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Feeding Rates of Two Species of Larger Foraminifera *Amphistegina lobifera* and *Amphisorus hemprichii*, from the Gulf of Eilat (Red Sea)

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Abstract

Radionuclide tracer methodology and microscopic observations were used to make an appraisal of feeding, factors which might affect feeding, egestion, and aspects of assimilated carbon flow in 2 species of larger Foraminifera, *Amphisorus hemprichii* and *Amphistegina lobifera*, from the Gulf of Eilat. Locally isolated species of food organisms were present in excess. Feeding and egestion were episodic in both species of Foraminifera. *A. hemprichii* fed more than *A. lobifera*. It consumed approximately 5% of its organic weight in 3 hr; *A. lobifera* consumed approximately 2.5%. Approximately 1.4% of the carbon entering *A. lobifera* through food was incorporated into the skeleton. In *A. hemprichii* the skeletal fraction received 3× more (4%). Feeding rates were approximately the same both in the light and in the dark. A significant fraction of the carbon ingested during feeding is egested within 24 hr. The amount egested after a meal is not uniform. Characteristics of the food are one factor affecting egestion. Neither species of foraminifera seemed to be able to digest the envelope of *Chlorella* sp. Its remains appeared as empty ghosts in the egesta.

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1. Introduction

The continuous tendency toward relative giantism in more than a score of families of foraminifera during their evolution since the mesozoic (Ross, 1974, 1982; Hottinger, 1982), suggests highly successful adaptation to their environment. One aspect of this adaption may be by symbiosis which many species of foraminifera have with algae. Although it is easy to suggest that algal-foraminferal systems seem well adapted for growth and reproduction in the well illuminated shallow tropical and semi-tropical seas in which they are found, most aspects of their adaptation still remain to be examined in detail (reviewed in Lee, 1980; 1983; Lee and McEnery, 1983). There is good reason to believe that algal-foraminiferal symbiotic systems have integrated and emergent characteristics that distinguish them from casual or temporary associations of animals and algae (reviewed in Cavalier-Smith and Lee, 1985; Lee et al., 1985a,b). What roles do each of the symbiotic partners play in the nutrition, maintenance, and growth or reproduction of the symbiotic system as a whole?

Various aspects of these symbioses have been studied. (e.g. Duguay and Taylor, 1978;Hallock, 1981; Lee et al., 1979; Röttger et al., 1980; ter Kuile and Erez, 1984). Light mediated processes seem to be a very important aspect of the biology of larger foraminiferal-algal symbiotic systems. It seems to be a factor influencing the depth distribution (reviewed by Reiss and Hottinger, 1984), shape (Hallock, 1979; Hallock and Hansen, 1979; Larsen and Drooger, 1977; ter Kuile and Erez, 1984), behavior (Lee et al., 1980; Zmiri et al., 1974) growth and calcification (Duguay and Taylor, 1978; Erez, 1978, 1983; Hallock, 1974; Röttger et al., 1980, Ter Kuile and Erez, 1984) of those species in which it has been examined. While it seems reasonable to suggest that light exerts effects on the system through photosynthesis of the endosymbiotic algae our knowledge of mechanisms by which they are accomplished is quite fragmentary. For example radionuclide tracers have been useful in showing that the endosymbiotic algae in axenic culture can release metabolites formed from photosynthetically assimilated inorganic carbon (Lee et al., 1974, 1984) which might be transferred to their hosts. It has also been shown that host homogenate can enhance metabolite release by symbiotic algae in axenic culture. Whether metabolite release is a major carbon pathway as some have suggested (e.g. Kremer et al., 1980) still lacks quantitative determination. Light may even have a direct effect on calcification as suggested by Erez (1983) and ter Kuile and Erez (1984). Many algal symbiont bearing foraminiferal species are known to feed on other species

of algae (Lee and Bock, 1976; Lee et al., 1980) and light influences the distribution of these food algae. While the growth of one species of larger foraminifera, *Heterostegina depressa*, seems to take place in the light in the absence of any obvious concentration of food (Röttger, 1976), feeding seems to be a major carbon source for other species (e.g. *Archaias angulatus*, *Sorites marginalis*, *Amphistegina lobifera* and *Amphisorus hemprichii*; Lee and Bock, 1976; Lee et al., 1980). It was clear to us during our previous studies of the latter 2 species (Lee et al., 1980) that our time-frame in the field did not make it practical to use abundant native algal species as food, except in agnotobiotic mixtures. New experiments with abundant native flora seemed to promise a much more realistic assessment of the role of feeding in the carbon pathway of these animals. While we recognize the difference between feeding (uptake) and nutrition (assimilation, maintenance, growth, fecundity; e.g. Lee, 1983) it seemed important to relate new data to the bulk carbon flow into and out of the animals. Although many workers have studied either the nutrition or feeding of foraminifera (reviewed in Lee, 1974, 1980), the feeding behavior of these animals does not easily lend itself to quantitative analysis.

Foraminifera seem to feed at irregular intervals. When they feed they may gather such large masses of food organisms that they may become imbeded within them (e.g. Arnold, 1954, 1974). There are several descriptions in the literature (Jepps, 1942; Angell, 1967, 1980; Myers, 1935) which show that after egesting food and diatom frustule fragments, new chambers are built. Afterwards they move to a new location leaving a fecal pellet of algal debris behind and begin to spread out their feeding nets again.

The purpose of the present study is to describe the feeding on algae in two symbiont-bearing species of foraminifera. Another goal was to estimate, if possible, what fraction of the carbon ingested is retained ($\approx$ assimilated) and how much is egested and if any food carbon is incorporated into the skeleton. We also hoped we would be able to discern whether feeding by these symbiont-bearing forms is an independent or light-linked (possibly through photosynthesis by the algal symbionts) process.

2. Materials and Methods

Isolation of food organisms

The processes of isolation, identification, and selection of the algae used in these experiments began during the period of our previous research on these animals (Lee et al., 1980) and was on-going for several years prior to the start of the experiments reported here. With the modifications noted

below the general procedures for the isolation and separation of epiphytic algae on agar-solidified media outlined by Lee and co-workers (1975) were followed. The salinity of the isolation media was raised to 4.0%. Since they had been shown to grow on a wide range of epiphytes, the following media were used: 1 (erdschreiber), 2 (Sea Water), 4 (d), 6 (A1), 14 (C2), 16 (C3), 17 (C4). Because many species of epiphytic algae are easily dislodged from their substrates, special care was taken not to cause too many currents or agitation once potential samples were identified during the SCUBA dives used to obtain materials for this part of the study. Forceps and scissors were used to hold and cut small samples of *Halophila* leaves with dense populations of large foraminifera. Each sample (~10–20 leaves) was placed in a separate resealable sterile plastic bag (whirl pack) along with small amounts of water from the collection site. As soon as practical, but generally within 1–2 hr, the samples were brought back to the laboratory and placed in sterile petri dishes with a 1% (V/V) antibiotic-antimycotic mixture (GIBCO. Cat. #600–5240, Chagrin Falls, Ohio) which in our experience (Lee et al., 1970) retards bacterial growth which might at a later stage hinder the axenic isolation of algal colonies. Individual *Halophila* leaves were held aseptically with sterile forceps and their epiphytes were dislodged with the aid of alcohol sterilized sable brushes. [Several leaves from each sample were not brushed but fixed in 4% glutaraldehyde in sea water (see below)]. The contents of each petri dish were aseptically transferred to sterile centrifuge tubes. After concentration by centrifugation, aliquotes of the epiphytes, were pipetted onto the surface of the agar-solidified media mentioned above. They were spread evenly on the surface of the media with the aid of bent sterilized glass rods. Inoculated petri-plates were placed in plastic bags to retard evaporation over the rather long incubation period (4–6 weeks). Incubation was at 20–25°C in chambers illuminated by fluorescent lights timed to provide a day/night cycle (16 hr/8 hr).

After location with the aid of a dissecting microscope (Lee et al., 1975) individual colonies were excised from the plates using a sterile small spatula and transferred to either medium "S" or erdschreiber (Lee et al., 1970).

Natural algal community samples and selection of food organisms

An aliquote of each epiphytic sample was placed in a screw-capped pyrex centrifuge tube and was oxidized with 30% H_2O_2 until the sample turned transparent. When required, the oxidation was facilitated by gently warming the samples in a water bath. Part of the diatom frustules prepared this way

were filtered through a Nucleopore filter. The remainder were dehydrated in a graded series of ethyl alcohol/water mixtures, passed through absolute alcohol, and then through toluene, and mounted on light microscope slides in Hyrax, a synthetic resin with a high index of refraction; (Custom Research and Development, Auburn, California). The Nucleopore filters were mounted on stubs, sputter coated with either Au or an Au-Pd mixture, and studied in a Cambridge Stereo Scan SEM (model 250). In the same way samples of the cultures begun from isolated colonies were also prepared and studied. Also prepared and studied were individual unbrushed *Halophila* leaves from each sample. They were fixed in 4% glutaraldehyde in sea water for 30 min. They were post-fixed in 1% OsO_4 for 1 hr and dehydrated in a graded series of ethyl alcohols to 70% alcohol. Just before examination in the SEM they were critical-point dried, sputter-coated, and mounted on stubs.

The organisms we isolated and cultured were compared to those in the natural epiphytic communities. Those which were most abundant in the natural communities, in which the foraminifera were also abundant, were selected for the feeding study.

Labelling of the algae

Axenic cultures of seven species of algae selected for tracer feeding experiments were inoculated into erdschreiber medium, 7–10 days before each experiment. After incubation for 4–5 days, 10 μCi of sterile $H^{14}CO_3^{-1}$ or $^{32}PO_4^{-3}$ was aseptically added to each 10 ml culture as a radionuclide tracer. The cultures were incubated for an additional 3–4 days, after which they were harvested by gentle centrifugation. In order to wash away unincorporated label, the algae were resuspended in filtered sea water and centrifuged again. This procedure was repeated overgain until the radioactivity in the wash water was much less than 1% of the initial label ($\sim$100 cpm). The concentration of the algae was measured by counting an aliquote in an A.O. Bright-line hemocytometer. The radioactivity of another aliquote was measured in a Packard Tricarb β liquid scintillation spectrometer (model 3255).

Experimental animals

Foraminifera used in our experiments were freshly collected while still attached to *Halophila* leaves roughly 2 km south of the H. Steinitz Marine Laboratory. The foraminifera were washed off the leaves into jars of seawater and left over-night. Only individuals that climbed on the wall by the next day and showed extensive pseudopodal nets were used in the experiments.

Prior to each experiment, the foraminifera were measured under dissecting binocular microscope fitted with an eyepiece micrometer. In addition, for all the ^{14}C label experiments, the average dry weight/individual was determined by weighing and counting a sample of 20 individuals. Relationship between size and weight were calculated based on a relationship determined earlier by ter Kuile and Erez (1984).

In several experiments the treatments of the animals prior to feeding were variable. In other experiments the size of the animals was a variable. The details of each experiment are outlined below.

Experimental procedures and variables studied

Nine-hole spot plates, 125 ml flasks, or deep (250 ml) petri dishes were used as experimental vessels. Inocula for each of these vessels were 10, 50, or 100 animals respectively. Animals were thoroughly brushed to eliminate adhered food particles and debris before being introduced into the experimental vessels. They were given time to develop pseudopodal net-works prior to the introduction of the potential food. Care was taken to make sure that the labeled food introduced into the experimental vessels was thoroughly mixed and equally available to all animals. This was accomplished by drawing up some of the algae and medium in a pipette and then releasing the material in a gentle swirling motion. The procedure was repeated until the algae were thoroughly dispersed. Incubations were carried out on a shelf near a north facing window where indirect light levels are roughly equivalent to those at a depth of 15 M (depending upon season) in the Gulf of Eilat.

At the conclusion of each experiment the animals were rinsed 3 times and brushed to remove all adhering algae. In several experiments the brushed animals were transferred to fresh experimental vessels with sea water and fed cold (unlabeled) food. ("Cold Chase"). Samples were taken to measure to release of radioactivity from the animals into the medium. The radioactivity was representative of the animal's carbon losses through egestion, respiration, and excretion.

Comparative feeding experiments

Only large (>3 mm) individuals of *Amphisorus hemprichii* were available for study at the time we did this experiment. A greater size range (0.9–1.5 mm) of *Amphistegina lobifera* was available and was used. After the examination of many natural populations on *Halophila* leaves (see results) we selected 7 of the most common algal species which we had been able to

isolate in axenic culture. Since many of the algae in the community epiphytic on *Halophila* are not readily identifiable with published species, they will be described in a separate later paper (work in progress). Some idea of the algal communities found on *Halophila* leaves can be seen in Figs. 18, 19 of Lee (1983). Two of the species, *Amphora* sp. (strain 29) (*Halophila* group) and *Navicula* sp. (strain RS9) are seen in the above figures. An *Amphora* tentatively identified as *bigibba* (RS) was also used. A *Cocconeis* sp. close to *C. placentula* var. *euglypta*, was also selected. A very small *Entomoneis* sp. was tentatively identified as *E. paludosa* var. *densistriata*. Two physiologically different clones of *Chlorella* were also tested as food for the foraminifera. In this experiment the food algae were labeled with ^{32}P and the incubations were in nine hole spot plates. The analysis followed the procedure described by Lee et al. (1980).

Duration and consistency of feeding

A comparison was made between uptake of ^{14}C labelled food by populations of either *A. hemprichii* or *A. lobifera* incubated with the food for either 3, 6, or 24 hr. In various experiments either *Cocconeis* or *Chlorella* were separately fed to the foraminifera. Food was always presented greatly in excess ($\sim 1 \times 10^6$ cells cm^{-2} of benthic surface) of the animals ability to gather it. DCMU [3-(3-4 dichlorophenyl)-1, 1-dimethyl urea] at 1×10^{-6} M concentration, an inhibitor of photosynthesis, and darkness were additional variables in the experiment. As a control for the technique, a batch of foraminifera were killed in 5% glutaraldehyde (V/V in sea water), washed through several changes of sea water, and then transferred to experimental vessels, where they were treated as all other experimental organisms.

The effects of captivity on feeding rates

In these experiments the protozoa were fed ^{14}C labelled *Chlorella*. The foraminifera were not all from the same field collection but they were all from the same site (marked location) in the field. Some animals were used within 2 hr of their harvest from the sea. Others were collected on either the previous day (24 hr) or 3 days earlier (72 hr). Those harvested earlier were brushed and placed into sterile filtered (0.45 μm) sea water in deep petri dishes. Some of the organisms in the 72 hr population were incubated in the presence of a dense population of *Chlorella* (2×10^3 cells/cm^2 of bottom surface).

After feeding on labelled food for 6 hr all of the foraminifera were harvested and brushed and washed free of adhering labelled algae. An aliquote of 50

animals was prepared for isotopic measurement. The rest of the foraminifera were placed in deep petri dishes with dense populations (1.5×10^5 cells/cm^2 bottom surface) of unlabeled *Chlorella*. These were harvested at 24, 48 or 72 hr respectively.

Duration of feeding was a variable in one experiment. Comparison was made between animals allowed to feed 3, 6 and 24 hr. When duration was not a variable, feeding was allowed for 6 hr.

Preparation of samples for β-liquid scintillation counting

After harvest, animals were dried in a warm oven (~40°C). The dried sample was crushed and thoroughly homogenized. A 5 mg sample was weighed precisely on a Cahn electrobalance, transferred to a scintillation vial and about 20 mg reagent grade $CaCO_3$ was added. This scintillation vial was put in a jar standing in an ice bath together with a magnetically stirred vial containing 2 ml of Oxisorb. The jar was sealed and 2 ml of 8.5% H_3PO_4 was added through a rubber port by means of a syringe into the $CaCO_3$ vial (Erez, 1978). After 45 min the icebath was removed and 15 min later the jar was opened, 10 ml Insta-gel (Packard) was added to the Oxisorb vial. The vial originally containing the sample was washed over a preweighed Nuclepore filter. The filter was dried in a petri dish in a vacuum-dessicator and the wash water containing the acid was evaporated to roughly 2 ml, then 10 ml Insta-gel was added. The dry filter was reweighed, transferred to the vial originally containing the sample, 10 ml was added. The samples so obtained are called respectively "organic matter" "acid" and "skeleton" (Erez, 1978).

Calculations and standardizations: As a biomass measurement we used the size or weight of the foraminifera.

When diameter was measured, the weight per individual was calculated and alternatively when weight/individual was measured the diameter was calculated (ter Kuile and Erez, 1984). In addition, the surface area, feeding area, total volume and "organic" volume were calculated for *A. lobifera* and *A. hemprichii* as follows:

1. Surface area of *A. lobifera* assuming that it is made of 2 short cones attached at their base. The height (h) of this species is $\sim 1/2D$ (where D is the diameter) therefore the surface area (SA) is

$$2\pi(\frac{D}{2}) \times \sqrt{(1/4D)^2 + (1/2D)^2} = 1.7562 \times D^2$$

The surface area of *A. hemprichii* is:
$2\pi(1/2D)^2 + 2\pi \times 1/2D \times 0.328 = 1.57 \times D^2 + 1.030 \times D$ (this assumes that the height is constant and is 0.328 mm (ter Kuile and Erez, 1984). The feeding area is assumed to be 15% of the total surface area. It represents the surface area of the aperture and its papillae which send the main web of pseudopods for feeding. Feeding area is the area of its vertical perimeter where the apertures are located $2\pi \times (1/2D) \times 0.03 \times D$.

2. Volume of *Amphistegina* is $\pi/24 \times D^3 = 0.13D^3$. Organic volume is 16% of the total volume based on density of this species. It represents the volume of the voids which are occupied by cytoplasm (ter Kuile and Erez, 1984). The volume is $0.258 \times D^2$ and its "organic" volume is 76% of its total volume based on its density (ter Kuile and Erez, 1984). Feeding rates were determined analytically on a basis of number of algae per weight of dried powdered sample. Various standardizations can then be made according to the weight/individual, surface area, feeding area, total volume and organic volume. Such standardizations are needed in order to compare feeding rates for populations of the same species having different shell size or differences in feeding rates between different foraminifera species.

Autoradiographic studies

Some organisms from each experimental flask in the time course study were fixed for microtechnique at the same times that others were harvested for counting by β liquid scintillation spectrometry. Treatment of the specimens (e.g. "Cold" food chase, washing, brushing) for each experiment was identical up to the step where some specimens were fixed for microtechnique.

The organisms were fixed in Zenkers for 1 hr and then dehydrated in a graded series of ethyl alcohol to 70% alcohol. They were placed in liquid scintillation vials and transported.

The forams were decalcified with 10% Poly-No Cal (catalog #16865, Polysciences, Inc.). Dehydration proceeded through a graded series of ethanol, 30–95%, 30 min each; 2 changes of absolute alcohol, 30 min each; 2 changes of toluence, 30 min each, and 2 changes of xylene, 30 min each. The organisms were transferred to a stainless steel mold, submerged in melted Paraplast (a mixture of paraffin and plastic polymers) at a temperature of 58.5°C and infiltrated at 15 pounds/in^2 in a vacuum infiltrator. Serial sections were cut at 7 μm on a microtome. The ribbons were mounted on slides coated with egg albumin and some drops of water, and were spread on a slide warmer.

The staining procedure used was Feulgen (Hamason, 1962; Thurston and Joftes, 1963). For this the slides were deparaffinized and hydrated to water. Hydrolysis in 1N HCl was 5 min at 60°C. The slides were rinsed at room temperature in 1N HCl and then with distilled water. Schiff's reagent was used to stain, 2 hr in the dark. The slides were transferred to bleaching solution, 3 changes, 1.5–2 min in each; washed in running water 10–15 min and rinsed in distilled water. Fast green was used to counterstain (10 sec). This was followed by dehydration through a graded series of ethanol to 70% alcohol.

Autoradiography followed the staining procedures. Fresh liquid emulsion was liquified in a water bath at 45°C. The processing of the emulsion was done in total darkness. The slides were dipped in the emulsion (Kodak NTB 2) and transferred to trays in a Conrad-Joftes incubation chamber. The slides were incubated for 3 weeks at 4°C. All preparations were developed in Kodak Dektol (1:1: 2 min), rinsed in distilled water (10 sec), fixed (5 min) and washed with distilled water (5 min). This was followed by dehydration through a graded series of ethanol 50–95%, 5 min each; 2 changes of absolute alcohol, 10 min each; absolute/toluene (1:1), 10 min; 2 changes of toluence, 10 min each.

Pictures of living foraminifera were taken with the aid of phase contrast mounted in a Zeiss photomicroscope II. All photographs were taken on the same microscope on Kodak Technical Pan Film, 2415 which was used at an exposure index of 100/21°(ASA/DIN). The pictures were printed on Kodak polyprint RC paper. Since there were considerable differences in the contrast of negatives taken in phase contrast and of different optical planes of the histological and radiographic sections, a very wide range of polycontrast filters (2–5) was used in printing the pictures. Compromise in focusing was necessary in some preparations so that the overlying radioautographic grains would appear in the same pictures as the histological sections.

3. Results

Microscopic observations

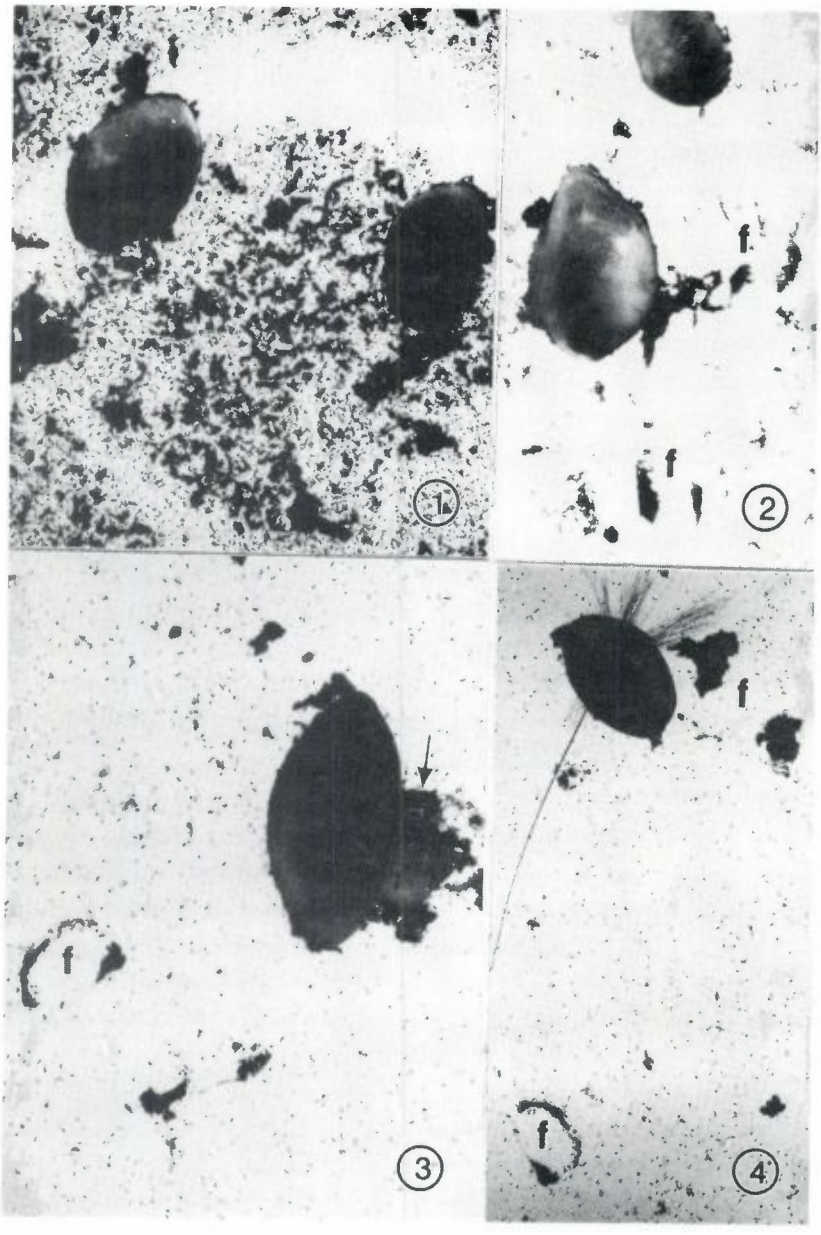
Visual observations under the binocular microscope showed that after the development of pseudopods most specimens of biconcave *A. lobifera* and *A. hemprichii* were oriented so that their aperture was near the surface of the dish and their dorsal ventral axis was almost parallel to the dish; their slightly concave ventral surface (radial axis) was perpendicular to the dish

(Figs. 1–4). The pseudopodial network emerged from the aperture in an 45–70 degree arc (Fig. 4). Each organism can literally cut a swath as it feeds in a dense algal lawn (Fig. 1). The disk shaped *Amphisorus*, on the other hand, generally lay flat with their radial axis parallel to the dishes. The pseudopodial network extended radially from the apertures which are located on the lateral circumferential edges of the shell (see Fig. 3, Lee (1983)). Rarely one encountered an *Amphisorus* standing up on one edge with the radial axis perpendicular to the dish.

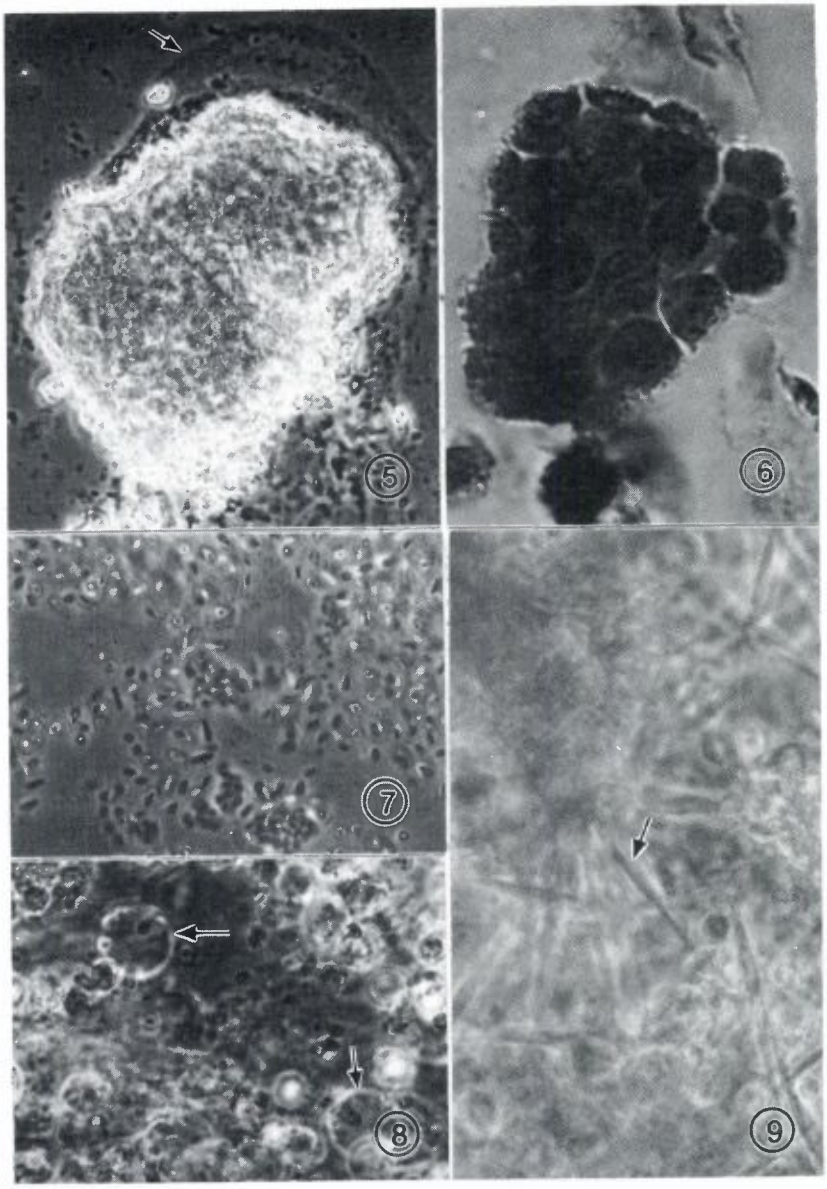
Feeding and egestion are episodic. Many of the brushed and cleaned organisms retracted portions of their pseudopodial nets after they had been feeding for several hours (3–6) in the algal lawns we provided for them. *Amphisorus* gathers food around the periphery of its shell (see Figs. 10, 11, Lee (1983)). Gradually the material takes on a reddish coloration indicating that much of it has been digested. In *Amphistegina* food is gathered into a small pellet near the aperture (Figs. 1,3). Reddish masses gradually appear here as well. Cytological and fine structural observations already published (e.g. McEnery and Lee, 1981; Koestler et al., 1985) indicate that food vacuoles are taken into the test. Evidence from radionuclide tracer studies (reported below) showed that much of the reddish residua was within the animal at the termination of the tracer feeding phase of the experiment. Thus the reddish material represents egestion or excretion of residua by the animal.

After a period in which the pseudopods are considerably retracted, the animal sends out new pseudopodia and moves away from its former location. It leaves behind either a ring (*Amphisorus* and some *Amphistegina*) or an irregular mass of red residua (some *Amphistegina*) (Figs. 2,3,4). We found 76 rings after 100 *Amphisorus* had been feeding for 24 hr. We found 516 (107–154/100) irregular masses of residua in 4 populations each of 100 *Amphistegina* (average 129/100 animals/24 hr).

We examined some residue in a phase-contrast compound microscope. Initially residue are membrane bound and contained granules and some recognizable remnants of cells (Fig. 5). Parts of cells eaten are not digested. When *Chlorella* sp. (strain AT) was eaten by *Amphistegina* we saw that the cell envelope was recognizable in the residua (Figs. 6,8). Whole frustules are observable in the residua when some diatom species are eaten and only fragments are observed when more weakly silicified species are fed (Fig. 9). After a short time the membrane surrounding the egesta brakes down and many bacteria are found in the residual mass (Figs. 7,8).



Figures 1–4.
(1) Two specimens of *A. lobifera* in a dense algal culture. Swath made in the algal population by the feeding and locomotion of the animal on the left. Magnification 10x. (2) Two specimens of *A. lobifera*. Fecal matter (f) deposited in small streaks on the bottom of a plate by a rapidly moving animal. 10x. (3) An animal producing a fecal pellet which will be attached to a small pebble (arrow). Semi-circular fecal remains (f) deposited by an animal. 20x. (4) An animal with its feeding net extended. Fecal pellets (f) next to the animal and a fecal ring at the lower left. 9x.



Figures 5–9.
(5) Freshly deposited fecal pellet. Arrow points to membrane which surrounds fresh fecal pellet. (6) Freshly deposited fecal pellet of an organism fed *Chlorella* st. (strain AT). Cell wall fragments obvious in pellet. (7) Bacteria on the surface of a fecal pellet membrane deposited several hours earlier. (8) A portion of a squashed fecal pellet similar to the one in Fig. 6 showing cell wall ghosts (arrows) and red pigment granules. (9) A portion of squashed fecal pellet of an animal which was fed *Cylindrotheca closterium*, a diatom. Arrow points to siliceous remnants of the diatom frustule.

Natural algal community

Halophila supports very rich and diverse assemblages of epiphytic microflora. With the exception of extensive unicellular chlorophyte blooms, the surfaces of the leaves and adjacent granitic rubble are carpeted with individuals and small colonies of bacteria, cyanobacteria, chlorophytes, diatoms, coccolithophorids, and fungi. Typical surfaces were illustrated in Lee (1983, Figs. 18,19). We have isolated a dozen chlorophyte clones from blooms on surfaces of leaves on which large numbers of large foraminifera were foraging. They all seem morphologically identical under the light microscope and seem to fall into 2 physiological groups based on growth rates of clones in the same media. The blooming chlorophytes are coccoid at all phases of the growth cycle of a batch culture in medium '5' and in erdschreiber (Lee et al., 1970). Since the isolates are azoosporic and meet our criteria, we have tentatively assigned them to the genus *Chlorella.* (Two clones (AG) and (AT) have been sent to our colleague E. Kessler, Universität Erlagen-Nürnberg, for further characterization and possible identification). The two most commonly observed Cyanobacteria were tentatively identified, on the basis of light microscope characteristics, as *Synechoccus* and *Oscillatoria.*

Our study of the natural populations of diatoms in the benthic and epibenthic habitats where foraminifera are abundant is still in progress. To date we have recognized more than 200 taxonomic entities (Table 1). A small number of species in each sample comprised more than 70% of the population in each of the 42 samples we have thus far examined. Between $1.0 - 1.2 \times 10^3$ diatoms in each sample were characterized. The following were, among the most numerous species (> 8%) in the majority of the samples studied: "*Fragilaria* sp., *Amphora* sp., *Cocconeis placentula* var. *euglypta, Cocconeis* sp., *Amphora bigibba, Amphora* spp. (*Halamphora* group) *Entomoneis* sp., *Navicula salinarum, Navicula* spp., *Nitzschia amphibia, Nitzschia frustulum, Nitzscha* spp., and *Cylindrotheca gracilis.* In designating one as "*Fragilaria*" sp. we are reserving taxonomic judgement on its proper designation. We are aware that fine marginal spines have been discussed as reasonable criteria which might be used to separate *Fragilaria* from *Synedra* but the argument remains unresolved (Poulin et al., 1986). At a light microscope level the organism is reasonably identified as a *Fragilaria* sp.

Table 1. Partial list of the most common diatoms found on the surfaces of *Halophila* and adjacent substrates

Thalassiosira nanolineata (Mann.) Fryxell & Hasle
Oestrupii var. *venrickae* Fryxell & Hasle

Melosira nummuloides (Dillw.) Agardh.
Coscinodiscus marginatus Ehr.
Actinoptychus undulatus (Bailey.) Ralfs
Cymatosira belgica Grun.
lorenziana Grun.

Delphineis uriella var. *Surirella* (Ehr.) Andrews
Dimerengramma minor var. *minor* (Greg.) Ralfs
Fragilaria gaillonii (Ehr.) Lange-Bertalot
Tabulata var. *tabulata* (Agadh.) Lange-Bertalot.

Synedra affinis Kutz.
barbatula Kutz
robusta Ralfs

Lichmophora gracilis (Ehr.) Grun.
paradoxa (Lyngb.) Agardh.
remulus Grun.
Opephora pacifica (Grun.) Petit.

Achnanthes brevipes var. *angustata* (Grev.) Cleve
Hauckiana Grun.
manifera Brun.

Cocconeis conveza Giffen.
cunoniae Chol.
disculoides Hust.
granulifer Grev.
japonica A. Schm.
pediculus Ehr.
placentula var. *euglypta* (Ehr.) Cleve
scutellum Ehr.

Amphora angusta var. *ventricosa* (Greg.) Cleve
bigibba Grun.
coffeiformis Agardh.
cymbelloides Grun.
eunotia Cleve
exigua Greg.
hamata Heiden and Kolbe
ovalis var. *pediculus* (Kutz.) V.H.
proteus var. *contigua* Cleve.
terroris Ehr.

Coloneis excentrica (Grun.) Boyer
liber (W. Smith) Grun.
disticha (A. Schm.) Halgelstein.

Cymbella pusilla Grun.
Diploneis papula (A. Schm.) Cleve
placida (A. Schm.) Hust.
smithi var. *adversa* Halgelstein
splenda (Greg.) Cleve
weissflogi (A. Schm.) Cleve

Entomoneis alata (Ehr.) Ehr.
triconvusa Van Landingham
Gyrosigma fasciola (Ehr.) Cleve
peisonis (Grun.) Hust.
variipunctatum Hagelstein
variistriatum Hagelstein
Mastogloia apiculata W. Smith
aspera Hust.
asperuloides Hust.
baltica Grun.
corsicana Grun.
cribrosa Grun.
cyclops Voigt
elegans Lewis
erythraea Grun
exigua Lewis
fimbriata (Bright w.) Cleve
hustedtii Meister
inaequalis Cleve
lanceolata W. Smith
paradoxa Grun.
peracuta janisch
pumila f. *africana* Giffen
pumila var. *papuarum* Chol.
punctifera Brun.
pusilla Lewis

Navicula abruptoides Hust.
approximata Grev.
carinifera Grun.
cancellata Donkin
clarata var. *Clarata* Greg.
directa Cleve
directa var. remota Cleve
forcipata Grev.
genifera Schmidt
gracilis Ehr.
hennedyi W. Smith

hagelsteinii Hust.
lacustris Greg.
lancellata Donkin
lyra var. *Lyra* Ehr.
mannii Hagelstein
multica Kutz.
platyventris Meister
praetexta Ehr.
salinarum Grun.
tubulosa Brun.
yarrensis Grun.

Pleurosigma intermedium W. Smith
portoricense Hagelstein

Auricla compleza (Greg.) Cleve
Rhopaloidia gibberula (Ehr.) O. Muller
operculata var. *operculata*
(Agardh.) Hakansson

Cylindrotheca gracilis (Brebisson) Grun.

Bacillaria paradoza var. *tumidula* Grun.
Denticula subtilis Grun.

Nitzschia acuta Hantzsch
amphibia Grun.
areolata Hust.
coarctata Grun.
constricta (Greg.) Grun.
dissipata (Kutz.) Grun.
divergens Hust.
frustulum (Kutz.) Grun.
granulata var. *granulata* Grun.
lanceolata W. Smith

lionella Chol.
longissima (Breb.) Ralfs
lorenziana Grun.
marginulata var. *didyma* Grun.
obtusa f. *parva* Hust.
ovalis Arnoff
panduriformis Gregory
sigma (Kutz) W. Smith
vidovichii Grun.

Rhopaloidia musculus Kutz.

Comparative feeding experiments

These experiments were carried out using ^{32}P labeled algae. Individuals of *Amphisorus* available in nature for this experiment were fairly large (~3.3 mm) and uniform in size (Table 2). The experiment was done in triplicates and the standard deviation from the mean is given in % of the mean value. In general all the algae fed were consumed by the foraminifera during 3 hr of feeding. On the basis of number of algae eaten per miligram of foraminifera there was a range from a low of 354/mg of *Amphora bigibba* to a high of 13,028/mg *Amphora* sp. (29), a 36 fold difference. Since the experimental animals were comparable in size, calculations of the number of algae eaten per individual, surface area, feeding area or volume reflect the same 36× range (Table 2). Both species of *Amphora* have approximately the same size range (7–16 μm) and are weakly silicified. Their biomass is comparable. Similar differential uptake was noticed with the 2 clones of *Chlorella* (AG and AT). There was approximately an 8 fold difference in the uptake of the strains of these morphologically identical green algae (Table 2). Three times more *Entomoneis* was taken up than *Cocconeis placentula* or *Navicula* sp. (8) (Fig. 3).

Table 2. Feeding of *Amphisorus hemprichii* on various species of algae abundant in its habitat

Food organisms	Average individual Foraminifera number					algae		Eaten per unit foraminifera		
	Size Diam. (mm)	Wt. (mg.)	Vol. (mm^3)	surface area (mm^2)	feeding area (mm^2)	mg	Individual	Surface area (mm^2)	Feeding area (mm^2)	Volume (mm^3)
Amphora bigibba	3.42 ±6.2	2.84 ±13.8	3.02 ±12.5	21.9 ±11.5	3.52 ±6.2	354 ±12.5	1,019 ±25.2	45.9 ±14.2	286.6 ±19	333 ±13.4
Amphora sp. (29)	3.41 ±8.2	2.84 ±17.2	3.02 ±15.8	21.9 ±14.6	3.51 8.18	13,028 ±8.4	36.776 ±17	1681 ±8.2	10,417 ±11	12,213 ±8.1
Cocconeis placentula	3.37 ±7.2	2.76 ±15.5	2.95 ±14	21.4 ±13	3.47 ±7.2	2.017 ±20.7	5,457 18.3	258.6 19.7	1,577 ±18.2	1,884 ±20.1
Entomoneis sp.	3.66 ±21	3.29 ±4.5	3.46 ±4.1	24.8 ±3.8	3.77 ±2.1	7,617 ±11.6	24,919 ±7.5	1009 ±10.9	6623 ±9.2	7,241 ±11.2
Navicula sp. (8)	3.47 ±5.4	2.49 ±2.1	3.1 ±10.6	22.5 ±9.8	3.57 ±5.4	2026 ±32	4,714 ±14.8	213 ±22	1330 ±18	1,548 ±235
Chlorella sp. (AG)	3.01 ±13.7	2.20 ±28	2.38 ±23.7	17.6 ±23.6	3.1 ±13.7	1,123 ±23.8	2,322 ±17.9	137 ±19	752 ±13	1,052 ±21.4
Chlorella sp. (AT)	3.29 ±4.7	2.6 ±10.3	2.79 ±9.4	20.4 ±8.6	3.38 ±4.7	8,300 ±18	21,563 ±19.4	1,058 ±16	6,630 ±18.4	7,728 ±18

The experiment was done in triplicate and the standard deviation from the mean is given in % of the mean value.

A greater range of sizes of *A. lobifera* was used for testing comparative feeding in this animal (Table 3, Figs. 1,2). Taken on average there was approximately an 11 fold difference between the uptake of *A. bigibba* (533/mg foram) than either *Amphora* sp. (29) (6057/mg foram) or *Chlorella* sp. (AT) (6,967/mg foram; Table 3). Again there was significant difference (8×) between uptake by the animal of the 2 isolates of *Chlorella* (AG and AT) and between the uptake of *Entomoneis* and either *Cocconeis placentula* and *Navicula* sp. (8) (Table 3). In general the number of algae eaten per individual was proportional to the size of the foram (Fig. 1). However, if we consider the data on the basis of the number of algae ingested per mg of foram against diameter size the rate declines (Fig. 2).

Table 3. Feeding of *Amphistegina lobifera* in various species of algae abundant in it's habitat

Food organisms	Average individual Foraminifera					Number Algae		Eaten per unit foraminifera		
	Size Diam. (mm)	Wt. (mg.)	Vol. (mm^3)	Surface area (mm^2)	Feeding area (mm^2)	mg	Individual	Surface area (mm^2)	Feeding area (mm^2)	Volume (mm^3)
Amphora bigibba	1.33 ±44.9	1.11 ±121	0.51 ±124	3.73 ±87.5	0.56 ±52	533 ±100	358 ±26.6	93.8 ±26.6	626 ±26.6	1,167 ±50.8
Amphora sp. (29)	1.39 ±39	1.14 ±101	0.52 ±104	3.93 ±73	0.59 ±73	6,057 ±58	4,162 ±67	1,101 ±11.5	7337 ±11.5	13,172 ±53.2
Cocconeis placentula	1.46 ±41.7	1.39 ±116	0.46 ±120	4.42 ±83	0.66 ±83	1,109 ±55	826 ±60.7	214 28.8	1,426 28.8	2,450 ±52.8
Entomoneis sp.	1.33 ±43	1.08 ±117	0.49 ±120	3.69 ±84	0.55 ±84	5,862 ±87	2,539 ±59	890 ±45	5,932 ±45	12,533 ±81
Navicula sp. (8)	1.33 ±37	0.96 ±93	0.43 ±95	3.35 ±68	0.53 ±68	2,432 ±86.8	982 ±42	375 ±46	2,503 ±46	5,295 ±82.8
Chlorella sp. (AG)	0.97 ±27	0.32 ±58	0.14 ±58	1.76 ±45	0.26 ±45	844 ±68	174 ±40	108 ±26	720 ±26	1,791 ±60
Chlorella sp. (AT)	1.14 ±40	1.17 ±107	0.54 ±110	3.98 ±77	0.60 ±77	6,967 ±72	3,963 ±51	1,183 ±22	7,885 ±22	15,084 ±66

The experiment was done in triplicate and the standard deviation from the mean is given in % of the mean value.

Duration and constancy of feeding

Even though the methodology for combining individuals into crushed homogenized pooled samples was expected to dampen variation we observed considerable variation (up to 10×) between some replicate samples in experiments done with small numbers of foraminifera in spot plates (Tables 4 and 5).

Differences between replicate samples were as great as differences between the number of algae ingested by either species of foraminifera in 3 hr or 24 hr. There did not seem to be any difference in uptake between groups of organisms incubated in the light or in the dark (Tables 4–8). The uptake of *Amphisorus* incubated in the light in the presence of DCMU was enhanced over those incubated without the inhibitor. We noted at the time of harvest that organisms in wells with DCMU had much more extensive pseudopodial

Table 4. The effects of length of exposure and conditions of incubation on the ingestion of *Chlorella* by *Amphisorus hemprichii*

Experimental conditions	Aver. feeding individuals diameter (mm)	Number of algae ingested calculated on basis of weight (mg)	individual feeding area (mm^2)
Fed 3 hr in light			
Sample 1	3.60	3,199	2,716
Sample 2	4.05	2,935	2,892
D C M U	3.68	9,567	8,357
Fed 24 hr			
a) in light			
Sample 1	4.05	2,468	1,957
Sample 2	4.16	10,215	10,394
D C M U	4.18	1,444	1,532
b) in dark			
Sample 1	3.77	6,557	5,870
Sample 2	3.81	5,338	4,924
c) Dead:			
Technique control	3.90	841	801

networks than did organisms without DCMU (Table 4). After 24 hr DCMU seemed to have the opposite effect. Uptake rates in DCMU treated samples were less than, or equal to, those incubated in the light (Table 4).

Distribution of tracer into the foraminiferal tests

Our chief interest in the rather crude separation of the cellular fractions was to gain insight into the fraction (if any) of the labeled food organisms which might have been channeled into the skeleton.

In *A. lobifera* approximately 1.4% of the total label which entered the organism in food was recovered from the skeletal fraction (Table 6). There were no differences in the fractionation of the label which entered with either of the algal species tested. In *A. lobifera*, where size was one of the variables tested, smaller organisms incorporated a higher fraction of the label into their shells than did medium sized animals. A higher level, approximately 4% of the initial level of label was incorporated into the skeletal fraction of *A. hemprichii*.

Table 5. The effects of light and photosynthesis on the feeding of 2 species of larger foraminifera on two food organisms *Cocconeis placentula* and *Chlorella* sp. (AT)

A. *Amphisorus hemprichii*

Exp.	Conditions	Food organisms	Average foram diameter (mm)	Number of algae ingested calculated on the basis of: Weight (mg)	Individual no.	Feeding area (mm^2)
Initial*	light	*Cocconeis*	3.68	935	3338	881
		Chlorella	3.67	6625	20916	5533
	DCMU	*Cocconeis*	4.28	1018	5019	1138
		Chlorella	3.82	6761	24678	6272
	dark	*Cocconeis*	4.21	643	2984	688
		Chlorella	4.04	5046	20890	5020
Final*	light	*Cocconeis*	3.70	1062	3621	950
		Chlorella	3.88	2660	10613	2656
	DCMU	*Cocconeis*	3.74	832	2870	745
		Chlorella	3.55	4907	15064	4120
	dark	*Cocconeis*	4.06	431	1815	434
		Chlorella	3.87	3144	11821	2966

B. *Amphistegina lobifera*

Exp.	Conditions	Food organisms	Average foram diameter (mm)	Number of algae ingested calculated on the basis of: Weight (mg)	Individual no.	Feeding area (mm^2)
Initial*	light	*Cocconeis*	1.23	988	553.3	1388
		Chlorella	1.20	6964	3621.3	9546
	DCMU	*Cocconeis*	1.17	1412	663.6	1840
		Chlorella	1.16	11259	5179.1	14611
	dark	*Cocconeis*	1.15	785	353.3	1014
		Chlorella	1.15	7415	3336.8	9578
Final*	light	*Cocconeis*	1.21	834	442.0	1146
		Chlorella	1.27	3520	2147.2	5054
	DCMU	*Cocconeis*	1.25	702	407.2	989
		Chlorella	1.22	7987	4313.0	11000
	dark	*Cocconeis*	1.30	581	383.5	861
		Chlorella	1.24	5183	2954.3	7294

* Initial — organisms harvested immediately after feeding with ^{14}C labeled algae.

* Final — organisms harvested from labeled food at same time as above and incubated with similar but unlabeled food ("cold chase"), for an additional 18 hr

Table 6. The distribution of label in crude fractions of the organisms used in the cold chase part of the experiment in the previous table

A. Number of algal equivalents per mg Forams*

	Total		Skeleton		Org. matter		Acid	
Exp. conditions	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Cocconeis light								
Ar*	915	924	54.5	69.6	509	683	351	171
Ag*	1067	746	3.95	13	732	513	331	220
Chlorella light								
Ar	6491	2726	128.5	216.2	4402	1818	1910	692
Ag	7806	3489	19.5	29.4	5295	2355	2492	1105
Cocconeis dark								
Ar	586	534	25.9	291	426	269	84	0
Ag	692	610	12.4	18	500	469	180	123
Chlorella dark								
Ar	4840	2171	134.5	130.2	3075	984	1530	1093
Ag	7051	6032	25.8	30.2	4740	4108	2285	1894
Cocconeis DCMU								
Ar	1161	832	28.1	16.3	832	676	301	155
Ag	949	690	51.4	17.7	617	516	281	156
Chlorella DCMU								
Ar	6584	5059	116.5	81.8	4302	3596	2165	1381
Ag	11180	8008	50.8	47.5	7824	5798	3305	2163
Average	4110	2652	58.5	57.8	2760	1826	1292	770
Stand. dev.	85.12%	90.61%	90.13%	100.83%	87.42%	93.61%	83.12%	92.67%

B. Number of algal equivalents per surface area (mm^2)

Cocconeis light								
Ar	130	125	7.77	9.38	72.5	92.1	50.0	23.0
Ag	225	154	0.83	2.68	154.3	105.8	69.8	45.4
Chlorella light								
Ar	927	394	25.49	31.21	628.6	262.5	272.7	99.9
Ag	1605	751	4.01	6.33	1088.7	507.1	512.4	237.9
Cocconeis dark								
Ar	77	75	3.39	3.35	38.1	59.7	35.2	11.8
Ag	134	136	2.40	4.00	96.9	104.3	34.9	27.4
Chlorella dark								
Ar	673	297	18.69	17.80	427.3	129.6	226.5	149.4
Ag	1366	1273	5.00	6.38	918.2	867.2	442.6	399.8
Cocconeis DCMU								
Ar	173	111	4.18	2.18	123.7	90.4	44.7	20.7
Ag	186	146	10.05	3.74	120.6	109.1	54.9	33.0
Chlorella DCMU								
Ar	895	663	15.84	10.71	585.0	471.0	294.4	180.9
Ag	2167	1654	9.89	9.81	1523.1	1198.8	643.4	446.8
Average	714	481	8.96	8.96	481	33	223	140
Stand. dev.	93.38%	102.44%	80.34%	88.76%	95.88%	105.26%	91.38%	103.77%

* *Chlorella* = *Chlorella* sp. (AT), * *Cocconeis* = *Cocconeis placentula*, * Ar = *Amphisorus hemprichii*, * Ag = *Amphistegina lobifera*

Algal equivalents means that the total dpm measured per mg of foraminifera has been divided by the counts per individual alga fed.

Effects of captivity on feeding rates

Amphisorus which were fed *Chlorella* sp. (AT) and kept in the laboratory for 72 hr, feed at a higher rate (6.4 μg) than those which were fed directly after collection (4.3 μg), or those maintained in the laboratory but starved (4.8 μg/mg; Table 8). Of those maintained in the laboratory the animals which were fed *Chlorella* sp. (AT) at a higher rate (4.4 μg/mg) in the experiment than did those which were starved (3.5 μg/mg; Table 9).

Egestion excretion and retention of carbon

Regardless of the initial rate of feeding in *Amphistegina* there was an initial period of rapid label loss in the first 24 hr (e.g. 5.97–1.98 μg/mg; Table 7). This coincided with egestion described earlier. Perhaps it is significant to recognize that the largest initial label loss was in the population which fed at the highest rates. After 24 hr the amount of carbon retained by the various pretreated populations of *Amphistegina* was approximately equal ($\sim$ 1.8 μg algal carbon/mg foram). The rate of loss changed and was more gradual over the next few days. The loss probably does not extrapolate to 0 gain. As indicated above some carbon gained by feeding is incorporated in the shell. Presumably it is not exchanged or lost. However, we did not incubate specimens longer than 72 hr to test this.

The loss of labeled carbon from within *Amphisorus* after they had ceased feeding was more gradual (e.g. 4.3–3.6 μg algal carbon/mg foram) than in *Amphistegina* (Table 9). In some of the pretreated groups (e.g. 72 hr in lab-fed, 24 and 72 hr in lab-starved) the loss in the first 24 hr was negligeable (Table 9), in the 72 hr-fed pre-treated group $\sim$1/3 of the carbon gained in feeding was egested between 24 and 48 hr after removal from labeled food (Table 9).

Autoradiography

Observations of sections of *Amphisorus hemprichii* harvested after 6 hr of incubation with ^{14}C labeled *Chlorella* showed that radionuclide labeled food was found in the outer chambers. No labeled food was found in the embryonic chambers and proloculum (Fig. 10). Food ingested was digested during the first hours. When *Chlorella* was fed, the cell walls were not digested by the foram (Fig. 13) and therefore the label in the wall was not dissipated in the cytoplasm of the foram. Transfer of ^{14}C label from the food to the symbionts must have been rapid. Label was already observed in the symbionts after 6 hr of incubation (Figs. 13,14).

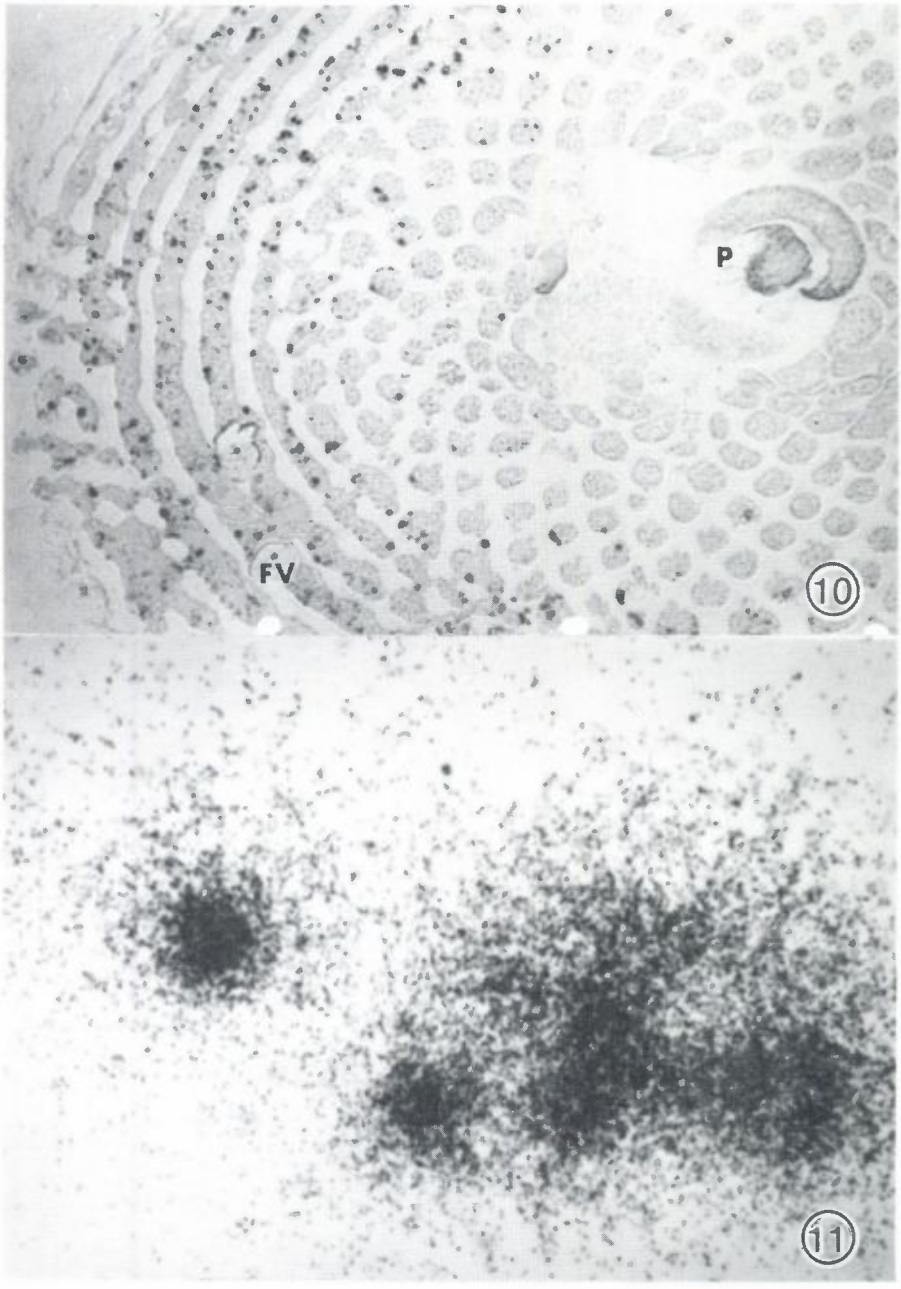


Figure 10. Autoradiographed of an horizontally sectioned *Amphisorus hemprichii* showing labelled food after 6 hr of feeding on ^{14}C labelled food. Proloculum, FV-food vacuole. 38×.

Figure 11. Higher optical plane, and enlarged portion Fig. 1 showing deposition of silver grains.

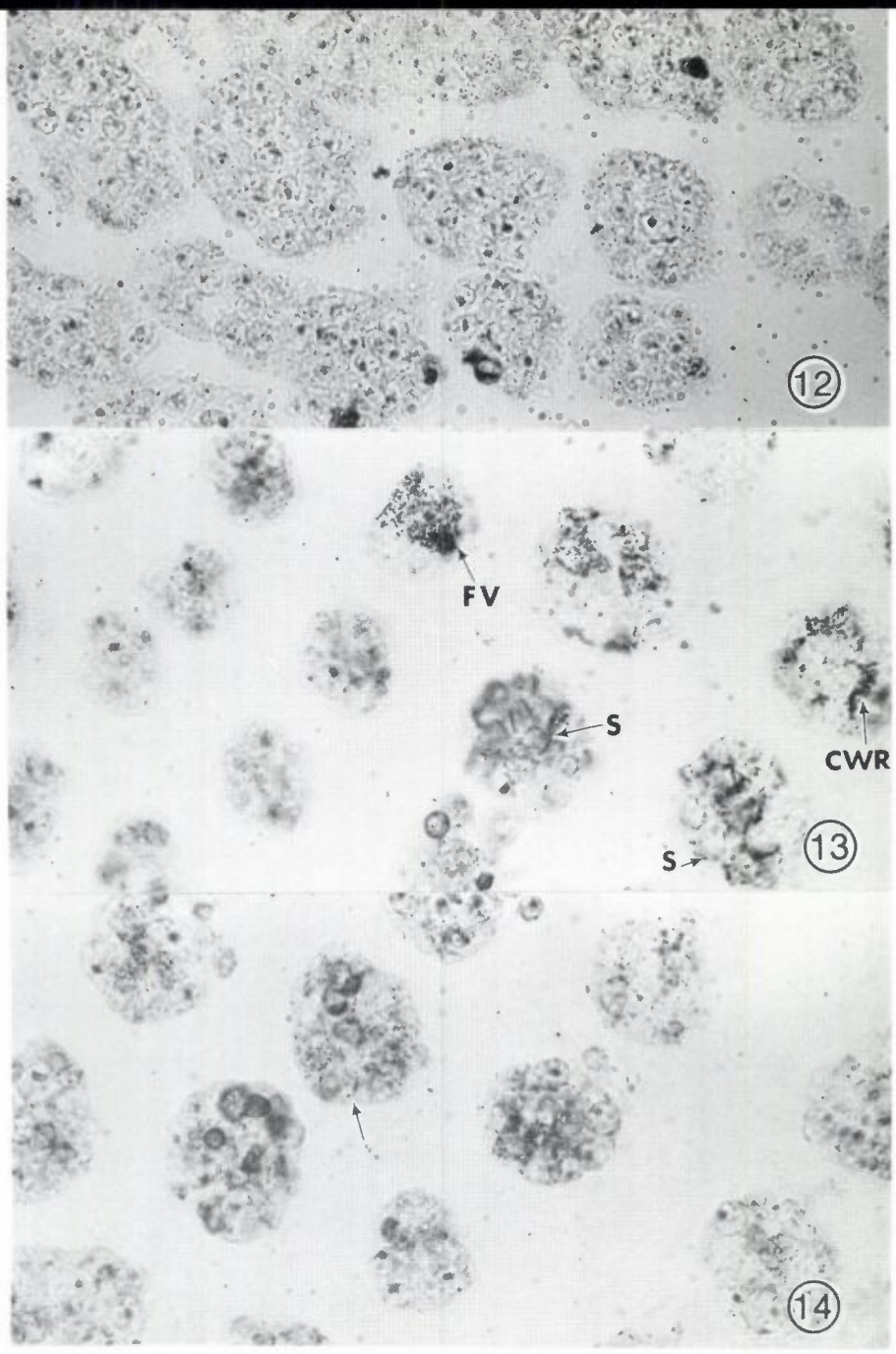


Figure 12. Optical section of chamberlets showing symbionts in the foram. 152×.

Figure 13. Chamber showing labelled food in food vacuole (FV), some labelled symbionts (S), and label in cell wall residua (CWR), after 6 hr of feeding on ^{14}C labelled food. 152×.

Figure 14. Chamberlet with labelled cell wall of *Chlorella*. Section after 6 hr of feeding on ^{14}C labelled food, showing that some of the label has already been transferred to the symbionts (arrow). 152×.

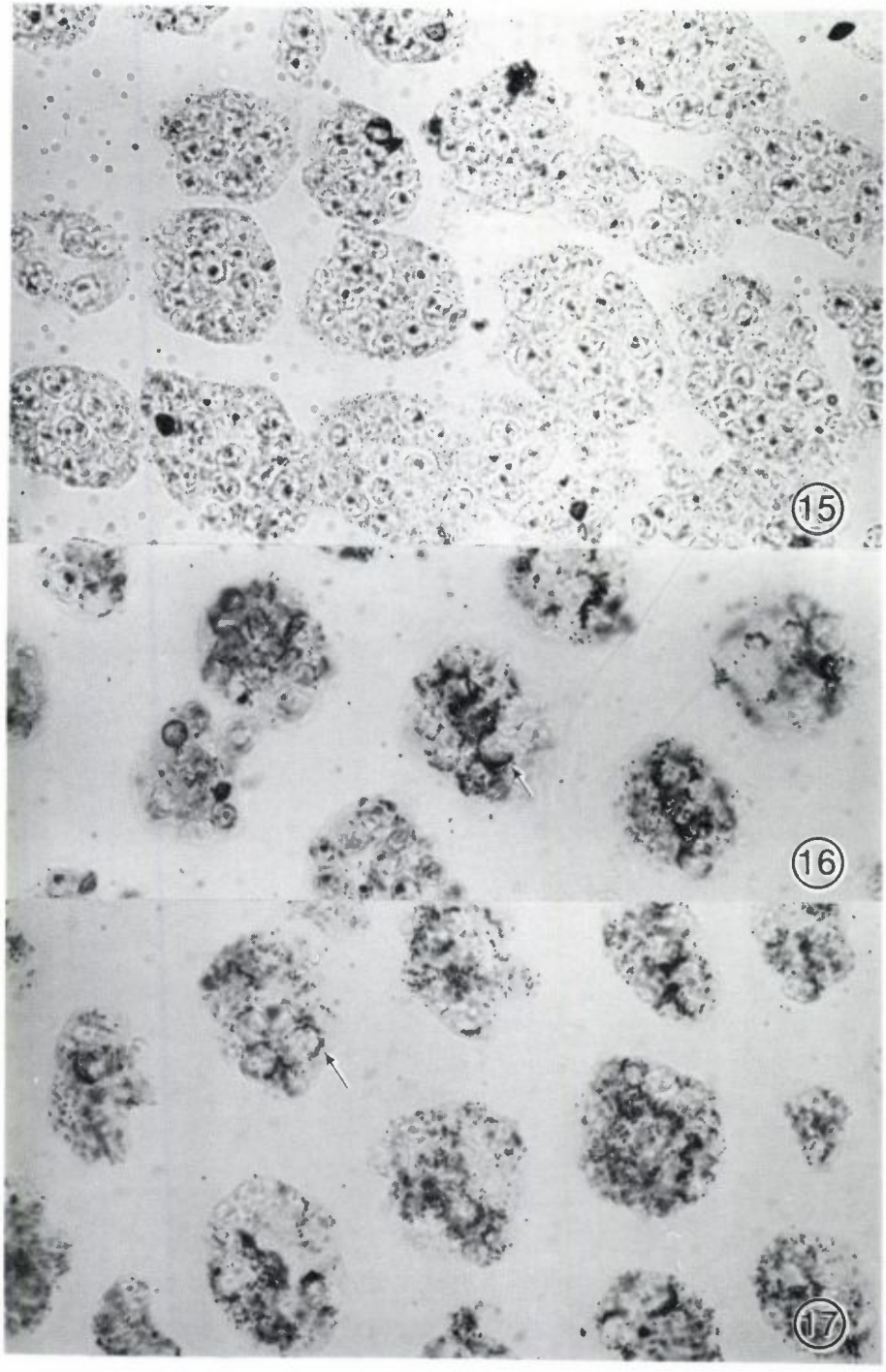


Figure 15. Optical section of chamberlets showing symbionts in the foram 152×.

Figures 16, 17. Chambers after 24 hr of incubation with unlabeled food, greater transfer of ^{14}C label from the foram's food to the symbionts. 152×.

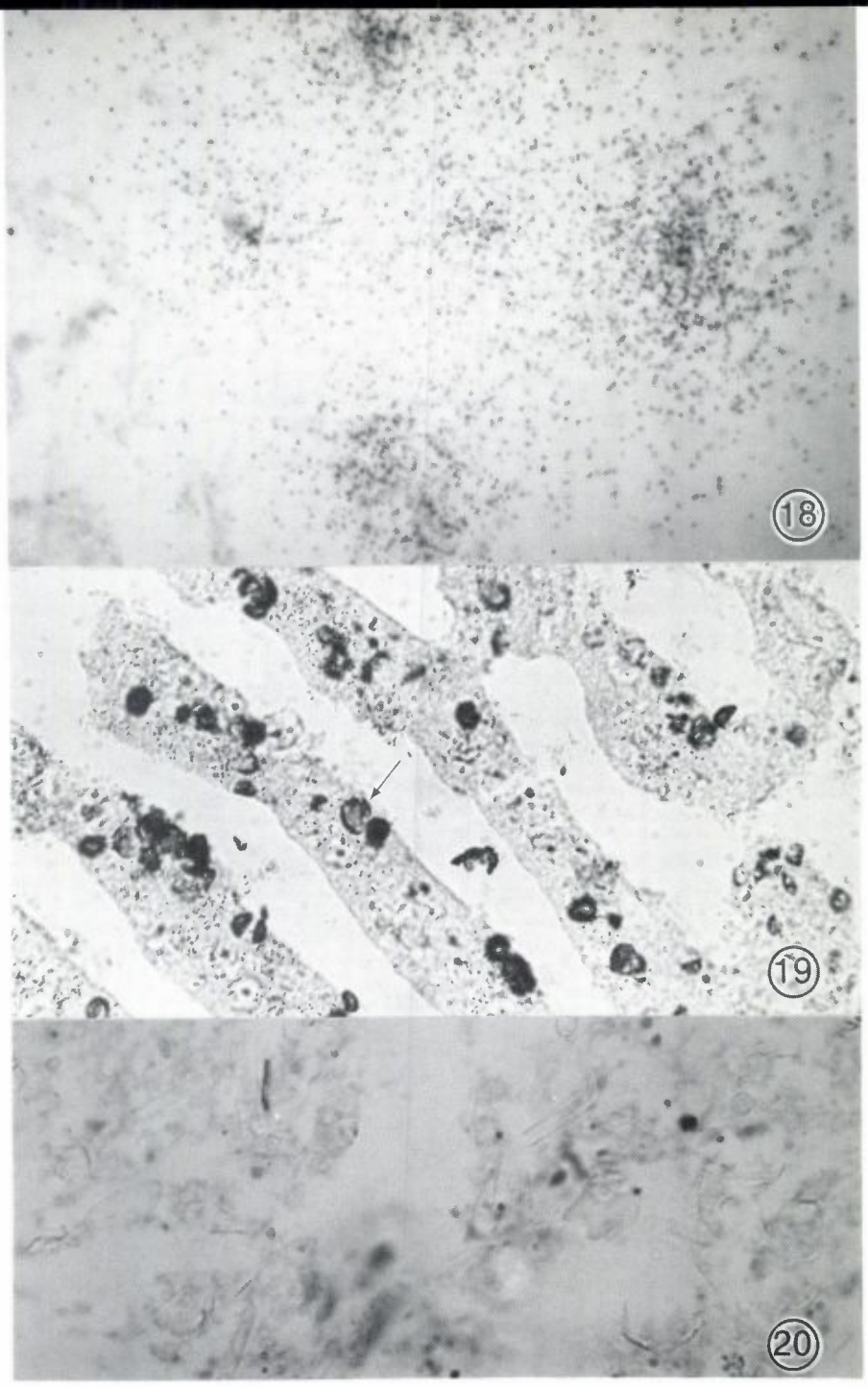
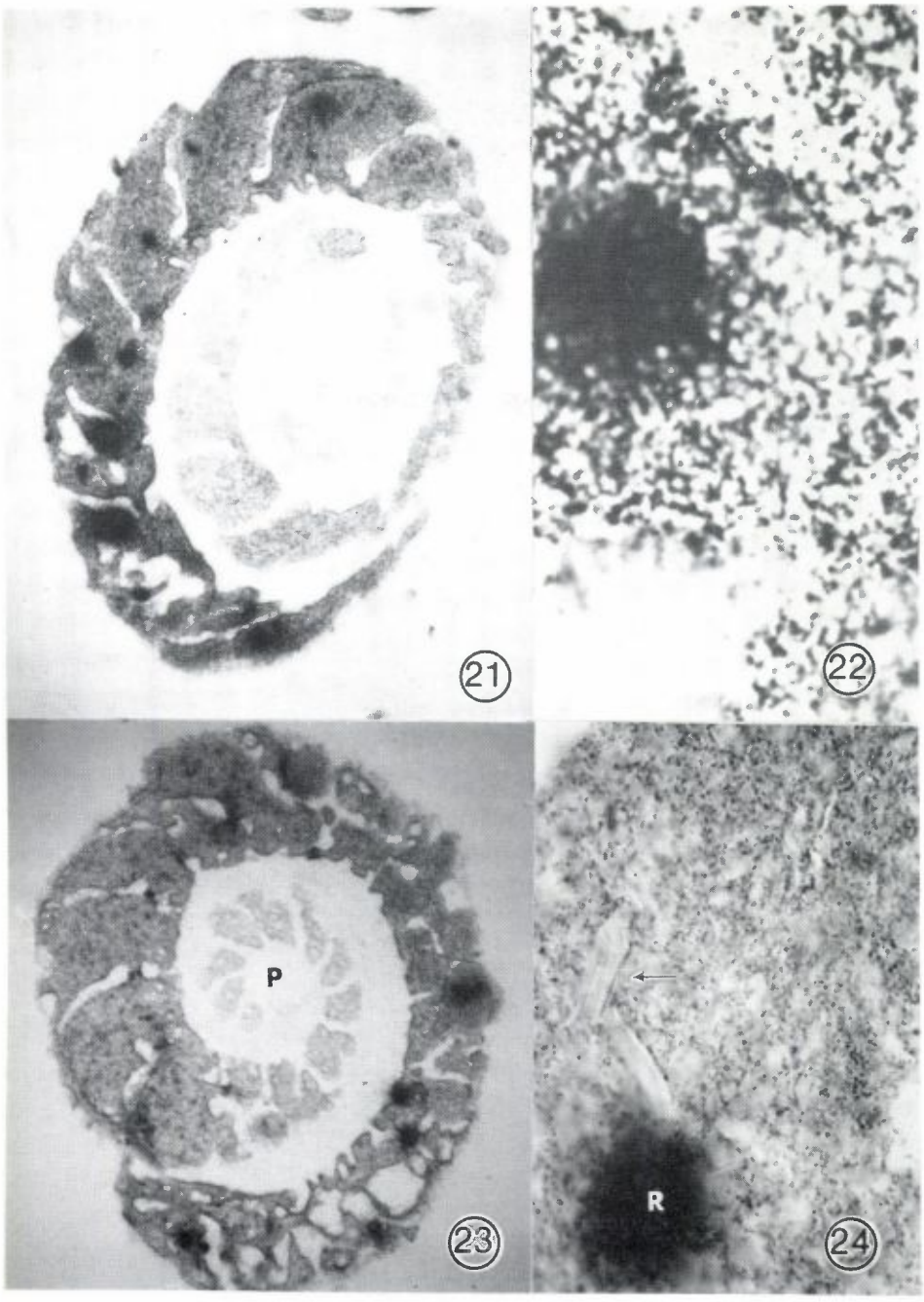


Figure 18. Higher optical plane of Fig. 19. Note that the label is more diffuse (= less concentrated) in comparison with Fig. 11.

Figure 19. Outer chambers showing radionuclide labelled envelope residue of *Chlorella* (arrow) after 24 hr of incubation with unlabeled food. 114×.

Figure 20. Section of *A. hemprichii* showing unlabeled frustules from diatoms and residua from food vacuoles.



Figures 21, 23. Autoradiographed of an horizontally sectioned *Amphistegina lobifera* showing labelled food after 6 hr of feeding on ^{14}C labelled food. P-proloculum. Fig. 21 is more ventral than Fig. 23. Note more label in the ventral section. 60×.

Figure 22. Higher optical plain of a portion shown in Fig. 21 showing the radioactivity in the food vacuole in the foram.

Figure 24. Section unlabeled diatom frustule (arrow). Some labelled non-described residua (R) is also shown.

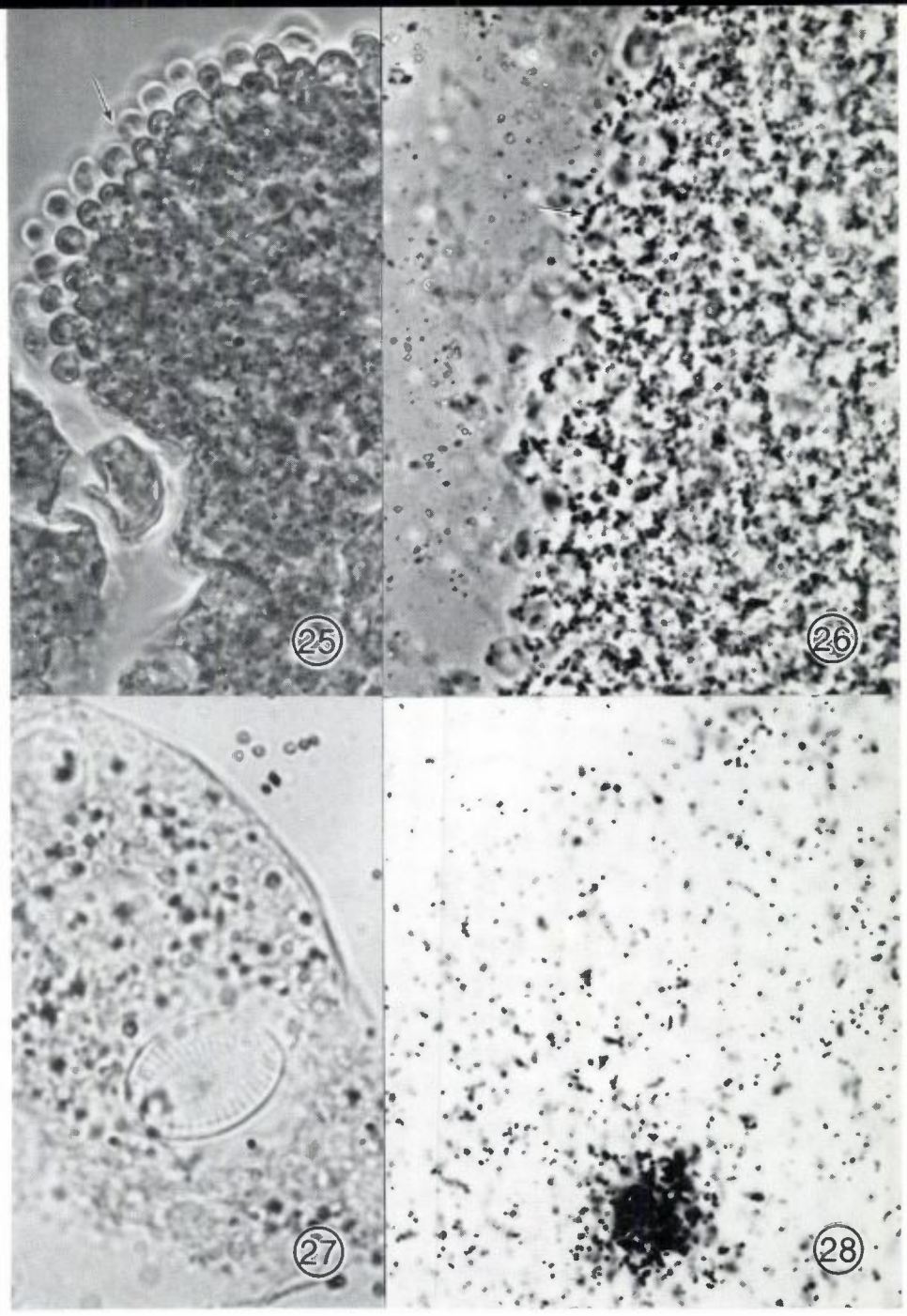


Figure 25. Section showing zooxanthellae in the cortex of the foram (arrow). 780×.

Figure 26. Higher optical plain of the cortex of *A. lobifera* after 48 hr of incubation with unlabeled food showing label distributed in this region. Many of the symbionts seem to be labelled.

Figure 27. *A. lobifera* showing an empty diatom frustule (*Cocconeis*).

Figure 28. Labelled residua in a food vacuole of *A. lobifera* after 48 hr of incubation with unlabeled food.

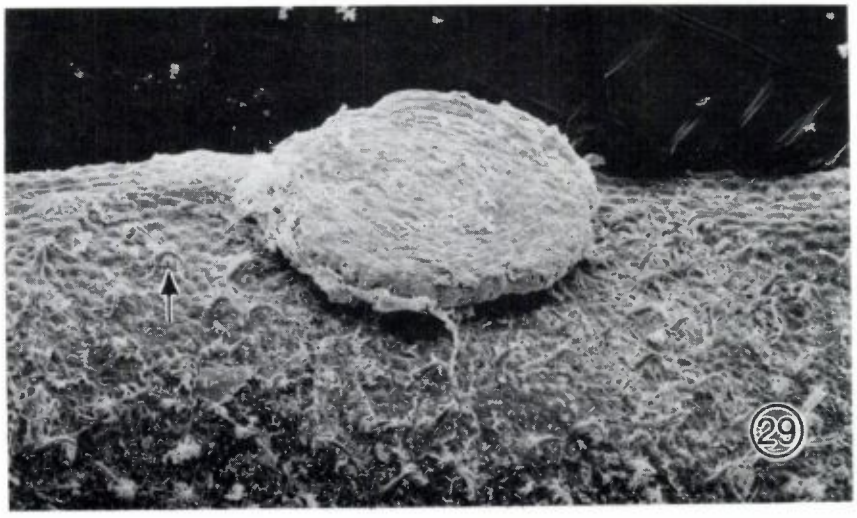


Figure 29. Relatively low magnification (15×) SEM of an *Amphisorus hemprichii* on the surface of an *Halophila* leaf. Note trichomes (arrow) keep the animal from direct contact with the leaf surface. (Preparation was critical-point dried before coating).

Table 7. The distribution of radiocarbon tracer label from ingested algae by *Amphisorus hemprichii* incubated with ^{14}C labeled *Cocconeis placentula* calculated on the basis of the number of algal equivalents per mg foram*.

	Light			Dark			DCMU		
Fraction	Initial	Final	Differ	Initial	Final	Differ	Initial	Final	Differ
Total	915	924	−9	586	534	52	1161	832	329
Acid	351	171	180	269	84	185	301	155	146
Organic	509	683	−174	291	426	−135	832	676	156
Skeleton	54	70	−16	26	2	28		16	12
Digested	1017			604			1114		
Egested	−102			−18			17		
Respired	93			70			282		
Calculated on the basis of number of algal equivalents per mm^2 of estimated feeding area.									
Total	862	827	35	627	538	90	1298	745	553
Acid	331	153	178	288	85	203	337	139	198
Organic	479	611	−137	311	429	−117	931	605	325
Skeleton	51	63	−12	28	24	4	31	14	17
Digested	958			646			1246		
Egested	−96			−19			53		
Respired	88			75			315		

* Algal equivalents means that the total dpm measured per mg of foraminifera has been divided by the counts per individual alga fed.

After 24 hr of incubation with unlabeled food the label in the radionuclide tagged food was more diffuse than after 6 hr (Figs. 11, 15). There was also less overall radioactivity observable in the entire animal (Figs. 16,17). After 24 hr, more ^{14}C label from the foram's food was transferred to the symbionts (Figs. 7,8). Radionuclide labeled envelope residua of *Chlorella* (Fig. 19, arrow) were more visible after 24 hr of incubation. This was because the labeled cell contents had been released and dissipated. Empty diatom frustules (non-labeled) were also commonly found in the forams. Thus we were clearly able to visualize differences in the efficiency of carbon transfer to the foram between diets of *Chlorella* and *Cocconeis*.

Autoradiographs made of *A. lobifera* at harvest supported the histological and fine structural observations made by McEnery and Lee (1981) and Koestler et al. (1985) (Figs. 21,23). Ventral sections (Fig. 21) contained more labeled food than dorsal sections of the foram (Fig. 23). At harvest most of the label was in food vacuoles which produced very dense autoradiographs.

Table 8. The distribution of radiocarbon tracer label ingested algae by *Amphisorus hemprichii* incubated on the basis of number of algal equivalents per mg foram*

	Light			Dark			DCMU		
Fraction	Initial	Final	Differ	Initial	Final	Differ	Initial	Final	Differ
Total	6491	2726	3765	4840	2171	2669	6504	5059	1525
Acid	1910	692	1218	1630	1093	537	2165	1381	784
Organic	4402	1818	2600	3075	948	2127	4302	3596	706
Skeleton	178	216	−38	134	130	4	116	82	34
Digested	3128			2700			5662		
Egested	3363			2140			992		
Respired	402			529			603		
Calculated on the basis of number of algal equivalents per mm^2 of estimated feeding area.									
Total	5770	2722	3048	4816	2048	2768	6107	4247	1860
Acid	1698	691	1007	1622	1031	591	2008	1159	819
Organic	3913	1815	2098	3059	894	2165	3990	3019	972
Skeleton	158	216	−57	133	123	11	108	69	39
Digested	2780			2686			5252		
Egested	2989			2129			855		
Respired	357			526			559		

* Algal equivalents means that the total dpm measured per mg of foraminifera has been divided by the counts per individual alga fed.

After 48 hr of incubation with unlabeled food, the label was released and broadly distributed to all regions of the organisms (Fig. 26). Zooxanthellae were located in the cortex of the animal (Fig. 25, arrow). Transfer of ^{14}C label from the foram's food to the symbionts was observed after 48 hr of incubation with unlabeled food. As expected most of the food was digested and the label was broadly distributed (Fig. 28). The symbionts were also very heavily labeled after 72 hr of incubation. Empty diatom frustules of *Cocconeis* and residue from food vacuoles were observed (Fig. 27).

4. Discussion

Radionuclide tracer methodology proved quite useful in providing quantitative data on carbon losses through egestion, respiration, and excretion. The new data correlated well with our own microscopic observations and those of earlier workers (e.g. Myers, 1935; Jepps, 1942, and Buchanan and Hedley, 1960). Both tracer and microscopic methods suggest that a sizable fraction of the potential energy present in items of food is not digested or assimilated. This of course must be considered when drawing up energy

Table 9. The effect of pretreatment on the feeding rates of *Amphisorus hemprichii*

	Sampling Pretreatment hrs	Animal time mm	Algal equivalents eaten or remaining* diameter μg/mg	μg/ind	Surface area mm^2	Feeding area mm^2	Organic volume mm^3
Direct from sea	0	3.26	4.315	10.516	0.526	3.136	5.06
	24	3.46	3.591	10.033	0.449	2.818	4.28
	48	3.22	2.583	6.129	0.313	1.849	3.02
Starved 24 hr in lab	0	3.11	2.866	6.274	0.342	1.961	3.32
	24	2.83	2.763	4.879	0.316	1.676	3.10
	48	3.06	2.761	5.823	0.327	1.850	3.18
	72	3.38	1.692	4.485	0.210	1.289	2.00
Fed cold food 72 hr in lab	0	3.05	6.401	13.423	0.757	4.276	7.37
	24	3.11	6.425	14.071	0.767	4.398	7.44
	48	3.23	4.305	10.336	0.523	3.103	5.04
	72	3.02	3.778	7.749	0.445	2.493	4.34
Starved 72 hr in lab	0	2.97	4.894	9.705	0.573	3.169	5.60
	24	2.73	4.432	7.264	0.499	2.579	4.95
	48	3.48	2.887	8.176	0.362	2.282	3.45
	72	3.02	1.968	4.036	0.232	1.298	2.26

* Algal equivalents means that the total dpm measured per mg of foraminifera has been divided by the counts per individual alga fed.

budgets or when calculating ecological efficiencies.

In our present tracer study of the *A. lobifera*, we obtained clear evidence that a significant fraction of the egested carbon is egested within 24 hr of feeding. The observations by Buchanan and Hedley (1960) on the extrathalmic digestion of small metazoan prey by *Astrorhiza limicola* with residual undigested integuments has to be viewed in a similar light. Still largely unknown are the ranges of digestive efficiencies (assimilation) of foraminifera when they are feeding on different diets.

The results of the tracer experiments just concluded suggest that there was selectivity in feeding by the two foraminiferal species tested. We caution not to infer too much from these results. The experiments were not designed to test this aspect of foram biology. The bias becomes obvious because the algal species tested were deliberately isolated from algal assemblages which supported abundant populations of one or both foraminiferan species. We did not test less abundant species of algae or those which were abundant in

Table 10. The effect of pretreatment on the feeding rates of *Amphistegina lobifera*

	Sampling Pretreatment hrs	Animal time mm	Algal equivalents eaten or remaining* diameter μg/mg	μg/ind	Surface area mm^2	Feeding area mm^2	Organic volume mm^3
Direct from	0	1.37	5.971	4.592	1.395	9.300	86.03
sea	24	1.31	1.976	1.334	0.442	2.945	28.45
	48	1.36	0.903	0.684	0.210	1.399	13.01
Starved	0	1.23	5.650	3.158	1.185	7.902	81.25
24 hr	1.27	1.664	1.027	0.361	2.406	23.94	
in lab	48	1.22	0.687	0.373	0.143	0.951	9.88
	72	1.46	0.520	0.483	0.129	0.863	7.50
Fed cold	0	1.28	4.448	2.802	0.972	6.477	64.01
food	24	1.23	2.045	1.147	0.430	2.864	29.41
72 hr	48	1.48	1.035	1.005	0.262	1.744	14.93
in lab	72	1.43	0.754	0.664	0.184	1.229	10.87
Starved	0	1.28	3.480	2.178	0.759	5.057	50.08
72 hr	24	1.27	1.670	1.015	0.360	2.403	24.03
in lab	48	1.37	1.322	1.013	0.308	2.056	19.05
	72	1.43	0.977	0.865	0.239	1.596	14.09

* Algal equivalents means that the total dpm measured per mg of foraminifera has been divided by the counts per individual alga fed.

assemblages where larger foraminifera were rate or absent. Cause and effect relationships, if there are any, still need to be established. We have very little idea whether foraminiferal grazing can effect algal assemblage population structure. It is possible that certain species of algae are abundant in communities with abundant foraminifera because they have not been eaten by the animals. Since most of the algae tested are eaten and partially assimilated by the two species of foraminifera they seem good candidates for future gnotobiotic nutritional studies. Judging from the results of nutritional experiments with other species of foraminifera (reviewed in Lee, 1974, 1980), food quality is likely to play a role in the growth and fecundity of *A. lobifera* and *A. hemprichii*. This facet of their biology remains as a target for future study.

We found the high levels of uptake of *Entomoneis paludosa* var. *densestriata* by both species of foraminifera rather interesting. One of us (J.J.L.) has identified the alga as the chloroplast donor in prepartions of organisms

made by E. Lanners in her studies of chloroplast husbandry in *Metarotaliella parva* (Lee, 1963, Figs. 14,15). *M. parva* was isolated from the Gulf of Eilat near the H. Steinitz Marine Biological Laboratory. It is possible that *E. paludosa*, which has numerous intercalary bands and is very delicately silicified, is easy prey for foraminiferal pseudopods. One could imagine that pseudopods might gain entry into the diatom through easily spread intercalary bands.

Radionuclide tracer methodology gave us a good quantitative confirmation of microscopic observations on the episodic nature of foraminiferal feeding. The two species studied do not continuously gather food. Microscopic observations suggest that there is a great range in feeding duration among individual animals. Perhaps more relevant is the feeding behavior of the population. Our data suggested that average individuals can gather their daily ration in dense algal cultures in ~3 hr. This experimental set-up might correspond to the algal blooms which we have encountered in our examination of natural communities in which foraminifera are particularly abundant. It is entirely likely that when food is less abundant food gathering would be more prolonged. Although we calculated uptake of algae on the basis of number per mg of foraminifera, per individual, per surface area, per feeding area, and per volume (Tables 2 and 3) some of the method may have more merit for comparative purposes. Many foraminiferal species become more heavily calcified, as they grow toward maturity. While not examined systematically, it is probable that the proportion of test to protoplasm differs among species. Thus inter-specific comparisons of uptake on the basis of weight need to be qualified by the stages of growth and the relative calcification of the species involved. Comparison of uptake on the basis of individual organisms ignores aspects of calcification and geometry and yet can be a meaningful measure when looking at ecosystem processes. Comparisons based on the numbers of algae eaten per surface area, feeding area, volume or organic volume seem quite attractive because they seem closer to characteristics which might affect the biology or physiology of the animal. Even these might be equivocal. For example, while the size of the feeding net might affect the number of food items captured at any moment in time, it might not have any bearing on the total amount of food captured per feeding episode. Forams with smaller nets might simply feed for a longer period of time. In an earlier paper, Lee et al. (1980) suggested that feeding rates might best be calculated on the basis of mg C/mg protein of foram/hr. Since the amount of carbon per alga was calculated rather than actually measured we refrained from using the method for this paper. With appropriate measurements, the latter might be

the most applicable comparative measure. More research on this point would certainly be justified but will not be easy. Diatoms by their very nature and method of division vary in size. The range of sizes for each species depends upon the interval since the last size rejuvination. Additionally, some genera such as *Entomoneis* and *Amphora* have variable volumes because individual specimens have a range of intercalary bands during the growth cycle.

Rough estimates of biomass based on dry weight of the food algae allow us to calculate the amount of food eaten on a dry weight basis. For *Amphisorus* for example, feeding on *Chlorella* of 30 μg algae we have ~2.5 μg food/mg foram. For *Amphisorus* we know that the dry weight of organic matter is roughly 8% of the total dry weight. Hence the feeding rate is ~2.5 μg algae/50 μg organic matter. This suggests that in 3 hr they feed 5% of their total organic weight. Similar calculation for *Amphistegina* feeding on *Chlorella* yields 2 μg/mg foraminifer which have roughly 8% organic matter hence 2.5% of their organic weight per 3 hr. One important conclusion may be that *Amphisorus* depends more on food for its carbon budget than *Amphistegina* (5% vs. 2.5%).

The most interesting observation arising from the tracer feeding experiments is that showing the distribution of the label into the skeleton. Regardless of the species of alga fed, *Amphistegina* incorporated 1.4% of the total algae ingested into the skeletal fraction and *Amphisorus* 4%. This is a small amount considering our estimate that feeding rate is roughly 5%/day of the total organic matter in these foraminifera. However, this is the first direct observation on incorporation of metabolic carbon originating from food into the skeleton.

Despite the large standard deviations it can be seen that in *Amphistegina*, small individuals transfer more food carbon (8–9%) into their skeleton than large individuals (6.6%) regardless of whether they fed on *Chlorella* or on *Cocconeis*. This observation is in good agreement with many observations on carbon isotope fractionations in foraminiferal shells which consistently show that small young individuals show lighter isotopic compositions than large ones (Berger, 1979).

It is not easy to compare the results of the comparative feeding experiment with the earlier tracer study done with the same foraminifera species from the Gulf of Eilat (Lee et al., 1980). In the earlier experiment the animals were starved for a week in either the light or the dark. The organisms offered as food were also not the same as used in the present experiment. Based on the results of our present experiments on the effects of pre-incubation

conditions we would have to regard the experimental animals used by us earlier as considerably stressed and atypical of natural populations.

Evidence gathered in this study on the effects of captivity on feeding by both species of foraminifera lends itself to cautious interpretation. Since *Amphisorus* feed at a higher rate after being kept in the laboratory for 72 hr than when freshly collected it seems reasonable to suggest that they may have been traumatized during collection and separation from their substrate. Even though the animals are raised by trichomes (leaf hairs) so that they are not directly in contact with the broad surfaces of the *Halophila* leaves on which we collected them (Fig. 29) they are strongly attached to the leaves. It often requires very vigorous brushing to dislodge them. Some idea of the extensive pseudopodial net of *Amphisorus* on *Halophila* were illustrated in our previously published SEM photographs (Figs. 10–12, in Lee, 1983). It is possible that the loss of all or much of the extrathalmic cytoplasm during removal from the substrate constitutes enough trauma to reduce feeding capacity. Amphistegina, on the other hand, are less tightly bound to the substrates from which they are harvested. In fact a diver has to be reasonably gentle in order not to loose some of the harvest of *Amphistegina* before it is placed into plastic bags. This might explain why *Amphistegina* fed at high rates just after harvest from the sea but not why they feed less after 72 hr in captivity. In both species we found that starvation did not increase feeding rates. We found just the opposite. Animals fed prior to their use as experimental organisms took up more algae during the experiments. Although this is open to several different interpretations, the simplest is that fed animals are healthier and more vigorous in their activities.

One aspect of the present tracer feeding experiments were disappointing, Dead technique controls (Tables 4,5) were much higher than expected. Since the physical manipulations were as close as possible to our earlier work with the same animals (Lee et al., 1980), we are led to suspect that our method of killing was less desirable for this type of experiment. In our previous experiment we killed the animals in formalin. In the present one we used glutaraldehyde. It is possible that excess glutaraldehyde leached out of the foraminifera during incubation and affected some of the algae making them loose some label. Perhaps the extrathalmic protoplasm is stickier after glutaraldehyde treatment. While this aspect might be studies systematically, it is probably simpler to revert to formalin fixation for controls in future experiments.

Although light mediated processes seem to be an important part of the biology of large foraminifera (cited in introduction) we were unable to demonstrate any direct effects by light on the feeding rates of either species tested. If there are subtler effects we did not detect them by our experimental design. The biological basis for enhanced pseudopodal development and feeding in the presence of DCMU is not obvious to us.

While many aspects still remain to be clarified before detailed carbon budgets can be drawn up for either *Amphisorus* or *Amphistegina* the data obtained in our present experiments seems to be roughly in consonance with our earlier published studies (ter Kuile and Erez, 1984; Lee et al., 1980; Lee and Bock, 1976) and the studies of others (e.g. Hallock, 1978; Duguay, 1983; Duguay and Taylor, 1978).

Growth rate maxima (3–6%/day measured *in situ* at the Gulf of Eilat (ter Kuile and Erez, 1984) seem to be fueled by feeding which shows acquisition of carbon at the same rates (2.5–5%/day) (data this study). The imperforate foraminiferan species studied to date, *A. hemprichii*, *Sorites marginalis*, and *Archaias angulatus* seem to acquire a higher percentage of their carbon budgets by feeding than do the perforte foraminifera, *A. lobifera* and *Heterostegina depressa.* Cultural experiments by Röttger and coworkers (Röttger et al., 1980) suggest that the latter species needs to feed very little and, given proper illumination, symbiont photosynthesis seemed to satisfy the nutritional needs of the host/symbiotic system.

Based on the evidence she obtained in ^{14}C radionuclide tracer experiments, Muller (1978) suggested that *Amphistegina* and their symbionts recycle about half of their carbon and that the animal depends upon its algal symbionts for growth and carbonate production. Paleoecologists interested in stable isotope fractionation will find it significant that *Amphistegina* incorporated 1.4% of the carbonate while *Amphisorus* recycled almost 3 times more (4%) from its food.

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