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# Project Summary

# Field Validation of Laboratory-Derived Multispecies Aquatic Test Systems

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**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 in Florida, and the York River estuary in Virginia, 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.**

***This Project Summary was developed by EPA's Environmental Research Laboratory, Gulf Breeze, FL, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).***

## Introduction

The basic question underlying 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

cific 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.

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; Florida State University) and Virginia (York River estuary; Virginia Institute of Marine Science). Our principal objectives were (a) 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 underway to determine the potential for extrapolation of validation results from one location to another.

## 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. 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.

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. 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 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 × 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. The approach was to sample replicated flow-through laboratory microcosms (0.8–1.0 $m^2$) derived from natural soft-sediment areas, simultaneously with field treatments (exclusion cages, inclusion cages, cage controls) (Figure 1).

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 no significant difference existed 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" and Czekanowski similarity coefficients and the flexible groping strategy with beta = −0.25.

## Results and Discussion

### *Experimental Program: Florida State University*

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 pro-

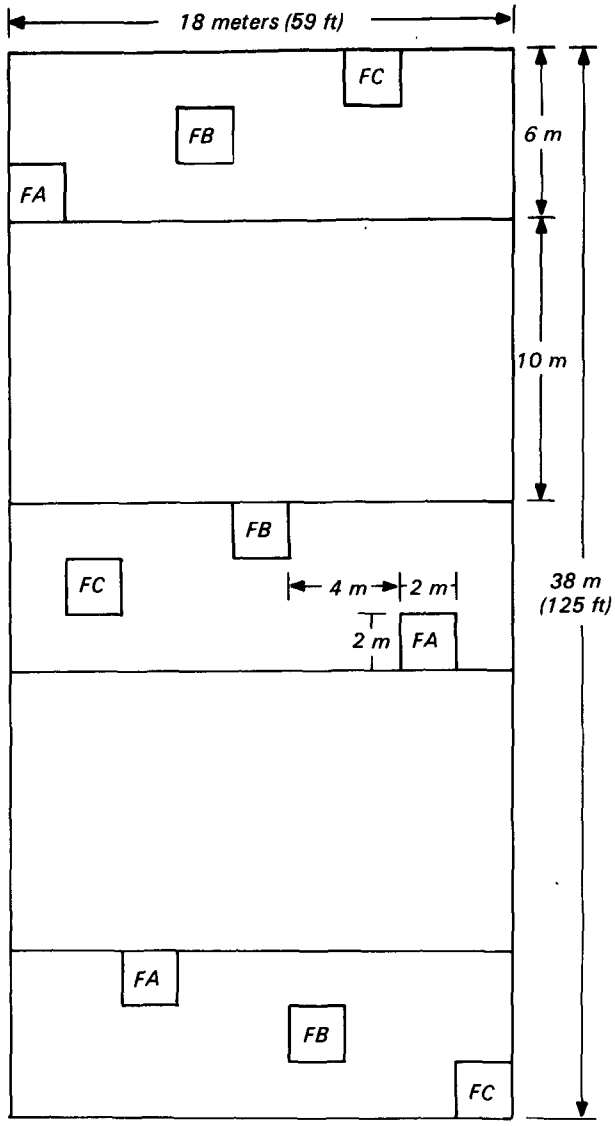


***Figure 1.*** *Diagram showing placement of cages (inclusion/exclusion cages), cage controls, and full-field sampling areas.*

vided 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 significance 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*

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 dynamics will result in periods when any given spespecies 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 remains unchanged. Microcosms need to capture this functional response to represent field response truly.

A broad view of all parts of the community was needed 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 evaluate 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 evaluate 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 showed 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.

## Conclusions and Recommendations

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.

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.

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 functional 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.

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.

## 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 1. 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 spespecies 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 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 plankton. Immigration is severely restricted. Patterns of recruitment and immigration are habitat- and time-dependent. Each species recruitment pattern should be

**Table 1.** *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 established. 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.* |
| *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.* |
| *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.* |

**Table 1.** *(Continued)*

| *Factor/Condition* | *Full-field* | *Semi-field cage* | *Microcosm* |
|---|---|---|---|
| *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.* |
| *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.* |

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-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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