THE EFFECTS OF ENVIRONMENTAL STRESS ON THE
COMMUNITY STRUCTURE AND PRODUCTIVITY OF SALT MARSH
EPIPHYTIC COMMUNITIES

Progress Report

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ABSTRACT

Significant advances have been made in a number of subprojects which, when published, will make substantial contributions to the field, and generally open up many new areas of research. One of these contributions is technological. We have successfully developed and tested an apparatus which can be used for in situ experiments with aufwuchs communities. It also seems to have application for use in estuarine water quality assessments. The environmental incubator does not use cellulose based filters or dialyzing membranes, which are rapidly digested and weakened by the cellulitic bacteria in the aufwuchs community.

The second major advance this year was the demonstration of information processing in food web transformations. Though ecosystem cybernetics has received some theoretical attention in the past, no practical methods had heretofore been developed to assess its importance. Spatial distribution of predators and their prey were also shown to be important. The "Cyberon" was developed as new unit of information gain in food web transformations. The theoretical and practical impact of this new assessment on ecosystem modeling is discussed in context with the data.

In keeping with the AEC review cycle, this report includes all work in progress and papers and manuscripts supported by the present contract (3 years). Not including abstracts, a total of 10 papers were published, 4 are in press, and 4 have been completed and have been, or shortly will be, under review for publication.

I.

COMPLETED PROJECTS

A brief synopsis of each of the full papers, all part of this report, follows:

1. Marie McEnery and John J. Lee. 1970. Tracer studies on Ca and Sr mineralization and mineral cycling in Rosalina leei and Spiroloculina hyalina. Limnol. Oceanogr. 15:173-182.

In this paper we provide data to calculate the rate at which ^{45}Ca and $^{89,90}\text{Sr}$ can be removed from coastal marine environments by calcareous foraminifera. Incorporation of the radionuclides increased directly as the concentration of these minerals was increased to the limit tested (0.1 MCi/ml). Once removed, the Ca and Sr are tightly bound under conditions of alkaline pH and low hydrostatic pressure. Two other interesting aspects of the study were the discovery that the mineralized portion of the foraminiferan shell can be removed and then remineralized and the anomalous effect of acetazolamide of foram mineralization. Acetazolamide [Na 2 acetyl-1,3,4 thiadiazole-5-sulfonamide] is a well known carbonic anhydrase inhibitor. Carbonic anhydrase regulates the carbonate ion necessary for calcareous mineralization in bone and various invertebrates such as molluscs and corals. The action of acetazolamide on the foraminifera tested, Rosalina leei, leads one to suspect that some biochemical aspects of Ca mineralization in foraminifera are different from other protozoa. ^{45}Ca but not ^{90}Sr incorporation was blocked by acetazolamide.

2. John J. Lee and Marie E. McEnery. 1970. Autogamy in Allogromia laticollaris. J. Protozool. 17:184-195.

This paper was coordinated with the quantitative study published in the Journal of Protozoology the previous year (J. Protozool. 16:377-395) in which we showed that Allogromia laticollaris life-cycle options were nutritionally dependent. We were motivated to

begin this study when it became clear that our hopes to learn more about the extent to which nuclear and cytoplasmic divisions are coordinated could not be fulfilled until the life cycle itself became better known. More new questions were raised by this research than answers to old ones. It is difficult to summarize the results of our findings without redetailing much of the background information given in the appended paper.

Very briefly, we found differences in fecundity, nutritional requirements, cytological and nuclear detail between the 2 strains studied. The basic life cycle is apogamic (without a sexual generation) but consists of a regular alternation of vesicularly nucleated and compactly multinucleated diploid generations. Occasional gamonts (sexually reproducing individuals) were formed. They were autogamous (self fertile) and fusion of the gametes took place within the parental test.

3. John J. Lee, John H. Tietjen, Robert J. Stone, William A. Muller, Judith Rullman and Marine McEnery. 1970. The cultivation and physiological ecology of members of salt marsh epiphytic communities. *Helgoland wiss. Meeres.* 20:136-156.

This is essentially a paper on the techniques we use to isolate and culture salt marsh micrometazoa and microorganisms. It was prepared to be included in a symposium volume on the cultivation of marine organisms. In it we detailed methods of collection; formulae of media and directions for their use; antibiotic mixtures; and techniques we have found useful for axenic isolation of a variety of organisms including sea weeds, diatoms, chrysophytes, dinoflagellates, ciliates, foraminifera, and marine free-living nematodes. Also included was comprehensive review of the results we obtained using the techniques described. The discussion contains a realistic revaluation of the status of our knowledge of

culture methods for salt marsh microorganisms and micrometazoa. Major emphasis was placed on tracer-feeding identification of potential food to use to synxenic cultures.

4. John J. Lee, Robert J. Stone, Marie McEnery and William A. Muller. 1970. Physiological perspectives of foraminifera in a Long Island salt marsh. Proc. L. I. Conference on shellfish culture.

This illustrated paper, written at a paraprofessional level, describes the rationale for some of our research and its relevance to commercial, regional, county, and town efforts on effective management of salt marsh ecosystems. It is a summation of an address given by Dr. Lee at the conference on shellfish culture held by the Regional Marine Resources Council of the Nassau-Suffolk Regional Planning Board.

5. Nicholas J. Matera and John J. Lee. 1972. Environmental factors affecting the standing crop of foraminifera in sublittoral and psammolittoral communities of a Long Island salt marsh. Marine Biology, 14:89-103.

This paper was an extension and elaboration of a study we had published earlier (Lee et al. 1969). The answers to 4 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 differences in temperature and salinity in the adjacent shallow drainage ditches? Is there a difference in the species composition of foraminiferal assemblages in "hot dry",

"wet", and "normal" summers?

Elphidium incertum (46.63%) and Protelphidium tisburyensis (25.64%) were dominant species in the epiphytic community. Ammonia baculites dilatatus, Ammonia beccarii, Ammonium salsum, Elphidium clavatum, Elphidium translucens and Trochammina inflata were subordinate species, < 10% of the total collected. There were fewer 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 for foraminifera in the epiphytic communities are not continuous with the psammolittoral communities below them.

The data on community structure show great differences between the assemblages from "hot dry", "wet", and "normal" summers. Communities under thermal stress had much lower species diversity.

6. John J. Lee, Marie E. McEnery, Eileen M. Kennedy and Howard J. Rubin. 1973. Educating the functional relationships among the diatom assemblages within sublittoral salt marsh communities. Presented at the IBP symposium on Modern Methods in Microbial Ecology, 19-23 June 1972 in Uppsala, Sweden. Bull. Ecol. Res. Comm. (Stockholm), 17:387-397.

In this paper we discuss the methods we have used to gain insight into the functional relationships within the algal assemblages within sublittoral salt marsh communities. The question being posed is: what are the bases which underly the huge diversity of microbial species on the same trophic level? If we understand this we would know much about the subtle variables which regulate population structure, succession and information within the community. 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 algae throughout the growing season. Diatom community structure, species abundance, and appearance correlations are discussed. The data obtained in this paper suggest that nutrient exchange and utilization is an important component of the carbon budget of the algal assemblage of the marsh community and that on a dry weight basis natural populations of algae can successfully compete with bacteria for organic substrates.

7. 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. Nat. Symp. Radioecol. Proc. 3:816-828.

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.

8. 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. Nat. Symp. Radioecol. Proc. 3:1023-1028.

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 foraminifera. 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.

9. Norman M. Saks and John J. Lee. The differential sensitivity of various species of salt marsh epiphytic algae to ionizing radiation and thermal stress. Presented at the symposium on The Interaction of Radioactive Contaminant with the Constituents of the Marine Environment, held in Seattle, Wash. on 10-14 July 1972. Radioactive Contamination of the Marine Environment IAEA-SM-158/35:565-571.

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 BL-17), Nitzschia sp (BL-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 (BL 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.

10. John J. Lee and William A. Muller. Trophic dynamics and niches of salt marsh sublittoral

foraminifera. It was presented as an invited paper at the International Congress of Zoology, Monaco, 18-26 September 1972, and printed in *American Zoologist* 13:215-223.

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. We conclude on the basis of this data and previously published data on tracer feeding, standing crops, competition and niche width, that the species of foraminifera we have studied are very opportunistic and are well adapted to play important trophic roles in the very rapidly changing food webs in the communities of which they are an important part. Evidence further suggests that the stability and high rates of productivity of the microbial and micrometazoan assemblages of salt marshes is achieved through species diversity rather than through a restricted number of generalized species as was suggested by Teal (1962) in an earlier study.

11. Norman M. Saks, John J. Lee, William A. Muller and John H. Tietjen. The growth of salt marsh microcosms subjected to thermal stress. Presented at the Thermal Ecology symposium held at Augusta, Georgia, 3-5 May 1972. To be published in the Proceedings.

In this paper we discuss some of our preliminary experiments on the effects of temperature and interspecific competition between pairs of organisms, a marine benthic feeding ciliate, Euplotes vannus, and either Allogromia laticollaris, a foraminifera, or Chromodorina germanica, a benthic nematode. At 15 C, neither Allogromia nor Chromodorina reproduce in the presence of Euplotes but the small numbers in the inoculum reduce Euplotes populations to $\leq 1/2$ that of controls. At 25 C each of the species was affected by the presence of another species. This was reflected by changes in both amplitude and phase of populations. The effect of temperature on the growth and survival of their food was also studied.

12. John J. Lee and William A. Muller. Culture of salt marsh microorganisms and micrometazoa. Presented at the Conference on Culture of Invertebrate Animals held at the Greenport Aquarium, Greenport, N. Y., Oct. 5-7 1972. To be published as a book by Pergamon Press.

The purpose of the conference was to bring together experts on the culture of microorganisms and micrometazoa of northeastern U. S. estuarine and coastal waters. In our paper we discuss the rationale and the techniques for collecting and setting up agnotobiotic and gnotobiotic cultures of salt marsh microorganisms and micrometazoa. The paper is sufficiently detailed so that a beginning graduate student could use it as a laboratory manual for

the study of near shore bacteria, algae, protozoa and micrometazoa. We also elaborate on our experience in this field and give helpful hints on pitfalls to be avoided.

13. John J. Lee. Towards understanding the niche of foraminifera. in: Foraminifera; (R. H. Hedley & G. Adams, eds) pp 207-260. Academic Press, London & New York. To be published this summer.

In this chapter, many facets of the niches of salt marsh foraminifera are discussed. The point of the review is to give a microbiologist's microscale perspective of the functional roles of a few littoral benthic foraminifera in their communities and the methods which can be used to gain insight into this aspect of their biology. Both field and laboratory approaches are stressed in the review. Patchiness, substrate preferences, seasonality, culture methods, nutrition and growth factors, interspecific and intraspecific competition, ecological efficiency and symbiosis in foraminifera are discussed in detail.

14. John J. Lee, John H. Tietjen, Norman M. Saks, George G. Ross, Howard Rubin, William A. Muller. Educing and modeling the functional relationships within sublittoral salt marsh aufwuchs communities - inside one of the black boxes. Presented at a symposium on Modeling Estuaries during the 2nd International Conference on Estuaries, Myrtle Beach, S. Car., Nov. 1973. To be published in the Proceedings.

The gist of our paper was that at the present time information on the population structure of at least one community, and by

inference other communities, is too fragmentary to be used in modeling and predicting community structure and function. Studies of salt marsh aufwuchs community foraminifera, nematodes, and microalgae grown in gnotobiotic culture suggest it is unreasonable to assume that measurements made on one species will be good estimators for similar parameters of other species or the assemblage as a whole. Nutritional experiments suggest many meiofauna are selective feeders and gain different nutritional values from the food they assimilate. Many problems relating to the population structure and dynamics of aufwuchs microbial and meiofaunal food webs were illustrated and discussed.

15. A nutritional analysis of a sublittoral epiphytic diatom assemblage from a Long Island salt marsh.
John J. Lee, Marie E. McEnery, Eilen M. Kennedy and Howard J. Rubin. Revision to be resubmitted for publication in Journal of Phycology.

This paper is the product of one of the most time consuming and difficult projects we have ever attacked in the laboratory. At the onset we hoped that a study of the auxotrophic profile of the microbial assemblage would give us insight into some of the subtle variables which regulate population succession and information within the assemblage. Several important technological gaps were bridged on the way to reaching our goals. We designed a series of differential media which are effective as tools for the isolation and nutritional characterization of the algae and bacteria from the community. Many diatom species can be recognized by their colony type or growth pattern on solidified differential media. A dichotomous key and illustrated plates have been developed as an aid to their identification. The changes in population structure of the community were also reflected by changes in the nutritional patterns in the community as judged by qualitative and quantitative differences in the growth of diatoms and bacteria on the differential

media tested. The comparative abilities of individual species isolated from the community to grow on the wide range of test media was evaluated. A measure, the Colony Band-Width Index (CBI) was developed as a comparative aid. A high CBI indicates that a species has a wide range of organic tolerance, possibly no macro- or micro-nutrient requirements, or is nutritionally quite versatile. The nutritional requirements and selectivity of media for individual species is also discussed. A rank order listing of media for the growth of individual species is given.

The distribution of many species in the community was seasonal. Distribution patterns were tested statistically. Many species showed high correlation with others in abundance or in their appearance in the community indicating possible common abiotic and biotic dependencies. These correlations are tabulated.

Perhaps the most significant advance is the development of new tools which we can use to isolate and study ecologically significant organisms in the laboratory. The new media and methods give promise that the functional interrelationships between diatoms from the same community assemblage may someday be accessible for detailed analysis. They may also be useful for the culture of many new species of protozoa and micrometazoan herbivores. The auxotrophic profile of the diatoms assemblage suggests that some measure of stability within this important component of the community is achieved through heterotrophic diversity. The same might be suggested as a key factor in community succession.

16. The taxonomic identity and physiological ecology of Chlamydomonas hedleyi (the algal flagellate symbiont from the foraminifer Archaias angulatus).
John J. Lee, Lawrence J. Crockett, Johnny Hagan
and Robert J. Stone. Submitted to the British
Phycological Journal.

Symbiosis between algae and invertebrates is a significant phenomenon in the marine environments in which it occurs. Although symbiotic associations between foraminifera and various species of algae have been known for over 65 years, the taxonomic identity of any of the symbionts or their metabolic contributions to their hosts has never been clarified. In this paper we discuss the fine structure, taxonomic identity, and physiological ecology of a symbiotic alga from a foraminifera which we recently isolated into axenic culture. The symbiont has no vitamin or organic requirements but growth is 3 x in the presence of thiamin, and 2 x in the presence of 1 μ M glutamic acid, histidine and methionine. Urea was the best nitrogen source. The symbiont fixed $\sim 7 \times 10^{-7}$ M carbon $\text{hr}^{-1} \text{gm}^{-1}$, 57% of which was released into the medium as a chromatographically homogeneous organic molecule provisionally identified as mannitol.

17. Niche width of salt marsh foraminifera. William A. Muller. Submitted to Marine Biology.

Several hundred species of foraminifera grow in various salt marshes throughout the world. Very little is known of the differences in their niches which allow some species to grow while others in the same marsh do not. 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 lati-
collaris 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 stable, a conspicuous species whose moderate numbers are relatively unaffected by physical stress and competition.

18. An Experimental Approach to the Assessment of the
 Informational Aspect of Energy Transfer. Howard A.
 Rubin. To be submitted to American Naturalist.

The role of information processing in food web energetic transformations was experimentally tested using 2 marine ciliates, Euplotes vannus and Uronema marinum, as experimental animals. In these experiments, caloric value of food was normalized so that information transfer could be measured. The informational quality of food was experimentally demonstrated by 3 independent means: 1) decay of synchrony in optimally reproducing cultures; 2) switching time lags; 3) comparison of generating times on calorically normalized diets. Information utilization was measured in terms of cell cycle advancement. Reproduction of the ciliates was often delayed when they were transferred from a diet of one algal species to a diet of another species. These switching time lags (≤ 2.5 hr) were interpreted as a consequence of adaptive enzyme formation, an information recognition response. Simple food web experiments tested whether the ciliates selected food to maximize their biotic potential. The information transfer system was modeled within the

conceptual framework of automata theory. The model was successful in predicting the outcome of 80% of the mixed diet experiments. It also predicted, because of switching time lags, that a patchy environment should be more productive than a homogeneous one. Food quality and spatial distribution of predators and their prey are therefore important factors in population dynamics. A measure of informational gain in food web transformations was developed. "Cyberons", a new unit of information utilization related to the energy saved per food organism processed, are defined and can be converted into Moles ATP or caloric equivalents.

WORK IN PROGRESS

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

- A. Comparative quantitative and qualitative studies on the role of diverse species of marine nematodes and ciliates in the detritus food chain of marine marshes including: mineral cycling, energy flow, biological half-life, trophic efficiency estimations, etc.

Some data on the mineral cycling ability of Rhabditis marina was published this year (NYO 3995-18). Some additional comparative experiments are in progress.

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 $\mu\text{Ci/ml}$ 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 weeks and at the end of the 2 week period all vials were counted on a Beckman liquid scintillation counter. The results were then plotted in CPM/ μgm nematode to determine biological half life.

The results of these experiments are summarized in the accompanying figures (1-10). 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

¹⁴C

Monhystera

^{14}C

Monhystera

- ADULT x
- - - INTERMEDIATE v
- - - JUVENILE o

dpm / nematode

80

40

10

5

2

TIME --- DAYS

2

4

6

8

10

12

14

^{35}S

Monhystera

^{35}S

Monhystera

— ADULT x
- - - INTERMEDIATE v
- - - JUVENILE o

dpm / nematode

^{32}P Monhystera

^{32}P

Monhystera

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

Adult Monhystera

^{14}C x
 ^{35}S v
 ^{32}P o

dpm / nematode

500

300

100

10

2

TIME --- DAYS

2

4

6

8

10

12

14

16

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

Intermediate
Monhystera

— ^{14}C x
— ^{35}S v
- - ^{32}P o

dpm / nematode

400

100

10

2

TIME --- DAYS

1 2 4 6 8 10 12 14 16

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. The T_b for S in Rhabditis marina adults was ~ 1.5 d while for intermediate stages it was ~ 7 d; for juveniles ~ 10 d.

The tracer feeding experiments were done in 3 stages. Bacteria or algae were inoculated with $10 \mu\text{Ci/ml } ^{32}\text{P}$; bacteria grew for 24 hrs and algae 48-72 hrs. The bacteria were spun down $3 \times 10,000$ rpm at 5 C in a Sorvall refrigerated centrifuge; the algae washed $3 \times$ 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.

In tracer feeding experiments with Monhystera denticulata, Chromadorina germanica and Rhabditis marina, we found a wide range in rates of consumption of different species of algae and bacteria (Tables 1 and 2). (Table 1 was presented at the Estuarine Conference.) Not all the potential food ingested was digested. Rates of assimilation ranged from a low of 22% to 100%. There was, for instance, 4 orders of magnitude difference in rates of consumption of 2 different but closely related species of algae by one worm. Rhabditis marina digested 1×10^{-4} mg of Dunaliella parva/day/worm and digested 88% of what it ate, while it digested only 31% or 6×10^{-8} mg/worm/day of Chlamydomonas hedleyi, a symbiotic alga isolated from a foraminifera. Rhabditis also ate fairly large amounts (2×10^{-5}) of two other species of algae, Nitzschia acicularis and Phaeodactylum tricornutum, and 5 species of bacteria, A 5-3, A 1-1, A 5-6, D 1-1 and D 5-2. Chromadorina consumed very low numbers of

TABLE 1

Consumption of algae by 3 species of nematodes.

ALGAE	<u>Rhabditis marina</u>					<u>Monhystera denticulata</u>				
	# algae/worm 24 hr	# algae/worm 48 hr	grazing factor % *	assimi- lation % **	mgm/ worm/day	# algae/worm 24 hr	# algae/worm 48 hr	grazing factor % *	assimi- lation % **	mgm/ worm/day
RF-1	2.4×10^1	2.7×10^1	0.003	100	3×10^{-8}	3.5×10^1	2.3×10^1	0.006	64	3×10^{-7}
BL-44	1.2×10^1	8.7×10^0	0.002	70	1×10^{-8}	2.3×10^1	7.5×10^0	0.002	32	9×10^{-6}
BL-42	9.8×10^2	6.6×10^2	0.37	67	3×10^{-6}	6.5×10^1	2.5×10^1	0.004	38	1.2×10^{-7}
39	3.5×10^2	3.4×10^2	0.04	95	2×10^{-5}	5.5×10^1	4.9×10^1	0.005	88	3×10^{-6}
8	2.7×10^2	1.5×10^2	0.01	58	2×10^{-5}	3.6×10^2	1.7×10^2	0.036	47	2×10^{-5}
13	1.8×10^3	1.5×10^3	0.19	88	1×10^{-4}	1.5×10^2	2.0×10^2	0.037	100	1.3×10^{-5}
38	4.9×10^2	3.4×10^2	0.07	70	3×10^{-7}	1.2×10^2	1.9×10^2	0.27	100	1×10^{-7}
41	9.2×10^2	6.4×10^2	0.09	70	4×10^{-6}	1.0×10^2	1.6×10^2	0.02	100	1×10^{-5}
RF-8	5.0×10^2	3.5×10^2	0.12	71	3×10^{-6}	4.4×10^1	2.4×10^2	0.033	100	2×10^{-6}
<u>Archaias</u> symbiont	2.2×10^2	7.1×10^1	0.01	31	6×10^{-8}	8.3×10^1	1.4×10^1	0.002	60	1×10^{-8}
94	8.3×10^2	2.0×10^2	0.25	24	4×10^{-6}	1.8×10^2	2.6×10^1	0.003	14	4.6×10^{-7}

* #of algae ingested/total inocula of food x 100

** %of algae digested of those ingested/worm

TABLE 1 (continued)

Chromadorina germanica

ALGAE	# algae/worm		grazing factor % *	assimi- lation % **	mgm/ worm/day
	24 hr	48 hr			
RF-1	1.5×10^3	1.9×10^3	0.75	100	2×10^{-5}
BL-44	9.4×10^3	7.8×10^3	20.5	83	9×10^{-6}
BL-42	9.4×10^3	4.5×10^3	1.8	47	2×10^{-5}
39	8.7×10^3	3.1×10^3	0.9	36	2×10^{-4}
8	6.3×10^3	5.2×10^3	1.7	82	6×10^{-4}
13	1.3×10^3	1.2×10^3	0.1	95	8×10^{-5}
38	2.7×10^3	2.2×10^3	0.6	79	2×10^{-6}
41	1.5×10^4	1.2×10^4	1.2	83	8×10^{-4}
RF-8	1.5×10^3	1.4×10^3	0.4	86	1×10^{-5}
<u>Archaias</u> symbiont	6.1×10^3	4.5×10^3	1.5	73	9×10^{-6}

TABLE 2

Consumption of bacteria by 3 species of nematodes.

BACTERIA	<u>Rhabditis marina</u>					<u>Monhystera denticulata</u>				
	# bact/worm 24 hr	# bact/worm 48 hr	grazing factor % *	assimi- lation % **	mgm/ worm/day	# bact/worm 24 hr	# bact/worm 48 hr	grazing factor % *	assimi- lation % **	# mgm/ worm/day
A3-1	6.1×10^3	3.5×10^3	0.37	57	1×10^{-7}					
A5-3	8.7×10^4	6.8×10^4	14.5	78	2×10^{-6}	4.6×10^4	3.0×10^4	29	63	9×10^7
A1-1	3.1×10^4	1.8×10^4	2.9	60	5×10^{-6}	8.9×10^3	4.5×10^3	0.56	51	1×10^{-7}
A5-6	8.3×10^3	6.9×10^4	39	78	2×10^{-6}	8.3×10^4	2.3×10^4	12.3	27	2×10^{-6}
D1-1	1.1×10^4	9.2×10^3	0.94	78	3×10^{-6}	9.1×10^5	6.0×10^5	100	66	2×10^{-5}
C1-4	3.6×10^4	2.2×10^4	4.7	61	6×10^{-7}	1.6×10^3	8.0×10^2	0.41	50	2×10^{-8}
D2-7	2.0×10^4	1.4×10^4	1.8	70	4×10^{-7}	8.8×10^4	7.0×10^4	100	79	2×10^{-6}
A5-7	3.2×10^4	2.0×10^4	1.9	61	5×10^{-4}	3.7×10^4	1.8×10^4	70.9	47	5×10^{-7}
D2-3	1.0×10^5	2.9×10^4	9.5	28	9×10^{-4}	1.4×10^4	1.1×10^4	4.1	100	3×10^{-7}
D5-2	1.2×10^5	6.2×10^4	9.7	52	2×10^{-6}	5.9×10^4	2.5×10^5	100	41	7×10^{-6}

* # of bacteria ingested/total inocula of food x 100

** % of bacteria digested of those ingested/worm

TABLE 2. (continued)

Consumption of bacteria by 3 species of nematodes.

Chromadorina germanica

BACTERIA	# bact/worm		grazing	assimi-	mgm/ worm/day
	24 hr	48 hr	factor % *	lation % **	
A3-1	1.6×10^3	1.1×10^3	0.21	64	3×10^{-8}
A5-3	3.9×10^3	2.4×10^3	0.39	61	7×10^{-8}
A1-1	9.3×10^2	3.3×10^2	0.04	35.8	9×10^{-9}
A5-6	2.0×10^3	4.7×10^2	0.05	22.8	1×10^{-8}
D1-1	1.7×10^3	5.5×10^2	0.07	31.7	2×10^{-8}
C1-4	2.2×10^3	8.1×10^2	0.08	36	2×10^{-8}
D2-7	3.1×10^4	1.4×10^4	18	44	4×10^{-7}
A5-7	6.0×10^3	2.8×10^3	1.6	47	8×10^{-8}
D2-3	1.0×10^3	1.0×10^3	0.1	100	3×10^{-8}
D5-2	1.6×10^3	1.1×10^3	0.1	66.8	1×10^{-8}

bacteria and generally consumed large quantities of most species of algae tested. The feeding patterns of Monhystera on algae were similar to those of Rhabditis. One hundred per cent of one species of bacterium (B1-1) fed to the worm was eaten while only 0.4% of another species (C1-4) was eaten.

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. $^{14}\text{CO}_2$ 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 turnover 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 3).

We have also studied the ecological efficiency ($E_e\%$) of populations of one worm, Rhabditis marina, as a function of time. For these experiments the worms were inoculated into large numbers of flasks with 4 species of bacteria in a medium which would promote the slow steady growth of the bacteria. Bacteria alone were inoculated into control flasks. Measurements were made weekly. At the beginning of each experiment a known quantity of labeled bacteria were added to each flask. Each week a census of the worm population was made and their rates of growth, respiration and ingestion were measured. The data (Table 4; Fig. 11) suggest that Rhabditis, at least grown this way, has a very low ecological efficiency. Additional experiments are in progress.

TABLE 3

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 1082 2050	321 μg		-	-	
<hr/>							
<u>Rhabditis marina</u>	12 grams	C ¹⁴ 23.35 μg	~12	1584 μg	~100	0.013	0.013
		G 23.35 2050	11.26 μg		~100	0.013	

TABLE 4

Energetic consideration of the growth of Rhabditis marina fed a mixture of 4 species of bacteria (un-identified species of Pseudomonas strains R 1, R 2, R 4 and R 6) at 25 C.

Week	Population	Ingestion $\mu\text{gC}/\text{worm}/\text{hr}$	Respiration $\mu\text{l O}_2/\text{hr}/\text{g}$	Production $\mu\text{g C}/\text{worm}$	$E_e = P/I$
1(10/31)	38	4×10^{-8}	1452	1.6×10^{-4}	0.0%
2(11/ 6)	1477	0.88×10^{-8}	375	1×10^{-5}	0.31%
3(11/10)	2886	1.02×10^{-8}	474	3.6×10^{-5}	-0.099%
4(11/13)	2713	12.3×10^{-8}	758	3.7×10^{-6}	-0.0002%
5(11/16)	2602	6.21×10^{-8}	142	4.6×10^{-6}	+0.004%

B. Microcosm experiments

A number of our experiments with model microcosms were directed toward competition between Euplotes vannus, a surface feeding ciliate with apparently few dietary requirements and known information processing abilities (COO 3254-17) and Allogromia laticollaris, a foraminifera with complex dietary requirements best satisfied by a mixture of food organisms. Experimental microcosms were gnotobiotic, but bacterized (monoxenic), since Allogromia, as do all foraminifera studied in this respect, has an absolute requirement for a growth factor (or factors) found only in living bacteria (see Muller and Lee 1974). In one important respect the competitors reported here differ from the pair in the completed subproject (COO 3254-17). In the completed study both ciliates had simple dietary requirements and neither seems to be able to combine food quality for growth enhancement. An unidentified chlorophyte (S 94) was used in the Allogromia-Euplotes microcosms since it is a good food organism for both species. Allogromia laticollaris has a lower threshold for feeding on most foods ($\sim 1 \times 10^2$ cells/ml; Lee et al. 1966) than does Euplotes vannus (Fig. 12). Above lower threshold, feeding generally follows Lotka-Volterra kinetics to a maximum of $\sim 1 \times 10^7$ or 10^8 cells/ml for Allogromia, whereas feeding saturation density is reached at a much lower level ($\sim 1 \times 10^5$) for Euplotes vannus (Fig. 12). Undoubtedly this is a reflection of the relative sizes of the 2 herbivores.

The experimental medium was limiting in nutrients required by the alga so that algal populations would themselves be limited during the experiment. Under experimental conditions control populations of Euplotes reached maximum density 2×10^3 in ~ 4 -5 weeks at 25 C and declined rapidly thereafter (Fig. 13). At 15 C the population grew slowly to $\sim 1/2$ that level over the 9 week incubation period. Allogromia populations also reached carrying capacity ($\sim 1.4 \times 10^3$) in ~ 4 -5 weeks at 25 C but characteristically did not decline (Fig. 14). Growth at 15 C was approximately 10% that at

Figure 12.

The effect of food concentration on feeding rate in Euplotes vannus.
(determined respirometrically)

<u>curve</u>	<u>concentration of food/ml</u>
1	10^2
2	10^4
3	10^6
4	10^8

Figure 13

A microcosm experiment with Euplotes vannus and Allogromia latcollaris. I. The effect of temperature and inoculum size on the growth of Euplotes.

<u>Curve</u>	<u>Incubation temperature</u>	<u>Number of organisms in inoculum</u>	
		<u>Allogromia</u>	<u>Euplotes</u>
1	25° C	10	10
2	25° C	control	10
3	25° C	10	20
4	15° C	10	10
5	15° C	10	20
6	15° C	control	10

Figure 14 (complementary to Figure 13)

A microcosm experiment with Euplotes vannus and Allogromia latcollaris. I. The effect of temperature and original inoculum on the growth of Allogromia.

<u>Curve</u>	<u>Incubation temperature</u>	<u>Number of organisms in inoculum</u>	
		<u>Allogromia</u>	<u>Euplotes</u>
1	25° C	10	control
2	25° C	10	10
3	15° C	10	control
4*	25° C	10	20
	15° C	10	10
	15° C	10	20

* Inoculum did not reproduce and remained same throughout.

Figure 15.

A microcosm experiment with Euplotes vannus and Chromadorina germanica. I. The effect of temperature and initial inoculum on the growth of Chromadorina.

<u>Curve</u>	<u>Incubation temperature</u>	<u>Number of organisms in inoculum</u>	
		<u>Chromadorina</u>	<u>Euplotes</u>
1	25° C	10	10
2	15° C	10	control
3	25° C	10	control
4	25° C	50	10
5	15° C	10	10
	15° C	10	50

Figure 16. (complementary to Figure 15)

A microcosm experiment with Euplotes vannus and Chromadorina germanica. I. The effect of temperature and inoculum size on the growth of Euplotes.

<u>Curve</u>	<u>Incubation temperature</u>	<u>Number of organisms in inoculum</u>	
		<u>Chromadorina</u>	<u>Euplotes</u>
1	25° C	control	10
2	15° C	control	10
3	25° C	10	10
4	15° C	10	10
5	25° C	10	50
6	15° C	10	50

Figure 17.

A microcosm experiment with Euplotes vannus and Chromadorina germanica. II. Competition when food concentration is not limiting.*

Curve

1	<u>Chromadorina</u> control
2	<u>Euplotes</u> control
3	<u>Chromadorina</u> in the presence of <u>Euplotes</u>
4	<u>Euplotes</u> in the presence of <u>Chromadorina</u>
(see curve 3)

* Algal and bacterial growth stimulated by 5% (v/v) soil extract and 10 mg% (w/v) nitrate; incubation at 25° C.

at 25C. When equal numbers of both Euplotes and Allogromia were incubated at 25 C, neither organism grew well (Figs. 13, 14). Allogromia was least effected; populations reached ~ 30% of their biotic potential in about 4-5 weeks. Initial population growth of Euplotes paralleled the controls for the first 2 weeks and then rapidly declined as the numbers of Allogromia increased. At maximum Euplotes populations were only ~ 13% of controls. When initial conditions were changed and twice as many Euplotes were inoculated as Allogromia the outcome of the competition was quite different. Allogromia did not reproduce at all. Euplotes populations reached ~ 25% control levels and then declined very slowly (Fig. 13). At 15 C Allogromia did not reproduce in the presence of Euplotes (Fig. 14). As a result, the populations of the latter grew better.

A similar microcosm experiment studied the interactions of Euplotes vannus and Chromadorina germanica. As do Euplotes populations at 25 C, Chromadorina populations also reach maximum density in 4-5 weeks. At 25 C when inocula were evenly matched, Chromadorina populations paralleled those of controls for the first 2 weeks and then slowly declined (Fig. 15). Under the same conditions, Euplotes populations bloomed (~ 70% of controls) in less than a week and then rapidly declined. Perhaps the more rapid early growth of Euplotes was due to the stimulation of bacterial growth by the feces of the worm but this was not measured. The same phenomenon was also observed when Euplotes initial inocula were increased 5 fold over Chromadorina (Fig. 16). Under the same conditions Chromadorina populations did not begin to increase until after Euplotes populations declined (Fig. 15). As with Allogromia in the previous experiment Chromadorina could not compete at all with Euplotes at 15 C. In the presence of Chromadorina, Euplotes populations at 15 C also grew initially at a more rapid rate than controls.

In a succeeding experimental microcosm, food was not limited because the medium (Erdschreiber) supported the growth of the algae. In this medium the growth of both control populations was similar

to growth in unenriched media. (Compare Figs. 15, 16, 17.) Synergistic effects were noted when both species were inoculated together. In the presence of Euplotes and excess food, Chromadorina populations reached maximum density (3 x controls) 3 1/2 weeks earlier than controls, declined, and grew again to almost twice the levels of controls (Fig. 17). Initial growth of Euplotes populations was retarded until Chromadorina numbers began to decline, but then growth was rapid, peaking one week before controls. In a comparable experiment, Chromadorina was stimulated by the presence of Uronema (Fig. 18). In a microcosm experiment with Allogromia laticollaris, Chromadorina germanica and Euplotes vannus under conditions which allowed food to be in excess, all three organisms grew more rapidly than in controls. (Figs. 15, 16, 17, 19).

Since competitive interaction seemed so closely coupled with food density, several experiments were designed to study the effect of food concentration on growth (Figs. 20, 21). In both the dark and light Euplotes populations were initially stimulated by the presence of additional food (Figs. 20 A, B). In the light, populations continued to increase in size, whereas in the dark they did not. The importance of the algal food in the diet was shown by the minimal population size developed in both the light and the dark. Grazing more greatly effected the algal populations in the dark than in the light (Fig. 20 C, D). As noted earlier (COO 3254-17) initial concentrations of predator and prey were extremely important characteristics of the system. When algal food was in low concentration in the dark, the ciliates were able to reduce the population to such a small size that they themselves became extinct. When ciliate populations were more balanced with their food (1×10^4 - 10^6 cells/ml) growth of the system seemed more stable (Figs. 20 A-D). When the initial concentration of food was high, eventually the ciliates became too numerous for the algal populations. In all cases the system, if unperturbed, is expected to eventually oscillate

Figure 18.

A microcosm experiment with Euplotes vannus, Chromadorina germanica and Uronema marinum. I. The effect of food concentration and competition on the growth of Chromadorina.

<u>Curve</u>	<u>Medium</u>	<u>Other Organisms</u>
1	Erdschreiber*	control
2	sea water	control
3	Erdschreiber	<u>Uronema</u>
4	sea water	<u>Euplotes</u>
5	Erdschreiber	<u>Euplotes</u>
6	sea water	<u>Uronema</u>

* Erdschreiber is a medium made from sea water enriched with 5 % soil extract (v/v) and 10 mg% (w/v) nitrate; incubation at 25° C.

Figure 19.

A microcosm experiment with Allogromia laticollaris, Chromadorina germanica and Euplotes vannus under conditions in which food is in excess.*

Curve

1	<u>Allogromia laticollaris</u>
2	<u>Euplotes vannus</u>
3	<u>Chromadorina germanica</u>

* Medium Erdschreiber, incubation 25°C.

Figures 20 A -D.

The effect of algal food concentration on the growth of monoxenic (bacterized) Euplotes vannus.*

<u>Curve</u>	<u>Algal Concentration</u>
1	non-fed control
2	10^4
3	10^5
4	10^6
5	10^8

Fig. 20

- A Growth of Euplotes in cultures incubated in the dark
- B Growth of Euplotes in cultures incubated in the light
- C Number of algae (S 94) in cultures incubated in the light
- D Number of algae (S 94) in cultures incubated in the dark

* medium Erdschreiber

Figure 21.

The growth of monoxenic (bacterized) Euplotes vannus as a function of food concentration.

<u>Curve</u>	<u>Incubation time</u>
1	20 days
2	30 days
3	40 days

Figure 22. A, B.

A microcosm experiment with Euplotes vannus and Allogromia latcollaris. II. Inoculum equal, food concentration varied. ***

<u>Curve</u>	<u>Organism Plotted</u>	<u>Initial concentration of alga</u>
1	<u>Euplotes</u>	no add
2	<u>Euplotes</u>	10^4
3	<u>Euplotes</u>	10^6
4	<u>Euplotes</u>	10^8
5	<u>Allogromia</u>	no add
6	<u>Allogromia</u>	10^4
7	<u>Allogromia</u>	10^6
8	<u>Allogromia</u>	10^8

* Fig. 22 A = unidentified chlorophyte (S 94);

Fig. 22 B = alga Chlorococcum sp (41) .

** inoculum = 5 Euplotes and 5 Allogromia; incubation 25° C
(in the light)

Figure 23 A-C

A microcosm experiment with Euplotes vannus and Allogromia latcollaris. II. Inoculum unequal, food concentration varied. * **

<u>Curve</u>	<u>Organism Plotted</u>	<u>Initial concentration of algae</u>
1	<u>Euplotes</u>	0, control
2	<u>Euplotes</u>	10^4
3	<u>Euplotes</u>	10^6
4	<u>Euplotes</u>	10^8
5	<u>Allogromia</u>	0, control
6	<u>Allogromia</u>	10^4
7	<u>Allogromia</u>	10^6
8	<u>Allogromia</u>	10^8

* Fig. 23 A - Allogromia inoculum 5 organisms
 B - Allogromia inoculum 10 organisms
 C - Allogromia inoculum 50 organisms

** Euplotes inoculum 100 organisms; incubation 25° C
 (in light); food organisms unidentified chlorophyte
 (S 94)

around some stable relationship. In the aufwuchs conditions change far too rapidly for this to occur.

The results of a microcosm experiment in which Euplotes vannus and Allogromia laticollaris were inoculated with varying concentrations of 2 species of food were quite interesting. When equal numbers of both species were inoculated, secondary production was quite low. Both species grew better on bacterial diets (though out of phase with each other) than they did with algae (Figs. 22 A, B). Allogromia reproduced fairly rapidly on an algal diet of 1×10^4 or more cells once Euplotes populations stabilized or diminished. When the ratio of Euplotes:Allogromia in the inoculum was increased to 20:1 Euplotes grew much better. For the first 5 weeks, numbers of Euplotes were proportional to food density. Allogromia populations could not compete with those of Euplotes at either high or low concentrations of food ($0, 10^8$) but grew after some delay (3 weeks) at intermediate levels (1×10^4 - 10^6). Changing the inoculum ratio of Euplotes:Allogromia to 10:1 did not greatly change the growth pattern of either species although populations of Euplotes were slightly smaller (Fig. 22 B). When the ratio was diminished to 2:1, Euplotes populations were much smaller and Allogromia populations were correspondingly higher (Fig. 23 C).

In another microcosm experiment, large inocula of each species were incubated in an illuminated thermal gradient block in the presence of excess food (S 94). Both species grew well over a range of 16-28 C (Figs. 24 A, B). In controls and in competition Allogromia did better at higher temperatures (>20 C) and Euplotes < 20 C (Figs. 24 C, D). Only at low food density did Euplotes successfully compete with Allogromia above 20 C. Population sizes were generally proportional to initial food concentration.

Figures 24 A, B.

A microcosm experiment with Euplotes vannus and Allogromia latcollaris. III. The effect of temperature and food on competition.* **

<u>Curve</u>	<u>Incubation period</u>
1	1 week
2	2 weeks
3	3 weeks
4	4 weeks

- * Fig. 24 A = Euplotes control;
 Fig. 24 B = Allogromia control

** inoculum 50 organisms of each species; food (S 94) in excess; incubation in thermal gradient block

Figure 24 C, D

A microcosm experiment with Euplotes vannus and Allogromia latcollaris. III. The effects of temperature and food on competition.*

<u>Curve</u>	<u>Incubation Period</u>	<u>Food concentration (S 94)</u>
1	2 weeks	10^2
2	4 weeks	10^2
3	2 weeks	10^4
4	4 weeks	10^4
5	2 weeks	10^6 & 10^8
6	4 weeks	10^6 & 10^8

* Fig. 24 C Euplotes populations plotted
 Fig. 24 D Allogromia populations plotted

C

C. Nutritional and stress experiments

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 vials. Dead worms and aliquots from the washing medium served as controls. No up-take of substrate was measured. The experiment was repeated with $3 \text{ M} \pm 15 \text{ } \mu\text{M}$ microspheres added to the experimental medium. No appreciable uptake of labeled substrate was measured. When $0.5 - 10 \text{ } \mu\text{M}$ 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.

Some progress was also made in the study of the special growth factors required by Rhabditis marina. We found that this organism is capable of digesting only a small number of species of bacteria and that it will grow in continuous monoxenic culture with very few. Growth stimulating factors were found in milk, brewers yeast, autoclaved bacteria, malt extract, gelysate peptone which led us to suspect that in addition to hemoglobin the worm requires either additional vitamins and/or some lipid growth factors.

R. marina was maintained axenically on Base B plus 25% (v/v) Graces Insect media (Grand Island Biological Company, L. I., N. Y.). Graces Insect media is a highly enriched nutritive media containing amino acids, vitamins, fatty acids and sugars. The pre-sterilized Graces Insect media was added aseptically to solidified Base B and slanted. The nematodes were transferred once a week with the weekly addition of antibiotics, and thus maintained for more than 75 generations. Before finding Graces Insect media best for maintenance,

we tested two other prepared organic media, M 199 and C. briggsae-medium (G.I.B. Co). In each case yeast extract, hemoglobin, case amino acids, trypticase and autoclaved bacteria were used as nutrient additives. Continuous growth and reproduction did not take place in either medium supplements.

The addition of various peptones, carbohydrates or yeast extract to Graces medium did not stimulate further growth. Crude natural materials rich in lipids (i.e. egg yolk, milk, whole egg, lactalbumin and brewers yeast) were stimulatory. Hexane and aqueous extracts of whole egg powder were more stimulatory than the other undefined supplements tested. Additional experiments with other lipid rich materials showed that a growth factor was also found in Tween 80, soy lecithin, cephalin and TEM 4 T (Table 5). Only 1 mg% (w/v) TEM 4 T and 0.5 mg% (w/v) Tween 80 stimulated growth after 7 serial point to point transfers. Experiments showed combinations of oleic, stearic, linolenic, linoleic and palmitic acids plus 0.01% (w/v) hemoglobin might satisfy the nutritional requirements of the worm (Table 6). Additional experiments are planned.

Preliminary nutritional experiments with some of the newly isolated aufwuchs harpacticoid copepods showed that they also have restricted nutritional capabilities. Nitocra typica, for instance, grew and reproduced well in gnotobiotic bacterized (2 species) culture supplemented with only 3 of 13 algal species tested (Table 7).

Preliminary experiments on the effects of heavy metal ions on the growth of epiphytic communities were begun this year. Choice of concentrations to be tested was based on Dr. Grant Gross' analyses of the levels of metallic ions present in the N.Y.C. Ambrose light tower sludge dumping area. Thus far we have measured LD₅₀ and MLD for 3 species of worms and their food when grown under optimal conditions. For both the algae and the worms the toxicity of the various ions was generally: Ag>Hg>Cu>Pb (Table 8, 9). Tolerance was generally lower for exposure to metals for long periods. The data for 30 days exposure are also tabulated (Table 10). In general,

TABLE 5

Assay for the nutritional requirements of *Rhabditis marina*. * I.

		A			B			C			D		
		no add			hexane extract whole egg powder 2.5 mg%			aqueous extract whole egg powder 1 mg%			aqueous extract whole egg powder 1 mg%		
		Init.	3 da	6 da	Init.	3 da	6 da	Init.	3 da	6 da	Init.	3 da	6 da
I.	No add	40	200	100	40	72	50	40	53	60	25	34	36
II.	TEM 1 mg%	40	71	20	42	12	14	55	77	25	30	64	75
III.	TWEEN 80 0.5 mg%	65	198	250	54	68	130	100	35	70	115	200	150
IV.	Soy lecithin 0.5 mg%	105	142	170	100	50	35	45	-	-	75	300	306
V.	Menadione 0.1 mg%	30	25	-	15	-	-	20	15	20	25	26	75
VI.	Animal lecithin 0.5 mg%	95	45	31	43	150	300	150	100	200	100	300	350
VII.	Cephalin 0.1 mg%	35	5	10	50	30	55	120	90	350	130	160	120
VIII.	Egg lecithin a) 0.1 mg%	130	80	100	100	25	15	65	120	200	60	180	200
	b) 0.5 mg%	30	70	80	50	70	90	100	200	100	110	50	50

* Medium was solidified Base B. (Lee et al., in press) and Graces Insect medium 15% (v/v); incubation 25° C; results of second serial transfer.

TABLE 6

Assay for the nutritional requirements of Rhabditis marina.* II.

		A		B		C		D	
		no add		whole egg powder 50 mg%		B+ hemoglobin 0.01%		hexane extract whole egg powder 2.5 mg%	
		Initial 6 day		Initial 6 day		Initial 6 day		Initial 6 day	
1.	Oleic acid								
	a) 0.1 mg%	4	14	8	3	5	20	9	8
	b) 0.5 mg%	7	5	3	16	4	18	6	21
	c) 1 mg%	4	10	4	8	2	5	2	6
2.	Palmitic								
	a) 0.1 mg%	3	16	3	13	4	12	3	--
	b) 0.5 mg%	15	20	5	3	6	14	3	6
	c) 1 mg%	6	8	6	11	14	28	13	20
3.	Oleic and palmitic								
	a) 0.1 mg%	10	8	5	9	12	16	3	10
	b) 1 mg%	10	20	11	50	9	30	6	10
4.	Linolenic, linoleic, stearic								
	a) 0.1 mg%	8	42	5	52	13	67	12	63
	b) 0.5 mg%	18	85	6	90	4	56	11	20
5.	Linolenic, linoleic, stearic, oleic, palmitic								
	a) 0.1 mg%	10	80	16	35	5	42	13	33
	b) 1 mg%	8	100	8	25	10	48	12	52

* Medium was solidified Base B (Lee et al., in press) and Graces Insect medium 15% (v/v); incubation 25° C; results of 7th serial transfer.

TABLE 7

The effect of diet on the reproduction of Nitocra typica

Alga	Number of copepods *	
	Initial	Final
<u>Dunaliella parva</u> (13)	25	22
<u>Dunaliella salina</u> (14)	22	108
<u>Nannochloris</u> sp (41)	36	143
unidentified chlorophyte (55)	28	131
<u>Dunaliella tertiolecta</u> (94)	25	69
<u>Chlorococcum</u> sp (39)	23	55
<u>Chlamydomonas subehrenbergii</u> (93)	34	14
BL-38	29	59
<u>Cylindrotheca closterium</u> (BL-35)	36	38
<u>Cylindrotheca fusiformis</u> (BL-27)	48	48
<u>Archaias</u> symbiont	42	55
<u>Amphora</u>	40	52
Control	29	30

* average of 4 replicates after 14 days incubation

TABLE 8

The toxicity of selected heavy metals to various salt marsh aufwuchs algae. *

Alga Tested	Ag		Cr		Cu		Hg		Pb	
	LD ₅₀	MLD	LD ₅₀	MLD	LD ₅₀	MLD	LD ₅₀	MLD	LD ₅₀	MLD
BL-1	7	10 (1)**	40	80 (4)	22	60 (2)	40	60 (3)	90	>100 (5)
SH-1	5	9 (1)	40	60 (4)	30	60 (3)	40-50	50 (2)	80	>100 (5)
8	3	5 (1)	70	80 (4)	9	60 (3)	20	30 (2)	100	>100 (5)
RF-8	2	5 (1)	--	--	--	50 (4)	1	6 (2)	20	30 (3)
9	3	5 (1)	70	80 (4)	9	-- (2)	10-20	40 (3)	100	>100 (5)
BL-15a	3	5 (1)	--	--	20	50 (3)	3	5 (1)	10	30 (2)
BL-17	4	6 (2)	--	--	20	40 (4)	2	5 (1)	20	30 (3)
38	7	10 (1)	20	90 (4)	40	60 (2)	40-60	60 (3)	>100	>100 (5)
50	10-20	-- (2)	40	80 (4)	30	80 (3)	4	8 (1)	>100	>100 (5)
55	0.8	1-10 (1)	80	80 (4)	69	100 (3)	4	10 (2)	>100	>100 (5)

*

all values in ppm

**

values in parenthesis represent relative toxicity on a scale of 1-5, where 1 = most toxic, 5 = least toxic.

TABLE 9

The acute toxicity of selected heavy metals to 3 species of salt marsh aufwuchs nematodes.*

Metal	<u>Chromadorina</u>			<u>Monhystera</u> **		<u>Rhabditis</u> **	
	LD ₅₀	MLD		LD ₅₀	MLD	LD ₅₀	MLD
Ag	0.25	0.5	24 hr	0.5-1	> 1	1	> 1
		0.4	3 d				
		0.3	6 d				
Hg	0.25	~ 1	24 hr	0.1	> 1	> 1	> 1
		0.8	3 d				
		0.75	6 d				
Pb	~5-10	> 100		> 100	> 100	> 1	> 1
Fe	>100	>100	24 hr	> 40	> 40	> 1	> 1
Cr	>100	>100	24 hr	~ 75	~ 100	> 1	> 1
Cu	2-3	> 75	24 hr	3	> 74	> 1	> 1
		75	3 d				

* all values in PPM

** data for 24 hr

TABLE 10

Preliminary study of the effects of heavy metal ions on the growth of selected salt marsh microorganisms and micrometazoa. *

Organism	Lead		Chromium		Mercury		Silver	
	LD 50/30	MLD	LD 50/30	MLD	LD 50/30	MLD	LD 50/30	MLD
<u>Algae</u>								
BL-1	6×10^{-4}	1×10^{-3}	1×10^{-4}	3×10^{-4}	2×10^{-4}	5×10^{-4}	1.5×10^{-6}	7×10^{-6}
RF-1	4×10^{-4}	5.5×10^{-4}	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-6}$	$< 1 \times 10^{-6}$
SH-1	5×10^{-4}	9×10^{-4}	1×10^{-4}	3×10^{-4}	1×10^{-4}	4×10^{-4}	2.5×10^{-6}	5×10^{-6}
RF-8	2×10^{-4}	6×10^{-4}	2×10^{-4}	3×10^{-4}	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-6}$	$< 1 \times 10^{-6}$
BL-15A	2×10^{-4}	5×10^{-4}	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-6}$	$< 1 \times 10^{-6}$
8	5.5×10^{-4}	9×10^{-4}	4×10^{-4}	5×10^{-4}	3×10^{-4}	4×10^{-4}	$< 1 \times 10^{-6}$	$< 1 \times 10^{-6}$
9	6×10^{-4}	6×10^{-4}	2×10^{-4}	3×10^{-4}	4×10^{-4}	5×10^{-4}	$< 1 \times 10^{-6}$	$< 1 \times 10^{-6}$
38	4×10^{-4}	7×10^{-4}	2×10^{-4}	3×10^{-4}	2×10^{-4}	3×10^{-4}	2×10^{-6}	3×10^{-6}
39	8×10^{-4}	1×10^{-3}	2×10^{-4}	4×10^{-4}	1×10^{-4}	1×10^{-4}	1×10^{-6}	4×10^{-6}
50	5×10^{-4}	7×10^{-4}	2×10^{-4}	3×10^{-4}	2×10^{-4}	3×10^{-4}	2×10^{-6}	3×10^{-6}
55	5×10^{-4}	9×10^{-4}	3×10^{-4}	4×10^{-4}	1×10^{-4}	2×10^{-4}	2×10^{-6}	$< 1 \times 10^{-6}$
<u>Nematodes</u>								
<u>Chromadorina</u>	$< 1 \times 10^{-4}$	2×10^{-4}	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-6}$	$< 1 \times 10^{-6}$
<u>Rhabditis</u>	2.5×10^{-4}	4×10^{-4}	1.5×10^{-4}	3×10^{-4}	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	2×10^{-6}	4×10^{-6}
<u>Monhystera</u>	2.5×10^{-4}	7×10^{-4}	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	1.5×10^{-6}	6×10^{-6}
<u>Foraminifera</u>								
<u>Spiroloculina hyalina</u>	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-6}$	$< 1 \times 10^{-6}$
<u>Rosalina leei</u>	5×10^{-4}	9×10^{-4}	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-6}$	$< 1 \times 10^{-6}$
<u>Allogromia laticollaris</u>	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	1.5×10^{-6}	3×10^{-6}

* MLD and LD 50/30 given in g/l.

metallic ions were more lethal to the foraminifera and nematodes than they were to the algae. With few exceptions they and 2 species of algae were killed by the lowest levels of metallic ions tested in the experiment (Table 8). The MLD for most algae was $\sim 5 \times 10^{-4}$ g/L (0.5 ‰) of lead, chromium and mercury. Chlorophyte B1-1 was a bit more resistant to lead. All the organisms were more sensitive to silver; MLD for this ion was $\leq 1 \times 10^{-6}$ g/L (0.005 ‰).

- D. 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 the progress report 3 years ago (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 well will we be able to estimate accurately the bacterial component. Three species grew very well on unenriched sea water alone (Fig. 25). 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. 27). Only 1-4 species were isolated on the sea water agar plates. Two species were consistently isolated on erdschreiber throughout the summer (Fig. 26). Although there were slight fluctuations in the populations, both species were quite abundant ($\sim 3 \times 10^6$ /g; Fig. 27). 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. 27), 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 [see 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% (w/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. 27). The growth of species b2, b3, c4, c5, d1, e3, e4 were possibly correlated

with 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. 28); 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. 29). 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. 28). The first isolation of species A 1-3 was in mid-August (Fig. 29). The pattern of colony growth on medium A 2 (enriched with NaNO_3) and A 3 (enriched with NH_4Cl) was similar. The same progressive general increase in number of bacteria/g was observed although the peak population was not as great as in medium A 1 (Fig. 29). The number of species growing on the media gradually increased throughout the summer (Fig. 28). A total of 7 species grew on both media (Figs. 31, 32). 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. 26, 28). 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. 26). 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. 33).

Throughout the season 2 species of bacteria were consistently isolated on medium B 1 (Fig. 34). Though there were minor fluctuations, populations of species B 1-1 numbered $\sim 2.5 \times 10^6$ /g of the epiphytic community while species B 1-2 numbered $\sim 2 \times 10^5$ /g. Small numbers of 6 other species were collected in late summer. In terms of total numbers of bacteria, the peptone enriched medium B 2, 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. 35, 36). Only 1 species, B 3-1, grew well on medium B 3, the medium enriched with urea (Fig. 37). Substantial growth was noted on this medium from the 6th field collection (July 23) until a decline which began on the 11th collection (Fig. 35). From mid-July on, there was a progressive increase in the number of species collected on this medium (Fig. 38).

The same trend of progressive increase in the number of species isolated from the community on successive collection which we observed on media A 5 and B 3 was observed on all the series C media (Fig. 38). 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. 39-42). 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. 43), 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. 27, 28, 38, 43) were quite dissimilar in respect to the seasonal trends in both the

total numbers and types of bacteria isolated. The growth of one organism, C 3-1, which was one of the most abundant species at the beginning of the season, gradually declines over the course of the summer (Fig. 44). 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 collection on medium C 4 (Fig. 45). During this period, the fluctuations in the number of organisms isolated on this vitamin enriched medium were minimal.

Through there were exceptions, there was a general trend of seasonal successional and increasing numbers of species which grew on the series D media (Figs. 38, 46, 47, 48). 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. 49). 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 of species which grew on this medium (Fig. 50). 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. 43, 46). It seems probable that autoclaved Enteromorpha stimulated the bacteria.

TIME

Number $\times 10^6 / g$

C1-7

C1-9

C1-10

TIME wks

NUMBER x 10⁻⁷ g

C2-9

C2-10

C2-11

TIME mts

TIME

The 125 salt marsh bacteria have been successfully maintained for four years. Identification procedures, developed for use with terrestrial organisms, have been or are being adapted for use with these marine isolates. Certain differential tests have been manipulated, modified and cross-tested in order to determine which methods provide the most consistently reliable results. To date, all of the isolates have been subjected to 12 taxonomically important tests. For clarity, the methods used are briefly outlined here:

1. Shape, arrangement and motility

determined by phase microscopy of hanging drop preparations of 24 hour cultures.

2. Starch hydrolysis

0.2% soluble starch (BBL) was added to out stock maintenance agar. Plates prepared with this medium were refrigerated for 2 days to increase opacity, brought to room temperature, and inoculated with single streaks of each culture. After 5 days incubation at room temperature, plates were flooded with Lugol's iodine to test for starch hydrolysis.

3. Gelatin hydrolysis

Nutrient gelatin (BBL) was prepared with stark basal medium, dispensed into tubes and inoculated by stabbing. Tubes were incubated at room temperature until complete gelatin liquifaction was noted or for a maximum of 30 days.

4. Indole

1.0% Tryptone (BBL) was added to stark basal medium. Tubes were inoculated and incubated at room temperature for 24 hrs and then tested with Kovac's reagent.

5. Carbohydrate metabolism

The method of Hugh and Leifson (1953) was modified by substituting our stark basal medium for the salts and distilled H₂O. Sterile Taxo discs (BBL) with glucose, sucrose, lactose,

mannose, galactose or mannitol were added aseptically to tubes of the media. Each organisms was stab inoculated into duplicate tubes of each sugar. One tube of each set was sealed with a 1 cm layer of a sterile petrolatum-vasoline mixture (= anaerobic tubes). All tubes were incubated at room temperature and examined daily for 10 days for fermentative or oxidative reactions. Acid production in both aerobic and anaerobic tubes is indicative of fermentative metabolism of the sugar. Acid production in only the aerobic tubes indicates oxidative metabolism.

6. Kovac's Oxidase

one drop of a 24 hour broth culture was placed on a Taxo N disc (BBL).

7. Catalase

10% hydrogen peroxide was added to 24 hour colonies of each organism.

8. Nitrate reduction

Liquid stock media were supplemented with 0.2% NaNO_3 . Inverted Durham tubes were placed in each tube to collect any gas produced. The media were inoculated with 0.1 ml of a 24 hour broth culture and incubated at room temperature. Detection of ammonia was done with Nessler's reagent and the presence of nitrite determined by the method of Griess-Llosvay. Initial tests were done after 24 hours incubation and repeated daily on those cultures giving negative results. Total incubation time was 2 weeks.

Results are tabulated (Tables 12, 13). As expected, and as can be seen by inspection of Table 11, most of the isolated organisms are gram negative rods which demonstrate diverse physical characteristics. Close inspection of the data strongly indicates that organisms isolated on our series of differential media demonstrate varied ability to attack glucose, sucrose and lactose (Table 11). Such results begin

to point out the utility and importance of diverse complex and synthetic media in the isolation and enumeration of the bacterial inhabitants of the salt marsh community. By employing such media, we have obtained a more comprehensive picture of the metabolic capabilities of salt marsh bacteria. A computer program is being worked out in an effort to get a more detailed analysis of these and future findings. We hope in the next year to complete this project as a companion to the diatom assemblage paper (COO 3254-9). Together they should give us a cohesive picture of auxotrophic pathways of carbon cycling in the microbial assembly of the marsh.

TABLE 11

Characteristics of bacteria isolated from epiphytic communities of Enteromorpha.

Organism	straight rod curved rod coccus	Singles Pairs Chains Clusters	Motility	Gram	Kovac's oxidase	Catalase	Starch hydrolysis	Gelatin hydrolysis	Indole	Glucose		Sucrose		Lactose		Mannose		Galactose		Mannitol		Nitrate Reduction		
										aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	NO ₂	NH ₄	gas
C3-705	+	+	+	-	+		+	++	-	-	-	-	-							-	-			
C3-706	+	+	-	+			-	+	-															
C3-707	+	++	+	-	+		+	++	+	A	A	A	A							-	-			
C3-707*	+	++	+	-	+		+	++	+	A	A	A	A							A	A			
C3-711	+	+	+	-	+		-	+	-	A	-	-	-			A	-	-	-	-	-			
C3-712	+	+	-	-	+		+	+	-	A	-	A	-											
C3-712*	+	++	-	-	+		-	+	-			-	-					-	-	-	-			
D1-701	+	++	+	-	+	+	+	++	-	A	-	A	-	A	-	A	-	A	-	A	-	+	+	-
D1-701*	+	++	+	-	+	+	-	+	-	A	-	A	-	A	-	A	-					+	+	-
D1-702	+	++	+	-	+	+	-	+	-	A	-	A	-	A	-	A	-					+	+	-
D1-703	+	+++	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
D1-704	+	++	+	-	+	+	+	++	+	A	A	A	A	A	A	A	A					+	+	-
D1-706	+	+	+	-	+	+	+	++	-	A	-	A	-	A	-	A	-					+	+	-
D1-707	+	++	+	-	+	+	+	+	+	A	A	A	-	A	-	A	-					+	+	-
D1-707*	+	+	+	-	+		+	+	-	-	A	-	-	-	-	A	-					+	+	-
D2-701	+	+	+	-	+	+	+	++	+	A	A			-	-					A	A	+	+	-
D2-702	+	++	+	-	+	+	+	+	-	A	A			A	A					-	-	+	+	-

Table 11 (continued)

Organism	straight rod curved rod coccus	Singles Pairs Chains Clusters	Motility	Gram	Kovac's oxidase	Catalase	Starch hydrolysis	Gelatin hydrolysis	Indole	Glucose		Sucrose		Lactose		Mannose		Galactose		Mannitol		Nitrate Reduction		
										aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	NO ₂	NH ₄	gas
C1-701*	+	+	+	-	-		+	++	-	A	A	A	-	-	-	A	A	-	-	-	-			
C1-701X	+	+	-	-	-		-	+	-	-	A			Alk	A	-	-	-	-	-	-	+	+	-
C1-702	+	+	+	-	-		+	+	-	Alk	A			Alk	A	A	A	A	A	A	A	+	+	-
C1-702'	+	+	+	-	+		+	++	+	A	A	A	Ag	A	A	A	A	A	A	A	A	+	+	-
C1-703*	+	+	+	-	+		-	+	-							A	A	A	A	A	A			
C1-704	+	+	+	-			+	++	-	A	A	A	A	A	A	-	-	A	-	-	-	+	+	-
C1-704*	+	++	+	-	+		+	+	-	A		A	-							A	-			
C1-705	+	++	+	-			+	++	-	A	-	A	-							A	-			
C1-708	+	+	+	-	+		+	++	-	-	-	-	-							-	-			
C1-710	+	++	+	-	+		+	+	+	A	A	A	A							A	A			
C2-702	+	++	+	-	-		-	+	-	A	-	A	-							A	-			
C2-703	+	+	+	-	+		-	-	-	A	-	A	-							A	-			
C2-704	+	+	-	-	+		-	-	-	-	-	-	-							-	-			
C3-701	+	++	+	-	+		+	++	+	A	A	A	A							A	A			
C3-702	+	+	+	-	+		+	++	-	-	-	-	-			A	-							
C3-704	+	+	+	-	+		-	-	-	A	-	-	-			A	-							
C3-704*	+	+	+	-	-		-	-	-	-	-	-	-							-	-			

Table 11 (continued)

Organism	straight rod curved rod coccus	Singles pairs chains clusters	Motility	Gram	Kovac's oxidase	Catalase	Starch hydrolysis	Gelatin hydrolysis	Indole	Glucose		Sucrose		Lactose		Mannose		Galactose		Mannitol		Nitrate Reduction		
										aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	NO ₂	NH ₄	gas
A5-705	+	++	+	-	+		+	++	+	A	A	A	A	-	-	A	A	A	A	A	A	-	+	-
A5-705*	+	++	+	-	+		+	++	+	A	A	A	A	A	-	A	A	A	A	A	A	-	+	-
A5-706	+	+	+	-	+		+	++	+	Ag	Ag	A	A	A	-	-	A	-	-	A	A	++	+	-
A5-707	+	+	+	-	+		-	-	-	A	A	A	A	-	-	-	A	-	-	A	A	+	+	-
B1-702	+	++	+	-	+		+	+	+	A	A					A	A	A	A			+	-	-
B1-706	+	++	+	-	+		-	+	+	A	A	A	A	-	-	A	A	A	A	A	A	++	+	-
B1-707	+	+	+	-	+		+	++	-	A	A	A	A	-	-	A	A	A	A	A	A	++	+	-
B2-702	+	++	+	-	+		+	+	-	Ag	A	A	A	A	A	-	-	A	-	-	-	+	+	-
B2-704	+	++	+	-	+		-	-	-	A	A	A	A	A	-	-	-	-	-	Alk	Alk	++	+	-
B2-Rh	+	++	+	-	+					A	A	A	A	-	-							++	+	-
B3-701	+	+	+	-	+		-	-	-	Alk	A	A	-	-	-	A	-	-	-	-	-	++	+	-
B3-705	+	++	+	-	+		-	+	-	A	A	A	-	-	-	-	A	-	-	-	-	-	+	-
B3-711	+	++	+	-	+		+	++	-							A	-	-	-	-	-			-
B3-712	+	++	+	-	+		+	++	-	A	A	A	-	-	-	A	A	A	A	A	-	++	+	-
B3-Ps	+	+	+	-	+		+	+	-			A	-	A	-					A	-			-
B3-Rh	+	+	+	-	+		+	++	-	A	A	-	-	-	-	A	-	A	-	-	-	++	+	-
C1-701	+	++	-	-	-		+	+	-	-	A	Ag	Ag	-	-	A	-	-	-	A	-	++	+	-

Table 11 (continued)

Organism	straight rod curved rod coccus	Singles pairs chains clusters	Motility	Gram	Kovac's oxidase	Catalase	Starch hydrolysis	Gelatin hydrolysis	Indole	Glucose		Sucrose		Lactose		Mannose		Galactose		Mannitol		Nitrate Reduction		
										aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	NO ₂	NH ₄	gas
e 8	+	++	+	-	-		+	+	-	A	A	A	A	-	-							+	+	-
A1-701	+	++	+	-	+		+	+	+	A	A	A	A	-	-	A	A	A	A	A	A			
A1-702	+	++	-	-	-		-	-	-	-	-	-	-	-	-	A	A					+	+	+
A1-712	+	++	+	-	+		-	-	-	-	-	-	-	-	-							+	+	-
A1-714	+	++	+	-	-		-	-	-	-	-	-	-	-	-							+	+	-
A1-714*	+	++	+	-	-		+	+	+	A	A	A	A	-	-	A	A	A	A			+	+	-
A2-701	+	++	+	-	+		+	+	-	-	-	-	-	-	-							-	+	-
A2-702	+	+	-	-	+		+	+	-	-	-	-	-	-	-							-	+	-
A2-703	+	+	+	-	+		+	+	-	A	A	-	-	-	-	-	-	A	A	-	-			
A2-703*	+	++	+	-	+		+	+	-	A	-	-	-	-	-	-	-	A	-	-	-	+	+	-
A2-704	+	+	+	-	+		+	+	-	A	A	-	-	-	-	-	-	A	-	-	-	+	+	-
A2-714*	+	+	+	-	+		+	+	-	-	-	-	-	-	-							+	+	
A3-701	+	++	+	-	+		+	+	-	-	-	-	-	-	-							+	+	-
A5-701	+	++	+	-	+		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-			
A5-701*	+	++	+	-	+		+	+	-	-	-	-	-	-	-	A	-	-	-					
A5-701	+	+	+	-	+		+	+	-	-	-	-	-	-	-									
A5-703	+	++	+	-	+		+	+	-	A	-	-	-	-	-	-	-	-	-	-	-			
A5-704	+	++	+	-	+		-	+	-	A	A					A	A	A	A			+	+	-

Table 11 (continued)

Organism	straight rod curved rod coccus	Singles pairs chains clusters	Motility	Gram	Kovac's oxidase	Catalase	Starch hydrolysis	Gelatin hydrolysis	Indole	Glucose		Sucrose		Lactose		Mannose		Galactose		Mannitol		Nitrate Reduction		
										aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	NO ₂	NH ₄	gas
a 3	+	+	+	-	+	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-
a 4	+	+	-	-	+	-	+	+	-	A	A	A	A	-	-	A	A	-	-	A	A	-	-	-
a 6	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	Alk	-	-	-
a 6*	+	+	+	-	+	+	-	-	-	-	-	A	-	-	-	A	-	-	-	Alk	-	-	-	-
a 7	+	+	+	-	+	+	-	-	-	-	-	A	-	-	-	-	-	-	-	A	-	-	-	-
c 2	+	+	+	-	+	-	+	+	-	A	A	A	A	-	-	A	A	A	A	A	A	-	-	-
c 4	+	+	+	-	+	-	+	+	-	-	-	-	-	A	-	A	-	-	-	A	-	-	-	-
c 6	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
c 7	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-
c 8	+	+	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
c 9	+	+	-	-	+	-	+	+	-	A	A	A	A	-	-	A	A	A	A	-	-	+	+	-
c 711	+	+	-	-	+	-	+	+	-	A	A	A	A	-	-	A	A	A	A	-	-	-	-	-
c 712	+	+	-	-	+	-	+	+	-	-	-	A	-	-	-	A	-	-	-	-	-	-	-	-
c 715	+	+	+	-	+	+	+	+	-	-	-	A	-	-	-	A	-	-	-	-	-	-	-	-
d 1	+	+	+	-	+	+	+	+	-	A	-	A	-	-	-	-	-	-	-	-	-	-	-	-
e 2	+	+	+	-	+	+	+	+	-	A	A	A	A	-	-	A	A	-	-	-	-	+	+	-
e 5	+	+	+	-	-	+	+	+	-	A	A	A	A	-	-	A	A	-	-	-	-	+	+	-
e 7	+	+	+	-	-	+	+	+	-	A	A	A	A	-	-	A	A	-	-	-	-	+	+	-

Table 11 (continued)

Organism	straight rod curved rod coccus	Singles pairs chains clusters	Motility	Gram	Kovac's oxidase	Catalase	Starch hydrolysis	Gelatin hydrolysis	Indole	Glucose		Sucrose		Lactose		Mannose		Galactose		Mannitol		Nitrate Reduction		
										aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	NO ₂	NH ₄	gas
D2-703	+	++	+	-	+	+	+	++	-	A	-											+	+	-
D2-703*	+	+++	+	-	+	+	+	++	+	A	A			A	-					A	A	+	+	-
D2-705	+	++	+	-	+	+	+	++	-	A	-			-	-					-	-	+	+	-
D2-705'	+	++	+	-	+	+	+	++	-	A	-			-	-					-	-	+	+	-
D2-706	+	++	+	-	+	+	+	++	+	A	A			-	-					A	A	+	+	-
D2-707	+	++	+	-	+	+	+	++	-	A	-			A	-					A	A	+	+	-
D2-711	+	+	+	-	+	+	+	++	+	Ag	Ag			-	-					A	A	+	+	-
D2-712	+	+	+	-	+	+	+	+	+	A	A			A	A					A	A	+	+	-
D4-701	+	+	+	-	+	+	+	-	-	A	-			A	-					A	-	+	+	-
D4-704	+	++	+	-	+	+	+	++	+	A	A			-	-					A	A	+	+	-
D4-704	+	+	-	-	+	+	+	+	-	A	-			-	-					-	-	+	+	-
D4-706	+	+	+	-	+	+	+	++	-	A	-			A	-					A	-	+	+	-
D4-707	+	+	+	-	+	+	+	+	-	A	-			A	-					A	-	+	+	-
D5-701	+	++	+	-	+		+	++	+	A	A	A	A	A	-					Ag	Ag	+	+	-
D5-701*	+	+	+	-	+		+	++	+	Ag	Ag	Ag	Ag	-	-					Ag	Ag	+	+	-
D5-702	+	+	+	-	+		+	++	+	A	A	A	A	-	-					A	A	+	+	-
D5-702*	+	++	+	-	+		+	++	-	A	A	A	A	A	A					A	A	+	+	-

Table 11 (continued)

Organism	straight rod curved rod coccus	Singles pairs chains clusters	Motility	Gram	Kovac's oxidase	Catalase	Starch hydrolysis	Gelatin hydrolysis	Indole	Glucose		Sucrose		Lactose		Mannose		Galactose		Mannitol		Nitrate Reduction		
										aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	NO ₂	NH ₄	gas
D5-704	+	+	+	-	+	-	-	-	-	-	A	-	A	-	-	-	-	-	A	-	A	+	+	-
D5-705	+	++	+	-	+	-	++	+	+	A	A	Ag	Ag	A	A	-	-	A	A	-	A	+	+	-
D5-705*	+	+	+	-	+	+	++	-	-	A	-	A	A	A	-	-	-	-	-	-	-	+	+	-
D5-708	+	++	+	-	+	-	+	-	-	-	-	-	A	-	-	-	-	-	-	-	-	+	+	-
D5-711	+	+	+	-	+	+	+	-	-	A	A	A	A	A	A	-	-	-	-	-	-	+	+	-
D7-701	+	++	+	-	-	+	++	-	-	A	A	A	A	A	A	-	-	-	-	-	-	+	+	-
D7-701*	+	++	+	-	-	+	+	-	-	-	-	A	A	-	-	-	-	A	-	-	-	+	+	-
D7-702	+	+	+	-	+	+	+	-	-	A	-	A	-	A	-	-	-	-	-	-	-	+	+	-
D7-703	+	+	-	-	+	+	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	+	+	-
D7-706	+	+	+	-	+	+	++	-	-	A	A	A	-	A	-	-	-	-	-	A	A	+	+	-
D7-706*	+	++	+	-	+	+	++	-	-	A	A	A	-	-	-	-	-	-	-	-	-	+	+	-
D7-707	+	++	+	-	+	+	+	+	+	A	A	A	-	-	-	-	-	-	-	-	-	+	+	-
D7-707*	+	++	+	-	+	+	++	+	+	A	A	A	A	-	-	-	-	-	-	-	-	+	+	-
D7-707'	+	++	+	-	+	+	++	-	-	A	A	A	A	-	A	-	-	-	-	-	-	+	+	-
D7-711	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
D7-Ps	+	+	+	-	+	+	+	-	-	A	-	-	-	-	-	-	-	-	-	-	-	+	+	-
D8-701	+	++	+	-	-	-	-	-	-	A	A	A	A	A	A	-	-	-	-	-	-	+	+	-
D8-702	+	++	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 11 (continued)

Organism	straight rod curved rod coccus	Singles pairs chains clusters	Motility	Gram	Kovac's oxidase	Catalase	Starch hydrolysis	Gelatin hydrolysis	Indole	Glucose		Sucrose		Lactose		Mannose		Galactose		Mannitol		Nitrate Reduction		
										aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic	NO ₂	NH ₄	gas
D8-702	+	++	-	-	-		+	+	-	A	A	A	A	A	A							+	+	-
D8-702'	+	+	+	-	+		+	++	-	A	A	A	A			A	A					+	+	-
D8-703	+	++	+	-	+		-	-	-	A	A	-	-	A	A							+	+	-
D8-706	+	++	+	-	+		-	-	-	A	A	-	-	-	-							+	+	-
D8-707	+	++	+	-	+		-	-	-	-	-	-	-	-	-							+	+	-
D8-711*	+	+	+	-	+		+	++	-	-	A	-	A	-	A							+	+	-
D8-712	+	++	+	-	+		+	++	+	A	A	A	A			A	A							-

TABLE 12

Synopsis of data obtained thus far on the characteristics of bacteria isolated from epiphytic communities living on Enteromorpha intestinalis.

Total number of isolates: 128

Characteristic (s)	# of organisms	% of organisms tested
rod	126	98
coccus	2	2
gram negative	127	99
gram positive	1	1
motile	111	87
non-motile	17	13
gram negative, motile rod	111	87
gram negative, non-motile rod	14	11
gram negative, non-motile coccus	2	2
ferment glucose	67	54
oxidize glucose	27	22
(-) glucose	29	24
ferment sucrose	45	42
oxidize sucrose	24	23
(-) sucrose	37	35
ferment lactose	21	20
oxidize lactose	17	17
(-) lactose	65	63
ferment mannose	27	
oxidize mannose	17	
(-) mannose	15	
ferment galactose	16	35
oxidize galactose	6	13
(-) galactose	24	52
ferment mannitol	30	39
oxidize mannitol	16	20
(-) mannitol	33	41
nitrate reduction to NO ₂	80	93
nitrate reduction to NH ₄	86	100
nitrate reduction to gas	2	2

TABLE 13

Synopsis of characteristics of isolates from individual media.

Medium	GLUCOSE				SUCROSE				LACTOSE			
	# tested	# fermenting	# oxidizing	# negative	# tested	# fermenting	# oxidizing	# negative	# tested	# fermenting	# oxidizing	# negative
a	5	1	0	4	5	1	3	1	5	0	0	5
c	9	3	0	6	9	3	2	4	8	0	1	7
d	1	0	1	0	1	0	1	0	1	0	0	1
e	4	4	0	0	4	4	0	0	4	0	0	4
A 1	5	2	0	3	5	2	0	3	5	0	0	5
A 2	6	2	2	2	6	0	0	6	6	0	0	6
A 3	1	0	0	1	1	0	0	1	1	0	0	1
A 5	2	5	1	3	3	4	0	4	3	0	2	3
B 1	3	3	0	0	2	2	0	0	2	0	0	2
B 2	3	3	0	0	3	3	0	0	3	1	1	1
B 3	4	4	0	0	5	0	4	1	5	0	1	4
C 1	10	7	2	1	8	4	3	1	6	4	0	2
C 2	3	0	2	1	3	0	2	1				
C 3	9	3	3	3	10	3	1	6				
D 1	8	3	4	1	8	1	5	2	8	1	5	2
D 2	10	6	4	0					10	2	2	6
D 4	5	1	4	0					5	0	3	2
D 5	9	7	1	1	9	9	0	0	9	3	2	4
D 7	11	6	3	2	10	4	4	2	10	2	2	6
D 8	8	7	0	1	8	5	0	3	6	4	0	2

E. Mixed culture studies on the synergistic or antagonistic effects of algae and bacteria from natural associations including:

- i) the effects of epiphytes on the growth and differentiation of Enteromorpha
- ii) the effects of Enteromorpha on its epiphytes
- iii) the isolation and identification of extra cellular, possibly community regulatory, substances released into the media.

1) Nitrogen budget of the aufwuchs community (Dissertation project of Ms Patricia Sullivan).

Preliminary experiments in the summer of 1973, using the acetylene reduction technique (Hardy et al. 1968), have suggested that N_2 fixation does occur among the epiphytes of Enteromorpha. Published data indicate that the blue green algae are the most important nitrogen fixers in the sea (Stewart, 1966; Brooks et al. 1971). Therefore, attempts are currently being made to isolate the blue-green epiphytes of Enteromorpha. The technique being followed is basically that of Van Baalen (1961) who succeeded in isolating a number of blue-greens occurring in coastal marine environments, many from the Long Island area. A small piece of Enteromorpha is transferred into an enrichment media (a modification of Provasoli's 1957, ASP-2 media) and incubated in a light bank with aeration. Later, plates are inoculated with 0.1 ml, sealed and incubated in the light for up to 3 months. After growth, algal colonies were picked and transferred.

Temperature has been used as a selective factor in isolating blue-greens from water and soil (Allen and Stainer, 1967). Incubation at 35 C suppressed eukaryotic algae development. This technique was not successful with the salt marsh Enteromorpha epiphytes, due to the ability of these organisms to tolerate high temperatures (Saks and Lee 1973).

The addition of cyclohexamide (actidione) to media is now being used to inhibit eukaryotic algae (McCurdy and Hodgson 1973). Pure cultures of blue-greens are difficult to obtain because bacteria live in the gelatinous sheaths. Vibration of algal samples with glass beads in a Bradson Sonifier followed by washing with sterile modified ASP-2 media and low pressure ($2\frac{1}{2}$ lbs/in²) filtration through 0.8 μ filters has been tried in an attempt to obtain axenic cultures. But these efforts have met with little success.

So far, the best results have been from picking algae which seem bacteria-free when viewed under the binocular microscope and streaking on new plates. Repeated transfers should yield axenic cultures.

2. Enteromorpha culture

Cultures of Enteromorpha intestinalis presently being maintained have been clones from one culture which demonstrated good growth, slight differentiation and zoospore production. Attempts at removing the bacterial contaminant by washing, dragging and using antibiotics have been unsuccessful. Cultures which may appear to be bacteria-free within 1-3 weeks of transfer (i.e., no visible turbidity in the liquid overlay or around the germlings) have been found to contain a fine film of bacteria on the solid substrate. In cultures where this film is extensive, the transferred germlings, as well as those resulting from zoospore production seem to be able to successfully attach to the substrate and survive but do not differentiate. In cultures where attachment does not occur, the algae become surrounded by a slight cloud of bacteria, bleach and die.

Routine maintenance of Enteromorpha has been bothered by an increasing proportion of unsuccessful transfers. Within the last few months, various changes have been introduced into the culturing program. Stock medium S is being used in place of Base B for the Enteromorpha medium and 150 ml Ehrlenmeyer flasks with some solid substrate and 25 ml liquid overlay have replaced tubes in a portion of the transfers. Both changes have been successful. We have obtained hundreds of germlings from the flask cultures alone.

Since the bacterial contaminants do not appear to enhance the growth of the algae and since our stock cultures of Enteromorpha have increased about 10-fold, pilot experiments have been started to test the effect of bacterial epiphytes of the macroalgae on its growth and development. Using S medium without added IAAA and 1 or 2 small, washed (3 serial washings with 10 ml sterile sea water) germlings, 17 bacteria have been tried. After 3 weeks incubation, only 1 of the bacteria seems to enhance the development of the Enteromorpha. With this organism (D4-701) present, growth and differentiation of the algae appear normal and better than in the control tubes with and without IAA and without bacteria. It is interesting to note that this bacterium was isolated from the same differential medium that yielded the greatest development of Enteromorpha zoospores from field samples. Germlings mixed with bacterium were morphologically abnormal while the algae mixed with the remaining 15 bacteria did not survive at all. In all cases, bacterial growth was moderately heavy. This series of experiments is being repeated and added to in order to determine if the effects observed can be consistently reproduced.

- F. Field experiments on transplanted and local natural communities aimed at testing the changes in community structure at a particular location due to: local water quality, heavy metal toxicity and water insoluble fractions of crude petrochemicals and insecticides.

For the past 3 years we have been attempting to develop an apparatus which would permit us to do some critical field experiments under ambient, or as close to ambient conditions as possible. The device we were seeking to develop had to have a reasonably free exchange of sea water and metabolites with the surrounding sea water, retain all the algae and meiofauna, and be able to withstand

the stresses imposed by natural environmental perturbations. Our initial studies in the summer of 1971 used dialyzing membranes to retain the organisms and allow exchange of soluble materials with the environment. The membranes were tied at both ends and suspended in racks above the marsh surface. Every design we tried during the summer had poor performance characteristics. It seemed to make little difference if the membranes were tightly suspended or loosely suspended, wind and wave action tore them in less than a week. Membranes of different manufacture were almost equally as fragile in the environment. In the summer of 1972, experimental vessels were made from 60 ml serum bottles with their bottoms cut off. The edges of the bottom were coated with paraffin and the bottoms were covered by a dialyzing membrane bound on nylon fishing line. The vessels were then secured in wooden racks which held 3 replicates of 10 different experimental situations. At each station the natural community from all 3 experimental stations, Sandy Hook, N. J., North Sea Harbor, L. I. and Jamaica Bay, N. Y., were incubated inside the experimental vessels as was the mixed inocula from all 3 areas. Other variables included inocula from the same station incubated with chunks of metallic Zn, Cu, Cr, Fe, Pb and crude oil. The dialysis membranes were obtained from the food products division of Union Carbide and have, according to the manufacturer, an average pore radius of 24 μ . The initial inocula and each of the 1 ml weekly samples were individually preserved in the field in small vials containing 95% alcohol. In the laboratory each sample was transferred to centrifuge tubes and washed 3 times with distilled water to remove the salt. The diatoms were then treated with H_2O_2 , dehydrated in ethanol, and placed on microscope slides. They were mounted using Zeiss phase contrast mounting medium (Einschlubmittel L 15).

On June 27, 1972, hurricane Agnes destroyed our experimental apparatus. The results of the initial experiment are tabulated (Tables 14-16). The experiment was begun again on July 12, 1972. By the time 3 weeks had passed, it became obvious that the dialysis

membranes were weakening and breaking. After the membranes were examined by phase microscopy, it became obvious that they were being attacked by cellulose digesting bacteria and were unsuitable for this type of long-term experiment. Consultation with many manufacturers led to the conclusion that none of the dialyzing membranes currently on the market would be suitable for this kind of experiment. After a careful survey of the materials commercially available, we chose nylon filters with a 1 μ M radius pore (Millipore NRWP 142 50) for additional experiments which were begun on August 15, 1972 and ran until September 19, 1972. The membranes proved durable and gave promise for utility in the experiments we did last summer. The diatom population structure of one set of experimental flasks, those containing iron, was examined. In addition to a reduction in population size in the experimental flasks we also found considerable changes in the structure of the community (Table 17).

This past summer we finally tested a device which meets performance criteria. The experimental vessels are made from falcon tissue culture flasks which are perforated by 12 1.25 cm holes, and to which 1 μ M pore size nylon membranes (Millipore, Duralon NRWP 142 50) have been bonded. These are held in a rugged apparatus constructed of lucite designed to hold each flask horizontally 3 cm from the substrate. The bottom of the flasks are not in direct contact with the holding apparatus. Below each flask is a 1 cm high chamber, the floor of which is perforated with many 0.6 cm holes (see attached photograph). The apparatus was then suspended between poles driven into the marsh substrate.

Two experiments were done this year using the experimental apparatus at Sippewisset Marsh, at Jamaica Bay, and at Towd Point in Southampton, New York. In the first experiment, populations of organisms from each of the field locations were transplanted to each of the other locations. A mixture of the inoculum from all 3 locations was incubated with reagent grade chunks of Fe, Cu, Zn, Cr, Pb and crude oil.

TABLE 14

Comparison of the inocula from the 3 field stations used in the study. * **

Species	Jamaica Bay		Sandy Hook		Southampton	
	#	%	#	%	#	%
<u>Cyclotella striata</u>	884	87.6	648	64.41	100	9.73
<u>Achnanthes hauckiana</u>	23	2.28			65	6.32
<u>Navicula cryptocephala</u>	26	2.58				
<u>Synedra gallioni</u>			75	7.46	152	14.79
<u>Achnanthes longipes</u>			94	9.34		
<u>Opephora martyi</u>					85	8.27
<u>Fragilaria construens</u>					42	4.09
<u>Skeletonema costatum</u>					32	3.11
<u>Achnanthes wellisiae</u>					33	3.21
<u>Nitzschia sigma</u>					36	3.50
<u>Navicula salinarum</u>					34	3.31
<u>Navicula mensicoides-like</u>					34	3.31
<u>Nitzschia apiculata</u>					25	2.43
<u>Navicula halophila</u>					27	2.62
<u>Cocconeis scutellum</u>					25	2.43
Total specimens counted	1009		1006		1028	
Σ %	89.88		81.21		67.1	
# of species identified	33		50		87	

* If species represented < 2%, it was not included in the results.

** Samples taken in the field June 9, 1972.

TABLE 15.

Seasonal succession in the diatom assemblage
of Enteromorpha growing in Jamaica Bay.

Date	6-9-72		6-15-72		6-23-72		6-27-72	
Count (species)	1009(33)		1026(67)		1009(49)		1002(54)	
Species	#	%	#	%	#	%	#	%
<u>Cyclotella striata</u>	884	87.6	479	46.69	530	52.53	624	62.28
<u>Cyclotella</u> sp			28	2.73	44	4.36	21	2.01
<u>Achnanthes hauckiana</u>	23	2.28	65	6.64	103	10.21	53	5.29
<u>Achnanthes wellsiae</u>			106	10.33	65	6.44	77	7.68
<u>Navicula cryptocephala</u>	26	2.58	34	3.31	30	2.97		
<u>Navicula incomposita</u> var.			35	3.41				
<u>Fragillaria construens</u>			23	2.24	21	2.08		
<u>Coscinodiscus</u> sp					36	3.57		
<u>Nitzschia proxima</u>					21	2.08		
Σ %		89.88		75.35		76.51		77.26

TABLE 16

Comparison of samples from the control flasks
and the inoculum after 1 week incubation. *

Species	Inoculum		Control flask	
	#	%	#	%
<u>Cyclotella striata</u>	884	87.6	181	41.9
<u>Achnanthes hauckiana</u>	23	2.28	12	2.78
<u>Navicula cryptocephala</u>	26	2.58		
<u>Melosira nummuloides</u>			48	11.11
<u>Synedra gallioni</u>			26	6.02
<u>Cocconeis scutellum</u>			19	4.40
<u>Melosira sulcata</u>			16	3.70
<u>Cyclotella</u> sp			16	3.70
<u>Opephora martyi</u>			14	3.24
<u>Coscinodiscus</u> sp			10	2.31
<u>Striatella unipunctata</u>			9	2.08
<u>Achnanthes longipes</u>			9	2.08
<u>Navicula incomposita</u> like			9	2.08
Total number of specimens counted		1009		432
Σ %		92.5		86.4
# of species identified		33		44

* If species represented < 2%, it was not included in the results.

TABLE 17.

The effect of iron on the growth and community structure of natural populations of diatoms.

Species	Control 1014 (79)		Iron Treatment *	
	#	%	#	% **
<u>Rhopalodia musculus</u> (+ " <u>gibberula</u> ")	299	29.48	11	5.85
<u>Navicula digitoradiata</u>	90	8.87	30	15.95
<u>Nitzschia sigma</u>	77	7.59	14	7.45
<u>Achnanthes hauckiana</u>	54	5.32	9	4.78
<u>Opephora martyi</u>	52	5.12	9	4.78
<u>Bacillaria paradoxa</u>	42	4.14	6	3.19
<u>Cyclotella</u> sp	35	3.45		
<u>Achnanthes longipes</u>	31	3.06		
<u>Synedra gallioni</u>	27	2.66	10	5.31
<u>Melosira nummuloides</u>	22	2.16	7	3.72
<u>Pleurosigma angulatus</u>			10	5.31
<u>Melosira sulcata</u>			8	4.25
<u>Nitzschia panduriformis</u>			6	3.19
<u>Caloneis westii</u>			5	2.65
<u>Pleurosigma affini</u>			4	2.12

* Fewer individual diatoms were present in these vessels (3 replicates).

** If species represented < 2%, it was not included in the results.

Each week, 5 ml samples were removed from each of the experimental vessels. Diatom community size estimates were made by hemocytometer. Peroxide cleaned frustules are being used for the study of the diatom population structure. Inocula for the experiments was 1×10^4 cells/ml. In the transplantation experiments all the communities seemed to thrive fairly well in Sippewisset. The community from Southampton grew best, in terms of total numbers of organisms/ml in Sippewisset marsh; Jamaica bay was the worst initially, but eventually it came up to the Sippewisset level.

At Sippewisset, Fe did not effect the overall growth of the diatom community initially, but eventually growth was < 30-50% of controls. Cu and Pb strongly inhibited growth. Population size in the presence of these ions was generally from 5-45% of controls. The effect of crude oil was similar. Except for an initial lag, Zn and Cr were less inhibitory. Zn was the least inhibitory of all those tested; populations fluctuated from 15-100% of controls. Cr populations varied from about 15-80% of controls.

About 250 species have been collectively found in the 3 marshes. The greatest diversity in any individual sample analyzed so far was 96 species. The analysis of the effects of individual ions on the community structure is very complex and very time consuming as any one who has studied diatom communities will tell you. Only initial results are available today. We hope to finish the complete study in the next ~ 2-3 years.

In the first week of incubation, crude oil completely inhibited the growth of Melosira nummuloides, whereas the growth of M. sulcata was enhanced. The growth of Synedra fasciculata was relatively enhanced in the presence of Fe, Cu, Zn and Cr. Cu depressed the growth of Opephora martyi. Zn more than doubled the proportion of A. hauckinana. Most ions depressed the growth of N. microcephala.

As mentioned in the accompanying renewal proposal, some of the preliminary data have already been published or is in press (see C00 3254-21 and C00 3254-16 in later sections of this progress report). The slide examination is itself extremely time consuming, and the bulk of the laboratory work remains to be done in the next year.