FIELD VALIDATION OF LABORATORY-DERIVED MULTISPECIES AQUATIC TEST SYSTEMS

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ABSTRACT

A three-year study was carried out to determine the feasibility of using multispecies microcosms of benthic microorganisms and infaunal macroinvertebrates to predict the responses of estuarine systems to toxic substances. Criteria were developed to evaluate the field validation of laboratory microcosms. Simultaneous laboratory/field experiments were carried out in the Apalachicola Bay system, Fla., and York River estuary, Va., to test the potential for extrapolation of validation results from one ecological system to another. The study demonstrated that microcosms of microorganisms and infaunal macroinvertebrates can be established for short periods (5-6 weeks) and that the microcosms can be used to simulate specific features of field assemblages within the range of uncertainty that is characteristic of natural systems. Moreover, validation results can be extrapolated from one system to another as long as the systems share common habitat features and dominance relationships of important populations.

Water quality in the microcosms essentially paralleled that in the field, although variation of certain water features and sediment characteristics was noted. These laboratory artifacts were apparently caused by the isolation of the microcosms from natural phenomena of the estuarine environment that were not replicable in the laboratory. Physical habitat features and biological responses in the respective study areas were extremely complex and highly variable in space and time. Factors such as water and sediment quality, predator-prey relationships, recruitment, and dominance relationships among infaunal populations influenced the community structure of benthic organisms in the laboratory and the field. However, the relative influence of physical and biological factors varied considerably between habitats and through time. Consequently, the extent to which the microcosms paralleled field conditions depended to a considerable degree on the time of testing and dominance/recruitment features of the system in the source area.

Specific populations of microorganisms and infaunal macroinvertebrates in the microcosms sometimes differed from field populations. These differences were often associated with known artifacts in the laboratory and could be qualified as such. The establishment of the microcosm was a crucial factor in the continuity and success of the microcosms. Microorganisms were particularly sensitive to disturbance during the establishment of the microcosms. Microbes in microcosms taken from polyhaline areas showed considerable divergence from field associations within two weeks after the intiation of the experiment. However, in microcosms taken from highly variable (physically dominated) oligohaline portions of the estuary, the microbial associations paralleled field conditions rather well over a six-week period. Microcosms of infaunal macroinvertebrates differed from field conditions depending on the timing of the experiment

and recruitment features of the subject populations. Thus, the predictive capacity of the laboratory microcosm depended to a considerable degree on habitat characteristics and recruitment trends in the source area at the initiation of the microcosm.

Microcosms of benthic organisms, although sometimes characterized by changes in individual populations, provided a broad spectrum of biological information for use in evaluating the impact of toxic wastes. Community indices such as species richness approximated field conditions quite closely. Criteria developed during this study can be used to determine the applicability of laboratory microcosms to toxicity tests.

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FOREWORD

The primary aim of this project was to carry out a series of experiments with multispecies microcosms of estuarine microbes and macroinvertebrates to develop criteria for the verification of laboratory test results relative to the field. The project was conducted in two completely different areas (i.e., the Apalachicola estuary in the northeast Gulf of Mexico and the York River estuary in the Chesapeake Bay system) to develop the data necessary for the extrapolation of results from one estuary to another. This work was undertaken by three separate research groups under the direction of Robert J. Livingston (Florida State University), Robert J. Diaz (Virginia Institute of Marine Science), and David C. White (Florida State University). The first year of the project (1982-1983) was devoted to the development of experimental protocols. Experiments were carried out during the subsequent two years to develop the criteria for the verification process. These criteria will be employed to evaluate the usefulness of multispecies laboratory tests in predicting the response of estuarine organisms to toxic substances under field conditions.

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SECTION 1

INTRODUCTION

The basic question that underlies the considerable effort to understand pollution-induced changes in aquatic systems is well established: what is required to predict the environmental effects of a toxicant or stimulatory substance on a given ecological system? With the recent development of sophisticated toxicological methods to evaluate acute and chronic effects of toxicants on laboratory populations, the question then becomes: what is required to establish a reliable measure of the capability of specific laboratory test systems to predict actual environmental effects of a given toxic agent?

We define the process of field validation as the testing of the capacity of specific laboratory test systems to predict the environmental responses of natural ecosystems, or portions thereof, to toxicants. Once a test system is validated, it provides a means of generating toxicological data that can be realistically correlated with expected field impacts. The process of validation necessitates two pursuits: selection of a particular test system and acquisition of knowledge about the natural variation and dynamics of field populations from which the test system is derived. Without knowledge of ecosystem structure and function, it is practically impossible to evaluate toxic effects.

There is a need to develop and use multispecies laboratory tests to evaluate the environmental implications of toxic waste disposal (Hammons et al., 1981). However, because of basic differences between laboratory and field conditions, the prediction of effects, in the field, based on laboratory results, is extremely complex and not well understood. Cairns et al. (1981) provided a thorough background for the need to verify laboratory test systems in the field; these authors have already provided procedures for determining the response of multispecies test systems to perturbation. A detailed review of the problems related to field verification of laboratory results is given by Livingston and Meeter (in press). Realistic simulation of field conditions and generality of test results from ecosystem to ecosystem require an identification of those aspects of the laboratory microcosm that "most accurately represent the natural prototype" (Hammons et al., 1981). In addition to the laboratory-to-field relationship, the question of generality of findings (i.e., location-to-location extrapolation of results) is a significant part of the verification process.

The focus of our three-year project has been microbial and infaunal macroinvertebrate communities of unvegetated soft sediments of shallow estuaries in Florida (Apalachicola Bay system) and Virginia (York River

estuary). Natural soft-bottom macroinvertebrate communities were selected for the test systems because of their trophic importance and the close association of such organisms with sediments, which mediate the fate of most toxicants. Also, there is a considerable body of literature concerning the response of the benthos to disturbances, and this information will facilitate the validation process. In terms of the ability to replicate, realism, generality, and potential application to protocol development, sediment cores as multispecies microcosms represent one of the most potentially useful methods in intermediate and advanced stages of hazardous waste assessment (Hammons et al., 1981).

Based on extensive background work in the respective estuaries by Florida State University and the Virginia Institute of Marine Science, a project was designed to answer the following questions:

1. What are the criteria for laboratory-to-field verification of multispecies test systems based on communities of estuarine microbes and macroinvertebrates?

2. What are the predictive capabilities of laboratory multispecies microcosms relative to natural processes under semi-field and full-field conditions?

3. Can results be extrapolated from one estuary to another?

Our principal objectives (a) were to evaluate the capacity of the laboratory test as a realistic analog or simulation of the natural community from which it was derived and (b) to develop criteria for field verification of laboratory results. The evaluation considered validation at three levels: physico-chemical differences, differences in population and community structure, and functional differences between full-field and semi-field treatments and laboratory microcosms. Results of such tests are being applied to current experiments that concern the predictive capability of microcosms exposed to toxic substances. Concurrently, a complete review is being undertaken to determine the potential for extrapolation of validation results from one location to another.

SECTION 2

CONCLUSIONS

A three-year study was conducted to determine the capability of specific laboratory test systems to predict environmental (field) effects of toxic substances. Multispecies microcosms of infaunal macroinvertebrates and microorganisms were used with simultaneous experiments in the Apalachicola Bay, Florida, system and the York River, Virginia, estuary to test for the feasibility of extrapolation of validation results from one ecological system to another.

Field conditions in the study areas were characterized by short-term disturbances (i.e., wind and tidal currents) and seasonal changes in the physical environment. The microcosms followed various physical aspects of the field habitat rather closely. However, storm-induced disturbances were not replicated and current regimes in the field were not simulated in the laboratory. Despite slightly increased accumulation of silt under laboratory conditions relative to the field, no significant changes were noted in various sediment properties among laboratory and field treatments.

Biological interactions in the field were complex and highly variable in space and time. Physico-chemical habitat changes, predation, and recruitment influenced the macroinvertebrate assemblages with differential effects exerted along habitat gradients and during different seasons of the year. Changes in the macroinvertebrate assemblages in the microcosms were due, in part, to alterations during transfer from field to laboratory, lack of motile predators in the laboratory, and altered recruitment. Such changes appeared to depend on the timing of the test and the natural assemblages of macroinvertebrates in the source areas at the initiation of the microcosm.

Experiments carried out in two different estuaries showed that the basic controlling features and microcosm response relative to the field were quite similar. The initial establishment of the microcosm and time-based alteration of recruitment in the laboratory microcosms were the most important elements contributing to changes in the microcosms relative to field conditions. The timing of the test, relative to seasonal changes in recruitment, was also an important aspect of the validation process. Thus, correct interpretation of microcosm results relative to field processes depends on an understanding of natural community processes. No single species in the laboratory was consistently representative of field conditions either because of laboratory artifacts or because of specific responses of individual populations to laboratory conditions.

The laboratory microcosm approach has considerable potential for evaluating microbial or macrobiological responses to natural disturbances or toxic effects in the field. Multispecies microcosms have the advantage of incorporating various forms of community level information into the experimental design; such information is not available in single-species tests. However, because of the extremely complex relationships of such associations, a thorough knowledge of the ecology of a given site is necessary for a reasonable application of laboratory-to-field or field-to-field extrapolations. For example, the microbial community in estuarine areas was particularly sensitive to both physical and biological sources of disturbance. In polyhaline areas, a relatively low variance was noted in field assemblages of microbes. Microcosms from polyhaline areas showed significant differences in the microbial associations after two weeks, and such differences between the laboratory and field increased substantially between week 2 and week 6 of the experiment. However, in physically driven oligohaline portions of the estuary, differences between laboratory and field assemblages were small and did not increase with time. Thus, although the microbial component of a laboratory microcosm was highly variable, the capacity of such a microcosm to predict natural trends depended on the habitat characteristics in the area of origin. It appeared that microcosms taken from areas subjected to continuous natural physical disturbances were better indicators of natural phenomena than were those taken from more physically stable systems.

Our experimental results demonstrated that microcosms of soft-sediment macroinvertebrates can be established for short periods (5-6 weeks) and that changes in the field populations can be either reflected in the overall response of the microcosms or accounted for in terms of specific laboratory artifacts. Moreover, extrapolation of such results from one system to another is possible within the range of uncertainty that is characteristic of natural systems. Just as extrapolation of results from the microcosm to the field cannot, by definition, be a direct process, so too is extrapolation from one ecosystem to another seriously qualified by funtional differences in community processes of such systems. With adequate qualification based on ecological knowledge of the areas in question, both verification and extrapolation are feasible within the limits of natural variation.

SECTION 3

RECOMMENDATIONS

The strength of the validation of a given microcosm depends on an assessment of the laboratory reaction of populations of individual species within the uncertainty that is natural to ecological systems. It is recommended that validation processes be evaluated according to criteria developed by our studies. Further analysis is needed to relate how well microcosms reflect the response of natural ecosystems to toxicants. The validation approach proposed by our research reflects the need to calibrate laboratory microcosms with established processes in the field. More work is needed to develop validation procedures for processes in natural communities in addition to structural aspects of the estuarine communities that have been emphasized in this research.

SECTION 4

MATERIALS AND METHODS

All field and laboratory operations in the respective study areas followed standardized methods. Aside from certain differences inherent in the two study sites, experimental procedures were carried out in a comparable manner. Detailed accounts of methods and material used in this project are given by Livingston et al. (1893) and Diaz et al. (1984).

Prior to the initiation of the project, all background field data from the study areas were updated and evaluated to establish a preliminary protocol for the full-laboratory, semi-field, and full-field treatments. Based on preliminary analyses of background data, the spatial limits and frequency and location of sampling were determined. A brief review of habitat parameters, biological characteristics, and methods of sampling appears in Appendix A.

The study sites in the Apalachicola Bay system (East Bay and St. George Sound) were shallow (1-2 meters (m)), unvegetated soft-bottom areas located in oligohaline (stations 3, 5A) and polyhaline (station ML) areas (Figure 1). Sediments in the oligohaline areas were silty sand, whereas sediments in the polyhaline zone were largely fine sands (1-2% silt-clay). The York River study site (Figure 2) was a shallow (1.5 m), unvegetated soft bottom located in the meso-polyhaline portion of the estuary.

Microcosms were constructed of a series of cores collected with hand-operated box corers (10 x 20 centimeters (cm); 10 cm deep). Core samples were placed in trays on sea-water tables in the same arrangement as the original field orientation of the cores. The size of each microcosm was 0.8 to 1.0 square meters (m^2). Light, temperature, and salinity regimes followed field conditions. Synoptic biological sampling of microcosms and field was done randomly with coring devices (5 cm, VIMS; 7.5 cm, FSU). Sieves of mesh sizes 250 and 500 micrometers (μm) were used for the infaunal macroinvertebrates. Microbial samples were taken from field areas and laboratory microcosms with a 3.2-cm-diameter corer and analyzed for lipids and fatty acids.

Four field-laboratory experiments were carried out over a 2-year period. The tests were conducted during spring and fall periods of peak biological activity and change in the respective study sites. Although some changes were made to the sampling program over the study period, a basic protocol was developed and followed for experiments at both sites (Appendix B). The approach was to sample replicated flow-through laboratory microcosms (0.8-1.0 m^2) derived from natural soft-sediment

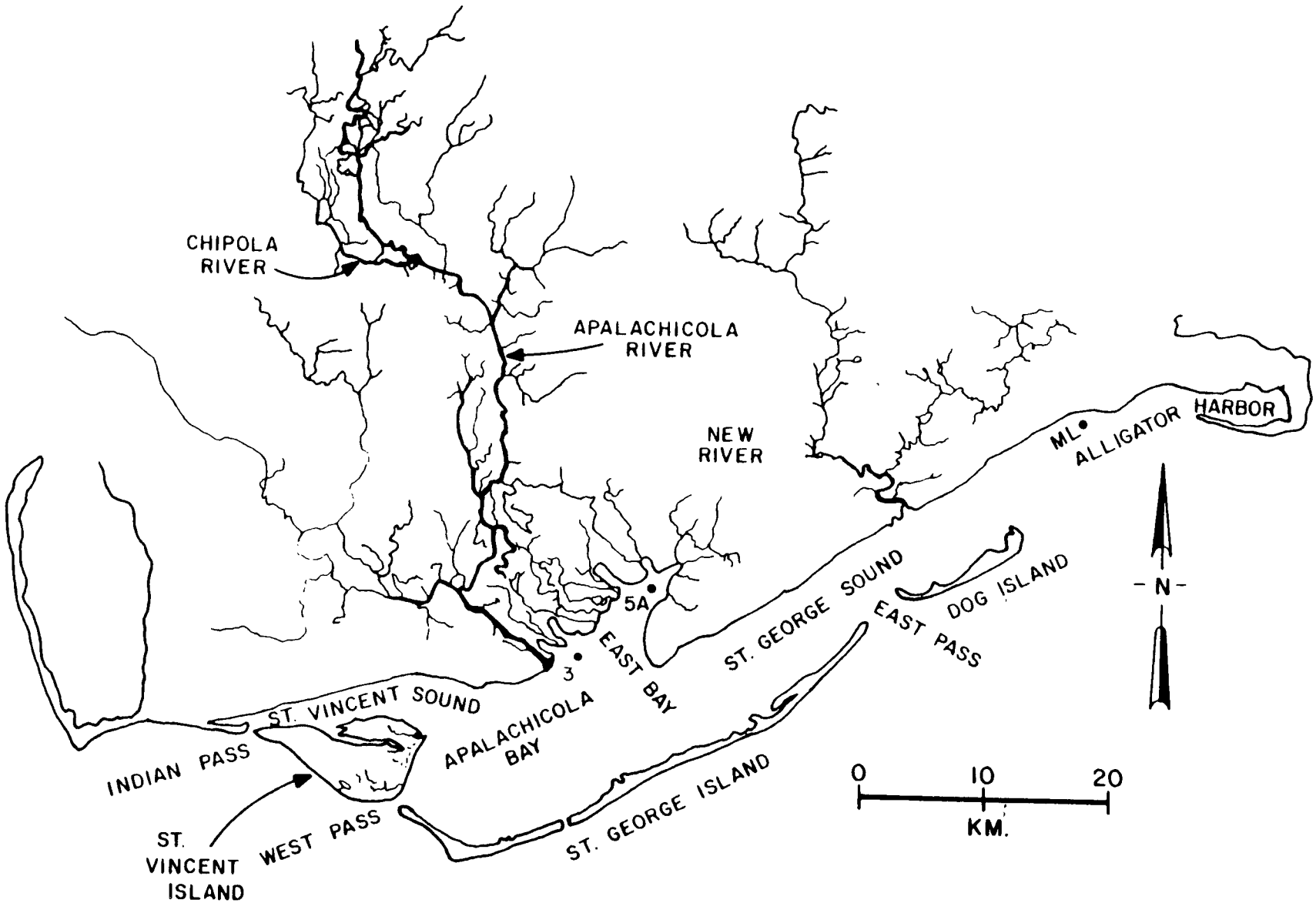


Figure 1. Study sites in the Apalachicola Bay system (Florida)

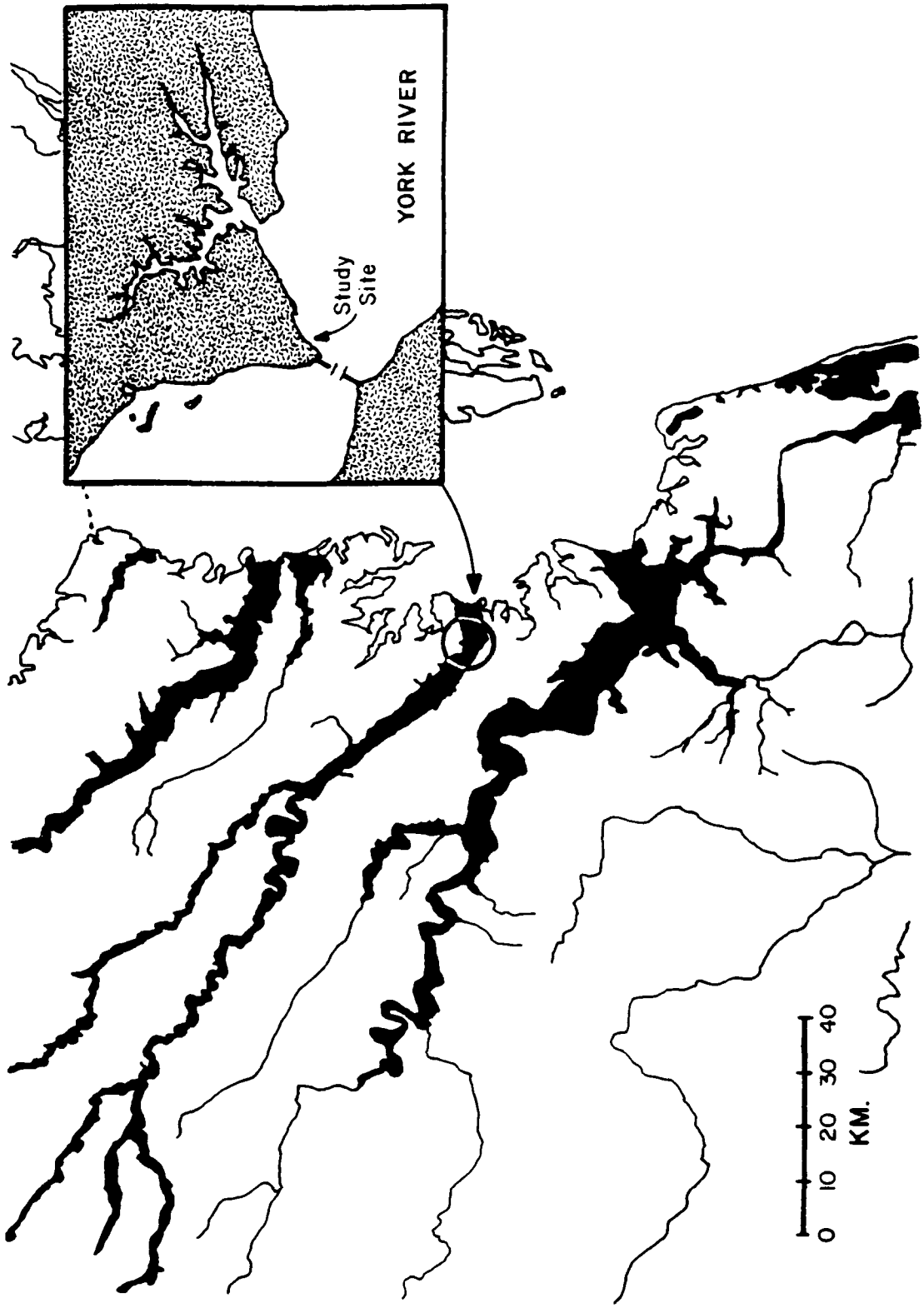


Figure 2. Polyhaline site in the lower York River (Virginia).

areas, simultaneously with field treatments (exclusion cages, inclusion cages, cage controls) (Figure 3). A sampling schedule is given in Appendix C.

Variables analyzed during the experimental series included numerical abundance (total number of individuals and dominant populations), numbers of species, and species diversity. All analyses were carried out with and without $\log_{10}(x+1)$ transformations. A nested ANOVA analysis to test for differences between laboratory microcosms was carried out with 250- and 500-μm sieve fractions (macroinvertebrates) and microbial parameters. To test the null hypothesis that there was no significant difference among field and laboratory treatments with respect to the variables listed above, selected ANOVA models were employed. A one-way ANOVA was run on all treatments by sampling period. A randomized block repeated-measures ANOVA was used with the field data with location as the blocking factor and time as the repeated measure. Tukey's method of multiple comparisons was used to test the differences between all possible pairs of means. Analyses of qualitative changes in infaunal assemblages were carried out using "rho" (Matusita, 1955; Van Belle and Ahmad, 1974) and Czekanowski (Bray-Curtis; Bloom, 1981) similarity coefficients and the flexible grouping strategy with beta = -0.25 (Lance and Williams, 1967).

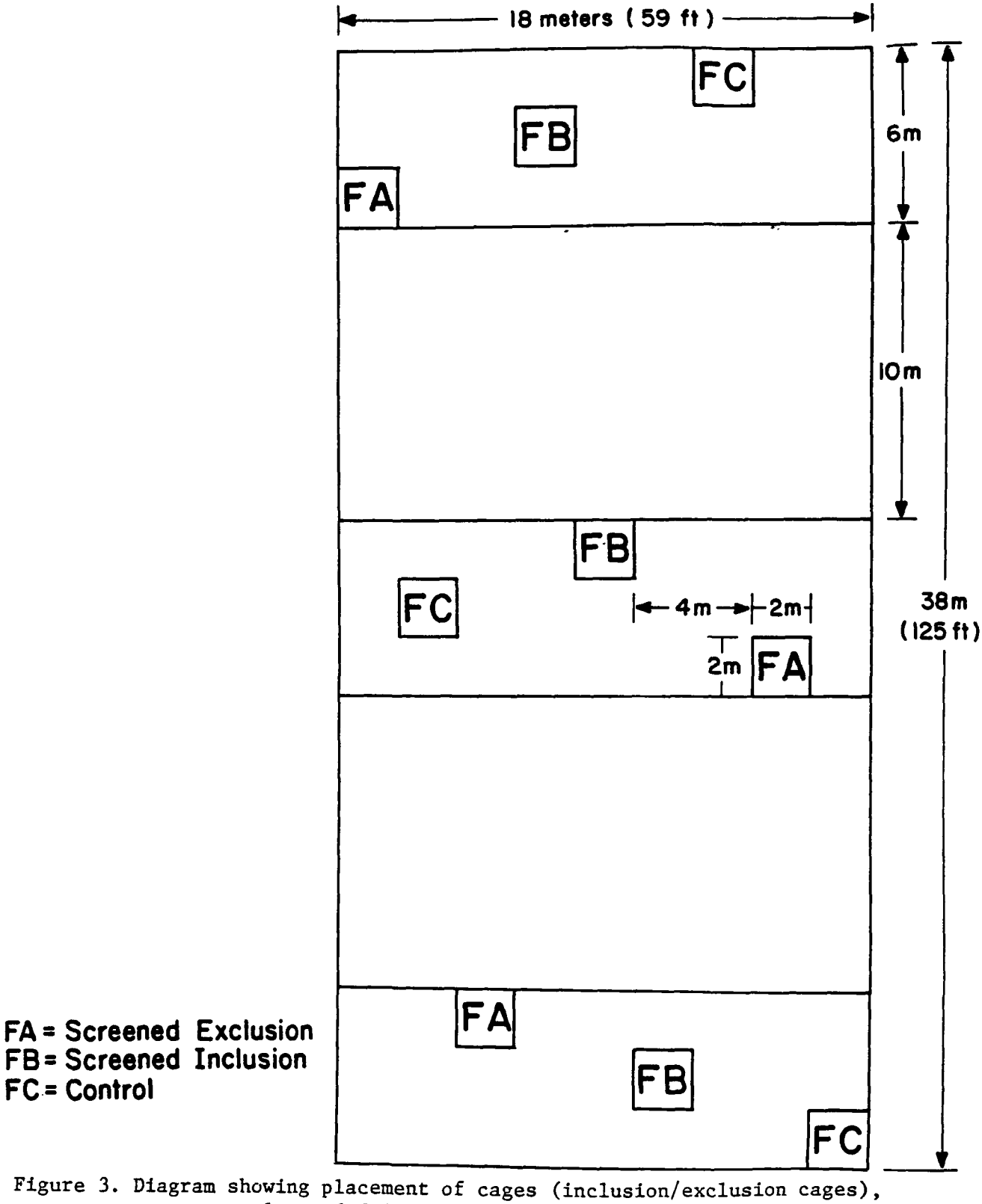


Figure 3. Diagram showing placement of cages (inclusion/exclusion cages), cage controls, and full-field sampling areas.

SECTION 5

RESULTS AND DISCUSSION

EXPERIMENTAL PROGRAM: FLORIDA STATE UNIVERSITY

Infaunal Macroinvertebrates: Background

Preliminary experiments were carried out to determine scaling parameters associated with the spatial distribution of infaunal macroinvertebrates. Data from these experiments were used to develop sampling methods. Tests were carried out in oligohaline and polyhaline areas of the Apalachicola Bay system. During October 1981, three sets of 100 core samples were taken from the sampling platforms at each of the study areas.

Species accumulation (with and without rare species) and rarefaction analyses indicated that 10 replicate cores per sample at stations 3 and 5A and 12 replicate cores per sample at station ML would take at least 85% of the available species. Oligohaline areas were characterized by moderately high numerical abundance, high dominance of a few polychaetes (primarily *Mediomastus ambiseta* and *Streblospio benedicti*), and low species richness and diversity. The polyhaline station was characterized by relatively low numerical abundance, low relative dominance (*Paraprionospio pinnata* and *Mediomastus ambiseta*), and high species richness and diversity. Analysis of variance of species richness and diversity and numbers of the two top dominants indicated relatively low within-station differences among replicates. Analyses of subsequent field experiments indicated statistically significant differences ($p < 0.05$) of one or more biological indices in at least one replicate in a given treatment. The results indicated that at least three samples per treatment were necessary for a given sample. Highly significant differences ($p < 0.05$) were noted between stations.

The oligohaline study area was characterized by low salinities that were highly variable (mean at station 3, 4.6°/oo; standard deviation (s.d.), 6.2) with peaks during summer-fall periods of low Apalachicola River flow. The polyhaline area was characterized by uniformly higher and less variable salinities (mean, 28.5; s.d., 3.5). Slight reductions of salinity were noted in this area during late winter-spring periods. Maximum water temperature occurred during July and August; winter lows were noted in December and January. The oligohaline experimental site (station 3) was an unvegetated, soft-sediment area that was quite turbid and dominated physically by tidal currents and Apalachicola River flow. Sediments were composed of silty sands. The polyhaline experimental area (station ML) was an unvegetated area dominated by sandy sediments and low to moderate tidal currents. Thus these stations represented significantly

different habitats in terms of major features such as salinity and sediment composition.

Overall, seasonal changes in macroinvertebrate assemblages at the oligohaline and polyhaline sites were somewhat similar although dominance hierarchies remained distinctive.

During the study period, oligohaline dominants included Paranais litoralis, M. ambiseta, S. benedicti, Cerapus sp., Hobsonia florida, and Grandidierella bonnieroides. Polyhaline dominants were M. ambiseta, tubificids, and P. pinnata. Community indices were seasonally and annually variable at the oligohaline site, with peak numerical abundance during the winter of 1981-82 (Figure 4). Species richness was high at the polyhaline station, with seasonal peaks during winter-spring periods. Most of the dominant species taken at the oligohaline site reached the highest numerical abundance during winter-spring periods. At the polyhaline site, there were complex dominance cycles with winter-spring peaks for M. ambiseta and spring-fall peaks for Brania wellfleetensis. Others, such as Aricidea fauvelli, showed no distinct seasonal patterns. Winter recruitment patterns were most common at both stations for species such as M. ambiseta, Paranais littoralis, and immature tubificids. Peak abundance of the meroplankton, dominated by bivalve and gastropod mollusks, occurred from May through November.

Field Experiments

Sediments from the oligohaline and polyhaline sites tend to differ in terms of the relative proportions of sand, silt, and clay. The polyhaline site was characterized by sandy sediments with relatively small components (1-2%) of silt and clay. Mean grain size ranged from 2 to 3 phi units. No seasonal trends in sediment characteristics were noted at the polyhaline site. Sediments in the oligohaline study area tended to have higher silt and clay fractions (usually ranging from 40% to 80% sand, 13% to 48% silt, and 3% to 10% clay). Average grain size was usually smaller (mean grain size, 3-4 phi units). Seasonal trends were apparent in the oligohaline system, with generally higher percentage of sand noted during spring periods of high river flooding.

An analysis of sediment characteristics (mean grain size, standard deviation, skewness, kurtosis; percent sand, silt, clay, and percent organics) as a function of treatment-related effects for each of the four experiments indicated no qualitative treatment effect on sediment composition. Analysis of variance for mean grain size and percent sand indicated no significant ($p < 0.05$) treatment-related effects on sediment composition of field samples of laboratory microcosms. No time-based differences were noted in sediments taken from field treatments or laboratory microcosms over the course of an individual experiment.

Field interactions are complex and highly variable in spatial as well as temporal terms. Benthic macroinvertebrate assemblages are influenced by complex series of physical, chemical, and biological stimuli and processes. In polyhaline areas during the spring of 1982, exclusion of predators led

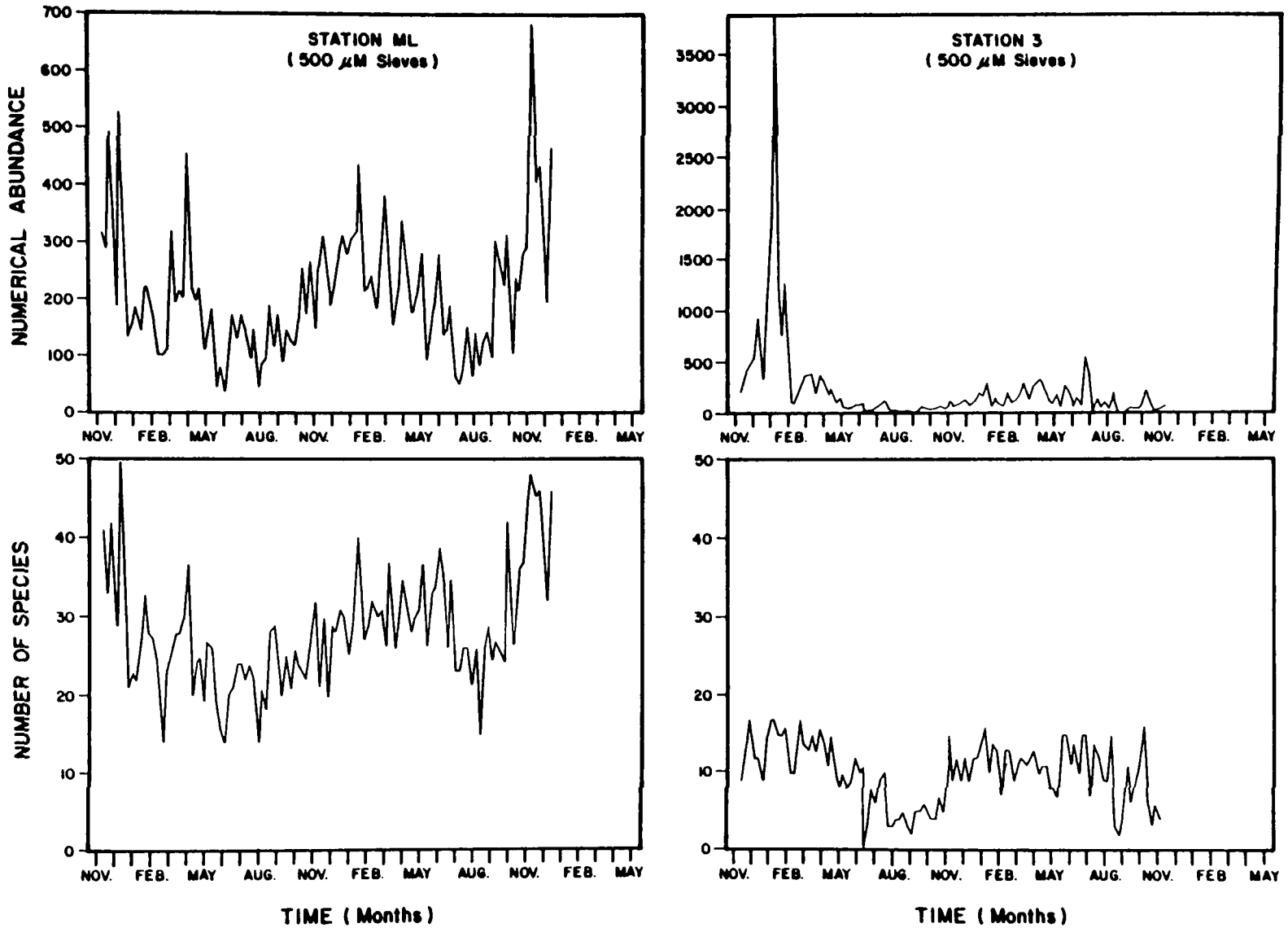


Figure 4. Numerical abundance and species richness of infaunal macroinvertebrates taken weekly at station 3 and ML in the Apalachicola Bay system over the study period. Numbers represent totals for 10-12-core samples.

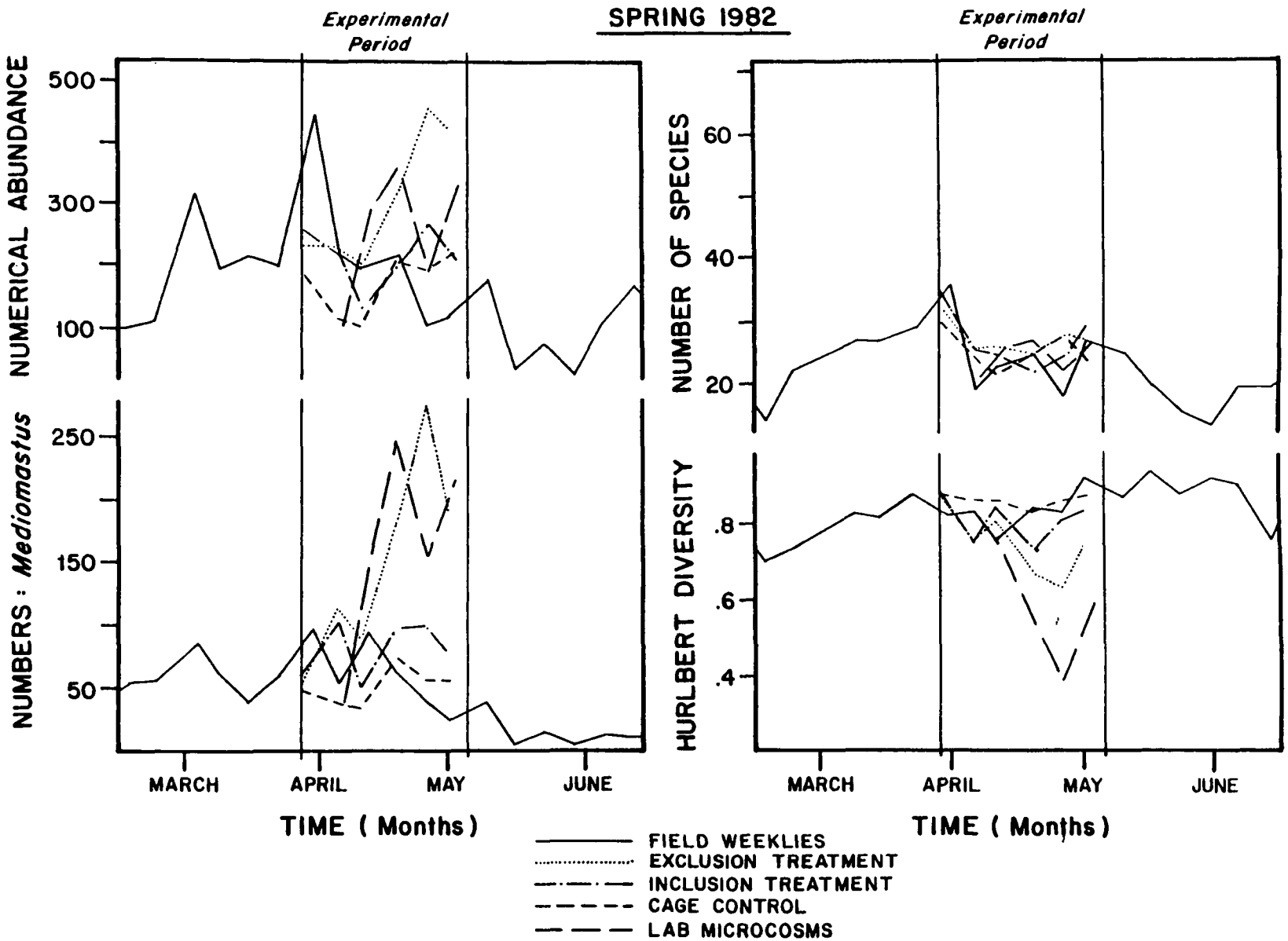


Figure 5. Experimental results in polyhaline area (station ML) showing long-term field changes and short-term (Spring 1982) treatment effects. Totals are for 12-core samples.

to increased numbers of the dominant polychaete Mediomastus ambiseta (Figure 5). Although species richness was not affected by predator exclusion, species diversity and evenness were significantly reduced, in large part because of the increased dominance of Mediomastus. Such effects were corroborated by the predator-inclusion treatments, which were not statistically different from the cage controls and uncaged areas. This predation effect occurred during the usual spring decline of the macroinvertebrate community (Figure 5). Randomized block ANOVA tests showed significant ($p < 0.05$) exclusion treatment effects for species diversity indices and log numbers of Mediomastus and total numbers of individuals. Time-based (week-to-week) effects were significant ($p < 0.05$) for all variables except species richness and numbers of individuals. The T x W interaction was significant, which indicated that different treatments had diverging temporal trends. Less significant effects ($p > 0.05$) were noted because of the disturbance of sampling. The main treatment-induced effects were shown for organisms taken in the top two centimeters of sediment, where the highest numbers of invertebrates are located. There were also indications of treatment-related effects on recruitment of young organisms in the field, with lower numbers of smaller organisms in the uncaged field controls. Recruitment of the cosmopolitan Mediomastus was an important factor in the relative abundance patterns of the macroinvertebrate assemblages. This species was a major colonizer of azoic sediments during the spring experimental period.

Subsequent experiments in oligohaline and polyhaline areas during different seasons of the year revealed no similar treatment-related effects in the field. The oligohaline tests indicated no treatment-related effects during fall or late winter-spring periods (Figures 6 and 7). These results confirmed previous findings by Mahoney and Livingston (1982). Tests during the spring indicated significant storm-induced effects on the macroinvertebrate assemblages although the effects were noted across all treatments. The lack of a predation effect in the field was tentatively attributed to the high dominance values, recruitment trends, and habitat variability in oligohaline portions of the study area. Analysis of 6-week experiments in the polyhaline area of study during the fall showed no treatment effects concerning either the quantitative or the qualitative distribution of organisms over the study period. There was no clear evidence of significant ($p < 0.05$) predation or sedimentation effects on the major population and community indices of infaunal macroinvertebrates. Time-based differences were noted for important species with high recruitment values (i.e., Mediomastus and immature tubificids).

Overall, results from the field tests indicated spatial and temporal gradients of habitat (e.g., salinity) on predation effects, dominance characteristics, and community indices. Predation was an important controlling variable in high-salinity areas during spring periods of low general recruitment and high predation pressure on infaunal species.

Laboratory Microcosms

The establishment of microcosms sometimes led to changes in the laboratory assemblages relative to the field assemblages from which they were derived. Such changes included a general reduction of numerical

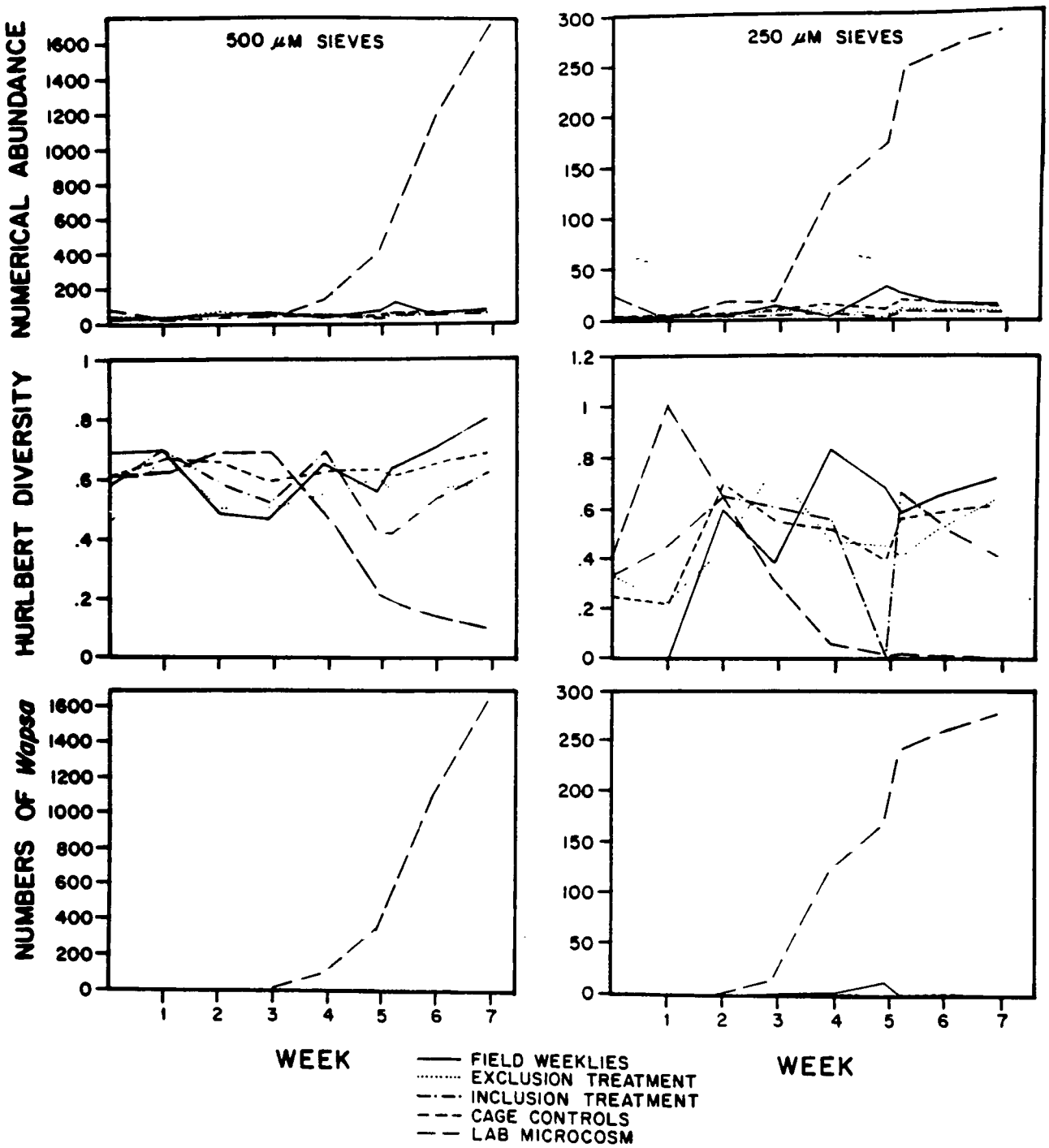


Figure 6. Experimental results in the oligohaline area (station 3) showing changes in numerical abundance, diversity, and top dominant (500- and 250-μm sieve fractions) in various treatments during Fall 1982. All numbers represent totals for 10-core samples.

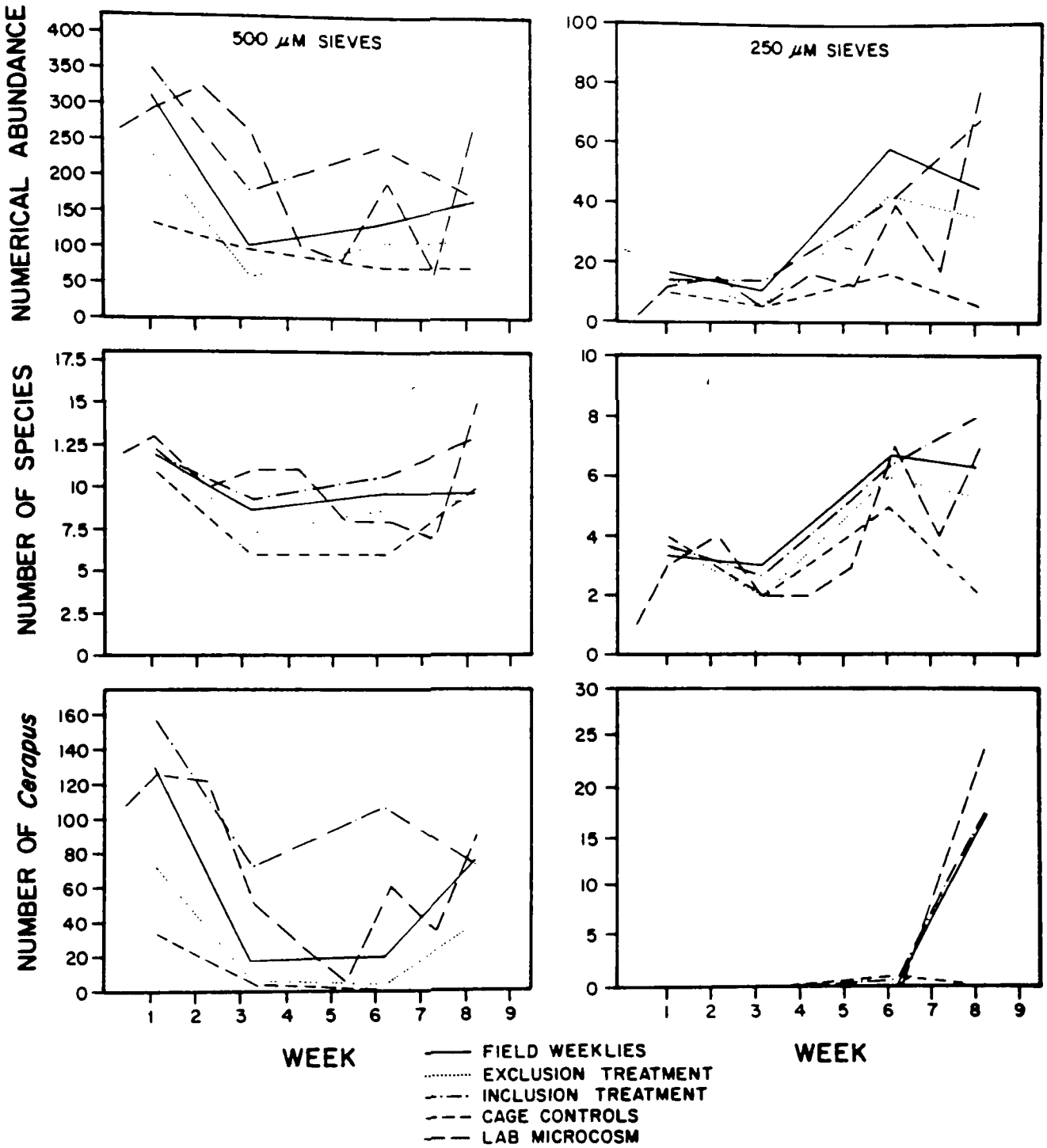


Figure 7. Experimental results in the oligohaline area (station 3) showing changes in numerical abundance, species richness, and a top dominant (500- and 250-μn sieve fractions) in various treatments during Spring 1983. Numbers represent totals for 12-core samples.

abundance and species richness due to the loss of certain species populations (Figures 8-9). However, the pattern of reduced abundance and loss of populations varied from experiment to experiment depending on the seasonally variable dominance relationships. Throughout each 6-9 week experimental period, the macroinvertebrate microcosms changed relative to the field. This change was caused by sampling activities and was more apparent in the microcosms than in the field treatments. Significant ($p < 0.05$) differences were noted between sampled and unsampled microcosms after weekly sampling over a six-week period. Such differences were also noted between the unsampled microcosms and the field samples. Sampling effects were noticeably reduced in the fall of 1982 when weekly coring was reduced to biweekly coring.

During the second week (T_1) of the Spring 1982 experiment at the polyhaline site, inclusion and exclusion cages were grouped together in a cluster analysis of treatments by week, as were cage controls and laboratory microcosms (Figure 10). By week 3 (T_2), the laboratory microcosms were somewhat different from the other treatments. However, for the balance of the experiment (weeks 4-6), field exclusions were grouped with laboratory microcosms. The inclusion treatment was intermediate between the above treatments and the two field controls. Although the laboratory microcosms differed from the field controls, the changes (i.e., numbers of Mediomastus with time) resembled the exclusion treatment, which indicates that, under the circumstances of the experiment, the lack of predation may have affected the laboratory microcosms (Figure 5). Subsequent experiments with predators in the laboratory microcosm indicated predation-related effects on the laboratory test systems. The effects varied according to the system (no effects were noted under oligohaline conditions) and the time of experimentation (no release of dominant species was noted during the fall experiment in the polyhaline system). Thus, predation was a complex but potentially important factor in the establishment and maintenance of laboratory microcosms.

Experiments in polyhaline areas during the fall showed significant differences in the total numerial abundance and species richness in the laboratory microcosms relative to the field (Figures 8-9). Differences between the lab and field were also noted in the oligohaline tests (Figures 6-7). The differences were caused by occasional increases in a given population or reduced numbers of organisms in the microcosms at the start of the experiment (Figure 7). Natural disturbances in the field led to responses in the infaunal macroinvertebrate assemblages that did not occur in the laboratory microcosms. The general increase in recruitment in the field following the storm in the spring of 1983 was not evident in the laboratory microcosms (see 250-μm sieve data, Figure 7). Specific changes in physical and biological features of the natural environment had complex effects on the laboratory test systems, which were largely dependent on the timing of the test and the nature of the associated macroinvertebrate assemblages. However, indices such as species richness often showed similar, if not parallel, trends in the lab microcosms and field treatments. Such indices were not appreciably affected by changes in a single population.

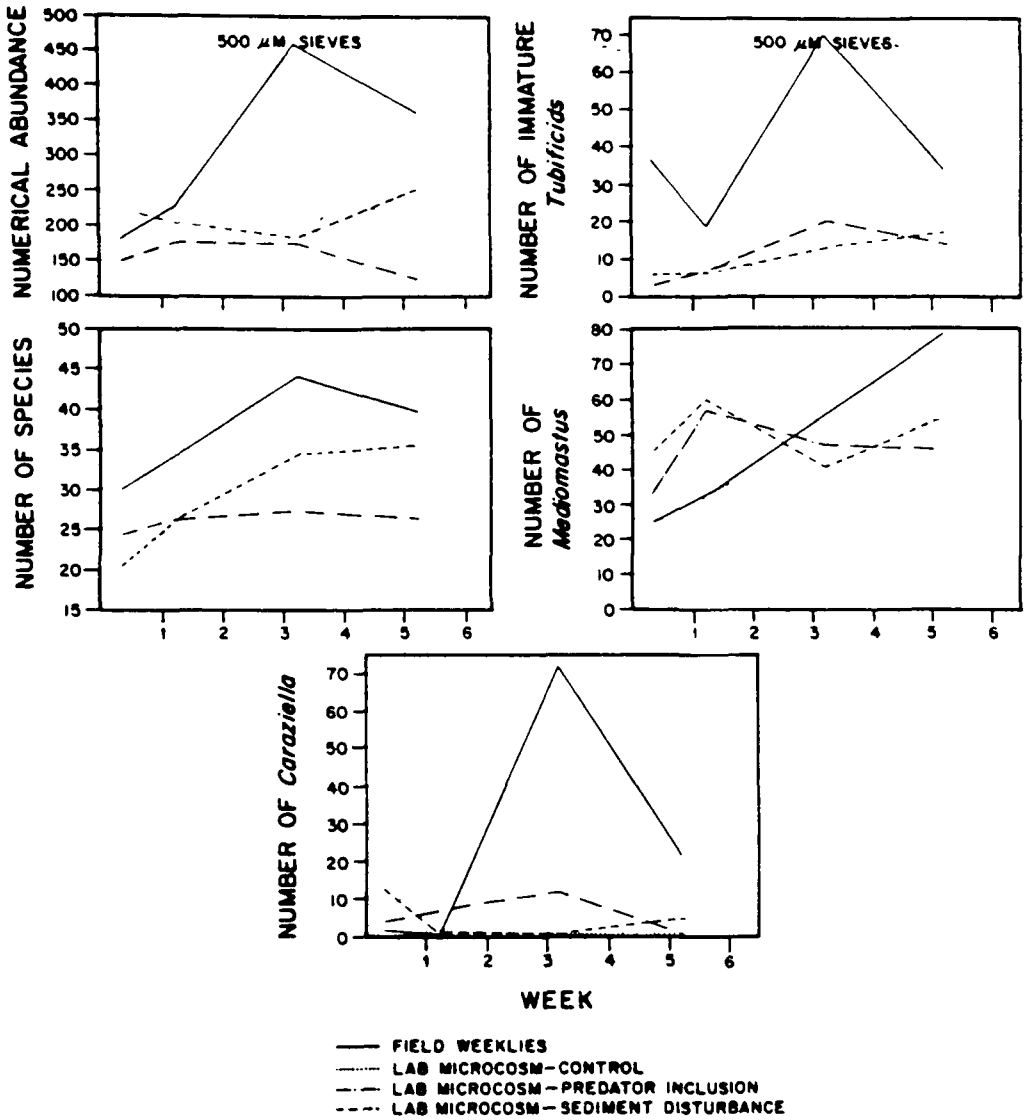


Figure 8. Comparison of weekly field collections (field weeklies) with laboratory microcosms (controls, predator inclusions, sediment disturbance) with organisms taken from station ML during Fall 1983 (500-µm sieve). Numbers represent totals for 12-core samples.

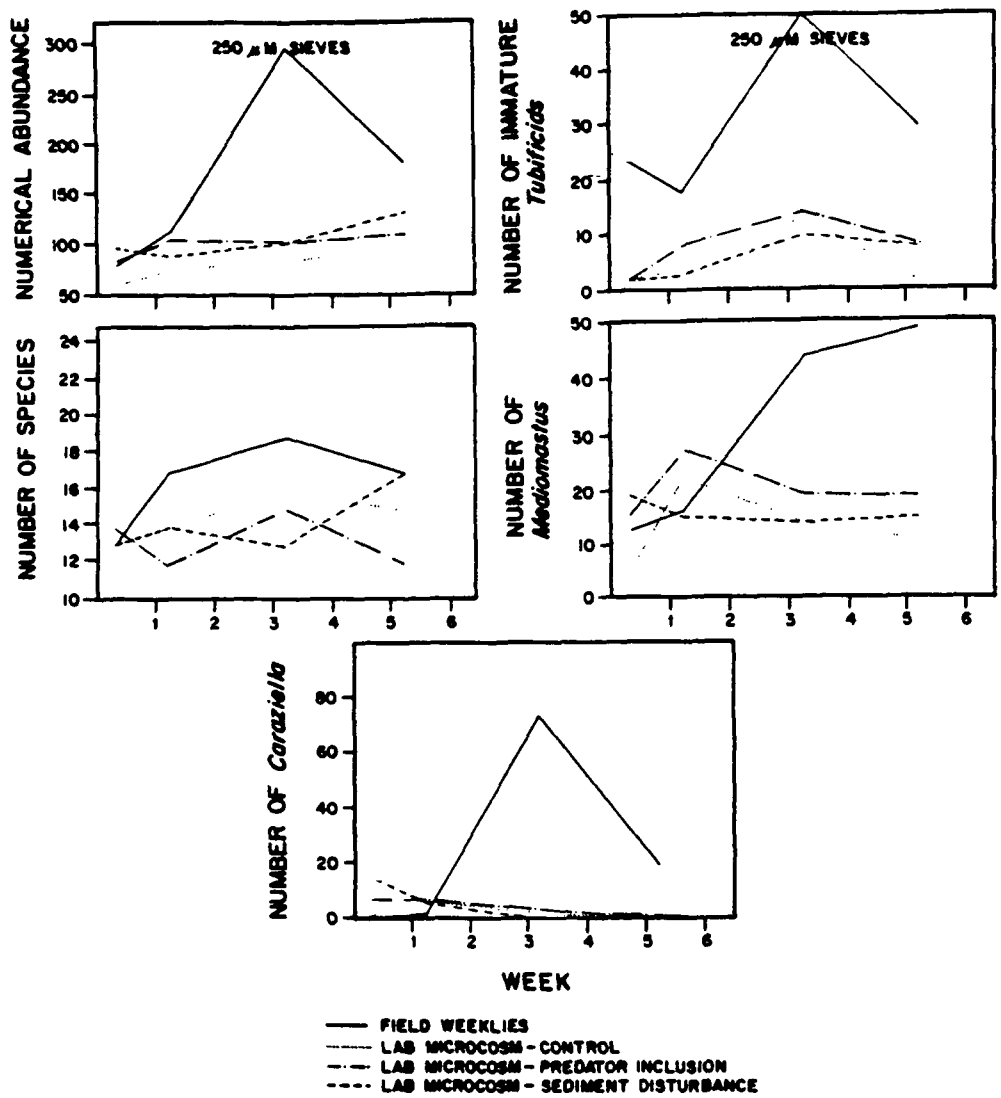


Figure 9. Comparison of weekly field collections (field weeklies) with laboratory microcosms (controls, predator inclusions, sediment disturbance) with organisms taken from station ML during Fall 1983 (250-µm sieve). All numbers represent totals for 12-core samples.

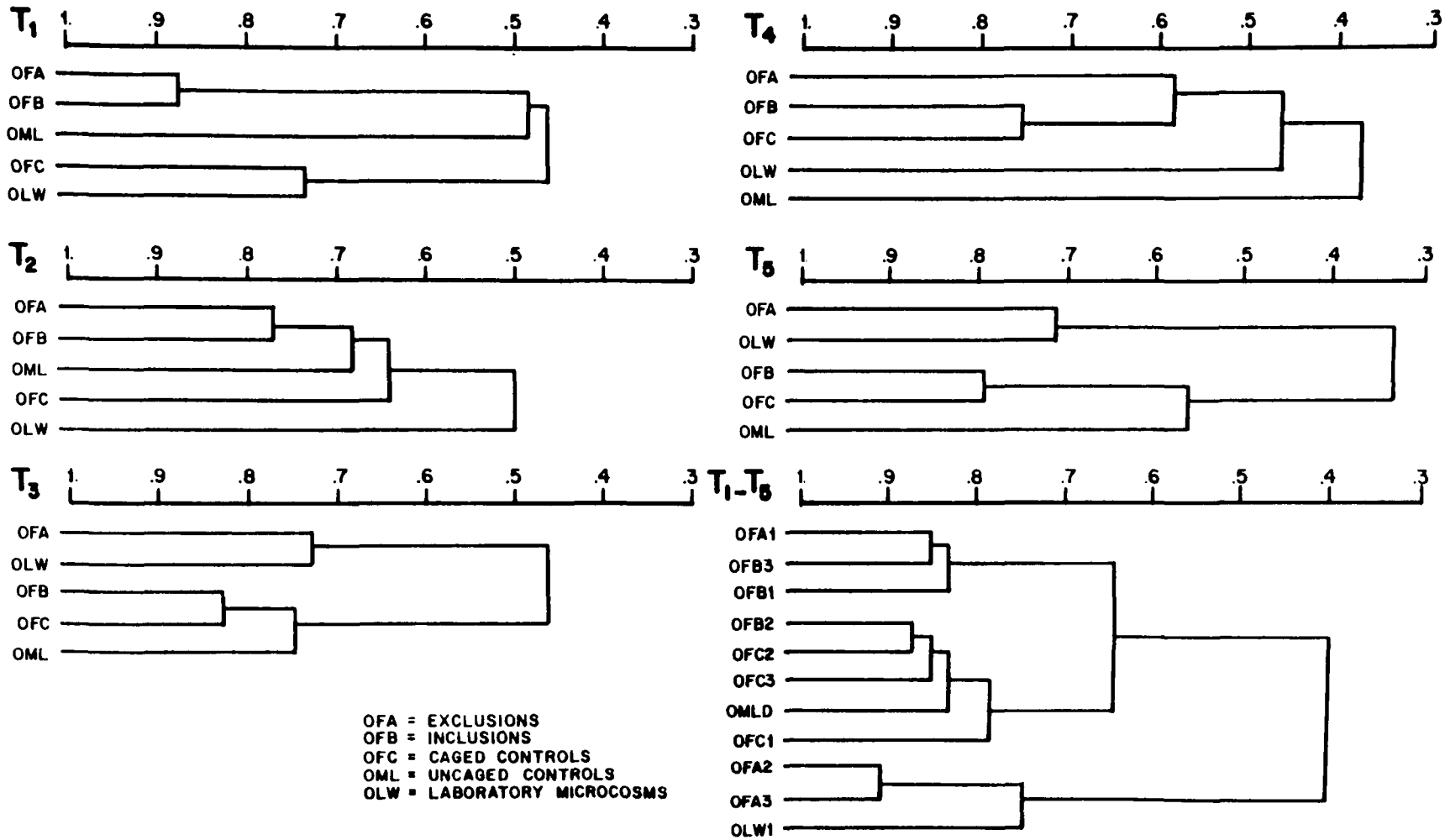


Figure 10. Cluster analysis of changes in species composition by treatment (summed cage results) for field and laboratory data over a six-week period in tests with polyhaline macroinvertebrates during spring 1982. Also shown is the cluster analysis by treatment for all replicates summed over the experimental periods (T_1-T_5).

Recruitment is one of the important processes of benthic communities. Recruitment of juveniles in the Apalachicola Bay system peaked from November through February (Figure 11). Recruitment was apparent for P. littoralis, M. ambiseta, S. benedicti, and G. bonnieroides at station 3; at station ML, such dominants included M. ambiseta, Brania wellfleetensis, Exogone dispar, Carrazziella hobsonae, immature tubuficids, and Tellina texana. Recruitment in the laboratory microcosms did not follow natural field conditions. Often, significant differences occurred in the recruitment of dominant populations in the microcosms. These differences may have led to increased recruitment in the laboratory for species such as Mediomastus (as a response to the disturbance of establishing the microcosm and sampling) or reduced recruitment of other species such as tubificid worms. Despite precautions taken with the seawater input from control areas, recruitment was particularly low in the laboratory microcosms during the fall experiments under polyhaline conditions (Figure 9). This result contrasted directly with results of the spring experiments, which were dominated by enhanced Mediomastus recruitment in the laboratory treatments relative to field controls (Figure 5). Other species such as Wapsa grandis showed higher recruitment in the laboratory during the fall experiments with oligohaline systems (Figure 6). Significant ($p < 0.05$) differences existed between laboratory microcosms and field conditions for various population and community features (numerical abundance, species richness, dominant populations) of the organisms taken in the 250-μm sieves. Such results indicated that recruitment of juveniles was affected by laboratory conditions, but that the exact effect was influenced by the nature of the source area and the time of sampling.

Microbiology

The microbial community structure in sediments of the polyhaline area was influenced by the activity of the epibenthic predators at the top of the food web. Analysis of the lipid components of the sedimentary microbiota showed reproducible shifts in the community composition in areas from which predators were excluded. After six weeks, the microeucaryotes and cis-vaccenic acid-containing microbes decreased in exclusion areas. Such disturbance effects could have been associated with observed increases in the deposit-feeding polychaete Mediomastus ambiseta. Bioturbation by invertebrate activity could have altered physical and chemical sediment characteristics. As a result, the growth and activity of certain microbes were stimulated. Predator exclusion experiments have demonstrated that intermediate benthic predators can control microbial biomass, community compositon, and trophic diversity through the immediate prey (infaunal macroinvertebrates) to the lowest trophic level of the benthic food web (Federle et al., 1983). Such findings could have implications involving the use of microcosms in analyzing microbial response to toxic substances in that removal of microbiota from natural predator-prey effects may influence the microbiological characteristics of microcosms relative to the field.

An attempt was made to compare the community structure of the field with laboratory microcosms meant to mimic the field. Oligohaline and polyhaline areas of the Apalachicola Bay system were used in this

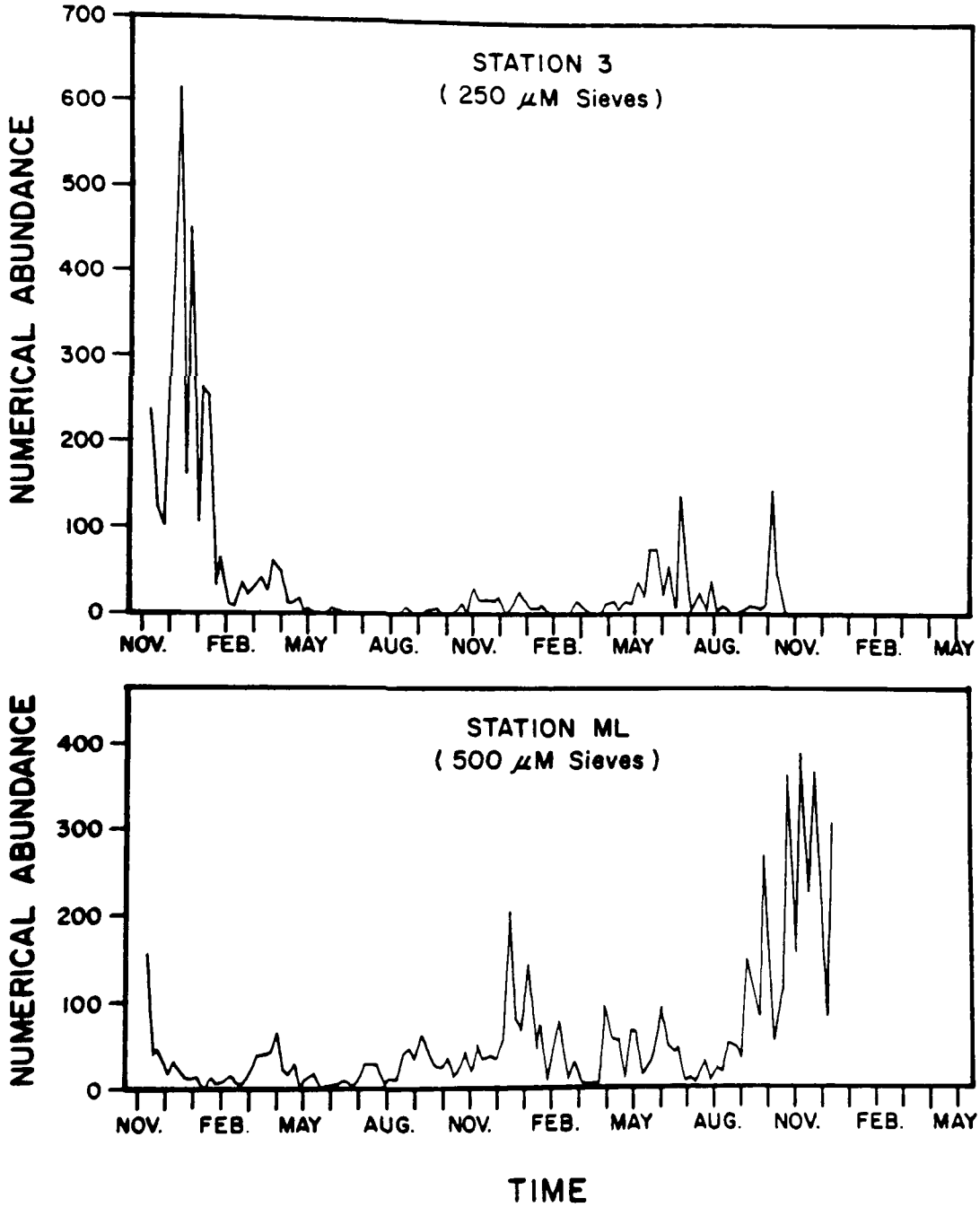


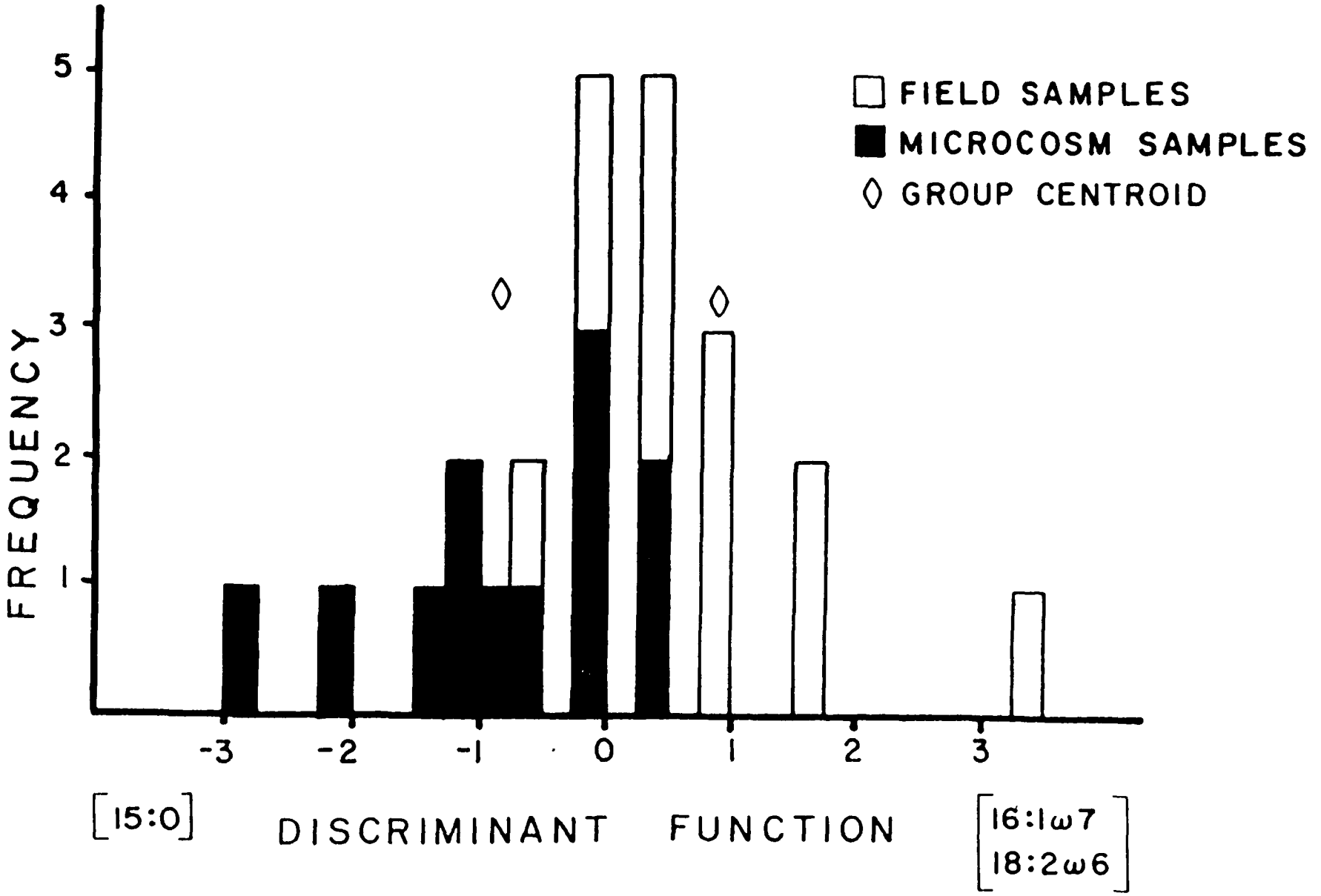
Figure 11. Numerical abundance (numbers per core sample) of macroinvertebrates on 250-μm sieves taken weekly at the study sites in the Apalachicola system from November 1981 through December 1983. Numbers represent totals for 11-12-core samples.

comparison. The polyhaline system (i.e. that with low temporal variability in salinity) was characterized by a highly organized trophic structure with little variance in the microbiota. In microcosms prepared from this area, significant differences existed between the laboratory and field microcosm after two weeks (Figure 12). Such differences were defined by the molar ratios of the fatty acids 15:0, 16:1ω7, and 18:2ω6. The differences increased substantially over the 6 weeks of the experiment. A discriminant analysis was carried out to compare regularly sampled microcosms, undisturbed microcosms, and field samples after six weeks. Two discriminant functions were generated in this analysis. Ten of the twelve possible variables contributed to the differentiation of the treatments. Samples from the field were differentiated from those from the microcosms primarily by discriminant function 1. Discriminant function 2 separated samples from the regularly sampled and undisturbed microcosms. Discriminant function 1 was defined principally by the molar ratios of the fatty acids 15:0 (Z = 1.14), 20:4ω6 (Z = -1.12), 20:5ω3 (Z = 1.09), and 18:1ω9 (Z = -0.88). Discriminant function 2 was defined largely by 20:4ω6 (Z = 2.14), 15:0 (Z = 0.83), and 18:2ω6 (Z = -0.82). Samples could be reclassified with 94.4% accuracy using the two discriminant functions. No field samples were classified incorrectly as being derived from microcosms or vice versa. It can be concluded that the classical stabilization period used in many microcosm studies would be highly misleading in a polyhaline system such as this one.

Further analysis of differences between the microbiota of micrcosms and field samples was carried out in oligohaline areas. The distribution of microcosm and field samples from station 3 along a single discriminant function after 3 weeks is given in Figure 13. This function was defined by the ratios of the fatty acids 15:0, 16:0, and 20:5ω3. The standardized discriminant function coefficients for these variables were 0.78, 0.52, and -0.51, respectively. Microcosm samples tended to be enriched in 15:0 and 16:0 and low in 20:5ω3 compared to those from the field. Using this single discriminant function, 81.5% of the samples could be correctly classified. Although only one field sample was misidentified, 33% of the microcosm samples were incorrectly classified. Thus, after 3 weeks, changes in fatty acid 20:5ω3 (common in marine algae) indicated shifts in algal populations. Also, higher levels of aerobic bacteria in the laboratory microcosms indicated a disturbance effect from the intial establishment of the microcosms.

A graphical representation of the distribution of microcosm and field samples along a single discriminant function after 6 weeks (Figure 14) indicated a high degree of variability within each treatment as shown by the spread of points around the centroid. The regularly sampled microcosms differed most from the field, and the undisturbed microcosms resembled more closely the field than did the regularly sampled microcosms. After six weeks, the differences between the sampled microcosms and the field were not much greater than they were after three weeks.

The oligohaline site was characterized by high and variable input of detritus from the Apalachicola River, low salinity, and high secondary productivity. There was also high temporal variability in the salinity and nutrient regimes. Epibenthic predators had little or no influence on



12. Distribution of field and microcosm derived samples of polyhaline (station ML) sediments along a single discriminant function after two weeks.

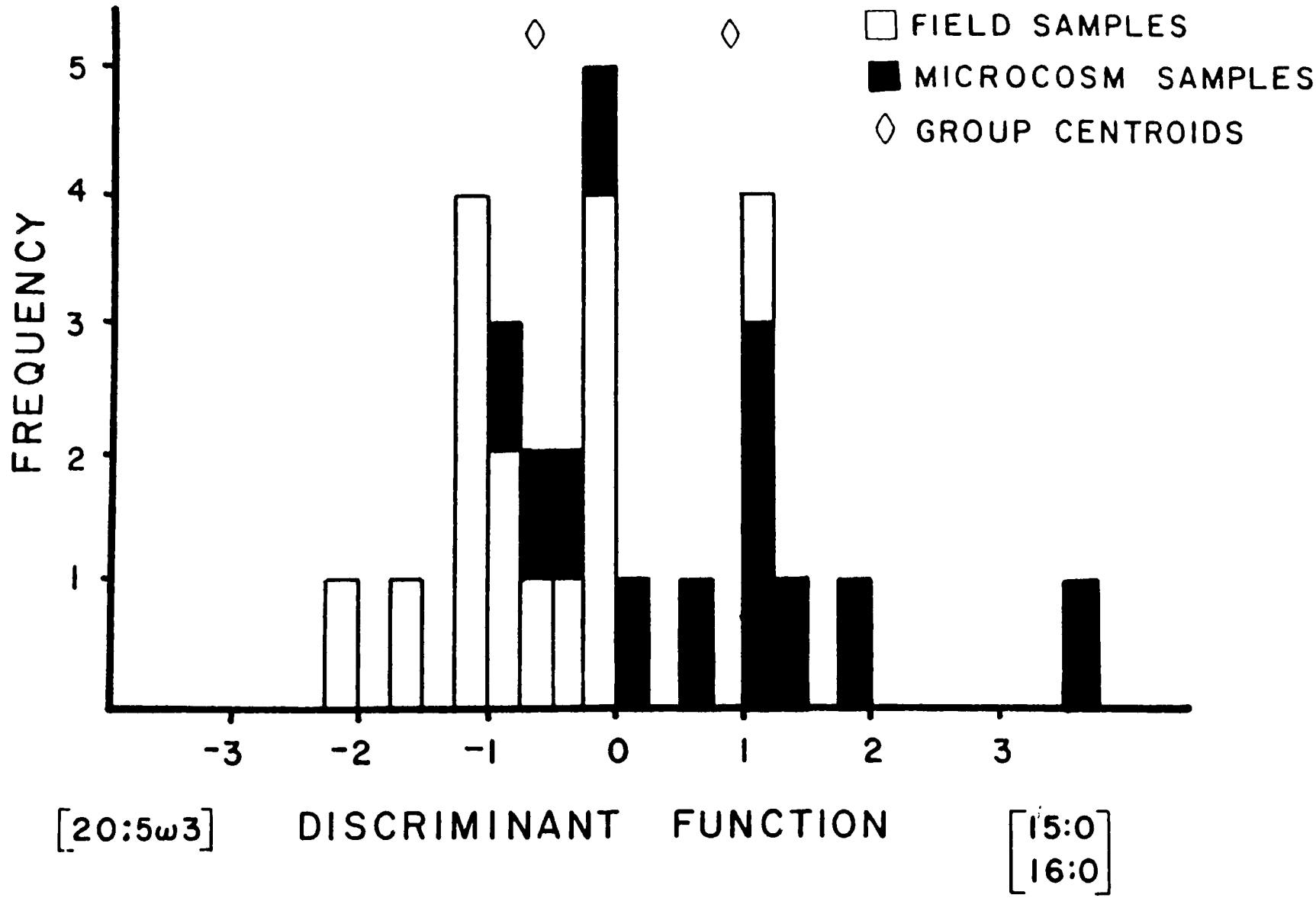


Fig. 13. Distribution of field- and microcosm-derived samples of oligohaline (station e) seidments along a single discriminant function after three weeks.

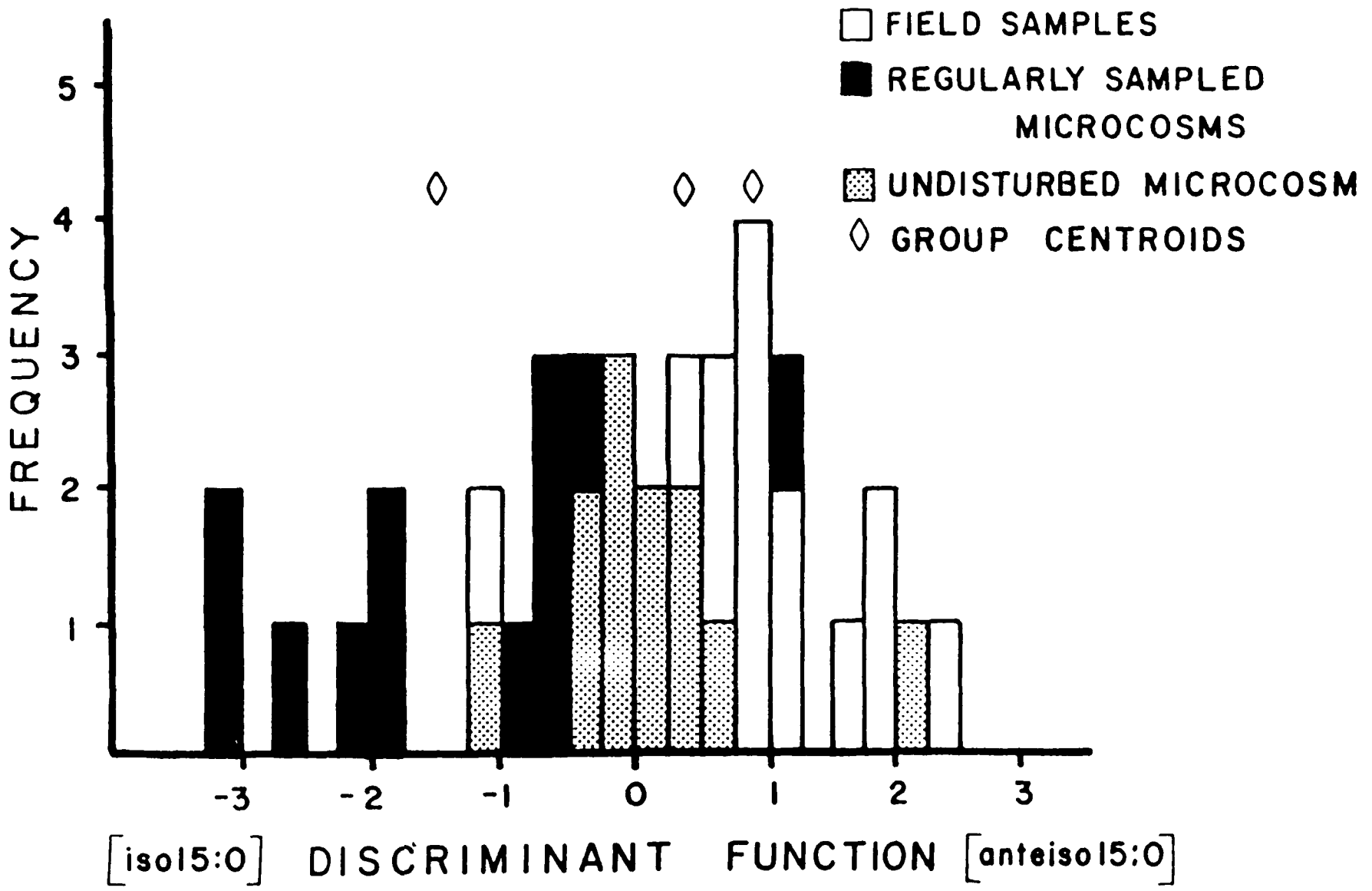


Figure 14. Discrimination of oligohaline (station 3) sediment samples from the field, regularly sampled microcosms, and undisturbed microcosms after six weeks.

deposit-feeding invertebrates or on the microbiota at station 3. Thus, this system was largely driven by physical and chemical inputs. Although there were detectable differences between the field microbiota and the laboratory microcosms, these differences were small and did not increase with time. In this oligohaline system, the classical "stabilization" period would have had little effect on the microbial community structure.

The microbiotic component in a laboratory microcosm is highly variable, and its capacity to predict natural trends depends on a combination of habitat characteristics in the area of origin. Overall, there is good evidence that, as time progresses, microbial elements in microcosms tend to deviate progressively from field associations; extended equilibration periods may be ill advised under certain environmental conditions. In microcosms of sediments from polyhaline areas, microbes did not follow field conditions as closely as those in microcosms from oligohaline portions of the estuary. As a result, without detailed knowledge of the ecology of the source area, an interpretation of results from laboratory microcosms could be misleading.

Summary of Findings

The relationship of laboratory microcosms to field conditions depended on a number of variables that changed depending on time and the location of the test. During the spring experiments in an oligohaline area, significant differences were noted for total numerical abundance and species richness of macroinvertebrates because of laboratory artifacts in recruitment. Similar experiments in the spring in polyhaline areas led to increases of the dominant polychaete, *M. ambiseta*, in the laboratory microcosms, paralleling changes in the field predator-exclusion treatments. Such changes in recruitment and possible predation effects could have led to significant differences of various community features between the laboratory and field assemblages of microorganisms and infaunal macroinvertebrates. The fall tests in oligohaline areas showed significant differences between laboratory and field treatments as a result of blooms of the oligochaete *Wapsa grandis* in the laboratory microcosms. These differences became significant after the fifth week of testing. Fall experiments in the polyhaline areas also resulted in significant differences because of low numbers of individuals and reduced recruitment in the laboratory treatments relative to the field.

Factors such as spatial habitat gradients, temporal changes in population processes, and changes in the influence of predation pressure all contributed to the complexity of the validation process. Also, the initial establishment of the microcosms and continued sampling led to observed differences between the laboratory microcosms and natural field conditions. However, the broad spectrum of information provided by microcosms produced indices such as species richness that were relatively conservative indicators of field conditions. Thus, field validation of macroinvertebrates can be qualified within known limits of spatial and temporal variability based on specific ecological conditions in a given area.

The results with microorganisms illustrated several points: (1) fatty acid analysis, combined with multivariate statistical techniques, was a powerful means of comparing the structure of different microbial communities; (2) microcosms may or may not mimic natural microbial communities; and (3) microbial communities from similar environments but different ecological conditions may show a wide range of response when isolated in the laboratory. This technique should have great potential in evaluating a microbial community's response to toxic substances. The major shortcoming of the microcosm approach is our current inability to interpret the signifiance of changes in particular fatty acids. Based on this study, it can be concluded that not all sediments will mirror the field to the same degree when placed in relatively complex microcosms. Our findings showed the importance of biological control of microbial communities in the estuarine environment and the need to include biological as well as physical factors in the design of model laboratory systems. A priori, without knowing the specific ecology of a particular site, one cannot conclude that a reasonably designed microcosm will always simulate the field.

EXPERIMENTAL PROGRAM: VIRGINIA INSTITUTE OF MARINE SCIENCE

Infaunal Macroinvertebrates: Background

Patterns of natural population variations from 1979-83 among the numerically dominant species indicated seasonal and non-seasonal changes that related in part to the life histories of the species and the estuarine environment (Figure 15). Two species, *Streblospio benedicti* and *Paranais littoralis*, exhibited a classic opportunistic life-style of extensive recruitment over a short period of time followed by mass mortality. The species *S. benedicti* recruited at the same times, spring and fall, each year. However, the magnitude of recruitment declined each year. For instance, *P. littoralis* was virtually nonexistent in the community from 1979 until early spring 1982, when it had a large and brief recruitment pulse. An equivalent pulse occurred at the same time in 1983.

The capitellids *Mediomastus ambiseta* and *Heteromastus filiformis* have also been cited as opportunists. *Heteromastus filiformis* had a regular recruitment period during the late spring each year. *Mediomastus ambiseta* had a large peak only in the spring of 1982. *Polydora ligni* had less dramatic changes in population densities due to recruitment. With some variation, *P. ligni* recruited essentially at the same time of the year. During 1981, the recruitment was weak, and populations were variable throughout the year. In contrast, 1980, 1982, and 1983 were years when *P. ligni* exhibited strong recruitments in the early part of each year. *Tubificoides* spp. had prolonged recruitment punctuated by periods of high mortality. This oligochaete had both summer and winter recruitments that were similar in magnitude from 1979 to 1984. Non-opportunistic, prolonged recruitment with low mortality was exhibited by *Phoronis* sp. Population densities decreased during the late winter but were otherwise maintained at fairly constant levels.

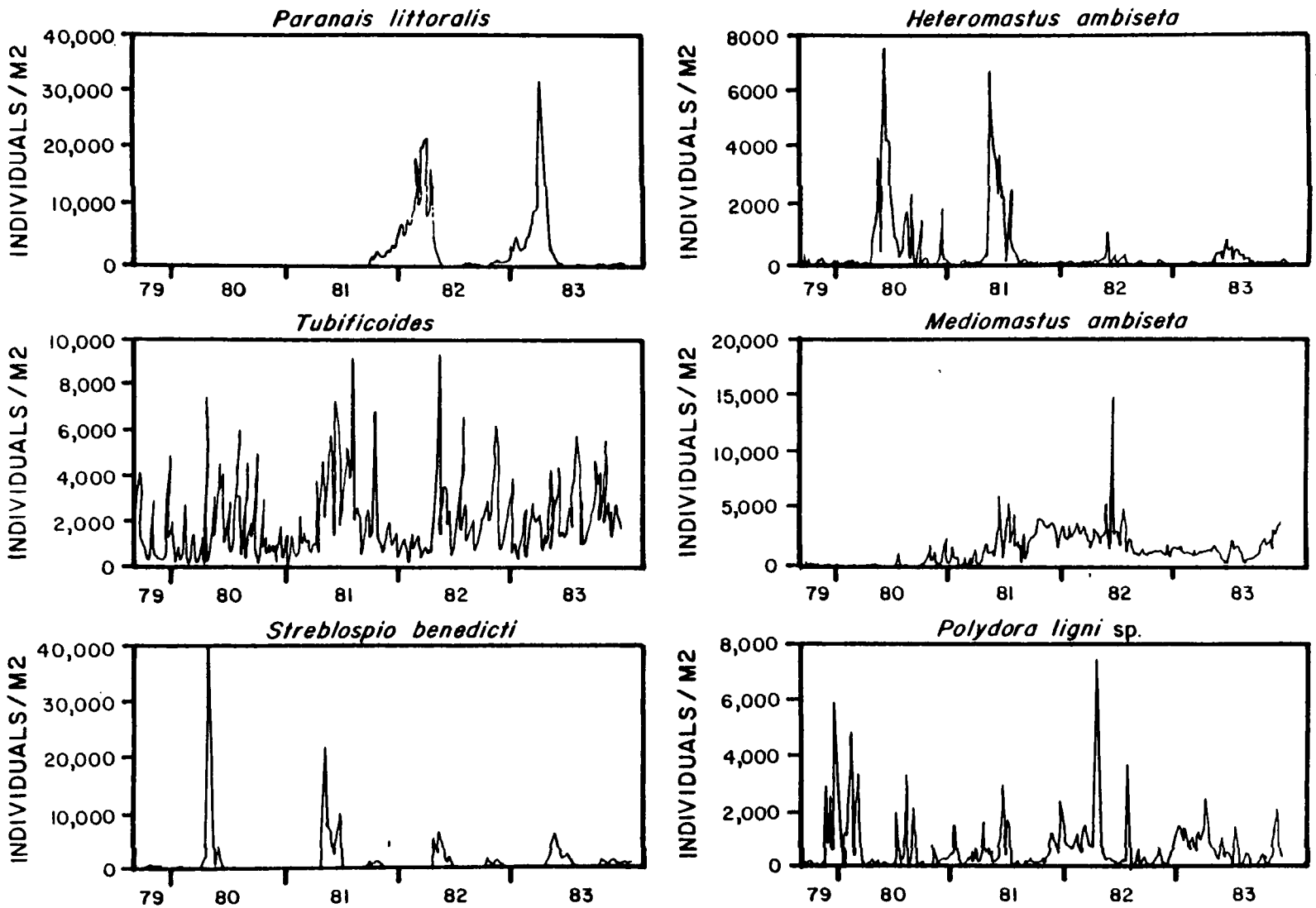


Figure 15. Long-term population variation of dominant annelids at the York River, Virginia, site.

Laboratory-field Experiments

Eleven dominant species were identified by cluster analysis for the first experiment (Spring 1982; Table 1). Approximately half of all the individuals involved in the test were recruited during the spring test period. The top numerical dominant, *P. littoralis*, was recruiting heavily before the test. By the end of the test, this species had almost vanished in all treatments (Figure 16). Both *S. benedicti* and *P. ligni* recruited heavily during the test, and to a greater degree in the semi-field treatments. Preferential recruitment into the cages was less dramatic, but still recognizable for *E. heteropoda* and *Scoloplos* sp. Taken together, the immature capitellids and *M. ambiseta* were more abundant in the full-field treatments. The greatest recruitment was also into the full-field treatments for *Tharyx* sp. The numbers of newly set bivalves were lowest in the microcosms. *Tubificoides* sp. recruited uniformly into all treatments. There were no apparent population changes due to recruitement for *Phoronis* sp. However, its abundance was considerably lower in the microcosms because of mortality caused by handling of the sediments in establishment of the laboratory microcosms.

The variance-to-mean ratios for all dominants were > 1, indicating aggregated spatial distributions of the individuals. *Polydora ligni*, *Tubificoides* sp., and *Paranais littoralis* had the highest ratios. Calculation of Morisita's indices showed that the greatest degree of clumping was for *P. ligni* and *Tubificoides* sp. in the full-field and semi-field treatments. The amount of aggregation was also lowest in the microcosm treatments for *Streblospio benedicti* and immature capitellids and bivalves. Similar spatial patterns were found in all treatments for *P. littoralis*, *Tharyx* sp., *Mediomastus ambiseta*, and *Phoronis* sp. The polychaetes *Scoloplos* sp. and *Eteone heteropoda* had less consistent patterns. *Eteone heteropoda* was highly clumped in only one semi-field treatment.

A two-way analysis of variance tested the hypothesis that there was no difference in the abundance of a particular species among treatments or through time. None of the two-way interactions between treatments and weeks was significant. For six of the eleven dominant species there were significant differences ($\alpha < 0.05$) in log abundance among treatments. There were significant differences with respect to time for five species. Only *P. littoralis* and newly set bivalves exhibited effects of both treatment and time. When *a priori* contrasts were used to evaluate treatment differences, the abundances were not consistently higher or lower in any one treatment. The lack of trend among the significant differences between treatments gave positive support to the field validity to this test.

The cluster analysis grouped species mainly on the basis of abundance and frequency of occurrence. A second analysis essentially separated stations (treatment replicates for each week) into three successive two-week periods. The onset of recruitment caused time to have the greatest influence in determining similarity. Community structure, as measured by diversity and evenness, did not change greatly through time or between replicates during Test 1 (Table 2). Even though community structure

TABLE 1. Dominant species (individuals/m^2) from the field validation experiments, York River, Va.

	Test 1			Test 2			Test 3			Test 4		
	FF*	SF	MC	FF	SF	MC	FF	SF	MC	FF	SF	MC
Paranais littoralis	7769	10398	17624		ND		1015	2400	2318		ND	
Streblospio benedicti	6272	11679	5663	714	1770	44	3211	9318	3729	1012	1234	43
Mediomastus ambiseta	3570	2547	3776	937	992	698	1104	1582	1422	2420	1877	2025
Immature Capitellidae	5472	4657	2131	750	1143	512	1526	2285	582	1444	1389	1203
Tubificoides sp.	2762	2650	2162	2385	2666	742	2481	2207	1248	2944	2634	1414
Phoronis sp.	1240	1632	697	1766	2103	627	896	522	374	2056	1895	512
Immature bivalves	772	874	315	933	1726	364	230	278	37	772	802	475
Scoloplos sp.	262	336	228	417	1237	64	487	452	1081	413	407	185
Tharyx sp.	6984	5267	3968		ND		189	285	156		ND	
Eteone heteropoda	1429	2366	869		ND		615	815	82		ND	
Polydora ligni	4198	16028	4175		ND		878	2396	896		ND	
Glycinde solitaria		ND**		357	500	385	126	215	337	358	247	401
Immature gastropods		ND		286	191	155		ND		302	241	216
Acteocina canaliculat		ND		183	151	306		ND			ND	
Paraprionospio pinnata		ND		159	187	242		ND		173	173	222
Heteromastus filiformis		ND			ND		252	663	248		ND	
Ampelisca abdita		ND			ND		48	315	111		ND	
Clymenella torquata		ND			ND		185	219	255		ND	
Scolelepis squamata		ND			ND			ND		543	352	142
Spiochaetopterus oculatus		ND			ND			ND		265	136	123

* FF -- Full-field
SF -- Semi-field
MC -- Microcosm
** ND -- Not a dominant species during this test.

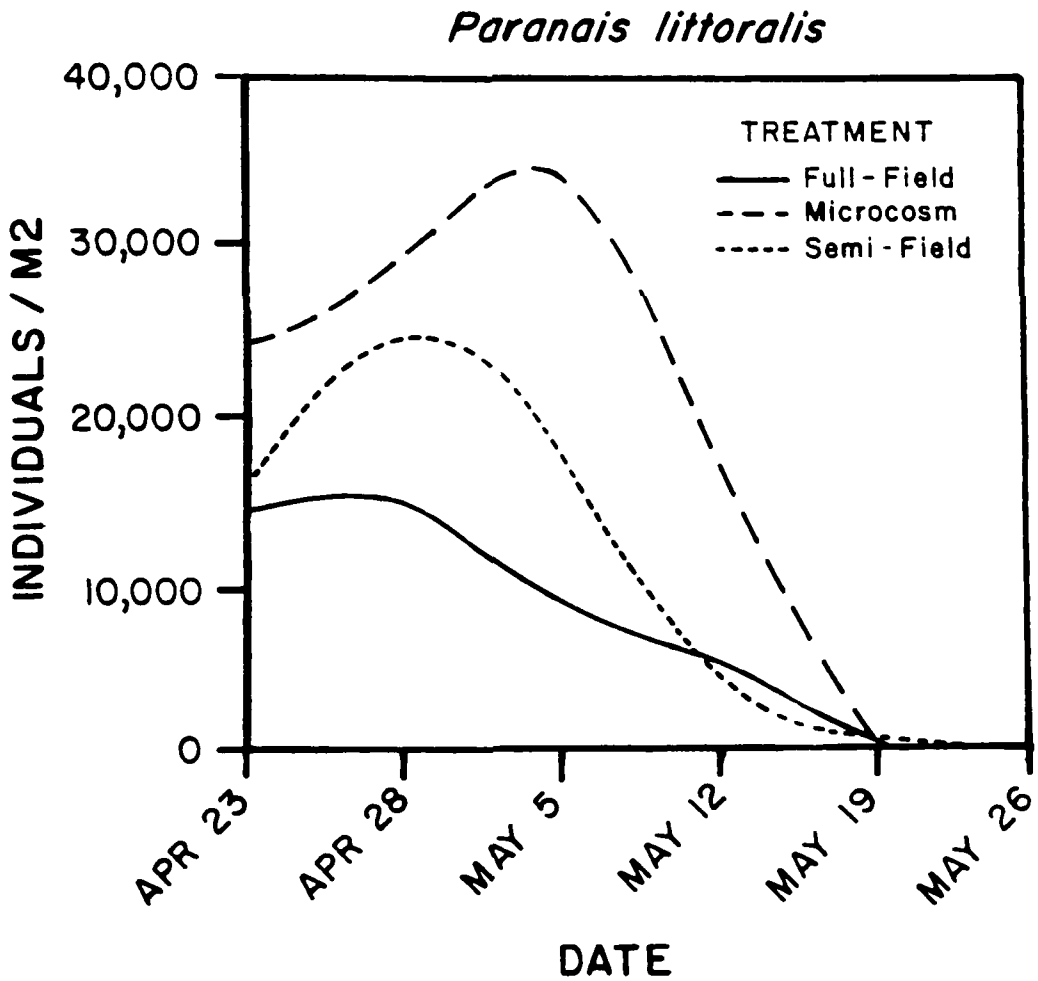


Figure 16. Patterns of Paranais littoralis abundance for Test 1.

measures did not change much during the test, there were considerable changes in individual species.

Of the eleven dominant species from Test 2, conducted in the fall, seven were annelids (Table 1). Approximately half of the individuals were recruited during the test period. Lower recruitment was observed in the microcosm treatments, exemplified by S. benedicti (Figure 17). As a result, the species populations were generally lower in the microcosms than in the field. All treatments, including the field, exhibited declining abundance as expected during the fall season.

The analysis of variance showed two-way interactions for two of the dominant species. Of the remaining species, two had significant among-week differences, and five had significant differences in log abundance among treatments. Evaluation of a priori contrasts showed that four of these five species had the lowest abundances in the microcosm treatments, emphasizing the difference between treatments.

Cluster analysis of weekly treatment replicates produced groups based on treatment. One cluster group contained the full- and semi-field treatments, and another group the microcosm treatments. The grouping of the microcosms together indicated a difference between the laboratory and field, which turned out to be primarily caused by differential recruitment. The measures of diversity and evenness did not change over time or between treatments (Table 2).

Of the fifteen dominant species from Test 3 in the spring (1983), three were not annelids (Table 1). In both full and semi-field treatments, approximately half of the total community was newly recruited. In the microcosm treatments, only one-fifth of the community was newly recruited. During Test 3, S. benedicti was recruiting heavily into the semi-field treatments. The populations were constant and smaller in the full field and microcosm treatments. The same pattern was seen for populations of H. filiformis. Both P. littoralis and P. ligni were declining in abundance in all treatments during the test periods, having reached their peaks before the test. For P. ligni, this decline was less rapid in the semifield treatments. Four species, E. heteropoda, Phoronis sp., immature capitellids, and Tubificoides sp., had the lowest average abundances in the microcosms. Of these four, only Tubificoides sp. was not recruiting. In contrast, Scoloplos sp. was twice as abundant in the microcosms.

Populations of Tubificoides sp., P. littoralis, P. ligni, and H. filiformis were consistently aggregated, yet less so in the microcosms. In contrast, Phoronis sp. was more aggregated in the microcosms. There were no pronounced differences between treatments for immature capitellids, M. ambiseta, or S. benedicti. Random distributions were indicated for E. heteropoda and immature bivalves in the microcosms, C. torquata in the semi-field, Ampelisca abdidta in the full-field treatments and microcosms, and G. solitaria and Tharyx sp. in all treatments.

Analysis of variance showed two-way interactions for two species. Of the remaining thirteen species, only two exhibited differences based on

TABLE 2. Community structure indices from the field validation experiments (York River, Va.).

	Grand Mean	Full-field Mean	Range	Semi-field Mean	Range	Microcosm Mean	Range
Test 1							
H' - Shannon	2.87	3.03	2.58-3.31	2.77	2.16-3.10	2.80	1.00-3.37
JPR - Evenness	.59						
H - Brillouin	2.80	2.96	2.53-3.25	2.72	2.14-3.07	2.73	1.95-3.25
J - Evenness	.59						
Test 2							
H' - Shannon	3.47	3.54	2.97-3.87	3.32	2.95-3.60	3.57	3.08-3.98
JPR - Evenness	.77						
H - Brillouin	3.18	3.26	2.76-3.59	3.10	2.75-3.46	3.17	2.72-3.46
J - Evenness	.77						
Test 3							
H' - Shannon	3.11	3.22	2.85-3.58	3.13	2.48-3.66	2.99	2.71-3.52
JPR - Evenness	.74						
H - Brillouin	2.84						
J - Evenness	.75	2.91	2.57-3.29	2.82	3.21-3.30	2.79	2.48-3.13
Test 4							
H' - Shannon	3.56	3.58	3.21-3.90	3.48	2.97-3.93	3.61	3.08-3.94
JPR - Evenness	.77						
H - Brillouin	3.39	3.63	2.99-3.63	3.25	2.77-3.68	3.30	2.81-3.66
J - Evenness							

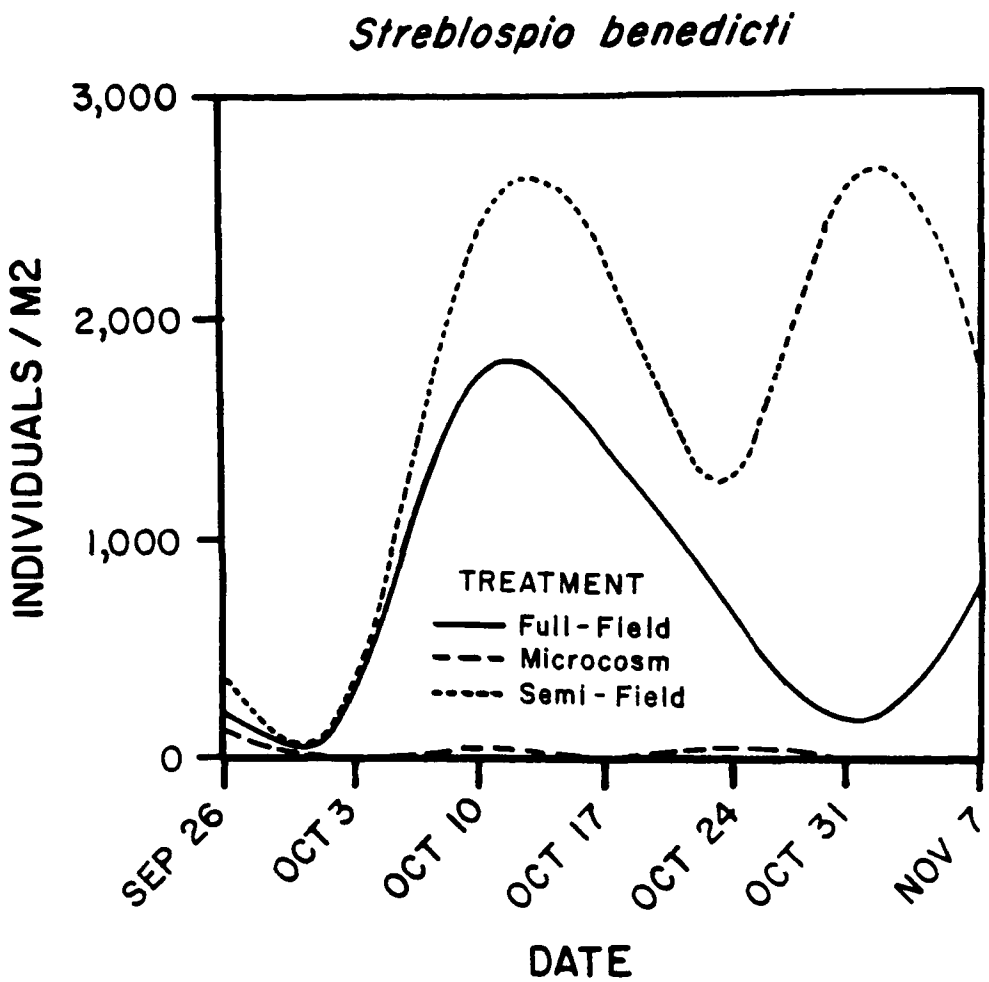


Figure 17. Patterns of Streblospio benedicti abundance for Test 2.

time. Differences among treatments were significant for nine species. Of these nine, a priori contrasts showed that five had the lowest abundances in the full-field treatments, three in the microcosms, and one in the semi-field.

Cluster analysis indicated that the effect of treatment rather than time was more important in forming groups (Figure 18). The microcosm treatments were separate from the group of full- and semi-field treatments. Averaged over time, the highest diversity was calculated for the full-field treatments, and the lowest in the semi-field treatments. The low diversity in the semi-field treatments was probably due to a decline in diversity during the last two weeks of the test.

Twelve species were identified as dominants from Test 4. Of these, nine were annelids. About half of the individuals were recruited during the test period. Recruitment was lower in the microcosms.

Analysis of spatial distribution patterns showed that six species were consistently aggregated. *Mediomastus ambiseta* exhibited no differences in the degree of aggregation between treatments. Immature bivalves, *Tubificoides* sp., and *Phoronis* sp. were all the least aggregated in the microcosm treatments. Species that were not highly aggregated and often approached a random distribution were immature gastropods, *S. squamata*, *Scoloplos* sp., and *S. oculatus*. Of these, the gastropods were most aggregated in the microcosms, and *Scoloplos* sp. and *S. squamata* were the most aggregated in the full-field treatments.

The trend evident from the cluster analysis was grouping by treatment, the microcosms being separate from the full- and semi-field treatments. With regard to community structure all treatments showed the same patterns as the other tests. On average, the diversity was slightly higher in the full-field treatments than in the microcosms. Diversity was often lowest in the semifield treatments.

Test Comparisons

Summing the average abundances of the top eleven numerically dominant species from each test illustrated a major difference in community abundance. The approximate numbers for each test were: 50,000 individuals m^{-2}, Test 1; 9,000, Test 2; 16,000, Test 3; and 11,000, Test 4. From the monitoring of the long-term dynamics it was expected that the fall tests (2 and 4) would have fewer individuals than the spring tests (1 and 3). The larger discrepancy between the two spring tests was due to two factors. First, the dominant species (*M. ambiseta*), the associated immature capitellids, and *P. ligni* had above-average recruitment during the spring of 1982 (Test 1). Below-average recruitment was seen for several species during the spring of 1983 (Test 3), especially for *Tharyx* sp. and *E. heteropoda*. Second, Test 1 was conducted during the peak in recruitment for many species, including the end of this period for *P. littoralis*, but, during Test 3, the short but considerable peak in the abundance of *P. littoralis* was missed. Test 3 included the end of the recruitment period for some of the other dominant species.

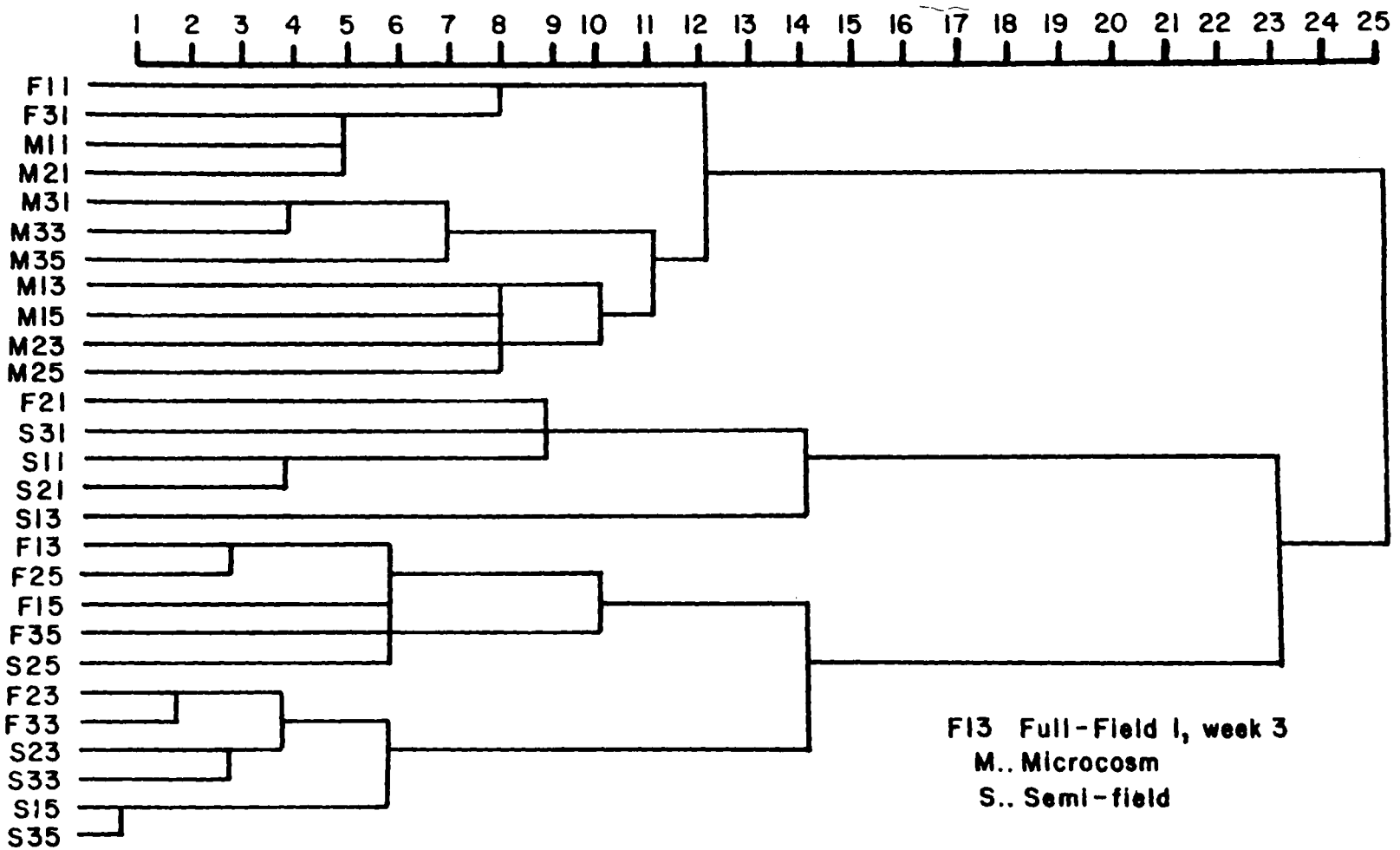


Figure 18. Cluster analysis of Spring 1983, Test 3 York River, Virginia, site.

The dissimilarity between Test 1 and the other three tests was also reflected in the lower diversity indices (Table 3). The large numbers of P. littoralis decreased the measurement of diversity. In comparisons of differences between treatments, the spring tests showed the highest diversity in the full-field treatments. Of the fall tests, Test 2 had lower diversity in the semi-field treatments, yet in Test 4 all treatments were similar.

A cluster analysis of all four tests identified nine dominant species based on abundance. Fifteen other species were identified as subdominants. The analysis of stations divided the treatment replicates from each week into two groups. The first group contained stations from the spring tests. This group was further divided into two subgroups, one of which contained all of Test 1, and the other, all of Test 3. The second group contained the two fall tests, which were not clearly divided within the group based on test, treatment, or time.

For comparison of species trends among the four tests, slopes of log abundance over time were calculated. The abundances were standardized to number per m^2, and the first 5 weeks of each test were used. The slopes were used to indicate only trends in abundance and not causal relationships. Several species exhibited changes caused by timing of the tests and to treatment. When recruitment was evident, such as for S. benedicti and Scoloplos sp. during both spring and fall tests and for immature bivalves in the spring, the rate of recruitment based on slope was either less pronounced or nonexistent in the microcosms. For some species, there were declines in abundances in the microcosms. This was consistently true for Phoronis sp. During the fall tests when populations of Phoronis sp. were decreasing in all treatments, it was more pronounced in the microcosms.

In some instances, a population trend was equivalent in all treatments. Such was the case for recruitment of immature capitellids during Test 1, and for the declining abundances of immature bivalves and P. littoralis during Test 1 and Test 4, respectively. The downward slope of the recruitment of P. littoralis was also included in the second spring test (Test 3). However, the populations in the microcosms did not decline. Two species, Tubificoides sp. and M. ambiseta, had fairly constant populations. There were no sizable increases or decreases in abundances, and no differences between treatments. Slopes of the dominant species indicated an effect of exposure to hydrocarbon-contaminated Elizabeth River sediment only in the microcosms. Scoloplos sp., immature bivalves, M. ambiseta, and immature capitellids all declined in the contaminated microcosms more rapidly than in the unexposed microcosms.

Summary of Findings

In the context of microcosm research, it is not necessary that we know the causes of population fluctuation but only that fluctuations occur. It is the interactive nature of the community and the environment that generates the fluctuations we observe. So, in evaluation of a microcosm

toxicity test, it is necessary to consider the broad, total-community approach. We should avoid singling out one species for assessing toxicity.

Long-term population dymamics will result in periods when any given species may be present in low abundance. This would make repeated testing difficult if those species in low abundance were needed. Also, at any laboratory conducting community microcosm tests, it is essential to know the natural population fluctuations. Otherwise, major changes in the community associated with natural cycles would be missed, making interpretation of microcosm results difficult or misleading. The total community represents a single energetic entity. From year to year, about the same amount of energy flows through the community. Although individual species patterns are different from year to year (and consequently the amount of energy flowing through each species is different), the total energy budget is relatively constant.

The following findings exemplify the need to consider the total system:

1. *Tharyx* sp. declined by a factor of 10 from 2,000 m^{-2} in 1983 to 200 m^{-2} in 1982. The *Mediomastus ambiseta* population increased from low abundances in early 1980 to peak in mid-1982 and declined through 1983. *Paranais littoralis* did not have successful recruitment in 1980 or 1981, and it was not even a community dominant until 1982. Most of the dominant species exhibited some year-to-year variation that might make repeated testing difficult if it were based on a single species.

2. The major natural fluctuations in the community were associated with recruitment. Should the initiation of a microcosm test unknowingly coincide with recruitment, populations could increase or decrease by orders of magnitude in test treatments. The onset of recruitment can generally be easily identified from the size of individuals. It is the subsequent decline of the recruitment peaks that could cause problems of interpretation. Without knowledge of the natural timing of these declines, it might be difficult to identify toxic effects. The species that consistently exhibited highest mortality after recruitment from 1980 to 1983 were *Paranais littoralis*, *Streblospio benedicti*, and *Heteromastus filiformis*.

3. Overall, there was about the same density of individuals in 1980, 1981, and 1982 (defining the year from October to September to better coincide with recruitment). In 1983, the density dropped by a third. In 1980 and 1981, populations of the dominant annelids were about the same size both years. Based on this fact and the assumption that total yearly production can be partitioned between species and still remain constant from year to year, it seems likely that the total production for the York River site was the same in both 1980 and 1981. We have not looked at the size of individuals in 1982 and 1983 to see whether this is the general trend. The importance of this productivity to microcosm testing is in understanding the interactive nature of the community. If one species is in low abundance for a given

year, then another may be more abundant and offset the loss in productivity. Although the community structure changes, the functioning of the community remain unchanged. Microcosms need to capture this functional response to represent field response truly.

It took a broad view of all parts of the community to see the relationship between the laboratory microcosm, which is the target of interest as a tool to judge environmental consequences of toxicants, and the field. Cluster analysis indicated that during Test 1 (spring of 1982) the microcosms behaved very much like the field, but in Test 3 (spring of 1983) they did not. Apparently, in the spring of 1983, recruitment into the microcosms was reduced relative to the field, possibly because of some laboratory artifact or timing of the test relative to recruitment peaks. Results of the fall tests were consistent, with recruitment being less in the microcosms. With this understanding that recruitment into the microcosms will likely be lower than in the field, because of the nature of the test system, we can more accurately interpret toxic effects in the microcosms.

No one species was able to carry consistently sufficient information about the validity of the microcosm test system. Analysis of the variation in individual species abundance within and between tests showed that most species did not have a consistent response to the full-field, semi-field, or microcosm treatments. The exception was Phoronis sp., whose populations were always lowest in the microcosms because of an artifact of the test system (larger individuals live deeper than 10 cm in the sediment and were damaged when the microcosms were established). It seems that the behavior of the natural system, and any portion of that system brought into the laboratory, has a stochastic component that precludes taking a few of the species and putting the whole back together again.

Preliminary Toxicology

Preliminary toxicity tests were conducted to evalute further the validation criteria developed in the previous tests. These experiments were carried out with contaminated sediments taken from the Elizabeth River (VA) to develop techniques for application of a toxicant to laboratory microcosms and field treatments. This sediment had (parts per thousand) concentrations of polycyclic aromatic hydrocarbons. Unpolluted sediments from the York River and Apalachicola estuary were used as treatment controls. Contaminated sediments were applied to enclosures over a twenty-four hour period to allow settling of this sediment. Even with nominal toxicant concentrations, certain problems were noted concerning the response of the laboratory microcosms and field treatments to the toxicants:

1. Overall, simultaneous laboratory-field experiments require close attention to the mode of application with comprehensive chemical analysis to evalute equivalence of exposure while specific objectives of the validation process are fulfilled.

2. Close chemical surveillance is necessary concerning the distribution of the toxicant.

3. Field treatments should be carried out in such a way that control areas are not contaminated.

4. Protocols for treatment should be developed so that recognizable but transient effects are noted without causing persistent adverse impact on the infaunal biota.

In summary, the nominal toxicant test indicated that the establishment of the microcosm treatment was the most sensitive part of the experiment. Most of the variation in abundance and changes in species could be attributed to the microcosm treatment. Through the course of the experiment, microcosms exposed to hydrocarbon-contaminated sediment did show the greatest degree of change. This sensitivity of the laboratory microcosms to toxic stress was documented even though there was a considerable contribution to the variance from the treatments. The exposure was possibly not as effective in the field treatments because of differences between laboratory and field conditions in terms of water volume and the even distribution of contaminated sediments. This problem may have reduced the component of variance caused by exposure to contaminated sediments.

SECTION 5

CRITERIA FOR VERIFICATION PROCEDURES

The simultaneous use of replicated multispecies microcosms (as defined in Giesy, 1980) and field mesocosms (Grice and Reeve, 1982) to test the validation hypothesis has led to specific observations concerning the relationships of full-field, semi-field, and controlled conditions. Criteria that relate the laboratory and field approaches to research of benthic estuarine associations are given in Table 3. Physical and chemical changes in the laboratory sea water quality relative to field conditions are unavoidable. Laboratory artifacts include changes in hydrostatic pressure, and current structure, which may lead to different sedimentation patterns. Procurement, transfer, and placement of sediments in the microcosms also sometimes leads to severe alterations of sediment conditions. Specific changes in the microcosm habitat arise from its isolation from the field and are enhanced by surface features of the laboratory enclosure. Although the effects of laboratory conditions can be avoided in varying degrees, duplication of field conditions is usually precluded by the conditions imposed on the microcosms. The real problem is to define those aspects of microcosm function that can be used to explain field conditions.

Some features of laboratory microcosms are especially difficult to control. Sudden changes of temperature or sedimentation in the field cannot be replicated in the laboratory microcosm. At the same time, the microcosm often acts as a silt trap through time, thus altering sediment and water column relationships relative to the field. Whereas natural physical disturbances such as storm effects are lacking in the laboratory microcosm, other features of the sediment and water column within the microcosm undergo a departure from natural conditions because of the limitations imposed by the size of the microcosm as compared to a virtually limitless natural environment.

Physical disturbance of the sediments in a given microcosm can be divided into two primary sources of impact: transference of sediments in the establishment of the microcosm and sampling during the course of an experiment. Our experiments indicated that establishment of the microcosm and separation from surrounding sediments can have an immediate impact on the macroinvertebrate assemblages in the microcosm. Often, certain sensitive species were lost in the transfer; the exact impact of this effect on numerical abundance and species richness varied according to seasonal patterns of relative abundance in the estuarine associations. Results of some experiments indicated a deviation from field conditions within time periods of 4-6 weeks. Too-frequent (i.e., weekly) sampling of the

TABLE 3: Criteria for review of the validation of infaunal macroinvertebrate microcosms with semi-field mesocosms and full-field conditions in estuarine systems.

Factor/Condition	Full-field	Semi-field cage	Microcosm
Physico-chemical			
Water source	No effect.	No effect.	Drawn from near bottom, 50-100 m from field and semi-field site.
Water supply	No effect.	Flow impeded by screen; some recruits set on screen.	In-pipe setting only in last 15 m; minimal reduction in O_2; alterations of larval setting.
Currents	Unaffected; variable in magnitude and direction because of tides and wind effects.	Slowed by screen mesh; variable in magnitude and direction because of tide. Effects minimized by choice of mesh and cage design.	Established by position of input and output and by sediment boxes; invariate once stablished. No true simulation of tidal and wind-driven currents.
Sampling effects	Damage to fauna during sampling; slumping of sediment to fill core holes.	Same as for full-field. Slumped holes may have trapped organics, making attractive site for larval setting and immigration.	Replacement of cores with azoic sediment; migration into azoic sediment led to dilution of populations. Because of scaling effect, sampling had more of an impact on the microcosm than under field conditions.
Light	Unshaded, but low light intensity due to water depth/turbidity.	Same as full-field.	Microcosm tanks varied from being partially shaded to a general replication of light intensity in the field.

(continued)

TABLE 3 (continued).

Factor/Condition	Full-field	Semi-field cage	Microcosm
Physical/Chemical (continued)			
Sedimentation	Frequent resuspension.	Possibly enhanced by reduced water flow. May be reduced near edges by scouring. No major effects noted in sediment characteristics over 4-9 week periods.	Enhanced by slow water flow in microcosm tank. May be changed by water intake system. Accumulation of silt may have been enhanced beyond the rate in the field.
Physical Disturbance	Bioturbation by epifauna (e.g., crabs and fishes). Enhanced microbial activity.	Large sediment disturbances excluded, activity of smaller species became more important. Enhanced microbial activity.	Certain forms of bioturbation were enhanced because of limited area compared to field. However, large-scale disturbance due to storms and tidal currents, not reproduced in microcosms.
Sediment Compaction/ pore water	No effect.	No effect.	Compaction reduced by removal from field and subsequent slumping; probably gradual compaction as experiment progresses. Flow of pore water restricted, possible changes in granulometric properties.
Sediment temperature	No effect.	No effect.	Temperature changes relatively rapid, no insulation; minor difference from field.

(continued)

TABLE 3 (continued).

Factor/Condition	Full-field	Semi-field cage	Microcosm
Physical/Chemical (continued)			
Sediment pH	No effect.	No effect.	Sedimentary processes affected pH by shallowness of microcosm, sedimentation enhancement, and changes in compaction.
Substrate depth	No effect.	No effect.	Limited escape routes, deep-dwelling organisms eliminated. Vertical organization of macroinvertebrates altered by depth restrictions.
Hydrostatic pressure	Variable because of tides, waves.	Same as full field.	Usually lower than field; less variable.
Biological			
Larval recruitment	No effect.	Possibly affected by mesh of cage. Solid substrate may have attracted some species. Affected by mesh size and type. No effects noted in exclusion cages in this series of experiments.	Possibly affected by difference in water source; potential change in available recruits due to passage through pipe; solid substrate attractive for setting of some species, selective (species-specific) mortality in lab.

(continued)

TABLE 3 (continued).

Factor/Condition	Full-field	Semi-field cage	Microcosm
Biological (continued)			
Predation (large, mobile epibenthic organisms)	Major impact under specific conditions of salinity and at certain seasons of the year.	Impact reduced by exclusion of large mobile predators.	Same as semi-field.
Immigration	No effect.	Possible effect of screen inserted into substrate and in water column.	Probably eliminated; most pelagic immigrants were probably destroyed by pumps.
Competition	Interference competition may have been important, although complexity precluded generalization.	Same as full field.	Same as full-field and semi-field.
Food source	No effect.	Possibly enhanced enrichment from cage mesh.	Possibly altered by seawater system, microbial effects.

microcosm during such experiments also led to alterations in the microorganism and macroinvertebrate assemblages.

If the laboratory microcosm was isolated from natural benthic assemblages, recruitment processes were altered, as shown in results from both research groups. Such changes can be enhanced by handling of water prior to entry into the microcosm. Another possible recruitment problem is isolation from surrounding populations that propagate through benthic transfer of larvae rather than through the plankton. Immigration is severely restricted. Patterns of recruitment and immigration are habitat- and time-dependent. Each species recruitment pattern should be evaluated to determine the potential for extrapolation of laboratory results to field conditions.

Biological processes other than recruitment may be altered under laboratory conditions. Isolation from natural field processes disconnects microcosm assemblages from interactions with various types of predators. Our experiments indicated that the impact of predation on field assemblages of macroinvertebrates was extremely complex. In addition to gradient effects of salinity on such impact, there were also seasonal differences in the predator influence in the field. During spring periods of maximal influence of predation impact in polyhaline areas of the Apalachicola Bay system, isolation of the microcosm in the laboratory led to increases of dominant populations that follow observed changes in exclusion cages in the field relative to inclusion cages and cage controls. Such changes were associated with altered microbial community structure. At other times of the year and under oligohaline conditions, no such effects were observed. Direct and indirect effects on natural energy relationships also occurred in the microcosms. Such effects may have given selective advantages to certain macroinvertebrate populations. Altered predation pressure, together with unavoidable restrictions in the depth (and vertical population distribution) of the microcosms, may alter competitive interactions that occur naturally in the field. The difficulty of demonstrating such complex competitive interactions under field conditions disallows strict generalization. Overall, simultaneous experiments with laboratory microcosms, semi-field conditions, and full-field conditions indicated that biological interactions comprised an important element in the verification of the predictive capability of microcosms to natural conditions.

The laboratory microcosms followed field conditions when viewed as groups of interacting populations rather than as sets of individual populations. Specific community parameters, such as species richness and diversity, and other indices of multispecies associations, when qualified by known changes caused by laboratory artifacts, were representative of field situations. Verification of both microbial and macrobiological assemblages was possible only within the bounds of our knowledge of the systems in question. Moreover, the critical factors that determined qualifications (i.e., recruitment, predator-prey interactions, relative species dominance) were relatively similar in two entirely different experimental areas. Thus, field to field extrapolation of results is also possible when it is based on a thorough knowledge of the subject systems.

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APPENDIX A

FIELD OPERATIONS FOR THE VERIFICATION PROJECT INCLUDING A LIST OF PHYSICAL/CHEMICAL AND BIOLOGICAL SAMPLING PARAMETERS TAKEN WEEKLY AT THE RESPECTIVE STUDIES SITES (FSU, OLIGOHALINE, POLYHALINE; VIMS, POLYHALINE).

I. Water quality (surface and bottom)
- depth (m)
- salinity (°/oo)
- color (Pt-Co units)
- turbidity (JTU)
- dissolved oxygen (ppm)
- pH

II. Meteorological data (FSU only)
- Apalachicola River flow (daily, m^3/sec)
- Apalachicola rainfall (daily-monthly, cm)

III. Sediment quality
- temperature
- water content
- Eh profile
- organic content
- grain size

IV. Biological factors
- microbiota
- meiofauna (VIMS, FSU)
- benthic macroinvertebrates (500- and 250-μm sieves)
- meroplankton (FSU only) 82-μm plankton net)
- epibenthic fishes and invertebrates (FSU only) (repetitive 5-m otter trawl tows)

APPENDIX B

GENERALIZED PROTOCOL FOR LABORATORY MICROCOSM/FIELD-SEMI-FIELD VALIDATION STUDIES.

I. Laboratory microcosms (0.7-1.0 m^2)
   A. Physical/chemical data
      1. temperature (°C)
      2. salinity (°/oo)
      3. color (Pt-Co units)
      4. turbidity (JTU)
      5. dissolved oxygen (ppm)
      6. pH
      7. sediment % organics
      8. sediment grain size
      9. sediment temperature, salinity, Eh
   B. Infaunal macroinvertebrates (500- and 250-μm sieves)
      1. repetitive cores (3 replicates, 1-3 treatments; FSU only)
      2. vertical distribution (2-cm intervals)
      3. azoic sediment samples (500- and 250-μm sieves
   C. Microbes
      1. repetitive cores (3 replicates, 1-3 treatments)

II. Field
   A. Treatments (3 replicates)
      1. unscreened platforms
      2. screened platforms (exclusion cages)
      3. screened platforms (predator-inclusion cages)
      4. weekly core samples (no platform)
      5. additional treatments (sepcific for individual experiments)
   B. Physical/chemical data (same as I.A.)
   C. Infaunal macroinvertebrates (same as I.B.)
   D. Microbes (same as I.C.)
   E. Epibenthic fishes and invertebrates (repetitive otter traw tows)
   F. Meroplankton (repetitive plankton net samples)

III. Variables analyzed
   A. Infaunal macroinvertebrates, epibenthic organisms
      1. numerical abundance (total and dominant species)
      2. ash-free dry weight biomass (total and dominant species)
      3. species richness
      4. species diversity and evenness indices
      5. productivity (growth per unit time of dominant species)
   B. Microbes
      1. total biomass (phospholipid, 16:0, 16:1ω7, 18:2ω6, 18:1ω9)
      2. bacteria (iso and anteiso 15:0, called Br 15:0, 15:0)
      3. photosynthetic microbes (16:1ω13t)
      4. microeukaryotes (20:4ω6, 20:5ω3, 20:3ω6, 22:4ω6, 22:6ω3)
      5. bacterial ecotype (18:1ω7)

IV. Experimental Methods

A. Weekly analyses

Weekly field samples (see Appendix A) provided background information for the combined laboratory-field tests carried out by personnel from FSU and VIMS. Samples of macrofauna were taken weekly from the study sites from October, 1982 (FSU), and September, 1979 (VIMS).

B. Laboratory-field Tests

The combined experiments were conducted in the spring and fall, 1982, and spring and fall, 1983. Each test was from five to nine weeks in duration. Test 1 was sampled weekly; all subsequent tests were sampled at two-week intervals after the initial two samplings (T_0, T_1), which were one week apart (T_0 = initial sampling).

For most of these tests, three basic field treatments were used:

Full-field -- either a 2 m x 2 m platform or an area delineated by a 30-m circle. No manipulation of the environment involved.

Semi-field -- a 2 m x 2 m caged platform, screened with 6-mm galvanized steel mesh, which extended 30 cm into the sediment. The cage excluded large predators that would also be excluded from the laboratory microcosms.

Laboratory microcosms -- 0.8-1 m x 1 m x 0.1 m fiberglass boxes placed in larger tanks with unfiltered water flowing through at the rate of 12 to 19 turnovers/day.

In addition, the FSU group used a predator-inclusion treatment using indigenous species in numbers resembling actual densities at the time of testing.

Sediments for the microcosms were obtained by SCUBA divers in the following manner. Rectangular metal cans (10 x 20 x 10 cm) with their bottoms removed and screw caps open were inserted into the sediment to a depth of 10 cm. The lids were tightened and intact sections of sediment were transferred in the can to the laboratory. Multiple cans containing sediment were placed adjacent to one another in the microcosms; the lids were opened and the cans removed leaving the sediment in the microcosms. In this way, relatively large areas of sediment were transferred to the laboratory with minimal disturbance of their vertical structure.

At weekly intervals, random samples of sediment were removed from three of the microcosms using plastic core tube. At each sampling four cores were removed from each microcosm. At the same time, similar samples were removed from the field. In the field, four or five random samples were taken from each of three 2 x 2 m control plot.

APPENDIX C

SAMPLING SCHEDULES FOR THE COMBINED (FSU-VIMS) EXPERIMENTAL PROGRAM (1981-1984).

I. Weekly samples
   A. FSU
      1. oligohaline stations 3, 5A (11/24/81-11/17/83)
      2. polyhaline station ML (11/25/81-3/15/84)
   B. VIMS
      1. polyhaline marine lab station (10-13-79-12/18/83)

II. Microbiological data
   A. FSU
      1. station 3 (fall, 1982; spring, 1983)
      2. station ML (spring, 1982)
   B. VIMS
      1. marine lab station (spring 1982)

III. Combined (field-laboratory) experiments
   A. FSU
      1. station 3 (fall, 1982; spring, 1983)
      2. station ML (spring, 1982; fall, 1983)
   B. VIMS
      1. marine lab station (spring, 1982; fall, 1982; spring, 1983; fall, 1983; spring, 1984)

Test 4 was carried out in a somewhat different way by the two research groups. The VIMS group used a nominal toxicant test using "naturally" hydrocarbon contaminated sediment from the Elizabeth River, Virginia. This sediment had parts per thousands concentrations of polycyclic aromatic hydrocarbons. Unpolluted York River sediment was also used as a control, being similar in compositoin (80-90% silt-clay) to the polluted Elizabeth River sediment. Expsure was accomplished by creating of an enclosed calm environment for 24 hours to allow settling of the sediments.

The FSU group conducted a five-week test with a protocol similar to that used during previous tests. Contamination of the field treatments and lab microcosms with the hydrocarbon-contaminated sediment from the Elizabeth River, Virginia, together with sediment controls from the VIMS and FSU study areas, was then undertaken subsequent to the T_5 sampling period. Exposure methods were the same as those described above for the VIMS program. These treatments were then sampled over a 4-week period (T_7, T_9).

GLOSSARY

Benthic -- of or pertaining to the bottom of any aquatic, estuarine, or marine habitat.

Bioturbation -- process of mixing, or otherwise disturbing, the physical structure of sediments by organisms.

Community -- a group of species living together in the same place.

Diversity -- a measure of the relationship between the number of species present and their relative abundances, based on Shannon's and Brillouin's formulas.

Dominant -- a species that is numerically most abundant in a sample or habitat. Usually there are several dominant species and many more less abundant species present in a habitat.

Evenness (equitability) -- A measure of the relative numbers of individuals of sepcies prsent in a given area.

Full-field -- a term used to describe the natural undisturbed field habitat; represents one of the three treatments used in the validation process.

Infauna -- species that live in the sediment.

Long-term -- time spans of years.

Macroinvertebrates -- individuals of species that are retained on 250-μm sieves.

Mesohaline -- salinities in the range of 5 to 18 °/oo.

Microcosm -- a portion of the natural field habitat that has been brought into the laboratory, with as little disturbance as possible.

Microorganisms -- individuals of species that pass through a 62-μm sieve; mainly bacteria and other unicellular forms.

Oligohaline -- salinities in the range of 0.5 to 5 °/oo.

Opportunistic species -- One whose life history includes rapid response to disturbance and underutilized resources.

Polyhaline -- salinities in the range of 18 to 32 °/oo.

Recruitment -- process whereby communities renew their numbers; usually involves reproduction and settlement of new individuals, but can also be accomplished by migration of adults.

Semi-field -- a term used to describe the areas in the field that are enclosed by 6-mm mesh cages. Each of these cages was 2 m on a side.

Scaling parameters -- number and size of replicates needed to give statistically reliable results.

Short-term -- time spans of weeks to months.

Species diversity -- index derived from species richness and evenness components

Species richness -- a component of diversity that measures the number of species relative to the number of individuals or simply the total number of species in a sample.

Validation -- determination of the capacity of a specific laboratory microcosm to predict the environmental response to toxic materials. A field validated microcosm would then provide a realistic means of correlating toxicological data with expected field results.

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