Order Number 9218232

The biology of *Peneroplis planatus* (Fichtel & Moll) Montfort

Faber, Walter W., Jr., Ph.D.

City University of New York, 1992

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

THE BIOLOGY OF *PENEROPLIS PLANATUS* (FICHTEL & MOLL) MONTFORT

by

WALTER W. FABER JR.

A dissertation submitted to the Graduate Faculty in Biology in Partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1992

This manuscript has been read and accepted for the Graduate Faculty in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

18 Oct 1991
date

Chairman of Examining Committee
Prof. John L. Lee, City College

12 Dec. 1991
date

Executive Officer
Dr. Peter C. Chabora

Prof. John H. Tietjen, City College

Prof. Linda H. Mantel, City College

Prof. Eva K. Hawkins, Mercy College

Prof. O. Roger Anderson, Lamont Doherty Geological Observatory

Supervisory Committee

The City University of New York

Dedicated to the memory of the late
Dr. Allan W. H. Bé,
who introduced me
to the wonders of the foraminifera.

ACKNOWLEDGEMENTS

I am grateful to the director and staff of the H. Steinitz Marine Biological Laboratory - Interuniversity Institute of Eilat, Eilat, Israel, where parts of this research were carried out. I especially thank Drs. Benno ter Kuile, Yehuda Cohen, and Jonathan Erez for all their assistance while in Eilat, Dr. Moritaka Nishihira and Mr. Joji Nakama for collecting and sending specimens from Kudaka Island, Japan, and Jack Downey for his assistance on the transmission electron microscope. I also thank Dr. Eva Hawkins for her discussions with me on *Porphyridium*, and the other members of my committee for their useful comments and suggestions. This research was supported in part by a grant from the Lerner Gray Fund for Marine Biology of the American Museum of Natural History.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

INTRODUCTION

Two characteristics of the endosymbioses of the foraminiferan species *Peneroplis* make these associations unique among all known algal-invertebrate (-protist) symbioses which have been examined. First, the endosymbionts are red algae (morphologically identical to *Porphyridium purpureum* ; Lee, 1990; Hawkins and Lee, 1991). The only other red algal endosymbiont was observed in the radiolarian *Spongaster tetras* (Anderson, personal communication). Secondly, the *Porphyridium* endosymbionts are not surrounded by perialgal vacuoles to separate them from the cytoplasm of the host cell (Lee and Hallock, 1987; Lee, 1990). In all other algal endosymbioses, vacuolar membranes of host origin (symbiosomes) surround the algal cells.

Although experimentation on endosymbiotic associations has increased in the last twenty years, most of the research on the functional biology (physiology and biochemistry) of endosymbioses focused on corals and other coelenterates (see review: Cook, 1983). The original dogma considered the endosymbiont species of all these organisms to be *Symbiodinium microadriaticum* Freudenthal, due to the similarity in gross morphology among the algal cells found in various host organisms. However, the recent increase in research has established several new species of *Symbiodinium* based on cell wall characteristics, karyotypes, physiology, host specificities, and isoenzyme patterns (Schoenberg and Trench, 1980a, b, c; Fitt, *et al.*, 1981; Blank and Trench, 1985 a, b; Blank, 1987; Trench and Blank, 1987).

The situation in the foraminifera is quite different. Many planktonic and benthic foraminifera possess a range of algal endosymbiotic species. In the case of the diatom-bearing forms the range of endosymbiotic species is wider. So far 19 different diatom species have been isolated from a number of foraminiferal hosts (Lee, *et al.*, 1989; ms submitted), although usually only one algal type has been observed within a single host at a particular time (Lee, *et al.*, 1985, 1989; ms submitted). However, the number of species involved is relatively small, even though we do not know the common characteristics of endosymbiotic diatom species. The endosymbionts of the planktonic foraminifera have been identified as dinoflagellates (Spindler and Hemleben, 1980; Spero and Parker, 1985) and chrysophytes (Gastrich, 1986, 1988; Faber, *et al.*, 1988); whereas the benthic foraminifera are associated with a much larger diversity of algal types (dinoflagellates, chlorophytes, diatoms and rhodophytes; see reviews: Leutenegger, 1984; Lee and Anderson, 1991; Lee, 1991).

The superfamily Soriticae (Foraminifera) has three families, each of which possesses a different algal class of symbionts: the Soritidae have dinoflagellate endosymbionts (Müller-Merz and Lee, 1976; Leutenegger, 1977b, 1984; McEnery and Lee, 1981; Lee and Lawrence, 1990); the Archaiadae have chlorophyte endosymbionts (Lee, *et al.*, 1974, 1979); and the Peneroplidae have rhodophyte endosymbionts (Leutenegger, 1984; Lee and Hallock, 1987; Lee, 1990). Adaptation for algal endosymbiosis in foraminifera is considered to have been the driving force in the evolution of the foraminifera (Lee, *et al.*, 1979; Lee and Hallock, 1987). And the fact that all recent larger foraminifera are hosts to such a diversity of algal types suggests that the foraminifera must possess some fundamental characteristics which make them good habitats for the establishment and maintenance of algal endosymbioses (Lee, 1990).

Algal endosymbionts utilize solar radiation to fix carbon by photosynthesis. These reduced carbon compounds can be used for algal respiration, growth and reproduction. The excess can be translocated to the host for its own respiration, growth and reproduction. Also, the host may ingest particulate food, or take in dissolved nutrients by diffusion to supplement the photosynthetically-derived carbon coumpounds, thus supplying the endosymbiotic algae with other necessary nutrients. The flux of ions and molecules during photosynthesis also could produce gradients that promote shell building.Therefore the endosymbiotic relationship could be viewed as a two compartment system, a microecosytem in itself (Reisser, 1986).

The dinoflagellate endosymbiont of the planktonic foraminifer *Orbulina universa* has a relatively high photosynthetic rate *in situ* (Spero and Parker, 1985). When *O. universa* were maintained in laboratory culture (25°C, 200-400 $\mu E\ m^{-2}s^{-1}$ white light, 12 h : 12 H light:dark cycle in f_{20} medium), 75% of the starved specimens (N=11) grew, and nearly half of them matured to gametogenesis (author's unpublished dtata). This implied that the endosymbionts were supplying the host with some nutrition.

The dinoflagellate endosymbionts of another planktonic foraminifer, *Globigerinoides sacculifer* , also enhanced the host's growth and calcification (Bé, *et al.*, 1982). Jørgensen *et al.* (1985) showed that *in situ* the net photosynthesis of the endosymbionts could provide all the reduced carbon required for growth and reproduction of the foraminifer. In contrast, the presence of the endosymbionts in *Globigerinella aequilateralis* did not affect the growth of the host (Faber, *et al.*, 1989). Those *G. aequilateralis* maintained in the dark and fed regularly (one *Artemia* nauplius every day or every other day) grew at the same rate as those fed regularly in the light (Faber, *et al.*, 1989).

In the benthic foraminifera, *Archaias angulatus*, the carbon derived from photosynthesis of the endosymbiont, *Chlamydomonas hedleyi*, was not necessary when external food sources were available (Lee and Zucker, 1969). Nonetheless, photosynthesis of the endosymbiont enhanced carbonate production in the host (Lee and Zucker, 1969; Duguay and Taylor, 1978). In light, the host calcification was directly proportional to the endosymbiont photosynthesis (Duguay and Taylor, 1978).

Within certain ranges, every laboratory experiment on larger foraminifera has shown a positive correlation between light intensity and foraminiferal growth (Lee and Zucker, 1969; Röttger, 1974, Duguay and Taylor, 1978; Muller, 1978; Caron, *et al.*, 1982). Based on *in situ* growth studies on *Amphistegina lobifera* and *Amphisorus hemprichii* , Kuile and Erez (1984) suggested this correlation was due more to some photobiological effect on the foraminiferal calcification and feeding than by the endosymbiont photosynthesis as other researchers have suggested.

Chlamydomonas provasoli, isolated from *Cyclorbiculina compressa*, released into the medium about 5.56% of the total carbon fixed by photosynthesis (Saks, 1982), while the endosymbionts from *Amphisorus hemprichii* and *Amphistegina lessonii* released less than 1% of the photosynthetically fixed carbon into the medium (Saks, 1981). In contrast, *Chlamydomonas hedleyi* releases 57% of its photosynthates into the medium (Lee, *et al.*, 1974; Saks, 1981, 1982). The presence of sterile host homogenate stimulated the release of more photosynthates by isolated diatom endosymbionts in laboratory log phase batch cultures (Lee, *et al.*,

1984).

In an experiment which used a radionuclide tracer, Kremer *et al.* (1980) suggested translocation of photosynthates to *Peneroplis pertusus* from its endosymbiotic *Porphyridium*. The levels of labeled (^{14}C)-floridoside, the storage product of *Porphyridium*, compared well with expected values for photosynthetic fixation of inorganic carbon by *Porphyridium*. Appreciable amounts of ^{14}C-glycerol and ^{14}C-galactose (two substances not usually seen in non-symbiotic red algae) were measured in the foraminifer, suggesting that*Porphyridium* -derived floridoside was metabolized by the foraminifer (Kremer, *et al.*, 1980). This was not proof of translocation since floridoside is composed of glycerol and galactose, and the methodology utilized, besides being rather vague, did not show movement of floridoside from the algae to the host. Nonetheless, this study was the first experimental study on any member of this genus, and it sparked my curiosity about these endosymbioses.

Recent studies on the growth, calcification and carbon flow within the endosymbiotic associations of two larger foraminifera (*Amphistegina lobifera* and *Amphisorus hemprichii*) from the Gulf of Aqaba/Eilat (Kuile and Erez, 1984, 1987, 1991; Kuile, *et al.*, 1987, 1989a,b; Lee, *et al.*, 1988b) prompted me to investigate various aspects of the biology of the *Peneroplis planatus /Porphyridium purpureum* association.

The goal of this study was to expand knowledge of the biology of this foraminifer and its endosymbiotic association:

1. To determine the dietary range and feeding rate of *P. planatus* ;
2. To determine the effect of light and nutrition on the growth of *P. planatus* in the laboratory;
3. To investigate the stability of the endosymbiotic association;
4. To gain insight into the rates of carbon flow in various compartments of the association; and
5. To expand knowledge of the life cycle of *P. planatus* .

CHAPTER 1

THE LIFE CYCLE AND REPRODUCTION OF *PENEROPLIS PLANATUS* (FICHTEL AND MOLL) MONTFORT

INTRODUCTION

Although there has been a flurry of interest in the biology of larger foraminifera in the past two decades (see review: Lee and Anderson, 1991), with some exception (Röttger, 1974, 1978; Leutenegger, 1977a; Kloos and MacGillavry, 1978; Kloos, 1981, 1984; Röttger, *et al.*, 1984, 1986, 1989, 1990a,b) little effort has been expended to increase our knowledge of the complete life cycles of these interesting protozoa. The groundwork for life cycle study of these apparently long lived foraminifera was done by Winter (1907) who studied *Peneroplis pertusus*. Following the "classical" life cycle model (reviewed in Lee, *et al.*, 1991a), which was the only model at the time, Winter described a dimorphic cycle consisting of a regular alternation of generations between a microspheric diploid multinucleate agamont, and a megalospheric haploid uninucleate gamont. Succeeding studies of living foraminifera and fossil remains led to the development of other models (reviewed in Lee, *et al.*, 1991a). Without much direct evidence from the study of living organisms, it has been believed generally that the life cycles of larger foraminifera follow a paratrimorphic model (Leutenegger, 1977a). This model has a morphologically distinct multinucleate diploid generation, the schizont, interposed between the agamontic and gamontic generations. Recent studies of *Heterostegina depressa* suggest that this is a good model for this organism (Röttger, *et al.*, 1984, 1986, 1989, 1990a,b).

Since larger foraminifera is a descriptive term, rather than one with taxonomic meaning (modern species belong to two different suborders and 8 different families), it is reasonable to ask whether they share other common traits. The dearth of additional studies on the life cycle of any species of *Peneroplis* since Winters' classic study (1907) and the easy availability of *Peneroplis planatus* in our lab prompted us to seek new insights into this aspect of their biology.

METHODS

Specimens of *Peneroplis planatus* (Fichtel and Moll) Montfort were collected at the *Halophila* meadow near Wadi Taba, Gulf of Aqaba/Eilat, Egypt, at depths of 10-25 m, from January to March 1988. The collection and initial processing of the foraminifera were previously described (Faber and Lee, 1991). Other specimens were collected from the back reef habitat of Kudaka Island, Okinawa Prefecture, Japan in December 1988 and July 1989. These were transported to New York in insulated plastic food containers.

Nine of the 36 specimens which released megalospheres in the laboratory were placed on microscope slides, decalcified with Poly No-Cal (Polysciences, Inc. # 16865), stained with methylene blue and mounted with Euperol. This procedure made viewing of the organic linings of the juvenile whorl of the test much easier

because they are covered by the second whorl.

Another 190 specimens were fixed in Zenker's solution, which decalcified the shells of the foraminifera. These specimens were rinsed in distilled water, dehydrated in an ethanol series, cleared in xylene and embedded in Tissuemat (Fisher Sci. # 12-647C), a paraffin polyester resin. Thick sections were cut at 7-10 μm, and stained with hematoxylin (Humason, 1962). Measurements were taken under 40X oil phase contrast microscopy and photographed on Kodak Technical Pan Professional Film (2415) on a Zeiss Photomicroscope II. Development was with Kodak HC-110 (dilution D).

RESULTS

Many of the freshly collected *P. planatus* reproduced in the laboratory by multiple fission. The maximum length of the asexually mature specimens from the Gulf of Aqaba/Eilat averaged 1471.6 ± 50.6 μm with a range from 837 to 2154 μm. At the onset of asexual reproduction, the early whorls of the specimens became clear suggesting possible withdrawal of the cytoplasm from this region. The cytoplasm concentrated in the latter whorls where multiple fission took place. Protoconchs or prolocular megalospheres were formed as a result of cytokinesis. The megalospheres were usually cleaved from each other so that they were in double rows within each chamber (Plate 1.1 Fig. 3), but in a few specimens with very large chambers, tetrads were observed. All the megalospheres were pink indicating the presence of the *Porphyridium* endosymbionts at this early stage. This was also observed by Röttger (1981). Before leaving the paternal shell, the megalospheres developed a flexostyle (Plate 1.1 Fig. 3). It is unclear whether calcification occurred at the prolocular stage or after the formation of the flexostyle.

Release of the juveniles occurred primarily at night, although actual release was not observed. Paternal shells appeared decalcified possibly this was a mechanism to obtain the calcium needed for the construction of the juvenile shells but it might also have been the result of a mechanism solely for the release of the offspring. The juveniles broke out along the region of the aperture, along the sides of the chambers, along the septal divisions of the chambers, and in some cases, directly across the brittle, and thin outer chamber walls (Plate 1.1 Fig. 1).

The newly released juveniles measured 62.5 μm in length. No deviation in this size of the juveniles was seen in any of the specimens which released juveniles in the laboratory. The ocular micrometer was calibrated at 15.5 μm per unit, so some variation in the length of the megalosphere of less than one unit may have been overlooked.

The number of juveniles released varied with the overall size of the parent. One specimen which measured 2030 μm released just over 750 juveniles while another large specimen from Japan, which measured 3503 μm, released 1439 juveniles. Based on the measured size of the adults which reproduced in the laboratory, the range in number of juveniles was between 500 and 1500.

At first, the released juveniles remained near the paternal shell, often in the residual food mat left behind by the adult (Plate 1.1 Fig. 2). Within hours they often moved away, and by the next day were spread throughout

the culture flask.

Growth of the juveniles was rapid, regardless whether the specimens were starved or fed algae. Specimens grew one chamber per day for the first week, completing their first whorls with 6 or 7 chambers. Each of these juvenile chambers (nos. 3-7) were usually smaller in size than the megalosphere. After the initial burst of growth, the growth rate decreased as the foraminifera increased in size. After the first whorl, growth averaged one chamber every twelve days (Faber and Lee, 1991).

Seven of the nine parental specimens, which released megalospheres in the laboratory and were studied after decalcification, were identified as megalospheric (Plate 1.1 Fig. 4), hence schizonts, while the other two specimens were microspheric agamonts. The schizonts may have arisen from other schizonts through multiple fission but the agamonts should have been derived from gamonts through sexual reproduction, although this was not observed.

The nuclear picture of the megalospheric and microspheric specimens was similar. Both types were multinucleate, and heterokaryotic (see: Plates 1.2 and 1.3). Small generative nuclei were in the early whorls (Plate 1.1, Fig. 4, Plate 1.2 Figs. 2 and 4, Plate 1.3 Figs. 1-4). Large somatic nuclei were either spherical (Plate 1.2 Fig. 1, Plate 1.3 Fig. 1) or irregularly shaped (Plate 1.2 Figs. 2 and 3, Plate 1.3 Fig. 2), and were always in the outer whorls. Several of the large nuclei appeared to have undergone division (Plate 1.3 Fig. 3). Few of the somatic nuclei were longer than they were wide.This was in contrast with Winter's (1907) illustration of the very long vermiform nucleus that he found in *P. pertusus* .

The examination of 190 specimens which were sectioned for light microscopy, yielded a range of prolocular sizes. The larger proloculi were on the smaller overall sized specimens, and the smaller proloculi on the largest specimens (Fig. 1.1). The relationship between size of the foraminifera and the size of the proloculi had a large area of overlap. These specimens were all alive at the time of collection and may not have reached their full adult sizes. The sizes of the proloculi formed two groups: 18.5 ± 0.3 µm; and 35 ± 1.3 µm, with only 20% of these specimens megalospheric. These measurements were made on the prolocular section with the largest diameter in serially sectioned specimens. The average thickness of the shell wall of *P. planatus* was about 15 µm. Adding the shell wall thickness to the measurements of the sections, the microspheres were about 40 µm and the megalospheres 65 µm in diameter. This latter value corresponded well with the measurements, made in the dissecting microscope, of megalospheres which were released in the laboratory.

The megalospheric specimens all possessed flexostyles and 5-7 chambers in their first whorl. Flexostyles were absent in the microspheric specimens, which had 8-11 chambers in the first whorl. The number of chambers in the first whorl showed no overlap between the megalospheric and microspheric specimens (Figure 1.2).

Cytotomy was also observed in one specimen of *P. planatus* from Japan. The juvenile whorl never emptied of cytoplasm. After releasing megalospheres, the residual cytoplasm expanded to the entire shell. Pseudopodia emerged. The organism began to feed and ultimately doubled in size to an extremely large 4128 µm in length. However, a second reproductive event never occurred, despite maintaining the organism for almost another year.

DISCUSSION

Asexual reproduction was similar between *P. planatus* and *P. pertusus* (Winter, 1907; Röttger, 1981). It is unclear due to changes in the species concepts of peneroplid foraminifera if Winter (1907) was working on *P. pertusus* or *P. planatus*. Based on Rhumbler's (1894) observations on perforations on the prolocular chambers of these foraminifera, Winter explained why he believed that *Nautilus planatus* of Fichtel and Moll (1803), and the *Peneroplis* of Montfort (1808) were the same species as that which was described by Forskål (1775). Cushman's (1917) concept of species in this genus was based on the shapes of the apertures and the ornamentations on the shell walls. Hofker (1951b) used the same criteria to separate *P. pertusus* (Forskål) and *P. planatus* (Fichtel and Moll). Hence, it is possible that Winter (1907) may have studied a mixed population of *P. pertusus* and *P. planatus*. This study distinguishes *P. pertusus* from *P. planatus* based on the ornamentation of the chamber walls: ornamentation of *P. pertusus* consists of shallow striations, with multiple rows of pits, which are sometimes partially fused, whereas the ornamentation of *P. planatus* consists of single to triple rows of irregularly, but distinct, pits (Gudmundsson, 1990).

It is not clear if there are slight differences in interpretation between Winter's (1907) observations and the present study. Cytokinesis occurred first in the *Peneroplis planatus* in the present study, which was followed by growth, and division to form two-chambered embryos (proloculus and flexostyle) prior to emergence from the

Table 1.1: Prolocular size of *Peneroplis planatus* and *P. pertusus*.

	Prolocular Size (µm)		Reference
	megalosphere	microsphere	
Peneroplis	62.5	45	this study
planatus	45-80		Hofker (1951a,1952a)
Peneroplis	27-34		Lister (1895)
pertusus	62	15-26	Winter (1907)
	23-90	10	Hofker (1952a)

parental shell. Winter (1907) described nuclear separation and growth prior to cytokinesis. The number of embryos formed, between 500 to 1500 offspring for *P. planatus* , may be a species characteristic related to the size of the mature adult (Röttger, 1974; Muller, 1974; Hallock, *et al.*, 1986; Pawlowski and Lee, in press).

Both putative "schizonts" and "agamonts" produced megalospheric juveniles in *P. planatus*. However no difference was seen in the prolocular sizes (62.5 µm) of their broods. Previous studies of *Peneroplis* shows the size of the proloculi varied greatly (Table 1.1). All of the megalospheres in the present study corresponded in size to the average size of the proloculus in the megalospheric specimens of Winter's (1907) study.

In some larger foraminifera, the megalosphere of the schizont was reported to be smaller than the megalosphere of the gamont (Hofker, 1950, 1951a,b, 1952a,b, 1953, 1976; Röttger, 1974; Röttger, *et al.*, 1986, 1990a; Krüger, 1990). Whereas in other species of larger foraminifera, the proloculi of the schizont and the gamont were not different (Ross, 1972; Kloos and MacGillavry, 1978; Kloos, 1984; Hallock, *et al.*, 1986). *Peneroplis* may not have bimodal prolocular size separation between the schizont and the gamont. Hofker (1952a) found a wide range of prolocular sizes of the megalospheres of *P. pertusus* (Table 1.1) and observed two peaks, one at 45 µm and the other at 55 µm. Röttger *et al.* (1989) explained the need to observe reproductive behavior in order to properly label specimens as either a schizont or a gamont. Without any empirical evidence, Hofker considered the 45 µm group to be A_2 or schizonts, and the 55 µm group A_1, gamonts. There is substantial overlap in Hofker's (1952a) data, making such assumptions unwarranted at this time. The variation in prolocular diameter may be influenced by as of yet unexamined environmental conditions. Fermont (1977) showed an increase in prolocular diameter with depth in *Heterostegina depressa* .

Sexual reproduction has only been observed in two species of larger foraminifera: *P. pertusus* (Winter, 1907); and *H. depressa* (Röttger, *et al.*, 1984, 1986, 1989). *P. pertusus* produced uniflagellated 1 µm isogametes which fused outside the parental shell (Winter, 1907). The small size of the gametes (< half the size of *H. depressa*) in Winter's (1907) report raises questions about them and begs confirmation. Although sexual reproduction has not been observed in *P. planatus* , the presence of microspheric specimens indirectly supports the probability this aspect of the life cycle will be discovered.

A surprisingly large number of the specimens sectioned in this study were microspheres. Hofker (1951b) only found megalospheric *P. planatus* , although he admitted small sample sizes precluded the use of statistics.

Gudmundsson (1990) examined over two thousand *P. planatus* , dissected 1.3% of them, and found only megalospheres. Although Gudmundsson (1990) did not actually measure the prolocular size for *P. planatus*, one of micrographs in his study showed a proloculus, flexostyle and first whorl from which the proloculus measured 68 µm. The lack of microspheric specimens in sediments, which were studied by Gudmundsson (1990), may have reflected seasonality of reproduction. Although *P. planatus* was often considered a minor constituent of foraminiferal assemblages, asexual reproduction seemed to occur once a year in the Spring (Faber, 1991). Megalospheric specimens that were released between February and June, predominated the assemblage of living *P. planatus* after their reproduction in the laboratory. The specimens collected between January and March, prior to reproduction, were microspheric. Since a second generation of this species never has been observed in the laboratory, the seasonal or yearly influences on the life cycle remain unexplained.

Because the parental shells become thin and brittle during the release of the megalospheric embryos, they may have been preserved poorly in the sediments. Paleontologists usually look at whole shells, rather than fragments, and hence the microspheric agamont and megalospheric schizont phases may be underrepresented in sediment samples. On the other hand, gamonts which release gametes through the apertures (Winter, 1907), leave behind intact megalospheric shells. This may also explain the lack of bimodality between usually smaller megalospheric schizonts and gamonts often seen in the size of the megalospheres.

Even if some residual cytoplasm remains, asexual or sexual reproduction by a foraminifer usually ends the life of the parental cell. Although cytotomy is common in the life cycles of some species of foraminifera (eg. Arnold, 1955; Lee and Pierce, 1963; Lee, *et al.*, 1969; McEnery and Lee, 1976), it is a rare asexual life cycle pathway in species of larger foraminifera. In this study, cytotomy was observed in one specimen of *P. planatus*. Röttger (1978) reported cytotomy in *Heterostegina depressa* where several individuals continued to live and grow after reproduction. One organism reproduced asexually a second time although the juveniles were irregular and failed to develop. Cytotomy also has been observed in studies of *Sorites orbiculus* , where it formed normal cyclic chambers after forming brood chambers (Kloos, 1981).

The nuclear picture that emerged from this study involved only multinucleate and heterokaryotic specimens. This has been observed in other species of larger foraminifera (Müller-Merz and Lee, 1976; McEnery and Lee, 1981). If one considers the definitions of the three phases of a paratrimorphic life cycle to be valid (Lee, *et al.*, 1991a), then the megalospheric specimens of this study were all schizonts, since gamonts, which are

megalospheric, would have been uninucleate. Most probably the specimens studied by Winter were schizonts and not gamonts. If this turns out to be so, then there is some confusion about what is a cytological picture of a gamont in this species. Winter (1907) described the presence of what now would be considered small generative nuclei and large somatic nuclei in the gamont of *P. pertusus* (Röttger, *et al.*, 1989). Winter even describes nuclear reproduction of the large nuclei, and Röttger and others (1989) considers large nuclei to be a common feature of all larger foraminifera. It is difficult to make more definitive statements until more cytological work is done on peneroplids. As previously noted, the specimens of this study were collected just prior to the major spring asexual reproductive event in the Gulf of Aqaba/ Eilat. One would expect a predominance in the natural assemblage of specimens which undergo asexual reproduction (agamonts and schizonts) at that time of year.

Direct observations of schizonts and agamonts releasing megalospheres suggests that trimorphic or paratrimorphic life cycle models seem to be applicable to *Peneroplis planatus* . The presence of gamonts has not been definitively shown, and is implied in the natural population only by the occurrence of the agamonts, which have microspheric proloculi. The small size of the proloculus implies fusion of gametes to form zygotes which later must acquire endosymbionts (4.91 μm in diameter). Sexual reproduction must occur but the time of year when it occurs in nature has not been determined. Specimens of the natural population must be collected and sectioned during other times of the year (eg. late summer to winter) to demonstrate the sexual phase of the life cycle. One would have to observe the reproductive behavior of successive generations in order to determine whether the cycle is trimorphic, or paratrimorphic or some other variant. As we improve our knowledge of the biology of these organisms, we may better our chances for maintaining them long enough to resolve these life cycle questions.

EXPLANATION OF FIGURES

Fig. 1.1. Size variation in proloculi in *Peneroplis planatus* as a function of the size of the foraminifera.

Fig. 1.2. Number of chambers in the first whorl in *Peneroplis planatus* as a function of the size of the proloculi.

Plate 1.1.

Fig. 1. Megalospheric juveniles of *Peneroplis planatus* beginning to emerge from the parental shell. Scale bar = 200 μm.

Fig. 2. The juveniles remain near the parental shell in the residual food mat after emerging from the parental shell. Many of the juveniles already have four or five chambers (arrows). Scale bar = 200 μm.

Fig. 3. A flexostyle has already developed on the megalospheric juveniles while inside the maternal shell. Scale bar = 100 μm.

Fig. 4. Prolocular section through a schizont. Specimen hydrolyzed for feulgen reaction, stained with Schiff's reagent and counterstained in fast green. Scale bar = 10 μm.

Plate 1.2. Transprolocular sections through schizonts of *Peneroplis planatus*. All specimens hydrolyzed for feulgen reaction, stained with Schiff's reagent and counterstained with fast green.

Fig. 1. Large somatic nucleus (arrow) in the outer whorl. Scale bar = 100 μm.

Fig. 2. Several micronuclei seen throughout the specimen. Scale bar = 100 μm.

Fig. 3. Close-up of another large somatic nucleus (arrow). Scale bar = 10 μm.

Fig. 4 Close-up of small micronuclei. Scale bar = 100 μm.

Plate 1.3. Transprolocular sections through agamonts of *Peneroplis planatus*. All specimens hydrolyzed for feulgen reaction, stained with Schiff's reagent and counterstained with fast green.

Fig. 1. Large somatic nucleus (s) in outer whorl with a small generative nucleus (g) seen in the inner whorl. Scale bar = 10 μm.

Fig. 2. Multiple somatic nuclei (s) seen in the outer whorl. Scale bar = 50 μm.

Fig. 3. Somatic nuclei (s) appear to have undergone division. Also seen in the section are generative nuclei (g) and endosymbionts (sy). Scale bar = 100 μm.

Fig. 4. Close-up of generative nucleus (g). Scale bar = 10 μm.

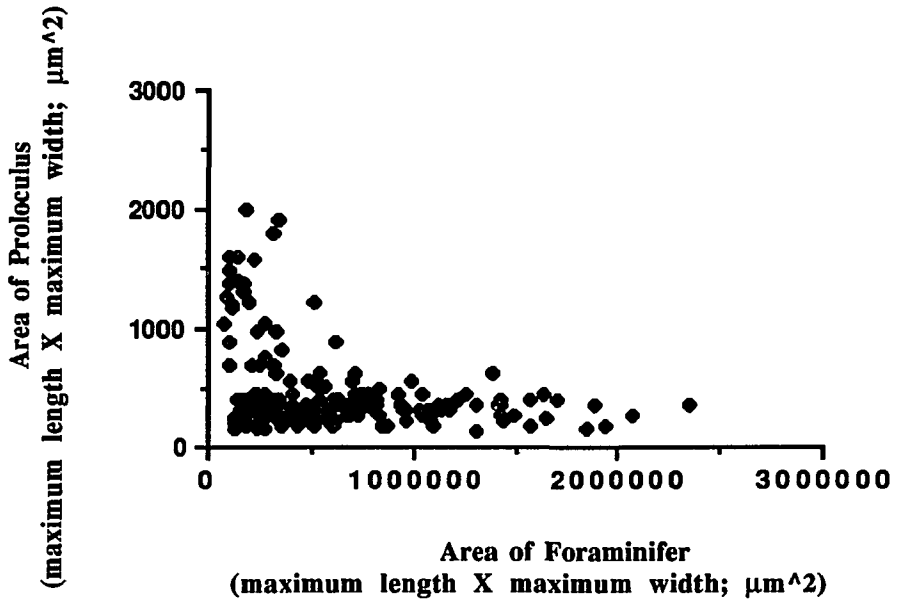


Figure 1.1

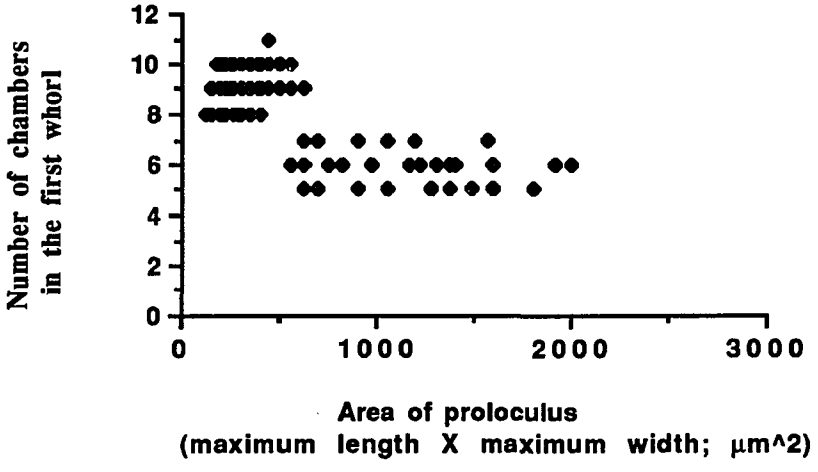


Figure 1.2

Plate 1.1

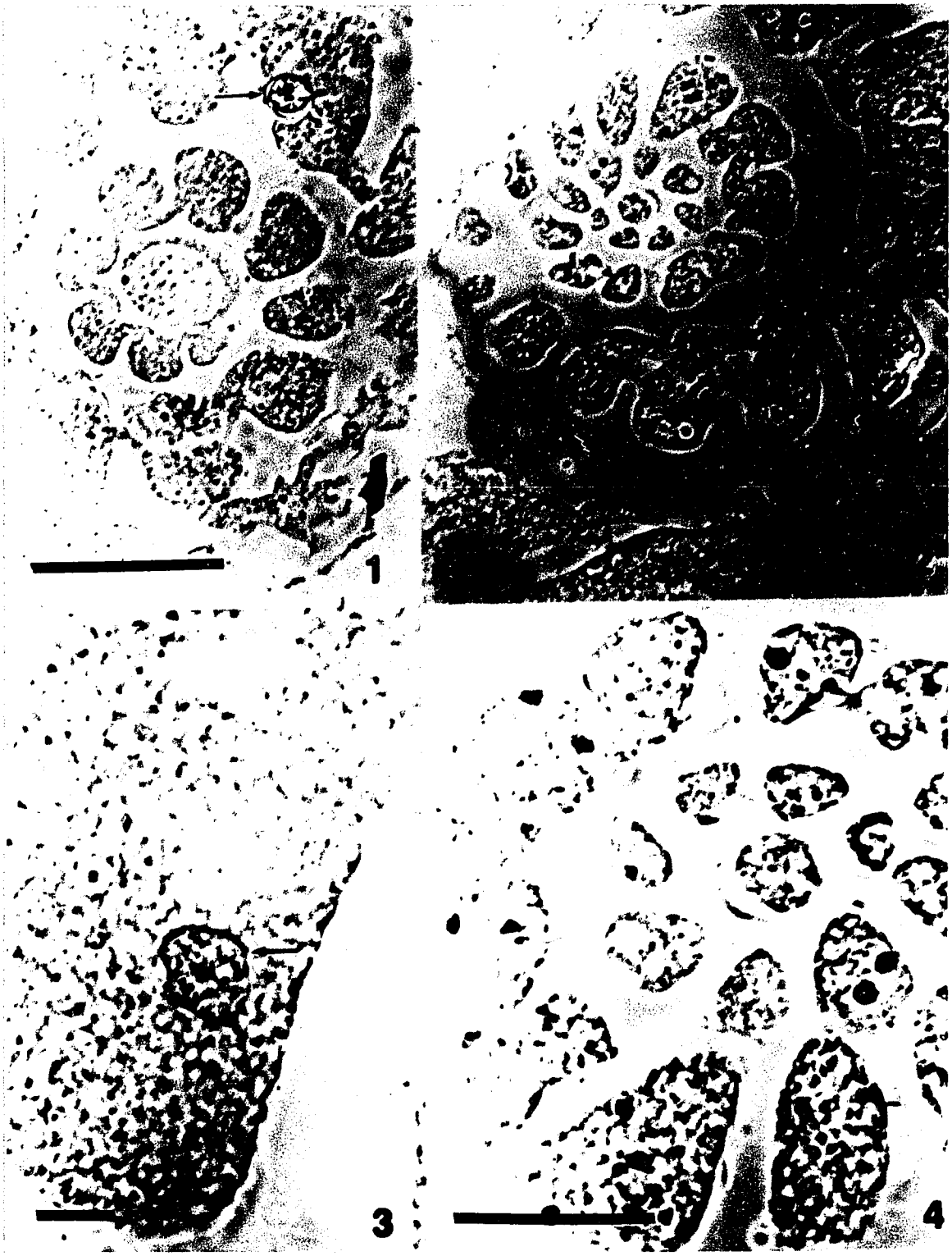


Plate 1.2

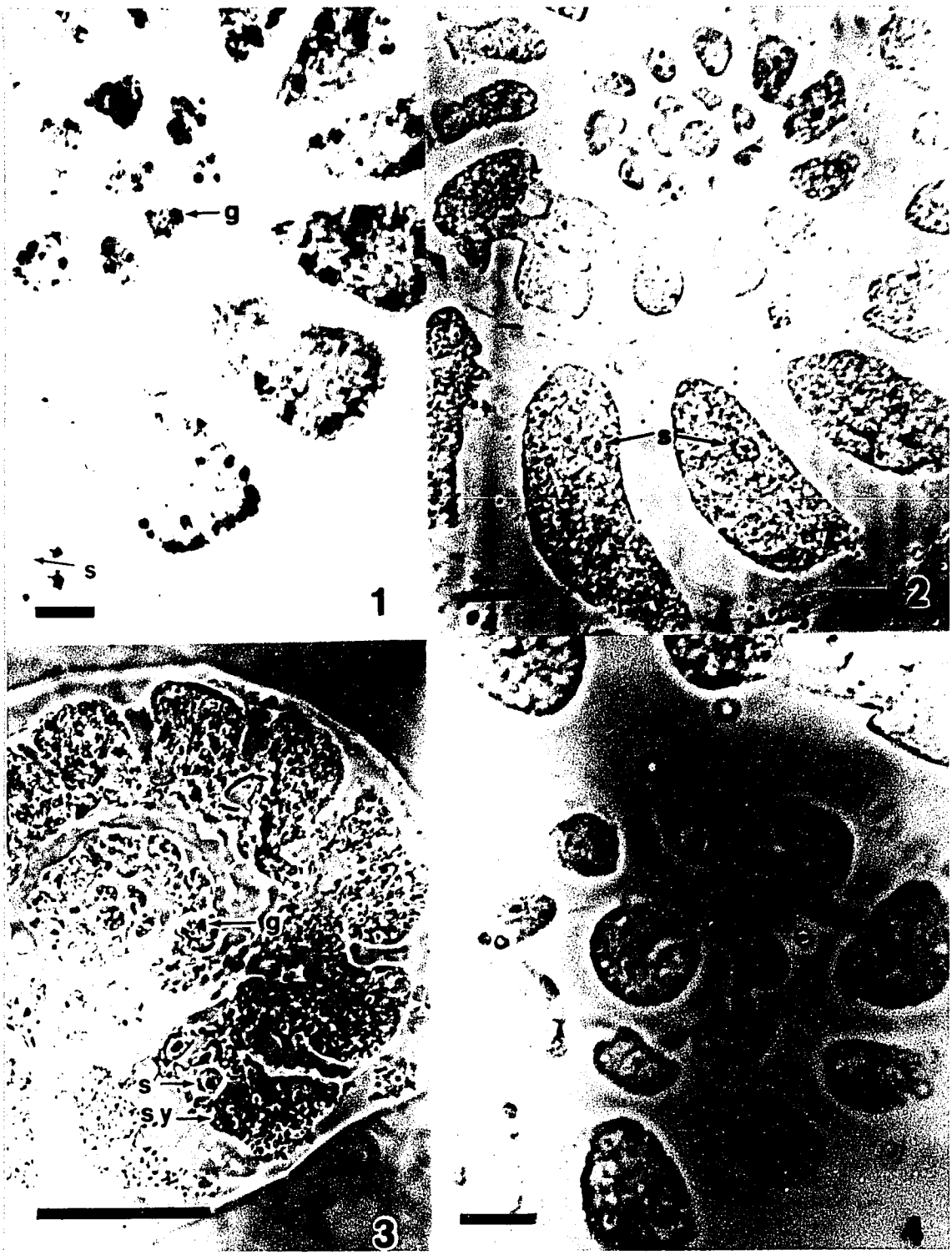


Plate 1.3

CHAPTER 2

STABILITY OF THE ENDOSYMBIOSIS BETWEEN *PORPHYRIDIUM PURPUREUM* (RHODOPHYTE) AND *PENEROPLIS PLANATUS* (FORAMINIFERA)

INTRODUCTION

The maintenance of an endosymbiotic association involves interactions between the host and the endosymbiont, which affect the life processes of the both partners and regulate the ecological fitness of association in the ecosystem (Reisser, 1988). In a stable association, as the host grows, the endosymbiont population must also increase (Muscatine and Pool, 1979). In foraminiferal-algal associations, as the foraminifer grows, an increase in the number of algal cells is only possible by sequestration of additional algal cells or by the growth of the population within the foraminifer. The former is unlikely because of the rarity of free-living conspecifics of the endosymbiont species in the environments where the foraminifera are found (for example see: Lee, *et al.*, 1988b,1989). A high correlation between the size of the foraminifera and the total numbers of symbionts implies reproduction of the algal cells within the foraminifera (Lee, *et al.*, 1983, 1985; Spero and Parker, 1985; Faber, *et al.*, 1988).

The growth of benthic foraminifera is episodic and slows down as the foraminifera increase in size (Chapters 6 and 7). When foraminiferal growth is reduced, the endosymbiont population must be regulated to prevent overgrowth of the algal cells. Such regulation is necessary to maintain a stable endosymbiosis (Taylor, *et al.*, 1989). The foraminifer has only three options available to regulate the endosymbiont population: 1. expulsion of excess endosymbionts; 2. digestion of excess endosymbionts; and 3. inhibition of the endosymbiont growth and reproduction (Reisser, 1988; Taylor, *et al.*, 1989).

Expulsion of the endosymbionts implies the release of viable algal cells into the environment, and has not been shown conclusively for any foraminifera. The only time when expulsion may be a possibility for foraminifera, is when foraminifera undergo gamete formation, since the algal endosymbionts tend to be larger than the gametes. Planktonic foraminifera expel their endosymbionts prior to gametogenesis, however, many of the expelled endosymbionts appeared fully or partially digested (Bé, *et al.*, 1983). The viability of the undigested expelled endosymbionts has never been established. And presumably, the endosymbionts were digested not as a form of regulation but as a mechanism to obtain the extra energy needed for reproduction (Bé, *et al.*, 1981).

In establishing an endosymbiosis, the algal cells initially must escape digestion by the foraminifer. They may accomplish this by inhibiting the digestive enzyme of the host, or by preventing host lysosome fusion with the vacuolar membrane (Muscatine, *et al.*, 1975). Foraminifera, as a rule, digest their food extracamerally so that once the potential endosymbionts pass into the test without the phagosomal vacuole being converted into a phagolysosomal vacuole, they are "safe" within the host cell. Evidence for this idea is based on acid

phosphatase assays on whole foraminifera (Lee, *et al.*, 1991b). The digestive enzymes were found in the external granuloreticulopodal network and not in the same regions within the foraminiferal cells as the endosymbionts. Periodic digestion of the endosymbionts as a means of regulation seems improbable unless the endosymbionts are expelled first into the external pseudopodial network. Nonetheless, planktonic foraminifera appear to digest their endosymbionts during host starvation (Caron, *et al.*, 1982; Faber, *et al.*, 1988). It is not clear in this situation whether the host is actually digesting some of its endosymbionts or whether they are senescing or autolysing.

Inhibition of endosymbiont growth and reproduction by the foraminiferal host seems the most likely means of regulation by the host, although this hasn't been proven. The host may secrete an algal growth inhibitor, secrete a substance which stimulates the release of organic materials from the endosymbionts which are necessary for algal reproduction (Taylor, *et al.*, 1989). Foraminiferal homogenate stimulated isolated axenic endosymbiotic diatoms to release more photosynthates in log phase batch cultures (Lee, *et al.*, 1984). Whether the foraminifer is producing another substance which could inhibit endosymbiont growth is unknown. Also, the host may control the algal population by denying the endosymbionts access to limiting nutrients (Taylor, *et al.*, 1989). Isolated diatom endosymbionts in batch culture exhibited optimum growth at levels of nitrogen and phosphorus which were several orders of magnitude greater than the concentrations of these nutrients in the environments where the foraminiferal hosts were collected (Lee, *et al.*, 1980a). Therefore the endosymbionts are constantly nutrient starved in their host. Nutrients must reach the endosymbionts after passage through the host membranes and cytoplasm. It is certainly conceivable that the symbiosome could be a nutrient regulating membrane. However, since the *Porphyridium* is not surrounded by a symbiosome, some other mechanism may be in operation within *Peneroplis*.

Benthic foraminifera are hosts to a wide range of endosymbionts (see review: Lee and Anderson, 1991). The endosymbionts of *Peneroplis arietinus, P. acicularis, P. pertusus and P. planatus* are unique among all the algal-invertebrate and -protist symbioses known since they are rhodophytes (Leutenegger, 1977b,1984; Lee and Hallock, 1988) morphologically identical to *Porphyridium purpureum* (Lee, 1990; Hawkins and Lee, 1991).

All peneroplids collected in nature possess endosymbionts. Except for one questionable observation (Winter, 1907), sexual reproduction of these species has not been observed and is considered rare (Hofker, 1951b). In asexual reproduction, the juveniles formed by multiple fission of the the agamont or schizont possess endosymbionts prior to their expulsion from the parent cell (Winter, 1907; Röttger, 1981; Chapter 8), hence the problems of recognition and establishment of the endosymbiosis are eliminated.

Another unique facet of the *Peneroplis/Porphyridium* association is the lack of perialgal vacuoles around the endosymbionts which lie free in the cytosol of the host (Lee and Hallock, 1987; Lee, 1990). The relationship between *Porphyridium* and *Peneroplis* may be quite different from other algal-foraminiferal associations. The aims of this research were: 1. To study the stability of the endosymbiosis in *Peneroplis planatus*; 2. To examine the possibility of reestablishing the association after rendering the specimens aposymbiotic.

METHODS

Between January and March 1988, *Peneroplis planatus* (Fichtel and Moll) Montfort were collected at the *Halophila* meadow near Wadi Taba, Elat, Israel, at depths from 10-25 meters. The collecting and processing of the foraminifera followed Kuile and Erez (1984) and were previously explained (Faber and Lee, 1991). Freshly collected specimens, which exhibited rhizopodal activity, were thoroughly brushed to eliminate adhered food particles and debris, then placed into the various experimental treatments.

Endosymbiont stability experiment

Groups of 40 individuals were placed into deep petri dishes with 200 ml of sterile Millipore-filtered (0.44μm) seawater (sfsw). The seawater was collected from the Gulf of Elat in front of the H. Steinitz Marine Biological Laboratory. Incubations were carried out on a shelf near an east facing window where incident light levels averaged daily 20-70 μE $m^{-2}sec^{-1}$. The room temperature was maintained at 24 ± 1°C. Specimens were harvested at t = 1, 2, 3, 4, 5, 7, 14, 21 and 28 days by either fixing them in Zenker's solution for light microscopy (LM) or in 5% glutaraldehyde, post-fixed in 2% osmium tetraoxide for transmission electron microscopy (TEM).

Reestablishment of endosymbionts experiment

To render the *Peneroplis planatus* aposymbiotic, specimens were either placed in 10^{-5} M DCMU (3-(3,4-dichlorophenyl)-1,1 dimethyl urea) for a 72 hour incubation in continuous light (40-60 μE $m^{-2}sec^{-1}$), or placed in continuous darkness for 3 weeks. After incubation, the specimens were brushed and rinsed with sfsw, then divided into groups of 20 individuals in petri dishes with 50 ml of sfsw. Five feeding regimes were utilized:

1. Starved;
2. Fed cultured endosymbionts (Pen-3);
3. Fed *Dunaliella salina* ;
4. Fed *Dunaliella salina* and cultured endosymbionts (Pen-3);
5. Fed *Porphyridium purpureum* (UTEX 161).

Three light regimes were utilized for the DCMU treated specimens. The specimens preincubated in the dark for 3 weeks were placed only in the first two regimes due to the small number of specimens available in the field:

1. Continuous light, 40-60 μE $m^{-2}sec^{-1}$;
2. Continuous darkness;
3. Ambient light and photoperiod, placed on shelf near an east facing window (approximately the same light intensity as at 25 m in the Gulf of Aqaba/Eilat).

After 12 days, all these specimens were harvested by fixing half in Zenker's solution for LM, and the rest in 5% glutaraldehyde, post-fixed in 2% osmium tetraoxide for TEM.

Light microscopic specimens

The acetic acid in the Zenker's solution decalcified the shells of the foraminifera within 24 hours, at which time the specimens were rinsed in distilled water and dehydrated in a graded series of ethanol to 70 % ETOH. These specimens were transported back to the laboratory in New York, and dehydrated to 100% ETOH, cleared with xylene and embedded in Tissuemat (Fisher Sci. # 12-647C), a paraffin polyester resin. Thick sections were cut at 7-10 μm. The specimens for the stability experiment were stained with hematoxylin (Humason, 1962). The specimens for the reestablishment experiment were hydrolyzed for a feulgen reaction and stained using Schiff's reagent and counterstained with fast green (Humason, 1962). Symbiont counts and measurements were made under 40x oil phase contrast microscopy, and photographed on a Zeiss Photomicroscope II using Kodak technical pan film 2415 and developed with Kodak HC-110 (dilution D).

Transmission electron microscopy

Specimens for examination in TEM were processed according to the procedures of Anderson and Bé (1976). The specimens were dehydrated in acetone and embed in LX-112 (Ladd Research Industries, Inc. #21210). Thin sections (70-90 nm) were cut with a diamond knife on a Reichert-Jung Ultracut E. The sections were examined and photographed on a Philips EM 300.

Calculations and statistical analyses

The shell of *P. planatus* resembles an ellipse (Faber and Lee, 1991) with a relative uniform thickness which is established early in the ontology of the organism (Hallock, 1979), hence size measurements consisted of the greater axis (maximum length) and the lesser axis (maximum width perpendicular to the greater axis). Transverse sections were located which passed through the prolocular chamber of each foraminifer.

The specimens sacrificed on the first day were viewed through a gridded reticle. The number of endosymbionts were counted in each quadrat that was completely filled by cytoplasm. Indices of dispersion were calculated for each of 15 specimens by dividing the variance between the counts by the mean number of endosymbionts in each quadrat, and the randomness of the indices were tested by a chi-square for a Poisson distribution. A random distribution would yield a dispersion close to unity (d=1.00). The dispersion values of the endosymbionts within *Peneroplis planatus* ranged from 0.52 to 1.50 (Table 2.1). The chi-square values for 11 of the 15 specimens confirmed a random distribution of the endosymbionts within the foraminifera (Table 2.1), since the chi-square differences were less than would have occurred by chance at $P = 0.05$. The other four specimens yielded values which were significant at $P \leq 0.05$. However, at $P = 0.01$, these values were not significant. With nearly three fourths of the specimens confirming randomness, I assumed all the endosymbionts of *P. planatus* were random throughout the host. The four specimens which lacked randomness (three tending toward a uniform dispersion and one toward an aggregated dispersion) may reflect the

movement of the endosymbionts within the cytoplasm of the host, or be due to biases related to the examination of only transprolocular sections.

Numbering the proloculus as chamber one, symbiont counts were made of chambers 7, 10, 14 and 21. Generally for both megalospheric and microspheric specimens, these chambers represented the first, second and third whorls of the foraminifer. The number of endosymbionts counted were pooled and divided by the overall surface areas of these chambers to obtain a value for the number of endosymbionts μm^{-2} foraminiferal cytoplasm.

Light micrographs were taken of 21 specimens which were sacrificed on the first day. Prints on Kodak Polyprint FM paper were then cut out along the outer organic lining, and the inner chamber areas (cytoplasm and endosymbiont fractions) were separated from the shell areas (shell fraction). Assuming a uniform weight throughout the photograph and the photographic paper, the shell and cytoplasm fractions were weighed on a Mettler H16 balance. A ratio of cytoplasmic area to overall foraminiferal area was calculated to be an overall mean of 0.535 ± 0.013. This ratio was multiplied by the overall area of the foraminifera and multiplied by the number of endosymbionts per μm^2 foraminiferal cytoplasm to estimate the overall number of endosymbionts.

Cricket Graph 1.10 (Cricket Software Inc.) was utilized to plot the data and calculate regressions. Analyses of variance were utilized to compare the endosymbiont densities for the stability and reestablishment experiments (SAS Institute Inc., Cary, North Carolina).

RESULTS

The endosymbionts are randomly distributed within all the chambers (except the ultimate and penultimate) of *Peneroplis planatus* (Plate 2.1 Fig. 1, Table 2.1) which is in agreement with previous observations on this genus (Leutenegger, 1977b). The randomness of endosymbiont distribution justified the sampling of endosymbionts in representative chambers as indicative of the entire organism. In specimens sacrificed on the first day, the endosymbiont densities were not significantly different as a function of host size (mean = $19.4 \pm 1.52 \times 10^{-3}$ endosymbionts μm^{-2} foraminiferal cytoplasmic area; $F = 0.10$, $P > 0.01$, $df = 1,28$). Since the density of endosymbionts is not influenced by the foraminifer's size, the population of endosymbionts increases as the foraminifer grows. A larger foraminifer possesses a larger endosymbiont population (Figure 2.1). Based on these estimates of endosymbiont densities and the actual sizes of the foraminifera, the endosymbionts occupy $\sim 49.4 \pm 3.31$ % of the foraminiferal cytoplasm.

The specimens starved in the laboratory showed an overall decrease in endosymbiont density with time (Fig. 2.2). Although the slope of the regression could be plotted, it was not a smooth one (Fig. 2.2). It was incrementally stepped as indicated by the significance between sequential days (Table 2.2).

As starvation continues, the foraminifera appear more vacuolated (Plate 2.1 Figs. 3 and 4).The endosymbionts also appear more vacuolated (Plate 2.1 Figs. 5 and 6). By the fourth week, many of the endosymbionts degenerated (Plate 2.2 Fig. 3-6). They appeared to have either undergone autolysis or had been digested by the host, although the later was not confirmed. None-the-less, somewhat "normal" looking

endosymbionts were still found (Plate 2.2 Fig. 2). They varied from the normal (Lee, 1990) by being much more vacuolated, by having reduced sheaths, and a less dense halo around each endosymbiont. The cytoplasm became less dense as starvation progressed.

Both the DCMU and complete darkness treatments reduced the endosymbiont population but neither eliminated it. Those specimens treated with DCMU, and then starved, had approximately 30 % of the normal endosymbiont density (Table 2.3). There was no statistical difference between populations treated with DCMU, starved, and incubated in the various light regimes (Table 2.4). Populations pretreated in complete darkness and then incubated in continuous light had endosymbiont densities similar to the starved populations, whereas populations which were kept in prolonged continuous darkness, but fed, seemed to have endosymbiont populations which were at or above the normal level on an endosymbiont:cytoplasm ratio (Table 2.3). However, the total volume of the cytoplasm shrank away from the outer chambers (Plate 2.2 Fig. 1).

All the treated specimens (DCMU, complete darkness, starved or fed) which were returned to the light, regardless of the initial treatment or subsequent feeding, averaged between 25 and 50 % of the normal endosymbiont density ($19.4 \pm 1.52 \times 10^{-3}$ endodosymbionts μm^{-1} foraminiferal cytoplasmic area; Table 2.3), however, none were significantly different from each other ($F = 2.27$, $P > 0.05$, $df = 5,59$). Most of the endosymbionts appeared "normal", although some degenerated. These reduced endosymbiont populations did not seem to increase when the host had the possibility of feeding on *Porphyridium* or benefit from nutrients obtained from food organisms consumed by the hosts incubated in continuous light. A similar picture emerged in specimens incubated in light with a normal photoperiod. The exception was specimens fed a unialgal diet of *Dunaliella salina*. They had a statistically higher density of endosymbionts (Table 2.3, 2.4). Even though the number of endosymbionts increased, some degenerated endosymbionts also were found in this fed population.

DISCUSSION

Stability of an endosymbiotic association requires specific and necessary interactions between the members of the association to maintain it. There is an implication of some form (positive or negative) of endosymbiont regulation to prevent overgrowth of the endosymbiont population. In the *Peneroplis planatus*/ *Porphyridium purpureum* endosymbiosis, there was a strong correlation between the total number of endosymbionts and the size of the foraminiferal host in specimens collected from the field (Fig. 2.1). A constant ratio of endosymbiont biomass to host cell biomass under a particular set of conditions, as has been seen in other foraminiferal-algal endosymbioses (Spero and Parker, 1985; Faber, *et al.*, 1988), is strong evidence of endosymbiotic regulation (Taylor, *et al.*, 1989).

The *Porphyridium* endosymbionts survived within their peneroplid host even when the host was stressed for 2 weeks. Darkness, starvation and herbicide treatment, all reduced the overall abundance of endosymbionts with time (Figure 2.2, Table 2.3). However, many of the endosymbionts appeared "normal" after each of these stresses. Studies of the diatom endosymbionts of *Amphistegina lessonii* showed that the same stresses reduced the endosymbiont populations, but the diatom cells were able to survive more than 6 weeks in the dark, and

after the stress, divide and repopulate their host (Lee, *et al.*, 1983, 1986; Koestler, *et al.*, 1985; Lee, *et al.*, 1991c). In *P. planatus*, the endosymbiont population also remained viable after the stresses to the host, however, unlike in the populations of endosymbionts in *Amphistegina lessonii* reported earlier (Lee *et al.*, 1986), we did not observe any sections which had obvious endosymbionts in stages of division while repopulating the foraminifer. Infuture studies *P. planatus* could be incubated with tritiated thymidine after treatment with the various stresses. If the endosymbiont populations were in normal cell cycles, the tritiated thymidine would be incorporated during the S phase, and autoradiographic analysis of these specimens would demonstrate a concentration of the label over the endosymbiont nuclei.

Continuous darkness, as a stress, had a detrimental effect. *P. planatus* failed to grow in complete darkness, even when fed external algal food (Faber and Lee, 1991). These same food organisms promoted growth in the species when specimens were placed in a light/dark cycle (Faber and Lee, 1991). Darkness may reduce the overall intake of external algal food by the foraminifer by reducing the rhizopodial web of the foraminifer. A future tracer feeding experiment with *P. planatus* incubated in the light and in the dark could be designed to test this idea. Darkness prevented the endosymbionts from photosynthesizing. The cytoplasm and total numbers of endosymbionts were reduced but the endosymbiont population was reduced proportionally even after 33 days in the dark. Bisalputra and Antia (1980) placed batch cultures of a free living isolate of *Porphyridium purpureum* into complete darkness for 8 weeks, and found no increase in mortality of the population. Shutting off of photosynthesis caused most of the cells to become dormant with minimal disruption of cellular structures, and a high capacity for rapid recovery upon restoration of light (Bisalputra and Antia, 1980). Within 72 hours of reillumination, the dormant cells reverted to "normal" cells (Bisalputra and Antia, 1980). A similar adaptive mechanism might have operated within populations of endosymbiotic *P. purpureum* while the host/endosymbiont systems were in continuous darkness. Most of the endosymbionts might have become dormant, and a proportional number of endosymbionts might have undergone autolysis or digestion. This would explain the reduction in cytoplasm which was previously seen in starved specimens (Faber and Lee, 1991). The details of this could be clarified by more extensive histochemical and fine structural analyses. The specimens examined in this study showed a loss of ER and granules, and an increase in cytoplasmic vacuolation.

The densities of the endosymbionts (cells/μm^2) in most of the specimens incubated in the darkness were the same or greater than as in specimens taken from the field (Table 2.3). In synchronous 12 h light/dark light pulsed cultures *Porphyridium purpureum* divides at the beginning of the dark phase (Gense, *et al.*, 1969; Knappe, 1972). However, earlier Jones *et al.* (1963) found that division took place during the light. None of these studies duplicated the conditions which we were using. The effect of continuous darkness as reported by Bisalputra and Antia (1980) is a more appropriate model. A better explanation for the increase in the density of the endosymbionts in the specimens incubated in the darkness would be a reduction of the host cytoplasmic volume which concentrated the endosymbiont population. The host appeared to be digesting its own cytoplasm while the endosymbiont population was diminishing at a slower rate. Although the method utilized in this study for enumerating endosymbionts by sampling specific chambers and extrapolating for the entire organism

was valid in estimating the overall endosymbiont population in unstressed, recently collected specimens, the method might over- or under-estimate the population when the density of the host cytoplasm changes. Future work should employ additional methods, such as the amount of chlorophyll, or chlorophyll to protein ratios, to increase the accuracy of estimates of endosymbiont numbers and their functional potential in the endosymbiont system.

The effects of prolonged starvation were similar to the effects of prolonged incubation in complete darkness, by reducing the total endosymbiont population (Fig. 2.2), and reducing host cytoplasm by becoming more vacuolated. External algal food is necessary for foraminiferal growth, and may provide other required nutrients (Faber and Lee, 1991), even though the endosymbionts can fix enough carbon to satisfy the energetic demands of the association (Chapter 8).

In attempts to render the specimens aposymbiotic by treatment with the herbicide DCMU (3-(3,4-dichlorophenyl)-1,1 dimethyl urea), the endosymbiont population was reduced but not eliminated (Table 2.3). These results were similar to those obtained in studies of *Amphistegina lessonii* when it was incubated in DCMU (the same concentration as this study) for 120 hours (Lee, *et al.*, 1983; Koestler, *et al.*, 1985). Since the endosymbionts can survive prolonged periods of darkness by becoming dormant, shutting off photosynthesis with a herbicide may have a similar response. DCMU inhibited photosystem II, thus shutting off photosynthesis in the endosymbionts but DCMU also increased rhizopodial activity in *Amphistegina lobifera* and *Amphisorus hemprichii* (Lee, *et al.*, 1988b). Experiments on planktonic species of foraminifer utilizing DCMU at the same concentration have encountered problems (Faber, *et al.*, 1988). This physiological and possibly detrimental effect on the planktonic foraminifer, *Globigerinella aequilateralis* , by DCMU, has not been studied in detail and suggests that DCMU may not be a good choice as a photosynthetic inhibitor in all algal/foraminiferal endosymbioses.

Although the experiments demonstrated the persistence of the association between *Peneroplis planatus/Porphyridium purpureum*, none of the post-stress treatments stimulated repopulation to the original levels. The post-stress treatments lasted only 12 days. Possibly, endosymbiotic *Porphyridium purpureum* needed more time to increase its population back to its original size. Under average conditions, and based on the growth of the *P. planatus*, the increase in the population of endosymbiotic *Porphyridium* was calculated at 1.40% (Chapter 8). At this low rate, the reduced endosymbiont population would need much longer than two weeks to restore its number, especially since there was no indication that the endosymbionts increased their division rate after the initial stresses.

Porphyridium purpureum seems the ideal endosymbiont. The isolated endosymbionts in batch culture required no vitamin supplements for growth and grew on very high concentrations of nitrogen and phosphorus (A. Delgado de Rodriquez, unpublished data). Free living *P. purpureum* survive in a wide range of pH and salinity levels (Jones, *et al.*, 1963), survive prolonged periods of darkness (Bisalputra and Antia, 1980), and exude large quantities of sulfated polysaccharides (Jones, 1962). How do the isolated endosymbionts compare with these free living conspecifics ? Cell counts, chlorophyll and protein analyses of the algae grown in different conditions, such as varying pH or inorganic nutrient quantities, could be utilized to compare the

growth of the isolated endosymbionts to the free-living forms, and to the values calculated for the endosymbionts within the host. The same stresses to which the foraminifera were exposed (nutrient deprivation, complete darkness and herbicide treatment), could be given to the isolated endosymbionts, to investigate the reaction of the endosymbiont population to the stress. Such experiments may elucidate the reactions of the entire endosymbiotic association to the stresses.

Although some of the algal cells appeared degenerated after the stresses to the foraminifer, there was no direct fine structural evidence that this degeneration was due to foraminiferal digestion (Plate 2.2 Figs. 3-6). Normally, foraminiferal digestion occurs externally, and in the last few chambers, and not near the endosymbionts (Lee, *et al.*, 1991b). The association appears to be obligatory for the foraminifer. The amount of reduced carbon obtained from external feeding by the foraminifer is inadequate to satisfy the energetic demands of the host, which must be at least supplemented by photosynthetic fixation of inorganic carbon by the endosymbionts (Chapter 8). Although the association appears facultative for the algae, since *Porphyridium* survives well outside of the host, the physiological adaptations of the endosymbionts and the intimate contact betwen the endosymbionts and the cytoplasm of the foraminifer is the first step necessary in developing a more obligatory relationship for the algae (Cavalier-Smith and Lee, 1987). The ability of *Porphyridium* to become dormant under adverse conditions, its freedom from the customary perialgal vacuolation (symbiosomes) found in other endosymbiont systems (Lee, 1990), and the lack of evidence of digestion of the endosymbionts by the host, implies that the *Porphyridium* endosymbionts may be imitating cellular organelles.The key difference is that cellular organelles share their genetic functions with the host genome. We imagine that first a xenosome becomes endosymbiotic and then loses parts of its genome to become an obligate organelle. Investigations into molecular and genetic controls between endosymbiotic algae and their hosts may empirically demonstrate how a sequestered cell may become an organelle. The *Peneroplis -Porphyridium* endosymbiosis affords a unique opportunity to study cellular evolution in the making.

Table 2.1. Analysis of the distribution of endosymbionts within a transprolocular light microscopic section of *Peneroplis planatus* fixed on the first day following collection. * $P < 0.05$, NS = not significant.

specimen	index of dispersion (s^2 / mean)	X^2 (Poisson)	df (n-2)
1	0.92	0.6455 NS	3
3	0.71	0.2627 NS	3
4	0.67	12.6470 *	4
5	0.92	1.8560 NS	2
6	0.52	6.7593 *	2
8	0.82	0.5359 NS	1
9	1.50	8.8858 *	3
10	1.06	5.8344 NS	5
12	0.84	5.1422 NS	3
13	0.62	2.2856 NS	3
14	0.77	0.8532 NS	2
16	0.78	3.5255 NS	4
17	0.82	2.7122 NS	5
22	0.95	0.7825 NS	3
23	0.62	6.4723 *	2

Table 2.2: ANOVA F-values for the number of endosymbionts per foraminiferal area from *Peneroplis planatus* starved in culture and then sacrificed at different days from collection†.

	Day sacrificed from collection								
	1	2	3	4	5	7	14	21	28
1	X	2.56 NS (1,46)	15.82 * (1,49)	14.72 * (1,50)	15.20 * (1,49)	22.99 * (1,52)	59.37 * (1,49)	37.10 * (1,53)	54.18 * (1,44)
2		X	30.23 * (1,37)	23.34 * (1,38)	28.32 * (1,37)	33.97 * (1,40)	83.60 * (1,37)	51.91 * (1,41)	75.69 * (1,32)
3			X	0.17 NS (1,41)	<0.01 NS (1,40)	1.44 NS (1,43)	22.83 * (1,40)	5.93 ** (1,44)	25.94 * (1,35)
4				X	0.15 NS (1,41)	0.38 NS (1,44)	8.94 * (1,41)	2.40 NS (1,45)	9.91 * (1,36)
5					X	1.31 NS (1,43)	19.91 * (1,40)	5.40 ** (1,44)	22.31 * (1,35)
7						X	6.30 ** ((1,43)	0.91 NS (1,47)	7.70 * (1,38)
14							X	2.82 NS (1,44)	0.56 NS (1,35)
21								X	4.45 ** (1,39)
28									X

† Degrees of Freedom are in parentheses under each F-value.
* = P ≤ 0.01, ** = P ≤ 0.05, NS = not significant.

Table 2.3: Relative abundances of endosymbionts in *Peneroplis planatus* after experimental treatments†.

Initial Treatment	Light Regime	Feeding Regime				
		Starved	Fed cultured endosymbionts	Fed *Dunaliella salina*	Fed *D. salina* and cultured endosymbionts	Fed *Porphyridium purpureum* UTEX 161
DCMU	Light	4.74 ± 0.83	7.20 ± 2.14	6.84 ± 1.15	10.80 ± 1.61	5.39 ± 0.86
DCMU	Dark	6.13 ± 0.95	21.68 ± 8.78	11.39 ± 2.26	17.57 ± 8.12	14.71 ± 2.34
DCMU	L/D	6.48 ± 2.02	7.61 ± 1.37	12.93 ± 2.06	5.19 ± 1.12	9.19 ± 1.63
Darkness	Light	6.30 ± 0.81	7.78 ± 1.27	6.45 ± 1.33	8.82 ± 0.93	10.25 ± 1.45
Darkness	Dark	19.37 ± 5.10	24.90 ± 1.78	27.64 ± 2.65	32.04 ± 3.74	42.27 ± 3.39

† The population density of the endosymbionts prior to the initial treatments was measured a $19.4 \pm 1.52 \times 10^{-3}$ μm^{-2} foraminiferal cytoplasm.
Values are in number of endosymbionts $\times 10^{-3}$ μm^{-2} foraminiferal cytoplasm.
DCMU treatment was a 72 h incubation in 10^{-5} M solution.
Darkness treatment was a 3 week incubation in complete darkness.
The feeding regimes followed the initial treatments and lasted 21 days.

Table 2.4: Analyses of variance between experimental groups of *P. planatus*, expressed as computed F-values†.

Initial treatment	Light regime	Feeding Regime	df	Dependent variable = Endosymbiont density
DCMU	V_l	Starved	2,16	0.57 NS
DCMU	V_l	Fed E	2,16	5.20 **
DCMU	V_l	Fed A	2,18	3.64 **
DCMU	V_l	Fed EA	2,14	4.22 **
DCMU	V_l	Fed P	2,22	5.44 **
Darkness	V_l	Starved	1,11	7.50 **
Darkness	V_l	Fed E	1,13	65.04 *
Darkness	V_l	Fed A	1,9	56.75 *
Darkness	V_l	Fed EA	1,9	13.43 *
Darkness	V_l	Fed P	1,11	50.54 *
DCMU	Light	V_f	4,30	2.52 NS
DCMU	Dark	V_f	4,26	3.10 **
DCMU	L/D	V_f	4,30	2.98 **
Darkness	Light	V_f	4,25	1.52 NS
Darkness	Dark	V_f	4,28	6.01 *

Table 2.4 continued:

Initial treatment	Light regime	Feeding Regime	df	Dependent variable = Endosymbiont density
V_t	Light	Starved	1,11	1.82 NS
V_t	Dark	Starved	1,13	9.69 *
V_t	Light	Fed E	1,14	0.06 NS
V_t	Dark	Fed E	1,7	0.26 NS
V_t	Light	Fed A	1,13	0.05 NS
V_t	Dark	Fed A	1,9	21.77 *
V_t	Light	Fed EA	1,8	0.58 NS
V_t	Dark	Fed EA	1,9	3.49 NS
V_t	Light	Fed P	1,9	9.00 **
V_t	Dark	Fed P	1,16	47.52 *

† V = independent variable, * = $P \leq 0.01$, ** = $P \leq 0.05$, and NS = not significant.

V_t Initial treatments: see Table 3.

V_l Light regimes: continuous light, 40-60 $\mu E\ m^{-2}\ s^{-1}$ (Light); continuous darkness (Dark); or ambient light and photoperiod (L/D).

V_f Subsequent feeding regimes: starved; fed cultured endosymbionts of strain PEN-3 (Fed E); fed *D. salina* (Fed A); fed cultured endosymbionts and *D. salina* (Fed EA); or fed *Porphyridium purpureum* UTEX 161 (Fed P).

EXPLANATION OF FIGURES

Fig. 2.1. The relationship between foraminiferal size and the number of endosymbionts.

Fig. 2.2. The change in density of endosymbionts within *Peneroplis planatus* after the host was starved for 28 days.

Plate 2.1.

Fig. 1, 2. Light micrograph of *Peneroplis planatus* sacrificed one day after harvest from the sea. Sections stained with hematoxylin. Scale bars = 50 μm.

Fig. 3, 5. Light micrograph of *Peneroplis planatus* sacrificed after three weeks of starvation, incubation in the light. Sections stained with hematoxylin. Endosymbionts are throughout the specimen (arrows), while the cytoplasm of the foraminifer becomes more vacuolated (V). For Fig. 3, the scale bar = 10 μm, and for Fig. 5, the scale bar = 100 μm.

Fig. 4. Transmission electron micrograph of an endosymbiotic *Porphyridium purpureum* within *Peneroplis planatus*. Sacrificed after one week of starvation, incubation in the light. Scale bar = 1 μm.

Fig. 6. Transmission electron micrograph of *Porphyridium purpureum* within *Peneroplis planatus*. Sacrificed after three weeks of starvation, incubation in the light. Scale bar = 1 μm.

Plate 2.2.

Fig. 1. Light micrograph of *Peneroplis planatus* placed in complete darkness for 3 weeks, and then starved in the dark for 12 days. Section hydrolyzed for the feulgen reaction, stained with Schiff's reagent and counterstained with fast green. Scale bar = 100 μm.

Fig. 2-6. Transmission electron micrographs of *Porphyridium purpureum* within *Peneroplis planatus*. Sacrificed after four weeks of starvation and incubation in the light. Remnants of thylakoids are seen within a degenerating endosymbiont (arrows). Scale bar = 10 μm for Fig. 3, and for Figs. 2, 4-6, the scale bars = 1 μm.

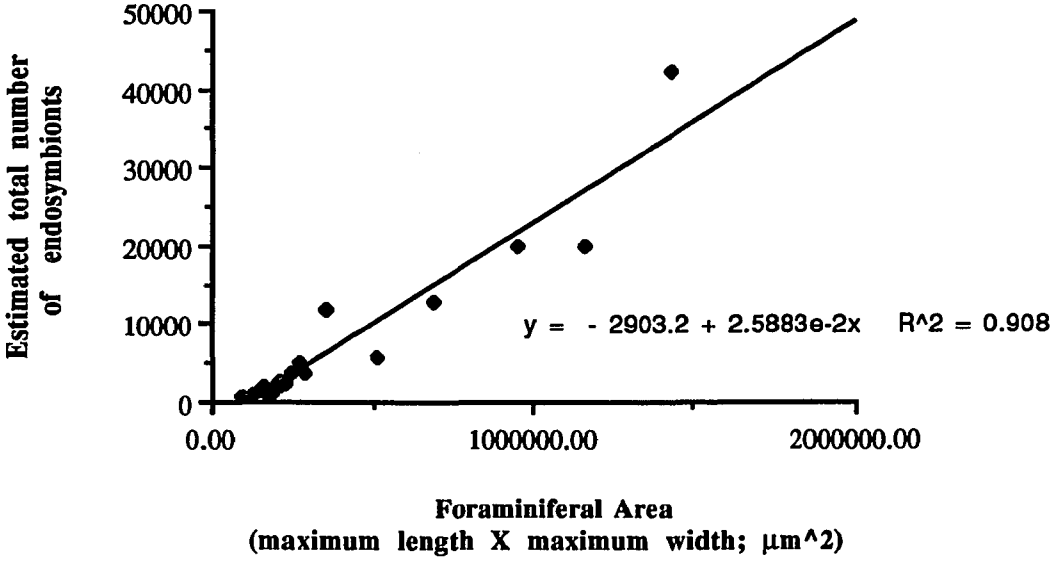


Figure 2.1

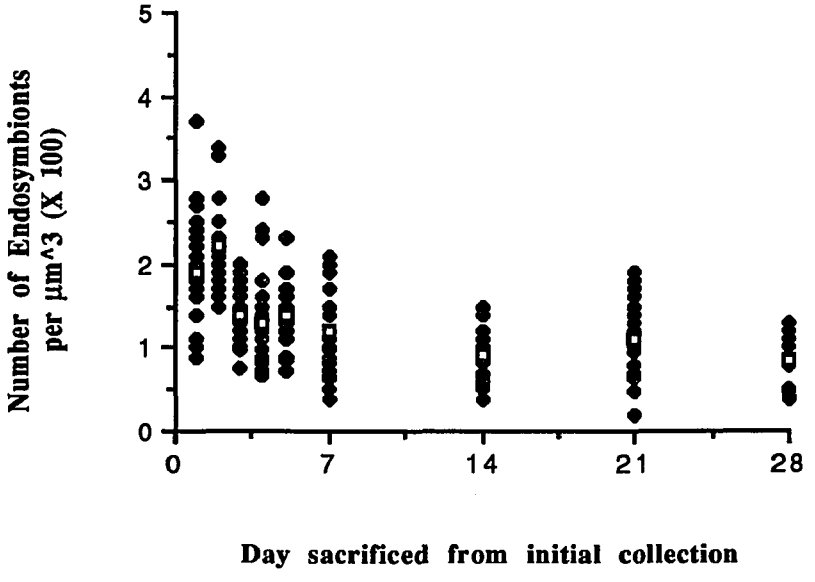


Figure 2.2

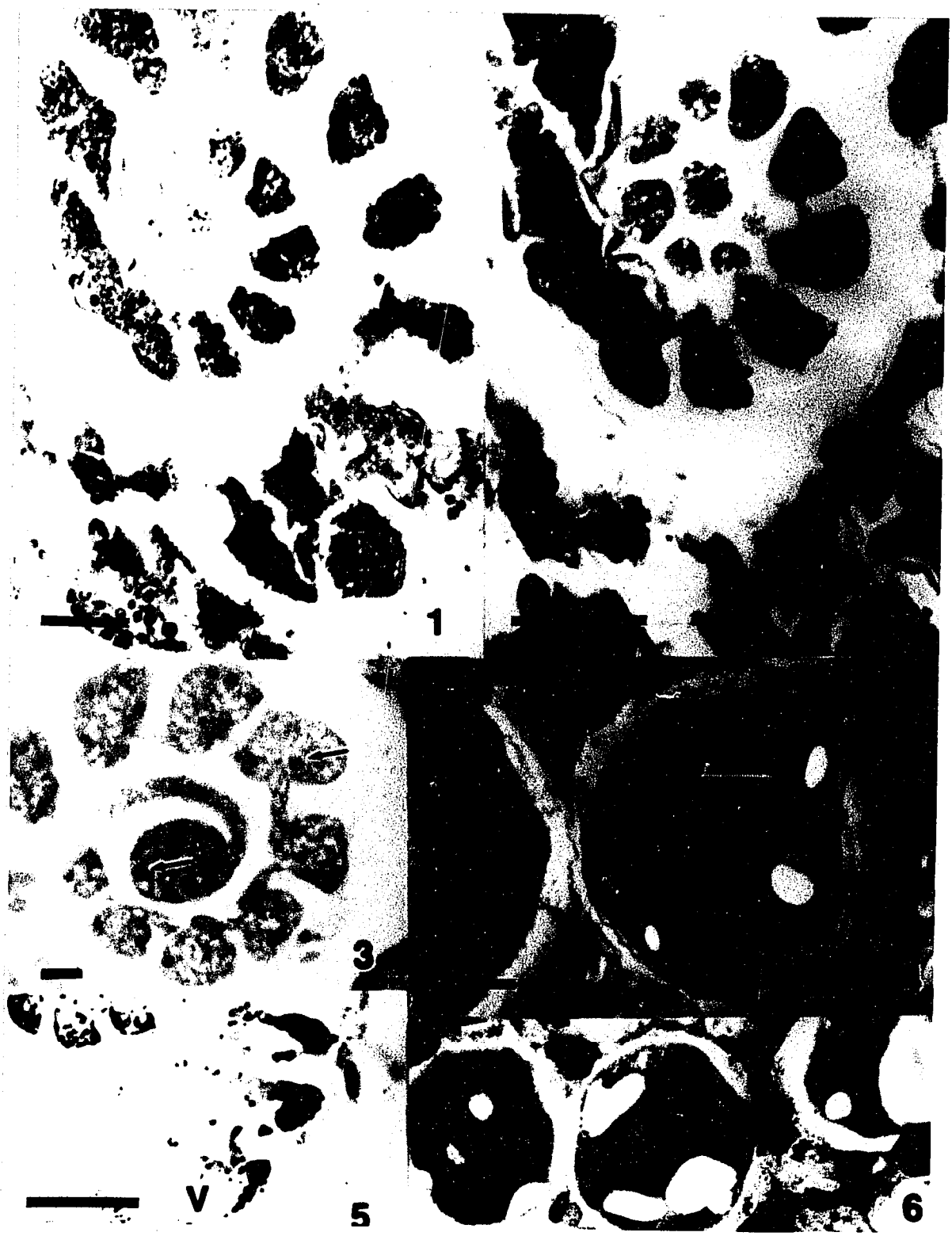


Plate 2.1

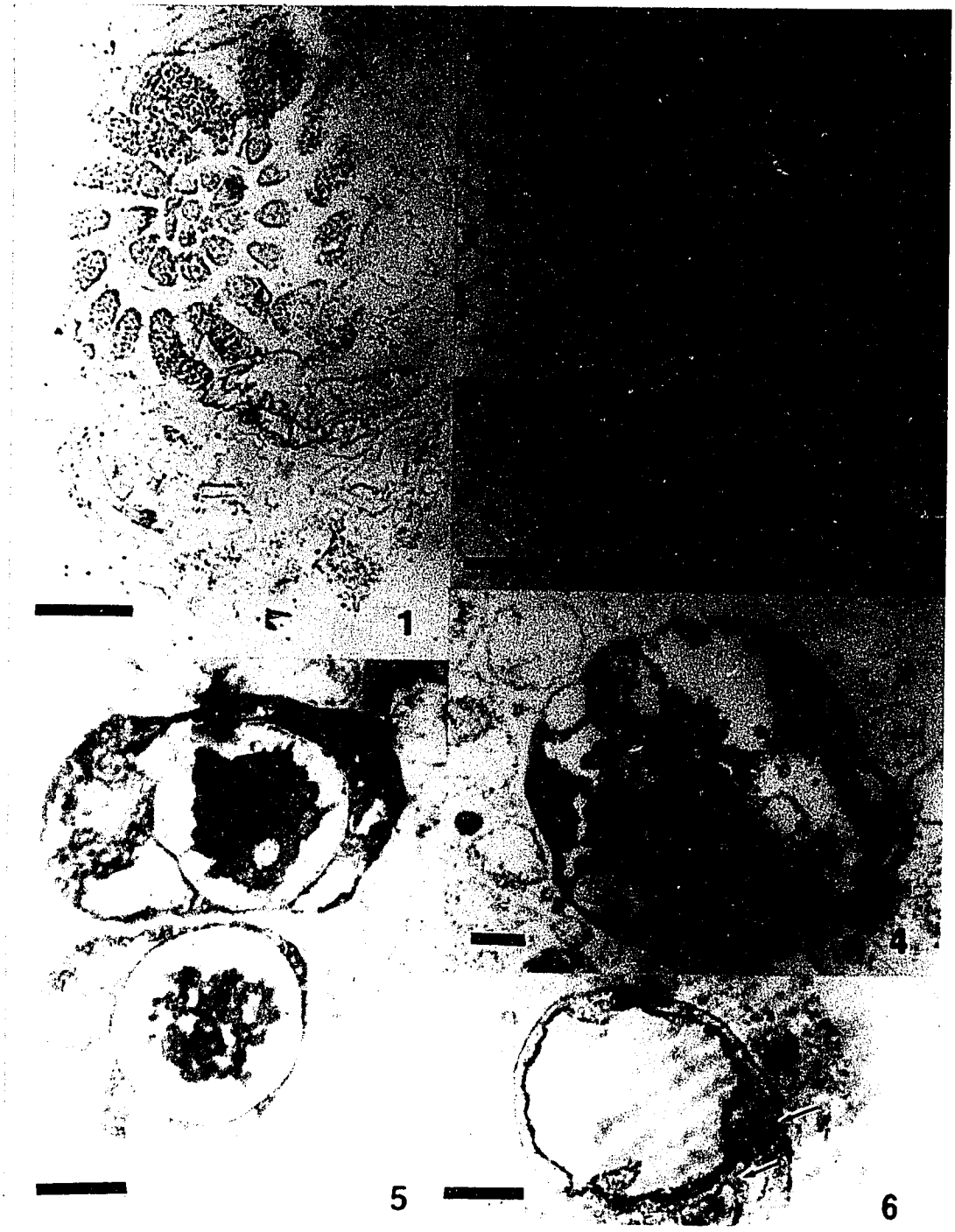


Plate 2.2

CHAPTER 3

DISTRIBUTION AND SUBSTRATE PREFERENCE OF *PENEROPLIS PLANATUS* AND *P. ARIETINUS* FROM THE *HALOPHILA* MEADOW NEAR WADI TABA, EILAT, ISRAEL.*

INTRODUCTION

In order for this thesis to be practical it was necessary to have fairly large number of organisms. A preliminary survey of the study site indicated that this would not be an easy task. The methods used by other investigators to harvest other foraminifera (Kuile and Erez, 1984) were yielding very few specimens. This study was undertaken to see if the methodology for harvesting or the microhabitats sampled could be modified to make higher yields.

Partitioning of microhabitats is often a means by which species separate their niches (Odum, 1983). Benthic foraminifera have a very patchy distribution, which allows them to utilize different microhabitats (Lee, 1974). Buzas (1970) contended that if the study area was sufficiently large, all organisms would exhibit a patchy or aggregated distribution, but even in small samples (~ 1 cm^3), benthic foraminifera are unevenly distributed (Lee, 1974).

Erskian (1972) found two benthic foraminiferal species to have an aggregated distribution, yet niche partitioning occurred in their location on *Thallassia* blades: *Planorbulina* sp. was always on the margins of the blades and *Sorites* sp. was on the medial portion.

Peneroplis species are most often found on soft substrates, such as seagrasses and algae, at depths ranging from 0 to 70 m (Murray, 1973; Hallock, 1984; Reiss and Hottinger, 1984). This depth range suggests that a wide range of microhabitats is available to the foraminifera.

Benthic foraminifera are ubiquitous and abundant in the Gulf of Eilat/Aqaba, where Reiss and Hottinger (1985) reported *Peneroplis* as a minor constituent of the total foraminifera. In the past, *Peneroplis* were found in collections from Wadi Taba, a site just south of the H. Steinitz Marine Biological Laboratory, although they were not abundant (J.J.Lee and B.ter Kuile, oral communication, 1988).

The *Halophila* meadow near Wadi Taba, Eilat, Israel, extends from 10 m to beyond 40 m depth. On January 7, 1988, SCUBA collections were made at depth intervals of 10, 15, 20, and 25 m. The collecting and processing followed Kuile and Erez (1984). *Halophila* leaves were gathered and placed in whirl-pack bags, which were prelabelled and sealable. In the laboratory, the samples were placed in a plastic colander in a shallow basin. Running seawater was washed over the leaves as they were rubbed to dislodge the foraminifera. The material in the basin was swirled and rinsed several times with fresh seawater. The concentrated material, comprising mostly foraminifera, was separated into either glass jars or deep petri dishes. This material was examined under a dissecting microscope to isolate *Peneroplis* . This collection yielded few *Peneroplis*

*Published in the Journal of Foraminiferal Research 21:218-221 (1991). Reproduced with permisssion.

regardless of the collection depth.

On a subsequent collection on January 25 from the same location, I focused on collecting the rhizosomal and other horizontal stems of the *Halophila* plants at a depth of 15-20 m. This collection yielded several hundred *Peneroplis* .

METHODS

On 31 January 1988, five quadrats were randomly sampled in the *Halophila* meadow at a depth of 20 m. A metal quadrat (10 cm X 10 cm) was carefully lowered over the plants. The erect, vertical leaves and stems were clipped with a small sharp scissor and placed into plastic whirl-pack bags (V sample). The horizontal leaves, stems and rhizomes were then clipped, and placed into bags (H sample). A sand sample (S sample) was collected by scooping up the bottom material, to a depth of one centimeter, with a teaspoon. Any dead or decomposing leaves lying on the bottom, unattached to the plants were placed in the S sample. The collecting was performed as meticulously as possible to prevent any foraminifera from being lost or placed in the wrong sample.

At the laboratory, for the V and H samples, all leaves, stems and rhizomes were vigorously brushed to remove the foraminifera. Then each plant piece was examined individually under a dissecting microscope to observe any foraminifera that may have remained attached. The removed foraminifera were washed into large petri dishes and examined under the microscope. These observations were completed within 24 hours of collection. The S samples were placed in large petri dishes and filled with seawater. These were sorted last under the dissecting microscope within two or three days of the collection. All the V, H and S samples were examined twice. Living specimens were distinguished by a dark pink color, characteristic of the endosymbionts, and by rhizopodial activity. Both the color and rhizopodial activity were absent in the dead specimens. Since the data were not normally distributed, a Kruskal-Wallis non-parametric one-way analysis of variance by ranks was applied to the counts (Daniel, 1978).

RESULTS

In the quadrat samples, I found only two species of peneroplids: *Peneroplis planatus* and *P. arietinus* . Few living Peneroplids were found (Table 3.1). A comparable number of dead peneroplids to the number of living specimens were found in the V and H samples, usually lodged in the ligulae of the *Halophila*. A large number of dead specimens, one to two orders of magnitude greater than the living counts, were found in the S sample. *Peneroplis planatus* made up a mere 0.74% and 1.80% of the total number of living foraminifera in the V or H samples, respectively (Table 3.2).

For *P. planatus*, the Kruskal-Wallis non-parametric one-way analysis of variance by ranks rejected the probability that the three samples did not differ ($H = 6.4035$, $P < 0.05$). Although *P. arietinus* was found only in the S sample, there was no significant difference between the substrates. This may be attributed to the low number of specimens encountered.

DISCUSSION

Peneroplis planatus lives on both the rhizomes of *Halophila* and the sand beneath the plants, preferring the horizontal substrates (Table 3.1), whereas *P. arietinus* lives only on the sand. These habitats may be partially shaded by the leaflets of *Halophila* . SCUBA collection that focused on picking the erect *Halophila* leaves rarely recovered *Peneroplis* .

This distribution may be partially controlled by light level. In the laboratory many specimens elevate their shells off the bottom of their culture dishes. Preliminary observations of the collected specimens of *P. planatus* (N=180) showed that 71 % of viable specimens (those with active rhizopodia) elevated their shells off the bottom of the culture flasks and 12 % climbed the sides of the culture flasks. Only 7 % lay flat on the bottom of the flasks. Possibly, this behavior is a response to expose both sides of the organisms to light as suggested by Leutenegger (1984), but this remains to be systematically investigated in carefully designed experiments. One would expect a light influenced distribution to be related to depth. However, *Peneroplis* showed no apparent preference for depth within the range of 10 to 25 m and succeeding collections yielded similar numbers of specimens.

The distribution of other species has been shown to be influenced by light intensity. In Key Largo Sound, Florida, *Sorites marginalis* and *Archaias angulatus*, which attach to *Thallassia testudinum*, exhibit a light-influenced partitioning (Lee and Zucker, 1969; Lee and Bock, 1976; Müller-Merz and Lee, 1976). At high tide, the *Thallassia* plants are about 2 m below the surface, whereas at low tide, the blades float at the surface. *Sorites* lies flat and cements itself on the distal portion of the blade, whereas *Archaias* orients the plane of its test at right angles to the blade at high tide (Lee and Bock, 1976).

In past years, *P. arietinus* was found abundant periodically on sand flats below the *Halophila* meadow at depths greater than 40 m (J.J. Lee, oral communication, 1988). Light may play a more important role in the distribution of *P. arietinus* than of *P. planatus*, since the *Halophila* plants may act as a light filter in the shallower waters, whereas in dim light at depth, the foraminifera live on exposed sand flats. A similar distribution to dim light is seen in *Heterostegina depressa* , which is distributed at depths greater than 40 m in the Gulf of Eilat, whereas it is found in shallower water in a tide pool at Makapuu, Hawaii, but only on the shaded side (Röttger, 1972b).

Halophila plants appear to be a good substrate for foraminifera, considering the coexistence of large numbers of different foraminiferal species (Table 3.2). Besides microclimate (ie: temperature, salinity, light intensity and quality), interspecific competition and/or resource partitioning may play a greater role in the distribution and abundance of these foraminifera. Dietary habits, nutritional requirements and seasonality may be important in the peneroplid distribution. Muller (1975) suggested nutrition may be functionally linked to seasonality. *Peneroplis* is a selective feeder with distinct differences in preference from *Amphistegina lobifera* and *Amphisorus hemprichii* (Faber and Lee, 1991), the two dominant foraminiferal species found in the *Halophila* meadow (Table 3.2). Possibly the rhizomes of the *Halophila* exude substances that are utilized directly as nutrients by the peneroplids, or these substances support a different diatom flora than that of the leaves, which may be consumed by the peneroplids. Neither of these possibilities have been investigated.

In February 1988, a macroalgal bloom was observed in the Gulf of Eilat/Aqaba. *Enteromorpha* sp. and *Cladaphora* sp. carpeted the sand flats from the H. Steinitz Marine Biology Laboratory to Wadi Taba at a few

centimeters in water depth to over 10 m. Within weeks, these algal mats were populated by *Peneroplis planatus* in large numbers. Hottinger (1977) had previously noted a correspondence between short lived algal growth and high productivity of foraminifera in the same area. Natural populations of *Peneroplis* in the Mediterranean and Pacific also exhibit peaks in abundance in spring or summer (Murray, 1973; Hallock, 1984), which may be related to seasonal production.

Reproduction and seasonality have never been adequately investigated. In the laboratory, of the initial 180 specimens of *P. planatus* collected in the summer of 1987 and maintained under constant culture conditions, four specimens reproduced asexually in March or April 1988. In February and March 1988, many *Peneroplis* collected at Wadi Taba during the algal bloom formed megalospheric juveniles and released them in the laboratory. Sexual reproduction among larger foraminifera is rarely observed (Kloos,1984; Röttger, 1974). It may occur in the deeper part of the range of a species (Kloos, 1984, Leutenegger, 1977a) .

To summarize its temporal distribution, *Peneroplis* is of minor abundance throughout most of the year (Reiss and Hottinger, 1984). The macroalgal blooms create new substrates and food sources stimulating *Peneroplis* to reproduce asexually and exploit this temporary habitat. Hence the reproductive seasonality of the *Peneroplis* may be an adaptation to interspecific competition.

Analogies are found in the salt marsh habitat where foraminiferal species dominance changes with the occurrence of algal blooms (Lee, 1974) and possibly in the deep sea where benthic foraminifera exploit areas of high surface productivity and high influx of phytodetritus (Gooday, 1988; Suess, 1988). However, little is known about the reproduction and seasonality of these deep sea foraminifera. Algal blooms and phytodetritus may create new substrates and food sources for foraminiferal species that otherwise cannot compete.

Peneroplis arietinus showed no population explosion in conjunction with the macroalgal bloom. The distribution of this species appears to be dependent on light intensity, whereas the distribution of *P. planatus* may be controlled by substrate and food availability in addition to light intensity.

Table 1.1. Standing crop of living peneroplids, 10 cm X 10 cm quadrat, from the vertical *Halophila* leaves (V), horizontal *Halophila* rhizomes (H) and sediment (S) samples collected on January 31, 1988, near Wadi Taba, Eilat, Israel, at a depth of 20 meters.

	Peneroplis planatus			*Peneroplis arietinus*		
Replicate	V	H	S	V	H	S
1	6	13	5	0	0	2
2	1	3	12	0	0	4
3	0	8	5	0	0	0
4	0	7	11	0	0	0
5	0	2	6	0	0	1
Totals	7	33	39	0	0	7

Table 1.2. The total counts of living larger foraminifera from the first replicate vertical *Halophila* leaves (V) and horizontal *Halophila* rhizomes (H) samples.

Species	V	H
Amphistegina lobifera	637	429
Amphisorus hemprichii	107	182
Peneroplis planatus	6	13
"*Quinqueloculina*" sp.	53	84
Other smaller foraminifera	11	16
Totals	811	724

CHAPTER 4

GRANULAR RETICULOPDAL DIGESTION-A POSSIBLE PREADAPTATION TO BENTHIC FORAMINIFERAL SYMBIOSIS ? *

INTRODUCTION

Algal-bearing foraminifera (larger and planktonic foraminifera) abound in the world's oceans, and in particular in the well illuminated tropical and semi-tropical seas. In the latter, "living sands" literally carpet the sea floor in back reef habitats and there are pacific islands where beach sand is almost entirely foraminiferal. Since the Pennsylvanian (3 X 10^8 yBP), the fossil record indicates bursts of evolutionary lines of larger foraminifera (sizes up to 15 cm) from ordinary sized (0.5 mm) ancestors. Their abundances were not random but generally corresponded to polytaxic episodes (Fisher and Arthur, 1977) which were periods of global warming, relative drought, raised sea levels, expansion of tropical and semitropical habitats, and reduced oceanic circulation (Lee and Hallock, 1987).

When one reflects on the pulsed abundances in the fossil record and the fact that larger and planktonic foraminifera as a collective group are hosts for a greater diversity of algal types (dinoflagellates, chlorophytes, rhodophytes, chrysophytes, and diatoms) than any other group of organisms in the sea, one wonders if the biological properties of foraminiferal organization do not make them particularly good habitats for the establishment and maintenance of algal symbionts. One could argue that the diversity of algal symbionts itself is strong evidence. Additionally, some of the associations, those between diatom-bearing and dinoflagellate-bearing hosts and their algal partners are not species specific (Lee, *et al.*, 1980a,b,c, 1983, 1985, 1989; Lee and Reimer, 1983; Reimer and Lee, 1984, 1988; Lee and Lawrence, 1990). The presence of rare or minor symbionts in some of the associations adds further evidence (Lee and Reimer, 1983; Reimer and Lee, 1984, 1988; Lee, *et al.*, 1983).

The complex life cycle of many foraminifera seems also to be a general preadaptation for symbiosis. Asexual reproduction of the agamont generation insures transmission of symbionts to their offspring. Although the life cycles of most larger foraminifera are not completely known, the sexual stages of some foraminifera are known to be gamontogamic (2 or more organisms fuse together or form a nuptual chamber around their umbilical surfaces). Gametes are released into this restricted space. If this happens in some of the larger foraminifera, the symbionts of the parents would be readily available to newly formed zygotes.

The survivability of symbiotic algae in a non-symbiont species (one experiment: *Chlamydomonas hedleyi* in *Rosalina leei* , reported in Lee and Zucker, 1969) suggests that the general cameral structure of foraminifera serves preadaptation for symbiosis. Cameral organization subdivides protoplasmic streams and promotes

*Published in the Symbiosis 10: 47-61 (1991). Reproduced with permisssion from Balaban Publishers.

regionalization of cellular activities and could separate algae into favorable microhabitats (eg. under pores in *Amphistegina* spp.) (reviewed in Lee and Hallock, 1987). In fact the fossil record of larger foraminifera could be interpreted as showing that foraminifera have been quite plastic in their responses to the algal symbionts by evolving complex subdivisions (chamberlets) and organelles for channeling protoplasmic streams (Hottinger, 1982; Lee and Hallock, 1987). This evidence led us to speculate that it was possible that the nature of digestion in foraminifera also could be a preadaptation to symbiosis.

The constant bidirectional movement of particles in granular reticulopodia has fascinated many observers (eg. Sandon, 1932; Jepps, 1942; Buchanan and Hedley, 1960; Hedley, 1964; Anderson and Bé, 1976). Several modes of behavior have been observed in living foraminifera. Several carnivorous species have been observed to digest their trapped prey directly in their granular reticulopodial networks (Buchanan and Hedley, 1960; Anderson and Bé, 1976). Other species habitually collect food and form balls of algae near their apertures (eg. Bowser, *et al.*, 1985). Sometimes the balls become so large that the foraminifera singly or in groups become embedded in them. Vacuoles with food have also been observed in cytological preparations of many species where they occur mainly in the youngest chambers (eg. Müller-Merz and Lee, 1976; McEnery and Lee, 1981; Koestler, *et al.*, 1985). Only a few researchers have attempted to detail the process of endocytic uptake and lysosomal fusion by cytochemical or ultrastructural methods. One of the first, Lengsfeld (1969) concluded that *Allogromia*, a monothalamous foraminifer, had a novel open digestive system consisting of intracellular anastomosing channels of seawater which could be traced from their origins in the aperture to sites of digestion within the cell. This lacunary system was interpreted as analogous to the open digestive tracts of several invertebrate phyla. This claim was disputed by further studies which suggested that the lacunary system was a fixation artifact (Anderson and Bé, 1976; Bowser, *et al.*, 1985). Interestingly, the latter two studies differed in their observations on the sites of lysosomal fusion with phagosomal vesicles. The former found fusion at sites of penetration of the reticulopodia into the naupliar prey. The latter found no lysosomes or acid phosphatase activity in the reticulopodia and concluded that the network in allogromiids serves mainly as a food gathering and transport organelle. From the perspective of symbiont maintenance the site(s) of lysosomal fusion with phagosomes is a key issue because symbionts are also surrounded by host vacuolar membranes. We began this study to broaden our knowledge of digestive processes in foraminifera. We reasoned that if lysosomal fusion with phagosomes is extra-cameral in the group, as a whole, then this might be a very good biological feature for the maintenance of symbiosis once it becomes established. Algae which escape the initial digestive challenge would be relatively immune from further challenge even though their cell envelopes are greatly reduced when they are in their hosts.

METHODS

Collections

Four species, *Amphisorus hemprichii* Ehrenberg, *Amphistegina lobifera* Larsen, *Peneroplis planatus* (Fichtel and Moll) Montfort, and *Planorbulina* sp. were collected between January and March, 1988 from the

Halophila meadow near Wadi Taba, Gulf of Eilat, Israel, at depths from 10-25 m. These specimens were brought to the H. Steinitz Marine Biological Laboratory-Interuniversity Institute of Eilat, and assayed about 24-48 hours after collection. *Baculogypsina sphaerulatus* (Lamarck) Parker & Jones emend Sacco, *Calcarina gaudichaudii* d'Orbigny, and *Marginopora kudakajimensis* Gudmundsson were collected on July 15, 1989 from Kudaka Island, Japan. The remaining species (*Allogromia laticollaris* Arnold, *Quinqueloculina costata* Walker & Jacob, *Elphidium incertum* Williamson, *E. translucens* Natland, *Protelphidium tisburyiensis* Butcher, *Haynesina germanica* (Ehrenberg) Banner and Culver , and *Nonion* sp.) were collected from Lackey's Bay, Naushon Island, Woods Hole, Massachusetts in August, 1989. The Japanese and Woods Hole specimens were transported back to the laboratory in New York, and assayed 1-2 weeks after collection. Several smaller foraminifera were cultured in the laboratory, and several of their offsprings were assayed at a later time.

Acid phosphatase assay

The foraminifera were placed either in a 9-well spot plate or a small petri dish, with or without an agar base. All were offered a mixture of diatoms, *Dunaliella salina* and *Chlorella* sp. (AT), and allowed from 1 to 12 hours to extend their pseudopodia and feed.

The foraminifera were fixed with cold 3% glutaraldehyde in an acetate buffer. The acid phosphatase assay (#180) followed the method given in the Lymphocyte Enzyme kit marketed by Sigma Chemical Co. The specimens were rinsed in distilled water for 30 seconds. A solution of Napthol AS-BL Phosphoric acid and Fast Garnet GBC with an acetate buffer was poured over the foraminifera, until covering the entire specimen, and placed in a 37 C oven or water bath for 1-2 hours. After incubation, the specimens were rinsed with distilled water and counterstained with methylene blue for 2 minutes. The foraminifera were gently decalcified with 5 % poly no-cal (Polysciences, Inc. #16865) for about 2 hours, then 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 also counterstained with hematoxylin (Humason,1962), since the methylene blue leeched out of most of the specimens during the dehydration in ethanol. The sections were observed and photographed on a Zeiss Photomicroscope II.

RESULTS

All the specimens had collected food balls and mats around their tests. Only a few specimens had extended rhizopodia out along the agar base after feeding. This episodic gathering of food close to the foraminiferal shell seems to be a general behavior of all investigated foraminifera (Lee, 1974; Lee, *et al.*, 1988; Faber and Lee, ms submitted), and can be observed *in situ* (Fig. 4.1). Often partially digested food particles and empty diatom frustules were found in these food mats (Fig. 4.2 - 4.3), and upon closer inspection, rhizopodia were seen intertwined (Fig. 4.4).

The acid phosphatase assay appeared to be a valid indication of digestion since every food vacuole observed within the foraminifera exhibited acid phosphatase activity, as noted by the red-diazo dye complex under light

microscopy (Fig. 4.8). In all the species examined, acid phosphatase activity was observed in the external food mats (Fig. 4.12), and in the extended rhizopodia (Table 4.1, Fig. 4.18). Some species, such as *Planorbulina* , showed only acid phosphatase activity outside the shell of the foraminifera in these mats.

Acid phosphatase was noted in all areas where the rhizopodia protrude from the organism. Often the activity was found near the apertures (Fig. 4.17), along the outer edge of the organism (Fig. 4.5, 4.7, 4.10, 4.15), or in the last few chambers (Fig. 4.19, 4.20). In *Baculogypsina sphaerulatus*, and *Calcarina gaudichaudii*, a strong reaction for acid phosphatase activity was present in the spines (Fig. 4.9 - 4.11, 4.13 - 4.15), and in the canal system along the tests (Fig. 4.10, 4.16).

In contrast, acid phosphatase activity was not seen in the vicinity of the endosymbionts (Table 4.1, Fig. 4.6). For example, *Peneroplis planatus* has a bilateral distribution of its endosymbionts throughout the foraminifera (Hallock, 1981). However, often in freshly collected *Peneroplis* , the outer few chambers appeared empty, lacking *Porphyridium* endosymbionts. The acid phosphatase assay showed activity only in these last chambers, which were devoid of endosymbionts (Fig. 4.19). Even in species which showed some food vacuoles distributed within the organism (eg. *Amphisorus*, and *Marginopora* , Fig. 4.5, 4.7), no enzyme activity was seen near the endosymbionts (Fig. 4.6).

The species lacking endosymbionts exhibited the same partitioning of cellular activity (Table 4.1). *Allogromia laticollaris* showed a distinct internal compartmentation with acid phosphatase activity confined to the outer cytoplasmic streams, along the apertural regions, and in the external food balls, whereas *Quinqueloculina costata* showed acid phosphatase activity only in the outer chamber.

DISCUSSION

The results of this study seem to support the hypothesis that the initial steps of digestion are generally extra-cameral in some benthic foraminifera. Therefore if living undigested algae are drawn into the shell they have potential to develop as endosymbionts. Very little is known about the digestive processes in symbiont-bearing forms. We found differences in the amount of carbon egested after *Amphisorus hemprichii* and *Amphistegina lobifera* had fed on different species of algae (Lee, *et al.*, 1988b). In particular we noted that the cell wall of *Chlorella* sp. (AT) was egested intact. The digestive enzymes of both hosts were unable to degrade this potential energy source during its residence time within the host. In an experimental study with nearly aposymbiotic *Amphistegina* Koestler, *et al.* (1985) found several interesting facts. Isolation techniques showed that various species of axenically cultured endosymbiotic diatoms could escape digestion and repopulate the host. Although free-living species were fed to the foraminifera in mixtures containing symbionts none escaped digestion. Fine structural studies revealed two additional facets of the relationship. Some of the diatoms in a chain of *Fragilaria shiloi*, an endosymbiotic species, were digested, others were not. This requires much more detailed examination. The other facet was shown in experimental groups of *Amphistegina* , which were starved and incubated in the dark, and specimens in groups starved and incubated in the light with DCMU. The cytoplasm, as time progressed, became greatly vaculated, less granular and eventually the pore rim cups, which

ordinarily are the sites for endosymbionts, gradually became vacant. Microbodies were seen in the vicinity of the symbionts but the study was not detailed enough to discriminate between possible autolysis of stressed symbionts and digestion by host lysosomal fusion with symbiont vacuoles.

The study just concluded whets the appetite for more detailed fine structural examination of the digestive processes in algal symbiont-bearing foraminifera, chloroplast husbanding foraminifera, and foraminifera in general. What are the recognition signals involved ? The diatom endosymbionts of many hosts belong to a very broad spectrum of pennate families. Some species which are digested belong to the same genera as others which are not. Do all endosymbiotic diatoms have common surface antigens not found in free-living forms ? Are the same antigens found on chloroplast envelopes or is the chloroplast recognition system in husbanding forms an entirely different recognition system ? Do they have some common property which changes phagosomes into symbiosomes (Jeon, 1987) ? Do foraminifera, in general, have a phagolysosomal system which is easily repressed, another possible preadaptation for symbiosis ? We eagerly look forward to the answers.

Table 4.1. Location of acid phosphatase activity (an indication of digestion), and of endosymbionts (when present) of various species of benthic foraminifera.

Species	Collection site	Acid Phosphatase Activity					Endosymbiont location
		Rhizopodial web	Aperture	Spines	Outer chambers	Canal system	
Amphisorus hemprichii	Elat	+	+		+		In concentric band around early chambers below the lateral surface.
Marginopora kudakajimensis	Kudaka Island	+	+		+		In wider concentric band around early chambers below the lateral surface.
Peneroplis planatus	Elat	+	+		+		Bilaterally throughout the shell.
Amphistegina lobifera	Elat	+	+				Below lateral chamber walls of last 1 or 2 whorls.
Calcarina gaudichaudii	Kudaka Island	+	+	+		+	Throughout the shell (not in the spines).
Baculogypsina sphaerulatus	Kudaka Island	+	+	+		+	In layers of outer chamberlets.
Elphidium incertum	Woods Hole	+	+				Along outer edges of chambers.
E. translucens	Woods Hole	+	+				Along outer edges of chambers.
Protelphidium tisburyiensis	Woods Hole	+	+				Along outer edges of chambers.
Haynesina germanica	Woods Hole	+	+				Along outer edges of chambers.
Nonion sp.	Woods Hole	+	?				Along outer edges of chambers.
Quinqueloculina costata	Woods Hole	+	+				-No known endosymbionts-
Planorbulina sp.	Elat	+	+		+		-No known endosymbionts-
Allogromia sp.	Woods Hole	+	+				-No known endosymbionts- (some acid phoisphatase activity at food plaque nodes).

EXPLANATION OF FIGURES

Figures 4.1 - 4.4 A microscopic approach to the food and potential food of *Amphisorus hemprichii.* All specimens critical point dried and examined with an SEM.

Figure 4.1. *Amphistegina hemprichii* on the surface of a *Halophila* leaf. Arrows point to residua ("midden heap") at periphery of animal. Scale bar = 600 µm.

Figure 4.2. A portion of the residuum of the animal in Figure 4.1. Note the empty *Amphora* frustules (F). Scale bar = 10 µm.

Figure 4.3. The surface of the *Halophila* leaf 1 cm from the animal. Note rich bacterial and diatom flora (*Cocconeis placentula, Cocconeis* sp., *Fragilaria* sp.). Scale bar = 10 µm.

Figure 4.4. Surface of a leaf closer to the animal showing peripheral pseudopodal network (arrows) intermeshed on the surfaces of diatoms (D) and bacteria. Scale bar = 10 µm.

Figure 4.5 - 4.20. All specimens after acid phosphatase assay, taken on a Zeiss photomicroscope II.

Figure 4.5. Decalcified *Amphisorus hemprichii* embedded in Tissuemat prior to sectioning. Several large food vacuoles are seen (arrow). Scale bar = 100 µm.

Figure 4.6. Dinoflagellate endosymbionts of *Marginopora kudakajimensis* . Note the lack of any acid phosphatase activity in this region. Scale bar = 10 µm.

Figure 4.7. *Marginopora kudakajimensis* , prior to decalcification, showing an outer edge of acid phosphatase activity (arrow). Specimen photographed in distilled water rinse. Scale bar = 100 µm.

Figure 4.8. Close-up of a food vacuole in *Marginopora kudakajimensis*, with strong acid phosphatase activity (arrow). Scale bar = 20 µm.

Figure 4.9.*Baculogypsina sphaerulatus*, prior to decalcification, showing attached food mat with acid phosphatase activity at end of spine (arrow). Specimen photographed in distilled water rinse. Scale bar = 200 µm.

Figure 4.10. Section of the specimen in Figure 4.9, showing activity along the outer edge of the specimen. Scale bar = 200 µm.

Figure 4.11 - 4.12. Close-up of food vacuoles (arrows) within the spine of *Baculogypsina sphaerulatus* . Scale bar = 10 µm.

Figure 4.13. *Calcarina gaudichaudii*, prior to decalcification, showing acid phosphatase activity at spine. Specimen photographed in distilled water rinse. Scale bar = 500 µm.

Figure 4.14. Cross section of *Calcarina gaudichaudii* spine showing food vacuoles (arrow). Scale bar = 25 µm.

Figure 4.15. Section of *Calcarina gaudichaudii* showing strong activity along spine (arrow), and along the edge of the shell. Scale bar = 200 µm.

Figure 4.16. Close-up of the acid phosphatase activity (arrow) following the canal system along the edge of the shell of Figure 4.15. Scale bar = 20 μm.

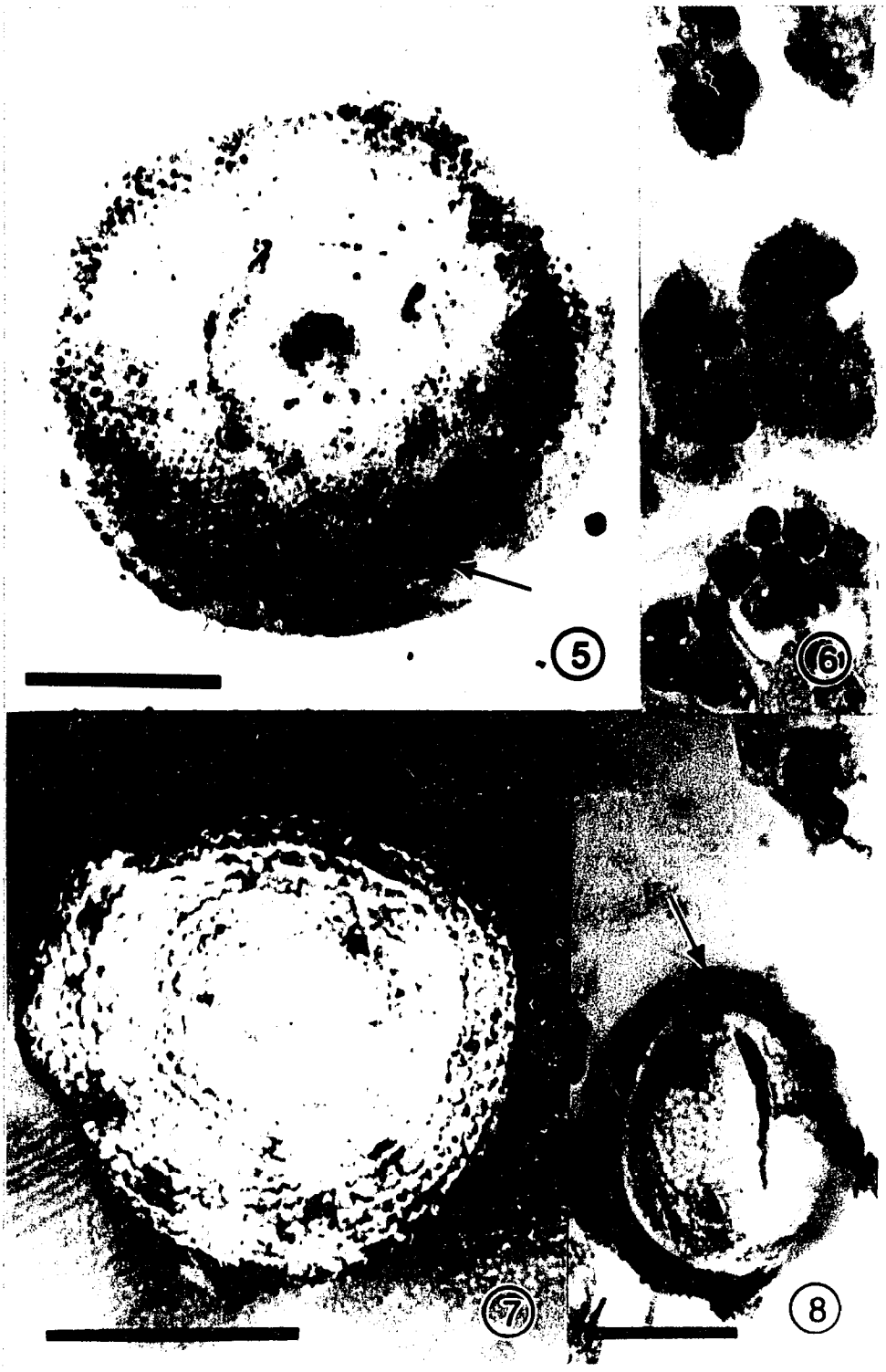
Figure 4.17. The apertural region of *Allogromia* sp. with attached food mass (F). Scale bar = 10 μm.

Figure 4.18. Food vacuoles (arrow) seen in the extended rhizopodia of *Amphistegina lobifera*. Scale bar = 10 μm.

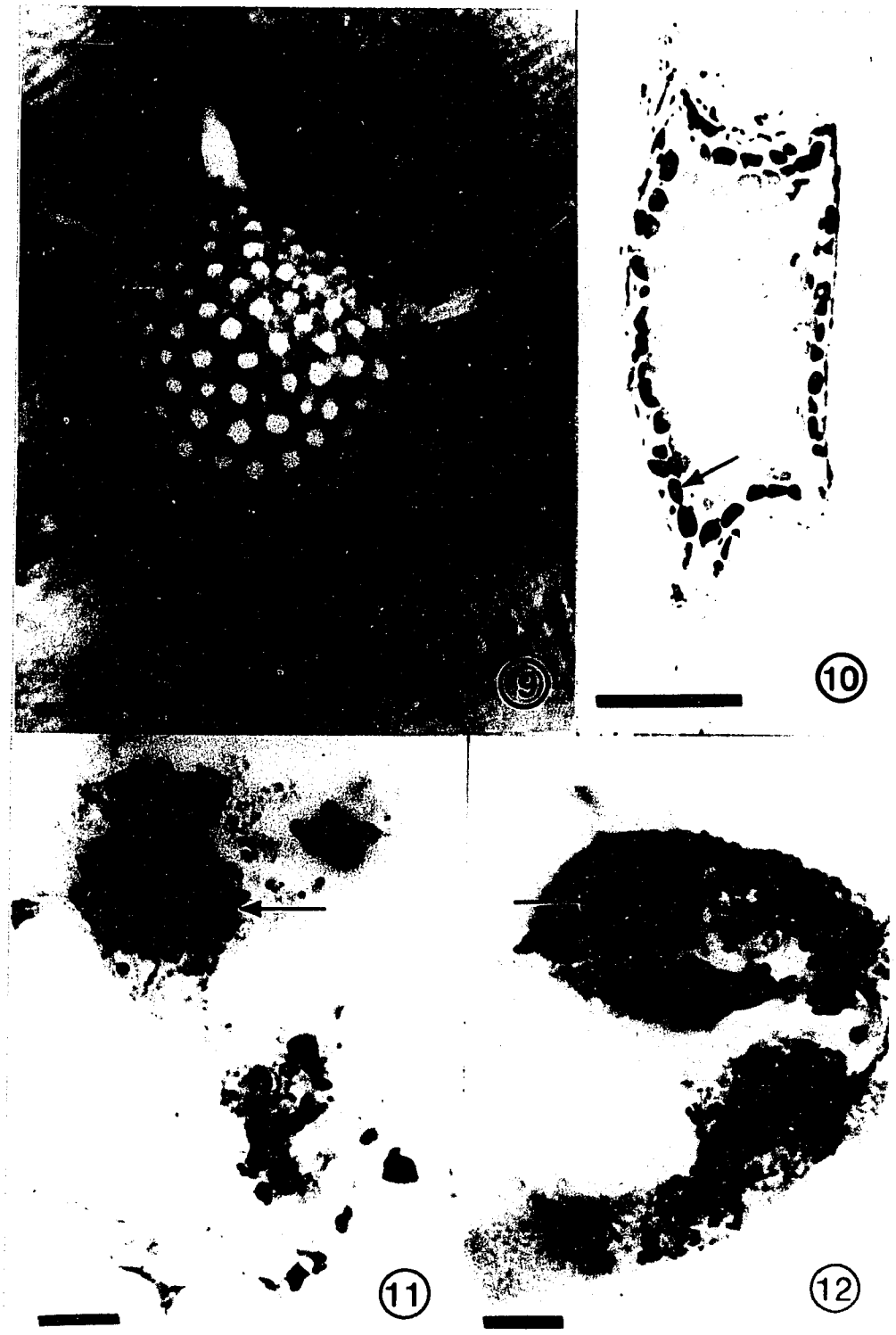
Figure 4.19. Decalcified *Peneroplis planatus* embedded in Tissuemat prior to sectioning. Acid phosphatase activity (arrow) seen along last chamber. Scale bar = 100 μm.

Figure 4.20. Strong enzyme activity (arrow) seen in the outer chamber of *Quinqueloculina costata*. Scale bar = 20 μm.

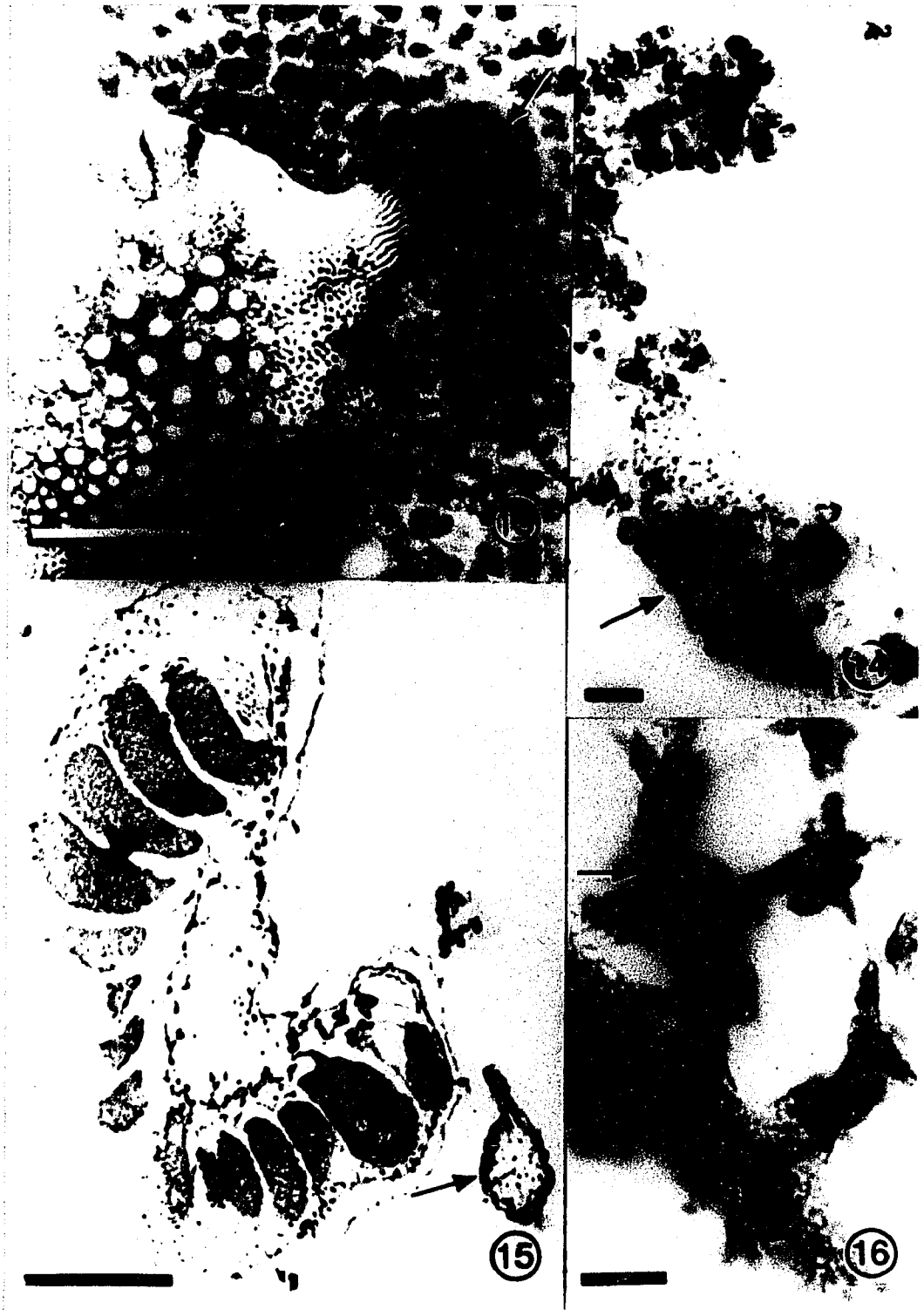
Figs. 4.1 - 4.4



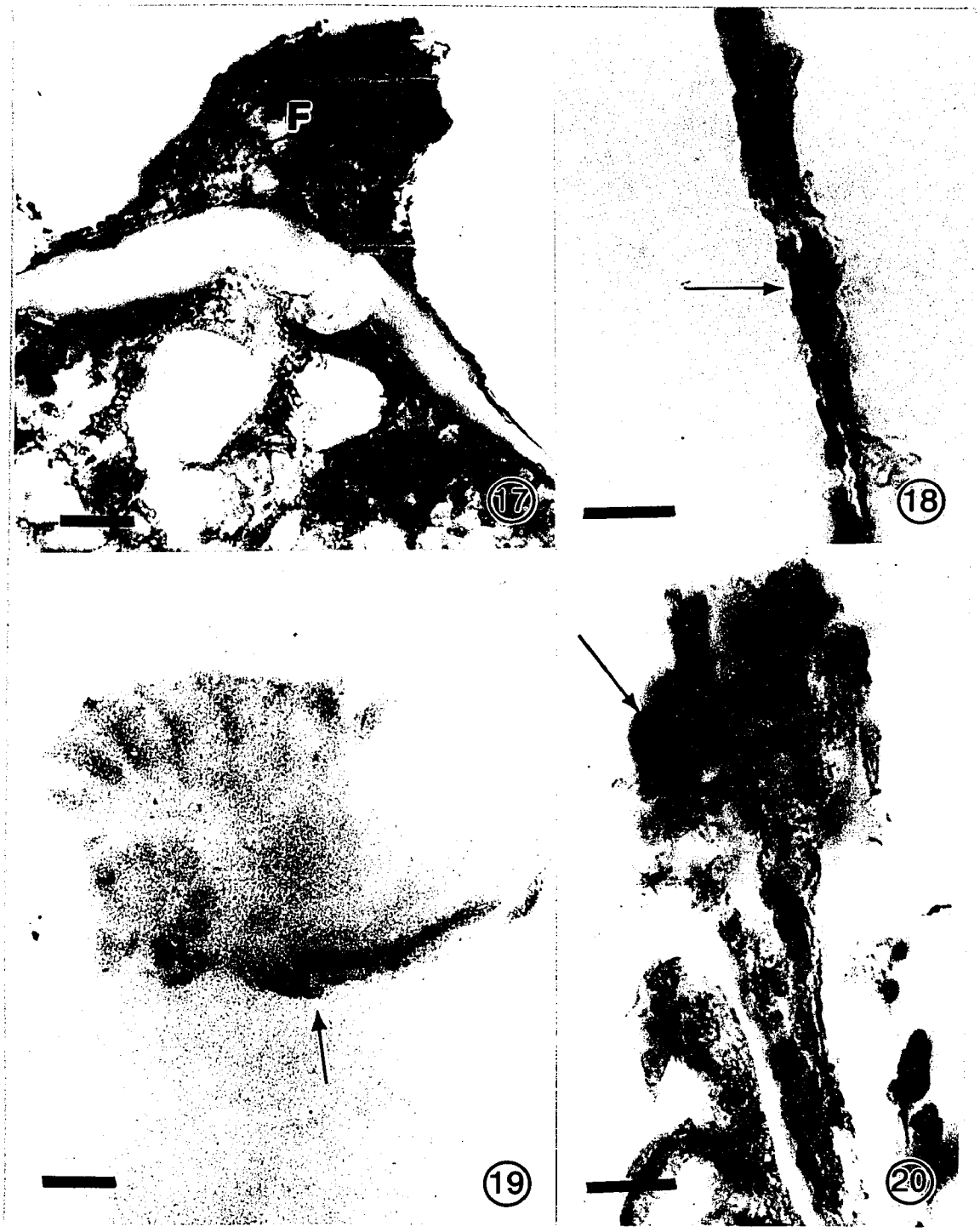
Figs. 4.5 - 4.8



Figs. 4.9 - 4.12



Figs. 4.13 - 4.16



Figs. 4.17 - 4.20

CHAPTER 5

HISTOCHEMICAL EVIDENCE FOR DIGESTION IN *HETEROSTEGINA DEPRESSA* AND *OPERCULINA AMMONOIDES* (FORAMINIFERA)*

INTRODUCTION

The diversity of endosymbiont types in larger foraminifera (Lee, *et al.*, 1974, 1985; Spindler and Hemleben, 1980; Leutenegger, 1984; Spero and Parker, 1985; Lee and Hallock, 1987; Faber, *et al.*, 1988; Gastrich, 1988) led us to speculate that regionalization of cellular activities could be a fundamental biological property which makes foraminifera particularly good habitats for the establishment and maintenance of algal endosymbionts (Lee and Anderson, 1991). This speculation, in turn, led us to investigate digestion in foraminifera (Lee, *et al.*, 1991b).

Benthic foraminifera are herbivores (Lee, 1974) and any potential algal symbiont ingested must escape digestion to establish itself as an endosymbiont. Our initial study examined the presence of acid phosphatase activity in six species of foraminifera which possess algal endosymbionts (four of which are diatom-bearing species), five species which husband chloroplasts, and three species with no known endosymbiont (Lee, *et al.*, 1991b). We found the initial steps of digestion to be extra-cameral in all the species examined. Therefore those living algae which can avoid the initial digestion and are drawn into the shell have the potential to develop as endosymbionts.

Recently, we examined digestive enzyme activity in three additional foraminiferal species: *Discorbis* sp. (Rotaliidae), a species with no known endosymbionts; and two diatom-bearing nummulitids, *Heterostegina depressa* and *Operculina ammonoides*. We were especially interested in *Heterostegina depressa* since there is strong evidence that this species grows and reproduces without obvious feeding on eukaryotes (Röttger, 1972a,b; Röttger and Berger, 1972; Röttger, *et al.*, 1980, 1990b).

METHODS

Samples were collected at the *Halophila stipulacea* meadow north of Wadi Taba, Egypt at depths from 15-25 m. The collecting and processing followed Kuile and Erez (1984). From these samples, specimens of *Discorbis* sp. were isolated. The next day, a sediment sample was collected in front of the H. Steinitz Marine Biological Laboratory-Interuniversity Institute, Eilat, Israel at a depth of 40 m. The sediment was placed into shallow dishes covered with seawater. The foraminifera were allowed to crawl to the top of the sediment where they were isolated. This sample yielded *Heterostegina depressa* d'Orbigny and *Operculina ammonoides* d'Orbigny.

The foraminifera were placed in deep petri dishes with fresh seawater and observed hourly to see if they

*Accepted for publication in Endocytobiosis and Cell Research (1991). Reproduced with permisssion.

extended their pseudopodia. Specimens which built up extensive rhizopodial nets were offered *Dunaliella salina* and several diatom species as food. Although the algae fed were axenic cultures, the foraminifera had many bacteria on their surfaces which reproduced during the experiment. After twenty-four hours, the foraminifera were fixed with cold 3% glutaraldehyde in an acetate (2.5 mol/L) buffer, pH 5.2. *Heterostegina depressa* had not yet formed a sheath during this interval. The acid phosphatase assay followed the method given in the Lymphocyte Enzyme Kit (#180) marketed by Sigma Chemical Co. The specimens were rinsed in distilled water for 30 seconds. A solution of Napthol AS-BL Phosphoric acid (5 ml of a 4g/L solution) and Fast Garnet GBC (15 g) with an acetate buffer (45 ml, 2.5 mol/L, pH 5.2) was poured over the foraminifera, covering the specimens, and placed in a 37 C water bath for 1-2 hours. After incubation, the specimens were rinsed with distilled water, dehydrated to 70% ETOH and transported back to the laboratory in New York.

The foraminifera were rehydrated and gently decalcified with 5% poly no-cal (Polyscience, Inc. #16865) overnight, then dehydrated in an ethanol series, cleared with xylene and embed in Tissuemat (Fisher Sci. #12-647C). The sections, cut at 7 μm, were counterstained with hematoxylin (Humason, 1962), observed and photographed on a Zeiss Photomicroscope II. The assay yielded a bright red stain in areas of enzyme activity. This stain was very noticeable under the microscope and in color micrographs. Because the assay results in a red stain which is not a distinctive gray when photographed on Kodak technical pan 2415, we found it necessary to use blue and green filters to intensify the contrast. The technical pan 2415 was treated as a high contrast film (ASA 100) and developed in HC110 (dilution D).

RESULTS

In both *Heterostegina depressa* and *Operculina ammonoides*, the location of the acid phosphatase activity was similar to the other larger foraminifera we investigated (see figures in Chapter 4; Figs. 5.1, 5.2 and 5.3). The enzyme activity appeared in food clumps around the specimens and in the youngest (outer) chambers (Fig. 5.8) and in the organic sheaths surrounding the specimens of *Operculina* (Fig. 5.1). These food clumps were made up of algal cells and bacteria. A strong reaction was present in the marginal canal system, oblique plexus and in the distal part of the septal canal (Figs. 5.5 - 5.9). Reaction was not found in secondary or lateral canals or in the chambers (Figs. 5.5 and 5.8). Although we looked for it we could not unequivocally demonstrate enzyme activity near the endosymbionts.

Discorbis sp. showed strong acid phosphatase activity in the rhizopodia extending from the shell, in the adjacent food balls, the umbilicus and in the outer chambers (Fig. 5.4). This corresponded well with our previous observations of smaller foraminifera which lack endosymbionts (Lee, *et al.*, 1991b).

DISCUSSION

The results of this study are interesting for two reasons. First, they broaden the test organisms upon which the idea that extra-cameral digestion and intra-cameral partitioning may be preadaptations to foraminiferal

endosymbioses is based (Lee, *et al.*, 1991b). All three species demonstrated external digestion and a lack of acid phosphatase activity near the endosymbionts in the diatom-bearing forms. Secondly, the results demonstrate the enzymatic potential of *Heterostegina depressa* to digest food even if it does not usually do so. Röttger (1972a,b) concluded that *H. depressa* depends solely on the photosynthetic activity of its endosymbiotic algae for growth and reproduction. However, it does need trace substances provided by epi- and peribiotic bacteria (Röttger, *et al.*, 1990b). This is probably true for a number of larger foraminifera in addition to *Heterostegina*. Recently comparative studies of *Amphisorus hemprichii* and *Amphistegina lobifera* from the Red Sea showed significant differences between the two species with respect to their dependence on feeding to their carbon budgets (Kuile and Erez, 1987; Kuile, *et al.*, 1987; Lee, *et al.*, 1988b). The conclusion reached was that *A. lobifera* like *H. depressa* could satisfy its bulk carbon needs with the aid of its symbionts. This was not true for *Amphisorus*. *Amphisorus hemprichii* must feed to satisfy its micronutrient and perhaps vitamin needs (Kuile and Erez, 1987; Kuile, *et al.*, 1987; Lee, *et al.*, 1988b). More important, however, both species perished in the dark even when they were fed (Kuile and Erez, 1987; Kuile, *et al.*, 1987; Lee, *et al.*, 1988b). Thus one must be careful in separating the light- and symbiont-mediated effects on host bulk organic carbon needs and other vital needs presumably mediated by symbiont photophysiology or release of lesser quantities of critically needed metabolites.

One aspect of the biology of *Heterostegina* was not unequivocally resolved by the experiments reported here. Acid phosphatase activity was reported near the symbionts in this species (Kremer, *et al.*, 1980). Using Gomori technique it was found that some of the endosymbiotic diatom population always degenerates and are used by the hosts. The activity found in TEM was on the surface of the degenerating symbionts and on the inner surfaces of the symbiosome. It is possible that the concentration of the acid phosphatase was not detectable at the light microscopical level in our experiments. Although we could see random stained granules in the cytoplasm of *Heterostegina* we did not detect concentrations of activity near some symbionts. It is possible that under the conditions of our experiments, *Heterostegina*, newly harvested from the sea, do not actively digest symbionts (nor do symbionts autolyse). Another possibility is that the technique we used on whole specimens did not permit reactants to reach digestion/autolysis sites in sufficient quantities so that they could be detected by light microscopy. This topic piques curiosity and begs further investigation by TEM.

EXPLANATION OF FIGURES

Fig. 5.1. *Operculina ammonoides*, prior to decalcification, showing an outer edge of acid phosphatase activity associated with an adjacent algal food mat and organic sheath (arrows). Specimen photographed in distilled water. Scale bar = 1 mm.

Figs. 5.2 and 5.3. *Heterostegina depressa*, prior to decalcification, showing an outer edge of acid phosphatase activity both externally and within the margin of the test (arrows). Specimen photographed in distilled water. Scale bar = 1 mm.

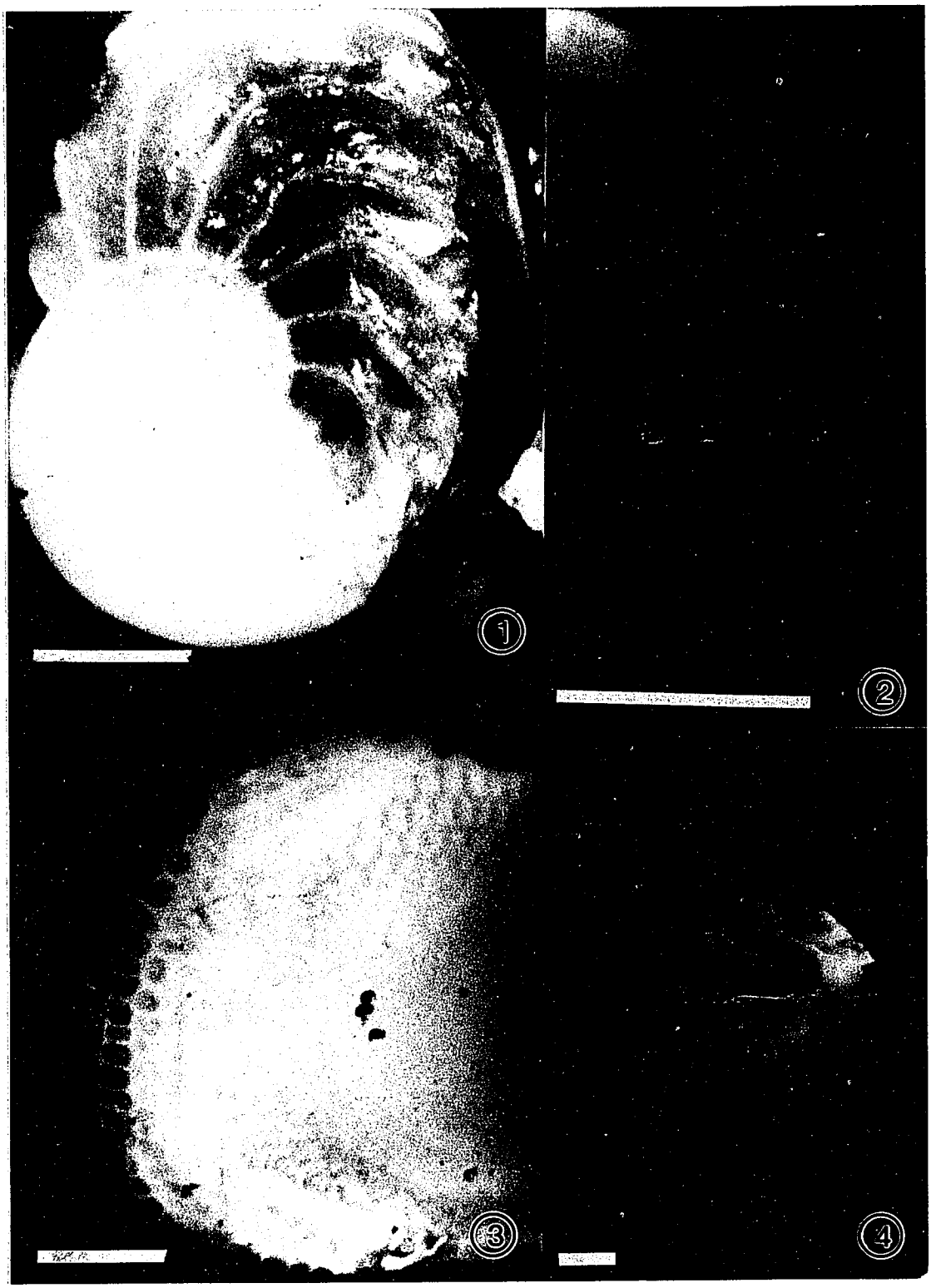
Fig. 5.4. *Discorbis* sp., prior to decalcification, showing an outer edge of acid phosphatase activity associated with an adjacent algal food mass and in the umbilicus near the apertures, slightly out of optical plane (arrow). Specimen photographed in distilled water. Scale bar = 250 μm.

Figs. 5.5 - 5.9. Photographed with the aid of green filters.

Figs. 5.5 and 5.6. Section of *Heterostegina depressa* showing acid phosphatase activity in the marginal canal system (arrows) whereas the intraseptal canal system lacks the enzyme. Scale bar for fig. 5.5 = 250 μm and scale bar for fig. 5.6 = 10 μm.

Fig. 5.7. Section of *Operculina ammonoides* showing acid phosphatase activity in the median marginal canal (arrow). Scale bar = 10 μm.

Figs. 5.8 and 5.9. Sections of *Operculina ammonoides* showing acid phosphatase activity in the median marginal canal and outer chamberlets (arrows). Scale bar = 10 μm.



Figs. 5.1 - 5.4

Figs. 5.5. - 5.9

CHAPTER 6

FEEDING AND GROWTH OF THE FORAMINIFER *PENEROPLIS PLANATUS* (FICHTEL AND MOLL) MONTFORT FROM THE GULF OF EILAT/AQABA*

INTRODUCTION

Despite an increase in research on the biology of larger foraminifera and their endosymbionts (see reviews: Lee, 1980, 1983; Lee and McEnery, 1983), the ability to maintain and culture larger foraminifera through successive generations has not yet been achieved. The larger foraminiferal-algal endosymbioses tend to deteriorate in the laboratory within a few days after the collection (Kuile and Erez, 1984; Koestler, *et al.*, 1985). Obviously typical culture conditions are not adequate for the organisms. Some have grown larger foraminifera for several months (Winter, 1907; Hallock, 1981b; Lee, *et al.*, 1991c), however, only one species, *Heterostegina depressa*, has been successfully cultured through successive generations (Röttger, *et al.*, 1986). If the needs of larger foraminifera could be satisfied, possibly they could be grown in continuous cultures to better study the entire association and eliminate the need for frequent collections of fresh organisms. Laboratory studies on the effects of environmental parameters on the life processes of foraminifera also help to define the ecological significance of the endosymbioses.

Since all of the larger foraminifera contain endosymbionts, the quality and quantity of light and food are expected to have the greatest effect on foraminiferal growth. Within certain ranges, laboratory experimentation on symbiotic foraminifera has shown a positive correlation between light intensity and foraminiferal growth (Lee and Zucker, 1969; Röttger, 1972b; Duguay and Taylor, 1978; Muller, 1978; Caron, *et al.*, 1982). Based on *in situ* growth studies on *Amphistegina lobifera* and *Amphisorus hemprichii*, Kuile and Erez (1984) suggest that this is due to some photobiological effect on the foraminiferal calcification and feeding rather than symbiont photosynthesis. Moreover, symbiont photosynthesis enhances carbonate production in the host (Lee and Zucker, 1969; Duguay and Taylor, 1978; Duguay, 1983). In light, host calcification in *Archaias angulatus* is directly proportional to the symbiont photosynthesis (Duguay and Taylor, 1978).

Although foraminifera consume a diverse diet of algae and bacteria, laboratory experiments have shown a selectivity in feeding and assimilation (Lee, *et al.*, 1966; Lee and Muller, 1973; Lee, 1980, 1983; Lee and McEnery 1983; Lee, *et al.*, 1988b).

Peneroplis planatus are cosmopolitan in tropical and subtropical lagoons and nearshore environments (Cushman, 1969; Murray, 1973; Loeblich and Tappan, 1988). The morphology of *Peneroplis* enhances light attenuation for the endosymbionts, although this has not been studied extensively (Hallock, 1979). *Peneroplis* have a wide range of salinity tolerance (3.7-5.3 %), temperature tolerance (18-27° C) and depth distribution (0-70 m) (Murray, 1973; Reiss and Hottinger, 1984). Natural populations seem to peak in spring and summer (Murray, 1973; Hallock,1984). The organisms are usually found on soft substrates, or on dead coral and coralline algal rubble (Winter, 1907; Murray, 1973; Hallock, 1984; Reiss and Hottinger, 1984; see Chapter 3).

*Published in Symbiosis 10: 63- 82 (1991). Reproduced with permisssion from Balaban Publishers.

The endosymbionts of *Peneroplis* are relatively unique among all the known algal-invertebrate (-protist) symbioses since they are rhodophytes (*Porphyridium*) (Leutenegger, 1977b, 1984; Lee and Hallock, 1987, Lee, 1990). Kremer, *et al.* (1980) identified tracer labeled photosynthates from the *Porphyridium /Peneroplis* system.

Although *Peneroplis* can be kept in the laboratory for a few days, no one has yet reported the culturing of the species from generation to generation. Since little was known about their dietary requirements, the main purpose of this study was to identify suitable food sources, by examining the feeding rates and retention of several species of algae; and to investigate the effect of light and food on the growth of *Peneroplis* in the laboratory.

METHODS

Collection of specimens

Between January 6 and March 26, 1988, *Peneroplis planatus* (Fichtel and Moll) Montfort were collected 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). The *Halophila* plants were haphazardly grabbed, along with the upper layer of sediment, and placed in whirl-pack or zip-lock bags. Back at the laboratory, the bags were emptied into a plastic colander, suspended in a flat plastic basin. Under running seawater, the plants were rubbed to dislodge foraminifera and the released foraminifera and sediment collected in the basin. This material was subdivided into several jars and dishes, and covered with fresh seawater. *Peneroplis* seem to prefer horizontal substrates such as the *Halophila* rhizomes, and the sediment (Faber, 1991). The material was allowed to sit several hours to overnight (in some cases) with frequent changes of the covering seawater. Specimens which were dark pink (indicative of their *Porphyridium* endosymbionts) were isolated under a dissecting microscope, and placed into deep petri dishes with fresh seawater until they were utilized.

Feeding experiment

The methodology of the tracer-labelled feeding experiment followed Lee *et al* . (1966, 1988b). Cultures of potential algal food sources, grown axenically in 10 ml of Erdschreiber's medium, to log phase, were inoculated aseptically with 10 µCi of sterile $NaH^{14}CO_3$ and incubated for an additional 3-7 days. The algal cells were harvested by gentle centrifugation, washed by resuspension in unlabeled sterile medium and recentrifuged ten times. Algal cell counts were made on a hemocytometer and the radioactivity measured by ß liquid scintillation counting (LSC). Within 24 hours after collection, healthy (with pseudopodia extended) *P. planatus* were fed these labeled algal cultures. The ten species of algae tested (*Chlamydomonas hedleyi*, *Chlorella* sp. (A.T.), *Chlorella* sp. (SUZY), *Cocconeis placentula*, *Entomoneis* sp., *Amphora* sp., *Nitzschia* sp., *Nitzschia* sp. (W521), *N. rhombica* (WH480) and *Navicula* sp.) resembled species of algae found in the *Halophila* beds where the *Peneroplis* were collected (Lee, *et al.* 1988b). After identification, the foraminifera were rigorously brushed clean, separated into groups of 10 organisms and placed into a well of a 9-spot plate,

with one milliliter of sterile, filtered seawater. The algae were evenly pipetted around the foraminifera to ensure uniform distribution throughout the spot plate. After a 24 hour feeding period, the foraminifera were brushed free of any adhering algae, and rinsed twice in seawater. In order to distinguish between ingestion and assimilation the specimens were split-sampled; half were harvested immediately while the others were returned to culture for a "cold" feeding period with the same algal species for an additional 24 hours. Three replicate, formalin-killed, and washed dead controls were treated as above.

Harvested foraminifera were rinsed in distilled water, and dried in a warm (40° C) oven overnight. The specimens were measured for maximum length and width, weighed on a Cahn 25 Electrobalance, then pooled and placed in a scintillation vial with 10 ml of Instagel, and counted on a Packard Tricarb LSC. Quench was corrected by the standard channels ratio method.

These measurements were converted, as in Lee *et al.* (1988b), to the mean algal equivalents eaten per microgram foraminifer (the total DPM measured per ug foraminifer divided by the DPM per individual alga fed or retained). Anovas were used to test mean differences between the initial amount eaten and the amount retained, for each of the algal species.

Growth experiment

Twenty six *Peneroplis* collected were undergoing multiple fission. The majority of these parents were megalospheric although a few were microspheric. The megalospheric juveniles were placed into Falcon flasks with 100 ml of sterile, filtered seawater, and fed *Dunaliella salina* . They received fresh seawater and food weekly. These specimens were transported back to the laboratory in New York. Only those which exhibited rhizopodial activity were brushed clean, separated into groups of 5 or 10 organisms, and placed in Falcon flasks with sterile, charcoal filtered seawater. The seawater was adjusted to 4.0% salinity, and a pH between 8.1 and 8.3, as described in Lee, *et al* . (1991c). Both salinity and pH were kept constant throughout the experiment. The culture temperature was a constant 23 ± 1° C.

Three light regimes were established: complete darkness (the specimens were placed in a dark incubator); low light of 30-50 $\mu E\ m^{-2}s^{-1}$ (Fluorescent tubes: F40CW/RS/EW-II); and high light of 200-400 $\mu E\ m^{-2}s^{-1}$ (Fluorescent tubes: F48T12.CWX.HO). Light measurements were taken with a LICOR (model LI-185B) quantum radiometer photometer. Seven feeding regimes were established: unfed control; and unialgal fed either *Dunaliella salina* , *Nitzschia* sp. (W521), *Chlorella* sp. (A.T.), *Navicula* sp., *Amphora* sp. or *Cocconeis placentula* .

Each week, the foraminifera were measured with a micrometer under a Wild M5 dissecting microscope, and transferred to fresh sterile, charcoal filtered seawater with fresh food (where applicable). The unfed specimens also received biweekly flask changes to minimize bacterial growth which may serve as food for the foraminifera.

The foraminifera were maintained until death. The criteria for death was either an empty test, or lack of rhizopodial activity for two consecutive weeks. This was directly observed or implied by the lack of an accumulated food ball or mat against the apertural region of the foraminifera. The formation of this mat is an indication of active feeding (Lee, *et al* ., 1988b). Such mats were removed in the weekly brushing of each foraminifera.

The shape of *P. planatus* resembles a raised ellipse. The thickness of the specimen is attained early in

the organism's development, and changes little as the organism grows by the addition of planispiral chambers (Hallock, 1979). The growth measurements were made along the greater axis (ie: the longest length), and the lesser axis (ie: the longest length perpendicular to the greater axis). These were multiplied to compare overall sizes and designated as an indication of the surface area. A plot of this area to the weight of the organism was constructed with data from 205 specimens. The regression obtained was utilized to convert the size measurements to weight:

$$\text{weight } (\mu g) = 0.4827 + (0.0001569 \times \text{surface area}), R=0.95.$$

After death, the specimens were rinsed twice in tap water and dried on paleontological slides. By measuring the size of the specimen at each chamber, the number of chambers grown in culture was estimated. Analyses of variance between comparable groups were performed to test significance.

RESULTS

Feeding experiment

Despite lower values for all the mean algal equivalents retained (final) per µg foraminifera, for six of the ten algal species tested, *P. planatus* statistically retained 100 % of the ^{14}C -labeled algae ingested (Table 6.1). The values are means of three replicates with standard errors. There appears to be some selectivity in feeding with *Cocconeis placentula* and *Amphora* sp. being ingested at a rate 5-6 times greater than any other species of algal food tested, with no statistical difference between the two species for the amount ingested and retained ($F=0.30$, $df=1,30$, and $F=0.96$, $df=1,25$, respectively; Table 6.1).

There was no statistical difference between the feeding rates using *Chlamydomonas hedleyi* , *Chlorella* sp. (A.T.), *Chlorella* sp. (SUZY), *Nitzschia* sp. (W521), *N. rhombica* , and *Navicula* sp ($F=0.99$, $df=5,92$), which averaged 6.6 algal equivalents per µg foraminifer d^{-1} (Table 6.1). Only one fourth the amount of *C. hedleyi* , about one third of the *Chlorella* sp. (A.T.), and almost one half of the *Navicula* sp. were retained (assimilated) after a 24 hour "cold" chase, whereas the carbon in the other species was completely retained (Table 6.1).

Except in the dead controls, the food organisms were drawn into mats around the apertural regions of the *Peneroplis*. We measured an overall uptake of a fraction of an algal equivalent (0.79 ± 0.18) per µg foraminifera d^{-1} in the dead controls. Hence, *Entomoneis* sp. and *Nitzschia* sp. may not have been eaten at all (Table 6.1). The other two *Nitzschia* species (*N. rhombica* (WH480), and clone W521) were ingested equally ($F< 0.01$, $df = 1,29$) at a rate 5 times greater than the former *Nitzschia* species or *Entomoneis*, and fully retained ($F=1.68$, $df = 1,22$; Table 6.1). The values obtained for *C. hedleyi* suggested that all of the algae ingested may have been egested (Table 6.1).

Growth experiment

The mean initial weights did not significantly vary among the experimental groups (Table 6.2, 6.3). Only those specimens which grew at least one chamber in their regime were used in calculating the mean final weight, mean number of chambers formed per individual and the mean weight gain. *P. planatus* did not grow

when starved, regardless of the presence or absence of light (Table 6.2). The six algal species used as food were chosen from the previous feeding experiment. Both *Amphora* sp. and *C. placentula* were heavily ingested and retained (Table 6.1). *Nitzschia* sp. (W521), *Chlorella* sp. (AT) and *Navicula* sp. represent moderate food species (Table 6.1). *Dunaliella salina*, although not employed in the feeding experiment, was shown in the past to be a good algal food for foraminifera (Lee, *et al.*, 1966). It produced one of the highest growth responses, 65.53 µg gain in high light (Table 6.2). When placed in complete darkness, regardless of the feeding regime, *P. planatus* grew few or no chambers (Table 6.2). The mean final weights were not significantly different (Table 6.3). Only seven specimens grew at all; four grew two chambers, and three grew one chamber. After 21 days, all the specimens in complete darkness had reduced cytoplasm with many empty chambers and weak rhizopodia, although the early whorl of each specimen retained the bright pink color of the endosymbionts.

Regardless of which species of alga fed, in low light, two thirds of the individuals grew (Table 6.2), with an overall mean gain in weight of 40.88 ± 4.24 µg. The foraminifera grew larger when fed *D. salina*, *Nitzschia* sp. (W521) and *Navicula* sp. than they did when they were fed *Chlorella* sp. (AT), *Amphora* sp. and *C. placentula* (Table 6.2). However, among the variables tested there was no statistical difference between the final weight, mean number of chambers per individual, or the survival time (Table 6.3). The specimens grew an average of over 8.8 ± 0.7 chambers in twenty weeks.

Most of the specimens (> 90 %) fed and placed in the high light regime grew much better (25 %) than those in the low light regime (Table 6.2). The mean final weights were not significantly different among the variables tested (Table 6.3) with an overall mean gain in weight of 51.78 ± 5.14 µg. However, the mean number of chambers grown were significantly different (Table 6.3). Those fed *C. placentula* grew on average only 4.17 ± 1.62 chambers per individual whereas on other algal diets *P. planatus* grew 12.58 ± 0.93 chambers per individual. This represents a fifty percent increase in the number of chambers formed in the high light compared to specimens in the low light. High variability of survival times of individuals within each group created very large standard deviations, hence growth rates (µg gain or number of chambers grown per week) were not calculated.

Although some of the specimens appeared "normal' (Fig. 6.1, 6.3), many of the specimens which grew, looked abnormal in shape (eg: Fig. 6.2). The new chamber growth was punctuated, which may be natural calcification (Hofker, 1951). Considerable variation was noted in the size of the chambers within a single specimen (Fig. 6.1). Other aberrant features included chambers lacking ornamentation (Fig. 6.4), the discontinuity of chambers (Fig. 6.5), rectilinear growth originating within the whorl (Fig. 6.6), small or partial chambers, chambers grown out of whorl (Fig. 6.7), the reversal of the whorl, chambers curling back over the specimen (Fig. 6.8), and fluting of the chambers.

Although there was large variability between the measurements (based on the standard errors), survival time seemed the most variable (Table 6.2). There are significant differences between some regimes, and no significant differences between others (Table 6.3). In general, except for the unfed specimens, those specimens in complete darkness lived shorter lives in the laboratory than those in either low or high light. The food source does not seem to be a factor. One might assume smaller specimens would live longer than larger specimens, but this too was not confirmed.

DISCUSSION

The feeding behavior of *P. planatus* resembles that of other larger foraminifera. They episodically gather large balls and mats of food around their shell, which does not necessarily coincide with digestion (Lee, 1974, Lee, *et al.*, 1988b). Often the excessive food is digested at a later time (Lee, 1974, Lee, *et al.*, 1988b). Lee, *et al.* (1988b), using tracer experiments and microscopic observations, suggested a large percentage of the potential energy present in the food is neither digested nor assimilated. The ^{14}C-labeled tracer pulse-chase experiment helps to distinguish between carbon in labeled food being ingested then egested, or being ingested and assimilated.

Peneroplis retained 100% of 6 algal food species in the first 24 hrs following ingestion (Table 6.1). This does not indicate complete digestion nor assimilation. Kuile, *et al.* (1987) found *Amphisorus hemprichii* to retain over 80% of *Chlorella* sp. (AT) ingested after the first 24 hr cold chase, but it retained only 50% after 10 days. The problem with more lengthy incubations is that while some molecules in the food can be channeled into anabolic pathways, others may be used catabolically. Still others may be recycled. Possibly even the 24 hour incubation was too long. When *P. planatus* is incubated in ^{14}C-bicarbonate, after one hour, the label is found throughout the entire specimen (Chapter 8). The labeled food algae may be respiring $^{14}CO_2$, which may be utilized by the endosymbionts. However, digestion in *Peneroplis* occurs extracamerally and in the last couple of chambers, not in the vicinity of the endosymbionts (Lee, *et al.* 1991b). And unlike the *Porphyridium* endosymbionts which lie free in the cytoplasm of the foraminifera, the food algae are enclosed in digestive vacuoles (Lee, 1990) which may limit interaction during a short incubation period. One should be very cautious about simplistic interpretations of this type of tracer experiment.

The experimental conditions of the feeding experiment do not simulate the foraminifera's true habitat. *Peneroplis* , collected from the *Halophila* meadow are not food limited, nor exposed to single species of algal food (Lee, *et al.*, 1988b). Nonetheless, the feeding rates are in the same order of magnitude as in other studies (Lee and Bock, 1976; Lee, *et al.*, 1988b), suggesting these experiments serve as a useful comparative tool for further laboratory work.

Our feeding experiment confirms that *Peneroplis*, like other foraminifera, are selective feeders, with a distinct preference for some algae over others (Table 6.1). *Amphora* sp. and *C. placentula* were heavily ingested and retained, whereas the *Chlorella* strains, *Navicula* sp., *Nitzschia* sp. (W521) and *N. rhombica* (WH480) were consumed and assimilated more moderately (Table 6.1). *Amphisorus hemprichii* also showed a strong bias toward ingesting and retaining the same strain of *Amphora* (Lee, *et al.*, 1988b), as did *Archaias angulatus*, which also ingested much *C. placentula* (Lee and Bock, 1976). Both *Amphisorus* and *Archaias* belong to the same superfamily (Alveolinacea) as *Peneroplis* (Loeblich and Tappan, 1988). The food preferences, although similar, show distinct differences. For example, *Amphisorus* ingested a substantial amount of *Entomoneis* sp. (Lee, *et al.*, 1988b) whereas *Peneroplis* ingested none (Table 6.1).

Clearly, *Peneroplis* needs ingested food, since the unfed specimens failed to grow (Table 6.2), but the type of algal food had little effect (Table 6.2, 6.3). Both *A. hemprichii* and *Marginopora kudakajimensis* grew poorly when starved in the light, and seemed to grow best on a mixed algal diet (Lee *et al.*, 1991c). The foraminifera were provided with large quantities of algae to eliminate the amount as a factor limiting growth.

Possibly a mixed algal species diet would increase the growth of *Peneroplis*. The overall increase in size, as well as the number of chambers formed was consistent when *Peneroplis* was fed, and light was constant (Table 6.3).

Hence light has a greater effect on *Peneroplis* growth than feeding. All the larger foraminifera studied either fail to grow or grow poorly in darkness, and grow in light: *A. hemprichii* (Kuile and Erez, 1984; Lee *et al.*, 1991c); *Amphistegina lessonii* (Muller, 1978; Röttger, *et al.*, 1980; Hallock, 1981b); *A. lobifera* (Hallock, 1981b; Kuile and Erez, 1984; Lee, *et al.*, 1991c); *Archaias angulatus* (Lee and Bock, 1976; Duguay and Taylor, 1978; Duguay, 1983); *Heterostegina depressa* (Röttger, *et al.*, 1980); *Marginopora kudakajimensis* (Lee, *et al.*, 1991b); and *Sorites marginalis* (Lee and Bock, 1976; Duguay, 1983). *P. planatus* also grew poorly in the dark yet grew well in 30-50 and 200-400 $\mu E\ m^{-2}sec^{-1}$(Table 6.2). The overall final sizes and the number of chambers formed increased in the higher intensity light. The primary production of other foraminiferal endosymbionts increases with light intensity (Lee and Bock, 1976; Duguay and Taylor, 1978; Röttger, *et al.*, 1980; Hallock, 1981b; Duguay, 1983) which may affect calcification and growth of the foraminiferal host. Kuile and Erez (1984) suggested that photosynthesis of endosymbionts and calcification may not be coupled. When food was not limiting, *Amphistegina lobifera* and *A. hemprichii* grew three times more in light than in darkness (Kuile and Erez, 1984). This difference indicates a direct photobiotic effect on calcification if one considers the contribution of endosymbiont photosynthetic carbon to the host to be less than the amount of carbon consumed by the foraminifera (Kuile and Erez, 1984). Another possibility may be that the spectral quality of the white light utilized in this study enhanced growth of *P. planatus* (Hemleben and Spindler, 1983).

Most of the *Peneroplis* grew when fed in light but some of the growth was abnormal. Aberrant chambers and structures were formed which varied in size and shape within individual specimens (see figures), although the cultures were maintained at constant temperature, salinity, pH, light intensity and food source. In planktonic foraminifera, the occurrence of kummerform chambers has been considered a result of environmental stress (Berger, 1970; Hecht and Savin, 1972, Caron, *et al.*, 1987). In *Peneroplis*, abnormal forms have been observed in the natural populations in the Persian Gulf (Basson and Al-Bahrani, personal communication). Hofker (1951b) considered abnormalities to be caused by nutritional conditions of the habitat. The effects of the other parameters, which may explain some of the abnormalities, remain uninvestigated. Also, none of the megalospheric juveniles grown in the laboratory reproduced. Several reached very large sizes (>1000 μm), well within the range of those specimens collected in the field which did reproduce in the laboratory. Our culture conditions, although sufficient to maintain and grow *Peneroplis*, are not adequate for continuous culturing. Only with further experimentation can the conditions be improved.

Transmission electron micrographs of the *Porphyridium* within *Peneroplis* show the algal cells lying free in the cytosol with envelope fibrils radiating from the endosymbionts (Lee, 1990). Kremer, *et al.* (1980) demonstrated primary productivity of the endosymbionts. In isolated cultures, *Porphyridium* exudes large quantities of sulfated polysaccharides (Jones, 1962; Hawkins and Lee, 1991). Tracer ^{14}C-labeled bicarbonate uptake experiments show a concentration of radioisotope around the endosymbionts in autoradiographs, and throughout the foraminifera after an hour of incubation (Chapter 8). It appears that the *Porphyridium* endosymbionts are providing the foraminifera with something, although the exact nature of the translocated substance needs further evaluation. Yet *Peneroplis* can not grow on this nutrition alone.

Heterostegina depressa has been shown to survive and grow without food (Röttger, 1972b). All the other larger foraminifera must supplement whatever is obtained from their endosymbionts with external food sources. The perforate foraminifera, such as *Heterostegina* and *Amphistegina* appear to rely less on food as an organic carbon source (Kuile, *et al.*, 1987; Lee, *et al.*, 1988b), whereas the imperforate soritids, *Amphisorus*, *Archaias*, *Sorites* and *Peneroplis*, require more algal food (Lee and Bock, 1976; Kuile, *et al.*, 1987, Lee, *et al.*, 1988b). This seems an anomaly, because *Peneroplis* and *Archaias* are so well adapted for endosymbiosis yet must have an external food source. Other studies suggest that feeding of external sources may be to obtain nitrogen or phosphorus which is needed by the foraminifera and algae (Jørgensen, *et al.*, 1985; Kuile, *et al.*, 1987; Lee, *et al.*, 1988b). Experiments on nitrate and phosphate removal from culture medium by foraminifera show uptake and an increase in the growth of the foraminifera (Lee, *et al.*, 1991c). *Amphisorus* showed a greater response to the nutrient enrichment than did *Amphistegina* (Lee, *et al.*, 1991c). This may explain the soritids' greater need for food.

In summary, *P. planatus* may derive some nutritional benefit from its endosymbionts. Also light plays an important role in the foraminifer's growth. Yet external feeding, which appears selective, is necessary. Now that we are able to maintain *Peneroplis* in culture for long periods, the obligatory relationship between the *Peneroplis* host and the its unique *Porphyridium* endosymbiont may be more easily experimentally probed.

Table 6.1. Feeding of *Peneroplis planatus* on various species of labeled algae in 24 hr (initial) and the retention of the label after a 24 hr "cold" feeding chase (final). The specimens were split sampled between the two groups. The values are means and standard errors for three replicate experiments, and are expressed in algal equivalents per µg foraminifer, which equals the total dpm measured per µg foraminifer divided by the dpm per individual alga fed. The F values are computed by ANOVA between the initial and final readings. * = $P < 0.01$, ** = $P < 0.05$, and NS = not significant.

Food organism	N	Average weight (µg)	Initial	N	Average weight (µg)	Final	df	F	Percent retained
Chlamydomonas hedleyi	28	87.46 ± 11.68	2.61 ± 0.70	29	70.66 ± 5.57	0.67 ± 0.24	1,30	5.58 **	25.7
Nitzschia sp. (W521)	30	66.23 ± 7.90	4.23 ± 0.59	27	75.22 ± 8.24	3.91 ± 0.77	1,30	0.11 NS	100.0
Entomoneis sp.	29	93.59 ± 19.06	0.22 ± 0.03	28	81.46 ± 7.82	0.10 ± 0.02	1,29	10.03 *	45.5
Chlorella sp. (AT)	29	59.79 ± 6.57	3.54 ± 0.95	28	82.00 ± 9.72	1.25 ± 0.22	1,30	5.21 **	35.4
Chlorella sp. (SUZY)	30	73.13 ± 9.08	6.84 ± 4.13	28	76.82 ± 10.03	4.39 ± 2.58	1,31	0.43 NS	100.00
Cocconeis placentula	29	80.66 ± 8.48	30.97 ± 5.38	29	66.55 ± 8.19	21.50 ± 3.86	1,29	1.27 NS	100.00
Amphora sp.	30	84.17 ± 13.49	33.65 ± 6.50	25	81.96 ± 9.68	27.78 ± 5.29	1,26	0.44 NS	100.00
Nitzschia sp.	26	93.65 ± 10.46	0.66 ± 0.23	30	64.83 ± 7.29	0.31 ± 0.08	1,30	2.03 NS	100.00
N. subcommunis (WH480)	30	89.30 ± 10.90	4.18 ± 0.94	26	87.81 ± 19.11	2.34 ± 0.70	1,21	1.75 NS	100.00
Navicula sp.	29	94.52 ± 17.43	6.11 ± 1.06	28	76.54 ± 8.31	2.98 ± 0.38	1,23	5.43 **	48.8

Table 6.2. Growth data for *Peneroplis planatus* for seven feeding regimes and three light regimes. D = complete darkness, L = low light intensity (30-50μE $m^{-2}s^{-1}$), H = high light intensity (200-400 μE $m^{-2}s^{-1}$). * averages of only those specimens which grew at least one chamber in culture.

Feeding regime	Light regime	N	Average initial weight (μg)	Percent which grew	Average final weight (μg)*	Average chambers per foraminifera*	Average μg gain*	Average survival time (days)	Range (days)
Unfed	D	5	20.24 ± 3.43	0	-	-	-	137 ± 10	97-147
Unfed	L	5	20.48 ± 4.65	0	-	-	-	79 ± 9	55-97
Dunaliella salina	D	20	17.82 ± 2.07	10.00	12.80 ± 3.96	1.50 ± 0.50	1.70	84 ± 5	42-105
Dunaliella salina	L	25	21.94 ± 2.39	72.00	78.46 ± 13.71	9.56 ± 1.86	56.96	123 ± 15	21-345
Dunaliella salina	H	15	17.19 ± 2.67	93.33	80.32 ± 11.79	15.50 ± 2.17	65.53	166 ± 27	21-280
Nitzschia sp. (W521)	D	20	19.51 ± 1.89	0	-	-	-	81 ± 5	35-112
Nitzschia sp. (W521)	L	27	23.81 ± 2.39	62.96	74.23 ± 6.80	11.18 ± 1.75	49.54	134 ± 15	14-294
Nitzschia sp. (W521)	H	11	19.21 ± 2.59	100.00	73.51 ± 5.82	13.36 ± 1.66	54.30	146 ± 33	28-294
Chlorella sp. (AT)	D	19	17.97 ± 1.81	21.05	16.96 ± 2.65	1.50 ± 0.29	3.42	90 ± 5	63-119
Chlorella sp. (AT)	L	26	24.17 ± 2.46	46.15	62.00 ± 11.05	9.25 ± 1.93	38.63	141 ± 21	14-462
Chlorella sp. (AT)	H	10	20.62 ± 3.14	80.00	63.90 ± 13.03	10.00 ± 2.25	44.38	128 ± 25	42-245
Navicula sp.	D	20	18.79 ± 2.00	5.00	10.68	2.00	2.34	80 ± 4	49-105

Table 6.2 continued:

Feeding regime	Light regime	N	Average initial weight (µg)	Percent which grew	Average final weight (µg)*	Average chambers per foraminifera*	Average µg gain*	Average survival time (days)	Range (days)
Navicula sp.	L	26	20.97 ± 1.97	69.23	67.33 ± 8.78	10.28 ± 1.77	45.98	146 ± 20	56-462
Navicula sp.	H	15	19.66 ± 2.01	100.00	81.82 ± 14.60	12.60 ± 2.06	62.15	100 ± 22	14-238
Amphora sp.	L	13	20.61 ± 2.32	100.00	46.87 ± 5.85	7.80 ± 0.91	26.26	156 ± 33	24-342
Amphora sp.	H	9	19.97 ± 3.24	100.00	62.93 ± 8.20	9.33 ± 1.56	43.15	106 ± 33	34-279
Cocconeis placentula	L	15	32.36 ± 4.34	86.67	45.54 ± 4.17	3 .92 ±0.71	16.95	124 ± 28	13-342
Cocconeis placentula	H	8	28.66 ±3.34	75.00	40.23 ±4.30	4.17 ±1.62	12.00	70 ±19	20-174

Table 6.3. Results of analysis of variance between comparable groups of *Peneroplis planatus*, expressed as computed F values. V = independent variable, * = P < 0.001, ** = P < 0.01, *** = P < 0.05, and NS = not significant.

			Dependent Variables			
Feeding regime	Light regime	df	Average initial weight	Average final weight	Average chambers individual^{-1}	Survival time
Unfed	V	1,8	<0.01 NS	<0.01 NS	N/A	19.77 **
Dunaliella salina	V	2,57	1.23 NS	10.22 *	19.31 *	5.49 **
Nitzschia sp. (W521)	V	2,55	1.26 NS	19.11 *	19.19 *	4.03 ***
Chlorella sp. (AT)	V	2,52	1.82 NS	7.03 **	7.12 **	2.12 NS
Navicula sp.	V	2,58	0.33 NS	12.17 *	16.42 *	4.20 ***
Amphora sp.	V	1,20	0.05 NS	2.69 NS	1.90 NS	1.07 NS
Cocconeis placentula	V	1,21	0.33 NS	2.10 NS	0.04 NS	1.80 NS
V	dark	4,79	0.16 NS	0.12 NS	1.33 NS	8.60 *
V	low	6,130	1.78 NS	1.39 NS	1.65 NS	0.60 NS
V	high	5,62	1.68 NS	1.85 NS	3.69 **	1.54 NS

EXPLANATION OF FIGURES

Fig. 6.1 - 6.8 SEM micrographs of *Peneroplis planatus* cultured in various laboratory regimes.

Fig. 6.1. *Peneroplis planatus* fed *Dunaliella salina* in the high light regime. Specimen grew 20 chambers in culture. Scale bar = 200 μm.

Fig. 6.2. *P. planatus* fed *Nitzschia* sp. (W521) in the high light regime. Specimen grew 16 chambers in culture. Scale bar = 100 μm.

Fig. 6.3. *P. planatus* fed *Dunaliella salina* in the high light regime. Specimen grew only one chamber in culture. Scale bar = 50 μm.

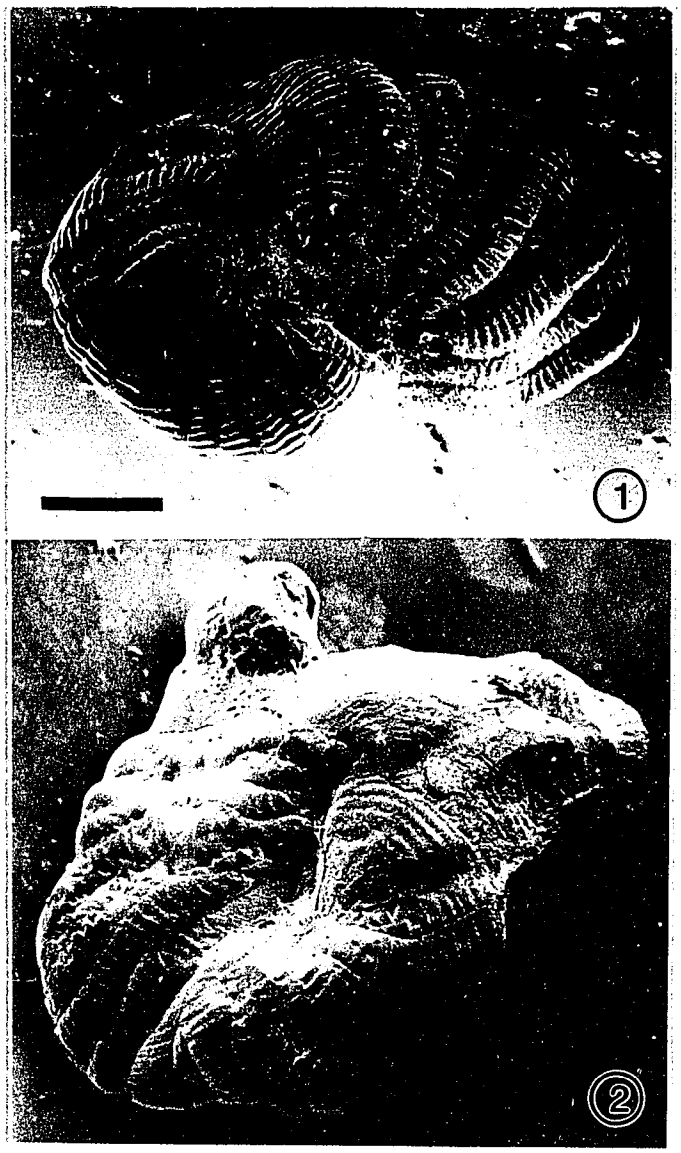
Fig. 6.4. *P. planatus* fed *Dunaliella salina* in the high light regime. Specimen grew 15 chambers in culture. Scale bar = 100 μm.

Fig. 6.5. *P. planatus* fed *Chlorella* sp. (AT) in the low light regime. Specimen grew 17 chambers in culture. Scale bar = 100 μm.

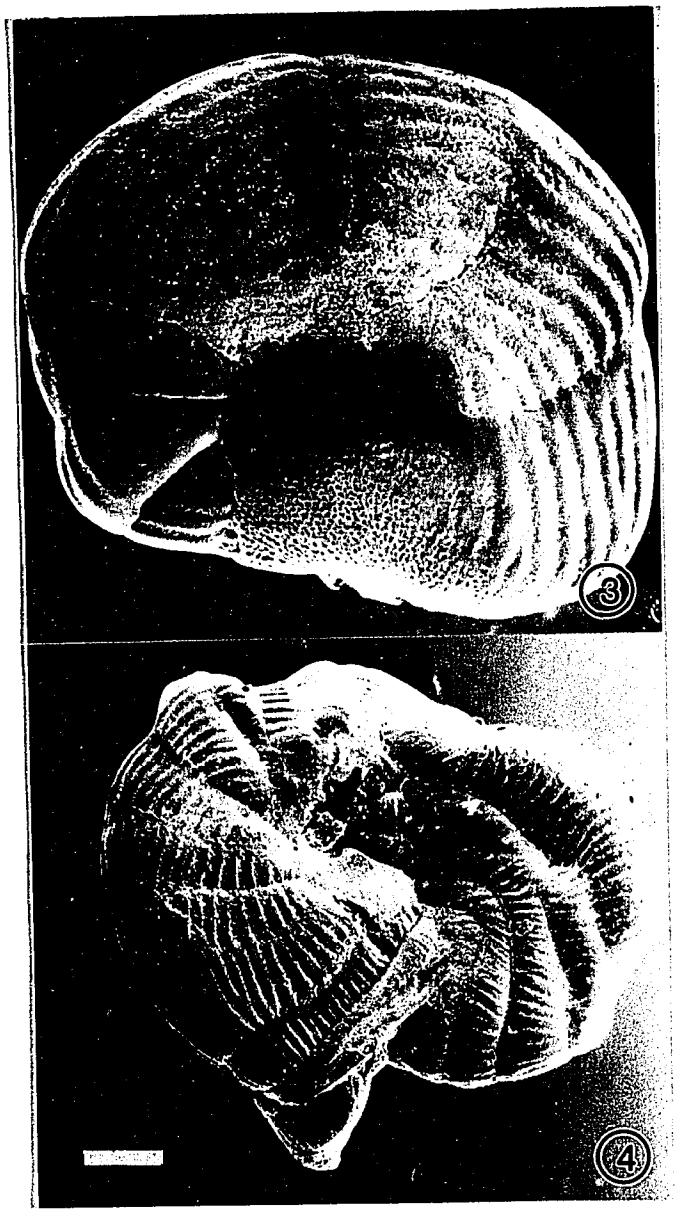
Fig. 6.6. *P. planatus* fed *Nitzschia* sp. (W521) in the low light regime. Specimen grew 22 chambers in culture. Scale bar = 200 μm.

Fig. 6.7. *P. planatus* fed *Dunaliella salina* in the high light regime. Specimen grew 2 chambers in culture. Scale bar = 100 μm.

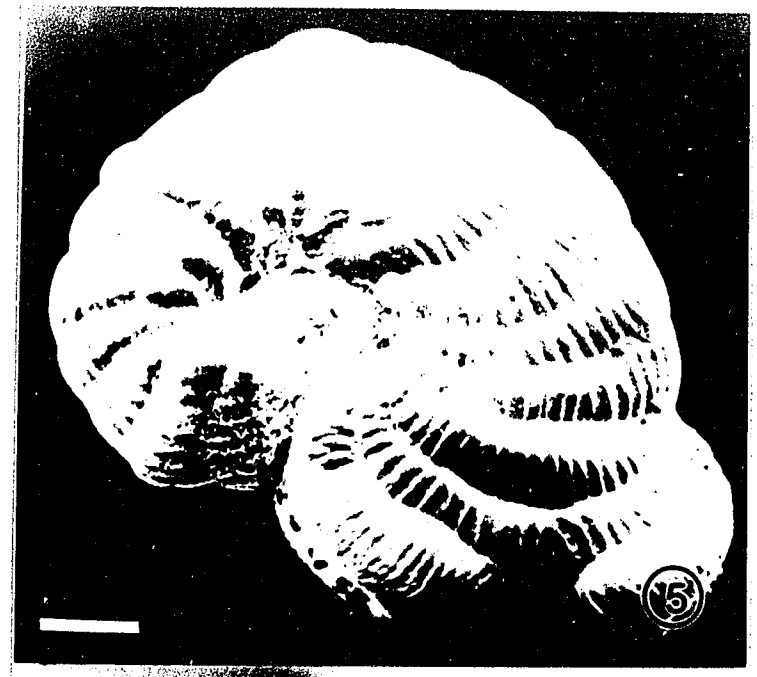
Fig. 6.8. *P. planatus* fed *Nitzschia* sp. (W521) in the low light regime. Specimen grew 12 chambers in culture. Scale bar = 100 μm.



Figs. 6.1 - 6.2



Figs. 6.3 - 6.4



Figs. 6.5 - 6.6

Figs. 6.7 - 6.8

CHAPTER 7

THE GROWTH IN THE LABORATORY OF *PENEROPLIS PLANATUS* (FICHTEL AND MOLL) MONTFORT AND *P. PERTUSUS* (FORSKÅL) FROM KUDAKA ISLAND, JAPAN

INTRODUCTION

The growth of foraminifera is influenced by many abiotic and biotic factors. Environmental conditions (eg. temperature, salinity, light) affect the physiology of the foraminifera, and often define their distribution. Nutrition, however, may be the most important factor affecting growth (Lee, 1974) and may determine the finer distributional patterns (Boltovskoy and Wright, 1976).

Our previous study (Faber and Lee 1991, Chapter 6) examined the feeding and growth in the laboratory of *Peneroplis planatus* collected near Wadi Taba, Gulf of Aqaba/Eilat, Egypt. Radioisotopic tracer experiments suggested selectivity in the diet of this foraminifer; *P. planatus* ingested and retained 100% of ^{14}C-labeled *Cocconeis placentula*, *Amphora* sp., *Chlorella* sp. (SUZY), *Nitzschia* sp., *Nitzschia* sp. (W521), and *N. subcommunis* (WH480) (Faber and Lee, 1991). Acid phosphatase assays demonstrated that digestion was external in the extended rhizopodia and the last few chambers (Lee, *et al.*, 1991b). Both light and food were needed for growth in this species, although light had a greater effect (Faber and Lee, 1991). Specimens failed to grow in the dark even when fed. Overall final size and the number of chambers produced increased with light intensity (Faber and Lee, 1991). Also, starved specimens in the light failed to grow; external algal food was necessary. However, all species of unialgal food tested supported growth equally.

Many of the foraminifera from the Gulf of Aqaba grew abnormally in the laboratory, forming aberrant chambers and final shell shapes, despite reaching final sizes comparable to those seen in the field, and none reproduced in the laboratory (Faber and Lee, 1991).In other species of larger foraminifera, light quantity seemed to control the shape of the foraminiferal shell, presumably by affecting the interactions between the endosymbionts and the foraminiferal host (Hallock, 1979). Although the quantity of light causes changes in the growth of *P. planatus*, it did not seem to be the cause of the deformities encountered (Faber and Lee, 1991). Murray (1963) speculated that food shortages lead to deformities in foraminiferal morphology. Hence, the diets we fed the foraminifera were likely to be incomplete. Although the algal food provided the foraminifera with enough bulk nutrients to grow, possibly some metabolite was missing or in insufficient quantity. Since in nature the foraminifera are exposed to a wide variety of potential algal foods (Lee, *et al.*, 1988b), possibly a mixed algal diet would better reflect the nutritional needs of these organisms.

Ingestion of external food by foraminifera possibly provides needed sources of nitrogen, phosphorus, required metabolites and nutrients, or vitamins (Jørgensen, *et al.*, 1985; Kuile, *et al.*, 1987; Lee, *et al.*, 1988b, 1991c). Laboratory experiments on removal of nitrate and phosphate from culture medium by the foraminifera, using batch and chemostat cultures, showed uptake and increased growth of the foraminifera (Lee, *et al.*, 1991c).

The goal of this study was to examine and compare the growth in the laboratory of *Peneroplis planatus* and *P. pertusus* from Kudaka Island, Okinawa Prefecture, Japan, to those from Wadi Taba. In addition we expanded the scope beyond the initial study by using multialgal food sources and nutritional supplements.

METHODS

Specimens of *Peneroplis planatus* (Fichtel and Moll) Montfort and *P. pertusus* (Forskål) were collected from the back reef habitat of Kudaka Island, Okinawa Prefecture, Japan, in December 1988 and July 1989. These were transported to New York in insulated plastic food containers.

The foraminifera were brushed clean and placed into Falcon flasks (5 to 10 specimens per flask) with 100 ml of sterile charcoal-filtered Long Island seawater, adjusted to 3.5% salinity and a pH between 8.1 and 8.3. Both the salinity and pH, which reflected natural conditions at the collection site, were constant throughout the experiment. The culture temperature was a constant 23 ± 1 °C. Eighteen feeding regimes were established:

1. starved controls;
2. unialgal-fed either
    a. *Dunaliella salina*,
    b. *Nitzschia* sp. (W521),
    c. *Chlorella* sp. (AT),
    d. *Navicula* sp., or
    e. *Amphora* sp.;
3. multialgal fed either
    a. *D. salina* and *Nitzschia* sp. (W521),
    b. *D. salina* and *Chlorella* sp. (AT),
    c. *D. salina* and *Navicula* sp.,
    d. *Nitzchia* sp. (W521) and *Chlorella* sp. (AT),
    e. *Nitzschia* sp. (W521) and *Navicula* sp., or
    f. *Chlorella* sp. (AT) and *Navicula* sp;
4. starved with a nitrogen supplement (see below);
5. starved with a phosphorus supplement (see below);
6. starved with both nitrogen and phosphorus supplements;
7. fed *D. salina* with a nitrogen supplement;
8. fed *D. salina* with a phosphorus supplement;
9. fed *D. salina* with both nitrogen and phosphorus supplements.

These specimens were kept in a low light regime of 30-50 $\mu E\ m^{-2}\ s^{-1}$ (Fluorescent tubes: F40CW/RS/EW-II). Also, a high light regime of 200-400 $\mu E\ m^{-2}\ s^{-1}$ (Fluorescent tubes: F48T12.CWX.HO) was used for the nutrient supplemented specimens.

The nitrogen supplement was the addition of $NaNO_3$ to a concentration of 1µg at l^{-1} NO_3^- and the phosphorus supplement was the addition of NaH_2PO_4 to a concentration of 0.1µg at l^{-1} PO_4^{-2} as in Lee *et al.* (1991c).

Each week the foraminifera were cleaned by brushing, measured with a calibrated ocular micrometer and transferred to fresh sterile charcoal-filtered seawater with fresh food or nutrient supplements (where applicable). The starved and nutrient (NO_3^- / PO_4^{-2}) supplemented specimens received biweekly flask changes to minimize bacterial growth which may have served as food for the foraminifera.

In addition to the specimens collected from the field and placed into the various feeding regimes, one schizont of *P. planatus* released megalospheric juveniles in the laboratory. These juveniles were also incorporated into the above growth test regimes.

The foraminifera were maintained until death as seen by an empty test or lack of rhizopodial activity for two consecutive weeks (Faber and Lee, 1991). The size measurements of *P.planatus* were converted to weight using the regression calculated earlier (Faber and Lee, 1991). A plot of surface area (estimated by the product of the maximum length and the largest perpendicular to this length) to the weight of the organism was constructed with data from 29 *P. pertusus* . The regression obtained was utilized to convert the size measurements of *P. pertusus* to weight:

$$\text{weight } (\mu g) = -15.7684 + (0.000172 \times \text{surface area}),\ R = 0.94.$$

After death, the specimens were rinsed twice in tap water and dried on paleontological slides. Since the initial and final sizes of each specimen were known, by measuring the sizes of the specimens at each chamber, the number of chambers grown in culture was estimated. Analyses of variance between comparable groups were performed to test significance. The specimens were divided into as many groups as possible, thus reducing the overall number of organisms in each group. This may have affected the statistical analyses of various groups since small sample sizes with large standard errors may not differ statistically.

RESULTS

Peneroplis planatus

All starved specimens grew, adding on average 4 chambers, with a range of 2-7 chambers (Table 7.1). These specimens only received nutrition from their endosymbionts or possibly from ingesting bacteria. Only 75% of the specimens given only one form of inorganic nutrient supplement grew. The specimens receiving inorganic nutrient supplements of either nitrogen or phosphorus grew less than the starved specimens, whereas those receiving both nitrogen and phosphorus grew nearly as many chambers as the starved specimens. However, there was no statistical difference in final size and number of chambers formed between the two groups ($F=0.41$ and $F = 1.31$, respectively, $P > 0.05$, $df=2,46$). Because we used specimens from natural

collections, the various experimental groups were not all uniform in initial size. This was not an experimental problem since the change in growth rate predictably slows with size in this genus (Faber and Lee, 1991). The initial sizes of the specimens given the inorganic nutrient supplements were not significantly different from each other ($F=0.68$, $P > 0.05$, $df = 2,46$) but they were nearly double the initial sizes of the starved specimens. Smaller foraminifera were expected to grow more chambers in the laboratory than larger specimens. Most probably, had the specimens given inorganic nutrient supplements been the same size as the starved specimens, their growth would have been more comparable. The percentage of increase in weight from the initial weight normalizes the weight gain for differences in initial weight. This percentage of increase for the starved specimens was close to those given the nutrient suppliments (Table 7.1).

The specimens fed *Dunaliella salina* , in addition to inorganic nutrient supplements, grew at a rate similar to those with just the inorganic nutrient supplements, with no statistical difference between the two groups with regard to final size ($F=0.54$, $P > 0.05$, $df=5,38$) and the number of chambers formed ($F=0.31$, $P > 0.05$, $df=5,38$) in low light, or in high light ($F=0.33$ and $F= 1.82$, respectively, $P > 0.05$, $df=2,17$).

There were no significant differences in the initial sizes of the foraminifera used in the unialgal feeding experiments ($F=0.48$, $P > 0.05$, $df=10,157$). Nearly all the specimens (98%) fed unialgal diets, without inorganic nutrient supplements, grew, and increased most in size when fed *D. salina* , *Nitzschia* sp. (W521) or *Navicula* sp. (Table 7.1). Specimens fed *Chlorella* sp. (AT) grew to final sizes which were less than 20% of the other specimens fed unialgal diets of other species, and formed less than 25% the number of chambers. Specimens fed *Amphora* sp. reached final sizes comparable to specimens fed on other unialgal diets but grew less than 75% the number of chambers.

All the specimens (100%) fed mixed algal diets also grew (Table 7.1). Those populations fed a mixture of *D. salina* and *Chlorella* grew less (<50%) than those fed mixtures of other algae. With the exception of those fed only *Chlorella* , and those fed the mixture of *D. salina* and *Chlorella* , specimens fed other unialgal or mixed algal diets grew the same number of chambers, 13.8 ± 6.7 (not significant, $F=1.85$, $P > 0.05$, $df=8,124$), and obtained similar final sizes with a mean of 189.4 ± 153.1 (not significant, $F=0.59$, $P > 0.05$, $df=8,124$). They also grew larger and built more chambers than any of the specimens fed on a diet of *D. salina* supplemented with inorganic nutrients, which grew only 2.53 ± 2.01 chambers on average.

The types of chambers formed varied. Starved specimens grew more dwarf (kummerform) chambers (25%) than all the other experimental groups. These chambers were less than 4% of the total number of chambers formed. Rectilinear (uncoiled chamber) growth was not seen in any specimen given an inorganic nutrient supplement or fed *D. salina* with an inorganic nutrient supplement (Table 7.1). With the exception of those fed *Amphora* sp., some degree of rectilinear growth was seen in every group of specimens fed unialgal and mixed algal diets. The greatest number of rectilinear chambers were formed by groups fed unialgal diets of *D. salina*, *Nitzschia sp.* (W521), or *Navicula* sp., and those fed mixed algal diets of *D. salina* and *Nitzschia* sp. (W521) (approximately 30%). At the other extreme groups fed a mixture of *D. salina* and *Navicula* sp. formed less than 6% of rectilinear chambers (Table 7.1).

The patterns of chamber formation varied widely, even in clone cultures. Several specimens grew normally

(Plate 7.1 Fig. 1). Rectilinear growth is normal when it appears within the whorl in a normal progression of chambers (Plate 7.3, Fig. 5). However, commonly two apertural regions grew from rectilinear chambers either out of the whorl (Plate 7.1 Fig. 2) or within the whorl (Plate 7.1 Fig. 3,4, Plate 7.2 Fig. 3,4). At times, these extra rectilinear chambers grew out of the planispiral (Plate 7.1 Fig. 6). Small adventitious chambers which lacked apertures were found (Plate 7.2 Fig. 1). These chambers may have begun with a secondary aperture which later closed in a blind end when calcification covered the aperture. Irregular growth included uneven chamber formation (Plate 7.2 Fig. 2), partial chamber formation (Plate 7.3 Figs. 1,2) and chambers which curved back onto other chambers (Plate 7.3 Figs. 3,4). *P. planatus* has an arrangement of single to triple rows of irregular pits on the surface of the chambers These rows are always perpendicular to the apertures (Plate 7.2 Fig. 6). Some chambers lacked normal ornamentation (Plate 7.3, Fig. 5), and others even took on strange shapes (Plate 7.4 Fig. 2).

Besides chamber irregularities, many specimens curved out of the plane of a normal planispiral (Plate 7.1 Fig. 5, Plate 7.4 Fig. 4). In some specimens; the growth of chambers backward onto other chambers led to the fluting of the specimen (Plate 7.3, Fig. 6).

The structure of the aperture of an adult *P. planatus* is either a single or double row of irregular dendritic apertures (Gudmundsson, 1990). Most laboratory reared specimens showed this arrangement (for example: Plate 7.3 Fig. 3) despite other morphological irregularities. Some of the rectilinear appendages which resulted in a second apertural region showed a single dendritic aperture (Plate 7.1, Fig. 6) which in nature is only found in *P. acicularis* (Plate 7.8, Figs. 2,4; Gudmundsson, 1990).

In adult *P. planatus* isolated from sand samples collected from the same location as the specimens which were utilized in this study, several specimens showed similar morphological aberations although the severity of the abnormalities was not as dramatic as those in laboratory reared specimens. Most specimens from the natural population appeared normal (Plate 7.5 Fig. 1). Others showed uneven chamber formation (Plate 7.5 Fig. 2), chambers grown over the previous chamber (Plate 7.4 Fig. 5, Plate 7.5 Figs. 3,4), partial chamber formation (Plate 7.5 Fig. 6), chambers curved back onto other chambers (Plate 7.5 Fig. 5), chambers grown out of the planispiral (Plate 7.4 Fig. 3) and branched apertures due to the chamber leaving the planispiral (Plate 7.4 Fig. 1).

Survival times in the laboratory were extremely variable with large standard errors (Table 7.1). As expected, there was a relationship between longevity and growth. The specimens with the shortest life span, grew the least.

Light intensity was a variable only in the inorganic nutrient supplemented experiments. The intensity of light had no effect on the overall final size obtained by the specimens (not significant, $F=0.03$, $P > 0.05$, $df=1,47$), however, there was a significantly different number of chambers formed ($F=4.29$, $P \leq 0.05$, $df=1,47$). Specimens incubated in high light grew fewer chambers (only 66%) than did those incubated at lower light.

Peneroplis pertusus

Starved specimens grew an average 5.20 ± 1.93 chambers during experimental incubation (Table 7.2). Those specimens incubated with inorganic nutrient supplements of either nitrogen or phosphorus, or both, and those fed *D. salina* with inorganic nutrient supplements grew less (about 73%) than starved controls, although the percentages of increase in weight for all of these specimens were within the same range as the starved controls (Table 7.2). They added an average of 2.86 ± 0.09 chambers during the experiment. No statistical difference was observed in the overall final sizes obtained or in the number of chambers formed between the groups in low light ($F=0.94$ and $F=1.38$, respectively, $P > 0.05$, df=5,34). Light had no effect on overall final sizes or number of chambers formed by the specimens receiving the inorganic nutrient supplements (not significant, $F=0.19$ and $F= 0.16$, respectively, $P > 0.05$, df=1,15).

Specimens fed either unialgal or mixed algal foods grew more rapidly than those starved or incubated with inorganic nutrient supplements. They obtained an average final size of 210 µg by adding 17 chambers in the laboratory. No statistical difference in final sizes or numbers of chambers formed was observed between any of the algal diets ($F=1.00$ and $F=1.08$, respectively, $P > 0.05$, df=10,79).

Relatively few final kummerform chambers were seen in specimens of laboratory grown *P. pertusus* . However, the number of rectilinear chambers was variable and high in some groups. Those fed *Nitzschia* sp. (W521) grew nearly 66% of its chambers rectilinearly (Table 7.2). Except for the population fed *D. salina* in a medium enriched with both nitrogen and phosphorus, which grew 12% rectilinear chambers, none of the other populations grown in media enriched with inorganic nutrients grew any rectilinear chambers.

Like *P. planatus* , many specimens of *P. pertusus* grew abnormally. Chambers grown out of the whorl resulted in rectilinear growth with two apertural regions (Plate 7.6, Figs. 1,2,7). A second apertural region also occurred when adventitious chambers left the planispiral (Plate 7.7 Figs 2,6). Blind adventitious chambers were formed (Plate 7.6 Fig. 4, Plate 7.7 Fig. 1) as well as partial chambers, chambers grown over previous chambers, and uneven chambers (Plate 7.6 Figs. 3,5,7,8, Plate 7.7 Fig. 4). Although adult *P. pertusus* usually have multiple irregularly dispersed dendritic apertures (Gudmundsson, 1990), several specimens showed single dendritic apertures (Plate 7.6 Fig. 6).

Specimens from the natural population showed similar abnormalities such as chambers grown over previous chambers (Plate 7.7 Fig. 3), rectilinear growth orginating within the whorl resulting in two apertural regions (Plate 7.7 Figs. 5,7) and uneven chambers (Plate 7.8, Figs. 3,5).

Survival times were extremely variable, ranging from 2 to 93 weeks in the laboratory (Table 7.2). Those given algal diets lived longer than the other specimens. As with *P. planatus* , those specimens which lived longer in the laboratory, grew more chambers and reached larger final sizes. However, none of the specimens of either species reproduced in the laboratory during the term of the experiment.

DISCUSSION

Historically, shell characteristics of the foraminifera have been used almost exclusively to delineate various taxonomic levels. Physiological differences in growth also could be important characters used to supplement morphological characters in species identification (Myers, 1936; Haynes, 1990). In the Peneroplidae, aperture shapes were considered the key characters for the separation of the various species of *Peneroplis* (Hofker, 1950). Due to variations seen in the apertures within each species, and the overlap of apertural shape between species, the ornamentation of the shell walls was used as a secondary character to define species (Hofker, 1950; Gudmundsson, 1990). In addition recent studies (Gudmundsson, 1990) have shown ontogenetic changes in apertural patterns. While populations of *P. planatus* and *P. pertusus* differed little in their physiological responses to nutrition in the laboratory, the Japanese populations of *P. planatus* varied in their growth from specimens of the same species collected from the Red Sea.

Populations from Japan of both *P. planatus* and *P. pertusus* grew when starved (Table 7.1, 7.2). This was in sharp contrast to the *P. planatus* harvested from the Red Sea which failed to grow when they were starved (Faber and Lee, 1991). Despite brushings, seawater changes and flask changes, there may have been sufficient populations of bacteria to provide food for the foraminifera but the experimental conditions for the Japanese populations were not noticeably different from those used for the poulations from the Red Sea.

The specimens incubated in inorganic nutrient supplemented media did not grow as well as the starved specimens of either species (Table 7.1, 7.2). If external algal food was utilized as a source of nitrogen and phosphorus as has been suggested (Jørgensen, *et al.*, 1985; Kuile, *et al.*, 1987; Lee, *et al.*, 1988b, 1991c; Faber and Lee, 1991), and not as a bulk carbon source then specimens grown in inorganic nutrient enriched media should have grown as well as those fed algal diets. This was not the case. Possibly the amount of nitrogen and phosphorus enriching the medium in which the foraminifera were incubated was inadequate. If this was true then the specimens given nutrient supplements and fed *D. salina* might have grown more than those specimens just given the nutrient supplements. For both species, there was no difference between those specimens receiving only the supplements and those receiving the supplements and *D. salina* . The amount of nitrogen and phosphorus added to the specimens reflected the nitrogen and phosphorus levels in the Gulf of Aqaba/Eilat (Lee, 1980). This amount may have been above the tolerance of the populations of foraminifera from Japan and thus might have had a toxic effect, reducing the overall growth.

Light intensity had a greater effect than feeding on populations of *P. planatus* from the Red Sea when they were fed unialgal foods. An increase in light intensity led to larger overall final sizes of the foraminifera, and an increased number of chambers that the foraminifera grew in the laboratory (Faber and Lee, 1991). Light had no effect on the overall final size, but did reduce the number of chambers grown by populations of Japanese *P. planatus* in nutrient enriched media in the laboratory (Table 7.1). The possible toxic effect of the nutrient enhacements may have overridden any potential beneficial effect of light on the foraminiferal growth.

The type of algal food had little effect on the overall growth of *P. planatus* and *P. pertusus* , although differences were seen between the two species. *P. pertusus* obtained overall final sizes larger than *P. planatus*

when fed the same algal diets, and grew nearly 25% more chambers, despite similar survival times. In addition, rectilinear chambers were more prevalent in *P. pertusus* which agrees with the data of Gudmundsson (1990), who examined natural populations and found rectilinear chambers present in only 3% of *P. planatus* and in 11% of *P. pertusus*. Mixed algal diets had no additional benefit for either species. Those *P. planatus* fed *Chlorella*, and those fed a mixture of *D. salina* and *Chlorella* grew less than specimens fed other algal diets. This was not seen in experimental protists harvested from the Red Sea (Faber and Lee, 1991) The *Chlorella* strain used in our experiments as a sole diet may have had some inhibitory or detrimental effect on the Japanese specimens, but when it was fed to the foraminifera in combination with diatom species, there was no reduction in growth (Table 7.1). Possibly the *Chlorella* was not being assimilated to the degree that the other algal species were (eg. Lee, *et al.*, 1988b) hence less nutrients were available for growth.

Japanese *P. planatus* grew much more than those from the Red Sea when fed an unialgal diet. The Red Sea specimens grew on average nearly 9 chambers with a resulting overall mean gain in weight of 40.88 µg (Faber and Lee, 1991), while those from Japanese under the same conditions (ignoring the specimens fed *Chlorella*, and those fed *D. salina* and *Chlorella*) grew on average 14 chambers with an overall mean gain in weight of 140.59 µg. Salinity, however, was different and may have affected the overall growth. The salinity of the Red Sea specimens was maintained at 4.0% whereas the Japanese specimens were maintained at 3.5% salinity seawater. These values reflected the ambient conditions where the respective specimens were collected. Future work should reverse the salinities for each location collected specimens to test whether a lower salinity was responsible for increased growth.

The past history of the specimens differed by more than salinity. The Red Sea specimens were all F1 generation individuals taken from field collected adult *P. planatus* which released megalospheric juveniles in the laboratory. In contrast, only half of the Japan *P. planatus* were F1 generation individuals; the others were taken directly from the field. The depth of the seawater where the specimens were collected also differed. The Red Sea specimens were collected at 20 meters whereas the Japan specimens were from shallower water. Hence the foraminifera were adapted to different light levels.

Morphological variability is common among all foraminiferal species and particularly in the genus *Peneroplis* (Hofker, 1951b). In his classic monograph on form and function, Thompson (1961) utilized *Peneroplis* to illustrate variation in foraminiferal morphology. This species starts as a simple series of chambers in a spiral and is "apt to be modified in various ways" as the foraminifer grows (Thompson, 1961). Hofker (1951b) described several common forms found in both *P. planatus* and *P. pertusus* : 1. totally coiled form with gradual increase in size; 2. totally coiled form with broad later chambers; 3. partially uncoiled form with compressed, spherical rectilinear chambers; 4. partially uncoiled form with fan-shaped rectilinear chambers.

Species with wide geographic distributions (like *P. planatus* and *P. pertusus*) often show variation in morphological characters (Frankel, 1989). The different environmental parameters found in these location are believed to alter the growth of foraminifera and cause changes in morphology (see review: Boltovskoy, *et al.*, 1991). Variations may be due strictly to physio-chemical forces and not consanguinity (Thompson, 1961). It is

difficult to determine whether the variations are due to genetic or environmental effects (Röttger and Berger, 1972). The ecophenotype concept perpetuates the idea that a particular morphology is an adaptation to a particular set of environmental conditions, however, several morphological forms may be present in the same location or between different locations (Haynes, ms submitted).

In this study, the specimens were kept under constant temperature, salinity, pH and light. These parameters are believed to be the cause of abnormalities and aberrant forms (Boltovskoy, *et al.*, 1991). Some of the *P. planatus* used in this study were clones from a single parental cell (the specimens in Plate 7.1 Figs 1,3,4,5 and 6, Plate 7.2 Figs. 1,2 and 4, and Plate 7.3 Figs 1-6 were all clones). These clones were subjected to the same conditions except for the algal food offered them, yet they exhibited a wide range of morphology. Often the abnormalities encountered in natural populations are not considered the result of existing environmental conditions but rather the result of a rapid change in the physical-chemical condition of the environment (Boltovskoy, *et al.*, 1991). Weekly seawater changes and sealed flasks attempted to maintain a constant salinity, pH and water chemistry in the laboratory environment.

Hofker (1951b) illustrated four abnormalities from the natural population which were similar to those seen in this study. He attributed these abnormalities to "brusing." Rhumbler (1911) considered abnormalities involving the formation of adventitious chambers to be the regeneration of damaged areas. Although the specimens in this study were removed weekly from their culture flasks and brushed, physical damage to the shells of the foraminifera did not occur, although damage to the pseudopodia, especially in those specimens with extended webs, may have resulted.

Foraminiferal growth involves episodic protoplasmic expansion, then chamber formation. Each chamber is formed separately from the rest of the organism (Thompson, 1961). Obstructions on the substrates have been shown to create abnormalities in the chamber shape of several foraminifera (Arnold, 1954; Kloos, 1981). The protoplasm simply grows around the obstruction forming an irregular chamber. Dents or wrinkles on a specimen may be due to such obstructions (Plate 7.5 Fig. 4). However, physical obstacles may not be necessary to cause abnormalities.

Pattern formation in protozoa is both an old, and a very exciting new, field (eg. Frankel, 1989). For example, the present cell and molecular interest in ciliate pattern formation emerged from the basic analyses of Lwoff (1950) and experimental studies of Tartar (sumarized in 1967) which showed that cortical development in ciliates depends on sets of spatial relationships in the cortex itself. Ciliates seem to provide the best examples of structural inheritance (Wolpert, 1990). Underlying this phenomenon is the fact that existing structural organization is inherited during vegetative growth and division and experimental cortical alterations do not seem to be reflected in DNA alterations. It is not only that inverted ciliary rows are inherited, but that once this is done other parts of the cortex within the altered domain are affected in their symmetry and development. Although we know even less about the development of the test patterns in foraminifera, some aspects may be gleaned from the present clone culture growth experiments with *Peneroplis.* : 1) The pattern of chamber addition is not completely linked to the pattern of the previous chamber (eg. Plate 7.2 Figs. 1-5); 2) The axis of growth may be altered in three dimensional space, even to the extent of being reversed (Plate 7.3

Fig. 3, Plate 7.4 Fig. 3); 3) The pattern of pseudopod streams may be an organizer of growth axes (Plate 7.1 Figs. 2-4); 4) The pattern of pits and furrows is fairly constant within a *Peneroplis* species and the axis of this pattern is always parallel to the main axis(es) of the pseudopodia.

The psuedopodia of *Peneroplis* extend only from the aperture and usually downward, elevating the shell off the substrate (Faber, 1991). Foraminifera episodically gather large balls and mats of food around their shells (Lee, 1974; Lee, *et al.*, 1988b; Faber and Lee, 1991). Sometimes the pseudopodial webs extend to several clumps or colonies of food. If the protoplasmic expansion follows the pseudopodia then the extension of two webs of pseudopodia may cause the formation of two chambers at the same time, which would lead to the two apertural regions (for example Plate 7.1 Fig. 2-4,6) The manipulations of the specimens in the laboratory (such as the transfers and brushings) are not experienced by the specimens in the natural environment. This weekly disturbance may account for the more severe abnormalities seen in the laboratory specimens.

The variation in morphology encountered for both species in this study suggest that the morphological characteristics used in the taxonomy of the species of *Peneroplis* need to be carefully reexamined. Hofker (1951b) recognized similarities between the various species and settled on the shape of the aperture and the ornamentation of the shell walls as the chief characters which separate the species. Ontogenetic changes seen in the apertural patterns make juvenile forms more difficult to separate (Gudmundsson, 1990). Both *P. planatus* (Plate 7.1 Fig. 6) and *P. pertusus* (Plate 7.6 Fig. 6) formed apertures in culture similar to those characteristic of *P. acicularis* (Plate 7.8 Fig. 4). This eliminates the single dendritic aperture as a morphological character that defines *P. acicularis* , and demonstrates that all three species have the genetic potential to produce this phenotype. Yet in natural populations, *P. planatus* and *P. pertusus* do not exhibit this apertural shape.

The *P. planatus* of this study collected from Japan were physiologically different from the specimens of *P. planatus* from the Red Sea (Faber and Lee, 1991). The differences in the particular environments at the time of collection may have caused particular physiological adaptations, which resulted in different growth patterns under the same laboratory conditions. This idea of physiological conditioning was supported by the larger degree of similarity between the two different species of *Peneroplis* collected from the same location than between the two populations of the same species from different locations. However, morphological variation, except in overall size and number of chambers formed, did not correspond with the physiological response. The morphological variability seen in the laboratory was not strictly a result of genetic factors, as evidenced by the differences seen in the clonal specimens, nor was the variability an adaptation to the environment. The fact that the specimens from both the Red Sea and Japan showed the same range of phenotypes and that other investigators have seen these variations (Hofker, 1951b; Basson and Al-Bahrani, personal communication), suggests that *Peneroplis planatus* has a very complex developmental repertoire which begs further investigation.

Table 7.1. The effect of diet, light and inorganic nutrient enrichment on the growth of *Peneroplis planatus* from Japanese waters. NO_3^- supplement = 1 µg at l^{-1}. PO_4^{-2} supplement = 0.1 µg at l^{-1}. L = low light intensity (30-50µE $m^{-2}s^{-1}$). H = high light intensity (200-400 µE $m^{-2}s^{-1}$). * averages of only those specimens which grew at least one chamber in culture.

Feeding regime	Light regime	N	Average initial weight (µg)	Percent which grew	Average final weight (µg)*	Average chambers per foraminifera*	Average µg gain*	Percent increase in growth	Average number rectilinear chambers*	Average survival time (days)	Range (days)
Starved	L	4	58.33 ± 12.56	100	80.19 ± 11.42	4.00 ± 1.22	21.87 ± 7.27	37	0	62±23	32-130
Dunaliella salina	L	21	49.66 ± 5.36	100	190.88 ± 24.17	16.14 ± 1.67	141.23 ± 23.33	284	5.95 ± 1.65	187±36	49-483
Nitzschia sp. (W521)	L	18	44.84 ± 5.25	94	199.17 ± 25.54	15.00 ± 1.51	154.81 ± 25.16	345	5.47 ± 1.65	134±35	36-364
Chlorella sp. (AT)	L	20	48.70 ± 9.26	80	85.27 ± 18.66	3.69 ± 0.46	30.43 ± 9.71	62	0.44 ± 0.44	123±23	21-351
Navicula sp.	L	17	56.24 ± 8.86	100	169.14 ± 18.46	13.41 ± 1.64	112.91 ± 13.20	201	4.59 ± 1.36	182±41	14-644
Amphora sp.	L	5	76.89 ± 12.88	100	195.66 ± 37.34	9.60 ± 1.36	118.7 ± 254.76	154	0	59±4	49-70
D. salina + *Nitzschia*	L	15	36.87 ± 6.58	100	161.83 ± 18.41	13.53 ± 1.42	124.96 ± 18.62	339	4.53 ± 1.10	182±43	14-427
D. salina + *Chlorella*	L	15	44.86 ± 12.77	100	104.32 ± 23.13	6.4 ± 0.71	59.45 ± 11.47	133	0.73 ± 0.73	91±7	28-126
D. salina + *Navicula*	L	13	50.33 ± 13.16	100	131.30 ± 19.31	11.78 ± 1.39	80.96 ± 12.72	161	0.69 ± 0.69	251±144	63-455
Nitzschia + *Chlorella*	L	15	54.92 ± 17.71	100	222.04 ± 49.58	12.53 ± 1.25	167.08 ± 36.82	304	3.80 ± 1.28	117±26	49-354
Nitzschia + *Navicula*	L	15	53.90 ± 16.17	100	228.86 ± 28.55	18.00 ± 2.38	174.96 ± 27.63	325	3.67 ± 1.09	248±41	21-427
Chloreela + *Navicula*	L	14	42.62 ± 11.62	100	213.34 ± 91.54	10.93 ± 1.77	170.72 ± 84.29	401	1.50 ± 0.80	136±40	49-525

Table 7.1 continued:

Feeding regime	Light regime	N	Average initial weight (µg)	Percent which grew	Average final weight (µg)*	Average chambers per foraminifera*	Average µg gain*	Percent increase in growth	Average number rectilinear chambers*	Average survival time (days)	Range (days)
NO_3^- supplement	L	9	118.24 ± 22.92	89	133.93 ± 21.34	2.38 ± 0.63	28.44 ± 9.36	24	0	45±7	14-77
NO_3^- supplement	H	7	102.45 ± 11.66	71	116.06 ± 11.38	2.20 ± 0.73	16.00 ± 6.62	16	0	77±34	21-266
PO_4^{-2} supplement	L	7	102.09 ± 11.41	71	122.97 ± 20.20	2.80 ± 1.11	25.40 ± 6.25	25	0	89±43	14-329
PO_4^{-2} supplement	H	8	127.70 ± 22.17	38	149.41 ± 60.02	1.00 ± 0.00	14.44 ± 6.36	11	0	39±6	14-56
NO_3^- /PO_4^{-2} supplement	L	13	89.72 ± 12.69	69	109.75 ± 17.63	3.56 ± 0.65	29.64 ± 4.54	33	0	108±28	14-256
NO_3^- /PO_4^{-2} supplement	H	5	114.81 ± 18.25	60	120.98 ± 22.15	2.67 ± 0.88	25.51 ± 9.56	22	0	59±11	21-91
D. salina + NO_3^- supplement	L	3	100.10 ± 39.84	100	117.13 ± 40.56	2.00 ± 1.00	17.03 ± 5.19	17	0	132±95	27-321
D. salina + PO_4^{-2} supplement	L	3	105.70 ± 6.29	100	143.33 ± 10.05	3.67 ± 1.20	37.63 ± 16.20	36	0	69±35	27-139
D. salina + NO_3^- /PO_4^{-2} supplement	L	9	102.83 ± 12.55	89	121.00 ± 19.31	2.63 ± 0.71	21.34 ± 7.04	21	0	66±14	20-153

Table 7.2. The effect of diet, light and inorganic nutrient enrichment on the growth of *Peneroplis pertusus* from Japanese waters. NO_3^- supplement = 1 µg at l^{-1}. PO_4^{-2} supplement = 0.1 µg at l^{-1}. L = low light intensity (30-50µE $m^{-2}s^{-1}$). H = high light intensity (200-400 µE $m^{-2}s^{-1}$). * averages of only those specimens which grew at least one chamber in culture.

Feeding regime	Light regime	N	Average initial weight (µg)	Percent which grew	Average final weight (µg)*	Average chambers per foraminifera*	Average µg gain*	Percent increase in growth	Average number rectilinear chambers*	Average survival time (days)	Range (days)
Starved	L	5	95.63 ± 16.29	100	131.64 ± 10.09	5.20 ± 1.93	36.02 ± 10.87	38	0	74±14	47-130
Dunaliella salina	L	19	70.54 ± 8.52	94	188.09 ± 27.53	15.61 ± 3.47	117.34 ± 29.25	166	6.83 ± 2.07	178±38	14-609
Nitzschia sp. (W521)	L	7	62.08 ± 15.28	100	262.52 ± 18.66	24.14 ± 3.70	200.14 ± 24.69	322	16.14 ± 2.87	225±68	119-630
Chlorella sp. (AT)	L	11	64.28 ± 11.01	100	187.10 ± 12.27	16.18 ± 3.19	122.82 ± 13.60	191	2.91 ± 1.12	182±48	63-651
Navicula sp.	L	13	66.98 ± 8.53	100	223.75 ± 40.79	22.50 ± 3.86	145.83 ± 35.21	218	6.48 ± 0.82	212±115	56-553
Amphora sp.	L	4	62.93 ± 12.74	100	207.88 ± 30.60	9.60 ± 1.36	118.77 ± 25.76	189	0	59±4	49-70
D. salina + *Nitzschia*	L	9	69.55 ± 8.53	100	206.26 ± 28.65	17.33 ± 2.74	136.71 ± 28.01	197	7.22 ± 2.33	163±7	133-211
D. salina + *Chlorella*	L	5	90.03 ± 17.90	100	264.95 ± 37.76	21.00 ± 3.36	174.92 ± 29.13	194	10.60 ± 3.14	172±25	119-252
D. salina + *Navicula*	L	5	98.05 ± 16.13	100	151.47 ± 30.59	7.80 ± 2.24	53.42 ± 27.13	54	4.00 ± 2.21	108±26	56-182
Nitzschia + *Chlorella*	L	5	79.39 ± 18.75	100	163.88 ± 28.49	11.60 ± 1.03	84.49 ± 18.51	106	3.20 ± 1.16	176±16	147-238
Nitzschia + *Navicula*	L	6	101.84 ± 19.05	83	257.46 ± 23.73	19.40 ± 7.16	161.56 ± 40.14	159	14.80 ± 8.06	247±100	63-609
Chlorella + *Navicula*	L	6	96.54 ± 17.77	100	234.20 ± 36.62	17.33 ± 3.39	137.66 ± 26.73	143	5.17 ± 2.86	238±81	77-546

Table 7.2 continued:

Feeding regime	Light regime	N	Average initial weight (µg)	Percent which grew	Average final weight (µg)*	Average chambers per foraminifera*	Average µg gain*	Percent increase in growth	Average number rectilinear chambers*	Average survival time (days)	Range (days)
NO_3^- supplement	L	1	86.37	100	98.66	9.00	12.29	14	0	35	
NO_3^- supplement	H	3	89.18 ± 6.70	67	109.55 ± 5.48	4.00 ± 0.00	27.06 ± 5.18	30	0	101±43	21-168
PO_4^{-2} supplement	L	2	110.22 ± 32.65	100	151.76 ± 4.27	3.50 ± 0.50	41.54 ± 6.25	38	0	60±10	50-70
PO_4^{-2} supplement	H	2	85.22 ± 22.17	50	141.08	1.00	28.92	34	0	60±10	50-70
NO_3^-/PO_4^{-2} supplement	L	7	107.66 ± 16.46	43	120.79 ± 21.29	3.67 ± 2.19	31.08 ± 16.61	29	0	70±14	14-125
NO_3^-/PO_4^{-2} supplement	H	2	86.83 ± 12.93	100	148.40 ± 24.17	3.00 ± 1.00	25.51 ± 9.56	29	0	92±22	70-113
D. salina + NO_3^- supplement	L	7	95.21 ± 17.46	71	99.57 ± 19.89	2.80 ± 0.49	25.99 ± 6.38	27	0	99±39	27-321
D. salina + PO_4^{-2} supplement	L	7	74.66 ± 6.28	86	85.83 ± 9.66	2.33 ± 0.49	15.35 ± 5.47	21	0	90±17	27-139
D. salina + NO_3^-/PO_4^{-2} supplement	L	16	86.25 ± 8.44	94	111.30 ± 12.24	4.60 ± 1.29	25.35 ± 8.58	29	0.53 ± 0.53	66±14	6-370

EXPLANATION OF FIGURES

Plate 7.1. *Peneroplis planatus* (Fichtel and Moll) Montfort. SEM micrographs of laboratory grown specimens. All scale bars = 200 µm.

Fig. 1. Normal specimen fed *Nitzschia* sp. (W521).

Fig. 2. Two apertural regions emerging from irregular rectilinear growth (arrows). Specimen fed *Navicula* sp.

Fig. 3. Two apertural regions (arrows) emerging from rectilinear growth originating in the whorl. Specimen fed *Nitzschia* sp. (W521) and *Chlorella* sp. (AT).

Fig. 4. Two apertural regions (arrows) emerging from rectilinear growth originating in the whorl. Specimen fed *Nitzschia* sp. (W521) and *Chlorella* sp. (AT).

Fig. 5. Dramatically curved specimen with chambers out of the planispiral. Note normal aperture (arrow). Specimen fed *Nitzschia* sp. (W521) and *Chlorella* sp. (AT).

Fig. 6. Rectilinear growth originating out of the planispiral resulting in two apertural regions (arrows). Specimen fed *Nitzschia* sp. (W521).

Plate 7.2. *Peneroplis planatus* (Fichtel and Moll) Montfort. SEM micrographs of laboratory grown specimens. Figs. 1-5 scale bars = 200 µm.

Fig. 1. Adventitious chamber without an aperture (arrow). Specimen fed *Nitzschia* sp. (W521) and *Chlorella* sp. (AT).

Fig. 2. Uneven chamber formation (arrow) in specimen fed *Nitzschia* sp. (W521).

Fig. 3. Two apertural regions (arrows) emerging from rectilinear growth originating in the whorl. Specimen fed *Navicula* sp.

Fig. 4. Two apertural regions (arrows) emerging from rectilinear growth originating out of the whorl. Specimen fed *Nitzschia* sp. (W521).

Fig. 5. Irregular chambers lacking ornamentation (arrow). Specimen fed *Navicula* sp.

Fig. 6. Close-up of specimen in Figure 3 showing arrangement of the pits. Scale bar = 50 µm.

Plate 7.3. *Peneroplis planatus* (Fichtel and Moll) Montfort. SEM micrographs of laboratory grown specimens of F1 generation megalospheric clones from the same parental cell. Figs. 1-4 and 6 scale bars = 200 µm.

Fig. 1. Partial chamber formation in specimen fed *Nitzschia* sp. (W521).

Fig. 2. Irregular chamber formation in specimen fed *Nitzschia* sp. (W521) and *Chlorella* sp. (AT).

Fig. 3. Curved specimen with chambers out of the planispiral. Specimen fed *Nitzschia* sp. (W521) and *Chlorella* sp. (AT).

Plate 7.3. continued:

Fig. 4. Chamber curved back over previous chamber (arrow) out of the planispiral resulting in a fluted specimen which was fed *Nitzschia* sp.

Fig. 5. Fan-shaped specimen fed *Nitzschia* sp. (W521) and *Chlorella* sp. (AT). Scale bar = 500 µm.

Fig. 6. Uneven chamber formation (arrow). Specimen fed *Nitzschia* sp. (W521).

Plate 7.4. *Peneroplis planatus* (Fichtel and Moll) Montfort. Figs. 1, 3, 5 and 6 SEM micrographs of specimens isolated from a sand sample collected at Kudaka Island, Japan in July 1989. Figs 2 and 4 SEM micrographs of laboratory grown specimens. All scale bars = 200 µm except for Fig. 3 where the scale bar = 100 µm.

Fig. 1. Forked aperture (arrow) due to final chamber curving back onto the other chambers.

Fig. 2. Irregular growth stucture lacking normal ornamentation with multiple openings (arrows) without a well defined aperture. Specimen fed *Navicula* sp.

Fig. 3. Specimen with chambers grown out of the original planispiral.

Fig. 4. Irregular chambers curved back on the specimen which was fed *Nitzschia* sp. (W521). Apertural region damaged during SEM preparation.

Fig. 5. Final chamber recurved over previous chamber creating the appearance of partial chambers (arrow).

Fig. 6. Irregular chamber lacking ornamentation (arrow).

Plate 7.5. *Peneroplis planatus* (Fichtel and Moll) Montfort. SEM micrographs of specimens isolated from a sand sample collected at Kudaka Island, Japan in July 1989. Figs. 1, 4-6 scale bars = 200 µm, and Figs. 2 and 3 scale bar = 100 µm.

Fig. 1. Normal morphology.

Fig. 2. Uneven chamber growth (arrow).

Fig. 3. Irregular chamber overlapping previous chamber (arrow).

Fig. 4. Wrinkled chamber growth with overlap of previous chambers (arrows).

Fig. 5. Final chamber beginning to leave planispiral by curving onto the previous whorl (arrow).

Fig. 6. Partial chamber (arrow).

Plate 7.6. *Peneroplis pertusus* (Forskål). SEM micrographs of laboratory grown specimens.

Fig. 1. Irregular whorl with two apertural regions (arrows). Specimen fed *Navicula* sp. Scale bar = 200 µm.

Fig. 2. Irregular rectilinear growth originating out of the whorl resulting in two apertural regions (arrows). Specimen fed *Dunaliella salina* and *Chlorella* sp. (AT). Scale bar = 200 µm.

Fig. 3. Irregular chamber growth (arrow) on specimen fed *Nitzschia* sp. (W521). Scale bar = 200 µm.

Fig. 4. Adventitious chamber without an aperture (arrow). Specimen fed *Navicula* sp. Scale bar = 200 µm.

Plate 7.6. continued:

Fig. 5. Irregular growth on rectilinear series (arrows). Specimen fed *Nitzschia* sp. (W521). Scale bar = 200 µm.

Fig. 6. Close-up of aperture of specimen in Fig. 8. Scale bar = 100 µm.

Fig. 7. Two apertural regions (arrows) emerging from rectilinear growth originating out of the whorl, with irregular chamber growth (arrow). Specimen fed *Nitzschia* sp. (W521). Scale bar = 200 µm.

Fig. 8. Extra calcification along planispiral whorl and uneven rectilinear growth. Specimens fed *D. salina* and *Chlorella* sp. (AT). Scale bar = 500 µm.

Plate 7.7. *Peneroplis pertusus* (Forskål). Figs. 1,2,4 and 6 SEM micrographs of laboratory grown specimens. Figs. 3, 5 and 7 SEM micrographs of specimens isolated from sand samples collected at Kudaka Island, Japan in July 1989. All scale bars = 200 µm.

Fig. 1. Adventitious chambers without apertures (arrows) surrounding rectilinear growth which originated out of the whorl. Specimen fed *Navicula* sp.

Fig. 2. Specimen with two outer whorls twisted around each other resulting in two apertural regions (arrows). Specimen fed *D. salina* and *Chlorella* sp. (AT).

Fig. 3. Uneven chamber growth (arrow).

Fig. 4. Irregular chambers out of whorl with aperture in midddle of chamber (arrow). Specimen fed *Navicula* sp.

Fig. 5. Rectilinear chambers originating within the whorl resulting in two apertural regions (arrows).

Fig. 6. Irregular rectilinear chambers originating out of the planispiral resulting in two apertural regions (arrows). Specimen fed *Navicula* sp.

Fig. 7. A lateral view of the specimen in Fig. 5 to show overall chamber growth.

Plate 7.8. SEM micrographs.

Fig. 1. Close-up of pits along the apertural region of a *Peneroplis pertusus* (Forskål) isolated from a sand sample collected at Wadi Taba, Gulf of Aqaba/Eilat, Egypt in Jan 1988. Scale bar = 20 µm.

Fig. 2. *Peneroplis arietinus* (Batsch) isolated from a sand sample collected at Wadi Taba, Gulf of Aqaba/Eilat, Egypt in Jan 1988. Scale bar = 200 µm.

Fig. 3. Lateral view of specimen in Fig. 1 showing irregular chamber growth (arrow). Scale bar = 200 µm.

Fig. 4. Close-up of aperture of specimen in Fig. 2. Scale bar = 100 µm.

Fig. 5. Uneven chamber formation (arrow) in a specimen of *Peneroplis pertusus* (Forskål) isolated from a sand sample collected at Kudaka Island, Japan in July 1989. Scale bar = 200 µm.

Fig. 6. Close-up of chambers in the spiral whorl of the specimen in Fig. 3. Scale bar = 20 µm.

Fig. 7. Close-up of pit arrangement of the specimen in Fig. 5. Scale bar = 10 µm.

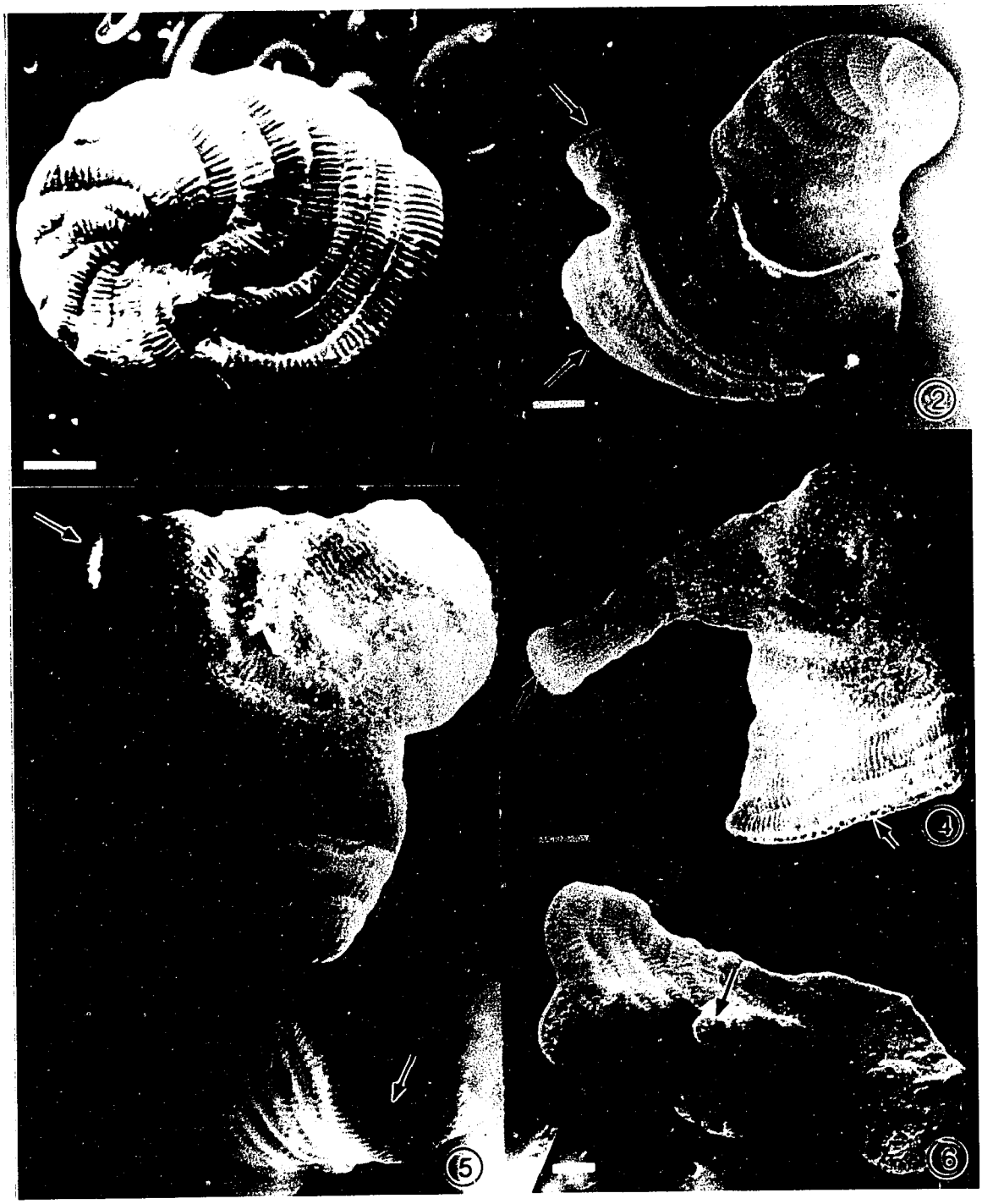


Plate 7.1

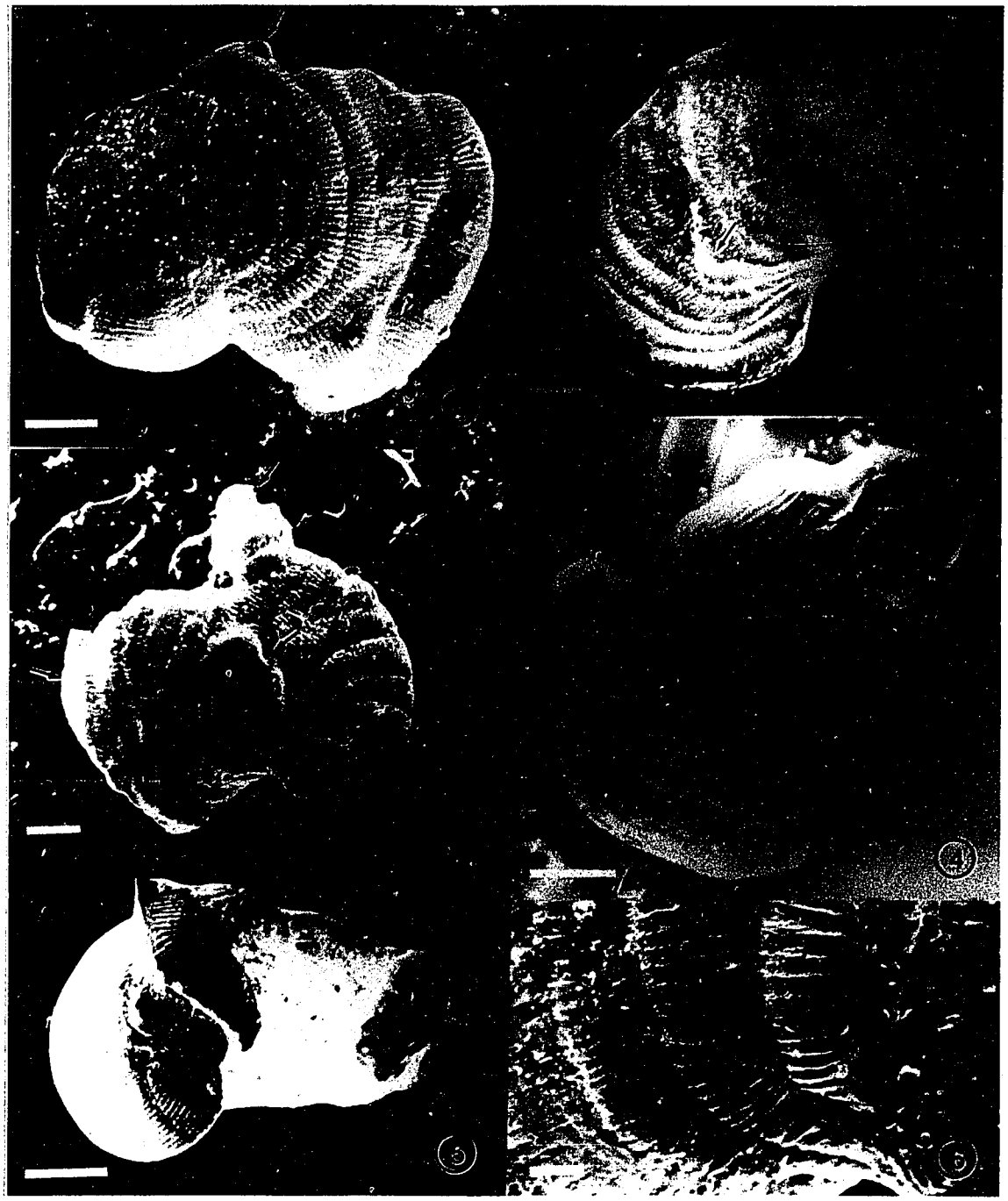


Plate 7.2

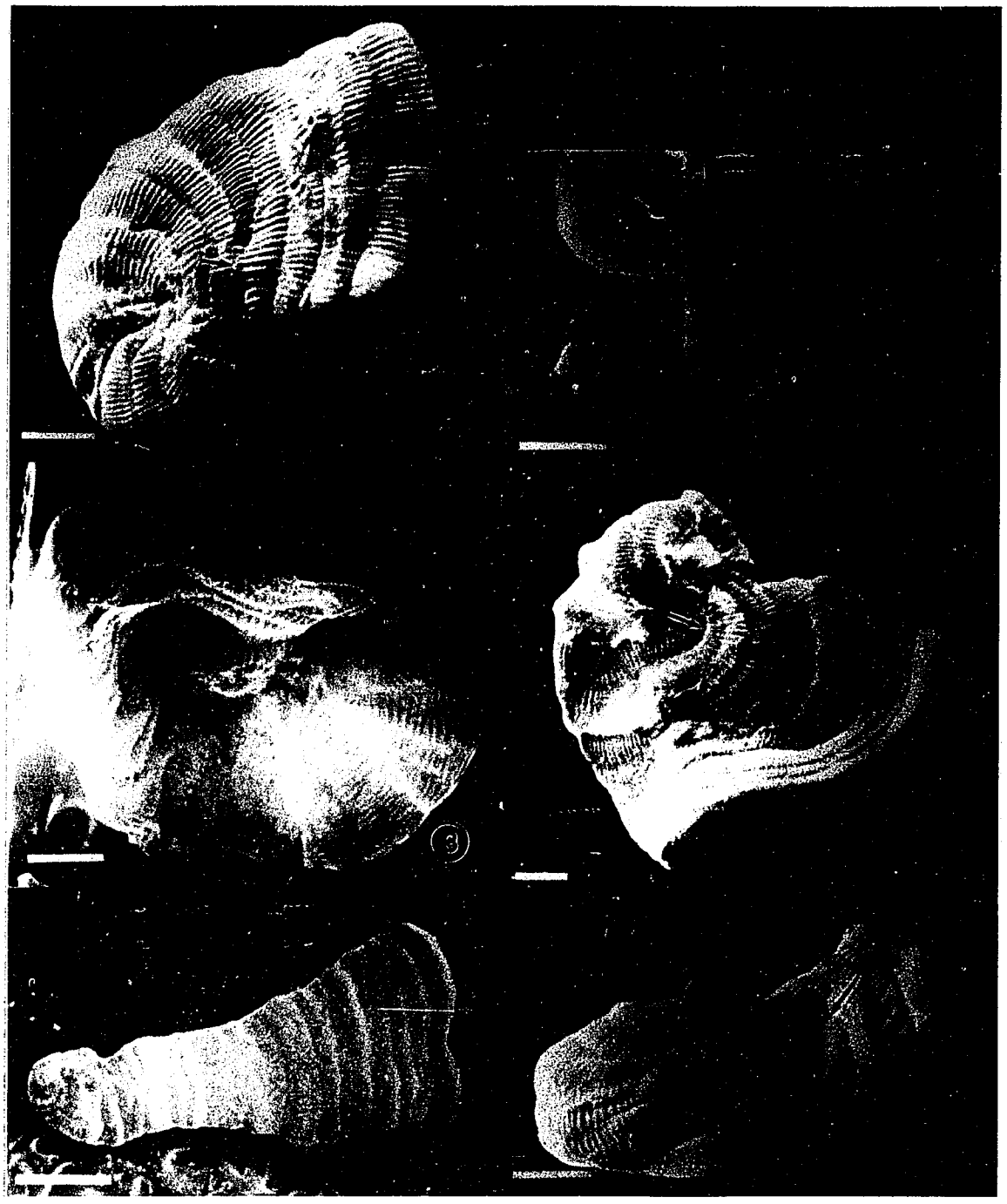


Plate 7.3

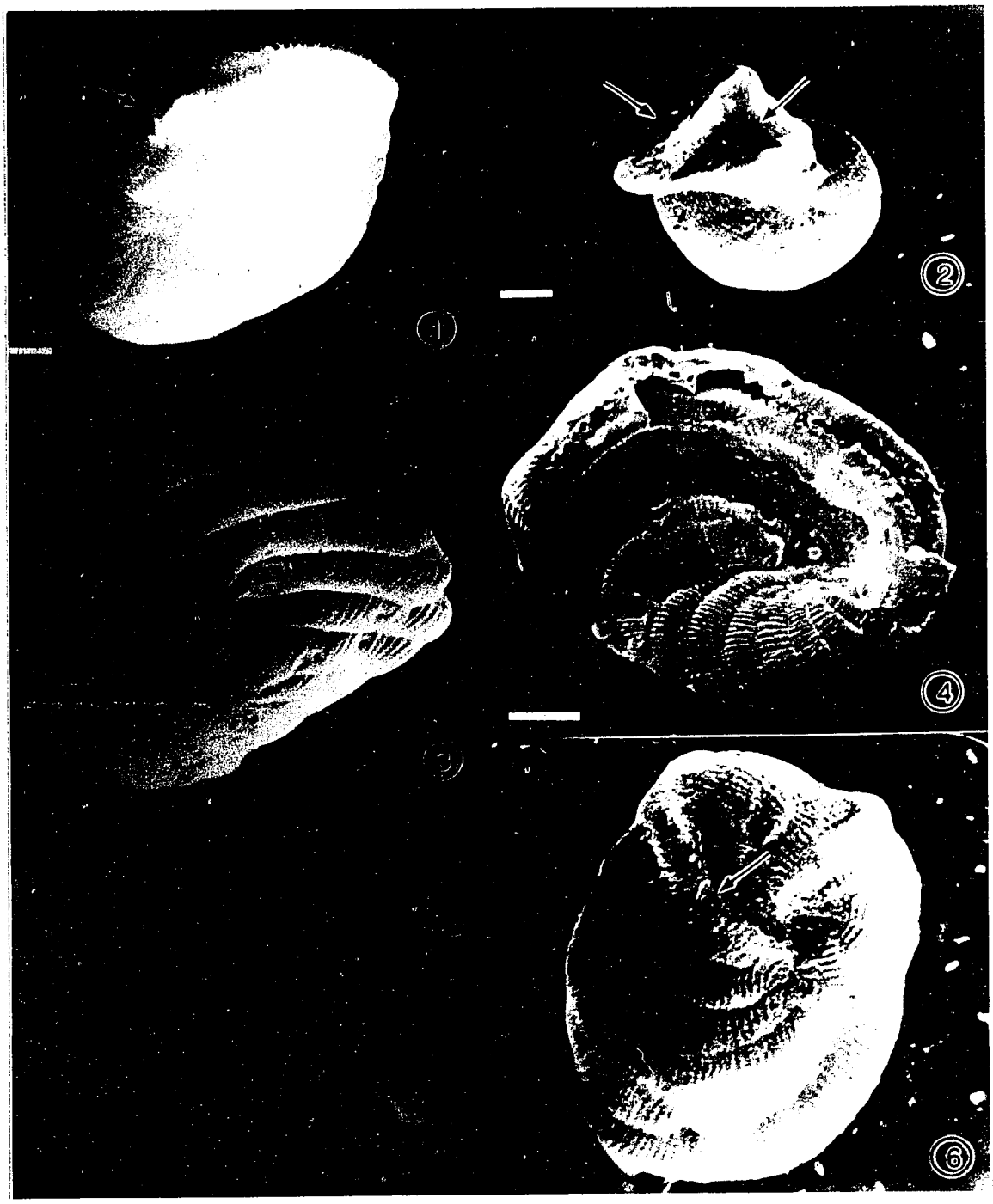


Plate 7.4

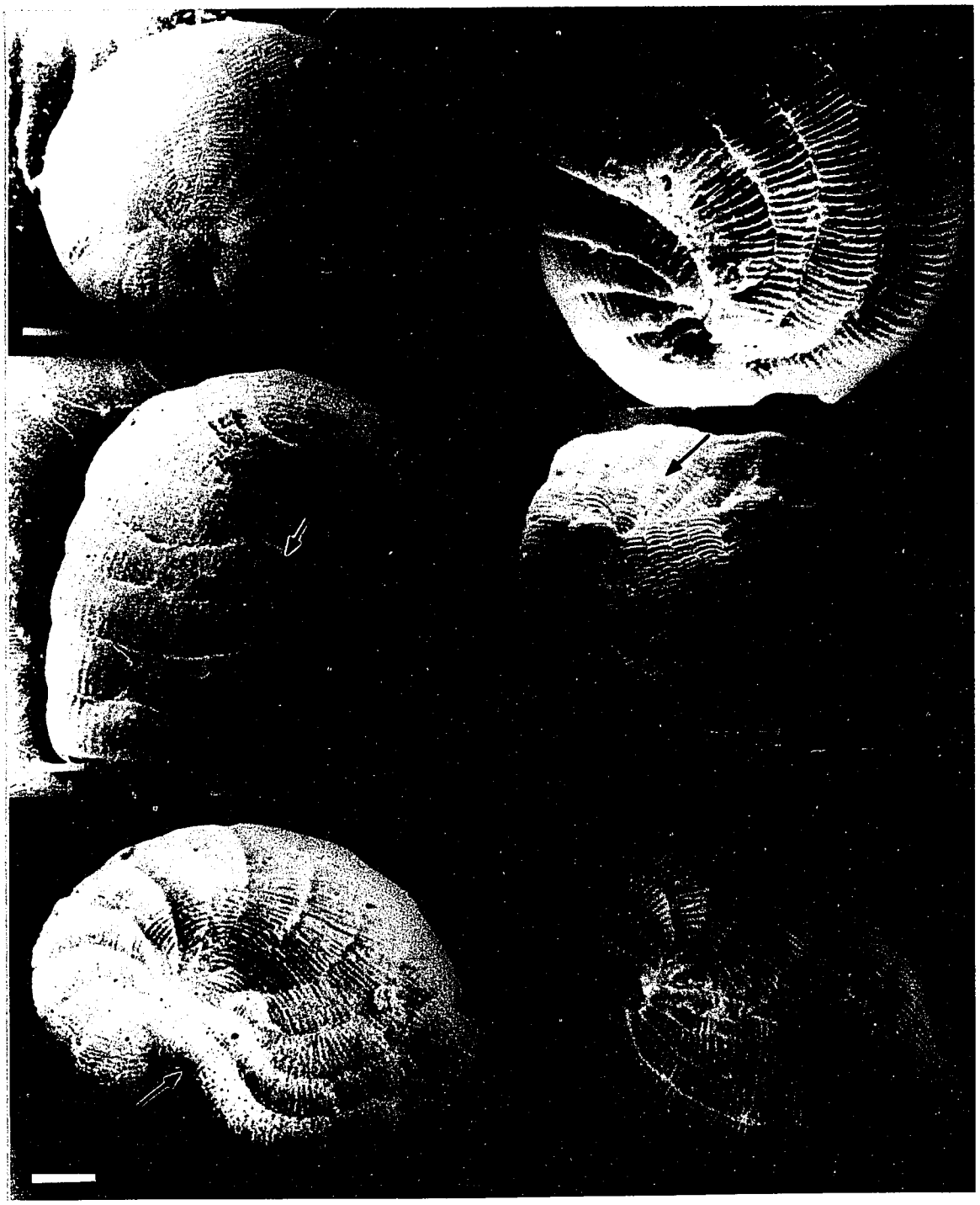


Plate 7.5

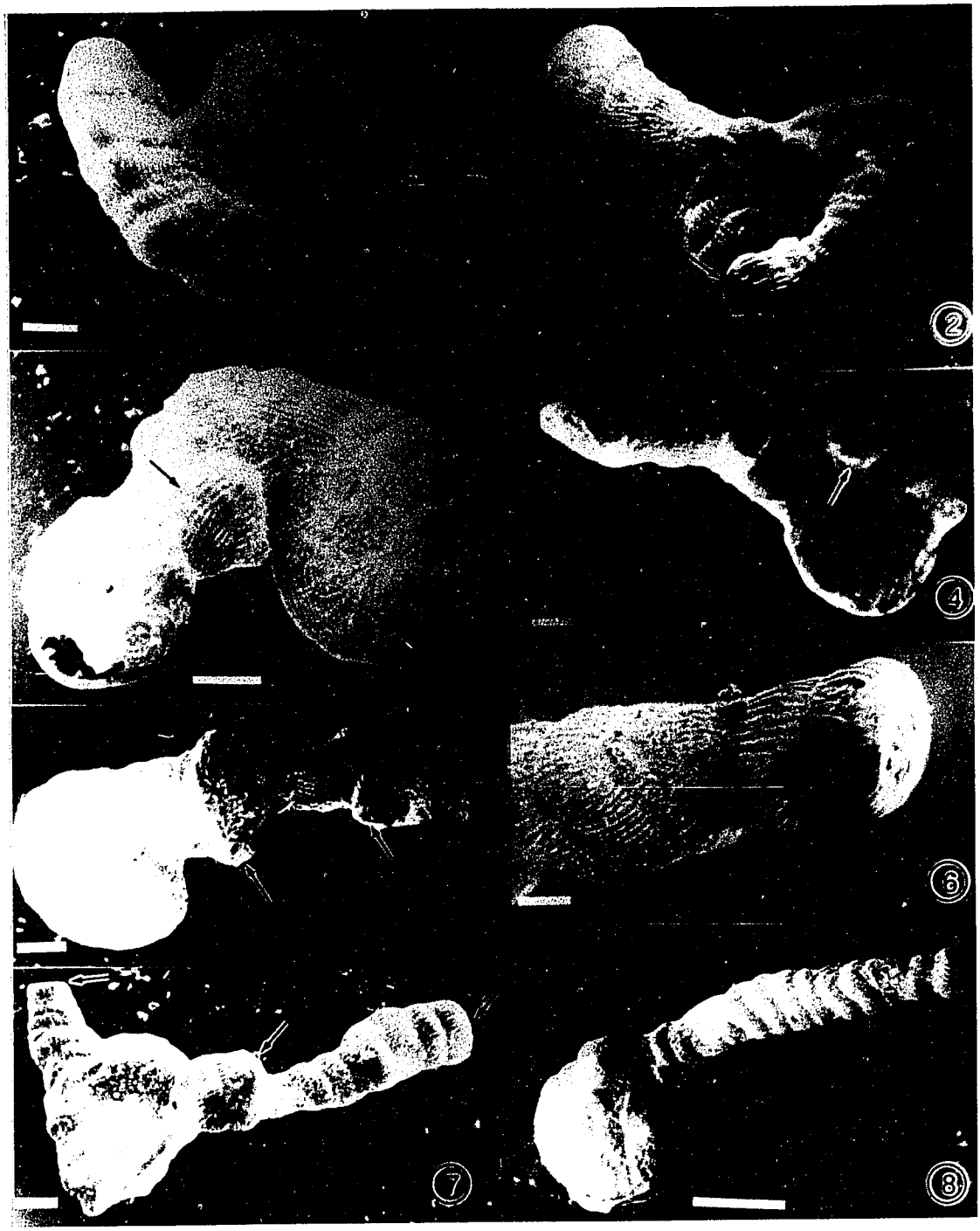


Plate 7.6

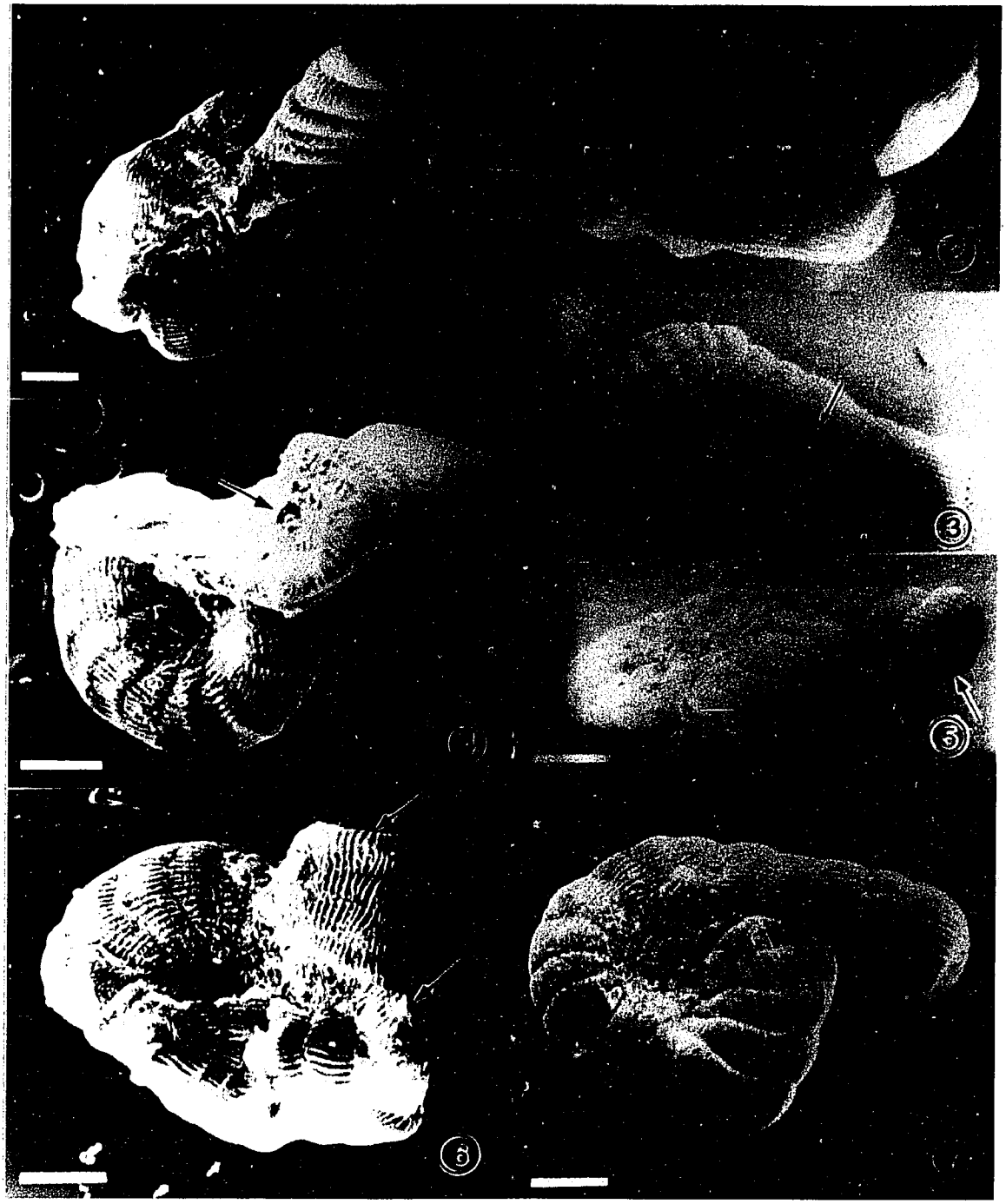


Plate 7.7

Plate 7.8

CHAPTER 8

PATHWAYS OF CARBON IN THE *PENEROPLIS PLANATUS* (FORAMINIFER) -*PORPHYRIDIUM PURPUREUM* (RHODOPHYTE) ENDOSYMBIOSIS

INTRODUCTION

The maintenance of algal endosymbioses in foraminifera has been viewed as an adaptation for survival and growth, implying an intimate relationship between the endosymbionts 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 endosymbiont- bearing foraminifera. The structure of the foraminiferal shell, and the location of the endosymbionts 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.*, 1980a), 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.*, 1991c).

Peneroplis planatus possess *Porphyridium purpureum* , a red algal endosymbiont (Leutenegger, 1977b; 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 to 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.*, 1980a; Duguay, 1983; Spero and Parker, 1985; Kuile and Erez, 1987; Kuile, *et al.*, 1987, 1989a,b; Gastrich and Bartha, 1988).

Chromatographic analysis of organic products within $H^{14}CO_3$-seawater incubated specimens of *P. pertusus* was reported by Kremer *et al.* (1980). They concluded that metabolites produced by the endosymbionts 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 endosymbiotic algae, and other

necessary nutrients from ingested food (Jørgensen, *et al.*, 1985; Kuile, *et al.*, 1987; Lee, *et al.*, 1988b; Faber and Lee, 1991).

This study utilized the *Peneroplis planatus - Porphyridium purpureum* endosymbiotic system 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 within this endosymbiotic association. Such a model, although only a first approximation, would be a starting point toward understanding the energetic interactions between the host and the endosymbionts.

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, with 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 minutes. 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 one hour in saturated buffered formaldehyde) were incubated for one hour in the light with $NaH^{14}CO_3$, and harvested at t=0. Dark controls, 10-20 living foraminifera and separately 5 formaldehyde-killed foraminifera, were performed by covering the inoculated dish in a double black plastic sheet and harvested at t = 0, 60 and 1440 minutes. This experiment was repeated in triplicate.

The foraminifera were harvested in two ways. Half were placed in Zenker's solution for one hour, then

rinsed six times with seawater. The foraminifera were rinsed in distilled water and transferred to 50 % ethyl alcohol for one half hour then into 70% ethyl alcohol. These specimens were transported from Eilat back to New York for autoradiography. The other half of the harvested specimens was placed in 10% formaldehyde for 10 minutes, 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, 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 hours. 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 hours in the light, and dark controls of living elphidids were incubated for 2 hours 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 meters 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. Dark controls were covered with aluminum foil and a black plastic sheet. Specimens were harvested at t= 24, 48 and 72 hours. Other specimens were transferred to "cold" sterile filtered seawater after an incubation of 24 hours. These specimens were harvested after an additional 24 and 48 hours. The Dark controls and dead controls were incubated in the radionuclide tracer labeled media for 48 hours 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, N.J.). The jar was sealed. Through a rubber port, 2 ml of 8.5% HPO_4 was injected into the original vial. After the digestion of the shell by the

acid, two hours 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% absorbence after two hours). Both scintillation vials then received 15 ml Instagel and were counted on a ß 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 a 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 ± 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 minutes for one hour, 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 (Valiela, 1984). This data were plotted against time and regressions were calculated with the aid of Cricket Graph 1.0 software (Cricket Software Inc.). Values for one hour were calculated from the regressions.

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). The total inorganic C content of the seawater was assumed to be 2.05 mM (Kuile and Erez, 1987).

Graphs were constructed with regressions utilizing Cricket Graph 1.10 (Cricket Software Inc.). Analyses of variance between comparable groups were performed to test for significant differences (SAS Institute Inc., Cary, North Carolina).

RESULTS

Experimental results

In the laboratory, *Peneroplis planatus* took up 0.336 ± 0.024 μg C mg^{-1} foraminifer h^{-1} after one hour pulse-labeled 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 8.1; $F = 1.06$, $P > 0.05$, $df = 8,237$) during forty eight hours. 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 8.1).

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. 8.1, 8.2). Some of the grains were in dense concentric patterns over the symbionts. After a 48 h "cold" chase, the grains in the autoradiographs were less evenly distributed and with many "hot spots" that were not over the symbionts (Fig. 8.3). The dark and dead controls had very few grains randomly scattered over the foraminifera and throughout the background of the slide (Figure 8.4).

There was close correspondence between the measurements of inorganic carbon taken up in the laboratory and measurements made in the natural environment (Table 8.1, 8.2).

The *in situ* experiment showed a steady increase in total uptake of ^{14}C with time (Table 8.2). 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 h and 48 h but no increase was seen when incubation was extended an additional 24 h ($F=4.29$, $P > 0.05$, $df=1,3$). 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 8.2) (ie. not statistically different; $F = 081$, P. 0.05, $df = 4,8$).

Those specimens incubated for 24 h in the radioisotope and then in "cold" chases took up slightly less carbon; this decrease was seen mostly in the organic fraction (Table 8.2). However, it was not significantly different from the 24 h 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 8.2) 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 8.1, 8.2). 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 absorbence of the radioisotope by the foraminiferal shell or possibly adhesion to the formaldehyde killed organic fraction. Most of the label (~75%) in dark controls was in the shell fraction.

The amount of carbon fixed by the endosymbionts estimated by respirometric methods was 0.0321 μg Carbon mg^{-1} foraminifera h^{-1} at 200-300 μE $m^{-2}s^{-1}$. Dark respiration of the foraminifera was 0.0402 μg Carbon mg^{-1} foraminifera h^{-1}. Assuming a RQ equal to 1.0 (Table 8.3), 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 8.4). They incorporated on average 1.00 ± 0.22 μg C mg^{-1} foraminifera in 24 hours.

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 the *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 8.5) and are modeled in Figure 8.5. 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 shell carbonate and the cytoplasm biomass are increasing at the same rate (ie. there is a constant ratio of cytoplasm biomass to shell carbonate); 4. the amount of carbon assimilated from ingested algal foods was based on measurements of protein content and we assumed a four to one ratio of carbon to nitrogen; 5. 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 weigh ca. 47.6 μg (Table 8.5; Faber and Lee, 1991). This size organism would possess approximately 4867 endosymbionts (Chapter 2). Assuming an average dry weight of each endosymbiont to be 8.0×10^{-5} μg (Jones, 1962), the total biomass of the endosymbionts would be 0.3894 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 4.8942 μg. Subtracting this from the average weight of the host minus the weight of the endosymbionts, the weight of the shell would equal 42.3164 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 2.4471 μg C and in the endosymbionts 0.1947 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 5.0780 μg C (Table 8.5).

Carbon flow

The amount of carbon taken up by the foraminifera, while the system was undergoing photosynthesis, was measured as 0.2846 μg C d^{-1}. The net respiration for the entire association was measured to be 0.0463 μg C d^{-1}. 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. 8.5).

Kuile *et al.* (1989b) suggested there were separate pathways for the uptake of inorganic carbon 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 carbon fixed by photosynthesis channeled into the organic fraction of the association was measured as 0.2237 μg C d^{-1}; and the amount incorporated into the shell was measured as 0.0609 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 (ie. all excess carbon fixed by the endosymbionts after fulfilling the metabolic needs of the endosymbionts must be translocated to the host). If the foraminifer 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 (Chapter 2). 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 0.2846 μg C d^{-1}, and needed 0.0027 μg C d^{-1} for their growth, we estimate that 0.2819 μg C d^{-1} would be translocated back to the host (Fig. 8.5).

The foraminifer also obtains reduced carbon from ingested food. Based on the feeding experiments reported earlier (Faber and Lee, 1991), 9.45 cells μg^{-1} foraminifer d^{-1} were ingested, and the carbon equivalent of 3.12 cells μg^{-1} foraminifer d^{-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 X 10^{-5}, the foraminifer would have gained 0.0723 μg C d^{-1} (the difference between the ingested and egested) for utilization, assuming the amount retained in 24 h 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 (Fig. 8.6). 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 0.0370 μg d^{-1} and the shell 0.3176 μg d^{-1} with carbon accounting for 0.0185 μg and 0.0381 μg, respectively (Table 8.5, Fig. 8.5).

DISCUSSION

The pathways and cycles of carbon are important in understanding of 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.*, 1980a, 1988b; 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.

As expected the *Peneroplis planatus* endosymbiont system incorporated significant quantities of inorganic carbon (Tables 8.1, 8.2). 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. 8.1-8.3). 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 8.3). 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.*, 1989 a,b). As evidence for this bipartite process, Kuile and Erez (1987) showed that after the first 24 h 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 h 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 (ie: added chambers) during the interval of the experiment. Foraminiferal growth involves episodic cytoplasmic expansion and the addition of chambers to the shell (Fig. 8.6). 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 8.5, Fig. 8.5), the uptake of 0.0609 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 0.0609 μg C d^{-1} for the entire 72 h incubation in the label. We really do not think that 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 h after being placed in "cold" chases (Table 8.1, 8.2). A small decrease was seen in the total retention of carbon with time which could be accounted for by respiration (measured, see Table 8.2). 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 8.1). Since we know the host and endosymbionts were respiring during the 48 h "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 8.3). 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 processess 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 0.0463 μg C d^{-1} (Table 8.5, Fig. 8.5) which corresponds well to the reduction of incorporated label during the "cold" chases of the *in situ* experiment (Table 8.2).

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} sec^{-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} sec^{-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 (eg. 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.*, 1988b, 1991c; 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. 8.5).

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 0.0584 μg C d^{-1} would be translocated to the host. In our model, the endosymbionts were translocating carbon at nearly four 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. 8.5). 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 hours for *Elphidium crispum* (Lee, *et al.*, 1988b). 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. 8.5). 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 0.1562 μg C d^{-1} from the incorporated inorganic carbon (Fig. 8.5). 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 (eg., 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 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. 8.5). 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 8.5, Fig. 8.5).

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 8.3) 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 0.0345 $\mu g\ C\ d^{-1}$. This amount creates a deficit of 0.0684 $\mu g\ C\ d^{-1}$ due to the carbon demands of the host. The net assimilation of reduced carbon from ingested food was calculated at 0.0723 $\mu g\ C\ d^{-1}$ (Table 8.5, Fig. 8.5). 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.

Table 8.1: The amount of carbon fixed in one hour 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
Light	0	30	0.336 ± 0.024
	5	33	0.278 ± 0.024
	10	32	0.322 ± 0.027
	60	29	0.342 ± 0.023
	120	30	0.377 ± 0.027
	240	29	0.333 ± 0.023
	480	19	0.331 ± 0.026
	1440	18	0.341 ± 0.047
	2880	26	0.352 ± 0.033
Controls			
Light Dead	0	25	0.098 ± 0.015
Dark	0	26	0.011 ± 0.002
	60	5	0.005 ± 0.001
	1440	4	0.006 ± 0.003
Dark Dead	0	5	0.012 ± 0.003

Table 8.2: 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 h incubation	1.28 ±0.16	4.70 ± 0.38	5.98	0.249
48 h incubation	1.85 ± 0.08	9.68 ± 0.60	11.53	0.240
72 h incubation	1.41 ± 0.27	14.63 ± 2.43	16.04	0.223
24 h incubation with 24 h "cold" chase	0.84 ± 0.32	3.66 ± 0.83	4.50	0.188
24 h incubation with 48 h "cold" chase	0.63 ± 0.54	2.90 ± 1.99	3.53	0.147
Dead controls 48 h incubation	0.42 ± 0.05	0.05 ± 0.01	0.47	0.020
Dark Control 48 incubation	0.51 ± 0.04	0.16 ± 0.02	0.67	0.014

Table 8.3: 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	0.0331 *
200-300	0.0326 *
30-40	0.0259 *
darkness	-0.0405 **

*PQ = 1.2
**RQ = 1.0

Table 8.4. The amount of carbon incorporated by elphidid foraminifera, which husband chloroplasts, in 24 hours.

	N	average weight foraminifer	µg C mg^{-1} foraminifera
Elphidium crispum			
Light	10	164.0 ± 28.3	0.44
	10	153.0 ± 13.0	0.56
	10	112.1 ± 7.9	0.61
Light Dead Control	5	86.8 ± 19.4	0
Dark Control	5	157.9± 50.0	0.01
Elphidium incertum			
Light	11	34.2 ± 3.3	0.21
	10	33.5 ± 4.2	0.36
	9	13.6 ±1.5	2.34
Light Dead Control	4	43.1 ± 3.3	0
Dark Control	4	35.0 ± 7.0	0.01
Elphidium williamsonii			
Light	10	51.3 ± 5.0	0.39
	10	12.2 ± 1.3	1.20
Light Dead Control	4	16.8 ± 3.6	0
Dark Control	5	15.7 ± 1.1	0
Haynesina germanica (England)			
Light	8	14.9 ± 3.1	1.43
	8	10.1 ± 1.1	0.64
	7	5.9 ± 1.2	2.31
Light Dead Control	4	13.3 ± 1.8	0
Dark Control	2	5.3 ± 0.8	0
Haynesina germanica (MA)			
Light	10	15.1 ± 1.5	0.45
	6	9.6 ± 1.2	2.03
Light Dead Control	4	8.8 ± 2.3	0
Dark Control	4	9.4 ± 3.1	0

TABLE 8.5: Measured, calculated and assumed values for a hypothetical *Peneroplis planatus* weighing 47.6 µg.

Measurement		Value	Reference
size of foraminifer	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	Chapter 2
symbiont biomass	calculated	0.3894 µg	
weight of foraminiferal cytoplasm	measured	4.8942 µg	
weight of foraminiferal shell	calculated	42.3164 µg	
C content of foraminiferal cytoplasm	calculated	2.4471 µg C	
C content of endosymbionts	calculated	0.1947 µg C	
C content of foraminiferal shell	calculated	5.0780 µg C	
respiration rate	measured	0.0463 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	0.0723 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}	Chapter 2
endosymbiont growth rate	calculated	0.0054 µg d^{-1}	
host cytoplasm growth rate	calculated	0.0370 µg d^{-1}	
host shell growth rate	calculated	0.3176 µg d^{-1}	
increase in C in host cytoplasm	calculated	0.0185 µg C d^{-1}	
increase in C in endosymbionts	calculated	0.0027 µg d^{-1}	
increase in C in host shell	calculated	0.0381 Mg C d^{-1}	

EXPLANATION OF FIGURES

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

Fig. 8.1: Juvenile whorl of a specimen fixed after one hour 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.

Fig. 8.2: Outer chamber of a specimen fixed after one hour incubation in the radioactive tracer. Circular pattern of grains are above the endosymbionts (arrows). Scale bar = 10 μm.

Fig. 8.3: Outer chambers of specimen fixed after a 48 hour "cold" chase following the one hour incubation in the radioactive tracer. The grains are in dense "hot" spots and not directly over the endosymbionts (arrow). Scale bar = 10 μm.

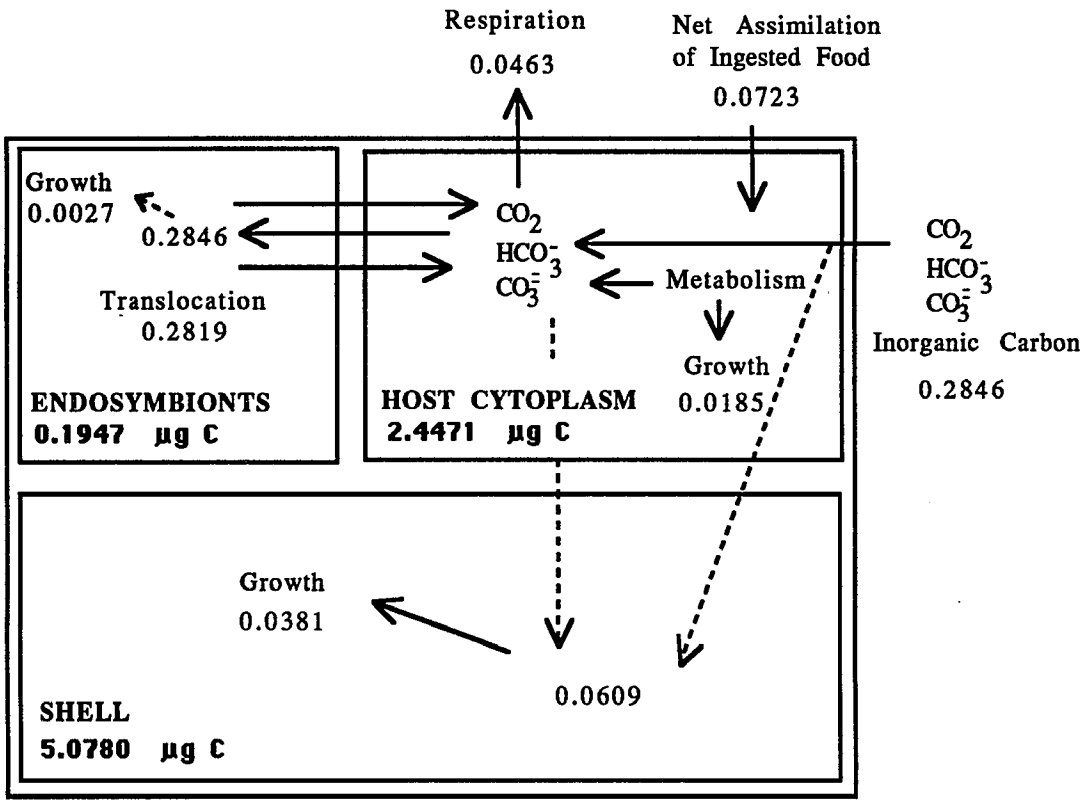
Fig. 8.4: Dark control specimen showing negligible grains. Scale bar = 10 μm.

Fig. 8.5: The carbon budget and flow of an average *Peneroplis planatus* weighing 47.6 μg. Values are in μg carbon per day, and found in Table 8.5.

Fig. 8.6: Laboratory growth over time of *Peneroplis planatus*, which were fed *Dunaliella salina* and kept in low light intensity (30-50 $\mu E\ m^{-2}\ s^{-1}$). The values (filled circles) are the means for 21 specimens from Chapter 7, with the standard errors bars shown.

Figs. 8.1 - 8.4

Fig. 8.5


Respiration
0.0463
Net Assimilation
of Ingested Food
0.0723
Growth
0.0027
0.2846
Translocation
0.2819
CO_2
HCO^-_3
$CO^=_3$
Metabolism
Growth
0.0185
CO_2
HCO^-_3
$CO^=_3$
Inorganic Carbon
0.2846
ENDOSYMBIONTS
0.1947 µg C
HOST CYTOPLASM
2.4471 µg C
Growth
0.0381
0.0609
SHELL
5.0780 µg C

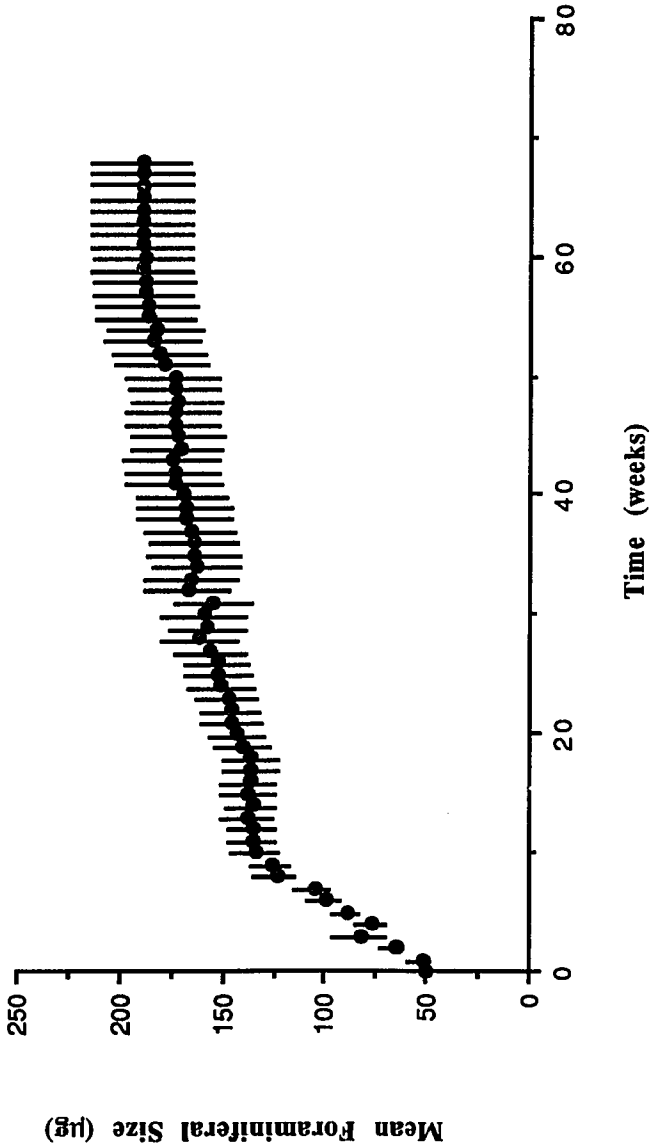

Mean Foraminiferal Size (µg)
250
200
150
100
50
0
0
20
40
60
80
Time (weeks)

Fig. 8.6

CONCLUSIONS

Direct observations of schizonts and agamonts releasing megalospheres clarified the asexual phase of the life cycle of *Peneroplis planatus* and made it most probable that this species has a paratrimorphic life cycle (Chapter 1). Specimens with maximum lengths between 837 and 3503 µm released about 500 to 1500 megalospheric juveniles, which possessed two chambers (proloculi and flexostyles) prior to emergence from the parental shell. The presence of gamonts was not shown and was only implied by the occurrence of the agamonts. Since agamonts and schizonts have been found from December to May and since asexual reproduction occurs in the spring in Eilat, sexual reproduction probably occurs at another time of the year (June to December). More detailed studies of this species need to be conducted throughout the year to improve our knowledge of the life cycle of this species.

The stability (regulation of endosymbiont population in the host) of the endosymbiosis between *Peneroplis planatus* and *Porphyridium purpureum* was examined (Chapter 2). Prolonged starvation, continuous darkness and herbicide treatment (DCMU) reduced the total number of endosymbionts but failed to completely eliminate the endosymbiont population. In the TEM, degenerated endosymbionts were seen often. However, we did not find fine structural evidence of symbionts surrounded by a symbiosome or phagosome vacuolar membranes or fusion of lysosomes with such vacuoles (convincing evidence of digestion of the endosymbionts by the foraminiferal host). Since endosymbiont photosynthesis is necessary for foraminiferal growth, the foraminifer may maintain the endosymbionts during periods of stress, rather than consume them.

It is difficult to make generalizations about any large group of organisms, such as the foraminifera, but one aspect of their biology piques curiosity. Why have so many families of foraminifera, at many separate times throughout geological history, formed endosymbiotic associations with algae ? Do foraminifera, in general, have some biological characteristics which make them particularly good hosts ? Fine structural observations of several species of larger foraminifera suggested that primary lysosomes are not near vacuoles containing symbionts (see review: Lee and Anderson, 1991). This fact gave rise to the speculation/hypothesis that foraminifera are regionally specialized and compartmentalized.. If potential symbionts escaped digestion and were transported away from lysosomal fusing regions they have an increased chance for survival within the host. It was further speculated that if such a system were in place in foraminifera, the food or symbiont screening activity would have to take place at, or near, the "front door" (pseudopodal net or aperture) of the host. To test this hypothesis, specimens of 17 different, and taxonomically widely distant, foraminifera were assayed using Napthol AS-BL Phosphate for the presence of acid phosphatase (an indication of digestion), then prepared for light microscopy (Chapters 4,5). Eight of the species investigated harbor endosymbionts, five species husband chloroplasts, and four species have no known endosymbiont. The acid phosphatase was located in the food mats around the specimens, around the periphery of the foraminifer, near the apertures, in the last few chambers, or along the marginal canal system and in the oblique plexis. This digestive enzyme, on the other hand, was not seen in the secondary or lateral canals, or near the endosymbionts. However, the location of acid phosphatase activity only explains one step in the establishment of algal endosymbioses. The

algal species found as endosymbionts are very rare species in the habitats where the symbiont-bearing foraminifera are located (eg. Lee, *et al.*, 1988b, 1990, ms submitted). Since the initial contact and recognition is in the pseudopodia, either in the rhizopodial net or near the apertures, it is reasonable to suspect signaling and recognition at the molecular level. Some initial immunochemical studies (Lee, *et al.*, 1988a) suggest that part of the answer may be found in the surface antigens of the endosymbionts. This aspect has great potential for future work.

The study of Peneroplids is particularly interesting because they are unique among protists and invertebrates by forming symbiotic associations with unicellular red algae. Unfortunately the phenomenon is not easy to study because they are not overly abundant in the habitats where they are located (see: Murray, 1973; Reiss and Hottinger, 1984). A pilot study on the distribution of *Peneroplis planatus* in the *Halophila* meadow near Wadi Taba, Eilat, Israel, showed statistically the foraminiferal species preferred horizontal rhizomes and stems of the *Halophila*, as well as the sediment beneath the plants, over the erect blades (Chapter 3). *P. arietinus* was only found on the sediment. Also, no apparent preference for depth was seen, as *Peneroplis* was collected from a few centimeters water depth to over 25 meters. *P. planatus* is a minor foraminiferal species in overall abundance except in the spring when the species reproduces asexually. This corresponds to a macroalgal bloom which is quickly exploited as a new substrate and food source.

The majority of endosymbiotic foraminifera are tropical or subtropical, and the expense of collecting specimens coupled with dwindling grant availability has prohibited extensive research. The ability to culture the organisms in the laboratory from generation to generation would alleviate these problems. However, to culture organisms successfully one needs to know details of their diets and environmental preferences.

Specimens of the foraminifer *Peneroplis planatus* collected from the *Halophila* meadow, at 15-20 m depth, near Wadi Taba were fed ^{14}C-labeled algal food for 24 hours (Chapter 6). The initial uptake of radionuclide tracer labeled food was measured in half of the specimens, and the rest were placed in a 24 hr cold food chase before retention was measured. Ten algal species were tested. *P. planatus* retained 100% ($P > 0.05$) of the ^{14}C-label of six of the ten algal species ingested. There appeared to be some selectivity in feeding; *Cocconeis placentula* and *Amphora* sp. were ingested at a rate 5 times greater than other species. Megalospheric juveniles released in the laboratory were maintained in culture and placed into several experimental regimes to investigate the effects of light and food on growth of the foraminifera. In culture, *P. planatus* did not grow when starved, and grew less (one or two chambers) when fed in complete darkness. As sole food organisms, *Dunaliella salina* and various diatoms promoted a higher total increase in weight of the foraminifera than did *Chlorella* sp. (AT). The mean number of chambers formed was the same with all the algal foods in light. Even though light was necessary for growth *P. planatus*, like other imperforate foraminifera, needed to feed in order to grow.

Specimens of *Peneroplis planatus* and *P. pertusus* collected from the back reef habitat of Kudaka Island, Okinawa Prefecture, Japan and megalospheric juveniles released in the laboratory were maintained in culture and placed into several experimental regimes to investigate the effects of feeding, and nutrient enhancement of the medium, on growth of the foraminifera in the laboratory (Chapter 7). In contrast to

specimens from Wadi Taba, Gulf of Aqaba/Eilat, *P. planatus* from Japan grew in the light when starved or given a nutrient supplement of 1.0 μg at l^{-1} NO_3^- and/or 0.1 μg at l^{-1} PO_4^{-2}. These specimens grew approximately 10-40% in size. Those Japanese specimens fed unialgal or multialgal diets grew an order of magnitude more than the specimens which were starved or given inorganic nutrient supplements.When fed the same algal diets *P. pertusus* grew larger (overall final size) than did *P. planatus*, and formed nearly 25% more chambers.

P. planatus collected from Japan were physiologically different from the specimens of *P. planatus* from the Red Sea. The idea of physiological ecotypes was supported by the large degree of similarity between the two different species from the same location. Morphological variation seen in the laboratory was not strictly a result of genetic factors, since clones from the same parental cell grew differently. This variability also was not simply an adaptation to the environment, since specimens treated the same way, grew differently. Perhaps *P. planatus* exhibits a wide range of phenotypes which are influenced by a mixture of genetic and environmental factors.

The carbon budget and flux of carbon through the *Peneroplis planatus - Porphyridium purpureum* endosymbiotic association were examined with the aid of radionuclide tracer experiments (Chapter 8). During primary production, *P. planatus* incorporated inorganic C at a rate of 0.336 ± 0.024 μg C mg^{-1} foraminifer h^{-1} when kept in continuous light. It did not significantly lose any of the carbon incorporated after a one hour pulse-labeling in the radionuclide, even after a "cold" chase of 48 h, Autoradiographs of the specimens showed dense concentric patterns of grains over the endosymbionts after the initial incubation in the radionuclide. After 48 h, the grains in the autoradiographs showed movement of the carbon label from the symbionts to the cytoplasm of the host. The organisms in experiments done *in situ* showed a steady increase in total uptake of C with time (0.249 to 0.223 μg C mg^{-1} foraminifer h^{-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 0.0405 μg C mg^{-1} foraminifer h^{-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* is ~ 600μm long, weighs 47.6 μg, and possesses ~ 4,900 endosymbionts. The foraminifer grows 0.36 μg d^{-1} and its endosymbiotic population increases by 68 endosymbionts d^{-1}. Feeding (net assimilated food) provided only 0.0723 μg C d^{-1} to the carbon budget of the growing endosymbiotic system. The symbiotic association tightly recycles carbon while in light, whereas in the dark, it looses some carbon by respiration. The endosymbionts fix enough 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.

It is reasonable to compare the carbon and nutritional budgets of the *Peneroplis planatus - Porphyridium purpureum* system to hermatypic corals, the best known tropical marine symbiotic systems. In spite of all the efforts that have been made to look at the carbon budgets of a coralline system, there is no agreement among all the workers on how to interpret the results. Nowhere was this more evident than at the most recent

symbiosis congress (International Symbiosis Congress, Jerusalem, Israel, November 17-22, 1991). The best consensus is that the endosymbionts provide far more photosynthetic carbon than is needed to satisfy the carbon budgets of the system. Most of the carbon fixed seems to be respired (45%; Cowen, 1988). The system requires feeding on zooplankton for growth. This is what Falkowski (1991) called the paradox of nutrient limitation in corals. The *Peneroplis - Porphyridium* system also obtains most of its reduced carbon from the photosynthesis of its endosymbiotic algae, and incorporates into its shell a similar percentage of carbon as the coral does for its skeletal growth, although the amount of respiratory loss of carbon by *P. planatus* is less (16% of the available carbon) than that of corals. The feeding by corals and foraminifera seems to supply nitrogen to the systems but even this may be a simplistic point of view. We know so little about the metabolism and nutritional needs of either of these systems. There is no way of knowing at this time whether there are other nutrients, such as essential fatty acids, amino acids, or lipids, which are not synthesized by either of the partners in both systems, and must be obtained from prey. Although there have been decades of research on corals, it is clear that these best investigated systems are still not well understood.

REFERENCES

Anderson, O. R., and Bé, A. W. H., 1976. A cytochemical fine structure study of phagotrophy in a planktonic foraminifer, *Hastigerina pelagica* (d'Orbigny). Biol. Bull. 151: 437-449.

Arnold, Z. M., 1954. *Discorinopsis aguayoi* (Bermudez) and *Discorinopsis radescens* Cushman and Bronnimann: a study of variation in cultures of living foraminifera. Cushman Found. Foram. Res. Contrib. 5: 4-13.

Arnold, Z. M., 1955. The unusual feature of miliolid reproduction. Cushman Found. Foram. Res. Contrib. 6: 94-96.

Bé, A. W. H., Anderson, O. R., Faber, W. W., Jr., and Caron, D.A., 1983. Sequence of morphological and cytoplasmic changes during gametogenesis in the planktonic foraminifera *Globigerinoides sacculifer* (Brady). Micropaleont. 29: 310-325.

Bé, A. W. H., Caron, D. A., and Anderson, O. R., 1981. Effects of feeding frequency on life processes of the planktonic foraminifer *Globigerinoides sacculifer* in laboratory culture. J. mar. biol. Ass. U.K. 61: 257-277.

Bé, A. W. H., Spero, H. J., and Anderson, O. R., 1982. Effectsof symbiont elimination and reinfection on the life processess of the planktonic foraminifer *Globigerinoides sacculifer* in laboratory culture. Mar. Biol. 70: 73-86.

Berger, W. H., 1970. Planktonic foraminifera: differential production and expatriation off Baja California. Limnol. Oceanogr. 15: 183-204.

Bisalputra, T., and Antia, N. J., 1980. Cytological mechanism underlying darkness-survival of the unicellular red alga *Porphyridium cruentum*. Bot. Mar. 23: 719-730.

Blank, R. J., 1987. Evolutionary differentiation in gymnodinoid zooxanthellae. Annals N.Y. Acad. Sci. 503: 530-533.

Blank, R. J., and Trench, R. K., 1985a. Speciation and symbiotic dinoflagellates. Science 229: 656-658.

Blank, R. J., and Trench, R. K., 1985b. *Symbiodinium microadriaticum*: a single species? Proc. 5th Int. Coral Reef Congr. 6: 113-117.

Boltovskoy, E., Scott, D. B., and Medioli, F. S., 1991. Morphological variations of benthic foraminiferal tests in response to changes in ecological parameters: a review. J. Paleont. 65: 175-185.

Boltovskoy, E.,and Wright, R., 1976. Recent Foraminifera. W. Junk, the Hague: 515 pp.

Bowser, S. S., McGee-Russell, S. M., and Rieder, C. L., 1985. Digestion of prey in foraminifera is not anomalous: a correlation of light microscopic, cytochemical, and HVEM technics to study phagotrophy in two allogromiids. Tissue Cell 17: 823-839.

Buchanan, J. B., and Hedley, R. H., 1960. A contribution to the biology of *Astrorhiza limicola* (Foraminifera). J. mar. biol. Ass. U.K. 39: 549-560.

Buzas, M. A., 1970. Spatial homogeneity: Statistical analyses of unispecies and multispecies populations of foraminifera. Ecology 51: 874-879.

Caron, D. A., Bé, A. W. H., and Anderson, O. R., 1982. Effects of variations in light intensity on life processes of the planktonic foraminifer *Globigerinoides sacculifer* in laboratory culture. J. mar. biol. Ass. U.K. 62: 435-451.

Caron, D. A., Faber, W. W., Jr., and Bé, A. W. H., 1987. Effects of temperature and salinity on the growth and survival of the planktonic foraminifer *Globigerinoides sacculifer*. J. mar. biol. Ass. U.K. 67: 323-341.

Caron, D. A., Goldman, J. C., and Fenchel, T., 1990. Protozoan respiration and metabolism. In: Ecology of Protozoa (Capriulo, G.M. ed.). Oxford U. Press, New York: 307-322.

Cavalier-Smith, T., and Lee, J.J., 1985. Protozoa as hosts for endosymbioses, and the conversion of symbionts into organelles. J. Protozool. 32: 376-379.

Cook, C. B., 1983. Metabolic interchange in algal-invertebrate symbioses. Int. Rev. Cytol. Suppl. 14: 177-210.

Cowen, R., 1988. The role of algal symbiosis in reefs through time. Palaios 3: 221-227.

Cushman, J. A., 1917. A monograph of the foraminifera of the North Pacific Ocean. U.S. Nat. Mus. Bull. 71: 1-596.

Cushman, J. A., 1969. Foraminifera: Their Classification and Economic Use. Harvard U. Press, Cambridge, MA: 242-248.

Daniel, W. W., 1978. Biostatistics: A Foundation for Analysis in the Health Sciences. J. Wiley & Sons, New York: 500 pp.

Darley, W. M., 1977. Biochemical Composition. In: The Biology of Diatoms (Werner, D., ed.). U. Calif. Press, Berkeley: 198-223.

Duguay, L. E., 1983. Comparative laboratory and field studies on calcification and carbon fixation in foraminiferal-algal association. J. Foram. Res. 13: 252-261.

Duguay, L. E., and Taylor, D. L., 1978. Primary production and calcification by the soritid foraminifera *Archaias angulatus* (Fichtel & Moll). J. Protozool. 25: 356-361.

Erskian, M. G., 1972. Patterns of distribution of foraminifera on *Thallassia testudinum.* Atoll Res. Bull. 152: 3.

Faber, W. W., Jr., 1991. Distribution and substrate preference of *Peneroplis planatus* and *P. arientinus* from the *Halophila* meadow near Wadi Taba, Eilat, Israel. J. Foram. Res. 21: 218-221.

Faber, W. W., Jr., Anderson, O. R., Lindsey, J. L., and Caron, D. A., 1988. Algal-foraminiferal symbiosis in the planktonic foraminifer *Globigerinella aequilateralis*: I. Occurrence and stability of two mutually exclusive chrysophyte endosymbionts and their ultrastructure. J. Foram. Res. 18: 334-343.

Faber, W. W., Jr., Anderson, O. R., and Caron, D. A., 1989. Algal-foraminiferal symbiosis in the planktonic foraminifer *Globigerinella aequilateralis*: II. Effects of two symbiont species on foraminiferal growth and longevity. J. Foram. Res. 19: 185-193.

Faber, W. W., Jr., and Lee, J. J., 1991. Feeding and growth of the foraminifer *Peneroplis planatus* (Fichtel and Moll) Montfort. Symbiosis 10: 63-82.

Falkowski, P.G., 1991. The paradox of nutrient limitation in zooxanthellate corals. (Abstract) International Symbiosis Congress Program and Abstracts, Jerusalem, Israel, November 17-22, 1991. p. 57.

Fermont, W. J. J., 1977. Biometrical investigation on the genus *Operculina* in recent sediments of the Gulf of Elat, Red Sea. Utrecht Micropaleont. Bull. 15: 111-147.

Fisher, A. G., and Arthur, M. A., 1977. Secular variations in the pelagic realm. Spec. Publ. Soc. Econ. Paleontol. Mineral. 25: 19-50.

Fitchel, L. von, and Moll, J. P. C. von, 1803. Testacea microscopica alaique minuta ex generibus Argonauta et Nautilis ad naturam delineata et descripta. Second edition. Wien.

Fitt, W. K., Chang, S. S., and Trench, R. K., 1981. Mobility patterns of different strains of symbiotic dinoflagellate *Symbiodinium* (=*Gymnodinium*) *microadriaticum* (Freudenthal) in culture. Bull. Mar. Sci. 31: 436-443.

Forskål, P., 1775. Descriptiones Animalium. Copenhagen.

Frankel, J., 1989. Pattern Formation: Ciliate Studies and Models. Oxford U. Press, New York: 314 pp.

Gastrich, M. D., 1986. The ecology of planktonic foraminifera and their symbiotic algae. PhD dissertation. Rutgers University: 268 pp.

Gastrich, M. D., 1988. Ultrastructure of a new intracellular symbiotic alga found within planktonic foraminifera. J. Phycol. 23: 623-632.

Gastrich, M. D., and Bartha, R., 1988. Primary productivity in the planktonic foraminifer *Globigerinoides ruber*. J. Foram. Res. 18: 137-142.

Gense, M.-T., Guérin-Dumartrait, E., Leclerc, J.-C., and Mihara, S. et, 1969. Synchronisation de *Porphyridium*: évolution des quantités de pigments et de la capacité photosynthétique au cours du cycle biologique. Phycologia 8:135-141.

Gooday, A. J., 1988. A response by benthic foraminifera to the deposition of phytodetritus in the deep sea. Nature 332: 70-73.

Gudmundsson, G., 1990. Systematics of recent species of the superfamily Soritidae Ehrenberg 1939 with notes on systematic prinicples. PhD dissertation, CUNY: 129 pp.

Hallock, P., 1979. Trends in test shape with depth in large, symbiont-bearing foraminifera. J. Foram. Res. 9: 61-69.

Hallock, P., 1981a. Algal symbiosis: a mathematical analysis. Mar. Biol. 62: 249-255.

Hallock, P., 1981b. Light dependence in *Amphistegina*. J. Foram. Res. 11: 40-46.

Hallock, P., 1984. Distribution of selected species of living algal symbiont-bearing foraminifera on two Pacific coral reefs. J. Foram. Res. 14: 250-261.

Hallock, P., Forward, L. B., and Hansen, H. J., 1986. Influence of environment on the test shape of *Amphistegina*. J. Foram. Res. 16: 224-231.

Hansen, H. J., and Dalberg, P., 1979. Symbiotic algae in milioline foraminifera: CO2 uptake and shell adaptions. Bull. Geol. Soc. Denmark 28: 47-55.

Hawkins, E. K., and Lee, J. J., 1991. Fine structure of the cell surface of a cultured endosymbiont strain of *Porphyridium* sp. (Rhodophyte). Trans. Amer. Microsc. Soc. 109: 352-360.

Haynes, J. R., 1964. Symbiosis, wall structure and habitat in foraminifera. Cushman Found. Foram. Res. Contrib. 16: 40-43.

Haynes, J. R., 1990. The classification of the foraminifera - a review of historical and philosophical perspectives. Paleont. 33: 503-528.

Haynes, J. R., 1991 ms submitted. What, if anything, are ecophenotypes? J. Foram. Res.

Hecht, A. D., and Savin, S. M., 1972. Phenotypic variations and oxygen isotope ratios in recent planktonic foraminifera. J. Foram. Res. 6: 295-311.

Hedley, R. H., 1964. The biology of foraminifera. Int. Rev. Gen. Exp. Zool. 1: 1-45.

Hemleben, C., and Spindler, M., 1983. Recent advances in research on living planktonic foraminifera. Utrecht Micropaleont. Bull. 30: 141-170.

Hofker, J., 1950. Recent Peneroplidae, Pt. 1. J. Microsc. Soc. 70: 388-396.

Hofker, J., 1951a. Recent Peneroplidae, pt. 1 (continued). J. Microsc. Soc. 71: 223-239.

Hofker, J., 1951b. Recent Peneroplidae, Pt. 2. J. Microsc. Soc. 71: 342-356.

Hofker, J., 1952a. Recent Peneroplidae, Pt. 3. J. Microsc. Soc. 71: 450-463.

Hofker, J., 1952b. Recent Peneroplidae, Pt. 4. J. Microsc. Soc. 72: 102-122.

Hofker, J., 1953. Recent Peneroplidae, Pt. 5. J. Microsc. Soc. 73: 40-46.

Hofker, J., 1976. Further studies in Caribbean Foraminifera. Studies on the Fauna of Curaçao and other Caribbean Islands 49: 1-256.

Hottinger, L., 1977. Distribution of larger Peneroplidae, *Borelis* and Nummulitidae in the Gulf of Elat, Red Sea. Utrecht Micropaleont. Bull. 15: 35-110.

Hottinger, L., 1982. Larger foraminifera, giant cells with a historical background. Naturwissenschaften 69: 361-371.

Humason, G. L., 1962. Animal Tissue Techniques. W.H. Freeman and Co., San Francisco: 124-141.

Jeeps, M. W., 1942. Studies on *Polystomella crispum* Lamark (Foraminifera). J. mar. biol. Ass. U.K. 25: 605-666.

Jeon, K. W., 1987. Change of cellular "pathogens" into required cell components. In: Endocytobiology III. (Lee, J.J. and Fredrick, J.F. eds.) N.Y. Acad. Sci., New York: 359-371.

Jones, R. F., 1962. Extracellular mucilage of the red alga *Porphyridium cruentum*. J. Cell. Comp. Physiol. 60: 61-64.

Jones, R. F., Speer, H. L., and Kury, W., 1963. Studies on the growth of the red alga *Porphyridium cruentum*. Physiol. Plant. 16: 636-643.

Jørgensen, B. B., Erez, J., Revsbech, N. P., and Cohen, Y., 1985. Symbiotic photosynthesis in planktonic foraminifera. *Globigerinoides sacculifer* (Brady), studied with microelectrodes. Limnol. Oceanogr. 30: 1253-1267.

Kinsey, D. W., 1985. Metabolism, calcification and carbon production: I. Systems level studies. Proc. 5th Int. Coral Reef Congr. 4: 505-526.

Kloos, D. P., 1981. Growth and embryogenesis of the foraminifer *Sorites orbiculus*. Paleont. Proc. B84: 145-159.

Kloos, D. P., 1984. Parents and broods of *Sorites orbiculus* (Forskål), a biometric analysis. J. Foram. Res. 14: 277-281.

Kloos, D. P., and MacGillavry, H. J., 1978. Reproduction and life cycle of *Sorites orbiculus* (Forskål) Foraminifera. Geologie en Mijnbouw 57: 221-225.

Knappe, J., 1972. Synchronkulturen von *Porphyridium cruentum* und ihr photosynthetyisches Verhalten. Ber. Deutsch. Bot. Ges. Bd. 85: 425-434.

Koestler, R. J., Lee, J. J., Reidy, J., Sheryll, R. P., and Xenophontos, X., 1985. Cytological investigation of digestion and re-establishment of symbiosis in the larger benthic foraminifera *Amphistegina lessonii*. Endocytobiol. C. Res. 2: 21-54.

Kremer, B. P., Schmaljohann, R., and Röttger, R., 1980. Features and nutritional significance of photosynthates produced by unicellular algae symbiotic with larger foraminifera. Mar. Ecol. Prog. Ser. 2: 225-228.

Krüger, R., 1990. Untersuchungen zur Biologie der Grossforaminifere *Calcarina gaudichaudii*, d'Orbigny, 1840. Diplomarbeit Universität Kiel: 65 pp.

Kuile, B. ter, and Erez, J., 1984. In situ growth rate experiments on the symbiont-bearing foraminifera *Amphistegina lobifera* and *Amphisorus hemprichii*. J. Foram. Res. 14: 262-276.

Kuile, B. ter, and Erez, J., 1987. Uptake of inorganic carbon and internal carbon cycling in symbiont-bearing benthonic foraminifera. Mar. Biol. 94: 499-509.

Kuile, B. ter, and Erez, J., 1991. Carbon budgets for two species of benthonic symbiont-bearing foraminifera. Biol. Bull. 180: 489-495.

Kuile, B. ter, Erez, J., and Lee, J. J., 1987. The role of feeding in the metabolism of larger symbiont bearing foraminifera. Symbiosis 4: 335-350.

Kuile, B. ter, Erez, J., and Padan, E., 1989a. Competition for inorganic carbon between photosynthesis and calcification in the symbiont-bearing foraminifer *Amphistegina lobifera*. Mar. Biol. 103: 253-259.

Kuile, B. ter, Erez, J., and Padan, E., 1989b. Mechanisms for the uptake of inorganic carbon by two species of symbiont-bearing foraminifera. Mar. Biol. 103: 241-251.

Lee, J. J., 1974. Towards understanding the niche of foraminifera. In: Foraminifera, Vol. 1. (Hedley, R.H. and Adams, G., eds. Academic Press, New York: 208-257.

Lee, J. J., 1980. Nutrition and physiology of the foraminifera. In: Biochemistry and Physiology of Protozoa 3. (Levandowsky, M. and Hutner, S. eds.). Academic Press, New York: 43-66.

Lee, J. J., 1983. Perspective on algal endosymbionts in larger foraminifera. Int. Rev. Cytol. Suppl. 14: 49-77.

Lee, J. J., 1990. Fine structure of the rhodophycean *Porphyridium purpureum in situ* in *Peneroplis pertusus* (Forskål) and *P. acicularis* (Batsch) and in axenic culture. J. Foram. Res. 20: 162-169.

Lee, J. J., 1991. Symbiosis in foraminifera. In: Algae and Symbioses: Plants, Animals, Fungi, Viruses, Interactions Explored (Reisser, W. ed.). Biopress, Bristol, U.K.:

Lee, J. J., and Anderson, O. R., 1991. The Biology of the Foraminifera. Academic Press, London: 368 pp.

Lee, J. J., and Bock, W. D., 1976. The importance of feeding in two species of soritid foraminifera with algal symbionts. Bull. Mar. Sci. 26: 530-537.

Lee, J. J., Chan Y., and Lagziel, A., 1988a. An immunofluorescence approach toward the identification of endosymbiotic diatoms in several species of larger foraminifera. In: Lecture Notes on Coastal and Estuarine Studies. (Yentsch, C.M., Mague, F.C., and Horan, P.K. eds.). Springer-Verlag, New York: 230-241.

Lee, J. J., Crockett, L., Hagen, J., and Stone, R., 1974. The taxonomic identity and physiological ecology of *Chlamydomonas hedleyi*, sp. nov., algal flagellate symbiont from the foraminifera *Archaias angulatus*. Br. J. Phycol. 9: 407-422.

Lee, J. J., Erez, J., Kuile, B. ter, Lagziel, A., and Burgos, S., 1988b. Feeding rates of two species of larger foraminifera, *Amphistegina lobifera* and *Amphisorus hemprichii*, from the Gulf of Elat (Red Sea). Symbiosis 5: 61-102.

Lee, J. J., Erez, J., McEnery, M. E., Lagziel, A., and Xenophontos, X., 1986. Experiments on persistence of endosymbiotic diatoms in the larger foraminifer *Amphistegina lessonii*. Symbiosis 1: 211-226.

Lee, J. J., Faber, W. W., Jr., Anderson, O. R., and Pawlowski, J., 1991a. Life-cycles of foraminifera. In: Biology of Foraminifera (Lee, J.J. and Anderson, O.R., eds.). Academic Press, London: 285-334.

Lee, J. J., Faber, W. W., Jr., and Lee, R. E., 1991b. Granuloreticulopodial digestion--a possible preadaptation to benthic foraminiferal symbiosis ? Symbiosis 10: 47-61.

Lee, J. J., Faber, W. W., Jr., Nathanson, B., Röttger, R., Nishihira, M., and Krüger, R., 1991 ms submitted. Endosymbiotic diatoms from larger foraminifera collected in Pacific habitats. Micropaleont.

Lee, J. J., and Hallock, P., 1987. Algal symbiosis as the driving force in the evolution of larger foraminifera. Annals N.Y. Acad. Sci. 503: 330-347.

Lee, J. J., and Lawrence, C., 1990. Identity to the dinoflagellate symbionts from soritids. In: Endocytobiology IV. (Nardon, P. ed.) Walter de Gruyter & Co., Berlin: 221-223.

Lee, J. J., and McEnery, M. E., 1983. Symbiosis in foraminifera. In: Algal Symbiosis. (Goff, L.J. ed.). Cambridge U. Press, Cambridge: 37-68.

Lee, J. J., McEnery, M. E., and Garrison, J. R., 1980a. Experimental studies of larger foraminifera and their symbionts from the Gulf of Elat on the Red Sea. J.Foram. Res. 10: 31-47.

Lee, J. J., McEnery, M. E., Kahn, E., and Schuster, F., 1979. Symbiosis and the evolution of larger foraminifera. Micropaleont. 25: 118-140.

Lee, J. J., McEnery, M. E., Koestler, R. J., Lee, M. J., Reidy, J., and Shilo, M., 1983. Experimental studies of symbiont persistence in *Amphistegina lessonii* -a diatom-bearing species of larger foraminifera from the Red Sea. In: Endocytobiology II. (Schenk H.E.A. and Schwemmler, W. eds.) Walter de Gruyter & Co., Berlin: 487-514.

Lee, J. J., McEnery, M. E., Kuile, B. ter, Erez, J., Röttger, R., Rockwell, R. F., Faber, W. W., Jr., and Lagziel, A., 1989. Identification and distribution of endosymbiotic diatoms in larger foraminifera. Micropaleont. 35: 353-366.

Lee, J. J., McEnery, M. E., Pierce, S., Freudenthal, H. D., and Muller, W. A., 1966. Tracer experiments in feeding littoral foraminifera. J. Protozool. 13: 659-670.

Lee, J. J., McEnery, M. E., Röttger, R., and Reimer, C. W., 1980b. The isolation, culture and identification of endosymbiotic diatoms from *Heterostegina depressa* d'Orbigny and *Amphistegina lessonii* d'Orbigny (larger foraminifera) from Hawaii. Bot. Mar. 23: 297-302.

Lee, J. J., McEnery, M. E., and Rubin, H., 1969. Quantitative studies on the growth of *Allogromia laticollaris* (Foraminifera). J. Protozool. 16: 377-395.

Lee, J. J., and Muller, W. A., 1973. Trophic dynamics and niches of salt marsh foraminifera. Amer. Zool. 13: 215-223.

Lee, J. J., and Pierce, S., 1963. Growth and physiology of foraminifera in the laboratory; Part 4. Monoxenic culture of an Allogromiid with notes on its morphology. J. Protozool. 10: 404-411.

Lee, J. J., and Reimer, C. W., 1983. Isolation and identification of endosymbiotic diatoms from larger foraminifera of the Great Barrier Reef, Australia, Makapuu Tide Pool, Oahu, Hawaii, and the Gulf of Elat, Israel with descriptions of three new species *Amphora roettgerii*, *Navicula hanseniana* and *Nitzschia frustulum* variety *symbiotica*. In: Proceedings of the 7th International Diatom Symposium, Philadelphia, August 22- 27, 1982 (D.G. Mann, ed.). Otto Koeltz, Konigstein: 327-343.

Lee, J. J., Reimer, C. W., and McEnery, M. E., 1980c. The identification of diatoms isolated as endosymbionts from larger foraminifera from the Gulf of Elat (Red Sea) and the description of 2 new species, *Fragilaria shiloi* sp. nov. and *Navicula reissii* sp. nov. Bot. Mar. 23: 41-48.

Lee, J. J., Saks, N. M., Kapioutou, F., Wilen, S. H., and Shilo, M., 1984. Effects of host cell extracts on cultures of endosymbiotic diatoms from larger foaminifera. Mar. Biol. 82: 113-120.

Lee, J. J., Sang, K., Kuile, B. ter, Strauss, E., Lee, P. J., and Faber, W. W., Jr., 1991c. Nutritional and related experiments on laboratory maintenance of three species of symbiont-bearing, larger foraminifera. Mar. Biol. 109: 417-425.

Lee, J. J., Soldo, A. T., Reisser, W., Lee, M. J., Jeon, K. W., and Görtz, H-D, 1985. The extent of algal and bacterial endosymbioses in protozoa. J. Protozool. 32: 391-403.

Lee, J. J., Tietjen, J. H., Stone, R. J., Muller, W. A., Rullman, J., and McEnery, M. E., 1970. The cultivation and physiological ecology of members of salt marsh epiphytic communities. Helgolander Wiss. Meeres. 20: 136-156.

Lee, J. J., and Zucker, W. H., 1969. Algal flagellate symbiosis in the foraminifera Archaias. J. Protozool. 16: 71-81.

Lee, M. J., Ellis, R., and Lee, J. J., 1982. A comparative study of photoadaptation in four diatoms isolated as endosymbionts from larger foraminifera. Mar. Biol. 68: =193-197.

Lengsfeld, A. M. 1969. Nahrungaufnahme und Verdaung bei der Foraminifere *Allogromia laticollaris*. Helgolander wiss. Meeresunters 19: 385-400.

Leutenegger, S., 1977a. Reproductive cycles of larger foraminifera and depth distribution of generations. Utrecht Micropaleont. Bull. 15: 27-34.

Leutenegger, S., 1977b. Ultrastructure de foraminiferes perfores et imperfores ainsi que de leurs symbiotes. Cahiers Micropaleont. 3: 1-52.

Leutenegger, S., 1983. Specific host symbiont relationship in larger foraminifera. Micropaleont. 29: 111-125.

Leutenegger, S., 1984. Symbiosis in benthic foraminifera: Specificity and host adaptations. J. Foram. Res. 14: 16-35.

Leutenegger, S., and Hansen, H., 1979. Ultrastructural and radiotracer studies of pore-function in foraminifera. Mar. Biol. 54: 11-16.

Lister, J. J., 1895. Contributions to the life-history of the foraminifera. Phil. Trans. R. Soc. Lond. 186: 401-450.

Loeblich, A. R., Jr., and Tappan, H., 1988. Foraminiferal Genera and Their Classification. Van Nostrand Reinhold, New York: 970 pp.

Lwoff, A., 1950. Problems of Morphogenesis in Ciliates. J. Wiley & Sons, New York:

McEnery, M. E., and Lee, J. J., 1976. *Allogromia laticollaris*: a foraminiferan with an unusual apogamic metagenic life cycle. J. Protozool. 23: 94-108.

McEnery, M. E., and Lee, J. J., 1981. Cytological and fine structural studies of 3 species of symbiont-bearing larger foraminifera from the Red Sea. Micropaleont. 27: 71-83.

Montfort, P. Denys de, 1908. Conchyliologie systematiqué et classification mëthodique des coquilles, I. F. Schoell, Paris: 410 pp.

Muller, P. H., 1978. ^{14}Carbon fixation and loss in a foraminiferal-algal symbiont system. J. Foram. Res. 8: 35-41.

Muller, W. A., 1975. Competition for food and other niche-related studies of three species of salt-marsh Foraminifera. Mar. Biol. 31: 339-351.

Müller-Merz, E., and Lee, J. J., 1976. Symbiosis in the larger foraminiferan *Sorites marginalis* (with notes on *Archaias* spp.). J. Protozool. 23: 390-396.

Murray, J. W., 1963. Ecological experiments on foraminiferids. J. mar. biol. Ass. U.K. 43: 621-642.

Murray, J. W., 1973. Distribution and Ecology of Living Benthic Foraminiferids. Heinemann Educational Books, London: 274 pp.

Murray, J. W., 1983. Population dynamics of benthic foraminifera: Results from the Exe estuary, England. J. Foram. Res. 13: 1-12.

Muscatine, L., Cook, C. B., Pardy, R. L., and Pool, R. R., 1975. Uptake, recognition and maintenance of symbiotic *Chlorella* by *Hydra viridis*. Symp. Soc. exp. Biol. 291:175-203.

Muscatine, L., Falkowski, P. G., and Dubinsky, Z., 1983. Carbon budgets in symbiotic associations. In: Endocytobiology II (Schenk, H.E.A. and Schwemmler, W. eds.). Walter de Gruyter & Co., Berlin: 649-658.

Muscatine, L., McCloskey, L. R., and Loya, Y., 1985. A comparison of the growth rates of zooxanthellae and animal tissue in the Red Sea coral *Stylophora pistillata*. Proc. 5th Int. Coral Reef Congr. 6: 119-123.

Muscatine, L., and Pool, R. R., 1979. Regulation of numbers of intracellular algae. Proc. R. Soc. Lond. B 204: 131-139.

Myers, E. H., 1936. The life-cycle of *Spirillina vivipara* Ehrenberg, with notes on morphogenesis, systematics and distribution of the foraminifera. J. Roy. Microsc. Soc. 56: 120-146.

Odum, E. P., 1983. Basic Ecology. Saunders College Publishing, New York: 613 pp.

Parsons, T. R., and Takahashi, M., 1973. Environmental control of phytoplankton cell size. Limnol. Oceanogr. 18: 511-515.

Pawlowski, J., and Lee, J. J., 1991 in press. The life cycle of *Rotaliella elatiana* n. sp.--a tiny macroagavorous foraminifer from the Gulf of Elat. J. Protozool.

Reimer, C. W., and Lee, J. J., 1984. A new pennate diatom: *Protokeelia hottingeri* gen. et sp. nov. Proc. Acad. Nat. Sci. Phil. 136: 194-199.

Reimer, C. W., and Lee, J. J., 1988. New species of endosymbiotic diatoms (Bacillariophyceae) inhabiting larger foraminifera in the Gulf of Elat (Red Sea). Proc. Acad. Nat. Sci. Phil. 140: 339-351.

Reiss, Z., and Hottinger, L., 1984. The Gulf of Aqaba: Ecological Micropaleontology. Springer-Verlag, Berlin: 354 pp.

Reisser, W., 1986. Endosymbiotic associations of freshwater protozoa and algae. Prog. Protistol. 1: 195-214.

Reisser, W., 1988. Signals in the *Paramecium bursaria-Chlorella* sp. association. In: Cell to Cell Signals in Plant, Animal and Microbial Symbiosis (Scannerini, S., Smith, D.C., Bonfoante-fasolo, P., Gianinazzi-Pearson, V., eds.). NATO ASI series H17: 271-282.

Rhumbler, L., 1894. Die Perforation der Embryonalkammer von *Peneroplis pertusus* Forskål. Zool. Anz. 16: 335-342.

Rhumbler, L., 1911. Die Foraminiferen (Thalamophoren) der Plankton Expedition; Teil 1 -- Die Allgemeinen Organisations-Verhältnisse der Foraminiferen. Plankton Exped. Humbolt-Stiftung, Ergebn. 3: 1-331.

Ross, C. A., 1972. Biology and ecology of *Marginopora vertebralis* (Foraminifera), Great Barrier Reef. J. Protozool. 19: 181-192.

Röttger, R., 1972a. Die Bedeutung der Symbiose von *Heterostegina depressa* (Foraminifera, Nummulitidae) für hohe Siedungsdichte und Karbonatproduktion. Verh. Dtsch. Zool. Ges. 65: 43-47.

Röttger, R., 1972b. Die Kultur von *Heterostegina depressa* (Foraminifera: Nummulitidae). Mar. Biol. 15: 150-159.

Röttger, R., 1974. Larger foraminifera: Reproduction and early stages of development in *Heterostegina depressa*. Mar. Biol. 26: 5-12.

Röttger, R., 1976. Ecological observations of *Heterostegina depressa* (Foraminifera, Nummulitidae) in the laboratory and in its natural habitat. Maritime Sediments Spec. Publ. 1: 75-80.

Röttger, R., 1978. Unusual multiple fission in the gamont of the larger foraminiferan *Heterostegina depressa* (Rhizopoda). J. Protozool. 25: 41-44.

Röttger, R., 1981. Vielteilung bei Grossforaminiferen. Mikrokosmos 70: 137-138.

Röttger, R., and Berger, W. H., 1972. Benthic foraminifera: morphology and growth in clone cultures of *Heterostegina depressa*. Mar. Biol. 15: 89-94.

Röttger, R., Fladung, M., Schmaljohann, R., Spindler, M., and Zacharias, H., 1986. A new hypothesis: the so-called megalospheric schizont of the larger foraminifera, *Heterostegina depressa* d'Orbigny, 1826, is a separate species. J. Foram. Res. 16: 141-149.

Röttger, R., Irwan, A., Schmaljohann, R., and Franzisket, L., 1980. Growth of the symbiont-bearing foraminifera *Amphistegina lessonii* d'Orbigny and *Heterostegina depressa* d'Orbigny (Protozoa). In: Endocytobiology. Vol. 1. (Schwemmler, W. and Schenk, H.E.A. eds.) Walter de Gruyter & Co., Berlin: pp. 125-132.

Röttger, R., Krüger, R., and Rijk, S. de, 1990a. Larger foraminifera: variation in outer morphology and proloculus size in *Calcarina gaudichaudii*. J. Foram. Res. 20: 170-174.

Röttger, R., Krüger, R., and Rijk, S. de, 1990b. Trimorphism in foraminifera (protozoa) - verification of an old hypothesis. Euro. J. Protist. 25: 226-228.

Röttger, R., Schmaljohann, R., and Zacharias, H., 1989. Endoreplication of zygotic nuclei in the larger foraminifer *Heterostegina depressa* (Nummulitidae). Euro. J. Protist. 25: 60-66.

Röttger, R., Spindler, M., Schmaljohann, R., Richwien, M., and Fladung, M., 1984. Functions of the canal system in the rotaliid foraminifer, *Heterostegina depressa*. Nature 309: 789-791.

Saks, N. M., 1981. Growth, productivity and excretion of *Chlorella* spp. endosymbionts from the Red Sea: implications for lost foraminifera. Bot. Mar. 24: 445-449.

Saks, N. M., 1982. Primary production and release of assimilated carbon by *Chlamydomonas provasoli* in culture. Mar. Biol. 70: 205-208.

Sandon, H., 1932. The food of protozoa. Publs. Fac. Sci. Egypt. Univ. 1: 1-187.

Schmaljohann, R., and Röttger, R., 1978. The ultrastructure and taxonomic identity of the symbiotic algae of *Heterostegina depressa* (Foraminifera, Nummulitidae). J. mar. biol. Ass. U.K. 58: 227-237.

Schoenberg, D. A., and Trench, R. K., 1980a. Genetic variation in *Symbiodinium* (=*Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates: I. Isoenzyme and soluble protein patterns of axenic cultures of *Symbiodinium microadriaticum*. Proc. Roy. Soc. Lond. B. 207: 405-427.

Schoenberg, D. A., and Trench, R. K., 1980b. Genetic variation in *Symbiodinium* (=*Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates: II. Morphological variation in *Symbiodinium microadriaticum*. Proc. Roy. Soc. Lond. B. 207: 429-444.

Schoenberg, D. A., and Trench, R. K., 1980c. Genetic variation in *Symbiodinium* (=*Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates: III. Specificity and infectivity of *Symbiodinium microadriaticum*. Proc. Roy. Soc. Lond. B. 207: 445-460.

Sheath, R. G., Hellebust, J. A., and Sawa, T., 1979. Floridean starch metabolism of *Porphyridium purpureum* (Rhodophyta) I. Changes during aging of batch culture. J. Phycol. 18: 149-163.

Smith, D. F., and Wiebe, W. J., 1977. Rates of carbon fixation, organic carbon release and translocation in a reef building foraminifera, *Marginopora vertebalis*. Aust. J. Mar. Freshwater Res. 28: 311-319.

Sournia, A., 1976. Primary production of sands in the lagoon of an atoll and the role of foraminiferan symbionts. Mar. Biol. 37: 29-32.

Spencer-Davies, P., 1984. The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. Coral Reefs 2: 181-186.

Spero, H. J., and Parker, S. L., 1985. Photosynthesis in the symbiotic planktonic foraminifer *Orbulina universa* and its potential contribution to oceanic primary productivity. J. Foram. Res. 15: 273-281.

Spindler, M., and Hemleben, C., 1980. Symbionts in planktonic foraminifera (protozoa). In: Endoctyobiology, Vol. 1. (Schwemmler, W. and Schenk, H.E.A. eds.). Walter de Gruyter & Co., Berlin: 133-140.

Stoecker, D. K., and Michaels, A. E., 1991. Respiration, photosynthesis and carbon metabolism in planktonic ciliates. Mar. Biol. 108: 441-447.

Strickland, J. D. H., and Parsons, T. R., 1968. A practical handbook of seawater analysis. Fisheries Res. Bd. Canada Bull. 167: 311 pp.

Suess, E., 1988. Effects of microbe activity. Nature 333: 17-18.

Sverdrup, H. U., Johnson, M. W., and Fleming, R. H., 1942. The Oceans. Prentice Hall, Englewood Cliffs, N.J.: 1087 pp.

Tartar, V., 1967. Morphogenesis in protozoa. In: Research in Protozoology vol. 2 (Chen, T.T. ed.). Pergamon Press, New York: 1-116.

Taylor, C. E., Muscatine, L., and Jefferson, D. R., 1989. Maintenance and breakdown of the *Hydra-Chlorella* symbiosis: a computer model. Proc. R. Soc. Lond. B. 238: 277-289.

Thompson, D. W., 1961. On growth and form. Abridged edition (Bonner, J.T., ed.). Cambridge U. Press, New York: 346 pp.

Thurston, J. M., and Joftes, D. L., 1963. Stains compatible with dipping radioautography. Stain Technol. 38: 231-235.

Trench, R. K., and Blank, R. J., 1987. *Symbiodinium microadriaticum* Freudenthal, *S. goreauii* sp. nov., *S. kawagutii* sp. nov. and *S. pilosum* sp. nov.: gymnodiniod dinoflagellate symbionts of marine invertebrates. J. Phycol. 23: 469-481.

Valiela, I., 1984. Marine Ecological Processes. Springer-Verlag, New York: 546 pp.

Winter, F. W., 1907. Zur Kenntnis der Thalamophoren I. untersuchung über *Peneroplis pertusus* (Forskål). Archiv. Protistenk. 10: 1-133.

Wolpert, L., 1990. Ordered growth. Nature 346: 711-712.

Zmiri, A., Kahan, D., Hochstein, S. and Reiss, Z., 1974. Photoaxis and thermotaxis in some species of *Amphistegina* (Foraminifera). J. Protozool. 21: 133-138.