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The effects of environmental stress on the community structure and productivity of salt marsh epiphytic communities.

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The microbial and micrometazoan assemblages in salt marshes and shallow embayments in the greater New York City metropolitan region are being studied to gain insight into the functional relationships which regulate the community. By means of studies on labeled uptake and the growth of algae and bacteria on a differential series of media, an auxotrophic profile of the community over a summer growing season was obtained. Evidence suggests that dissolved organic nutrients are recycled and play a central role in the nutrition, community regulation and succession within the unicellular algal compartment. The algae in the community vary widely in their sensitivity to ionizing radiation and thermal stress. At 40 C, some of the thermally more resistant species have an MLD > 135 min. Many of the same species are also more resistant to UV (MLD $> 4.5 \times 10^4$ ergs) and γ irradiation (MLD $\sim 1 \times 10^7$ ergs). This combination of resistance to stresses seems to be an adaptation to growth or repeated exposure at ebb tide in hot shallow tide pools or in interstitial waters. Light quality is also important in selectively regulating growth and succession among the algae. The trophic dynamics, mineral cycling, and niches of salt marsh sublittoral foraminifera were also studied. The ecological growth efficiency was estimated to be 21.5% in Rosalina leei, 12% in Allogromia laticollaris and only 5% in Spiroloculina hyalina. Other energetic measurements were also made. Tracer feeding and microcosm experiments provided some of the data used for testing a graphic representation of the Hutchinsonian niche. An equation based on competitive feeding experiments was used to reduce data to a single coefficient.

All papers
removed

This year has been exceedingly fruitful and rewarding. One subproject on the standing crop and succession of diatoms in a salt marsh represents the culmination of 4 years' laboratory and field studies - the success of which has surpassed our expectations and has made many difficult problems more easily accessible to laboratory study. In total this year we published 3 papers supported by this project. Abstracts of 4 papers presented at national meetings were also published. Six manuscripts have been completed; 2 have been scheduled for publication and the others will be sent to appropriate journals.

A brief synopsis of each of the full papers (which are appended to this report) follows:

1. Nicholas J. Matera and John J. Lee. 1972.
Environmental factors affecting the standing
crop of foraminifera in sublittoral and
psammonlittoral communities of a Long Island
salt marsh. Marine Biology, in press.
(next issue)

This paper was an extension and elaboration of a study we had published earlier (Lee et al. 1969). The answers to 3 basic questions were sought. How do the standing crops of living foraminifera in the sediments compare with those in the overlaying epiphytic communities? Do the foraminifera migrate through the sediments as a function of tidal cycle as do some of the littoral diatoms? Is the distribution of foraminifera in our very small study area affected by the small rivulets of currents occurring during tidal excursions in and out of the adjacent shallow drainage ditches?

Elphidium incertum (46.63%) and Protelphidium tisburyensis (25.64%) were dominant species in the epiphytic community. Ammonobaculites dilatatus, Ammonia beccarii, Ammonium salsum, Elphidium clavatum, Elphidium translucens and Trochammina inflata were subordinate species, <10% of the total collected. There were fewer

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species of foraminifera but larger standing crops of foraminifera in the sediments. With the exception of Elphidium incertum, a dominant generalist throughout the water column, the species of foraminifera in the epiphytic communities are not continuous with the psammonlittoral communities below them.

2. John J. Lee, John H. Tietjen, Robert J. Stone, William A. Muller, Marie McEnery, Norman Saks, Carmine Mastropaolo and Eileen Kennedy. The effects of environmental stress on the community structure, productivity, energy flow, and mineral cycling in salt marsh epiphytic communities. Presented at the 3rd National Symposium on Radioecology, May 10-12, 1971, Oak Ridge, Tennessee. (in press).

In this paper we summarize the overall research effort of our laboratory on the salt marsh epiphytic communities. The new data in the paper is on carbon cycling and the effects of competition among pairs and small groups of herbivores in the community. The rates of primary productivity and respiration of the community and individual organisms are given. The herbivores in the community seem to consume most of the carbon fixed. Only during 2 months of the year is net O_2 production > community respiration. The caloric values of 8 unicellular algae and the foraminiferan Allogromia laticollaris were also measured. The values ranged from a low of 7.7×10^2 c/g for Cylindrotheca closterium to 3.9×10^3 c/g for an unidentified chlorophyte (BL-55); Allogromia laticollaris had a caloric value of 6.2×10^3 c/g. Nutritional studies of the algae suggest that the carbon budget of the community is augmented by heterotrophy and that algae can compete successfully with bacteria for labeled substrates. The biological half life of ^{14}C in Rhabditis marina, a marine nematode, is ~ 2 days.

The effect of competition among pairs and small groups of herbivores was shown to be reflected as phase and amplitude changes.

3. Norman M. Saks and John J. Lee. Radiosensitivity and light quality responses to salt marsh epiphytic algae. Presented at the 3rd National Symposium on Radioecology, May 10-12, 1971, Oak Ridge, Tennessee. (In press).

Salt marsh littoral and sublittoral communities are subjected to a great range of light intensity and light quality. In this paper we studied the effects of 2, possibly coupled, physical community regulating factors, light quality and radiation resistance. Representative algae from salt marsh littoral epiphytic communities varied in their sensitivity to gamma radiation and in their growth responses to different visible light spectra. In general, those organisms which grew best in the less energetic visible range were more radioresistant. Some exceptions among the more heterotrophic species were noted. Radiosensitivity of the algae ranged from a MLD of 78,285-169,000 rad. The MLD's for 2 animals from the community were 143,524 rad for Rhabditis marina, a marine nematode, and 208,760 for Allogromia laticollaris, a foraminiferan. With respect to light quality some algae grew over 800% better in certain spectra than in white light of the same total energy. Minimal growth by 1-5% of controls was noted in other regimes. As shown by these studies, light quality as regulated by depth in the water column is important in selectively regulating growth and succession in salt marsh epiphytic communities. Resistance to high energy natural radiation during ebb tide exposure is also an important ecological factor.

4. Robert J. Stone and John J. Lee. Carbon budget in a marine salt marsh community. II. Heterotrophy in representative diatoms and chlorophytes. (ms completed; will be submitted for publication after Mr. Stone's thesis defense).

The concept of heterotrophy in algae, particularly marine algae, is well established. Our previous study suggested that the carbon budget of the community required a large input of organic metabolites. We felt that one of the keys to unravelling the trophic relationships in the epiphytic community would be a study of the nutritional requirements of representative components. Metabolites could be regulatory factors controlling energy flow and succession.

The heterotrophic abilities of 11 species of unicellular algae from the epiphytic community of Enteromorpha intestinalis were studied. Each of the algal species tested has some heterotrophic capabilities and each has a different nutritional pattern. Crude natural products, such as soil and yeast extracts, were the most growth stimulating factors tested. Monosaccharides and disaccharides inhibited the growth of 5 chlorophytes but some of the diatoms were stimulated. The growth of 7 of the 11 species tested was doubled by the addition of 1 or more amino acids. One diatom was stimulated by any of 7 amino acids.

Many of the organisms were inhibited by relatively low concentrations (25 mM) of substrates which suggests that organic ion pollutants in concentrations as low as this could depress the growth of algae in salt marshes. Several organisms required a delicate balance of nutrients for best growth. The picture which emerged from this study is one in which dissolved organic nutrients are recycled and play a central role in the nutrition, community regulation, and succession within the unicellular algal compartment.

5. Niche width of salt marsh foraminifera. William A. Muller. Dissertation. To be revised shortly and submitted to Limnology and Oceanography.

Foraminifera are very important organisms in the mineral cycling of Ca^{++} and Sr^{++} in the sea and as biological indicators

pf water quality. Several hundred species of foraminifera have been isolated from various salt marshes throughout the world. Very little is known of the differences in niche which allow the growth of some species and not others in the same marsh. Tracer feeding and microcosm experiments provided some of the data used for testing a graphic representation of the Hutchinsonian niche. A polygon was constructed using 6 factors: temperature, salinity, pH, feeding rate, interspecific competition, and intraspecific competition as niche determinants. The effects of these biotic and abiotic factors on the generation time of 4 species of foraminifera were tested. Biotic factors were more difficult to evaluate than the abiotic ones. An equation, based on competitive feeding experiments, was developed to reduce data to a single coefficient. One species, Spiroloculina hyalina, did not compete with the other species for food, but Allogromia laticollaris and Rosalina leei were competitive. Intraspecific competition (crowding) seems to be an important factor limiting the exploitation of available community space by Allogromia laticollaris but it was not a limiting factor for the other 2 species. A. laticollaris was characterized as a rare species which may become locally abundant when dominant species are missing. S. hyalina is also a rare species with a high biotic potential but with very specialized niche requirements. It will bloom in physically unstressed environments provided that moderate numbers of appropriate species of bacteria are present. R. leei is a stable, conspicuous species whose moderate numbers are relatively unaffected by physical stress and competition.

6. Norman M. Saks and John J. Lee. The differential sensitivity of various species of salt marsh epiphytic algae to ionizing radiation and thermal stress. To be presented at the symposium on The Interaction of Radioactive Contaminants with the Constituents of the

Marine Environment, to be held at Seattle, Washington, on 10-14 July 1972.

Many biotic and abiotic factors play an important role in determining the population structure and stability of the littoral benthic microflora in the salt marshes we have studied. At ebb tide the community is exposed to strong ionizing radiation, temperature, and osmotic stress. In this paper we report the results of these forms of stress on 12 algae from the same community. The species tested varied widely in their sensitivity to ionizing radiation and thermal stress. Seven of the species tested, Amphora spp (RF 8 and B1-17), Nitzschia sp (B1-15a), Cylindrotheca closterium (9), unidentified chlorophytes (50 and 55), and Chlorococcum sp (38), were relatively resistant to thermal stress at 40 C (MLD > 135 min). Four of the same species, Amphora spp (B1 17 and RF 8), Cylindrotheca closterium (9) and Chlorococcum sp (38) were among the more resistant to ultraviolet (MLD > 4.5×10^4 ergs). All of the algae tested were almost equally sensitive to ^{60}Co irradiation (MLD $\sim 1 \times 10^7$ ergs). This combination of resistance to stresses seems to be an adaptation to growth or repeated exposure at ebb tide in hot shallow tide pools or in interstitial waters. Four of the epiphytic algae were more radio- and thermal sensitive, which would indicate that they may be better adapted for growth deeper in the water column.

7. John J. Lee, Marie E. McEnery, Eileen M. Kennedy and Howard Rubin. Educing the functional relationships among the diatom assemblages within sublittoral salt marsh communities. To be presented at the IBP symposium on Modern Methods in Microbial Ecology to be held 19-23 June 1972 in Uppsala, Sweden, and published in the Proceedings.

In this paper we discuss the methods we have used to give insight into the functional relationships within the algal assemblages within sublittoral salt marsh communities. Included

are: measurements of primary productivity by the community and the epiphytes, labeled uptake studies by the algae and the bacterial assemblages and the results of studies using differential media to study the auxotrophic profile of the algae throughout the growing season. Diatom community structure, species abundance, and appearance correlations are also discussed.

8. John J. Lee, Marie E. McEnery, Eileen M. Kennedy and Howard Rubin. Nutritional relationships and population structure of diatom assemblages from a Long Island salt marsh epiphytic community. Paper in final stages of preparation; will be submitted to Marine Biology or Limnology and Oceanography.

This paper summarizes the results of an important subproject which has finally been completed after 4 years of intensive study. Some of the overall results are included in the shorter paper prepared for the Uppsala symposium. Here we detail the diatom community structure and correlate species abundance and appearance. The series of differential media used to give an auxotrophic profile is detailed, as is the dichotomous key we used to separate colony types from one another. The nutritional requirements of individual species, genera, and the entire community are discussed. An overall view of our present state of knowledge on the subtle niche relationships of the diatom flora found in salt marsh sublittoral communities is presented.

9. John J. Lee and William A. Muller. Trophic dynamics and niches of salt marsh sublittoral foraminifera. To be presented as an invited paper at the International Congress of Zoology, Monaco, 18-26 September 1972, and printed in the Proceedings.

In this paper we use data on generation time, carrying capacity, respiration, and feeding rates to characterize the carbon cycling and

ecological efficiency of 3 species of salt marsh foraminifera. The ecological growth efficiency was highest in Rosalina leei (21.5%); Allogromia laticollaris was 12%; and Spiroloculina hyalina was 5%. The tissue growth efficiency was higher in A. laticollaris (18%); the value for S. hyalina was only 5%. Assimilation efficiencies for S. hyalina and A. laticollaris were estimated to be as high as 97 and 82% respectively. At 20 and 25 C, respiration values for populations at carrying capacity were also estimated. At 25 C Allogromia populations required ~ 5,000 $\mu\text{g C/week}$, and at 20 C 1,200 $\mu\text{g C/week}$; R. leei required ~ 16,000 $\mu\text{g C/month}$ at 20 C, and 4,500 $\mu\text{g C/month}$ at 25 C; S. hyalina, the smallest of all the species tested, required only 600 $\mu\text{g C/week}$ at 20 C and 700 $\mu\text{g C/week}$ at 25 C.

10. The abstracts of 4 papers presented at the Annual Meetings of The Phycological Society of America, The Society of Protozoologists, The American Society of Limnology and Oceanography, and the Marine Biological Laboratory are also included in this progress report.

Work in progress

In addition to completing the aforementioned studies, we are pleased to report substantial progress on other subprojects.

1. Field and laboratory studies aimed at characterizing the changes in the bacterial and unicellular algal communities over a summer growing season.

For the last few years we have been broadening and extending our knowledge of the natural community. The study of the diatom assemblage was finally completed this year and is included in this progress report. The bacterial population structure of the community is still being studied by one of our graduate students,

Miss Eileen Kennedy. Her methods for collection and enumeration of the bacterial colonies were detailed in last year's progress report (NYO 3995-20; pp 14-15).

About 18 hrs after incubation, all plates were examined and any bacterial growth recorded. They were then incubated at 20 C in a 12 L/12 D environmental chamber and rechecked for growth after 4 and 10 days.

Bacterial colony types were described on the basis of relative size, pigmentation, whole colony appearance, edge and surface. The frequency of each was noted. Each different type was isolated by restreaking on a fresh plate of its initial isolation medium. Pure cultures were originally maintained on slants of the same medium but as a matter of practicality were transferred to a medium containing the following: yeast extract (Difco), 0.5%; Bactotryptone (Difco), 0.5%, Base B without B₁₂ (Lee et al. 1966), 100 ml. pH was adjusted to ~ 7.5.

The results were quite dramatic. The natural sea water based media, which are the standard media for this type of work, were quite inferior to the artificial sea water in both the isolation of bacteria and algae. The algal component of the microbial community was simpler to evaluate than the bacterial, since we were able to easily recognize the growth of the same species on different media. The total number of bacteria per gram (dry weight) of the community was estimated variously by individual media. Only after we identify each species and see the range of media on which each species grew will we be able to accurately estimate the bacterial component. Three species grew very well on unenriched sea water alone (Fig. 1). The number of bacteria growing on sea water agar fluctuated from $< 1 \times 10^6$ - 2×10^6 /g in early summer to a peak of $\sim 6 \times 10^6$ /g on field trip #9 in early August (Fig. 3). Only 1-4 species were isolated on the sea water agar plates. Two species were consistently isolated on erdschreiber throughout the summer (Fig. 2). Although there were slight fluctuations in the populations, both species were quite abundant ($\sim 3 \times 10^6$ /g, Fig. 3).

Fig. 1

TOTAL NUMBER OF BACTERIA $\times 10^6$ /GRAM

TIME

TOTAL NUMBER OF BACTERIA X 10⁶/GRAM

A -

A3

A4

A5

TIME

The number of different species isolated on this medium gradually increased over the summer from 1 in early June to 7 in mid-August. Among the natural sea water based media, Marine nutrient agar (Difco, Bacto Nutrient Agar 23 g/L, distilled sea water 0.5 L, sea water 0.5 L) and marine trypticase soy agar (B.B.L. trypticase soy agar 40 g/L, distilled water 0.5 L, sea water 0.5 L) best showed fluctuations in the natural community. Two peaks in the total number of bacteria/g were observed (Fig. 3), a peak in late June (field trip # 3) and another peak in early August (field trips 8, 9). These peaks coincide in time with the 2 annual summer blooms of foraminifera (see Matera and Lee, ms in this report). The 2 peaks were observable in all of the sea water based media except erdschreiber [sea water KNO_3 0.01% (w/v), $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 0.01% (w/v), and soil extract 5% (v/v)] and medium d. Growth on erdschreiber was depressed during the first peak. The second summer peak on other media was preceded by a late July peak on these latter media (Fig. 3). The growth of species b2, b3, c4, c5, d1, e3, e4 were possibly correlated with 1 or both peaks. Species a2, d3, e1, e3 and e4 were depressed in numbers during the first peak. The greatest total numbers of species were isolated on marine trypticase agar (13 colony types; Fig. 4); on marine nutrient agar 10 types were isolated. Five colonial types were isolated on unenriched sea water agar and 7 types were isolated on erdschreiber. Medium d (sea water enriched with KNO_3 10 mg% and $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 10 mg%) was more selective, since it promoted the growth of 2 species of bacteria and only a few other species grew on the medium all summer.

The seasonal changes in the community reflected by growth on the series A media are quite interesting. Many species which grew on this medium were agar digesters. On medium A 1, unenriched artificial sea water, there was a steady increase in total number of bacteria/g throughout the summer (Fig. 5). Of the 3 species which grew on the medium only 2, A 1-1, and A 1-2, grew in the early part of the summer (Fig. 4). The first isolation of species A 1-3 was in mid-August (Fig. 5). The pattern of colony growth

on media A 2 (enriched with NaNO_3) and A 3 (enriched with NH_4Cl) was similar. The same progressive general increase in numbers of bacteria/g was observed although the peak population was not as great as in medium A 1 (Fig. 5). The number of species growing on the media gradually increased throughout the summer (Fig. 4). A total of 7 species grew on both media (Figs. 7, 8). We found nitrite inhibited bacterial growth. Only on August 19 did we grow a small number of colonies of a single species on medium A 4 (Figs. 2, 4). The growth on medium A 5, which is enriched with NH_4NO_3 and NaH_2PO_4 , was very interesting since it paralleled the growth pattern observed in medium d, the sea water media also enriched with NO_3^- and H_2PO_4^- . Growth on medium A 5 was minimal during the 2 seasonal peaks on other media (Fig. 2). Two peaks, one in week 7 and one in week 9, immediately preceding and immediately following those observed on other media, were noted. Species A 5-1 was present in both peaks and greatly depressed in the collections on August 5. Species A 5-3 was present in large numbers only during weeks 6 and 7 (Fig. 9).

Throughout the season 2 species of bacteria were consistently isolated on medium B1 (Fig. 10). Though there were minor fluctuations, populations of species B 1-1 numbered $\sim 2.5 \times 10^6/\text{g}$ of the epiphytic community while species B 1-2 numbered $\sim 2 \times 10^5/\text{g}$. Small numbers of 6 other species were collected in late summer. In terms of total numbers of bacteria, the peptone enriched medium, B2, was the best. On it 4 species grew well ($> 1 \times 10^6$). Peaks in the population were observed on field trips 3, 7, and 10 (Figs. 11, 12). Only 1 species, B 3-1, grew well on medium B3, the medium enriched with urea (Fig. 13). Substantial growth was noted on this medium from the 6th field collection (July 23) until a decline which began on the 11th collection (Fig. 11). From mid-July on, there was a progressive increase in the number of species collected on this medium (Fig. 14).

The same trend of progressive increase in the number of species isolated from the community on successive collections which we observed on media A 5 and B 3 was observed on all the

Fig. 8

Fig. 9

Fig. 10

Fig. 11

TOTAL NUMBER OF SPECIES

series C media (Fig. 14). Ten species grew on media enriched with B_{12} (C 1) and 11 species grew on the media enriched with thiamin, biotin and B_{12} (C 2) (Figs. 15-18). Organisms with these requirements were not isolated from the community until the 6th or 5th field collections respectively. Though the population trends on both media at first seem quite similar (Fig. 19), closer examination of the growth patterns of individual species on both media shows that different species were isolated on each of the media. Peaks in the portion of the community isolated on these media were observed on either the 6th and 9th collections in medium C 1 or the 5th and 8th weeks on medium C 2. Although medium C 3 could be described as an artificially based erdschreiber, the growth on both media (Figs. 3, 4, 14, 19) were quite dissimilar in respect to the seasonal trends both in the total numbers and types of bacteria isolated. The growth of one organism, C3-1, which was one of the most abundant species at the beginning of the season, gradually declined over the course of the summer (Fig. 20). Another species, C 3-4, bloomed from mid-July to the end of August. One species, C 4-1, grew well from the 4th to the 11th field collections on medium C 4 (Fig. 21). During this period, the fluctuations in the number of organisms isolated on this vitamin enriched medium were minimal.

Though there were exceptions, there was a general trend of seasonal succession and increasing numbers of species which grew on the series D media (Figs. 14, 22, 23, 24). On medium D 1 which contained acetate, lactate and glucose as substrates, only 1 species, D 1-1, was found in quantity throughout the summer (Fig. 25). Species D 1-2 was abundant in early summer and gradually diminished in numbers. Species D 1-4 was found from weeks 3-8, species D 1-5 from 7-8, species D 1-6 from 8-10, and species D 1-7 was found only in weeks 11 and 12. The increase in the total numbers of bacteria isolated on the glutamate enriched medium (D 2) as the summer progressed was due both to the increase in the populations of species D 2-1 and D 2-3 and to the increase in the total

Number $\times 10^6 / g$

CI-7

CI-9

CI-10

TIME wks

NUMBER x 10⁵/g

C2-9

C2-10

C2-11

TIME wks

TOTAL NUMBER OF BACTERIA X 10⁶/GRAM

Fig. 24

number of species which grew on this medium (Fig. 26). Peak growth on medium D 2 was observed on collections from weeks 7, 9, 10. We observed peak growth on medium D 4 during weeks 3, 5-7, and 10. Though the species isolated were different the population trends observed on medium D 2 were also observed on media 4-8. Mannitol, glycerol, and extracts of Enteromorpha each stimulated the growth of 7 species while only 5 species grew on the acrylic acid enriched plates. The pattern of growth observed on medium D 7 (autoclaved Enteromorpha community) was similar to the growth observed on medium C 4 (vitamins 8A; Figs. 19, 22). It seems probable that autoclaved Enteromorpha stimulated the bacteria by providing required vitamins.

2. Studies on the factors which are thought to be responsible for the succession of unicellular algae in the epiphytic salt marsh community.

Some of the methods used and results of this subproject are included in the papers appended to this report. Additional experiments are reported here.

In one series of experiments we tested 11 species of algae for their ability to grow heterotrophically in the dark. Cultures were grown in the dark in a cardboard box (15 x 9 x 9) covered with aluminium foil and taped closed. The light seal of the box was tested by enclosing photographic print paper with the incubating cultures. At the termination of incubation, after 2 weeks, the paper was developed and no light leaks were found.

Dark cultures were serially transferred in darkness with the blue flame of the bunsen burner providing the only illumination. Cultures were not exposed directly to the light except during flaming.

Only one organism tested, Nitzschia brevirostris (RF-1) was able to reproduce in complete darkness. All other organisms tested grew poorly or not at all in reduced light.

Fig. 26

Nitzschia brevirostris (RF-1) showed a threshold effect for light intensity. At maximum light the lag phase of growth was shortest. At light levels from only slightly below maximum all the way to total darkness the lag phase was lengthened by a fairly uniform amount regardless of light intensity (Table 1).

After the second serial transfer in complete darkness, N. brevirostris (RF-1) grew only on media containing a substantial carbon source. Glucose, fructose, sucrose and maltose supported high levels of growth in the dark. Galactose, mannose, and lactose were not utilized (Fig. 27).

In the dark the salinity tolerance of N. breivorstris was substantially less than in the light (Fig. 28).

Temperature optima ranged from 16 C for an unidentified chlorophyte (strain 50) to 25 C for another unidentified chlorophyte (55) and the diatom Phaeodactylum tricornutum (39; Figs. 29, 30). Of the group of algae tested, only the latter 2 species were well adapted to grow in the warm tide pools which occur at our field station in late July. Most of the organism tested were able to grow well although not optimally at temperatures of 16-25 C. Chlorophyte BL-1 has a very narrow growth range.

In another series of experiments we were interested in the nutritional effects of the extracellular algal products of various algae on the growth of other algae. Preliminary tests of the effects of released metabolites were performed with the aid of filter paper disks. The disks were soaked overnight and autoclaved in 10 ml of supernatant from mature centrifuged algal cultures. They were then aseptically placed on agar plates which were evenly inoculated with the test algae. The test plates were incubated in the light at 23 C. Each alga was tested against the extracellular products of all other algae and its own.

Liquid cultures were also used to test the effects of extracellular metabolites. Algae were grown for 2 weeks in 300 ml of base B and then filtered through a millipore AA filter. The filtrate was diluted 1:1 with fresh base B in test tubes, autoclaved and

Table 1

The effect of light on the growth of Nitzschia brevirostris

Light Intensity ($\mu\text{W}/\text{cm}^2/\text{nm}$ at 580)	Growth (cells/ml $\times 10^4$) (± 2 S.E.)
225.9	111.2 $\pm$ 13.6
77.7	63.2 $\pm$ 11.0
16.5	66.2 $\pm$ 10.2
0	72.0 $\pm$ 10.2

LIGHT
DARK

Fig. 27

N. brevirostris

dark —

light —

inoculated with test algae.

In the first experiment with NH_4NO_3 provided as a nitrogen source (Table 2), 11 of the 12 organisms tested were stimulated by at least 1 other culture. Nitzschia sp (BL-15a) was stimulated by 4 others while a chlorophyte (SH-1), Amphora sp (RF-8) and Phaeodactylum tricornutum (39) were each stimulated by the filtrates from 3 cultures. Phaeodactylum tricornutum (39) was inhibited by its own culture media under these conditions.

In the next experiment an amino acid mixture was used as a nitrogen source. This completely altered the pattern of stimulation. In most cases the stimulatory effects seen in the media with the inorganic N sources were replaced by entirely new patterns of stimulation or inhibition (Table 3). One chlorophyte (SH-1) was inhibited by media from 3 other organisms. Filtrates from an unidentified chlorophyte (50) stimulated the growth of 5 other species. Nitzschia sp (BL-15a), Phaeodactylum tricornutum (39), and Amphora sp (RF-8) were stimulated by the filtrates of at least 3 other species.

The results of the quantitative studies made in liquid media are summarized (Table 4; Figs. 31-37). The growth of Nitzschia acicularis (8) was unaffected by the filtrates of other cultures (Fig. 34). Chlorophyte 50 was inhibited by the culture filtrates from 2 other species of chlorophytes (SH-1 and 55) and was stimulated by 1 other (38; Fig. 32). The growth of chlorophyte SH-1 was depressed by the filtrate from Phaeodactylum tricornutum (Fig. 31). Phaeodactylum tricornutum was stimulated by chlorophyte 50 (Fig. 37).

The culture filtrates of the algae were also assayed on thin layer 2 dimensional chromatographs. Monosaccharides and peptides were identified in the filtrates of almost all the algae tested (Table 5). Glutamic acid was also commonly found.

Some additional nutritional experiments were suggested by some of the filtrate stimulation experiments. The methods used were the same as those reported previously (see Stone and Lee in this progress report).

Table 2

The effects of extracellular algal products on the growth of other species of algae. I. Tests in media supplemented with NH_4NO_3 .

Test organisms	Donor organisms											
	<u>Nitzschia brevirostris</u> (RF-1)	<u>Nitzschia acicularis</u> (8)	<u>Nitzschia sp</u> (BL-15a)	<u>Phaeodactylum tricornutum</u>	<u>Amphora sp</u> (RF 8)	<u>Amphora sp</u> (BL-17)	<u>Chlorococcum sp</u> (38)	chlorophyte (SH-1)	chlorophyte (BL-1)	chlorophyte (50)	chlorophyte (55)	<u>Cylindrotheca closterium</u> (9)
<u>Nitzschia brevirostris</u> (RF-1)	0	0	0	0	0	0	0	0	0	+	0	0
<u>Nitzschia acicularis</u> (8)	0	0	0	+	0	0	+	0	+	0	0	0
<u>Nitzschia sp</u> (BL-15a)	++	0	+	0	0	+	+	0	0	0	0	0
<u>Phaeodactylum tricornutum</u> (39)	0	++	0	-	0	0	0	0	0	0	++	++
<u>Amphora sp</u> (RF-8)	0	0	0	0	+	0	0	0	0	++	0	+
<u>Amphora sp</u> (BL-17)	0	0	0	++	0	0	+	0	0	0	0	0
<u>Chlorococcum sp</u> (38)	0	0	0	+	0	0	0	0	+	0	0	0
Chlorophyte (SH-1)	0	0	+	0	0	+	+	0	0	0	0	0
Chlorophyte (BL-1)	+	0	0	0	0	0	0	0	0	0	0	0
Chlorophyte (50)	0	0	0	+	0	0	0	0	0	0	0	0
Chlorophyte (55)	0	0	0	0	0	0	0	0	0	0	0	0
<u>Cylindrotheca closterium</u> (9)	0	0	0	0	0	0	0	+	0	0	0	0

Table 3.

The effects of extracellular algal products on the growth of other species of algae. II. Tests in media supplemented with amino acid mixture 18a.

Test organisms	Donor organisms											
	<u>Nitzschia brevirostris</u> (RF-1)	<u>Nitzschia acicularis</u> (8)	<u>Nitzschia</u> sp (BL-15a)	<u>Phaeodactylum tricornutum</u> (39)	<u>Amphora</u> sp (RF-8)	<u>Amphora</u> sp (BL-17)	<u>Chlorococcum</u> sp (38)	chlorophyte (SH-1)	chlorophyte (BL-1)	chlorophyte (50)	chlorophyte (55)	<u>Cylindrotheca closterium</u> (9)
<u>Nitzschia brevirostris</u> (RF-1)	0	0	0	0	0	0	0	0	0	+	0	0
<u>Nitzschia acicularis</u> (8)	0	0	0	0	0	0	0	0	0	+	0	0
<u>Nitzschia</u> sp (BL-15a)	+	0	0	+	0	0	+	0	0	+	0	0
<u>Phaeodactylum tricornutum</u> (39)	+	0	+	0	0	0	0	+	0	+	0	+
<u>Amphora</u> sp (RF-8)	+	+	0	0	0	0	0	+	0	0	0	0
<u>Amphora</u> sp (BL-17)	0	0	0	0	0	0	+	0	0	0	0	0
<u>Chlorococcum</u> sp (38)	0	0	0	0	0	+	0	0	0	++	0	0
chlorophyte (SH-1)	0	0	0	-	0	0	0	0	0	-	-	++
chlorophyte (BL-1)	+	0	0	0	0	0	0	0	0	0	0	0
chlorophyte (50)	0	0	0	0	0	0	0	0	0	++	0	0
chlorophyte (55)	0	0	0	0	0	0	0	++	0	0	++	0
<u>Cylindrotheca closterium</u> (9)	0	0	0	0	0	0	+	0	0	0	0	0

Table 4

The effects of extracellular algal products on the growth
of other species of algae. III. Quantitative tests.

organisms tested	control (Base B)	Donor organisms						
		BL-1	SH-1	9	38	39	50	55
B2-1	1.0	0.4	1.1	0.7	0.8	1.3	1.0	0.6
RF-1	1.0	2.3	0.6	5.3	1.7	1.0	6.7	1.7
SH-1	1.0	1.0	1.1	1.0	1.0	0.7	0.9	1.0
8	1.0	0.8	0.8	0.8	0.8	0.9	0.7	0.9
9	1.0	1.2	0.8	1.0	1.0	0.8	1.2	0.8
38	1.0	1.2	1.3	0.9	1.2	1.1	1.1	0.9
39	1.0	1.3	1.0	1.0	1.2	1.1	0.8	1.2
50	1.0	1.0	0.7	1.2	1.3	0.8	0.9	0.5
55	1.0	1.1	0.9	0.7	0.8	0.7	0.9	0.6
Average Growth Factor		1.1	0.9	1.4	1.1	0.9	1.6	0.9

Table 5

Qualitative identification by thin layer chromatography of the extracellular products found in the culture filtrates of various algae.

Organism filtrate	Extracellular products									
	monosaccharide	polysaccharide	peptide	cysteine	glutamic acid	alanine, serine or aspartic acid	histidine	lysine	asparagine or glutamine	arginine
<u>Enteromorpha</u>										
<u>intestinalis</u>	+	+	-	+	+	+	-	-	-	-
<u>Nitzschia</u>										
<u>brevirostris</u> RF-1	+	+	+	-	-	-	-	-	+	+
<u>Cylindrotheca</u>										
<u>closterium</u> (9)	+	-	+	-	+	-	-	+	-	-
<u>Phaeodactylum</u>										
<u>tricornutum</u> (39)	+	-	+	-	+	-	-	+	-	-
chlorophyte (BL-1)	+	-	+	-	+	-	-	-	+	-
Chlorophyte (SH-1)	+	-	+	-	+	-	+	-	-	-
chlorophyte (50)	+	-	+	-	+	-	+	-	-	-
chlorophyte (55)	+	-	+	+	+	-	+	-	-	-
<u>Chlorococcum</u> sp (38)	+	-	+	-	-	-	-	+	-	-

Table 6

The effect of nitrate and phosphate limitation on the growth of chlorophyte 50 in the presence of a crude organic stimulant

		no add	yeast extract 10.0 mg%	yeast extract 25 mg%	galactose 10 mM
A.	NO ₃ 1 mM	5	236	26	4.2
	PO ₄ 0.1 mM				
B.	NO ₃ 0.1 mM	1	20	0.4	0.6
	PO ₄ 0.01 mM

Cylindrotheca closterium (9)

Phaeodactylum tricornutum (39)

Chlorophyte 50 has an optimum N:P ratio of 10:1 at relatively high levels of N ($\text{NO}_3 > 1.0 \text{ mM}$). When low levels of yeast extract were added (10.0 mg% yeast extract) growth was increased by 1 to 2 orders of magnitude (Table 5). Slightly greater amounts of yeast extract were not as stimulatory. Individual carbohydrates did not stimulate the growth of this organism.

Cylindrotheca closterium (9) utilized glutamate and arginine, alanine + phenylalanine as a nitrogen source. Growth was improved by 1 order of magnitude by thiamin ($> 1.0 \mu\text{M}$) in unstarved cultures. An absolute thiamin requirement has been shown in vitamin depleted cultures.

Maltose was the only sugar tested which doubled the growth of Cylindrotheca closterium (9).

Amphora sp (RF-8) used a wide variety of amino acids as nitrogen sources including serine, arginine, valine, aspartic acid, lysine, methionine and histidine. Two amino acids, methionine and leucine, stimulated growth even in the presence of an adequate nitrogen source. Of the 2, methionine was also used as a nitrogen source.

Nitzschia brevirostris (RF-1) utilized aspartic acid, glycine and histidine as nitrogen sources. Aspartic acid and histidine also stimulated growth in the presence of an adequate nitrogen source. Glucose and sucrose are the only individual carbohydrates tested which more than doubled growth.

Several preliminary algal succession experiments were conducted this year. Axenic laboratory cultures of Enteromorpha intestinalis were incubated in medium containing $^{14}\text{CO}_2$ as sodium bicarbonate for 3 days. The Enteromorpha was then washed and placed in fresh medium with mixtures of bacteria isolated from the field and the diatoms Nitzschia brevirostris (RF-1), Amphora sp (RF-8), Cylindrotheca closterium (9) and chlorophytes BL-1 and 50.

In all cases tested after 10 days only 1 diatom, Cylindrotheca closterium (9), 1 chlorophyte (50) and large numbers (10^7 cells/ml) of bacteria remained. The chlorophytes conformed to the

growth pattern which it follows in pure culture but diatom growth was slightly depressed, especially in those cultures containing bacteria group III.

After 1 month only 17% of the Enteromorpha was alive; 83% was either dead or apochlorotic. We normally subculture Enteromorpha bimonthly. In tubes of living Enteromorpha, chlorophyte 50 was very numerous both on the surface and at the bottom of the tube. Many motile individuals were present indicating that the population was not declining but was in a phase of cyclic stability. Sometimes the chlorophyte and the diatom Cylindrotheca closterium (9) were found in ~ equal numbers in tubes of living Enteromorpha. In this case most of the diatoms formed a pellet at the bottom of the tube. Chlorophytes were also found at the bottom dispersed around the pellet. Chlorophytes and diatoms were mixed in sheets on the surface of the water.

When Enteromorpha was dead the diatom was either dominant or very numerous in 80% of the cases. The chlorophyte was dominant only 20% of the time and was numerous in another 20% of the cases studied.

In some cases the diatom and chlorophyte populations were mixed, most commonly in the surface film. When a ring was formed on the tube wall at the surface it consisted of almost all 1 population, either diatoms or chlorophytes.

The 3 groups of bacteria added promoted differential growth on the part of the diatom and chlorophyte. When bacteria group I was present the diatom Cylindrotheca closterium (9) was dominant in 50% of the cases and was numerically equal in another 25% while the chlorophyte (50) was dominant in the remaining 25%.

When bacteria group II was present the diatom was dominant in 50% of the cases and was numerically equal to the chlorophyte in the remaining 50%.

When bacteria group III was present a markedly different pattern appeared. The chlorophyte was dominant in 75% of the cases and was numerically equal in the remaining 25%. The diatom was not dominant in any case with bacteria group III.

The bacteria themselves did not dominate any cultures no matter how much the Enteromorpha had decayed. In all cases the bacterial numbers were estimated at 10^4 - 10^5 cells/ml. By weight the algal population exceeded the bacterial population after 1 month by 1-2 orders of magnitude. This contrasts to the short term results. After 10 days the bacterial biomass was ~ equal to that of the algae (not counting Enteromorpha).

3. Quantitative mineral cycling and energy flow studies.

The data of foraminifera from this subproject has been included in previously discussed papers which are part of this report. Some experiments on the mineral cycling abilities of nematodes were begun this year. In these experiments 5 μ Ci/ml of label (^{14}C , ^{35}S , ^{32}P) was inoculated into a solidified nematode growth medium with a shallow liquid overlay. Large populations of each of the various stages of the nematodes were transferred onto the labeled media and were allowed to grow 1-2 weeks depending on the life cycle of the worm. After growing in the labeled medium, the worms were transferred onto "cold" (unlabeled) media and 10 adults, intermediate stages and juveniles were picked, washed 3 x and placed in scintillation vials. An aliquot of the final wash also was placed in a vial as a control, reflecting relative carry-over as a check on procedure. The three stages of worms were picked successively every 1-2 days and at the end of the 2 week period all vials were counted on a Beckman liquid scintillation counter. The results were then plotten in CPM/ μ gm nematode to determine biological half life.

The tracer feeding experiments were done in 3 stages. Bateria or algae were inoculated with 10 μ Ci/ml ^{32}P ; bacteria grew for 24 hrs and algae 48-72 hrs. The bacteria were spun down 3 x 10,000 rpm at 5 C in a Sorvall refrigerated centrifuge; the algae washed 3 x at 5,000 rpm for 10 min in a Sorvall GLC-1

centrifuge. The remaining pellets were resuspended in 0.3 ml of sea water; 0.1 ml was placed in a scintillation vial; 0.1 ml was counted using a hemocytometer or a B & L spectronic 20 spectrophotometer; the final 0.1 aliquot was added to tubes of 50 nematodes.

The nematodes were allowed to feed for 24 hrs, after which time they were picked, washed and placed on unlabeled media for 2-8 hrs in order to allow any undigested foods remaining in their gut to be voided. The worms were then washed and counted on a Beckman liquid scintillation counter.

The results of these experiments are summarized in the accompanying figures (38-44). The biological half life of ^{14}C in *Monhystera* differs in each age class. The C T_b for adults was ~ 2 d; intermediate stages ~ 5 d; and newly hatched juveniles 2.3 d. The T_b for S was: 2 d for adults; 1 d for intermediate stages; and 3.6 d for juveniles. The T_b for P was: 9.6 d for adults; 5 d for intermediates; and 1 d for juveniles (Figs. 38-40; 42-44). The T_b for S in *Rhabditis marina* adults was ~ 1.5 d while for intermediate stages it was ~ 7 d and for juveniles ~ 10 d (Fig. 41).

In ^{32}P tracer feeding experiments with *Monhystera denticulata* we found a wide range in rates of consumption. Various species of small green algae were eaten in the range of $\sim 3 \times 10^4$ - 2×10^2 . *Nannochloris* sp was eaten in the greatest amounts and species of *Dunaliella* and *Chlamydomonas* were eaten in the lowest numbers (Table 7). Some clones of bacteria, D 1 701, A 3 711, A 5 703, and D 2 703 were eaten at a greater rate ($\sim 1 \times 10^5$) than others such as D 2 7, A 3 111, D 5 2, which were eaten at a rate which was an order of magnitude or less lower. (Table 8)

Several interesting labeled experiments were done using axenic *Rhabditis marina*. The worms were exposed to mM amounts of labeled low mW substrates, glycine, glucose, sucrose, aspartic acid, Na acetate, and Na bicarbonate. The worms were incubated for 2 hrs in labeled substrate and then transferred to unlabeled medium for an additional 2 hrs. They were then harvested into

TABLE 7

Consumption of bacteria by Monhystera.

<u>Bacteria</u>	<u>Bacteria cells/nematode x 10⁴</u>
A ₅ 7	5.375
D ₁ 701	16.95
A ₃ 711	22.35
A ₁ 711	3.60
A ₅ 703	47.90
D ₂ 703	33.65
A ₃ 111	3.90
A ₅ 6	93.50
D ₂ 7	0.45
D ₅ 2	4.30

TABLE 8

Consumption of algae by Monhystera.

<u>Species</u>	<u># algae/worm x 10³</u>
<u>Amphora</u> sp	0.944
unidentified chlorophyte (SH 1)	11.645
<u>Dunaliella salina</u> (13)	0.830
<u>Dunaliella parva</u> (14)	1.775
unidentified chlorophyte (93)	44.255
unidentified chlorophyte (50)	0.235
unidentified chlorophyte (BL-1)	3.166
<u>Nannochloris</u> sp (41)	75.225
<u>Cylindrotheca closterium</u> (9)	3.295
<u>Chlorococcum</u> sp (BL 38)	15.715
unidentified chlorophyte (55)	2.702
<u>Archaias</u> symbiont	2.120

vials. Dead worms and aliquots from the washing medium served as controls. No uptake of substrate was measured. The experiment was repeated again with 3 M 15 $\pm$ 5 μ M microspheres added to the experimental medium. No appreciable uptake of labeled substrate was measured. When 0.5 - 10 μ M microspheres were used, some uptake of most substrates was measured. These results suggest to us that the cuticle of the worms does not permit absorption of even low MW compounds and that dissolved nutrients probably do not play much of a role in the nutrition of Rhabditis.

We have also begun to test trophic efficiency and mineral cycling in another series of experiments. Into the vessels of a Gilson respirometer, worms and bacteria or bacteria alone were inoculated into media containing various 14 C labeled substrates. 14 CO₂ was trapped in 40% KOH moistened filter paper placed in the center well of the flask. At the end of each experiment (3 hrs) the substrate turn over was measured. Overall respiration was measured by means of the Gilson apparatus. Initial results with Rhabditis marina and Monhystera sp indicate that substrate turn-over is ~ 125-150% of the rate of the flasks with bacteria alone. Preliminary estimates of nematode ecological efficiencies and associated trophic dynamic measurements are tabulated (Table 9). Additional experiments are in progress.

TABLE 9

Preliminary estimates of marine nematode efficiencies

	Ingestion $\mu\text{gC/pop/gen}$	Respiration $\mu\text{gC/pop/gen}$	Assimilation $\mu\text{gC/pop/gen}$	Production	% Ea=A/I	% Et=P/A	% Ee=P/I
<u>Monhystera</u> sp	980 μg	C^{14} 1082 μg	-102	50 μg	-	-	5.10
		G H I N 321 μg	-121		-	-	
<u>Rhabditis marina</u>	12 grams	C^{14} 23.35 μg	~12	1584 μg	~100	0.013	0.013
		G H I N 11.26 μg	~12		~100	0.013

^{32}P

Monhystera

Fig. 39a

^{35}S Monhystera

^{35}S Monhystera

^{35}S Rhabditis
marina

^{14}C vs. ^{35}S vs. ^{32}P Intermediate
Monhystera