Aquatic Microcosms for Assessment of Effluent Effects

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ELECTRIC POWER RESEARCH INSTITUTE

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ABSTRACT

Laboratory freshwater lake microcosms are studied with respect to their usefulness in providing a tool for environmental impact assessment. The microcosm system studied consists of a diverse assemblage of phytoplankton, zooplankton, and microbes in four sets of sextuplet 50-liter tanks initiated under four different nutrient level conditions. Three four-month studies were carried out, each with a different pollutant expected to be found in significant concentration in the aqueous effluent from coal gasification operations. These studies have led to: i) identification of those biological and chemical parameters most useful for evaluating the responses of microcosms to chemical pollutants, ii) characterization of the difficulties in obtaining replicate microcosms that approximately simulate natural systems, iii) a test of the usefulness of microcosms in evaluating hypothetical stability indicators, iv) evaluation of the relative usefulness of the bloom and the quiescent stages of microcosm development for impact assessment studies, v) a specific research plan for determining optimum procedures for microcosm design, initiation, and operation.

EPRI PERSPECTIVE

PROJECT DESCRIPTION

This final report, Aquatic Microcosms for Assessment of Effluent Effects, describes the first element of a research effort to develop and apply microcosm systems to the assessment of ecological questions related to electric power production. Microcosms are essentially model ecosystems that have been assembled in the laboratory. A microcosm study represents a middle ground between experimental field studies and laboratory bioassay studies of toxicity effects: It permits manipulation and experimental controls not possible under field conditions, while having a higher degree of "reality" than bioassay experiments. It is hoped that this line of research will help in designing field experiments as well as in developing an independently useful data base.

PROJECT OBJECTIVES

The overall objective of the study was to evaluate the feasibility of using small (50-liter) freshwater microcosms and ecological stability indicators to assess the effects of coal conversion effluents on freshwater ecosystems. Specific questions addressed included

- Identification of biological and chemical parameters that would be most useful for evaluating the responses of microcosms to constituents of coal conversion effluents,
- Assessment of replicability of freshwater microcosms simulating natural systems,
- Testing of a hypothesized, predictive, stability indicator against data derived from microcosm experiments.

CONCLUSIONS AND RECOMMENDATIONS

The overall conclusion of the feasibility study was that chemically closed, small (50-liter) freshwater microcosms are a potentially convenient experimental tool for intermediate- and short-term assessment studies if standard procedures for design and operation are established. A number of problems identified during the study must be overcome before this type of microcosm can become an established technique. Recommendations for research to improve aquatic microcosm methodology were developed.

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EXECUTIVE SUMMARY

Continuing social concern over the effects of coal gasification and liquefaction on aquatic systems demands strategies for reconciling our needs for new energy sources with the necessity for preserving our natural environment. Part of the solution lies in choosing sites for coal conversion facilities where the environment is sufficiently robust, or buffered to absorb the impact of these activities. In order to aid in site selection we have been working towards the development of practical methods for predicting the robustness, or conversely sensitivity, of freshwater ecosystems.

Our long-term goal for expediting the process of rational site selection can be illustrated with the following hypothetical example. A coal gasification facility is to be located in the vicinity of one of several lakes. To the extent that the effluents have been characterized, it is now desired to determine which of the lakes will be least disturbed by the facility. Ideally, one would measure certain ecological properties (which we call stability indicators) of the lakes and on the basis of the results draw conclusions concerning the relative future robustness of each lake should it be selected as the site for the facility. Needless to say, we are far from this goal today; ecology lacks the requisite predictive capability. Described here is an assessment of one approach to the attainment of this goal.

Ecosystem impact assessment has available four basic methods of research: experimental field studies, laboratory dose-response studies of toxicity effects on single organisms, laboratory studies of whole ecosystems effects (the microcosm approach), and theoretical analysis (which can range from simple models designed to aid in the search for correlations and assist in data analysis, to complex models which attempt to provide a tool for detailed ecological prediction).

Each of these approaches has both drawbacks and advantages. No one of them alone is adequate to the task of impact assessment, in general, and the development of a predictive capability, in particular. Field studies suffer from i. the large expense involved in data acquisition, ii. the absence of experimental controls in experiments carried out on disturbed systems, and iii. the difficulty in readily manipulating the experimental system. Species-specific dose-response studies in the laboratory, while valuable for the purpose for which they are designed, do not provide enough information to assess potential impacts at the system level which can arise from exposure to toxic substances. Moreover, the effects of certain substances, even on individual organisms, may be mitigated or enhanced during transit through an active and rich aquatic ecosystem. Laboratory microcosms may suffer from three potential drawbacks: i. their relatively large surface-to-volume ratio (as compared with natural systems) may lead to distortion of behavior, ii. a realistic diversity of species, particularly among the larger organisms at the top of the food chain, may be impossible to maintain except, again, by introducing distortion of behavior, and iii. the absence of realistic environmental variability in the laboratory may lead to the lack of opportunity to observe synergistic effects occurring as a result of the collusion of stresses arising from toxic substances and those arising from natural environmental variability. Finally, the disadvantages of a purely theoretical approach to impact assessment are, as in all sciences, self-evident.

An increasing number of research groups have employed microcosms in recent years, motivated to a great extent by the possibility that they can provide a non-trivial interacting ecosystem for assessing effects of toxic materials - a system which is easily manipulated and monitored, is much more similar to natural systems than single-organism cultures, is relatively inexpensive to use compared to field studies, and results in no damage to natural systems. Yet few, if any, standard methods for the design, installation, and operation of microcosms have resulted from this interest. Moreover, the influence of the previously mentioned potential drawbacks of microcosms on the validity of results obtained from their use has been given scant attention. Our ultimate objective, the development of predictive indicators that allow prediction of the sensitivity of a system to disturbance, would be greatly facilitated if microcosms could be used to generate data necessary for development and testing of such indicators. Our first priority, therefore, was to assess the microcosm method itself and determine the conditions under which microcosms can be useful for understanding effects of toxic substances and for evaluating possible stability indicators.

The specific environmental stress with which we have been concerned is the effluent waste water from coal conversion processes. This water contains substances known to be toxic to many organisms, including man, above certain concentrations. We have sought:

- To identify those biological and chemical parameters most useful for evaluating the responses of microcosms to constituents of coal conversion effluents.
- To characterize the difficulties in obtaining replicate microcosms that approximately simulate natural systems.

- To test the use of microcosms in evaluating a hypothetical predictive stability indicator derived from theoretical studies.

Our experiments used four sets of freshwater lake microcosms, differing in their nutrient levels. The four lake conditions created in the laboratory correspond to four different broad types of lakes found in nature. A range of nutrient conditions was studied in order to characterize problems of microcosm design and operation common to diverse systems or specific to particular conditions. In addition, a range of microcosm conditions was necessary in order to create systems which would exhibit differing responses to each of the substances (ammonia, iron, and phenol) used as perturbations. The microcosms consist of an assemblage of phytoplankton, zooplankton, and microbes in 50-liter aerated cylinders. Their diversity and population levels resemble that of natural lakes.

For each of the three perturbations studied, a four month experiment was carried out. In the first two months of each experiment, six replicate microcosms of each of the four nutrient types were observed. At the end of the two months, three of each of the six systems were perturbed and the remaining three left as controls. A two month period of observation of the perturbed and control systems followed. A set of sensitivity indices, one for each of the chemical and biological parameters measured weekly throughout the experiments, was calculated and used to quantify both the replicability of the systems and their responses to the toxic substances.

A hypothesis, to be tested in these experiments, was that a particular indicator, to be measured prior to the disturbance of the systems, would allow prediction of the relative responses of the four types of systems to each of the perturbations. The indicator, denoted by K_D, is a parameter characterizing one aspect of the decomposition and nutrient cycling processes in an ecosystem. Earlier theoretical studies of nutrient flow in ecosystems had suggested its relevance to stability. As part of the test of our hypothesis, the value of the indicator was measured at the end of the first two months of each experiment, for each of the four types of lake conditions represented. These measurements were carried out in small subsystems derived from the larger microcosms. The values of the predictive indicators were then compared with the robustness of the perturbed systems as measured by the sensitivity indices. In the review of this series of experiments we examine what we have learned about the design and operation of microcosms as tools for toxic substance impact assessment and we evaluate the hypothesized stability indicator.

An assessment of the use of microcosms for the study of impacts on freshwater lakes has indicated that microcosms have the potential of being convenient experimental tools for short or intermediate term studies of effluent effects and of predictive indicators provided that standard procedures for design and operation are established. Key problems in the development of these procedures have been identified in this study and the use of microcosms is recommended, contingent upon the resolution of these problems. More specifically, interpretation of the results of these studies leads to the following points:

- Microcosms of the type employed here, with no influx or outflux of nutrients or organisms, can simulate the behavior of natural lakes at best for periods generally not exceeding two to three months. Longer term studies will require solution to the problem of growth of algae on the surface of the containers.

- Microcosms are most useful during a dynamic growth or bloom phase, in contrast with the prevalent viewpoint that microcosms become useful when having reached a static state.

- A progressive decrease in the replicability of the microcosms in successive experiments suggests a systematic problem that correlates with the age of the large microcosm used for initiating our test systems. It is thus likely that microcosms become adversely conditioned by laboratory environments.

- The relatively high proportion of nutrient uptake by surface growth in microcosms diminishes the importance of water column nutrient measurements; quantification of biota is indispensable. The need for determination of biota is reinforced by experiments in which nutrient levels were similar over time in different systems but the biota were quite different.

- The means by which microcosms are initiated affects replicability and ecosystem community structure. Further study of initiation procedures is required.

Direct observations of the effects of three different constituents of coal conversion effluent water on microcosm systems corresponding to four different trophic types of natural systems revealed significant responses to the perturbation in all experiments. As expected, the specific responses of the individual systems depended upon the nature of the perturbation. The phosphorus-rich, nitrogen-limited systems were most affected by the additions of ammonia and phenol; the phosphorus-rich systems were most affected by the addition of iron. The most significant effects of the ammonia perturbation were lowered inorganic carbon and zooplankton levels as compared to the controls. This is consistent with the ammonia stimulating primary production while being toxic to the zooplankton. In the iron addition experiment, the most significant effects were the decrease in phytoplankton and zooplankton. The formation of insoluble iron-phosphate complexes and the chelation of algal surface compounds, resulting in flocculation, were primarily responsible. The addition of phenol to the microcosms resulted in suppression of zooplankton in all systems and of the alga Anabaena in the nitrogen-limited systems.

These results confirm our intuitively held belief and that of many other researchers that:

- Microcosms can be used to simulate specific trophic types of natural systems in order to assess non-trivial effects of perturbations. Because they provide a diverse assemblage of interacting biota, upon which a perturbation can be tested, phenomena are observed which would be missed in single-organism studies.

Our analysis of the validity of our hypothesized stability indicator suggests that the perturbation experiments were not of sufficiently long duration to test the long-term nutrient cycling stability implications of K_D. Without some effective means of controlling side growth of algae, sufficiently long-term microcosm studies for that purpose would not be advised. On the other hand, the results of our experiments do suggest a new and promising approach to the use of short-duration decomposition studies in microcosms for predicting longer-term effects in natural systems. After further studies to determine optimum microcosm design and operation are completed, high priority should be assigned to a rigorous evaluation of this new approach to the problem of ecosystem impact prediction.

- Microcosms hold forth promise for identifying and assessing possible stability indicators for freshwater lakes. Although the quest for a predictive capability in ecology is beset with great difficulties, the ultimate benefit to the site selection procedure of reliable stability indicators warrants further effort in this direction.

Finally, further studies to determine the factors influencing replication and tracking of natural systems by microcosms are indicated. Specific subjects for immediate study, suggested by our experiments, include:

- Optimization of initiation strategy.
- Control of surface activity and/or optimal use of microcosms in the presence of surface activity.
- Determination of the effects of size, nutrient levels and temperature on replicability among microcosms and between microcosms and natural systems.
- The behavior of microcosms during blooms and the effects of perturbation during this dynamic phase, prior to the onset of laboratory conditioning.

Section 1
INTRODUCTION

Continuing social concern over the effects of energy production and conversion on aquatic systems demands strategies whereby our needs for new energy sources can be reconciled with the necessity for preserving our dwindling aquatic resources. Part of the solution lies in choosing sites for energy facilities where the environment is sufficiently robust, or buffered, to absorb the impact of energy related processes. In order to aid in site selection we have been working towards the development of practical methods for predicting the robustness or, conversely, sensitivity, of aquatic systems.

The specific problem with which we have been concerned is the effluent waste water from coal conversion processes. This water contains substances known to be toxic to many organisms, including man, at certain concentrations. The usual approach to this kind of problem has been to study the direct effect of these substances on specific organisms. We are convinced that species-specific toxicological studies, while most valuable for protecting against critical intoxications, do not provide enough information to assess other potential impacts which can arise from exposure to toxic substances at the system level. The effects of certain substances may be mitigated during transit through an active and rich aquatic system, while the effects of others may be amplified. The problem is further aggravated by the fact that many substances interact synergistically to produce large net effects at low concentrations of each. Whole system studies obviously cannot be conducted with the same facility as can toxicological laboratory studies,and for this reason we have chosen to evaluate freshwater microcosms as a means of testing the stability, or sensitivity, of a given aquatic system.

Our ultimate objective is to develop predictive indicators that can reveal the sensitivity of a system to disturbance prior to the imposition of the disturbance. The attainment of this objective would be greatly facilitated if microcosms could be used to generate the data necessary for the development and testing of such indicators. An increasing number of research groups have employed microcosms in recent years,but few, if any, standard methods for the design, installation and operation of microcosms have resulted from this expanded interest. Our first priority, therefore, has been to consider the conditions under which microcosms can be useful for helping to understand system behavior in natural bodies of water and to develop an effective protocol which would necessarily result in reproducible microcosm behavior for its use. We have sought:

- To characterize the difficulties in obtaining replicate microcosms that simulate natural systems;
- To explore the use of microcosms for simulating perturbations of natural aquatic systems by coal conversion effluents;
- To identify those biological and chemical parameters most useful for evaluating the responses of microcosms to stress;
- To test the use of microcosms in evaluating a hypothetical stability indicator derived from theoretical studies.

We have conducted our experiments with four sets of microcosms that correspond to four different types of natural systems in order to identify both general problems in microcosm design and phenomenological parameters common to diverse systems. One of our initial hypotheses, which we have tested using the microcosms, was that the decomposition process could be analyzed to predict the sensitivity to perturbation of the system; a parameter, K_D, reflecting the carrying capacity of the system for decomposition, was identified from theoretical studies conducted previously in an ERDA sponsored project. (1) We measured this carrying capacity and assessed its relationship to the sensitivity of each system, as determined by subsequent perturbation, in order to test its value as a predictive indicator.

Each of the three rounds of principal experiments involved replicate microcosms for each condition and lasted four months. During the first two months of each experiment data were collected and used to assess reproducibility in the microcosm systems for the purposes of locating problems particular to microcosm operation and providing trend lines by which to evaluate the sensitivity indices. At the end of the first two months of each experiment, sub-experiments were conducted to measure the carrying capacity of the decomposers. Immediately following the commencement of each sub-experiment, a common constituent of coal conversion was added to half the microcosms and all systems were monitored for an additonal two months.

At the conclusion of each experiment,sensitivity indices were calculated for various parameters in the microcosms and K_D values were determined from the sub-experiments. Particular problems related to microcosms, such as side growth, were noted. Finally, an additional detrital experiment was conducted to clarify the relationship between an increase of detritus in a system and the subsequent increase in decomposition activity.

In the review of this first series of experiments we examine what we have learned about the design and operation of microcosms and attempt to determine whether we have had a valid test, using this microcosm methodology, of the stability indicator initially suggested by our theoretical work.

Section 2 is a description of our approach to the problem of studying the feasibility of using microcosms as experimental probes. In Section 3 we describe our experimental observations and discuss the results of the perturbation experiments. We then relate conclusions drawn from our data and qualitative observations to problems in the design and operation of microcosms (Section 4). Included in this section is a critique of our methods in which we also consider problems in our approach to monitoring the systems. Section 5 is a statement of theoretical developments. Results and conclusions are summarized in Section 6. Specific details are provided in the appendices; these include write-ups of the three principal experiments, abstractions from which are contained within the main text of this report.

Section 2

OPERATIONAL DESIGN

In this section we will discuss the basis and format for our work. Details of our experimental methods and results are contained in Section 3 and the appropriate appendices.

EXPERIMENTAL

Rationale

Our primary experimental concern has been evaluating the feasibility of using microcosms to determine the impact of energy conversion and production upon aquatic systems. Laboratory studies are motivated by the observation that nature operates by certain inviolable rules, and the likelihood of discovering these rules is increased when the system under scrutiny is complicated by fewer uncontrolled properties and influences. However, laboratory systems cannot be expected to imitate natural systems in all aspects of their biological processes; thus the ultimate usefulness of laboratory systems depends upon the identification and quantification of a sufficient number of properties that are common to both natural and laboratory systems. Quantification of these common properties depends upon the development of theoretical and experimental methods.

We have undertaken a wide range of observations of our microcosms in order to look for indications of properties that may be common to most, if not all, aquatic systems; the breadth of our observations has also been motivated by the need to identify and correct problems that are specific to laboratory systems and thus obscure fundamental properties. We have found, for example, that the exceptionally high underwater surface (sides and bottom) to volume ratios in small laboratory systems result in disproportionately high percentages of sessile biomass: long term operation of microcosms will be possible only if means are found to control this problem. We have also demonstrated that it is unrealistic to attempt to maintain fish in any but exceptionally large laboratory systems. These issues will be discussed, in context, below.

Facilities

All experimental systems were housed in a constant-temperature room, maintained at 18C (62F), and were provided with controlled, high-intensity fluorescent lighting on a 12 hour light:dark cycle. The incident irradiance on the water surface of the microcosms was adjusted to 63 ± 2 μmol m^{-2} s^{-1} PAR. A system of filtered compressed air distribution was used to maintain mixing and aeration in the experimental containers. Oligotrophic microcosms of 700 liter capacity were established prior to the onset of the first experiment and were maintained during experiments in order to provide inocula for each of the three experiments.

Strategy

The strategy in the conduct of the 1977 series of experiments was to establish four sets of microcosms corresponding to a range of trophic states found in nature. We chose to inoculate the microcosms with water from a larger established microcosm with the intent of obtaining a greater degree of uniformity among the different serial experiments than would be obtained using inocula from natural lakes; the choice of type and volume of inocula was of necessity arbitrary, due to lack of information on the subject, and is discussed further in Sections 4 and 6. The decomposition activity in the microcosms was evaluated in a set of sub-experiments carried out in aliquots derived from the microcosms. The resistance of the different types of microcosms to perturbation was tested by introducing into the microcosms one of several common constituents of coal conversion processes.

The Basic Microcosm Experiment. Three sets of microcosms were established in the range of oligotrophic (1 μmol l^{-1} N), mesotrophic (10 μmol l^{-1} N), and eutrophic (100 μmol l^{-1} N) systems, respectively; N:P molar ratios in these sets were between 5:1 and 10:1. A fourth set was oligotrophic and N-limited with an N:P ratio of 1:10. The precise values are given, in context, in the appendices to each experiment. The composition of the nutrient enrichment media used to establish the microcosms in correspondence with different trophic types of natural systems has been described (2).

Each set consisted of two triplets of 50 liter microcosms; one triplet of each set was eventually subjected to contamination by one of the pollutants to be tested while the other triplet remained as a base line, or control, for comparison.

Each experiment was divided into two 2-month phases. The purpose of the first phase was to assess the reproducibility of the sets to be used in the perturbation phase of the experiment and to allow the microcosms to pass through an initial bloom before subjecting them to perturbation; the purpose of the second phase was to determine whether the effects of perturbation by particular constituents of coal conversion effluents, at the levels tested, could readily be seen in microcosms, to assess these effects, and to evaluate the relationship between our original predictive indicator and these effects in different systems.

The pollutants added in each of the three experiments were chosen because of their prevalence in coal conversion effluent water, their ready availability, and their relatively low hazard to laboratory personnel. They were added at concentrations chosen to simulate possible levels in the receiving water. The actual downstream water exposed to effluent discharges would depend upon the size of the body of water so exposed, the through-put of unpolluted water, and the operating level of the conversion facility. We chose 1.6 mM (28.8 ppm) NH_4, 0.12 mM (6.5 ppm) Fe(III), and 5.3 μM (0.5 ppm) phenol. Ammonium was added as a mixture of NH_4Cl and $(NH_4)_2SO_4$ so as to minimize pH modification of the perturbed tanks because we were interested in the effects of the ammonia compound as a source of nutrient. Iron was added as $FeCl_3 \cdot 6H_2O$. In this experiment we decided to allow the pH to respond to the imposition of the acidic chloride salt in order to observe the response of the system to the coupled imposition and because artificial maintenance of the pre-perturbation pH would have resulted in a loss of much of the iron as $Fe(OH)_3$ precipitate. Phenol was added from aqueous solution.

Near the end of the first phase of each microcosm experiment, a beaker sub-experiment was conducted to establish the quantitative values of our predictive indicator. This predictive indicator, K_D, and the quantitative effects of perturbation, S^{-1}, are calculated from the relationships explained below in this section.

Parameters Measured. In both phases of the basic microcosm experiments we monitored a number of biological and chemical parameters on a weekly basis; in the beaker sub-experiments some of these parameters were monitored on a daily basis for the first week and subsequently on an irregular basis for several more weeks. The parameters monitored included inorganic carbon (IC), organic carbon (OC), NH_4, NO_3+NO_2, total phosphorus (TP) phytoplankton (number, volume and dominant species), zooplankton (number, volume and dominant species), chlorophyll *a* flourescence, pH, and temperature. The pH and temperature were measured at two different times during the day on samples maintained under dark and light conditions, in order to derive values of CO_2 exchange for dark respiration and net productivity. The methods utilized have been described elsewhere (1).

The Beaker Experiments. The predictive indicator, K_D, is determined from measured changes in the rate of decomposition in a system when its level of detritus changes.

Prior to perturbation of the microcosms by the introduction of a toxic substance, small aliquots were removed from the microcosms. The use of small aliquots insures that the original systems will not be significantly alterea. It is absolutely necessary to conduct the experiment in this manner because any predictive indicator, if it is to be of value in field application, must be determinable without modifying the natural system.

Four liter beakers were set up for each of the four different trophic microcosm systems. A volume of 2 2/3 liters was removed from each microcosm of each triplet, pooled and divided into two 4 liter aliquots. One aliquot was enriched 40% with respect to organic carbon by a detrital preparation, obtained as described in Appendix A, while the other aliquot served as a control. Thus, in each system, both the original control triplet as well as the original treatment triplet was subjected to this test, providing duplication of the test itself, as the treatment triplet was not yet perturbed.

One method of quantifying the response of decomposers to addition of detritus is to measure the variation in respiratory gas exchange following the addition. In all of these experiments we monitored altered and control respiration rates in every aliquot. In the first beaker experiment, which accompanied the ammonia perturbation experiment, we also monitored bacterial plate counts as well as many of the parameters monitored in the basic microcosm experiments. In the beaker experiments related to the iron and phenol perturbation experiments, we closely examined changes in phytoplankton and zooplankton in selected aliquots, primarily to determine if our experimental approach has further application in understanding decomposition processes in planktonic systems.

The specific details of each experiment are given in the appropriate appendices.

The Indices

The Sensitivity Index. This parameter, S, is used to reflect the effects of perturbation and is defined by the equation:

$$S^{-1} = \frac{1}{n} \int |T - C| \, / \, \langle T + C \rangle_n \qquad \text{(EQ-1)}$$

where T is the value of a particular measurement, such as zooplankton volume or ammonia concentration, in the perturbed tanks, C is the value of the same measurement in the controls and n is the number of days over which the integral is taken.

Calculation of S^{-1} for the period prior to the perturbation serves as a measure of replicability with values of S^{-1} approaching 0 representing high replicability during that period.

The Stability Index. This is the parameter determined from the decomposition beaker experiments and compared with S^{-1} to assess its value as a predictive indicator. It is defined by the equation:

$$K_D = \left(\int(E - C)/\int C\right)/(\Delta D/D_0) \qquad \text{(EQ-2)}$$

where E is the value of a particular measurement in the beakers that were enriched by a detrital preparation, C is the value of the same measurement in the controls, D_0 is the initial amount of detritus (characterized by the level of organic carbon) and ΔD is the change in the amount of the detritus.

DATA PROCESSING SYSTEM

Data analysis is aided by the laboratory's interactive data storage and retrieval system. Experimental data may be stored using a Tektronix 4001 graphics terminal, which is linked to LBL's CDC 6000 computer. The data is stored on-line, using interactive programs written by our group, and is available at any time for correction or review. Any of the data may be analyzed or displayed graphically, and permanent hard copies of the results may be produced. The programs for data analysis and storage have been integrated into an easy to use system, making the computer accessible to all members of the group regardless of computer skills.

Section 3

EXPERIMENTAL OBSERVATIONS

A generalization of the results from each of the three perturbation experiments, including the three beaker sub-experiments, is presented here. More complete summaries of the data for each experiment can be found in the corresponding appendices.

The trophic characteristics of each of the systems were defined by the initial nutrient levels used to establish the microcosms, as shown in Table 3-1.

Table 3-1

CHARACTERISTICS OF THE MICROCOSM SYSTEMS

System	System Type	Limited by N or P	Excess N*	Excess P*	Ratio N:P	Use
A	Oligotrophic	N and P	1x	1x	10:1	Treatment
B	Oligotrophic	N and P	1x	1x	10:1	Control
C	Mesotrophic	P	10x	10x	10:1	Treatment
D	Mesotrophic	P	10x	10x	10:1	Control
E	Eutrophic	Neither	100x#	200x	5:1#	Treatment
F	Eutrophic	Neither	100x#	200x	5:1#	Control
G	Oligotrophic, N-limited	N	1x@	100x@	1:10	Treatment
H	Oligotrophic, N-limited	N	1x@	100x@	1:10	Control

Notes: *Excesses of N and P are relative to tanks A and B; #Excess N was 200x and the N:P ratio was 10:1 in phenol experiment; @Excess N was 2x and excess P was 200x in the iron experiment.

REPLICABILITY

The data acquired during the first two months of each experiment and during the beaker experiments, which were conducted on each system prior to perturbation, provided the opportunity to assess replicability in the microcosms and in the beakers. As explained in the description of sensitivity index, a value of $S^{-1} = 0$ for the period prior to perturbation represents perfect replication between the two triplet sets of a system, for the parameter being measured. The range of S^{-1} is 0 to 1. Calculated values for the pre-perturbation period are presented in Table 2. The values of K_D for each triplet set of a system are derived prior to perturbation and therefore can be compared to provide another parameter for the purpose of assessing replication. A new value, S', is obtained from the same type of relationship used to calculate S:

$$S'^{-1} = |T_K - C_K| \;/\; (T_K + C_K) \qquad \text{(EQ.-3)}$$

where T_K and C_K are K_D for the two sets of each system. No integral is involved because K_D is taken only once in each experiment, but K_D itself is an integral of measurements taken over a series of time intervals. The index, S^{-1}, reflects replication in the beaker experiments while values of S^{-1}, prior to perturbation, reflect replication between the triplet sets.

Table 3-2

REPLICABILITY

S^{-1} and S'^{-1} x 100

Experiment	A-B I	A-B II	A-B III	C-D I	C-D II	C-D III	E-F I	E-F II	E-F III	G-H I	G-H II	G-H III
Parameter												
$S'^{-1}CO_2EV$	nd	2	nd	15	0	nd	33	43	nd	72	5	nd
$S'^{-1}NH_2$	7	nd	2	22	nd	13	27	nd	9	26	nd	33
$S^{-1}CO_2EV$	10	1	11	14	1	1	7	7	5	9	2	4
$S^{-1}NH_4$	7	12	26	5	3	22	6	3	39	5	14	21
$S^{-1}IC$	0	1	2	1	1	2	2	3	1	2	1	6
$S^{-1}OC$	3	5	12	3	5	5	4	5	8	6	10	13
$S^{-1}TP$	6	26	nd	3	18	nd	5	6	nd	4	4	nd
S^{-1}Phyto*	15	27	11	11	25	17	13	13	13	39	56	8
S^{-1}Zoopl	23	nd	2	25	nd	2	6	nd	8	11	nd	2
S^{-1}Rotif	nd	6	18	nd	27	28	nd	81	60	nd	42	85
S^{-1}Crust	nd	71	2	nd	17	6	nd	32	5	nd	24	4
Avgs, S^{-1}												
nutrients	5	8	10	4	5	6	4	4	11	4	5	9

Notes: *Determined from Chlorophyll a flourescence in Experiment I; nd, no data; Experiments: I, NH_4 perturbation, II, Fe(III) perturbation, III, Phenol perturbation. All values are for periods prior to perturbations.

Generalization of the above data must consider the fact that the relative error for a given measurement increases when the levels of the quantity being measured approach the limits of sensitivity of the method. This is the case for phosphorus in the phosphate limited systems. Two apparent trends merit discussion and are dealt with in Section 4: i)measurements of the biota reflect lower replicability than do nutrient measurements and ii) replication as reflected by the nutrients is poorest in the Phenol Perturbation Experiment.

BIOTIC GROWTH IN THE SYSTEMS

Increases in biomass, as reflected by increases in organic carbon, fluorescence, and phytoplankton numbers, began immediately following inoculation in all systems in each of the three experiments. In the first (ammonia) experiment, phytoplankton blooms occurred on the same days both before (days 14-22, 42-56) and after (days 90-111) the perturbation regardless of the initial nutrient conditions. In the second (iron) experiment, phytoplankton blooms occurred in the low phosphorus systems before (days 14-22) the perturbation; in the high nitrogen and high phosphorus systems the blooms occurred at about the time of the perturbation (day 50), while in the low nitrogen high phosphorus systems the perturbed systems had a phytoplankton maximum before the perturbation and the unperturbed systems had one afterward. These maxima were dominated by *Cryptomonas* and *Ankistrodesmus*. In the third (phenol) experiment, the phytoplankton maxima all occurred after the perturbation except in the high nitrogen and phosphorus systems. All the above maxima occurred on different days for different systems, and consisted mainly of *Chlorella*, *Anabaena*, and *Ankistrodesmus*. In all the experiments phytoplankton volume maxima and OC levels were usually highest in the high phosphorus systems.

Following initiation, rotifers were usually the first zooplankton to significantly increase their numbers. Total zooplankton volumes usually achieved their maxima following phytoplankton blooms, the 2nd largest maxima were found in the high phosphorus systems.

THE PERTURBATION EXPERIMENTS

Ammonia

All systems underwent a phytoplankton bloom, on days 90-111, following the perturbation. The peaks of all phytoplankton blooms in this experiment occurred on the same days, regardless of initial nutrient levels and perturbation; the magnitude of the blooms, however, reflected the particular conditions. Zooplankton levels were depressed by a factor of 10 in perturbed sets as compared to controls. Maximum zooplankton levels in the controls were correlated with the initial nutrients as shown in Table 3.

Table 3-3

MAXIMUM ZOOPLANKTON LEVELS

	System Characteristic	$\log cm^3/l$
B	N and P limited	-5
D	P limited	-4
H	N limited	-4
F	N and P excess	-3

The other striking result was a large decrease, at least 50% over 60 days, in IC in the perturbed tanks; the levels of organic carbon and flourescence were approximately the same in the perturbed and control sets except for system G-H. Three possible explanations are discussed in the appendix for the behavior observed in this experiment.

The sensitivity indices for IC, OC, flourescence, net productivity, dark respiration, and NO_3+NO_2 all reflected the greatest response to perturbation in systems G-H (Table 7A-1).

Iron

The addition of Fe(III) resulted in an order of magnitude drop in IC levels as compared to the controls (Fig. 7B-1). This response accompanies a drop in pH from 7 to 4, due to the addition of the acidic chloride salt, and can be entirely accounted for by the lowered atmospheric equilibrium value for CO_2 at pH 4.

The levels of total phosphate in E and G decreased abruptly to those of the phosphate limited systems, A-B, C-D (Table 7B-1). This may also be explained in terms of chemical phenomena by the formation of insoluble iron-phosphate complexes.

Phytoplankton volume decreased in all treated sets immediately following the addition of iron but eventually began to recover in E (Fig. 7B-5). The major cause of the decreases was probably due to flocculation of algae by the trivalent iron. Rotifer and crustacean populations were destroyed by the addition of iron and thus could not have been responsible for the failure of the phytoplankton to recover from the initial drop.

Phenol

The initiation of this experiment differed from the first two experiments in that 10 liters, rather than 25 liters, of inoculum water from the 700 liter parent tank were used to initiate each of the 50 liter microcosms.

Although replicability in this experiment was below that in the previous experiments, it was still possible to observe effects due to the introduction of phenol. The greatest differences between the perturbed tanks were in the zooplankton volumes (Fig. 7C-2). Cladoceran blooms were suppressed relative to the controls in all perturbed tanks, and copepod blooms were suppressed relative to the controls in A and E (Table 7C-2). Rotifers and ostracods did not appear to be affected at the concentration of phenol employed. In general, G-H again appeared to elicit the greatest relative response to perturbation, at least with respect to zooplankton, but the difference between the responses of G-H and E-F was minimal. In the low nitrogen systems (A and B, G and H), *Anabaena* was suppressed by the phenol.

THE INDICATOR EXPERIMENTS

The Measurement of K_D

There were no essential differences in experimental logistics among the three indicator experiments because they were all conducted prior to perturbation of their parent microcosm. Following enrichment of the detrital pool of half the beakers with a preparation of heat-killed *E. coli*, the parameters directly indicative of decomposition generally showed a rise and subsequent fall in all the treated beakers relative to the controls. These parameters were ammonia, dark respiration and bacterial counts. The increase in organic carbon in the water column of the treated beakers, which resulted from the detrital addition, generally underwent a sharp drop during the first two days, a continued more gradual decline for several more days, and then a subsequent rise. The initial drop was most likely the result of the settling of larger particles, while the more gradual decline correlated with increases in dark respiration levels. The subsequent rise reflects stimulation of phytoplankton growth by the nutrients released in the decomposition process.

The values of K_D derived from the parameters indicative of decomposition tended to be highest in system G-H in the first experiment (Table 7A-2). These results were not borne out by the second and third indicator experiments.

The Effects of Detrital Enrichment

An additional experiment was conducted in order to evaluate the relationship between the size of the increase in the amount of detritus and the response of the decomposers. This experiment is described in Appendix 4. It was found that progressively greater additions of detritus led to a greater than linear response in primary decomposition activity, suggesting a need for further understanding the basic decomposition processes in aquatic ecosystems.

Section 4

GENERAL DISCUSSION

A substantial amount of diverse data has been generated during the course of our exploratory studies with microcosms. At the onset of one experimental series we considered two procedural alternatives: i) conducting few kinds but large numbers of repetitive measurements in order to obtain the statistical parameters necessary for a rigorous evaluation of our hypothetical indicators, or ii) conducting relatively few measurements of a larger number of chemical and biological parameters in order to obtain a broader overview of microcosm behavior. Our choice of the latter was determined by the need to firmly establish the reasonableness of microcosms as experimental systems for study of the effects of waste water from energy conversion processes on aquatic systems. In our discussion below, we have focused primarily on specific problems in the design and operation of microcosms and, secondly, on the choice of parameters to be eventually monitored in detail.

DESIGN AND OPERATION OF MICROCOSMS

Some of the problems discussed herein have apparent solutions while others must be investigated in more depth.

Initiation

A 700 liter microcosm, which had been established several months prior to the commencement of the first experiment of this series, was used for inoculation of the 50 liter microcosms. Because the three perturbation experiments were to be conducted sequentially, at different times of the year, we anticipated difficulties in comparing results if the three experiments were initiated by natural lake water, which would contain seasonally variable biota. We found, however, that the biota in our 700 liter microcosm continued to change during the course of the experiments and became progressively less diversified. This problem, which causes us to believe that the use of a long term microcosm for stock cultured biota is not an optimal method, is discussed further in the next sub-section.

Initiating microcosms by enriching demineralized water with nutrients and inoculating it with a small proportion of natural lakewater is not considered a likely alternative. Experience has shown that organisms of low abundance in the inocula may develop profusely in a microcosm environment, often resulting in poor replication, due to significant random variations in the initial levels of organisms present in low numbers in the inoculum. The same problem may occur when proportionately small inocula are derived from a large microcosm and diluted into small microcosms. Problems of scale are discussed below in Appendix E.

A need exists for testing microcosm initiation using a "semi-gnotobiotic" inoculum, one in which all organisms present occur either in large or precisely defined numbers, in order to avoid significant variation for any organism among different inocula. We plan to accomplish this, and a testing of the influence of other aspects of initiation strategy, such as dilution factors and successional stage of the parent system, in a further series of experiments.

Conditioning

It was noted in the previous section that the best replication was obtained between identical sets of microcosms in the first of the three experiments. Replication in the second experiment was acceptable, while that of the third experiment was disappointing. This progressive decline in replicability suggests a systematic problem. In the case of the third experiment improper tank cleaning prior to the experiment probably caused the poor replication.

The communities present in microcosms invariably deviate, with respect to the natural system from which the microcosms were derived, as a function of time in the laboratory. One of the first changes in our microcosms was the disappearance of diatoms. We observed the emergence of Ankistrodesmus, a needle-like chlorophyte that is a common inhabitant of aquaria, early in our experiments. This species, along with Cryptomonas, dominated the phytoplankton communities in the second experiment. Ankistrodesmus was co-dominant with Chlorella and Anabaena in the third experiment.

The loss of species diversity among aquatic communities maintained for prolonged periods in the laboratory may be due to artificial lighting, seasonal and spatial homogeneity, lack of temperature variations, particular surface problems, and other factors that must be understood in order to conduct long term microcosm studies. Conditioning may also be intimately related to the initiation strategy of using proportionately small inocula. The feasibility of reinoculating microcosms on a frequent basis as a means of alleviating the problem of laboratory conditioning is another aspect of initiation and maintenance strategy to be explored.

Resolution of the problem of conditioning, however, necessitates tracking studies, in which the microcosms and the systems from which they were derived are followed concurrently. Manipulations of basic design, such as microcosm size, and of operating procedures, such as periodic reinoculation or semi-gnotobiotic initiation, in tandem with tracking studies, will help to establish standard procedures for microcosm research, enhancing their usefulness and defining the limits of their applicability.

Problems of Scale

Among the most important problems facing aquatic microcosm research are those resulting from the small size of laboratory microcosms, because not all biological and physical processes present in natural ecosystems can be scaled down to laboratory size. Three major problems of scale are:

- The dominance of chemical and biological activity occurring on the sides and bottoms of microcosms;
- The difficulty of including higher trophic levels;
- Problems arising from the shallow depth of most microcosms.

Side Growth. The most severe of the above problems is the first: it is, in particular, the growth of periphyton on the sides of the container. Periphyton exerts a significant effect on the metabolism of microcosms within 40 to 50 days after inoculation. Biological control of periphyton growth has been largely unsuccessful. We intend to study mechanical means of effecting this control.

A possible way around the problem of periphyton growth is to focus on the plankton bloom which occurs shortly after inoculation. Because side growth does not become firmly established for nearly two months after inoculation, little interference with the activity of the water column during that period is expected. Bloom phenomena in natural systems play an important role in determining the overall behavior of the ecosystems. This viewpoint contrasts with the notion that microcosms are useful only after they have progressed to a static configuration in which net biotic growth has ceased.

Further research into the problem of periphyton growth is strongly warranted.

Higher Trophic Levels. Inclusion of larger fauna such as fish, snails and decapod crustaceans in relatively small systems can lead to significant alterations of nutrient levels and nutrient cycles as a consequence of the substantial utilization and excretion of carbon and nitrogen compounds by these organisms. If the relative proportion of biomass in the higher trophic levels is too large, these animals may simply overgraze the available food supply and starve.

It initially appeared that a 1000 liter microcosm could readily support 1 gram of fish biomass. We found, however, in a study of microcosm trophic structure (4) that 3g of fish biomass in a 700 liter microcosm resulted in a fish to zooplankton biomass ratio higher than would be expected in a natural situation. Abnormally high ratios of carbon to chlorophyll *a* were also observed. For these, and other reasons (detailed in Appendices E and F) we chose to exclude higher trophic levels from the microcosms.

Depth. One of the problems in shallow microcosms is that losses of phytoplankton from the water column due to sinking are abnormal. The death and decomposition rates of phytoplankton, which results in accumulation on the bottom of the container, are difficult to measure. These rates affect the rate at which nutrients are recycled to the water column; consequently, measurements of nutrients in the water column cannot always be used to characterize pelagic systems. This point is further explored in the sub-section on monitoring methods below.

CRITIQUE OF MONITORING METHODS

The acquisition of meaningful data in microcosm studies is a twofold problem. First, one must consider what kinds of information are deducible from the specific properties measured. One must next consider whether the methods used to measure those properties are sufficiently precise.

The Types of Data

Measurement of nutrients in the water column is relatively facile and, in large scale operations, can be automated. It is not clear, however, that expanded nutrient measurements can be substituted for biotic data. One striking result from our perturbation experiments was the disparity between the sensitivity indices derived from plankton counts and those derived from nutrient levels. We frequently observed only small differences in some nutrient levels between the perturbed and unperturbed sets of the same system, while differences in number and composition of pelagic communities clearly reflected the effects of perturbation. An example of this is the similarity of organic carbon (Fig. 7B-2) and flourescence levels between the perturbed and unperturbed sets in the first (ammonia) experiment, while zooplankton levels were depressed by an order of magnitude in the perturbed tanks (Fig. 7B-6; 7B-7).

It is apparent that the nutrient levels can suggest when one system has different surface activity from another, even though the pelagic communities appear similar. Pelagic nutrient measurements do not appear to be conclusive, however, in distinguishing one pelagic system from another. This point is of paramount importance because many microcosm studies have invoked nutrient levels to compare the microcosms with natural systems as well as with other laboratory systems.

Enumeration of the biota thus seems to be a requisite for monitoring pelagic systems. It also appears to be the best means of determining the degree to which microcosm communities have become conditioned by their residence in the laboratory.

Measurement Techniques

Our methods have been those employed in standard limnological studies, and references to their sources are provided in the relevant appendices. In our experience, however, some of these methods do not provide the sensitivity required to make their results statistically significant. In the case of phosphorus measurements in the phosphate limited systems, for example, the data vary around a very low mean - the phosphate concentrations in those systems are not above the limits of sensitivity of the method. It is not likely that increased repetition of these measurements would provide meaningful data. Similarly, the method by which light and dark CO_2 uptake or evolution is determined depends upon a pH measurement precision of .01 when the uptake of CO_2 is on the order of 1 μmol per hour, a rate common in oligotrophic and mesotrophic systems that are not in a bloom phase. Few pH measuring systems are sensitive to this degree. For these reasons, as well as those discussed above, we advocate more thorough study of the biota in conjunction with nutrient analyses.

In aquatic systems, many biological processes are well reflected by changes in nutrient levels. Valuable information about decomposition activity and nitrification can be obtained from measurements of ammonia, nitrate and nitrite. In subsequent perturbation experiments it would also be useful to employ chemical methods to monitor pelagic levels of pollutants. Nevertheless, there appears to be no convenient substitute for biotic data.

MICROCOSM USE IN PERTURBATION STUDIES

The studies of the plankton communities again appear to have provided the most useful data for the analysis of the feasibility of using microcosms to study the effects of coal conversion effluents on aquatic systems. In all three experiments we observed significant changes in at least some of the pelagic communities following the imposition of the pollutant.

The one question that arises concerns the approach in these experiments: that of using small aliquots of the aquatic system to predict, by an indirect method such as the evaluation of decomposition activity, the eventual response of the system to perturbation. The rationale for not applying the pollutant directly to the small aliquots is that the recovery of the system is as much of interest as the initial effects of toxification. The small aliquots do not provide the opportunity for intermediate or long term observations. As discussed below, subsequent analysis of our results showed that combinations of detrital and toxic additions to our small aliquots may provide a predictive tool for assessing perturbations.

Direct assessment of the effects of perturbation on the microcosms themselves yielded results interpretable in terms of what would be expected in natural systems. Rectification of the problem of microcosm conditioning will further enhance the utility of microcosms for perturbation studies. We are more able to focus on the particular problems of microcosm design and operation as a result of these preliminary perturbation studies. Although microcosms can be used for intermediate term perturbation studies, given the current state of the art, efforts to improve microcosm methodology are necessary.

TEST OF THE PREDICTIVE INDICATOR

Our analysis of the detrital spike experiments and the perturbation experiments suggests (1) that the perturbation experiments were not of long enough duration to test the theoretically suggested decomposition and long term nutrient cycling stability implications of K_D, and (2) a new method appears promising for using detrital spike experiments to predict ranges of ecosystem behavior in cases both with and without stress. In both the ammonia and phenol perturbation experiments, relative ranges of behavior were successfully indicated by the detrital spike experiments. In the iron perturbation experiment, the effects of the perturbation were so overwhelming in all the systems that ranking the effects according to systems does not seem meaningful, and hence the iron experiment cannot be compared meaningfully with the spike experiments.

K_D was selected as a hypothetical predictive indicator because theoretical studies suggest that it reflects the existence of positive feedback effects in ecosystems that would be likely to retard recovery of the system subsequent to perturbation. These feedback effects can be understood from the following example: If a perturbation causes an initial increase (decrease) in the growth of phytoplankton in the system, then the eventual increase (decrease) in organic detritus will provide a larger (smaller) source for decomposition. The resulting increase (decrease) in decomposition will be large if K_D is large, and small if K_D is small. If K_D is large, and the increase (decrease) in decomposition is large, then a large increase (decrease) in available inorganic nutrients for phytoplankton growth will result.

Thus the effect of the perturbation will not damp out in time but will persist. It is clear from this example that the type of self-damping or stability properties reflected by the K_D indicator are pertinent to a time scale for recovery which is of the order of the time scale for nutrient cycling. However, the major contributions to the sensitivity indices as evaluated in our perturbation experiments arise from the direct effects of the effluent. Therefore, we do not believe that the potential validity of K_D as a stability indicator related to long term nutrient cycling was tested by our relatively short term experiments. Unless problems of side growth are solved, it will not be possible to conduct such long term experiments in microcosms.

Although the spike experiments were designed to study decomposition properties related to long term nutrient recycling, they may also be useful as guides to ecosystem behavior over moderate (2-6 mos.) lengths of time; to elucidate this possibility, we consider an aquatic ecosystem initially in a relatively quiescent state. At some later time, increased availability of both organic and inorganic nutrients, due to natural processes in the system, will stimulate phytoplankton and/or zooplankton blooms and subsequent decomposition activity. In many cases, the size and duration of these blooms is one of the most ecologically significant features of the ecosystem.

Consider now the detrital addition experiments in our beakers. Imagine taking an aliquot of water from our originally quiescent ecosystem and adding a spike of rich detrital material to it. Primary and secondary decomposition of the added organic material provides a diverse spectrum of organic and inorganic nutrients for many of the biota in the aliquot to utilize and react to. The subsequent growth (blooms) or non-growth of particular species provides a preview of the range of behavior to be expected in the original ecosystem over moderate time periods.

In trying to assess the effects of a perturbing agent, a modified version of the above detrital addition experiment performed in addition to the standard spike experiment would be very useful. This experiment would use aliquots taken from the original system to which the perturbing agent, along with the detrital spike, is added. The perturbing agent would be added before the spike in one aliquot and afterward in the other. Effects on the decomposition activity as well as various species of phytoplankton and zooplankton by the perturbing agent would result in the significant growth or non-growth of certain species of biota. Comparison of significant blooms in the two types of detrital spike experiment would provide a preview of gross differences in terms of significant species to be expected over moderate time periods between the perturbed and non-perturbed systems.

One key advantage of the detrital spike experiments over just watching a natural ecosystem evolve is that the spike experiments take very little time compared to most natural ecological time scales.

Section 5
THEORETICAL DEVELOPMENTS

Although the basic thrust of this project has been experimental, it was found necessary to develop certain theoretical tools in order to interpret our data. Much of our concern has been with the stability of ecosystems that have been disturbed by such stresses as pollutants. We have therefore developed a definition or measure of stability that can be evaluated in a perturbation experiment and used to compare the stability of one system with another. We describe here some theoretical advances we have made in interpreting this measure and relating it to other, more familiar, quantities in ecology.

BACKGROUND

All notions of stability in ecology pertain to the relation between a stressed state of a system and the state the system would have been in had there been no stress. Where these notions differ are in the aspects of that relation which are selected as being especially important. No single measure of ecosystem stability can be suitable for all purposes and satisfy the interests of all ecologists. The notion of resistance to stress is very useful if one's interest is in the maximum extent of the deviation between the stressed and unstressed system; resilience is of relevance to those concerned with the rate at which a system returns to pre-stress conditions; asymptotic stability is a useful concept for those concerned with whether or not a system will eventually return to its pre-stressed state. Other concepts of stability likewise single out certain aspects of perturbed behavior for emphasis.

A shortcoming of much theoretical work in ecology is that results often are not expressed or expressible as relations among readily measurable quantities. A familiar example is the often-quoted result that a necessary and sufficient condition for asymptotic stability of a system described by a community matrix is the negativity of the real parts of all the eigenvalues of that matrix. While mathematically rigorous, this result unfortunately is not very useful in such situations of practical concern as environmental impact prediction or assessment. Suppose an ecosystem is disturbed by some activity; for example, effluent is dumped in a lake or a watershed is partially timbered. On the one hand, asymptotic stability is too stringent a condition in the sense that the eventual return of a system to precisely its pre-disturbed state is neither likely (even if the stress is removed) nor essential to environmental acceptability. On the other hand, the condition may be too lenient if the system takes a very long time to return to predisturbed conditions, or is displaced quite far from its original state prior to its eventual return. Asymptotic stability is a mathematically convenient notion because there is a precise mathematical prescription for determining whether or not a system is asymptotically stable. But the relevant question in much environmental impact assessment work is not one of <u>whether</u> a system is stable, but rather of <u>how</u> stable it is.

Our work in this area was motivated by our perceived need to bring theoretical stability analysis more in alignment with experimental constraints and practical needs. Our objective was to introduce a definition of stability which offers a quantitatively useful measure of ecosystem (or more generally any system) response to stress. Although the definition of stability we developed <u>appears</u> to be mathematically ungainly, it has the advantage of quantifying what is often of most interest to those concerned with the responses of ecosystems to environmental stress. Moreover, with rigorous analytical methods, we have now been able to show how the value of this stability measure depends upon certain potentially measurable parameters characterizing the predisturbed system.

<u>Results</u>

The definition of stability we employ provides a measure of the deviation between a disturbed system and a control. In most general form it is given by:

$$S^{-1} = \Sigma\, c_i \int_0^T dt \;\; f(\, x_i(t)\;,\; \bar{x}_i(t)) \qquad \text{(EQ.-4)}$$

where the x_i are the variables that describe the disturbed system, the $\bar{x}_i$ describe the undisturbed or control system, $t = 0$ is the time at which the disturbance begins, and $t = T$ is the duration of the perturbation experiment. The c_i are weight constants, and f is a non-negative function that vanishes when $x_i = \bar{x}_i$ and is a monotonically increasing function of $|x_i - \bar{x}_i|$. Note that these properties of the function, f, insure that our stability measure, S, is biggest when the time-integrated deviation of the perturbed systems from the control system is least. A particular form for f that we have used in our data analysis is described in Section 2.

While Eq. 1 provides an index or measure of stability which is empirically convenient (or can be made so by appropriate choices of the c_i and f) and appears to have the flexibility to reflect well many of the realities of environmental impact concerns, it does not relate readily to methods of mathematical analysis of theoretical models other than computer simulation. And yet if it is to provide more than just an empirical property of stressed ecosystems, and is to be related in some way to properties of the pre-stressed system, such as diversity, then methods of mathematical analysis of this measure would be useful.

In a recent article (3), we have developed an analytic approach to the study of this stability index. We picked a reasonable choice for the form of f in Eq. 1 and performed a random average over all initial stresses. Our analysis then showed that the stability measure, S^{-1}, correlates unexpectedly well with two parameters (the mean and the variance) characterizing the distribution of residence times in the ecosystem. Because it may be possible to measure these parameters by means of a tracer experiment, we are encouraged that predictive indicators for the stability measure, S^{-1}, will be achievable.

To summarize, the stability index, S^{-1}, is of interest for three reasons. First, it is of phenomenological value in that it provides a convenient way to express experimental data obtained from ecological perturbation experiments carried out under controlled conditions. Second, it is amenable to theoretical analysis, as we have now shown. Third, there are encouraging suggestions that our stability measure correlates with other quantities that are more customarily measured in ecology and that, therefore, there exist measurable properties of undisturbed ecosystems that will allow prediction of S^{-1} for certain kinds of stresses.

In other theoretical studies carried out this year in connection with the microcosm feasibility study, we have been developing improved approximation techniques for handling systems of equations describing nutrient flow in ecosystems under conditions in which compartment sizes are changing rapidly. The motivation for this work lies in our general observations that microcosms appear to be of most use during the initial bloom period in an annual lake cycle. During this period of intense growth and decline of phytoplankton and herbivorous zooplankton, numerical and analytical mathematical methods customarily used in theoretical ecology are least effective. We have begun work on an analytical approximation method designed particularly for this period in which ecological parameters are rapidly changing. Using model equations as a "laboratory," we have compared the analytical approximations with computer-generated solutions and are encouraged by the preliminary results. Further work is now in progress to refine and further test the method.

Section 6

CONCLUSION

An evaluation of the use of chemically closed microcosms for assessing the impact of energy conversion on aquatic systems has indicated that such systems offer potentially convenient experimental tools for intermediate term (less than three months) studies, provided that standard procedures for design and operation are established. Key problems in the development of these procedures have been identified in this study, and the use of microcosms is recommended contingent upon the resolution of these problems.

Our analysis of the detrital spike experiments and the perturbation experiments suggests (1) that the perturbation experiments were not of long enough duration to test the theoretically suggested decomposition and long term nutrient cycling stability implications of K_D, and (2) a new method appears promising for using detrital spike experiments to predict ranges of ecosystem behavior in cases both with and without stress.

Direct observations of the effects of three different constituents of coal conversion effluent water on microcosm systems corresponding to four different trophic types of natural systems revealed significant responses to the perturbation in all experiments. The specific responses of the individual systems depended upon the nature of the perturbation. For most measurements, the phosphorus-rich, nitrogen-limited systems were most affected by the additions of ammonia and phenol; the phosphorus-rich systems were most affected by the addition of iron.

Interpretation of the results of these studies leads to the following points:

- Microcosms can simulate the behavior of natural systems for periods generally not exceeding three months. Longer term studies will require solution of the problem of side growth.
- Microcosms are most useful during a dynamic growth phase, in contrast to the prevalent viewpoint that microcosms become useful when having reached a static state.
- A progressive decrease in the replicability of the microcosms in successive experiments suggests a systematic problem that correlates with the age of the parent microcosm from which all inocula were derived. It is thus likely that microcosms become conditioned by laboratory environments.

- The relatively high proportion of surface to pelagic activity in microcosms diminishes the importance of water column nutrient studies; quantification of pelagic biota is indispensable. The need for determination of pelagic biota is reinforced by experiments in which nutrient levels as well as IC and OC were similar over time in different systems but the biota were quite different.

- The means by which microcosms are initiated is a degree of freedom that affects replicability and community structure. Further study of initiation procedures is required.

- Microcosms can be used to simulate specific trophic types of natural systems in order to assess the effects of particular perturbations on a given system. Because they provide a diverse assemblage of interacting biota, upon which a perturbation can be tested, phenomena are observed which would be missed in single-organism studies.

- The most significant effects of the ammonia perturbation were lowered inorganic carbon and zooplankton levels as compared to the controls. This is consistent with the ammonia stimulating primary production while being toxic to the zooplankton.

- In the iron addition experiment, the most significant effects were the decrease in phytoplankton and zooplankton. The formation of insoluble iron-phosphate complexes and the chelation of algal surface compounds, resulting in flocculation, were primarily responsible.

- The addition of phenol to the microcosms resulted in suppression of zooplankton in all systems and of the alga *Anabaena* in the nitrogen-limited systems.

- A new strategy for using detrital spike experiments to predict ecosystem behavior was suggested by our experiments. In both the ammonia and phenol perturbation experiments relative ranges of behavior were successfully indicated by this method.

Further studies to determine the factors influencing tracking of natural systems by microcosms are indicated. Objects for immediate study, suggested by the preceding experiments, include:

- Optimization of initiation strategy;

- Control of surface activity and/or optimal use of microcosms in the presence of surface activity;

- Determination of the effects of size, nutrient levels and temperature on replicability among microcosms and between microcosms and natural systems;

- The behavior of microcosms during blooms and the effects of perturbation during this dynamic phase, prior to the onset of laboratory conditioning.

Many of the problems impeding the use of microcosms for short and intermediate term simulations of natural systems appear to be potentially resolvable. Our experiences indicate that efforts to obtain steady state conditions in microcosms are of marginal value and that the greatest utility of microcosms well may be the opportunity for studying the sensitivity of aquatic systems during dynamic phases.

APPENDIX-A

GENERAL BACKGROUND FOR PERTURBATION AND STABILITY INDICATOR EXPERIMENTS

AND

THE AMMONIA PERTURBATION EXPERIMENT

Preliminary studies designed to evaluate the feasibility of using microcosms to obtain predictive information about the resistance of freshwater systems to disturbances have been in progress, under EPRI support, since October 1976. Replicability, with respect to nutrient concentrations and the distribution of plant and animal species, between microcosms of identical starting conditions has been good enough to allow the preliminary evaluation of short term stability properties of our systems.

The particular stability indicator that has been under investigation in the current series of experiments is a parameter characterizing the elasticity of the decomposition process, denoted by K_D. It is described by the response of an ecosystem (or an aliquot derived from an ecosystem) to an artificially induced change in the amount of detritus that serves as substrate for the decomposition process: $K_D = \int(T - C)/C)/(\Delta D/D_0)$, where T and C are measured values of parameters characterizing the decomposition process (see below) for the treated and control systems, respectively. D_0 is the initial amount of detritus (characterized by the level of organic carbon) and ΔD is the change in the amount of detritus.

Four series of 6 tanks, each of 50 liters capacity, comprise the microcosm facility. Each of the four sets differs only with respect to initial N and P concentrations. Within a given set, all six tanks are identical at the outset of each 4 month experiment and are allowed to develop under identical conditions for 2 months. Key species and nutrients, along with dark and light bottle CO_2 exchange, are monitored weekly in each tank. At the end of two months, half the tanks in each sextet are subjected to some by-product of coal gasification deemed likely to disturb natural freshwater systems. The evolution of the perturbed (treated) and control tanks is followed for an additional two months.

Just prior to the disturbance, 8 liters are removed from each triad of tanks, divided into 2 four liter aliquots, and used for the determination of K_D as follows: an amount of bacterial detritus equal to 40% of the ambient organic carbon is added to one of the 2 four liter aliquots; bacterial plate counts, chlorophyll _a_ flourescence, ammonia, nitrate + nitrite, carbon concentrations, and dark respiration are monitored daily for one week.

Some of the above parameters are then measured for two more weeks on an irregular basis. The quantity $\int(T - C)/\int C$ is determined for the different parameters. This constitutes the predictive measurement. It uses only small aliquots taken from the original 50 liter microcosms.

Subsequent to the disturbance, all tanks are monitored weekly for two more months. A sensitivity index is determined for each of the monitored parameters:

$$S^{-1} = \frac{1}{n} \int |T - C| / \langle T + C \rangle_n .$$

Below we describe and discuss what was observed in the ammonia perturbation experiment and the associated K_D experiment.

RESULTS OF NH_4^+ PERTURBATION EXPERIMENT

The general features observed in our first perturbation experiment are described here. The 24 systems used in this experiment were divided into 8 sets (A,B,C,D, E,F,G,H) of 3 replicates each. Every successive pair (i.e., A and B, C and D, E and F, G and H) was initiated with the same concentrations of N and P; thus, there were 4 different sets of initial conditions, with 6 systems in each set (Table 3-1). Sets A and B had low N and P levels, sets C and D had moderate N and low P levels, sets E and F had high N and P levels, and sets G and H had low N and high P levels. Each tank was initiated by using a 25 liter inoculum from a 700 liter system. The inoculum was added to 25 liters of deionized water and inorganic nutrients added to bring the levels up to the values shown in the table. The 24 tanks were unperturbed for 59 days. On day 60, 1.6 mM of NH_4^+ was added to the set to tanks A,C, E, and G, and followed for another 60 days. Throughout the 4-month duration of the experiment, weekly monitoring of selected chemical and biological properties of the systems was carried out.

Prior to the perturbation, all tanks went through two phytoplankton blooms on days ∿14-22 and on days ∿42-56. After the perturbation, all tanks exhibited a final bloom on days 90-111. The peaks of all these blooms for all tanks, as measured by OC and fluorescence, occurred on the same days, regardless of the initial nutrient levels and independent of whether the systems were perturbed or not.

In most cases, zooplankton blooms approximately coincided with the second and third phytoplankton blooms. The size of the peaks of the zooplankton blooms was dependent on the initial nutrient levels and on whether the tanks were perturbed or not. Zooplankton levels were depressed by a factor of 10 in the perturbed systems as compared to the controls. In addition, the maximum zooplankton levels in the controls (reduced by a factor of 10 for the perturbed tanks) were correlated with the initial nutrients in the following way: excess N and P (F) $\sim 10^{-3} cm^3 \ell^{-1}$ excess N or P (D or H) $\sim 10^{-4}$ $cm^3 \ell^{-1}$, and no excess nutrients B $\sim 10^{-5}$ $cm^3 \ell^{-1}$.

Besides the decrease in zooplankton levels in all the perturbed systems, the other striking significant change was the large decrease in IC (at least a 50% decrease over the 60 day period) in the perturbed systems. Three possible explanations for this drop in IC are consistent with the data:

1) The perturbed systems synthesized significantly more organic material from the IC than did the controls. However, since the organic carbon and fluorescence levels in the water column were approximately the same in the perturbed and control systems, except for G and H, much of this activity must have either resulted in biomass which sank to the bottom or in growth activity which took place on the sides of the containers in the form of periphyton. Our measurements of net productivity in the pelagic zone are not sensitive enough to distinguish between these two alternatives.

2) The reduction of zooplankton in the perturbed tanks decreased the IC since their contribution to respiration would be less than in the controls.

3) Finally, it is possible that the addition of NH_4^+, which is an end product of decomposition, decreased decomposition in the perturbed tanks so that there was less IC produced. Further analysis of our data, and some subsidiary experiments, will be required to clarify this situation and suggest which combinations of these possibilities are most likely.

The fact that a sizeable decrease of zooplankton occurred in the perturbed tanks suggests the first two explanations, and is not inconsistent with the third. Such a decrease in zooplankton could have reduced grazing pressure on phytoplankton, allowing larger amounts of biomass to accumulate on the bottom and sides of the perturbed tanks.

The extent to which algal growth in any of our systems was limited by grazing cannot be deduced from the available data. In future studies, subsidiary experiments involving the comparison of small aliquots where zooplankton are filtered out with aliquots where the zooplankton are not filtered will be made in order to obtain more information on grazing properties of our systems. In addition, direct phytoplankton counts of the main tanks will be made. This information, combined with zooplankton-phytoplankton grazing models we are developing, will shed light on the grazing properties of our system.

The data on total phosphorus (TP) is interesting and quantitatively consistent with results of phosphorus measurements in our microcosm studies previously carried out in 700 ℓ tanks (LBL-5965). In all tanks with excess phosphorus (E,F,G, and H), TP left the water column at a steady rate until it reached a level of 1.5 M. The first order kinetic rate constant for this loss was 0.03, 0.02, 0.02 and 0.02 d^{-1} ($r^2 \approx 0.9$ in all cases) in E,F,G, and H, respectively. Previous experiments (LBL-5965) suggest that this loss of TP can be mediated either by uptake and sinking of phytoplankton or directly by periphyton uptake. In tanks with low levels of P (A,B,C,D) the TP patterns were the same with minima on days 0,42, and 90. These minima correspond to blooms and probably reflect the sinking of grazed or ungrazed phytoplankton.

The increase in $NO_3 + NO_2$ in the perturbed tanks reflects the oxidation of some of the excess NH_4^+ combined with net uptake due to biosynthesis by phytoplankton and perhaps bacteria (Table 7A-1). One interesting result is that the magnitude of the $NO_3 + NO_2$ increase in tank E subsequent to the perturbation was considerably higher than that in A or C. This is suprising in light of the considerably higher levels of $NO_3 + NO_2$ that we suspect were removed from E in the form of algal growth. The inhibition of nitrification in the phosphorus-limited perturbed systems (A and C) as suggested by these phemomena may be related to the somewhat similar effects observed in the K_D experiment.

Dark bottle respiration measurements showed small differences between all the systems (Table 7A-1). Net productivity measurements showed a large difference between G and H, where G was markedly more productive after the addition of NH_4^+, and a relatively small percent difference between E and F. There were two bacterial blooms which correspond to the first and third phytoplankton blooms which were not significantly correlated to NH_4^+ or dark respiration numbers.

In part, this may indicate that most bacterial action takes place on the bottom or that our plate counts do not favor the autotrophic nitrifying bacteria.

In summary, the most important biotic changes were reflected in the IC and zooplankton level changes (Table 7A-1); namely, in the perturbed tanks both the IC and zooplankton levels were significantly lowered. The net productivity and flourescence data show the expected results that biosynthesis was highest in the spiked tanks with nitrogen limitation and excess phosphorus since they can utilize large amounts of nitrogen when it is present. Comparison with the phosphorus limited tanks (A and C), shows that the set with more initial nitrogen (C) responded more to the perturbation than the low nitrogen set (A). This reflects the fact that there was more biological material in the set (C) at the beginning of the perturbation than the low nitrogen set (A). In the phosphorus rich but nitrogen poor tanks (G and H), the large increase in biosynthesis in G after the perturbation was probably due mainly to the presence of utilizable nitrogen from the NH_4^+ addition. In keeping with this picture, these systems (G & H) exhibited the largest absolute and fractional changes. The smallest fractional increase in net productivity was observed in tanks with high initial levels of nitrogen and phosphorus (E and F). Because of the large amounts of biological materials in tanks E and F, the absolute change in zooplankton and IC levels was large; however, the fractional change in IC was not as large as in G & H. All these changes are well described by our sensitivity indices (Table 7A-1) which represent fractional differences in the tanks both before and after the perturbation.

Table 7A - 1

SENSITIVITY VALUES

	IC		Zoo		OC	
Time Period	I	II	I	II	I	II
Tanks						
(A,B)	.002	.187	.47	.89	.033	.070
(C,D)	.006	.363	.49	.83	.025	.039
(E,F)	.020	.256	.13	.84	.036	.051
(G,H)	.015	.468	.22	.10	.055	.223

	Fluor		NO_3 + NO_2		NH_4	
Time Period	I	II	I	II	I	II
Tanks						
(A,B)	.149	.110	.20	.46	.070	1.0
(C,D)	.107	.148	.01	.33	.054	1.0
(E,F)	.134	.116	.07	.29	.059	1.0
(G,H)	.387	.837	.04	.44	.045	1.0

	Net Prod		Dark Res.		Bact.	
Time Period	I	II	I	II	I	II
Tanks						
(A,B)	.36	.55	.14	.13	1.20	1.48
(C,D)	.16	.43	.65	.21	.58	1.00
(E,F)	.08	.14	.10	.11	.37	1.81
(G,H)	.21	.86	.21	.35	.70	.92

Note: Sensitivity values for measured parameters during EPRI 1. Time period I represents 60 days before perturbation while time period II represents 60 days after perturbation.

MEASUREMENT OF THE PREDICTIVE INDICATOR

Theoretical work has suggested that the response of decomposers to additions of detritus may be related to the ability of an ecosystem to resist stress.

The spike used for the detrital additions was prepared from E. Coli grown at 37°C in the following medium:

	g/l
NaCl	8
Dextrose	5
Tryptose	8
Peptone	8

Cells were harvested by centrifugation and adjusted with distilled water to 42 mM organic carbon, 1mM inorganic carbon. Analysis of the spike showed:

inorganic carbon	1	mM
organic carbon	42	mM
NH_4	.4	mM
Total PO4	.4	mM
Phosphatase	0	mM

During the first week subsequent to the addition of the spike, the parameters directly indicative of decomposition (ammonia, dark respiration, and bacteria) generally showed a rise and subsequent fall in the spiked systems relative to the controls, the only exception to this being the absence of a rise in ammonia in E-F.

Replication between pairs of supposedly identical aliquots (taken from A-B, C-D, E-F, G-H) was generally quite good with the exceptions of zooplankton volume in all systems, dark respiration and bacteria in G-H, and organic carbon, bacteria, and dark respiration in E-F.

Of the parameters characterizing secondary effects of detrital decomposition, nitrate-nitrite levels showed a rise in the spiked E-F and G-H systems but not in A-B or C-D (the phosphorus-limited systems). This result is indeed surprising, given that the nitrogen-limited systems, in which we can deduce that the greatest absolute amount of inorganic nitrogen uptake occurred in new algal growth, also showed the highest nitrate-nitrite levels. This cannot be explained by differences in induced ammonia levels; it may signify that the nitrifying bacteria in A-B and C-D were phosphorus limited.

Organic carbon showed a rise, a fall, and subsequent rise in all spiked systems, relative to controls, due presumably to the addition of organic carbon in the spike, its subsequent decomposition, and the consequent increase in algal biomass induced by the release nutrients. Zooplankton biomass differed between treatment and control only in A, where the spiked system had a significantly higher level than the control.

The values of the K_D indices for our measured decomposition parameters and fluorescence are shown in table 7A-2. The greater the value of this index, the greater the percent difference between the spiked and control systems. Ammonia, which directly measures decomposition activity, shows E-F exhibiting the least response and G-H the greatest. Large standard deviations between some of the replicate pairs with respect to dark respiration make the similar ordering of this measurement only suggestive. The largest bacterial response occurred in G-H. Overall, the K_D index for decomposition tends to suggest that G-H is the most labile of the systems, while E-F is the least.

Table 7A - 2

VALUES OF K_D

	NH_4	$NO_3 + NO_2$*	Dark Resp.	Bact.	Fluorescence
A	12.0	-.1		6.7	4.4
B	13.7	-.1	.66	3.7	3.7
C	8.8	-.1	.25	1.1	10.4
D	13.8	-.2	.34	2.8	5.4
E	-3.1	1.6	.12	1.1	1.6
F	-1.8	1.8	.24	13.5	1.4
G	17.0	5.0	1.23	>30	1.9
H	28.7	8.0	.20	>30	1.7

NOTE: Values of K_D for the detrital spike experiment. *NH_4^+ index calculated by taking $\frac{T - \langle C \rangle}{\langle C \rangle}$ on day 3, where $\langle C \rangle$ is the average over the first three days. *$NO_3 + NO_2$ index is calculated by taking $\frac{T - C}{C}$ on day 8.

APPENDIX B

THE IRON PERTURBATION EXPERIMENT

One of the major metals appearing in the aqueous effluent from coal conversion plants is Fe. In this experiment, the potential effects of high Fe discharge into aquatic systems is explored with the use of 8 triplicated sets of aquatic microcosms. In addition, the extent to which each treated set of microcosms responds to Fe discharges is compared with the corresponding values of a hypothesized stability indicator, K_D.

RESULTS OF PERTURBATION

Methods

Each of 24 50-ℓ tanks was filled with 23 ℓ of deionized H_2O plus 23 ℓ from an oligotrophic 700-ℓ microcosm in our laboratory. The water from the 700-ℓ microcosm had been given the usual nutrient enrichment and was screened through a 64-μm net before distribution among the tanks. Temperature was maintained at 19 ± 1 °C and irradiance incident on the water surface at 63 ± 2 μmol m^{-2} s^{-1} PAR.

The tanks in each of the 8 triplicates (designated A,B,.....H) were enriched with $NaNO_3$ and Na_2HPO_4 as follows:

	A	B	C	E	F	G	H
N (μmol l^{-1})	0	0	11	110	110	1.1	1.1
P (μmol l^{-1})	0	0	1.1	22	22	22	22

On day 57, 31.5 mgℓ$^{-1}$ $FeCl_3 \cdot 6H_2O$ was added to triplicates A,C,E,G, equivalent to 0.12 mmol l^{-1} or 6.5 mgℓ$^{-1}$ Fe(III).

The following parameters were monitored: inorganic carbon (IC), organic carbon (OC), pH, CO_2 exchange in the light (P_L) and the dark (P_D), NH_4, NO_3 + NO_2, total phosphorus (TP), and phytoplankton and zooplankton species and numbers.

Results and Discussion

Carbon. IC values dropped precipitously in the systems with added Fe (Fig. 7B-1).

The general features of this decrease were similar in all cases, resulting in an order-of-magnitude less IC in systems A,C,E,G. The reasons for this decrease are clear. The added Fe (III) has a strong tendency to form complexes with other ions;

complex formation with OH^-, PO_4^{-3}, and organic bases is well established, although the exact forms of the complexes may remain obscure in specific instances. The formation, in particular, of iron-hydroxide complexes will lead to a decrease in OH^- activity and a drop in pH, and indeed a change from approximately pH 7 to pH 4 was observed in the systems to which Fe was added. The lower pH implies, in turn, a lower IC concentration in equilibrium with the atmosphere. In an ideal solution at 25°C, a change in pH from 7 to 4 does result in approximately an order-of-magnitude decrease in IC.

In all systems there was a buildup of OC over the first 2 months (Fig. 7B-2), the levels reached reflecting the relative levels of initial N and P. Replication within triplicate pairs, i.e., within A-B, C-D, E-F, and G-H, is good up to Day 57 when the Fe was added. After Day 57, the treated systems differ in OC from their respective controls, although there is no obvious pattern in the way the treated systems differ.

CO_2 Exchange. The introduction of $FeCl_3$ in to A,C,E,G resulted in an altered buffering capacity that made it impossible to interpret the pH changes in terms of CO_2 exchange.

Nutrients. In systems A,B,C,D, the TP levels varied around a low mean with no obvious systematic pattern (Table 7B-1). Much of the variation may represent random error, as we were working near the limits of sensitivity for the TP method used. In E,F,G, and H, there was a gradual decay from the initial levels of 22 $\mu mol\ l^{-1}$. The addition of Fe to E and G on Day 57 resulted in drastic decreases in TP to levels similar to those of A,B,C,D. The decrease represents the formation and precipitation of insoluble iron-phosphate complexes of the form $Fe_x(H_nPO_4)_4^{3x-(3-n)4}$, adsorption to the surfaces of other insoluble products, and precipitation of phosphorus-containing algal flocs.

Ammonia levels (Table 7B-2) remained on the order of 1 $\mu mol\ l^{-1}$ in all systems, except for E following the Fe addition. The phytoplankton levels attained highest values in the pair E-F before the Fe addition, and the NH_4 production undoubtedly represents the decomposition of precipitating algae flocculated by the Fe.

Several difficulties were encountered with the hydrazine sulfate reduction method for NO_3 + NO_2, and we do not consider the data reliable enough to report for this experiment. Interference of the reduction step by particulate matter appears to be a major problem.

Plankton. Algal communities were dominated by *Cryptomonas* and *Ankistrodesmus*. Other taxa occasionally present included *Scenedesmus*, *Gloeocystis*, *Synedra*, and *Oscillatoria*. After the Fe addition, numerous small dark flakes with an effective diameter of 10^2 µm were present. Masses of detritus containing these flakes and decomposing *Cryptomonas* were also observed. The numbers of small (< 5 µm d.), spherical green algae increased significantly at this time. *Ankistrodesmus* was not associated with these flakes to the same extent as *Cryptomonas*. Perhaps the flagella made it more susceptible to flocculation by these flakes, presumably some iron-hydroxyl-phosphate complex.

Total phytoplankton volume decreased immediately after the Fe addition in A, C,E,G (Fig. 7B-5). In A and C, the decline was followed by a similar decline in the respective controls, B and D (Fig. 7B-5). In all treated systems but E, phytoplankton levels remained low for the duration of the experiment. The fact that the phytoplankton remained suppressed cannot be attributed to increased grazing pressure in the treated systems, as both the rotifer and crustacean populations also were destroyed by the Fe addition (Figs. 7B-6,7).

Sensitivity Indicators. The sensitivity of each parameter to Fe addition was quantified by computing:

$$S^{-1} = \frac{1}{n} \int |T - C| \; / \; < T + C >_n$$

for the periods prior to (i=1) and after (i=2) the spike, where T is the treatment parameter value and C is the control parameter value. S_1^{-1} represents the lack of replication between paired triplicates before the spike, while S_2^{-1} also incorporates the differences caused by Fe addition. Values for S_1^{-1} and S_2^{-1} are included in Table 7B-3.

Conclusion

Addition of Fe to the aquatic microcosms has the following repercussions:

(i) decrease in IC due to the lower pH caused by iron-hydroxyl complexing and percipitation;

(ii) a decrease in TP due to complex formation, adsorption, and particle flocculation and sedimentation;

(iii) a decrease in total algal volume due to flocculation and sedimentation, and a change in community composition because of selective flocculation;

(iv) a decrease in rotifer and crustacean populations.

MEASUREMENT OF THE PREDICTIVE INDICATIOR

One method of quantifying the decomposer response is to measure the change in respiratory gas exchange following a change in the amount of detrital substrate. In this set of K_D experiments, we examined the altered respiration rates arising from additions of organic matter to aliquots of systems A through H. In addition, we took a close look at changes in phytoplankton and zooplankton in the aliquots from E and F, primarily to determine if our experimental approach has further application in understanding the mechanisms underlying planktonic systems.

Methods

From each triplicate system on Day 48, 4 liters of water (composed of equal amounts from each triplicate) was removed and distributed between 2 2-liter beakers. The beakers, designated A1, A2, B1, B2,.....,H1, H2 were aerated and illuminated in the same manner as the microcosms A through H. Opaque paper barriers prevented light from entering the beakers except through the top.

A stationary phase E. coli culture was harvested by centrifugation and autoclaved. The autoclaved pellet was suspended in water to form a concentration of 281 mmol liter^{-1} organic carbon (OC). Portions of the suspension then were added to the first of each pair of beakers (i.e., A1, B1,....H1) to cause an increase of approximately 40% in the detrital carbon pool (Table 7B-4).

IC and OC were measured on Days 0-4 and 64 in all beakers. On the same days, respiratory CO_2 efflux was estimated from changes in pH over 4 h in opaqued 125-ml reagent bottles. Phytoplankton and zooplankton were identified and enumerated microscopically in E1, E2, F1, and F2.

The response of decomposer activity to detrital additions was estimated as:

$$K_D = (\int(T - C)/ \int C)/(\Delta D/Do)$$

where T, C are respiration rates in enriched and control beakers, respectively. The greater the effects of added detritus, the larger will be K_D. A first-order rate constant also was estimated for the decay in added OC over the 2 days following the detrital addition.

Results and Discussion

Carbon. The IC data were used in the estimation of gas exchange rates. The actual in situ variation in IC is uninteresting, as we have no idea of the extent to which aeration and air-water exchange affects this variation.

A malfunctioning carbon analyzer limited the collection of reliable OC data to the 4 days following the detrital spike. The general trend was a decrease in ΔY (the difference between treatment and control OC concentrations) over the first two days following the spike followed by an increase (Table 7B-5). This trend is consistent with the hypothesis that mineralization of detritus dominated the initial carbon transformations while phytoplankton photosynthesis dominated beginning on the third day (cf. Figs. 7B-8,9).

Phytoplankton. Beginning 2 days after the spike, the treated systems (E1, F1) showed enhanced phytoplankton increase rates over the respective controls (Figs. 7B-8, 9). The enhancement lasted for 5-7 days, after which the control and treatment communities grew (E1, E2) or decreased (F1, F2) at the same rate.

The communities in both E and F were dominated by *Cryptomonas*, a biflagellated cryptophyte, and *Ankistrodesmus*, a needle-like chlorophyte that often assumes an S-shape and is a common inhabitant of aquaria. In all 4 beakers (E1, E2, F1, F2), *Ankistrodesmus* was increasing while *Cryptomonas* was decreasing throughout the experiment. In E1 and F1, both species exhibited a transient increase in growth rate over control populations during days 2 through 7-9. After day 9, *Oscillatoria*, a non-nitrogen-fixing blue-green filament, appeared in all 4 beakers, higher populations occurring in E1 and F1 with respect to controls (E2, F2).

The transient growth-stimulation in the spiked beakers represents either a nutrient-enhanced reproduction rate or a decrease in zooplankton grazing pressure. Because E and F are so high in N and P levels, the first mechanism is unlikely, although it must be kept in mind that the introduction of the killed bacteria may have resulted in growth stimulation by increasing concentrations of available trace metals (directly or by increased chelating materials), vitamins, other growth factors, and organic substrates for heterotrophic growth.

Zooplankton. A transient stimulation of zooplankton increase rates also occurred between days 2 and 7, after which zooplankton levels in the spiked beakers (Figs. 7B-8,9) gradually returned to control levels. The only zooplankter present was *Cyclops vernalis*, a cyclopoid copepod capable of predatory (as opposed to filter-) feeding in the later stages of its life cycle.

If the transient stimulation of zooplankton growth rates was due to the transient increase in phytoplankton growth rates, it would be difficult to explain why the zooplankton in E1 decrease after day 9 while the phytoplankton are still increasing. More plausibly, the zooplankton were engaging in selective feeding on the added detritus and/or the bacterial population that was enhanced visibly (i.e. as observed microscopically) by the added detritus. Grazing pressure on the algae was relieved, allowing them to increase in the spiked beakers.

Why do the zooplankton return so quickly to control levels? The nauplii produced during the period of transient stimulation may have been overgrazing their bacterial-detrital food source, been too small to switch over to feeding on the phytoplankton, and died. Alternatively, the adult copepods may have preyed on the nauplii when the bacterial-detrital food source disappeared.

Why do the zooplankton decrease in E and increase in F after day 14? We have no explanation, but it should be noted that the inverse correlation between phytoplankton and zooplankton after day 14 does support the notion that the phytoplankton are responding to the zooplankton changes and not vice-versa.

Gas Exchange. CO_2 efflux (Table 7B-6) is subject to errors of at least ± 0.2 μmol l^{-1} h^{-1}, assuming that our pH measurements are accurate to within ± 0.01 units. Errors of this size imply that some of the estimates made for A and B may be off by a factor of 2. Calculation of the parameter K_D (Table 7B-6) leads to the following ordering for K_D for pairs of systems with identical initial nutrient conditions: $(E,F) < (C,D) \approx (G,H) < (A,B)$. A glance at the standard error for means of paired systems illustrates that this ranking has only a low level of significance.

Predictive Value

A comparison of the K_D values (Table 7B-6) with the sensitivity indices for the various parameters measured in the Fe perturbation experiments (Table 7B-3) shows that K_D could not be used to order or predict the relative size of the effects in the perturbed tanks. However, it should be noted that all four sets of K_D values were similar as were the perturbation effects in all four sets of tanks. Hence K_D had, in a very weak sense, a correlation with the perturbation effects.

Table 7B-1

TOTAL PHOSPHORUS LEVELS IN THE MICROCOSMS

Total Phosphorus
(μmol l^{-1})

Day	A	B	C	D	E	F	G	H
0	0.0	0.0	0.2	0.3	22	22	22	22
7	0.4	0.5	0.5	0.6	21	22	21	22
14	0.9	0.4	0.5	1.2	25	26	22	23
21	0.4	0.3	0.4	0.4	18	21	20	19
35	0.1	0.2	0.3	0.3	14	17	15	18
42	0.2	0.4	0.3	0.2	13	16	14	16
49	0.2	0.1	0.4	0.2	17	16	16	15
70	0.2	1.0	0.5	1.1	0.7	10	0.8	10
77	0.7	1.9	0.5	2.6	1.0	9.9	1.5	12
84	0.2	0.3	0.5	0.3	0.6	9.3	0.2	8.0

Table 7B-2

AMMONIUM LEVELS IN THE MICROCOSMS

NH_4
($\mu mol\ l^{-1}$)

Day	A	B	C	D	E	F	G	H
0	2.6	2.1	1.4	1.8	1.8	1.8	0.9	1.6
7	4.1	2.6	2.5	2.4	2.8	2.8	3.0	2.7
21	2.4	1.9	2.0	1.8	1.8	1.8	1.6	1.6
28	3.5	3.2	2.8	2.7	3.1	2.9	0.1	2.5
35	1.4	1.5	1.4	1.4	1.7	1.7	1.3	1.2
42	1.2	1.1	1.3	1.3	2.0	1.8	1.3	1.3
49	1.6	1.6	1.6	1.6	2.3	2.1	1.6	1.6
63	2.6	1.7	4.0	2.6	17	2.6	1.9	0.3
70	1.0	1.3	0.4	1.2	0.4	1.3	1.6	0.3
77	1.6	1.6	0.4	1.1	8.0	2.1	2.3	1.6
84	3.9	1.8	2.0	2.1	25	2.4	2.2	2.2
91	1.8	0.5	2.2	0.0	8.0	2.5	0.2	0.1
98	3.4	2.2	1.3	1.4	20	1.1	0.5	0.6
105	0.7	1.5	1.9	1.0	1.5	1.1	2.1	1.5

Table 7B-3

SENSITIVITY INDICES FOR Fe PERTURBATION

Parameter	S_1^{-1}				S_2^{-1}			
	A-B	C-D	E-F	G-H	A-B	C-D	E-F	G-H
IC	.009	.012	.031	.010	.83	.80	.89	.83
OC	.046	.049	.050	.095	.21	.14	.13	.26
TP	.26	.18	.045	.043	.89	.51	.72	.68
NH_4	.12	.028	.032	.14	.26	.20	.75	.17
Phytoplankton	.27	.25	.13	.56	.59	.82	.57	1.0
Zooplankton								
Rotifers	.06	.27	.81	.42	.91	.89	.88	.91
Crustaceans	.71	.17	.32	.24	1.0	1.0	.83	.98

Table 7B-4

DETRITAL ENRICHMENT*

Beaker	Initial OC ($mmol\ l^{-1}$)	Suspension added (ml)	Expected OC ($mmol\ l^{-1}$)	Observed OC ($mmol\ l^{-1}$)
A1	0.21	1.2	0.29	0.30
B1	0.24	1.4	0.34	0.34
C1	0.28	1.6	0.39	0.41
D1	0.27	1.5	0.38	0.42
E1	1.07	6.7	1.54	1.49
F1	1.12	6.9	1.60	1.53
G1	0.25	1.4	0.35	0.31
H1	0.25	1.4	0.35	0.35

Notes: *Amount of OC derived from _E.coli_ used to increase the detrital substrate in the beakers. _E.coli_ suspension was 281 mmol $liter^{-1}$ OC.

Table 7B-5

DECAY OF ORGANIC CARBON*

Beaker	ΔY ($mmol\ liter^{-1}$) on Days 51	54	55	56	57	k d^{-1}
A	0.00	0.12	0.08	0.05	0.07	0.36 ± 0.12
B	0.01	0.12	0.12	0.07	0.13	
C	0.00	0.14	0.10	0.07	0.05	0.33 ± 0.04
D	0.01	0.20	0.12	0.11	0.12	
E	0.05	0.55	0.32	0.08	0.37	1.5 ± 0.7
F	0.03	0.51	0.30	-0.06†	0.25	
G	0.00	0.13	0.09	0.06	0.14	0.35 ± 0.06
H	0.00	0.13	0.09	0.07	0.09	

Notes: *Decay of OC is treated with respect to control beakers. ΔY is the difference in OC between treatment and control, and k is the rate-constant for decrease in ΔY over Days 54-57.

†Assumed 0.01 for rate-constant calculations.

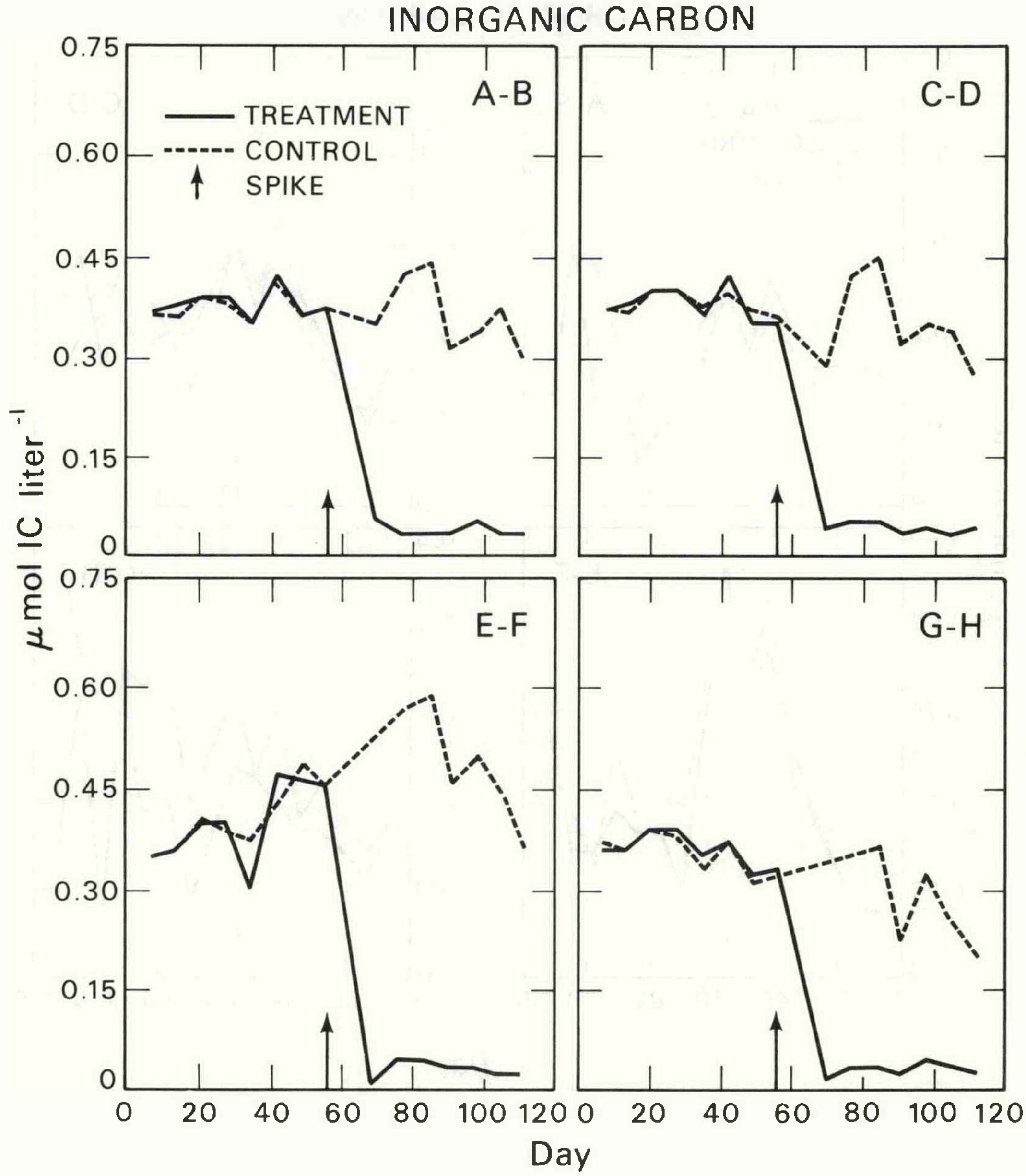
TABLE 7B-6

CO_2 EFFLUX*

Beaker	Dark CO_2 efflux (mol^{-1} h^{-1}) on Days 54	55	56	57	58	64	K_D
A1	-0.31†	0.12	-0.092	1.2	0.37	0.76	
A2	-0.24	0.022	0.030	0.80	0.087	0.74	0.55 ± 0.20
B1	-0.50	0.28	0.46	0.74	0.20	0.91	
B2	-0.32	0.059	0.30	0.35	0.11	0.76	
C1	0.80	0.46	0.76	1.2	0.37	0.62	
C2	0.51	0.12	1.1	0.95	0.44	0.55	0.33 ± 0.05
D1	0.38	0.28	1.2	0.76	0.24	0.59	
D2	0.24	0.030	0.72	0.87	0.23	0.51	
E1	6.6	9.7	5.9	6.5	2.4	5.0	
E2	5.7	4.4	4.9	5.7	2.4	5.0	0.22 ± 0.06
F1	5.4	7.4	13	7.0	3.0	5.8	
F2	11	3.5	6.3	5.5	2.1	2.9	
G1	0.48	1.3	0.55	0.81	0.49	1.1	
G2	0.026	0.57	0.69	0.74	0.41	1.7	0.34 ± 0.12
H1	0.72	1.8	1.7	1.3	0.58	1.7	
H2	0.81	0.31	0.93	0.64	0.43	1.4	

Notes: *Estimated pH changes and the value of the parameter K_D.
†Negative numbers may represent chemolithotrophy.

Figure 7B-1



INORGANIC CARBON
TREATMENT
CONTROL
SPIKE
A-B
C-D
E-F
G-H
0.75
0.60
0.45
0.30
0.15
0
μmol IC liter ⁻¹
0
20
40
60
80
100
120
Day

Figure 7B-2

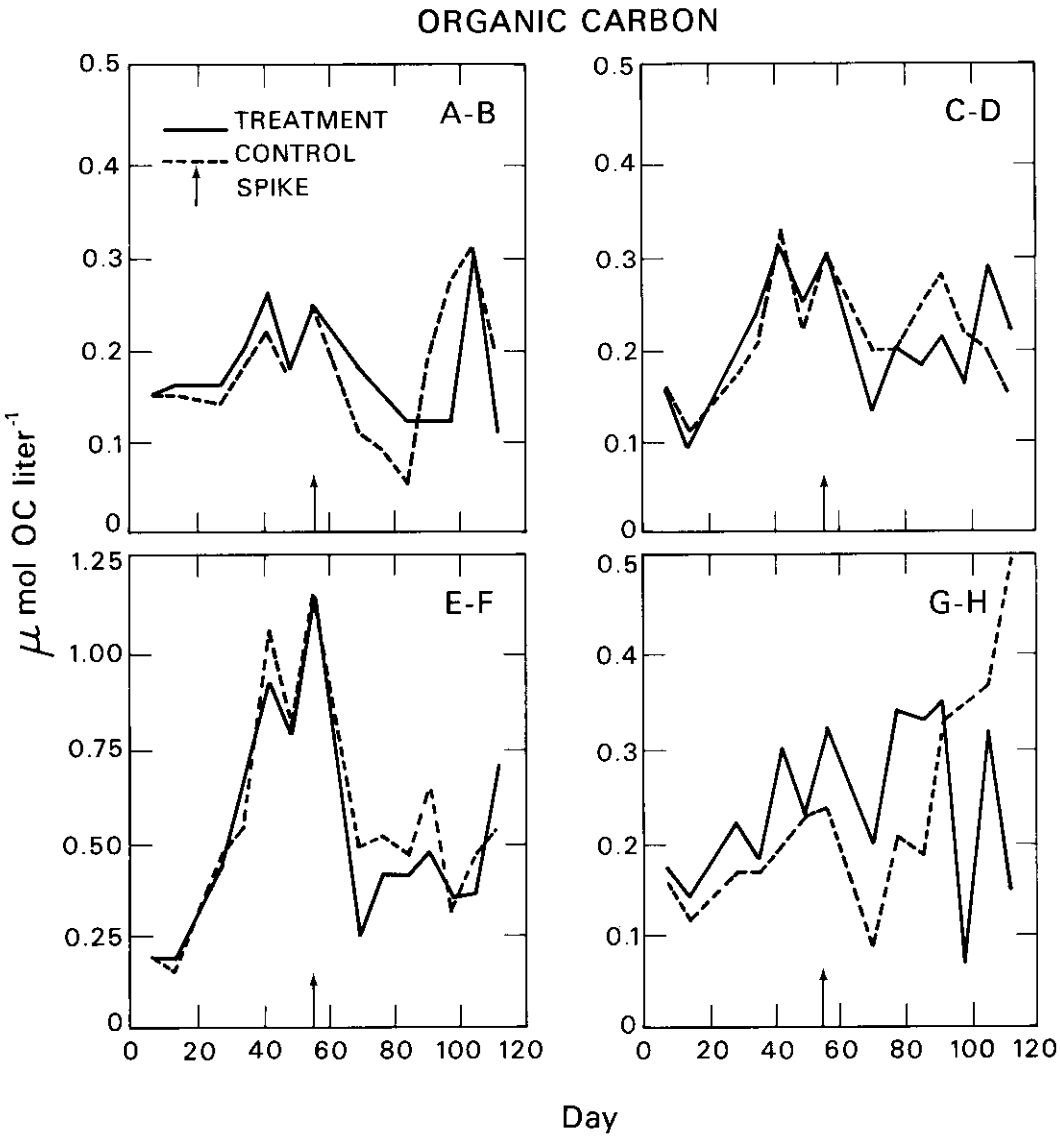

ORGANIC CARBON
TREATMENT
CONTROL
SPIKE
A-B
C-D
E-F
G-H
μ mol OC liter⁻¹
Day
0
20
40
60
80
100
120
0.1
0.2
0.3
0.4
0.5
0.25
0.50
0.75
1.00
1.25

Figure 7B-3

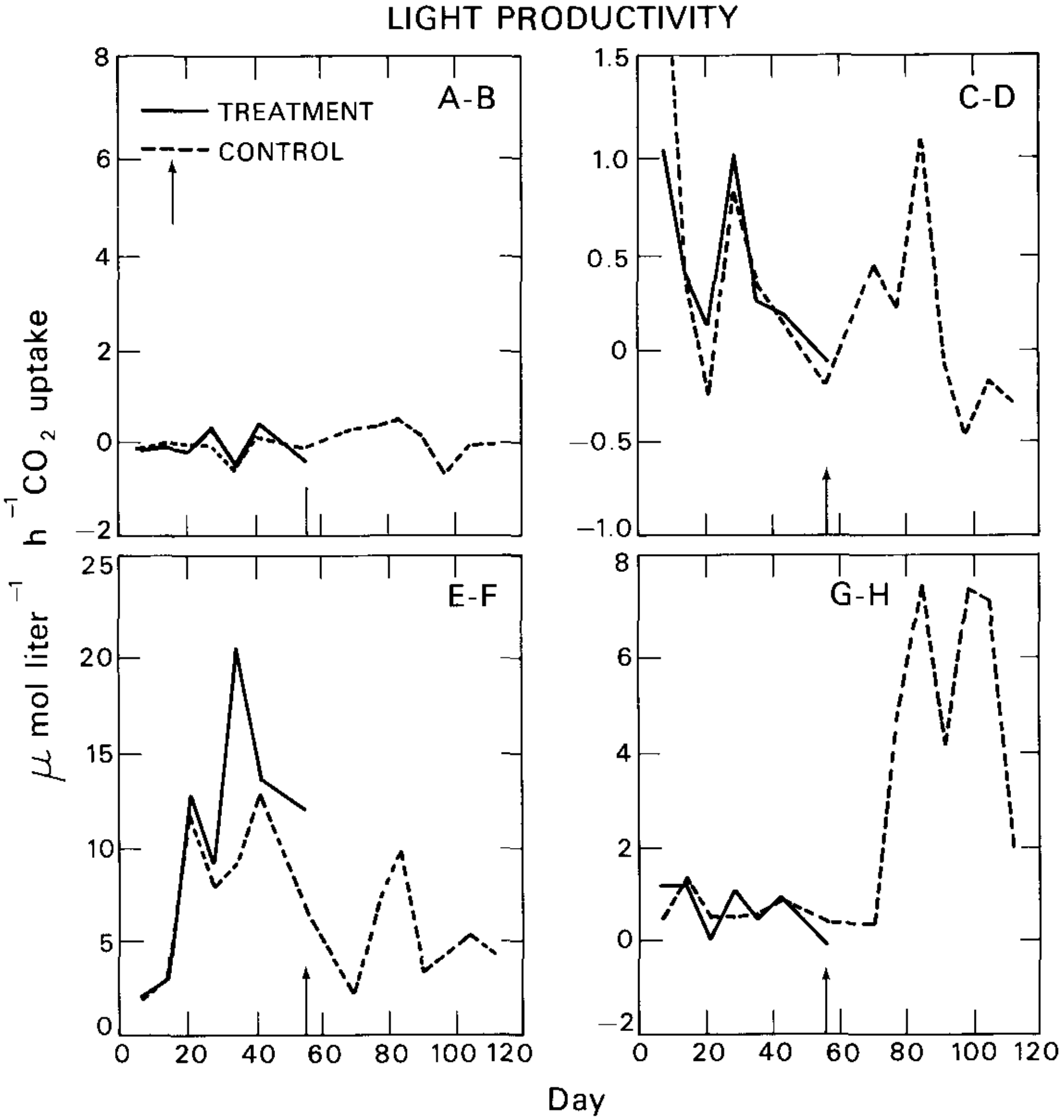

LIGHT PRODUCTIVITY
TREATMENT
CONTROL
A-B
C-D
E-F
G-H
μ mol liter^{-1} h^{-1} CO_2 uptake
Day
8
6
4
2
0
−2
1.5
1.0
0.5
0
−0.5
−1.0
25
20
15
10
5
0
8
6
4
2
0
−2
0
20
40
60
80
100
120

Figure 7B-4

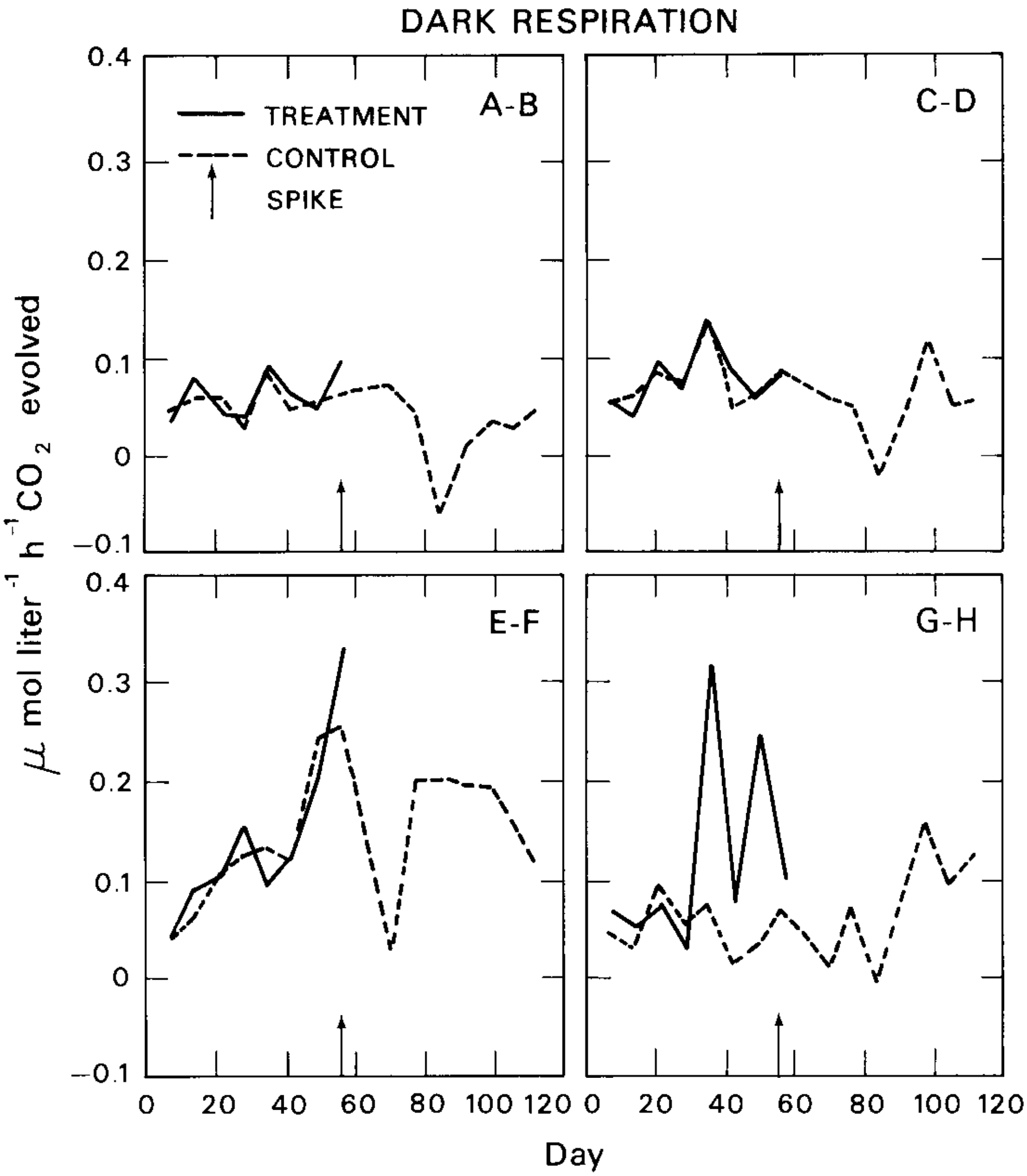

DARK RESPIRATION
TREATMENT
CONTROL
SPIKE
A-B
C-D
E-F
G-H
0.4
0.3
0.2
0.1
0
−0.1
μ mol liter^{-1} h^{-1} CO_2 evolved
0
20
40
60
80
100
120
Day

Figure 7B-5

PHYTOPLANKTON

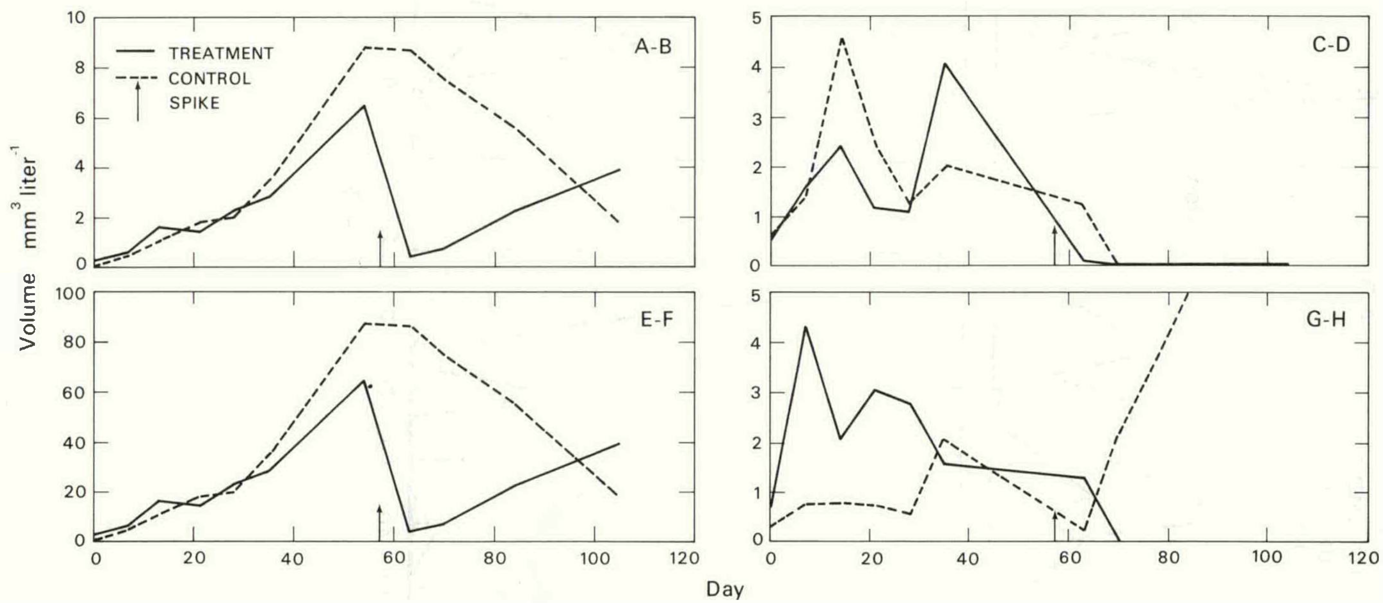
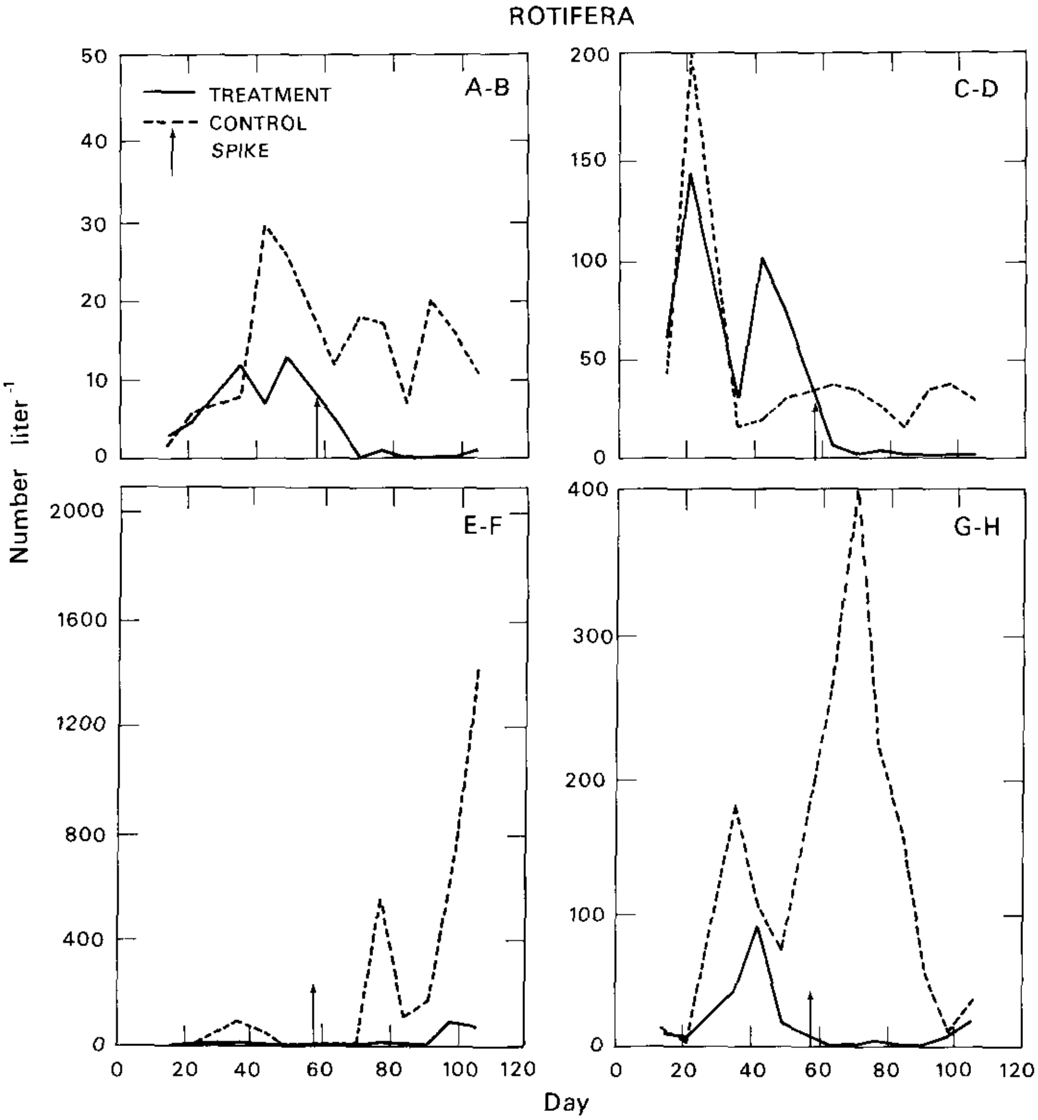


Figure 7B-6



ROTIFERA
TREATMENT
CONTROL
SPIKE
A-B
C-D
E-F
G-H
Number liter⁻¹
Day

Figure 7B-7

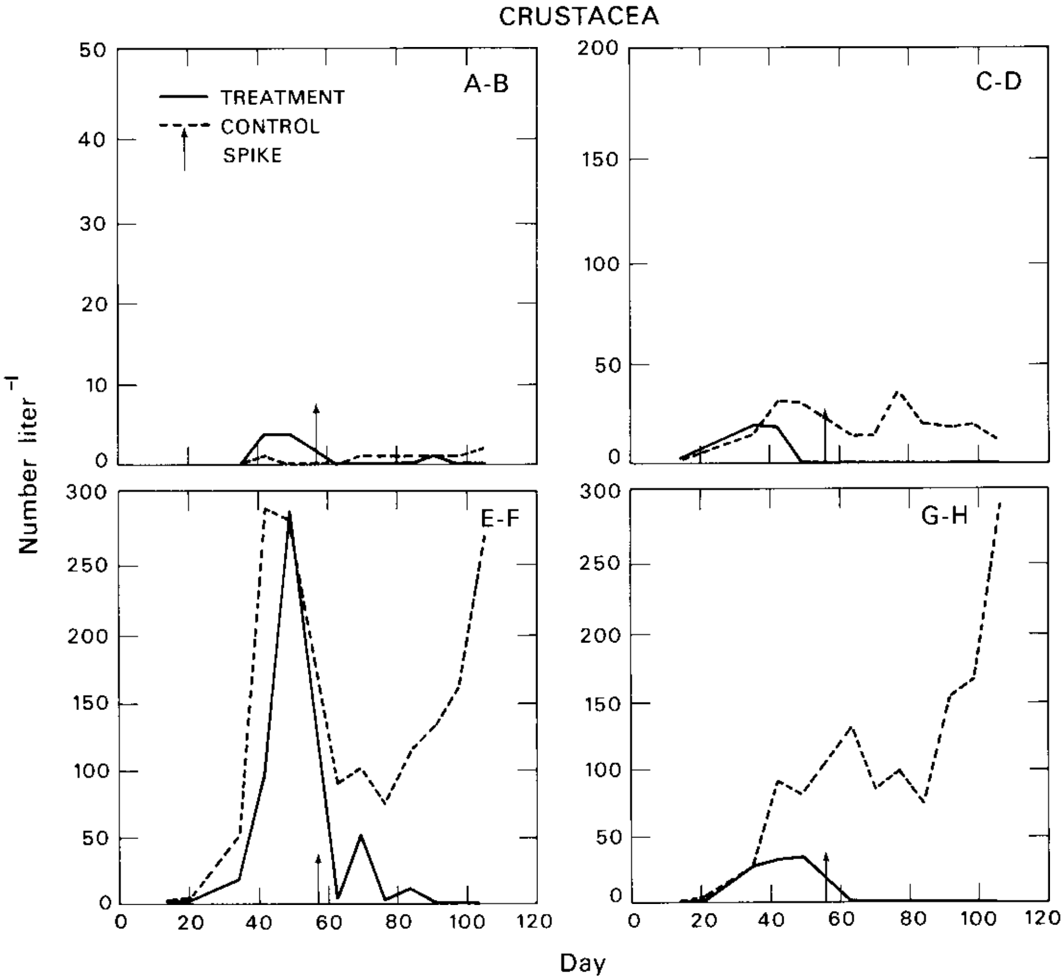
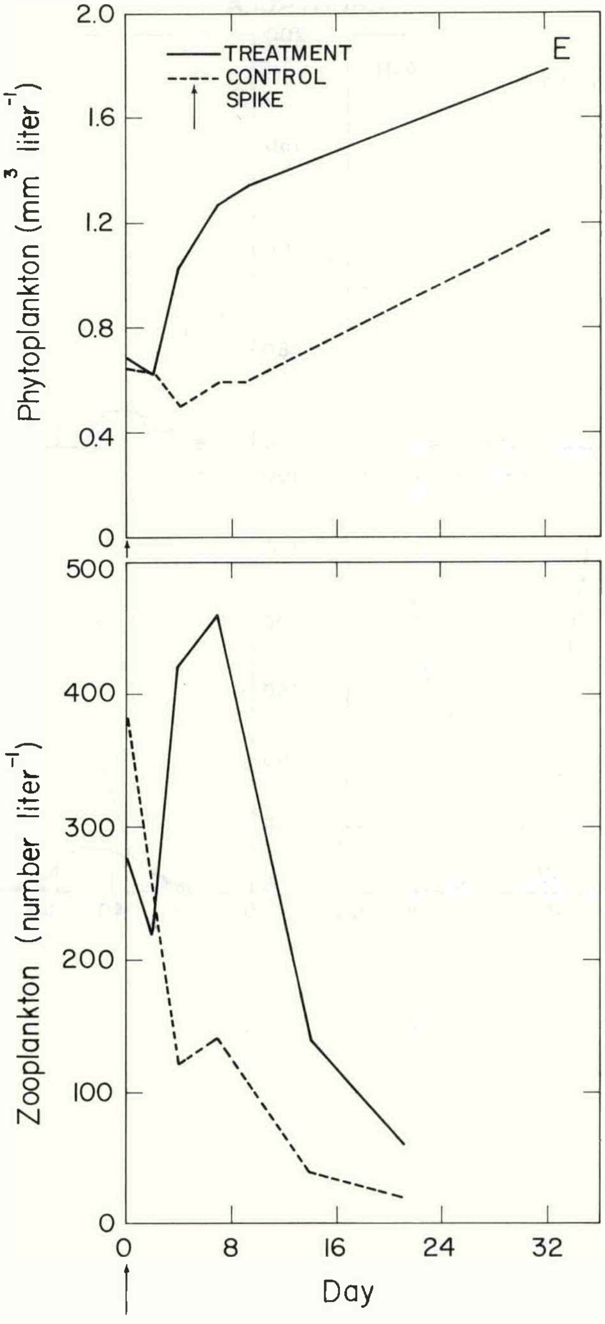
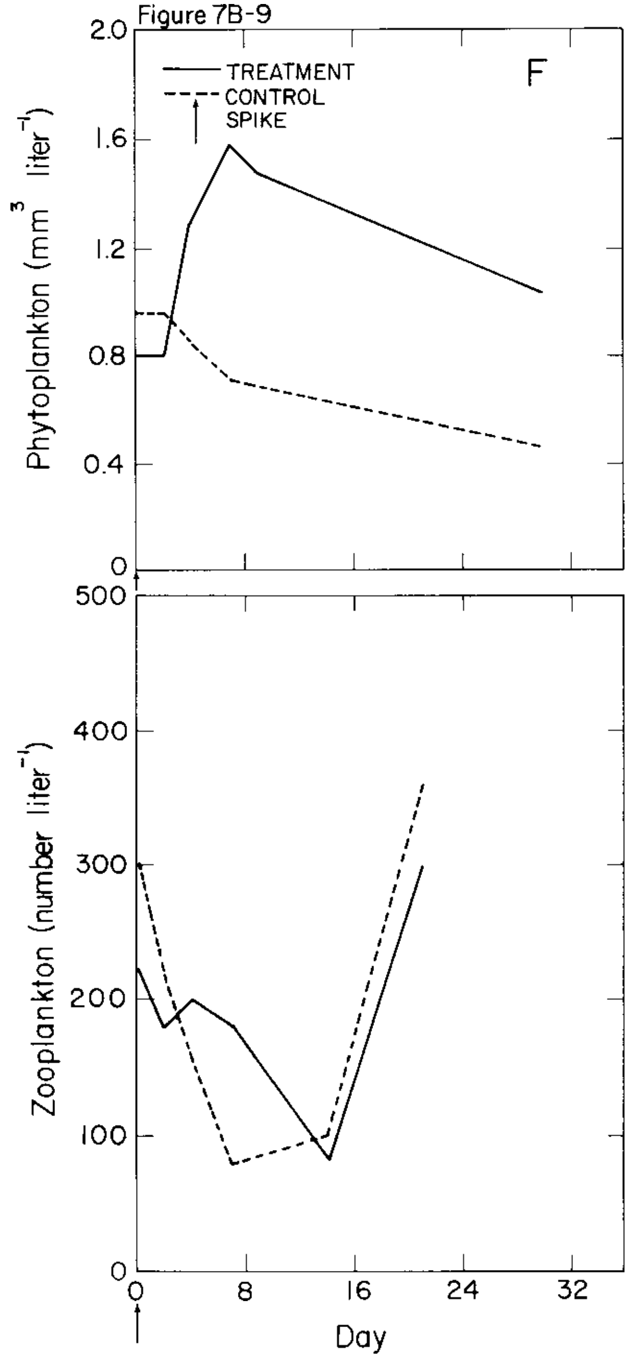

CRUSTACEA
TREATMENT
CONTROL
SPIKE
A-B
C-D
E-F
G-H
Number liter^{-1}
Day
0
10
20
30
40
50
100
150
200
250
300
0
20
40
60
80
100
120

Figure 7B-8



TREATMENT
CONTROL
SPIKE
E
2.0
1.6
1.2
0.8
0.4
0
Phytoplankton (mm^3 liter^{-1})
500
400
300
200
100
0
Zooplankton (number liter^{-1})
0
8
16
24
32
Day

Figure 7B-9



2.0
1.6
1.2
0.8
0.4
0
TREATMENT
CONTROL
SPIKE
F
Phytoplankton (mm³ liter⁻¹)
500
400
300
200
100
0
Zooplankton (number liter⁻¹)
0
8
16
24
32
Day

APPENDIX C

THE PHENOL PERTURBATION EXPERIMENT

RESULTS OF PERTURBATION

Except for initiation procedure, this experiment was similar in design to the first two perturbation experiments. It was initiated by adding to each tank a 10 liter inoculum, taken from an already existing 700 liter system, along with 40 liters of nutrient and mineral enriched deionized water. In previous experiments, a 25 liter inoculum was used. Following initiation, the phytoplankton and zooplankton increased in all tanks. Rotifers were the first zooplankton to increase markedly, and their growth paralleled the first rise in phytoplankton, which in most cases was Chlorella. On day 60, one half of the tanks (A,C,E, and G) were perturbed by the addition of 0.5 ppm phenol. The experiment was then run another 56 days and was terminated on day 112. In all tanks, except E and F, the phytoplankton and zooplankton volumes reached their measured maximum values after the perturbation (Table 7C-1). These maxima occurred on different days for different systems.

The dominant phytoplankton in our systems were Chlorella, Ankistrodesmus, and Anabaena. In the low nitrogen tanks (A,B) and (G,H), Chlorella and Anabaena, the latter of which has nitrogen fixing capabilities, were the principal species. In the moderate nitrogen and high nitrogen tanks (C,D) and (E,F), Chlorella and Ankistrodesmus were the most abundant species. Also present for brief periods were dinoflagelletes, euglenoids, and the green alga Gloeocystis. In terms of volume, cladoceranswere the dominant zooplankton; however, rotifers, copepods, and ostracods also appeared in significant numbers at various times (see Table 7C-2).

It was noted that the total phytoplankton volumes in tanks (C,D) differed significantly both before and after the perturbation. This was due to the presence of high levels of Ankistrodesmus in C as compared to D throughout the experiment. Because of this early non-replicability, we will not include the pair of tanks (C,D) in most of the following discussion.

The largest differences between perturbed and unperturbed tanks were in zooplankton volumes (Table 7C-2). The cladocerans in all the unperturbed tanks, including D, exhibited significant blooms, while the perturbed tanks, including C, did not. In other words, the cladocerans in the perturbed tanks were suppressed relative to the unperturbed tanks. Similarly, the copepods were suppressed in A and E. The rotifers and ostracods were not significantly different in the perturbed and unperturbed systems. Our sensitivity indices can be used to quantitatively describe the fractional or percentage differences between the perturbed and unperturbed tanks after the perturbation. For zooplankton volume, we can rank in decreasing magnitude these percentage differences between the pairs of tanks as (G,H) $\gtrsim$ (E,F) > (C,D)> (A,B) (see Table 7C-3 for sensitivity indices). In terms of absolute numbers (both peak as well as time averaged values) highest total zooplankton volumes were obtained in the unperturbed high phosphorus tanks. In terms of descending magnitudes of total zooplankton volumes, we can rank the unperturbed tanks as $F \simeq H > D > B$.

In the low nitrogen tanks (A,B), (G,H) after the perturbation, the total phytoplankton volumes (peak and time averaged) were greater in the unperturbed systems (B and H) than the perturbed ones (A and G), and were dominated by Anabaena (Fig. 7C-1). In the low nitrogen and low phosphorus systems (A,B), ther was a significant Anabaena bloom in the unperturbed system B and none in the perturbed system A; in addition, during this bloom, there were low and approximately equal volumes of zooplankton in both systems. In the low nitrogen and high phosphorus systems (G, H), in addition to increased levels of Anabaena, the unperturbed system H, during this time, had higher zooplankton levels and most likely experienced somewhat greater grazing pressure than its perturbed sister system G (Fig. 7C-2) (Anabaena, reportedly, is not effectively grazed by zooplankton and may not reflect grazing pressure). In both sets of low nitrogen systems, we see that the volume of Anabaena was greater in the unperturbed systems as compared to the perturbed ones, even when there was some grazing pressure in the unperturbed system. Thus, our data suggest that phenol depresses the growth of Anabaena as well as cladocerans (Table 7C-3).

In the high nitrogen and high phosphorus systems (E,F), the total phytoplankton volume reached its maximum in both systems before the perturbation. After the perturbation, the time averaged total phytoplankton volume was greater in the perturbed systems (E) than in the unperturbed system (F).

In terms of the fractional or percentage differences in the total phytoplankton volumes between perturbed and unperturbed systems, we can rank the systems in decreasing magnitude of these percentage differences using the sensitivity indices as (A,B) > (G,H) > (E,F). This ordering of the magnitude of these differences for phytoplankton was the same as was obtained in the spike experiment, where the spiked systems exhibited phytoplankton growth relative to the unspiked systems.

The IC, OC, and CO_2 evolution data were all unremarkable in this experiment.

Conclusions

1) Phenol suppressed cladocerans in all tanks and copepods in tanks A and E.

2) After the perturbation in the low nitrogen systems, the total phytoplankton volume (time averaged and peak) was greater in the unperturbed tanks (B and H) as compared to the perturbed tanks (A and G). In the high nitrogen and high phosphorus tanks, the phytoplankton were greater in the perturbed tank (E).

3) Phenol suppressed *Anabaena* in A and G.

4) Care needs to be taken that initiation procedures replicate biota between desired replicate systems.

Table 7C-1

MAXIMUM VALUES FOR TOTAL PHYTOPLANKTON AND ZOOPLANKTON VOLUME AND DAY OF OCCURRENCE

PHYTOPLANKTON

Tank	Volume (x 10^9 μ^3/l)	Day
A	.8	63
B	2.4	77
C	3.7	84
D	1.0	63
E	1.6	56-63
F	2.0	49
G	.5	77
H	.6	63

ZOOPLANKTON

Tank	Volume (x 10^9 μ^3/l)	Day
A	1.4	112
B	2.45	98
C	1.4	98-105
D	3.7	63
E	3.0	84
F	8.0	63
G	2.1	105
H	6.9	77

Table 7C-2

AVERAGE ZOOPLANKTON VOLUMES (x 10^6 μ^3/l)*

Tanks	Rotifera	Copepoda	Cladocera	Ostracoda
		BEFORE PERTURBATION		
A	4.4	3.3	14	--
B	1.6	2.1	43	.1
C	6.1	4.5	291	1.1
D	10.4	30.7	232	--
E	3.5	8.0	69	--
F	13.3	23.0	377	2.2
G	3.9	2.3	78	1.2
H	3.8	5.1	101	.7
		AFTER PERTURBATION		
A	6.2	.7	643	2.4
B	3.3	23.4	1030	14.1
C	.7	14.6	1044	13.7
D	.6	161.0	1635	15.5
E	.6	64.0	973	27.4
F	.2	396.0	3161	13.3
G	.2	39.0	718	10.4
H	.1	70.0	3136	18.5

*The averages in this table were calculated by performing an integration over time of the data, while in Table 7C-1 the data points were arithmetically averaged. This accounts for any apparent discrepancies.

Table 7C-3

SENSITIVITY INDICES

	(A,B)	(C,D)	(E,F)	(G,H)
		Zooplankton Volumes		
Before	.03	.03	.16	.03
After	.43	.51	.86	1.06
		Phytoplankton Volumes		
Before	.22	.34	.25	.17
After	1.16	.97	.43	.57
		NH_4 Volumes		
Before	.26	.22	.39	.21
After	.12	.10	.25	.70
		NO_3 + NO_2 Volumes		
Before	.18	.16	.19	.07
After	.09	.03	.15	.10
		Rotifera Volumes		
Before	.36	.57	1.2	1.7
After	.42	.06	.08	.05
		Total Phosphorus		
After	.16	.32	.26	.56

Table 7C-3

SENSITIVITY INDICES (cont.)

	(A,B)	(C,D)	(E,F)	(G,H)
		Copepoda Volumes		
Before	.1	.24	.06	.07
After	1.6	1.6	1.3	.68
		Cladocera Volumes		
Before	.08	.02	.12	.14
After	.47	.31	.97	1.13
		Ostracoda Volumes		
Before	--	.07	.09	--
After	1.5	.92	1.01	.70

MEASUREMENT OF THE PREDICTIVE INDICATOR

Detrital spike experiments were carried out on day 60 in a manner similar to those carried out in earlier experiments. From these experiments, K_D values were calculated using phytoplankton and zooplankton data. For each set of aliquots coming from a given pair of tanks, say A and B, we note the largest percentage change induced by the detrital enrichment, using the sensitivity indices.* We then use this value of the sensitivity index for our value of K_D. In Table 7C-5, the K_D values obtained from phytoplankton and zooplankton are given.

In Table 7C-6, we rank in descending order the pairs of tanks according to their K_D values or, in other words, in terms of the percentage (fractional) changes induced in the indicator experiment.

Table 7C-5

K_D VALUES FOR PHYTOPLANKTON AND ZOOPLANKTON

Tanks	K_D (from Phytoplankton)
(A,B)	.5
(C,D)	.5
(E,F)	.36
(G,H)	.47
	(from Zooplankton)
(A,B)	.59
(C,D)	.38
(E,F)	.35
(G,H)	.82

*We used the form of sensitivity index, in which the denominator is $\langle T+C \rangle_n$, rather than that of K_D, in which the denominator is $\int C$, because the systems were in a bloom phase.

Table 7C-6

TANK PAIRS RANKED BY DECREASING SIZE OF PERCENTAGE CHANGES (K_D) INDUCED IN THE INDICATOR EXPERIMENTS

PHYTOPLANKTON
(CD,AB)
(G,H)
(E,F)
ZOOPLANKTON
(G,H)
(A,B)
(C,D)
(E,F)

In Table 7C-7, we rank the pairs of perturbed and unperturbed tanks from the Phenol Experiment, according to descending order, the percentage (fractional) differences between perturbed and unperturbed systems. Here we exclude tanks (C,D) from our consideration, because of non-replication.

Table 7C-7

TANK PAIRS RANKED BY DECREASING SIZE OF PERCENTAGE CHANGES (USING SENSITIVITY INDICES)

PHYTOPLANKTON	ZOOPLANKTON
(A,B)	(G,H)
(G,H)	(E,F)
(E,F)	(A,B)

From comparison of Table 7C-6 with 7C-7 we see that the values of K_D obtained using the detrital experiments, phytoplankton data rank various sets of tanks in the same order as the sensitivity indices rank the fractional changes between the perturbed and unperturbed systems in the perturbation experiments. This seems significant because the induced changes in the phytoplankton in the detrital experiments were all increases, whereas there were both increases and decreases observed in the perturbation experiments.

Except for the fact that the percentage changes in zooplankton volumes were greatest in (G,H), for both the perturbation and the indicator experiments the zooplankton data from the indicator experiments were not of useful predictive value. The NH_4^+ data was not useful for comparison with the phenol perturbation experiments.

Taken as a whole, the indicator experiment suggested the correct ordering of the phytoplankton changes in the perturbation experiment, but not the zooplankton changes.

Figure 7C-1

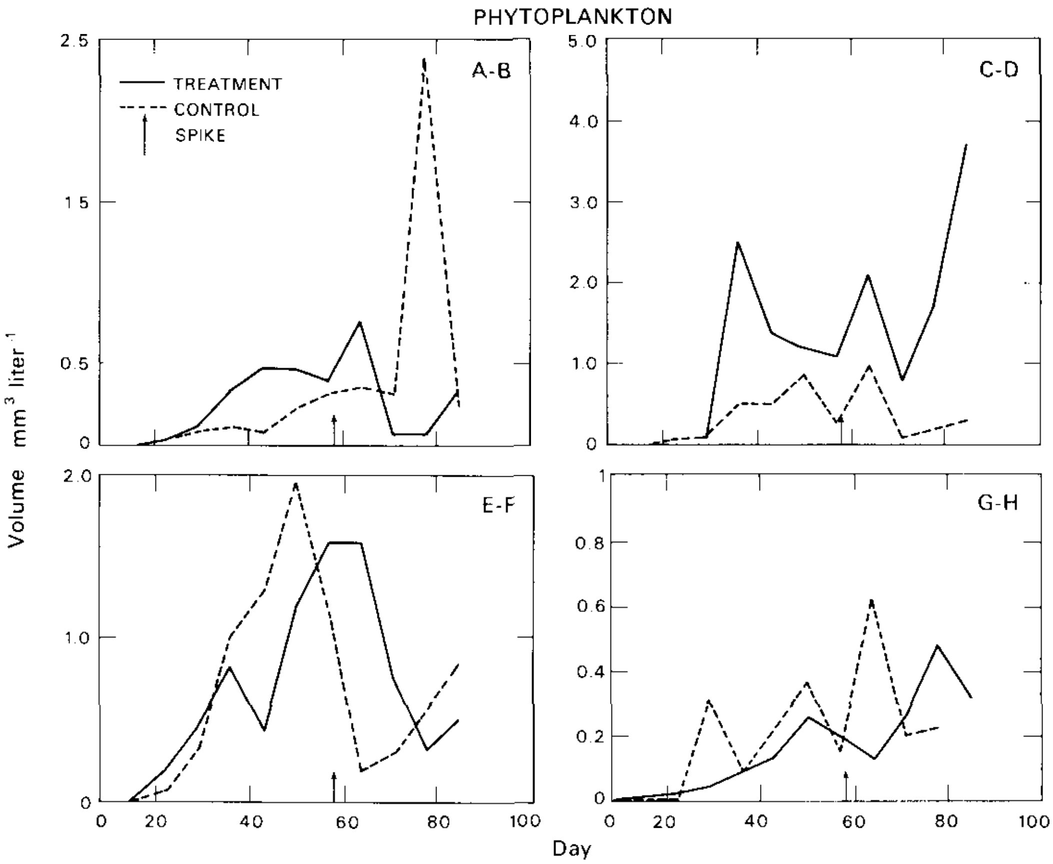
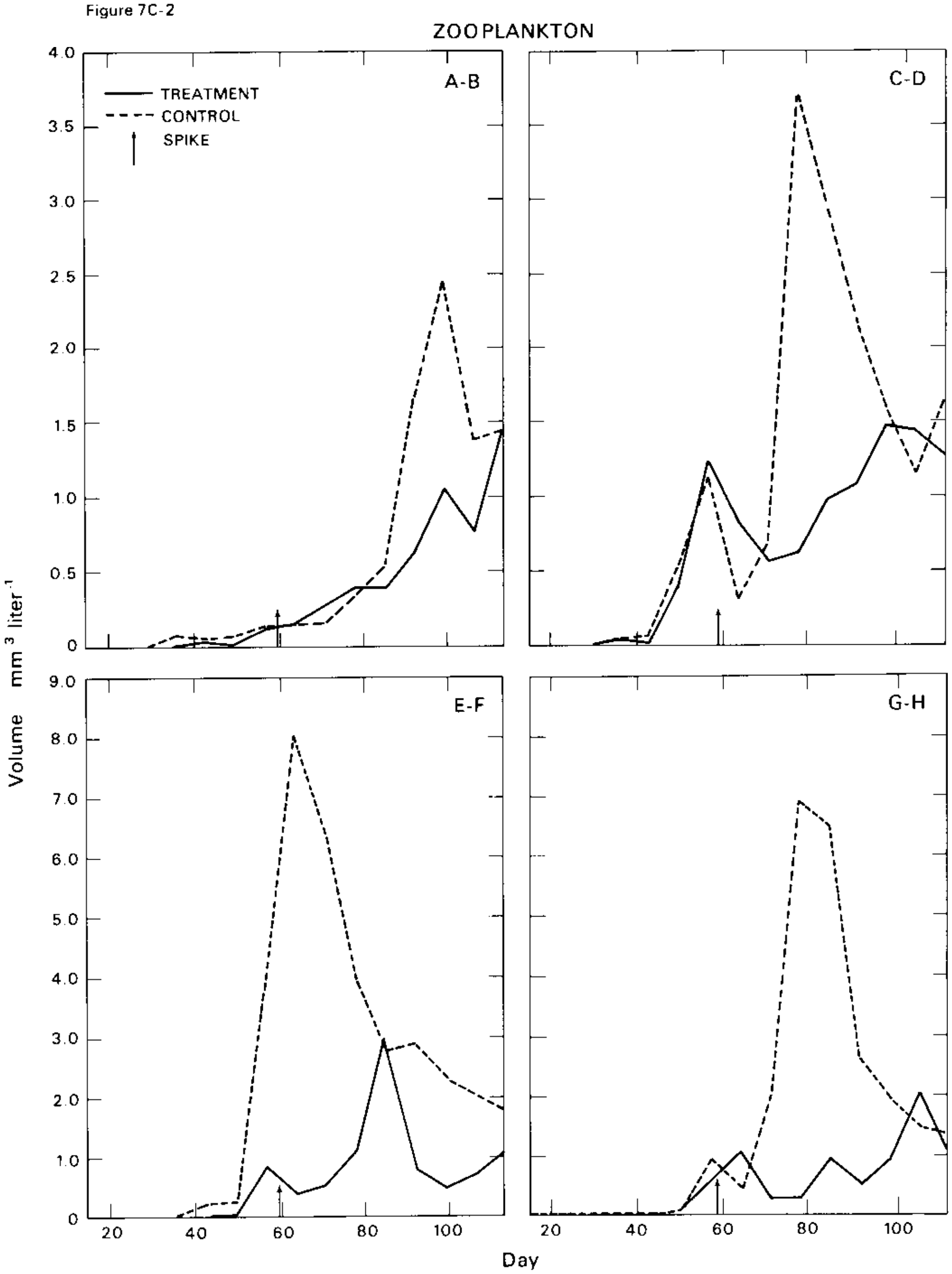

PHYTOPLANKTON
TREATMENT
CONTROL
SPIKE
A-B
C-D
E-F
G-H
Volume mm³ liter⁻¹
Day
2.5
1.5
0.5
0
2.0
1.0
5.0
4.0
3.0
0.8
0.6
0.4
0.2
0 20 40 60 80 100

Figure 7C-2


ZOOPLANKTON
TREATMENT
CONTROL
SPIKE
A-B
C-D
E-F
G-H
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
20
40
60
80
100
Volume mm ³ liter ⁻¹
Day

Appendix D

EFFECTS OF DETRITAL ENRICHMENT

The hypothetical stability indicator, $K_D = (\int(T-C)/\int C)/(\Delta D/D.)$, derived from theoretical studies, would be particularly simple if the response term, $\int(T-C)/\int C$, had a direct linear relationship to the change in detritus, ΔD. In that case, K_D would be a constant, independent of the size of the detailed addition. The purpose of the following experiment we performed was to determine whether this relationship is exact, i.e., whether the measured value of K_D is independent of the detrital increase used in its experimental determination.

METHOD

Four duplicate pairs of 4 liter beakers were filled with well-mixed samples of microcosm water derived from the twelve control tanks used in the phenol perturbation experiment. The beakers were maintained as described for the indicator experiments in Appendices I, II, III. The system's organic carbon was measured and found to be 0.43 mM, and a preparation of simulated detritus, also described in the preceding appendices, was added in varying amounts to three of the four pairs, as follows:

Table 1

Addition of Detritus

	μMOC added	% increase
1A,1B	0	0
2A,2B	117.5	27
3A,3B	235.0	54
4A,4B	470.0	109

Dark and light CO_2 changes, organic carbon, $NO_3 + NO_2$, and NH_4 levels were measured daily for the first week following addition of the detrital preparation and then irregularly for another two weeks.

RESULTS

The values of $\int(T-C)/\int C$ were calculated for the interval day 0 to each day measurements were taken and were found to vary with time. The greatest dark respiration responses were observed during the first two days, and the greatest light productivity responses were observed on the last measurement day. The data from the NH_4^+ + and NO_3^+ + NO_2^+ measurements after day 7 are questionable because significant turbidity interfered with the assays after this point. Values of $\int(T-C)/\int C$ are presented in Table 2 for the period 0-7 and 0-18 days.

Table 2

Effects of Detrital Enrichment on $\int T-C/\int C$

		percent increase in detritus		
		27%	54%	109%
OC	0-7	15	25	35
	0-18	33	37	62
DK RESP	0-7	14	19	65
	0-18	10	10	31
Lt Prod	0-7	235	287	395
	0-18	276	401	488

In Table 3, maximum values of NH_4^+ produced are presented.

Table 3

Maximum Values for NH_4^+ (occurred on day 2)

	0%	27%	54%	109%
NH_4^+ (μM(N))	1	1.1	7.2	24.5

In general, the results suggest nonlinearly increasing dependence of the direct response terms (DK PROD and NH_4^+) to the size of the detrital addition.

These results are surprising and interesting. Further experimental analyses will be required to confirm and clarify the nonlinear relations observed here and to assess their significance to nutrient cycling, stability, and response to perturbation. Mathematical models customarily used to describe bacterial decomposition of detritus do not lead to the type of nonlinear behavior suggested here. We point out here that these results from an admittedly preliminary study do underscore the potential usefulness of microcosms for identifying interesting mechanisms and phenomena.

APPENDIX E

PROBLEMS OF SCALE*

INTRODUCTION

A central methodological problem in aquatic microcosm research concerns the question of realism: To what extent are the biological phenomena which are observed in laboratory microcosms either representative of or similar to phenomena observed in natural systems, or when is a microcosm an adequate model for a large ecosystem? It is essential that this question be answered, because of the increasingly important role that microcosm research will play in the assessment of the transport and ultimate effects of toxic chemicals released to the environment.

The response of a microcosm to the addition of a toxic substance must resemble the response of the natural system of interest. It is not enough to demand that the microcosm display a trophic structure similar to the natural system. A similarity in magnitude of the underlying biological and chemical components and the rates of transformation of these components is also required. Consequently, microcosms should be designed so that processes are not too distorted and extraneous effects are minimized. The relatively small size of laboratory microcosms makes some distortion of properties inevitable since it is not possible to scale down to laboratory size many natural physical and biological processes. In some cases this distortion may be acceptable (e.g., the relative lack of spatial heterogeneity in microcosms is an asset insofar as it decreases the sampling necessary to characterize the system). Other problems of size may be more severe and may pose serious obstacles to the usefulness of microcosms. Three serious size related constraints encountered in microcosm research are: (i) the shallow depth of most laboratory microcosms; (ii) the difficulty of including higher trophic levels, such as fish, in small microcosms, without severe distortion of nutrient cycles; and (iii) the dominance of chemical and biological activity occurring on the sides and bottom of microcosms, due to the relatively high surface to volume ratio of the container. This report examines these constraints and provides possible methods for resolving some of their disadvantageous effects.

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PROBLEMS OF SCALE

Depth

Unless one is attempting to simulate a shallow pond ecosystem, the relatively shallow depth of most laboratory microcosms will introduce unavoidable problems of scale. Zooplankton behavioral responses will be altered, due to the restriction of the range for vertical migration. Vertical irradiance changes will be smaller than in most natural systems, and may affect the growth rates of phytoplankton. In addition, losses of phytoplankton from the water column due to sinking will be increased due to reduced depth. Most of these effects are difficult to include in a model of microcosm dynamics. Increased phytoplankton sinking rates, for example, imply more than increased quantities of phytoplankton lying on the bottom of the microcosm; it also increases the rate that nutrients are recycled to the water column, leading to effects which are difficult to model, and these rates determine the rate that nutrients are recycled to the water column.

Higher Trophic Levels

Another problem of scale concerns the difficulty of including such macrofauna as fish, snails, and larger crustacea in microcosms. If the microcosms are too small, the disproportionate consumption and excretion of carbon and nitrogen compounds by the macrofauna leads to a significant alteration of nutrient levels and of the nutrient cycle itself. In extreme cases, the larger animals may overgraze the food supply and consequently starve. A few simple calculations will serve to make the point. Consider a small mosquitofish of 1 g wet weight. It may be estimated that a fish of this size will excrete at $20^{\circ}C$. 0.25 mmol C per day, and 0.5 mmol N per day, primarily in the form of NH_4^+(4). In a 20 l system, those daily amounts correspond to concentrations of carbon and nitrogen, respectively, of 12.5 μM and 2.5 μM, both significant fractions of the typical values for these quantities in most lakes. It is likely that in a microcosm of that size, the fish could not sustain feeding rates high enough to live and would slowly starve. In larger systems, the problem does not appear to be quite so severe, the amounts of carbon and nitrogen excreted by 1 g of fish in a single day being equivalent to concentrations of 0.25 μM and 0.05 μM respectively in a 1000 l microcosm. However, in a study of microcosm trophic structure, a 700 l microcosm with 3 g of fish biomass displayed a fish to zooplankton biomass ratio higher than would be expected in a natural situation (5). Abnormally high ratios of particulate organic carbon to dissolved organic carbon and of carbon to chlorophyll a were also observed, while the corresponding ratios in a control microcosm without fish were within the range commonly observed in large lakes.

For other macrofauna the arguments are similar, and care must be excercised when adding any macroscopic organisms to be sure that the microcosm can accommodate the organisms without significant distortion of those properties of interest.

Side and Bottom Effects

The final major scaling problem is the large ratio of side and bottom surface area to the volume of the microcosm. A biological or chemical process which depends on a solid substrate may exert a disproportionate influence on the dynamics of a small microcosm. This is explained more precisely by considering the dynamics of a chemical substance of concentration x in a cylindrical microcosm of radius r and depth z. Assuming that the substance is being transformed in the water column and has a flux to or from the sides and bottom, we may write the conservation equation:

$$(1)\quad (\pi r^2 z)\frac{dx}{dt} = (\pi r^2 z)C_v + (2\pi r z)J_s + (\pi r^2)J_b$$

where:

C_v is the net production rate of x per unit volume

J_b is the net flow rate to the bottom per unit area

J_s is the net flow rate to the sides per unit area

Dividing both sides of Eq. 1 by $(r^2 zx)$ we obtain:

$$(2)\quad \frac{1}{x}\frac{dx}{dt} = \frac{1}{x}C_v + \frac{1}{x}\frac{2}{r}J_s + \frac{1}{x}\frac{1}{z}J_b$$

It is seen that the second and third terms depend upon the dimensions of the container while the first does not. For natural systems z is typically 10 - 100 m, r is 100 - 10^4m, so that even in large laboratory microcosms (z, r = 1 m), we might expect size related terms up to 10^4 times as large as in many natural systems.

If one is interested in nutrient cycles or energy flow, or the partitioning of a toxic substance among the different trophic levels, the activity on the container surfaces cannot be ignored. It may prove extremely difficult to interpret the behavior of a microcosm if the influence of the sides or bottom is large. For example, the effect of sediments on the overlying water is not simply accounted for by the relative increase in bottom area. In many lakes stratification of the water column serves to isolate the benthos from the epilimnion, reducing the effect of the benthos throughout much of the year. Although it is not difficult to create a thermocline ina small microcosm, such attempts suffer from scaling problems (e.g., achieving realistic mixing rates, and the highly illuminated benthos and hypolimnion that would result).

Population dynamics are also affected by problems of scale. There is the sampling problem of finding a species in a part of the microcosm that is not its preferred habitat, such as algae sloughing off the sides into the water column or normally side-grazing zooplankton in the center of the microcosm. Some species may occupy several "zones" and have several, possibly distinct, food sources. Interpretation of population fluctuations in these species may require complicated considerations of food supply. One of the primary advantages of microcosms, the relative lack of complicating spatial heterogenity, is weakened by biological activity on the sides or bottom.

The growth of periphyton on the sides of microcosms is the most important of the side related effects. In past experiments some degree of side growth became established 40-50 days after inoculation (2). The appearance of such periphyton can have a marked effect on the cycling of nutrients. In one experiment (1), chlorophyll a densities of 60 mg m^{-2} were observed on the sides after 84 days. This density represented an amount of chlorophyll a on the sides more than 10 times higher than the total amount found in the plankton.

Attempts at biological control of periphyton growth have been largely unsuccessful. Snails (Physa sp.) and South American catfish (Plecostomus sp.) were added to 700 l microcosms in an attempt to keep periphyton levels low by grazing (2). Plecostomus were the most successful at reducing the growth, but the difficulties mentioned above about fish must apply. Even so, the addition of Plecustomus caused a shift from unicellular to filamentous forms of periphyton, which were not eaten. The catfish were successful in maintaining complete absence of periphyton growth only in one microcosm with low levels of nutrients. The snails were less effective initially, but they grew in size until they were able to exert a noticable effect on the periphyton. They then reproduced rapidly, and there was soon high mortality resulting from the lack of food due to overgrazing. Control of periphyton by macroscopic herbivores is impractical because of the disproportionate size of the herbivores, and because the much faster reproductive rate of periphyton allows it to quickly outstrip its grazer when conditions favor its growth.

Mechanical means of control may be more successful. Scraping the sides of tanks regularly may prevent periphyton growth, but this is a tedious procedure when done on many microcosms, and an automated means may be necessary. Even if this method of control is attempted, it will have to be done with great precision. A close-packed layer of cells only 1 μm in thickness on the walls of a microcosm 1 m in diameter and 1 m deep, will have a volume equivalent to 4 cm^3 m^{-3}, about the same as the volume density of phytoplankton found in a mesotrophic lake (6). Other methods of control may be necessary, such as transferring the microcosms to new containers at regular intervals. This method has the additional advantage of allowing the simulation of algae sinking out of the epilimnion. The water column could be decanted into a new container, letting the settled algae and detritus remain.

The above considerations of scaling imply that microcosms can be expected to give realistic quantitative information only during those times when the first term in Eq. 2 dominates the second and third terms. This is usually the case during the period of intense production or decomposition such as occurs during and immediately following a phytoplankton bloom.

IMPORTANCE OF STUDYING BLOOMS

During the first 40 or 50 days after microcosm initiation, most chemical and biological activity takes place in the water column, rather than on the sides or the bottom (5,6). As described elsewhere, this activity resembles a spring bloom and subsequent decomposition of the produced material. Referring to Eq. 2, this means that for chemical or biological quantities, the first term is large and the second and third terms small. After this 50 day period, much of the biological activity shifts to the sides and bottom. Thus, microcosms with no side treatment progress from planktonic systems to littoral-benthic systems during the course of their development. Once established, side growth and bottom activity can exert large effects on the rates of transformation of chemicals in the water column. Hence, it is our belief that the most fruitful period to study aquatic microcosms is during the first month or two, i.e., during that period of activity analogous to the spring bloom in many natural systems, and not that period when side growth and bottom decomposition dominate the metabolism of the microcosm. This is in contrast with the widely held view (7) that the time to study microcosms is long after their inoculation - when they have passed from an "immature" to a "mature" state.

It is possible that the behavior of an aquatic microcosm during the spring bloom may be strongly connected to its behavior throughout the rest of the year. An indication of this connection may be seen in a model by Cushing of zooplankton-phytoplankton dynamics (8). The model was first proposed for temperate marine waters and then extended to polar and tropical marine waters. While this is not an adequate model for lakes, a brief discussion will help to point out the possible importance of studying bloom phenomena. In Cushing's model, temperature differences affect the delay time between the spring phytoplankton increases and the onset of effective grazing by zooplankton. In polar areas, after the phytoplankton bloom begins, there is a relatively long delay before zooplankton rise to levels sufficiently high to graze down the phytoplankton. Because of the long delay, phytoplankton rise to levels relatively higher than in temperate or tropical areas. The efficiency of conversion of primary to secondary production is low because much of the production takes place during the bloom, when grazing is low; thus, the percentage lost to sinking is high. By the time the zooplankton die, low temperature and light levels have ended the growing season, and no fall bloom can occur. In contrast to polar oceans is the seasonal cycle found in tropical oceans, in which the temperature is relatively high, leading to shortened delay times for zooplankton reproduction. As a consequence, the phytoplankton is grazed down before it reaches a high level, and the maximum zooplankton level is lower than in temperate or polar regions. The efficiency of conversion from primary to secondary production is higher because more primary production takes place when zooplankton levels are high, and a large percentage is grazed.

A simple phenomenon, the temperature dependence of zooplankton reproductive rates and delay times before reproduction, can change the entire seasonal behavior of an aquatic ecosystem. The effects of this and other similar phenomena can be investigated in and for microcosms as well. Other quantities, such as the initial numbers of zooplankton present at the time of inoculation may serve to affect the long term behavior of a microcosm in very marked ways, determining whether a system will become grazing limited or nutrient limited. An aquatic microcosm, just like a naturally occurring aquatic ecosystem, is a dynamic system which undergoes strong oscillations, the nature of which may depend in detail upon the nature of zooplankton-phytoplankton-nutrient interactions as well as upon the initial conditions of the microcosm.

APPENDIX F

WHAT MINIMUM SIZE MICROCOSM IS NEEDED TO ACCOMMODATE A FISH?

To answer this question, two points have to be stated clearly. The first is the criteria which are used to determine minimum acceptable size. The second is the purpose for having fish in a microcosm. Below, we will list possible criteria and estimate what minimum size is suggested by each. We will then discuss, qualitatively, how the estimated lower bounds should be interpreted depending upon the planned use of the microcosm with fish. We conclude with a brief note about the use of shad in microcosms.

We cannot overemphasize that in referring to natural systems for background data on fish, one is hampered by a paucity of reliable measurements. In particular, the utilization or flow-through rate of carbon through the fish community is poorly determined, and total fish biomass information in natural lakes is scarce. These numbers are important since they reflect the fish community's impact on the nutrient through-put of the system.

CRITERIA

Criterion 1. Survival of the Fish

Survival depends upon a number of factors, most important of which is adequate oxygen and adequate food supply. Even in our relatively small (50 liters) systems, oxygen supply is generally not a problem. Ours are lightly aerated, but that is done primarily to provide mixing and to avoid having to sample waters from a heterogeneous system. But if fish food is not provided externally, and thus the fish is to survive solely on food provided by primary and secondary production within the system, then microcosm size is a relevant factor. We have found that 50 liter systems, varying from oligotrophic to mesotrophic conditions, could not support a single 1 gram _Gambusia_ (mosquito fish) for more than a few weeks, after which they died of apparent starvation. On the other hand, 700 liter microcosms supported five 1 gram _Gambusia_ for five continuous months. We have no data on survival for longer periods. During that 5-month period the _Gambusia_ appeared to increase in length at roughly normal rates. On the basis of survival, 50 liters is inadequate for a single 1 gram fish; 700 liters is adequate. However, an important caveat is necessary here. Side growth, rather than pelagic productivity, _may_ have been responsible for the fish being able to survive. In microcosms without this side growth, 700 liters might be too small to support such a fish population. But survival is clearly not the sole condition for acceptability. It is also important that the survival of the fish not be at the expense of a distortion of other properties of the system. Our next three criteria reflect this consideration.

Criterion 2. Ratio of Fish Biomass to Lower Trophic Level Biomass

In order that the presence of the fish in the microcosm not represent an unduly large influence on the rest of the system, one possible restriction might be that the trophic pyramid resemble that of natural systems. More precisely: The ratio of fish biomass to either phytoplankton or zooplankton biomass should not be significantly larger than the corresponding ratios in natural systems of interest. The phytoplankton and zooplankton biomass in both lakes and microcosms varies tremendously during the seasonal cycle. Fish biomass tends to be more constant. We will use the average biomass throughout the cycle for our comparisons. Typical concentrations of phytoplankton vary from one lake to another and from one microcosm to another depending upon, among other factors, available nutrients and the extent of grazing pressures. In our microcosms with fish (two 1 gram Gambusia), phytoplankton concentrations averaged about 3.5 mg (wet weight)/liter, typical of phytoplankton concentrations in mesotrophic natural lakes. This then corresponds, in a 700 liter system, to about 2.5 g (wet weight) phytoplankton biomass. In that instance, with two 1 gram fish, the microcosm had a fish to phytoplankton biomass ratio of 4/5. Our fish to zooplankton biomass ratios were larger by about a factor of two.

In comparison, in natural lakes, fish to phytoplankton or fish to zooplankton biomass ratios are rarely this close to unity. Typical values rarely exceed 1/10 for either ratio and are often no larger than 1/100 for fish to phytoplankton. Based on these considerations, we estimate that an absolute minimum size microcosm to contain one 1 gram fish would be somewhere in the neighborhood of 10 m^3. Otherwise, studies would have to be confined to highly eutrophic systems.

Criterion 3. Nutrient Flow Needed to Support the Fish

If a fish is placed in a microcosm in which its presence is a potentially overbearing influence, two possibilities can arise. The fish may develop normally but consume a far larger share of primary or secondary production than would be characteristic of a natural system, or the fish may not receive adequate food supply and then either die or live under stress and not grow at normal rates. While it is difficult to predict in advance whether the fish will obtain its food supply at the expense of distorting nutrient cycles or whether it will starve, some simple considerations allow us to deduce conditions under which it is likely that one or the other possibility will arise.

First we consider the case of a fish which is not in its growth phase and is utilizing a fraction of the carbon through-put commensurate with what it would consume in a natural system. In a variety of natural systems the percent of primary productivity ultimately utilized by the fish community is in the range .06% to 1%. The 1% number comes from a study of Dalnee Lake (9), and we are somewhat leery of this large number because it does not represent an annual average for the lake, but a 3-month mid-summer period. By way of contrast, a very careful study of Lake Erken yielded a value of .06% for this ratio (10). Other more general trophic studies have yielded numbers on the order of .1% (11,12). We want to stress that the paucity of data from natural systems makes these estimates very rough, and so we will use the range of .1% to 1% in making our calculations. Consider now an idealized 1 gm (wet weight) fish added to a microcosm with an average pelagic primary productivity of .01 mmol (C)/l/day. This number can vary by a factor of 5 in either direction, but it represents a rough average for a mesotrophic system and is typical both for our microcosms as well as natural lakes. Such a fish would require ~ .1 mmol (C)/day for metabolism and maintenance (13,14). If we do not require that the fish exhibit a normal growth rate, then under the above conditions, a 1000 liter microcosm would enable a ratio of fish utilization of C to total primary productivity = 1% to be obtained. A 10^4 liter microcosm would be needed to establish a ratio of .1%.

In order to study the uptake of toxic substances and/or their effects, it is important that the fish exhibit their normal growth rates, particularly as young rapidly growing fish are often the most severely affected by toxins. We consider an idealized young rapidly growing fish of 1 gm (wet weight)--equivalent to 4 mmol (C) which utilizes 20% of its assimilation for growth (13,14,15). It is to be placed in a microcosm with an average primary productivity of .01 mmol (C)/l/day. We take the optimal case, where the fish can assimilate 1% of the primary productivity, which implies that it uses .2% of the primary productivity for growth. If we place the above fish in a 1000 l microcosm, it will grow at a rate of .02 mmol (C)/l/day; or, in other words, it will have a doubling time of 200 days. Both our data as well as other data suggest that a more realistic doubling time is ~ 25 days, so that we see that growth-related phenomena would be severely distorted in a 1000 l purely pelagic microcosm. In order to obtain a doubling time of 25 days, an 8 M^3 microcosm would be needed.

Criterion 4. Effect of the Presence of Fish on Ambient Chemical and Physical Conditions

A possible effect which could arise in a microcosm which is too small for its fish population is that certain chemical and physical properties of the system will be distorted relative to what they would be in a natural lake. In our studies with 700 liter systems (5), we found that two such properties differed considerably between microcosms with and without fish. Moreover, these properties resembled those of natural lakes when the fish were absent from the microcosm and differed from those of natural lakes when the fish were present in the microcosm. One of these is the ratio of dissolved organic carbon (DOC) to particulate organic carbon (POC) in the water column. In our 700 liter systems without fish and in many natural lakes, this ratio is typically about 10/1(16). In other microcosms with two 1 gram fish, the DOC/POC ratio was about 3/1. The second property was the chl a/phytoplankton carbon ratio, which was abnormally low (17) in our systems with fish. While we are able to advance plausible explanations for these observations (5), we cannot predict the minimum size of a microcosm which would be necessary to avoid these abnormal conditions.

DISCUSSION

From two perspectives (criteria 2 and 3 above) a lower bound on the size of a freshwater microcosm supporting a single 1 gram growing fish is 10-20 m^3. To support a larger fish or to support more fish, the minimum microcosm size would have to be proportionally larger. A fish can survive in an order of magnitude smaller system for periods of at least 5 months, but to do so the fish, if it is in its growth phase, must either suffer starvation stress or exert an unnaturally large influence on the trophic flow in the system. A crucial ingredient in our estimates is the fraction of phytoplankton productivity in a natural lake which is utilized by the fish in that lake. Further experimental determination of that fraction for a variety of lakes would be most useful in obtaining better estimates of acceptable microcosm size. If the experimental goal is to study fish response to a pollutant or other stress, the complications arising from food stress could seriously affect the validity of any deductions drawn from the data. In particular, synergies could enhance the apparent sensitivity of the fish to external perturbation. If the fish is not starving, but is consuming an abnormally high proportion of primary or secondary productivity, any experiments concerned with concentration in fish of toxic materials, such as pesticides or heavy metals, may fail to give realistic results. If the experimental goal is to study components of the system other than fish, then the abnormal influence of the fish on other components could again destroy the possibility of interpreting the data in a manner that would provide results valid for natural systems. We see no way in which the addition of an external supply of fish food could avoid these problems without creating others of an even more difficult nature. We are therefore confident that a minimum microcosm volume of about 10 m^3/gram of fish represents a practical lower bound.

A Note on Shad in Microcosms

The use of shad or any members of the herring-shad-alewife group (Clupeiformes) in microcosms presents difficulties. The gizzard shad (*Dorosoma cepedianum*), for example, is a phytoplankton feeder, straining food from the water by means of gill rakers. In terms of trophic function, its presence in the microcosm thus would not fulfill the purpose of providing a carnivorous trophic level, nor would it serve any purpose different from that of herbivorous zooplankton. If the purpose of their inclusion is not to provide trophic diversity, but rather to allow fish impact studies under controlled conditions, then problems still arise. Adult *D. cepedianum* would probably be too big for any laboratory microcosm (adults attain a foot in length), while the juveniles, which would rapidly attain more than a gram in weight, would require a microcosm greater than 10 m^3. Moreover, all herring-like fishes swim continually, even while not feeding, and would most probably require larger microcosms with a continual supply of mixing water for a proper feeding environment than expected from the above considerations.

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