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Feeding and Growth of the Foraminifer *Peneroplis planatus* (Fichtel and Moll) Montfort

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Abstract

Specimens of the foraminifer *Peneroplis planatus* (Fichtel and Moll) Montfort collected from a *Halophila* meadow, at 15–20 m depth, near Wadi Taba, Elat Israel were fed ^{14}C-labeled algal food for 24 hr. Half of the specimens were counted for uptake, and the rest placed in a 24 hr cold food chase then counted for retention. For six of the ten algal species tested, *P. planatus* statistically retained 100% of the ^{14}C-labeled algae ingested. There appears to be some selectivity in feeding, with *Cocconeis placentula* and *Amphora* sp. being 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* does not grow when starved, and grows 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. *P. planatus*, like other imperforate foraminifera, acquires much of its energy for growth from external food sources, and cannot grow solely on carbon substrates produced by endosymbiotic algae, even though light seems to be necessary for growth.

Keywords: *Peneroplis planatus*, endosymbiosis, growth, feeding, diet, light

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1. 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 for most species. 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 studies have grown larger foraminifera for several months (Winter, 1907; Hallock, 1981), however, only one species, *Heterostegina depressa*, has been successfully cultured through successive generations (Röttger et al., 1986). If the needs of large 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, 1972; 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., 1988).

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, 1985). 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, 1985).

The endosymbionts of *Peneroplis* are relatively unique among all the known algal-invertebrate (-protist) symbioses since they are rhodophytes (*Porphyridium*) (Leutenegger, 1977, 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.

2. Materials and 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, Elat, 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 whirlpack 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 between our hands 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, in press). 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, 1988). 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 β liquid scintillation counting (LSC). Within 24 hr 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. subcommunis* (WH480), and *Navicula* sp.) resembled species of algae found in the *Halophila* beds where the *Peneroplis* were collected (Lee et al., 1988). 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 hr feeding feed 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 of 24 hr. 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, weighted 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. (1988), to the mean algal equivalents eaten per microgram foraminifer (the total DPM measured per μg 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. (in press). Both salinity and pH were kept constant throughout the experiment. The culture temperature was a constant $23 \pm 1°C$.

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 feed 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., 1988). 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 (i.e. the longest length), and the lesser axis (i.e. 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.

3. Results

Feeding experiment

Despite lower values for all the mean algal equivalents retained (final) per μg foraminifera, for 6 of the 10 algal species tested, *P. planatus* statistically retained 100% of the ^{14}C-labelled algae ingested (Table 1). The values are means of 3 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 2 species for the amount ingested and retained (F=0.30, df=1.30, and F=0.96, df=1.25, respectively; Table 1).

There was no statistical difference between the feeding rates using *C. hedleyi*, *Chlorella* sp. (A.T.), *Chlorella* sp. (SUZY), *Nitzschia* sp. (W521), *N. subcommunis*, and *Navicula* sp. (F=0.99, df=5.92), which averaged 4.6 algal equivalents per μg foraminifer d^{-1} (Table 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 hr "cold" chase, whereas the carbon in the other species was completely retained (Table 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 1). Both other 2 *Nitzschia* species (*N. subcommunis* (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 1). The values obtained for *C. hedleyi* suggested that all of the algae ingested may have been egested (Table 1).

Growth experiment

The mean initial weights did not significantly vary among the experimental groups (Tables 2,3). 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 2).

Table 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	0.11NS	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	0.43NS	100.0
Cocconeis placentula	29	80.66 ±8.48	30.97 ±5.38	29	66.55 ±8.19	21.50 ±3.86	1.29	1.27NS	100.0
Amphora sp.	30	84.17 ±13.49	33.65 ±6.50	25	81.96 ±9.68	27.78 ±5.29	1.26	0.44NS	100.0
Nitzschia sp.	26	93.65 ±10.46	0.66 ±0.23	30	64.83 ±7.29	0.32 ±0.08	1.30	2.03NS	100.0
N. subcommunis (WH480)	30	89.30 ±10.90	4.18 ±0.94	26	87.81 ±19.11	2.34 ±0.70	1.21	1.75NS	100.0
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

The 6 algal species used as food were chosen from the previous feeding experiment. Both *Amphora* sp. and *C. placentula* were heavily ingested and retained (Table 1). *Nitzschia* sp. (W521), *Chlorella* sp. (AT) and *Navicula* represent moderate food species (Table 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 2). When placed in complete darkness, regardless of the feeding regime, *P. planatus* grew few or no chambers

Table 2. Growth date of *Peneroplis planatus* for seven feeding regimes and three light regimes. D = complete darkness, L = low light intensity (30–50 μE m^{-2}sec^{-1}, H = high light intensity (200–400 μE m^{-2}sec^{-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 foraminifer*	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	62.0 ±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 ±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
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.	9	19.79	100.00 ±3.24	62.93	9.33 ±8.20	43.15 ±1.56	106	34–279 ±33	
Cocconeis placentula	L	15	32.36 ±4.34	86.67	45.51 ±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 ±1.63	12.00	70 ±19	20–174

Table 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

Feeding regime	Light regime	df	Dependent Variables: 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 selina	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

(Table 2). The mean final weights were not significantly different (Table 3). Only 7 specimens grew at all; 4 grew 2 chambers, and 3 grew 1 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 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 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 3). The specimens grew an average of over 8.8 ± 0.7 chambers in 20 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 2). The mean final weights were not significantly different among the variables tested (Table 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 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 50% 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.

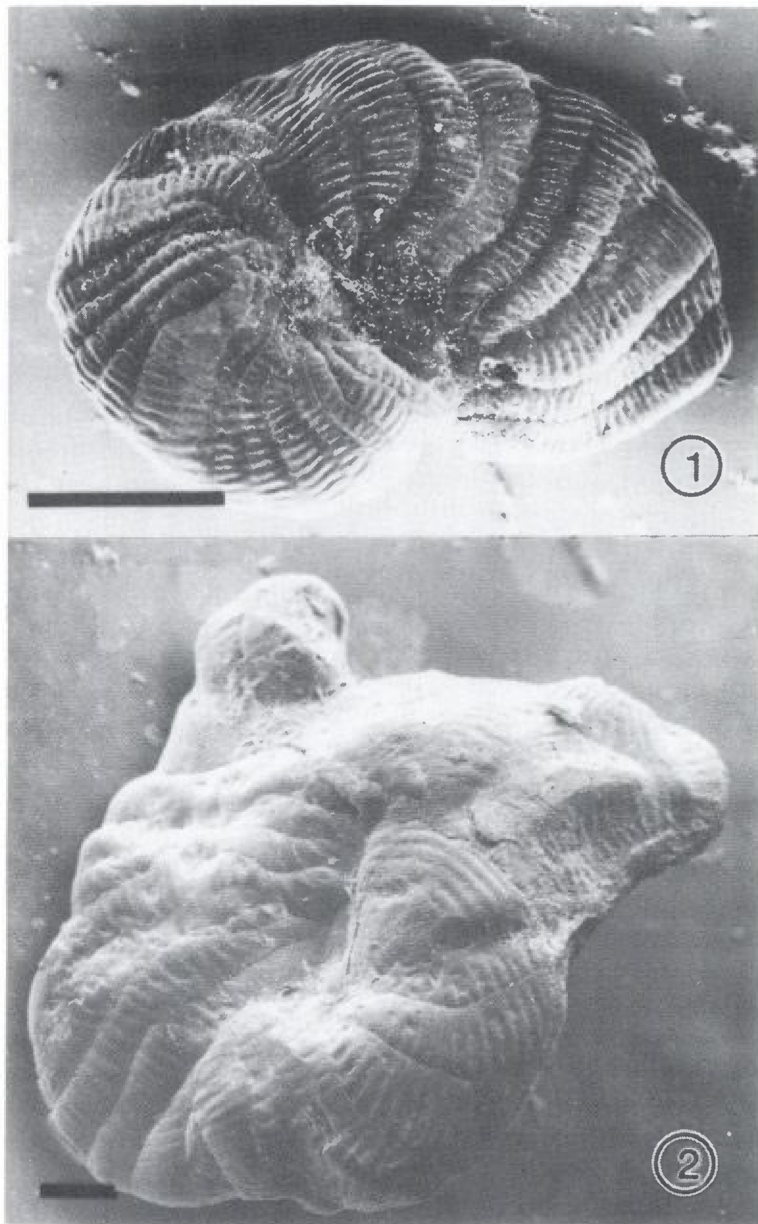
Although some of the specimens appeared "normal" (Figs. 1, 3), many of the specimens which grew, looked abnormal in shape (e.g. Fig. 2). The new chamber growth was punctuated, which may be natural calcification (Hofker, 1951). Considerable variation was noted in the size of the chambers within a single specimen (Fig. 1). Other aberrant features included chambers lacking ornamentation (Fig. 4), the discontinuity of chambers (Fig. 5), rectilinear growth originating within the whorl (Fig. 6), small or partial chambers, chambers grown out of whorl (Fig. 7), the reversal of the whorl, chambers curling back over the specimen (Fig. 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 2). There are significant differences between some regimes, and no significant differences between others (Table 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.

4. Discussion

The feeding behaviour 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., 1988). Often the excessive food is digested at a later time. Lee (1974; Lee et al., 1988), 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 hr following ingestion (Table 1). This does not indicate complete digestion nor assimilation. Kuile et al. (1987) found *Amphisorus hemprichii* to retain over 80% of



Figures 1–8. SEM micrographs of *Peneroplis planatus* cultured in various laboratory regimes.

Figure 1. *P. planatus* fed *Dunaliella salina* in the high light regime. Specimen grew 20 chambers in culture. Scale bar = 200 μm.

Figure 2. *P. planatus* fed *Nitzschia* sp. (W521) in the high light regime. Specimen grew 16 chambers in culture. Scale bar = 100 μm.

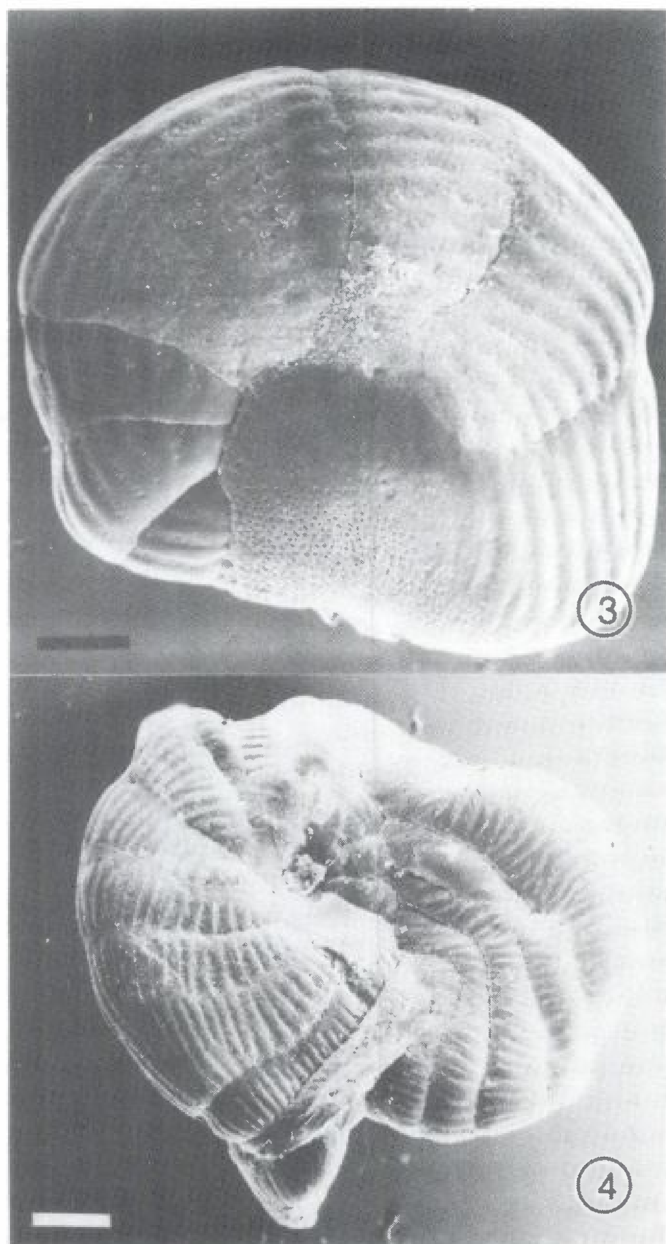


Figure 3. *P. planatus* fed *Dunaliella salina* in the high light regime. Specimen grew only one chamber in culture. Scale bar = 50 μm.

Figure 4. *P. planatus* fed *Dunaliella salina* in the high light regime. Specimen grew 15 chambers in culture. Scale bar = 100 μm.

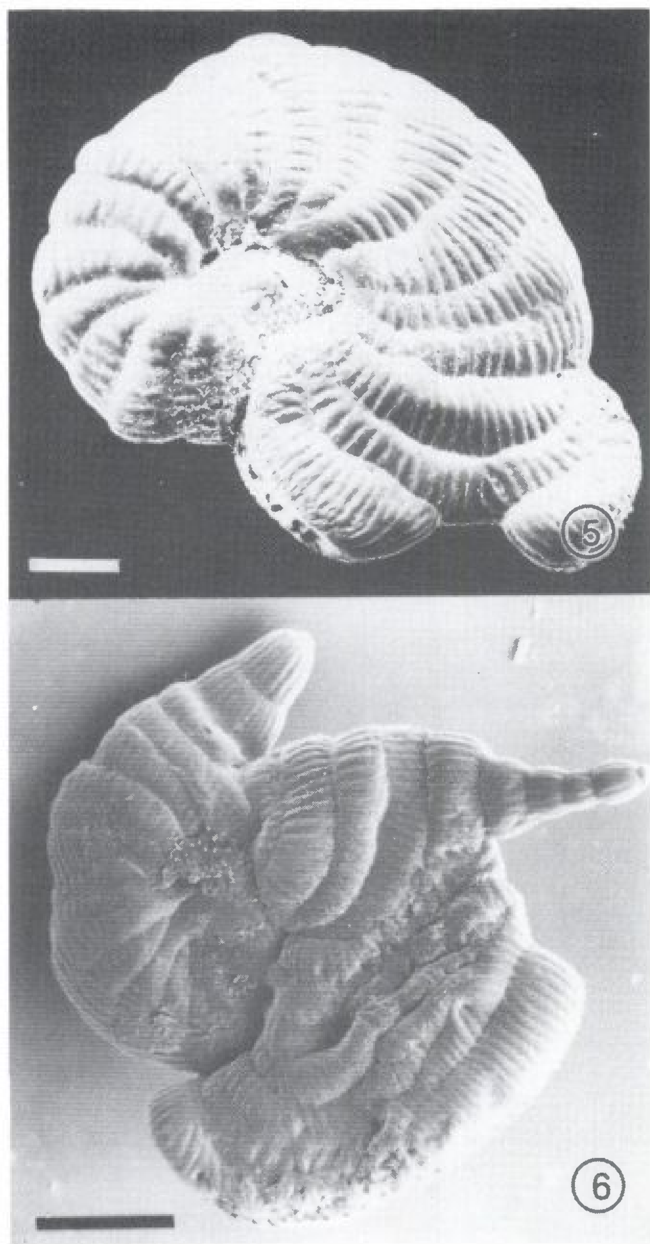


Figure 5. *P. planatus* fed *Chlorella* sp. (AT)in the low light regime. Specimen grew 17 chambers in culture. Scale bar = 100 μm.

Figure 6. *P. planatus* fed *Nitzschia* sp. (W521) in the low light regime. Specimen grew 22 chambers in culture. Scale bar = 200 μm.

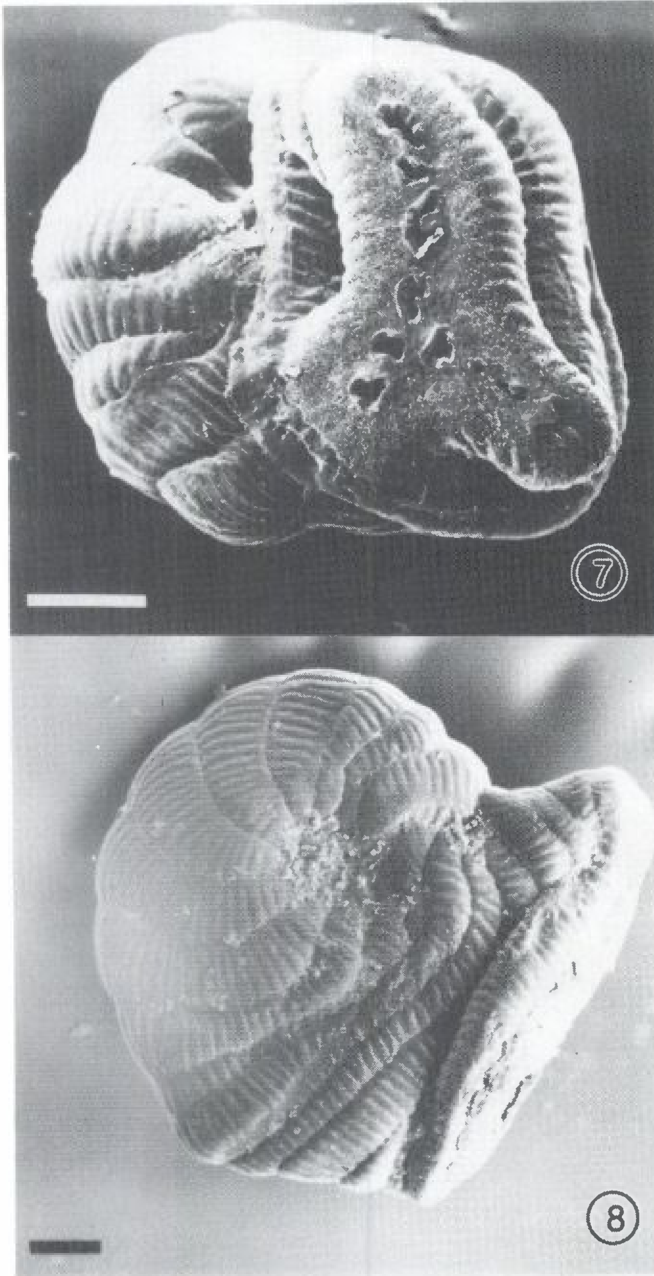


Figure 7. *P. planatus* fed *Dunaliella salina* in the high light regime. Specimen grew 2 chambers in culture. Scale bar = 100 μm.

Figure 8. *P. planatus* fed *Nitzschia* sp. (W521) in the low light regime. Specimen grew 12 chambers in culture. Scale bar = 100 μm.

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 hr incubation was too long. When *P. planatus* is incubated in ^{14}C-bicarbonate, after 1 hr, the label is found throughout the entire specimen (Faber, unpublished observation). 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., submitted). 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., 1988). Nonetheless, the feeding rates are in the same order of magnitude as in other studies (Lee and Bock, 1976; Lee et al., 1988), 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 over some algae to others (Table 1). *Amphora* sp. and *C. placentula* were heavily ingested and retained, whereas the *Chlorella* strains, *Navicula* sp., *Nitzschia* sp. (W521) and *N. subcommunis* (WH480) were consumed and assimilated more moderately (Table 1). *Amphisorus hemprichii* also showed a strong bias toward ingesting and retaining the same strain of *Amphora* (Lee et al., 1988), 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., 1988) whereas *Peneroplis* ingested none (Table 1).

Clearly, *Peneroplis* needs ingested food, since the unfed specimens failed to grow (Table 2), but the type of algal food had little effect (Tables 2,3). 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., in press). 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 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., in press); *Amphistegina lessonii* (Muller, 1978; Röttger et al., 1980; Hallock, 1981); *A. lobifera* (Hallock, 1981; Kuile and Erez, 1984; Lee et al., in press); *Archaias angulatus* (Lee and Bock, 1976; Duguay and Taylor, 1978; Duguay, 1983); *Heterostegina depressa* (Röttger et al., 1980); *Marginopora kudakajimensis* (Lee et al., in press); 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 μE m^{-2}sec^{-1} (Table 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, 1981; 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 in 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 (1951) 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, in press). 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 (Faber, unpublished observation). 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, 1972). 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., 1988). Whereas the imperforate soritids, *Amphisorus, Archaias, Sorites* and *Peneroplis*, require more algal food (Lee and Bock, 1976; Kuile et al., 1987; Lee et al., 1988). 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 (Jörgensen et al., 1985; Kuile et al., 1987; Lee et al., 1988). 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., in press). *Amphisorus* showed a greater response to the nutrient enrichment than did *Amphistegina* (Lee et al., in press). 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 its unique *Porphyridium* endosymbiont may be more easily experimentally probed.

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