orbigny.

Equally predictably, *Am. lessonii* and *Am. gibbosa* exhibited photosynthetic efficiencies (α) that were 50–100% higher than those of the chlorophyte-bearing taxa. All of our medians are higher than the means reported by Nobes and other (2008) for a suite of diatom-bearing taxa that included *Am. lessonii* and *Am. radiata*. They reported α values of ~0.5 for high-light trials (up to 1200 μmol photon m^{-2} s^{-1}) and ~0.8 for intermediate- and low-light trials (375 and 60 μmol photon m^{-2} s^{-1}). However, their methods were unlike ours, so the implications of those differences are difficult to interpret.

TABLE 2. Physical parameters, including maximum diameter (Diam), chl *a* extracted, mass, and chl *a* normalized to mass, for each amphisteginid foraminifer used in the photosynthesis to irradiance observations shown in Figures 2 and 3 (AG = *Amphistegina gibbosa*; AL = *Am. lessonii*; AR = *Am. radiata*). Because not all data are normally distributed, data are summarized as median values.

Specimen ID	Diam (mm)	Chl a (μg)	Mass (mg)	μg Chl *a* /mg foram
AG01-1	0.90	0.07	0.27	0.26
AG01-2	0.90	0.04	0.23	0.19
AG01-3	0.90	0.04	0.29	0.13
AG01-4	1.00	0.05	0.28	0.19
AG01-5	1.15	0.07	0.44	0.16
AG02-1	0.95	0.06	0.36	0.18
AG02-2	0.90	0.04	0.27	0.14
AG02-3	1.30	0.12	0.47	0.25
AG02-4	1.00	0.06	0.33	0.17
AG02-5	1.20	0.08	0.52	0.15
AG03-1	0.90	0.03	0.23	0.13
AG03-2	1.05	0.09	0.36	0.24
AG03-3	1.20	0.08	0.60	0.13
AG03-4	1.10	0.10	0.49	0.20
AG03-5	1.40	0.14	0.83	0.17
AG04-1	1.00	0.05	0.20	0.27
AG04-2	1.15	0.07	0.38	0.18
AG04-3	1.10	0.10	0.34	0.29
AG04-4	1.30	0.11	0.60	0.18
AG04-5	1.35	0.12	0.65	0.19
AG05-1	1.00	0.05	0.27	0.19
AG05-2	1.10	0.06	0.42	0.14
AG05-3	1.10	0.10	0.42	0.23
AG05-4	1.10	0.10	0.51	0.19
AG05-5	1.10	0.09	0.40	0.23
AG06-1	0.85	0.03	0.24	0.11
AG06-2	0.80	0.03	0.24	0.12
AG06-3	1.00	0.07	0.34	0.22
AG06-4	1.05	0.05	0.29	0.17
AG06-5	1.15	0.11	0.38	0.28
AG07-1	0.75	0.03	0.10	0.26
AG07-2	0.85	0.04	0.12	0.33
AG07-3	0.95	0.04	0.16	0.25
AG07-4	1.10	0.10	0.30	0.32
AG07-5	1.25	0.09	0.53	0.17
AG08-1	0.75	0.03	0.10	0.27
AG08-2	0.80	0.03	0.10	0.34
AG08-3	1.00	0.05	0.24	0.19
AG08-4	1.10	0.04	0.37	0.12
AG08-5	1.10	0.05	0.40	0.13
AG09-1	0.85	0.03	0.14	0.23
AG09-2	0.90	0.05	0.20	0.25
AG09-3	1.20	0.11	0.54	0.20
AG09-4	1.20	0.14	0.45	0.30
AG09-5	1.35	0.12	0.58	0.20
AG10-1	0.80	0.04	0.15	0.24
AG10-2	0.80	0.04	0.16	0.25
AG10-3	0.85	0.05	0.21	0.22
AG10-4	0.95	0.08	0.22	0.35
AG10-5	1.25	0.07	0.40	0.19
AG median	1.03	0.06	0.34	0.20
AL01-3	1.40	0.21	0.97	0.21
AL02-1	1.00	0.10	0.31	0.33
AL02-2	1.25	0.14	0.65	0.22
AL02-3	1.15	0.12	0.56	0.21
AL03-1	1.00	0.08	0.26	0.31
AL03-2	1.40	0.14	0.71	0.19
AL03-3	1.45	0.19	0.83	0.23
AL04-1	1.00	0.07	0.22	0.33
AL04-2	1.15	0.08	0.30	0.25
AL04-3	1.25	0.11	0.51	0.22
AL05-1	1.15	0.12	0.29	0.40
AL05-2	1.20	0.12	0.38	0.32
AL05-3	1.25	0.14	0.39	0.35
AL median	1.20	0.12	0.39	0.25
AR01	1.90	0.29	1.73	0.17
AR02	1.75	0.26	1.53	0.17
AR03	1.80	0.24	1.51	0.16
AR04	1.80	0.32	1.41	0.23
AR05	1.70	0.20	0.98	0.20
AR06	1.75	0.32	1.54	0.21
AR07	1.90	0.35	1.56	0.22
AR08	1.85	0.28	1.53	0.18
AR09	2.15	0.59	2.12	0.28
AR10	1.80	0.40	1.75	0.23
AR median	1.80	0.31	1.54	0.21

In our study, all three amphisteginids approached light saturation (E_k) at relatively low intensities of PAR: 13 μmol photon m^{-2} s^{-1} for *Am. gibbosa* and *Am. radiata*, and 26 μmol photon m^{-2} s^{-1} for *Am. lessonii*. Full sunlight reaching the tropical sea surface is typically on the order of 1200–1500 μmol photon m^{-2} s^{-1}, so these foraminifers can be at maximum photosynthetic production in about 1–2% of sea-surface light intensity. These results are consistent with previously reported experimental responses to light. In growth experiments under different PAR intensities, Hallock and others (1986b) reported that *Am. gibbosa* exhibited comparable increases in mean diameter at 14 and 40 μmol photon m^{-2} s^{-1}, while *Am. lessonii* grew slightly faster at 40 μmol photon m^{-2} s^{-1}. *Amphistegina gibbosa* exhibited some bleaching after three months at 14 μmol photon m^{-2} s^{-1}, and both species exhibited partial bleaching at 40 μmol photon m^{-2} s^{-1}, indicating chronic photo-oxidative stress (e.g., Talge and Hallock, 2003). Our results are also consistent with those reported for *Amphistegina* spp. and other diatom-bearing species by Nobes and others (2008), who used pulse-amplitude modulated fluorometry and found E_k values of 27–42 μmol photon m^{-2} s^{-1} across treatments, as well as ~60% mortality in their full-sunlight treatments.

Differences in maximum photosynthetic rates have many potential causes including differences in P/E responses of diatom symbionts and differences in metabolic rates of foraminifers. *Amphistegina lessonii* had the lowest initial dark respiration and the highest metabolic scope of the three *Amphistegina* spp. These species may possess different suites of diatom endosymbionts, with the deeper-dwelling species utilizing symbionts that are adapted to lower light intensities (Lee and others, 1980). Generally, low-light-adapted phytoplankton species exhibit lower P_{max} values than those seen in species adapted to higher light intensities (Richardson and others, 1983), which is consistent with our observations; our lowest P_{max} values were from *Am. radiata*. In future studies of photosynthesis in diatom-bearing taxa, it may be useful to identify the dominant symbiont species.

Photosynthesis/irradiance responses can change with photoacclimation (Köhler-Rink and Kühl, 2001), so the relatively low light in which we held most specimens prior to experiments may have depressed the E_k values we recorded. However, Köhler-Rink and Kühl (2001) reported

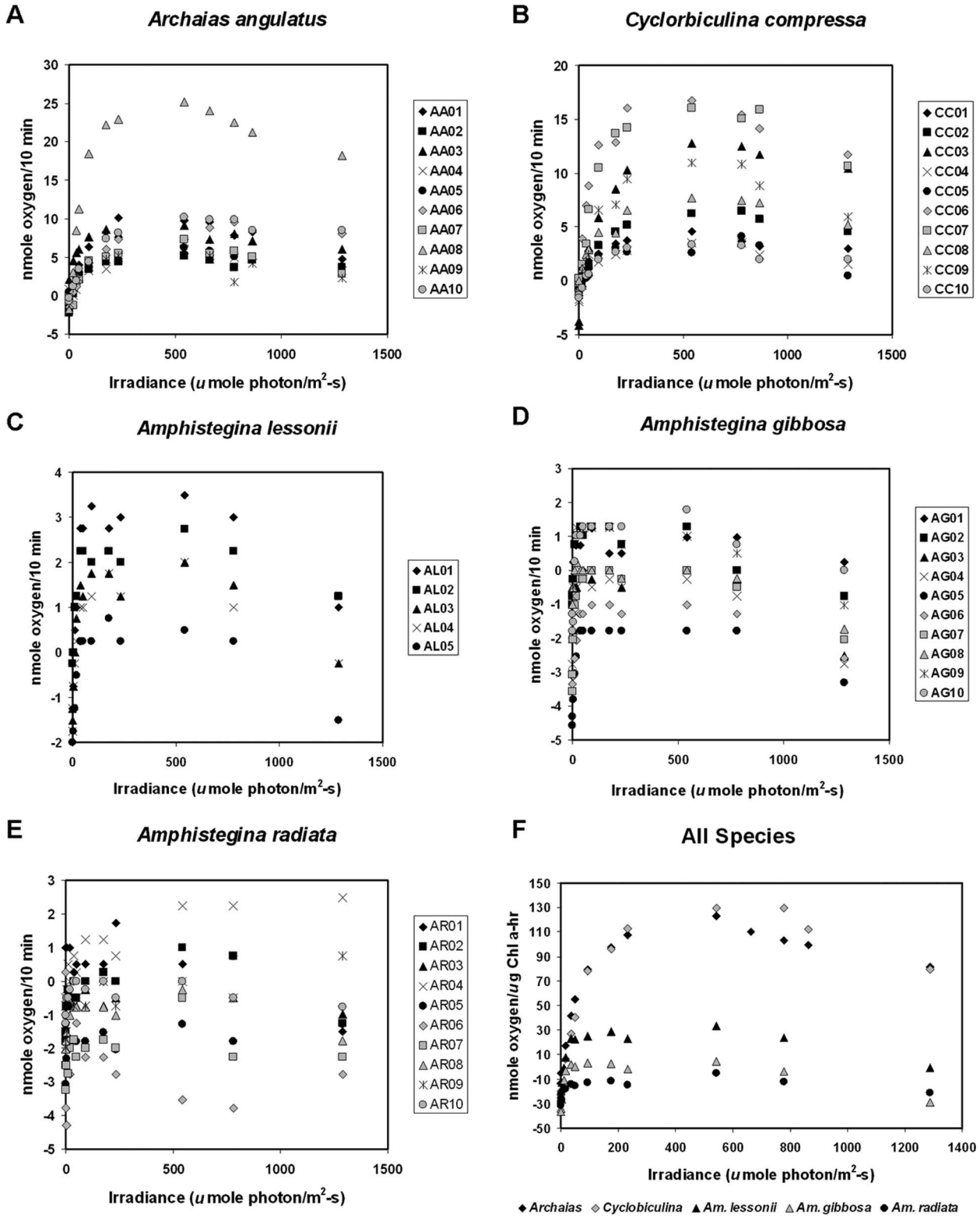

FIGURE 2. Oxygen production/consumption measured sequentially beginning with darkness (0 μmol photon m^{-2} s^{-1}) up to 1300 μmol photon m^{-2} s^{-1}, for the specimens described in Tables 1 and 2: A) ten *Archaias angulatus* specimens; B) ten *Cyclorbiculina compressa* specimens; C) five groups of three *Amphistegina lessonii* specimens; D) ten groups of five *Am. gibbosa* specimens; E) ten *Am. radiata* specimens. F) Median data from A–E, normalized to chl *a*, by species.

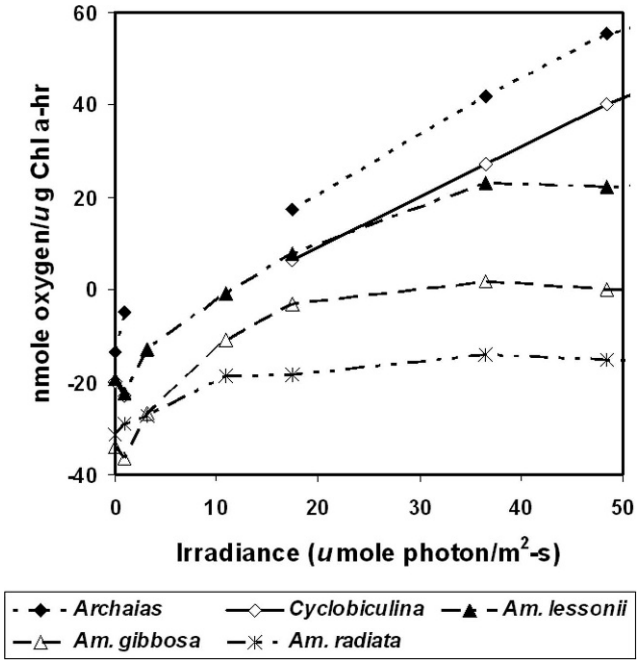

FIGURE 3. Expanded view of a portion of Fig. 3F, showing median oxygen production/ consumption data at 0–50 μmol photon m^{-2} s^{-1}. Data points for *Cyclorbiculina compressa* coincide with those of *Am. lessonii* at 0 and 0.96 μmol photon m^{-2} s^{-1}.

an E_k value for *Am. lobifera* of 95 μmol photon m^{-2} s^{-1}, which is similar to the values we found for *Ar. angulatus* and *C. compressa* (Table 3) and substantially higher than what we recorded for *Am. lessonii*. *Amphistegina lobifera* is a shallow-dwelling Indo-Pacific species with a depth distribution similar to that of the soritid species we examined (e.g., Hallock, 1999). Thus, finding that the E_k of *Am. lobifera* is much more similar to Caribbean shallow-dwelling species than to its Indo-Pacific competitor, *Am. lessonii*, is not surprising.

Moreover, E_k values for *Am. lessonii* are roughly double those for *Am. gibbosa* and *Am. radiata* (Table 3), despite similar pre-experiment treatment. This difference is also consistent with results noted above from the culture experiments comparing light tolerances and growth rates of *Am. lessonii* and *Am. gibbosa* (Hallock and others, 1986b). In subsequent laboratory experiments, growth rate in *Am. gibbosa* reached saturation at light intensities of 6–8 μmole photon m^{-2} s^{-1}, and the protists showed increased frequency of bleaching at higher light intensities (William and Hallock, 2004). Talge and Hallock (2003) also observed deterioration of symbionts and endoplasm in *Am. gibbosa* at experimental light levels as low as 13–15 μmol photon m^{-2} s^{-1}. Although *Am. lessonii* only exhibit a slightly shallower depth distribution than *Am. gibbosa*, they apparently can better utilize higher light intensities. If the two species were competing in the same geographic area, there might be a more significant difference in their depth ranges. Interestingly, calculated median values for E_k are very similar for *Am. gibbosa* and *Am. radiata*, indicating a potential for competition if they co-occurred.

For *Ar. angulatus*, we obtained median values of E_k of 86 μmole photon m^{-2} s^{-1} (Table 3). Duguay (1983) reported its maximum calcium and carbon uptake at 200–250 μmole photon m^{-2} s^{-1}. However, E_k values are inherently lower than the intensity at which maximum photosynthesis is observed (Richardson and others, 1983). Data averaged over *Ar. angulatus* specimens indicated light saturation at ~200 μmole photon m^{-2} s^{-1} (Fig. 2F), which is consistent with values reported by Duguay (1983). Moreover, based on the E_k values calculated, we agree with Duguay (1983) that this species is likely light saturated for most of the day in shallow water.

All three amphisteginid species exhibited similar fluctuations in the P/E curves (Fig. 2). Oxygen production peaked at three intensities, 37, 180, and 540 μmole photon m^{-2} s^{-1}, with a subsequent decline in production at the next higher light intensity. The first drop in oxygen production between light intensities of 37 and 48 μmole photon m^{-2} s^{-1} was not statistically significant in any of the three species. However, the decline in oxygen production recorded between 180 and 230 μmole photon m^{-2} s^{-1} was significant in *Am. lessonii* and *Am. gibbosa*, and the decline above 540 μmole photon m^{-2} s^{-1} was significant in all three species. Potential causes

TABLE 3. Median values of measured parameters and derived-photosynthetic parameters normalized to chl *a* for five foraminiferal species. *Chl *a* normalized to foraminiferal mass; **negative values indicate net oxygen consumption.

Species:	*Ar. angulatus*		*C. compressa*		*Am. lessonii*		*Am. gibbosa*		*Am. radiata*	
Number of trials:	10		10		5		10		10	
Parameter	Range	Median	Range	Median	Range	Median	Range	Median	Range	Median
Max diameter (mm)	2.15–4.25	2.87	3.50–5.60	3.90	1.00–1.50	1.2	0.75–1.40	1.03	1.70–2.15	1.80
Mass (mg)	1.06–3.85	1.74	2.10–7.70	3.00	0.22–1.03	0.51	0.10–0.83	0.34	0.98–2.12	1.54
Chl a (μg)/mg foram*	0.14–0.39	0.24	0.08–0.14	0.09	0.19–0.40	0.24	0.11–0.35	0.20	0.16–0.28	0.20
P_{max} (nmoles O_2 μg chl a^{-1} hr^{-1})	54.8–202	120	115–189	140	39.0–61.8	41.8	14.8–58.6	34.5	4.31–49.2	17.1
Alpha (α)	0.55–5.74	1.18	0.92–1.83	1.23	1.48–2.09	1.85	1.50–4.26	2.37	0.07–3.16	1.27
E_k (μmol photon m^{-2} s^{-1})	35.3–163	85.7	70.8–160	122	21.8–29.6	26.4	8.95–27.2	13.2	7.49–179	13.5
Initial respiration (nmoles O_2 μg chl a^{-1} hr^{-1})	0.00–43.1	9.21	0.00–54.8	15.2	4.12–32.4	18.3	5.48–69.6	27.4	9.31–70.9	30.3
Post Trial Respiration (nmoles O_2 μg chl a^{-1} hr^{-1})	50.2–129	88.6	107–316	157	44.8–104	73.9	43.8–104	65.0	0.00–132	73.4
Metabolic Factorial Scope	1.0–14	8.8	1.0–62	7.9	2.8–18	3.4	1.1–14	1.9	0.0–4.7	2.3
Daily net production** (nmoles O_2 $foram^{-1}$)	**	450	**	320	**	20	**	−2.9	**	−78

of these fluctuations include: 1) increased metabolic rate of foraminifers; 2) increased metabolic rate of symbionts as they become more light stressed; 3) decreased oxygen output due to photoinhibition; 4) ramped-up short-term photo-protective mechanisms; 5) presence of multiple species of endosymbionts with different light requirements; and 6) multiple layers of symbionts in inner chambers of the foraminiferal test. In the latter case, symbionts in outer chambers may initially shade those in inner chambers. As light levels increase and more light penetrates to the inner chambers, inner-chamber symbionts may increase their oxygen production accordingly, making up for the decline in photosynthesis by photoinhibited symbionts in outer chambers.

Amphisteginids are known to exhibit cryptic behavior and are phototaxic (Zmiri and others, 1974; Lee and others, 1980). The decrease in oxygen production at higher light levels could be associated with increased metabolic activity by the foraminifer as it migrates to a shaded location. To facilitate movement, foraminifers have mechanisms for rapid assembly and disassembly of microtubules that allow for rapid extension and retraction of rhizopodia (Welnhofer and Travis, 1996). Furthermore, during exposure to higher light intensities, *Am. gibbosa* has been observed to relocate electron-opaque cytoplasmic material into pore cups (Talge and Hallock, 2003). The metabolic cost of this internal mobilization of cytoplasm may reduce net O_2 production. Phototaxic behavior and the foraminifer's ability to mobilize cytoplasm in response to changing light intensities implies its ability to sense when light intensity exceeds what is optimal for survival. It is possible that the foraminifer is responding to changes in the cytoplasm resulting from release of oxygen and other photosynthetic products by its endosymbionts in response to changing photon flux densities. Although pursuing this hypothesis was beyond the the capabilities of the instrumentation used, they suggest possible avenues for future study.

Photoinhibition is a time-dependent process that occurs on the same time scale needed to produce P/E curves (Geider and Osborn, 1992). Two previous studies did not report photoinhibition in *Amphistegina lobifera* and *Amphisorus hemprichii*, both shallow-dwelling species, at light intensities as high as 3,300 μmole photon m^{-2} s^{-1} (Lee and others, 1980; Köhler-Rink and Kühl, 2001). However, Nobes and others (2008) clearly found both photoinhibition and high mortality in full sunlight in *Amphistegina* spp. from Australia.

Nobes and other (2008) sought to determine if diatom-bearing foraminifers are light limited. Both their results and ours indicate that, in clear shallow waters, the amphisteginids are more likely to experience photoinhibition than light limitation.

"Optimum" summer depth distributions for *Amphistegina* spp. and *Ar. angulatus* can be predicted (Fig. 4), based on calculated light intensities at which they should photosynthesize at near maximum rates, experiencing neither light limitation (based on E_k) nor photoinhibition (from Fig. 2F). Curves in Figure 4 represent extinction coefficients (per m) of photosynthetically active radiation for a range of water transparencies (see, e.g., Yentch and others, 2002), including those typical of a patch reef (~0.2), a Florida forereef (~0.1), and subtropical ocean waters (~0.05). Distribution for *C. compressa*, based on such calculations, would be essentially indistinguishable from that shown for *Ar. angulatus,* although Baker and others (2009) found that *C. compressa* often extends somewhat deeper than *Ar. angulatus.* Photosymbiotic foraminifers survive in shallower, higher-light environments by moving into local shade (negatively phototaxic behavior). They can also live in deeper, lower-light environments, but their growth rates are reduced.

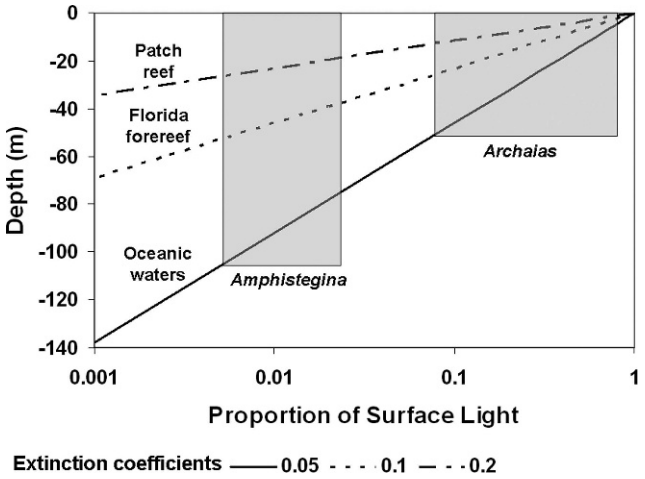

FIGURE 4. "Optimum" depth distributions for *Amphistegina* spp. and *Archaias angulatus*, assuming surface light intensities of ~1500 μmol photon m^{-2} s^{-1}, based on calculated light intensities at which they should experience neither light limitation ($<E_k$) nor photoinhibition. Curves represent extinction coefficients (per meter) of photosynthetically active radiation for a range of water transparencies. Distribution for *C. compressa* would be very similar to that shown for *Ar. angulatus.* All of these foraminifers can live in shallower, higher-light environments by local movement to shaded environments. They can also live in somewhat deeper, lower-light environments, although at reduced growth rates.

Evidence of increased oxygen consumption in the light can be seen by examining the oxygen production or consumption data from the initial dark trial and the lower-intensity light trials (Fig. 3). In *C. compressa*, *Am. lessonii*, and *Am. gibbosa*, oxygen consumption increased at the lowest light intensity (0.96 μmole photon m^{-2} sec^{-1}) compared with the initial dark trial. Although mean oxygen consumption was not significantly different between the dark and the lowest-light exposure, the consistency indicated metabolic change in either the symbiont or the foraminifer at the onset of photosynthesis. Similar increases in respiration were seen in some individuals of the other two species but were not reflected in median values.

Metabolic factorial scope commonly ranges 2–10 in marine invertebrates (Hernández-León and Ikeda, 2005), which is similar to the range we found in these foraminifers. Post-illumination rates were as much as an order of magnitude higher than pre-illumination metabolic rates in the soritids and only 2–4× higher in the amphisteginids (Table 3). This difference may be associated with a fundamental difference in the feeding strategies between the two groups, as active organisms tend to exhibit higher metabolic scopes than sedentary ones (Gordon, 1977). Previous studies have reported that *Ar. angulatus* grazes to

obtain most of its organic carbon (Lee and Bock, 1976; Duguay and Taylor, 1978) and both *Ar. angulatus* and *C. compressa* digest their endosymbionts when starved in the light (Hallock and Peebles, 1993). In contrast, the amphisteginids can survive in very low-nutrient environments apparently supplemented by photosynthate provided by their endosymbionts (Hallock, 1999 and references therein). *Amphistegina* spp. readily go dormant and can tolerate darkness for up to a year (Talge, 2002), while *Ar. angulatus* can only survive a few weeks without feeding (Hallock, unpublished observations).

A question that commonly emerges when working with organisms that host algal endosymbonts is: ''Is the holobiont a net primary producer or net consumer?'' We attempted to address that question by calculating the median daily net production of each species, the results of which are presented in Table 3. *Archaias angulatus* and *C. compressa* are clearly net primary producers, as individual foraminifers in the size range examined ranged 300–500 nmoles O_2/day (~13–21 nmoles O_2 hr^{-1}). *Amphistegina lessonii* can also be a net producer, though at a much more modest 20–27 nmoles O_2 per individual (~1 nmoles O_2 hr^{-1}).

Fujita and Fujimura (2008) tabulated data from previous studies of primary production and respiration by LBF, nearly all of which were reef-flat or otherwise shallow-dwelling species. Most data were reported in units of carbon rather than O_2, or were standardized to units more difficult to compare. Fujita and Fujimura (2008) estimated net production ranging 2.4–26 nmol C mg^{-1} dry wt hr^{-1}, with *Am. lessonii* (6.6–10 nmol C mg^{-1} dry wt hr^{-1}) and *Am. lobifera* (5.9–9.3 nmol C mg^{-1} dry wt hr^{-1}) appearing more productive than *Ar. angulatus* (4.5–5.8 nmol C mg^{-1} dry wt hr^{-1}) when production was standardized to dry weight. Adult *Am. lessonii* are considerably smaller than those of *Ar. angulatus,* which accounts for some but not all of the relative difference between the net production rates we calculated versus those obtained from ^{14}C tracer studies. Köhler-Rink and Kühl (2001), whose methodology was most comparable to ours, reported rates of 13 nmoles O_2 $foram^{-1}$ hr^{-1} for both *Am. lobifera* and *Amphisorus hemprichii*, both Indo-Pacific shallow-dwelling taxa and therefore living in light regimes similar to those of the soritids that we examined.

Amphistegina gibbosa and *Am. radiata* appear to be net consumers, at least under our experimental conditions. Given their light saturation at quite low irradiance (13 μmole photon m^{-2} s^{-1}) and therefore relatively low maximum photosynthesis rates, their role as net consumers is not surprising. Rough calculations based on comparing initial respiration rates over 24 hours with maximum rates of photosynthesis over 10 hours indicates that symbiosis may provide up to 40% of the energy budget of *Am. gibbosa* compared with up to 20% for *Am. radiata*. These estimates raise interesting questions about the energy budgets of LBF that occupy middle- and outer-shelf depths; these are the depths at which the shells of Paleogene LBF accumulated in biohermal thicknesses (e.g., Hallock and Pomar, 2008 and references therein). Under what environmental conditions is algal symbioses energetically advantageous when the primary production by the symbionts is so light limited? Certainly more studies should examine the physiology of these taxa, both the holobionts and their symbionts in isolated cultures.

CONCLUSIONS

- The chlorophyte-bearing foraminifers *Archaias angulatus* and *Cyclorbiculina compressa* produced 3–4× more oxygen normalized to chl *a* at maximum rates of photosynthesis (P_{max}) than did the diatom-bearing species *Amphistegina gibbosa*, *Am. lessonii*, and *Am. radiata.*
- The diatom-bearing *Am. gibbosa* and *Am. lessonii* exhibited higher photosynthetic efficiencies (α) than did chlorophyte-bearing species, consistent with photosynthetic efficiencies in related free-living algae.
- Calculated irradiance at P_{max} (E_k) was estimated at 13 μmole photon m^{-2} s^{-1} in *Am. gibbosa* and *Am. radiata*, confirming their very low light requirements. The E_k value calculated for *Am. lessonii,* though slightly higher, 26 μmole photon m^{-2} s^{-1}, was also consistent with a shade-adapted existence.
- *Archaias angulatus* and *C. compressa* reached P_{max} at E_k = 86 and 122 μmole photon m^{-2} s^{-1} respectively, consistent with their shallower maximum depth distributions.
- *Archaias angulatus* and all amphisteginids exhibited photoinhibition at light intensities above 660 μmole photon m^{-2} s^{-1}; photoinhibition in *C. compressa* was observed above 780 μmole photon m^{-2} s^{-1}.
- Metabolic factorial scope (the ratio of post-trial to pre-trial metabolic rate) was 2–4× higher in the chlorophyte-bearing soritid species than in the diatom-bearing amphisteginids.
- *Archaias angulatus, C. compressa*, and *Am. lessonii* appear to be net primary producers; *Am. gibbosa* and *Am. radiata* are net consumers receiving about 20–40% of their energy from their algal symbionts.

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