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Pathways of Carbon in the *Peneroplis planatus* (Foraminifer)-*Porphyridium purpureum* (Rhodophyte) Endosymbiosis

WALTER W. FABER JR.[1] and JOHN J. LEE[2]
[1] *Department of Biology, City College of CUNY, Convent Avenue at 138th St. New York, NY 10031, USA*
Tel. (212) 650-8545, Fax (212) 650-8585
[2] *Department of Invertebrates, American Museum of Natural History Central Park West at 79th St., New York, NY 10024, USA*

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Abstract

The carbon budgets and flows of the *Peneroplis planatus–Porphyridium purpureum* endosymbiotic association were examined by radionuclide tracer experiments both *in situ* and in the laboratory. During primary production, *P. planatus* incorporated inorganic C at a rate of 34 ± 0.02 μg C mg^{-1} foraminifer hr^{-1}. After a one hour pulse-labeling in the radionuclide, it did not significantly lose any of the incorporated carbon even after a "cold" chase of 48 hr, when kept in continuous light. Autoradiographs of the specimens showed dense concentric patterns of grains over the endosymbionts after the initial incubation in the radionuclide. After 48 hr, the grains in the autoradiographs showed movement of the carbon label from the symbionts to the cytoplasm of the host. The *in situ* experiments showed a steady increase in total uptake of C with time (0.25 to 0.22 μg C mg^{-1}foraminifer hr^{-1}). A small reduction in the total label retained in the organic fraction was seen in those specimens which were placed in "cold" chases. This decrease corresponded to respirometry measurements of 0.04 μg C mg^{-1}foraminifer hr^{-1}. Drawing on the values obtained in these experiments, and on data from feeding and growth experiments published previously by the authors, a carbon flow model has been developed which describes C fluxes under constant conditions. The average sized adult *P. planatus*

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is $\sim$ 600 μm long, weighs 47.6 μg,and possesses $\sim$ 4,900 endosymbionts. The foraminifer grows 0.36 μg d^{-1} and its endosymbiotic population increases by 68 endosymbionts d^{-1} (mitotic index 1.4%). Feeding (net assimilated food) provided only 0.07 μg C d^{-1} to the carbon budget of the growing endosymbiotic system. The symbiotic association tightly recycles carbon under illuminated conditions, whereas in the dark, it looses some carbon through respiration. The endosymbionts fix sufficient inorganic carbon to satisfy the energetic demands of the entire system. However, in times of reduced photosynthesis, feeding on external algae must supplement this amount, and provide other limiting nutrients.

Keywords: larger foraminifera, *Peneroplis planatus*, *Porphyridium purpureum*, endosymbiosis, carbon budget model, red algal symbiosis

1. Introduction

The maintenance of algal endosymbioses in foraminifera has been viewed as an adaptation for survival and growth, implying an intimate relationship between the symbionts and the foraminiferal hosts (see reviews: Lee, 1983; Lee and Hallock, 1987; Lee and Anderson, 1991). Most of the evidence for this concept lies in the significant effects of light on the morphology, behavior and metabolism of symbiont-bearing foraminifera. The structure of the foraminiferal shell, and the location of the symbionts within the host, optimize the illumination for the endosymbionts (Haynes, 1964; Hansen and Dalberg, 1979; Leutenegger and Hansen, 1979; Lee, 1983; Leutenegger, 1984; Lee and Hallock, 1987). Some foraminifera are phototactic (Zmiri et al., 1974; Lee et al., 1980), and others elevate their shells off substrates toward light (Leutenegger, 1984; Faber, 1991). The strongest evidence is that all larger foraminifera require light for growth and survival even when they are fed (Röttger and Berger, 1972; Lee and Bock, 1976; Röttger, 1976; Duguay and Taylor, 1978; Muller, 1978; Röttger et al., 1980; Hallock, 1981b; Duguay, 1983; Kuile and Erez, 1984; Hallock et al., 1986; Faber and Lee, 1991; Lee et al., 1991).

Peneroplis planatus possess *Porphyridium purpureum*, a red algal symbiont (Leutenegger, 1977; Lee, 1990). The endosymbionts are not enclosed in symbiont vacuoles (symbiosomes) but lie free in the cytoplasm of the foraminifer (Lee and Hallock, 1987; Lee, 1990; Hawkins and Lee, 1991). TEM micrographs show envelope fibrils emanating from the endosymbionts and dissipating in the host cytoplasm (Lee, 1990). Since the endosymbionts are photosynthetic algae, a major point of interest is the algal contribution to the host's carbon needs. Radionuclide tracer experiments have been employed to measure the primary

production of the algae and estimate the carbon budgets within foraminiferal associations (Lee and Zucker, 1969; Lee and Bock, 1976; Smith and Wiebe, 1977; Duguay and Taylor, 1978; Muller, 1978; Kremer et al., 1980; Lee et al., 1980; Duguay, 1983; Spero and Parker, 1985; Kuile and Erez, 1987; Kuile et al., 1987, 1989a,b; Gastrich and Bartha, 1988).

Chromatographic analyses of organic products within H $^{14}CO_3$-seawater incubated specimens of *P. pertusus* were reported by Kremer et al. (1980). They concluded that metabolites produced by the symbionts were translocated to the host, *Peneroplis pertusus*. They obtained expected amounts of ^{14}C-floridoside with appreciable amounts of ^{14}C-glycerol and ^{14}C-galactose. Since the latter two substances were not usually seen in non-symbiotic red algae, Kremer et al. (1980) suggested the foraminifer metabolized the *Porphyridium*-derived floridoside. Although laboratory experiments have shown the importance of light on the growth of *Peneroplis planatus*, these protists also require external algae as food (Faber and Lee, 1991). Possibly the foraminifer obtains reduced carbon by translocation from the endosymbionts and other necessary nutrients from ingested food (Jørgensen et al., 1985; Kuile et al., 1987; Lee et al., 1988).

This study utilized the *Peneroplis planatus-Porphyridium purpureum* endosymbiosis to examine the uptake of inorganic carbon *in situ* and in the laboratory. Its aim was to apply the experimental values to a possible model of carbon flow. Such a model, although only a first approximation, would be a starting point toward understanding the energetic interactions between the host and the endosymbionts.

2. Materials and Methods

Collection of specimens

Peneroplis planatus (Fichtel and Moll) Montfort were collected between January 24 and March 26, 1988, at the *Halophila* meadow near Wadi Taba, Eilat, Israel, at depths from 10–25 m. The collecting and processing of the foraminifera followed Kuile and Erez (1984), and is explained in Faber and Lee (1991). The laboratory experiments were carried out at the H. Steinitz Marine Biological Laboratory-Interuniversity Institute of Eilat, Eilat, Israel.

Specimens of *Elphidium crispum* were collected by dredge at Drake's Island, England, and *E. williamsonii* and *Haynesina germanica* (England) were collected from the Lihne River, England in August 1988. These were transported back to the laboratory in New York in insulated plastic food containers. Specimens of *E. incertum* and *Haynesina germanica* (MA) were collected at Naushon Island, Massachusetts, at the same time. Experiments on these elphidids were conducted at the laboratory at City College.

Laboratory experiments

The *P. planatus* were vigorously brushed clean of external algae, and placed in a covered petri dish with 200 ml sterile filtered seawater in a temperature controlled culture room (23±1°C) in front of a light bank (white Sylvania F40 T12/CW; 40–60 μE $m^{-2}sec^{-1}$). The dish containing 200 foraminifera was inoculated with 60 μCi NaH $^{14}CO_3$ between 10:00–11:00 A.M., and incubated for one hour. Some specimens were harvested at this time (t=0), while the remainder were rinsed twice with "cold" sterile filtered seawater, and returned to the culture room. These foraminifera were harvested at t=5, 10, 60, 120, 240, 480, 1440 and 2880 min. After each harvesting, the remaining foraminifera were placed in fresh unlabeled sterile filtered seawater. Aliquots of the initial tracer labeled medium, and each rinse and subsequent media were taken for radioactivity measurements. Dead controls (10 foraminifera placed for 1 hr in saturated buffered formaldehyde) were incubated for 1 hr in the light with NaH $^{14}CO_3$, and harvested at t=0. Dark controls consisted of two groups containing either 10–20 living foraminifera or 5 formaldehyde-killed foraminifera. These latter control groups were inoculated with NaH $^{14}CO_3$ and placed in a double black plastic sheet and harvested at t=0, 60 and 1440 min. The entire experiment was repeated in triplicate.

The foraminifera were harvested in two ways. Half were placed in Zenker's solution (Humason, 1962) for 1 hr, then rinsed six times with seawater. The foraminifera were rinsed in distilled water and transferred to 50% ethyl alcohol for 30 min, then into 70% ethyl alcohol. These specimens were transported from Eilat back to CCNY for autoradiography. The other half of the harvested specimens was placed in 10% formaldehyde for 10 min, then rinsed six times with seawater, once with distilled water, and placed in a drying oven (40°C) overnight. These foraminifera were measured for maximum length and width, and weighed on a Cahn 25 electrobalance, then placed individually into scintillation vials with 10 ml of Instagel XF (Packard Cat#6013394), and counted on a Packard Tricarb liquid scintillation counter. Quench was corrected by the standard channels ratio method.

The elphidids were placed in a covered petri dish with 100 ml of sterile filtered seawater in a culture room (23±1°C) in front of a light bank (F40CW/RS/EW-II, 30–50 μE $m^{-2}s^{-1}$). Each dish, containing 10 specimens, was inoculated with 20 μCi NaH $^{14}CO_3$ and incubated for 4 hr. Aliquots of the radionuclide tracer labeled media were taken at the start and completion of the incubations. Dead controls were incubated in radionuclide tracer labeled media for 4 hr in the light, and dark controls of living elphidids were incubated for 2 hr in radionuclide tracer labeled media. After incubation, the

foraminifera were rinsed twice in sterile filtered unlabeled seawater and once in distilled water, and placed in a 40°C oven to dry overnight. The specimens were measured and weighed on a Cahn ratio electrobalance model G, then pooled into scintillation vials with 15 ml of Instagel and counted on a Beckman LS 5801 liquid scintillation counter. Quench was corrected by the standard channels ratio method.

In situ *experiments*

Cleaned (sable brushed) foraminifera were placed in 300 ml sealable jars, 40 specimens per jar, with sterile filtered seawater and inoculated with 20 μCi NaH $^{14}CO_3$. The jars were placed upside down in a metal cage, which was taken to a depth of 20 m in front of the H. Steinitz Marine Biological Laboratory. The cage was tied to a small coral head. The jars were not shading each other. At 20 m depth, light has been measured in April to be approximately 18 μE $m^{-2}s^{-1}$ at 9 AM increasing to 60 μE $m^{-2}s^{-1}$ at noon (Lee et al., 1982). The natural photoperiod was close to 12.5 hr light. Dark controls were covered with aluminum foil and a black plastic sheet. Specimens were harvested at t=24, 48 and 72 hr. Other specimens were transferred to "cold" sterile filtered seawater after an incubation of 24 hr. These specimens were harvested after an additional 24 and 48 hr. The dark controls and dead controls were incubated in the radionuclide tracer labeled media for 48 hr before harvesting.

After harvesting, the specimens were brought into the laboratory and kept in the dark for a few minutes until they could be rinsed five times in seawater and once in distilled water. The specimens were then dried at room temperature, and returned to the laboratory in New York. The specimens were measured for maximum length and width, and weighed on a Cahn ratio electrobalance model G. The specimens were pooled into scintillation vials. Following the methods of Kuile and Erez (1987), the foraminifera were separated into an organic fraction and a shell fraction. Twenty milligrams of reagent grade $CaCO_3$ was added to the scintillation vial containing the foraminifera. This vial was placed in a larger jar with another scintillation vial which contained 2 ml of Oxymix (Reich solution; IN/US Service Corp., Fairfield, NJ). The jar was sealed. Through a rubber port, 2 ml of 8.5% H_3PO_4 was injected into the original vial. After the digestion of the shell by the acid, 2 hr were allowed for the $^{14}CO_2$ to be fully absorbed by the Oxymix (an initial test of the absorbent with a known sample of dissolved NaH $^{14}CO_3$ showed nearly 100% absorbance after 2 hr). Both scintillation vials then received 15 ml Instagel and were counted on a β liquid scintillation counter (Beckman LS 5801). Quench was corrected by the standard channels ratio method.

Autoradiography

The specimens brought back to the laboratory in New York in 70% ETOH were dehydrated in an ethanol series, cleared with xylene, and embedded in Tissuemat (Fisher Sci.#12-647C), a paraffin polyester resin. Thick sections were cut at 7–10 μm. The sections were stained utilizing the Raflako modification of the feulgen method (Thurston and Joftes, 1963). The slides were dipped into liquefied photographic emulsion (Kodak NTB2), and transferred to a Conrad-Joftes incubation chamber where they incubated for one month under refrigeration. The slides were developed using Kodak Dektol, followed by dehydration through an ethanol series, cleared with toluene, and mounted with Permount. The finished slides were observed and photographed with the aid of a Zeiss Photomicroscope II.

Respirometry

Photosynthetic rates were measured as μl oxygen evolved μg^{-1} foraminifer in an illuminated Gilson Differential Respirometer (model GRP-20) at 24$\pm$1°C. KOH saturated glass fiber filter paper was placed in the center well of the respirometry flasks to absorb CO_2. Light intensity was measured with a LICOR Quantum Photometer (model LI-185B). Light levels were adjusted by the addition of nile blue dye to the respirometer's water bath. Readings were taken every 15 min for 1 hr, with 5 foraminifera per flask. Dark measurements were achieved by placing double aluminum foil over the respirometry flasks with black plastic over the entire respirometer in a darkened room. The photosynthetic rates were measured twice and the dark respiration in triplicate. The values were converted to μg carbon mg^{-1} foraminifera, assuming a RQ equal to 1.0, and a PQ = 1.2 (Valiela, 1984). These values for the amount of carbon fixed by the foraminifera were plotted against time, and regressions were calculated (Cricket Software, Inc.). Values for the uptake of carbon during 1 hr were calculated from the regression.

Statistics

The data for both the laboratory and *in situ* experiments were divided by the measured weights of the foraminifera then converted to μg C uptake per mg foraminifera based on the formula in Kuile and Erez (1987):

$$\mu \text{ C mg}^{-1} \text{ foraminifer} = \frac{\text{DPM}}{\text{sample weight}} \times \frac{\text{inorganic carbon content}}{\text{specific activity}}$$

The total inorganic C content of the seawater was assumed to be 2.05 mM (Kuile and Erez, 1987). Analyses of variance between comparable groups were performed to test for significant differences (SAS Institute, Cary, NC).

3. Results

Experimental results

In the laboratory, *Peneroplis planatus* took up 0.34 ± 0.02 μg C mg^{-1}foraminifer hr^{-1} after 1 hr pulse-labelled incubation in the radionuclide. After rinsing with unlabeled medium and post "cold" chase incubation, in unlabeled seawater, the amount of ^{14}C in the foraminifera did not significantly change (Table 1: $F = 1.06$, $P > 0.05$, df = 8,237) during 48 hr. The living dark controls incorporated less than 4% of the amount of ^{14}C incorporated by experimental flasks in the light. The dead control in the dark had a similar low value whereas the dead control in the light were higher than dark controls (Table 1).

The autoradiographs of specimens from the initial incubation without a "cold" chase showed grains throughout the specimens, but they were more concentrated around the outer chambers of the *Peneroplis*, and less concentrated over the early chambers (Figs. 1,2). Some of the grains were in dense

Table 1. The amount of carbon fixed in 1 hr which is retained by the host/endosymbiont system after "cold" chase incubations up to 2 d

	Chase Time (min)	N	μg C mg^{-1}foraminifer hr^{-1}
Light	0	30	0.34 ± 0.02
	5	33	0.28 ± 0.02
	10	32	0.32 ± 0.03
	60	29	0.34 ± 0.02
	120	30	0.38 ± 0.03
	240	29	0.33 ± 0.02
	480	19	0.33 ± 0.03
	1440	18	0.34 ± 0.05
	2880	26	0.35 ± 0.03
Controls			
Light Dead	0	25	0.10 ± 0.02
Dark	0	26	0.01 ± 0.00
	60	5	0.01 ± 0.00
	1440	4	0.01 ± 0.00
Dark Dead	0	5	0.01 ± 0.00

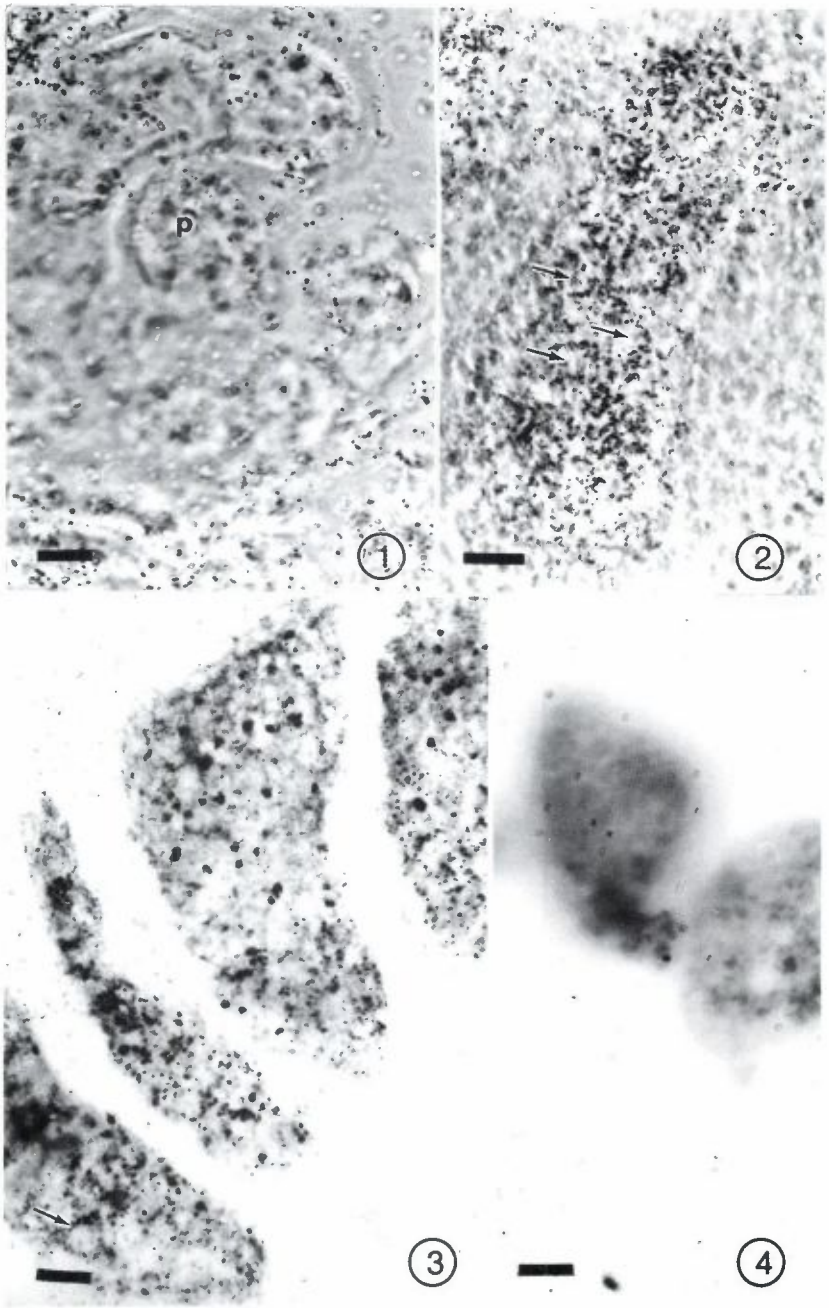
p
1
2
3
4

Figure 1–4. All figures are autoradiographs of *Peneroplis planatus*, which are focused on the grains above the specimens.

Figure 1. Juvenile whorl of a specimen fixed after 1 hr incubation in the radioactive tracer. Few grains are seen over the proloculus (p) and the other chambers of the first whorl. Scale bar = 10 μm.

Figure 2. Outer chamber of a specimen fixed after 1 hr incubation in the radioactive tracer. Circular pattern of grains are above the endosymbionts (arrows). Scale bar = 10 μm.

Figure 3. Outer chambers of specimen fixed after a 48 hr "cold" chase following the 1 hr incubation in the radioactive tracer. The grains are in dense "hot" spots and not directly over the endosymbionts (arrow). Scale bar = 10 μm.

Figure 4. Dark control specimen showing negligible grains. Scale bar = 10 μm.

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concentric patterns over the symbionts. After a 48 hr "cold" chase, the grains in the autoradiographs were less evenly distributed and with many "hot spots" that were not over the symbionts (Fig. 3). The dark and dead controls had very few grains randomly scattered over the foraminifera and throughout the background of the slide (Fig. 4).

There was close correspondence between the measurements of inorganic carbon taken up in the laboratory and measurements made in the natural environment (Tables 1,2). Often algae reach their photosynthetic maximum in the late morning. Although the diel pattern of photosynthesis of the endosymbiont was not determined, the specimens of the laboratory experiment were inoculated with the radioactive tracer in the morning between 10:00–11:00 AM. The intent was to incubate the specimens in the tracer during a time period when photosynthesis of the endosymbiont might approach the maximum possible incorporation. The *in situ* experiment showed a steady increase in total uptake of ^{14}C with time (Table 2). When the total uptake is partitioned between an organic fraction and a shell (inorganic) fraction, this steady increase was seen only in the organic fraction. A significant increase was observed in the shell fraction ($P \leq 0.01$, $F = 44.83$, $df = 1,4$) from the radioisotope incubations of 24 hr and 48 hr but no increase was seen when incubation was extended an additional 24 hr (not significant, $F = 4.29$, $P > 0.05$, $df = 1,3$). When uptake is converted to the amount of carbon per hour of incubation in ^{14}C, the rates (uptake/hr) measured in all the incubations and "cold" chases were identical (Table 2) (i.e. not statistically different; $F = 0.32$, $P > 0.05$, $df = 4,8$).

Those specimens incubated for 24 hr in the radioisotope and then in "cold" chases took up slightly less carbon; this decrease was seen mostly in the organic fraction (Table 2). However, it was not significantly different from the 24 hr

Table 2. The *in situ* experiment uptake of inorganic carbon by *Peneroplis planatus* incubated in ^{14}C. Values are in μg C mg^{-1} foraminifer.

Treatment	Shell fraction	Cytoplasm fraction	Total uptake	Uptake per hour incubation in ^{14}C
Ambient illumination				
24 hr incubation	1.28 ± 0.16	4.70 ± 0.38	5.98	0.49*
48 hr incubation	1.85 ± 0.08	9.68 ± 0.60	11.53	0.47*
72 hr incubation	1.41 ± 0.27	14.63 ± 2.43	16.04	0.47*
24 hr incubation with 24 hr "cold" chase	0.84 ± 0.32	3.66 ± 0.83	4.50	0.37*
24 hr incubation with 48 hr "cold" chase	0.63 ± 0.54	2.90 ± 1.99	3.53	0.28*
Dead controls 48 hr incubation	0.42 ± 0.05	0.05 ± 0.01	0.47	0.00*
Dark control 48 hr incubation	0.51 ± 0.04	0.16 ± 0.02	0.67	0.01**

* First the values for total uptake were corrected for the amount of ^{14}C incorporated in the dark control. The hourly uptake values were calculated for a 12 hr light phase and reflect uptake during illumination.

** This value was calculated for the entire 48 hr in the dark.

incubation in the label without a "cold" chase ($F = 0.88$, $P > 0.05$, $df = 2,5$). The amount of carbon retained by the host/endosymbiont system (in organic fraction), regardless of the length of incubation with the label (24–72 hr), declined significantly after a "cold" chase ($F = 13.14$ and $F = 16.34$ respectively; $P \leq 0.01$, $df = 4,8$; Table 2) whereas the incorporation into the shell fraction was not significantly different ($F = 3.14$, $P > 0.05$, $df = 4.8$). This suggests there is metabolic turnover and loss of carbon (excretion, respiration) in the host organic compartment but not in the shell.

The dark and dead controls for both the laboratory and *in situ* experiments incorporated only 4–6% of the total label of living *Peneroplis* incubated in a light/dark cycle or continuous light (Table 1,2). A significantly larger amount was found in the organic fraction of the dark control than the dead control ($P \leq 0.05$, $F = 17.72$, $df = 1,3$). In the *in situ* dead controls, nearly 90% of the label was in the shell fraction suggesting absorbance of the radioisotope by the foraminiferal shell or possibly adhesion to the formaldehyde killed organic fraction. Most of the label ($\sim$ 75%) in dark controls was in the shell fraction.

The amount of carbon fixed by the endosymbionts estimated by respirometric methods was 0.03 μg C mg^{-1}foraminifer hr^{-1} at 200–300 μE $m^{-2}s^{-1}$. Dark respiration of the foraminifera was 0.04 μg C mg^{-1}foraminifer hr^{-1}. Assuming a RQ equal to 1.0 (Table 3), this value for respiration is about 12% of the amount of inorganic carbon incorporated in the radioisotope experiments.

The smaller, chloroplast-husbanding elphidids in this study incorporated more carbon than the larger specimens on a per weight basis except in *Haynesina germanica* (Table 4). They incorporated on average 1.00 ± 0.22 μg C mg^{-1}foraminifer hr^{-1} in 24 hr.

Model of carbon budget and flow in Peneroplis planatus

This model attempts to quantify the relative contributions of photosynthesis and feeding to the overall carbon budget of *Peneroplis/Porphyridium*, host-endosymbiont, system. Because the system grows at different rates during the life of the individual host, we assume the data calculated from the experimental results would give a first approximation of the carbon budgets and flows of an average sized specimen of *Peneroplis planatus* under the stated conditions. All the data for this model are tabulated (Table 5) and are modeled in Fig. 5. The data were converted to a daily basis (24 hr period) for this mature adult specimen. The model is not dynamic or responsive to fluctuations in environmental or biological variables. Several assumptions were used to estimate the sizes of the carbon compartments and flows: (1) the percentage of carbon in each compartment is based on the size (measured weight); (2) the amount of photosynthetically fixed carbon translocated from the endosymbionts to the host is the amount of carbon incorporated into the foraminifer and not counted for by the increase in endosymbiont biomass; (3) the value of carbon incorporation integrated the total photosynthesis of the endosymbionts over the 12 hr light phase; (4) the shell carbonate and the cytoplasm biomass are

Table 3. Respirometry reading of *Peneroplis planatus* for primary production and dark respiration

Incident light (μE m^{-2} s^{-1})	Productivity (μg C mg^{-1} foraminifer hr^{-1})
500–600	0.03*
200–300	0.03*
30–40	0.03*
darknes	–0.04**

* PQ = 1.2
**RQ = 1.0

Table 4. The amount of carbon incorporated in elphidid foraminifera, which husband chloroplasts, in 24 hr

	N	Average weight foraminifer	μg C mg^{-1} foraminifera
Elphidium crispum			
Light	10	164.00±28.30	0.44
	10	153.00±13.00	0.56
	10	112.10±7.90	0.61
Light dead control	5	86.90±19.40	0
Dark control	5	157.90±50.00	0.01
Elphidium incertum			
Light	11	34.20±3.30	0.21
	10	33.50±4.20	0.36
	9	13.60±1.50	2.34
Light dark control	4	43.10±3.30	0
Dark control	4	35.00±7.00	0.01
Elphidium williamsonii			
Light	10	51.30±5.00	0.39
	10	12.20±1.30	1.20
Light dead control	4	16.80±3.60	0
Dark control	5	15.70±1.10	0
Haynesina germanica (England)			
Light	8	14.90±3.10	1.43
	8	10.10±1.10	0.64
	7	5.90±1.20	2.31
Light dead control	4	13.30±1.80	0
Dark control	2	5.30±0.80	0
Haynesina germanica (MA)			
Light	10	15.10±1.50	0.45
	6	9.60±1.20	2.03
Light dead control	4	8.80±2.30	0
Dark control	4	9.40±3.10	0

increasing at the same rate (i.e. there is a constant ratio of cytoplasm biomass to shell carbonate); (5) the amount of carbon assimilated from ingested algal food was based on measurements of protein content and we assumed a four to one ratio of carbon to nitrogen; (6) the recycling of respired carbon within the endosymbionts was not separated from other compartments because it was not experimentally measured or calculable from the data at hand.

Sizes of compartments

The average size of an adult *Peneroplis planatus* with a maximum length of 600 μm would weight ca. 47.6 μg (Table 5; Faber and Lee, 1991). This size organism would possess approximately 4867 symbionts. Assuming an average

Table 5. Measured, calculated and assumed values for a hypothetical *Peneroplis planatus* weighing 47.6 μg

Measurement		Value	Reference
Size of foraminfer	measured average	600 μm length	
Weight of foraminifer	calculated	47.6 μg	Faber and Lee (1991)
Size of endosymbiont	measured	4.91 μm diameter	
Weight of endosymbiont	assumed	8.0×10^{-5} μg	Jones (1962)
Total number of endosymbionts	calculated	4867	
Symbiont biomass	calculated	0.39 μg	
Weight of foraminiferal cytoplasm	measured	4.89 μg	
Weight of foraminiferal shell	calculated	42.32 μg	
C content of foraminiferal cytoplasm	calculated	2.45 μg C	
C content of endosymbionts	calculated	0.19 μg C	
C content of foraminiferal shell	calculated	5.08 μg C	
Respiration rate	measured	0.05 μg C d^{-1}	
Protein content of algal food	measured	6×10^{-5} μg $cell^{-1}$	
C:N ratio of algal food	assumed	4:1	
Ingestion rate	measured	9.45 cells μg^{-1} foram. d^{-1}	Faber and Lee (1991)
egestion rate	measured	3.12 cells μg^{-1} foram. d^{-1}	Faber and Lee (1991)
Net assimilation of algal food	calculated	0.07 μg C d^{-1}	
Growth rate of foraminifer	measured	0.36 μg d^{-1}	Faber and Lee (1991)
Endosymbiont daily increase	calculated	68 endosymbionts d^{-1}	
Endosymbiont growth rate	calculated	0.01 μg d^{-1}	
Host cytoplasm growth rate	calculated	0.04 μg d^{-1}	
Host shell growth rate	calculated	0.32 μg d^{-1}	
Increase in C in host cytoplasm	calculated	0.02 μg C d^{-1}	
Increase in C in endosymbionts	calculated	0.0027 μg d^{-1}	
Increase in C in host shell	calculated	0.04 μg C d^{-1}	

dry weight of each endosymbiont to be 8×10^{-5} μg (Jones, 1962), the total biomass of the endosymbionts would be 0.39 μg. The relationship between the organic material (host cytoplasm plus endosymbionts) to the total dry weight for *P. planatus* was $11.1 \pm 1.3\%$. The weight of the host cytoplasm would be 4.89 μg. Subtracting this from the average weight of the host minus the weight of the endosymbionts, the weight of the shell would equal 42.32 μg. Assuming that half the organic dry weight is carbon (Sverdrup et al., 1942; Parsons and Takahashi, 1973; Kuile and Erez, 1991), then the amount of carbon in the host cytoplasm would be 2.45 μg C and in the endosymbionts 0.19 μg C. Ignoring the organic components of the shell, the carbon in the $CaCO_3$ of the shell is 12% of its dry weight or 5.08 μg C (Table 5).

Carbon flow

The amount of carbon taken up by the foraminifera, while the system was undergoing photosynthesis, was measured as 0.28 μg C d^{-1}, as converted from the hourly uptake value for the *in situ* experiment specimens which were incubated for 24 hr (Table 2). The net respiration for the entire association was measured to be 0.05 μg C d^{-1}, as converted from the respirometry (Table 3).

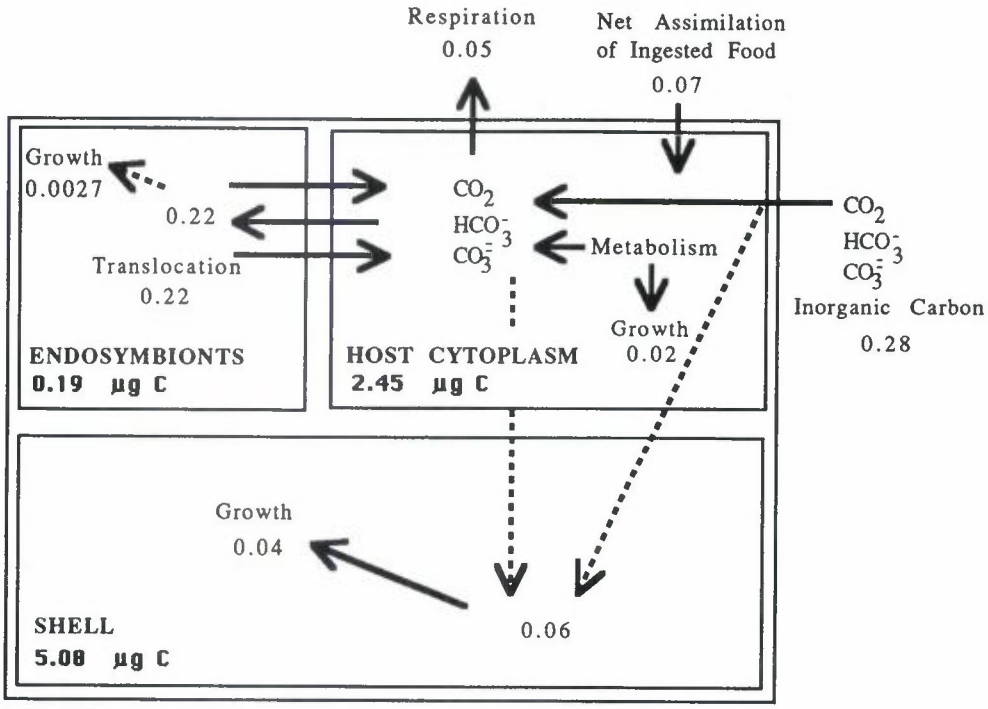

Figure 5. The carbon budget and flow of an average *Peneroplis planatus* weighing 47.6 μg. Values are in μg carbon per day, and in Table 5.

This value is the net loss of carbon to the environment from the host/endosymbiont system and does not reflect the probability that some carbon respired by the foraminifer or endosymbionts is recycled by the endosymbionts or the exchanges which take place during the "cold" chase. The latter are indicated by separate vectors (Fig. 5).

Kuile et al. (1989b) suggested that there were separate pathways for the uptake of inorganic C which was fixed in photosynthesis or was incorporated into the test of *Amphisorus hemprichii*. We have no evidence for the operation of separate pathways in *P. planatus* but we do have measurements for each of these functions: the C fixed by photosynthesis channeled into the organic fraction of the association was measured as 0.22 μg C d^{-1}; and the amount incorporated into the shell was measured as 0.06 μg C d^{-1}.

The present model treats the endosymbiotic algae as if they were in continuous culture. The translocated carbon is equal to the amount of carbon

photosynthetically fixed by the endosymbionts, minus the net amount of carbon needed for endosymbiont growth (i.e. all excess carbon fixed by the endosymbionts after fulfilling the metabolic needs of the endosymbionts must be translocated to the host). If the foraminifera was growing at 0.36 μg d^{-1} (Faber and Lee, 1991), the increase in the endosymbiont population would be 68 symbionts d^{-1}, based on the relationship between the foraminiferal size and endosymbiont number. This resulted in an increase of 1.40%. The resulting increase in weight of the endosymbionts would be 0.0054 μg d^{-1}. Since the endosymbionts fixed 0.28 μg C d^{-1}, and needed 0.0027 μg C d^{-1} for their growth, we estimate that 0.28 μg C d^{-1} would be translocated back to the host (Fig. 5).

The foraminifer also obtains reduced carbon from ingested food. Based on the feeding experiments reported earlier (Faber and Lee, 1991), 9.45 cells μg C mg^{-1}foraminifer hr^{-1} were ingested, and the carbon equivalent of 3.12 cells μg C mg^{-1}foraminifer hr^{-1} was egested. Using an average C:N ratio of 4:1 for coastal phytoplankton (Darley, 1977), and an average μg protein $cell^{-1}$ of 6×10^{-5}, the foraminifer would have gained 0.07 μg C d^{-1} (the difference between the ingested and egested) for utilization, assuming the amount retained in 24 hr is assimilated, or metabolized, and is not egested at a later time. We also assume the nitrogen content is equal to the amount of protein.

We know that these foraminifera do not add a new chamber every day but grow episodically. At 600 μm, a large chamber is added every 12 days (Faber and Lee, 1991). On the average, however, utilizing a growth rate of 0.36 μg d^{-1} (Faber and Lee, 1991) and assuming a steady cytoplasm to shell ratio, the foraminiferal cytoplasm would increase 0.04 μg d^{-1} and the shell 0.32 μg d^{-1} with carbon accounting for 0.02 μg and 0.04 μg, respectively (Table 5, Fig. 5).

4. Discussion

The pathways and cycles of carbon are important in understanding the overall relationship between endosymbionts and their foraminiferal host (Kuile and Erez, 1987). Measurements in nature of photosynthesis by the endosymbiont/larger foraminiferal systems suggest that they are major primary producers in shallow oligotrophic tropical and semitropical marine environments (Sournia, 1976). The uptake and fixation of inorganic carbon from seawater by these associations have been demonstrated by other researchers (Lee and Bock, 1976; Smith and Wiebe, 1977; Duguay and Taylor, 1978; Muller, 1978; Kremer et al., 1980; Röttger et al., 1980; Duguay, 1983; Kuile and Erez, 1987). The uptake of organic carbon by feeding supplements the photosynthetic reduction of

inorganic carbon and remains a second major input of reduced carbon in larger foraminifera (Lee and Bock, 1976; Lee et al., 1988; Faber and Lee, 1991).

The relationship between the organic material (host cytoplasm plus the endosymbionts) to the total dry weight for *Peneroplis planatus*, measured at over 11%, was larger than the amounts reported for other larger foraminifera (5.2–8.0% organic dry weight; Kuile and Erez, 1991). The amount of carbon in small benthic foraminifera, which lack endosymbionts, was estimated at 30% of their total dry weight (Lee and Muller, 1973). The total carbon in *P. planatus* was only 16% of its dry weight. It was approximately the same in *Amphistegina lobifera* and *Amphisorus hemprichii* (15% of the total dry weight; Kuile and Erez, 1991). The percentage of carbon was assumed to be 50% of the total organic dry weight, for both the host and the endosymbionts. The model would be enhanced with better measurements of the actual amount of carbon present.

There is no reasonable simple explanation for the higher absorption of the tracer-labelled inorganic carbon by the illuminated dead controls. The illuminated and dark incubated dead controls were killed the same way. Curiously, Muller (1978) reported similar results without comment. None-the-less, as expected the *Peneroplis planatus* endosymbiont system incorporated significant quantities of inorganic carbon (Tables 1,2). The measurements obtained by Röttger and coworkers (1980), for CO_2 fixation by *P. pertusus* and *P. arietinus*, are within the range reported here for *P. planatus*. Both laboratory and *in situ* experiments showed that the endosymbionts were the source of fixed inorganic carbon. Autoradiography further confirmed that the endosymbionts were photosynthezing by showing the label first over the algae, and later, that the label was translocated to the host by the presence of "hot spots" over the foraminiferal cytoplasm (Figs. 1–3). If the endosymbionts fixed more carbon than they needed for respiration, growth and reproduction, then the excess was translocated to the host.

Photosynthetic rates of the *Peneroplis planatus-Porphyridium purpureum* association measured by respirometry yielded values one order of magnitude less than the values obtained with ^{14}C incorporation. Inherently, photosynthetic rates are underestimated using respirometry, obtaining values somewhere between gross and net photosynthesis (Strickland and Parsons, 1968). With only 5 specimens in each respirometry flask, the sensitivity of the respirometer, which was 0.2 μl, was not adequate to achieve valid estimates of the amount of oxygen liberated by photosynthesis, since our measurements were lower than this amount (Table 3). Radioisotope incubation experiments also underestimate productivity due to dilution of the specific activity caused

by recycling of respired carbon, and equilibration with ambient non-radioactive carbon dioxide (Kuile and Erez, 1987; Stoecker and Michaels, 1991).

The discrepancy in the amount of carbon between the two methods also may indicate that the uptake of inorganic carbon was not solely for photosynthesis. It has been suggested that *Amphisorus hemprichii* took up different forms of inorganic carbon (CO_2, HCO_2^-, or CO_3^-) directly from seawater independently for photosynthesis compared to calcification (Kuile and Erez, 1987, 1991; Kuile et al., 1989a,b). As evidence for this bipartite process, Kuile and Erez (1987) showed that after the first 24 hr radioisotopic tracer incubation, the division of the radioactive tracer between shell and cytoplasmic fractions remained constant. Also they treated the foraminifera with the herbicide DCMU, which inhibits the photosystem II of the algal endosymbionts, and thus shuts off photosynthesis, and showed no effect on the uptake of carbon by the skeletal fraction (Kuile et al., 1989b). In the present experiments, there was no statistical difference in the amount of carbon taken up by the shell fraction of *P. planatus in situ*, between the 24–72 hr incubations and those with "cold" chases. If growth was continuous, one would expect a linear relationship between uptake and time. Many of the foraminifera in this experiment may not have grown in size (i.e. added chambers) during the interval of the experiment. Foraminiferal growth involves episodic cytoplasmic expansion and the addition of chambers to the shell. Maturing *P. planatus* grew a chamber, on average, every 12 days (Faber and Lee, 1991), but the *in situ* experiment lasted only 3 days. Although it might have been desirable to run the tracer experiment longer from the standpoint of this aspect, we did not do so because recycling, equilibration and loss from the system would have introduced additional unknown error and complexity to an already multivariate system. In the model (Table 5, Fig. 5), the uptake of 0.06 μg C d^{-1} into the skeletal fraction would result in a slight inorganic carbon deficiency if all carbon for $CaCO_3$ for foraminiferal shell growth is taken up directly from the sea. The model simplifies uptake for inorganic carbon as a steady quantity measured as 0.06 μg C d^{-1} for the entire 72 hr incubation in the label. We really do not think this linear rate vector is an adequate reflection of the carbon taken up for shell growth. Our data show the need for additional measurements to fine tune this vector for the model. Because *P. planatus* and *A. hemprichii* are both imperforate disc-shaped larger foraminifera with peripheral apertures, they may utilize similar mechanisms for carbon uptake. However, if the amount of inorganic carbon taken up directly and added to the shell fraction is inadequate for shell growth, some carbon derived from translocated photosynthates, ingested food, or recycled from respired carbon dioxide must be cycled into the carbonate of the test.

P. planatus retained most of the radionuclide label (^{14}C) which traced inorganic uptake for 48 hr after being placed in "cold" chases (Tables 1,2). A small decrease was seen in the total retention of carbon with time which could be accounted for by respiration (measured, as Table 2). However, statistically the reduction was not significant. This lack of significance could be attributed to the small number of samples and the large variance between specimens, the change in specific activity of the respired carbon lost to the seawater, or it could be explained by a tight internal carbon-recycling within the association, as suggested in other foraminiferal endosymbioses (Muller, 1978; Kuile and Erez, 1987).

The *Peneroplis/Porphyridium* endosymbiont system seems to involve tighter recycling than other foraminiferal systems because we did not see loss of radionuclide tracer from the system during the "cold" chase when the specimens were incubated in continuous light (Table 1). Since we know the host and endosymbionts were respiring during the 48 hr "cold" chase we should have seen a loss from the system if tight recycling was not occurring. The ^{14}C loss in the experiments incubated *in situ* which were exposed to an ambient light/dark cycle was good evidence the respired CO_2 was recycled within the host endosymbiont system during periods of active photosynthesis, preventing the loss of carbon to the environment. Whereas in the dark, since the endosymbionts are not photosynthesizing, some carbon leaves the association by respiration. A mixotroph (such as this endosymbiotic association) in appropriate levels of ample sunlight is limited only by the availability of nutrients, and may overcome this limitation by the ability to recycle nutrients with minimal loss (Hallock, 1981a). Maintaining the specimens in continuous light may also have overstimulated the endosymbionts to photosynthesize more than they would in a normal light/dark cycle, requiring larger amounts of inorganic carbon than was taken up by the host, hence demanding the use of all the respired carbon dioxide. The photosynthesis of the endosymbionts may have satisfied the respiratory reduced carbon needs of the association. The respirometry experiments demonstrated the possibility that the endosymbionts are providing enough reduced carbon for the basal metabolism of the association within certain light intensities (Table 3). If however the photosynthetic reduction of carbon was indeed less than the respiratory needs of the association, then an external source of reduced carbon would be required.

It is difficult to determine the respiration rate of an organism with algal endosymbionts (Caron et al., 1990). The endosymbionts utilize respired carbon dioxide during photosynthesis, whereas in the dark, both the host and the endosymbionts respire carbon dioxide. This diel change in carbon dioxide within the association makes extrapolation of dark respiration difficult (Caron

et al., 1990). Also, respiration may be higher in the light due to increased energetic requirements of anabolic processes of photosynthesis (Stoecker and Michaels, 1991). Our respiration rate was lower than published rates for other foraminifera (Lee and Muller, 1973; Kuile and Erez, 1991). However, based on the respirometry, the model respired 0.05 μg C d^{-1} (Table 5, Fig. 5) which corresponds well to the reduction of incorporated label during the "cold" chases of the *in situ* experiment (Table 2).

Besides energetic needs which were measured by respiration, the foraminifer needed reduced carbon metabolites for growth of its cytoplasm and calcification of its shell. Protozoans, in general, require more energy for growth than for any other process (Caron et al., 1990). Specimens of *P. planatus* fed in low light (30–50 μE $m^{-2}s^{-1}$) collected from the same location as the specimens in this study (Faber and Lee, 1991), grew at an average rate of 0.21 ± 0.3 μg day^{-1} and those specimens fed in high light (200–400 μE $m^{-2}s^{-1}$) grew 0.36 ± 0.07 μg day^{-1}. Foraminiferal growth rates are not steady and decrease with increasing size (Lee et al., 1969; Murray, 1983; Kuile and Erez, 1984; Faber and Lee, 1991). *P. planatus* fed in low light grew 0.39 μg day^{-1} in the first week following release of megalospheric juveniles. The amount of inorganic carbon fixed, as measured by respirometry, would not satisfy this carbon need as well as fulfilling the respiration requirements. *P. planatus* does not grow when starved (Faber and Lee, 1991). Feeding may supply the extra reduced carbon needed for foraminiferal growth and reproduction, as well as a source of nutrients other than carbon (e.g. fixed nitrogen, sulfur compounds, metabolites, essential amino acids, polyunsaturate fatty acids and vitamins) which the foraminifera may be unable to synthesize from simpler organic molecules (Jørgensen et al., 1985; Kuile et al., 1987; Lee et al., 1988, 1991; Faber and Lee, 1991). However, if one quality factors and assumes that the amount of carbon taken up by the cytoplasm is equal to net photosynthesis then the endosymbionts are indeed fixing enough carbon to fulfill gross (bulk, "black box") needs (Fig. 5).

Jones (1962) showed that after stationary phase in batch cultures that the extracellular secretions of *Porphyridium purpureum* were, at most, 15% of the total dry weight of the algae. If the endosymbionts behaved the same within the foraminifer, a postulated maximum of 0.06 μg C d^{-1} would be translocated to the host. In our model, the endosymbionts were translocating carbon at nearly 4.5 times the postulated maximum for this species in batch culture. Experiments with a different foraminiferal/algal endosymbiont system, have shown that the presence of a sterile host (*Amphistegina lobifera*) homogenate stimulated isolated axenic endosymbiotic diatoms to release more photosynthates in log phase batch cultures (Lee et al., 1984). The eight diatoms tested

released 0.3 to 39.4% of photosynthates to the medium when grown without host cell extracts, and increased to 25.5 to 76.6% in the presence of host cell extracts (Lee et al., 1984). It is possible that the *P. planatus* host stimulated the endosymbiotic *Porphyridium* to release more extracellular secretions than the algae measured by Jones (1962). Just because the endosymbionts from *Peneroplis* are morphologically identical to *Porphyridium purpureum* (Lee, 1990), does not rule out the possibility that there are physiological differences between the endosymbionts and their free-living cogeners.

The amount of inorganic carbon taken up by the foraminifer for photosynthesis is more than adequate to satisfy the carbon needs of the system (Fig. 5). Lee and Muller (1973) showed that salt marsh elphidids, which husband chloroplasts, only rely on photosynthesis of these chloroplasts for 10% of the carbon needed by the foraminifer. The elphidids in this study incorporated one-sixth the amount of carbon incorporated by *P. planatus* which agreed with a previous published value of 0.765 μg C mg^{-1} foraminifera in 24 hr for *Elphidium crispum* (Lee et al., 1988). The elphidids required a greater external carbon food source to satisfy their carbon needs.

The only experimental models of carbon flow in foraminifera, currently available for comparison, are those for *Amphistegina lobifera* and *Amphisorus hemprichii* (Kuile and Erez, 1987, 1991; Kuile et al., 1989b). *Amphistegina lobifera*, a perforate foraminifera, possesses an inorganic carbon pool not seen in the imperforate foraminifer *Amphisorus hemprichii*. Kuile et al. (1989a) proposed that *Amphisorus hemprichii* incorporated carbonate ions (CO_3^{-2}) directly for calcification. In *P. planatus*, 20% of the total incorporated inorganic carbon was deposited in the shell fraction (Fig. 5). This amount corresponds well with the data from corals which deposit 10–30% into their skeletons (Kinsey, 1985) but is less than half the amount *Amphisorus hemprichii* incorporated into its shell (Kuile and Erez, 1991). The amount incorporated by *P. planatus* falls short of the amount of carbon needed for shell growth. Hence, differential uptake of inorganic carbon for photosynthesis and calcification would create a deficit for the shell growth.

There is ample carbon in the host's cytoplasm from the translocation of endosymbiont-derived photosynthates to accommodate the extra carbon needed by the shell. All the inorganic carbon taken up by the foraminifer may be fixed by the endosymbionts, and then translocated as reduced carbon to the host where it is used for respiration, growth and calcification. In our model, the host obtains a surplus of 0.16 μg C d^{-1} from the incorporated inorganic carbon (Fig. 5). This surplus may supply the shell with the extra carbon needed during calcification, or it may be utilized during increased respiration. Other than respiration, growth and reproduction, foraminifera do not lose carbon in other

ways (e.g. mucous secretion in corals). Possibly the excess carbon is stored somehow within the host cytoplasm for times when demand is greater or in anticipation of reproduction. Organic storage in the form of starch has been found in the cytoplasm of *Sorites marginalis* (Müller-Merz and Lee, 1976).

The endosymbionts in both *A. hemprichii* and *P. planatus* translocate most of the photosynthetically-derived carbon to the host (Kuile and Erez, 1991; this study). Coral endosymbionts translocate large quantities of their photosynthates to the host tissues (Spencer-Davies, 1984; Muscatine et al., 1985; Cowen, 1988). In corals 40–67% of the translocated reduced reduced carbon is utilized to satisfy respiratory needs (Muscatine et al., 1983; Cowen, 1988). *A. hemprichii* lost 20–80% of its reduced carbon from translocated photosynthates and from assimilated carbon derived from external algal food, to respiration (Kuile and Erez, 1991), whereas *P. planatus* respired only 16% of the available carbon (Fig. 5). The overall uptake of inorganic carbon by *P. planatus* was double that of *A. hemprichii* (Kuile and Erez, 1991), which may account for the lower percentage utilized for respiration (Table 5, Fig. 5).

The amount of carbon ingested by *P. planatus* did not balance the demands of carbon needed for respiration and growth of the foraminifer. The algal food ingested by *P. planatus* may not be utilized for reduced carbon (Faber and Lee, 1991). Likewise, the *A. hemprichii* model (Kuile and Erez, 1991) does not require ingested food to satisfy the bulk carbon requirements of the foraminifera. However, if one substitutes the primary production value derived from respirometry (Table 3) for the radionuclide tracer derived value in this study's model, then the amount of carbon translocated from the endosymbionts to the host is only 0.03 μg C d^{-1}. This amount creates a deficit of 0.07 μg C d^{-1} due to the carbon demands of the host. The net assimilation of reduced carbon from ingested food was calculated at 0.07 μg C d^{-1} (Table 5, Fig. 5). The amount of reduced carbon from ingested food, coupled with the productivity value from the respirometry would be ample to satisfy the demands of respiration and growth of the foraminifer. In times of reduced photosynthesis, the ingested food must serve as a source of reduced carbon as well as required metabolites.

Peneroplis planatus probably has alternate rates of carbon flow to satisfy overall bulk carbon needs of the association depending on the conditions in which the foraminifer is found. We acknowledge that the present model is only a snapshot at a particular point in time in the life cycle of *P. planatus*. It is valid for a medium sized schizont under specific conditions of light, nutrients and food. Hallock's (1981a) conceptual modeling of algal symbiosis provides ideas which suggest we should consider the models developed in this, and previous papers (Kuile and Erez, 1991), as only first approximations. The next

steps in modeling should approach the host/endosymbiont system as dynamic and responsive. For example: assimilation efficiencies probably change as a function of prey density and prey quality; within particular ranges, the contribution of feeding to the carbon budget may be a function of light (quality or quantity) or dissolved inorganic nutrients. Any other factors which affect growth of the system will also affect the values for rates in future models. Experiments will have to be designed to test the ranges so that models will become more realistic instruments to help us understand the niches of these interesting protistan symbiotic systems.

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