AD-A239 433

(2)

AD_____

CONTRACT NO: DAMD17-88-C-8068

TITLE: NATURALLY-DERIVED MICROCOSMS FOR ESTIMATING STRESS EFFECTS IN AQUATIC ECOSYSTEMS

PRINCIPAL INVESTIGATOR: James R. Pratt, Ph.D.
AUTHORS: James L. Rosenberger
Charles T. Morrow

CONTRACTING ORGANIZATION: Pennsylvania State University
School of Forest Resources
University Park, PA 16802

REPORT DATE: May 31, 1991

TYPE OF REPORT: Annual and Final Report

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

20030241041

91 8 13 049

91-07741

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Pennsylvania State University School of Forest Resources; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): University Park, PA 16802	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: U.S. Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: Contract No. DAMD17-88-C-8068
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, Maryland 21702-5012	10 SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO. 3E1-	TASK NO.	WORK UNIT ACCESSION NO.
62720A	62720A835	C1	WUDA314044

11. TITLE (Include Security Classification)

NATURALLY- DERIVED MICROCOSMS FOR ESTIMATING STRESS EFFECTS IN AQUATIC ECOSYSTEMS

12. PERSONAL AUTHOR(S)

James R. Pratt; James L. Rosenberger; Charles T. Morrow

13a. TYPE OF REPORT	13b. TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual and Final	FROM 1/15/88 TO 5/1/91	1991 May 31	

16. SUPPLEMENTARY NOTATION

Annual is for the period of time 1/15/90 - 5/1/91.

17. COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Aquatic Microcosm; Production; Respiration; RAIII; Protozoans; Biomonitoring; Enzyme; Ecotoxicology; Microcosms; Artificial Substrates
06	06		
06	aa		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Traditional methods of estimating hazards of toxic stressors in aquatic ecosystems have fail to account for ecologically important interactions within complex systems. A microcosm testing method was developed to assess the effects of chemical stresses (including complex mixtures) on naturally derived freshwater microbial communities. Microbial communities were collected on polyurethane foam artificial substrates and used to establish replicate laboratory ecosystems to be stressed with toxicants. Responses measured included taxonomic richness, standing crop biomass, enzyme activities, and gross system production and respiration. Both pure compounds and complex mixtures were used as stressors. Results were compared to single species tests and to measured ecosystem impacts. Measures of ecological structure (species richness, biomass) were consistently sensitive indicators of stress. Measures of ecological processes were often less sensitive than measures of structure. Microcosm responses were sensitive at environmental levels of toxicants and were more sensitive to conservative toxicants than to degradable toxicants.

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Mrs. Virginia M. Miller	301-663-7325	SGRD-RMI-S

DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

___ Where copyrighted material is quoted, permission has been obtained to use such material.

___ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

___ Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

___ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

___ For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

___ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

PI Signature 91.5.31 Date

Accesion For	
NTIS CRA&I	[X]
DTIC TAB	[ ]
Unannounced	[ ]
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

DTIC COPY INSPECTED 4

Table of Contents

Volume 1

Executive summary ii

Introduction

1.0 INTRODUCTION 1

Body

2.0 METHODS

2.1 Artificial substrate-microcosm..... 7
2.2 Dissolved oxygen.....27
2.3 Coninuous pH data.....27
2.4 Single species tests.....28
2.5 Receiving stream analysis.....29
2.6 Statistical analysis.....29

3.0 RESULTS

3.1 Copper.....31
3.2 Pentachlorophenol.....43
3.3 Trinitrotoluene.....54
3.4 Chlorpyrifos.....64
3.5 Selenium.....75
3.6 Copper/Zinc.....99
3.7 Publically owned treatment works.....105
3.8 Copper forming point source.....141
3.9 Zinc point source.....157
3.10 Heavy ash effluent.....166
3.11 Fly ash effluent.....184
3.12 Analysis of continuous pH data 201

Conclusions

4.0 SYNTHESIS

4.1 Microcosm sensitivity 208
4.2 System comparisons 219
4.3 General conclusions 233

5.0 REFERENCES 234

Appendices

A. Dissolved oxygen system information
B. pH system information
C. Published papers
D. Submitted papers and draft manuscripts
E. Personnel and publications

Contract DAMD17-88-C-8068

EXECUTIVE SUMMARY

Traditional methods of estimating the hazards of chemicals and mixtures in freshwater ecosystems have relied on testing individual representative species. These methods have been criticized for ignoring the potential of ecological interactions to affect the expression of adverse, toxic effects. This study examined the utility of laboratory microecosystems (microcosms) developed from natural microbial communities in the assessment of chemical effects on freshwater communities.

Standard methods

The study involved several tasks including developing a standard protocol for conducting tests, testing individual pure compounds, testing complex mixtures, and comparing the effects of pure compounds and mixtures to results from traditional toxicity tests and field studies of potential chemical impacts. Additionally, automated and semiautomated methods were developed for nondestructive acquisition of microcosm dissolved oxygen and pH data. Collaborative studies were undertaken to train other research personnel and to compare estimates of adverse effects by different laboratories. The experimental systems were designed to be compatible with existing research capabilities and equipment of the aquatic toxicology group of the US Army Biomedical Research and Development Laboratory (BRDL).

Pure chemical tests

Experiments testing the ecological toxicity of copper, pentachlorophenol, trinitrotoluene, chlorpyrifos, and selenium were conducted. Results showed that microcosms were sensitive to copper at levels below current water quality criteria. Experiments with organic toxicants showed that microcosms were less sensitive than traditional test species. Experiments with selenium showed adverse effects near current water quality criteria, and a field validation studied confirmed the sensitivity of microbial communities to selenium. Conservative toxicants such as metals produced greater effects than expected in microcosms, while effects of the more labile organic toxicants were less than expected.

Mixture tests

Experiments examining the effects of complex mixtures made use of a mobile laboratory for site specific evaluations of toxicity. Experiments examining effects of copper and zinc singly and in combination at water quality criteria demonstrated

additive toxicity of the metals and confirmed the greater toxicity of copper to microbial communities. Repeated experiments on a high-nutrient chlorinated waste water failed to show evidence of toxicity, probably because of the overriding influence of nutrients on the tested communities. These experiments conflicted with observed effects on the receiving ecosystem and with acute toxicity to daphnids.

Experiments examining the effects of industry waste streams were compared to laboratory effects, standard test species, and in-stream indicators. Effluent from a copper forming point source was chronically toxic to daphnids and had adverse effects on microbial communities. Measurement of in-stream effects did not confirm toxicity because toxicity occurred at levels greater than the expected low-flow dilution. A zinc point source effluent showed limited toxicity to daphnids and microcosms, although measures of in-stream effects were consistent with stream degradation. Stream degradation was attributed to an upstream nonpoint source. Microcosm estimates of toxicity were consistent with estimates from daphnid tests. Microcosm tests of two coal ash basins also showed similarity between microcosm responses and the responses of standard test species. However, a heavy ash basin considered non-toxic to daphnids and fish adversely affected microcosms. Interpretation of in-stream effects was complicated by upstream degradation. Microcosm tests of a fly ash basin confirmed toxicity measured independently as effects on daphnids and fish. Microcosms were adversely affected at high waste concentrations. In-stream effects were clearly observed as downstream recovery of aquatic communities below the headwater reservoir outfall.

Conclusions

The sensitivity of microcosm measures of adverse effects was estimated by examining variability of microcosm measures and by conducting a statistical power analysis. These analyses used additional literature information on the outcomes of microcosm experiments. For structural measures of adverse effects, species richness were the least variable. Biomass measures and measurements of standing stocks of nutrients were more variable. Process measures such as enzyme activities were the most variable, although estimators of whole-microcosm conditions (dissolved oxygen, pH) were the least variable of all measures examined. Power analyses showed that the experimental design of triplicate microcosms tested at six toxicant concentrations were associated with acceptable minimum detection differences from controls. The lease variable measures such as species richness, biomass, and dissolved oxygen levels had high detection potential.

Comparison of microcosm measures of toxicity showed that microcosms responded to toxicants in a range similar to that of traditional toxicological tests. However, microcosm experiments provided qualitatively different estimates of the kinds of effects observed in environmental contamination. Adverse effects on communities can be stimulatory as well as inhibitory. Process measures, while having acceptable detection power, often do not show community responses to stress, probably because the supply of materials to processes is unchanged by toxic action. Continuous monitoring of effects using pH displayed qualitative similarities to other measured effects, but inferential methods could not be used to statistically compare time series among treatments.

These studies demonstrated that appropriate standard methods can be applied to ecological toxicity testing using natural communities. Experiments produced repeatable results with sensitive responses to environmental stressors. Laboratory estimates of effects were usually consistent with observed effects in the field, although microcosms are not sensitive to all toxicants. Toxicants with specific actions on biological components not present in microcosm cannot be observed to have adverse effects. Toxicant effects are sometimes affected by other biologically active materials such as nutrients, and these interactions can alter the expression of adverse effects.

1.0 INTRODUCTION AND RATIONALE

The toxicity of chemicals introduced into aquatic ecosystems is commonly evaluated using simple single species toxicity tests. These tests lack the complexity of biological interactions within ecosystems (Cairns, 1981). To assess the environmental risk of a chemical, many single species toxicity tests are needed to determine the range of responses to the chemical, to determine which organisms are most sensitive to the chemical, and to estimate the levels of chemical producing adverse environmental effects. According to USEPA guidelines for new chemicals, a minimum of eight species representing at least five families must be tested (Stephan et al., 1981). This limited array of test species provides little information about effects on interactions in the more complex ecosystem that may contain hundreds or thousands of species. Additionally, measurements made in single species tests often relate to either death of the test species or effects on growth or reproduction of a small population of laboratory tolerant organisms. Tests on individual chemicals fail to account for interactions among toxicants in complex waste streams.

Microcosms serve as surrogates of complex natural systems,include many species, and demonstrate many of the same interactions present in natural systems. Microcosms dominated by microorganisms such as bacteria, algae, and protozoa are representative of complex communities found in streams, rivers, and lakes. A microcosm developed from bottom and surface dwelling communities might easily contain 200 species (exclusive of bacteria). These communities can display emergent ecological properties such as competition, succession, and nutrient cycling that cannot be measured in simple single species tests. Microcosms often are representative of communities from the most common points of contact of toxics and biota: shallow, near-shore waters.

Many microcosm designs have been developed (e.g., Giesy, 1980) and a few are routinely used for research purposes (e.g., Taub, 1977; Leffler, 1981; Giddings, 1978). These microcosms are generally static systems which are dosed once or regularly with a toxic material. However, shallow water communities in nature often receive toxic materials continually. In fact, the development of microcosms has paralleled the development of single species tests: static tests with single doses have given way to systems capable of studying continuous dosing of toxicant (e.g., Hendrix et al., 1981).

Microcosms developed by using biological communities on artificial substrates can receive continuous toxicant replacement without "washing out" the natural biological community. By carefully designing microcosms to simulate continuous water movement in the shallow water communities of most ecosystems, a large number of measurements can be made on a biologically realistic complex system to evaluate the health of the system in response to chemical stress. Environmental health can be measured using both structural and functional changes in the test system (Cairns and Pratt, 1986). Comparable measurements can be made in natural ecosystems receiving the same or similar stess to validate the adequacy of microcosm-based predictions.

1.1 HYPOTHESIS

The hypothesis upon which this research is based is that chemical stress causes the removal of sensitive system components resulting in changes in biological structure and function. These changes are measurable in terms of alteration of photosynthetic production and community respiration which result in changes in oxygen production and consumption on a diurnal cycle. Effects on particular populations and individuals can result in differential production in community components measurable as shifts in the allocation of biomass to photosynthetic and heterotrophic compartments, including bacteria, fungi, and other compartments. These effects can also be observed as changes in the abundance of microbial populations capable of assimilating organic and inorganic nutrients and can be measured by differences in enzyme activities per unit of biomass (Lanza and Burton, 1989).

Experiments were designed to detect significant changes in community structure and function due to the influence of toxic stress measure the resistance of the community to displacement. That is, the relative dose required to induce displacement in two communities can be compared and used as a measure of resistance. When a toxic stress does not act continuously, however, a second component of community response to stress becomes important. The resilience of the community can be measured as the time required for the stressed community structure and function to return to the normal range. The second component measures the relative rate at which the community recovers when the stress is eliminated and is an indication of both the biotic potential of the community and the depth of displacement by the toxic stress. The relationship between resistance and resilience is not well understood (c.f., Yount and Shannon, 1989). Experiments in the proposal research focused on resistence of communities to toxic stress.

1.3 OBJECTIVES

The three objectives of this research are listed below with relevant research questions. Collaboration with USABRDL personnel was essential for developing and implementing microcosm testing methodology. Interaction of technical personnel in the two research groups improved the overall design of the test systems, reduced the time required to reach certain technical goals, and improved the transferability of the methods and techniques developed in this research to other research laboratories.

Objective 1. Determine the response range of microcosm systems dosed with pure toxic compounds. Experiments conducted using pure compounds of known toxicological properties addressed the questions how sensitive is the microcosm testing system, and are there differences in the sensitivity of the several measured parameters? Research evaluated the response ranges of test systems by monitoring several biological structure and function variables. Results of these experiments were used to compare the relative sensitivities of measured responses, to compare results to results from other complex test systems, and to determine if the testing systems respond as expected based on the known toxicology of the pure compounds selected for testing.

Objective 2. Determine the consistency of response of the microcosm testing system. By comparing responses of microcosms developed from differing source ecosystems, research addressed the questions do ecosystems differ in their responses to the same toxicant, and, if so, what is the magnitude of the difference in response? As part of the collaborative research program, parallel testing was initiated to evaluate responses to a select set of toxic chemicals tested in microcosms developed from different ecosystems. These experiments were a first step in comparing both interlaboratory variability and ecosystem variability to the same toxic stress.

Objective 3. Compare the results of microcosm experiments to alterations in real ecosystems receiving toxic stress. This portion of the research addressed the question do naturally derived microcosms display qualitatively and/or quantitatively similar responses to real receiving ecosystems under toxic stress? This research evaluated both transient and persistent stress responses in ecosystems and examined the nature of the adaptation that takes place when toxic stress is present and the rate of recovery of the system from displacement when the toxic stress is removed.

1.4 MILITARY SIGNIFICANCE

This project developed environmentally realistic testing systems for evaluating the impact of toxic stress on aquatic ecosystems. This research is significant to the operation of military systems because it can reduce the need for developing extensive toxicological data bases. The application of the research to military systems is explained below.

Military installations produce wastes that impact aquatic ecosystems. In many ways, these impacts are very similar to those attributable to any other residential community. However, military installations may produce unique waste streams and the impact of these waste streams on receiving ecosystems require evaluation. The microcosm testing system can account for the site specific nature of discharges and can test in an environmentally realistic manner the many interactions among toxicants in waste streams that might impact aquatic life. This information is not obtainable by standard testing methods.

Speciality chemicals developed for military materiel or chemicals adapted from one use to an alternative use such as training purposes may enter aquatic ecosystems and impact the resident biota. Frequently the toxicological data base for these compounds is not particularly well developed. In fact, the toxicological data for a large number of potential pollutants outside the 129 priority pollutants identified for most industrial discharges lacks certain data elements for making an ecological risk assessment. Compounds for which there is only modest toxicological information can be screened in using the microcosm testing system to evaluate their potential ecological risk without requiring great amounts of time and expense that would be required to conduct the normal suite of tests such as those required for new chemicals (c.f., Stephan et al., 1981). The components of dyes, smokes, explosives, propellents, and chemical agents may include speciality compounds for which standard toxicological evidence is not available. Testing these compounds singly or in the complex formulations in which form they may enter the environment will provide important information regarding the selection of particular materials for training or testing given the potential for environmental damage.

In addition to making general predictions about the environmental risk of releasing certain compounds in military materiel, microcosm tests can evaluate the site specific nature of stress effects. That is, certain sites may be more resistant to stress effects from particular compounds and precautions that might be necessary to avoid environmental damage in one geographic area might not be required in another area. Similarly, site specific evaluations may reveal that the

potential for environmental damage is so great at a given site that it would be unwise to test or train with certain compounds.

1.5 SCOPE OF WORK

This report represents the product of the research and includes a detailed description of construction, development, monitoring, and response range of small aquatic microcosms for evaluating ecological effects of chemicals and complex mixtures on intact natural biological communities. A standard method for conducting continuous dilution/replacement microcosm tests was developed, documented, tested, and validated.

The test system was designed to be compatible with existing biomonitoring systems (automated respiration and carcinogenicity testing) in USABRDL mobile biological laboratory which is equipped with automated systems for determining select water chemistry parameters and has dedicated microcomputer for monitoring fish respiratory response. The microcosm system can use existing hardware and automated monitoring and data analysis capabilities of USABRDL aquatic toxicology lab and provide information not available by standard testing on the potential for ecosystem damage and the nature of any hypothesized adverse effects by using simple, biologically meaningful measurements.

The specific research tasks included:

(1) System development - Developing a microcosm system using microbial communities on artificial substrates to examine stress responses of biological systems using non-taxonomic measures of adverse effect based on energy flow (production/respiration), allocation of biomass to system compartments, and uptake of nutrients and using automated data collection methods to examine cyclic behavior in systems.

(2) System calibration - Determing the response range and sensitivity of the test system by comparing results of experiments to responses of organisms in standard toxicological tests. Similar microcosm systems have shown excellent sensitivity to pure toxic compounds and complex mixtures. Nontaxonomic analyses are attractive because of ease of measurement, the potential for greater replication, the speed of analysis, use of automated equipment, and reduction in "noise" attributable to random variation in taxonomic composition of communities.

(3) System validation - Predicting the magnitude and kinds of effects that might result from toxic stress by tests of complex effluents coupled with upstream/downstream analyses of demonstrable effect in actual receiving ecosystems. A mobile

laboratory with the developed test system was used in experiments at specific sites with toxic discharges.

(4) Collaboration - Cooperating in development of system features based on existing design features of USABRDL mobile laboratory test systems. To ensure that existing work in developing automated data collection and non-taxonomic methods for evaluating system-level effects maximize existing expertise of personnel for our and the USABRDL research group, joint efforts were undertaken to cooperatively solve experimental design, sampling, and analysis problems. This involved exchange of information on hardware specifications and software design and short-term training of personnel in analytical methods and system operation. Taxonomic analyses of USABRDL experiments was done at PSU by exchange of physical samples and tape recordings made using videomicroscope.

2.0 METHODS

This section includes detailed accounts of the technical methods used for the experiments conducted by our laboratory. The methods are presented as a generalization of our experimental design. Methods will only be discussed for individual experiments when they deviate from the standard procedures described below.

2.1 ARTIFICIAL SUBSTRATE-MICROCOSM PROCEDURE

2.1.1 Objectives

The artificial substrate-microcosm method is intended for use in evaluating direct and indirect effects of chemicals and chemical mixtures on whole, naturally derived, aquatic microbial communities (ecosystem surrogates). The method includes examination of effects on microbial production, respiration, nutrient dynamics, enzyme activities, and species richness. Endpoints can be added or eliminated, depending on the experimental design and the direct objectives of the test.

The artificial substrate-microcosm serves as a link between highly standardized tests on individual species and effects on whole ecosystems. Unlike natural ecosystems, these microcosms can be replicated and controlled for testing. The method utilizes an experimental design similar to standard single species toxicological tests to determine both the level of chemical which produces adverse biological effects and the specific kind of effects.

2.1.2 Scope

The method is designed for testing responses of substrate associated microbial communities from both lentic and lotic systems to:

(i) Pure compounds
(ii) Mixtures of chemicals
(iii) Complex mixtures, such as effluents.

2.1.3 Principle

Methods for predicting the effects of chemicals on individual species may not account for indirect or unexpected responses which occur in diverse ecosystems (Cairns, 1983; Kimball and Levin, 1985). Standardized testing of sensitive single species does not examine important interactions among species and cannot examine collective and emergent properties of comples systems.

The testing procedures presented here assumes that different ecosystems have functional equivalency. That is, many ecological functions are common to ecosystems even though the constituent species may differ. Communities and ecosystems are not simply a collection of species but are interacting, co-evolving systems (O'Neill and Wade, 1981). New properties emerge from interactions among organisms as biological complexity increases. Properties such as predation, succession, competition, and nutrient and mineral cycling result from biological interactions and cannot be effectively examined by testing species in isolation. Ecosystem studies typically evaluate conditions using descriptors of collective properties (e.g., species richness, diversity, biomass of functional components), and such measures of ecosystem health have no correlates in single species testing.

Microbial communities developing on surfaces (Aufwuchs) are diverse and have properties similar to those of the larger ecosystem: nutrient cycling, energy and matter processing, succession, high species richness. These communities can be collected on inert artificial substrates and manipulated in the laboratory in replicate microcosms (Niederlehner et al., 1985; Pratt et al., 1987 a, b; Pratt et al., 1988 a, b).

The artificial substrate-microcosm method can be summarized as follows. Natural aquatic microbial communities are collected on polyurethane foam (PF) artificial substrates and exposed to various concentrations of chemical(s) or effluent in laboratory microcosms either statically or under continuous flow. Specific microbial responses are monitored, and are chosen to include ecologically meaningful estimates of primary production, community respiration, biomass allocation, nutrient cycling, and community structure. Microbial communities are tested in replicate microcosms and controlled with respect to most environmental conditions, allowing powerful statistical analyses of results. A full description of the artificial substrate-microcosm method follows.

2.1.4 Materials, Reagents, and Apparatus

2.1.4.1 Materials

2.1.4.1.1 Materials for Construction of Test System

Tanks (glass, polyethylene, stainless steel; capacity 6-12L)
Medium density polyurethane foam, washed
String or nylon line
Plastic hooks
Lights (CRI>90, e.g., Vita Lites, DuroTest, Corp.)
Timers (for lights)
Overflow boxes, drains

Activated carbon dechlorinator or alternative source of diluent
Silicone sealant
Peristaltic pump (0.5-10 ml/min, 1-20 heads)
Diluter or flow splitter with mixing chambers
Reservoir(s) for toxicant solutions
Headbox
Tygon tubing

2.1.4.1.2 Materials for Laboratory Analysis

Automatic pipettes (10-200 µl, 200-1000 µl, 1-5 ml, and 5-10 ml)
Test tubes (13x100 mm and 16x125 mm)
Microscope slides and cover glasses
60 ml BOD bottles (18 light, 18 dark)
0.2 µm polycarbonate filters (optional)
1.5 microfuge tubes (optional)
Protistan taxonomic references (optional)

2.1.4.2 Reagents

Reagent grade chemicals and glass distilled, deionized water (dd water) or equivalent should be used unless otherwise stated.

2.1.4.2.1 Reagents for Total Protein Determination

BioRad Protein Determination Kit or,
Bradford dye binding reagent (DBR):
  100 mg Coomassie Brilliant Blue
  50 ml methanol
  100 ml phosphoric acid
  Bring up to 1 L with dd water
  Filter through Whatman No.1 filter paper
0.5 N NaOH
1.0 mg/ml bovine serum albumin (BSA) in 0.5 N NaOH

2.1.4.2.2 Reagents for Chlorophyll Analysis

Saturated magnesium carbonate (1.0 g $MgCO_3$ in 100 ml dd water)
Buffered aqueous acetone (spectrograde, 90 parts acetone plus 10 parts saturated magnesium carbonate)
0.1 N HCl

2.1.4.2.3 Reagents for Hexosamine Analysis

1.0 mg/ml glucosamine in dd water
Concentrated HCl
2 M $NaCO_3$; 1.5 M $NaCO_3$
2% acetylyacetone in 1.5 M $NaCO_3$
Absolute ethanol

Ehrlich's Reagent (1.0 g dimethylaminobenzaldehyde in 15 ml concentrated HCl and 15 ml absolute ethanol)

2.1.4.2.4 Reagents for Alkaline Phosphatase Analysis

1.0 M Tris-HCl buffer, pH 8.6
0.2 M Tris-HCl buffer, pH 7.6
1 mg/ml p-nitrophenyl phosphate (p-NPP) in 0.2 M Tris-HCl
10 µmole p-nitrophenol/ml (p-NP, Sigma Chemical Co.)
1.0 N NaOH

2.1.4.2.5 Reagents for Carbohydrate Analysis

1.0 mg/ml glucose in dd water
75% (v/v) sulfuric acid
Anthrone reagent (200 mg anthrone/100 ml 75% sulfuric acid)

2.1.4.2.6 Reagents for Bacterial Counts (optional)

38% formaldehyde (filtered, 0.2 µm)
0.2% irgalan black in 2% acetic acid
10 µg/ml 4', 6-diamidino-2-phenylindole (DAPI)
100 ml sterilized water

2.1.4.3 Apparatus

Dissolved oxygen meter and probe
pH meter
Conductivity meter
Table top centrifuge (250 x g; approx. 1000 rpm)
Spectrophotometer (visible)
Water bath (up to 100 C)
Compound microscope
Epifluoresence microscope
Vacuum filtration apparatus (optional)
Autoclave (optional)

2.1.5 Microcosm Design

2.1.5.1 Microcosm Components

2.1.5.1.1 Tanks

Shallow tanks, pans, or aquaria are used to contain the biological components and medium. These containers should have a capacity of 6-12 L and should be made of a material compatible with the intended toxicant. In general, polyethylene pans can be used for metals or metal-containing mixtures. Glass containers (small aquaria or hand-made tanks) can be used for organic

toxicants that are not strongly adsorbed to glass. Tanks should be cleaned with dilute (5-10%) acid or a metal chelator, rinsed with water, rinsed with pesticide-free acetone, rinsed again, and air-dried prior to use. Small plastic hooks used to attach artificial substrates to the bottom of the tanks should be affixed with nontoxic silicone sealant. The number and placement of the hooks will be determined by the number of substrates used, typically 4-6. In general, the hooks should be equidistant from each other and far enough from the sides of the tanks to prevent attached substrates from touching the sides.

Each test consists of triplicates of 4-5 exposure groups and a control group, for a total of 15-18 microcosms. If a carrier solvent is used, a solvent control must also be included.

2.1.5.1.2 Artificial Substrates

Artificial substrates are cut from a single sheet of medium density polyurethane foam (PF). Each substrate should be a hexahedron measuring 4x5x6 cm. Sufficient PF substrates should be cut for 4-6 substrates per microcosm, depending on the experimental design. One to two substrates per microcosm will be placed in a natural ecosystem to accumulate the native microbiota for use as species sources ("epicenters") in the microcosms. PF substrates should be washed overnight in a bath of distilled water to remove nitrogenous by-products of urethane polymerization.

Each PF substrate is tied tightly in the middle, perpendicular to the longest dimension using either cotton string or nylon monofilament line. A method of attaching the substrate to hooks on the bottom of the microcosm tanks should be incorporated during the tying of the substrates. A loop or ring should be large enough to pass over a plastic hook on the bottom of the tank, but not so large as to allow the substrate to float at the surface of the water. Substrates for placement in a source ecosystem require additional line (15-20 cm) for attachment to an anchor line.

2.1.5.1.3 Collection of Source Communities

PF substrata can be placed in a source ecosystem for colonization by microbiota using one of several methods. Substrates should be placed in shallow water (<1 m). Anchors and line will be determined by local conditions, especially flow rates. Tying substrates to nylon lines or reinforced plastic lines attached to one or more cinder blocks is recommmended. In flowing water, these substrates should be left to accumulate microbiota for 3-10 d, depending on the flow rate and sediment load. In lentic ecosystems, exposure times should be increased

to 14-21 d (Cairns et al., 1979). Where site conditions do not permit wading, substrates can be tied to a longer, weighted anchor line secured to a float, buoy, or pier. Placement of extra substrates is recommmended in case of damage or loss, and three additional substrates are needed for examination prior to the start of each test. For a typical experiment, the number of PF substrates placed for colonization should exceed the expected need by at least 20 %.

After a sufficient colonization period (3-21 d), substrates are collected en masse and returned to the laboratory in an insulated container partially filled with source water. Care should be taken in removing the substrates from the water to avoid letting them "drain" too much. This can be accomplished by filling the insulated container with source water and float the cluster of substrates into the container while it is under water. The excess wwater can then be carefully drained. After being returned to the laboratory, substrates are randomly allocated to microcosms at the start of the experiment. These colonized substrates are referred to as "epicenters" and provide the source of microorganisms for the colonization of barren "island" substrates which are placed in microcosms at the start of the test. The island substrates are then collected at intervals (e.g., weekly) after the start of a test, and the colonized microbial communities are removed and evaluated.

2.1.5.1.4 Test Medium

The test medium may be water, or water and sediment. Guidelines for the collection, storage, and use of dilution water should be followed (Horning and Weber, 1985 ? or USEPA, 1982). Sediment should be collected in a manner that will allow it to be homogenized before distribution. Any large debris (leaves, twigs, stones) should be removed. All microcosms should receive equal volume or depth of sediment. Where sediment contains appreciable amounts of organic matter, it may be necessary to aerate microcosms prior to the introduction of toxicant. Aeration is necessary if the dissolved oxygen concentration is below 5.0 mg/L after water has been added and the sediment has settled.

Laboratory water (i.e., dechlorinated tap water or reconstituted water) or site-specific water may be used as a diluent. Flow-through tests conducted in a laboratory away from a natural source of surface water require suitable diluent such as well water or dechlorinated tap water. Construction of a dechlorinator using a 30 gallon trash can and granular activated carbon is shown in Fig.2.1. It is important to check dechlorinated tap water for the presence of chlorine at the start and end of every test. The amperometric method (APHA, 1985) is

satisfactory if a micropipette (5 µl) is used to deliver the phenylarsenine oxide titrant, since total residual chlorine should never exceed 10 µg/L.

2.1.5.1.5 Static Tests

Typically, in static tests, a concentrated stock solution of toxicant is prepared and the amount needed to spike a given volume of diluent water to produce the chosen test concentrations are calculated. To start the test, microcosms are filled to the selected volume with diluent. A volume of water equal to the volume of toxicant to be added is removed from each microcosm and replaced with the appropriate volume of stock solution, and the microcosms are gently mixed. The water level is marked on the side of each microcosm and water lost through evaporation should be replaced with distilled, deionized water.

Barren island PF substrates are placed around the perimeter of the test tank and squeezed to fill them with medium. The colonized epicenter PF substrate(s) are then added to the center of the tank (Fig. 2.2). It is advisable to place the microcosms where they will remain for the duration of the test before adding any substrates, since movement tends to dislodge substrates from the anchor hooks.

2.1.5.1.6 Continuous Flow Tests

Toxicant solutions of differing concentrations can be supplied to test microcosms by several methods. A gravity-fed proportional diluter, constructed from glass (Benoit et al., 1982), is relatively simple to build, requires only a single concentration of primary stock toxicant solution, and can be used for effluent testing. It produces 5 toxicant dilutions (100, 50, 25, 12.5, 6.25%) and a control. A dosing system comprised of a flow splitter (Maki, 1977) and 15-18 mixing chambers may also be used, but requires as many primary toxicant stock solutions as the number of microcosms used. This system, however, allows testing of a greater range of toxicant concentrations.

The rate of flow through the system should be such to provide a minimum of 5 volume turnovers per day. Greater flows may be used depending on experimental design, but should not exceed the drainage capacity of the microcosms.

In the continuous flow tests, island substrates are placed towards the drain or overflow end of experimental tanks, and the colonized epicenter substrate(s) at the influent end (Fig. 2.2). Toxicant-ammended dilution water flows over and past the source substrates prior to draining through holes in the end wall of the tank. Island substrates should be in place and test medium

should be flowing through the microcosms several hours before placement of the colonized substrates to ensure that the system is fully operational before adding the source of microorganisms (epicenter substrates).

2.1.5.1.7 Environmental Conditions for Microcosm Tests

Microcosms should be lighted with daylight equivalent bulbs (CRI > 80; 1000-2000 lux) to permit growth of autotrophic organisms. Automatic timers for turning the lights on and off at prescribed times should be used. If productivity/respiration (P/R) ratios are to be determined, the photoperiod should be 12L:12D. Normally, ambient tempertures are sufficient to permit continuous replacement tests to be conducted under uncontrolled temperatures. Microcosms in static tests can be placed in an environmental chamber or water baths of specific temperatures if temperature control is needed.

2.1.6 Microcosm Test Procedure

2.1.6.1 Starting

Prior to the start of the test, three of the colonized substrates from the source ecosystem should be examined microscopically to ensure adequate species richness and biomass. There should be > 15 genera of protozoa (includes flagellates, ciliates, and sarcodines), > 15 genera of algae (includes greens, blue-greens, and diatoms), and rotifers or other micrometazoa. Organisms need not be identified to species, unless taxonomic composition has been chosen as an endpoint. In addition, subsamples of the epicenter substrates should be anlayzed for the responses chosen to be monitored in the island substrates (see Section 2.1.6.2.2). These analyses need not be conducted at the start of the test, as long as subsamples are properly preserved for the chosen responses. Colonized substrates are then added to the microcosms which already contain the island substrates, constituting the start of the experiment (see Sections 2.1.5.1.6 and 2.1.5.1.7).

Temperature, pH, dissolved oxygen, and conductivity should be measured in each tank immediately after the start of the test (APHA, 1985). A sample of water should be collected from the control and highest concentration tanks for hardness and alkalinity determinations. These measurements should be repeated weekly for the duration of the test and should be collected at the same time of the day (e.g., just after lights on). Special care should be taken not to contaminate the control test tanks, and all measurements should start with the control tanks and then proceed with tanks of increasing concentration. Water samples should be collected from each microcosm or from the influent tube

(in flow-through systems) of each microcosm for toxicant analysis at the start of each test. In the flow-through system in which there is an unlimited supply of test medium, toxicant concentrations should be monitored at least weekly, and more frequently if possible. In static systems, it is not desirable to remove more than 10-15% of the initial volume. If chemical analysis requires a large volume of sample, toxicant concentrations can be measured at the start of each test.

2.1.6.2 Microbial Responses

Responses such as pH, dissolved oxygen, and production/respiration ratios, which are measured in individual microcosms, should be measured frequently at the start of the test (daily for the first week, weekly thereafter). Effects on these responses are rapidly manifested and easily monitored. Although typically measured by hand, pH and dissolved oxygen data can be automatically collected using automated, computer-driven methods.

2.1.6.2.1 Production/Respiration Ratio

Gross photosynthesis/respiration (P/R) ratios within each microcosm are determined weekly using the three point dissolved oxygen method of McConnell (1962). Microcosms are covered with clear plastic to reduce oxygen diffusion (Giddings and Eddlemon, 1978) and the dissolved oxygen in each microcosm is measured at the beginning of a dark cycle (D1), the beginning of the following light cycle (D2), and the beginning of the next dark cycle (D3):

$$D1 - D2 = \text{nighttime respiration}$$
$$D3 - D2 = \text{net production}$$

$$\frac{D3 - D2}{D1 - D2} = \text{Estimated P/R ratio}$$

2.1.6.2.2 Biochemical Analyses of Artificial Substrates

Although the colonization of island substrates by microorganisms is a rapid process, there typically is not enough biomass accumulated on island substrates for biochemical analyses until after one week of exposure. Therefore, island substrates are usually sampled on a weekly basis for 3-4 weeks. A single island substrate is removed from each microcosm and placed into a sterile Whirl-Pak bag, beaker, or plastic cup. The substrate is then squeezed to remove as much of its contents as possible (> 50 ml). The substrate is then discarded. At the end of the test, epicenter substrates are removed from the control microcosms and

analyzed in the same manner as island substrates.

Subsamples are removed for each assay by gentle, but thorough mixing of each sample and removing the specified volume using an automatic pipettor. The volume necessary for each assay varies depending on the amount of biomass on the artificial substrates. The amount of sample needed may also vary if the toxicant has decreased the amount of island biomass available. The procedures for assays typically performed are outlined below. Unless otherwise specified, all centrifugation is carried out at 250 x g (approx. 1000 rpm) for 5 min.

2.1.6.2.2.1 Total Protein

Protein analysis provides an estimate of the total biomass accumulated on island substrates. The material collected from the substrate is extracted (Rausch, 1981) and protein concentration measured using the method of Bradford (1976) or a commercial kit.

1. Place 4-8 ml of sample in 13x100 mm test tubes and centrifuge.
2. Discard supernatant and resuspend pellet in 0.5 ml 0.5 N NaOH.
3. Place samples in a 90 C water bath for 10 min.
4. Remove and centrifuge. Save the supernatant and resuspend the pellet in 0.5 ml 0.5 N NaOH.
5. Repeat steps 3 and 4 and pool supernatants for a total extraction volume of 1.5 ml. The extract may be stored frozen up to six months before protein analysis.
6. Mix 0.1 ml extract and 5.0 ml DBR or BioRad reagent with a vortex mixer and wait 15 min.
7. Measure the absorbance at 595 nm.
8. Standards (10-100 ug BSA) are made by appropriate dilutions of the 1.0 mg BSA/ml stock with 0.5 N NaOH and are analyzed the same as samples.
9. Calculate µg protein/ml in each sample by accounting for volume of original sample used, extract volume (1.5 ml), and assay sample size (0.1 ml, need to multiply by 10 to get per ml):

µg protein/ml = 10 x (µg protein/ml x 1.5ml)/mlsample

2.1.6.2.2.2 Chlorophyll a

Chlorophyll analysis provides an estimate of the biomass of autotrophic organisms which have accumulated in the island substrates. The method of chlorophyll analysis may be found in Standard Methods (Method 1002G; APHA, 1985), and is summarized below.

1. Place 15-30 ml of sample in 16x125 mm tubes or 50 ml tubes if necessary and centrifuge.
2. Discard the supernatant and resuspend the pellet in 10 ml 90% buffered aqueous acetone. Extract overnight at 4 C in the dark.
3. Clarify samples by centrifugation and measure the absorbance of 3 ml of extract at 750 nm and 664 nm.
4. To correct for phaeopigments add 0.1 ml 0.1 N HCl to the sample and mix gently. After exactly 90 s, measure the absorbance at 750 nm and 665 nm.
5. Correct the A664 and A665 readings by subtracting the appropriate A750 readings and use the corrected values in the subsequent calculations:

$$\mu g/L \text{ Chl a} = [26.7 (A664 - A665) \times V1/ V2 \times L] \times 1000$$
$$\mu g/L \text{ Phaeo.} = \{26.7 [1.7(A665) - A664] \times V1/ V2 \times L\} \times 1000$$

where V1 = extract volume (0.01 L)
V2 = sample volume (0.015-0.030 L)
L = path length of cuvette (1, 5, or 10 cm)

2.1.6.2.2.3 Hexosamine

Hexosamine (chitin) analysis provides an estimate of the biomass of chitin-containing orgnaisms in the sample (Gatt and Berman, 1966). This typically includes some species of aquatic fungi and micro-arthropods.

1. Place 4-8 ml of sample in 13x100 mm test tubes and centrifuge.
2. Discard supernatant and resuspend pellet in 1.0 ml distilled deionized water. The samples may be frozen at this point for up to 6 months.
3. Split the sample into duplicate 0.5 ml subsamples and add 0.1 ml concentrated HCl.
4. Cover samples with foil and place in a boiling water bath for 4 hrs. Remove and cool.
5. Add 0.4 ml 2.0 M $NaCO_3$ to each tube and mix.
6. Add 0.5 ml 2.0% acetylacetone to each tube, mix, and place in a boiling water bath for 20 min.
7. Remove and cool. Add 1.0 ml absolute ethanol and mix.
8. Slowly add 0.5 ml Ehrlich's Reagent and wait for the precipitate to dissolve.
9. Measure the absorbance at 630 nm.
10. Standards (10-100 ug glucosamine) should be carried through the entire procedure and are made by diluting the stock 1.0 mg/ml glucosamine with distilled, deionized water.
11. Calculate ug hexosamine/ml in each sample by

accounting for the volume of original sample used and assay sample size (0.5 ml):

μg hexosamine/ml = 2 x (μg hexosamine/ml sample used)

2.1.6.2.2.4 Alkaline Phosphatase Activity

The measurement of alkaline phosphatase activity provides an estimate of the rate of cleavage of organic phosphorus compounds by the microbial community. The method is a modification of the one used by Sayler et al. (1979).

1. Place 2-4 ml of sample in 13x100 mm test tubes and centrifuge.
2. Discard supernatant and resuspend pellet in 1.0 ml 1.0 M Tris-HCl buffer (pH 8.6).
3. Add 0.25 ml of 1.0 mg/ml p-NPP and incubate at room temperature for 1 hr.
4. Add 0.25 ml 1.0 N NaOH to each sample and mix.
5. Centrifuge and transfer supernatant to clean test tubes. Measure the absorbance 420 nm.
6. Standards (1-100 nmole p-NP/ml) are made by appropriate dilutions of the stock p-NP with 1.0 M Tris-HCl buffer.
7. Initially calculate nmole p-NP/ml/hr by:

   nmole p-NP/ml/hr = (nmole p-NP/ml/hr)/ ml sample
   (in sample) (from assay)

8. Using the protein data, calculate nmole p-NP/mg protein/hr by:

   nmole p-NP/mg pro./hr = (nmole p-NP/ml/hr)/(mg pro./ml)

2.1.6.2.2.5 Carbohydrate

Carbohydrate analysis gives an estimate of storage material within the community. The ratio of protein to carbohydrate has been used as an indication of whether or not the community is nutrient limited (Pick, 1987).

1. Place 4-8 ml of sample in 13x100 mm test tubes and centrifuge.
2. Discard supernatant and resuspend pellet in 1.0 ml distilled water. Samples may be stored frozen at this point up to 6 months.
3. Place 0.5 ml of resuspended sample in 13x100 mm test tubes and place in an ice bath.
4. Prepare fresh anthrone reagent daily as described in

Section 2.2.4.1.5 and keep cold.
5. Add 3.0 ml anthrone reagent to each sample and mix with a vortex mixer. Wait 5 min.
6. Place samples in a boiling water bath for 10 min.
7. Remove samples and cool under running water.
8. Measure the absorbance at 625 nm.
9. Standards (10-100 µg glucose) are made by the appropriate dilutions of the stock 1.0 mg/ml glucose with distilled, deionizes water. Analyze standards the same as samples.
10. Calculate µg carbohydrate/ml in each sample by accounting for the volume of original sample used and assay sample size (0.5 ml):

µg carbohydrate/ml = 2 x (µg carbohydrate/ml sample used)

2.1.6.2.3 Optional Analyses of Artificial Substrates

Communities collected on artificial substrates are comprised of bacteria, fungi, algae, protozoa, and micrometazoa. Species identification of any or all of these groups inhabiting artificial substrates can be followed throughout the test, depending on the specific objectives of the test and the taxonomic expertise of the personnel.

2.1.6.2.3.1 Protozoan Species Composition

Protozoan species composition has been shown to be a useful indicator of toxic stress (Niederlehner et al., 1985; Pratt et al., 1987a,b; Pratt et al., 1988a,b). Identification of protozoa must be carried out within 12 hrs of sampling and must be done with live organisms, since fixation distorts many species of protozoa beyond recognition. Subsampling for taxonomic analysis should be done after sampling for the quantitative measures. To prepare slides for examination, it is desirable to obtain as much detrital material from each sample as possible, since many of the organisms are substrate-associated. This material settles rapidly to the bottom of the sample container, and therefore, the sample should not be mixed before preparing the slide. Each sample is subsampled 2-4 times with a Pasteur pipette and 1-2 drops placed on a microscope slide and examined at 200-400 x. Protozoa encountered are identified to genus or species when possible. There are several excellent guides to the protzoa which can be used (see Cairns et al., 1979).

If necessary, preserved samples can be used for quantitative analyses of broad categories of protozoa (since not all species are identifiable in the fixed state), such as ciliates and flagellates. Algal species can be identified and quantified using a Palmer-Maloney counting chamber (APHA, 1985). Identification and quantification of rotifers and other micrometazoa can

be made with a Sedgwick-Rafter counting chamber (APHA, 1985).

2.1.6.2.3.2 Bacterial Counts

The advent of epifluorescent DNA stains such as acridine orange and 4',6-diamidino-2-phenylindole (DAPI) has permitted the rapid enumeration of viable bacteria in water samples (Porter and Fieg, 1980). Direct bacterial counts can be made on artificial substrate samples to evaluate toxicant effects on the bacterial component of the microbial community.

Place 1.2 ml of sample in a sterile 1.5 ml microfuge tube using and automatic pipettor with a sterile tip, and fixed with 0.07 ml 37% formaldehyde (filtered through a 0.2 μm filter). Filter samples onto prestained polycarbonate filters and stain with DAPI as outlined in Porter and Feig (1980) and count using epifluoresence microscopy.

2.1.7 Expression of Results

2.1.7.1 Chemical Analysis

Both the nominal (target) and the average measured toxicant concentration for each treatment should be reported. Actual measured concentrations should be used in subsequent data analysis and any large deviation from the nominal concentrations should be explained. If an effluent is tested, then percent dilutions of the original effluent are used.

2.1.7.1 Analysis of Variance and Multiple Comparisons

Differences in average responses among control and treatment groups on a particular sampling day for a given response are determined using analysis of variance (ANOVA). If ANOVA indicates a significant difference for a response ($p < 0.05$), multiple comparisons are carried out to determine at which concentration(s) the response differs using Fisher's LSD, Duncan's Multiple Range (Sokal and Rohlf, 1983) or Dunnett's test (Dunnett, 1955). Dunnett's test references all treatment differences to the controls.

2.1.7.2 Dose-response Estimators

Ordinary least squares regression of a response variable against log toxicant concentration can be used to investigate the dose-response relationship. When a significant regression is determined, the predicted regression equations can be used to inversely predict toxicant concentrations producing a given proportion in reduction in a response, commonly called an effective concentration (EC) The EC is commonly reported for EC05, EC20, and EC50, representing concentrations at which a 5,

20, and 50% reduction in response (relative to controls) are predicted. The regression equation is determined by excluding control responses from the regression analysis. The procedure is explained more fully (including methods for estimating confidence intervals) by Sokal and Rolhf (1983).

NOTE: Nonlinear responses may complicate such anlayses. Where low toxicant doses produce enhanced responses by microcosm biota, ordinary least square regression analyses are inappropriate.

2.1.7.3 Calculation of NOEC, LOEC, and MATC

For responses significantly affected by toxicant exposure, the no effect concentration (NOEC, the highest toxicant concentration at which the response is not significantly different from controls) and the lowest effect concentration (LOEC, the lowest toxicant concentration at which the response differs significantly from controls) are reported. The maximum allowable toxicant concentration (MATC), the geometric mean of the NOEC and LOEC, is also reported. The sensitivities of the various responses can be compared using these estimators.

2.1.8 PERSONAL REMARKS

2.1.8.1 Quality Assurance

Typical exposure conditions for collecting natural communities on artificial substrates make failure to achieve appropriate microcosm conditions at the beginning of an experiment unlikely. However, no experiment should begin before representative artificial substrate communities are examined (see Section 2.1.6.1). Similarly, daily observation of the experimental system and weekly sampling are needed to ensure against unexpected results. Monitoring should identify any microcosms which deviate greatly within a replicate group. The use of natural communities on artificial substrates provides extremely high taxonomic richness, so the potential for unusual responses is somewhat less than for simpler systems.

During the experiment, periphytic growth should appear on the sides and bottoms of the test vessels. Day-night dissolved oxygen differences should be at least 1mg/L in control and typically reach 1-5 mg/L as the communities develop. At the end of an experiment, anlaysis of epicenter substrates from control microcosms should show increases in biomass estimators when compared to reference substrates examined at the start of an experiment. Further, epicenter taxonomic richness in controls shuld not be less than 90% of that at the beginning of an experiment. Failure to achieve such performance should

invalidate a given experiment.

2.1.8.2 Polyurethane Foam

Care should be taken to use the same lot of polyurethane foam for a given experiment. Washing of cut substrates to remove nitrogenous compounds is essential. Dry foam will yellow upon exposure to sun or fluorescent light. This is a normal reaction and does not affect experiments. The yellow coloring can be removed by washing in Contrad 70 or other laboratory detergent.

Pf substrates can be cut in several ways. We mark sheets of foam with an indelible marker and then cut substrates on a standard band saw. This is efficient and allows good quality control on substrate size. Where substrate size is not closely controlled, corrections for differences in sample volume should be made. PF substrates may also be cut using a serrated knife. Cutting the flexible material with scissors is not recommended because substrates tend to rip from the action of the scissors and substrate size becomes more variable.

2.1.8.3 Time to Complete Tasks

We have estimated the time involved in the various aspects of setting up and conducting an artificial substrate-microcosm test (Table 2.1). The values shown are man-hours (m-h) and, therefore, the absolute time involved may vary according to the number of individuals involved and the level of expertise. We typically can conduct an artificial substrate-microcosm experiment with one full-time skilled technician and an additional parttime worker to assist with taxonomic identification.

2.1.8.4 Selection of Community Responses

We have conducted over 30 artificial substrate-microcosm experiments in which a range of responses have been measured. The choice of responses to be monitored in an artificial substrate-microcosm test depends on several factors, one of which is the inherent variability of the response, and subsequent ability to detect differences among treatments based on that response. We have calculated the average coefficient of variation (CV) of control group responses from all of our artificial substrate-microcosm experiments (Table 2.2). We believe control data alone is a more accurate indicator of the potential variability of a given response, since we have tested toxicants that both increased and decreased variability. We have also calculated the minimal detectable distance (MDD, as a percentage of the control) that would be expected for each response based on its CV at alpha = 0.05 and beta = 0.2

(Conquest, 1983). MDDs were calculated for 2 experimental designs: 6 treatments (k=6) with 3 replicates (n=3) or 5 treatments (k=5) with 3 replicates (n=3).

2.1.8.5 Acknowledgments

Several scientists contributed to the development of these procedures: J. Cairns, Jr. and Barbara R. Niederlehner are specifically acknowledged for their assistance. Rita Ciresi provided editorial assistance.

Table 2.1 Estimated time to set up and conduct a 21-day artificial-substrate microcosm test, in which there are 15 microcosms and island substrates are sampled weekly.

Task	Time (m-h)
Test set-up and monitoring	
Preparation and test start up	20
Routine checks and microcosm monitoring	35
Toxicant concentration analysis	32[a]
Artificial substrate analyses	
Collection and preparation of samples	16
Protein extraction and measurement	9
Alkaline phosphatase analyses	6
Chlorophyll analyses	9
Carbohydrate analyses	6
Hexosamine analyses	8
End of test	
Clean up	8
Data analyses and report preparation	40
Secretatial support	24
Total	213
Optional artificial substrate analyses	
Protozoan species identification	45
Bacterial counts	36

[a] - This time can vary from 0 (effluents) to > 32 m-h (difficult to analyze chemicals).

Table 2.2. Coefficients of variation (CV) for several microcosm responses and the minimal detectable distance (as a percentage of the control value) for each response based on an alpha = 0.05 and beta = 0.2 under two experimental designs (Conquest, 1983)

Response	n	CV	Minimal Detectable Distance (%) k=6, n=3	k=5, n=3
Protozoan Species	28	9.6	23.9	24.3
Protein	27	19.8	49.3	50.2
Alkaline Phosphatase	25	17.2	42.9	43.6
Chlorophyll	21	24.5	61.0	62.1
Hexosamine	13	27.8	69.3	70.5
Carbohydrate	15	22.5	56.1	57.1
Fluorescence Units	7	16.3	40.6	41.3
Potassium	9	16.1	40.1	40.8
Magnesium	8	7.2	18.0	18.3
Calcium	8	9.6	23.9	24.3
Ash Free Dry Weight	4	29.9	74.5	75.9
Phosphate	5	25.9	64.5	65.7
P/R	5	8.4	21.0	21.4
ATP	2	39.7	98.9	101
Production	2	25.0	62.3	63.4

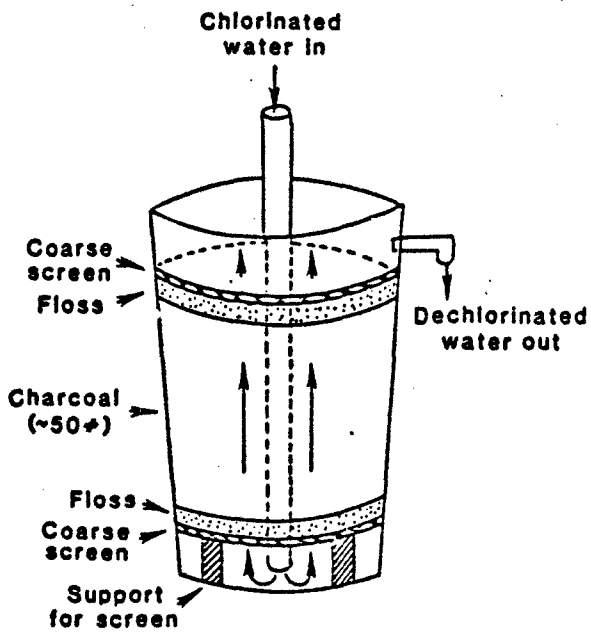


Figure 2.1. Construction of a carbon dechlorinator using a 30-gallon trash can.

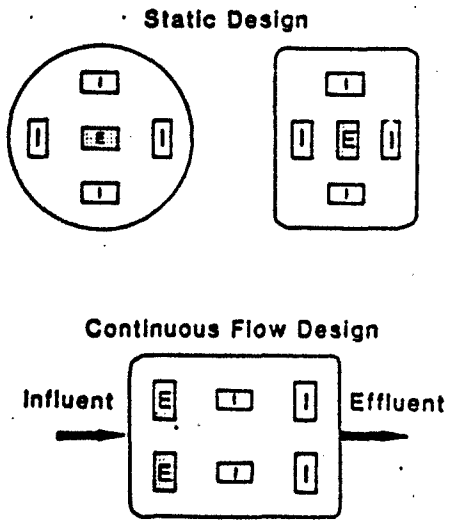


Fig. 2.2. Possible substrate arrangements in static and continuous flow microcosm experiments. E=epicenter substrate, I=island substrate.

2.2 DISSOLVED OXYGEN

2.2.1 Manual readings

Daily readings were taken at basically the same time each day (between 4-5 pm) when levels are thought to be at a maximum. When applicable, production/respiration ratios were determined using the three point dissolved oxygen method of McConnell (1962). See Section 2.1.6.2.1.

2.2.2 Computerized System Overview

The dissolved oxygen data acquistion system is designed to acquire, digitize, and store data from a YSI Model 58 Dissolved Oxygen meter. The analog output of the YSI is sampled with an analog-to-digital (A/D) converter board installed in the computer. Software is provided to control the data input, review and modify data, and store the data for analysis in typical MS-DOS spreadsheet software. (Dissolved oxygen system documentation is included as Appendix A).

2.3 CONTINUOUS pH MONITORING

Measurement of diurnal patterns of production and respiration was measured indirectly by continuous collection of microcosm pH using a 16 channel Fisher Scientific 935 Scanner (see Appendix B) interfaced to a dedicated microcomputer (IBM PS/2 Model 30). The pH scanner and software was configured to collect pH from 15 microcosms every 15 minutes. Collected pH was corrected for temperature, and temperature was determined on the same 15 minute schedule in one randomly selected microcosm using a temperature probe attached to the scanning unit. Scanner software was used to standardize the pH electrodes as a group using two ph buffers (pH 7 and pH 10). The efficiency and slope function for each electrode was determined, and electrodes with efficiencies less than approximately 90% were replaced. The electrodes were then restandardized.

Electrodes used were identical refillable combination electrodes with KCl electrolyte. The electrolyte leaked through the electrode walls gradually (approx. 100 ul/d) and had to be replaced regularly. Electrodes were cleaned in 10% HCl before and after each experiment, and electrodes with low efficiencies were discarded.

The pH scanner stored files in comma and quote delimited format suitable for importing into spreadsheet software. Initial studies showed that the stored files were, in fact, not compatible with spreadsheet software (e.g., Lotus 123). A

conversion program was written to modify the files and recode them in ASCII format for use in developing SAS data sets. During the last project year, a new data editor (QEdit) was used to modify the files to sizes readable by conventional software. Files of pH data were changed daily, and the files were concatenated during analysis. This required recalculating the time step number from the start of experiments for each file, but otherwise no file modifications were made unless out-of-range data were encountered in which case data points were set to missing data.

The pH data was analyzed descriptively by plotting the time series and by developing periodograms using SAS procedures for spectral analysis (PROC SPECTRA) in the ETS module (see 3.12). Prototype SAS files for these analyses are given in the appendix. Data were not corrected for diffusion because all microcosms were assumed to be the same.

2.4 SINGLE SPECIES TESTS

2.4.1 Acute Toxicity Tests

Acute toxicity tests were conducted with two species of daphnids (Daphnia magna, Ceriodaphnia dubia) over a 48 hr period. Neonatal daphnids (<24 hr old) were randomly allocated to treatment groups and exposed to dilutions of effluent/toxicant plus dilution water controls. The dilution water was charcoal dechlorinated PSU tap water (used as culture medium for daphnids) with calcium carbonate hardness approx. 180 mg/L and ph approx. 7.4. Ten neonate D. magna were exposed in sterile plastic cups to 100 ml test medium. Duplicate groups of five C. dubia were exposed in disposable beakers to 15 ml of test medium. Organisms were not fed and the medium was not changed during the tests. The number of living organisms was determined at 24 and 48 hr. Probit analysis was used where appropriate. No confirmation of toxicant levels or other water quality chemistry was done.

2.4.2 Chronic Toxicity Testing

Chronic toxicity testing with daphnids was done according to standard methods (Weber et al., 1989). Daphnid tests are conducted in 20 ml disposable polystyrene beakers at 25 C in a low-temperature incubator. Each test concentration is represented by 10 replicate test containers. Beakers are filled with 15 ml of test medium which was changed daily. One daphnid neonate was randomly allocated to each test container. After daily survival and reproduction were recorded, treatment was renewed by transferring individuals to test chambers containing fresh solutions. Once distributed to fresh solutions, daphnids

were fed standard trout chow-yeast-alfalfa (TYA) medium and algae (Selenastrum capricornutum). Each test beaker received 100 µl of both foods daily.

One cooperating company conducted chronic toxicity tests using fathead minnows (Pimephales promelas) according to standard methods (Weber et al., 1989).

2.5 FIELD SAMPLING METHODS

The biological health of the receiving stream was evaluated by collecting both microbial and invertebrate communities upstream and downstream of the discharge (and receiving stream). When possible, more than one upstream location was examined to evaluate intrinsic variability of community measures.

Microbial communities were collected at each site using polyurethane foam artificial substrates identical to those used in microcosm studies. Artificial substrates were exposed for a seven day colonization period prior to collection. Invertebrate communities were evaluated by collecting triplicate samples using both a kick net for qualitative samples and a Surber sampler for quantitative samples at each sampling location.

Microbial samples were collected by placing replicate artificial substrates in a small amount of stream water in individual collecting bags and returning the live material immediately to the laboratory. Analyses of microbial community samples were identical to those analyses done on laboratory material. Invertebrate samples were preserved in the field in 70% ethanol and returned to the laboratory for later processing (separation from debris, sorting, identification). Fish were collected by electroshocking until no new species were captured. Fish samples were preserved in the field in formaldehyde and returned to the laboratory. Ambient water chemistry was evaluated concurrent with sample collection and included routine analyses for dissolved oxygen, temperature, pH, hardness, and alkalinity.

2.6 STATISTICAL ANALYSIS

Hypothesis tests (analysis of variance and multiple comparisons) were used to determine concentrations at which responses differ significantly from controls (Weber et al., 1989). Dose-response relationships were determined by ordinary least-squares regression followed by inverse prediction (Sokal and Rohlf, 1983). Inverse predictions were obtained for effective concentrations (ECs), the concentration of toxicant or

effluent resulting in a given percentage change in response relative to controls.

Supplemental analyses include examination of changes in community structure based on the taxonomic identification of component organisms (Smith, et al., 1990). In addition, time-series analyses can be used to evaluate continuously collected pH data in microcosms; these data are an index of diurnal relative production and respiration.

3.0 RESULTS

Four types of experiments were conducted by our laboratory: pure chemical tests, a pure chemical test with field validation, mixture experiments, and mixture experiments with field validation. The results from all of the experiments are presented below.

3.1 COPPER

High levels of copper in surface and ground waters are usually found as a result of anthropogenic inputs such as industrial effluents, dissolution of brass and and copper pipes, burning of coal, and the use of copper as an algicide (USEPA, 1984). The current national water quality criterion for copper states that the average 4-day concentration (µg/L) should not exceed the numerical value given by exp(0.8545[ln hardness] - 1.465) in µg/L more than once every three years and the 1-hr average concentration should not exceed the numerical value given by exp(0.9422[ln hardness] - 1.464) in µg/L more than once every three years (USEPA, 1986). Based on an average hardness of 180 mg $CaCO_3$/L in our inflow water, this translates into an acute criterion of 19.5µg/L. These criteria are based on total recoverable copper.

LOEC's estimated from endpoints measured in weekly island substrates and day 29 epicenter substrates exposed to copper are shown in Table 3.1. A complete set of results can be found in Tables 3.2-3.9. Microbial communities were very sensitive to copper exposure, with the productive component of the community initially eliminated at the two highest concentrations (day 14). By day 28, however, producers appeared to have recovered slightly and were affected at only the highest concentration tested (205 ug/L). On day 28, protozoan species richness, proportion of bacterivores, chlorophyll *a*, and dissolved oxygen were all affected at $\geq$ 40 ug/L. These results suggested that producers were functionally more sensitive to copper exposure than structurally. The most sensitive response to copper appeared to be nutrient cycling. Phosphorus levels in substrates exposed to 9.9 to 90 ug copper/L were reduced or nondetectable compared with controls, and at 205 ug copper/L, phosphorus levels were almost 12 times greater than that found in control substrates and more than double that of inflow water. The mechanism of this disruption of nutrient cycling is unknown, but alkaline phosphatase results did not reflect the changes observed in substrate phosphate levels.

Epicenter substrates appeared to be more sensitive to copper exposure than the island substrates. Biomass (both protein and chlorophyll *a*) was reduced at the lowest copper concentration tested (9.9 ug/L). Alkaline phosphatase activity was increased in substrates exposed to $\geq$ 19.9 ug/L, which reflected the disruption in nutrient cycling and decreased phosphorus levels observed in island substrates.

For comparative purposes, chronic toxicity data from the literature are shown in Table 3.10. Single species responded to chronic copper exposures in the range of < 4 to 60.4 ug/L (USEPA, 1984), which corresponded well with our results. In a previous laboratory microcosm test (Pratt et al., 1987a), in which the experimental design differed slightly from that of our current design, we saw similar effects at similar copper concentrations (LOEC's ranged from 6.6 to 59.5 ug/L). The water quality criterion based on water hardness in that test was 8.2 ug/L. Chlorophyll a, ATP biomass, and protozoan species composition were the most sensitive endpoints in that test (LOEC = 6.6 ug/L) and species richness and potassium were also affected. Based on microcosm results, it would appear that naturally derived microbial communities responded to copper at a level slightly lower than the current water quality criterion of 19.5 ug/L, but within the same range as sensitive single species tests.

Table 3.1. Effect of copper on microbial communities from laboratory microcosms. LOEC's (ug/L) for each significant response are shown.

Response	Island Substrates Day 14	Day 21	Day 28	Epicenter Substrates
Species richness	40(I)	9.9(I)	19.9(I)	-
% Bactivores	90(I)	90(I)	40(I)	-
% Producers	19.9(I)	90(I)	90(I)	-
Protein	40(I)	9.9(S) 90(I)	9.9(S) 90(I)	9.9(I)
Chlorophyll	19.9(I)	19.9(I)	90(I)	9.9(I)
Alk. Phos.	90(S)	NS	NS	19.9(S)
Calcium	-	-	90(I)	-
Magnesium	-	-	NS	-
Potassium	-	-	90(I)	-
Phosphorus	-	-	9.9(I) 90(S)	-
DO	19.9(I)	90(I)	40(I)	-
pH	9.9(I)	205(I)	205(I)	-
P/R	NS	NS	NS	-

Table 3.2. Average copper concentration in laboratory microcosms. Values are mean (SD) in ug/L. Coefficients of variation (C.V.) are also shown.

Target	Mean	SD	c.v. (%)
Control	0.0	-	-
10.0	9.9	1.3	13.6
20.0	19.9	2.7	13.6
40.0	40.0	5.4	13.5
80.0	90.0	9.6	10.7
160	205	16.9	8.2

Table 3.3. Alkaline phosphatase activity on artificial substrates from microcosms dosed with copper. Values are mean (SD) in nmole p-nitrophenol/mg protein/hr.

Treatment	Day 14	Day 21	Day 28
Control	13.5 (3.77)	23.3 (4.04)	19.0 (3.60)
9.9 ug/L	11.1 (1.80)	17.3 (2.51)	15.3 (1.53)
19.9	17.0 (3.46)	40.3 (30.9)	15.0 (2.64)
40.0	36.0 (10.4)	21.0 (5.20)	14.7 (0.58)
90.0	58.7[a] (12.6)	30.3 (4.62)	17.0 (3.60)
205	130[a] (44.7)	14.0 (1.00)	14.5 (3.53)
p	0.0001	0.2302	0.3875

[a] Significantly different from control at $\alpha = 0.05$

Table 3.4. Biomass (protein) accumulation on artificial substrates from microcosms dosed with copper. Values are mean (SD) in ug/ml.

Treatment	Day 14	Day 21	Day 28	Epicenters
Control	59.3 (16.2)	125 (33.8)	253 (38.2)	315 (99.9)
9.9 ug/L	77.7 (27.4)	187[a] (15.7)	331[a] (6.43)	208[a] (8.50)
19.9	48.3 (14.0)	144 (11.0)	281 (17.4)	155[a] (14.8)
40.0	16.7[a] (2.08)	97.3 (12.6)	252 (11.8)	162[a] (19.9)
90.0	9.67[a] (1.52)	38.7[a] (15.5)	154[a] (20.5)	149[a] (31.8)
205	3.47[a] (0.25)	51.0[a] (4.36)	44.5[a] (0.71)	116[a] (15.0)
p	0.0001	0.0001	0.0001	0.0021

[a] Significantly different from control at $\alpha = 0.05$

Table 3.5. Chlorophyll concentrations on artificial substrates from microcosms dosed with coppper. Values are mean (SD) in ug/L.

Treatment	Day 14	Day 21	Day 28	Epicenters
Control	172 (56.5)	389 (83.1)	552 (264)	4138 (1185)
9.9 ug/L	141 (22.6)	387 (87.7)	731 (164)	2373* (148)
19.9	87.7* (16.9)	148* (24.0)	321 (88.3)	1557* (598)
40.0	45.3* (14.6)	175* (66.7)	185* (42.0)	1038* (206)
90.0	22.7* (2.31)	29.7* (51.3)	104* (37.0)	667* (311)
205	13.7* (2.31)	< 10.0* -	45.5* (7.78)	482* (78.2)
p	0.0001	0.0039	0.0007	0.0001

* Significantly different from control at $\alpha = 0.05$

Table 3.6. Protozoan species richness on artificial substrates from microcosms dosed with copper. The proportion of bactivores and producers is also shown. Values are mean (SD).

Treatment	Day 14 Total	Day 14 %B	Day 14 %P	Day 21 Total	Day 21 %B	Day 21 %P	Day 28 Total	Day 28 %B	Day 28 %P
Control	34.0 (1.73)	74.7 (5.08)	18.6 (1.41)	50.0 (3.51)	71.7 (3.05)	14.0 (1.85)	41.0 (2.64)	69.2 (4.86)	16.3 (2.10)
9.9 ug/L	31.0 (2.64)	78.6 (3.02)	17.1 (3.18)	38.3[a] (1.52)	74.2 (8.66)	16.3 (5.43)	38.7 (4.61)	71.5 (8.95)	21.8 (3.51)
19.9	29.0 (5.57)	76.9 (9.25)	14.2[a] (2.05)	37.7[a] (4.51)	77.6 (6.70)	12.6 (2.89)	28.7[a] (2.08)	72.1 (5.14)	15.1 (0.97)
40.0	19.0[a] (2.64)	84.5 (15.2)	9.08[a] (4.03)	26.3[a] (5.69)	73.3 (9.60)	15.5 (4.68)	29.7[a] (2.51)	82.3[a] (12.7)	10.0 (3.05)
90.0	10.0[a] (1.00)	99.2[a] (21.7)	0.00[a] -	16.3[a] (3.05)	90.1[a] (5.13)	8.25 (3.29)	17.0[a] (1.00)	90.1[a] (9.29)	9.93 (3.86)
205	10.7[a] (0.58)	99.0[a] (24.6)	0.00[a] -	11.0[a] (1.00)	99.0[a] (24.6)	0.00[a] -	10.3[a] (0.58)	90.6[a] (1.15)	6.37[a] (5.53)
p	0.0001	0.0008	0.0001	0.0001	0.0002	0.0008	0.0001	0.0001	0.0017

[a] Significantly different from control at $\alpha = 0.05$

Table 3.7. Phosphorus, calcium, magnesium, and potassium in artificial substrates from microcosms dosed with copper on day 28. Values are mean (SD).

Treatment	Phosphorus (ug/L)	Calcium (mg/L)	Magnesium (mg/L)	Potassium (mg/L)
Inflow	112	43.1	21.7	1.98
Control	21.3 (11.5)	102 (9.91)	23.3 (0.36)	7.00 (1.27)
9.9 ug/L	14.3* (4.04)	110 (15.6)	23.3 (0.59)	8.40 (0.40)
19.9	< 10.0* -	76.0 (27.5)	19.9 (3.69)	6.73 (0.84)
40.0	< 10.0* -	79.4 (12.6)	21.6 (0.25)	5.90 (0.15)
90.0	< 10.0* -	44.1* (1.00)	21.3 (1.07)	3.67* (0.67)
205	24* (53.7)	38.5* (2.12)	20.6 (0.99)	2.75* (1.20)
p	0.0001	0.0008	0.1730	0.0001

* Significantly different from control at $\alpha = 0.05$

Table 3.8. Analysis of epicenter substrates from microcosms dosed with copper after 29 days of exposure. Values are mean (SD).

Treatment	Total Species	% B	% P	APA	Pro. (ug/ml)	Chl. a (ug/L)	Carb. (ug/ml)
Control	54	77.8	11.1	99 (5.1)	315 (99.9)	4138 (1184)	41.0 (13.5)
9.9 ug/L	52	86.5	3.80	114 (5.1)	208[a] (8.50)	2373[a] (148)	29.5 (5.72)
19.9	52	92.3	1.18	147[a] (7.9)	155[a] (14.8)	1557[a] (598)	24.5 (2.68)
40.0	47	83.0	10.0	150[a] (12)	162[a] (19.8)	1038[a] (206)	21.9 (8.07)
90.0	38	81.6	10.5	142[a] (36)	149[a] (31.8)	667[a] (311)	34.1 (18.6)
205	26	80.8	11.5	130 (20)	116[a] (15.0)	482[a] (78.2)	21.8 (4.54)
p				0.0163	0.0021	0.0001	0.2251
Day 0 Epicenters	52.0 (3.60)	75.6 (1.82)	7.73 (2.21)	277 (16)	129 (14.7)	537 (93.4)	25.0 (5.29)

[a] Significantly different from control at $\alpha = 0.05$

Table 3.9. Afternoon dissolved oxygen (DO) concentration and pH in microcosms dosed with copper. Values are mean (SD), with DO in mg/L and pH in pH units.

Treatment	Day 14 DO	Day 14 pH	Day 21 DO	Day 21 pH	Day 28 DO	Day 28 pH
Control	9.87 (0.11)	8.07 (0.12)	12.1 (1.40)	8.45 (0.32)	15.7 (0.81)	8.35 (0.17)
9.9 ug/L	10.0 (0.10)	8.30[a] (0.17)	13.6 (0.90)	8.60 (0.12)	16.3 (0.98)	8.53 (0.17)
19.9	9.63[a] (0.11)	-	12.4 (0.47)	-	15.0 (0.35)	-
40.0	9.53[a] (0.15)	8.04[a] (0.06)	12.0 (1.01)	8.43 (0.08)	14.4[a] (0.84)	8.29 (0.10)
90.0	9.40[a] (0.20)	7.82[a] (0.06)	10.3[a] (0.40)	8.17 (0.14)	12.7[a] (0.65)	8.14 (0.10)
205	9.33[a] (0.06)	7.69[a] (0.01)	9.50[a] (0.00)	7.96[a] (0.24)	11.1[a] (0.20)	7.91[a] (0.13)
p	0.0003	0.0002	0.0007	0.0183	0.0001	0.0030

[b] Significantly different from control at $\alpha = 0.05$

Table 3.10. Chronic toxicity data for copper from the literature. Values are mg/L.

Species/System	Duration	Response	Effect Level	Ref.
Champia parvula	2 wks	# tetra-sporangia	13.3 (MATC)	a
		Growth	3.9-4.7 (MATC)	a
Microbial community	28 d	Species Richness	6.6 (LOEC)	b
		Species composition	12.7 (LOEC)	b
		Chlorophyll	6.6 (LOEC)	b
		ATP	6.6 (LOEC)	b
		Potassium	19.5 (LOEC)	b
Daphnia pulex	7 d	Survival	5 (LOEC)	c
Juga plicifera	30 d	Survival	8 (LOEC)	d
Lithoglyphus sp.	30 d	Survival	< 4 (LOEC)	d
Fathead minnow	7 d	Growth	28.5 (LOEC)	e

a Steele and Thursby, 1983

b Pratt et al., 1987

c Ingersoll and Winner, 1982

d Nebeker et al., 1986

e Norberg and Mount, 1985

3.2 PENTACHLOROPHENOL

Pentachlorophenol (PCP) has been used extensively as a wood preservative due to its fungicidal and bactericidal properties (Pignatello et al., 1983). PCP acts as an uncoupler of the oxidative phosphorylation of mitochondria (Weinbach, 1954), but its toxic effects are extremely species specific, with some sensitive algae affected in the µg/L range (Gotham and Rhee, 1982). Point source contamination of water from both manufacturing and wood preservation sites, and possibly, non-point source contamination where there is runoff from PCP-treated lumber products may occur (USEPA, 1980). The current USEPA ambient water quality criteria states that the four-day average concentration of PCP (µg/L) should not exceed the numerical value given by exp[1.005(pH)-5.280] more than once every three years and the one-hour average should not exceed the numerical value given by exp[1.005(pH)-4.830] more than once every three years (USEPA, 1986). Results suggest that PCP toxicity is dependent on pH due to the ratio of the nonionized and undissolved forms, with decreasing pH (Smith et al., 1987). Based on an average pH of 7.70 for diluent water used in our laboratory microcosm tests, the chronic water quality criterion for PCP would be 11.6 µg/L and the acute value would be 18.3 µg/L.

A summary of LOEC's based on day 21 island substrates and day 22 epicenters substrates is shown in Table 3.11. A complete set of results can be found in Tables 3.12-3.19. Protozan species richness was reduced by 24-41% at $\geq$ 228 ug/L (MATC = 159 ug/L), but there was no effect on functional group composition, suggesting the nonselective toxic effect of PCP. Microbial nutrient cycling was sensitive to PCP with increased phosphorus levels at $\geq$ 228 ug/L and decreased calcium levels at $\geq$ 456 ug/L (MATC = 322.4 ug/L). Day 22 epicenter communities appeared less sensitive to PCP than the day 21 island communities, with respect to protozoan species richness, but more sensitive with respect to nutrient cycling (LOEC for APA = 939 ug/L).

Chronic toxicity data from the literature for PCP are shown in Table 3.20. Chronic toxicity data for PCP is limited, but it would appear that the responses we observed in laboratory microcosms occured at PCP levels corresponding to chronic toxicity in single-species tests. Microbial communities in laboratory microcosms do not appear to be as sensitive to PCP as higher organisms. At the time of publication of this report, data from the collaborative study with USABRDL was not available, so the consistency of our results cannot be evaluated.

Table 3.11. Effect of pentachlorophenol on microbial communities from laboratory microcosms. LOEC's (ug/L) for each significant response are shown.

Response	Day 21	Day 22 Epicenters
Species richness	228	939
% Bactivores	NS	NS
% Producers	NS	NS
Protein	NS	NS
Chlorophyll a	NS	NS
Hexosamine	NS	NS
Carbohydrate	NS	NS
Alkaline phosphatase	NS	939
P/R	NS	-
Net productivity	NS	-
Phosphorus	228	-
Potassium	NS	-
Magnesium	NS	-
Calcium	456	-
pH	939	-
DO	NS	-

Table 3.12. Pentachlorophenol concentration (ug/L) in laboratory microcosms. Values are mean (SD). Also shown is the coefficient of variation (CV) for each treatment.

Treatment	Average	SD	CV
Control	< 0.034	-	-
63.5	56.0	9.0	16.1
125	111	11.5	10.4
250	228	17.7	7.8
500	456	29.4	6.4
1000	939	64.9	6.9

Table 3.13. Effect of pentachlorophenol on protozoan species richness and composition after 21 days of exposure. The proportion (%) of bactivores and producers is given. Values are mean (SD).

Treatment	Total Species	Bactivores	Producers
Control	57.0 (3.60)	77.2 (2.15)	9.9 (1.61)
56.0 ug/L	50.0 (6.93)	82.0 (3.33)	7.6 (4.90)
111	48.8 (4.51)	73.3 (3.89)	10.4 (2.43)
228	43.3[a] (3.79)	79.4 (5.71)	7.6 (2.14)
456	36.7[a] (3.51)	78.1 (2.75)	8.1 (1.95)
939	33.3[a] (4.93)	75.7 (3.35)	14.4 (5.06)
p	0.0004	0.1819	0.1581
Day 0 Epicenters	32.3 (4.16)	76.4 (12.7)	12.7 (4.59)

[a] Significantly different from control at $\alpha = 0.05$

Table 3.14. Effect of pentachlorophenol on protozoan species richness and composition on epicenters at the end of the test (day 22). The proportion (%) of bactivores and producers is shown. Values shown are mean (SD).

Treatment	Total Species	Bactivores	Producers
Control	52.7 (6.11)	78.7 (3.50)	7.5 (1.05)
939 ug/L	32.7[a] (5.69)	80.3 (4.69)	11.2 (2.67)
p	0.0143	0.6473	0.2124

[a] Significantly different from control at $\alpha = 0.05$

Table 3.15. Effect of pentachlorophenol on microbial productivity to respiration ratios (P/R) and light:dark bottle productivity. Values are mean (SD).

Treatment	P/R (Week 2)	P/R (Week 3)	L:D Productivity (mg O_2/6 hr)
Control	1.07 (0.18)	1.04 (0.01)	5.85 (1.99)
56.0 ug/L	1.09 (0.07)	0.98 (0.06)	4.59 (2.54)
111	1.11 (0.12)	0.97 (0.07)	5.20 (1.20)
228	1.11 (0.08)	1.00 (0.04)	4.69 (0.62)
456	1.09 (0.37)	1.02 (0.09)	4.68 (0.75)
939	1.11 (0.11)	1.04 (0.04)	5.04 (0.51)
p	0.7655	0.3434	0.8963

Table 3.16. Afternoon dissolved oxygen (mg/L) in microcosms dosed with pentachlorophenol after two and three weeks of exposure. Values are mean (SD). pH values at the start of the test (Day 0) are shown to illustrate the effect of PCP on microcosm pH.

Treatment	Day 14 DO	Day 14 pH	Day 21 DO	Day 21 pH	Day 0 pH
Control	14.3 (1.18)	8.32 (0.04)	14.8 (0.35)	8.42 (0.10)	7.71 (0.09)
56.0 ug/L	14.1 (0.51)	8.39 (0.10)	13.7 (0.74)	8.45 (0.04)	7.72 (0.10)
111	13.2 (2.01)	8.35 (0.21)	13.6 (1.44)	8.49 (0.30)	7.84* (0.05)
228	13.3 (0.49)	8.39 (0.10)	14.0 (1.11)	8.50 (0.08)	7.93* (0.05)
456	12.3 (0.75)	8.39 (0.10)	14.1 (0.93)	8.61 (0.09)	8.07* (0.06)
939	13.0 (0.15)	8.77* (0.18)	15.1 (0.17)	8.91* (0.06)	8.48* (0.03)
p	0.2402	0.0142	0.3094	0.0101	0.0001

* Significantly different from control at $\alpha = 0.05$

Table 3.17. Effect of pentachlorophenol on microbial nontaxonomic responses measured on day 21. Values are mean (SD).

Treatment	Protein (ug/ml)	Chl a (ug/L)	Carbohydrate (ug/ml)	Hexosamine (ug/ml)	APA[a]
Control	93.8 (26.3)	1497 (548)	13.8 (3.91)	5.63 (1.42)	285 (93)
56.0 ug/L	87.6 (26.0)	1579 (501)	13.6 (4.76)	5.50 (2.68)	387 (133)
111	104.1 (38.3)	1758 (781)	15.7 (5.74)	3.86 (0.42)	321 (135)
228	86.4 (14.2)	1476 (425)	15.1 (3.72)	4.09 (0.20)	251 (97)
456	82.0 (20.6)	1646 (345)	13.7 (2.80)	5.01 (1.76)	205 (16)
939	107.1 (15.2)	1995 (290)	20.4 (2.95)	6.65 (1.77)	146 (41)
p	0.7689	0.8133	0.3672	0.3486	0.1057
Day 0 Epicenters	65.9 (8.5)	164 (57)	16.6 (3.42)	-	468 (21)

[a] nmole p-nitrophenol/mg protein/hr

Table 3.18. Effect of pentachlorophenol on phosphate, potassium, magnesium, and calcium levels in artificial substrate samples after 21 days of exposure. Values are mean (SD).

Treatment	Phosphorus (ug/L)	Potassium (mg/L)	Magnesium (mg/L)	Calcium (mg/L)
Control	41.0 (16.5)	2.30 (0.34)	39.0 (7.0)	46.5 (4.4)
56.0 ug/L	167 (64.9)	2.13 (0.45)	38.7 (8.4)	46.2 (3.7)
111	249 (62.5)	2.29 (0.48)	36.7 (11.6)	44.7 (3.5)
228	344[a] (82.5)	2.09 (0.02)	35.0 (5.3)	45.2 (2.0)
456	500[a] (236)	2.07 (0.18)	28.3 (7.8)	37.5[a] (1.5)
939	521[a] (178)	2.27 (0.34)	30.7 (8.4)	38.5[a] (1.5)
p	0.0045	0.9069	0.5528	0.0087

[a] Significantly different from control at $\alpha = 0.05$

Table 3.19. Effect of pentachlorophenol on microbial nontaxonomic responses measured on day 22 epicenters. Values are mean (SD).

Treatment	Protein (ug/ml)	Chl a (ug/L)	Carbohydrate (ug/ml)	Hexosamine (ug/ml)	APA[a]
Control	146.2 (29.2)	1379 (284)	22.8 (5.10)	25.6 (9.86)	250 (26)
56.0 ug/L	173.0 (54.8)	1219 (346)	32.6 (15.9)	35.9 (16.8)	269 (31)
111	136.5 (5.41)	1438 (404)	24.4 (2.19)	23.5 (6.45)	240 (33)
228	174.5 (35.8)	1483 (143)	29.0 (11.2)	27.7 (11.6)	264 (35)
456	176.0 (8.91)	1654 (158)	33.5 (8.30)	34.4 (7.21)	215 (52)
939	207.6 (6.58)	1327 (210)	42.0 (11.2)	46.3 (7.38)	127[b] (22)
p	0.1297	0.5293	0.2730	0.1610	0.0028

[a] nmole p-nitrophenol/mg protein/hr
[b] Significantly different from control at $\alpha = 0.05$

Table 3.20. Chronic toxicity data for pentachlorophenol from the literature. Values are ug/L.

Species	Duration	Response	Effect Level	Ref.
Daphnia magna	30 d	Survival, reproduction	240 (MATC)	a
Rotifers	8 wks	Survival	300 (LOEC)	b
Fathead minnow	28 d	Survival	176 (LOEC)	c
Physa gyrina	36 d	Growth	102 (LOEC)	c
		Egg Production	25.7 (LOEC)	c
Ceriodaphnia dubia/affinis	7 d	Survival	504 (LOEC)	c
		Reproduction	161 (LOEC)	c
Ceridaphnia reticulata	7 d	Survival	161 (LOEC)	c
		Reproduction	4.1 (LOEC)	c
Fathead minnow	32 d	Survivial, number of larve, weight	161 (LOEC)	d
		Embryo hatchability	327 (LOEC)	d

a Adema, 1978

b Feind et al., 1988

c Hedtke et al., 1986

d Spehar et al., 1985

3.3 TRINITROTOLUENE

Trinitrotoluene (2-methyl-1,3,5,-trinitrobenzene, TNT) is a munitions compound which may find its way into surface or ground waters through runoff or industrial discharges (Ryon, 1987). Although microbial degredation occurs, degredation due to photolysis occurs at a much faster rate (half life of 3 - 22 hrs, Lu et al., 1987). Using available TNT toxicity data and USEPA water quality criteria guidelines, Ryon (1987) estimated that 40 µg TNT/L would protect freshwater aquatic life under continuous exposure. A criterion data for estimating a safe level of chronic TNT exposure, however, was insufficient to meet all of the USEPA guideline requirements.

A summary of the LOEC's estimated from endpoints measured in weekly island substrates and day 22 epicenter substrates is shown in Table 3.21. A complete set of results can be found in Tables 3.22-3.28. After 14 days, both protozoan species richness and dissolved oxygen production was reduced at $\geq$ 76.3 ug/L, but by day 21, communities recovered slightly. On day 21, protozoan species richness was significantly reduced by 23% and the proportion of producer species increased by 54% at $\geq$ 362 ug TNT/L (MATC = 166 ug/L). Calcium levels were reduced and alkaline phosphatase activity increased at the highest concentration tested (711 ug/L, MATC = 507 ug/L). There was no effect of TNT on protozoan species richness or composition in epicenter substrates on day 22, but alkaline phosphatase was affected at the highest concentration tested (711 ug/L).

Chronic toxicity data from the literature for TNT are shown in Table 3.29. Effect levels in our microcosms occured at the lower range of TNT concentrations shown to be toxic, but we believe our values to be more accurate than some of the literature values. Much of the chronic data is based on tests in which TNT concentrations were either not measured of were only measured at the start of the test. TNT is extremely photoreactive and degredation over the duration of a test could lead to an underestimation of toxic concentrations. We monitored TNT concentrations twice weekly and provided a continuous input of toxicant. Based on our results, the safe concentration estimated by Ryon (1987) of 40 ug/L would protect freshwater microbial communities.

Table 3.21. Effect of TNT on microbial commmunities from laboratory microcosms. LOEC's (ug/L) for each significant response are shown.

Response	Island Substrates Day 7	Day 14	Day 21	Epicenter Substrates
Species richness	NS	76.3	362	NS
% Bactivores	NS	NS	NS	NS
% Producers	NS	NS	362	NS
Protein	NS	NS	NS	NS
Chlorophyll a	-	-	-	NS
APA	NS	NS	711	711
Carbohydrate	-	-	-	NS
Calcium	-	-	711	-
Magnesium	-	-	NS	-
Potassium	-	-	NS	-
DO	NS	76.3	NS	-
pH				
P/R	NS	NS	NS	-

Table 3.22. TNT concentration (ug/L) measured in laboratory microcosms. Values are mean (SD) and coefficients of variation are shown.

Target Concentration	Average	SD	CV (%)
Control	< 14	-	-
62.5 ug/L	32.6	10.4	32.0
125	76.3	21.3	27.9
500	362	57.8	16.0
1000	711	107	15.3

Table 3.23. Protozoan species richness on island substrates from laboratory microcosms dosed with TNT. Values are mean (SD). Also shown is the proportion of bactivores and producers on each sampling day.

Treatment	Day 7 T	Day 7 %B	Day 7 %P	Day 14 T	Day 14 %B	Day 14 %P	Day 21 T	Day 21 %B	Day 21 %P
Control	28.7 (5.51)	81.6 (5.73)	6.91 (7.57)	47.0 (2.64)	76.4 (6.72)	9.20 (0.95)	62.7 (5.51)	79.4 (3.28)	6.31 (1.15)
32.6 ug/L	26.7 (4.51)	80.9 (15.0)	6.85 (5.88)	44.0 (4.58)	78.9 (1.24)	9.10 (2.22)	57.3 (1.15)	75.5 (2.02)	8.72 (1.73)
76.3	24.7 (3.21)	86.4 (2.22)	4.15 (3.88)	33.7[a] (4.04)	78.8 (5.44)	9.13 (3.51)	60.3 (2.52)	72.4 (3.06)	9.41 (1.99)
362	29.0 (6.08)	83.6 (2.69)	2.21 (1.98)	36.0[a] (3.60)	75.9 (5.90)	9.86 (1.33)	48.0[a] (4.00)	76.5 (6.85)	9.76[a] (2.47)
711	26.3 (6.11)	78.8 (7.88)	8.03 (6.97)	32.7[a] (2.52)	76.6 (9.55)	11.1 (4.00)	40.7[a] (2.08)	73.6 (3.93)	11.6[a] (4.15)
p	0.836	0.942	0.713	0.002	0.954	0.776	0.001	0.323	0.010

[a] Significantly different from control at $\alpha = 0.05$

Table 3.24. Protozoan species richness on epicenter substrates from laboratory microcosms dosed with TNT at the start of the test (d 0) and on day 22. Also shown is the proportion of producers and bactivores. Values are mean (SD).

Treatment	Total Species	Bactivores (%)	Producers (%)
Control	56.0 (5.20)	82.6 (3.39)	6.06 (2.39)
711 ug/L	52.3 (2.89)	80.4 (4.94)	7.66 (0.44)
p	0.3455	0.5765	0.3206
Day 0	52.3 (2.08)	76.6 (6.45)	5.08 (2.87)

Table 3.25. Dissolved oxygen (DO), pH and production/respiration ratios in microcosms exposed to TNT. Values are mean (SD).

Treatment	DO (mg/L) D 7	D 14	D 21	pH D 7	D 14	D 21	P/R Wk 1	Wk 2	Wk 3
Control	10.4 (0.38)	12.7 (0.19)	13.1 (0.12)	7.84 (0.14)	8.10 (0.18)	8.29 (0.14)	3.13 (2.08)	1.30 (0.13)	1.00 (0.04)
32.6	10.3 (0.11)	12.2 (0.18)	12.8 (0.76)	7.78 (0.05)	8.05 (0.12)	8.15 (0.05)	4.72 (3.54)	1.17 (0.09)	1.02 (0.04)
76.3	10.4 (0.50)	11.7[a] (0.80)	12.6 (0.21)	7.84 (0.12)	7.92 (0.12)	8.18 (0.17)	7.18 (6.94)	1.13 (0.15)	0.98 (0.01)
362	10.2 (0.27)	11.3[a] (0.38)	12.1 (0.34)	7.96 (0.04)	8.10 (0.10)	8.27 (0.17)	7.79 (3.34)	1.11 (0.06)	1.01 (0.15)
711	10.2 (0.45)	11.1[a] (0.38)	12.1 (0.34)	7.82 (0.09)	7.96 (0.12)	8.10 (0.05)	7.02 (5.73)	1.22 (0.08)	1.05 (0.07)
p	0.9278	0.0186	0.0617	0.3060	0.3426	0.3986	0.7147	0.2837	0.8840

[a] Significantly different from control at $\alpha = 0.05$

Table 3.26. Total protein and alkaline phosphatase activity on artificial substrates from microcosms dosed with TNT. Values are mean (SD).

Treatment	Protein (ug/ml) Day 7	Day 14	Day 21	APA (nmole p-NP/mg Pro/hr) Day 7	Day 14	Day 21
Control	2.3 (0.57)	30.3 (4.72)	70.7 (11.1)	158 (35.4)	120 (5.3)	64.3 (11.9)
32.6 ug/L	1.6 (0.40)	27.3 (18.8)	77.3 (12.5)	191 (22.2)	131 (16.5)	59.7 (11.9)
76.3	1.3 (0.60)	29.7 (3.78)	88.7 (10.0)	205 (20.5)	128 (16.1)	59.0 (2.64)
362	1.6 (0.34)	22.0 (8.71)	68.0 (9.84)	179 (40.2)	129 (8.5)	65.0 (5.30)
711	1.2 (0.71)	22.7 (1.53)	91.7 (10.8)	221 (56.4)	122 (5.2)	153[a] (18.2)
p	0.3065	0.7610	0.1032	0.3497	0.7397	0.0001

[a] Significantly different from control at $\alpha = 0.05$

Table 3.27. Calcium, magnesium, and potassium in island substrates from laboratory microcosms dosed with TNT after 21 days of exposure. Values are mean (SD) in mg/L.

Treatment	Calcium	Magnesium	Potassium
Control	65.5 (0.85)	18.4 (0.15)	2.21 (0.44)
32.6 ug/L	69.3 (2.48)	18.4 (0.06)	2.16 (0.33)
76.3	64.4 (0.98)	18.3 (0.06)	1.88 (0.17)
362	61.8 (4.38)	18.2 (0.15)	1.89 (0.10)
711	58.0[a] (4.38)	18.2 (0.32)	1.83 (0.26)
p	0.0116	0.3142	0.3932

[a] Significantly different from control at $\alpha = 0.05$

Table 3.28. Nontaxonomic responses in epicenter substrates from laboratory microcosms dosed with TNT after 22 days of exposure. Values are mean (SD).

Treatment	Protein (ug/ml)	Alkaline Phosphatase (nmole p-NP/mg pro/hr)	Chl a (ug/L)	Carbohydrate (ug/ml)
Control	231.0 (71.9)	136 (17.1)	2275 (213)	32.5 (14.2)
32.6	216.7 (19.3)	121 (14.5)	2714 (391)	27.3 (6.03)
76.3	175.6 (12.0)	167 (19.8)	2568 (88)	30.3 (10.0)
362	191.3 (30.2)	140 (20.5)	2594 (644)	29.0 (6.21)
711	151.1 (34.0)	215[a] (27.5)	1999 (335)	22.7 (2.08)
p	0.1754	0.0016	0.2213	0.7087
Day 0	95.8 (8.92)	534 (78.5)	352 (73.8)	24.3 (2.65)

[a] Significantly different from control at $\alpha = 0.05$

Table 3.29. Chronic toxicity data for trinitrotoluene from the literature. Values are ug/L.

Species/system	Duration	Response	Effect Level	Ref.
Daphnia magna	14 d	Mortality	200 (LC50)	a
Algae	14 d	Growth	5600 (LOEC)	a
Selenastrum	7 d	Growth	2500 (LOEC)	b
		Mortality	10000 (LOEC)	b
Fathead minnow	180 d	Mortality	40 (LOEC)	c
		Productivity	40 (LOEC)	c
Fathead minnow	30 d	Fry survival	1400 (LOEC)	d
Rainbow trout	60 d	Fry growth	300 (LOEC)	d
Daphnia magna	30 d	Reproduction	2500 (LOEC)	d

a Bailey, 1982

b Won et al., 1976

c Bailey et al., 1985

d Liu et al., 1983

3.4 CHLORPYRIFOS

Chlorpyrifos [o,o-diethyl-o(3,5,6-trichloro-2-pyridyl) phosphorothioate] is the active ingredient in several commercial formulations of organophosphate insecticides used in the control of a variety of agricultural, biting-fly, and domestic-indoor pests (Marshall and Roberts, 1978). Chronic toxicity tests suggest chlorpyrifos to be toxic at less than 1 µg/L, with its anti-acetylcholinesterase effects being the most potent. The ambient water quality criterion states that the four-day average concentration should not exceed 0.041 µg/L more than once every three years and the one-hour average should not exceed 0.083 µg/L more than once every three years (USEPA, 1986). The recommended application rate for chlorpyrifos use is 0.014-0.056 kg a.i./ha. If 0.03 kg a.i. was applied to a 3 hectare pond, the realized water concentration would be estimated at 1 µg/L.

A summary of LOEC's estimated from endpoints measured in island substrates and day 22 epicenter substrates exposed to chlorpyrifos is shown in Table 3.30. A complete set of results can be found in Tables 3.31-3.37. Due to lack of biomass in day 7 island substrates, no chlorophyll a analysis was done. Results from this test indicate that microbial communities are tolerant toward chlopyrifos at the levels which are chronically toxic to some species (see Table 3.38 for LOEC comparisons).

The occurance of algal blooms have been reported in ponds immediately after treatment with chlorpyrifos (Butcher et al., 1977; Roberts and Marshall, 1978; Papst and Boyer, 1980; Brazner et al., 1988), suggesting tolerance of this group to low levels of chlorpyrifos. Sewage sludge communities have been shown to be tolerant of chlopyrifos concentrations as high as 100 mg/L, with nitrification stimulated at 10 mg/L (Lieberman and Alexander, 1981). Brown et al. (1976) exposed freshwater phytoplankton to 1.2, 2.4, 24, and 240 ug chlorpyrifos/L and found the growth of some species (i.e., Ceratium) to be unaffected even at the highest concentration. Eaton et al. (1984) examined the effect of chlorpyrifos on "community level" characteristics in outdoor artificial streams. Continuously dosed streams received 0.22 ug chlorpyrifos/L and pulsed dosed streams received 3.1 ug chlorpyrifos/L for 24 h every 14 days. These concentrations were increased to 11.5 and 1.01 ug/L, respectively, after 14 days due to lack of observed effects. Community level effects monitored include production/respiration ratios, plant litter decomposition, organic matter decomposition, microbial biomass production, and heterotrophic activity. None of these community functions were affected by chlorpyrifos exposure, even after 100 days of dosing.

Based on our laboratory microcosm results and those found in the literature, it would appear that single-celled organisms (i.e., bacteria, fungi, algae, protozoa) are fairly tolerant to chlorpyrifos exposure, possibly due to the lack of acetylcholinesterase, the primary target of chlorpyrifos' toxic effect. Additional studies evaluating the effect of chlorpyrifos on microbial communities in a field situation would be useful to corroborate our microcosm results, sonce there is a paucity of information on the chronic toxicity of chlorpyrifos.

Table 3.30. Effect of chlorpyrifos on microbial communities from laboratory microcosms. LOEC's (ug/L) for each significant response are shown.

Response	Island Substrates Day 7	Day 14	Day 21	Epicenter Substrates
Species richness	NS	NS	NS	-
% Bactivores	NS	NS	NS	-
% Producers	NS	NS	NS	-
Protein	NS	NS	NS	NS
Chlorophyll	-	NS	NS	NS
Alk. Phos.	NS	NS	NS	NS
Calcium	-	-	NS	-
Magnesium	-	-	NS	-
Potassium	-	-	NS	-
Phosphorus	-	-	NS	-
Diss. Oxygen	NS	NS	NS	-
pH	NS	NS	NS	-
P/R	NS	NS	NS	-

Table 3.31. Chlorpyrifos concentration (ug/L) in laboratory microcosms. Difficulty with analysis resulted in only one measurement for the duration of the test. Values shown are from a single sample.

Target Concentration	Measured Concentration
Control (0.0)	<0.02
0.05	0.01[a]
0.10	0.02[a]
0.20	0.04
0.40	0.07
0.80	0.12

[a] These values were estimated based on diluter performance, actual values were below the detection limit of the analysis.

Table 3.32. Protozoan species richness and composition on island substrates from microcosms dosed with chlorpyrifos. Values are mean (SD).

Treatment	Day 7 Total	Day 7 % B	Day 7 % P	Day 14 Total	Day 14 % B	Day 14 % P	Day 21 Total	Day 21 % B	Day 21 % P
Control	29.0 (5.06)	81.0 (2.12)	9.00 (4.24)	34.7 (2.89)	73.1 (4.58)	15.9 (3.46)	31.7 (3.76)	79.5 (9.54)	11.3 (5.57)
0.01 ug/L	26.5 (4.95)	79.6 (7.07)	13.0 (0.00)	32.3 (5.77)	76.4 (14.8)	14.9 (7.21)	35.0 (7.94)	72.9 (5.51)	20.8 (7.21)
0.02	23.0 (1.41)	78.3 (8.48)	10.5 (2.12)	33.3 (4.04)	72.9 (10.5)	18.2 (5.51)	30.0 (3.60)	77.1 (9.54)	14.6 (4.51)
0.04	25.0 (4.24)	74.1 (6.36)	15.9 (2.83)	31.3 (2.08)	75.3 (5.13)	17.9 (5.20)	33.3 (3.05)	78.5 (2.08)	14.3 (3.05)
0.07	23.0 (1.41)	87.2 (14.0)	8.99 (7.07)	30.7 (4.16)	82.3 (12.8)	10.7 (1.53)	34.7 (1.15)	80.0 (8.14)	9.65 (2.08)
0.12	26.0 (4.24)	81.6 (13.4)	19.9 (2.83)	26.3 (1.53)	79.8 (4.04)	11.0 (3.00)	27.0 (1.73)	80.2 (7.00)	9.65 (5.86)
p	0.6724	0.4053	0.3902	0.1831	0.3958	0.2576	0.2268	0.8168	0.2375

Table 3.33. Total protein on island and epicenter substrates (day 22) from microcosms dosed with chlorpyrifos. Values are mean (SD).

Treatment	Island Substrates Day 7	Day 14	Day 21	Epicenter Substrates
Control	14.0 (3.60)	107 (35.2)	245 (75.5)	224 (72.8)
0.01 ug/L	18.0 (3.00)	111 (14.1)	274 (42.3)	209 (74.2)
0.02	13.7 (3.78)	113 (27.6)	277 (23.1)	247 (33.9)
0.04	13.2 (7.80)	105 (33.6)	275 (48.2)	278 (32.6)
0.07	13.0 (10.6)	78.0 (64.6)	225 (92.1)	267 (20.7)
0.12	23.3 (8.02)	113 (37.6)	289 (64.1)	314 (30.0)
p	0.4113	0.8651	0.7986	0.1817

Table 3.34. Alkaline phosphatase activity on island and epicenter substrates from microcosms dosed with chlorpyrifos. Values are mean (SD) in nmole p-nitrophenol/mg protein/h.

Treatment	Island Substrates Day 7	Day 14	Day 21	Epicenter Substrates
Control	77.1 (31.2)	54.0 (3.40)	31.7 (6.80)	237 (32.5)
0.01 ug/L	70.7 (34.5)	50.6 (6.85)	40.7 (16.9)	257 (9.39)
0.02	81.0 (12.2)	43.0 (9.60)	35.7 (3.51)	264 (13.9)
0.04	77.7 (29.7)	48.5 (8.90)	32.3 (1.53)	248 (18.5)
0.07	161 (135)	76.5 (35.4)	45.0 (5.20)	277 (25.5)
0.12	60.7 (11.6)	53.5 (6.00)	45.0 (5.20)	291 (73.6)
p	0.4029	0.2354	0.3137	0.5155

Table 3.35. Chlorophyll a biomass on island and epicenter substrates from microcosms dosed with chlorpyrifos. Values are mean (SD) in ug/L.

Treatment	Island Substrates Day 14	Island Substrates Day 21	Epicenter Substrates
Control	231 (23.0)	315 (140)	3315 (812)
0.01 ug/L	254 (44.5)	322 (107)	3901 (434)
0.02	237 (49.0)	318 (107)	4332 (909)
0.04	210 (55.0)	316 (47.8)	3456 (522)
0.07	146 (86.1)	221 (109)	4420 (771)
0.12	163 (20.2)	254 (73.1)	4383 (1751)
p	0.1329	0.7484	0.5192

Table 3.36. Dissolved oxygen (DO), pH and production/respiration ratios (P/R) in microcosms dosed with chlorpyrifos. Values are mean (SD).

Treatment	DO (mg/L) d 7	DO (mg/L) d 14	DO (mg/L) d 21	pH d 7	pH d 14	pH d 21	P/R d 7	P/R d 14	P/R d 21
Control	11.8 (1.40)	14.9 (0.69)	15.3 (1.79)	8.14 (0.23)	8.37 (0.28)	8.32 (0.22)	2.93 (2.97)	1.45 (0.13)	1.26 (0.18)
0.01 ug/L	12.4 (0.98)	16.2 (1.34)	16.3 (1.52)	8.04 (0.25)	8.27 (0.25)	8.33 (0.33)	4.59 (3.94)	1.42 (0.15)	1.32 (0.09)
0.02	11.8 (1.02)	16.0 (0.65)	16.1 (1.52)	7.99 (0.11)	8.12 (0.11)	8.13 (0.07)	3.10 (2.21)	1.45 (0.14)	1.24 (0.05)
0.04	11.5 (1.04)	15.4 (1.15)	15.7 (1.57)	-	-	-	3.05 (1.78)	1.49 (0.18)	1.35 (0.07)
0.07	11.4 (2.01)	14.3 (1.38)	14.5 (2.42)	8.05 (0.30)	8.23 (0.29)	8.22 (0.21)	1.31 (0.44)	1.47 (0.28)	1.40 (0.28)
0.12	11.9 (1.21)	14.7 (0.78)	15.2 (1.34)	8.05 (0.23)	8.19 (0.18)	8.14 (0.13)	1.51 (0.56)	1.43 (0.38)	1.31 (0.15)
p	0.9572	0.2403	0.8162	0.9485	0.7534	0.6286	0.6876	0.9986	0.8467

Table 3.37. Phosphorus, calcium, magnesium, and potassium levels on artificial island substrates (d 21) from microcosms dosed with chlorpyrifos. Values are mean (SD).

Treatment	Phosphorus (ug/L)	Calcium (mg/L)	Magnesium (mg/L)	Potassium (mg/L)
Control	76.3 (4.93)	84.6 (11.8)	18.2 (0.81)	6.27 (0.96)
0.01 ug/L	65.0 (4.36)	97.2 (14.1)	18.3 (3.73)	5.28 (0.75)
0.02	66.0 (14.9)	101 (14.9)	18.3 (3.72)	6.20 (0.53)
0.04	61.0 (9.64)	99.9 (22.1)	20.7 (7.48)	5.53 (1.07)
0.07	63.0 (12.1)	96.5 (37.9)	24.4 (6.60)	5.11 (2.28)
0.12	70.3 (16.4)	87.5 (24.3)	19.2 (6.16)	6.38 (1.64)
p	0.6213	0.9299	0.7131	0.7648

Table 3.38. Chronic toxicity data for chlorpyrifos from the literature. Values are ug/L.

Species/system	Duration	Response	Effect Level	Ref.
Estuarine benthic communities	8 wks	Species Richness	0.1 (LOEC)	a
Fathead minnows	60 d	Growth	1.21 (LOEC)	b
		Reproduction	0.63 (LOEC)	b
		AChE Activity	0.12 (LOEC)	b
Small pond phytoplankton	17 d	Growth	1.2 (LOEC)	c
Fathead minnow	7 d	Growth	7.4 (LOEC)	d
Fathead minnow	10(d	AChE Activity	0.14 (LOEC)	e

a USEPA, 1986

b Jarvinen et al., 1988

c Brown and Chow, 1975

d Norberg and Mount, 1985

e Eaton et al., 1984

3.5 SELENIUM

Selenium is a metalloid which enters surface water from several sources, including runoff from coal plant ash settling basins, effluent discharges from the electrolytic refining of copper and the manufacture of semiconductors, photoelectronic, and xerographic materials (Fishbein, 1983). Natural deposits of uranium ores and selenium-containing rocks also contribute a significant amount of selenium to surface waters (Fishbein, 1983). Natural waters typically contain 0.0001 to 0.160 mg selenium/L and may be as high as 0.6 mg/L due to geologic or anthropogenic inputs (Saiki and Lowe, 1987; Fishbein, 1983). Initially, the USEPA ambient water quality criterion was 35 ug selenium/L as a 24-hr average and not to exceed 260 ug/L at any given time (USEPA, 1986). Recently, this criterion has been lowered in respose to field studies which indicated that effects were occurring below the recommended criterion level (Lemly, 1985). The current USEPA chronic criterion for the protection of freshwater aquatic life is 5 ug selenium/L as a 4-day average not to occur more than once every 3 years and the acute criterion a 1-hr average of 20 ug selenium/L not to occur more than once every 3 years (USEPA, 1987).

A summmary of LOEC's for the various endpoints monitored in laboratory microcosms and outdoor experimental streams exposed to selenium is shown in Table 3.39. A complete set of results can be found in Tables 3.40-3.59. The most sensitive endpoint in the laboratory microcosm test to the inhibitory effect of selenium was protozoan species richness and hexosamine content. The predicted LOEC of 20 ug selenium/L (maximum allowable toxicant concentration, MATC = 14.2. was similar to some of the other chronic LOEC values obtained for trout and some species of algae (see Table 3.60 for chronic selenium toxicity data from the literature). We saw no effect of selenium on the distribution of protozoa species between the two dominant functional groups (bactivores and producers). We observed some stimulation of nutrient retention at both low and high selenium concentrations. Potassium levels were increased in day 21 island substrates at 10.0 ug/L and calcium and magnesium were both increased at 161 ug/L.

In the outdoor experimental streams, the effect of selenium on microbial communities collected on artifical substrates appeared to be transient and no effects could be detected after 10 days of exposure, despite the prediction of an effect at 20 ug/L on protozoan species richness, based on laboratory microcosm test. It is possible that negative effects on microbial communities would have been sustained if we had increased the duration of the exposure (only 10 days in the field vs. 21 days in the laboratory; see Owlsey and McCauly, 1986). Established epilithic algal communities in the 30 ug/L streams exhibited reduced photosynthetic

effeciency, suggesting that these communities, which were exposed to selenium for longer than 10 days, were in fact sensitive to a prolonged exposure of selenium. Alternately, differences observed between field and laboratory experiments may have been due to differences in source ecosystems. The microbial community collected from Spring Creek and tested in laboratory microcosms may have possessed a different tolerance toward selenium than the microbial community from the Mississippi River.

Based on these results, we concluded that microbial communities in the laboratory microcosm experiment responded to selenium in the ranges found chronically toxic in single species toxicity tests. Although results from the laboratory microcosm experiment did not predict all of the effects observed in the field, algal communitis in experimental streams responded at levels which were predicted to cause an effect based on laboratory results.

Table 3.39. Effect of selenium on naturally derived microbial communities in laboratory microcosms and MERS outdoor experimental streams. LOEC 's (ug/L) for each significant response are shown. Stimulatory (S) and inhibitory (I) responses are differentiated.

Response	Laboratory Microcosm d 7	d 14	d 21	MERS Artificial Streams d 1	d 3	d 5	d 10
Species Richness	NS	40(I)	20(I)	10(S)	NS	10(S)	NS
% Bactivores	NS	NS	NS	NS	NS	NS	NS
% Producers	NS	NS	NS	NS	NS	NS	NS
Alk. Phos.	NS	160(I)	NS	NS	NS	NS	NS
Protein	NS	NS	NS	NS	30(I)	10(S)	NS
Chlorophyll a	NS	NS	80(I)	NS	NS	NS	NS
Hexosamine	NS	NS	20(I)	NS	NS	NS	NS
Carbohydrate	NS	NS	NS	NS	NS	NS	NS
Potassium	-	-	10(S)	-	-	-	-
Magnesium	-	-	160(S)	-	-	-	-
Calcium	-	-	160(S)	-	-	-	-
P/B	NS	NS	20(S)		10(S), 30(I)		
P/R	NS	NS	NS	-	-	-	-
D.O.	NS	NS	NS	-	-	-	-
pH	NS	NS	NS	-	-	-	-

Table 3.40. Selenium concentration (ug/L) in laboratory microcosms. Values are mean (SD).

Treatment	Day 0	Day 7	Day 14	Day 21	Average	SD	CV(%)
Control	< 2.0	< 2.0	< 2.0	< 2.0	-	-	-
10 ug/L	9.9	9.1	10.3	10.9	10.0	0.75	7.55
20	20.9	17.6	20.5	21.5	20.1	1.73	8.62
40	43.3	33.2	43.3	44.6	41.1	5.30	12.9
80	86.9	77.5	89.9	85.4	84.9	5.29	6.23
160	155	155	161	175	161	9.55	5.45

Table 3.41. Protozoan species richness and composition on artificial substrates from laboratory microcosms dosed with selenium. Values are mean (SD).

Treatment	d 7 Total	d 7 % B	d 7 % P	d 14 Total	d 14 % B	d 14 % P	d 21 Total	d 21 % B	d 21 % P
Control	33.3 (0.58)	75.1 (9.22)	10.9 (3.55)	63.0 (3.60)	74.0 (5.00)	10.0 (0.61)	61.7 (3.21)	74.1 (0.20)	9.65 (2.91)
10.0 ug/L	30.7 (2.08)	79.8 (2.54)	8.17 (3.31)	55.0 (2.64)	76.1 (4.84)	9.51 (1.47)	57.0 (3.60)	75.3 (5.24)	10.6 (4.49)
20.1	30.7 (9.02)	73.8 (3.56)	8.16 (3.17)	55.3 (3.21)	77.1 (10.5)	8.56 (5.35)	50.7[a] (6.03)	74.9 (5.09)	9.13 (2.46)
41.1	27.0 (2.64)	71.1 (10.7)	12.1 (4.66)	53.3[a] (6.35)	67.5 (1.15)	13.1 (0.90)	54.0 (5.57)	73.4 (3.98)	9.81 (4.14)
84.9	26.0 (6.08)	80.1 (6.95)	5.66 (5.23)	47.3[a] (8.14)	70.7 (8.70)	11.3 (1.71)	49.3[a] (6.81)	79.1 (6.33)	7.63 (2.04)
161	21.3 (3.21)	69.3 (6.74)	13.9 (3.43)	47.7[a] (2.51)	68.5 (2.91)	10.4 (3.13)	47.3[a] (2.08)	76.7 (4.87)	7.76 (3.16)
p	0.104	0.080	0.223	0.019	0.089	0.453	0.031	0.434	0.850

[a] Significantly different from control at $\alpha = 0.05$.

Table 3.42. Response of microbial communities to selenium after 7 days of exposure in laboratory microcosms. Values are mean (SD). Alkaline phosphatase activity is in nmole p-nitrophenol/mg protein/hr.

Treatment	Protein (ug/ml)	Chl a (ug/L)	APA	Hexosamine (ug/ml)	Carb. (ug/ml)	P/R
Control	11.5 (3.07)	244 (67)	127 (10.5)	4.3 (0.35)	2.7 (0.55)	1.2 (0.37)
10.0 ug/L	11.5 (4.16)	306 (75)	136 (7.5)	3.9 (0.92)	2.9 (0.46)	1.3 (0.07)
20.1	4.5 (1.79)	163 (67)	181 (70.7)	2.4 (0.62)	2.2 (0.79)	1.3 (0.19)
41.1	8.8 (7.65)	241 (179	210 (88.4)	4.1 (1.89)	1.9 (0.78)	1.2 (0.05)
84.9	5.3 (0.94)	175 (52)	159 (60.0)	3.2 (0.55)	1.9 (0.61)	1.3 (0.13)
161	8.9 (3.47)	278 (109)	130 (27.3)	4.1 (1.97)	2.5 (1.32)	1.3 (0.06)
p	0.238	0.496	0.328	0.559	0.585	0.962

* Significantly different from control at $\alpha = 0.05$.

Table 3.43. Response of microbial communities to selenium after 14 days of exposure in laboratory microcosms. Values are mean (SD). Alkaline phosphatase activity is in nmole p-nitrophenol/mg protein/hr.

Treatment	Protein (ug/ml)	Chl a (ug/L)	APA	Hexosamine (ug/ml)	Carb. (ug/ml)	P/R
Control	52.6 (5.6)	955 (126)	156 (13.3)	1.9 (0.35)	12.6 (0.93)	1.0 (0.01)
10.0 ug/L	46.9 (10.8)	896 (160)	135 (11.1)	1.4 (0.49)	12.0 (1.80)	1.0 (0.01)
20.1	36.1 (7.1)	558 (152)	153 (26.3)	0.98 (0.37)	11.4 (2.51)	1.0 (0.01)
41.1	39.2 (19.3)	676 (287)	162 (11.7)	1.5 (0.63)	12.2 (3.73)	1.0 (0.03)
84.9	34.6 (10.9)	694 (155)	154 (8.4)	0.91 (0.08)	14.0 (1.74)	1.0 (0.04)
161	45.9 (8.57)	736 (156)	112[a] (20.3)	1.4 (0.28)	13.7 (2.87)	1.0 (0.06)
p	0.3829	0.1485	0.0258	0.1147	0.7589	0.3590

[a] Significantly different from control at $\alpha = 0.05$.

Table 3.44. Response of microbial communities to selenium after 21 days of exposure in laboratory microcosms. Values are mean (SD). Alkaline phosphatase activity is in nmole p-nitrophenol/mg protein/hr.

Treatment	Protein (ug/ml)	Chl a (ug/L)	APA	Hexosamine (ug/ml)	Carb. (ug/ml)	P/R
Control	115.7 (22.1)	2136 (137)	130 (11.5)	3.7 (0.76)	34.8 (5.98)	1.1 (0.07)
10.0 ug/L	96.4 (26.0)	1804 (592)	129 (28.4)	2.9 (1.35)	26.4 (5.81)	1.1 (0.02)
20.1	73.0 (11.1)	1420 (330)	141 (44.8)	2.0[a] (0.06)	22.9 (6.74)	1.1 (0.05)
41.1	98.7 (9.50)	1793 (450)	125 (20.8)	2.8 (0.32)	22.2 (4.76)	1.1 (0.04)
84.9	79.5 (18.9)	1131[a] (206)	112 (14.7)	1.8[a] (0.26)	20.9 (4.15)	1.2 (0.03)
161	80.0 (17.6)	1354[a] (275)	121 (29.4)	2.0[a] (0.45)	22.4 (8.14)	1.1 (0.02)
p	0.1163	0.0181	0.8489	0.0334	0.1256	0.1338

[a] Significantly different from control at $\alpha = 0.05$.

Table 3.45. Afternoon dissolved oxygen (DO, mg/L) and pH in microcosms dosed with selenium. Values are mean (SD).

Treatment	d 7 DO	d 7 pH	d 14 DO	d 14 pH	d 21 DO	d 21 pH
Control	12.8 (1.09)	8.78 (0.53)	13.5 (0.60)	8.57 (0.16)	13.8 (1.10)	8.48 (0.27)
10.0ug/L	13.6 (0.32)	8.48 (0.04)	13.8 (0.16)	8.57 (0.09)	14.2 (0.17)	8.48 (0.07)
20.1	13.0 (0.64)	8.45 (0.10)	13.7 (0.44)	8.58 (0.06)	14.3 (0.64)	8.53 (0.18)
41.1	13.2 (0.46)	8.42 (0.20)	14.4 (0.46)	8.73 (0.09)	14.4 (0.28)	8.63 (0.07)
84.9	12.1 (0.03)	8.42 (0.11)	13.8 (0.17)	8.55 (0.18)	14.3 (0.14)	8.60 (0.03)
161	12.8 (0.16)	8.46 (0.13)	14.4 (0.47)	8.61 (0.15)	14.7 (1.08)	8.72 (0.09)
p	0.0868	0.4797	0.1014	0.5849	0.7472	0.4148

Table 3.46. Production to respiration and production to biomass (chlorophyll) ratios in laboratory microcosms dosed with selenium. Values are mean (SD).

Treatment	Production to respiration d 7	d14	d21	Production to biomass d7	d14	d21
Control	1.21 (0.37)	1.01 (0.01)	1.09 (0.07)	6.30 (3.02)	1.92 (0.41)	0.87 (0.17)
10.0 ug/L	1.28 (0.08)	0.99 (0.01)	1.07 (0.07)	6.02 (1.20)	2.19 (0.30)	1.31 (0.56)
20.1	1.34 (0.19)	1.00 (0.01)	1.08 (0.05)	12.1 (5.65)	3.65 (1.13)	2.00[a] (0.41)
41.1	1.25 (0.05)	1.01 (0.03)	1.12 (0.04)	9.30 (5.22)	3.52 (1.51)	1.27 (0.32)
84.9	1.27 (0.13)	1.04 (0.04)	1.16 (0.03)	7.62 (1.29)	2.80 (0.54)	1.90[a] (0.39)
161	1.29 (0.06)	1.05 (0.06)	1.14 (0.02)	5.90 (2.34)	2.97 (0.71)	1.65[a] (0.09)
p	0.9620	0.3590	0.1338	0.2985	0.1692	0.0406

[a] Significantly different from control at $\alpha = 0.05$.

Table 3.47. Calcium, magnesium, and potassium in island substrates from laboratory microcosms dosed with selenium after 21 days of exposure. Values are mean (SD) in mg/L.

Treatment	Calcium	Magnesium	Potassium
Control	76.7 (17.4)	23.5 (2.83)	3.96 (0.25)
10.0 ug/L	75.1 (5.77)	20.8 (0.70)	6.50[a] (1.15)
20.1	66.2 (7.84)	21.8 (1.02)	3.37 (0.25)
41.1	74.9 (3.12)	25.0 (0.49)	4.43 (0.84)
84.9	62.6 (4.74)	24.3 (0.75)	3.20 (0.66)
161	91.9[a] (6.55)	28.8[a] (3.91)	3.33 (0.70)
p	0.0241	0.0114	0.0009

[a] Significantly different from control at $\alpha = 0.05$.

Table 3.48. Selenium concentration measured in MERS outdoor streams. Water samples were analyzed twice during the duration of the field study. Values are in ug selenium/L.

Stream	13 June 1988	16 June 1988
Control-1	< 2.0	< 2.0
Control-2	< 2.0	< 2.0
10 ug/L-1	8.2	7.3
10 ug/L-2	9.3	8.3
30 ug/L-1	25.5	29.5
30 ug/L-2	26.9	29.3

Table 3.49. Routine water chemistry in MERS artificial streams collected 10 June 1988 (day 1 of field study).

Treatment	Sta. #	DO (mg/L) 0630 h	DO (mg/L) 1330 h	mg O_2/ L/8 h	pH	Hard. (mg CaCO3/L)	Alk.	Cond. (umho/cm)
Control-1	6	5.2	11.6	6.4	8.40	164	150	295
	7	4.7	12.8	8.1				
Control-2	6	4.8	11.2	6.4	8.44	166	150	285
	7	4.3	12.6	8.3				
10 ug/L-1	6	4.9	10.1	5.2	8.15	162	148	320
	7	3.9	12.9	9.0				
10 ug/L-2	6	5.4	12.0	6.6	8.80	180	164	310
	7	5.9	12.8	6.9				
30 ug/L-1	6	4.8	7.7	2.9	8.18	160	148	250
	7	4.3	9.5	5.2				
30 ug/L-2	6	5.1	9.7	4.6	8.33	164	152	280
	7	4.2	10.3	6.1				

Table 3.50. Protozoan species richness on artificial substrates over time from MERS outdoor experimental streams dosed with selenium. Values are mean (SD). Results of ANOVA are shown for comparsions between replicate streams (p str(trt)) and among treatments (p trt).

Treatment	Day 1	Day 3	Day 5	Day 10
Control	44.7 (4.99)	80.5 (7.59)	81.0 (2.71)	83.5 (6.24)
10 ug/L	57.5[a] (4.12)	82.5 (4.04)	91.0[a] (2.16)	82.5 (5.00)
30 ug/L	57.7[a] (2.87)	78.5 (1.73)	87.2[a] (3.40)	98.7 (9.21)
p str(trt)	0.5796	0.9730	0.5366	0.0944
p trt	0.0235	0.0894	0.0271	0.1624

[a] Significantly different from control at $\alpha = 0.05$.

Table 3.51. Protozoan functional group composition on artificial substrates over time from MERS outdoor experimental streams dosed with selenium. Values are mean (SD). Results of ANOVA are shown for comparsions between replicate streams (p str(trt)) and among treatments (p trt).

Treatment	Day 1		Day 3		Day 5		Day 10	
	% B	% P	% B	% P	% B	% P	% B	% P
Control	69.6 (6.38)	21.5 (6.07)	74.3 (4.99)	13.9 (2.58)	76.4 (6.27)	9.75 (3.20)	74.5 (6.05)	10.7 (2.63)
10 ug/L	75.1 (7.48)	21.3 (14.6)	75.3 (3.30)	10.9 (3.56)	78.3 (9.83)	9.25 (8.13)	77.5 (4.03)	9.25 (2.22)
30 ug/L	70.1 (7.92)	23.9 (10.5)	73.6 (2.21)	14.7 (4.92)	77.4 (5.32)	9.75 (3.30)	76.1 (2.38)	11.2 (0.96)
p str (trt)	0.3649	0.1983	0.7679	0.6420	0.5474	0.4303	0.2285	0.8304
p trt	0.5249	0.9763	0.1166	0.2085	0.9114	0.6678	0.7139	0.2428

Table 3.52. Total biomass (protein) on artificial substrates from MERS outdoor experimental streams dosed with selenium. Values are mean (SD in ug/ml. Results of ANOVA are shown for comparisons between replicate streams (p str(trt)) and among treatments (p trt).

Treatment	Day 1	Day 3	Day 5	Day 10
Control	164.2 (68.3)	189.3 (45.2)	90.7 (18.1)	81.8 (20.2)
10 ug/L	216.2 (55.9)	184.8 (34.4)	136.5[a] (20.7)	74.0 (15.2)
30 ug/L	138.5 (85.3)	119.2[a] (21.7)	84.4 (12.4)	73.5 (13.9)
p str(trt)	0.0165	0.8270	0.6155	0.0001
p trt	0.5761	0.0164	0.0146	0.9011

[a] Significantly different from control at $\alpha = 0.05$.

Table 3.53. Chlorophyll content on artificial substrates from MERS outdoor experimental streams dosed with selenium. Values are mean (SD) in ug/L. Results of ANOVA are shown for comparisons between replicate streams (p str(trt)) and among treatments (p trt).

Treatment	Day 1	Day 3	Day 5	Day 10
Control	2560 (1090)	2510 (714)	1513 (309)	952 (169)
10 ug/L	3304 (846)	2128 (486)	2058 (618)	923 (205)
30 ug/L	2458 (1677)	1824 (308)	1259 (303)	1023 (24)
p str(trt)	0.0163	0.6015	0.1475	0.0015
p trt	0.7676	0.1573	0.1917	0.8380

[a] Significantly different from control at $\alpha = 0.05$.

Table 3.54. Carbohydrate concentrations on artificial substrates from MERS outdoor experimental streams dosed with selenium. Values are mean (SD) in ug/ml. Partial results of ANOVA are shown for comparsions between replicate streams (p str(trt)) and among treatments (p trt).

Treatment	Day 1	Day 3	Day 5	Day 10
Control	43.3 (9.83)	48.3 (11.5)	24.8 (5.60)	14.2 (2.64)
10 ug/L	47.5 (8.62)	48.5 (9.52)	38.0 (9.74)	17.3 (4.76)
30 ug/L	26.8 (17.1)	37.7 (8.59)	19.8 (7.47)	12.7 (4.41)
p str(trt)	0.0096	0.4780	0.2627	0.0401
p trt	0.3470	0.2257	0.0833	0.5084

Table 3.55. Hexosamine content on artificial substrates from MERS outdoor experimental streams dosed with selenium. Values are mean (SD) in ug/ml. Results of ANOVA are shown for comparisons between replicate streams (p str(trt)) and among treatments (p trt).

Treatment	Day 1	Day 3	Day 5	Day 10
Control	12.7 (6.19)	17.3 (6.12)	13.3 (3.14)	7.83 (1.47)
10 ug/L	18.0 (6.36)	24.5 (7.17)	23.0 (4.43)	10.8 (2.99)
30 ug/L	8.83 (6.34)	13.0 (5.76)	11.0 (5.76)	7.17 (3.19)
p str(trt)	0.0581	0.3495	0.1260	0.4459
p trt	0.3677	0.1318	0.0840	0.1720

Table 3.56. Alkaline phosphatase activity on artificial substrates from MERS outdoor experimental streams dosed with selenium. Values are mean (SD) in ug/ml. Results of ANOVA are shown for comparisons between replicate streams (p str(trt)) and among treatments (p trt).

Treatment	Day 1	Day 3	Day 5	Day 10
Control	131 (18.2)	406 (72.2)	430 (38.6)	395 (44.8)
10 ug/L	136 (21.8)	378 (57.8)	426 (59.0)	461 (41.4)
30 ug/L	140 (18.2)	452 (70.9)	437 (49.2)	468 (81.4)
p str(trt)	0.3224	0.3947	0.9063	0.0583
p trt	0.8109	0.3279	0.7106	0.4012

Table 3.57. Periphyton production and photosynthetic efficiency (P/B) in MERS artificial streams dosed with selenium. Values are mean (SD).

Treatment	Production (P) (mg C/m^2/h)	Chlorophyll *a* (B) (mg/m^2)	P/B (mg C/mg Chl/h)
Control	529 (45.2)	121 (8.48)	4.37 (0.07)
10 ug/L	571 (22.6)	110 (5.66)	5.19[a] (0.06)
30 ug/L	454 (21.9)	124 (13.4)	3.66[a] (0.22)
p	0.0759	0.4124	0.0037

[a] Significantly different from control at $\alpha = 0.05$

Table 3.58. Results of ANOVA of responses between replicate streams. The p values are shown for significant differences between streams. NS = not significant ($p > 0.05$).

Response	d 1	d 3	d 5	d 10
Protozoan Species	NS	NS	NS	NS
Alk. Phosphatase	NS	NS	NS	NS
Chlorophyll	0.0163	NS	NS	0.0015
Protein	0.0165	NS	NS	0.0001
Carbohydrate	0.0096	NS	NS	0.0401
Hexosmaine	0.0581	NS	NS	NS

Table 3.59. Coefficients of variation for control responses measured in laboratory microcosms and outdoor artificial streams.

Response	Laboratory	Outdoor Streams
Hexosamine	30.0	31.6
Protein	18.8	27.6
Chlorophyll	15.7	27.3
Carbohydrate	15.1	21.9
Alkaline phosphatase	8.5	13.0
Species richness	4.2	7.8

Table 3.60. Chronic toxicity data for selenium from the literature. Values are ug/L.

Species	Duration	Endpoint	Effect Level	Ref.
Larval rainbow trout	90 d	Survival	47 (LOEC)	a
		Bone calcium	12 (LOEC)	a
Ankistrodesmus	2 wks	Biomass	10 (LOEC)	b
Scenedesmus	2 wks	Biomass	100 (LOEC)	b
Selenastrum	2 wks	Biomass	300 (LOEC)	b
Microcoleus	2 wks	Biomass	10,000 (LOEC)	b
Daphnia pulex	7 d	Mortality, growth, reproduction	280 (MATC)	c
Rainbow trout	90 d	Survivial	31 (NOEC)	d
		Weight	68 (NOEC)	d
		Length	68 (NOEC)	d

a Hunn et al., 1987

b Vocke et al., 1980

c Reading and Buikema, 1983

d Mayer et al., 1986

3.6 COPPER/ZINC MIXTURE TESTS

3.6.1 Introduction

Single toxicants rarely occur in waste streams. While water quality criteria (WQC) are derived for individual compounds, complex mixtures of toxic materials can appear in the environment. We conducted two short-term experiments examining the toxicity of copper and zinc mixtures using the mobile laboratory and located at the Penn State Waste Water Treatment Plant. In these experiments we tested the hypothesis that the EPA derived water quality criteria are protective of aquatic life and that any toxicity was independent of the mixture of toxicants.

3.6.2 Methods

Microcosm experiments were conducted using a standard protocol (Pratt and Bowers, 1990). Briefly, the microcosm protocol involved colonization of artifical substrates by microbiota at a reference site in Thompson Run, the stream originating from Thompson Spring, a hardwater spring. The cololonized substrates served as source communities in microcosms which were exposed to differing doses of toxicant mixture using continuous replacement of test medium. Diluent water, obtained from Thompson Spring near the site of the PSU WWTP, was supplied to the microcosms by a mini-diluter (Benoit et al. 1982) in which the serial diluter was replaced with five sets of nested (150 and 50 ml) beakers. A multi-channel peristaltic pump delivered copper and zinc stocks to the beakers at concentrations based on water quality criteria. Toxicant and diluent were mixed in the beakers, and the mixed overflow passed to the flow booster cells, the flow splitter cells of the diluter system, and then into the microcosms. Water hardness was 280 mg $CaCO_3$/L. Target concentrations were 28.5 ug Cu/L and 253.6 ug Zn/L and were calculated from hardness-based water quality criteria (USEPA 1986). Treatments were control, copper only, zinc only, copper and zinc, and copper and zinc at double their water quality criterion with microcosms dosed every other day.

3.6.3 Results

Effects of copper were much greater than those of zinc (Tables 3.61, 3.62). Copper exposure reduced species richness by nearly 50% in the first experiment and by 27% in the second. Zinc exposure reduced species numbers by between 15 and 20% in the two experiments. The effects of mixtures of the two toxicants were similar, and mixtures of the two metals did not result in significant increases in adverse effects. Mixture exposure frequency (continuous versus pulsed) did not alter community responses in the microcosms.

Permutation procedures for comparing community similarity (Pratt and Smith, 1991) also showed that alterations in community structure were primarily a result of copper exposure (Table 3.63). While Jaccard coefficients for average control similarity were similar between experiments, similarities of treatments to controls differed markedly. Copper exposed communities were less similar to each other and differed from controls. In the first experiment, there was little evidence of progressive, additive toxicity. However, in the second experiment, community structure continued to change with added toxicant. That is, copper effects were prominent and zinc effects were less severe. The two metals together decreased community similarity further and the variable exposure also adverse affected communities. These analyses are consistent with decreasing species richness in these communities and demonstrate dissimilarity among stressed communities.

Biomass measures were somewhat more variable. Total (protein) biomass was insufficient to detect, and chlorophyll biomass had to be estimated by in vivo fluorescence. Fluorescence analysis showed similar patterns to those found for species. Copper adversely affected biomass measures more than zinc, although zinc reduced algal biomass in the second experiment but not in the first. The combined activity of the two toxicants was heavily influenced by copper toxicity which was only slightly exacerabated by added zinc.

Process measures (dissolved oxygen, pH) were not effective in separating treatments, but gross alkaline phosphate activity declined with toxic input in both experiments. Because of our inability to detect protein, it was impossible to correct alkaline phosphatase activity for total biomass. If protein values had followed the same pattern as chlorophyll fluorescence, then the expected stimulation of activity with toxicity would have been observed.

3.6.4 Discussion

Based on these experiments, the interaction between copper and zinc at WQC levels shows modest evidence of additivity of effect. In general, however, the effect of copper was much greater than the effect of zinc, even though zinc exposure was much higher. Copper exposure at WQC significantly reduced taxa and biomass, while zinc was not consistently associated with adverse effects.

There are few experiments on toxicant interaction at metal levels near WQC. For example, McFarlane and Franzin (1978) studied effects of in place metal contaminants on white suckers, but did not separate the individual effects of cadmium, copper, and zinc. Clements et al. (1988) examined effects of combined doses of copper and zinc (12 ug/L of each metal) on macroinvertebrates in soft-water fed artificial streams and concluded that even these low

level exposures altered community structure. However, the zinc exposure in these experiments was over an order of magnitude below the present WQC, and the separate effects of the two metals were not determined. Therefore, it is impossible to use previous studies to distinguish between the toxicity of the two metals. A hypothesis of primary copper toxicity with little zinc effect could not be tested using published experimental data.

These experiments demonstrated that effects on community structure, and to a lesser degree community biomass, were easily discernable in microcosm tests. Given that these experiments were conducted by exposing natural communities and using naturally occurring water as diluent, it is clear that metal toxicity is extremely important in adversely affecting aquatic ecosystems. Combinations of toxicants suggest additive toxicity, even at low metal exposure.

Table 3.61. Results for experiments examining the effects of copper and zinc on microbial communities, Experiment 1. Data are treatment means (SD) for triplicate microcosms. Chl a units are fluorometric units. APA (alkaline phosphatase activity) units are nmole p-nitrophenol/hr and are corrected for protein. Protein units are mg/ml.

Treatment	Chl a	APA	PRO	Species richness
Control	2.95 (0.85)	1530.52 (356.12)	0.0044 (0.0012)	41.7 (1.2)
Copper	0.62 (0.12)	25751.08 (38324.71)	0.0009 (0.0007)	20.7 (2.1)
Zinc	1.12 (0.08)	4878.57 (30.30)	0.0009 (0.0002)	35.7 (1.5)
Copper + Zinc	0.54 (0.04)	7405.56 (2359.16)	0.0008 (0.0004)	18.7 (2.3)
Copper + Zinc (2x)	0.56 (0.06)	8301.59 (3563.91)	0.0005 (0.0002)	16.0 (0.0)

Table 3.62. Results for experiments examining the effects of copper and zinc on microbial communities, Experiment 2. Data are treatment means (SD) for triplicate microcosms. Chl a units are fluorometric units. AP(alkaline phosphatase) units are nmole p-nitrophenol/hr. Protein levels were not detectable.

Treatment	Chl a	AP	Species richness
Control	44.8 ('4.79)	3.91 (0.58)	45.7 (2.1)
Copper	17.6 (3.73)	2.17 (0.53)	33.3 (4.2)
Zinc	20.7 (1.48)	2.01 (0.16)	36.7 (3.8)
Copper + Zinc	14.3 (3.13)	2.79 (0.56)	32.7 (2.1)
Copper + Zinc (2x)	14.5 (1.84)	2.51 (0.22)	29.5 (0.7)

Table 3.63. Community structure comparison for copper-zinc mixture experiments. Comparisons show mean community similarities to controls. Statistical tests are based on permutation procedures.

	Experiment 1		Experiment 2	
Treatment	Jaccard coef.	Lambda value	Jaccard coef.	Lambda value
Contol	0.44	1.00	0.46	1.00
Copper	0.23	0.54	0.25	0.63
Zinc	0.27	0.63	0.36	0.77
Copper + Zinc	0.26	0.65	0.21	0.46
2 Copper + 2 Zinc	0.25	0.66	0.12	0.33
	$p < 0.001$		$p < 0.001$	

3.7 PUBLICALLY-OWNED TREATMENT WORKS (POTW)

3.7.1 Effluent characterization

Spring 1989. Total residual chlorine (TRC), free chlorine, monochloramines, dichloramines, and ammonia levels in the incoming effluent were monitored three times per week (Figs. 3.1-3.4). Based on temperature and pH, the concentration of un-ionized ammonia for each sampling period was calculated (Fig. 3.4). No free chlorine was ever detected.

Fall 1989. TRC levels were significantly reduced after new monitoring requirements were in effect. Effluent TRC was commonly undetectable and was always less than 0.06 mg/L.

3.7.2 Bioassays

During the spring study, the POTW effluent was acutely toxic to daphnids (100% mortality) at effluent concentrations $\geq$ 30%; at concentrations less than 30% there were no deaths. Although no effort was made to estimate an LC50 (as percent effluent), the toxic levels corresponded to TRC concentrations of at least 0.1 mg/L, well above the 0.01 criterion recommended by USEPA (EPA 1986). In fall of 1989, studies were resumed on this effluent after new reporting requirements for TRC were added to the NPDES permit. During this period, effluent TRC was often not detectable. Daphnid acute toxicity tests of 3-100% showed no acute toxicity (Table 3.64).

3.7.3 Microcosm experiments

Spring 1989. Epicenters used as species sources were colonized for 7 days at Station 2 in Spring Creek, located immediately upstream of the POTW discharge. See Tables 3.65 and 3.66 for species numbers and biomass data for the day 0 epicenters. Production in the microcosms was not adequate for the evaluation of P/Rs and this data is not presented.

Species richness on artificial substrates was elevated in response to effluent treatment at $\geq$ 25% on all sampling days (Table 3.65). Species survival on epicenters in the control microcosms was not satisfactory. Day 0 epicenters contained 42 species, whereas day 21, control epicenters contained only 27 species (65% of day 0). Epicenters from the 100% treatment contained 39 species (93% of day 0). Total microbial biomass (as protein) was also greater in microcosms receiving effluent (Table 3.66). On day 21, protein was significantly higher than control at $\geq$ 12.5% effluent. Alkaline phosphatase activity (APA) was not very sensitive to effluent treatment, although on day 21, APA was greater than control at 100% effluent. Calcium, magnesium, and potassium

concentrations responded in a similar manner as total biomass (Table 3.67). On day 21, concentrations of these elements were significantly greater than control at >25% effluent.

There were no differences in protozoan species richness among treatments in the epicenter experiment based on the examination of a single substrate from each treatment (Table 3.68). There were also no differences in protein, alkaline phosphatase, chlorophyll, or metals, and epicenter species survival was adequate. Total reactive phosphorus concentrations in all microcosms receiving effluent were greater than in control microcosms (Table 3.69), but phosphorus concentrations in epicenter samples were significantly greater than controls in only the highest concentration (100% effluent)

In the third experiment, protozoan species survival in laboratory microcosms was adequate using Spring Creek water as diluent. However, we observed no effect of POTW effluent on protozoan species richness (Fig. 3.5). These results are in agreement with results from the two previous microcosm tests using well water as diluent. In both the ambient stream survey and PFU transfer experiment, however, we found protozoan species numbers to be reduced directly downstream of the POTW discharge (see below). The difference between field and laboratory results could be related to several factors, such as abiotic components of the environment which affect community response which were missing in laboratory microcosms.

Total biomass was stimulated at high effluent concentrations (25-100%) in island substrata, but there was no effect on epicenter substrata biomass (Fig. 3.6). Alkaline phosphatase (APA) was elevated at 50 and 100% effluent in island substrata and at 100% effluent in epicenter substrata (Fig. 3.7). This stimulation of APA is unusual considering the high phosphorus content of the effluent (typically greater than 100 ug/L) and may reflect a disruption in metabolic function in response to effluent exposure. This response was observed in the 21 day microcosm experiment, but not in the 7 day experiment, in which well water was used as diluent. Elevated APA in substrata collected immediately downstream of the POTW discharge was observed in the ambient survey, but not in substrata transferred from above to below the discharge. There was no effect of effluent exposure on the chlorophyll a content of either island or epicenter substrata (Fig. 3.8).

The concentration of calcium and magnesium in island and epicenter substrata was unaffected by effluent exposure (Fig. 3.9). Potassium, however, was elevated in both island and epicenter substrata at 100% and ≥ 50%, respectively, reflective of the elevated biomass at these effluent concentrations.

Fall 1989. A final testing of the effects of the UAJA effluent was conducted in September 1989 using upstream water and the diluent and communities from station 2 as the epicenter material in the microcosms. Results of these experiments are summarized in Table 3.70. As before, no significant toxic effects of the effluent were observed, although increasing effluent dose produced increases in the numbers of species, biomass estimates, and enzyme activity. Dissolved oxygen was significantly reduced in higher effluent doses, but this effect corresponded to increased loading of organic carbon to the microcosms.

The direction and amount of change in microcosms corresponded during both study periods and appeared to be primary a response to nutrient inputs. The nutrient response was clear in Fall 1989 microcosm studies which mirrored the clear lack of toxicity based on instream effects and acute toxicity. Microcosm responses were similar during both the Spring and Fall study periods, even though instream effects and acute toxicity were obvious during spring.

3.7.4 Receiving stream analyses

Spring 1989. Spring creek microbial communities below the POTW discharge were clearly adversely affected. Species numbers decreased by over 80%, relative to upstream, at the first downstream station (3) and were still depressed at station 4 (Figs. 3.10, 3.11). Recovery was apparent at stations 5 and 6 with some enhancement evident. Downstream microbial biomass (protein, chlorophyll, Figs. 3.12, 3.13) did not differ relative to upstream, but stimulation of microbial biomass was evident at stations 5 and 6 which were affected by a phosphorus containing effluent from the Benner Springs fish hatchery. Community macronutrients reflected this stimulation even though inputs of phosphorous did not reach maximum until the most downstream station (Figs. 3.14-3.16). Comparable studies in the fall of 1989 showed evidence of significant adverse alteration from the POTW effluent (Table 3.71).

Macroinvertebrate communities did not show the same strong response to the effluent as microbial species (Fig. 3.17). Qualitative samples showed greater densities of isopods downstream of the POTW discharge. Isopods are known to be tolerant of organic pollution. Toxic effects such as the elimination of taxa and reduced diversity were not observed.

Transfer experiment. Protozoan species richness, total protein, chlorophyll *a*, and APA were 61.3 $\pm$ 4.04 species, 145 $\pm$ 14.0 ug/ml, 5325 $\pm$ 795 ug/L, and 576 $\pm$ 44.3 nmole p-nitrophenol/mg protein/h, respectively. Seven days after transfer, all of the substrates were collected and sampled for the same endpoints as above and electron transport system activity and total reactive

This experiment was conducted to examine the impact of the POTW discharge on microbial communities from an upstream, unimpacted source. We found protozoan species richness to be the most sensitive indicator of adverse effects, with species numbers being reduced approximately 50% at Station 3, the most immediate downstream station (Fig. 3.18). Species numbers recovered rapidly and were elevated at the last station. This pattern of protozoan species richness is similar to that observed in the instream survey.

We found no effect of transfer on protein biomass (Fig. 3.19), electron transport system (Fig. 3.20) but did find reduced chlorophyll a (Fig. 3.21) and alkaline phosphatase activity (Fig. 3.22) which we had not observed in the ambient stream survey. The effect on the transferred communities and not on the indigenous communities may indicate an adaptation of the indigenous communities to the UAJA discharge.

Fall 1989. Microbial communities showed no evidence of earlier perturbation after reduction of effluent TRC levels. Downstream communities generally fell within the range of upstream variability. There was some enhancement of chlorophyll biomass and concomitant increases in potassium content.

Macroinvertebrate samples (Table 3.72) did not show evidence of significant changes in downstream communities.

3.7.5 Summary and Conclusions

Studies of the POTW effluent, which contained potentially toxic levels of chlorine and unionized ammonia, showed acute toxicity to daphnids and significant instream effects on microbial communities during Spring 1989. Instream analyses of invertebrate communities showed changes in faunistic composition but no obvious effects on numbers of species. Microcosm tests did not provide evidence of the assumed toxicity of the effluent. No significant adverse changes were measured despite several variations of methods used to assess microcosm toxicity. The origin of negative results in the microcosms is unclear because previous microcosm studies of chlorine toxicity (Pratt et al. 1988) and chlorine-ammonia interaction (Niederlehner et al., 1990) have shown the sensitivity of the microcosm design to very low levels of perturbation. However, neither of the previous studies has examine the effect of increasing nutrient levels on the expression of toxicity. In the case reported here, nutrient effects overwhelmed any toxic effects despite relatively high levels of toxic materials in the effluent mixture.

Table 3.64 Water chemistry of diluent water and POTW effluent used in the 48h Daphnia magna test conducted in September 1989.

Sample	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Cond. (umho/cm²)	Hardness (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)
Diluent	21.3	8.65	8.54	531	368	223
Effluent	20.7	7.05	6.95	845	320	165

Table 3.65 Protozoan species richness in artificial substrates from microcosms exposed to UAJA effluent with well water as the diluent. Values are mean (SD) and values significantly different than control are designated with an asterisk. Also shown for comparison is the species richness in day 0 epicenters.

Treatment	Day 7 Islands	Day 14 Islands	Day 21 Islands	Day 21 Epicenters
Control	15.7 (2.89)	16.3 (1.15)	17.0 (1.00)	27.5 (0.07)
6.25%	18.0 (1.00)	16.7 (2.89)	22.0 (1.73)	
12.5	17.0 (1.73)	22.0 (1.00)	26.7* (1.15)	
25	25.3* (2.89)	36.7* (3.21)	35.7* (1.15)	
50	25.3* (1.53)	38.0* (6.08)	40.0* (4.58)	
100	33.7* (4.51)	36.0* (5.29)	40.0* (5.69)	39.0 (4.24)
p	0.0001	0.0001	0.0001	
Day 0 Epicenters	42.3 (3.21)			

Table 3.66 Microbial biomass (protein) and alkaline phosphatase activity in artificial substrates from microcosms exposed to UAJA effluent with well water as the diluent. Values are mean (SD) and values significantly different than control are designated with an asterisk.

Treatment	Protein (ug/ml) Day 7	Day 14	Day 21	Alkaline Phosphatase[a] Day 7	Day 14	Day 21
Control	1.80 (0.28)	2.13 (0.38)	10.8 (0.72)	570 (10.6)	620 (144)	420 (155)
6.25%	1.67 (0.78)	4.77 (2.16)	13.3 (4.88)	1103 (382)	809 (363)	512 (92)
12.5	2.10 (0.80)	6.00 (1.38)	32.6* (2.43)	1157 (307)	770 (230)	869 (115)
25	4.90 (1.21)	12.5 (3.88)	42.6* (12.1)	1113 (222)	793 (89)	775 (161)
50	10.2* (3.52)	41.0* (10.4)	48.8* (18.4)	1074 (466)	619 (125)	840 (160)
100	16.7* (4.27)	56.9* (24.9)	49.6* (3..31)	809 (118)	928 (305)	1345* (366)
p	0.0001	0.0002	0.0015	0.3735	0.5725	0.0112
Day 0 Epicenters		436 (88.9)			388 (36.0)	

[a] nmole p-nitrophenol/mg protein/h

Table 3.67 Calcium, magnesium, and potassium in artificial substrates from microcosms exposed to UAJA effluent with well water as diluent (Day 21). Values are mean (SD) and values significantly different than control are designated with an asterisk.

Treatment	Calcium (mg/L)	Magnesium (mg/L)	Potassium (mg/L)
Control	7.80 (0.90)	8.93 (2.75)	3.87 (0.32)
6.25%	9.67 (1.33)	11.8 (3.67)	3.47 (1.50)
12.5	12.7 (0.98)	15.5 (6.72)	4.70 (0.85)
25	19.3* (3.46)	21.7* (0.47)	6.00* (0.66)
50	34.9* (1.78)	21.6* (6.46)	9.93* (0.45)
100	62.8* (5.58)	39.6* (4.52)	18.4* (1.36)
p	0.0001	0.0001	0.0001

Table 3.68 Microbial parameters measured in artificial substrates from microcosms exposed to UAJA effluent for 7 days with well water as diluent. Values are mean (SD) and values significiantly different than control are designated with an asterisk. Protozoan species richness was determined in a single sample.

Treatment	Species	Protein	APA	Chl *a*	Ca	Mg	K
Control	47	116 (20.4)	433 (86.7)	1772 (289)	413 (87.8)	36.3 (11.6)	16.9 (4.06)
6.25%	40	132 (32.0)	392 (37.5)	2225 (694)	410 (108)	38.1 (15.8)	12.8 (3.04)
12.5	42	133 (24.8)	409 (15.1)	1887 (398)	403 (75.2)	42.9 (10.4)	15.5 (2.44)
25	41	155 (12.5)	386 (36.7)	2396 (227)	452 (17.4)	48.6 (9.58)	13.1 (5.02)
50	44	147 (22.7)	442 (11.7)	2385 (1174)	495 (110)	55.1 (2.52)	18.0 (2.82)
100	49	120 (20.1)	476 (26.0)	1973 (612)	421 (53.1)	56.3 (2.20)	20.5 (0.84)
p		0.3263	0.1737	0.7693	0.7304	0.1240	0.1015
Day 0 Epienters	53.0 (7.00)	112 (17.5)	1212 (127)	5377 (572)	-	-	-

Units: Protein ug/ml
APA nmole p-nitrophenol/mg protein/h
Chl *a* ug/L
Ca, Mg, K mg/L

Table 3.69 Total reactive phosphorus levels in microcosms dosed with UAJA effluent with well water as diluent. Also shown are total reactive phosphorus in epicenter substrates collected after 7 days of exposure. Values are mean (SD) and values significantly different than control are designated with an asterisk.

Treatment	Total Reactive Phosphorus (ug/L) Epicenter	Microcosm
Control	94.0 (12.8)	< 10
6.25%	72.0 (10.8)	16.0* (4.35)
12.5	76.7 (14.0)	17.7* (5.86)
25	91.3 (10.5)	28.0* (2.64)
50	94.0 (7.00)	52.3* (2.52)
100	129* (15.3)	104* (4.62)
p	0.0014	0.0001

Table 3.70 Effect of POTW effluent on microbial communities in laboratory microcosms after 7 days of exposure. Values are mean (SD) and values significantly different from control are designated with an asterisk. The units for alkaline phosphatase activity are nmole p-nitrophenol/mg protein/hr.

Treatment	Protozoan Species Total	% B	% P	Protein (ug/ml)	Alk. Phos.	Chl a (ug/L)	Fluor. Units	DO (mg/L)
Control	43.7 (3.79)	74.7 (2.93)	11.4 (3.62)	11.2 (8.09)	1412 (531)	256 (106)	36.9 (8.62)	9.23 (0.37)
6.25%	48.0 (6.24)	71.8 (6.31)	13.0 (3.24)	12.7 (7.87)	1568 (549)	397 (109)	66.2 (12.8)	8.54 (0.22)
12.5	53.3 (9.71)	76.2 (5.20)	11.2 (2.96)	13.7 (6.01)	1330 (261)	375 (152)	54.8 (23.3)	8.94 (0.08)
25	52.3 (9.29)	73.6 (1.94)	12.6 (0.72)	19.2 (8.01)	1021 (229)	516 (188)	69.4 (25.3)	8.16* (0.05)
50	59.7 (9.50)	72.7 (3.96)	10.0 (0.08)	18.8 (6.64)	1094 (140)	530 (152)	74.7 (18.2)	7.16* (0.50)
p	0.233	0.698	0.637	0.585	0.425	0.201	0.000	0.182

Table 3.71. Microbial parameters measured from artificial substrata placed in Spring Creek. Values are mean (SD).

Site	Species	Protein (ug/ml)	APA[a]	Chl a (ug/L)	Ca (mg/L)	Mg (mg/L)	K (mg/L)
1	43.7 (3.51)	273 (35.7)	379 (52.3)	987 (114)	1748 (63.1)	115 (12.4)	4.33 (0.98)
2	42.3 (4.62)	317 (38.4)	374 (39.1)	1068 (324)	1659 (55.9)	109 (14.1)	4.85 (0.69)
3	--[b]	304 (50.5)	373 (8.89)	638 (100)	1800 (40.2)	80.3 (5.86)	4.04 (0.58)
4	38.7 (1.15)	250 (19.1)	442 (59.9)	1112 (58.8)	1287 (107)	141 (9.50)	6.02 (0.60)
5	44.3 (2.08)	274 (30.7)	503 (36.1)	1068 (190)	1138 (142)	109 (18.9)	10.4 (0.68)
6	56.3 (2.89)	319 (48.9)	468 (52.6)	1261 (167)	1563 (108)	143 (15.4)	8.25 (0.24)
7	48.0 (2.65)	407 (14.3)	405 (16.0)	1187 (279)	1774 (109)	95.7 (20.6)	8.80 (1.05)
8	41.3 (2.08)	309 (24.9)	533 (16.1)	1617 (290)	1284 (240)	117 (23.1)	9.63 (1.60)

[a] nmole p-nitrophenol/mg protein/h
[b] -- no data available

Table 3.72. Spring Creek macroinvertebrates. Number of indivduals/taxonomic group per site.

Taxa	1	2	3	4	5
Oligochaeta			2		1
Isopoda					
Aselidae					
Caecidotea sp.		2	3		
Lirceus sp.	15		5	163	24
Amphipoda					
Gammaridae					
Gammarus sp.	372				
Hydracarina	2	7	11	20	62
Ephemeroptera			3		
Baetidae				15	
Baetis sp.	222	4	5	180	322
Ephemerllidae					
Serratella sp.	59				
Heptageniidae					
Stenacron sp.		1	4		1
Stenonema sp.	9				
Tricorythidae					
Tricorythodes sp.			1	1	1
Hemiptera	1	1	1	1	
Tricoptera				2	
Hydropsychidae	190	20	1	348	724
Hydropsyche sp.		1	1	141	294
Ceratopsyche sp.	480	20	8	144	256
Cheumatopsyche sp.	7	3	2	50	23
Beraeidae					2
Brachycentridae					
Micrasema sp.	1				
Hydropsychidae					
Hydroptila sp.				1	
Rhyacophilidae					
Rhyacophilia sp.	41				
Hymenoptera	1		1		2
Coleoptera					
Elmidae					
Optioservus sp.	96		1		1
Psephenidae					
Ectopria sp.			1		
Diptera	3	7		2	3
Chironomidae	187	102	148	241	322
Empididae					
Chelifera sp.		1			
Hemerodromia sp.	5		10	2	1
Ephydridae	1				
Simulidae					
Simulium	56	4	5	2	2
Tipulidae		6			18
Antocha sp.	119	80	29	23	98
Gastropoda					
Ancyclidae					
Ferissia sp.	1				
Total Individuals	1868	259	242	1336	2157
Total Taxa	21	15	20	17	19

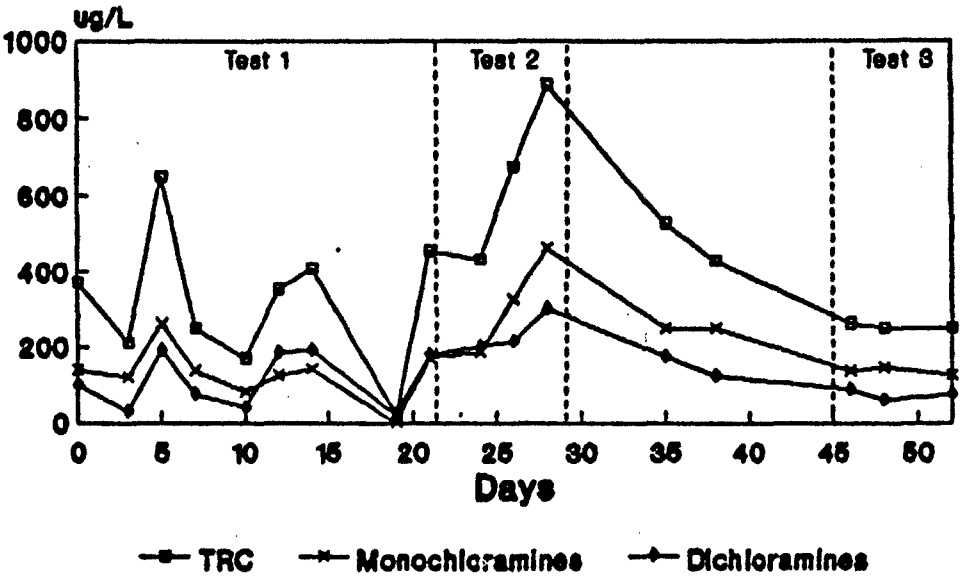


Fig. 3.1. Total residual chlorine (TRC), mono-, and dichloramine concentrations in incoming effluent during continuous flow microcosm experiments at the UAJA facility. No tests were conducted from day 28 to day 45.

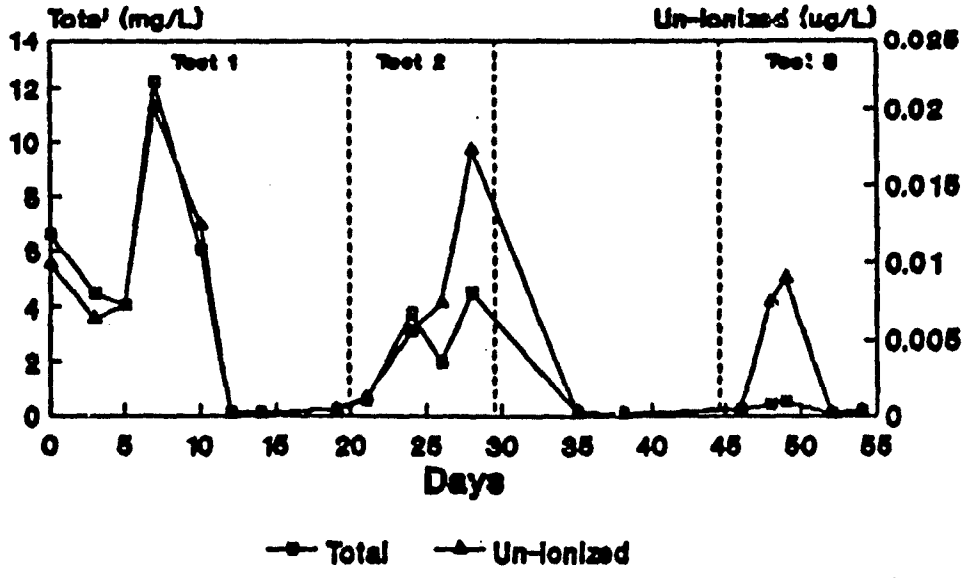


Figure 3.2. Total and un-ionized ammonia in incoming effluent during continuous flow microcosm experiments at the UAJA facility. No tests were conducted from day 28 to day 45.

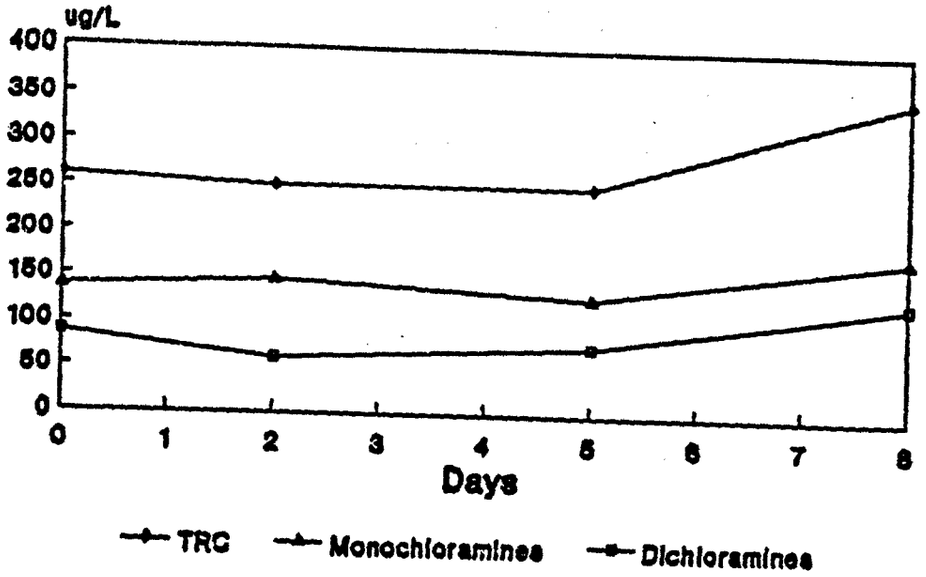


Figure 3.3. Total residual chlorine (TRC), mono- and dichloramine concentration in UAJA effluent during the microcosm experiment.

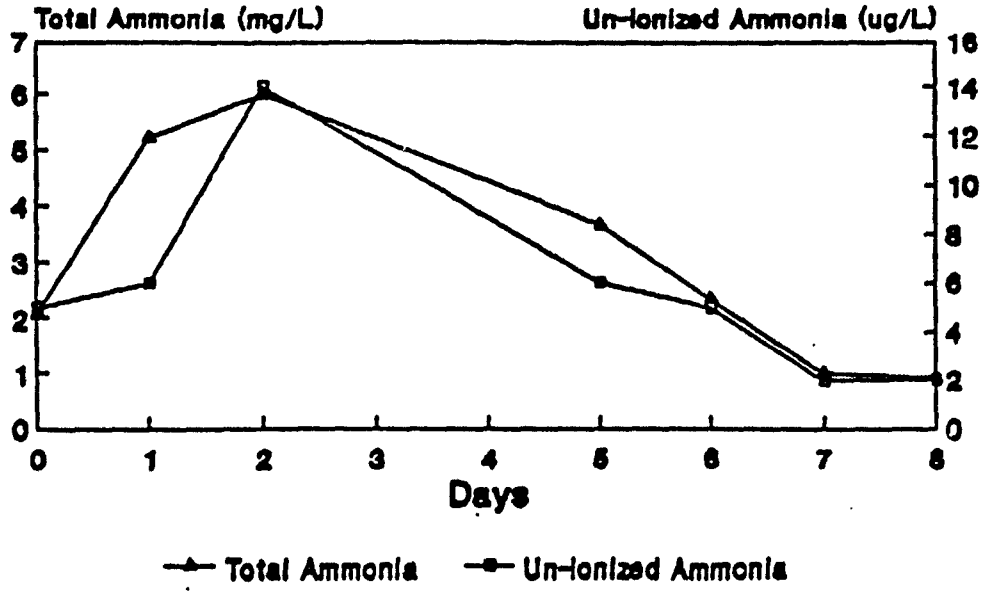


Figure 3.4. Total ammonia and un-ionized ammonia in UAJA effluent during the microcosm experiment.

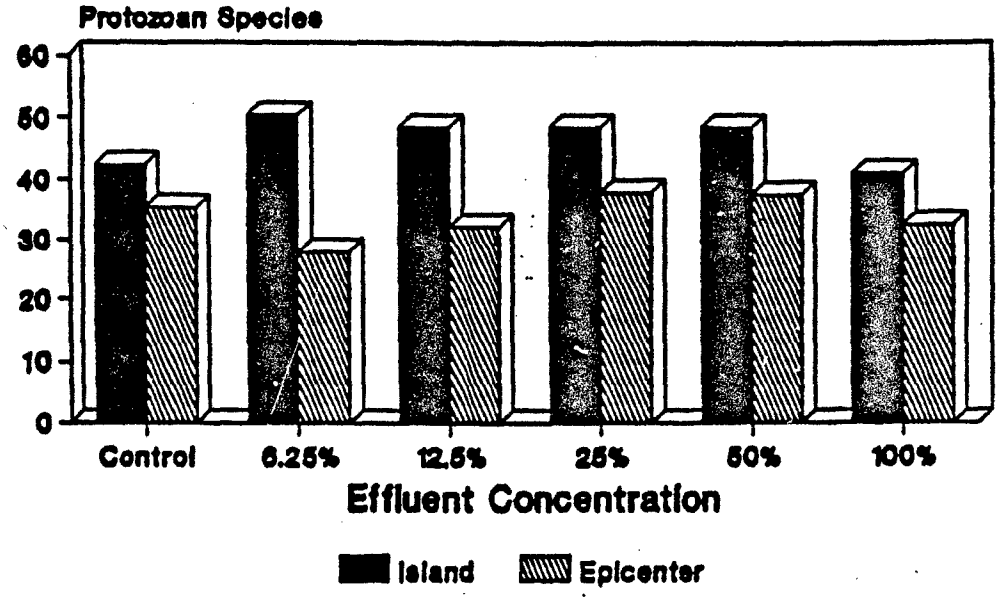


Figure 3.5. Protozoan species richness on island and epicenter substrata after 7 and 8 days, respectively.

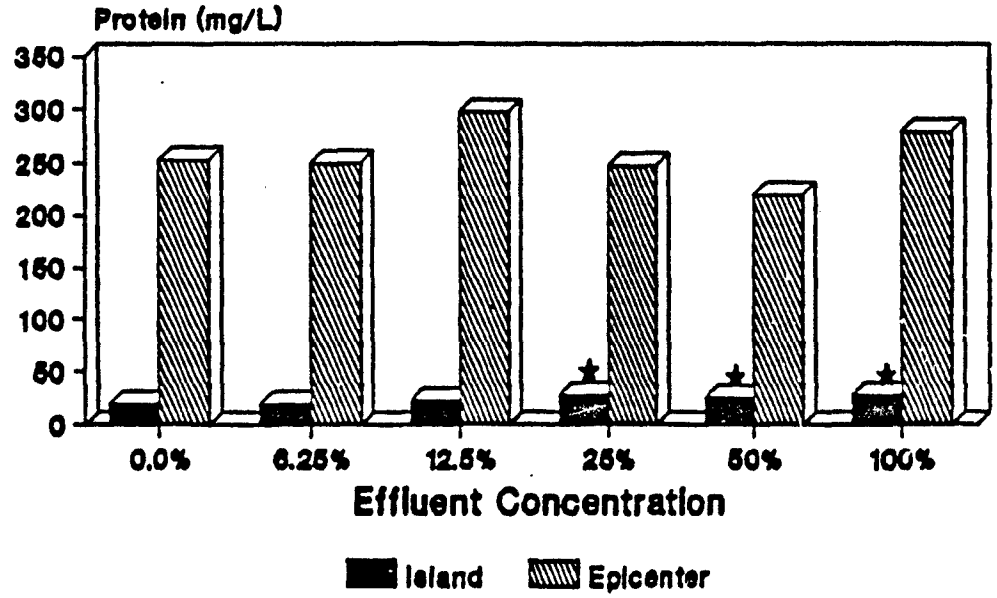


Figure 3.6. Total protein on island and epicenter substrata after 7 and 8 days, respectively. Values significantly different than control are designated with a star.

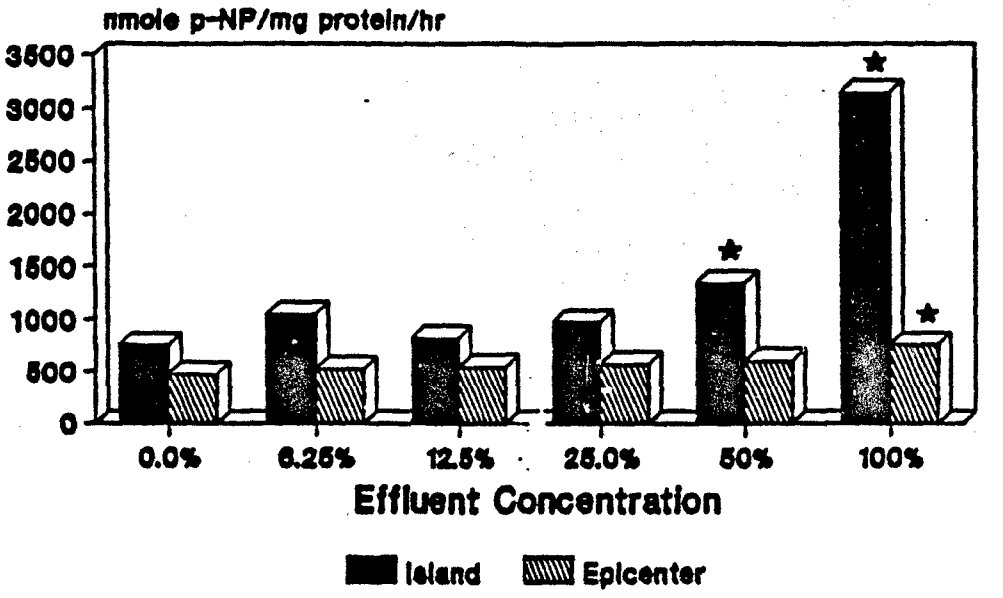


Figure 3.7. Alkaline phosphatase activity in island and epicenter substrata after 7 and 8 days, respectively. Values significantly different than control are designated with a star.

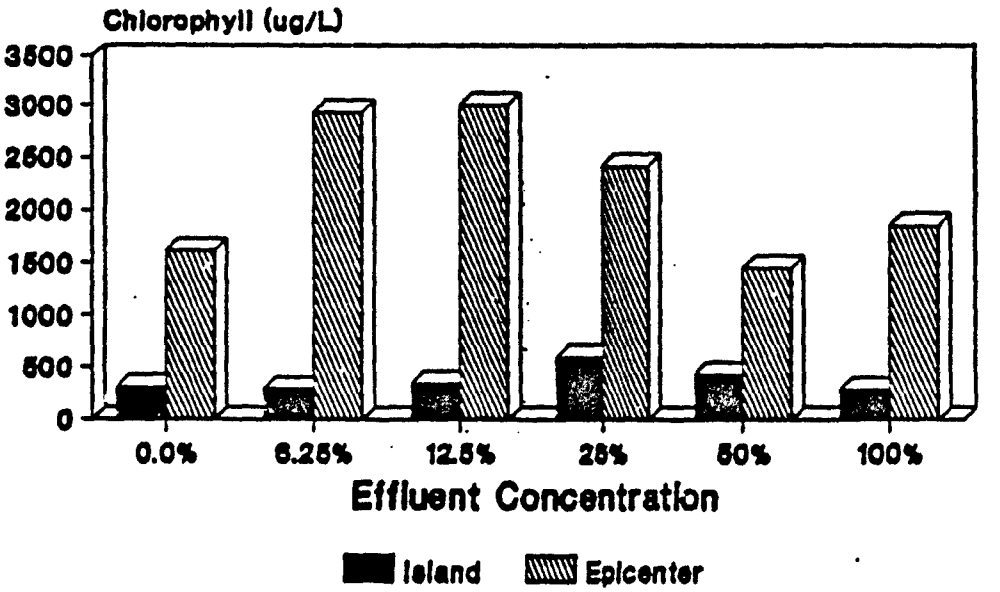


Figure 3.8. Chlorophyll a content on island and epicenter substrata after 7 and 8 days, respectively.

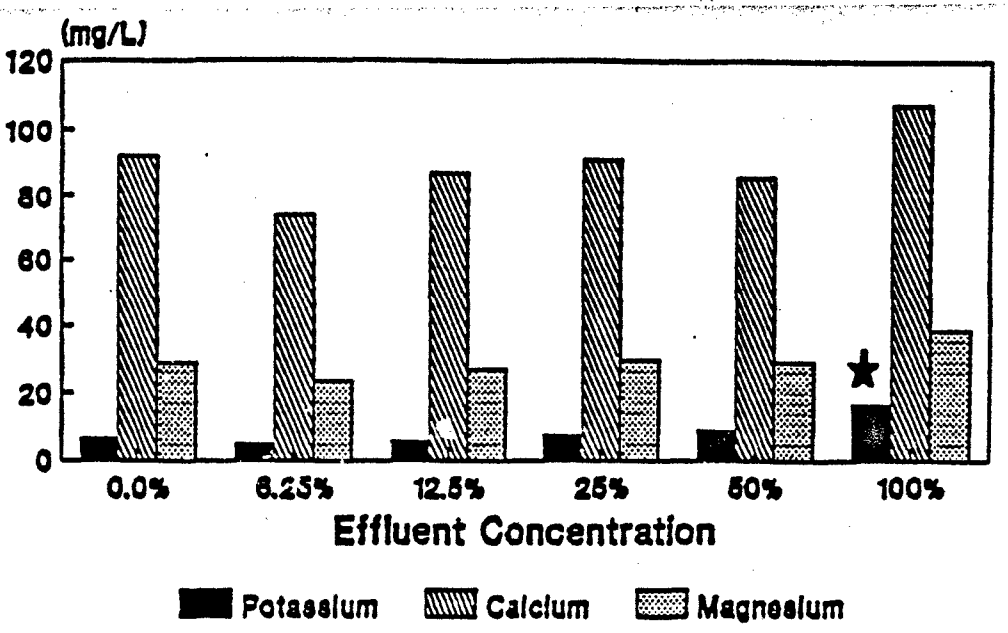


Figure 3.9.. Potassium, calcium, and magnesium concentration on island substrata after 7 days. Values significantly different than control are designated with a star.

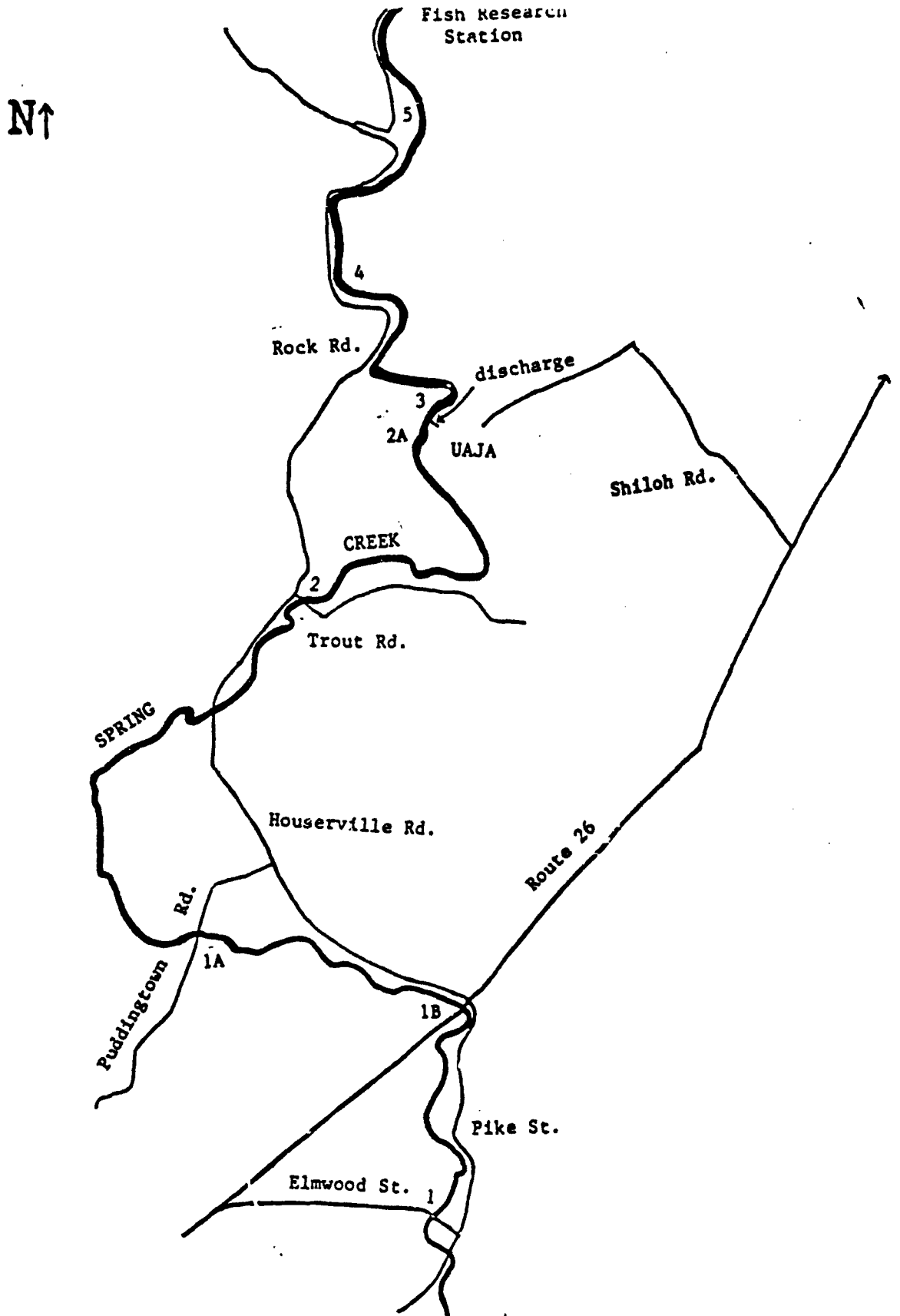


Figure 3.10. Sampling sites in Spring Creek associated with UAJA discharge. See site descriptions that follow.

Site Descriptions

Spring Creek Macroinvertebrates

Site 1 = The riffles on the north side (downstream) of the Elmwood Street bridge. The substrate primarily ranges from sand to gravel. The water depth ranges from ~0.1m-0.5m. Partial sun blockage (~20% sun blockage) due to tree cover.

Site 1A = Near Spring Creek Park on the east side (upstream) of the Puddingtown Road bridge. The substrate is primarily muck and sand. Water depth ranges from 0.5m-0.75m. The stream receives partial shade from trees overhanging the banks.

Site 2 = The riffle area on the north side (downstream) of the Trout Road bridge. The substrate primarily ranges from gravel to cobble. The water depth ranges from ~0.2m-0.5m. The stream is partially shaded by trees on the northwest bank, where as the southeast bank is completely open.

Site 2A = Just upstream of the UAJA discharge. The substrate ranges from muck to sand. Water depth is ~0.5m. The stream is relatively open with sparse cover from a few trees.

Site 3 = Samples were collected in the riffles ~20m downstream of the UAJA discharge. The substrate ranged from gravel to cobble. Water depth ranged from 0.1m-0.3m. The stream bank is open on the east side and partially shaded by overhanging trees on the west bank.

Site 4 = Samples were collected in the riffled area along Rock Road, about 1.2 miles downstream from the previous site. The substrate is mostly cobble. Water depth ranges from ~0.3m to 0.7m. The middle portion of the stream is open with sparse shading along the banks.

Site 5 = Samples were collected in the riffle areas off Rock Rd. about 100m downstream of the blue railroad bridge approximately 1.5 miles downstream of the previous site. Water depth ranges from ~0.3m-0.5m. The substrate ranges from sand to gravel with some cobble thrown in.

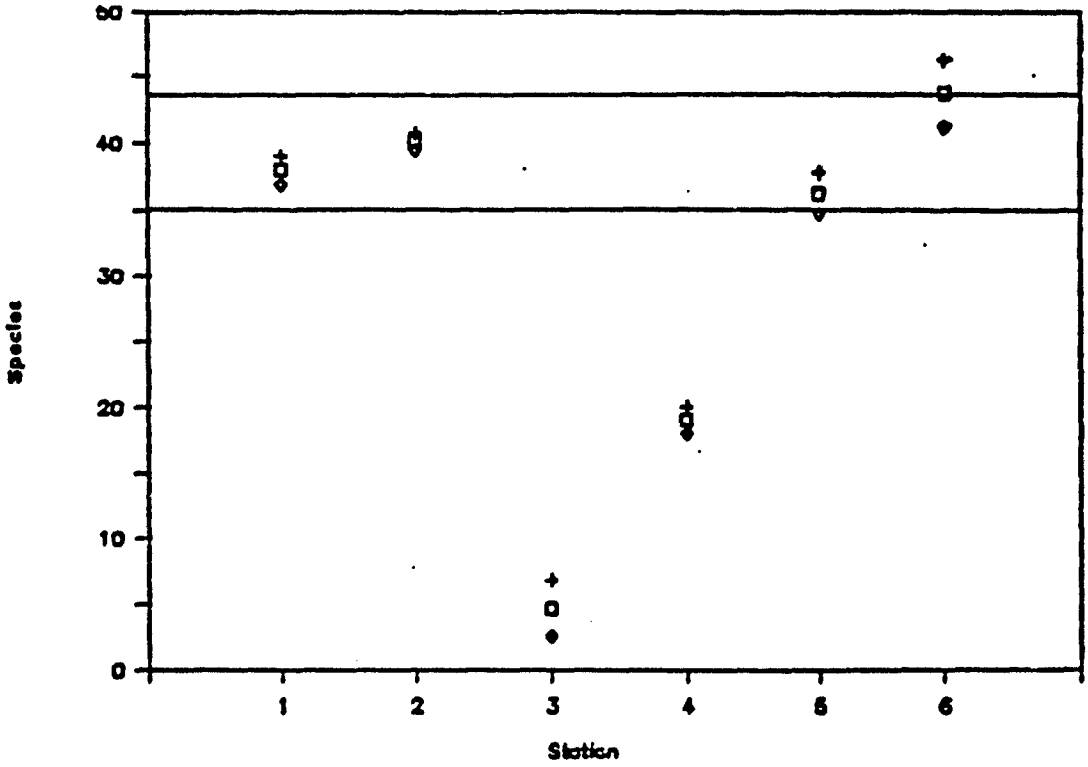


Figure 3.11. Protozoan species richness in artificial substrates from Spring Creek. Solid lines represent the 95% confidence interval around the mean value for the two stations upstream of the STP. The squares are mean and the plus and diamond represent +1 SD and -1 SD, respectively.

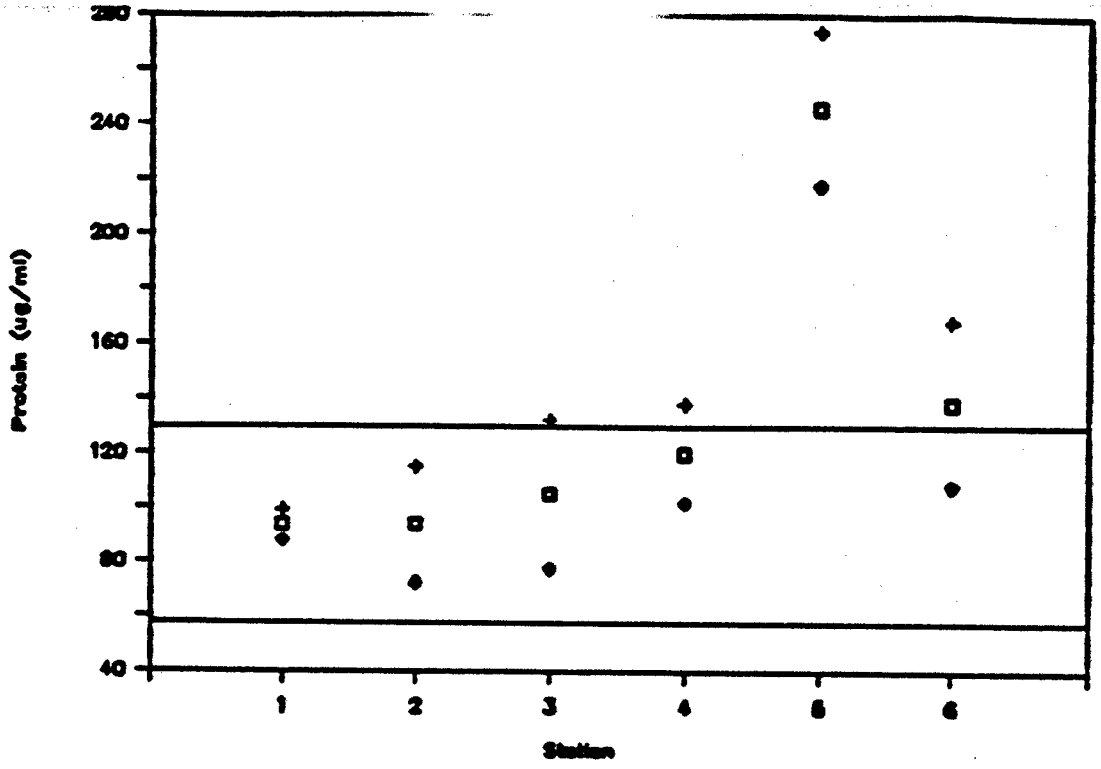


Figure 3.12. Protein biomass on artificial substrates from Spring Creek. See Fig.3.11 for explanation of symbols.

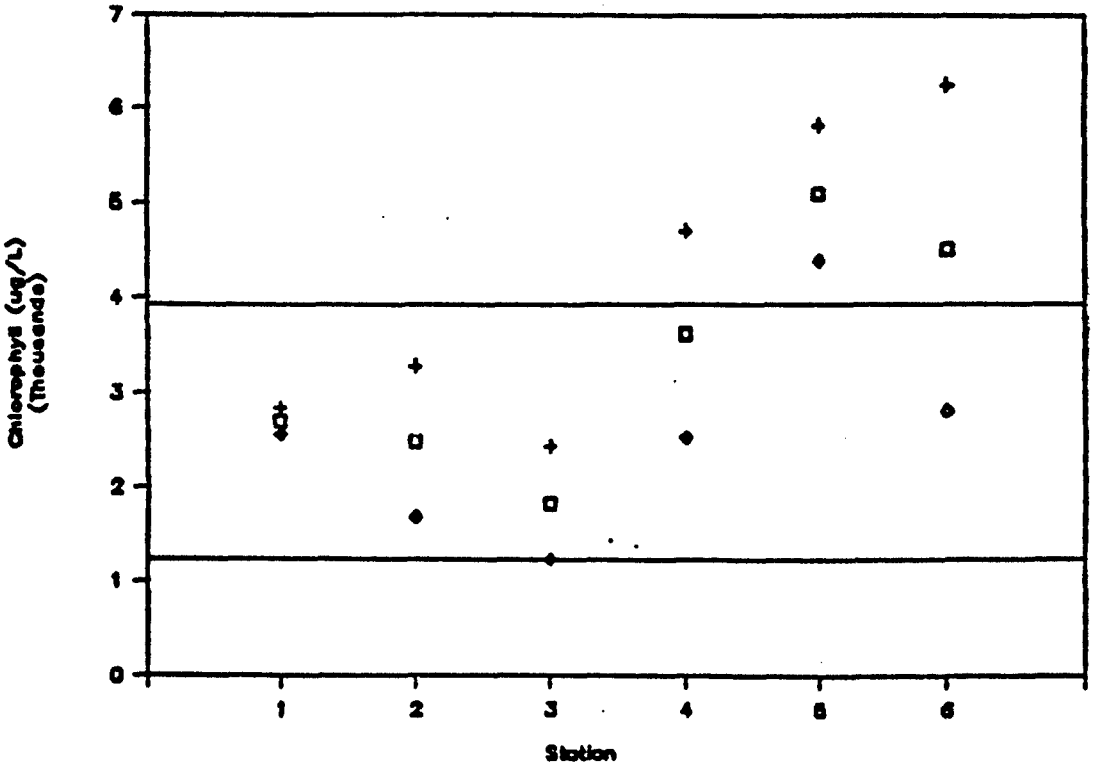


Figure 3.13. Clorophyll a biomass on artificial substrates from Spring Creek. See Fig.3.11 for explanation of symbols.

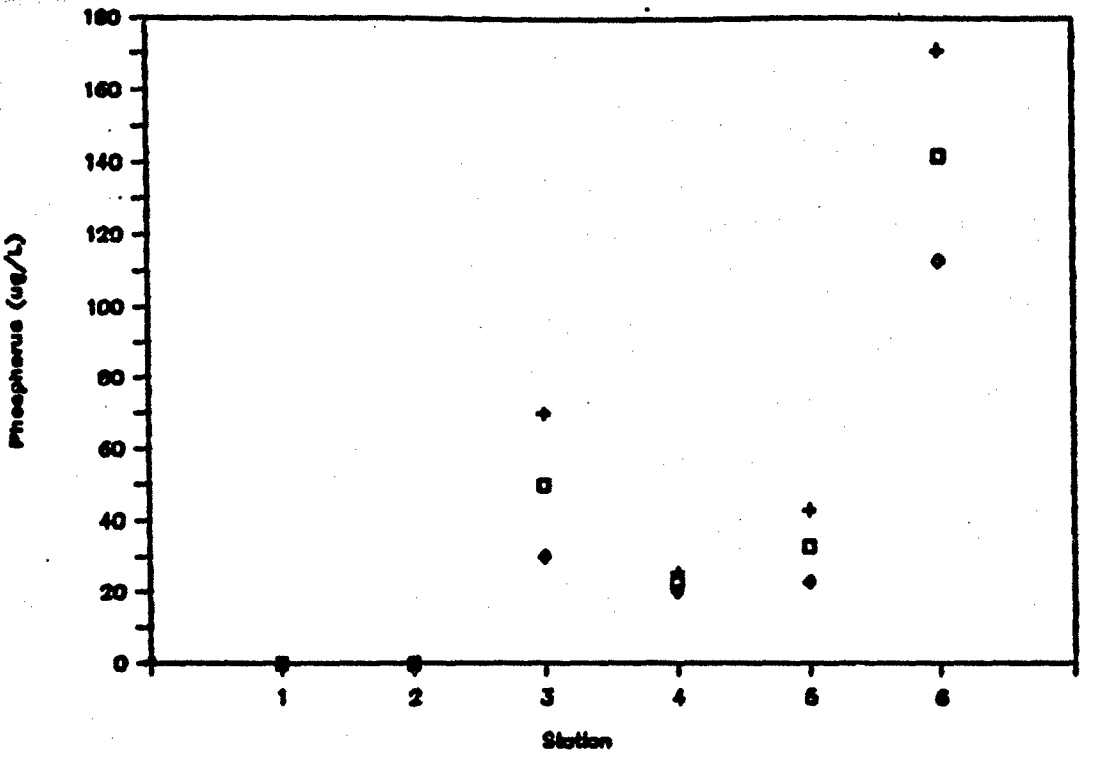


Figure 3.14. Soluble reactive phosphorus in artificial substrates from Spring Creek. See Fig. 3.11 for explanation of symbols.

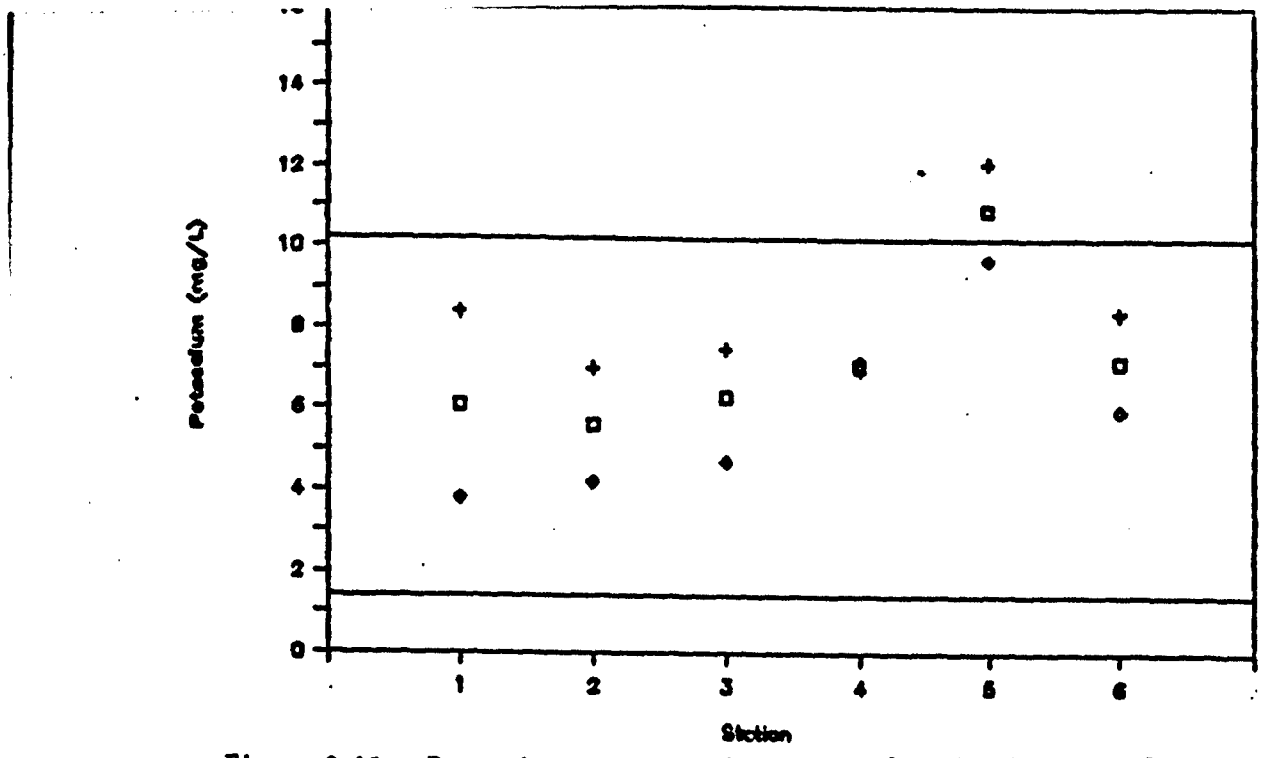


Figure 3.15. Potassium concentration in artificial substrates from Spring Creek. See Fig. 3.11 for explanation of symbols.

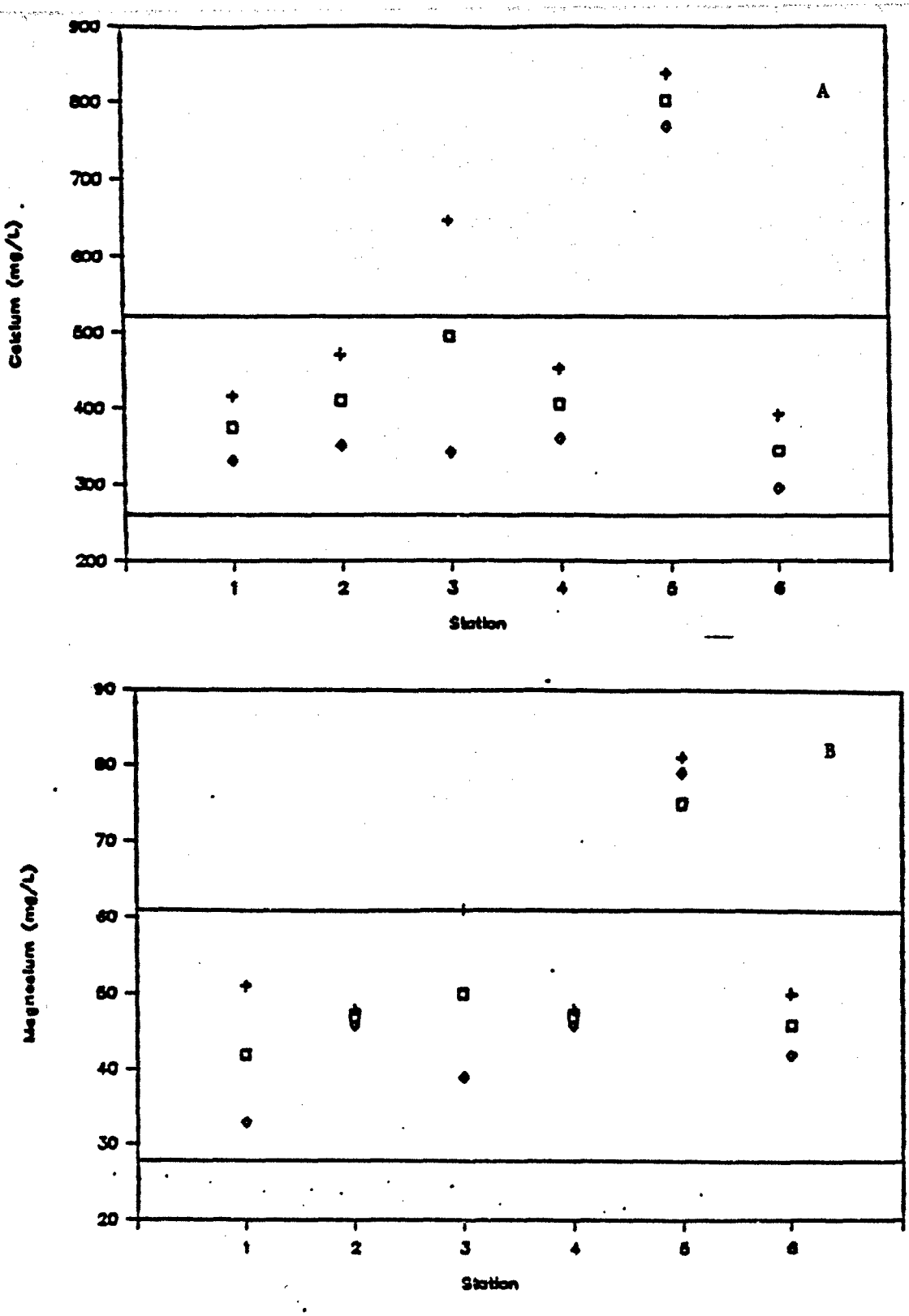


Figure 3.16. Concentrations of macronutrients in artificial substrates from Spring Creek. A. Calcium. B. Magnesium. See Fig. 3.11 for explanation of symbols.

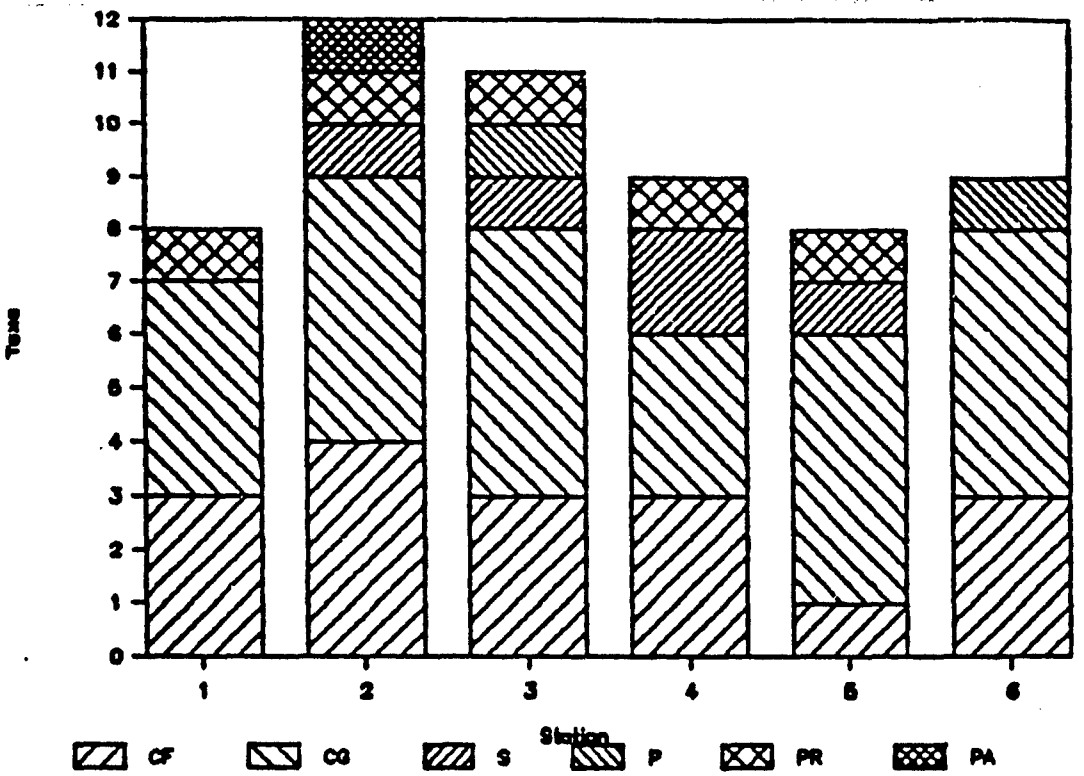


Figure 3.17. Macroinvertebrate species richness and functional group compostition in Spring Creek. Discharge from the UAJA STP occurs between Stations 2A and 3. CF = collector, filters; CG = collector, gatherers; S = scrapers; P = piercers; PR = predators, scavengers; and PA = parasites. No shredders were found at any of the stations.

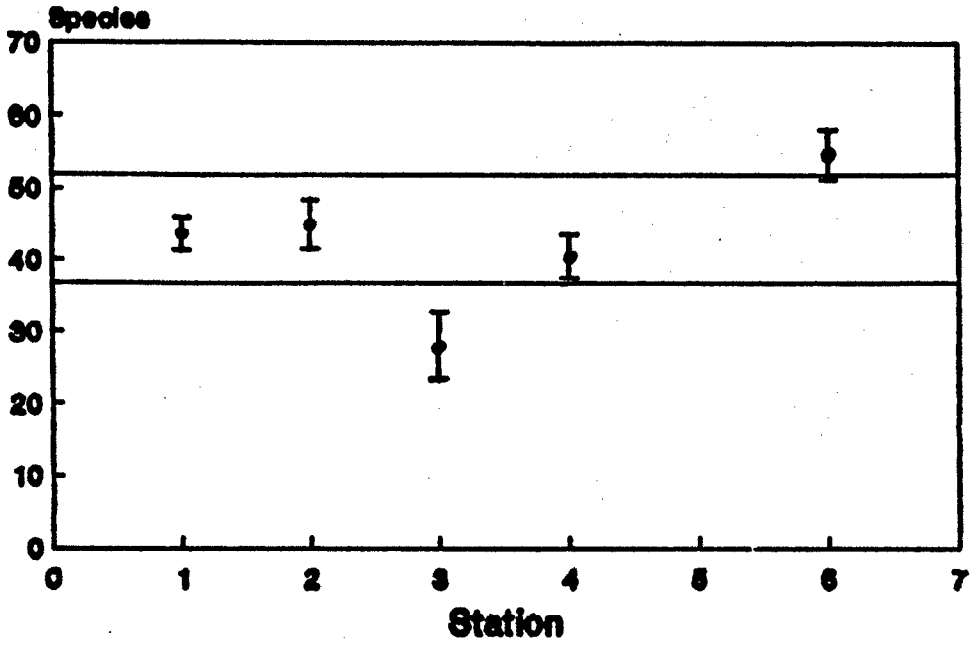


Figure 3.18. Protozoan species richness on PFU substrates seven days after transfer from Station 2A. Values are mean with one standard deviation shown. The solid lines represent the 95% confidence interval around the mean value for Station 1A and 2A.

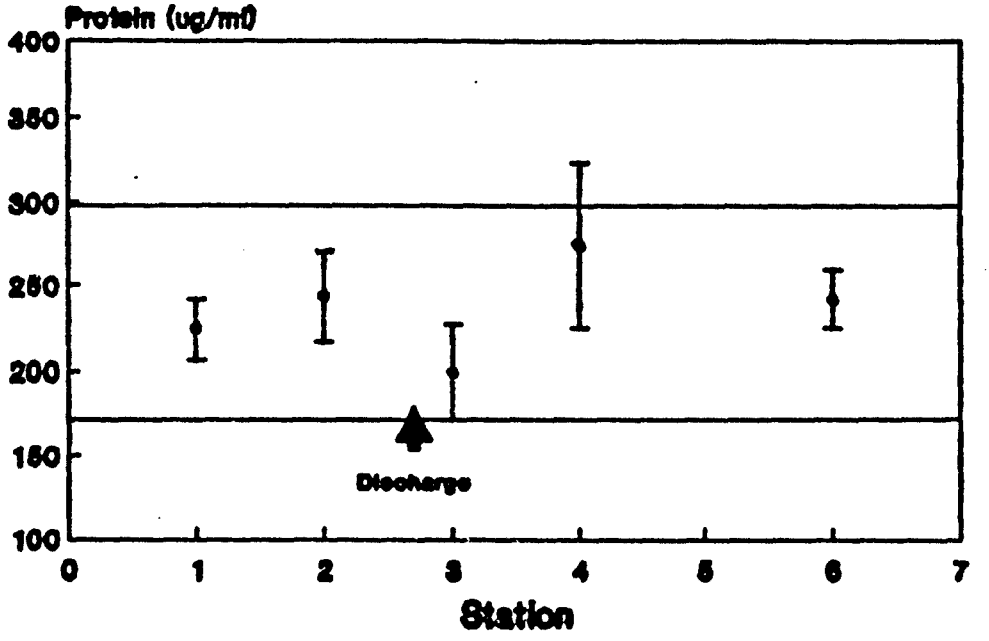


Figure 3.19. Microbial protein biomass on PFU substrates seven days after transfer from Station 2A. Values are mean with one standard deviation shown. The solid lines represent the 95% confidence interval around the mean value for Station 1A and 2A.

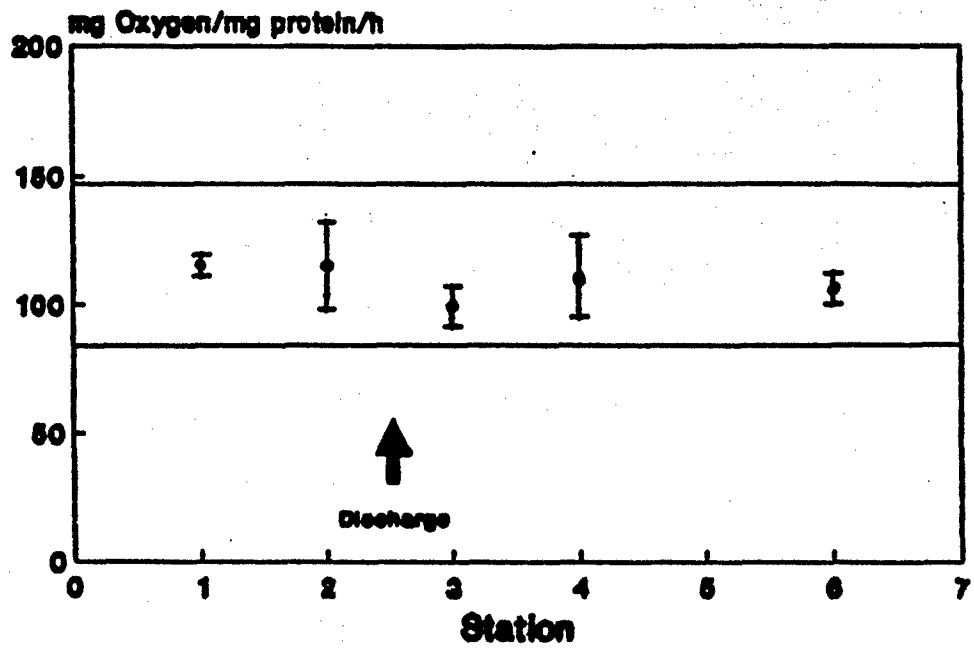


Figure 3.20. Microbial electron transport system activity on PFU substrates seven days after transfer from Station 2A. Values are mean with one standard deviation shown. The solid lines represent the 95% confidence interval around the mean value for Station 1A and 2A.

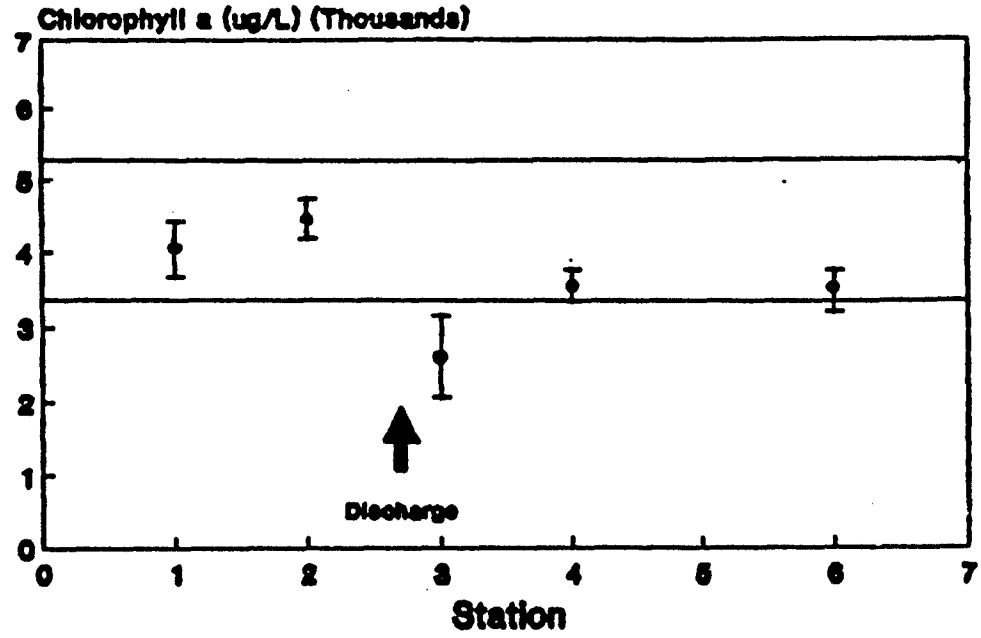


Figure 3.21. Chlorophyll a concentrations on PFU substrates seven days after transfer from Station 2A. Values are mean with one standard deviation shown. The solid lines represent the 95% confidence interval around the mean value for Station 1A and 2A.

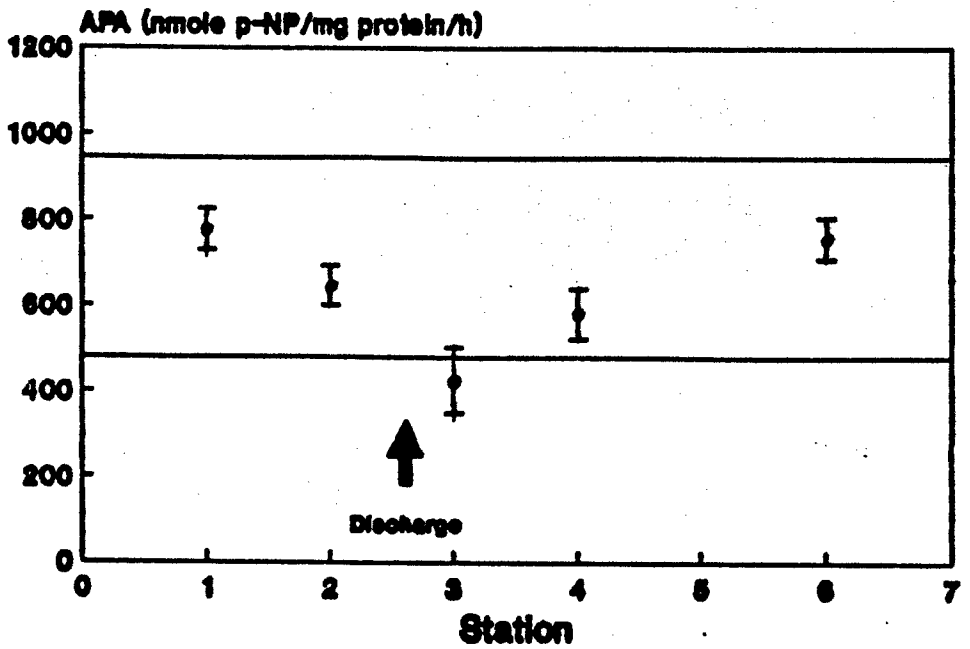


Figure 3.22. Microbial alkaline phosphatase activity on PFU substrates seven days after transfer from Station 2A. Values are mean with one standard deviation shown. The solid lines represent the 95% confidence interval around the mean value for Station 1A and 2A.

3.8 COPPER FORMING POINT SOURCE

3.8.1 Effluent composition

Analyses and NPDES permit values for the copper forming point source (CFPS) effluent during the periods of study are shown in Table 3.73. Effluent metal concentrations varied by a factor of approximately two during the study period. Effluent concentrations of toxic metals are sufficient to produce toxic effects in 100% effluent. Effluent dilutions of 10-20% correspond to concentrations of copper - the primary toxic metal - near the national water quality criteria (EPA 1986) of 20 ug/L for receiving stream hardness. Similarly, zinc concentrations in 50% effluent would be expected to be chronically toxic. Design flows for permit purposes predict that the maximum expected instream waste concentration will be 1.9 to 4%, depending on stream flow assumptions.

3.8.2 Bioassays

Acute mortality tests conducted to establish the upper boundary of toxicity showed that the CFPS effluent was acutely toxic to D. magna (90% mortality in 100% effluent). However, no deaths occurred at effluent concentrations $\leq$ 10% effluent. The effluent had very low dissolved oxygen (<0.5 mg/L) and required aeration before testing. Characteristics of the effluent and dilution water are shown in Tables 3.74 & 3.75. Because of acute toxicity and low dissolved oxygen, chronic tests were conducted beginning with effluent dilutions of 50%.

Short-term chronic toxicity tests using C. dubia showed reduced survival at 50% effluent, the highest tested dilution (Table 3.76). Survival and young production were adequate in controls. The effluent was chronically toxic with young production showing a strong dose-response relationship. Young production was reduced by approximately 85% at the highest tested dilution (50% effluent). Effluent concentrations > 12.5% (approximately 12 ug Cu/L and 42 ug Zn/L) resulted in significantly lower numbers of young ($p<0.05$, Dunnett's Test). Young production in effluent dilutions $\leq$ 6.25% did not differ from controls. The no observable effect concentration (NOEC) for daphnid reproduction was 6.25% and the lowest observed effect concentration (LOEC) was 12.5%. These values compare to the design instream waste concentration and are consistent with, but lower than, numerical water quality criteria.

Previous toxicological studies of the CFPS effluent conducted by USEPA in September 1987 found 70% survival for C. dubia in 100% effluent and young production at least as great as controls in 4% effluent, the design instream waste concentration.

3.8.3 Microcosm experiments

Microcosm experiments showed adverse ecological effects of the CFPS effluent at concentrations similar to those revealed by daphnid testing. A strong dose-response was apparent. Developing communities in microcosms showed reduced species numbers; reduced chlorophyll content (as in vivo fluorescence) and elevated calcium (Table 3.77) were apparent and significantly different from controls at all tested dilutions. However, protein biomass was unaffected, and retention of other nutrients showed no dose-response pattern. Alkaline phosphatase activity was not significantly affected, although the highest effluent dose produced expected elevated activity.

Epicenter communities in microcosms did not reflect effects on developing island communities (Table 3.78). A strong dose effect on species numbers was not substantiated by changes in other parameters with the exception of potassium retention which was significantly reduced at all tested effluent dilutions.

A second test substantiated observed effects on epicenter species numbers (Table 3.79); species numbers were significantly reduced at all tested effluent dilutions. No significant effects on other measured variables were observed. The effluent was enriched in calcium and magnesium when compared to dilution water (Tables 3.80 & 3.81).

Species numbers were consistently depressed by >10% at the lowest effluent dilution (6.25%, approximately 10 and 11 ug/L for the two respective experiments, Table 3.79). Biomass changes in both experiments were similar in response to increasing effluent but no significant differences in responses relative to controls were detected with the exception of in vivo fluorescence estimates of algal biomass on islands in the first test. Variability of replicates was at the high end of the expected range for controls. Significant differences among treatments for individual nontaxonomic and process parameters did not show expected dose-response relationships.

3.8.4 Receiving stream analyses

Evaluation of the receiving stream was complicated by two factors. First, the distance from the point of contact of the effluent discharge with the receiving stream to a larger stream is not large (< 1 km). This prevented some evaluation of effect and recovery downstream of the plant. Second, the quality of the receiving stream upstream of the plant is compromised by impacts from other dischargers (see Fig. 3.23 and Table 3.82).

Stream microbial parameters identical to those evaluated in the laboratory are shown in Table 3.82. Upstream areas are highly variable in biological quality. The most upstream stations (7,8) were first and second order streams and had low diversity. Biological quality was unclear at station 6 (below the confluence with a heavy metal containing waste stream) and deteriorated rapidly following confluence with a waste stream (oil and grease) above station 5. Samples at station 6 contained no detectable chlorophyll, and water and samples at station 5 were noticeably oily and measures of biological health reflected these adverse impacts. Recovery between this station and stations upstream of the CFPS was supplemented by wastewater nutrients from a state fish hatchery above station 4. This impact stimulated species numbers and algal biomass with the greatest algal biomass being found above the CFPS at station 3. Biological measures below the CFPS are within the range of upstream variability.

Invertebrate samples showed similar patterns (Table 3.83) to those reported for microbes. Upstream communities in the low order, high gradient stream segments at stations 7 and 8 showed high diversity and low density. Diversity declined rapidly at station 6 where the heavy metal containing waste water entered the stream and density increased. Diversity improved somewhat below station 5 and showed moderate recovery at station 3 above the facility. Diversity declined again below the point of contact with the CFPS effluent and recovered slightly downstream before confluence with a larger stream. Composition of the invertebrate community showed reductions in the numbers of isopods and amphipods below the plant; however, these changes are also consistent with assimilation of upstream sources of nutrients and organic carbon. Typical sensitive taxa such as mayflies occurred both above and below the plant discharge, suggesting that any impact associated with the CFPS effluent is modest.

3.8.5 Summary and Conclusions

The CFPS effluent mixture showed chronic toxicity to C. dubia and adverse effects on microcosm communities. Species richness was reduced and a "no effect" concentration could not be determined. The observed adverse effects occurred at concentrations near the design low flow instream waste concentration suggesting that there is little margin for error in operation of the treatment facility.

Stream communities showed some evidence of adverse effects from the effluent even though flow was sufficient to thoroughly dilute the effluent to concentrations below the design instream waste concentration confirming expectations based on toxicity testing. However, receiving stream communities were significantly impaired upstream of the facility, making any interpretation of downstream impacts problematic.

Table 3.73. Survey of NPDES average monthly permit limits and CFPS daily effluent analysis. Values are mean (SD) in mg/L.

Week	Total Suspended Solids	Chromium Total	Copper Total	Lead Total	Nickel Total	Zinc Total
NPDES limits	50.0	0.26	0.26	0.19	1.85	0.89
Nov. 6-10	0.53 (0.26)	0.01 (0.00)	0.13 (0.04)	0.05 (0.01)	0.02 (0.00)	0.17 (0.08)
Nov. 13-17	0.48 (0.17)	0.03 (0.01)	0.20 (0.09)	0.06 (0.01)	0.02 (0.00)	0.18 (0.08)
Nov. 20-24	0.55 (0.07)	0.06 (0.04)	0.26 (0.10)	0.08 (0.02)	0.03 (0.00)	0.37 (0.26)
Nov. 27 -Dec. 4	0.70 (0.35)	0.03 (0.01)	0.14 (0.05)	0.04 (0.01)	0.04 (0.01)	0.25 (0.10)
Dec. 4-8	0.48 (0.15)	0.04 (0.04)	0.23 (0.13)	0.05 (0.02)	0.02 (0.00)	0.28 (0.29)
Dec. 11-15	0.85 (0.42)	0.02 (0.00)	0.09 (0.02)	0.04 (0.01)	0.03 (0.01)	0.11 (0.02)
Dec. 18-22	0.74 (0.27)	0.02 (0.00)	0.11 (0.03)	0.06 (0.01)	0.02 (0.00)	0.23 (0.09)

Table 3.74. Ranges of hardness and alkalinity measures of diluent and 50% effluent during the Ceriodaphnia dubia chronic test of CFPS effluent. The units for both measures are mg $CaCO_3$/L).

Treatment	Hardness		Alkalinity	
	New	Old	New	Old
Diluent	250-280	265-293	206-235	216-252
50% Effluent	640-760	645-784	176-192	185-206

Table 3.75. Range in water chemistries monitored during the chronic Ceriodaphnia dubia test in which CFPS effluent was tested. Effluent was aerated before use. Ranges are shown for freshly made dilutions (New) and replaced solutions (Old).

Treatment	pH	Temperature (°C)	Diss. Oxy. (mg/L)	Cond. (ohm/cm^2)
Control				
New	7.45-8.40	21.3-24.0	7.54-9.48	522-583
Old	8.30-8.45	20.3-21.6	8.01-8.57	530-687
3.125%				
New	7.50-8.35	21.4-23.3	7.95-9.44	552-610
Old	8.35-8.45	20.5-21.5	7.90-8.94	557-725
6.25%				
New	7.50-8.35	21.5-23.8	8.15-9.45	588-651
Old	8.35-8.50	20.6-21.4	7.99-8.73	582-754
12.5%				
New	7.55-8.35	21.5-23.5	8.30-9.33	612-671
Old	8.35-8.40	20.6-21.5	8.13-8.84	641-826
25%				
New	7.60-8.30	21.5-24.0	8.26-9.11	723-808
Old	8.30-8.35	20.4-21.3	8.16-8.67	722-983
50%				
New	7.65-8.40	21.9-23.4	8.00-9.23	870-1053
Old	8.20-8.25	20.5-21.1	8.17-8.81	901-1099

Table 3.76. Survival and reproduction of Ceriodaphnia dubia exposed to CFPS effluent for 7 d. Ten organisms were exposed to each concentration. The average number of young per individual (mean ± SD) and the p value from analysis of variance are shown.

Treatment	% Survival	No. Young
Control	100	20.6 (5.31)
3.125%	100	19.4 (3.53
6.25	100	19.7 (3.13)
12.5	100	14.8 (4.71)[a]
25	100	13.2 (6.58)[a]
50	80	3.5 (3.55)[a]
p	-	0.0001

[a] Significantly different from control at $\alpha = 0.05$ (Dunnett's Test).

Table 3.77 Microbial parameters measured from island artificial substrates from microcosms exposed to brass mill effluent (Test #1) with tributary water as diluent. Values are mean (Standard Deviation).

Trt	Spp	Protein	APA	INVFL	ortho-Ca	Mg	K	Phos.
Control	24.0 (0.00)	2.42 (0.58)	459 (233)	70.9 (16.2)	39.1 (3.70)	18.7 (0.80)	4.41 (2.87)	0.45 (0.46)
6.25%	24.0 (0.00)	3.28 (0.78)	344 (22.7)	44.8[a] (17.8)	55.0[a] (0.81)	19.48 (0.34)	1.45 (0.05)	0.12 (0.01)
12.5	23.0 (0.00)	1.83 (0.55)	444 (139)	26.7[a] (4.01)	69.9[a] (1.40)	18.7 (0.13)	1.12 (0.16)	0.06 (0.02)
25%	24.0 (0.00)	2.00 (0.72)	432 (126)	23.3[a] (1.89)	103[a] (1.62)	19.3 (0.22)	1.48 (0.16)	0.08 (0.02)
50%	11.0 (0.00)	2.36 (0.76)	496 (136)	22.7[a] (8.78)	171[a] (1.62)	20.1 (0.26)	2.27 (0.05)	0.04 (0.00)
100%	7.00 (0.00)	1.81 (1.46)	1283 (897)	12.0[a] (5.41)	302[a] (0.81)	21.5[a] (0.13)	3.72 (0.19)	0.03 (0.01)
p	0.000	0.355	0.097	0.0003	0.0001	0.0001	0.023	0.124

Units: Protein ug/mL
APA nmole p-nitrophenol/mg protein/h
INVFL fluorescence units
Ca, Mg, K mg/L
ortho-Phos. mg/L

[a] = significantly different than control

Table 3.78. Microbial parameters measured from epicenter artificial substrata exposed to CFPS (Test #1) with tributary water as diluent. Values are mean (SD).

Treatment	Species	Protein (ug/ml)	APA[a]	Chl a (ug/L)	Ca (mg/L)	Mg (mg/L)	K (mg/L)	o-Phos. (mg/L)	Production/ Respiration
Control	54.7 (1.15)	290 (135)	330 (102)	1862 (603)	313 (138)	62.7 (24.1)	19.2 (7.44)	0.86 (0.24)	0.38 (0.03)
6.25%	38.3[b] (6.66)	436 (110)	228 (53.7)	1535 (438)	454 (93.9)	82.0 (13.8)	10.0[b] (1.44)	0.71 (0.14)	0.19 (0.16)
12.5%	43.7[b] (7.23)	397 (114)	228 (50.6)	1535 (124)	385 (77.8)	66.7 (12.7)	8.65[b] (1.44)	0.79 (0.26)	0.16 (0.06)
25%	33.0[b] (3.00)	326 (40.8)	333 (23.5)	1291 (491)	386 (23.9)	62.7 (6.47)	8.00[b] (0.76)	0.64 (0.05)	0.01 (0.03)
50%	24.3[b] (2.89)	246 (76.5)	418 (49.5)	1395 (450)	397 (50.3)	55.4 (11.7)	7.86[b] (1.04)	0.63 (0.15)	0.09 (0.09)
100%	15.3[b] (1.15)	199 (23.6)	501[b] (51.8)	1009 (136)	511 (32.5)	50.9 (2.75)	8.53[b] (0.34)	0.64 (0.14)	0.34 (0.29)
p	0.0001	0.066	0.0007	0.287	0.127	0.176	0.006	0.533	0.057

[a] nmole p-nitrophenol/mg protein/h
[b] Significantly different from control at $\alpha = 0.05$

Table 3.79 Microbial parameters measured from epicenter artificial substrates from microcosms exposed to copper forming point source effluent (Test #2) with tributary water as diluent. Values are mean (SD).

Trt.	Spp	Pro.	APA	Chl a	Ca	Mg	K	ortho Phos
Control	51.7 (2.08)	321 (129)	140 (29.5)	2203 (862)	475 (119)	61.0 (9.16)	4.54 (1.01)	0.40 (0.09)
6.25%	45.7[a] (2.08)	273 (139)	115 (17.3)	1469 (445)	427 (203)	50.0 (13.5)	3.17 (1.34)	0.37 (0.15)
12.5%	41.3[a] (2.52)	577 (248)	74.4 (38.7)	2863 (823)	521 (000)	94.4 (28.3)	7.75 (2.92)	0.64 (0.25)
25%	43.3[a] (2.89)	282 (173)	93.9 (3.85)	2055 (1210)	292 (59.9)	54.3 (30.2)	4.13 (2.19)	0.36 (0.17)
50%	30.3[a] (1.53)	204 (70.0)	129 (12.5)	1328 (604)	463 (137)	47.2 (10.6)	3.93 (1.25)	0.26 (0.06)
100%	13.7[a] (3.06)	345 (190)	118 (31.9)	1624 (1000)	399 (000)	62.0 (25.6)	6.32 (2.05)	0.38 (0.18)
p	0.0001	0.195	0.074	0.320	0.753	0.164	0.097	0.171

Units: Protein ug/mL
APA nmole p-nitrophenol/mg protein/h
Chl a ug/L
Ca, Mg, K mg/L
ortho-Phos. mg/L

[a] = significantly different than control

Table 3.80 Calcium, magnesium, and potassium from microcosms exposed to copper forming point source effluent (Test #1) with tributary water as diluent (Day 7). Values are mean (SD).

Treatment	Calcium (mg/L)	Magnesium (mg/L)	Potassium (mg/L)
Control	39.1 (1.40)	17.9 (0.13)	0.97 (0.14)
6.25%	53.6 [a] (0.81)	19.0 [a] (0.26)	1.00 (0.05)
12.5%	68.1 [a] (2.91)	18.3 [a] (0.13)	0.97 (0.05)
25%	107 [a] (6.47)	18.5 [a] (0.13)	1.30 [a] (0.00)
50%	176 [a] (2.42)	19.0 [a] (0.26)	2.06 [a] (0.05)
100%	303 [a] (5.66)	21.7 [a] (0.13)	3.35 [a] (0.11)
P	0.0001	0.0001	0.0001

[a] = significantly different than control

Table 3.81 Calcium, magnesium, and potassium from microcosms (Test #2) exposed to copper forming point source effluent with tributary water as diluent (Day 7). Values are mean (SD).

Treatment	Calcium (mg/L)	Magnesium (mg/L)	Potassium (mg/L)
Control	45.7 (0.81)	18.2 (0.38)	0.75 (0.31)
6.25%	58.7 [a] (2.42)	18.4 (0.22)	0.72 (0.26)
12.5%	67.6 [a] (2.14)	18.7 (0.13)	1.11 (0.25)
25%	93.7 [a] (2.42)	18.7 (0.13)	1.52 (0.43)
50%	144 [a] (1.40)	19.0 (0.13)	2.34 [a] (0.46)
100%	234 [a] (4.50)	19.6 [a] (0.13)	3.48 [a] (0.17)
p	0.0001	0.0001	0.0001

[a] = significantly different than control

Table 3.82 Microbial parameters measured from artificial substrata placed in the copper forming point source effluent receiving stream. Values are mean (Standard Deviation).

Site	Spp	Protein	APA	Chl a	Ca	Mg	K
1	40.7 (2.08)	213 (40.8)	814 (74.9)	1305 (206)	341 (49.1)	59.8 (7.10)	5.84 (0.74)
2	41.0 (2.65)	130 (39.7)	1041 (51.8)	979 (350)	227 (82.0)	44.9 (12.4)	4.61 (0.89)
3	48.7 (3.06)	783 (80.7)	456 (41.6)	4042 (906)	645 (42.5)	131 (6.05)	15.5 (1.56)
4	51.0 (4.36)	568 (111)	965 (78.5)	1788 (187)	396 (80.9)	73.3 (12.4)	10.7 (1.91)
5	21.7 (1.53)	32.9 (4.12)	1156 (164)	126 (90.0)	130 (5.83)	26.7 (1.94)	2.97 (0.00)
6	42.7 (4.16)	202 (63.1)	2691 (397)	0 (0)	231 (45.0)	42.5 (6.88)	56.3 (8.04)
7	24.3 (4.04)	93.5 (38.7)	129 (21.7)	653 (360)	11.6 (3.52)	8.16 (2.99)	2.77 (0.56)
8	15.3 (4.04)	81.8 (19.6)	75.5 (3.07)	85.2 (33.8)	8.84 (3.16)	5.91 (0.94)	2.01 (0.17)

Units: Protein ug/mL
APA nmole p-nitrophenol/mg protein/h
Chl a ug/L
Ca, Mg, K mg/L

Table 3.83. Macroinvertebrates collected by Surber sampler from CFPS receiving stream and tributary. Total number of individuals/taxonomic group per site.

Taxa	Station Number 1	2	3	4	5	6	7	8
Tricladida								
Planaridae	4	2	113	359		2		
Oligochaeta	1	37	41	206	76	942	2	8
Hirudinea								
Erpobdellidae					28	1		
Nematoda			2	4		1		
Isopoda	1							
Asellidae								
Lirceus sp.	1		457	4060	2			
Amphipoda								
Gammaridae								
Gammarus sp.	1	2	584	22	1			
Hydracarina			3	3				
Collembola			1					
Ephemeroptera								
Baetidae								
Baetis sp.	53	70	149				4	1
Ephemerellidae	74							
Serratella sp.	108	209	210					
Ephemarella sp.	3	1						
Heptageniidae								
Epeorus sp.							3	16
Stenacron sp.							1	
Stenonema sp.							15	
Nixe sp.							1	1
Leptophlebiidae							2	
Siphlonuridae								
Ameletus sp.								1
Plecoptera								
Capniidae								
Allocapnia sp.								1
Chloroperlidae								
Suwallia sp.							4	
Sweltsa sp.							2	
Peltoperlidae								
Tallaperla sp.								3
Perlidae								
Beloneuria sp.							1	
Perlodidae								1
Cultus sp.							6	1
Isoperla sp.								2
Yugus sp.							1	
Pteronarcyidae								
Pteronarcys sp.							1	7
Odonata								
Gomphidae							1	
Dromogomphus sp.					1			
Gomphus sp.							1	
Hemiptera			1		1	1		
Lepidoptera								
Pyralidae								
Crambus sp.						1		
Tricoptera				3	4			
Brachycentridae			1					
Micrasema sp.			4					

Table 3.83. (Continued)

Taxa	1	2	3	4	5	6	7	8
Glossosomatidae								
Glossosoma sp.			3	1				1
Hydropsychidae								
Ceratopsyche sp.			75				13	
Cheumatopsyche sp.			6					
Diplectrona sp.							3	
Parapsyche sp.								14
Potamyia sp.							2	
Hydroptilidae								
Hydroptila sp.							2	
Limnephilidae							16	11
Philopotamidae							1	
Dolophilodes sp.							3	
Polycentropidae								
Polycentropus sensu lato							4	
Rhyacophilidae								
Rhyacophilia sp.			9					1
Coleoptera								
Elmidae								
Optioservus sp.	1		41				1	1
Oulimnius sp.							4	3
Promoresia sp.			1					
Stenelmis sp.								1
Haliplidae								
Haliplus sp.					1			
Hydrophilidae			1					
Psephenidae								
Ectopria sp.							1	
Diptera	1				1			
Ceratopogonidae						1		
Chironomidae	69	275	97	57	90	134	20	4
Empididae								
Chelifera sp.	3	5						
Wiedemannia sp.	1							
Ephydridae					1			
Muscidae	1	2						
Tipulidae				1				
Antocha sp.	2	7	39				1	
Dicranota sp.								1
Hexatoma sp.							1	3
Molophilus sp.								1
Polymera sp.								1
Tipula sp.								1
Simulidae								
Prosimulium sp.							1	
Simulium sp.	9	6	2	1				
Gastropoda								
Lymnaeidae								
Stagnicola sp.					2			
Physidae								
Physella sp.				1	1			
Stylommatophora								1
Pelecypoda								
Sphaeriidae				1	1		14	
Total Individuals	333	616	1840	4719	210	1083	132	86
Total Taxa	17	11	22	13	14	8	31	25

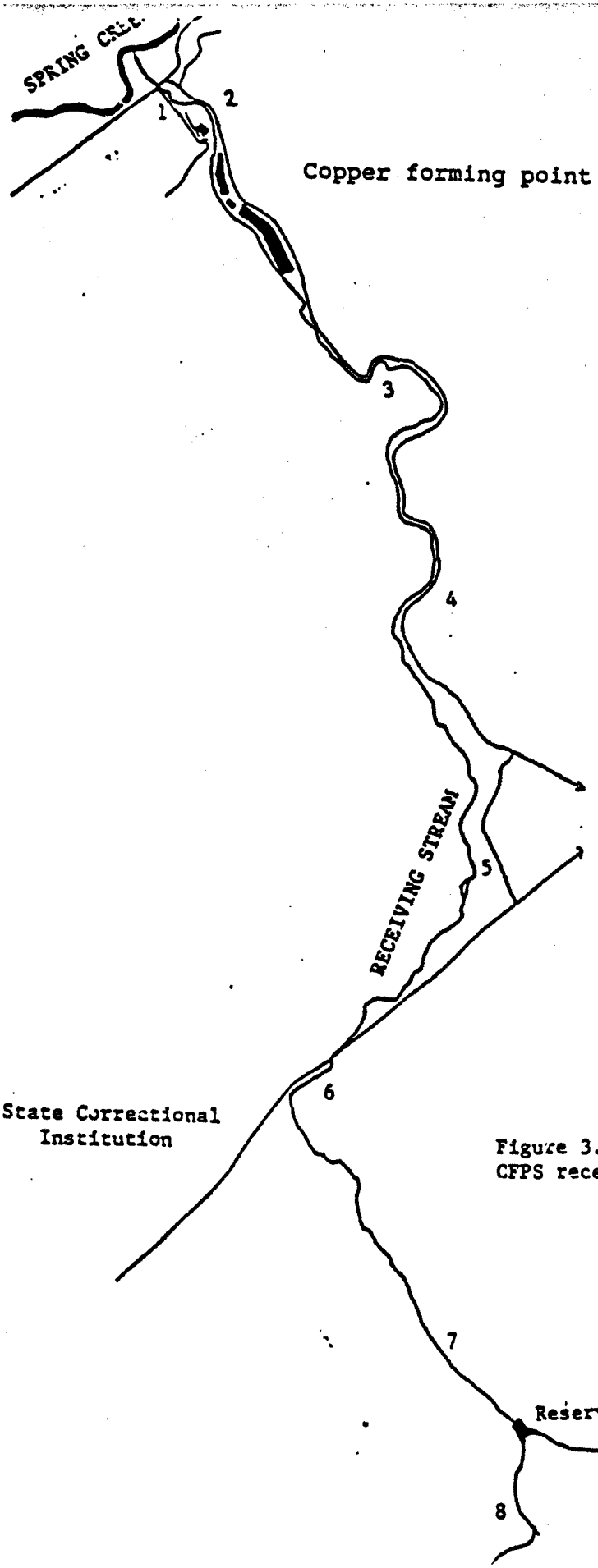


Figure 3.24. Sampling locations for the CFPS receiving stream.

3.9 ZINC POINT SOURCE

3.9.1 Effluent Composition

Analyses and NPDES permit values for the zinc point source (foundry) effluent during the periods of study are shown in Table 3.84. Zinc concentrations varied by a factor of less than two (0.27-0.38 mg/L) during the study period. Lead concentrations were more variable ranging from 0.005 to 0.043 mg/L. Total ammonia concentrations ranged from 1-11 mg/L. Effluent concentrations of toxic metals are sufficient to produce chronic toxic effects in 100% effluent. Effluent dilutions of 50% correspond to concentrations of zinc - the primary toxic metal - near national water quality criteria (EPA 1986) and Pennsylvania standards. The lagoon water has abundant populations of euglenid flagellates (Euglena, Phacus) known to be tolerant to toxic metals. The lagoon also contains rooted aquatic plants and filamentous algae.

3.9.2 Bioassays

Acute mortality tests conducted to establish the upper boundary of toxicity showed acute toxicity (75% mortality) to D. magna in 100% effluent. At effluent concentrations below 30% there was no mortality (Table 3.85). Two C. dubia tests were conducted, one in December 1989 and one January 1990. During the December test one composite effluent sample was acutely toxic to all test organisms at concentrations $\geq$ 1% effluent. This was considered to be an anomaly representing an isolated event in the plant, although extreme acute toxicity was evident in sample obtained on the following day as well.

The January short-term chronic toxicity test using C. dubia showed no reduced survival (Table 3.86). Young production was low in controls, but chronic toxicity was apparent. Young production showed a strong dose-response relationship and was reduced by approximately 60% relative to controls at effluent concentrations > 30% ($p<0.05$, Dunnett's Test). Young production in effluent dilutions $\leq$ 10% did not differ from controls. The no observable effect concentration (NOEC) for daphnid reproduction was 10% and the lowest observed effect concentration (LOEC) was 30%. These values correspond to average effluent zinc concentrations of between 0.03 and 0.1 mg/L, lower than current water quality criteria (USEPA 1986) and consistent with the presence of other toxicants in the effluent.

3.9.3 Microcosm Experiments

Microcosm experiments showed few adverse ecological effects. Developing communities in microcosms showed reduced species numbers in 100% effluent and some enhancement of species numbers at

intermediate effluent concentrations (Table 3.87). Although species numbers changed significantly in response to effluent dose, these changes can be interpreted in two ways. We have previously observed enhancement of species numbers at low levels of toxicity (Pratt et al. 1987; Pratt et al. 1989); however, the effluent itself contained relatively high numbers of species which could have supplement naturally occurring taxa.

Enhancement of microbial communities by the effluent is supported by elevated protein and chlorophyll biomass in effluent amended microcosms (Table 3.87). Retention of nutrients showed a dose-response pattern with microcosm calcium retention reduced at all effluent dilutions and magnesium retention reduced in microcosms dosed at $\geq$ 25% effluent. Island community retention of nutrients was less sensitive to effluent amendment. Small enhancements of potassium concentrations reflect the input of algal biomass in the effluent. Epicenter retention of nutrients was less sensitive to effluent effects. Alkaline phosphatase activity was not consistently affected; effluent doses produced decreased activity consistent with the input of additional phosphorus in the effluent.

Species numbers, depressed at the highest effluent dilution, and retention of calcium and magnesium were the only sensitive indicators of stress that corresponded to effects in the toxic range identified from daphnid tests. Effluent zinc concentrations were higher during the period of daphnid testing than during the microcosm tests by a factor of approximately two (Table 3.84).

3.9.4 Receiving Stream Analyses

Stream microbial parameters identical to those evaluated in the laboratory are shown in Table 3.88. Upstream samples from the receiving stream (Site 1) and the unnamed tributary (Site 7) were similar in species numbers, although biomass estimators were higher at Site 1. The impact of the effluent was observable at Site 6 directly below the effluent outfall where species numbers were slightly reduced. However, this modest direct impact did not correspond to observations of more serious impacts at other sites. Site 2, above the confluence of the unnamed tributary, had the lowest species numbers and showed reduced chlorophyll biomass and enhanced protein biomass relative to the Site 1 reference station. This site received runoff from a slag pile between the unnamed tributary and the receiving stream. Depression of species numbers and biomass estimators was apparent at Sites 3 and 4 below the entry of the foundry effluent, although both of these sites had higher species numbers than Site 2. Recovery of the microbial community to the upstream range was apparent at Site 5. These data suggest a greater impact from the slag pile than from the direct effects of the foundry effluent observed in the unnamed tributary.

Invertebrate samples collected in both the receiving stream and unnamed tributary showed comparatively low diversity (Table 3.89). This low diversity was, in part, attributable to the time of year that sampling occurred. Appropriate habitat was limiting in the unnamed tributary; the stream was very small and had several reached of fine, silted bed and other reaches of bedrock. There were few areas of cobble or gravel. The receiving stream had acceptable bed material to support a diverse benthic invertebrate community.

There were no differences in numbers of taxa above and below the effluent discharge in the unnamed tributary, although invertebrate density was low. Invertebrate communities differed considerably in the receiving stream. Numbers of taxa dropped sharply between Sites 1 and 2, consistent with observations on microbial communities and reflective of the impact of runoff from the slag pile. Further impacts were apparent at Site 3 below the entry of the foundry effluent, but there was little evidence of downstream recovery. Again, the severity of impacts in the receiving stream was much greater than impact in the unnamed tributary which received direct input of effluent.

3.9.5 Summary and Conclusions

Evaluation of the foundry effluent mixture showed only moderate toxicity with adverse chronic effects on daphnids above 30% effluent. Microcosm evaluation of the foundry effluent also revealed only moderate toxicity at effluent concentrations higher than those used in daphnid tests; concentration of the primary toxicant - zinc - was greater by a factor of about two during daphnid testing than during microcosm tests. This difference in concentration was sufficient to account for the observed differences in effluent toxicity.

Degradation of receiving stream communities was measured but was not consistent with the presence of the effluent. Significant deterioration, first recognized by Pennsylvania DER biologists, occurred before the foundry effluent entered the receiving stream. Analyses showed adverse effects on both microbial and invertebrate communities upstream of the regulated point of contact of the effluent-unnamed tributary and the receiving stream. The area between the unnamed tributary and the receiving stream is occupied by a large, apparently unmanaged slag pile. Runoff from this slag pile would enter the stream in the area of observed degradation. Microcosm predictions of moderate toxicity coupled with observations of no deterioration of communities in the unnamed tributary immediately below the effluent outfall confirmed the likelihood of other causes of observed degradation of receiving stream biota.

Table 3.84 Foundry effluent mixture chemistry for selected parameters during two study periods. Missing values (---) indicate that all measurements were the same.

		pH	TSS	mg/L Cu	Fe	Al	Zn	Pb
Ave. Mon. NPDES limits		6-9	30	0.05	1.5	1.1	1.0	0.06
October 1989	max.	9.1	7	0.01	5.7	0.1	0.51	0.01
	min.	8.8	5	--	0.01	---	0.27	----
January 1990	max.	8.5	12	0.01	0.55	0.3	0.38	0.043
	min.	7.3	6	----	0.20	0.1	0.27	0.005

Table 3.85 Results of a 48h D.magna test on Foundry effluent conducted in January 1990. Dechlorinated tap water was used as diluent and test conducted at 20 C. Only 8 organisms were exposed in each concentration.

Treatment	24 h (# of Dead/# at start)	48 h
Control	0/8	0/8
1%	0/8	0/8
3%	0/8	0/8
10%	0/8	0/8
30%	0/8	1/8
100%	2/8	6/8

At > 10% effluent, a compound present in the effluent caused the daphnia to "float" on the surface. Some died, some were still alive. They could be poked back under, but after 48 h, they would be floating again. Possibly a surfactant.

Table 3.86 Survival and reproduction of Ceriodaphnia dubia exposed to Foundry effluent for 7 days. Ten organisms were exposed to each concentration. The average number of young per individual (mean ± SD) and the p value from analysis of variance are shown.

Treatment	% Survival	No. Young
Control	100	9.2 (1.40)
1.0 %	100	8.9 (1.10)
3.0	100	8.1 (0.99)
10.0	100	7.6 (1.17)
30.0	100	3.7 (2.41)[a]
100	100	3.1 (2.38)[a]
p	-	0.0001

[a] = significantly different than control at alpha = 0.05, Dunnett's Test.

Table 3.87. Microbial parameters measured in island artificial substrata exposed to foundry effluent for 7 days with tributary water as diluent. Values are mean (SD).

Treatment	Species	Protein (ug/ml)	APA[a]	Chlorophyll (ug/L)	Prod./ Resp.
Control	49.3 (2.52)	16.5 (5.65)	589 (338)	200 (62)	1.44 (0.44)
6.25%	53.7 (3.51)	31.5[b] (7.64)	173[b] (28.9)	371 (110)	1.20 (0.25)
12.5%	51.0 (2.65)	31.5[b] (3.29)	157[b] (1.15)	360 (119)	5.33 (7.16)
25%	59.7 (5.69)	44.1[b] (4.53)	103[b] (10.1)	505[b] (101)	1.72 (0.40)
50%	49.7 (3.06)	43.0[b] (3.58)	86.7[b] (17.6)	638[b] (113)	1.37 (0.10)
100%	29.0[b] (5.00)	46.4[b] (3.82)	66.3 (11.0)	515[b] (74)	2.13[b] (0.20)
p	0.0001	0.0001	0.005	0.003	0.536

[a] nmole p-nitrophenol/mg protein/h
[b] significantly different from control at $\alpha = 0.05$

Table 3.88. Microbial parameters measured from artificial substrata exposed to water from zinc point souce receiving stream and unnamed tributary. Values are mean (SD).

Site	Species	Protein (ug/ml)	APA[a]	Chl a (ug/L)
1	42.7 (3.51)	847 (88.6)	426 (72.8)	1758 (118)
2	30.0 (2.65)	1056 (112)	358 (21.2)	1387 (130)
3	32.0 (1.00)	569 (106)	492 (80.2)	1046 (96.8)
4	33.7 (5.69)	871 (144)	417 (34.5)	1135 (146)
5	42.0 (2.65)	713 (68.5)	620 (89.1)	1914 (328)
6	37.3 (5.13)	492 (48.2)	703 (59.2)	994 (164)
7	42.3 (5.69)	338 (24.5)	756 (49.4)	401 (66.5)

[a] nmole p-nitrophenol/mg protein/h

Table 5.89. Macroinvertebrates collected by Surber sampler from foundry receiving tributary and its unnamed tributary. Total number of individuals/taxonomic group per site.

Taxa	1	2	3[a]	4	5	6[b]	7
Tricladida							
Planaridae							
Dugesia tigrina					20	3	
Oligochaeta	7			1		1	1
Nematoda					1		
Amphipoda							
Gammaridae							
Gammarus sp.	264	2					
Hydracarina				1			
Decapoda		2					
Ephemeroptera							
Baetidae							
Baetis sp.	17	1			1		
Ephemerellidae							
Serratella sp.	125						
Heptageniidae							
Stenonema sp.	1						
Plecoptera							
Perlidae							
Beloneuria sp.							1
Hemiptera				1			1
Corixidae							
Corisella sp.						2	
Tricoptera							
Glossomatidae							
Glossosoma sp.	4						
Hydropsychidae	1						
Ceratopsyche sp.	2						
Cheumatopsyche sp.	1	2					
Hydropsyche sp.	22	18	4	2		1	
Hymenoptera			1		1		
Megaloptera							
Sialidae							
Sialis sp.		1					
Coleoptera							
Elmidae							
Optioservus sp.	141	6	1		3	19	
Stenelmis sp.	14				2	5	
Lampyridae				1			
Diptera							
Chironomidae	21	16	2	115	31	36	43
Empididae				2			
Hemerodromia sp.				2	1	2	1
Simulidae					1		
Tipulidae							
Antocha sp.	5	15					6
Total Individuals	627	61	8	125	56	50	77
Total Taxa	15	7	4	8	7	8	8

[a] Site in the tributary below the confluence with the unnamed tributary.
[b] Site in unnamed tributary just below the foundry discharge.

3.10 HEAVY ASH EFFLUENT

3.10.1 Effluent Composition

On-site experiments were conducted to evaluate the toxicity of a power plant effluent mixture containing a suite of heavy metals. The effluent is monitored for metals which are included in the current NPDES permit. Effluent composition was monitored regularly by plant personnel for required reporting.

The power plant discharges effluent into two receiving streams from two sources - overflow from the heavy ash settling basin and overflow from the fly ash settling basin (see Section 3.11). The toxicity of the two waste streams was evaluated separately.

Analyses of effluent during the study period are shown in Table 3.90. Effluent metal concentrations were below detection for the duration of the testing period. Concentrations of metals in the receiving stream are at or below concentrations at which toxic effects would be observed both above and below the effluent outfall (Table 3.91). The data for copper are reported as < 50 µg/L, the detection limit for the method of analysis. However, there may still be sufficient copper to result in adverse ecological effects in the receiving stream. Water chemistry data for diluent and discharge are listed in Table 3.92.

3.10.2 Bioassays

Chronic fathead minnow and Ceriodaphnia dubia tests were conducted by laboratories under contract in 1990, as part of NPDES permit compliance. There were no effects on the survival or reproduction of C. dubia at any tested concentration. The LOEC was reported as > 100% effluent. There were no observed effects on the survival and growth of fathead minnows at any concentration of effluent and the LOEC was reported as > 100% effluent. Therefore, the effluent was determined to be non-toxic on the basis of these tests and the instream waste concentration (100%).

3.10.3 Microcosm experiments

Artificial substrates were colonized at reference site 1 (see Fig. 3.24). Test concentrations for the heavy ash effluent were: control, 12.5%, 25%, 50%, and 100%.

Microcosm tests showed adverse ecological effects from the heavy ash basin at concentrations of effluent which were lower than those estimated from daphnid and minnow tests (100%). Developing communities in the microcosms showed reduced species numbers at 100% effluent (Table 3.93). Decreased fluorescence (a measure of photosynthetic biomass) was observed at all tested effluent

concentrations, and there was a significant dose response (Table 3.94). Protein biomass was unaffected by the effluent. Magnesium and dissolved oxygen concentrations were affected at 50% effluent, and alkaline phosphatase activity was stimulated at 100% effluent. For affected variables with significant dose resposes EC_{20}s were calculated, and these are reported in Table 3.95. There were no other effects of the basin 1 effluent on the developing microbial community.

3.10.4 Receiving stream analyses

The biological health of the receiving stream was evaluated by collecting both microbial and invertebrate communities upstream and downstream of the discharge. More than one upstream location was examined to evaluate variability of community measures. A sketch map of the receiving streams' sampling stations is shown in Fig. 3.24. Water chemistry data for the reference sites are listed in Table 3.96.

Analyses identical to the laboratory were conducted on the stream microbial community. There were no apparent effects of the effluent on the receiving stream microbial community as indicated in Tables 3.97 and 3.98. Values for protein, alkaline phosphatase activity, fluorescence, and species richness were not outside the range of variability for sites upstream of the effluent outfall.

The effects of the effluent on the macroinvertebrate community are more difficult to assess. There are observable effects on both species numbers and abundance in the receiving stream, however these effects are observed at sites above the confluence with the effluent (Table 3.99). Therefore, the integrity of the receiving stream was compromised before any effects of the effluent, probably due to runoff from nearby roadways and a golf course. Due to this uncertain impact, it was impossible to detect any other effects on the macroinvertebrate community downstream of the effluent confluence. There was no observed recovery of the community downstream of the effluent, because the receiving stream enters a large river approximately 400 m downstream of the effluent. Macroinvertebrates collected from the receiving stream in August, 1990, by the cooperating company do not corroborate the results obtained from our sampling (Table 3.100).

Species richness and abundance of fish did not reflect the observed effects on macroinvertebrates in the receiving stream. The number of fish taxa increased with movement downstream (Table 3.101). This effect may be due to the influence of the nearby river. The river may supply a source of taxa which move upstream on encountering the stream. There is an unusually high number of Cyprinella spiloptera at site 3 which accounts for the high number

of total individuals of all taxa at this site. Otherwise, there were no adverse effects of the effluent on the receiving stream.

3.10.5 Summary and Conclusions

The heavy ash basin showed no toxicity to fathead minnows or C. dubia. Adverse effects were detected using microbial communities in microcosm tests. Species richness was reduced at 100% effluent, fluorescence was reduced at all tested concentrations, and alkaline phosphatase activity was stimulated at 100% effluent. Dissolved oxygen was reduced at 50% effluent. Therefore, effects occurred at concentrations below the design low flow instream waste concentration suggesting that adverse ecological effects could result in the receiving stream from the effluent.

Stream communities were generally unaffected by the effluent. However, detection of adverse effects on the stream was made difficult because the biological integrity of the receiving stream was compromised upstream of the effluent confluence. Recovery of the receiving stream was not detected because the receiving stream flows into a larger river shortly after the heavy ash basin discharge.

Table 3.90. Characteristics of cooperating company's heavy ash effluent. Values were obtained from cooperating company's discharge monitoring reports (DMRs) for the testing period. Values are mg/L except pH which is standard units, and are monthly averages.

Parameter	September	October	November	December
pH	6.6-8.5	6.9-7.9	7.0-8.5	6.6-8.0
TSS[a]	5.6	8.2	8.5	9.5
Oil and Grease	< 1.0	< 1.0	< 1.0	< 1.0
Beryllium, total	< 0.001	< 0.001	< 0.001	< 0.001
Lead, total	< 0.0148	N.D.[b]	N.D.	N.D.
Thallium, total	0.0035	< 0.0015	N.D.	< 0.0015
Antimony, total	< 0.1	< 0.1	< 0.0055	< 0.005
Selenium, total	< 0.0135	N.D.	N.D.	N.D.
PCBs[c]	< 0.001	N.D.	N.D.	N.D.
Hydrazine	< 0.002	0.0	< 0.002	< 0.002

Notes
a - Total suspended solids
b - Not detectable
c - Polychlorinated biphenyls

Table 3.91. Characteristics of heavy ash receiving stream at two sites (reference and impact) and effluent measured in August, 1990.

Parameter	Reference	Effluent	Impact
pH	7.72	8.49	7.85
Alkalinity (mg/L $CaCO_3$)	66	18	48
Aluminum (mg/L)	0.4	0.4	0.4
Ammonia (mg/L)	0.08	0.14	0.06
Arsenic, total (µg/L)	1.0	25.0	10.0
Cadmium, total (µg/L)	< 0.2	< 0.2	< 0.2
Chromium, hexavalent (µg/L)	< 10.0	< 10.0	< 10.0
Copper, total (µg/L)	< 50.0	< 50.0	< 50.0
Hardness, as $CaCo_3$ (mg/L)	101.0	134.3	115.0
Lead, total (µg/L)	4.0	< 4.0	4.0
Dissolved oxygen (mg/L)	8.5	8.6	8.5
Selenium, total (µg/L)	< 1.0	3.0	1.0
Total suspended solids (mg/L)	34.0	4.0	26.0
Conductance (µmhos/cm)	210.0	330.0	250.0
Water temperature (°C)	18.0	21.5	19.3
Zinc, total (µg/L)	20.0	< 10.0	10.0

Table 3.92. Water chemistry data for diluent water and basin discharges from microcosm experiments conducted in the mobile laboratory.

Variable	Diluent	Heavy ash effluent	Fly ash effluent
Hardness[a]	116	136	144
Alkalinity[a]	54.9	35.3	39.2
Conductivity[b]	180-230	230-280	250
Nitrate[c]	2.6	1.0	–
Nitrite[c]	0.3	0.09	–
Phosphate[d]			
ortho	28.4	34.0	–
total			

a = mg $CaCo_3$/L
b = µmhos
c = mg/L
d = µg/L

Table 3.93 Species data from island substrates after 7 day field test of cooperating company's heavy ash discharge.

Treatment	SpTot	B	P	N	A	S	R
Control	49.3 (2.5)	32.7 (3.5)	10.0 (1.0)	4.7 (1.5)	1.0 (0)	1.0 (0)	0 (0)
12.5%	54.3 (1.5)	36.7 (0.6)	10.0 (1.0)	3.7 (0.6)	3.0 (0)	1.0 (0)	0 (0)
50%	44.3 (3.2)	31.7 (3.2)	9.0 (1.7)	1.3 (1.5)	1.3 (1.5)	1.0 (0)	0 (0)
100%	29[a] (3.5)	18.3 (2.5)	8.7 (1.5)	0.3 (0.6)	1 (1)	0.7 (0.6)	0 (0)

Definition of functional groups: B-bacterivores, P-producers, N-omnivores, A-algivores, S-saprobes, R-raptors.

a = significantly different from control

Table 3.94 Island substrate results from 7 day field test of cooperating company's heavy ash discharge. Data are treatment means (SD) for triplicate microcosms. Chl a units are fluorometric units. Protein units are mg/ml. APA (alkaline phosphatase activity) units are nmole p-nitrophenol/mg prot/hr.

Treatment	Protein	APA	FU (Chla)
Control	.0176 (.0062)	407 (112)	45.4 (5.8)
12.5%	.0151 (.0088)	771 (323)	30.8[a] (4.9)
25%	.0148 (.00750	1049 (464)	23.1[a] (2.2)
50%	.0167 (.0051)	1007 (387)	17.0[a] (0.4)
100%	.0124 (.0043)	1707[a] (434)	16.1[a] (1.8)

[a] = significantly different from control

Table 3.95 Regression data for test of cooperating company's heavy ash discharge. R^2, p-value, and slope direction are listed for several ecologically important variables. Significant regression is determined by $p < 0.05$.

Variable	R^2	p	equation	EC20
APA	41.5%	0.024	290+399(LC)	.
FU	78.7%	0.0001	47.6-7.26(LC)	5%
Protein		NS		
Species (Total)	84.1%	0.0001	88.5-12.2(LC)	56%
Bacterivores	77.8%	0.0001	59.7-8.27(LC)	58%
Producers		NS		
Omnivores	54.0%	0.006	9.10-1.88(LC)	17%
Algivores	48.8%	0.012	5.26-0.962(LC)	103%

LC=$\log_e$ concentration

Table 3.96 Water chemistry data for reference sites of streams receiving heavy ash effluent (sites 1-5) and fly ash effluent (sites 6-10). Temperature units are degrees Celcius. Conductivity units are μmhos. Dissolved oxygen units are mg/L.

Site	Temperature	Conductivity	Dissolved oxygen
1	8.5	240	10.56
2	8.5	200	10.70
3	8.0	210	11.57
4	9.5	249	11.50
5	8.0	242	11.70
6	11.0	280	10.65
7	9.7	282	10.68
8	9.0	258	11.37
9	8.5	249	10.95
10	9.0	180	11.25

Table 3.97 Species data from survey of stream receiving discharge from cooperating company's heavy ash basin.

Site	SpTot	B	P	N	A	S	R
1	38.7 (3.5)	27.3 (2.1)	6.7 (1.2)	3 (1)	0.7 (0.6)	1 (0)	0 (0)
2	39 (1)	27.3 (2.5)	6.3 (2.1)	3.3 (1.2)	1 (0)	1 (0)	0 (0)
3	46 (1.7)	33.3 (3.1)	5.3 (1.5)	3.7 (0.6)	2.7 (0.6)	1 (0)	0 (0)
4	40.7 (1.2)	24 (1)	9.7 (1.5)	3.3 (1.5)	1.3 (0.6)	2.3 (0.6)	0 (0)
5	41 (2.6)	26 (3)	9.7 (4.6)	2.7 (0.6)	1.7 (1.2)	1 (0)	0 (0)

Definition of functional groups: B-bacterivores, P-producers, N-omnivores, A-algivores, S-saprobes, R-raptors.

Table 3.98 Results from survey of stream receiving discharge from cooperating company's heavy ash drainage basin. Data are means (SD) for three polyurethane foam substrates. Chl *a* units are fluorometric units. Protein units are mg/ml. APA (alkaline phosphatase activity) units are nmole p-nitrophenol/mg prot/hr.

Site	Protein	APA	FU (Chla)
1	.0470 (.0080)	367 (2.6)	22.3 (5.5)
2	.0784 (.0128)	436 (6.1)	76.7 (1.2)
3	.0585 (.0092)	427 (59)	58.8 (17)
4	.0471 (.0072)	523 (116)	37.1 (8.3)
5	.0601 (.0060)	741 (116)	38.1 (9.1)

Table 3.99 Summary of macroinvertebrates collected from streams receiving heavy ash discharge (sites 1-5) and fly-ash discharge (6-10). Station 7 samples were lost and thus are not included).

Taxon	Station									
	1	2	3	4	5	6	7	8	9	10
Platyhelminthes										
Turbellaria										
Planariidae					1			7	1	
Mollusca										
Ancylidae	5		1	3						
Gastropoda	4									8
Pelecypoda										
Sphaeridae	4	3			2			17	9	1
Annelida										
Oligochaeta	84	10	1	14	15	1		14	6	2
Hirudinea			7							
Isopoda										
Asellidae										
Asellus	1		1	1						
Amphipoda										
Gammaridae										
Gammarus			7	2	2				1	137
Diplopoda			1							
Ephemeroptera										
Baetidae										
Baetis	11	8	1			6		2	2	6
Caenidae										
Caenis					5					
Ephemerellidae										
Danella	1	4								
Ephemerella		15						2	2	11
Eurylophella	7									
Serratella										
Heptageniidae			1							
Leucrocuta					1					
Stenonema	9	2							1	
Leptophlebiidae										
Leptophlebia	12	7								
Potomanthidae										
Potomanthus					2					
Siphlonuridae										
Ameletus						1				
Odonata										
Anisoptera										
Calopterygidae										
Calopteryx	1									
Zygoptera										

Table 3.99 continued:

	Station									
	1	2	3	4	5	6	7	8	9	10
Cordulegastridae										
Cordulegaster										1
Gomphidae										
Lanthus								1		
Plecoptera										
Capniidae		4						1		
Allocapnia	11									
Perlidae										
Acroneuria		2								
Beloneuria	1									
Perlodidae										
Isoperla	1									
Megaloptera										
Sialidae										
Sialis	19	13								
Trichoptera										
Glossosomatidae										
Glossosoma						3		2	3	
Hydropsychidae										
Cheumatopsyche	69	19		9	1	4		31	43	10
Hydropsyche	15	31		5		25		49	97	16
Hydroptilidae					1					
Leucotrichia									4	
Limnephilidae										
Hydatophylax	3									1
Neophylax	3							5	1	3
Philopotamidae										
Chimarra	1	27						9		
Psychomyiidae										
Lype	1									
Coleoptera										
Elmidae	9	32		1						
Dubiraphia	1	2		1						
Optioservus	23	120	5	3						4
Stenelmis	1	24	3	2	2			6	13	1
Gyrinidae										
Dineutus					1					
Hydrophilidae									1	
Psephenidae										
Psephenus			2		2				1	
Diptera										
Ceratopogonidae	2									
Chironomidae	64	4	4			8		10	8	11
Empididae										
Hemerodromia	1									
Psychodidae										
Pericoma					1					

Table 3.99 continued:

	Station									
	1	2	3	4	5	6	7	8	9	10
Simuliidae										
Prosimulium		2								
Simulium			1			1				
Tabanidae										
Chrysops	15	1								
Tipulidae										
Cryptolabis									1	
Dicranota	20	7						1		4
Hexatoma	3	5						3	3	1
Limnophila	8									
Tipula	1					1				4
total individuals	411	342	35	41	36	50		160	197	221
number of taxa	33	22	13	10	13	9		16	18	17

Table 3.100 Summary of macroinvertebrates collected from cooperating company's heavy ash discharge receiving stream above (station 9) and below (station 7) outfall. Samples were collected and identified by cooperating company in August, 1990.

Taxa	Station		Total Number
	9	7	
Oligochaeta	20	5	25
Gammaridae			
Gammarus sp.	1	-	1
Astacidae			
Cambarus sp.	1	-	1
Hydropsychidae			
Macronema sp.	-	38	38
Hydropsyche sp.	1	195	196
Corydalidae			
Corydalus cornutus	-	3	3
Simuliidae			
Simulium sp.	63	1	64
Chironomidae			
Cricotopus sp.	3	2	5
Eukiefferiella sp.	-	1	1
Polypedilum sp.	1	1	2
Thienemannimyia complex	-	1	1
Elmidae			
Stenelmis sp.	99	30	129
Corbiculidae			
Corbicula sp.	1	-	1
Pisiidae			
Pisidium sp.	-	1	1
Number of taxa	9	11	14
Abundance	190	278	468

Table 3.101 Results of the fish survey of heavy ash receiving stream (sites 1-5) and two fly ash receiving streams (sites 6-10) .

Species	1	2	3	4	5	6	7	8	9	10
Salmo trutta	0	0	0	0	1	0	0	0	0	0
Esox niger	0	0	0	1	0	0	0	0	0	0
Campostomus anomalum	0	0	0	2	0	0	0	0	0	0
Cyprinella spiloptera	0	0	250	80	0	0	0	0	0	0
Luxilus cornutus	0	0	0	0	2	0	0	0	0	0
Notemigonus chrysocephalus	0	0	0	1	0	0	0	0	0	0
Cyprinella spiloptera	0	0	0	0	21	0	0	0	0	0
Notropis amoenus	0	0	1	0	0	0	0	0	0	0
Notropis procne	0	0	2	0	0	0	0	0	0	0
Pimephales notatus	0	0	13	22	0	0	0	0	0	0
Pimephales promelas	0	0	0	0	0	29	6	0	0	6
Rhinicthys atratulus	38	34	8	0	0	11	0	18	36	68
Semotilus atromaculatus	32	39	7	0	2	0	0	16	16	88
Semotilus corporalis	0	0	12	1	80	0	0	0	0	0
Catostomus commersoni	0	0	10	3	4	0	0	0	0	0
Hypentelium nigricans	0	0	0	1	0	0	0	0	0	0
Amieurus natalis	0	0	0	1	1	0	0	0	0	0
Noturus insignis	0	0	0	1	0	0	0	0	0	0
Ambloplites rupestris	0	0	0	2	1	0	0	0	0	0
Lepomis auritus	0	0	0	1	0	0	0	0	0	0
Lepomis cyanellus	0	1	2	0	9	0	0	0	0	0
Lepomis gibbosus	0	0	5	5	4	0	0	0	0	0
Lepomis macrochirus	0	0	0	0	3	0	0	0	0	0
Micropterus dolomieui	0	0	0	10	10	0	0	0	0	0
Micropterus salmoides	0	0	0	0	3	0	0	0	0	0
Etheostoma olmstedi	0	0	0	0	1	0	0	0	0	0
Total Taxa	2	2	10	14	14	2	1	2	2	3
Total Individuals	70	73	310	131	242	40	6	34	52	162

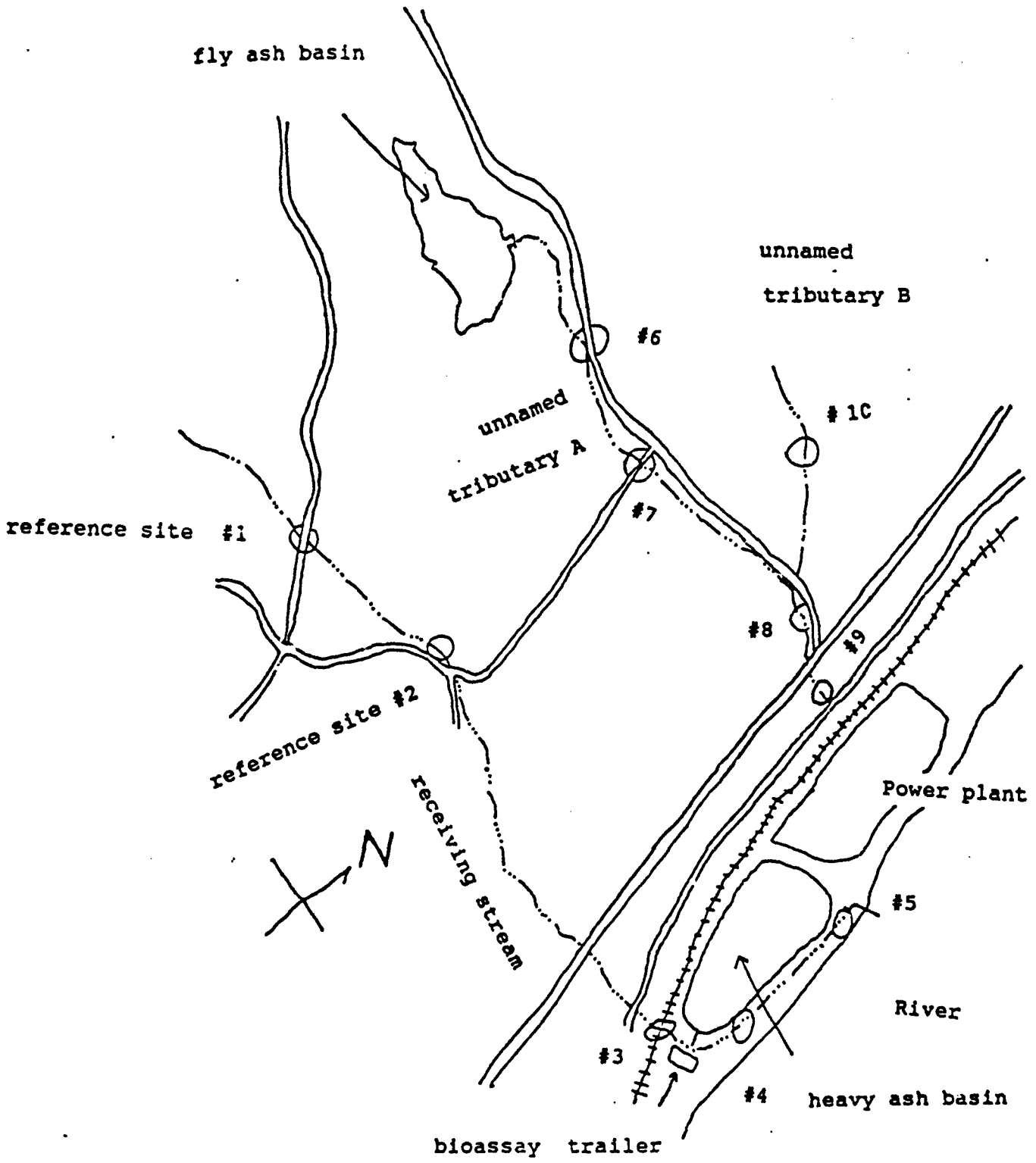


Fig. 3.24 Sketch map of test area for heavy ash and fly ash discharge experiments.

3.11 FLY ASH EFFLUENT

3.11.1 Methods

We conducted our microcosm experiment of the fly ash basin form the trailer's original location because the property near the fly ash basin was not suitable for the operation of our bioassay trailer (insufficient level ground, no supply of electricity). Each day, a supply of toxicant was pumped from the basin into plastic garbage cans and immediately transported to the trailer. The same diluent water was used for the second experiment because the outfall from the fly ash basin is actually the head waters of the receiving stream.

3.11.2 Effluent composition

Analyses of effluent during the study period are shown in Table 3.102. Effluent metal concentrations were below detection for the duration of the testing period. Concentrations of metals in the receiving stream are at or below concentrations at which toxic effects would be expected (Table 3.103), but are elevated relative to nearby reference waters. Water chemistry data for diluent and discharge are listed in Table 3.92.

3.11.3 Bioassays

Chronic fathead minnow and Ceriodaphnia dubia tests were conducted by outside laboratories in 1990 as part of the cooperating company's compliance with NPDES. Toxic effects on C. dubia were observed at 50% for both survival and reproduction (Table 3.104). The EC_{50}s for survival and reproduction of C. dubia were calculated to be 40% and 29.6% effluent, respectively. There were no observed toxic effects on either survival or growth of fathead minnows (Table 3.105).

Before conducting microcosm experiments, the PSU lab conducted acute Ceriodaphnia dubia and Daphnia magna survival tests. The LOEC for both of these tests was 100% (Table 3.106). The data were not amenable to probit analysis so LC_{50}s could not be calculated.

3.11.4 Microcosm experiments

Artificial substrates were colonized at reference site 1 (see Fig. 3.24). Test concentrations for the fly ash effluent were: control, 6.25%, 12.5%, 25%, 50%, and 100%. The lower concentration was included for the fly ash discharge because the cooperating company's monitoring reports indicated possible acute toxicity.

Results from day 0 epicenters indicated that replicability was acceptable (Tables 3.107 and 3.108). Adverse effects for non-

taxonomic measures on day 7 island substrates were only observed for fluorescence and potassium concentration, which were inhibited and stimulated, respectively, at 100% effluent (Table 3.109). Protozoan species numbers in developing communities were affected at 50 and 100% effluent (Table 3.110). These effects are at equivalent effluent concentrations which produced effects on Daphnids. For variables in which regressions were significant at $p < 0.05$, EC_{20}s were calculated and are reported in Table 3.111. EC_{20}s ranged from 29-82% effluent for species richness related variables. Regressions were not significant for non-taxonomic variables.

Day 7 epicenters were analyzed for non-taxonomic measures. Adverse effects were observed at 25% effluent for protein and chlorophyll a (Table 3.112). Alkaline phosphatase activity was unaffected. The effects on protein and chlorophyll a were both stimulatory effects, and may be due to the large numbers of photosynthetic flagellates grossly observed from the samples. EC_{20}s were calculated when the regression for a given variable was significant at $p < 0.05$, and these are reported in Table 3.113.

3.11.5 Receiving stream analyses

The biological health of the receiving stream was evaluated by collecting samples at five reference points downstream of the basin overflow (see Fig. 3.24). Water chemistry data for reference sites are listed in Table 3.96.

Analyses identical to those conducted in the laboratory were conducted on the receiving stream microbial community. Non-taxonomic measures appear to improve as one moves away from the basin, however there is some additional loss of biotic integrity at site 3 where there is a confluence with another stream (Table 3.114). The effect is not likely due to toxic effects in the tributary that joins with the discharge because the tributary microbial community appears healthy based on the site 5 data. Protozoan species richness improves as one moves away from the discharge, and this does not seem to corroborate the non-taxonomic information (Table 3.115).

The macroinvertebrate community improved continuously with downstream movement from the discharge point, in terms of both species richness and abundance (Table 3.99). Samples for site 2 were lost and could not be analyzed. Site 3 does not seem to adversely affected for macroinvertebrates, which confirms the data for protozoan species richness.

Fish survey data indicate that these low-order streams do not support a diverse fish assemblage (Table 3.101). There is only a total of 3 taxa collected from the discharge stream and from the

tributary. Therefore, it is difficult to assess any impact of the discharge on the fish community, unless the only source of fish to the tributary is movement up the discharge stream. In terms of numbers of individuals, there does not seem to be any effect at site 3 where the non-taxonomic microbial community was affected.

3.11.6 Summary and Conclusions

Daphnids and the developing microbial community were adversely affected at effluent concentrations of 50% under chronic exposure. Day 7 epicenters were affected at 25% effluent although the effects were stimulatory. Biotic integrity of the receiving stream improves as one moves away from the effluent source, in terms of protozoan species richness, and macroinvertebrate species richness and abundance. The low order streams do not support a diverse fish community, so it is difficult to assess any impact of the effluent.

Effects were observed at concentrations of effluent which are lower than the instream waste concentration (100%). Therefore, there is the possibility for adverse ecological effects in the receiving stream from the effluent.

Table 3.102 Characteristics of cooperating company's fly ash effluent. Values were obtained from cooperating company's discharge monitoring reports (DMRs) for the testing period. Values are mg/L except pH which is standard units, and are monthly averages.

Parameter	September	October	November	December
pH	7.0-7.7	7.3-8.9	7.4-8.5	7.1-7.7
TSS[a]	7.7	9.2	17.1	15.1
Oil and Grease	< 1.0	< 1.0	< 1.0	< 1.0
Beryllium, total	< 0.001	< 0.001	< 0.001	< 0.001
Lead, total	< 0.0148	N.D.[b]	N.D.	N.D.
Thallium, total	0.0065	< 0.0035	0.004	0.003
Antimony, total	< 0.1	< 0.1	0.0135	0.013
Selenium, total	< 0.0135	N.D.	0.0135	N.D.
PCBs[c]	< 0.001	N.D.	N.D.	N.D.

Notes
a - Total suspended solids
b - Not detectable
c - Polychlorinated biphenyls

Table 3.103. Characteristics of fly ash discharge at four sites. Site A is immediately after discharge, site B is downstream of discharge, site C is downstream of an inactive basin that merges with the active fly ash basin's discharge upstream of the confluence, site D is downstream of the confluence of the active and inactive basin streams (see sketch map).

Parameter	A	B	C	D
pH	7.2	7.2	7.6	7.3
Alkalinity (mg/L $CaCO_3$)	13	20	36	28
Aluminum (mg/L)	0.4	1.0	0.2	0.9
Ammonia (mg/L)	0.04	0.11	0.09	0.31
Arsenic, total (µg/L)	68	77	3	61
Cadmium, total (µg/L)	0.6	0.5	0.2	0.5
Chromium, total (µg/L)	20.0	17.0	2.0	15.0
Copper, total (µg/L)	12.0	12.0	2.0	12.0
Hardness, as $CaCo_3$ (mg/L)	148.9	149.3	94.6	133.8
Dissolved oxygen (mg/L)	8.2	8.2	9.4	8.0
Selenium, total (µg/L)	9.0	8.0	2.0	7.0
Total suspended solids (mg/L)	9.0	73.0	14.0	62.0
Conductance (µmhos/cm)	387.0	378.0	216.0	343.0
Water temperature (°C)	21.6	20.6	15.2	19.8
Zinc, total (µg/L)	17.0	27.0	10.0	31.0

Table 3.104 Survival and reproduction of C. dubia exposed to fly ash basin effluent for 7 days. Ten organisms were exposed to each concentration.

Treatment	% Survival	No. Young
Control	80	15.6
6.25	70	16.9
12.5	90	21.0
25	80	17.2
50	10	1.4*
95	10	0*
100	0	0*

* = significantly different than control

Table 3.105 Fathead minnow survival data from chronic toxicity test of cooperating company's fly ash discharge. The mean number of surviving organisms per replicate and the mean weight of the larva per replicate are given for each test concentration.

Test concentration (% effluent)	mean # of surviving organisms	mean wgt. (mg)
Control	8.0	1.16
6.25%	7.7	1.02
12.5%	6.0	1.48
25%	5.7	1.04
50%	10.0	1.15
95%	10.0	1.31
100%	9.0	1.38

NOEC = 100%
LOEC cannot be determined
LC50 > 100%

Table 3.106 Results of 48 hr acute toxicity tests on cooperating company's fly ash discharge collected on 90.11.21. Data are numbers of living organisms.

Daphnia magna

Test conc.(%)	No. tested.	Number alive 24 hr	48 hr
control	10	10	10
1	10	10	10
3	10	10	10
10	10	10	9
30	10	10	10
100*	10	0	0
100 filtered*	10	1	0

* 7 alive at 16 hr.

Ceriodaphnia dubia

Test conc.(%)	Rep.	No. tested.	Number alive 24 hr	48 hr	Total
control	A	5	5	5	
	B	5	5	5	10
1	A	5	5	5	
	B	5	5	5	10
3	A	5	4	4	
	B	5	5	4	8
10	A	5	5	4	
	B	5	4	4	8
30	A	5	5	5	
	B	5	5	5	10
100	A	5	0	0	
	B	5	0	0	0
100 filtered	A	5	3	1	
	B	5	0	0	1

Table 3.107 Results from day 0 epicenters for test of cooperating company's fly ash waste discharge. Data are treatment means (SD). Chl a units are fluorometric units. Protein units are mg/ml. APA (alkaline phosphatase activity) units are nmole p-nitrophenol/mg prot/hr.

Treatment	Protein	APA	FU (Chla)
Control	.0427 (.0118)	359 (7)	62.8 (22.3)

Table 3.108 Species data for day 0 epicenters for test of cooperating company's fly ash waste discharge.

Treatment	SpTot	B	P	N	A	S	R
Control	45.7 (2.5)	31.3 (5.0)	6.7 (2.3)	5.7 (1.2)	1.3 (1.5)	0.7 (0.6)	0 (0)

Definitions of functional groups: B-bacterivores, P-producers, N-omnivores, A-algivores, S-saprobes, R-raptors.

Table 3.109 Results from 7 day on-site microcosm test of cooperating company's fly ash waste discharge. Data are treatment means (SD) for triplicate microcosms. Chl a units are fluorometric units. Protein units are mg/ml. APA (alkaline phosphatase activity) units are nmole p-nitrophenol/mg prot/hr.

Treatment	Protein	APA	FU (Chla)
Control	.0205 (.0019)	295 (60)	44.4 (7.3)
6.25%	.0129 (.0048)	428 (199)	44.8 (5.4)
12.5%	.0156 (.0021)	310 (2.0)	38.3 (2.6)
25%	.0165 (.0039)	341 (14)	48.9 (10.8)
50%	.0167 (.0046)	336 (38)	42.5 (9.4)
100%	.0184 (.0058)	362 (45)	24.8[a] (9.1)

[a] = significantly different from the control ($p < 0.05$).

Table 3.110 Species data from 7 day on-site microcosm test of cooperating company's fly ash waste discharge.

Treatment	SpTot	B	P	N	A	S	R
Control	42 (6.9)	27.3 (12.7)	9.7 (4.5)	1.7 (1.2)	2 (1)	1.3 (0.6)	0 (0)
6.25%	42 (2.8)	28 (2.8)	6.5 (2.1)	4.5 (2.1)	1.5 (0.7)	1.5 (0.7)	0 (0)
12.5%	35 (1)	24.7 (0.6)	5 (1)	2 (0)	2 (0)	1 (0.6)	0 (0)
25%	35.7 (3.2)	26 (1)	5.3 (1.2)	2.7 (1.2)	0.3 (0.6)	1.3 (0.6)	0 (0)
50%	34[a] (2)	25 (4.6)	3.7 (0.6)	2 (1.7)	2.3 (0.6)	1 (0)	0 (0)
100%	25[a] (3)	19.7 (2.5)	4.3 (0.6)	0.7 (0.6)	0 (0)	0.3 (0.6)	0 (0)

Definition of functional groups : B-bacterivores, P-producers, N-omnivores, A-algivores, S-saprobes, R-raptors.

[a] = significantly different from the control ($p < 0.05$).

Table 3.111 Regression data for test of cooperating company's fly ash discharge (d.7 islands). R^2, p value, regression equation, and EC 20 are listed for several ecologically important variables. Significance is determined by $p < 0.05$.

Variable	p	R^2	equation	EC 20
Protein	NS	-	-	-
Fluorescence units	NS	-	-	-
Alkaline phosphatase activity	NS	-	-	-
Species total	0.0001	69.6%	y=50.3-4.97(L.C.)	28.8
Functional groups				
Bacterivores	0.014	40.7%	y=32.2-2.35(L.C.)	82.1
Producers	0.030	33.7%	y=7.41-0.769(L.C.)	.63
Omnivores	0.014	40.8	y=5.64-1.03(L.C.)	63.8
Algivores	NS	-	-	-
Saprobes	0.015	40.3	y=2.40-0.40(L.C.)	29.7

L.C. = $\log_e$ concentration
EC 20 = effective concentration at which 20% of the community would be affected

Table 3.112 Day 7 epicenter results from on-site microcosm test of cooperating company's fly ash waste discharge. Data are treatment means (SD). Chl a units are fluorescence units. Protein units are mg/ml. APA (alkaline phosphatase activity) units are nmole p-nitrophenol/mg protein/hr.

Treatment	Protein	APA	Chla
Control	.0273 (.0067)	446 (75)	11.7 (2.89)
6.25%	.0379 (.0112)	559 (228)	12.6 (3.08)
12.5%	.0387 (.0055)	494 (29)	17.9 (3.38)
25%	.0542[a] (.0130)	469 (18)	22.1[a] (3.14)
50%	.0512[a] (.0076)	435 (34)	22.1[a] (7.26)
100%	.0489[a] (.0054)	414 (38)	21.4[a] (1.03)

[a] = significantly different than control ($p < 0.05$)

Table 3.113 Regression data for test of cooperating company's fly ash waste discharge (d.7 epicenters). R^2, p value, regression equation, and EC 20 are listed for several ecologically important variables. Significance is determined for $p < 0.05$.

Variable	p	R^2	equation	EC20
Protein	0.042	28.1%	y=0.0373+0.0093(L.C.)	.6137
Fluorometric units	0.004	47.5%	y=40.4+18.1(L.C.)	.233
Alkaline phosphatase activity	NS	-	-	-

L.C. = $\log_e$ concentration
EC20 = effective concentration at which 20% of the community would be affected

Table 3.114 Results from survey of stream receiving discharge from cooperating company's fly ash basin. Data are treatment means (SD) of three PFU's at each site. Chl *a* units are μg chl *a*/L. Protein units are mg/ml. APA (alkaline phosphatase activity) units are nmole p-nitrophenol/mg prot/hr.

Site	Protein	APA	Chl *a*
6	.1047 (.0285)	574 (213)	1203 (502)
7	.2423 (.0301)	309 (57)	1893 (87)
8	.1600 (.0078)	331 (27)	344 (107)
9	.1613 (.0438)	246 (45)	1062 (275)
10	.2087 (.0749)	261 (37)	1952 (42)

Table 3.115 Species data from survey of stream receiving discharge from cooperating company's fly ash basin. See sketch map (Fig. 3.24) for site location.

Site	SpTot	B	P	N	A	S	R
6	19	14.7	4	0	0.3	0	0
	(2.6)	(4)	(1.7)	(0)	(0.6)	(0)	(0)
7	18	13	4.7	0	0.3	0	0
	(2.6)	(2.6)	(1.2)	(0)	(0.6)	(0)	(0)
8	30	22.3	4	2.3	1.3	0	0
	(2)	(2.1)	(1)	(0.6)	(1.2)	(0)	(0)
9	25.7	21.7	3	1	0	0	0
	(1.2)	(1.2)	(1)	(1)	(0)	(0)	(0)
10	26.3	20.3	4.7	0.7	0.7	0	0
	(1.5)	(3.2)	(1.5)	(0.6)	(0.6)	(0)	(0)

Definition of functional groups: B-bacterivores, P-producers, N-omnivores, A-algivores, S-saprobes, R-raptors.

3.12 ANALYSIS OF CONTINUOUS pH DATA

3.12.1 Background

The dynamics of ecological processes such as primary production and respiration are coupled to the availability of light, nutrients, and substrates and to the efficiencies of species. Diurnal patterns of net oxygen production from photosynthesis and net oxygen consumption from repiration follow the light-dark cycle. In microcosm experiments, the light-dark cycle is controlled (12L:12D). If respiration equals primary production, then microcosm oxygen levels should oscillate in a sinusoidal pattern following the light cycle. This cycle has been used previously to examine the effects of stress (Van Voris et al. 1980).

In aquatic ecosystems, inorganic carbon for primary production is obtained by algal cells as bicarbonate ion, the common dissolved form of carbon dioxide under circumneutral conditions. While the carbon dioxide-bicarbonate-carbonic acid equilibrium of surface waters is somewhat complex (Wetzel 1982), removal of carbon dioxide for primary production displaces the equilibrium and results in increasingly alkaline pH. This pH change is buffered more strongly in hard water than in soft water, but the photosynthesis-related rise in pH during the day can be readily observed as a result of the dominance of carbon dioxide removal over its replacement by respiration. In the dark, respiration dominates, carbon dioxide is replenished, and pH falls.

Changes in carbon dioxide and oxygen are equimolar as a result of production and respiration. Measuring changes in pH is less problematic than measuring oxygen. Polarographic oxygen measurement requires diffusion of oxygen through a celluloid membrane which can become colonized by bacteria and other Aufwuchs, resulting in poor repeatability of measurements. Additionally, oxygen measurement requires frequent recalibration. Therefore, automating oxygen measurement for continuous data collection requires either the simultaneous calibration of many probes or the pumping of microcosm water to a single probe. The former alternative is impractical because of variability among probes. The latter choice presents difficulties in collecting comparable data because of differences in timing of oxygen measurement due to pumping, line clearance, and probe response times. Semi-automated point in time measurements of oxygen are repeatable (see section ZZ.) but continuous measurement is impractical.

Automated pH measurement is a practical, indirect method for examining diurnal production-respiration cycles. The measurment of pH is well-understood. Multiple pH probes can be standardized en masse, daily drift is small, and several probes can be read quickly

and accurately using a multiplexed series of meters. We chose the Fisher Scientific 935 Scanner for this purpose because the number of probes was large (16) and because the system could be managed from a small computer using vendor-supplied software. The collected data is stored in ASCII format so that is can be read and manipulated using commonly available software.

3.12.2 Principles of time series analysis

The daily pattern of pH changes, related to oxygen production and consumption, is expected to change predictably. Alterations in biological integrity associated with toxicity can alter this pattern. If data are collected frequently and routine, statistical techniques can be used to measure the frequency of oscillations and the trend in the measured response (pH) through time. We collected pH data on a 15 minute cycle (96 measurements per day). Therefore, the cycle should repeat at 96 time step intervals each day. However, to adequately characterize the cycling pattern, data collection must be regular and frequent.

Spectral analysis was used to examine pH cycles and compare among treatments. The analysis tests the hypothesis that strong periodicity exists in the data. For the pH data, the frequency of repeating periods was expected to coincide with the 96 daily data collection intervals, and data showing cyclic behavior would be expected to have strong periods at integer multiples of the 96 daily time steps (192, 288,...). Toxic stress is hypothesized to alter the diurnal pattern: the strength of repeating patterns should be less and cycling frequencies both shorter and longer than the stong diurnal period should appear.

3.12.3 Results

Initial efforts to collect pH data continuously were met with repeated software and hardware failures which resulted in significant data gaps. However, qualitative analyses of daily patterns showed that toxic stress was associated with added short-wave frequencies in the diurnal pattern (Fig. 3.25). Field studies were conducted over shorter periods (typically 7d) than laboratory microcosm studies, so adequate characterization of the cyclic pattern was limited to deducing periodograms (visual displays of the strength of repeating frequencies, Fig. 3.25A-F).

A longer-term laboratory microcosm experiment of copper toxicity (0-160 ug/L, see 3.1) was conducted to allow the building of an adequate time-series model. Spectral analysis showed the progressive effects of copper on microbial communities and demonstrated the utility of the analysis to detect process changes in stressed communities. Summaries of the descriptive spectral analysis are shown in Figs. 3.25A-F. The summary spectral density

plots show that the average pH of control microcosms was strongly linked to the diurnal cycle controlled by the light-dark cycle which repeated every 96 time steps. All other microcosms did not show dominance of this cycle. Longer time cycles became more dominant in higher treatments.

3.12.4 Conclusions

The descriptive analysis of the power spectrum of pH frequencies in microcosms provided a qualitatively sensitive measure of changes in community processes as a result of toxic exposure. However, short-term data collections (7d) from experiments conducted in the mobile laboratory were insufficient to conduct statistical tests of the signficance of the spectral analysis. That is, without a sufficiently large time series it was not possible to determine if the periodicity of the data set was other than random. Comparisons among treatment were, therefore, more problematic. Additionally, harware and software failures in early designs of the data collection system resulted in large data gaps that limited our ability to utilize pH periodograms or spectral analysis for comparisons among treatments.

Previous use of spectral analysis in determining contaminant effects on terrestrial soil core microcosms (Van Voris et al. 1980) showed results congruent with those presented here. Frequencies of carbon dioxide flux patterns were altered in microcosms dosed with toxicants, and these changes were suggested to be a direct measure of the loss of functional complexity in the microcosm. In our experiments with copper, we found general agreement between increasing stress, decreasing species richness and biomass, and alterations in the pH frequency spectrum. Van Voris and colleagues interpreted changes in the power spectrum as indirect evidence for changes in funcitonal complexity. Clearly, in our microcosm tests, loss of the dominant diurnal frequency was related to losses in species richness and biomass attributable to copper toxicity.

Continuous data collection over a long (21 d) experiment can provide a sensitive indication of stress as measured by functional changes in microcosm communities. However, inferential tests of hypotheses of treatment differences were not possible, although indirect tests (e.g., comparisons of total areas in periodogram curves) might be useful for comparing treatments. However, such comparisons would be unlikely to distinguish between treatments with qualitatively different periodograms but similar areas under the periodogram trace. Therefore, analysis of continuously monitored data requires additional research before it can be integrated into chemical hazard assessments.

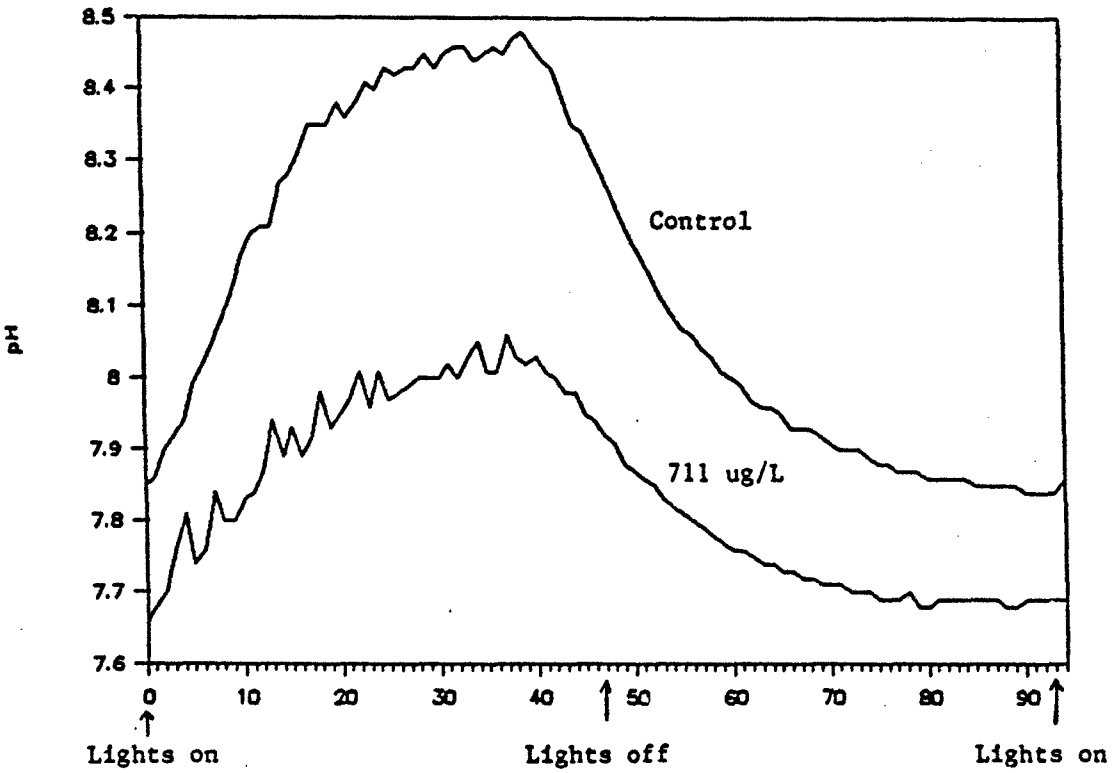


Fig 3.25. Twenty-four hour record of pH in two of the microcosms in the TNT test.

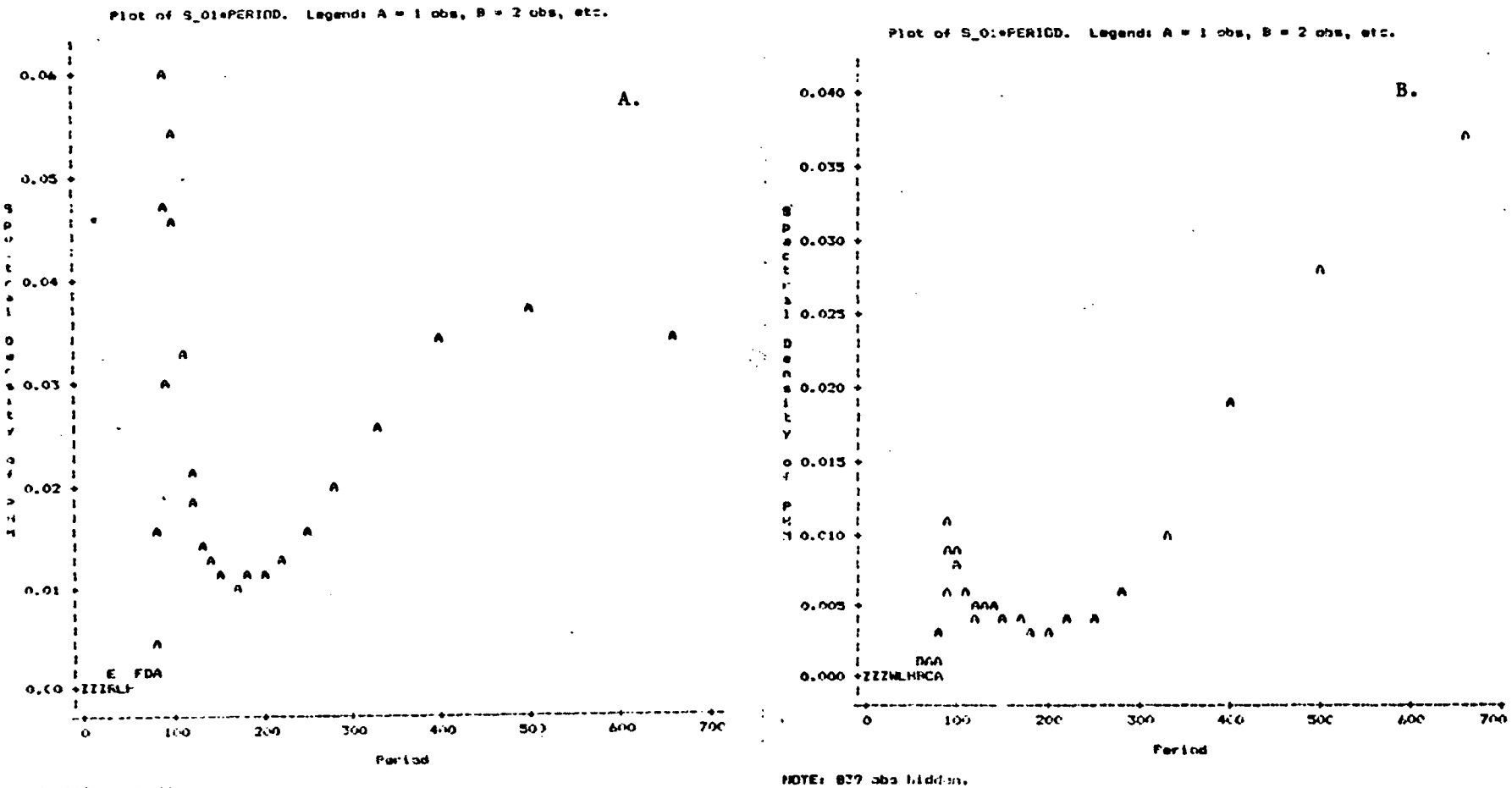
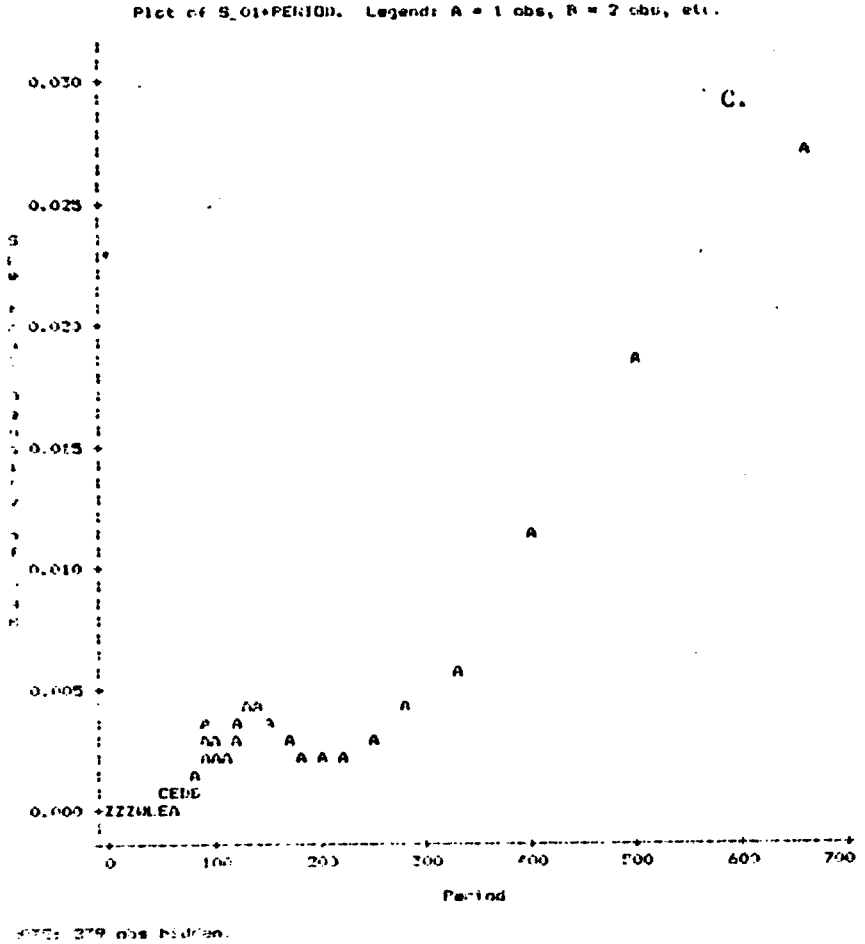


Fig. 3.26. Spectral density of continuous pH records for microcosms treated with copper (0-160 ug/L). A. Control (0 ug Cu/L). B. 10 ug Cu/L.



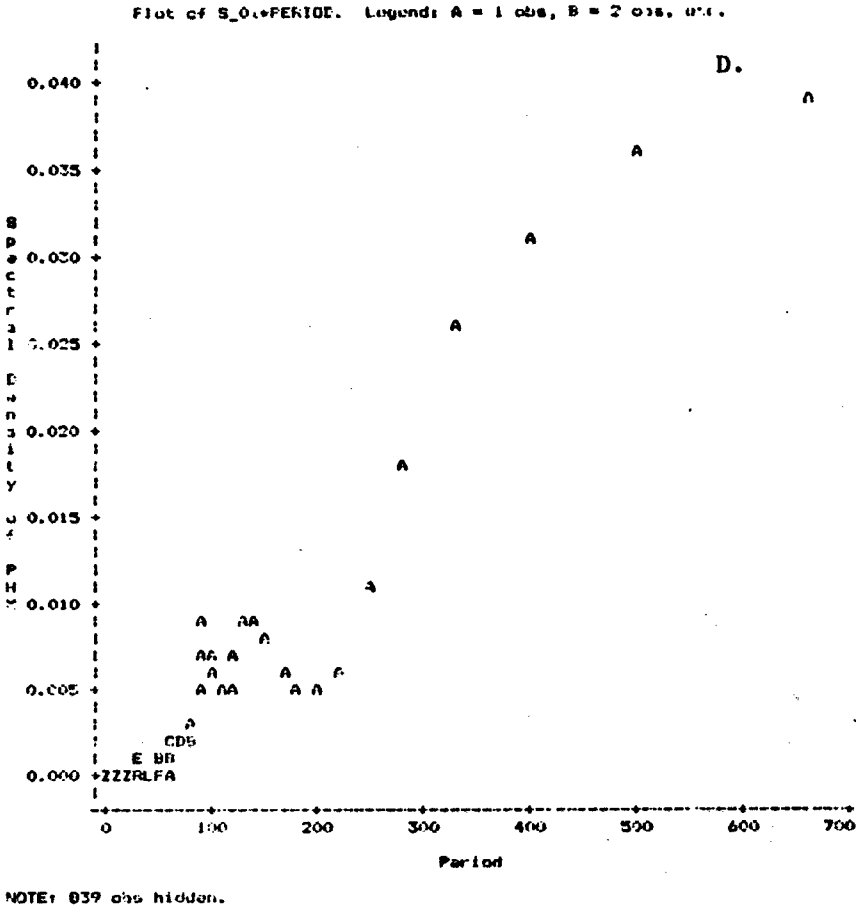
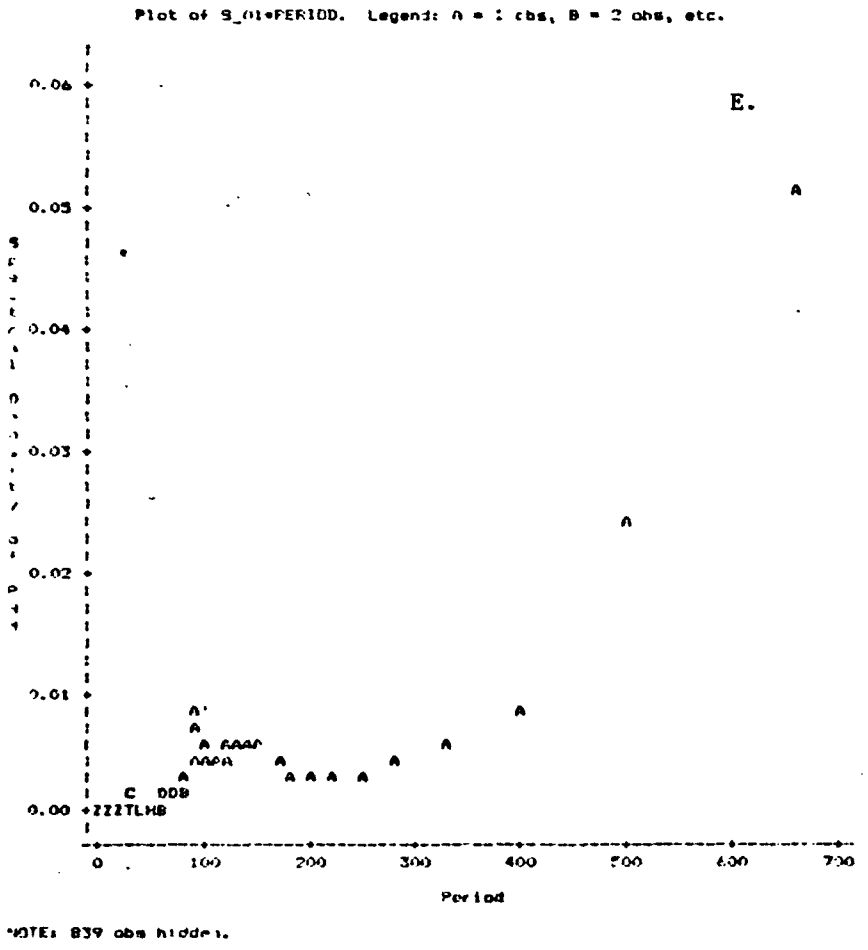


Fig. 3.26, continued. C. 20 ug Cu/L. D. 40 ug Cu/L.



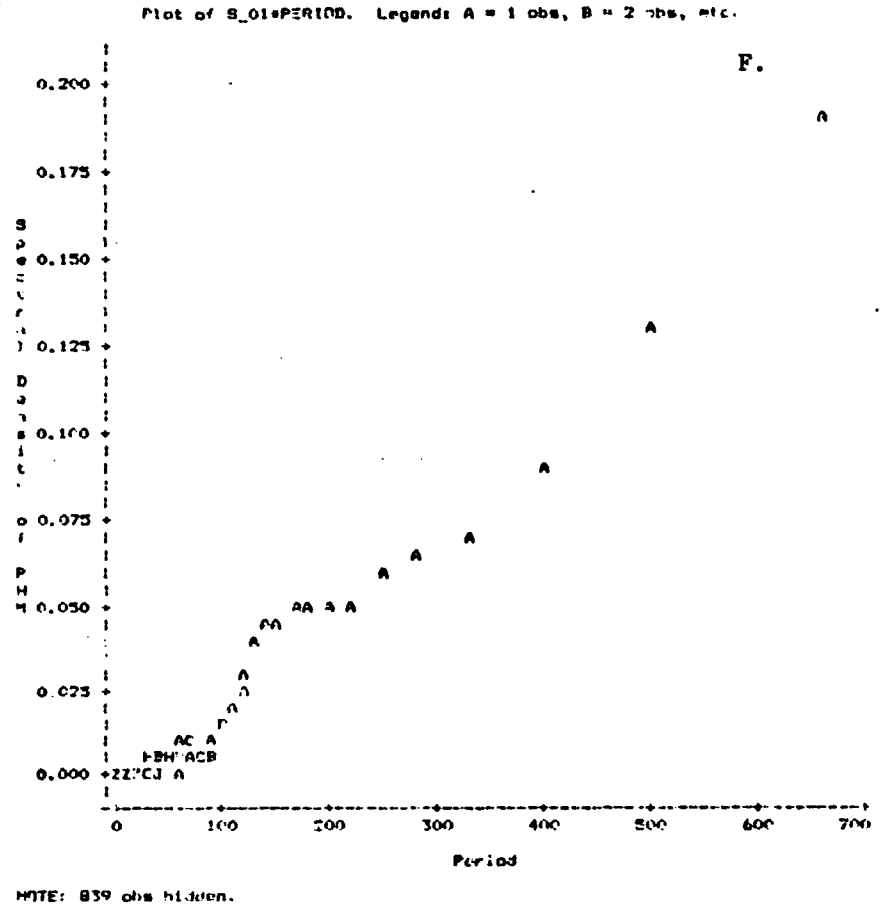


Fig. 3.26, continued. E. 80 ug Cu/L. F. 160 ug Cu/L.

CONCLUSIONS

4.0 SYNTHESIS

4.1 Microcosm sensitivity

4.1.1 Sensitivity and detectability of microcosm responses

The stress responses of complex biological communities in artificial ecosystems may be estimated using indices of change in community structure, nutrient cycling, and energy flow (Odum 1985). The choice of variables to be measured (metrics) is critical in any experiment directed at detecting adverse effects. Choosing metrics with high variability reduces the probability of detecting effects and can result in erroneous conclusions, namely a statistical type II error (incorrectly accepting a null hypothesis as true). Ecologically meaningful variables are often expected to have high variability, and variability is expected to increase with stress (Odum et al. 1979). Because of the natural variability, or noise, of even simple laboratory ecosystems, experiments attempting to examine stress effects under environmentally realistic conditions may lack appropriate statistical design, complicating the interpretation of stress effects (Mount 1985, Tebo 1985, Giesy 1985). Depending on the variability of the metrics chosen, the number of replicates required to detect differences among exposed, affected communities may be prohibitively large, suggesting that metrics with low variability and ecological meaning should be selected for measurement.

This chapter summarizes information on the detectability of community-level responses measured in laboratory microcosms using single-factor analysis of variance designs which resemble traditional, single-species methods for evaluating chemical stress effects. Statistical procedures for testing hypotheses in analysis of variance designs assume equality of variances among treatments and this assumption is also necessary for the analysis that follows. Coefficients of variation (CV's) for several commonly measured responses are summarized and minimal detectable differences (as a percentage of control values) possible based on different experimental designs are calculated. A limited number of comparisons with other reports of response variability are provided along with recommendations for the selection of measurement variables and suggested additional studies needed to assess community metrics.

4.1.2 Analysis

Data from several experiments evaluating the effects of toxic chemicals on microbial community structure and function in

laboratory microcosms have been summarized (Table 4.1). These include experiments from the present study and other experiments conducted using naturally derived microbial communities collected on artificial substrata from several different ecosystems. Effects on community structure (taxonomic richness, standing crop) and processes (production, respiration, nutrient cycling) are summarized. Because each physical sample was limited in volume and biomass and because the purposes of each experiment differed, the same suite of variables was not always measured in each experiment.

The purposes and dosing regimes differed among experiments, so only results from control communities were used in estimating the detectability of differences. Toxicant exposure can alter response variability, and we have observed response variability to increase, decrease, or remain the same depending on the response measured and the type of toxicant tested. Therefore, we have chosen to use control community data with the understanding that response variability under toxicant exposure may be altered. Variability of microbial community responses was estimated as the coefficient of variation (as a percent) for measures from triplicate control microcosms.

4.1.3 Results

Coefficients of variation for metrics collected under laboratory and field conditions are shown in Table 4.1. Dissolved oxygen and pH were the least variable metrics, but the low CV's are due in part to the limited range of these parameters (pH 6-10 and dissolved oxygen 5-20 mg/L). Protozoan species richness also had low variability despite the availability often of over 100 species of protozoa in laboratory experiments and a greater species pool in the field. All of the metrics in Table 4.1 exhibited CV's of no more than 30% under both laboratory and field conditions.

Statistical power curves for several possible experimental designs at $\alpha = 0.05$ level were generated (see Figs. 4.1 and 4.2) using the relationship (from Conquest 1983):

$$(\delta/\sigma)^2 > 2/n(t_{\alpha,\nu} + t_{2(1-\rho),\nu})^2$$

where $t_{\alpha,\nu}$ = two-tailed value from a t-distribution with ν degrees of freedom corresponding to a level of significance α, $t_{2(1-\rho),\nu}$ = two-tailed value from a t-distribution that cuts off probability ρ to the left of the value and $\rho = 1-\beta$ is the power of the test, δ = an estimate of $|\mu_i-\mu_j|$ (minimum detectable difference), s= an estimate of standard deviation, $\nu = k(n-1)$ (k=number of treatments), and n = the number of replicates.

With a knowledge of the variability (CV) of a given metric, the minimum detectable difference (MDD) between control and treatment means can be calculated by choosing a level of statistical power and estimate delta/sigma from the appropriate curve based on the experimental design using the relationship (from Conquest 1983):

$$MDD = CV/(\delta/\sigma)$$

MDD's for several community responses measured in laboratory microcosms are shown in Tables 4.2 and 4.3 under several experimental designs using CV's based on control response. When the number of treatments is held constant (e.g., k = 6), increasing the number of replicates from two to three decreases the MDD by approximately 9.1%, whereas increasing the number of replicates from three to four decreases the MDD by 16%. With an experimental design of six treatments and three replicates, the MDD for nine of the 14 metrics is less than 50%. The effect of increasing the number of treatments while maintaining the same number of replicates on the MDD is shown in Table 4.3. Increasing the number of treatments from two to three dramatically decreases the MDD by approximately 20%, although further increases in the number of treatments have much less effect (< 6%).

4.1.4 Discussion

One of the major concerns of increased environmental realism in toxicity testing has been increased variability of measured variables and, therefore, difficulty in establishing effect levels (Giesy 1985). There are three sources of metric variability: organismal, environmental, and methodological. The investigator has little or no control over the first two sources, but with prudent selection of metrics, careful analytical techniques, and replicate systems, variability can be managed so that community-level responses can be detected as statistically significant differences.

Based on our results from over 20 laboratory microcosm tests using naturally derived microbial communities, the variability of several ecosystem-level responses was sufficiently low to detect differences between control and exposed communities with simple experimental designs using minimal replication. Coefficients of variation for responses were similar whether collected in laboratory microcosms or under field conditions. An experimental design consisting of 5 to 6 treatments with 3 to 4 replicates provided enough statistical power to establish treatment effects using community metrics with CV's as high as 25%. Variability was similar for both structural and functional responses, although we did not evaluate species abundance data.

Crow and Taub (1979), however, reported that in their microcosms, measures of community "metabolism" (e.g., chlorophyll a, ash free dry weight [AFDW], dissolved oxygen) had less variability than organism counts.

Although the response variabilities reported refer to the artificial substrate microcosm method, other investigators using other laboratory microcosms have found similar response variability. Giddings and Eddlemon (1979) monitored sediment/water microcosms inoculated with a natural source of algae and macrophytes for 5 weeks and found that CV's for most microcosm measures were 10 to 30% and that CV's decreased as microcosms matured. Brockway et al. (1979) developed laboratory microcosms using pond sediment as a species source and measured nutrients, production/respiration (P/R), pH, and algal biomass. Coefficients of variation ranged from 0 to 99%, with the highest variability associated with orthophosphorus and nitrite measures, and static microcosms exhibited greater variability than flow-through microcosms. Coefficients of variation for chlorophyll a and AFDW measures of Aufwuchs collected on glass tubes in artificial streams were 13.6 and 6.96%, respectively (Krieger et al. 1988).

4.1.5 Cautions

Observations of the detectability and importance of ecosystem changes correspond well to most of the measured variables reported, but the possibility of detecting differences because of low variability does not predict the probability of detecting ecological effects. Changing community structure and standing crop biomass are important and useful measures of adverse ecological effects (Schaeffer et al. 1988). Changes in nutrient cycling and primary production are more problematic and may not be detectable in stressed ecosystems (Schindler 1987, Levine 1989). Additional environmental factors such as substrate supply affect processing rates more than the effects of toxicants. For example, we have observed comparatively low variability for alkaline phosphatase activity (APA), a measure of cycling rates of organic phosphorus. APA is affected by other variables such as the supply of inorganic phosphorous in dilution water. Ecological effects which might be interpretable as adverse effects of a toxicant on nutrient cycling often are observable only at extreme levels of stress (e.g., Pratt et al. 1987).

A knowledge of sampling and measurement precision is essential to understanding which variables will provide both the statistical power to detect ecological effects and heuristic power in interpreting results. Some variables have inherently low variability; for example, the numbers of species vary over

relatively narrow limits. Where variables are measured according to carefully controlled standard methods or standard operating procedures, variability will be reduced. Some response variables of inherent biological interest have low measurement precision because of methodological difficulties. Additionally, derived variables such as ratios often ignore the variability of the response variables used in their construction (Sokal and Rohlf 1981, Green 1989). The challenge in selecting response variables is to understand inherent variability. When the variability of response variables is estimated from pilot studies, the number of replicates can be controlled to achieve acceptable statistical power. When required replication is impractical, measurement error can often be controlled by improving sampling and analysis methods to achieve acceptable power.

Detection of ecosystem-level effects in multispecies laboratory microcosms has been shown to be possible, given prudent selection of metrics and experimental design. Microcosm experiments using natural communities optimize the probability of detecting effects (O'Neill and Waide 1981). Synthetic ecosystems relying on culture media and organisms that may not be co-adapted in any way should be expected to have higher variability. Further, results reported here utilize sampling from artificial substrata and standard methods to further reduce variability. Our experiments have been short-term measures of community response. Where longer experiment times and repeated measures have been used to characterize responses to pollutants, variability should be expected to be greater (Giesy and Allred 1985). The repeatability of community-level experiments has yet to be demonstrated (Giesy and Allred 1985), although response variability is sufficiently low that standardization of laboratory testing and field measurements should allow sufficient power to detect adverse community responses to stress.

Experimental designs based on the analysis of variance assume that variances among treatments are equal (Sokal and Rohlf 1981). Given the expectation of changing variance with stress (Odum et al. 1979), tests for variance homogeneity (e.g., Bartlett's test) should be conducted to assure that the assumptions of analysis of variance are validated. A pattern of increasing or decreasing variability can also be detected by plotting residuals from regression of response on dose or exposure (treatment). Such a pattern might be reflective of exposure variability or of important qualitative changes in the nature of the measured response. Although the number of replicates needed to detect effects of a given magnitude can be determined a priori using reasonable estimates of variability, such estimates of numbers of replicates do not always match the availability of research resources (personnel, space, time). Therefore, sampling must be focused on those variables that

convey ecotoxicological meaning and provide investigators with resolving power for finding differences. At the present time, these variables are primarily structural measures.

Table 4.1. Coefficients of variation (CV) for several microbial community metrics measured under laboratory and field conditions.

Metric	Laboratory Microcosms n	CV		Field Conditions n	CV	
Protozoan Species	23	7.29	(4.33)	11	6.57	(3.74)
Total Protein	24	17.6	(10.6)	11	19.5	(11.4)
Chlorophyll a	18	22.11	(12.0)	11	24.2	(11.1)
Alkaline Phosphatase Activity	23	18.0	(11.3)	11	13.2	(12.2)
Carbohydrate	6	26.0	(14.1)	9	20.2	(4.12)
Hexosamine	8	25.8	(11.7)	5	30.6	(11.8)
Ash Free Dry Weight	4	30.1	(18.1)	13	21.2	(16.8)
Electron Trans. System Act.		--		4	10.4	(6.51)
Magnesium	12	13.3	(10.8)			
Calcium	12	11.4	(6.38)			
Potassium	13	16.5	(7.43)			
Phosphate	5	25.6	(20.1)			
Dissolved Oxygenb	14	4.53	(3.33)			
pHb	11	2.22	(1.44)			
P/Rb	8	13.9	(7.61)			

a Values in parentheses are S.D. of CV
b Measured directly in microcosm

Table 4.2. Calculated minimum detectable differences (MDDs as percent of control) for several microbial community metrics based on coefficients of variation from laboratory microcosm measurements. MDDs are shown for experimental designs of 6 treatments and n replicates using $\alpha = 0.05$ and $\beta = 0.2$.

Metric	n = 2	n = 3	n = 4	n = 6	n = 8	n = 12
Protozoan Species	20.0	18.1	15.2	12.1	10.4	8.46
Protein	48.2	43.8	36.8	29.4	25.2	20.4
Chlorophyll a	60.5	55.0	46.2	36.9	31.6	25.6
APA[a]	49.3	44.8	37.6	30.1	25.7	20.9
Carbohydrate	71.2	64.7	54.3	43.4	37.2	30.2
Hexosamine	70.6	64.2	53.9	43.1	36.9	29.9
AFDW[b]	82.5	74.9	62.9	50.3	43.0	34.9
Magnesium	36.4	33.1	27.8	22.2	19.0	15.4
Calcium	31.2	28.3	23.8	19.0	16.3	13.2
Potassium	45.2	41.1	34.5	27.5	23.5	19.1
Phosphate	70.1	63.7	53.5	42.7	36.6	26.7
Dissolved Oxy.	12.4	11.3	9.47	7.56	6.48	5.25
pH	6.08	5.53	4.64	3.71	3.17	2.57
P/R	38.1	34.6	29.0	23.2	19.9	16.1

a Alkaline phosphatase activity
b Ash free dry weight

Table 4.3. Calculated minimum detectable differences (MDDs as percent of control) for several microbial community metrics based on coefficients of variation from laboratory microcosm measurements. MDDs are shown for experimental designs with 3 replicates and k treatments using $\alpha = 0.05$ and $\beta = 0.2$.

Metric	k = 2	k = 3	k = 4	k = 6	k = 8	k = 12
Protozoan Species	24.8	19.9	18.7	18.1	17.5	17.2
Protein	59.8	48.0	45.2	43.8	42.2	41.5
Chlorophyll a	75.1	60.3	56.8	55.0	53.0	52.1
APA[a]	61.2	49.1	46.3	44.8	43.2	42.5
Carbohydrate	88.3	71.0	66.8	64.7	62.4	61.4
Hexosamine	87.7	70.4	66.3	64.2	61.9	60.9
AFDW[b]	102.3	82.2	77.4	79.9	74.9	71.0
Magnesium	45.2	36.3	34.2	33.1	31.9	31.4
Calcium	38.7	31.1	29.3	28.4	27.4	26.9
Potassium	56.1	45.0	42.4	42.4	39.6	38.9
Phosphate	87.0	69.9	65.8	63.7	61.4	60.4
Dissolved Oxy.	15.4	12.4	11.6	11.3	10.9	10.7
pH	7.54	6.06	5.70	5.53	5.33	5.24
P/R	47.2	37.9	35.7	34.6	33.4	32.8

a Alkaline phosphatase activity
b Ash free dry weight

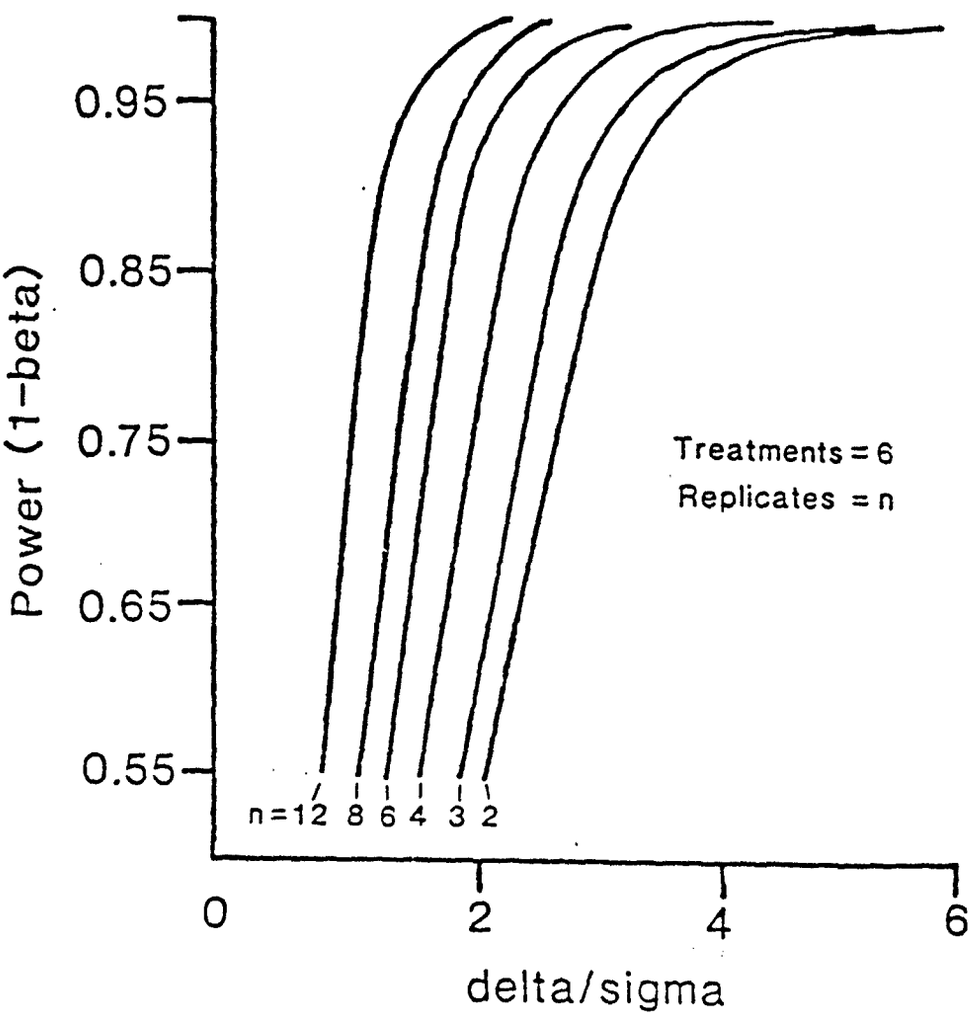


Fig. 4.1. Relationship among number of replicates (n), statistical power, and δ/σ for experiments with six treatment levels (k=6).

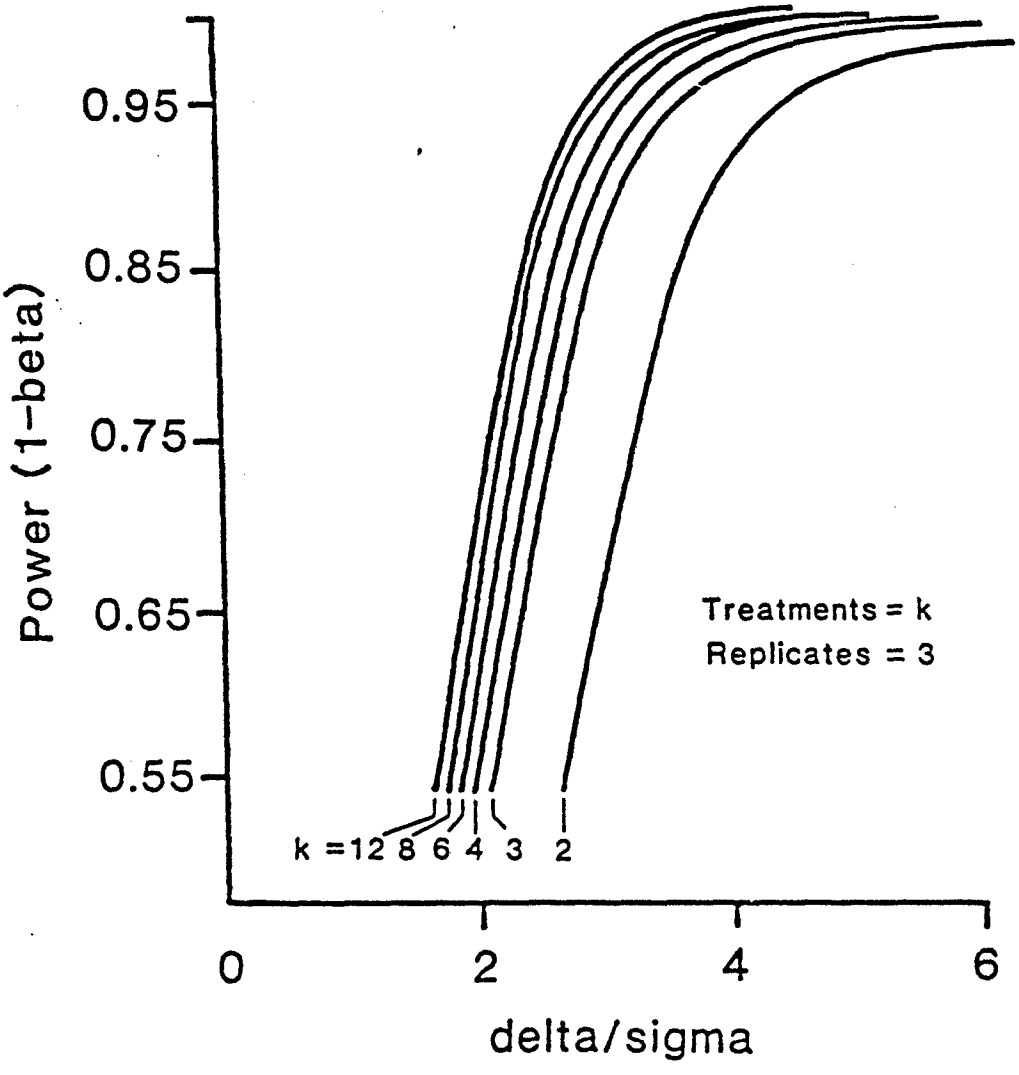


Fig. 4.2. Relationship among number of treatments (k), statistical power, and delta/sigma (δ/σ) for experiments with three replicates per treatment (n=3).

4.2 System Comparisons

A useful microcosm testing system should have the following qualities.

1. Respond at environmentally realistic levels of contaminants expected to produce adverse effects in ecosystems.
2. Display qualitatively or quantitatively different responses from those measurable in surrogate species testing.
3. Display repeatable responses to the same [reference] toxicant when conducted using standard procedures.
4. Have sufficiently low variability to achieve acceptable statistical detection power.

In this chapter, we examine the responses of a naturally derived laboratory microcosm system to chemical stresses and compare these responses to results from standard single species tests and other field and laboratory investigations.

4.2.1 Analysis

Response levels

For microcosm tests to be useful predictors of community and ecosystem effects, variables measured should be affected at toxicant levels similar to those derived for single species tests, and should be affected near concentrations expected to be encountered in stressed ecosystems. The results reported below are for a limited array of organic and inorganic compounds and complex effluents. Comparisons of the levels of toxicant producing the most sensitive adverse response, the reported effective concentrations in single species experiments, and numerical water quality criteria were made.

Inorganics

AS-M tests of five different inorganic compounds are summarized in Table 4.4. The reported MATCs from AS-M experiments agree with literature values from single-species tests. This is not surprising because these toxicants are generally broad spectrum biocides with multiple modes of action. Most of the MATCs from microcosm experiments are at or below the water quality criteria for the respective chemical. Particularly striking is the effect of zinc on biomass and function at levels two orders of magnitude lower than water quality criteria. This demonstrates the sensitivity of the microcosm system, and emphasizes the possibility that single-species tests may be inadequate for the protection of the entire ecosystem (Cairns 1984, Kimball and Levin 1985).

Structural and functional measures of the microbial community tend to be equally affected by these inorganic toxicants, although information on functional measures is limited. In general, measured variables were affected at concentrations in the lower portion of the range reported from single species tests.

Organics

The results from tests using organic compounds are more ambiguous and variable than those for inorganic chemicals (Table 4.5). This may be due to the selective modes of action of these compounds, the absence of toxicant targets in the microcosms, or the degradation of toxicant by components of the microbial community. The MATCs reported from the microcosm tests are within the range of reported literature values from single-species tests, but water quality criteria for these chemicals are limited by the relatively small amount of available data.

Structural variables were adversely affected at lower concentrations than the functional variables. Four of the seven experiments had lower MATCs for a structural measure than for functional variables. These data lend support to the contention that structural effects will be detected before a functional change (Odum 1990, Odum 1985, Schindler 1987). Ecosystems have many functionally equivalent organisms; that is, there are many organisms in an ecosystem which perform the same function, and elimination of some sensitive taxa may not result in a detectable adverse effect due to compensation by the functional equivalents. Additionally, functional changes should only be affected when the supply of chemical substrates changes (Levine 1989).

There are some unusual results from these experiments. First, species richness and biomass were stimulated at concentrations of atrazine an order of magnitude lower than literature values for chronic toxicity to animals. This stimulatory response, though different from the traditional inhibitory response, could be an indirect toxic effect on certain "keystone" or controlling species, whose elimination from the community results in the elaboration of previously rare species. Second, there were no detectable effects of chlorpyrifos. Chlorpyrifos is a highly toxic substance, as evidenced by the low water quality criterion, but its mode of action is specific. Chlorpyrifos is an acetylcholinesterase inhibitor, and the majority of organisms tested in these microcosms lack any neural function. Therefore, one must consider the mode of action of the toxicant to be tested, and realize the limitations of the microcosm system before drawing any conclusions (which in this case would be erroneous) about the toxicity of a substance.

Effluents

Four complex effluents were studied with the AS-M: a foundry effluent containing zinc and ammonia, a brass mill effluent contain copper and zinc, a sewage treatment effluent containing chlorination products and nutrients, and a fly ash basin effluent containing several heavy metals. The microcosm experiments were compared to results obtained in single-species tests. Effects were observed at or below the levels at which effects were observed in single-species chronic tests (Table 4.6). Adverse effects were also observed at effluent levels below the estimated instream waste concentration (IWC, the ratio of effluent flow to stream and effluent flow, as a percent). The lowest adverse effects were detected in the metal containing effluents from the brass mill and foundry. The sewage treatment effluent was apparently non-toxic, probably due to the interfering effects of the high nutrient levels in the effluent.

These experiments indicate that variables measured in the AS-M system respond at environmentally realistic levels of chemicals and mixtures expected to result in adverse effects on ecosystems. Both structural and functional variables can be useful in predicting adverse effects, although effects are dependent on the mode of action of the toxicant tested. Some substances may result in stimulatory responses, which may be considered an adverse effect on "keystone" organisms that is expressed indirectly. No one variable was always sensitive to toxicant action, although structural measures (both species richness and biomass measures) were typically more sensitive than functional measures of adverse effects

Qualitative response differences

Measurement of adverse effects in typical single species tests used for regulatory purposes focuses on reduced survival in acute tests and on reduced growth or reproduction in short-term chronic tests. Commonly, increasing dose results in a decrease in the response variable so that the dose-response relationship is usually log-linear (Rand and Petrocelli 1984). While stimulation at low doses (i.e., hormesis) can be observed it is not common.

The variables measured in microcosms show greater potential response ranges than single species tests, including stimulation as well as expected monotonic dose-response patterns. In short-term chronic tests, effects are measured on only one species. In microcosm experiments, the total number of species in the artificial ecosystem typically declines in response to toxicity (Figs. 4.3, 4.4). Microcosm experiments limit the potential recolonization and recovery of the experimental systems, but a

comparison of Figs. 4.3 and 4.4 shows that the level of toxic material eliminating 20% of the microcosm taxa is approximately the same as water quality criteria (see Table 4.4). This suggests not only that criteria may not be protective, but demonstrates a qualitative difference in response types. Elimination of taxa (local extinction) corresponds to levels of chronic (sub-lethal) responses of laboratory populations.

Community and ecosystem responses need not be monotonically negative with increasing toxicant dose. For example, the effect of atrazine on AS-M communities resulted in increased biomass at intermediate toxicant levels (Fig. 4.5), although higher levels resulted in an eventually negative response to the toxicant. This unexpected response (termed the subsidy-stress gradient, Odum et al. 1979), further demonstrates that adverse effects may not always result in a reduction of response. As previously mentioned, selective toxicants such as atrazine (a photosynthetic inhibitor) may affect controlling factors in communities, releasing some species to proliferate. It would be incorrect to categorize such a response as "enhancement" because it is difficult to understand how photosynthetic inhibition could be a positive influence on natural communities.

In addition to apparent subsidy effects, toxicant can sometimes affect community responses in a positive, dose-related manner. For example, inhibition of communities by zinc (Fig. 4.6) resulted in reduced total phosphate and increased activity of phosphorus recovering enzymes. The geometric increase in alkaline phosphatase activity (APA) is clearly a dose-related adverse effect of the toxicant, but such responses are not incorporated into current risk (or hazard) assessment methods.

Microcosms display qualitatively and quantitatively different responses than those of single species toxicity tests. These responses include changes in community composition and collective measures such as standing crop biomass. Functional responses, such as enzyme activities, may not fit neatly into expectation of adverse effects as reduced community performance.

Repeatability of results

If microcosm methods are to be useful in assessing toxicity, then procedures must be standardized to achieve repeatable results. The AS-Microcosm protocol was evaluated for repeatability using copper as a reference toxicant. Six experiments were conducted at three different locations: Pennsylvania, Virginia, and Maryland (Table 4.7) using community sources from local ecosystems. The source ecosystems were, respectively, Spring Creek (Centre County, PA), Pandapas Pond (Montgomery County, VA), and a spring-fed pond in Frederick

County (MD). The water quality criteria for dilution water ranged from 8-22 µg/L Cu at the water hardness at which experiments were conducted. For nearly all tests, adverse effects were observed at or below the water quality criteria and effect levels are all similar to each other.

Effect levels are similar with classes of measures (species richness, biomass, community function) in all experiments. Biomass measures are more variable, and this may be due to seasonal differences. Results of experiments conducted using microbial communities derived from the same source ecosystem are similar, suggesting repeatability of results within an ecosystem. Interecosystem comparisons are also similar. Variability is similar to or less than that reported for single species acute tests of many compounds (Mayer and Ellersieck 1986) using methods that are simple and standardized. These experiments indicate that the AS-Microcosm test system is repeatable when standard procedures are followed. Similar results were obtained by Taub and colleagues (Taub et al. 1989, Taub et al. 1986) when testing the Standard Aquatic Microcosm among several laboratories, although the concentrations tested were much higher (lowest copper concentration 300 µg/L).

Differences among ecosystems in response to toxic dose are more problematic. For example, the speciation of copper in these experiments clearly varied due to different water hardness and pH, but results do not vary greatly even though free cupric ion is probably not similar among experiments. In contrast, the relative sensitivity of communities from differing ecosystems is not understood, although it is widely assumed to be similar for purposes of regulation.

Measurement variability

Variables measured as microcosm responses to toxicants differ in their precision. Some measures have significant variability with both biological and procedural sources. For example, the measurement of chlorophyll a varies because replicate communities vary and because the methods for concentrating cells, extracting the pigment, and measuring the extract introduce additional error. Other variables can be measured with less error. For example, spectrophotometric determination of macronutrients such as calcium have low variability. Other measures may be discrete data, such as the enumeration of species, also associated with low variability, so some measures will be naturally less variable than others.

The effect of measurement error, in a statistical sense, is a reduction in the power to detect differences among treatments. The importance of measurement error in ecotoxicology is the error

that it may introduce into conclusions drawn from experiments. Adequate understanding of the effect of measurement error on the potential to detect effects is important in experimental design and interpretation of results (Giesy and Allred 1985, Conquest 1983).

4.2.2 Conclusions

Ecological assessment of toxic chemicals requires estimating effects on complex ecological structures. If the goal of environmental protection is to conserve ecological diversity and ensure the continued integrity of ecosystems, then laboratory ecosystems (microcosms) can provide a rapid and sensitive means of evaluating the adequacy of conclusions drawn from traditional hazard assessments. Microcosms containing diverse communities display the predicted symptoms of ecosystem disease (Schaeffer et al. 1988) in a manner that is both repeatable and sensitive to many stressors. However, microcosm experiments, like single species tests, are not globally sensitive to all stresses. Where microcosms lack appropriate target species for toxicants with specific modes of action, little effect can be detected. Toxicant effects are the result of complex interactions between the toxicant, the available biota, and abiotic factors resulting in responses that are not predictable from single species tests. Microcosms provide an opportunity to test hypotheses of environmental safety and harm in a manner that is rapid, sensitive, repeatable, and capable of demonstrating unexpected, adverse ecological consequences of toxic materials.

Table 4.4. MATCs for microcosm toxicity experiments of inorganic toxicants. Values shown are µg/L. Letters in parentheses identify the most sensitive variable in a class of responses: H = Hexosamine, A = Alkaline phosphatase activity, DO = Dissolved oxygen, C = Chlorophyll a, DW = Ash-free dry weight, ATP = Adenosine triphosphate biomass. Literature values are from USEPA (1986) unless otherwise noted. Water quality criteria (WQC) are for a hardness of 100 mg $CaCO_3$/L. N.S. = not significant.

Compound	Variable (Microcosm)			Literature[3]	WQC
	Species Richness	Biomass	Function		
Cadmium[1]	0.9	1.2(ATP)	-	0.15-156	1.1
Chlorine	3.6	2.1[2](C)	3.6(A)	< 3.4-26	11
Copper[1]	9.2	6.6[2](C)	-	3.9-60.4	12
Selenium	14.1	14.1(H)	N.S.	10-10,000	35
Zinc[1]	51.5	4.2[2](DW)	4.2[2](DO)	46.7-5243	110

1 - Hardness based criteria
2 - LOEC
3 - References: Hunn et al. (1987), Reading and Buikema (1983)

Table 4.5. MATCs for microcosm experiments using organic toxicants. Table values are µg/L. Letters in parentheses identify the most sensitive variable in a response group: A = Alkaline phosphatase activity, P = Protein, DO = Dissolved oxygen, C = Chlorophyll a, D = Dehydrogenase activity, PO_4 = Orthophosphate. Literature ranges and water quality criteria are from USEPA (1986) unless otherwise noted. N.S. = not significant.

Compound	Variable (Microcosm)			Literature	WQC
	Species Richness	Biomass	Function		
Organics					
Atrazine[1]	$3.2^{S,2}$ 192^{I}	$3.2^{S,2}$(P) 192^{I}	32 (DO)	71-3400	--
Chlorpyrifos	N.S.	N.S.	N.S.	0.12	0.041
PCP[3,4]	228	N.S.	228(PO_4)	<1.8-79.7	13
Phenol	5700	$300^{S,2}$(P) 5700^{I}(C)	9200(DO)	2560	2560
TNT[5,6]	208	N.S.	507(A)	40-10,000	--

S = Stimulation, I = Inhibition
1 - References: Johnson (1986), Kemp et al. (1985), Macek (1976), Shaw et al. (1985), Stay et al. (1985), Ward and Ballantine (1985).
2 - LOEC
3 - Pentachlorophenol
4 - pH dependent criteria
5 - Trinitrotoluene
6 - Reference: Ryan (1987)

Table 4.6. MATCs for microcosm experiments and single species tests (SST) using complex effluents. Table values are %. Letters in parentheses indicate the most sensitive variable in a response group: P = Protein, I = *In vivo* fluorescence, A = Alkaline phosphatase activity, DO = Dissolved oxygen, C = Chlorophyll a. Instream waste concentration (IWC) estimates vary with assumed low flow estimates. N.S. = no significant difference.

Effluent	Variable (Microcosm)			SST	IWC
	Species Richness	Biomass	Function		
Fly Ash	35	71(C)	35(DO)	35	100
Sewage Treatment	N.S.	N.S.	18(DO)	10[1]	37
Foundry	71	6[2](P)	6[2](A)	17(R)	10-30
Brass Mill	6[2]	6[2](I)	N.S.	8.8(R)	1-4

1 - acute toxicity test
2 - LOEC

Table 4.7. Summary of copper toxicity by response class in microcosms. Table values are μg Cu/L. Letters in parentheses identify the most sensitive variable in each response class: P = Protein, C = Chlorophyll a, A = Alkaline phosphatase activity, DO = Dissolved oxygen, ATP - Adenosine triphosphate biomass. The water quality criteria are calculated for dilution water hardness.

Test/ Date	WQC	MATC or LOEC Species Richness	Biomass	Function
Pennsylvania				
Nov88	19.5	19.9	9.9(P)	40 (DO)
Nov89		--	113 (C)	40 (DO)
Feb90		20[1]	73.3(C)	N.S.
Apr90		9.1[1]	53.9(C)	25.8(DO,A)
Maryland				
Mar89	22.1	13.9	6.8(P,C)	4.7[1] (DO)
Virginia				
Feb86	8.2	9.2	6.6(C,ATP)	--

1 - LOEC

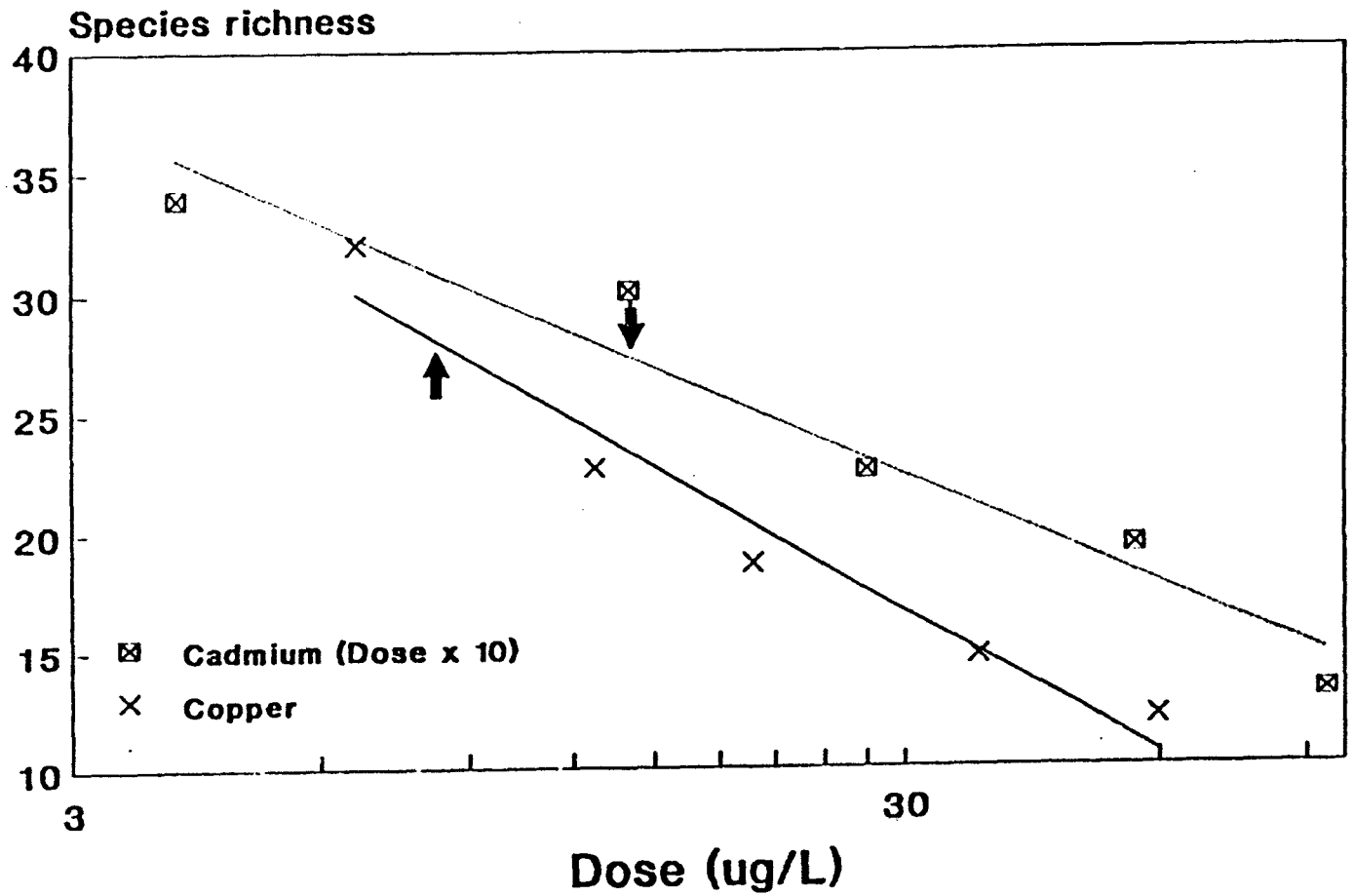


Fig. 4.3 Effects of cadmium and copper on the species richness of protozoa in artificial substrate microcosms. Plotted points are means of triplicates. Fitted lines are ordinary least squares regression lines. Arrows denote points on the fitted lines corresponding to a 20% reduction in species richness compared to controls. Data for cadmium are plotted as dose X10 for scale. Data from Niederlehner et al (1985) and Pratt et al. (1987).

Contract DAMD17-88-C-8068

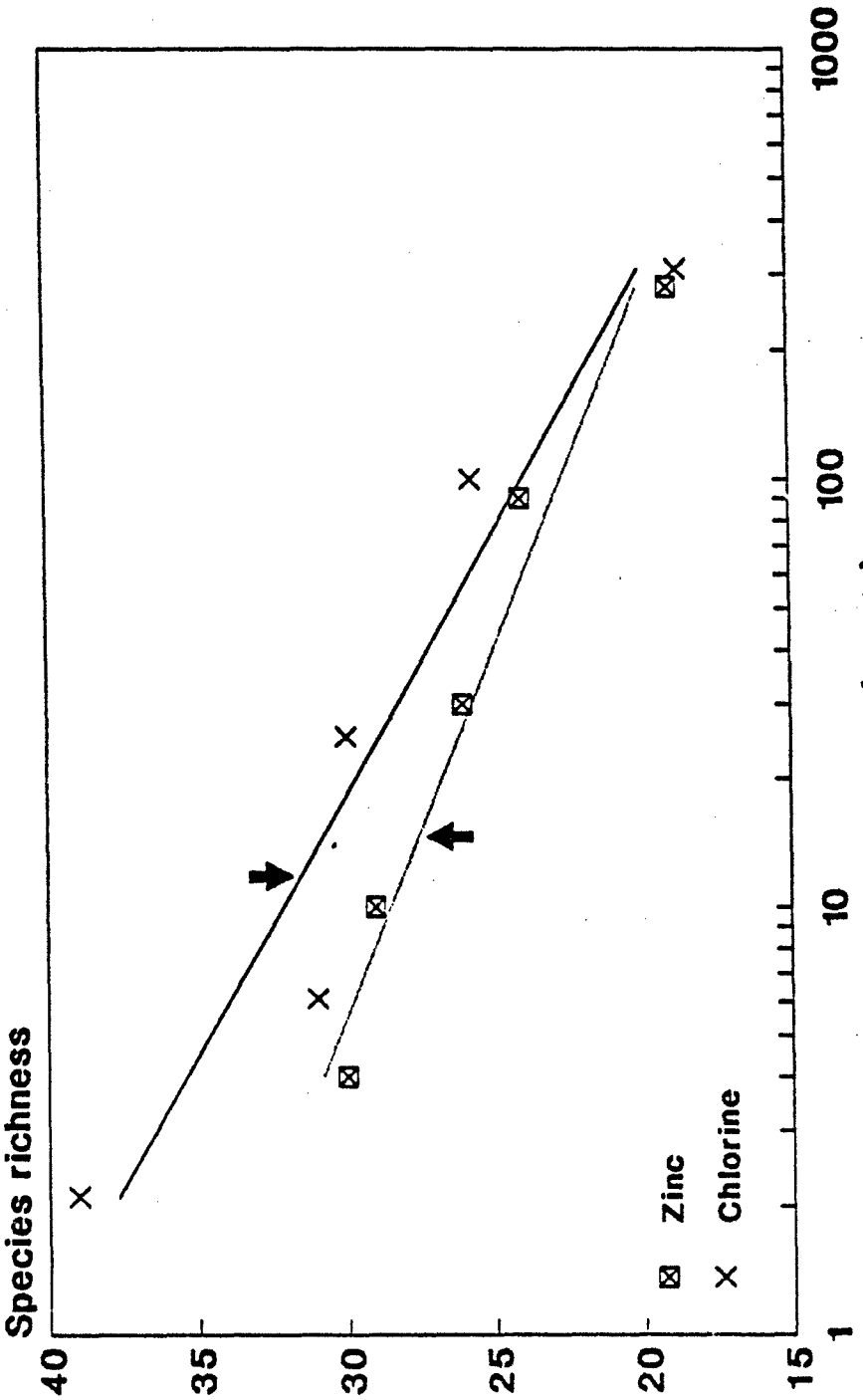


Fig. 4.4 Effects of zinc and total residual chlorine (chlorine) on the species richness of protozoa in artificial substrate microcosms. Plotted points are means of triplicates. Fitted lines are ordinary least squares regression lines. Arrows denote points on the fitted lines corresponding to a 20% reduction in species richness compared to controls. Data from Pratt et al. (1988) and Pratt et al. (1986).

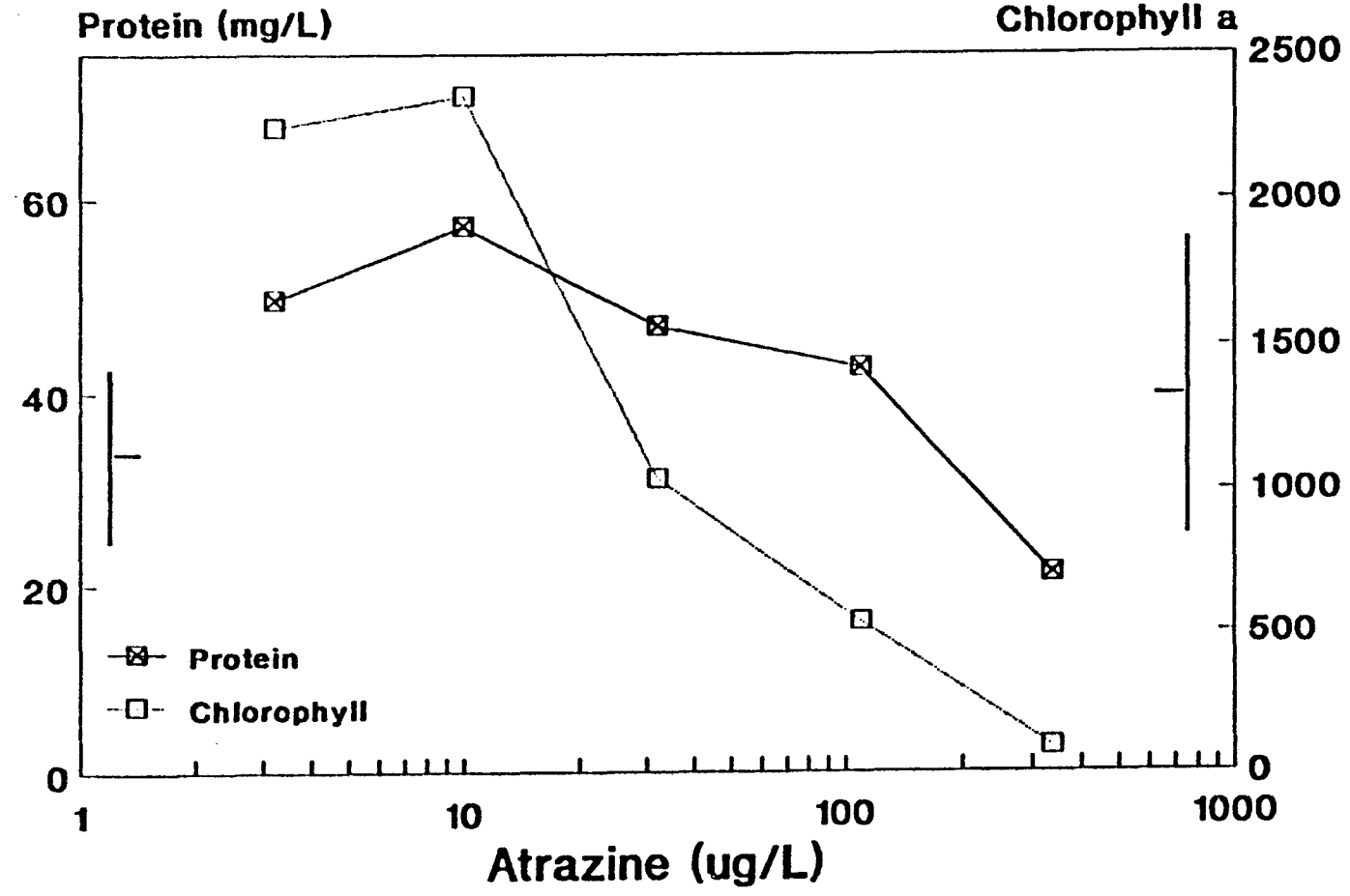


Fig. 4.5 Stimulation of protein and chlorophyll a biomass in artificial substrate microcosms by atrazine. Plotted points are means of triplicates. Heavy bars along axes show the mean (± standard deviation) of control values. Data from Pratt et al. (1988).

Contract DAMD17-88-C-8068

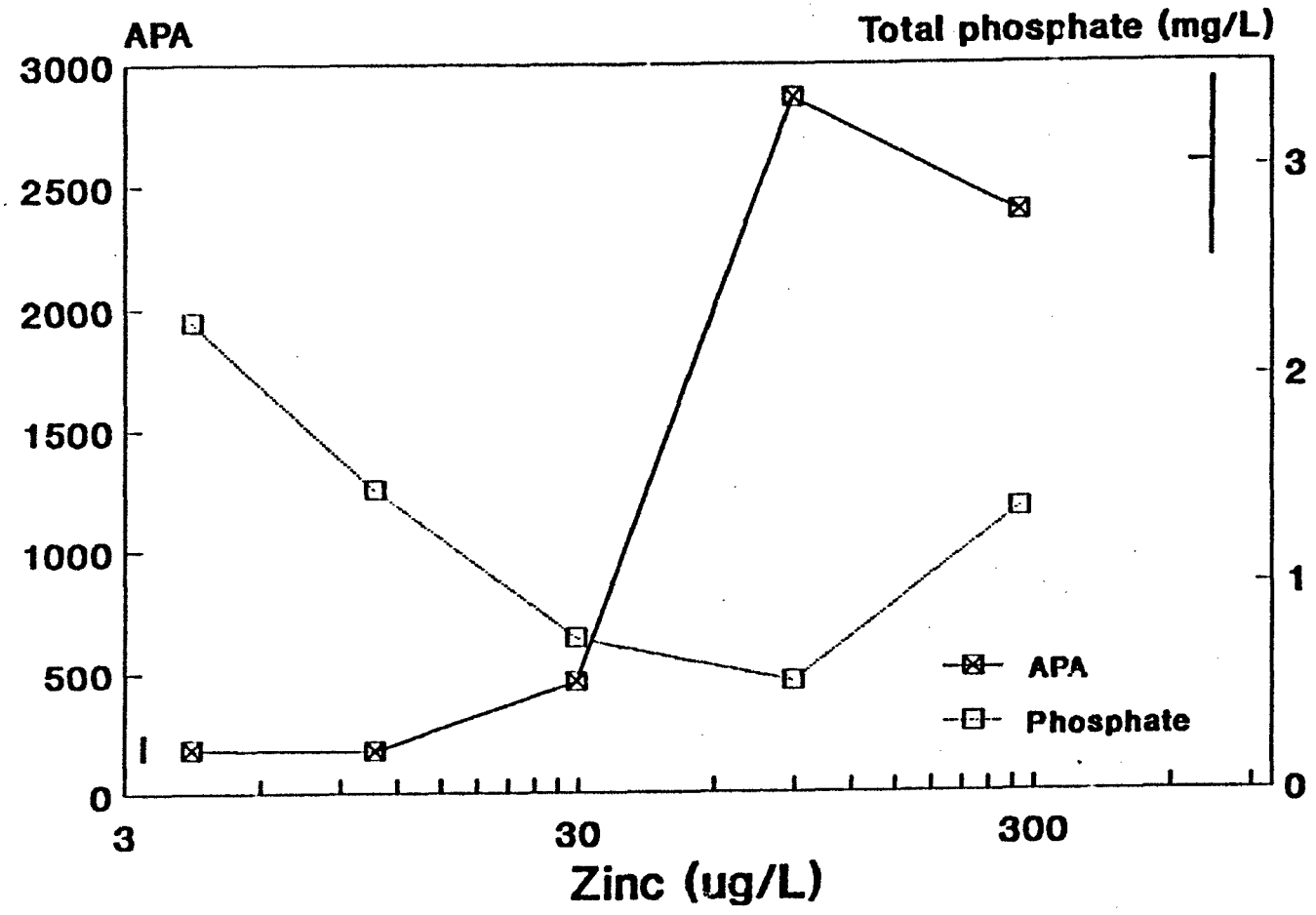


Fig. 4.6 Interaction of total phosphate and alkaline phosphatase activity (APA) in response to zinc toxicity. APA increases geometrically as communities lose phosphate. Heavy bars along axes show control values (± standard deviation). Units for APA are nmoles p-nitrophenyl phosphate/mg protein/hr. Data from Pratt et al. (1986).

Contract DAMD17-88-C-8068

4.3 General conclusions

These studies have shown the applicability of microcosm testing ecological hazard assessment. Microcosms can be developed quickly and easily using naturally occurring microbial communities. These communities respond to many toxicants at environmentally realistic levels of contaminants. Microbial community responses are sensitive to toxic action and include changes in species richness and composition, standing crop biomass, and processes such as production, respiration, and activity of nutrient transporting enzymes. Changes in species richness and standing crop biomass were broadly sensitive to toxicants, while changes in process measures were less sensitive or more variable responses to stress. Changes in indirect measures of ecosystem condition, such as changes in continuously monitored pH, require additional development before they can be applied to hazard assessments.

Microcosms were limited in detecting adverse effects of some materials. When microcosms lacked the target organisms of some specifically toxic compounds, their responses were less sensitive than those of single species. For example, microcosms were insensitive (in terms of contaminant level producing effects) to chlorpyrifos, an organophosphorus insecticide. Microcosms were also insensitive to chlorine-containing waste water when high levels of nutrients were present. Nutrient effects masked any adverse effects of toxicants, even though these effects were observed in the receiving ecosystem.

Microcosms can play an important role in ecological hazard assessment, but further research is needed to elucidate important ecosystem differences in response to toxicants. Ecosystem vary in their characteristic standing crops, species composition, and cyclic behavior. The interaction between these characteristics and the effects of toxic contaminants is not understood. Microcosm testing methods provide a means for evaluating site-specific effects of contaminants and complex mixtures, and they should provide information that cannot be obtained from single species toxicity testing. Microcosms are not universal answers to questions about toxicity, but they can provide rapid and inexpensive estimates of ecological effects. Response levels are comparable to those for single species tests; microcosms are somewhat more sensitive to conservative pollutants like heavy metals and contain organisms that can degrade labile pollutants. These characteristics make them more environmentally realistic indicators of environmental stress than tests of individual species and suggest that microcosm studies can complement traditional hazard assessment methods.

5.0 REFERENCES

Adema, D.M.M. 1978. Daphnia magna as a test animal in acute and chronic toxicity tests. Hydrobiologia 59:125.

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1985. standard Methods for the Examination of Water and Wastewater, 16th ed., Washington, D.C., 1193 p.

Bailey, H.C. 1982. Development and testing of a laboratory model ecosystem for use in evaluating biological effects and chemical fate of pollutants, pp. 221-233 in J.G. Pearson, R.B. Foster, and W.E. Bishop, eds., Aquatic Toxicology and Hazard Assessment, ASTM STP 766. American Society for Testing and Materials, Philadelphia, PA.

Bailey, H.C., R.J. Spanggord, H.S. Javitz, and D.H.W. Liu. 1985. Toxicity of TNT wastewaters to aquatic organisms. Volume Chronic Toxicity of LAP wastewater and 2,4,6-Trinitrotoluene. Final Report. SRI International, Menlo Park, CA. DAMD17-75-C-5056.

Benoit, D., V. Mattson, and D. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Res. 16:457-464.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein, utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Brazner, J.C., Lozano, S.J., M.J. Knuth, S.L. Bertelsen. L.J. Heinis, D.A. Jensen, E.R. Kline, S.L. O'Halloran, K.W. Sargent, D.K. Tanner, and R.E. Siefert. 1988. The effects of chlorpyrifos on a natural aquatic system: a research design for littoral enclosure studies. Final Research Report. U.S. EPA, ERL-Duluth, MN.

Brockway, D.L., J. Hill, J.R. Maudsley, and R.L. Lassiter. 1979. Development, replicability and modelling of naturally derived microcosms. Int. J. Environ. Stud. 13:149-158.

Brown, J.R., and L.Y. Chow. 1975. The effects of Dursban on microflora in non-saline waters. Environ. Qual. Safety, Pesticides Suppl. 3(155):774-779.

Brown, J.R., L.Y. Chow, and C.B. Deng. 1976. The effect of Dursban upon freshwater phytoplankton. Bull. Environ. Contam. Toxicol. 15:437-441.

Burton, G.A., Jr., D. Nimmo, D. Murphey, and F. Payne. 1987a. Stream profile determinations using microbial activity assays and Ceriodaphnia. Environ. Toxicol.Chem. 6:505-513.

Burton, G.A., Jr., J.M. Lazorchak, W.T. Waller, and G.R. Lanza. 1987b. Arsenic toxicity changes in the presence of sediment. Bull. Environ. Contam. Toxicol. 38:491-499.

Butcher, J.E., M.G. Boyer, and C.D. Fowle. 1977. Some changes in pond chemistry and photosynthetic activity following treatment with increasing concentrations of chlorpyrifos. Bull. Environ. Contam. Toxicol. 17:752-758.

Cairns, J., Jr. 1983. Are single species tests alone adequate for estimating environmental hazard? Hydrobiologia 100:47-57.

Cairns, J. Jr. 1981. Biological monitoring, part VI - Future needs. Water Res. 15:941-952.

Cairns, J., Jr., D. Kuhn, and J. Plafkin. 1979. Protozoan colonization of artificial substrates, p. 34-57 in R.L. Weitzel, ed., Methods and Measurements of Periphyton Communities: A Review, STP 690. American Society for Testing and Materials, Philadelphia, PA.

Cairns, J. Jr., B.R. Niederlehner, and J.R. Pratt. 1990. Evaluation of joint toxicity of chlorine and ammonia to aquatic communities. Aquat.Tox. 16:87-100.

Cairns, J. Jr. and J.R. Pratt. 1986. Ecological consequence assessment: effects of bioengineered organisms. Water Res. Bull. 22:171-182.

Conquest, L. 1983. Assessing the statistical effectiveness of ecological experiments: utility of the coefficient of variation. Int. J. Environ. Stud. 20:209-221.

Crow, M.E. and F.B. Taub. 1979. Designing an microcosm bioassay to detect ecosystem level effects. Int. J. Environ. Studies. 13:119-123.

Dunnett, C. 1955. Multiple comparison procedure for comparing several treatments with a control. J. Amer. Stat. Assoc. 50:1096-1121.

Eaton, J.G., J. Arthur, R. Hermanutz, R. Kiefer, L. Mueller, R. Anderson, R. Ericksen, B.Nordling, J. Rogers, and H. Pritchard. 1984. Biological effects of Dursban on an outdoor experimental stream ecosystem. Project Report. U.S. EPA, ERL-Duluth, MN.

Feind, D., Zieris, F.S., and W. Huber, 1988. Effects of sodium pentachlorophenate on the ecology of a freshwater model ecosystem. Environ. Pollut. 50:211-224.

Fishbein, L. 1983. Environmental selenium and its significance. Fund. Appl. Toxicol. 3:411-419.

Gatt, R. and E. Berman. 1966. A rapid procedure for the estimation of amino sugars on a microscale. Anal. Biochem. 15:167-171.

Giddings, J.M. and G.K. Eddlemon. 1978. Photsynthesis/respiration ratios in aquatic microcosms under arsenic stress. Water Air Soil Pollut. 9:207-212.

Giddings, J.M. and C.K. Eddelmon. 1979. Some ecological and experimental properties of complex aquatic microcosms. Int. J. Environ. Stud. 13:119-123.

Giesy, J.P. 1985. Multispecies tests:research needs to assess the effects of chemicals on aquatic life, pp.66-77 in R.C. Bahner and D.J. Hansen, eds., Aquatic Toxicology and Hazard Assessment:Eight Symposium, STP 891. American Society for Testing and Materials, Philadelphia, PA.

Giesy, J.P. Jr., ed. 1980. Microcosms in Ecological Research. DOE Symposium Series 52, 1110 pp.

Giesy, J.P. and P.M. Allred. 1985. Replicability of aquatic multispecies test systems, pp.187-247 in J. Cairns, Jr., ed., Multispecies Toxicity Testing, Pergamon Prss, New York, NY.

Gotham, I.J., and G.Y. Rhee. 1982. Effects of hexachlorobiphenyl and pentachlorophenol on growth and photosynthesis of phytoplankton. J. Great Lakes Res. 8:323-335.

Green, R.H. 1989. Power analysis and practical strategies for environmental monitoring. Environ. Res. 50:195-205.

Hedtke, S.F., C.W. West, K.N. Allen, T.J. Norberg-King, and D.I. Mount. 1986. Toxicity of pentachlorophenol to aquatic organisms under naturally varying and controlled environmental conditions. Environ. Toxicol. Chem. 5:531-542.

Hendrix, P.F., C.L. Langer, E.P. Odum, and C.L. Thomas. 1981. Microcosms as test systems for the ecological effects of toxic substances: An appraisal with cadmium. EPA-600/3-81-036. National Technical Information Service. Springfield, VA.

Henebry, M.S. and P.E. Ross. 1989. Use of protozoan communities to assess the ecotoxicological hazard of contaminated sediments. Tox. Assess. 4:209-227.

Horning, W.B. and C.I. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, EPA/600/4-85/C14. Environmental Monitoring and Support Laboratory, Cincinnati, OH, 162 pp.

Hunn, J., S. Hamilton, and D. Buckler. 1987. Toxicity of sodium selenite to rainbow trout fry. Water Res. 21:233-238.

Ingersoll, C.G., and R.W. Winner. 1982. Effect on Daphnia pulex (deGeer) of daily pulse exposure to copper or cadmium. Environ. Toxicol. Chem. 1:321-327.

Jarvinen, A.W., D.K. Tanner, and E.R. Kline. 1988. Toxicity of chlorpyrifos, Endrin, or fenvalerate to fathead minnows following episodic or continuous exposure. Ecotox. Environ. Safety 15.

Johnson, B.T. 1986. Potential impact of selected agricultural chemical contaminants on a northern prairie wetland: a microcosm evaluation. Environmental Toxicology and Chemistry 5:473-485.

Jones, J. and B. Simon. 1979. The measurement of electron transport system activity in freshwater benthic and planktonic samples. J. Appl. Bact. 46:305-315.

Kemp, W.M., W.R. Boynton, J.J. Cunningham, J.C. Stevenson, T.W. Jones, and J.C. Means. 1985. Effects of atrazine and linuron on photosynthesis and growth of the macrophytes Potamogeton perfoliatus L. and Myriophyllum spicatum L. in an estuarine environment. Marine Environmental Research 16:255-280.

Kimball, K. and S. Levin. 1985. Limitations of laboratory bioassays: the need for ecosystem-level testing. BioScience 35:165-171.

Krieger, K.A., D.B. Baker, and J.W. Krammer. 1988. Effects of herbicides on stream Aufwuchs productivity and nutrient uptake. Arch. Environ. Contam. Toxicol. 17:299-306.

Lanza, G., G.A. Burton, and J.M. Dougherty. 1989. Microbial enzyme activity tests: potential use to monitor sediment decomposition processes, pp. 41-54 in J. Cairns, Jr. and J.R. Pratt, eds. Functional Testing for Hazard Evaluation, American Society for Testing and Materials, Philadelphia.

Leffler, J. 1981. Tentative protocol of an aquatic microcosm screening test for ecosystem-level effects of chemicals. Battelle Columbus Laboratories Subcontract T6411 (7197).

Lemly, A. 1985. Toxicology of selenium in a freshwater reservoir: implications for environmental hazard evaluation and study. Ecotoxicol. Environ. Saf. 10:314-338.

Levine, S.N. 1989. Theoretical and methodological reasons for variability in the resposes of aquatic ecosystem processes to chemical stresses, pp.145-180, in S.A. Levin, M.A. Harwell, J.R. Kelly, and K.D. Kimball, eds., Ecotoxicology: Problems and Approaches, Springer-Verlag.

Leberman, M.T., and M. Alexander. 1981. Effects of pesticides on decomposition of organic matter and nitrification in sewage. Bull. Environ. Contam. Toxicol. 26:554-560.

Liu, D.H.W., R.J. Spanggord, H.C. Bailey, H.S. Javitz, and D.C.L. Jones. 1983. Toxicity of TNT Wastewaters to Aquatic Organisms. Volume I. Acute toxicity of LAP wastewater and 2,4,6-Trinitrotoluene. Final Report, AD A142 144. Stanford Research Institute, Menlo Park, CA. DMAD17-75-C-5056.

Lobban, C., D. Chapman, and B. Kremer. 1988. Experimental Phycology: A Laboratory Manual, p. 197-202. Cambridge University Press, Cambridge.

Lu, M., K. Wang, Y. Gang, W. Lin, and F. He. 1987. Photodegradation of alpha-trinitrotoluene in aqueous solution. Huanjing Kexue 8:15-20.

Macek, K.J. 1976. Chronic Toxicity of Atrazine to Selected Aquatic invertebrates and Fishes. U.S. Environmental Protection Agency Ecological Research Series, EPA-600/3-76-047.

Maki, A. 1977. Modifications of a continuous-flow toxicity test methods for small aquatic organisms. Prog. Fish-Cult. 39:172-174.

Marshall. W.K., and J. R. Roberts. 1978. Ecotoxicology of Chlorpyrifos. National Research Council of Canada, Crown Copyright Reserved, Ottawa.

Mayer, F.L. and M.R. Ellersieck. 1986. Manual of Acute Toxicity: Interpretation and Data Base for 410 Chemicals and 66 Species of Freshwater Animals, US Department of Interior, Fish and Wildlife Service, Resource Publication 160, Washington. 506pp.

Mayer, F.L., K.S. Mayer, and M.R. Ellersieck. 1986. Relation of survival to other endpoints in chronic toxicity tests with fish. Environ. Toxicol. Chem. 5:737-748.

McConnell, W. 1962. Productivity relations in carboy microcosms. Limnol. Oceanogr. 7:335-343.

Mount, D. 1985. Scientific problems in using multispecies toxicity tests for regulatory purposes, pp.13-18 in J. Cairns, Jr., ed., Multispecies Toxicity Testing. Pergamon Press, New York, NY.

Nebeker, A.V., A. Stinchfield, C. Sowonen, and G.A. Chapman. 1986. Effects of copper, nickel, and zinc on three species of freshwater snails. Environ. Toxicol. Chem. 5:807-811.

Niederlehner, B., J. Pratt, A. Buikema, Jr., and J. Cairns, Jr. 1985. Laboratory tests evaluating the effects of cadmium on freshwater protozoan communities. Environ. Toxicol. Chem. 3:155-165.

Niederlehner, B.R., J.R. Pratt, A.L. Buikema, Jr., and J. Cairns, Jr. 1986. Comparison of estimates of hazard derived at three levels of complexity, pp. 30-48 in J. Cairns, Jr., ed., Community Toxicity Testing, ATSM STP 920. American Society for Testing and Materials, Philadelphia, PA.

Norberg, T.J., and D.I. Mount. 1985. A new fathead minnow (Pimephales promelas) subchronic toxicity test. Environ. Toxicol. Chem. 4:711-718.

Nordlie, K., and J. Arthur. 1981. Effects of elevated water temperature on insect emergence in outdoor experimental channels. Environ. Pollut. Ser. A 25:53-56.

Odum, E.P. 1985. Trends expected in stressed ecosystems. BioScience 35:419-422.

Odum. E.P. 1990. Field experimental tests of ecosystem-level hypotheses. Trends in Ecology and Evolution 5:204-205.

Odum, E.P., J.T. Finn, and E.H. Franz. 1979. Perturbation theory and the subsidy-stress gradient. BioScience 29:349-352.

O'Neill, R. and J. Waide. 1981. Ecosystem theory and the unexpected: implications for environmental toxicology, p.43-73 in B.W. Cornday, ed. Management of Toxic Substances in Our Ecosystems, Ann Arbor Science, Ann Arbor, MI.

Owsley, J., and D. McCauley. 1986. Effect of extended sublethal exposure to sodium selenite on Ceriodaphnia affinis. Bull. Environ. Contam. Toxicol. 36:876-880.

Pabst, M.H., and M.G. Boyer. 1980. Effects of two organophosphorus insecticides on the chlorophyll a and phaeopigment concentrations of standing ponds. Hydrobiologia 69:245-250.

Peltier, W.H. and C.I. Weber. 1985. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, EPA/600/4-85/013. Environmental Monitoring Systems Laboratory, Cincinnati, OH, 216 pp.

Pick, F. 1987. Carbohydrate and protein content of lake seston in relation to plankton nutrient deficiency. Can. J. Fish. Aquat. Sci. 44:2095-2101.

Pignatello, J.J., M.M. Martinson, J.G. Steiert, R. E. Carlson, and R.I. Crawford. 1983. Biodegradation and photolysis of pentachloro-phenol in artificial freshwater streams. Appl. Environ. Microbiol. 46:1024-1031.

Porter, K. and Y. Feig. 1980. The use of DAPI for identificiation and enumeration of aquatic microflora. Limnol. Oceanogr. 25:943-948.

Pratt, J.R., and J. Cairns, Jr. 1985. Functional groups in the protozoa: roles in differing ecosystems. J. Protzool. 32:415-423.

Pratt, J.R., and N.J. Bowers. 1990. A microcosm procedure for estimating ecological effects of chemicals and mixtures. Tox. Assess. 5:189-205.

Pratt, J.R., B.R. Niederlehner, N.J. Bowers, and J. Cairns, Jr. 1987a. Prediction of permissible concentrations of copper from microcosm toxicity tests. Tox. Assess. 2:417-436.

Pratt, J., B. Niederlehner, N. Bowers, and J. Cairns, Jr. 1987b. Effects of zinc on freshwater microbial communities, pp. 324-326 in S.E. Lindberg and T.C. Hutchinson, eds., International Conference on Heavy Metals in the Environment, CEP Consultants, Edinburgh.

Pratt, J.R., B.R. Niederlehner, N.J. Bowers, and J. Cairns, Jr. 1988a. Effect of chlorine on microbial communities in naturally derived microcosms. Environ. Toxicol. Chem. 7:679-687.

Pratt, J.R., N.J Bowers, B.R. Niederlehner, and J. Cairns, Jr. 1988b. Effects of atrazine on freshwater microbial communities. Arch. Environ. Contam. Toxicol. 17:449-457.

Rand, G. and S. Petrocelli. 1984. Fundamentals of Aquatic Toxicology, Hemisphere Publishing, New York.

Rausch, P. 1981. The estimation of microalgae protein content and its meaning to the evaluation of algal biomass, V: Comparison of methods for extracting protein. Hydrobiologia 78:237-251.

Reading, J., and A. Buikema, Jr. 1983. Chronic effects of selenite-selenium on Daphnia pulex. Arch. Environ. Contam.Toxicol. 12:399-404.

Rodgers, J.H., Jr., K.L. Dickson, and J. Cairns, Jr. 1979. A review and analysis of some methods used to measure functional aspects of periphyton, pp. 142-167 in R.L. Weitzel, ed., Methods and Measurements of Periphyton Communities: A Review, ASTM STP 690. American Society for Testing and Materials, Philadelphia, PA.

Ryon, M.G. 1987. Water quality criteria for 2,4,6-trinitrotoluene (TNT). Oak Ridge National Laboratory. National Technical Information Service, Springfield, VA, 138 pp.

Saiki, M., and T. Lowe. 1987. Selenium in aquatic organisms from subsurface agricultural drainage water, San Joaquin Valley, California. Arch. Environ. Contam. Toxicol. 16:657-670.

Sayler, G., M. Puziss, and M. Silver. 1979. Alkaline phosphatase assay for freshwater sediments: application to perturbed sediment systems. Appl. Environ. Microbiol. 38:922-927.

SAS Institute, Inc. (SAS). 1980. SAS User's Guide: Statistics. SAS Institute, Cary, NC.

Schaeffer, D., E.E. Herricks, and H.W. Kerster. 1988. Ecosystem health: I. Measuring ecosystem health. Environmental Management 12:445-455.

Schindler, D.W. 1987. Detecting ecosystem response to anthropogenic stress. Canadian Journal of Fisheries and Aquatic Science 44(Suppl. 1):6-25.

Schindler, J.E., J.B. Waide, M.C. Waldron, J.J. Hains, S.P. Schreiner, M.L. Freedman, S.L. Benz, D.R. Pettigrew, L.A. Schissel, and P.J. Clark. 1980. A microcosm approach to the study of biogeochemical systems, I. Theoretical rationale, in J.P. Giesy, Jr., ed., Microcosms in Ecological Research, DOE

Symposium Series 52, National Technical Information Service, Springfield, VA.

Shaw, D.R., T.F. Peiper, and D.L. Nofziger. 1985. Weed Science 33:29-33.

Smith, E.P., and D. Mercante. 1989. Statistical concerns in the design and analysis of multispecies microcosms and mesocosm experiments. Tox. Assess. 4:129-148.

Smith, E.P., K.W. Pontasch, and J. Cairns, Jr. 1990. Community similarity and the analysis of multispecies environmental data: a unified statistical approach. Water Res. 24:507-514.

Smith, P.D., D.L. Brockway, and F.E. Stancil 1987. Effects of hardness, alkalinity, and pH on the toxicity of pentachlorophenol to Selenastrum capricornutum (Printz). Environ. Toxicol. Chem. 6:891-900.

Sokal, R. and F. Rohlf. 1983. Biometry. W.H. Freeman, New York, NY, 859 pp.

Spehar, R.L., and H.P. Nelson, M.J. Swanson, and J.W. Renoos. 1985. Pentachlorophenol toxicity to amphipods and fathead minnows at different test pH vlaues. Environ. Toxicol. Chem. 4:389-397.

Stay, F.S., D.P. Larsen, A. Katko, And C.M. Rohm. 1985. Effects of atrazine on community level responses in Taub microcosms. pp. 75-90, in T.P. Boyle, ed., Validation and Prediction of Laboratory Methods for Assessing the Fate and Effect of Contaminants in Aquatic Ecosystems, STP 865, American Society for Testing and Materials, Philadelphia, PA.

Steele, R.L., and G.B. Thursby. 1983. A toxicity test using life stages of Champia parvula (Rhodophyta), pp. 73-89 in W.E. Bishop, R.D. Cardwell, and B.B. Heidolph, eds., Aquatic Toxicity and Hazard Assessment: Sixth Symposium, ASTM STP 802, American Society for Testing amnd Materials, Philadelphia, PA.

Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. 1983. Guideline for deriving numerical national water quality criteria for the protection of aquatic life and its uses. U.S. Environmental Protection Agency, ERL-Duluth.

Taub, F., and M. Crow. 1980. Synthesizing aquatic microcosms, pp. 69-104 in J.P. Giesy Jr., ed., Microcosms in Ecological Research. DOE Symposium Series 52, National Technical Information Service, Springfield, VA.

Taub, F.B., A.C. Kinding, L.L. Conquest. 1986. Preliminary results of interlaboratory testing of a standardized aquatic microcosm. pp. 93-120 in J. Cairns, Jr, ed., Community Toxicity Testing, ASTM STP 920, American Society for Testing and Materials, Philadelphia, PA.

Taub, F., A.C. Kinding, L.L. Conquest, and J.P. Meador. 1989. Results of interlaboratory testing of the standardized aquatic microcosm protocol. pp.368-394 in G.W. Sutter II and M.A. Lewis, eds., Aquatic Toxicology and Environmental Fate: Eleventh Volume, ASTM STP 1007, American Society for Testing and Materials, Philadelphia, PA.

Tebo, L.B., Jr. 1985. Technical considerations related to the regulatory use of multispecies toxicity tests, pp. 19-26, in J. Cairns, Jr., ed., Multispecies Toxicity Testing. Pergamon Press, New York, NY.

United States Environmental Protection Agency (USEPA). 1980. Ambient water quality criteria for pentachlorophenol. EPA 440/5-80-065. National Technical Information Service, Springfield, VA.

United States Environmental Protection Agency (USEPA). 1983. Methods for the chemical analysis of water and wastewater, EPA 600/4-79-020. National Technical Information Service, Springfield, VA .

United States Environmental Protection Agency (USEPA). 1984. Ambient water quality criteria for copper. EPA 440/5-84-031. National Technical Information Service, Springfield, VA.

United States Environmental Protection Agency (USEPA). 1986. Quality Criteria for Water 1986, EPA 440/5-86/001. Office of Water Regulations and Standards, Washington, DC.

United States Environmental Protection Agency (USEPA). 1987. Ambient water quality criteria for selenium. EPA 440/5-87-006. National Technical Information Service, Springfield, VA.

Van Voris, R., R.V. O'Neill, W.R. Emanuel, and H.H. Shugart, Jr. 1980. Functional complexity and ecosystem stability. Ecolog 61:1352-1360.

Vocke, R., K. Sears, J. O'Toole, and R. Wildmon. 1980. Growth responses of selected freshwater algae to trace elements and scrubber ash slurry generated by coal-fired power plants. Water Res. 14:141-150.

Ward, G.S. and L. Ballantine. 1985. Acute and chronic toxicity toxicity of atrazine to estuarine fauna. Estuaries 8:22-27.

Weber C.I. and colleagues. 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 2nd ed. EPA 600/4-89-001, Environmental Monitoring Systems Laboratory, Cincinnati, OH, 258 pp.

Weinbach, E.C. 1954. The effect of pentachlorophenol on the oxidative phosphorylation. J. Biol. Chem. 210:545-550.

Won, W.D., L.H. DiSalvo, and J. Ng. 1976. Toxicity and mutagenicity of 2,4,6-trinitrotoluene. Appl. Environ. Microb. 27:513-516.

Yount, J.D. and L.J. Shannon. 1989. State changes in laboratory microecosystems in response to chemicals from three structural groups, pp. 86-96 in J. Cairns, Jr. and J.R. Pratt, eds., Functional Testing of Aquatic Biota for Estimating Hazards of Chemicals, STP 988, American Society for Testing and Materials, Philadelphia, PA.

Zischke, J., J. Arthur, K. Nordlie, R. Hermanutz, D. Standen, and T. Henry. 1983. Acidification effects on macroinvertebrates and fathead minnows (Pimphales promelas) in outdoor experimental channels. Water Res. 17:47-63.

APPENDIX A

Dissolved Oxygen System Information

APPENDIX A

DISSOLVED OXYGEN DATA ACQUISITION

1.0 SYSTEM OVERVIEW

The dissolved oxygen data acquisition system is designed to acquire, digitize, and store data from a YSI Model 58 Dissolved Oxygen meter. The analog output of the YSI is sampled with an analog-to-digital (A/D) converter board installed in the computer. Software is provided to control the data input, review and modify data, and store the data for analysis in typical MS-DOS spreadsheet software.

1.1 HARDWARE

The analog signal to be read is the recorder output of the YSI Model 58, which has a 0-1 volt range corresponding to a 0-20 mg/l dissolved oxygen measurement span (50 mV per mg/l). The data acquisition board incorporates a single channel 12-bit A/D converter, as well as digital I/O capability (Model ADA100, Real Time Devices, Inc., State College, PA). Both the ADA100 board and the DO software are designed for the XT/AT bus architecture, so this system cannot be used on computers with the Micro Channel1 bus. The ADA100 board occupies a half-length expansion slot and connects to the real world with a 40-pin ribbon cable connector. A connection interface box (also supplied by Real Time Devices) is used to facilitate connections and to provide breadboard space for signal conditioning circuitry. Data acquisition is controlled by the user with a remote pendant, allowing movement around the lab in order to reach various solutions. The remote control is a single button used like a computer mouse; that is, the number of consecutive clicks triggers data storage, as well as review or modification of previously stored data. The button signal is conditioned with simple circuitry (a "debouncing" circuit) located in the interface box, and is read with a digital input line on the ADA100 board.

1.2 SOFTWARE

The DO software is coded in C and compiled for MS-DOS computers; it requires just under 100K of memory plus graphics capability. Supported graphics drivers are CGA, MCGA, EGA, VGA, and Hercules monochrome. The software provides three major functions: I/O port setup, data manipulation, and data storage. The I/O port setup is necessary since the ADA100 board may be installed at a number of I/O port addresses. Data manipulation functions include providing a large screen display of the current data (since the user may be some distance from the computer), as well as processing the user's commands for data storage and modification. Finally, the data storage functions format the

3.0 NORMAL USE

3.1 STARTUP AND DATA MANIPULATION

Change to the subdirectory containing the DO files and, at the DOS prompt, type DO <cr> to start the program. Dip the YSI probe into a solution. After the screen is initialized, the current DO value will be sampled once each second and displayed. Above the current DO value on the screen, the active treatment cell and its stored data will be displayed together within a box (see Figure A.1). Data for all cells are initialized to zero. Eighteen treatment cells are provided, identified as follows:

0A, 0B, 0C
1A, 1B, 1C
2A, 2B, 2C
3A, 3B, 3C
4A, 4B, 4C
5A, 5B, 5C

Data storage is controlled with the pendant button. The treatments may be scrolled sequentially up or down, and the current DO value from the YSI may be stored in any cell. Data manipulation is summarized below:

- To save the current DO value in the displayed treatment cell, click the button once.
- To advance to the next treatment cell, click the button twice.
- To review a previous treatment cell, click the button three times.
- Data in any cell may be changed by selecting the cell, then clicking the button once to save the current DO value over the old data.

Scrolling past the first or last treatments (0A or 5C) simply rolls the display over within the 18 cells. For example, clicking twice at treatment 5C will display treatment 0A. Clicking three times at 0A will display treatment 5C.

DISSOLVED OXYGEN

TREATMENT = 0A

D.O. = 0.00 mg/l

CURRENT D.O. = 0.00 mg/l

Wed Dec 19 17:57:04 1990

CLICK ONCE TO SAVE VALUE

CLICK TWICE FOR NEXT TREATMENT

CLICK THREE TIMES FOR PREVIOUS TREATMENT

ESCAPE TO SAVE AND EXIT

TYPICAL SCREEN LAYOUT

3.2 DATA STORAGE AND RETRIEVAL

After data storage is complete, press the ESC button to end data acquisition and store the data. The software will prompt for a filename; any valid DOS filename is acceptable (include path information, if desired). Do not add a file extension (.ABC, etc.) because a .PRN extension will automatically be added to identify printable data files. If an invalid filename is entered, an error message will appear and the software will prompt for a filename until it receives a valid one. After successfully storing the data, the program will exit to DOS.

The procedure described above is the normal exit process, under which the program will not exit to DOS until the data has been safely stored to disk. However, the program may be aborted at any time by pressing CTRL-BREAK, BUT ALL DATA FROM THE SESSION WILL BE LOST AND CANNOT BE RETRIEVED.

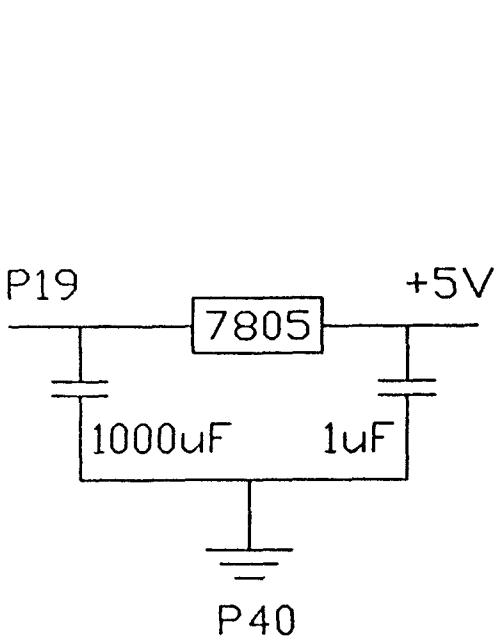
APPENDIX A.2: SOFTWARE ARCHITECTURE

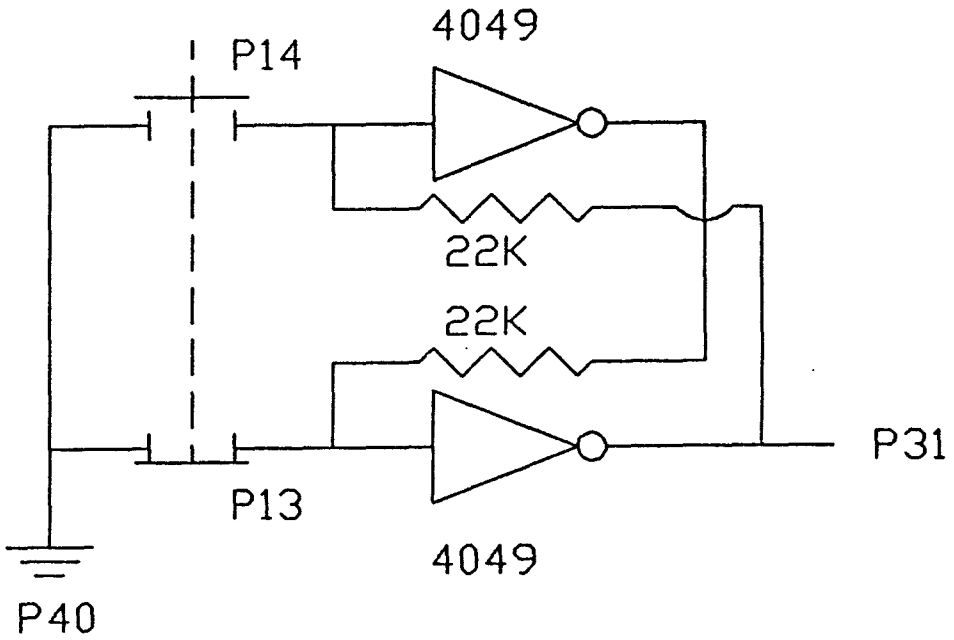
The DO_DATA software is coded and compiled using Borland Turbo C version 2.0. The source code is open and the individual code modules are listed below.

The code is straightforward; the only twist is the revectoring of the PC's IRQ0 interrupt (which is the system timer interrupt) in order to provide a real time base from which to monitor the user control button clicks. The interrupt is revectored to a service routine which polls an ADA100 digital input port for a button click during the previous time period. If a click has occurred, the routine sets up a time window for subsequent clicks to accumulate (up to a total of three) as a single user command. After the time window has closed, the routine totals the clicks which occurred and makes that total available (through an external variable) for the DO_EXEC routine to interpret as a user command. The interrupt service routine then begins to poll for a new click sequence.

DO_DATA CODE MODULES:

DOX.H -	Header file containing constants & file declarations.
DO.C -	Simple calling routine to start program.
DO_INTRO.C -	Introduction screen display.
DO_EXEC.C -	Main routine; calls all program functions.
DO_CFG.C -	Stores and retrieves A/D base address
GPRINT.C -	Graphics print routine.
GRDRVR.C -	Graphics driver loading routine.
DO_ADINI.C -	Initializes the A/D board.
DO_ADCON.C -	Converts a single analog data point.
DO_ADINT.C -	Interrupt routine to provide real time base for control pendant.
FILENAM.C -	Gets a filename for data storage.
DO_SAVE.C -	Stores data to disk.
TEXTBRK.C -	Provides clean ^BREAK handling in text mode.
GRPHBRK.C -	Provides clean ^BREAK handling in graphics mode.
DO_BRK.C -	Provides clean ^BREAK handling while interrupts are revectored





XB40 PIN	NORMAL STATE	WIRE COLOR	ADA100 FUNCTION
P19	+12V	NA	NA
P40	GND	BLK	NA
P13	LOW	RED	NA
P14	HI	WHT	NA
P31	HI	NA	DIG IN
P6	NA	~~BLUE~~ RED	DIFF IN+
P26	NA	~~BRWN~~ BLK	DIFF IN-

DO DATA ACQUISITION
POWER SUPPLY &
SWITCH DEBOUNCER

T. RUSCITTI
12/20/90

APPENDIX B

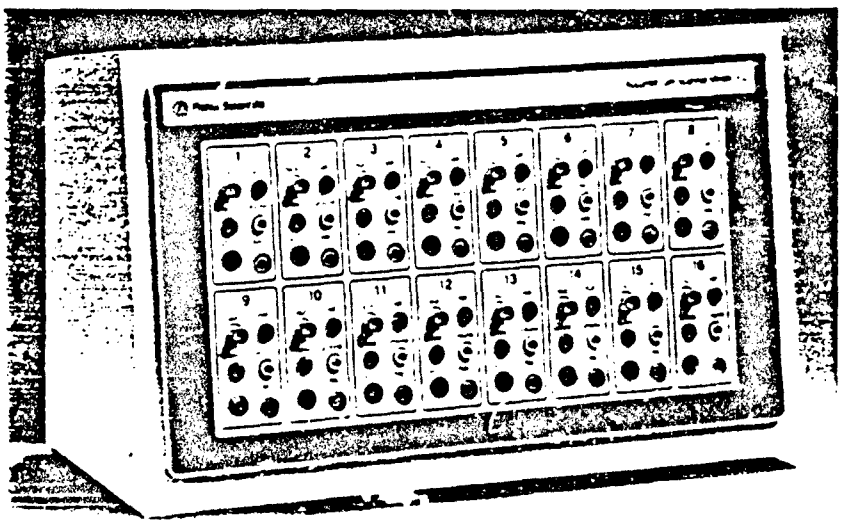
pH System Information

CONTENTS

Title	Page
PERFORMANCE CHARACTERISTICS	i
SPECIFICATIONS	i
INTRODUCTION	1
ACCESSORIES	1
PC COMPATIBILITY	1
CONNECTORS	1
Front Panel	2
Rear Panel	2
INSTALLATION	4
Unpacking	4
Scanner	4
Scanner PC Card	5
Printer	7
Controller	7
Electrodes	7
One Combination Electrode Per Solution	8
One Electrode Pair Per Solution	8
Multiple Combination Electrodes Per Solution	8
Multiple Electrode Pairs Per Solution	8
Multiple Indicating Electrodes One Common Reference Per Solution	8
Other Configurations	8
OPERATIONS	10
CALIBRATION	10
MAINTENANCE	11
Technical Information	12
Electrode Characteristics	12
Slope	12
Efficiency	13
Isopotential	14

ILLUSTRATIONS

Figure	Page
1. Front and Rear Panel Connectors	3
2. Rack Brackets Installation	4
3. PC Card Installation and Jumper Settings	6
4. Communication Cable Connections	7
5. Electrode Cable Configuration	9
6. Calibration Accessory Setup	11
7. Isopotential Point	14
8. Isopotential Area	14



PERFORMANCE CHARACTERISTICS

Ranges	electrode limited in pH/pX concentration, 0.0 to ±1999.9 mV, −4.0 to 104.0°C
Resolution	three significant figures in concentration 0.001 pH/pX 0.1 mV 0.1°C
Relative Accuracy	±0.3 n% (concentration) ±0.003 pH/pX ±0.2 mV ±0.2°C (0 to 100)
Repeatability	±least significant digit
Drift (24-hour)	±0.25 n% (concentration) ±0.0025 pH/pX ±0.15 mV ±0.2°C
Input Impedence	$>10^{13}$ohms
Channel Update Cycle	<6 seconds for all channels
Recorder Output	±1999.9 mV
Temperature Compensation	
ATC probe	0 to 100°C

SPECIFICATIONS

Power Requirements	Derives power from the PC.
Environmental	
Humidity	0 to 90% RH, non-condensing operating
Temperature	
Storage Temperature	−25 to +55°C
Operating Temperature	+10 to +40°C
Dimensions	17"W x 7½"D x 11"H
Weight	16 lb.
Case Construction	Chemically resistive paint on steel.

INTRODUCTION

The Fisher Accumet® Model 935 PC Electrode Scanner is an advanced-design PC interfaced instrument having the following features and functions:

- Scans up to 16 channels for pH, concentration or millivolts and temperature.
- Fast scan rate — 0.3 seconds per enabled channel.
- Displays, prints (on- or off-line), or writes data to disk files.
- Stores developed methods and standardization constants in files.
- Remote operation with up to 100 feet of cable between the PC and Scanner.
- Operates with IBM* PC, XT, or compatible systems.
- Channel Isolation (electrically) for all sixteen input channels.
- ATC (automatic temperature compensator) Probe may be used for each of the sixteen electrode channels; or a single probe may be shared between channels.
- Controller Module available for use with the Scanner to provide external load controlling capabilities. The controller utilizes solid state relays.
- Numeric tabular display on the CRT screen shows channel number, channel parameter (pH, mV, etc.), channel temperature, last scan parameter value, switch number, and switch state.
- Common reference allows use of a common reference probe for multiple channels via jumper cables.
- Setup and operation using menus and function keys.
- Single electrode channel standardization or standardization in user defined batches, with the option of one or two-point standardization.
- Isopotential correction for pH or concentration (activity) measurements according to operator entered isopotential. A function key causes display of isopotential of each currently enabled channel, standardized in pH or concentration.
- Temperature compensation for the temperature dependence of electrode response as predicted by the Nernst equation when operating in the pH or concentration mode.

ACCESSORIES

The following is a list of accessories that may be used with this instrument. Consult the current Fisher Catalog or local sales representative for information on a full line of accessories that are available for this Instrument.

Item	Cat. No./Part No.
Calibration Accessory	09-313-937
Model 936 Controller	09-313-936
Jumper Cables (pkg of 8) 4"	69595
8"	69596
Extra System Disk (5¼")	F.S. 69611

PC COMPATIBILITY

The Scanner can be interfaced with an IBM* PC, XT or compatable system having a standard monochrone monitor and at least 256K RAM and one disk drive.

NOTE: The Scanner will not operate with an IBM* AT PC.

CONNECTORS

The front panel is sectionized into an array of 16 electrode-input channels. The nomenclature for each channel is further described below. The rear panel contains two identical communication connectors.

**IBM is a registered trademark of International Business Machines.*

Front Panel (Figure 1)

The jacks located on the front panel include connectors for electrodes, temperature probes, potentiometric recorders, electrical ground and shield

INPUT JACK — Sixteen standard BNC electrode jacks that accept the BNC plugs of indicating or combination electrodes.

REFERENCE JACK — Sixteen pin jacks that accept the pin connectors of reference electrodes or jumper cables.

TEMPERATURE JACK — Sixteen jacks that accept the plugs of ATC probes (Cat. No. 13-620-16)

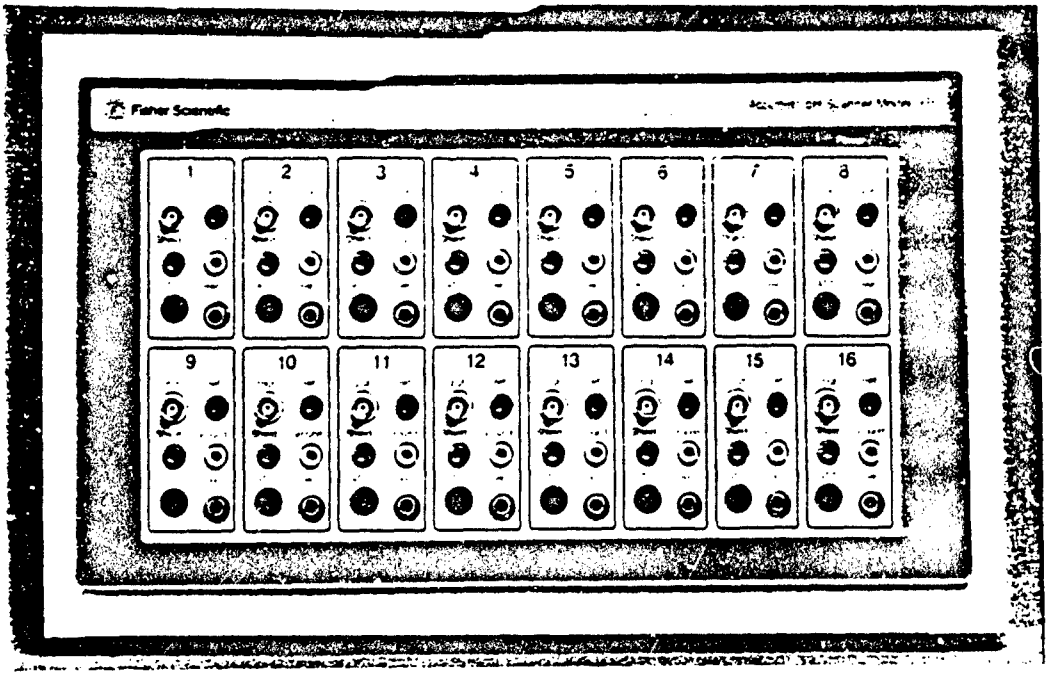
GROUND JACK — Sixteen pin jacks that accept the pin connectors of jumper cables used with the shield jack to reduce electrical noise.

SHIELD JACK — Sixteen pin jacks that accept the pin connector of jumper cables that must be connected to the REFERENCE Jack when using electrodes.

RECORDER JACK — Sixteen phono jacks that accept phono plugs from external recorders.

Rear Panel (Figure 1)

COMMUNICATION CONNECTORS — Two connectors used to provide communication between the Scanner and PC; and Scanner and Controller. Both connectors are identical and can be used for either connection.



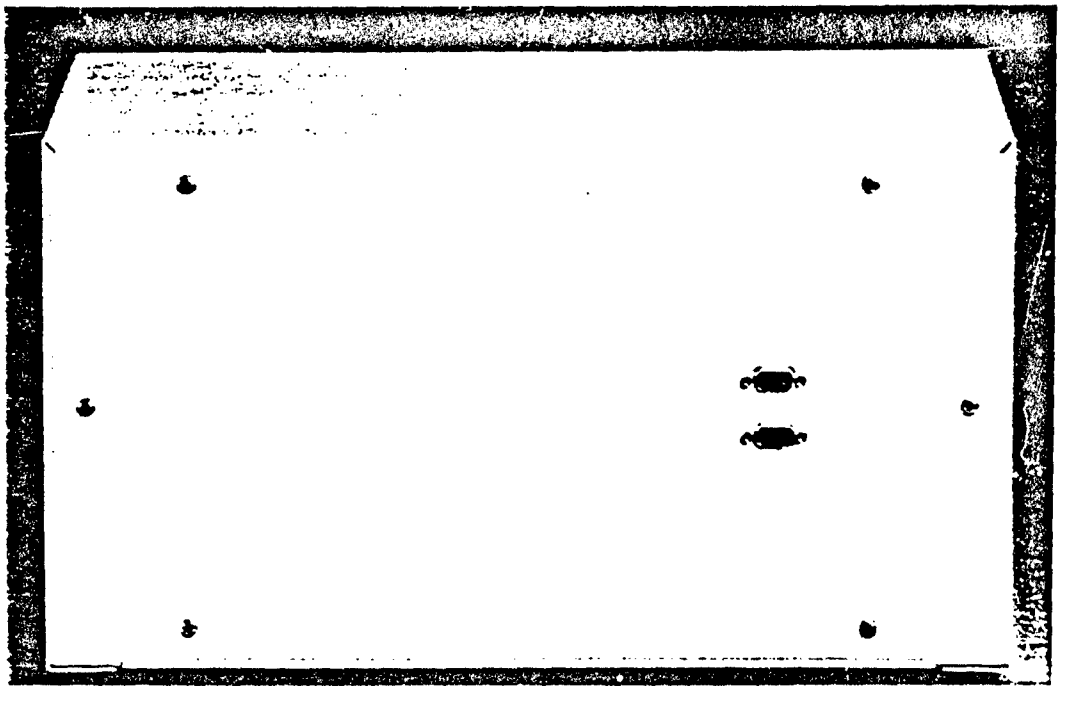


Figure 1. Front and Rear Panel Connectors

INSTALLATION

Installation and setup of this instrument is straight forward. It is necessary to install a PC card (part of Scanner system) into the host PC. Thus, the cover of the PC must be removed to complete this installation. Refer to the host's PC technical manual for details.

Unpacking

The Scanner is shipped in a single carton containing the items listed in the enclosed packing list. If a shortage exists, notify your Fisher branch or representative, giving the name and number of the missing item. Also, fill out and return the warranty card.

NOTE: In the event that shipping damage has been observed, retain the carton and packing material intact with the unit, and file a claim with the final carrier. Usually, the firm will send an inspector to ascertain liability.

Scanner

The Model 935 Scanner can be used as a free standing unit, or it can be rack mounted with the supplied mounting brackets. When used as a free standing unit, no additional preparation is necessary. To rack mount, perform the following:

1. Attach the mounting brackets (part no. 69585) to the Scanner with the supplied screws (part no. 15816) and lock washers (part no. 14004) as shown in figure 2 (round holes are attached to the 935 case).
2. Insert the Scanner into the rack and attach with the screws and lock washers.

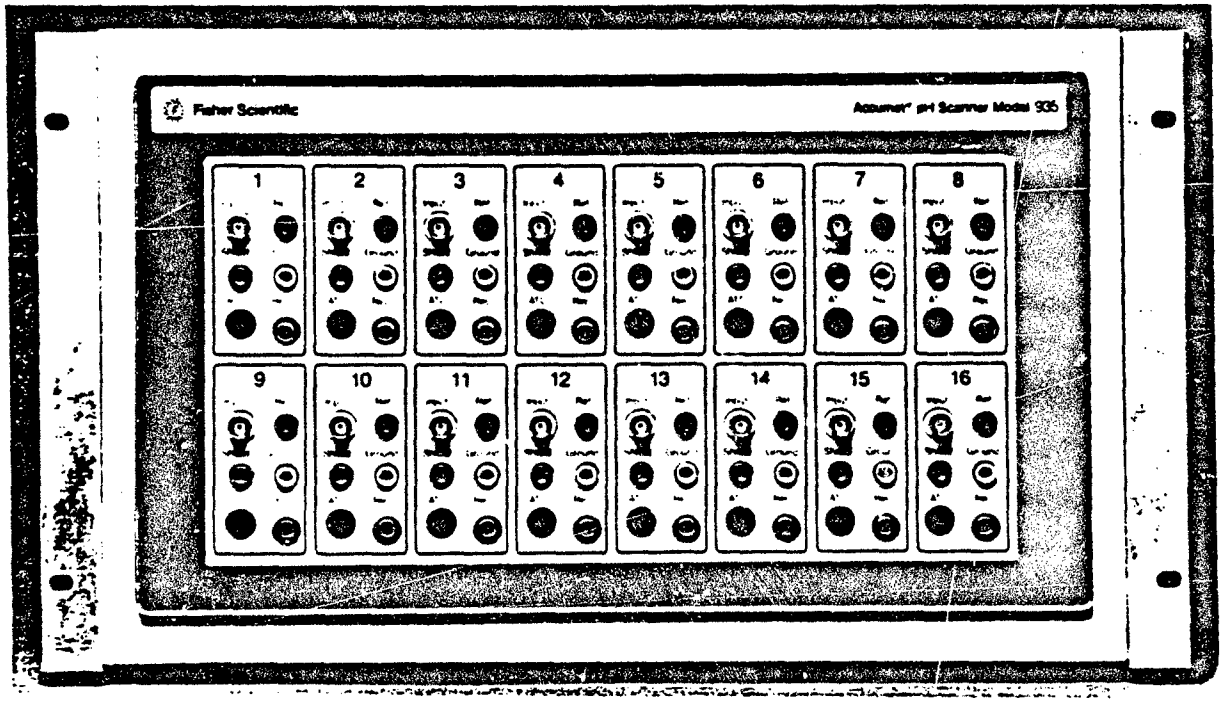


Figure 2. Rack Brackets Installation

Scanner PC Card

NOTE: Handle the Scanner PC Card only by its edges.

1. Set the power switch on the PC to off.
2. Set all external power switches (printer, monitor, etc.) to off.
3. Unplug the PC's power cord.
4. Remove the cover of the PC. Refer to the PC instruction manual for specific directions.
5. Determine if a serial port has been installed in the PC.
6. If no serial ports are occupied, then set the jumpers of the Scanner PC Card onto pins labeled COM1 and I4. If only one serial port is occupied then set the jumpers of the Scanner PC Card onto pins labeled COM2 and I3. See figure 3 for pin location.

   CAUTION: The Scanner will not function if more than one serial port is occupied. Therefore, additional occupied serial ports **must** be removed. Then set the jumpers of the Scanner PC Card onto pins labeled COM2 and I3.
7. Locate an empty expansion slot on the PC and remove the expansion slot cover. Hold the Scanner PC Card by its top edge and firmly press it into the expansion slot. Then attach (screw) the card to the retaining bracket. See figure 3 for details. Refer to the PC instruction manual for specific directions for installing a card.
8. Replace the cover of the PC. Refer to the PC instruction manual for specific directions.
9. Connect the Scanner to the PC with the cable assembly (part no.69093). See figure 4 for details.

   NOTE: Either communication connector on the rear of the Scanner may be used for cable connections to the PC.
10. Plug in the PC's power cord.

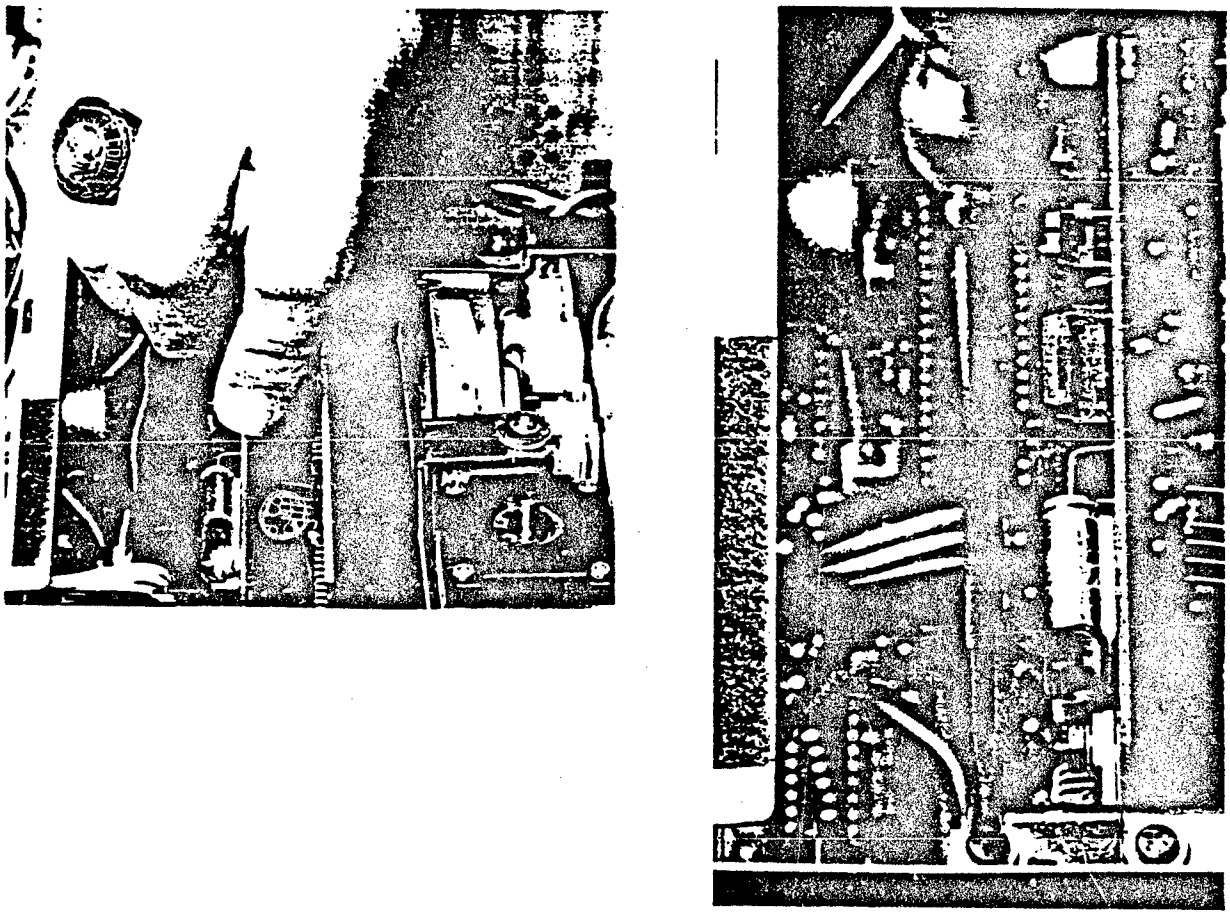


Figure 3. PC Card Installation and Jumper Settings

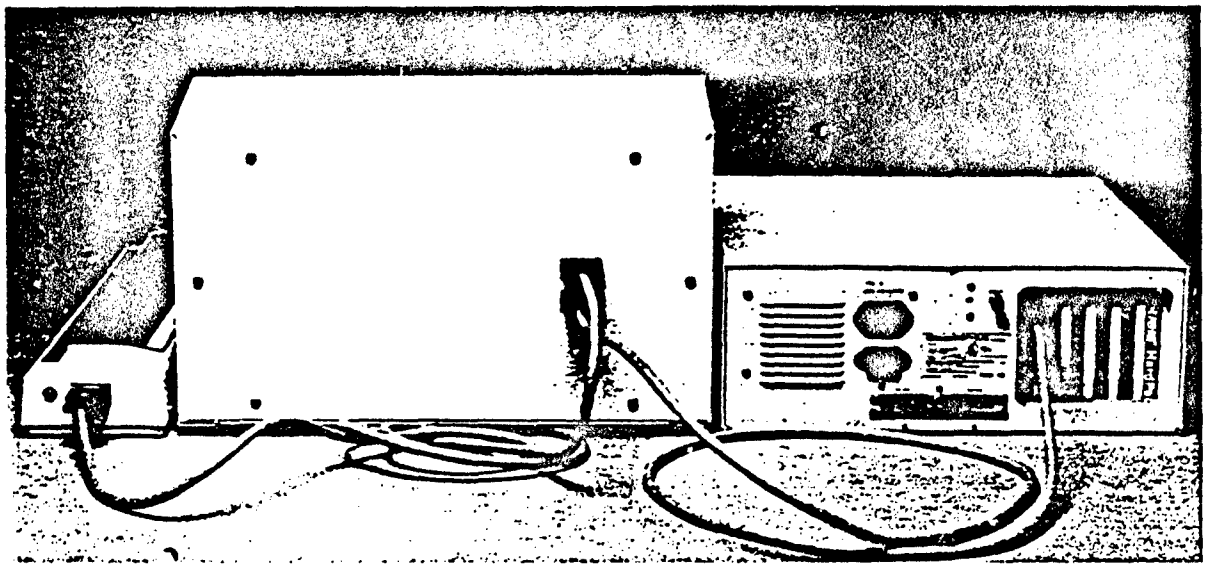


Figure 4. Communication Cable Connections

Printer

Any IBM PC compatible printer may be used. Refer to the PC manual for specific instructions.

Controller

1. Set the power switch of the PC to off.
2. Set all external power switches (printer monitor, etc.) to off.
3. Connect the Controller to the Scanner with the cable assembly (part no. 69093). See figure 4.

   NOTE: Either communication connector on the rear of the Scanner may be used for cable connections to the controller

Electrodes

NOTE: For specific electrode care and handling information, refer to the instructions supplied with the electrode.

The jumper cable configuration on the scanner is dictated by the number and type of electrodes present. When measurements are being made, it is necessary that the solution being measured is grounded. This must be done with the reference electrode by connecting the reference jack to ground with a jumper cable. However,if more than one channel is used to monitor a solution, only one reference should be connected to ground. Failure to observe this setup will result in an average reference value to be used which may lead to measurement error. Instructions for connecting various electrodes follow. For clarity, the possible arrangements are shown in figure 5.

CAUTION: When electrodes are to be batch standardized, and then used to take measurements in separate solutions, the electrodes should have only one channel connected to ground during standardization. When measurements are made in the separate solutions, the individual ground wire (jumper cables) should be connected.

One Combination Electrode Per Solution

When only one combination electrode is to be used in a solution, the electrode should be connected as shown in figure 5(a).

One Electrode Pair Per Solution

When only one electrode pair is to be used in a solution, the electrodes should be connected as shown in figure 5(b).

Multiple Combination Electrodes Per Solution

When multiple combination electrodes are to be used in the same solution, one of the combination electrodes must be connected as shown in figure 5(a). The remaining combination electrodes must be connected as shown in figure 5(c).

Multiple Electrode Pairs Per Solution

When multiple electrode pairs are to be used in the same solution, one of the pairs must be connected as shown in figure 5(b). The remaining electrode pairs must be connected as shown in figure 5(d).

Multiple Indicating Electrodes One Common Reference Per Solution

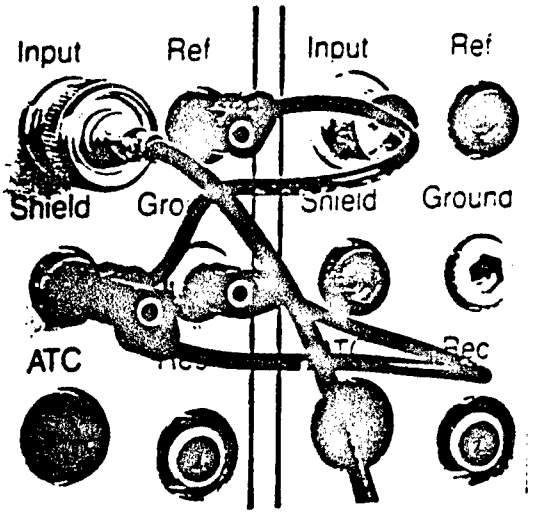
When multiple indicating electrodes sharing a common reference electrode are to be used in the same solution, the electrodes should be connected as shown in figure 5(e).

Other Configurations

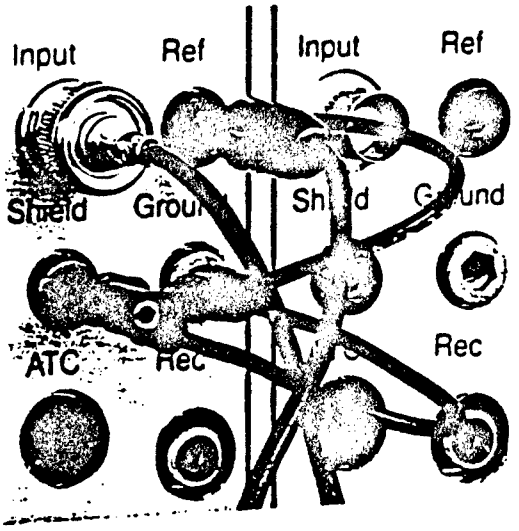
When mixed electrode types are used in the same solution, only one type (combination or separate reference) should be connected to ground. The remaining electrodes are attached as previously shown (for combinations see figure 5(a) and (c); for pairs see figure 5(b), (d) and (e).

Figure 5. Electrode Cable Configurations

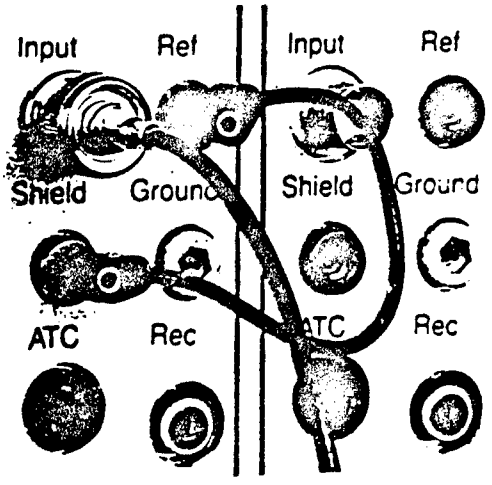
5 A



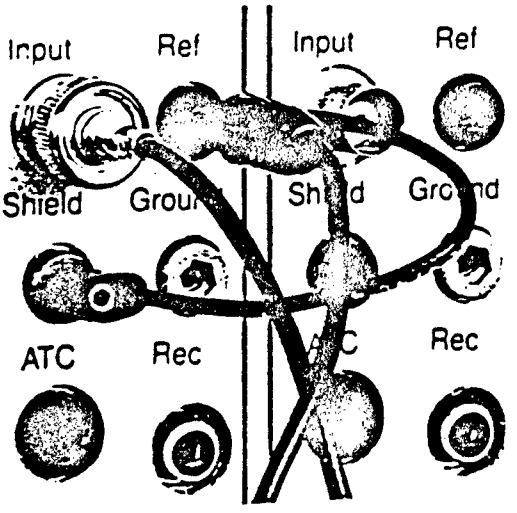
5 B



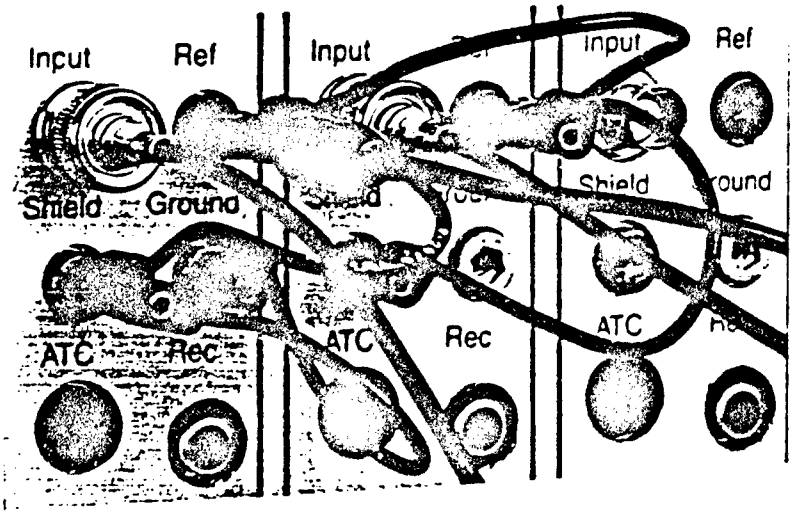
5 C



5 D



5 E



OPERATION

To operate the Scanner, perform the following:

1. Boot the system with a DOS diskette.
2. Replace the DOS diskette with the Scanner diskette.
3. Load the Scanner program.

The system now presents menu-driven software for standardizing probes, sequentially reading input channels in standardization units (mV, pH, concentration (activity), or degrees centigrade), displaying data on the CRT screen, printing and writing data to files. The Controller module permits external (on/off) control based on up to sixteen different user defined switching levels in any channel and in the following units mV, pH, concentration (activity), or degrees centigrade.

CALIBRATION

The calibration procedure for calibrating the millivolt measurement of each channel is built into the Scanner software. The **Calibration** procedure is brought up from the **Main Menu** by pressing F7. Only a DVM and the Calibration Accessory is required to perform the procedure (see figure 6). A channel is calibrated when the millivolt value displayed by the DVM (connected to the Calibration Accessory) is entered when prompted by the display. The scanner must be connected to a PC. That is, the scanner must be fully operational in a system as described in the operator's manual. Electrodes are not required to perform the calibration.

1. Bring up the **Calibration** category from the Main Menu by pressing key F7 at the PC keyboard.
2. Bring up the **Calibrate Channels** procedure by pressing F2.
3. Follow the procedure given on the screen.
4. If an **OUT-OF RANGE** is continually displayed on the screen after entering the millivolt value, then a malfunction exists.
5. Exit the system when calibration is completed.

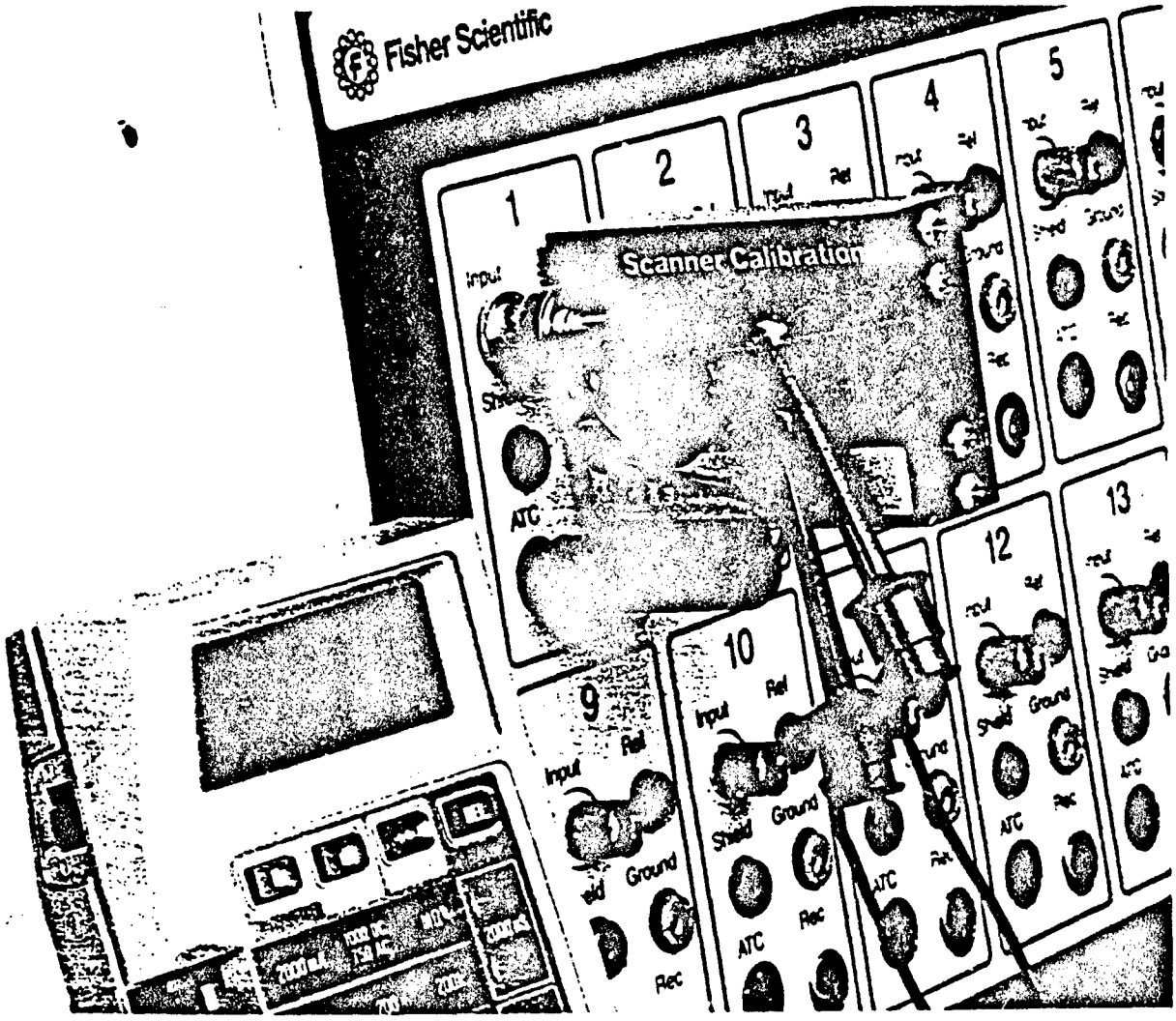


Figure 6. Calibration Accessory Setup

MAINTENANCE

The Scanner is constructed and finished with materials that provide long maintenance-free service. All that is normally required is a routine cleaning of the exterior surfaces. Use a mild detergent for this purpose. The separate service manual provides detailed information for servicing this equipment. To obtain a service manual for this instrument, contact Literature Services, telephone number: (412) 562-2101.

TECHNICAL INFORMATION

This section provides additional information regarding some of the more advanced capabilities of the Model 935.

Electrode Characteristics

The Model 935 includes features that consider the characteristics of slope, efficiency, and isopotential point for pH and other ion selective electrodes. The following paragraphs briefly define these characteristics and outline how the Model 935 uses them.

Slope

Slope is a constant that relates electrode potential to the logarithm of the concentration, as can be seen in the following equation.

$$E_{meas} = K + 2.3\,RT/nF \log C$$

Where	E_{meas} =	measured electrode output usually in millivolts.
	C =	concentration* of the species being measured.
	K =	constant which is dependent on the electrode system in use.
	R =	the gas constant.
	T =	temperature in degrees Kelvin.
	F =	Faraday.
	n =	number of electrons.

The terms RT/nF define the theoretical slope for an electrode system. Inspection of this definition shows that the slope is dependent on temperature. The theoretical slope for some species at 25°C follows:

Species	Slope
H_3O^+ (pH)	+59.16
F^- (fluoride)	−59.16
Cl^- (chloride)	−59.16
Ca^{2+} (calcium)	+29.58
S^{2-} (sulfide)	−29.58

The slope for a given electrode is calculated by the Model 935 once all data for a two-point standardization has been entered.

Concentration rather than activity is used for simplification (ignoring ionic strength effects).

Efficiency

Efficiency is the actual electrode slope divided by the theoretical slope. Ideal efficiency is assumed to be unity (1.000), and the calculated efficiency of an actual electrode should result in values near unity.

The Model 935 substitutes values into the following equation:

$$EFF = \frac{Smeas}{2.3\ RT/nF}$$

Where:

EFF =	electrode efficiency
Smeas =	measured slope from a two-point standardization.
R =	gas constant.
T =	temperature in degrees Kelvin.
F =	Faraday.
n =	Assumed to be one in all cases.

The Model 935 assumes n = 1 in all cases. when calculating efficiency. Therefore, the calculated efficiency for systems where n = 1 should be near unity, and for systems when n = 2 should be near one half. Some examples follow:

Species	Calculated Efficiency (if perfect)
H_3O^+ (pH)	+1.00
F^- (fluoride)	−1.00
Cl^- (chloride)	−1.00
Ca^{2+} (calcium)	+0.500
S^{2-} (sulfide)	−0.500

The algebraic sign of the efficiency indicates the direction of the slope.

As an example, assume a pH electrode has a measured slope of 58.9 at 25°C. Since ideal slope at 25°C is 59.157, then:

$$\text{Efficiency} = 58.9 / 59.157 = 0.9956$$

The efficiency factor for a given electrode is calculated by the Model 935 once all data for a two-point standardization has been entered.

Once the efficiency factor is known for a given electrode, the value may be used for greater accuracy in a one-point standardization procedure.

However, as electrodes age, efficiency decreases at a usually slow but unpredictable rate. To use the calculated efficiency reliably, perform a two-point standardization periodically. This automatically replaces the old efficiency value with the new one.

Isopotential

The isopotential point of an electrode system is the point at which electrode potential is unaffected by a change in temperature, having coordinates of (pX_{iso}, E_0).

Where:

pX_{iso} =	negative of the log of the concentration* of the species being measured (X) at the isopotential point.
E_{iso} =	the potential usually expressed in millivolts at the isopotential point.
E_0 =	zero potential of the system.

In real electrode systems, however, this coincidence rarely occurs, and for some systems there is no true isopotential point but a general isopotential area. If a system exhibits an apparent isopotential point, or at least an isopotential area with relatively small spread, isopotential coordinates may by established and possibly used to some advantage.

Isopotential correction may be used only in conjunction with a one- or two-point standardization; it is required only when both accuracy over a significant temperature range as well as operation with an symmetric electrode system are contemplated. However, isopotential correction is never necessary if all measurements will be performed on samples which are at approximately the same temperature.

Most conventional pH electrode systems are designed and manufactured to be highly symmetrical cells. Consequently, for most pH work, unless ultimate accuracy over broad temperature ranges is required, isopotential may be ignored. Specifically, this is accomplished by setting the isopotential at its reset value of zero millivolts.

Most ion selective electrode systems are not symmetric. Thus, isopotential correction should always be considered in ion selective work over significant temperature ranges.

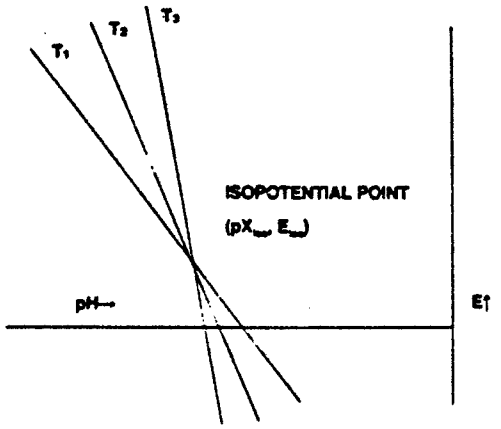


Figure 7. Isopotential Point

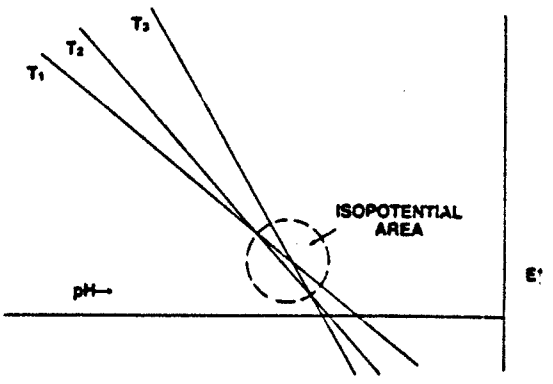


Figure 8. Isopotential Area

**Concentration rather than activity is used for simplification (ignoring ionic strength effects).*

CONTENTS

Title	Page
INTRODUCTION	1
TROUBLE ANALYSIS	1
Special Precautions	1
Required Tools and Test Equipment	2
Trouble Analysis Chart	2
SUPPLEMENTAL INFORMATION	3
Performance Characteristics and Specifications	3
Supply Voltages	4
Impedance and Offset Problems	4
Cleaning Front End	4
BNC Input Jacks	4
Circuit Boards	5
CIRCUIT DESCRIPTION	5
DISASSEMBLY AND REASSEMBLY	6
CALIBRATION	9
REPLACEMENT PARTS	9

ILLUSTRATIONS

Figure	Page
1. Removing Chassis Assembly	8
2. Connecting Calibration Accessory	9
3. Overall Interconnect D69154	11
4. PC Card Schematic C67084	12
5. Analog Board Schematic D64236	13
6. Processor Board Schematic D64319	14
7. Main Assembly D69550	15
8. Analog Input Assembly D69532	16
9. Processor PC Board Assembly D64319	17
10. Analog PC Board Assembly D64236	18
11. PC Card Assembly C67084	19
12. Communication Cable Assembly B69093	20

INTRODUCTION

This manual provides service information for the Model 935 Accumet* pH Scanner. The information is intended primary for use by Fisher service representatives or other qualified personnel. Fisher Scientific maintains fully-staffed service centers to further assist with any service problems.

The instrument is constructed of high grade materials and components which, under normal working environments, should provide trouble-free operation for many years. As with any high usage instrument, however, malfunctions may occur as a result of aging components or airborne contaminants. Recognizing this fact, Fisher engineers have intentionally designed the instrument for easy field checkout and repair.

The use of microprocessor technology in the design of this instrument has greatly simplified operator performance of sophisticated electro-chemical measurements. In addition, it has also greatly reduced the number of electronic components and **eliminated internal adjustments** for the instrument, resulting in a highly reliable system. The internal electronics for the instrument are contained on just three printed circuit boards. A fourth circuit card must be installed in an available slot within the host personal computer.

The service information begins with a generalized trouble analysis section which includes special precautions, required test equipment and tools, and a trouble analysis chart. The chart provides a listing of observable trouble symptoms, their probable cause, and their recommended corrective actions. Supplemental information is provided at the end of the chart to clarify corrective actions where necessary. Following the chart and its supplemental is a brief circuit description, but of sufficient depth to provide service personnel with a basic understanding of the instrument operation for trouble analysis purposes. Disassembly and replacement procedures (there are no adjustments for this instrument) along with calibration and replacement parts information, follow in order. As a convenience, the replacement parts are located with their assembly drawing. Also, the schematics for the equipment are provided at the end of the manual.

NOTE: Service personnel will find the Operating Instruction Manual and extensive on screen help and diagnostic messages invaluable in troubleshooting this instrument. It is essential to be familiar with the operation of this instrument in order to evaluate its performance.

TROUBLE ANALYSIS

The following chart is provided as a reference aid to assist in defining and evaluating possible service problems. Preceding the chart are special precautions to be observed, along with a list of required tools and test equipment. When using the chart, select the SYMPTOM category(s) that best describes the observable malfunction. Then proceed to check the PROBABLE CAUSE description(s) and take the necessary CORRECTIVE ACTION directive(s).

NOTE: The top and bottom analog boards are identical. Each analog board is associated with 8 input channels. The top board is associated with channels 1 through 8, as marked on the front panel; whereas, channels 9 through 16 apply to the bottom board. Each channel includes an electrode/reference and ATC input. Refer to the overall schematic at the rear of this manual for the system layout.

Special Precautions

Before performing trouble analysis, service personnel should carefully read the precautions concerned with servicing the instrument.

NOTE: There are no hazardous voltages used within this equipment. The maximum voltages used throughout the instrument are ±12V which is derived from the PC.

- Use care in handling a MOS solid-state device. Never touch its leads or solder in place without using a grounded soldering iron. Although MOS gates are Zener protected, they can be damaged by static discharges. Also, avoid touching the board landings; handle a board by its edges.
- When replacing a plug-in IC, be certain that its pin orientation is correct. Refer to the circuit board drawing for proper pin determination.
- Before removing or replacing a plug-in IC, the rear cables of the instrument, or the card contained in the host PC; be certain PC power is turned off.
- Be sure all internal screws are snug tight on board mounting posts, since certain posts are used for grounding the RFI coating to the circuit. DO NOT OVER TIGHTEN THE SCREWS.
- Although the instrument uses a standard RS-422 protocol for communication with the host PC, the cabling is modified to carry operating power to the instrument.

Required Tools and Test Equipment

The following tools and test equipment should be on hand for servicing the instrument. Alternately available test equipment may be used in lieu of that listed. Be sure that test equipment are calibrated according to manufacturer's instructions.

Item	Model and/or Application
Calibration Accessory.	Fisher accessory (see instruction manual). Calibrate millivolt measurement of each channel.
Precision digital multimeter.	John Fluke Model 8060A DVM. Field testing and general troubleshooting.
Millivolt source.	Datel Model DVC-350 Hand Held Battery Powered Voltage Calibrator. Zero to ±2 volt source, accurate to 0.1%. Field test.
Dual in-line extraction tool.	Removal of dual in-line ICs from board sockets.
Cleaner.	Isopropyl alcohol (Fisher A-416) or Freon based cleaner. Cleaning of critical connections, Teflon standoffs, etc.
Logic probe.	COS/MOS compatible. General troubleshooting.

Trouble Analysis Chart

A subject denoted with an asterisk (*) indicates that additional service information on the subject matter is provided at the end of this chart, in the section headed **SUPPLEMENTAL INFORMATION**. This analysis assumes that external equipment (PC, electrode system, buffers, etc.) are in working order — CHECK THAT THIS ASSUMPTION IS TRUE AS A FIRST STEP IN TROUBLE ANALYSIS. Many problems can occur, such as noise interference, from incorrect electrode installation — see Operating Instruction Manual.

NOTE: Tantalum type capacitors have a tendency to short from an over voltage condition. Also, metal film type resistors will generally defect to an open condition.

In general, the symptom-cause method of troubleshooting can be performed on microprocessor based circuits in only a very few situations. Usually a malfunction in this area manifests itself in total garbage output, which is not possible to relate to a specific cause in the field. Thus, troubleshooting in this area is left to the device of substitution.

It is desirable to have a complement of "chips" on hand for troubleshooting by substitution. In lieu of this complement, "chips" may be interchanged between circuits.

Additional information is provided on the replacement parts drawings located in the **REPLACEMENT PARTS** section at the rear of this manual. Special attention should be given to any notations on these drawings.

Symptom	Probable Cause	Corrective Action
Note: All circuit boards are accessible by removing the supporting chassis from the front of the scanner. Refer to the **DISASSEMBLY AND REPLACEMENT** Procedures for additional details.		
Inaccurate readings on all electrode channels and or inability to calibrate any electrode channel.	Incorrect reference A/D voltage.	Refer to processor board schematic and check reference voltage at pin 2 of IC6. It should be +1.0245 volts.
	Defective PIO port.	Check IC5 and IC8.
	Defective A/D converter.	Check IC6 by swapping chip with IC7.
Inaccurate readings on one of the two input banks of electrode channels.	Defective electrode input multiplexer.	Check corresponding input multiplexer or IC2 on analog board.
Inaccurate readings on either bank of temperature channels.	Defective temperature input multiplexer.	Check input multiplexer IC3 on corresponding top or bottom analog board.

	Incorrect reference ATC voltage.	Refer tc analog board schematic and check reference voltage at J2-1. It should be +1.87 ±0.01 volts.
Inaccurate readings on a single channel.	Defective input amplifier.	Check associated input amplifier pair on analog board for channel in question.
Inaccurate readings on all temperature channels and/or inability to calibrate any temperature channel.	Incorrect reference A/D voltage.	Refer to processor board schematic and check reference voltage at pin 2 of IC7. It should be +1.0245 volts.
	Defective PIO port.	Check IC5 and IC8.
	Defective A/D converter	Check IC7 by swapping chip with IC6.
Flagged communication error codes #1, #2, #5, #6, and #8.	General communications failure between PC and scanner.	Check all supply voltages including the main bus supply from the PC. Also, check fuses F1 and F2 on PC card.
	Improper jumper connections.	Required jumper connections for this version of the scanner system are shown in the schematic diagrams at the rear of this manual.*
Error code #4 occurs.	Defective real time clock.	Check the real time clock on processor board. This chip is designated IC17 and is located beneath RAM chip IC3.*
Error code #3 or #11 occurs.	Defective cabling associated with optional controller.	Check cabling for continunity. Also, refer to optional controller.

SUPPLEMENTAL INFORMATION

This section contains supplemental service information for the scanner. Also, certain corrective actions listed in the preceding **Trouble Analysis Chart** were marked with an asterisk (*), indicating additional clarifying information on those subjects would be found in this section.

Certain information from the **Performance Characteristics and Specifications** provided in the operator's instructions are repeated here as a reference for service personnel.

Performance Characteristics and Specifications

Range

Activity/pH/pX	Electrode limited
Millivolts	0.0 to ±1999.9
Temperature	−4.0 to 104.0°C

Resolution

	3 significant digits
pH/pX	0.001
Millivolts	0.1
Temperature	0.1°C

Accuracy

Activity	0.3n*%
pH/pX	0.003
Millivolts	0.2
Temperature	0.2°C (from 0 to 100°C)

Drift (24 hours)

Activity	0.25n*%
pH/pX	0.0025
Millivolts	0.15
Temperature	0.2°C
Input Impedance (all channels)	$>10^{12}$ Ohms
Communication Cable Length	Up to 100'

* n = number of electrons exchanged in electrode reaction.

Supply Voltages

Supply voltages and their test points are listed below:

Voltage	Location	Test Point	Comment
±12 Vdc (reg.)	PC Card	P1-3,4	Fused for ½A.
+5 Vdc (reg.)	PC Card	IC2 pin 14	
±5 Vdc analog	Processor Bd	TP3 (+5) TP4 (−5)	Voltage presence indicated by LED1.
+5 Vdc digital	Processor Bd	TP2	Voltage presence indicated by LED1.
+12 Vdc	Processor Bd	TP5	Voltage presence indicated by POWER indicator (LED1 on front panel).

NOTE: Digital ground reference located at TP1.

+5 Vdc	Analog Bd	J2-20
−5 Vdc	Analog Bd	J2-10

Impedance and Offset Problems

The analog circuit input channel is designed to measure the output voltage from a high impedance sensor, namely the electrode pair (or combination electrode). The scheme uses two unity-gain input amplifiers as buffers — one for the measurement electrode and one for the reference electrode. The combined outputs of these buffers provide a differential input voltage for the instrument. The impedance of the electrodes is generally in the vicinity of 100 megohms. Thus, the input impedance of its amplifier must be at least 5 to 10 times higher so as not to affect the characteristics of the electrode. In this instrument, the circuit is designed around a low-leakage, low-noise op-amp which has a guaranteed input impedance greater than 10^{12} ohms. This high input impedance is degraded somewhat by the circuit wiring, components, and board. The low noise specification is necessary to assure accurate measurement by the instrument in this high impedance environment. Any leakage currents will degrade the electrode system output. Ideally, the noise generated by the op-amp and associated input components must be much less than the noise produced by the electrode system. This condition will result in a noise figure that approaches the ideal value of one for the instrument.

Cleaning Front End

From the above brief discussion, it can be seen that it is necessary to have high grade components in the front end, along with cleanliness of assembly, to achieve the intended measurement accuracy (pH, mV, etc.).

BNC INPUT Jacks

The BNC jack (one per channel) is the least likely source of possible impedance problems. This jack configuration has been in industry for many years and has evolved into an extremely reliable component. Originally intended for shielded, high frequency use, the jack provides high DC isolation between its inner and outer conductors, together with positive locking action with its mating connector. The BNC jack is an ideal connector for this application.

The jack should not require any maintenance, unless liquids or other contaminants are allowed to enter the jack opening. A shorting cap is supplied with the instrument. This cap is intended to double as a protective

cover for this jack. The jack should never be left unconnected — use the shorting cap to protect the jack when it is disconnected from an electrode.
The BNC jack is difficult to clean; it is better to replace the jack. If replacing a jack, be sure to use a high quality part. If replacement is not feasible, clean the jack with a high-grade Freon based cleaner or isopropyl alcohol. Dry out interior of jack with compressed air. Do not attempt to use any other cleaner as a substitute, as the materials involved may become adversely affected.

Circuit Boards

A more likely candidate for impedance problems is the input circuit associated with each input buffer. Pin 3 of each buffer is intentionally soldered directly to a standoff with a Teflon insulator to minimize leakage currents. This insulator is used to bridge a resistor to its input jack. The resistor is intended to provide some degree of static discharge protection to the input gate of IC1 when the input jack is left floating. Thus, the input circuit employs a low leakage design to maintain the high impedance integrity at the instrument input. It is a simple procedure to clean the circuit board. Actually, it is only necessary to clean the Teflon insulator and the board around insulator. This area is depicted by the absence of the solder resist coating. Use isopropyl alcohol for cleaning purposes; dry with compressed air (oil free).

CIRCUIT DESCRIPTION

The scanner operates as a dedicated instrument to an IBM or compatible PC to provide up to 16 channels of pH and or ion selective electrode inputs for evaluation by the operator. Provided software (5¼" floppy disk) includes extensive on-screen help messages and diagnostics to enable the operator to quickly begin instrument use. A PC based instrument offers all of the advantages of mass disk storage (ASCII format), real-time print out (on a variety of printers), off-line print out, and spreadsheet manipulation (Lotus, dBase, etc.). Also, method setups can be stored on disk.

The scanner allows multiple channel readings with either common references or electrically isolated individual references. Electrically isolated inputs are required when measuring multiple electrode inputs from the same solution, but with different references. Measurements may be in millivolt, pH, temperature, or activity units. Temperature correction for pH and activity measurements require either an operator-entered fixed temperature input or a continuously updated temperature by an accessory ATC (automatic temperature compensator) probe. More than one channel can be designated to operate with the same ATC probe. Each channel also can be monitored (front panel jack) with a chart recorder. The system provides a four and one-half digit resolution.

The scanner employs a battery backed RAM (IC3) and real time clock (IC17) which are mounted together on the same socket on the processor board. The battery is integral to the RAM socket, with an approximate 10 year life. Calibration values for the A/D converters are hardware specific. They are stored in RAM. The real time clock is set via software. Communication with the PC is by means of an RS-422 protocol, modified to carry the ±12 Vdc scanner power from the PC. The RS-422 protocol was selected for its following inherent features: (1) Noise-free communication over long distances (up to 4000 feet). However, the power carring modification limits this distance to about 100 feet. (2) Ability to daisy-chain devices (e.g., accessory controller).

An accessory device is available to greatly assist in calibrating the scanner channels. This device is called the Calibration Accessory. Only a DVM is required with this accessory. Complete calibration procedures are provided in this manual. (Procedures are part of scanner software for on-screen display.)

The buffered analog signals from the electrodes are multiplexed by IC1 and IC2 on the analog boards for application to the processor board. Both the measurement and reference inputs of a channel are multiplexed. Each multiplexer is capable of multiplexing 8 inputs. Note that IC1 is associated with the measurement electrode inputs, and IC2 is associated with the reference. Each analog board handles eight channels. IC3 of the analog boards is used to multiplex the ATC probe input. The recorder output for each channel uses an attenuated differential output from the buffers of its channel. The two input resistors and common capacitor for each channel increases noise immunity. The use of patch cords (supplied) allows electrical isolation.

On the processor board, the selected multiplexed analog signals (measurement, reference, and ATC) are digitized by A/D converters IC6 and IC7. IC6 is responsible for electrode signals (measurement and reference) and IC7 is for the ATC signal. Both A/D converters process at a rate of 7 conversions per second. The frequency that a channel is updated is determined by the total number of enabled channels (by operator). For example, when 7 channels are enabled, a complete update of each channel will require approxi-

mately one second.

Channel data is stored in the scanner RAM (IC3) and held for access by the PC. The PC instructs the scanner as to which channels are to be enabled (analog input digitized, stored and made available) and which channel data is to be actually sent to the PC. The PC also controls the setting and reading of the real time clock (in the scanner), and the storage and retrieval of calibration variables.

The RS-422 communications protocol permits multiple devices (up to 32) to use the same communications line in a daisy chain fashion. The first character in any message is known as the address character which designates the specific device to receive the message. The scanner recognizes the sign "<" (less than symbol) as its address character. Thus, all communication from the PC to the scanner must be preceded by the "<" character. Also, each message must be terminated by a carriage return/linefeed sequence.

In order to enable a given channel, the PC must send a "<E" character sequence, then the channel number + 95. For example, in BASIC, to enable channel 2 the following statement is used: PRINT #1,"<E + CHR$(2 + 95). The same format is used to disable a channel. However, the "E" character in the message is replaced with a "D" character for disable.

Sending the command "<R" + CHR$(96) instructs the scanner to return its last millivolt reading for channel 1. The scanner then sends an ASCII coded integer that represents the channel reading in tenths of a millivolt. The PC multiplies this integer by 0.1 (0.1 x integer value) to obtain the actual decimal millivolt value (raw channel millivolt data). Raw channel millivolt data does not take into account the error introduced by non-unity slope of the A/D converters or the offset of the input buffers. These errors are corrected by calibration.

Two slope values are calculated for each electrode input channel. A high slope (for millivolts greater than zero) and a low slope value (for millivolts less than zero). The ATC input for a channel requires only a high slope since its millivolt readings is always greater than zero or positive. The electrode inputs require an additional calibration constant which represents the offset voltage introduced by the input buffers. This constant is equivalent to the millivolt reading returned from the A/D converter when the electrode inputs are shorted (no input). All calibration variables are stored in an array within the RAM of the scanner by the following command:

"<U" + (record number – 96) + 0I (file number – 96) + (value to be stored)

Record 0, file 0 through 15 corresponds to the 16 electrode channel offset values or constants. Record 1, file 0 contains the electrode channel high slope value; file 1 contains the low slope value. Finally, record 1, file 2 holds the ATC values.

Other commands which may be sent to the scanner from the PC are: (1) reset "<Z", (2) set clock "<S – DATE – TIME", and (3) read clock "<T". In all cases, the communication parameters are: 9600 BAUD, no parity, 8 data bits, one stop bit, and no handshaking.

At the present time (see publishing date on rear cover of this manual), four characters are reserved for addressing different devices on the RS-422 communications line. As previously mentioned, the command "<" addresses the scanner. The character command ">" addresses the accessory controller, and "@" or "?" is reserved for addressing two future accessories that are presently being planned. Additionally, for future considerations, the following characters are reserved for addressing devices: "=", ";", and ":". These six characters comprise the characters between 9 and A in the ASCII character set. Data can be stored on disk or hard copy (PC attached printer).

The scanner is interfaced with the PC communication port by means of a card which is installed in any vacant PC slot. Only one other port can be present in the PC in this configuration. If one port is already present (such as a modem), then the card communication jumpers must be set to COM2 and I3. The remaining jumpers locations on the card are not used. Likewise, if two ports are present, then the existing COM2 port must be removed and the card installed with its jumpers set as above. If no ports are present, then set the communication jumpers for COM1 and I4. Complete installation procedures are provided in the operator's manual. The card provides communication between the PC and scanner via an asynchronous communication adapter (IC4). Differential line transeivers are used at the cable interface.

DISASSEMBLY AND REASSEMBLY

In order to obtain unobstructed access to the circuit boards for tests and troubleshooting, the chassis assembly (not broken down as an assembly in the replacement parts) must be removed from the instrument case. The chassis is removed from the front of the case, along with the front panel as shown in figure 1. Perform this disassembly as follows:

1. Turn off the PC and disconnect the communication cable end at the scanner.
2. Remove the back panel of the scanner.
3. From the rear of the scanner, remove the four nuts from within the case that secures the chassis to the front of the case assembly.
4. Carefully pull the chassis out of and free of the case.
5. Reconnect the communication cable to the scanner. The PC can now be turned on and the system booted for operation.

Any other disassembly that may be required is apparent from inspection of the scanner and parts location drawings at the rear of this manual. Reassembly is always in the reverse order of disassembly.

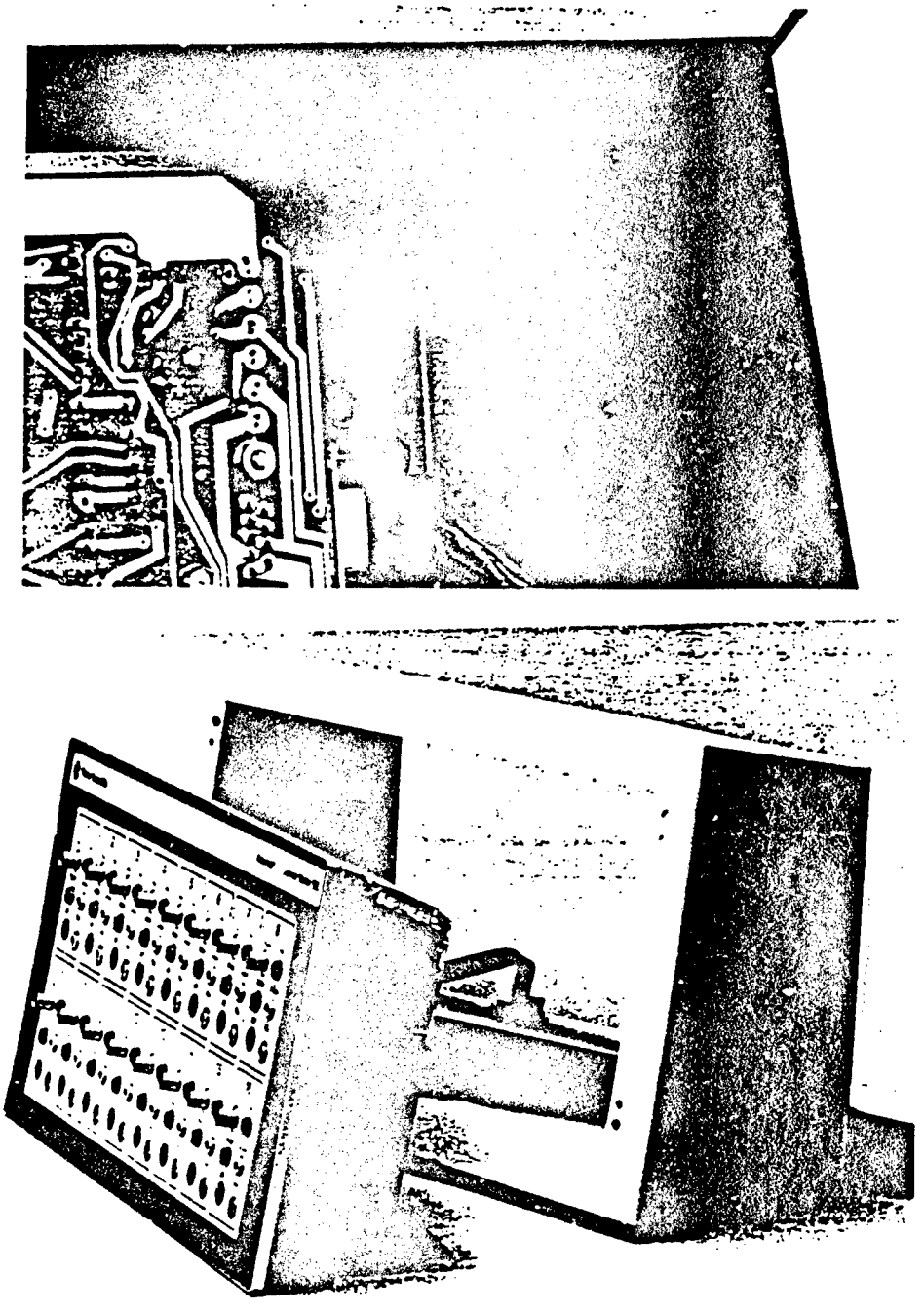


Figure 1. Removing Chassis Assembly

CALIBRATION

The calibration procedure for calibrating the millivolt measurement of each channel is built into the scanner software. The Calibration procedure is brought up from the Main Menu by pressing F7. Only a DVM and the Calibration Accessory is required to perform the procedure (see figure 2). A channel is calibrated when the millivolt value displayed by the DVM (connected to the Calibration Accessory) is entered when prompted by the display. The scanner must be connected to a PC. That is, the scanner must be fully operational in a system as described in the operator's manual. Electrodes are not required to perform the calibration.

1. Bring up the Calibration category from the Main Menu by pressing key F7 at the PC keyboard.
2. Bring up the Calibrate Channels procedure by pressing F2.
3. Follow the procedure given on the screen.
4. If an OUT-OF RANGE is continually displayed on the screen after entering the millivolt value, then a malfunction exists.
5. Exit the system when calibration is completed.

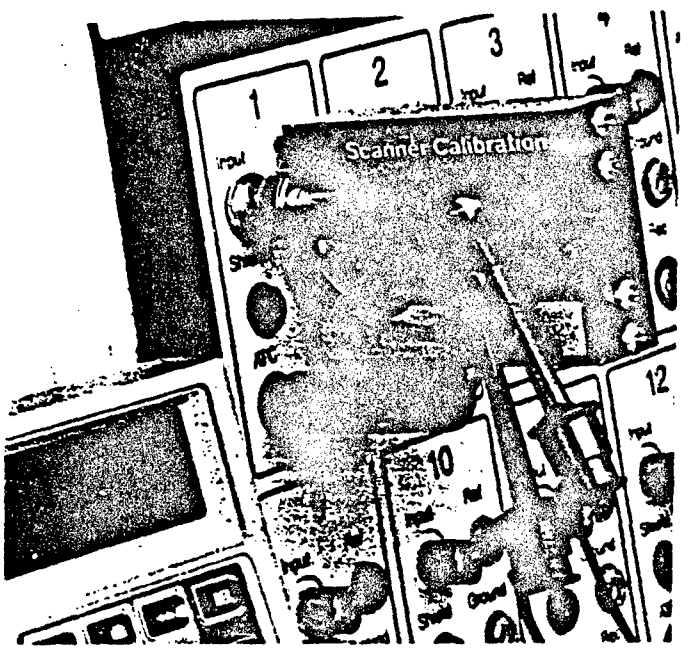
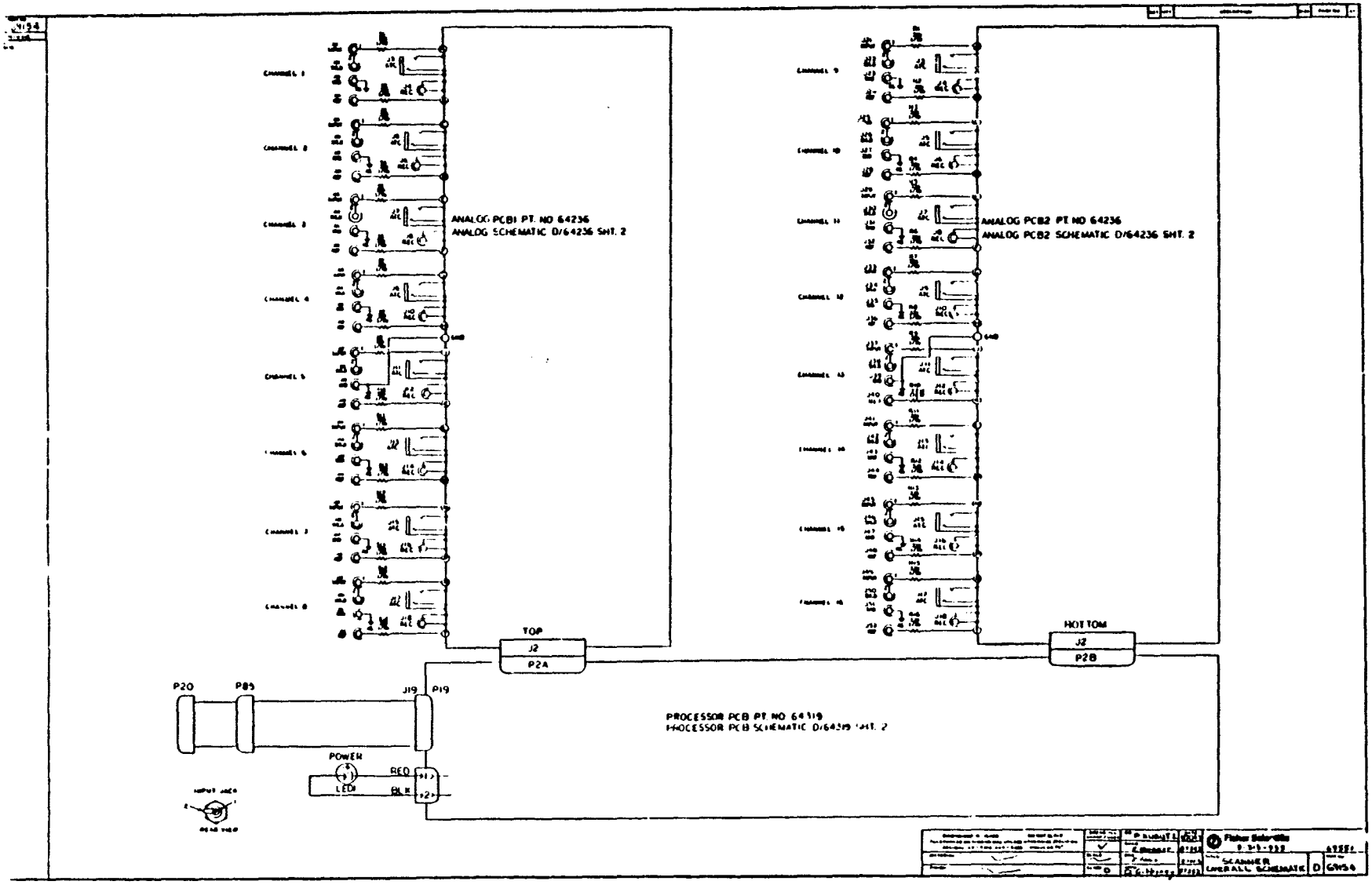


Figure 2. Connecting Calibration Accessory

REPLACEMENT PARTS

The replacement parts, along with their location drawings, are provided in this section. The section is arranged in assembly to subassembly breakdown. That is, the highest order assembly appears first, followed by its subassembly make-up. Non-field repairable assemblies or subassemblies are not broken down into their component parts. Note that parts information is only valid at the publication date (see back cover) of this manual, and subsequent revisions may have occurred after publication.



igure 3. Overall Interconnect D69154

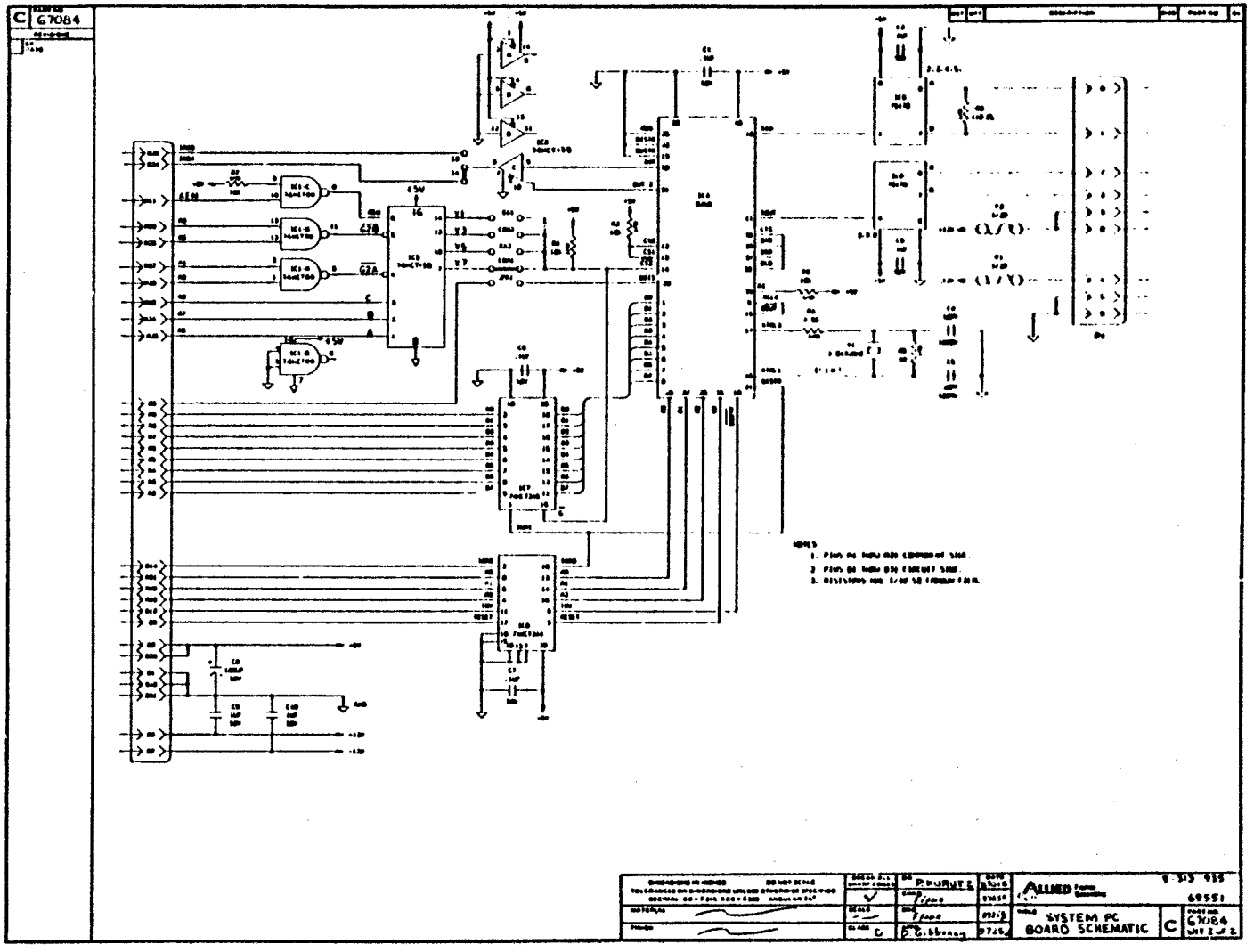
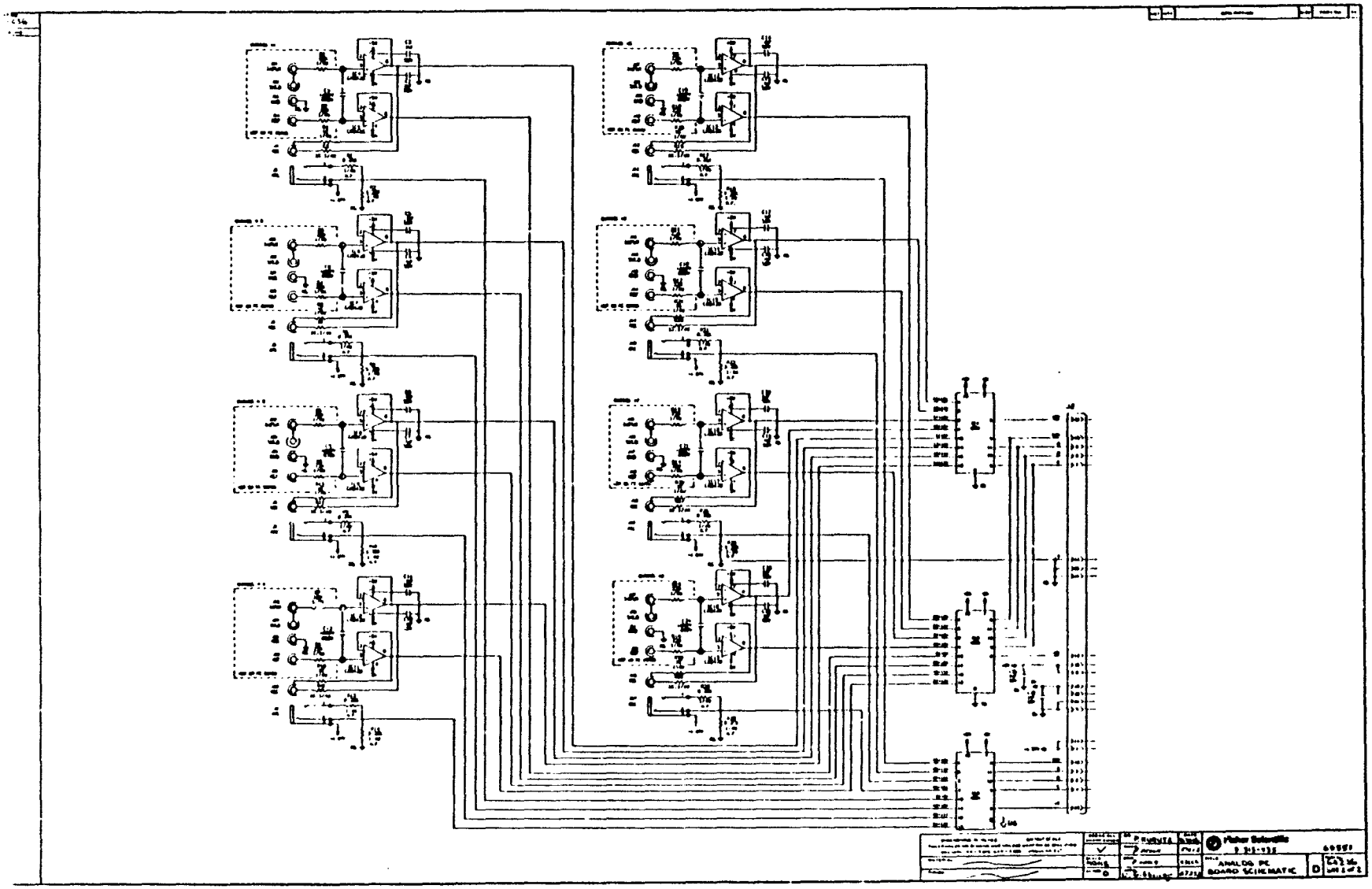


Figure 4. PC Card Schematic C67084



igure 5. Analog Board Schematic D64236

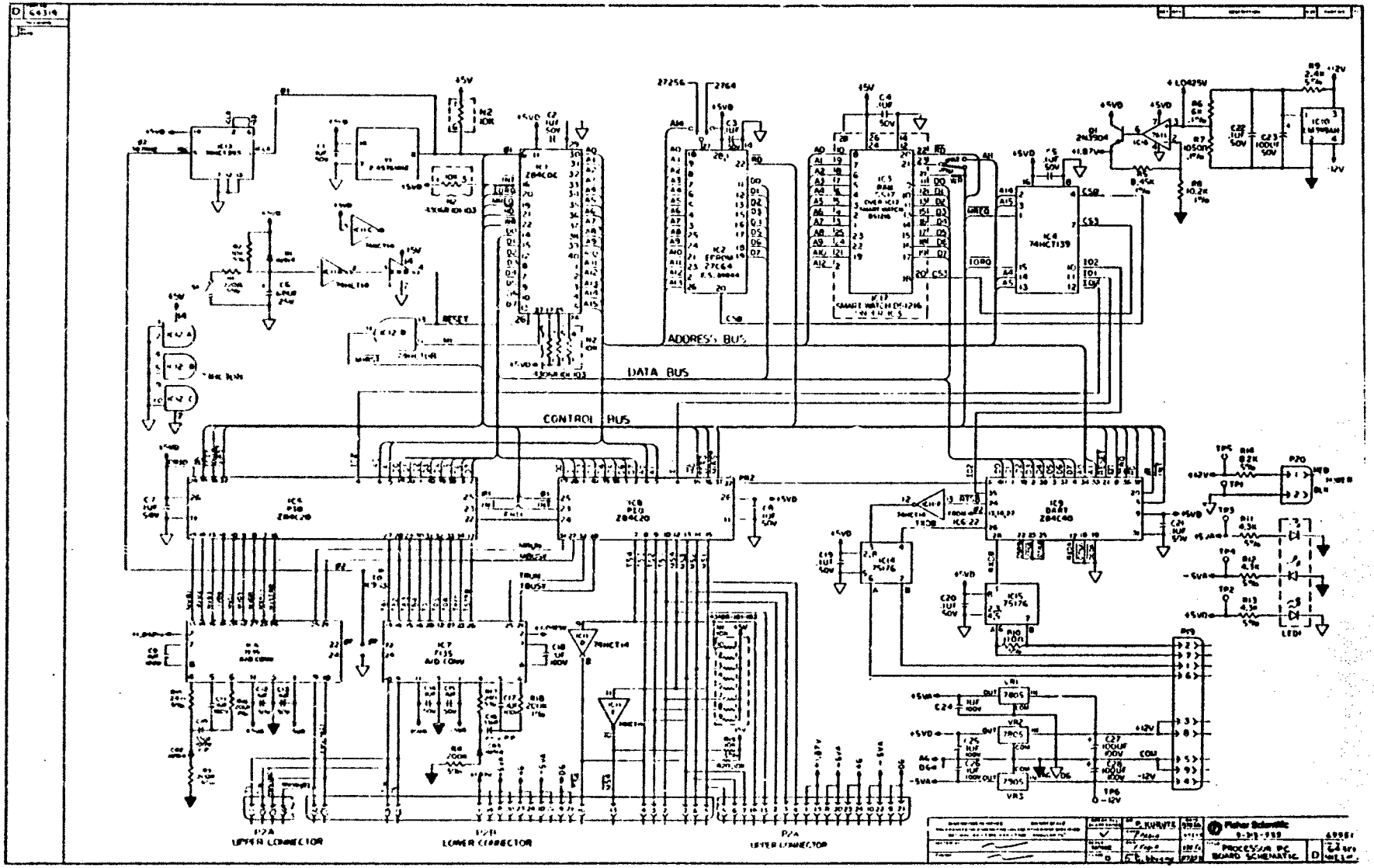


Figure 6. Processor Board Schematic D64319

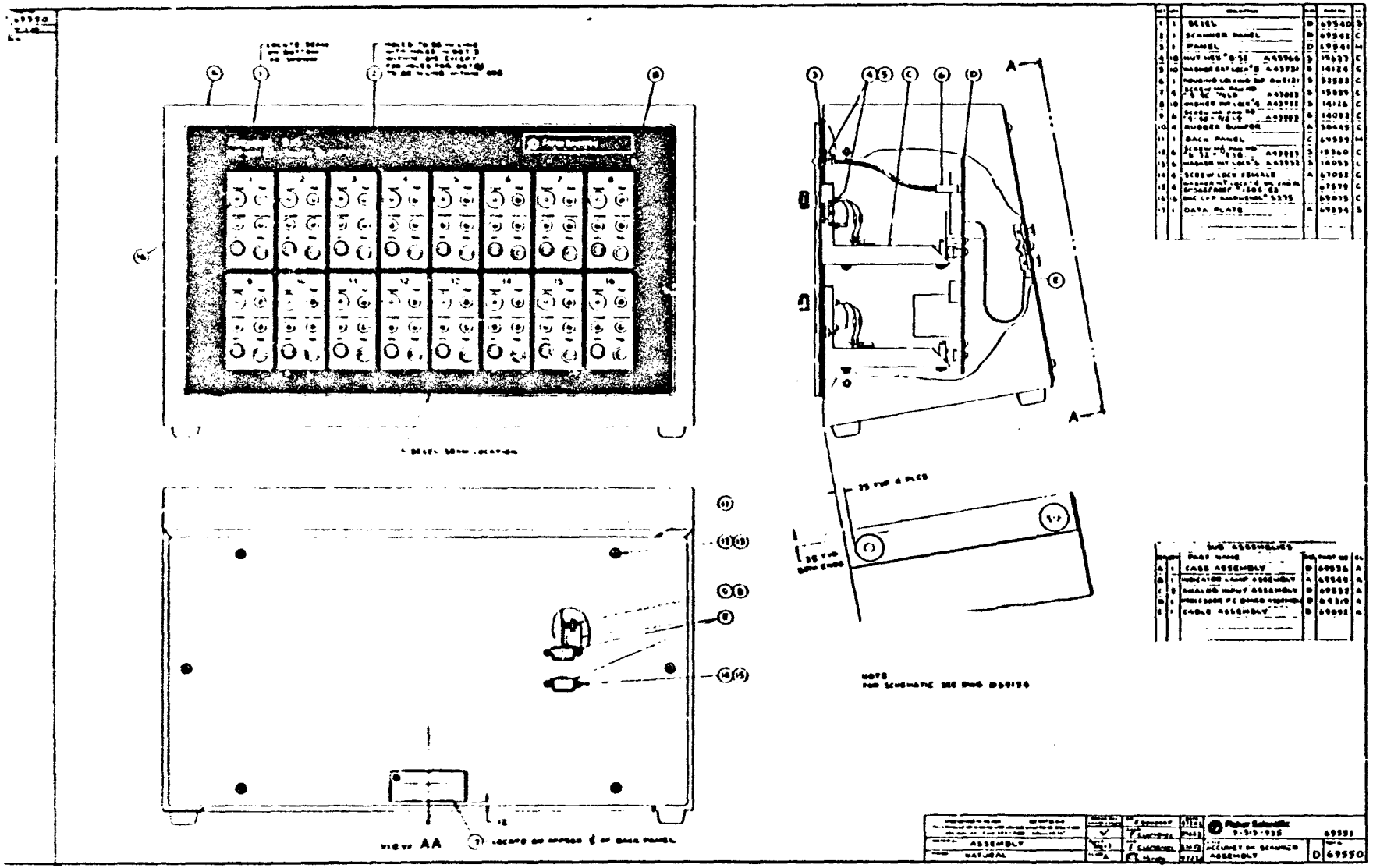


Figure 7. Main Assembly D69550

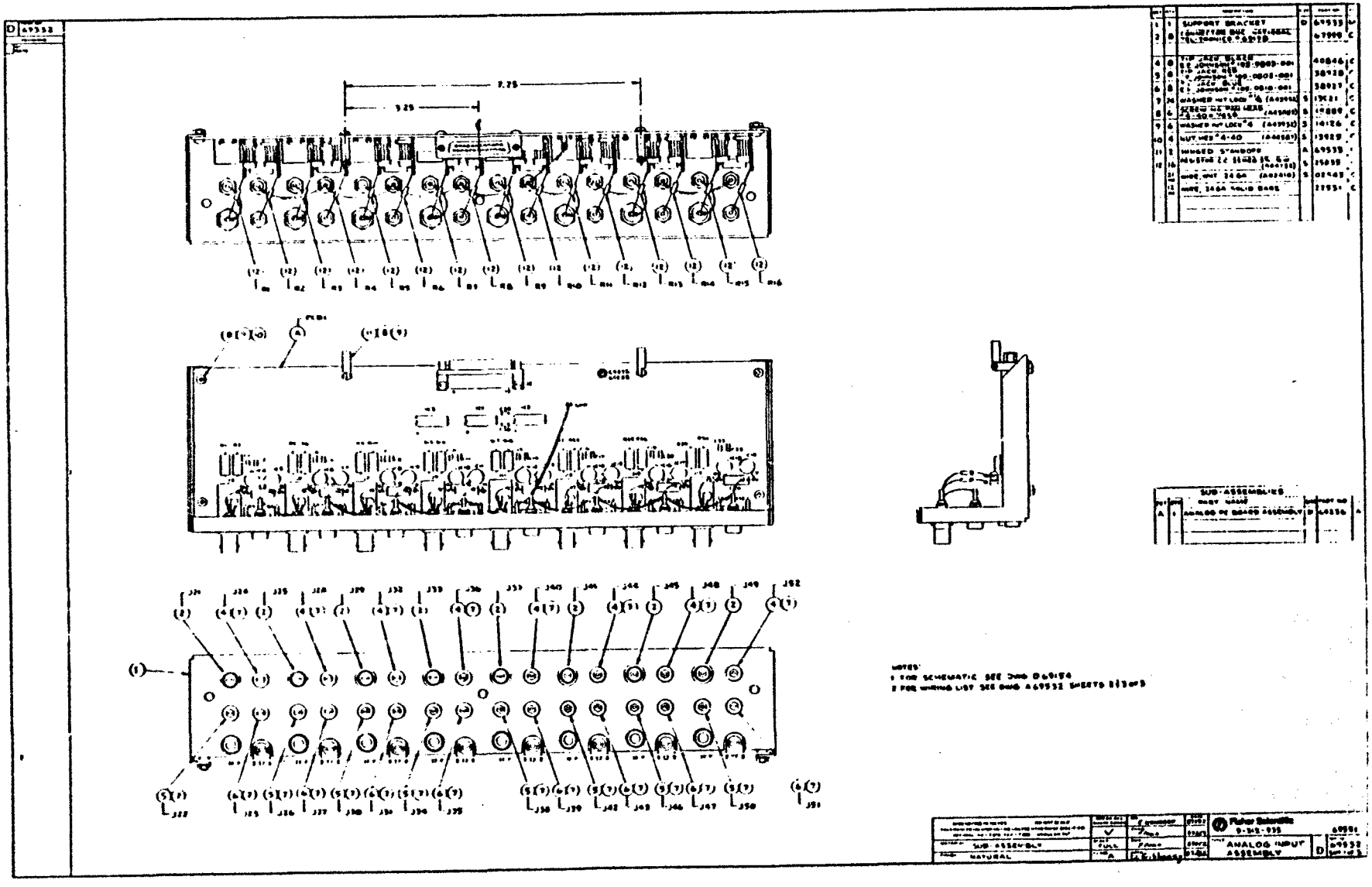


Figure 8. Analog Input Assembly D69532

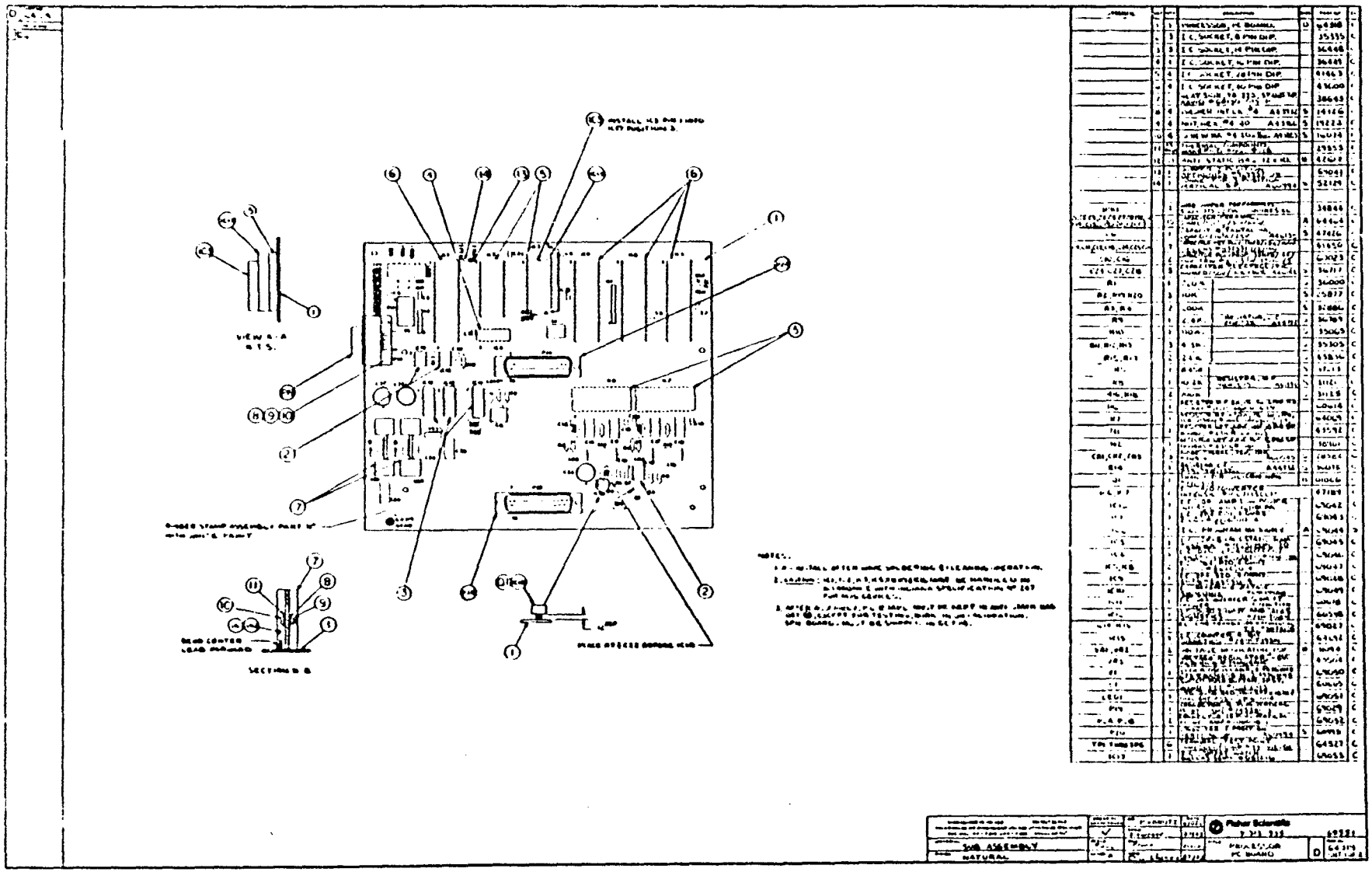


Figure 9. Processor PC Board Assembly D64319

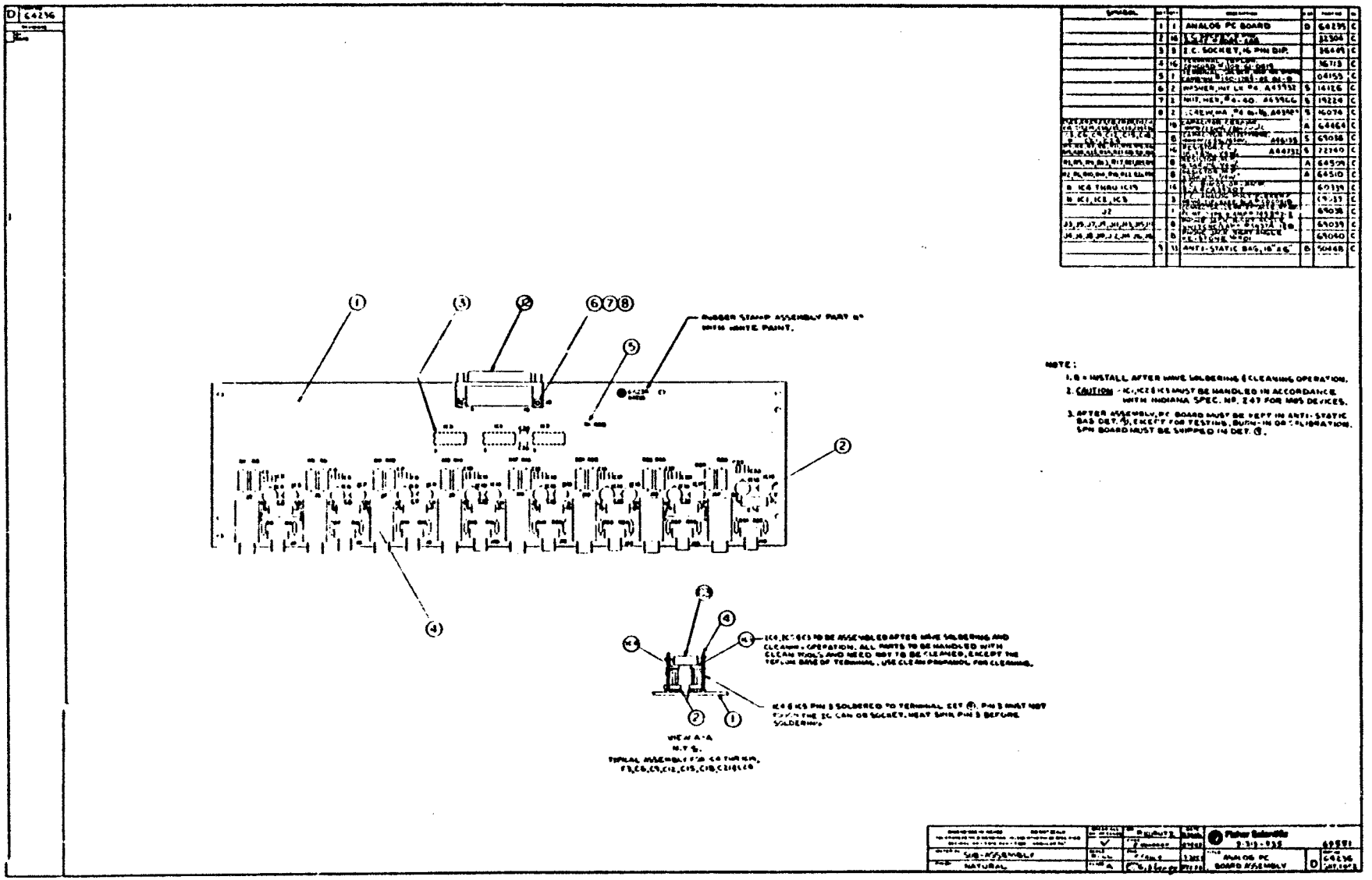


Figure 10. Analog PC Board Assembly D64236

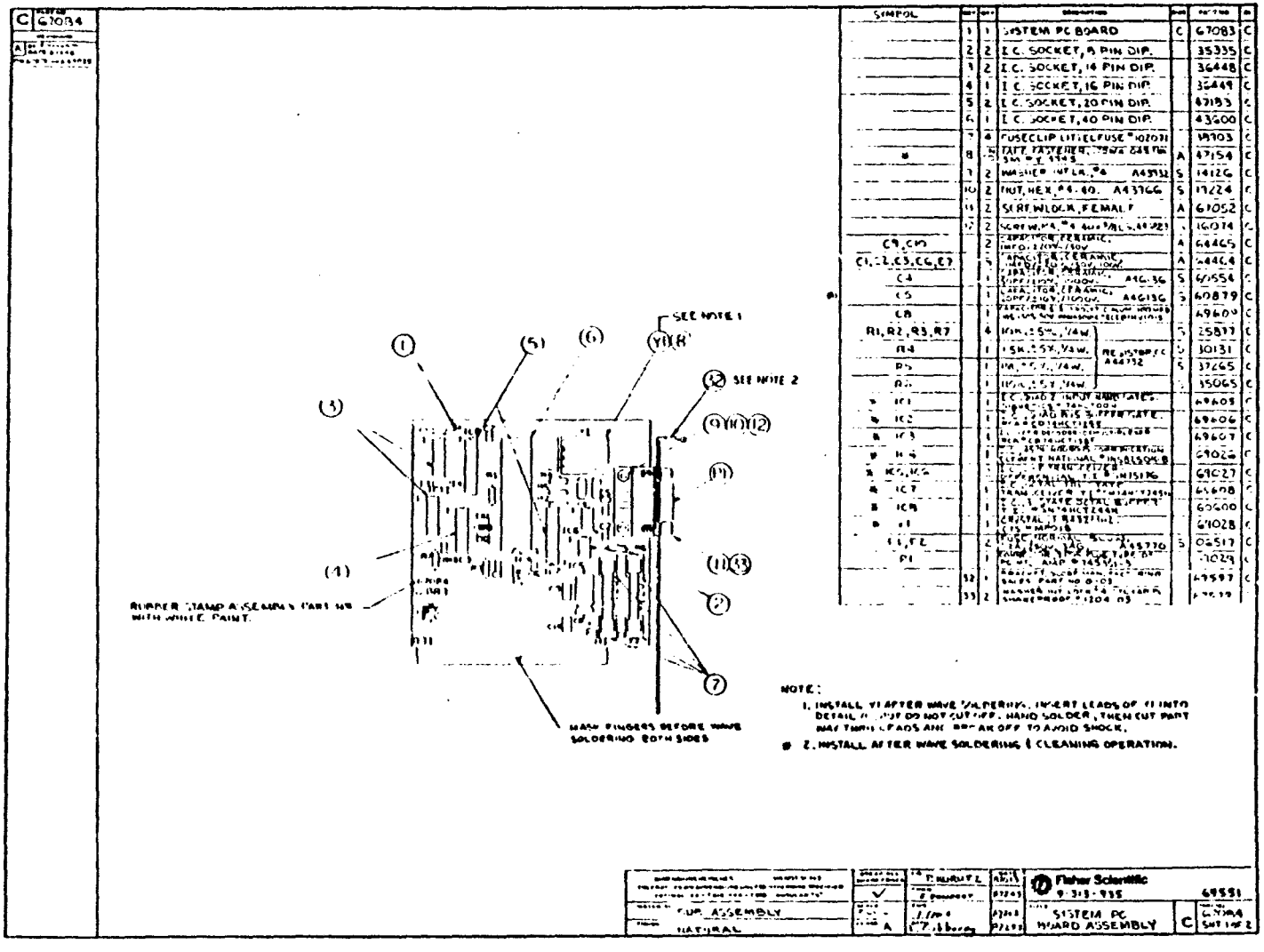


Figure 11. PC Card Assembly C67084

Figure 12. Communication Cable Assembly B69093

APPENDIX C

Published Papers

MAKING THE TRANSITION FROM TOXICOLOGY TO ECOTOXICOLOGY

James R. Pratt

School of Forest Resources and
Graduate Program in Ecology
Penn State University
University Park, PA 16802

ABSTRACT

Aquatic toxicology is rapidly becoming an ecological science with the need to make predictions about the effects of human influences on ecosystem structures and processes. Present methods for assessing chemical hazard rely on testing a few sensitive surrogate species but these methods fail to consider effects on untested population, community, or ecosystem properties. Failure to consider the ecological perspective of chemical hazard can result in both over- and under-regulation of chemicals. Ecotoxicologists use ecologically meaningful endpoints to estimate chemical effects. For example, the use of laboratory ecosystems to simulate and replicate ecosystems shows promise for characterizing not only the chemical concentrations associated with adverse ecological effects but also for predicting the kinds of adverse effects expected. Future work is needed to characterize ecosystem vulnerability and to develop standard methods for assessing ecological effects in critical risk assessments.

INTRODUCTION

The sciences of toxicology and ecology are at a crossroads. Toxicological methods have served as quick, convenient, and pragmatic ways of estimating the effects of chemical stressors and mixtures on aquatic life. Ecological risk assessment as practiced by regulatory agencies is, however, nothing more than a series of increasingly sensitive toxicity tests linked together in a tiered or sequential fashion.[1] While these tests examine effects on species of different ecological positions, different life histories, and different longevities, present risk assessment practices in no way evaluate any ecologically meaningful interactions.

Field ecologists have played a major role in assessing environmental damage through biological surveys of the presence, absence, and abundance of a variety of taxa ranging from microorganisms to fish. Concurrent with the "toxicological era" of environmental regulation, a great deal has been learned about the important structures and processes in aquatic ecosystems. The flow of energy through stream ecosystems has been estimated and the functional role of a variety of taxa within the ecosystems that normally receive our wastes is increasingly better understood.[2] Additionally, our concept of ecosystems has changed to include the importance of energy and nutrient flow and cycling so that the traditional focus on indicator species[3] holds very little relevance for most ecosystem ecologists.

My purpose is not to review toxicology and applied ecology. Rather, my purpose is to provide a pedagogical review of environmental toxicology as practiced and applied in the regulatory framework, to compare toxicological responses with ecological responses to toxic chemicals, to examine the applicability of ecotoxicological testing, and to make recommendations for adapting and adopting ecological toxicity testing.

A number of professionals like to be known as ecotoxicologists, but the number of practicing ecotoxicologists is small. In fact, it may be a set with no members. An ecotoxicologist applies ecological principles, measures, and information in the evaluation of chemical hazards and risks.[4] I define hazard according to a dose-response relationship in which the relative hazard of toxicants is ranked by the dose required to produce a significant, adverse response. A risk is

defined by hazard and by the probability of exposure of organisms to hazardous concentrations. In protecting aquatic life, ecotoxicological information is not being used. Because of the reliance on sensitive, surrogate species some chemicals are over regulated with respect to their ecological hazards and risks and some chemicals are under regulated. That is, the best scientific information is not being used to regulate chemicals. This brief chapter in no way summarizes the vast toxicological literature or even the debate on the appropriateness of tests for regulation. The interested reader is referred to texts which can put these fields in some scientific perspective.[5-7]

Toxicity and Bioassays

Toxicity is usually defined as the inherent property of a chemical to produce adverse biological effects. These effects usually are conceived of ranging from acute responses (occurring over a small part of an organisms life cycle), such as mortality or behavioral responses to chronic effects (occurring over a long portion of an organism's life cycle), such as impaired reproduction or reduced growth. The definition of toxicity is critical in the regulatory framework since the general intent based on the Clean Water Act has been to eliminate toxic chemicals in toxic amounts from our surface waters. With respect to aquatic life, a chemical is not of concern if it does not appear in the environment or if it appears in such a low concentration that its biological effects are not measurable. Clearly, the definition of toxicity and the means by which it is measured are crucial in environmental protection. If toxicity were defined only as acute toxicity then allowed environmental concentrations of chemicals would be much higher than if toxicity is defined over a chronic exposure. The ratio of the concentration of chemical that produces acute toxicity to the concentration that produces chronic effects can vary from almost 1 to several orders of magnitude. A variety of now standard procedures for assessing the toxicity of a chemical has been developed. These procedures - bioassays - use biological material to assess the potential for a chemical to cause damage. The specific mode(s) of action of most toxic chemicals is not known and so healthy, sensitive organisms must be used to assess biological effects.

Over the years a small group of organisms has been used to assess toxicity (Table 1). These organisms serve as surrogates for the larger assemblage of species in an ecosystem, the overwhelming majority of which can never be tested. The surrogates selected are expected to be broadly sensitive, well-studied, and commercially or ecologically important.[7] Ecosystems are quite diverse and very few species are truly representative of a large number of ecosystems. Perhaps more important criteria in the selection of test species beyond their sensitivity is their availability and ease of culture. This presents a fundamental conflict in toxicology. Test organisms that are sensitive may be fastidious and, therefore, difficult to rear in sufficient numbers for routine testing. The selected group of surrogates is really quite small when one considers that there are probably between 5 and 50 million species on earth and that several thousand of these occur in any ecosystem. Further, the selection of surrogates strongly favors familiar organisms such as fish in deference to smaller, less familiar, but extremely abundant invertebrates and algae.

Two approaches to using bioassays might be envisioned. One would be to test indigenous or representative species from a particular ecosystem that might receive toxic chemicals. Such an approach could lead to regionally specific limits on the exposure of indigenous species to particular chemicals. A second approach, the one that is widely used in regulation today, is to use a fixed set of biological sensors (the standard test species) to test every chemical or chemical mixture. In this way, the biological material is a constant and the relative hazards of individual stressors can be evaluated. Since the procedures are standardized, there is a reasonable assumption of comparability among laboratories, although inter-laboratory comparisons have been equivocal.

Deriving water quality criteria or standards is based on the assumption that the sensitivities of species are normally distributed (Fig. 1) and that by testing species at the sensitive end of the distribution a relationship between dose and the cumulative effects on an array of species can be used to extrapolate to the larger assemblage of species found in ecosystems. While these assumptions might be acceptable if one examines the sensitivities of individual species, it lacks the ability to predict effects on ecological interactions. Further, many compounds must be regulated in the absence of sufficient information. For example, prior to 1980 water quality standards for

chronic zinc discharges were based on the toxicity of zinc to rainbow trout because there was insufficient data to relate chronic responses and dose and because rainbow trout are recreationally (and therefore economically) important.[8] Because of the egalitarian nature of regulation zinc standards were the same all over the country, meaning that even ecosystems which lacked rainbow trout were regulated at the same zinc concentrations as which did contain rainbow trout. We know understand that zinc toxicity is affected by water hardness and zinc criteria differ among soft and hard water streams.[9]

Bioassay information has been used in a standard fashion to develop water quality criteria for over 100 pollutants; however, 6500 chemicals are in common use and may commonly appear in the environment. The bioassay model has been extended to complex mixtures as a way of regulating effluent streams,[10] but because the chemical constituents of an effluent may vary or may not be known the "dose" can only be expressed in terms of the relative dilution of the effluent by clean water. Nevertheless, the bioassay can differentiate between the relative toxicities evidenced by different compounds or mixtures. Bioassays do not, however, provide direct predictions of ecological effects.

Most toxicological evaluations focus on the responses of small laboratory populations of the surrogate species. While the laboratory populations might serve as surrogates of ecological populations, testing usually focuses on a sensitive life stage (typically juveniles) and so lacks much of the population realism that occurs in the field where populations have variable age structures and growth is limited by the availability of food and other life requisites. If ecosystems are viewed hierarchically,[11] most of the important ecological processes are as result of interacting populations (Fig. 2). For example, nutrients are cycled by decomposer food chains. Food chains link species together by predator-prey interactions. Species compete for resources and those with superior competitive abilities under existing conditions typify certain environments. A large number of ecologists consider themselves to be community ecologists[12] and, although communities as interacting units in the biological hierarchy probably do no exist, communities do form understandable subsets of the larger ecosystem.

From a human perspective, ecosystems provide a variety of essential services ranging from biomass production (food and fiber) to waste assimilation. It has never been clearly demonstrated that chemical regulation and the regulation of discharges has resulted in the protection of these ecosystem services. In fact, ecosystems are providing mixed messages to environmental scientists because there are signs of both improvement and continued degradation in related systems. For example, body burdens of DDT and PCB's in lake trout have decreased in Lake Michigan[13] but have either remained the same or increased in Lake Ontario over the past decade (Fig. 3). Commercial fisheries landings show similar confounding responses: catches of some fish have improved while catches of other fish have decreased significantly (Fig. 4). It is well known that human influences on terrestrial ecosystems have decreased overall productivity,[14] so it is reasonable to assume that in certain heavily used aquatic ecosystems similar effects might be expected.

Bioassays have been criticized because they lack the ability to predict effects of chemicals at the community and ecosystem level.[5] While the responses of standard test species may vary considerably to a toxic challenge, it would be surprising if the responses of interacting groups of species differed widely from the responses of sensitive surrogate test organisms. In other words, there should be sufficient similarity in the biological machinery that exposures toxic to standard test species would also be toxic to multi-species assemblages. From an evolutionary perspective, metabolic and physiological processes are remarkably constant across all living things. I believe the major point of contention lies not in the prediction of the concentration of a particular chemical that might be toxic, but rather prediction of the magnitude and type of effects that might be observed at the ecosystem level. Since most testing focuses on organisms representative of only a few functions in ecosystems, effects of toxicants on the typically untested species such as bacteria, other microbes, and plants is more problematic.

Both toxicologists and ecologists have been somewhat unwilling to accept the concept of laboratory controlled, ecological test systems where species interactions and ecologically meaningful variables could be measured. Over the past decade it has become clear that laboratory

manipulation of species assemblages is not only possible but achievable with sufficiently low variability that important ecological responses can be discerned both in the investigation of basic questions of ecosystem structure and function and in the applied aspects of anthropogenic influences on ecological phenomena.[15] Ecological test systems should not be expected to be used in the routine way that bioassays are used in chemical regulation.[16] However, laboratory scale ecosystems can provide important "quality controlled" checks on predictions from single species bioassays and can also identify those properties of ecosystems that might be reasonable to measure both in the laboratory and field.

Ecosystems have both collective and emergent properties that result from the occurrence of species in the same place at the same time. Collective properties include measures that express the state of an ecological system at a particular time and include population, community, and ecosystem variables. Emergent properties are those properties which emerge from the interactions among species and include such processes as predator-prey interactions, nutrient cycling, competition, and succession. These emergent properties are typically measured as rate processes. In a general way, the collective properties of systems can be thought of as measures of the structure of the system and the emergent properties can be considered measures of system function. In the next section of this paper, examples of ecological responses to stress are given and related to conceptual models of expected changes in ecosystems under stress.

Ecological Toxicity Testing

The diversity of laboratory scale systems that display ecological properties is quite amazing, ranging from sediment-water systems of only a few milliliters to large channels, tanks, or ponds of around one million liters.[15] Smaller systems have usually been termed microcosms and larger systems are called mesocosms. The most intensively tested systems are usually a few liters and rarely include organisms as large as fish.[17] Mesocosms which are closer to field scale usually include fish and other large, long-lived organisms.[18] Typically, as the size and longevity of the component organisms increases experiments must be run over a longer period of time and the cost

increases significantly. Artificial pond mesocosms are currently being used to evaluate pesticides.[19]

The criteria for establishing a successful laboratory scale ecosystem are generally considered to be demonstration of several ecological properties. Artificial ecosystems should display energy and matter processing, nutrient cycling, and succession. That is, the system should be sustainable and should change with time. For purposes of experimental manipulation and interpretation of results, laboratory scale systems must be replicable, meaning not that each system is an identical copy of the other but that variability among systems is sufficiently low that measured properties can be considered similar in the replicates and that during experiments systems develop in approximately the same way.

Approaches to studying the effect of toxic materials on ecosystem structure and function in microcosms have ranged from completely synthetic systems to naturally derived communities. For example, the standard aquatic microcosm (SAM) developed by Taub and colleague[20] assembles an ecosystem from cultured components including protozoa algae and crustaceans. The assembled microcosms are dosed with toxic chemical and are periodically reinoculated from the cultures. This system is nearly totally defined from the culture medium to the component taxa. A second approach, the mixed flask culture (MFC) originally developed by Leffler assembles a test system from a cultured collection of pond or lake water in a defined medium. Only taxa capable of surviving in the defined medium are eventually apportioned to the microcosms for testing. In the SAM, population dynamics and nutrient pools are measured in response to the toxic dose. In the MFC, functional responses such as primary production and respiration are measured along with non-destructive measures of biomass.

A very different approach which does not involve culturing has been used by Giddings and colleagues[21] who developed microcosms in large aquaria using sediment, plants, and water from a pond. In this microcosm the amount of water and sediment are fixed and a constant wet weight of plants added to each microcosm. No attempt is made to culture or select species, rather the community is allowed to develop after the microcosm is assembled. By thoroughly mixing

sediment and water, there is a good probability of equitable distribution of taxa among replicates. Following the addition of the toxic chemical, microcosms are studied for numbers and kinds of selected taxa, diurnal production and respiration, and major nutrient pools.

Each of the above microcosms uses a static test system. Typically, although not always, the toxic material is supplied in a single dose or a series of pulses. Our laboratory has taken an alternative approach using natural communities and incorporating a continuous input of the diluted toxic material.[22] Microcosms are developed by collecting natural communities of microorganisms on artificial substrata (polyurethane foam) at a reference or unimpacted site in a stream. These communities form the seed material for the replicate microcosms. The toxic chemical is added from a serial dilution device so that the 4-7 L contents of the microcosms are replaced at least five times per day. Communities from the artificial substrata are sampled by removing a substratum and squeezing it into a collecting container. Microcosms are evaluated for species richness of protista, community biomass (protein chlorophyll) activity of nutrient transporting enzymes, major nutrient pools, and diurnal production and respiration patterns. Each microcosm design has unique advantages and disadvantages, and there is not general agreement among ecologists or toxicologists as to the biological significance of certain responses. However, it seems clear that microcosms play an important intermediate role between surrogate species testing and the release of chemicals in ecosystems. Examples of the effects of toxicants on microbial communities in microcosms follow.

Zinc

Zinc is a heavy metal ubiquitous in waste streams. It is known to be bioaccumulated by algae. The water quality criteria for zinc are hardness dependent, but for an intermediate water hardness of 100 mg/l, the chronic zinc criterion for freshwaters is approximately 100 ug/l.[9] Effects of zinc on microbial communities and microcosms showed that species numbers responded quickly and significantly to zinc inputs resulting in significant depressions of the biota at zinc concentrations above about 90 ug/l (Fig. 5). Concurrent with the loss of species, both protein and

chlorophyll biomass decreased with zinc dose, with chlorophyll showing extremely high sensitivity to the zinc input (Fig. 6). In other words, algal biomass was severely depressed by zinc addition over ambient. The rate of alkaline phosphatase activity, a measure of the ability of the microbiota to recover phosphorous from organic compounds, was enhanced as phosphate pools in the test systems dropped (Fig. 7).

It is worth noting that most of the significant responses in the test systems occurred at zinc levels below current water quality criteria and that biomass responses were significant at levels more than one order of magnitude lower than the current standard (Table 2). Summaries of previous research have shown that microcosm responses are comparatively sensitive when compared to the responses of surrogate species.

Atrazine

Atrazine is not a priority pollutant whose environmental levels is regulated as are many compounds discharged from point sources. Atrazine enters the environment primarily from agricultural and horticultural uses because it is a commonly used herbicide. Atrazine comprises a significant proportion (greater than 10%) of the annual poundage of pesticide used in the United States. It is widely used as a pre-emergence herbicide. Related triazine herbicides find diverse uses from clearing rights of way to aquatic plant control. All of these herbicides are photosynthetic inhibitors affecting the Hill reaction and electron flow in photosystem II.

Our investigations of atrazine toxicity in microcosms showed unexpected patterns including the stimulation of biomass production and increases in species number (Fig. 7). At elevated doses of atrazine, alkaline phosphatase activity increased dramatically as phosphorus was lost from experimental systems (Fig. 8). Although biomass was stimulated, the production of oxygen in experimental systems did not increase, suggesting that although more chlorophyll was present, no additional primary production was taking place. Presumably, the elaboration of chlorophyll biomass was a response to the inhibition of photosynthesis by the added atrazine. Increases in

species richness can only be interpreted as indicative of the breakdown of normal control mechanisms in communities. Interestingly, these effects of atrazine occur at concentrations that have been measured in the field in areas where atrazine is widely used as an agricultural chemical.

These examples are not intended to show the superiority of a particular testing system, but demonstrate the potential for ecologically meaningful measures of complex interacting communities to be made under laboratory conditions using experimental designs similar to those used in surrogate species testing. Modeling the dose response of toxic chemicals is critical to conducting quantitative risk assessments, and it is now clear that ecological measures can be used to differentiate adverse responses from normal variability in communities. Ecological responses are not as variable as had once been anticipated assuming that adequate and experimental design, sampling, and analysis are exercised to improve detection power.

Expected Changes in Stressed Ecosystems

If microcosms are valid test systems, their responses should be congruent with predictions and observations of ecosystem response to stress. This congruence does not validate the microcosm approach to evaluating ecological toxicity, but does show that microcosms can respond in a manner that might be anticipated by observers of larger systems. Several recent papers have reflected on the types of changes that might be anticipated in stressed systems, although not all of these anticipated changes are readily observed.[23-25]

Species richness

Species are usually considered to be normally distributed (Fig. 9) in most communities.[26, 27] Chemicals affecting communities usually reduce the abundance and diversity of taxa. Occasionally, species numbers are stimulated and both effects result in a deviation from the nominal state, indicating adverse ecological effects.

Biomass

Toxic influences usually reduce standing crop, but stimulation is not unknown since resistant species or groups of species may be capable of exploiting stressed environments when normal competitive controls are released.

Primary production

The photosynthetic machinery of most ecosystems is sensitive to the abundance of photosynthetic individuals and the availability of nutrients. Like biomass changes, productivity may be depressed by toxic chemicals or enhanced by the removal of competitors. While primary productivity response to both nutrient enrichment and toxicity, primary production is usually limited by the availability of nutrients. In aquatic systems affected by toxicants primary productivity may respond more to nutrient limitation than to toxicity. For example in a stream that had fifty algal taxa whose primary productivity was limited by phosphate availability the effect of a toxicant that removed all but ten taxa but had no effect on the supply of phosphate might be observed to have no effect on production.

Productivity measures are further complicated by methodological limitations so that only relatively gross changes can be detected. Where biomass is low, radio tracer techniques may require relatively long incubation times to achieve detectable uptake of labeled compounds. The resulting measure of primary production often has a coefficient of variation as high as 100 percent. We have attempted to measure diurnal production and respiration patterns indirectly in microcosms using continuous measures of pH and have discovered that not only is production and respiration affected by toxic action, but the coupling of processes clearly decreases as toxicity increases.

Energy flow

As primary production is inhibited in ecosystems, there are increasing trends toward heterotrophy. In most stable ecosystems there is a balance between production and respiration. However, as toxicity depresses primary production, decomposer pathways can dominate.

Macronutrients

Ecosystems under stress commonly are unable to recover nutrients that are usually tightly cycled and the systems become "leaky" (e.g. Fig. 6). Similarly as systems shift from plant dominated (where potassium levels are high) to animal dominated or decomposer dominated systems pools of a variety of nutrients can shift.

Homeostasis

At the extremes of stress effects, systems are no longer able to compensate for small perturbations. The inability of systems to maintain structure and function under stress is simply the cumulative effect of all of the responses listed above: changing species richness, changing standing crop, changing productivity dynamics.

Unfortunately, ecosystem stress is widespread and ecological studies have not characterized a sufficient diversity of ecosystems for us to understand the normal operating range of most ecosystems. Additionally, it must be remembered that the development of every ecosystem is a unique, historical process and that although ecosystems share many common structures and functions, the diversity of even neighboring ecosystems is a result of numerous processes that are not understood. Regional approaches to understanding the normal structures in terms of species composition of ecosystems may provide some baseline against which to compare future changes or current conditions, but it is unlikely that we will have good information on the range of processes and the normal community structure at a variety of taxonomic and trophic levels for very many ecosystems unless there is a significant change in the research interests of funding organizations.

To a cynical mind, this ignorance is blissful because we have considerable difficulty detecting when ecosystem changes have occurred. Evidence now clearly points to the importance of understanding the community and population dynamics of small, rapidly reproducing, poorly dispersing species as indicators of ecosystem change. Interestingly, the biology and ecology of these species has been wontonly ignored by all but a small number of professionals so that the

expertise is not available to assess many ecosystems. Further, taxonomic skill is rapidly disappearing in the biological sciences so that our ability to monitor ecosystems by the species present is compromised. Even if we were to suddenly return to the biological survey as a way of evaluating ecosystem conditions, it would be difficult to identify many taxa since work on their systematic position, identity, tolerances, and distribution essentially stopped twenty years ago.

Summary and Conclusions

The single species bioassay remains the workhorse of environmental toxicology. Surrogate species are used to rank the relative hazards of chemicals in both acute and chronic exposures. Such standard assays when coupled with information about the environmental fate of chemicals is a useful first step for establishing standards for allowable environmental concentrations of potentially toxic materials. However, ecological information is increasingly needed both in sensing the condition of the environment and in checking the accuracy of predictions based on surrogate species testing. Surrogate species cannot be used to predict the kinds of ecological changes that might occur from the exposure of complex ecosystems to toxic materials even though there is some consistency in the innate biological machinery of all living organisms. Some toxic materials may be bioaccumulated and bioconcentrated and produce harmful effects that cannot be predicted from short term laboratory tests. Other chemicals may be relatively harmless in the environment because they may be rapidly metabolized or in other ways sequestered in the environment. Ecological toxicity testing can serve as an intermediate step between surrogate species testing and environmental release in ecological toxicity testing. Laboratory scale ecosystems are used to evaluate ecological responses in ecosystem-like setting. For conservative chemicals such as heavy metals or organic materials with long environmental persistence, ecological testing can serve as a necessary quality control step in standard setting. For non-conservative pollutants, ecological testing can reveal the degree to which toxicity can be ameliorated by ecosystem processes such that presumptions of hazard may be reduced.

At the present time, ecological testing is sufficiently well developed to be used in site specific cases or in cases where additional, ecologically meaningful information is needed to evaluate environmental effects of chemicals. However, considerably more study is needed to understand the relative vulnerability of differing ecosystems so that standards may vary regionally based on ecological characteristics rather than simple measures such as water hardness. The factors that influence ecosystem variability are not well understood, but ecological toxicity testing using natural communities could certainly unravel these problems. Additional work is needed to understand the natural variability of ecosystems so that ecologists and environmental scientists can distinguish between anthropogenic changes and natural oscillations in ecosystems. Despite the ability to measure a variety of biochemical changes in systems, the identities of component species continue to be important and failure to adequately characterize the taxonomic composition of ecosystems will result in poor detection power for scientists endeavoring to protect ecosystem structure and function.

ACKNOWLDGEMENT

This work was supported in part by grants from the Air Force Office of Scientific Research (AFOSR085-0324), the US Environmental Protection Agency (R-812813-01-0), and the US Army Biomedical Research and Development Laboratory (DAMD17-88-C-8068) and an award from the Air Force Office of Scientific Research and the Society of Environmental Toxicology and Chemistry. Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the granting agencies. The assistance of N.J. Bowers is gratefully acknowledged.

REFERENCES

1. Urban, D.J. and N.J. Cook. Hazard evaluation division standard evaluation procedure ecological risk assessment. U.S. EPA Report-540/9-85-001 (1986).

2. Vannote, R., G.W. Minshall, K.W. Cummings, J.R. Sedell, and C.E. Cushing. "The River Continuum Concept," Can. J. Fish. Aquat. Sci. 37:130-137 (1980).

3. Metcalfe, J. "Biological water quality assessment of running waters based on macroinvertebrate communities: history and present status in Europe," Environ. Pollut. 60:101-139 (1989).

4. Cairns, J. "Will the real ecotoxicologist please stand up," Env. Toxicol. Chem. 7:843-844 (1989).

5. Testing for Effects of Chemicals on Ecosystems, (Washington: National Academy Press, 1981) p. 103.

6. Moriarty, F. Ecotoxicology, the study of pollutants in ecosystems (London: Academic Press, 1989) p. 289.

7. Rand, G. and S. Petrocelli. Fundamentals of Aquatic Toxicology, Methods and Applications (Washington, Hemisphere Publishing Corp., 1984) p. 666.

8. "Ambient water quality criteria for zinc," U.S. EPA Report-440/5-80-079 (1980).

9. "Quality criteria for water," U.S. EPA Report-440/5-86-001 (1986).

10. Technical support document for water quality-based toxics control," Office of Water, U.S. EPA (1974) p. 74.

11. Webster, J.R. "Hierarchical organization of ecosystems," Theoretical Systems Ecology, A. Halfon, Ed. (New York: Academic Press, 1979) p. 119.

12. Travis, J. "Results of the survey of the membership of the Ecological Society of America: 1987-1988," Bull. Ecol. Soc. Am. 70:78-88 (1989).

13. Environmental Trends (Washington: Council of Environmental Quality, 1989) p. 152.

14. Turner, M.G., E. P. Odum, R. Costanza, and T. M. Springer. "Market and nonmarket values of the Georgia landscape," Environ. Manage. 12:209-217 (1988).

15. Giesy, J., Ed. Microcosms in Ecological Research. (Washington: Technical Information Service, U.S. Department of Energy, 1980) p. 1110.

16. Harrass, M.C. and P.G. Sayre. "Use of microcosm data for regulatory decisions," in Aquatic Toxicology and Hazard Assessment, 12th Volume, U.M. Cowgill and L.R. Williams, Eds., (Philadelphia: American Society for Testing and Materials, 1989) p. 204.

17. Sheehan, P.J. "Statistical and nonstatistical considerations in quantifying pollutant-induced changes in microcosms", in Aquatic Toxicology and Hazard Assessment, 12th Volume, U.M. Cowgill and L.R. Williams, Eds., (Philadelphia: American Society for testing and Materials, 1989) p. 178.

18. deNoyelles Jr., F. and W. D. Kettle. "Experimental ponds for evaluating bioassay predictions," in Validation and Predictability of Laboratory Models for Assessing the Fate and Effects of Contaminants in Aquatic Ecosystems, T.P. Boyle, Ed. (Philadelphia: American Society for Testing and Materials, 1985) p. 91.

19. Touart, L. "Simulated aquatic ecosystems to support pesticide registrations," U.S. EPA Draft Report (1986) p. 18.

20. Taub, F.B., A.C. Kindig, and L.L. Conquest. "Preliminary results of interlaboratory testing on a standardized aquatic microcosm protocol," in Community Toxicity Testing, J. Cairns, Jr., Ed. (Philadelphia: American Society for Testing and Materials, 1986) p. 158.

21. Giddings, J.M. and P. Franco. "Calibration of laboratory bioassays with results from microcosms and ponds," in Validation and Predictability of Laboratory Models for Assessing the Fate and Effects of Contaminants in Aquatic Ecosystems, T.P. Boyle, Ed. (Philadelphia: American Society for Testing and Materials, 1985) p. 104.

22. Pratt, J.R. and N.J. Bowers. "A microcosm procedure for estimating ecological effects of chemicals and mixtures", Tox. Assess. 5:189-205 (1990).

23. Odum, E.P. "Trends expected in stressed ecosystems," BioScience 35:419-422 (1985).

24. Schindler, D.W. "Detecting ecosystem responses to anthropogenic stress," Can. J. Fish. Aquat. Sci. 44(Suppl. 1):6-25 (1987).

25. Schaeffer, D.J., E.E. Herricks, and H.W. Kerster. "Ecosystem Health I. Measuring ecosystem health," Environ. Manage. 12:445-455 (1988).

26. Preston, H. "The commonness, and rarity, of species," Ecology 29:254-283 (1948).

27. Patrick, R. "The effect of invasion rate, species pool, and size of area on the structure of the diatom community," Proc. Acad. Nat. Sci. (Phila.) 58:335-342 (1967).

Table 1. Representative standard test organisms used to assess toxicity.

Freshwater	Marine
Fish	Fish
Fathead minnow	Sheepshead minnow
Bluegill sunfish	Mummichog
Rainbow trout	Silverside
Invertebrates	Invertebrates
Daphnids (water fleas)	Grass shrimp
	Mysid shrimp
Algae	Algae
Selenastrum	Skeletonema

Table 2. Summary of zinc toxicity in naturally derived microcosms. Table values are μg Zn/L. The chronic value (ChV) is the geometric mean of the lowest observable effect concentration (LOEC) and the no observable effect concentration (NOEC) determined by comparisons to controls. The EPA water quality criterion is shown for water hardness of 65 mg $CaCO_3$/L, the water hardness of the experimental systems. The NOEC could not be calculated when all responses differed from controls.

Variable	NOEC	LOEC	ChV
Species richness	89.2	280	158
Dry weight	---	4.2	---
Chlorophyll *a*	4.2	10.7	6.7
Dissolved oxygen	---	4.2	---
Alkaline phosphatase activity	29.8	89.2	51.6
EPA criterion			73.6

Figure Legends

Fig. 1. Hypothetical distribution of sensitivities to a toxicant. Arrows denote the assumed relative sensitivities of common toxicity test species.

Fig. 2. The biological hierarchy. Each higher level is assumed to consist of several components from the next lower level.

Fig. 3. Changes in levels of contaminants in lake trout from the Great Lakes. LO=Lake Ontario, LH=Lake Huron. (Redrawn from ref. 13).

Fig. 4. Changes in domestic fish landings. (Redrawn from ref. 13).

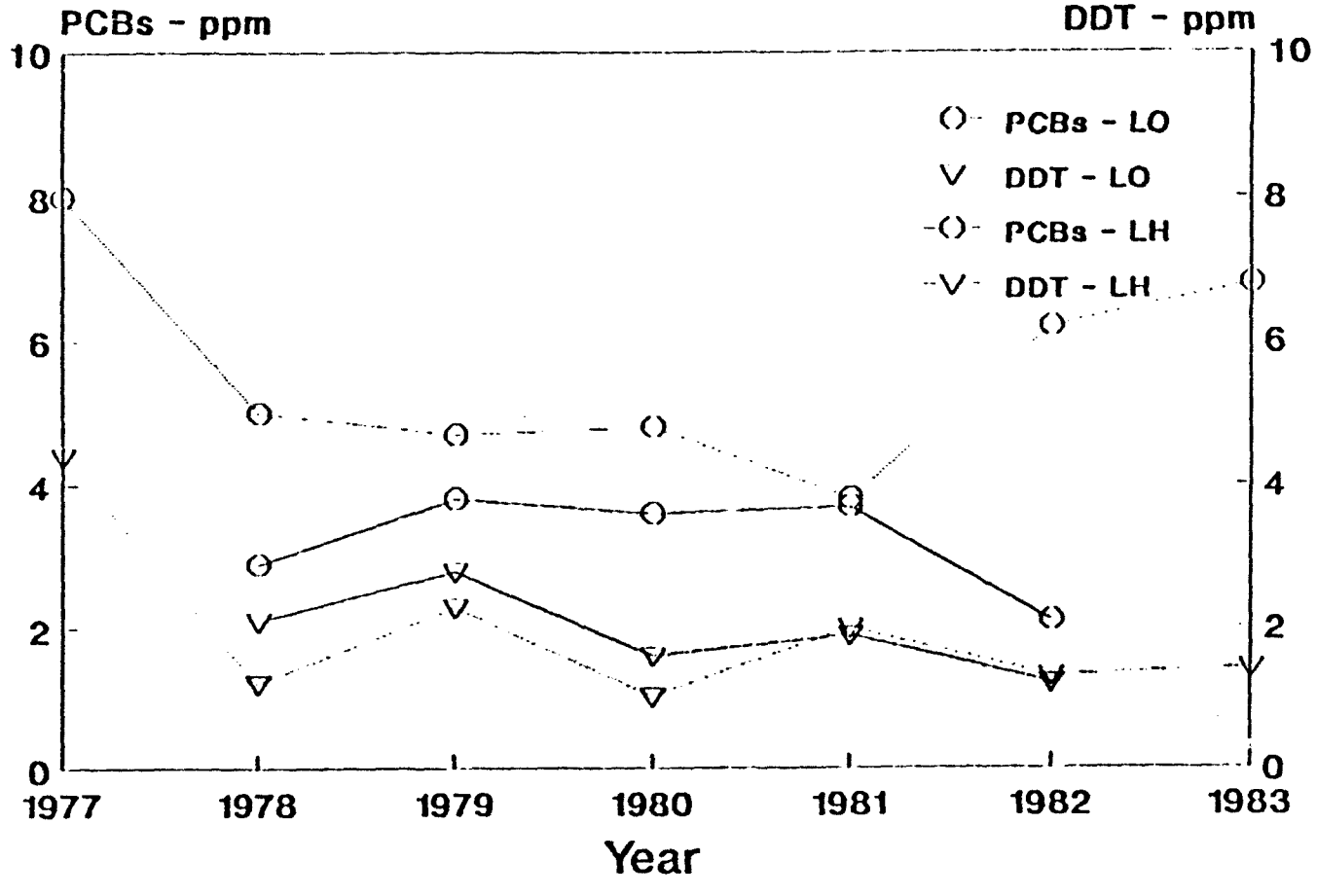
Fig. 5. Effect of added zinc on community structure of laboratory microcosms. Species richness data are for protozoa. Dry weight units are ug/ml. Chlorophyll was measured as fluorometric units. Controls had , 2 ug Zn/L but are plotted as 2 ug/L. Points are means of triplicates.

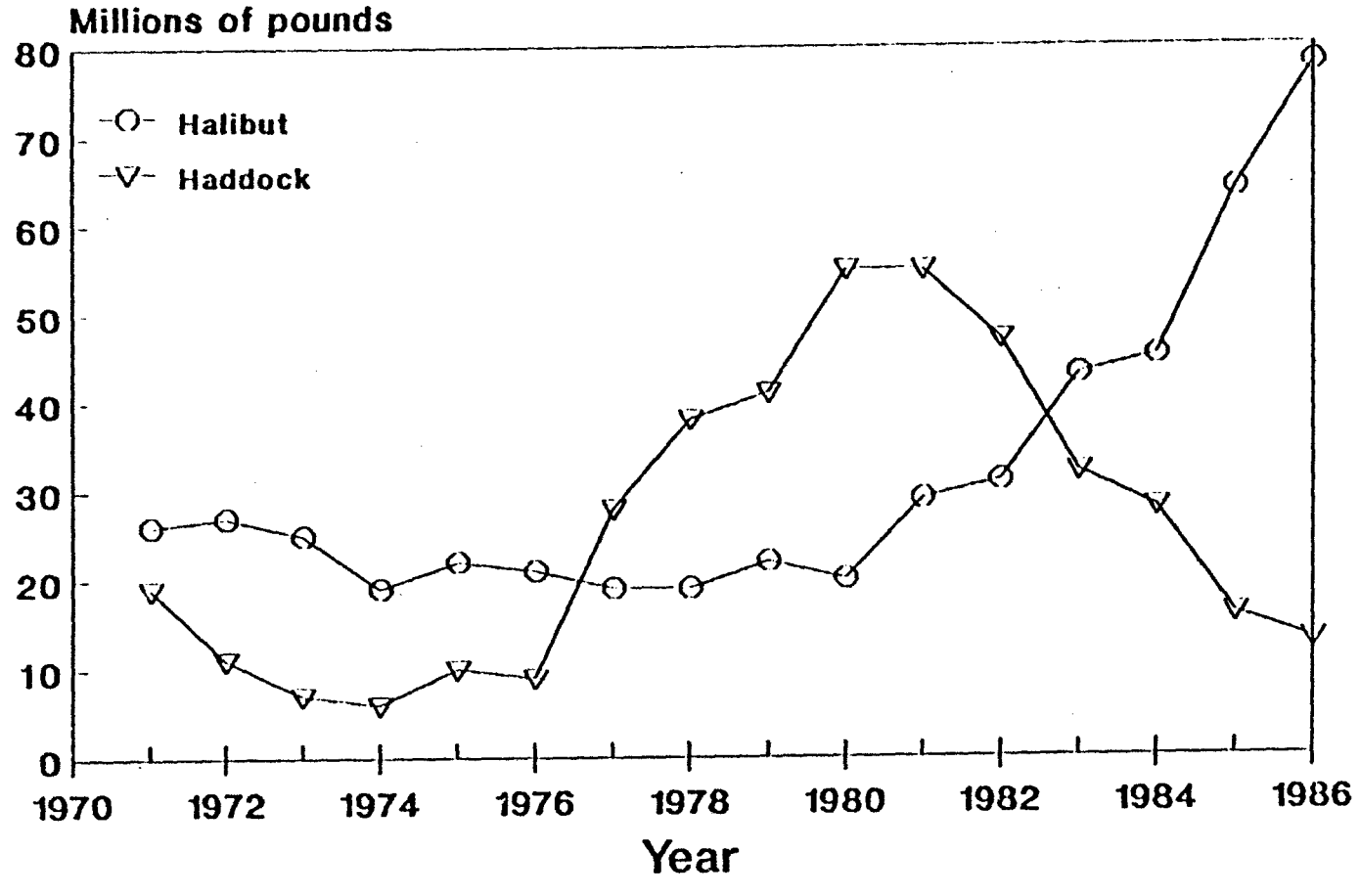
Fig. 6. Effect of added zinc on nutrient cycling in laboratory microcosms. Units for alkaline phosphatase activity (APA) are nmoles p-nitrophenol/mg protein/hr. Controls had < 2 ug Zn/L but are plotted as 2 ug/L.

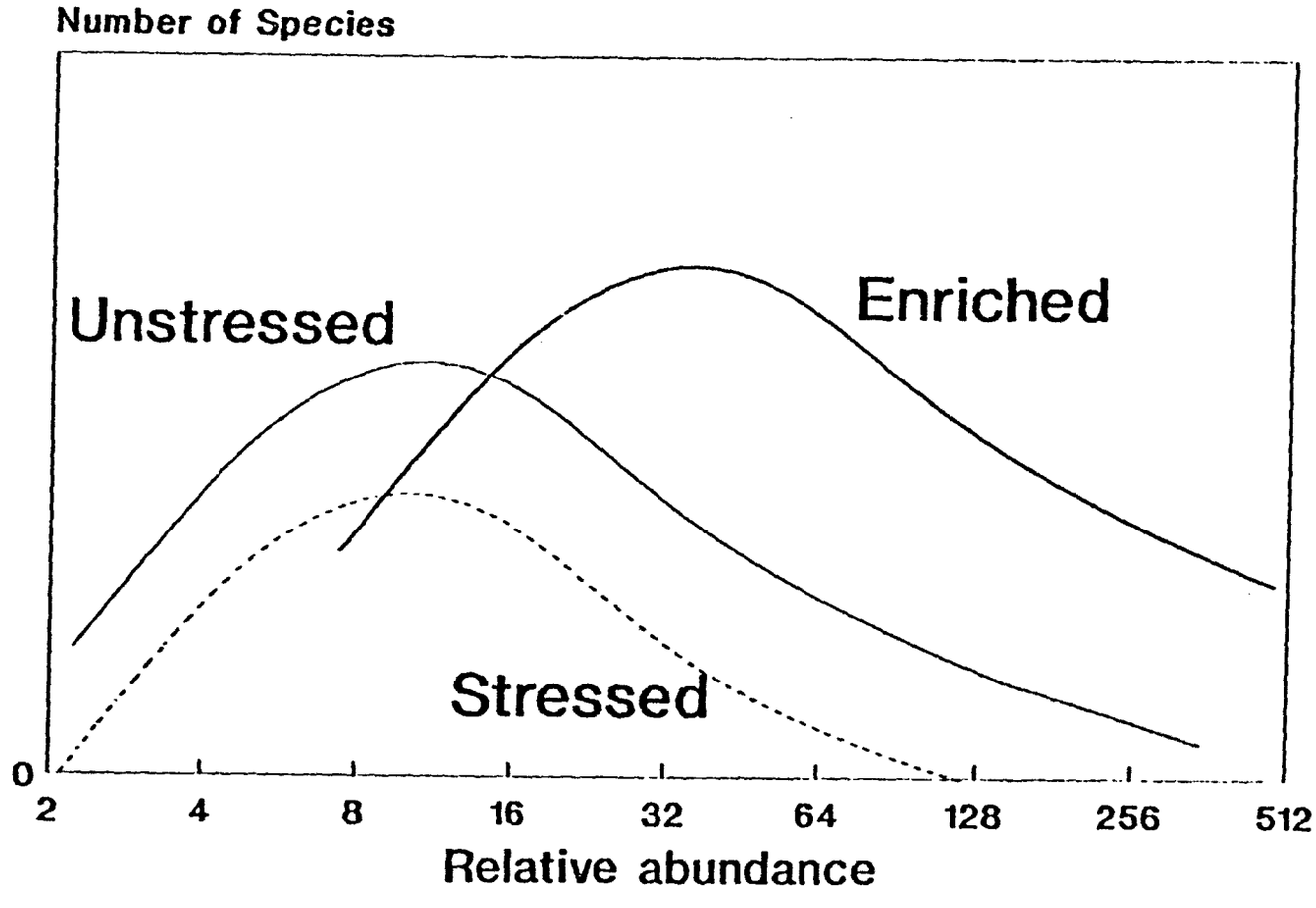
Fig. 7. Effect of atrazine on community structure of laboratory microcosms. Species richness data are for protozoa. Biomass data are total protein (ug/ml). Controls are plotted as 1 ug atrazine/L.

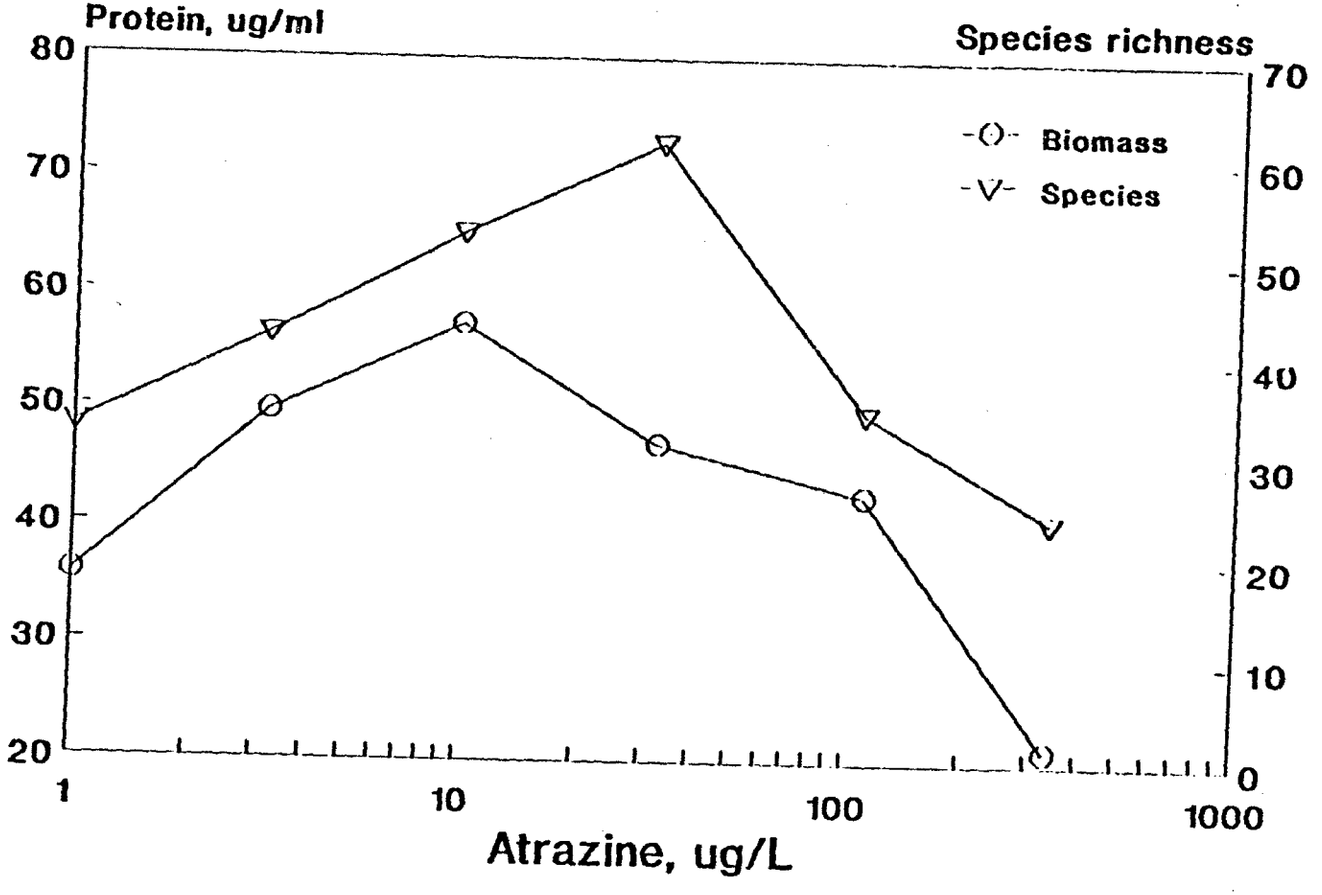
Fig. 8. Effect of atrazine on chlorophyll biomass and mid-morning dissolved oxygen levels in laboratory microcosms. Chlorophyll units are ug chlorophyll a/L. Dissolved oxygen units are mg/L. Controls are plotted as 1 ug atrazine/L.

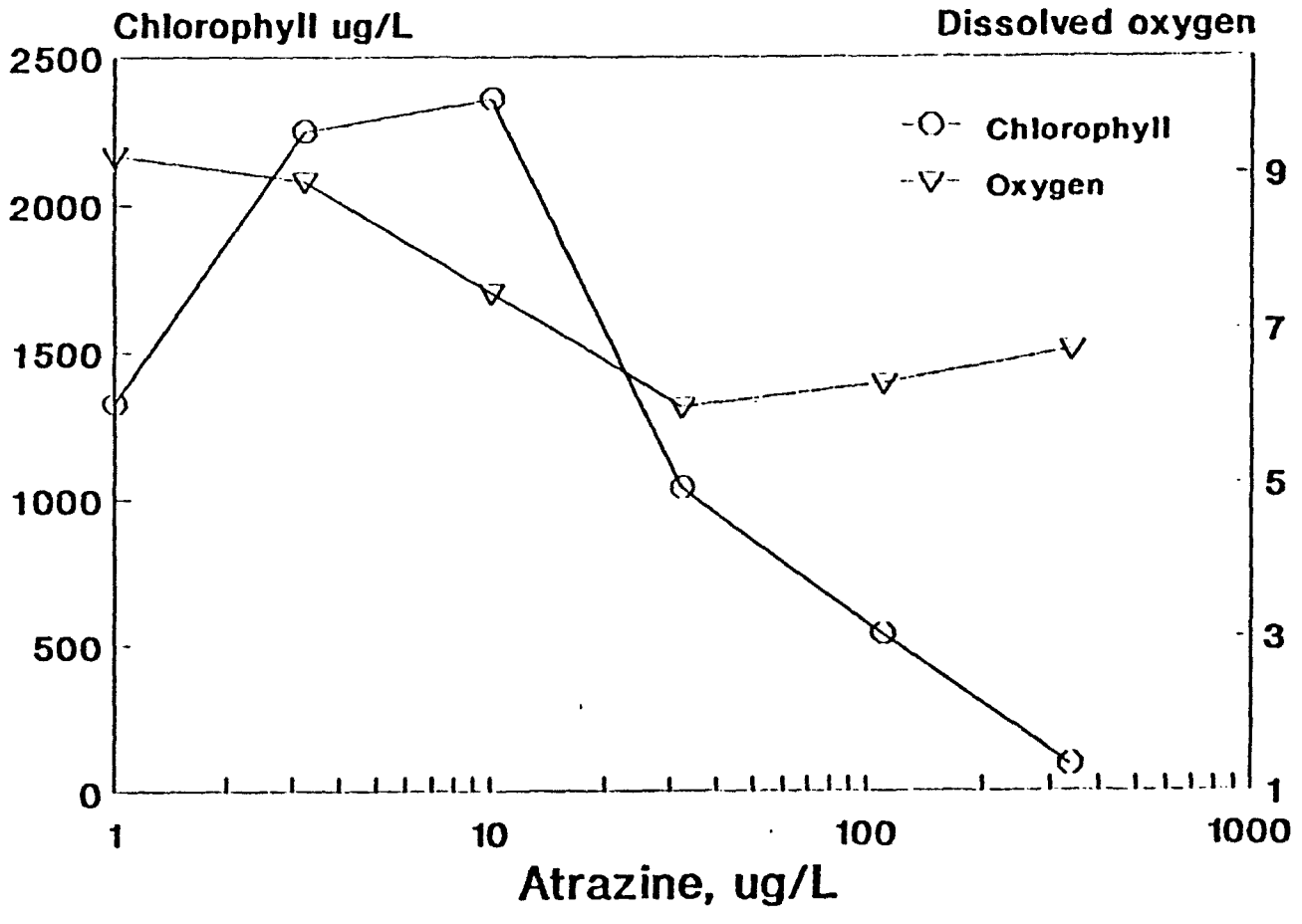
Fig. 9. Changes in relative abundance and numbers of species in communities under stress. Enriched communities often show large population sizes of insensitive species. Stressed communities show depressed population sizes and species richness when compared to unstressed reference communities (after ref. 26, 27).

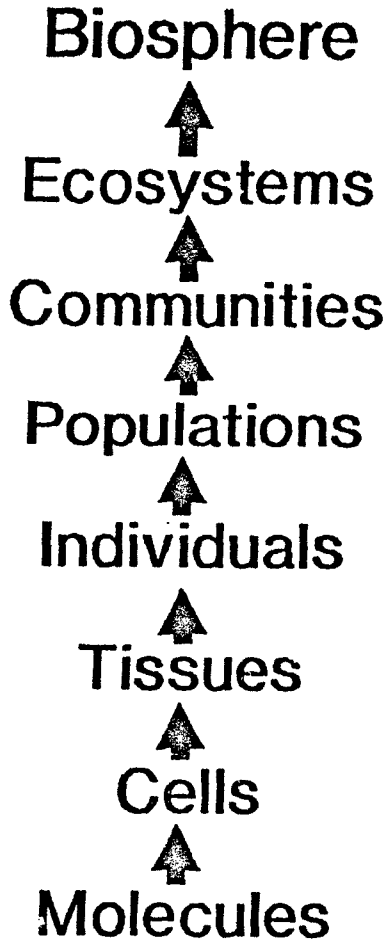

PCBs - ppm
DDT - ppm
10
8
6
4
2
0
PCBs - LO
DDT - LO
PCBs - LH
DDT - LH
1977
1978
1979
1980
1981
1982
1983
Year

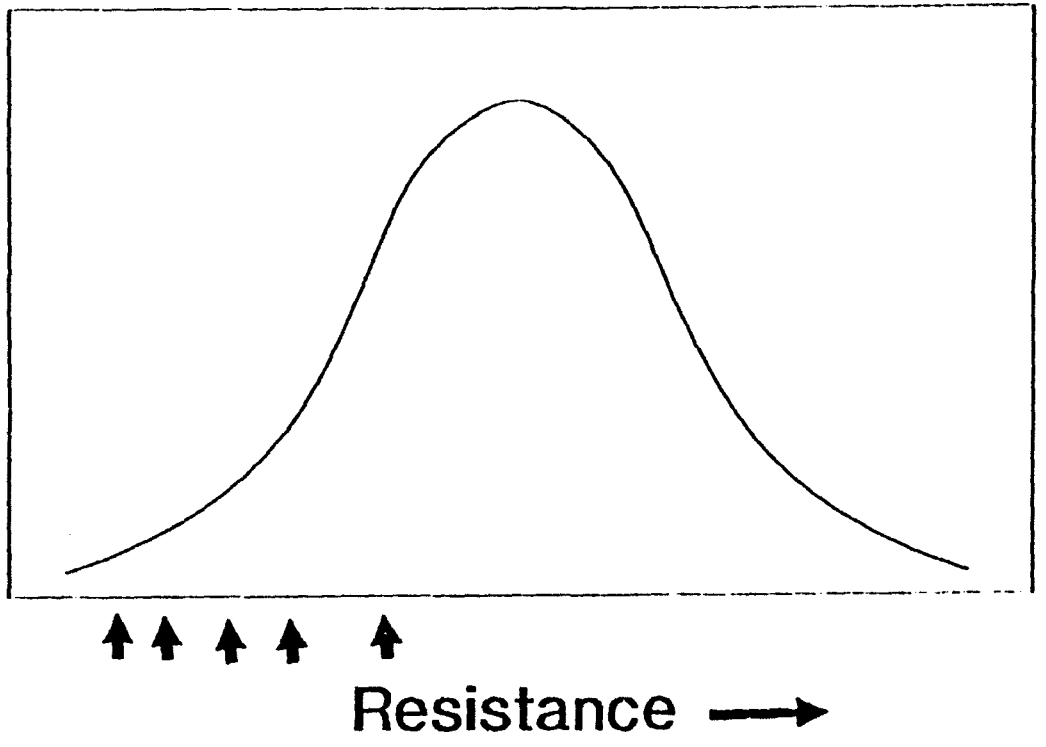

Millions of pounds
80
70
60
50
40
30
20
10
0
Halibut
Haddock
1970
1972
1974
1976
1978
1980
1982
1984
1986
Year

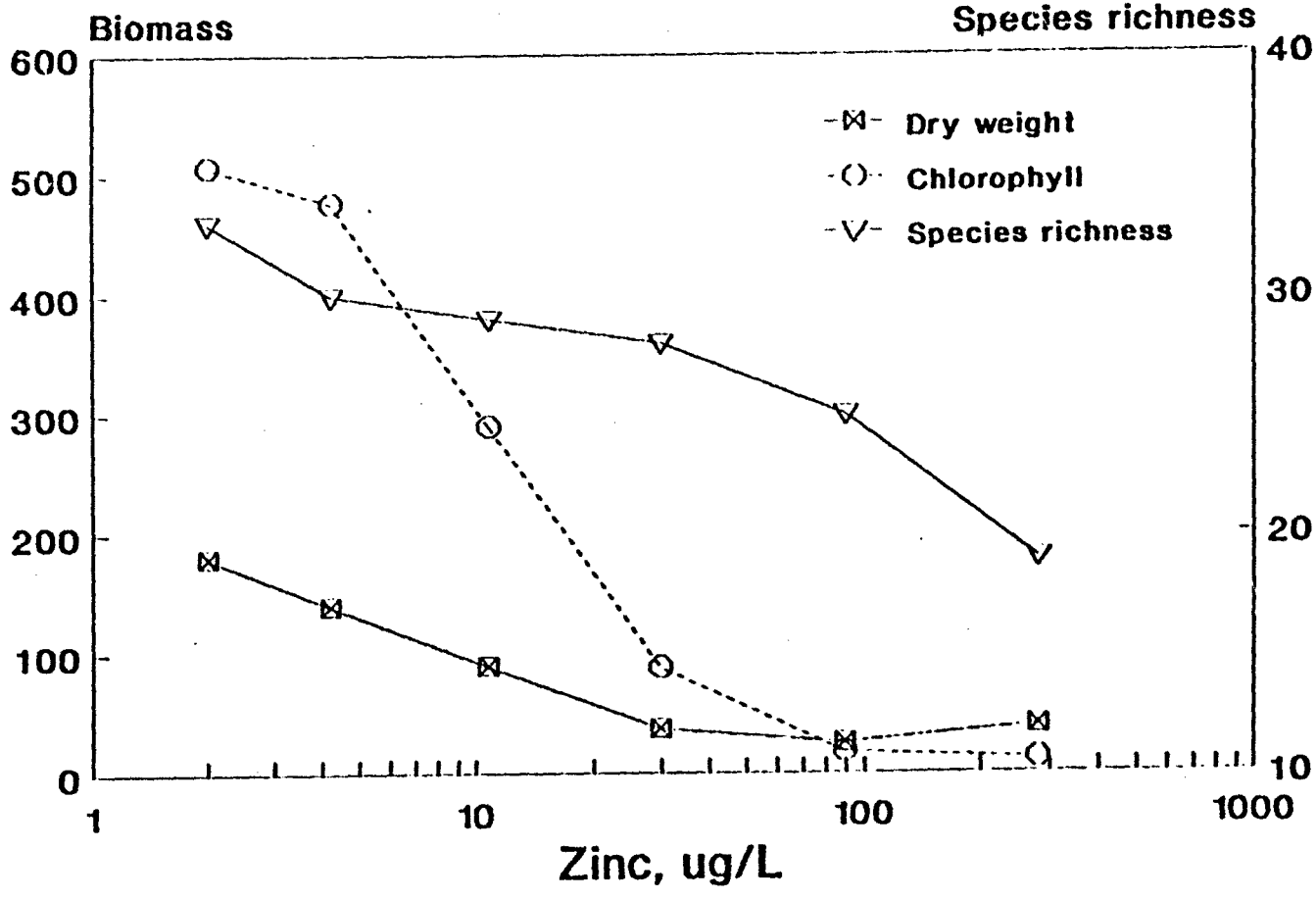

Number of Species
Unstressed
Enriched
Stressed
0
2
4
8
16
32
64
128
256
512
Relative abundance

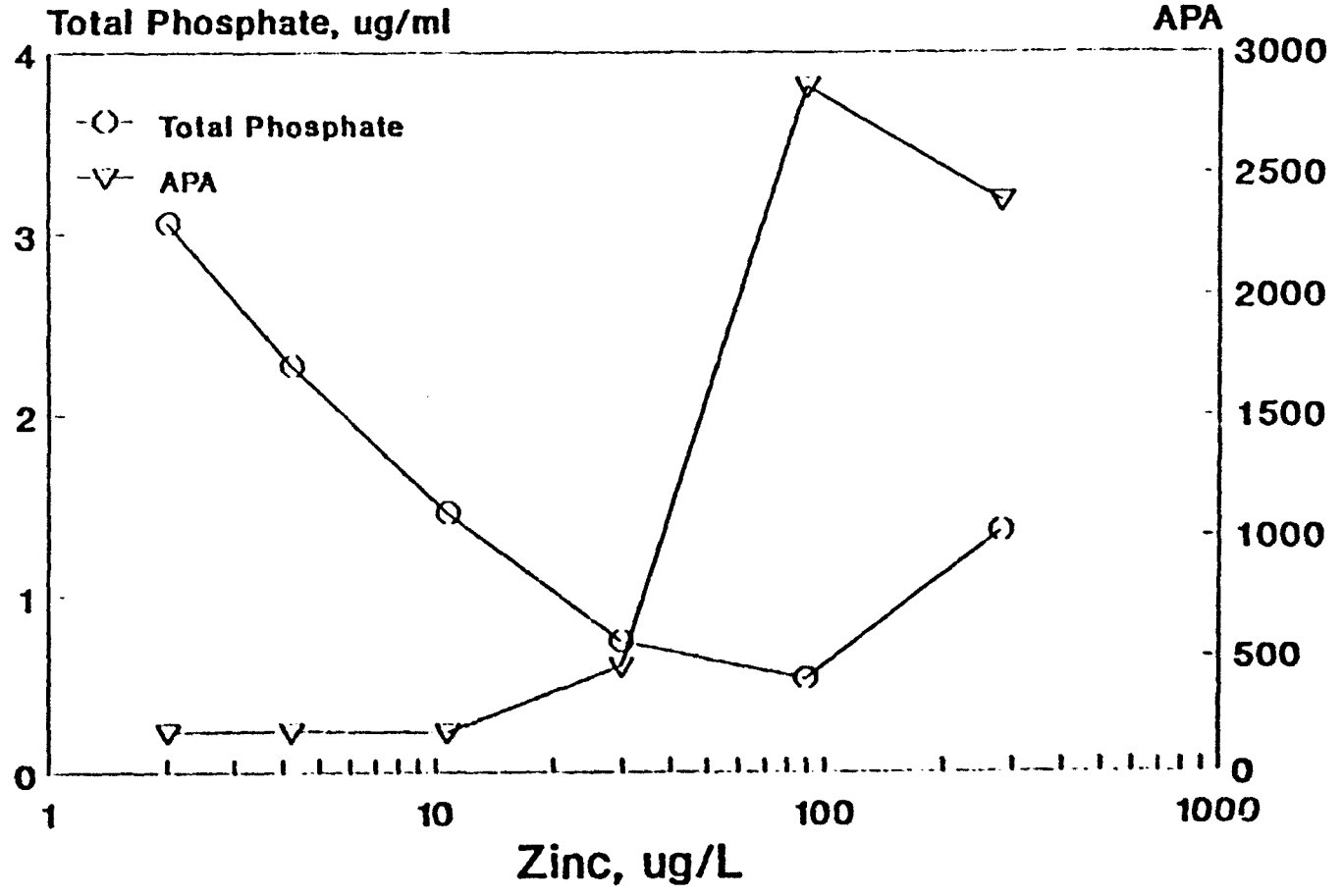

Protein, ug/ml
Species richness
80
70
60
50
40
30
20
70
60
50
40
30
20
10
0
Biomass
Species
1
10
100
1000
Atrazine, ug/L


Chlorophyll ug/L
Dissolved oxygen
2500
2000
1500
1000
500
0
9
7
5
3
1
Chlorophyll
Oxygen
1
10
100
1000
Atrazine, ug/L


Biosphere
Ecosystems
Communities
Populations
Individuals
Tissues
Cells
Molecules


Resistance


Biomass
Species richness
600
500
400
300
200
100
0
40
30
20
10
Dry weight
Chlorophyll
Species richness
1
10
100
1000
Zinc, ug/L


Total Phosphate, ug/ml
APA
Total Phosphate
APA
4
3
2
1
0
3000
2500
2000
1500
1000
500
0
1
10
100
1000
Zinc, ug/L

A microcosm using natural microbial communities: comparative ecotoxicology

J.R. Pratt, N.J. Bowers, J.M. Balczon
School of Forest Resources and Graduate Program in Ecology
Penn State University
University Park, PA 16802

First Symposium on
Environmental Toxicology and Risk Assessment

Running head:

PRATT ET AL. ON MICROCOSM COMPARATIVE ECOTOXICOLOGY

Correspondence: Dr. J.R. Pratt
213 Ferguson Bldg.
Penn State University
University Park, PA 16802
814/865-6942
FAX 814/865-3725

ABSTRACT: Natural microbial communities are species rich assemblages which display ecosystem properties in the laboratory. Chemical perturbations are expected to alter species richness, reduce standing crop biomass, affect nutrient pools, and alter community primary production and respiration. Experiments using microcosms developed from natural microbial communities on artificial substrata and continuously dosed toxicants (including heavy metals, simple organics, and pesticides) and complex mixtures (effluents) showed that microcosm variability was sufficiently low to detect adverse effects on species richness, standing crop biomass, and measures of ecological function, but critical toxicant concentrations often differed from expectations based on single species testing. Experiments assessing heavy metal toxicity, including effluent mixtures with heavy metals, showed adverse effects at toxicant levels at or below current water quality criteria. For example, copper reduced species richness and biomass at 10-20 ug/L in microcosms; zinc reduced chlorophyll biomass at <10 ug/L. Microcosm responses to toxicants differed both quantitatively and qualitatively from responses of standard test species and showed that compensatory mechanisms in complex assemblages sometimes prevented the expression of toxic effects, especially when non-conservative toxicants affected communities. Zinc toxicity elevated microbial recovery of organic phosphate. Low levels of atrazine (<100 ug/L) stimulated species richness and standing crop biomass, but oxygen production did not increase. Chlorpyrifos did not affect

microcosms even when concentrations exceeded water quality criteria by three orders of magnitude. TNT only affected microcosms at >400 ug/L although provisional water quality criteria predicted chronic effects at 40 ug/L. Microcosms were sensitive to chlorine in the laboratory (6 μg/L) but were insensitive to a nutrient rich, chlorinated effluent (>400 μg/L) which was acutely toxic to daphnids. Reference toxicant experiments showed that effects on naturally derived microcosms are repeatable within and between ecosystems. Differences between microcosm responses and expected toxicity based on standard bioassays are a result of complex interactions including toxicant degradation, the interaction of toxicants and nutrients, and the lack of species sensitive to some toxicants, and these interactions allow microcosms to display a range of ecological responses not predictable from single species tests.

Keywords: microcosm, aquatic toxicology, risk assessment, hazard evaluation

INTRODUCTION

For many years, toxicologists, ecotoxicologists, and ecologists have debated the adequacy of basing predictions of chemical safety and harm on short-term tests conducted with a small number of test species [1-3]. The surrogate species approach is widely used in human toxicology where many animal models are used as surrogates for one, untestable species. The same approach, when applied to ecosystems, requires a significantly greater number of assumptions because several hundred to several thousand species are in need of protection.

A fundamental, usually unstated, assumption in aquatic toxicology is that the few "surrogates" are sufficiently sensitive representatives of other species. This assumption has been validated by numerous reviews showing that species such as fathead minnows, blue gill sunfish, and rainbow trout respond at lower toxicant doses than many other fishes. Evaluation of the sensitivity of cladocerans (daphnids) has shown similar results, although the relationship between fish and invertebrate sensitivity is variable. Toxicants affect the cellular machinery, and it would be surprising if toxicants with a particular mode of action did not affect many species. Based on this assumption of sensitivity to toxicants, a few sensitive species could be tested and environmental limits established based on a few test results; protecting one fish species should protect many.

An alternative approach based on a holistic or hierarchical

view of systems assumes that ecosystems have important properties and characteristics that cannot be predicted (or protected) by studying lower levels in the hierarchy [4]. Some properties of complex systems are collective, the sum of the properties of components. Properties such as energy flow, standing crop biomass, or species diversity result from the entire collection of parts. Other properties, emergent properties, result from interactions among individuals and species and are not predictable from the properties of the interacting parts [5]. For example, the results of interference competition, the preference of predators for prey, or the activities of microbial consortia may not be predictable by independently studying the structure and activity of system components. With this view of ecosystems, uncertainties exist about the action of toxic chemicals that can only be answered by studying a multispecies biological system with ecosystem properties.

A number of laboratory-scale aquatic ecosystems have been designed, and many have been used for studying the effects of chemical stressors [6]. These systems range from those assembled from cultures of individual species [7] to laboratory mul'_species cultures [8] to portions of natural ecosystems maintained in the laboratory [9, 10]. These experimental systems have been used to explicitly or implicitly test the hypothesis that systems are more sensitive indicators of ecological stress than individual taxa.

A useful microcosm testing system should have the following

qualities.

1. Respond at environmentally realistic levels of contaminants expected to produce adverse effects in ecosystems.

2. Display qualitatively or quantitatively different responses from those measurable in surrogate species testing.

3. Display repeatable responses to the same [reference] toxicant when conducted using standard procedures.

4. Have sufficiently low variability to achieve acceptable statistical detection power.

In this report, we examine the responses of a naturally derived laboratory microcosm system to chemical stresses and compare these responses to results from standard single species tests and other field and laboratory investigations. We address the question of sensitivity of response from two aspects, the statistical power of endpoint variables and the level of toxicant producing adverse effects.

METHODS

The experimental methods for testing the effects of chemicals and mixtures in laboratory microcosm have been described in detail elsewhere [10] and are summarized here. The artificial substrate-microcosm (AS-M) method was designed to test the responses of surface-dwelling microbial communities to challenges by toxic materials. Microbial communities were

collected on polyurethane foam (PF) artificial substrata at a reference site in a natural ecosystems. Several different reference sites have been used in the reported experiments. The naturally derived communities on PF substrates were returned to the laboratory and placed in small (4-7 L) microcosms filled with dechlorinated tap water. Experiments used triplicate microcosms dosed at six levels (including controls). For tests of complex effluents, upstream water from the stream receiving the effluent was used for effluent dilution. The naturally derived communities served as species sources for the colonization of additional PF substrata which served as sampling devices over the 21 d course of experiments.

Toxic materials were supplied to the microcosms continuously from a proportional dilution system or from individual concentrated toxicant stock solutions. Depending on microcosm size (4-7 L) and flow rate (20-60 ml/min), there were 7-12 volume turnovers per day. For tests of pure compounds, toxicant concentrations were confirmed at least weekly and results were expressed using the mean measured toxicant concentration.

The variables measured in experiments were intended to characterize changes in species richness and composition, community biomass, and the dynamic performance of communities. Species richness was estimated by microscopic examination of protists (protozoa and algae) collected from the PF substrates. Substrate collections were also used for determination of total biomass (protein, adenosine triphosphate [ATP], or dry weight)

and chlorophyll a biomass. When biomass was sufficient to obtain additional measures, hexosamine and carbohydrate content were determined. The ability of communities to process organically bound phosphate was used to assess nutrient cycling ability and was measured as the activity of alkaline phosphatase enzymes. The macronutrient content of collected communities was determined by measuring total phosphate, calcium, magnesium, and potassium. Diurnal changes in dissolved oxygen and pH were measured to estimate net primary production and community respiration. For effluent experiments, effluent toxicity was also estimated from short-term chronic tests [11] using daphnids.

Weekly data were analyzed using analysis of variance followed by multiple comparisons to locate treatment differences. although only results from samples taken after either 7 or 21 d of exposure are reported here. The relationship between toxicant dose and response was also examined using ordinary least squares regression of the response on the logarithm of dose. The lowest observable effect concentration (LOEC) was defined as the lowest toxicant concentration producing a response significantly different from controls ($p<0.05$). Similarly, a no observable effect concentration (NOEC) was defined as the highest toxicant concentration producing responses which could not be differentiated from controls. A maximum allowable toxicant concentration (MATC) or chronic value (ChV) was defined as the geometric mean of the NOEC and LOEC.

RESULTS AND DISCUSSION

Response levels

For microcosm tests to be useful predictors of community and ecosystem effects, variables measured should be affected at toxicant levels similar to those derived for single species tests, and should be affected near concentrations expected to be encountered in stressed ecosystems. The results reported below are for a limited array of organic and inorganic compounds and complex effluents. Comparisons of the levels of toxicant producing the most sensitive adverse response, the reported effective concentrations in single species experiments, and numerical water quality criteria were made.

Inorganics

AS-M tests of five different inorganic compounds are summarized in Table 1. The reported MATCs from AS-M experiments agree with literature values from single-species tests. This is not surprising because these toxicants are generally broad spectrum biocides with multiple modes of action. Most of the MATCs from microcosm experiments are at or below the water quality criteria for the respective chemical. Particularly striking is the effect of zinc on biomass and function at levels two orders of magnitude lower than water quality criteria. This demonstrates the sensitivity of the microcosm system, and emphasizes the possibility that single-species tests may be inadequate for the protection of the entire ecosystem [1, 2].

Structural and functional measures of the microbial

community tend to be equally affected by these inorganic toxicants, although information on functional measures is limited. In general, measured variables were affected at concentrations in the lower portion of the range reported from single species tests.

Organics

The results from tests using organic compounds are more ambiguous and variable than those for inorganic chemicals (Table 2). This may be due to the selective modes of action of these compounds, the absence of toxicant targets in the microcosms, or the degradation of toxicant by components of the microbial community. The MATCs reported from the microcosm tests are within the range of reported literature values from single-species tests, but water quality criteria for these chemicals are limited by the relatively small amount of available data.

Structural variables were adversely affected at lower concentrations than the functional variables. Four of the seven experiments had lower MATCs for a structural measure than for functional variables. These data lend support to the contention that structural effects will be detected before a functional change [29, 30, 31]. Ecosystems have many functionally equivalent organisms; that is, there are many organisms in an ecosystem which perform the same function, and elimination of some sensitive taxa may not result in a detectable adverse effect due to compensation by the functional equivalents. Additionally, functional changes should only be affected when the supply of

chemical substrates changes [32].

There are some unusual results from these experiments. First, species richness and biomass were stimulated at concentrations of atrazine an order of magnitude lower than literature values for chronic toxicity to animals. This stimulatory response, though different from the traditional inhibitory response, could be an indirect toxic effect on certain "keystone" or controlling species, whose elimination from the community results in the elaboration of previously rare species. Second, there were no detectable effects of chlorpyrifos. Chlorpyrifos is a highly toxic substance, as evidenced by the low water quality criterion, but its mode of action is specific. Chlorpyrifos is an acetylcholinesterase inhibitor, and the majority of organisms tested in these microcosms lack any neural function. Therefore, one must consider the mode of action of the toxicant to be tested, and realize the limitations of the microcosm system before drawing any conclusions (which in this case would be erroneous) about the toxicity of a substance.

Effluents

Four complex effluents were studied with the AS-M: a foundry effluent containing zinc and ammonia, a brass mill effluent contain copper and zinc, a sewage treatment effluent containing chlorination products and nutrients, and a fly ash basin effluent containing several heavy metals. The microcosm experiments were compared to results obtained in single-species tests. Effects were observed at or below the levels at which effects were

observed in single-species chronic tests (Table 3). Adverse effects were also observed at effluent levels below the estimated instream waste concentration (IWC, the ratio of effluent flow to stream and effluent flow, as a percent). The lowest adverse effects were detected in the metal containing effluents from the brass mill and foundry. The sewage treatment effluent was apparently non-toxic, probably due to the interfering effects of the high nutrient levels in the effluent.

These experiments indicate that variables measured in the AS-M system respond at environmentally realistic levels of chemicals and mixtures expected to result in adverse effects on ecosystems. Both structural and functional variables can be useful in predicting adverse effects, although effects are dependent on the mode of action of the toxicant tested. Some substances may result in stimulatory responses, which may be considered an adverse effect on "keystone" organisms that is expressed indirectly. No one variable was always sensitive to toxicant action, although structural measures (both species richness and biomass measures) were typically more sensitive than functional measures of adverse effects

Qualitative response differences

Measurement of adverse effects in typical single species tests used for regulatory purposes focuses on reduced survival in acute tests and on reduced growth or reproduction in short-term chronic tests. Commonly, increasing dose results in a decrease in the response variable so that the dose-response relationship

is usually log-linear [33]. While stimulation at low doses (i.e., hormesis) can be observed it is not common.

The variables measured in microcosms show greater potential response ranges than single species tests, including stimulation as well as expected monotonic dose-response patterns. In short-term chronic tests, effects are measured on only one species. In microcosm experiments, the total number of species in the artificial ecosystem typically declines in response to toxicity (Figs. 1, 2). Microcosm experiments limit the potential recolonization and recovery of the experimental systems, but a comparison of Figs. 1 and 2 shows that the level of toxic material eliminating 20% of the microcosm taxa is approximately the same as water quality criteria (see Table 1). This suggests not only that criteria may not be protective, but demonstrates a qualitative difference in response types. Elimination of taxa (local extinction) corresponds to levels of chronic (sub-lethal) responses of laboratory populations.

Community and ecosystem responses need not be monotonically negative with increasing toxicant dose. For example, the effect of atrazine on AS-M communities resulted in increased biomass at intermediate toxicant levels (Fig. 3), although higher levels resulted in an eventually negative response to the toxicant. This unexpected resposne (termed the subsidy-stress gradient, [34]), further demonstrates that adverse effects may not always result in a reduction of response. As previously mentioned, selective toxicants such as atrazine (a photosynthetic inhibitor)

may affect controlling factors in communities, releasing some species to proliferate. It would be incorrect to categorize such a response as "enhancement" because it is difficult to understand how photosynthetic inhibition could be a positive influence on natural communities.

In addition to apparent subsidy effects, toxicant can sometimes affect community responses in a postive, dose-related manner. For example, inhibition of communities by zinc (Fig. 4) resulted in reduced total phosphate and increased activity of phosphorus recovering enzymes. The geometric increase in alkaline phosphatase activity (APA) is clearly a dose-related adverse effect of the toxicant, but such responses are not incorporated into current risk (or hazard) assessment methods.

Microcosms display qualitatively and quantitatively different responses than those of single species toxicity tests. These responses include changes in community composition and collective measures such as standing crop biomass. Functional responses, such as enzyme activities, may not fit neatly into expection of adverse effects as reduced community performance.

Repeatability of results

If microcosm methods are to be useful in assessing toxicity, then procedures must be standardized to achieve repeatable results. The AS-Microcosm protocol was evaluated for repeatability using copper as a reference toxicant. Nine experiments were conducted at four different locations: Pennsylvania, Michigan, Virginia, and Maryland (Table 4) using

community sources from local ecosystems. The source ecosystems were, respectively, Spring Creek (Centre County, PA), Douglas Lake (Cheboygan County, MI), Pandapas Pond (Montgomery County, VA), and a spring-fed pond in Frederick County (MD). The water quality criteria for dilution water ranged from 8-22 µg/L Cu at the water hardness at which experiments were conducted. For nearly all tests, adverse effects were observed at or below the water quality criteria. Effect levels are all similar with the exception of the third experiment using Douglas Lake microbial communities. The second and third Douglas Lake experiments were conducted using at Penn State University using a different dilution water, and this may have contributed to high variability in effect levels. The first experiment was done at Douglas Lake using Douglas Lake dilution water, and effect levels were in the range of the other experiments.

Effect levels are similar with classes of measures (species richness, biomass, community function) in all experiments. Biomass measures are more variable, and this may be due to seasonal differences. Results of experiments conducted using microbial communities derived from the same source ecosystem are similar, suggesting repeatability of results within an ecosystem. Interecosystem comparisons are less similar due to the second and third Douglas Lake experiments. However, results both within and among regions are quite similar with effect levels varying approximately an order of magnitude. This variability is similar to or less than that reported for single species acute tests of

many compounds [35] using methods that are simple and standardized. These experiments indicate that the AS-Microcosm test system is repeatable when standard procedures are followed. Similar results were obtained by Taub and colleagues [7, 36] when testing the Standard Aquatic Microcosm among several laboratories, although the concentrations tested were much higher (lowest copper concentration 300 μg/L).

Differences among ecosystems in response to toxic dose are more problematic. For example, the speciation of copper in these experiments clearly varied due to different water hardness and pH, but results do not vary greatly even though free cupric ion is probably not similar among experiments. In contrast, the relative sensitivity of communities from differing ecosystems is not understood, although it is widely assumed to be similar for purposes of regulation.

Measurement variability

Variables measured as microcosm responses to toxicants differ in their precision. Some measures have significant variability with both biological and procedural sources. For example, the measurement of chlorophyll *a* varies because replicate communities vary and because the methods for concentrating cells, extracting the pigment, and measuring the extract introduce additional error. Other variables can be measured with less error. For example, spectrophotometric determination of macronutrients such as calcium have low variability. Other measures may be discrete data, such as the

enumeration of species, also associated with low variability, so some measures will be naturally less variable than others.

The effect of measurement error, in a statistical sense, is a reduction in the power to detect differences among treatments. The importance of measurement error in ecotoxicology is the error that it may introduce into conclusions drawn from experiments. Adequate understanding of the effect of measurement error on the potential to detect effects is important in experimental design and interpretation of results [37, 38].

To assess the variability of measures from the AS-M experiments, the responses of control microcosms were summarized and the median coefficient of variation (CV, the ratio of the standard deviation to the mean) was determined. Using this estimate of the variability of a particular measure, the minimum detectable difference (MDD) was determined as the smallest percentage difference between control and treatment means that could be detected given the expected measurement variability and the experimental design [39]. The assumed design was six treatments of three replicates each. Using an alpha of 0.05 (the probability of a Type I error) and beta of 0.2 (the probability of a Type II statistical error), MDDs were determined for several measured variables (Table 5).

The CVs and MDDs reported in Table 5 are similar to the variability often observed in single species tests and are no greater than those predicted [36] as typical for ecosystem experiments. In fact, many measures have low variability and

detection power is high, so that differences of only 20% in means between toxicant treated microcosms and control microcosms can reveal significant differences, assuming variance is similar among treatments. Expectations that microcosm replicability is poor and large variability would confound interpretation of results are unfounded.

CONCLUSIONS

Ecological assessment of toxic chemicals requires estimating effects on complex ecological structures. If the goal of environmental protection is to conserve ecological diversity and ensure the continued integrity of ecosystems, then laboratory ecosytems (microcosms) can provide a rapid and sensitive means of evaluating the adequacy of conclusions drawn from traditional hazard assessments. Microcosms containing diverse communities display the predicted symptoms of ecosystem disease [40] in a manner that is both repeatable and sensitive to many stressors. However, microcosm experiments, like single species tests, are not globally sensitive to all stresses. Where microcosms lack appropriate target species for toxicants with specific modes of action, little effect can be detected. Toxicant effects are the result of complex interactions between the toxicant, the available biota, and abiotic factors resulting in responses that are not predictable from single species tests. Microcosms provide an opportunity to test hypotheses of environmental safety and harm in a manner that is rapid, sensitive, repeatable, and

capable of demonstrating unexpected, adverse ecological consequences of toxic materials.

Acknowlegements

Portions of this work were supported by the US Army Biomedical Research and Development Laboratory (BRDL) contract DAMD17-88-C-8068 and by previous research support from the US Air Force Office of Scientific Research and the Environmental Protection Agency. Opinions, conclusions, and recommendations are those of the authors and are not necessarily endorsed by these agencies. BRDL experiments were directed by W.H. van der Schalie and T.R. Shedd, and their assistance is grateful.y acknowledged. B.R. Niederlehner and J. Cairns, Jr. provided valuable assistance and advice.

REFERENCES

1. Cairns, J. Jr. Hydrobiologia, Vol. 100, 1984, pp. 47-57.
2. Kimball, K.D. and Levin, S.A. BioScience, Vol. 35, 1985, pp.165-177.
3. Mount, D.I. In Cairns, J., Jr., Ed., Multispecies Toxicity Testing, Pergamnon Press, New York, 1985, pp. 13-18.
4. Allen, T.F.H. and Starr, T.B. Hierarchy, University of Chicago Press, Chicago, 310 pp.
5. Salt, G. American Naturalist, Vol. 113, 1979, pp. 144-146.
6. Giesy, J.P., Ed. 1980. Microcosms in Ecological Research, Technical Information Center, US Department of Energy, Oak Ridge, 1110 pp.

7. Taub, F.B., Kindig, A.C. and Conquest, L.L. In J. Cairns, Jr., Ed., Community Toxicity Testing, ASTM STP 920, American Society for Testing and Materials, Philadelphia, 1986, pp. 93-120

8. Stay, F.S., Katko, A., Rohm, C.M., Fix, M.A., and Larsen, D.P. Archives of Environmental Contamination and Toxicology, Vol. 18, 1989, pp. 866-875.

9. Giddings, J. In Cairns, J., Jr., Ed., Community Toxicity Testing, ASTM STP 920, American Society for Testing and Materials, 1986, pp. 121-132.

10. Pratt, J.R. and Bowers, N.J. Toxicity Assessment, Vol. 5, 1990, pp. 189-205.

11. Weber, C. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, 2nd. edition, EPA 600/4-89-001, 1989.

12. US Environmental Protection Agency. Quality Criteria for Water, EPA 440/5-86-001 , 1986.

13. Niederlehner, B.R., Pratt, J.R., Buikema, A.L., Jr., and Cairns, J., Jr. Environmental Toxicology and Chemistry, Vol. 5, 1985, pp. 155-165.

14. Pratt, J.R., Niederlehner, B.R., Bowers, N.J., and Cairns, J., Jr. Environmental Toxicology and Chemistry, Vol. 7, 1988, pp. 679-687.

15. Pratt, J.R., Niederlehner, B.R., Bowers, N.J., and Cairns, J., Jr. Toxicity Assessment, Vol. 2, 1987, 417-436.

16. Pratt, J.R. and Bowers, N.J. Toxicity Assessment, Vol. 5, 1990, pp. 293-307.

17. Pratt, J.R., Niederlehner, B.R., Bowers, N.J., and Cairns, J., Jr. In Lindberg, S.E. and Hutchinson, T.S., Eds. International Conference on Heavy Metals in the Environment, Vol. 2, CEP Consultants Ltd., Edinburgh, 1986, pp. 324-326.

18. Hunn, J., Hamilton, S. and Buckler, D. Water Research, Vol. 21, 1987, pp. 233-238.

19. Reading, J., and Buikema, A., Jr. Archives of Environmental Contamination and Toxicology, Vol. 12, 1983, pp. 399-404.

20. Pratt, J.R., Bowers, N.J., Niederlehner, B.R., and Cairns, J., Jr. Archives of Environmental Contamination and Toxicology, Vol. 17, 1988, pp. 449-457.

21. Pratt, J.R., Bowers, N.J., Niederlehner, B.R., and Cairns, J., Jr. Toxicity Assessment, Vol. 4, 1989, pp. 161-174.

22. Johnson, B.T. Environmental Toxicology and Chemistry, Vol. 5, 1986, pp. 473-485.

23. Kemp, W.M., Boynton, W.R., Cunningham, J.J., Stevenson, J.C., Jones, T.W. and Means, J.C. Marine Environmental Research, Vol. 16, 1985, pp. 255-280.

24. Macek, K.J. Chronic Toxicity of Atrazine to Selected Aquatic Invertebrates and Fishes. U.S. Environmental Protection Agency Ecological Research Series, EPA-600/3-76-047, 1976.

25. Shaw, D.R., Peiper, T.F., and Nofziger, D.L. Weed Science, Vol. 33, 1985, pp.29-33.

26. Stay, F.S., Larsen, D.P., Katko, A. and Rohm, C.M. In Boyle, T.P., Ed., Validation and Prediction of Laboratory Methods for Assessing the Fate and Effect of Contaminants in Aquatic Ecosystems, STP 865. American Society for Testing and Materials, Philadelphia, 1985, pp.75-90.

27. Ward, G.S., and Ballantine, L. Estuaries, Vol. 8, 1985, pp. 22-27.

28. Ryon, M. Water Quality Criteria for 2,4,6-Trinitrotoluene (TNT). Report AD-A188 951, ORNL-6304, Oak Ridge National Laboratory, Oak Ridge, TN, 1987.

29. Odum, E.P. Trends in Ecology and Evolution, Vol. 5, 1990, pp.204-205.

30. Odum, E.P. BioScience, Vol. 35, 1985, pp.419-422.

31. Schindler, D.W. Canadian Journal of Fisheries and Aquatic Science, Vol. 44 (Suppl. 1), 1987, pp.6-25.

32. Levine, S.N. In Levin, S.A., Harwell, M.A., Kelly, J.R. and Kimball, K.D. Eds. Ecotoxicology: Problems and Approaches, Springer-Verlag, 1989, pp. 145-180.

33. Rand, G. and Petrocelli, S. Fundamentals of Aquatic Toxicology, Hemisphere Publishing, New York, 1984.

34. Odum, E.P., Finn, J.T., and Franz, E.H. BioScience, Vol. 29, 1979, pp. 349-352.

35. Mayer, F.L. and Ellersieck, M.R. Manual of Acute Toxicity: Interpretation and Data Base for 410 Chemicals and 66 Species of Freshwater Animals, US Department of Interior, Fish and Wildlife Service, Resource Publication 160,

Washington, 1986, 506 pp.

36. Taub, F., Kindig, A.C., Conquest, L.L., and Meador, J.P. In Suter II, G.W. and Lewis, M.A., Eds., Aquatic Toxicology and Environmental Fate: Eleventh Volume, ASTM STP 1007, American Society for Testing and Materials, Philadelphia, 1989, pp. 368-394.

37. Giesy, J.P. and Allred, P.M. In Cairns, Jr., J., Ed., Multispecies Toxicity Testing, Pergamon Press, New York, 1985, pp. 187-247.

38. Conquest, L.L. International Journal of Environmental Studies, Vol. 20, 1983, pp. 209-221.

39. Sokal, R.R. and F.J. Rohlf. Biometry, 2nd ed., W.H. Freeman, New York, 1983.

40. Schaeffer, D. Herricks, E.E., and Kerster, H.W. Environmental Management, Vol. 12, 1988, pp.445-455.

Table 1. MATCs for microcosm toxicity experiments of inorganic toxicants. Values shown are μg/L. Letters in parentheses identify the most sensitive variable in a class of responses: H = Hexosamine, A = Alkaline phosphatase activity, DO = Dissolved oxygen, C = Chlorophyll *a*, DW = Ash-free dry weight, ATP = Adenosine triphosphate biomass. Literature values and water quality criteria are from [12] unless otherwise noted. Water quality criteria (WQC) are for a hardness of 100 mg $CaCO_3$/L. N.S. = not significant.

Compound	Variable (Microcosm)			Literature	WQC
	Species Richness	Biomass	Function		
Cadmium[1][13]	0.9	1.2(ATP)	-	0.15-156	1.1
Chlorine[14]	3.6	2.1[2](C)	3.6(A)	< 3.4-26	11
Copper[1][15]	9.2	6.6[2](C)	-	3.9-60.4	12
Selenium[3][16]	14.1	14.1(H)	N.S.	10-10,000	35
Zinc[1][17]	51.5	4.2[2](DW)	4.2[2](DO)	46.7-5243	110

1 - Hardness based criteria
2 - LOEC
3 - References [18, 19]

Table 2. MATCs for microcosm experiments using organic toxicants. Table values are μg/L. Letters in parentheses identify the most sensitive variable in a response group: A = Alkaline phosphatase activity, P = Protein, DO = Dissolved oxygen, C = Chlorophyll *a*, D = Dehydrogenase activity, PO_4 = Orthophosphate. Literature ranges and water quality criteria are from [12] unless otherwise noted. N.S. = not significant.

Compound	Variable (Microcosm)			Literature	WQC
	Species Richness	Biomass	Function		
Organics					
Atrazine[1][18]	$3.2^{S,2}$ 192^{I}	$3.2^{S,2}$(P) 192^{I}	32 (DO)	71-3400	--
Chlorpyrifos	N.S.	N.S.	N.S.	0.12	0.041
PCP[3,4]	228	N.S.	228(PO_4)	<1.8-79.7	13
Phenol[19]	5700	$300^{S,2}$(P) 5700^{I}(C)	9200(DO)	2560	2560
TNT[5,6]	208	N.S.	507(A)	40-10,000	--

S = Stimulation, I = Inhibition
1 - References [22-27]
2 - LOEC
3 - Pentachlorophenol
4 - pH dependent criteria
5 - Trinitrotoluene
6 - Reference [28]

Table 3. MATCs for microcosm experiments and single species tests (SST) using complex effluents. Table values are %. Letters in parentheses indicate the most sensitive variable in a response group: P = Protein, I = *In vivo* fluorescence, A = Alkaline phosphatase activity, DO = Dissolved oxygen, C = Chlorophyll a. Instream waste concentration (IWC) estimates vary with assumed low flow estimates. N.S. = no signficant difference.

Effluent	Variable (Microcosm)			SST	IWC
	Species Richness	Biomass	Function		
Fly Ash	35	71(C)	35(DO)	35	100
Sewage Treatment	N.S.	N.S.	18(DO)	10[1]	37
Foundry	71	6[2](P)	6[2](A)	17(R)	10-30
Brass Mill	6[2]	6[2](I)	N.S.	8.8(R)	1-4

1 - acute toxicity test
2 - LOEC

Table 4. Summary of copper toxicity by response class in microcosms. Table values are μg Cu/L. Letters in parentheses identify the most sensitive variable in each response class: P = Protein, C = Chlorophyll a, A = Alkaline phosphatase activity, DO = Dissolved oxygen, ATP - Adenosine triphosphate biomass. The water quality criteria are calculated for dilution water hardness.

Test/ Date	WQC	MATC or LOEC Species Richness	Biomass	Function
Pennsylvania				
Nov88	19.5	19.9	9.9(P)	40 (DO)
Nov89		--	113 (C)	40 (DO)
Feb90		20[1]	73.3(C)	N.S.
Apr90		9.1[1]	53.9(C)	25.8(DO,A)
Michigan				
Aug89[2]	15.7	18.9	82.1(C)	18.9 (DO)
May90[3]	19.5	66.2	12.1[1](C)	17.1 (A)
Aug90[3]	19.5	95.8	95.8(C)	N.S.
Maryland				
Mar89	22.1	13.9	6.8(P,C)	4.7[1] (DO)
Virginia				
Feb86	8.2	9.2	6.6(C,ATP)	--

1 - LOEC
2 - Test conducted with Douglas Lake dilution water.
3 - Test conducted with Pennsylvania State Univ. dilution water.

Table 5. Estimates of variability of response variables in laboratory microcosm toxicity tests. Tables values are median coefficients of variation (CV) or minimum detectable distance as a percent of the mean.

Variable	n	CV (%)	Minimum distance %
Structure variables			
Species richness	27	7.3	18.1
Total protein	28	17.6	43.8
Chlorophyll *a*	22	22.1	55.0
Calcium	16	11.4	28.3
Magnesium	16	13.3	33.1
Potassium	16	16.5	41.1
Function variables			
Dissolved oxygen	18	4.5	11.3
pH	15	2.2	5.5
Alkaline phosphatase activity	26	18.0	44.8

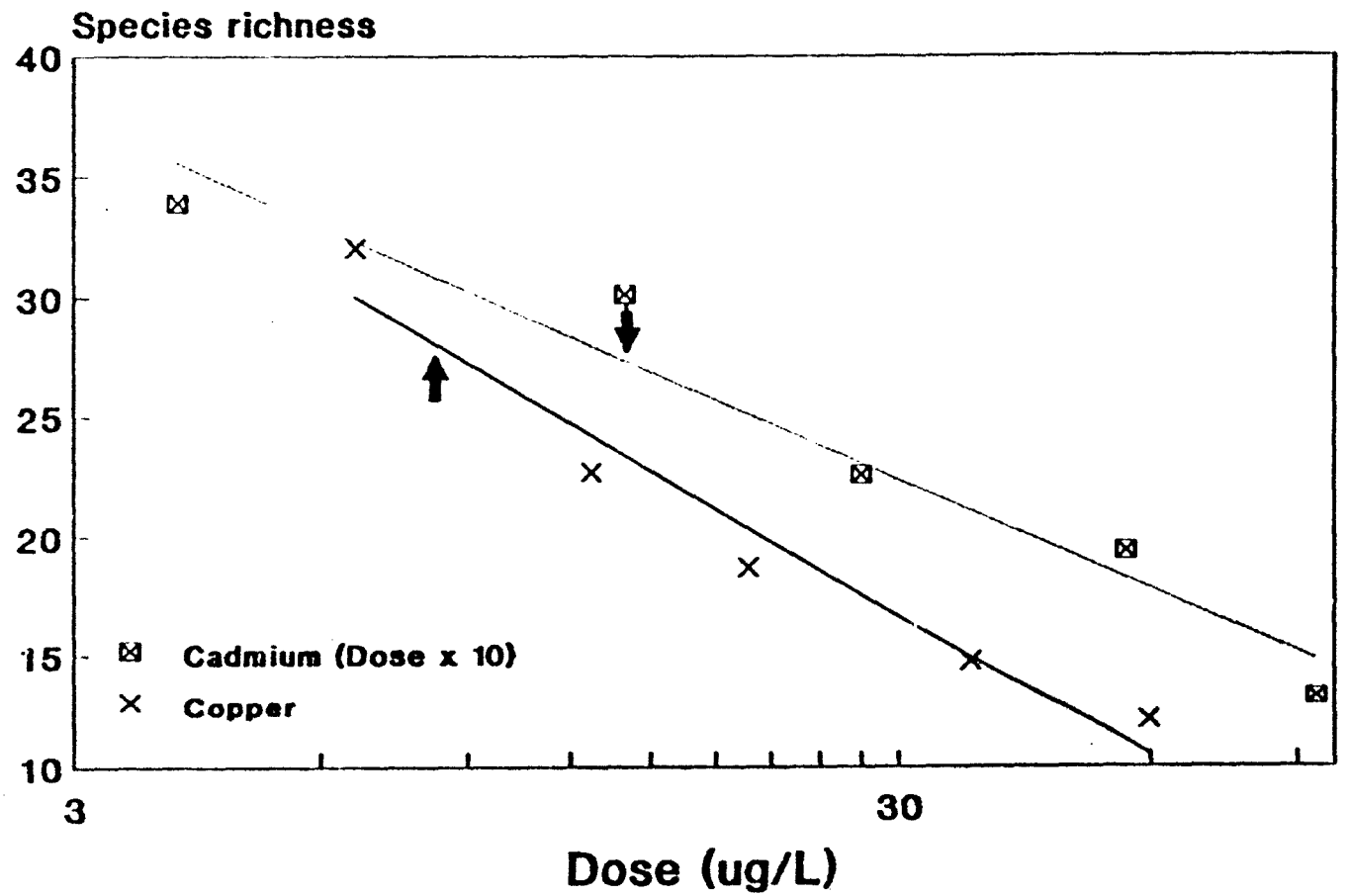
Figure legends

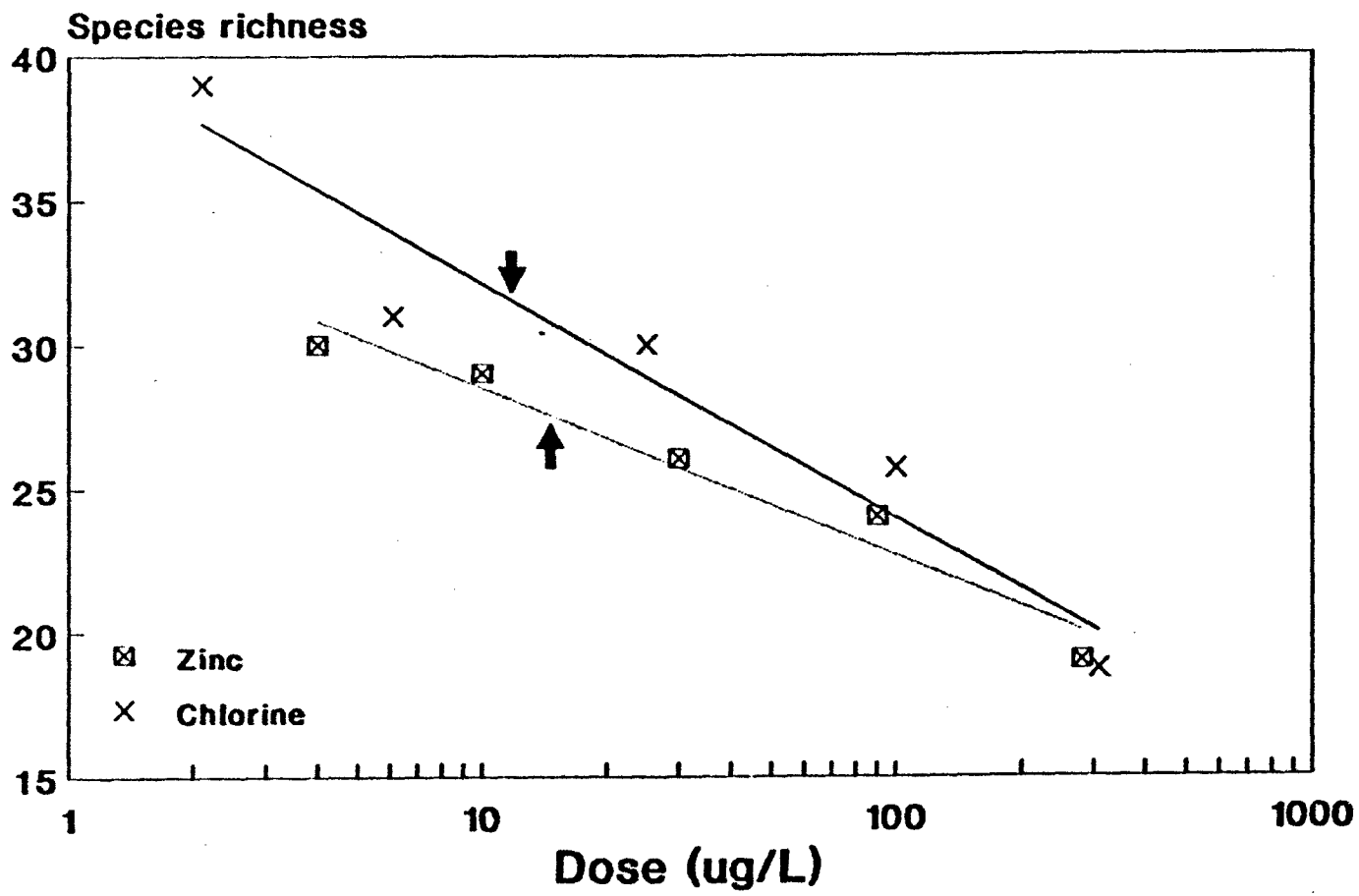
Fig. 1. Effects of cadmium and copper on the species richness of protozoa in AS-M. Plotted points are means of triplicates. Fitted lines are ordinary least squares regression lines. Arrows denote points on the fitted lines corresponding to a 20% reduction in species richness compared to controls. Data for cadmium are plotted as the mean measured dose X 10 for scaling purposes. Data from [13, 15].

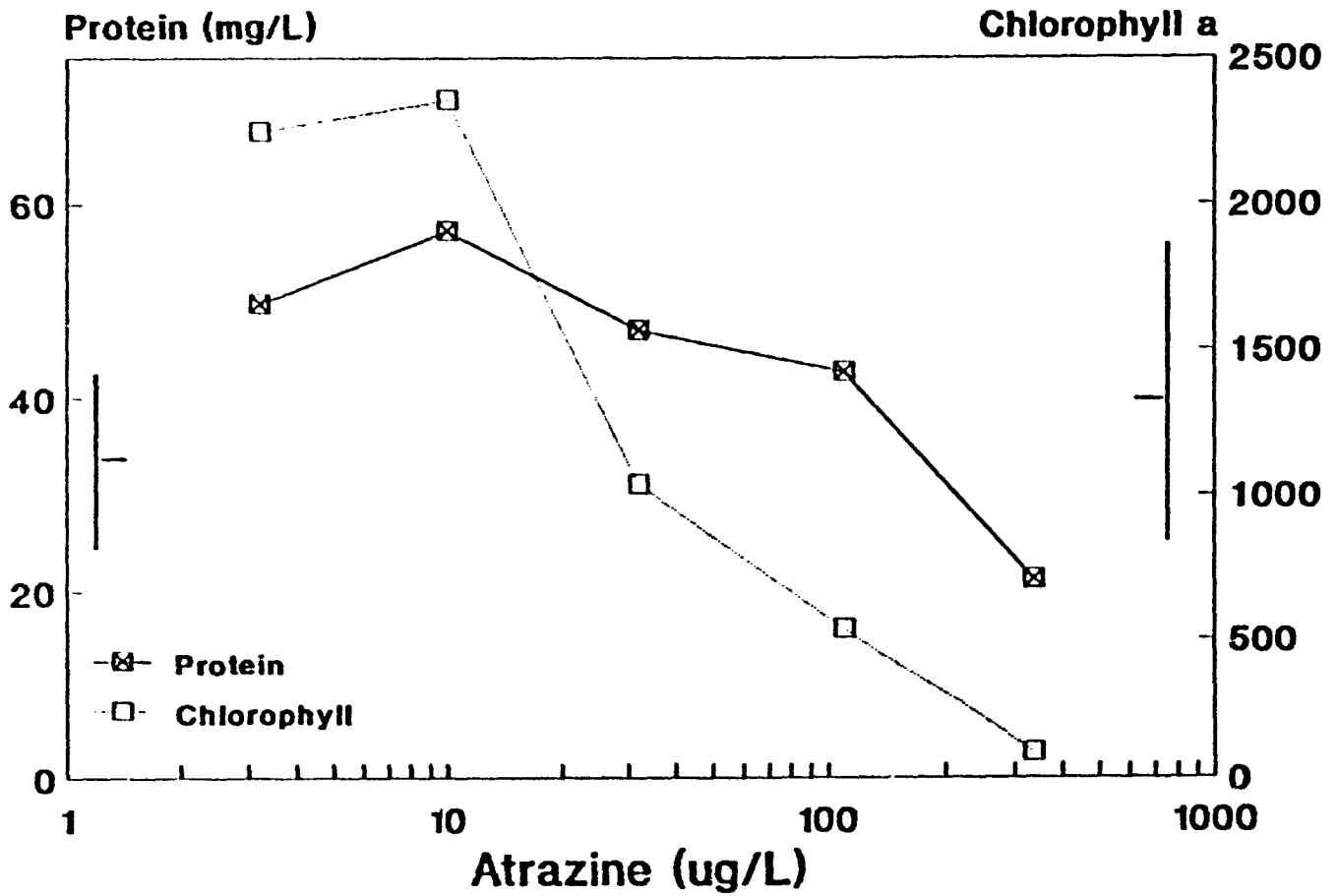
Fig. 2. Effects of zinc and total residual chlorine (chlorine) on the species richness of protozoa in AS-M. Plotted points are means of triplicates. Fitted lines are ordinary least squares regression lines. Arrows denote points on the fitted lines corresponding to a 20% reduction in species richness compared to controls. Data from [14, 17].

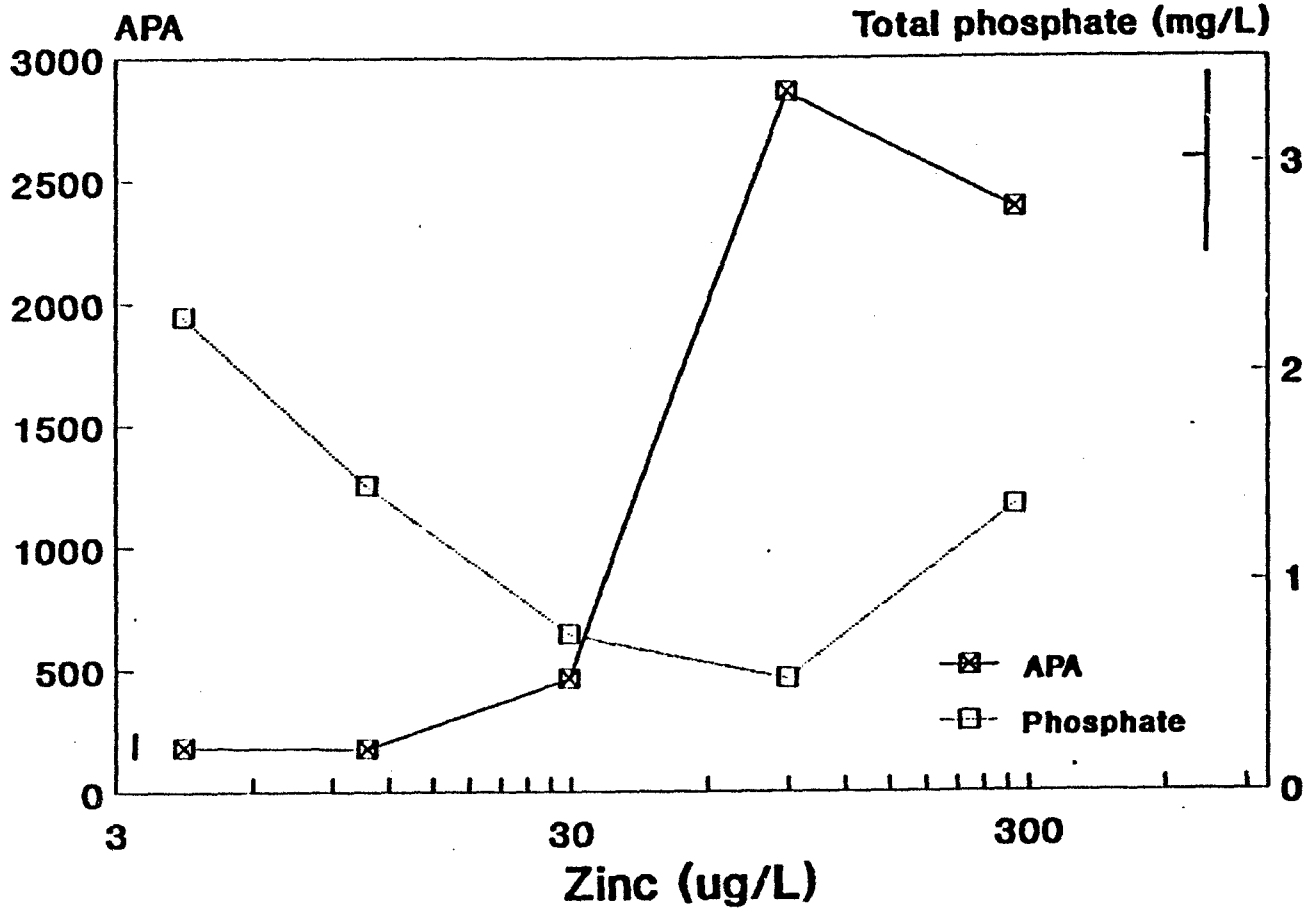
Fig. 3. Stimulation of protein and chlorophyll *a* biomass in AS-M by atrazine. Plotted points are means of triplicates. Heavy bars along axes show the mean (± standard deviation) of control values. Data from [20].

Fig. 4. Interaction of total phosphate and alkaline phosphatase activity (APA) in response to zinc toxicity. APA increases geometrically as communities lose phosphate. Heavy bars along axes show control values (± standard deviation). Units for APA are nmoles p-nitrophenylphosphate/mg protein/hr. Data from [17].


Species richness
40
35
30
25
20
15
10
3
30
Cadmium (Dose x 10)
Copper
Dose (ug/L)


Species richness
40
35
30
25
20
15
Zinc
Chlorine
1
10
100
1000
Dose (ug/L)


Protein (mg/L)
Chlorophyll a
60
40
20
0
2500
2000
1500
1000
500
0
Protein
Chlorophyll
1
10
100
1000
Atrazine (ug/L)


APA
Total phosphate (mg/L)
3000
2500
2000
1500
1000
500
0
3
2
1
0
3
30
300
Zinc (ug/L)
APA
Phosphate

Effect of Selenium on Microbial Communities in Laboratory Microcosms and Outdoor Streams

JAMES R. PRATT and N.J. BOWERS,

School of Forest Resources,
The Pennsylvania State University

ABSTRACT

Ecological effects of selenium (as sodium selenite) on naturally derived microbial communities were evaluated in laboratory microcosms and in outdoor experimental streams at the U.S. Environmental Protection Agency (U.S. EPA) Monticello Ecological Research Station (MERS). Microcosms were continuously dosed for 21 days at selenium concentrations ranging from 0 to 160 μg Se/L. Outdoor streams were continuously dosed at 0, 10, and 30 μg Se/L, the highest concentration approximating the current U.S. EPA water quality criterion. In laboratory microcosms, protozoan species richness was reduced by 20%, and chlorophyll and hexosamine levels were reduced by 40% at $\geq$80 μg Se/L. Total biomass and carbohydrate levels decreased with increasing Se, but these effects were not significant. Selenium had no effect on microcosm production to respiration ratios. In outdoor streams, microbial community biomass collected on artificial substrata was 2–3 times greater than in the laboratory. In general, adverse effects were not observed, confirming laboratory estimates of no adverse effects at <80 μg Se/L. However, low doses (10 μg Se/L) consistently stimulated microbial biomass (protein, chlorophyll, hexosamine) and elevated production to biomass (P/B), consistent with reports of Se stimulation of algal growth. The highest Se dose (30 μg/L) caused decreased primary production and decreased P/B compared to controls. Experiments showed that ecological responses of laboratory microcosms and outdoor experimental ecosystems are similar, and are at least as sensitive as standard toxicological responses.

INTRODUCTION

The potential use of microcosms in predicting the fate and effect of hazardous chemicals has led to the development of a variety of protocols employing multiple trophic levels and increased environmental realism over that of traditional single-species toxicity tests. Microcosms are defined as "artificially bounded subsets of naturally occurring environments which are replicable [and] should exhibit system-level proper-

Toxicity Assessment: An International Journal Vol. 5, 293–307 (1990)
© 1990 John Wiley & Sons, Inc.
0884-8181/90/050293-015$0.400

ties" (Giesy and Odum, 1980, p. 4), and may vary greatly in size and complexity. We have used naturally derived microbial communities in laboratory microcosms to evaluate the toxicity of several pure chemicals and complex effluents (Pratt *et al.*, 1988a,b; Pratt *et al.*, 1987; Niederlehner *et al.*, 1985) and found effect levels that approximated those of single-species tests. Unfortunately, applying of results from relatively simple laboratory microcosms to complex natural ecosystems is complicated and the predictive capabilities of laboratory microcosms in environmental risk assessment often questioned. Validation of laboratory microcosm results with outdoor mescosm-scale experiments or whole-ecosystem experiments is critical to evaluate the usefulness and applicability of laboratory microcosms.

We conducted a laboratory flow-through microcosm experiment in which naturally derived microbial communities were exposed to selenium. Adverse effect levels were estimated based on changes in microbial community structure and function. The validity of predictions of specific adverse effects on microbial responses was tested under field conditions using outdoor artificial streams continuously dosed with selenium, in which microbial communities were collected and analyzed in a similar manner as in the laboratory experiment. Both experiments examined several categories of ecosystem responses to stress (Odum, 1985). Although the microbial communities tested in the two experiments were derived from two different ecosystems, we predicted that the two communities should have similar sensitivities and resistances.

METHODS

Laboratory Microcosms

Naturally derived microbial communities were exposed to a continuous input of selenium as sodium selenite in 4-L glass microcosms for 21 days. A stock solution of selenium was mixed with carbon-dechlorinated tap water and proportionally diluted by a gravity-fed minidiluter (Benoit *et al.*, 1982) to produce the treatments of 10, 20, 40, 80, and 160 μg Se/L. Concentrations were tested in triplicate and a control group receiving unamended dechlorinated tap water was included (see Fig. 1 for a schematic of the experimental design). Water samples were collected weekly and fixed to pH < 2 with trace pure HNO_3 for selenium analysis. Selenium was analyzed using a Perkin-Elmer Model 100 atomic absorption spectrophotometer with a HGA 2100 Graphite Furnace [Method 270.2, U.S. Environmental Protection Agency (U.S. EPA), 1985]. Temperature was uncontrolled and fluctuated between

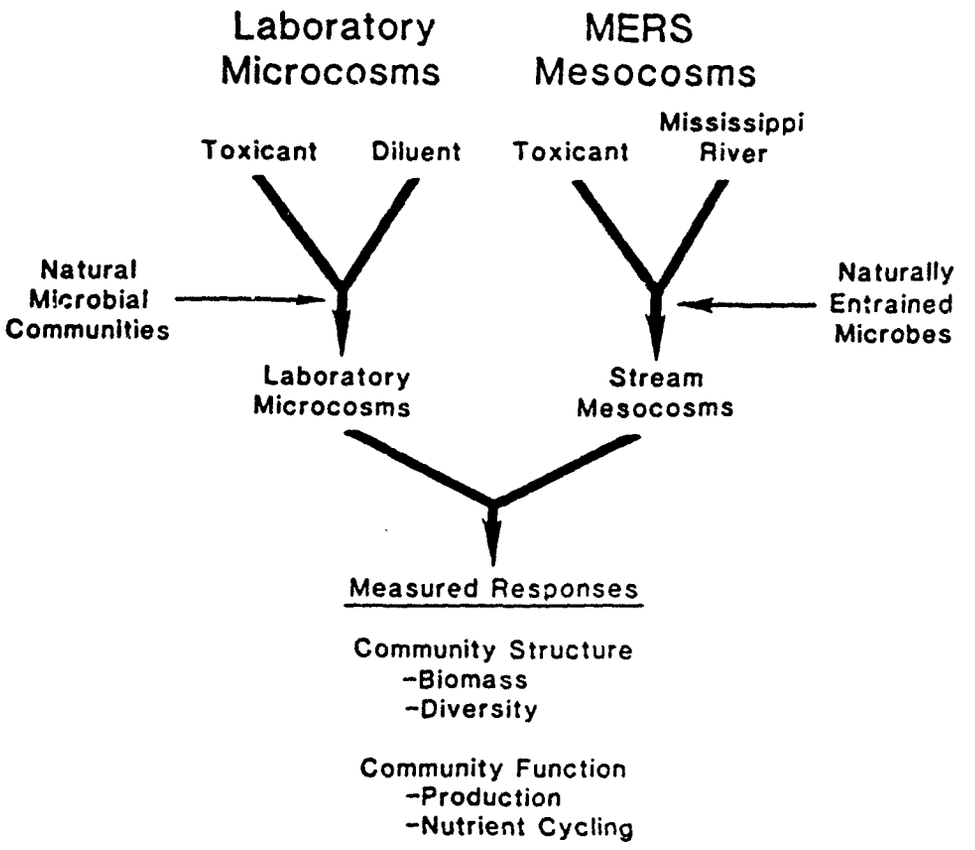


Fig. 1. Schematic of laboratory microcosm and the outdoor artificial stream experiments showing the relationship of tested communities and measured responses.

15.3 and 18.0°C. Lighting was provided by Vita-Lites (Color Rendering Index > 90; DuroTest, Corp.) at an intensity of 5000 lux and a photoperiod of 12 h of light/12 h of dark.

Microbial communities were collected on polyurethane foam artificial substrata (e.g., Cairns, 1982) that had been exposed for seven days in a small, moderately hardwater stream in Centre County, Pennsylvania. Communities collected in this manner typically contain hundreds of species of bacteria, fungi, algae, protozoa, and micrometazoa. Exposed substrata were collected, and a single colonized substratum ("epicenter") was placed at the influent end of each microcosm as a microorganism source, and three additional unexposed ("island") substrata were placed toward the effluent end to provide colonizable habitat.

A single island substratum was removed from each microcosm after 7, 14, and 21 days of exposure, and the microbial community contained within each substratum analyzed for total protein, chlorophyll *a*, hexosamine, carbohydrate, alkaline phosphatase activity, and

protozoan species richness and composition. Protein provided an estimate of total microbial biomass accumulated on island substrata. Subsamples were extracted with 0.5 N NaOH (Rausch, 1980) and protein were analyzed by the method of Bradford (1976). The algal component of the community was quantified using chlorophyll *a* (American Public Health Association, 1985). Hexosamine content provided an estimate of chitin-containing organisms, and was measured using the method of Gatt and Berman (1966). Carbohydrate was measured to estimate the amount of stored material within the community (Pick, 1987). Alkaline phosphatase estimated the microbial community's ability to cycle inorganic phosphate (Sayler *et al.*, 1979), and results were expressed as nmole *p*-nitrophenol (p-NP)/mg protein/h. Subsamples were also examined for protozoan species richness by repeated microscopic observation at 200–400× (see Cairns *et al.*, 1979). Protozoa were identified to the lowest practical taxonomic unit, usually species.

Production to respiration (P/R) ratios were estimated weekly in each microcosm using the three-point dissolved oxygen method of McConnell (1962). The ratio of primary production to chlorophyll *a* was used as an estimate of photosynthetic efficiency. Production was converted from mg O_2/L/12 h to total mg C/h and P/Bs were expressed as mg C/mg chl*a*/h.

MERS Outdoor Streams

The Monticello Ecological Research Station (Monticello, MN; MERS) has eight outdoor experimental streams that receive water from the Mississippi River. The streams have been described in detail by Nordlie and Arthur (1981) and Zischke *et al.* (1983). Four of the eight streams began receiving a continuous input of selenium as sodium selenite in March 1987 to achieve 10 μg/L in two streams and 30 μg/L in two streams (the criterion at that time was 35 μg/L; U.S. EPA, 1986). Two streams served as controls and received no selenium. Each stream consisted of a series of pools (30.5 m long × 3.7 m wide) and riffles (30.5 m long × 0.8 m wide). Microbial communities were collected and tested in a similar manner as in the laboratory microcosm experiment, except that the source of microbial species was Mississippi River water instead of epicenter substrata (see Fig. 1). Artificial substrata were placed at the start of the first riffle in each stream and exposed for 1, 3, 5, and 10 days.

Periphyton productivity within the streams was analyzed using plexiglass productivity chambers (Rodgers *et al.*, 1979). Twenty-four rocks of similar size were collected near the end of the first riffle in each stream and divided evenly into duplicate chambers. The chambers were

TABLE I
Selenium concentrations (μg/L) in laboratory microcosms

Treatment (μg/L)	Average	*SD*	Coefficient of variation (%)
Control	—	—	—
10	10.0	0.75	7.55
20	20.1	1.73	8.62
40	41.1	5.30	12.9
80	84.9	5.29	6.23
160	161	9.55	5.45

filled with filtered (20 μm) stream water, and one chamber was covered with black cloth to exclude light and the other left exposed. Chambers were incubated in their respective streams for 4 h (1000–1400 h), each with a battery-operated pump to generate a continuous flow of water over the rocks. After incubation, chambers were removed from the streams and dissolved oxygen (DO) was measured immediately using a YSI Model 58 oxygen meter. The difference in DO between the light and dark chamber was used as an estimate of net primary production. Rocks were scraped to remove periphyton for chlorophyll *a* analysis and total rock surface area was calculated for each chamber. Oxygen production was converted to carbon fixation and results were expressed as mg C/m^2/h and mg C/mg chl*a*/h.

Data Analyses

In the laboratory experiment, responses among treatments were compared using analysis of variance (ANOVA). Multiple paired comparisons were made using Fisher's Least Significant Difference (Sokal and Rohlf, 1983) for significant responses ($p < 0.05$). In the field experiments, nested ANOVA was used to compare treatments and multiple comparisons were made when the ANOVA was significant. Differences between replicate streams within the same treatment were also evaluated.

RESULTS

Laboratory Test

Average selenium concentrations in laboratory microcosms closely approximated target concentrations and remained constant for the duration of the experiment (Table I).

TABLE II
Production to respiration and production to biomass (chlorophyll) ratios in laboratory microcosms dosed with selenium [values are mean (*SD*)]

Treatment (μg Se/L)	Production to respiration			Production to biomass		
	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
Control	1.21	1.01	1.09	6.30	1.92	0.87
	(0.37)	(0.01)	(0.07)	(3.02)	(0.41)	(0.17)
10	1.28	0.99	1.07	6.02	2.19	1.31
	(0.08)	(0.01)	(0.07)	(1.20)	(0.30)	(0.56)
20	1.34	1.00	1.08	12.1	3.65	2.00[a]
	(0.19)	(0.01)	(0.05)	(5.65)	(1.13)	(0.41)
40	1.25	1.01	1.12	9.30	3.52	1.27
	(0.05)	(0.03)	(0.04)	(5.22)	(1.51)	(0.32)
80	1.27	1.04	1.16	7.62	2.80	1.90[a]
	(0.13)	(0.04)	(0.03)	(1.29)	(0.54)	(0.39)
160	1.29	1.05	1.14	5.90	2.97	1.65[a]
	(0.06)	(0.06)	(0.02)	(2.34)	(0.71)	(0.09)
p	0.9620	0.3590	0.1338	0.2985	0.1692	0.0406

[a] Significantly different from control at $\alpha = 0.05$.

Production to respiration (P/R) ratios in microcosms were consistent over the three-week period and did not differ among treatments at any of the time periods measured (Table II). Communities tended toward autotrophy, with the average P/R ratio slightly greater than 1 (1.13 ± 0.12). The photosynthetic efficiencies (P/B) of microbial communities are also shown in Table II. There was a trend toward increased photosynthetic efficiency with increased selenium concentration and this effect was significant at 20.1 and ≥84.9 μg Se/L on Day 21.

Microbial biomass accrual on artificial substrata over time is shown in Fig. 2. Selenium had no effect on microbial protein or carbohydrate accumulation at any of the concentrations tested. After 21 days, however, chlorophyll *a* was significantly reduced by 37–47% at ≥84.9 μg Se/L. Hexosamine content was also reduced on Day 21 at 20.1 and ≥84.9 μg Se/L. Protozoan species richness was reduced by 15–25% on Day 14 at ≥43.3 μg Se/L and by 18–23% on Day 21 at ≥20.1 μg Se/L (Table III). Selenium had only a slight effect on microbial alkaline phosphatase activity, which was reduced on Day 14 at the highest concentration (161 μg Se/L, Table III).

Field Test

Water samples from MERS artificial streams were analyzed for selenium on a bimonthly schedule, and the average (*SD*) selenium concen-

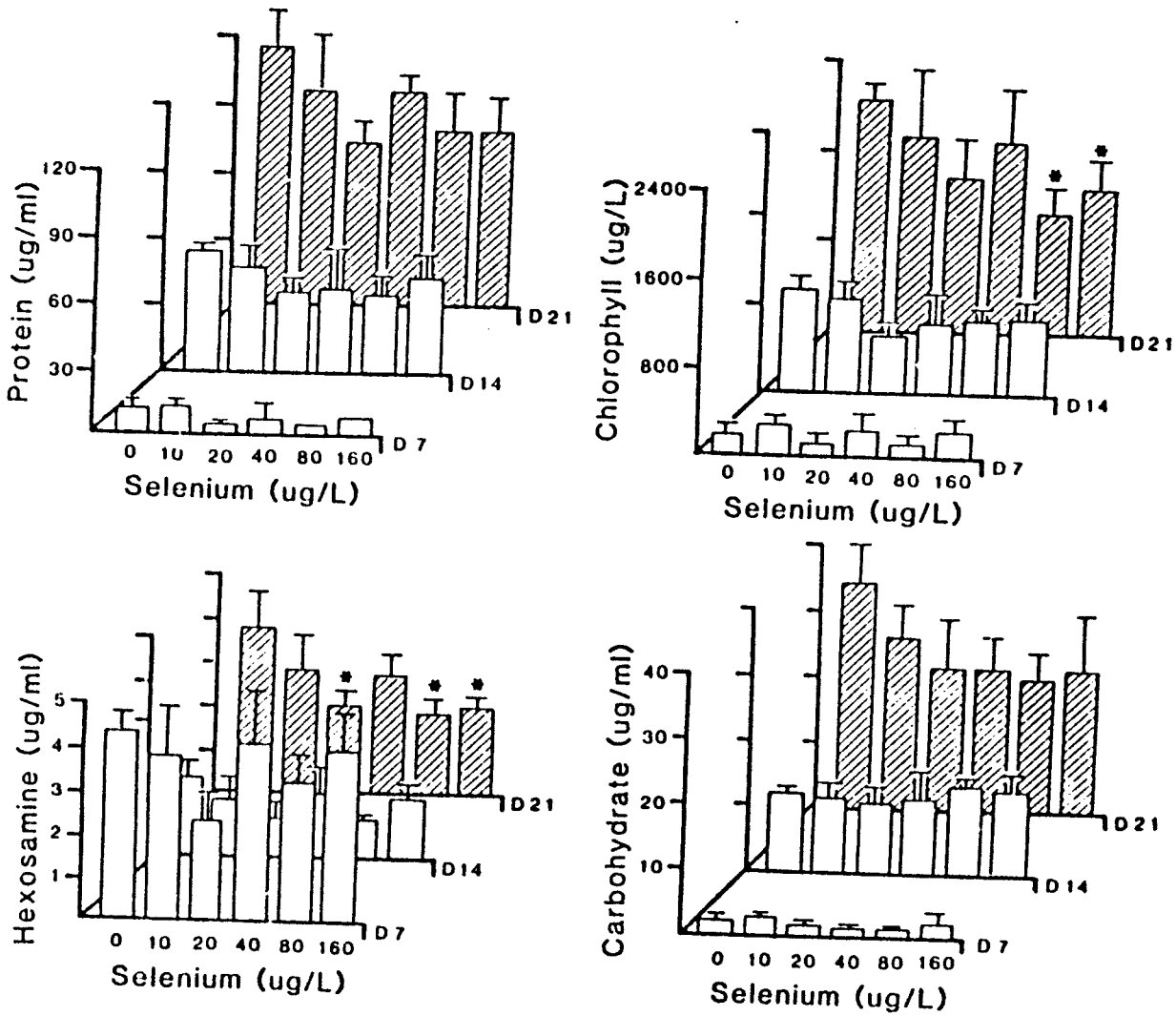


Fig. 2. Accumulation of microbial biomass (protein, chlorophyll *a*, hexosamine, and carbohydrate) on artificial substrata in laboratory microcosms. Error bars show one standard deviation and asterisks indicate values significantly different from control.

TABLE III
Protozoan species richness and microbial alkaline phosphatase activity on artificial substrates from laboratory microcosms dosed with selenium

Treatment (μg Se/L)	Protozoan species			Alkaline phosphatase activity (nmole *p*-NP/mg protein/h)		
	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
Control	33.3	63.0	61.7	127	156	130
	(0.58)	(3.60)	(3.21)	(10.5)	(13.3)	(11.2)
10	30.7	55.0	57.0	130	135	129
	(2.08)	(2.64)	(3.60)	(7.5)	(11.1)	(28.4)
20	30.7	55.3	50.7[a]	181	153	141
	(9.02)	(3.21)	(6.03)	(70.7)	(26.7)	(44.8)
40	27.0	53.3[a]	54.0	210	162	125
	(2.64)	(6.35)	(5.57)	(88.4)	(11.7)	(20.8)
80	26.0	47.3[a]	49.3[a]	159	154	112
	(6.08)	(8.14)	(6.81)	(60.0)	(8.38)	(14.8)
160	21.3	47.7[a]	47.3[a]	130	112[a]	121
	(3.21)	(2.51)	(2.08)	(27.3)	(20.3)	(29.4)
p	0.1037	0.0191	0.0313	0.3281	0.0258	0.8489

[a] Significantly different from control at $\alpha = 0.05$.

trations during our field study were 8.27 (0.82) and 27.8 μg Se/L (1.94) for the 10 and 30 μg/L dosed streams, respectively. The level of selenium in the control streams was < 2.0 μg Se/L. Selenium concentrations had been consistent over the duration of the selenium dosing period (approximately one year at the time of our study).

Variability in microbial responses between replicate streams was a problem on some of the sampling days for some of the responses. Statistically, more replicate streams would have increased our ability to detect differences among treatments, but we were limited by the number of available streams. Results of ANOVA on replicate stream data are shown in Table IV. Most of the variability between replicate streams was due to differences between the highest treatment streams.

The effect of selenium on microbial communities is shown in Fig. 3. There was a tendency toward increased protozoan species richness in both treatments, and increased biomass in the 10 μg Se/L streams. There was a transient reduction in microbial biomass (as protein) on artificial substrata in the 30 μg Se/L streams, but no differences were detected after 10 days of exposure.

Periphyton production measured *in situ* was greater at 10 μg Se/L and reduced at 30 μg Se/L, compared to control streams (Table V). Although there was no difference in periphyton chlorophyll biomass

TABLE IV
Results of ANOVA of responses between replicate MERS streams[a]

Response	Day 1	Day 3	Day 5	Day 10
Protozoan species	NS	NS	NS	NS
Alkaline phosphatase	NS	NS	NS	NS
Chlorophyll	0.0163	NS	NS	0.0015
Protein	0.0165	NS	NS	0.0001
Carbohydrate	0.0096	NS	NS	0.0401
Hexosamine	0.0581	NS	NS	NS

[a] The p values are shown for significant differences between streams.
NS: not significant ($p > 0.05$)

periphyton photosynthetic efficiency (P/B) was stimulated in the low-treatment streams and decreased in the high-treatment streams.

In laboratory microcosms, variability between replicate samples for most of the responses were low (Table VI). Coefficients of variation (%) ranged from 4.2 to 18.8, except for hexosamine, which was 30.0%. In the outdoor experimental streams, variability between replicate samples increased slightly, but generally remained below 30%. The lowest observeable effect concentrations for several of the responses measured in the laboratory and field experiments are shown in Table VII. In some cases, the observed effect was stimulation (S), while in other cases, it was inhibition (I). In general, the laboratory results predicted that there should be minimal negative effects of selenium on microbial communities at the concentrations tested in the outdoor experimental streams. This prediction was valid based on field results.

TABLE V
Periphyton production in MERS artificial streams dosed with selenium
[Values are mean (SD)]

Treatment	Production (P) (mg C/m2/h)	Chlorophyll a (B) (mg/m2)	P/B (mg C/mg Chl/h)
Control	529	121	4.37
	(45.2)	(8.48)	(0.07)
10 μg Se/L	571	110	5.19[a]
	(22.6)	(5.66)	(0.06)
30 μg	454	124	3.66[a]
	(21.9)	(13.4)	(0.22)
p	0.0759	0.4124	0.0037

[a] Significantly different from control at $\alpha = 0.05$

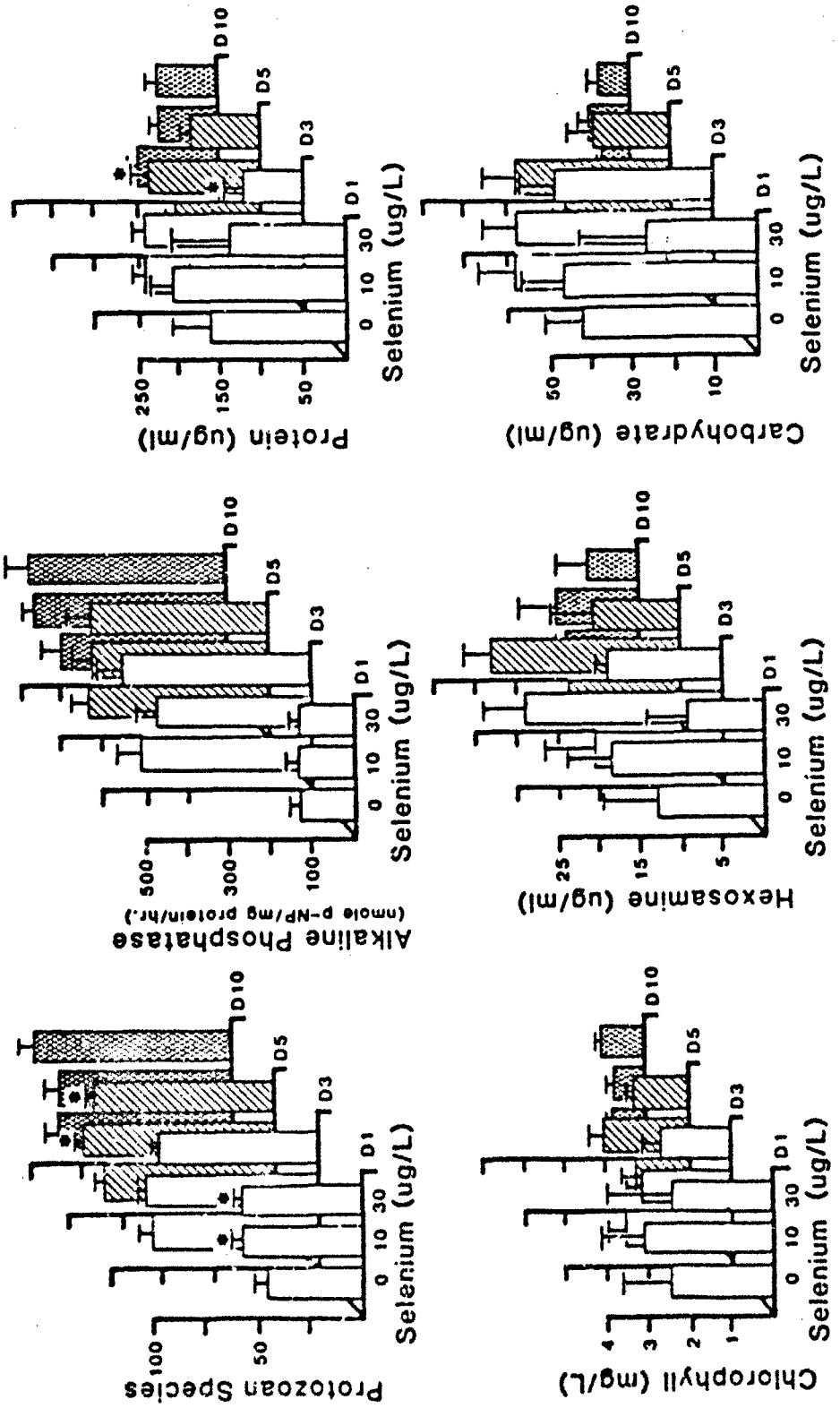


Fig. 3. Protozoan species richness and biomass on artificial substrata from MERS outdoor experimental streams. Error bars show one standard deviation and asterisks indicate values significantly different from control.

TABLE VI
Coefficients of variation for control responses measured in laboratory microcosms and outdoor artificial streams

Response	Laboratory	Outdoor streams
Hexosamine	30.0	31.6
Protein	18.8	27.6
Chlorophyll	15.7	27.3
Carbohydrate	15.1	21.9
Alkaline phosphatase	8.5	13.0
Species richness	4.2	7.8

However, results from the microcosm experiment did not predict the stimulation effect on protein and species numbers observed at low levels of selenium.

DISCUSSION

In 1986, U.S. EPA criteria for the protection of freshwater aquatic life for selenium (as selenite) were 35 μg/L as a 24-h average, not to exceed 260 μg/L at any time (U.S. EPA, 1986). Chronic laboratory tests have shown selenium to be moderately toxic toward aquatic life, with maximum allowable toxicant concentrations (MATCs) for daphnids and fish of 180–360 μg/L (Adams and Johnson, 1981; Reading and Buikema,

TABLE VII
Summary of results from the laboratory microcosm experiment and the MERS artificial stream experiment[a]

	Laboratory microcosm			MERS artificial streams			
Response	Day 7	Day 14	Day 21	Day 1	Day 3	Day 5	Day 10
Protozoan species	NS	40(I)	20(I)	10(S)	NS	10(S)	NS
Alkaline phosphatase	NS	160(I)	NS	NS	NS	NS	NS
Protein	NS	NS	NS	NS	30(I)	10(S)	NS
Chlorophyll	NS	NS	80(I)	NS	NS	NS	NS
Hexosamine	NS	NS	20(I)	NS	NS	NS	NS
Carbohydrate	NS	NS	NS	NS	NS	NS	NS
P/B	NS	NS	20(S)		10(S), 30(I)		

[a] The lowest observable effect concentrations (LOEC, μg Se L) for each significant response are shown. NS: not significant.

1983: Owsley and McCauly, 1986). However, severe impacts on fish survival and reproduction under field conditions have been observed at concentrations below those thought to produce direct effects. Selenium was found to be rapidly bioconcentrated by all components of the aquatic food chain, ranging from 500 times background in phytoplankton and periphyton to as high as 4000 times in some species of fish (Lemly, 1985). Lemly (1985) estimated the MATC for selenium to be 2–5 μg/L based on fish populations in Belews Reservoir (U.S.A.). Hunn *et al.* (1987) found decreased bone calcium in larval rainbow trout exposed to selenium at $\leq$12 μg/L and suggested 10 μg/L to be the criterion for selenium. In 1987, the U.S. EPA revised the freshwater selenium criteria to 5 μ Se/L as a 4-day average and 20 μg Se/L as a 1-h average (U.S. EPA, 1987).

In the present study, microbial communities in laboratory microcosms were moderately sensitive to selenium, with increased sensitivity corresponding to longer exposure time. Protozoan species richness and microbial hexosamine concentrations were the most sensitive indicators of selenium toxicity (MATCs = 14.4 μg Se/L), but photosynthetic efficiency (P/B) was stimulated at low selenium concentrations (MATC = 14.4 μg Se/L). There was no effect of selenium on total microbial biomass (protein) or alkaline phosphatase activity. Based on laboratory results, we estimated 14.4 μg Se/L to be the maximum acceptable toxicant concentration.

In outdoor experimental streams, response variability was slightly greater than observed under more controlled laboratory conditions, and some differences were observed between replicate streams. Based on laboratory results we would have expected decreased protozoan richness and hexosamine in the 30 μg/L treatment, but after 10 days, no persistent adverse effects of selenium on microbial communities collected on artificial substrata was observed. It is possible that a longer exposure period may have resulted in more persistent effects. Prolonged exposure to selenium in single-species tests has been found to increase the sensitivity of test organisms to the toxic effects of selenium (Owsley and McCauley, 1986; Adams and Johnson, 1981).

The effect of long-term selenium exposure was evaluated by the examination of mature epilithic algal biofilms. Water flow in the artificial streams was controlled, and therefore periphyton assemblages had been undisturbed and exposed to selenium for a longer period of time than communities collected on artificial substrata. Periphyton photosynthetic efficiency was stimulated at 10 μg Se/L, which was predicted based on laboratory results. However, efficiencies were decreased at 30 μg Se/L, which was not predicted from laboratory results, even though

communities in the laboratory were exposed to selenium levels as high as 161 μg Se/L.

Based on the most sensitive response in the outdoor stream experiment (periphyton P/B) we would estimate the MATC for selenium to be 17.3 μg/L. This value is very similar to the value obtained in the laboratory microcosm experiment (14.4 μg Se/L), but microbial communities in the outdoor streams were, in general, less sensitive to selenium than communities in laboratory microcosms. One of the major differences in experimental design between the laboratory microcosms and the outdoor artificial streams that possibly affected system response is the source of microorganisms. In microcosms, colonized epicenter substrata provided a species source for the colonization of island substrata. The same epicenter was used throughout the test with no other source of test organisms. In the outdoor experimental streams, however, the Mississippi River provided a continual source of unexposed organisms, a much more realistic situation. Other studies have found laboratory tests to be "overprotective" of the environment (Lewis *et al.*, 1986; Boyle *et al.*, 1985; Davies and Wooding, 1980), which is not unexpected, since laboratory microcosms, although more complex and realistic than single-species tests, still only represent a subset of the natural environment.

Several other differences exist between laboratory and field testing, including differences in community exposure history, system biomass, duration of testing, and experimental design. As expected, changes in community structure (species richness, standing crop) were generally more sensitive to selenium effects than process measures (Odum, 1985). Alteration in processing rates was attributable to changing standing crop, although the selenium concentration at which inhibition of responses was not evident. Standing crop is clearly a function of nutrient regimes that differed between the two experiments. This suggests that the relationship between the expected selenium subsidy (*sensu* Odum *et al.*, 1979) of algal selenium needs and adverse ecological effects is imprecise. In neither the laboratory nor field experiments were effects of bioaccumulation apparent in microbial indices of stress. However, even long-term dosing of the MERS streams with selenium has produced no obvious ecological effects, although some evidence now indicates bioaccumulation effects in fish (R. Hermanutz, MERS, personal communication).

Despite discrepancies between laboratory microcosms and field experiments, microcosm tests remain a valuable tool in identifying chemicals that may produce adverse or indirect effects in the environment. They permit the evaluation of sublethal effects and account for some of

the complex ecosystem interactions that cannot be measured in single-species tests. One of the major problems with microcosm testing is the inherent measurement variability in monitoring ecological processes. Improved techniques and methods for evaluating effects on several ecologically meaningful end points, especially those relating to nutrient cycling, are needed. While unpredicted ecological effects may be discovered in microcosm tests, artificial ecosystem sensitivities are still not well understood.

This work was supported by the U.S. Army Medical Research and Development Command under Contract No. DAMD17-88-C-8068. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army. Selenium analyses were performed by Kathleen Allen at MERS. Special thanks go to all of the personnel at MERS for their support and assistance in the outdoor experimental stream test.

References

Adams, W., and H. Johnson. 1981. Selenium: A hazard assessment and a water quality criterion calculation, P. 124–137. *In* D. Branson and K. Dickson (Eds.), Aquatic Toxicology and Hazard Assessment: Fourth Conference, ASTM STP 737. American Society for Testing and Materials, Philadelphia, PA.

American Public Health Association. American Water Works Association, and Water Pollution Control Federation. 1985. Standard Methods for the Examination of Water and Wastewater (16th ed.). Washington, DC. 1193 p.

Benoit, D., V. Mattson, and D. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Res. *16*:457–464.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein, utilizing the principle of protein-dye binding. Anal. Biochem. *72*:248–254.

Cairns, J., Jr. 1982. Freshwater protozoan communities, P. 249–285. *In* A. Bull and A. Watkinson (Eds.), Microbial Interactions and Communities (Vol. 1). Academic Press, London.

Cairns, J., Jr., D. Kuhn, and J. Plafkin. 1979. Protozoan colonization of artificial substrates, P. 34–57. *In* R. Weitzel (Ed.), Methods and Measurement of Periphyton Communities: A Review, ASTM STP 620 American Society for Testing and Materials, Philadelphia, PA.

Davies, P., and J. Wooding. 1980. Importance of laboratory-derived metal toxicity results in predicting in-stream response of resident salmonids, P. 181–299. *In* J. Eaton, P. Parrish, and A. Hendricks (Eds.), Aquatic Toxicology: Third Symposium, ASTM STP 707. American Society for Testing and Materials. Philadelphia, PA.

Gatt, R., and E. Berman. 1966. A rapid procedure for the estimation of amino sugars on a microscale. Anal. Biochem. *15*:167–171.

Giesy, J., and E. Odum. 1980. Microcosmology: Introductory comments, P. 1–13. *In* J. Giesy (Ed.), Microcosms in Ecological Research, National Technical Information Service, Springfield, VA.

Hunn, J., S. Hamilton, and D. Buckler. 1987. Toxicity of sodium selenite to rainbow trout fry. Water Res. *21*:233–238.

Lemly, A. 1985. Toxicology of selenium in a freshwater reservoir: Implications for environmental hazard evaluation and study. Ecotoxicol. Environ. Safety. *10*:314–338.

Lewis, M., M. Taylor, and R. Larsen. 1986. Structural and functional response of natural phytoplankton and periphyton communities to a cationic surfactant with considerations of environmental fate, P. 241–268. *In* J. Cairns, Jr. (Ed.), Community Toxicity Testing, ASTM STP 920. American Society for Testing and Materials, Philadelphia, PA.

McConnell, W. 1962. Productivity relations in carboy microcosms. Limnol. Oceanogr. *7*:335–343.

Niederlehner, B., J. Pratt, A. Buikema, Jr., and J. Cairns, Jr. 1985. Laboratory effects evaluating the effects of cadmium on freshwater protozoan communities. Environ. Toxicol. Chem. *3*:155–165.

Nordlie, K., and J. Arthur. 1981. Effects of elevated water temperature on insect emergence in outdoor experimental channels. Environ. Pollut. Ser. A *25*:53–56.

Odum, E. 1985. Trends expected in stressed ecosystems. BioScience *35*:419–422.

Odum, E., J. Finn, and E. Franz. 1979. Perturbation theory and the subsidy-stress gradient. BioScience *29*:349–352.

Owsley, J., and D. McCauley. 1986. Effect of extended sublethal exposure to sodium selenite on *Ceriodaphnia affinis*. Bull. Environ. Contam. Toxicol. *36*:876–880.

Pick, F. 1987. Carbohydrate and protein content of lake seston in relation to plankton under nutrient deficiency. Can. J. Fish. Aquat. Sci. *44*:2095–2101.

Pratt, J., N. Bowers, B. Niederlehner, and J. Cairns, Jr. 1988a. Effects of atrazine on freshwater microbial communities. Arch. Environ. Contam. Toxicol. *17*:449–457.

Pratt, J., B. Niederlehner, N. Bowers, and J. Cairns, Jr. 1988b. Effect of chlorine on microbial communities in naturally derived microcosms. Environ. Toxicol. Chem. *7*:679–687.

Pratt, J., B. Niederlehner, N. Bowers, and J. Cairns, Jr. 1987. Prediction of permissible concentrations of copper from microcosm toxicity tests. Toxic. Assess. *2*:417–436.

Rausch, P. 1981. The estimation of microalgae protein content and its meaning to the evaluation of algal biomass, V: Comparison of methods for extracting protein. Hydrobiologia *78*:237–251.

Reading, J., and A. Buikema, Jr. 1983. Chronic effects of selenite-selenium on *Daphnia pulex*. Arch. Environ. Contam. Toxicol. *12*:399–404.

Rodgers, J., Jr., K. Dickson, and J. Cairns, Jr. 1979. A review and analysis of some methods used to measure functional aspects of periphyton, P. 142–167. *In* R. Weitzel (Ed.), Methods and Measurements of Periphyton Communities: A Review, ASTM STP 690. American Society for Testing and Materials, Philadelphia, PA.

Sayler, G., M. Puziss, and M. Silver. 1979. Alkaline phosphatase assay for freshwater sediments: Application to perturbed sediment systems. Appl. Environ. Microbiol. *38*:922–927.

Sokal, R., and F. Rohlf. 1983. Biometry. W.H. Freeman, New York. 859 p.

U.S. Environmental Protection Agency. 1985. Methods for the Chemical Analyses of Water and Wastewater, EPA 600/4-79-020. National Technical Information Service, Springfield, VA.

U.S. Environmental Protection Agency. 1986. Quality Criteria for Water 1986. EPA440/5-86-001. Office of Water Regulations and Standards, Washington, DC.

U.S. Environmental Protection Agency. 1987. Ambient Water Quality Criteria for Selenium—1987. EFA440/5-87-006. Office of Research and Development, Duluth, MN.

Zischke, J., J. Arthur, K. Nordlie, R. Hermanutz, D. Standen, and T. Henry. 1983. Acidification effects on macroinvertebrates and fathead minnows (*Pimphales promelas*) in outdoor experimental channels. Water Res. *17*:47–63.

TECHNICAL METHODS SECTION

A Microcosm Procedure for Estimating Ecological Effects of Chemicals and Mixtures*

JAMES R. PRATT and N.J. BOWERS,

School of Forest Resources,
The Pennsylvania State University,
University Park, Pennsylvania 16802

1.0 OBJECTIVES

The artifical substrata–microcosm method is intended for use in predicting direct and indirect effects of chemicals and chemical mixtures on whole, naturally derived, aquatic microbial communities (ecosystem surrogates). The method includes examination of effects on microbial production, respiration, nutrient dynamics, enzyme activities, and species richness.

2.0 SCOPE

The method is designed for testing responses of surface-dwelling microbial communities from lentic or lotic systems to pure compounds, mixtures of chemicals, or complex mixtures such as effluents.

3.0 PRINCIPLE

Microbial communities developing on surfaces (Aufwuchs) are diverse and exhibit properties analogous to those of ecosystems: nutrient cycling, energy and matter processing, succession, and high species richness. These communities have been collected on inert artificial substrata and manipulated in the laboratory

* Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army.

Toxicity Assessment: An International Journal Vol. 5, 189–205 (1990)
© 1990 John Wiley & Sons, Inc. CCC 0884-8181/90/020189-017$04.00

in replicate microcosms (Niederlehner *et al.*, 1985; Pratt *et al.*, 1987a,b; Pratt *et al.*, 1988a,b).

The microcosm method using natural, surface-dwelling microbial communities is based on several assumptions. First, source ecosystems are assumed functionally equivalent. This does not mean that ecosystem functions occur at the same rate in all systems, but that systems share comparable functions mediated by unique combinations of taxa. Second, the method assumes the microcosms represent some of the properties of the source ecosystem that may be sensitive to toxic stress, such as energy flow, nutrient cycling, and biological structure. The operational hypothesis for testing toxic effects is that as toxic effects occur, stressed microcosms will display altered properties relative to controls.

3.2 The artificial substrata-microcosm method can be summarized as follows. Natural aquatic microbial communities collected on polyurethane foam (PF) artificial substrata are exposed to various concentrations of chemical or effluent in laboratory microcosms either statically or under continuous flow. Community-level responses are monitored and chosen to include ecologically meaningful variables. Microbial communities are tested in replicate microcosms and controlled with respect to most environmental conditions, allowing powerful statistical analyses of results and predictions of environmental impacts as a result of chemical exposure.

4.0 MATERIALS, REAGENTS, AND APPARATUS

4.1 Materials

4.1.1 Materials for Construction of Test System

Tanks (glass, polyethylene, or stainless steel)
Medium-density PF, washed
String or nylon line
Plastic hooks
Lights (CRI > 90, e.g., Vita Lites, DuroTest, Corp.)
Timers (for lights)
Overflow boxes, drains
Activated carbon dechlorinator or alternative source of diluent water
Silicone sealant

Peristaltic pump (0.5–10 mL/min, 1–20 heads)
Diluter or flow splitter with mixing chambers
Reservoir(s) for toxicant solutions
Headbox
Tygon tubing

4.1.2 *Materials for Laboratory Analysis*

Automatic pipettes (10 μL to 10 mL)
Test tubes (13 × 100 mm and 16 × 125 mm)
Microscope slides and cover glasses
Protistan taxonomic references

4.2 Reagents

Reagent grade chemicals and glass-distilled deionized water (dd water) or equivalent should be used unless otherwise stated.

4.2.1 *Reagents for Total Protein Determination*

BioRad Protein Determination Kit *or*,
Bradford dye binding reagent (DBR):

100 mg Coomassie Brilliant Blue
50 mL methanol
100 mL phosphoric acid
Dilute to 1 L with dd water
Filter through Whatman No. 1 filter paper

0.5 N NaOH
1.0 mg/mL bovine serum albumin (BSA) in 0.5 N NaOH

4.2.2 *Reagents for Chlorophyll Analysis*

Saturated magnesium carbonate (1.0 g $MgCO_3$ in 100 mL dd water)
Buffered aqueous acetone (spectrograde, 90 parts acetone plus 10 parts saturated magnesium carbonate)
0.1 N HCl

4.2.3 *Reagents for Hexosamine Analysis*

1.0 mg/mL glucosamine in dd water
Concentrated HCl

2 M $NaCO_3$; 1.5 M $NaCO_3$
2% acetlyacetone in 1.5 M $NaCO_3$
Absolute ethanol
Ehrlich's reagent (1.0 g dimethylaminobenzaldehyde in 15 mL concentrated HCl and 15 mL absolute ethanol)

4.2.4 Reagents for Carbohydrate Analysis

1.0 mg/mL glucose in dd water
75% (v/v) sulfuric acid
Anthrone reagent (2 mg/mL anthrone in 75% sulfuric acid)

4.2.5 Reagents for Alkaline Phosphatase Analysis

1.0 M Tris-HCl buffer, pH 8.6
0.2 M Tris-HCl buffer, pH 7.6
1 mg/mL *p*-nitrophenyl phosphate (p-NPP) in 0.2 M Tris-HCl
10 μmole *p*-nitrophenol/mL (p-NP, Sigma Chemical Co.)
1.0 N NaOH

4.3 Apparatus

Dissolved oxygen meter and probe
pH meter
Conductivity meter
Tabletop centrifuge (300 × *g*; approximately 1500 rpm)
Spectrophotometer (visible)
Water bath (up to 100°C)
Compound microscope

5.0 MICROCOSM DESIGN

5.1 Tanks

Shallow tanks, pans, or aquaria are used to contain the biological components and medium. These containers should have a capacity of 6–12 L and should be made of a material compatible with the intended toxicant. Small plastic hooks used to attach artificial substrata to the bottom of the tanks should be affixed with nontoxic silicone sealant. The number and placement of the hooks will be determined by the number of substrata used, typically 4–6. Each experiment consists of 4–5 exposure groups

and a control group tested in triplicate, for a total of 15-18 microcosms.

5.2 Artificial Substrata

Artificial substrata are cut from a single sheet of medium density polyurethane foam (PF). Each substratum should be a hexahedron measuring $4 \times 5 \times 6$ cm. One to two substrata per microcosm will be placed in a natural ecosystem to accumulate the native microbiota for use as species sources ("epicenters") in each microcosm. PF substrata should be washed overnight in distilled water to remove nitrogenous by-products of urethane polymerization. An additional 4–5 substrata per microcosm are needed as colonizable habitat ("islands"). A typical experiment with 6 treatments and 1 epicenter and 4 island substrata per treatment, would require 90 substrata.

Each substratum is tied tightly in the middle, perpendicular to the longest dimension using either cotton string or nylon monofilament line. A method of attaching the substratum to hooks on the bottom of the microcosm tanks should be incorporated, such as a loop or ring, large enough to pass over a hook, but not so large as to allow the substratum to float at the surface of the water.

5.3 Collection of Source Communities

PF substrata are placed in shallow water (<1 m) for colonization by littoral microbiota using a concrete block or similar weight to hold substrata under water. In lotic systems, maximum species accumulation on substrata occurs in about 3–10 days and in lentic systems, in about 14–21 days (Cairns *et al.*, 1979). The number of substrata placed in the source ecosystem should exceed the number required by 20%, to account for possible loss of substrata. Also, at the start of the experiment, three extra colonized substrata are needed for community evaluation (see Section 8.1).

After the colonization period, substrata are collected en masse and returned to the laboratory in an insulated container partially filled with source water. These colonized substrata are referred to as "epicenters" and provide microbiota for the colonization of barren "island" substrata that are placed in micro-

cosms at the start of the test. The island substrata are then collected at intervals (e.g., weekly) after the start of a test to monitor the development and function of microbial communities.

5.4 Test Medium

The test medium may be water or water and sediment. Guidelines for the collection, storage, and use of dilution water should be followed (Horning and Weber, 1985). Laboratory water (i.e., dechlorinated tap water or reconstituted water) or site-specific water may be used as a diluent. It is important to monitor dechlorinated tap water for the presence of chlorine at the start and end of every test. The amperometric method [American Public Health Association (APHA), 1985] is satisfactory if a micropipette (5 μL) is used to deliver the phenylarsine oxide titrant. Total residual chlorine should never exceed 10 μg/L.

6.0 MICROCOSM TEST PROCEDURE

6.1 Start-Up

Prior to the start of the test, three of the colonized substrata should be randomly selected for evaluation. A decision must be made about the adequacy of the source community on the substrata before the experiment is started (see Section 8.1). If the communities are adequate, then the collected epicenters are randomly allocated to microcosms containing island substrata and media. Additionally, subsamples of reference substrata should be saved for later analysis of the same response variables to be monitored in island substrata (see Section 6.5.2).

6.2 Static Tests

Microcosms are filled with appropriate test solutions and the level marked on the side of each microcosm. Water lost through evaporation should be replaced with dd water as needed. Barren island PF substrata (4 or 5) are placed around the perimeter of the microcosms and squeezed at the start of the test to fill them with test medium. One or two colonized epicenter substrata are added to the center of the microcosm.

6.3 Continuous Flow Tests

Toxicant solutions of differing concentrations may be supplied to test microcosms by several methods. A gravity-fed proportional diluter, constructed from glass (Benoit *et al.*, 1982), is relatively simple to build, requires only a single concentration of primary stock toxicant solution, and can be used for effluent testing. Alternatively, a flow splitter (Maki, 1977) and 15–18 mixing chambers may also be used. The rate of flow should provide a minimum of 5 volume turnovers per day.

In continuous flow tests, 4 or 5 island substrata are placed toward the overflow end of experimental tanks, equidistant from each other and the colonized epicenter substratum, which is placed at the influent end. Toxicant-amended dilution water flows over and past the substrata prior to draining through holes in the end wall of the microcosms. Daily maintenance is necessary to ensure proper flow rates and adequate toxicant stock solution.

6.4 Environmental Conditions

Microcosms should be lighted with daylight-equivalent bulbs (Color Rendering Index >90; 1000–2000 lux) to permit growth of autotrophic organisms. Automatic timers for photoperiod regulation should be used. Ambient laboratory temperatures are typically sufficient to permit continuous flow tests to be conducted at uncontrolled temperatures. An environmental chamber or water bath may be needed for static tests to prevent over heating due to lights.

Temperature, pH, dissolved oxygen, and conductivity in each tank should be recorded at the start of the test (APHA, 1985), and weekly thereafter. Hardness and alkalinity should be determined weekly on water collected from the control and highest concentration microcosms. At the start and end of the experiment, water samples should be collected from each microcosm for toxicant analysis, or more often if possible.

6.5 Microbial Community Responses

6.5.1 Production/Respiration Ratio

Gross photosynthesis/respiration (P/R) ratios within each microcosm are determined weekly using the three point dissolved

oxygen method of McConnell (1962). Microcosms must be maintained on a 12-h light:12-h dark cycle for these comparisons. Microcosms are covered with clear plastic to reduce oxygen diffusion (Giddings and Eddlemon, 1978) and the dissolved oxygen in each microcosm is measured at the beginning of a dark cycle (D1), the beginning of the following light cycle (D2), and the beginning of the next dark cycle (D3):

$$\begin{aligned} D1 - D2 &= \text{nighttime respiration} \\ D3 - D2 &= \text{net production} \\ \frac{D3 - D2}{D1 - D2} &= \text{Estimated P/R ratio} \end{aligned}$$

6.5.2 *Biochemical Analyses of Microbial Communities*

Each island substratum represents an experimental unit, and the development and function of the microbial communities on them monitored for stress effects due to chemical exposure. A number of community-level variables can be monitored, but here we present a selection of variables that we have found sensitive to toxic stress. Additional end points that may be monitored are listed at the end of this section.

Island substrata are usually sampled on a weekly basis for three to four weeks, since sufficient biomass for biochemical analysis does not accumulate on island substrata until after at least one week of exposure. A single island substratum is removed from each microcosm, placed into a clean collecting bag or beaker, and squeezed to remove as much of its contents as possible (>50 mL). This volume is recorded and the substratum discarded. At the end of the experiment, epicenter substrata are removed from control microcosms and analyzed in the same manner as island substrata.

Subsamples are removed for each assay by gentle but thorough mixing of each sample using an automatic pipette. The volume necessary for each assay varies depending on the amount of biomass accumulated on the substrata. The amount of sample needed may also vary if the toxicant has substantially decreased the amount of biomass available. Samples are concentrated for each analysis by centrifugation at 300 × *g* (approximately 1500 rpm) for 5 min. This gentle centrifugation does not collect the entire microbial community, but it collects many

of the fragile protistans that would be damaged under faster centrifugation.

6.5.2.1 *Protein.* Total biomass accumulated on island substrata is estimated by measuring protein. Material collected from substrata is extracted (Rausch, 1981) and protein concentration measured using the method of Bradford (1976) or a commercial kit. The detection limit is 5 μg protein, but the actual sensitivity is dictated by the amount of sample used.

1. Place 4–8 mL of sample in 13 × 100 mm test tubes and centrifuge.
2. Discard supernatant and resuspend pellet in 0.5 mL 0.5 N NaOH.
3. Place samples in a 90°C water bath for 10 min.
4. Remove and centrifuge. Save the supernatant and resuspend the pellet in 0.5 mL 0.5 N NaOH.
5. Repeat steps 3 and 4 and pool supernatants for a total extraction volume of 1.5 mL. The extract may be stored frozen up to six months before protein analysis.
6. Mix 0.1 mL extract and 5.0 ml DBR or BioRad reagent with a vortex mixer and wait 15 min.
7. Measure the absorbance at 595 nm.
8. Standards (5–100 μg BSA in 0.5 N NaOH) are analyzed the same as samples.
9. Calculate μg protein/mL in each sample by accounting for volume of original sample used, extract volume (1.5 mL), and assay sample size (0.1 mL):

$$\mu\text{g protein/mL} = 10 \times (\mu\text{g protein/mL} \times 1.5\ \text{mL})/\text{mL sample}$$

6.5.2.2 *Chlorophyll* a. The biomass of autotrophic organisms accumulated on island substrata is estimated by measuring chlorophyll *a*. The spectrophotometric method (Method 1002G; APHA, 1985) is summarized below. The sensitivity of this method is determined by the amount of sample used. A 1, 5, or 10 cm path-length cuvette may be used.

1. Place 15–30 mL of sample in 16 × 125 mm tubes or 50 mL tubes if necessary and centrifuge.
2. Discard the supernatant and resuspend the pellet in 10 mL 90% buffered aqueous acetone. Extract overnight at 4°C in the dark.
3. Clarify samples by centrifugation and measure the absorbance of 3 mL of extract at 750 and 664 nm.
4. To correct for phaeopigments, add 0.1 mL 0.1 N HCl to the

sample and mix gently. After exactly 90 s, measure the absorbance at 750 and 665 nm.

5. Correct the A_{664} and A_{665} readings by subtracting the appropriate A_{750} readings and use the corrected values in the subsequent calculations:

$$\mu g/L \text{ Chl a} = [26.7\ (A_{664} - A_{665}) \times V_1/V_2 \times L] \times 1000$$

$$\mu g/L \text{ Phaeo.} = \{26.7\ [1.7(A_{665}) - A_{664}] \times V_1/V_2 \times L\} \times 1000$$

where V_1 is the extract volume (0.01 L), V_2 is the sample volume (0.015–0.030 L), and L is the path length of cuvette (1, 5, or 10 cm).

6.5.2.3 Hexosamine. The biomass of chitin-containing microbiota on artificial substrata is estimated by measuring hexosamine (= chitin; Gatt and Berman, 1966). This typically includes some species of bacteria, fungi and micro-arthropods. The detection limit is 0.5 μg hexosamine, but the actual sensitivity is dictated by the amount of sample used.

1. Place 4–8 mL of sample in 13 × 100 mm test tubes and centrifuge.
2. Discard supernatant and resuspend pellet in 1.0 mL distilled deionized water. The samples may be frozen at this point for up to 6 months.
3. Split the sample into duplicate 0.5 mL subsamples and add 0.1 mL concentrated HCL.
4. Cover samples with foil and place in a boiling water bath for 4 h. Remove and cool.
5. Add 0.4 mL 2.0 M $NaCO_3$ to each tube and mix.
6. Add 0.5 mL 2.0% acetylacetone to each tube, mix, and place in a boiling water bath for 20 min.
7. Remove and cool. Add 1.0 mL absolute ethanol and mix.
8. Slowly add 0.5 mL Ehrlich's Reagent and let the precipitate to dissolve.
9. Measure absorbance at 630 nm.
10. Standards (10–100 μg glucosamine) are analyzed the same as samples.
11. Calculate μg hexosamine/mL in each sample by accounting for the volume of original sample used and assay sample size (0.5 mL):

$$\mu g \text{ hexosamine/ml} = 2 \times (\mu g \text{ hexosamine/mL sample used})$$

6.5.2.4 Carbohydrate. Carbohydrate analysis estimates the amount of

to carbohydrate has been used as an indication of nutrient limitation (Pick, 1987). The detection limit of the assay is 1 μg carbohydrate, but the actual sensitivity is dictated by the amount of sample used.

1. Place 4–8 mL of sample in 13 × 100 mm test tubes and centrifuge.
2. Discard supernatant and resuspend pellet in 1.0 mL distilled water. Samples may be stored frozen at this point up to 6 months.
3. Place 0.5 mL of resuspended sample in 13 × 100 mm test tubes and place in an ice bath.
4. Prepare anthrone reagent (Section 4.2.4), keep cold.
5. Add 3.0 mL anthrone reagent to each sample and mix with a vortex mixer. Wait 5 min.
6. Place samples in a boiling water bath for 10 min.
7. Remove samples and cool under running water.
8. Measure the absorbance at 625 nm.
9. Standards (10–100 μg glucose) are analyzed the same as samples.
10. Calculate μg carbohydrate/mL in each sample by accounting for the volume of original sample used and assay sample size (0.5 mL):

μg carbohydrate/mL = 2 × (μg carbohydrate/mL sample used)

6.5.2.5 Alkaline Phosphatase Activity. Alkaline phosphatase activity measures the rate of cleavage of organic phosphorus compounds by the microbial community. A modification of the method of Sayler *et al.* (1979) is used. The level of detection is 1 nmole p-nitrophenol/mL.

1. Place 2–4 ml of sample in 13 × 100 mm test tubes and centrifuge.
2. Discard supernatant and resuspend pellet in 1.0 mL 1.0 M Tris-HCl buffer (pH 8.6).
3. Add 0.25 mL of 1.0 mg/mL p-NPP and incubate at room temperature for 1 h.
4. Add 0.25 mL 1.0 N NaOH to each sample and mix.
5. Centrifuge and transfer supernatant to clean test tubes. Measure the absorbance 420 nm.
6. Standards (1–100 nmole p-NP/mL) are made by appropriate dilutions of the stock p-NP with 1.0 M Tris-HCl buffer.

7. Initially calculate nmole p-NP/mL/h by

$$\underset{\text{(in sample)}}{\text{nmole p-NP/mL/h}} = \underset{\text{(from assay)}}{\text{(nmole p-NP/mL/h)/mL sample}}$$

8. Using the protein data, calculate nmole p-NP/mg protein/h by

$$\text{nmole p-NP/mg pro./h} = \text{(nmole p-NP/mL/h)/(mg pro./mL)}$$

6.5.3 *Taxonomic Analyses of Microbial Communities*

Depending on the available taxonomic expertise, one or more components of the microbial community may be monitored. Protozoan species composition has been shown to be a sensitive indicator of toxic stress (Niederlehner *et al.*, 1985; Pratt *et al.*, 1987a,b; Pratt *et al.*, 1988a,b). Identification of protozoa must be done within 12 h of sampling and must be done with live organisms, since fixation distorts many protozoan species beyond recognition (see Cairns *et al.*, 1979, for taxonomic references). Algal species in fixed samples can be identified and enumerated using a Palmer-Maloney counting chamber (APHA, 1985).

Identification and quantification of rotifers and other micrometazoa can be made with a Sedgwick–Rafter counting chamber (APHA, 1985).

6.5.4 *Optional Analyses of Artificial Substrata*

Ash free dry weight (APHA, 1985)
Nutrients (APHA, 1985)
Adenosine triphosphate (APHA, 1985)
Electron transport system (Jones and Simon, 1979)
Nitrate reductase (Lobbar. *et al.*, 1988)
Bacterial enumeration (Porter and Feig, 1980)

7.0 EXPRESSION OF RESULTS

Measured toxicant concentrations should be used in data anal-

be explained. If an effluent is tested, then percent dilutions of the original effluent are used.

7.1 Analysis of Variance and Multiple Comparisons

Response differences among control and treatment groups on a particular sampling day for a given response are determined using analysis of variance (ANOVA; Sokal and Rohlf, 1983). If ANOVA is significant ($p < 0.05$), multiple comparisons are made to determine at which concentration(s) the response differs using Duncan's Multiple Range test (Sokal and Rohlf, 1983) or Dunnett's test (Dunnett, 1955). Dunnett's test references treatment differences to the controls.

For responses significantly affected by toxicant exposure, the no effect concentration (NOEC, the highest toxicant concentration at which the response is not significantly different from controls) and the lowest effect concentration (LOEC, the lowest toxicant concentration at which the response differs significantly from controls) are reported. The maximum allowable toxicant concentration (MATC) is the geometric mean of the NOEC and LOEC.

7.2 Dose-Response Estimators

Ordinary least squares regression of a response variable against toxicant concentration (exclusive of controls) can be used to investigate the dose-response relationship. Usually the regression will be linear with respect to the logarithm of the toxic dose. When a significant regression is determined, the predicted regression equation can be used to inversely predict toxicant concentrations producing a given proportional reduction in response, commonly called an effective concentration (EC; Sokal and Rohlf, 1983). The EC is commonly reported for concentrations at which a 5, 20, and 50% reduction in response relative to controls is predicted (EC_{05}, EC_{20}, and EC_{50}, respectively). In some cases, low toxicant doses may produce an enhanced response, which may complicate analyses based on linear regression. In such cases, prediction of ECs using linear regression would be inappropriate unless an adverse response was defined as a response which is less than control. When enhancement of a response differs significantly from control, this difference can be interpreted as an adverse response if

"adverse" is defined as any significant deviation from the nominal (control) state.

8.0 PERSONAL REMARKS

8.1 Quality Assurance

Microcosm experiments must be conducted under conditions that allow valid interpretation of findings. We have conducted numerous experiments with natural microbial communities and provide recommendations for evaluating the quality of experiments. Most of our sampling and experimentation has been restricted to ecosystems in the eastern United States, and we have not examined community dynamics for the full range of conditions likely to be encountered by other investigators.

At the beginning of a microcosm experiment, three reference artificial substrata should be examined to determine adequate taxonomic richness and acceptable intersubstratum variability. Colonizing communities usually have a minimum of 25 genera of protozoa or algae (including filamentous, colonial, and unicellular forms of blue-greens, greens, diatoms, and other algae). We have found the coefficient of variation for taxonomic measures is typically less than 10%. We recommend that if at least 15 genera of protozoa or algae cannot be identified, or if the coefficient of variation for taxonomic measures is greater than 20%, consideration should be given to abandoning the experiment until more representative communities can be obtained. These suggestions are arbitrary limits that need to be interpreted in light of knowledge of the source ecosystem.

At the end of an experiment, analysis of epicenter substrata from control microcosms should show increased biomass compared to reference substrata examined at the start of an experiment. Epicenter species richness should not be less than 90% of that on the reference substrata at the beginning of the experiment. Again, these are arbitrary limits, but our experience shows that failure to achieve these conditions may be cause to invalidate a given experiment.

8.2 Selection of Community Responses

The choice of community responses monitored in an artificial substrata-microcosm test depends on several factors, one of

TABLE I

Coefficients of variation (CV) for several microcosm responses and the minimal detectable distance (as a percentage of the control value) for each response based on an $\alpha = 0.05$ and $\beta = 0.2$ under two experimental designs (Conquest, 1983)

Response	n	CV	Minimal detectable distance (%)	
			$k = 6, n = 3$	$k = 5, n = 3$
Protozoan species	28	9.6	23.9	24.3
Protein	27	19.8	49.3	50.2
Alkaline phosphatase	25	17.2	42.9	43.6
Chlorophyll	21	24.5	61.0	62.1
Hexosamine	13	27.8	69.3	70.5
Carbohydrate	15	22.5	56.1	57.1
Fluorescence units	7	16.3	40.6	41.3
Potassium	9	16.1	40.1	40.8
Magnesium	8	7.2	18.0	18.3
Calcium	8	9.6	23.9	24.3
Ash free dry weight	4	29.9	74.5	75.9
Phosphate	5	25.9	64.5	65.7
P/R	5	8.4	21.0	21.4
ATP	2	39.7	98.9	101

which is the inherent variability of the response, and subsequent ability to detect differences among treatments based on that response. The average coefficient of variation (CV) of control group responses measured in previous artificial substrata-microcosm experiments are shown in Table I. The minimal detectable distance (MDD, as a percentage of the control) expected for each response based on its CV at $\alpha = 0.05$ and $\beta = 0.2$ (Conquest, 1983) is also shown. MDDs were calculated for two experimental designs: six treatments ($k = 6$) with three replicates ($n = 3$) or five treatments ($k = 5$) with three replicates ($n = 3$).

Several scientists contributed to the development of these procedures: J. Cairns, Jr., Barbara R. Niederlehner, and Frank S. Stay are specifically acknowledged for their assistance. Rita Ciresi provided editorial assistance. This work was supported in part by the U.S. Army Medical Research and Development Command under contract No. DAMD17-88-C-8068.

References

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1985. Standard Methods for the Examination of Water and Wastewater (16th ed.). Washington, DC, 1193 p.

Benoit, D., V. Mattson, and D. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Res. *16*:457–464.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein, utilizing the principle of protein-dye binding. Anal. Biochem. *72*:248–254.

Cairns, J., Jr., D. Kuhn, and J. Plafkin. 1979. Protozoan colonization of artificial substrates, P. 34–57. *In* R.L. Weitzel (ed.), Methods and Measurements of Periphyton Communities: A Review, STP 690. American Society for Testing and Materials, Philadelphia, PA.

Conquest, L. 1983. Assessing the statistical effectiveness of ecological experiments: utility of the coefficient of variation. Intern. J. Environ. Stud. *20*:209–221.

Dunnett, C. 1955. Multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. *50*:1096–1121.

Gatt, R., and E. Berman. 1966. A rapid procedure for the estimation of amino sugars on a microscale. Anal. Biochem. *15*:167–171.

Giddings, J., and G. Eddlemon. 1978. Photosynthesis/respiration ratios in aquatic microcosms under arsenic stress. Water Air Soil Pollut. *9*:207–212.

Horning, W., and C. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Environmental Monitoring and Support Laboratory. United States Environmental Protection Agency, Cincinnati, OH.

Jones, J., and B. Simon. 1979. The measurement of electron transport system activity in freshwater benthic and planktonic samples. J. Appl. Bacteriol. *46*:305–315.

Lobban, C., D. Chapman, and B. Kremer. 1988. Experimental Phycology: A Laboratory Manual. Cambridge University Press, Cambridge. pp. 197–202.

Maki, A. 1977. Modifications of continuous-flow toxicity test methods for small aquatic organisms. Prog. Fish-Cult. *39*:172–174.

McConnell, W. 1962. Productivity relations in carboy microcosms. Limnol. Oceanogr. *7*:335–343.

Niederlehner, B., J. Pratt, A. Buikema, Jr., and J. Cairns, Jr. 1985. Laboratory tests evaluating the effect of cadmium on freshwater protozoan communities. Environ. Toxicol. Chem. *3*:155–165.

Pick, F. 1987. Carbohydrate and protein content of lake seston in relation to plankton nutrient deficiency. Can. J. Fish. Aquat. Sci. *44*:2095–2101.

Porter, K. and Y. Feig. 1980. The use of DAPI for identification and enumeration of aquatic microflora. Limnol. Oceanogr. *25*:943–948.

Pratt, J., B. Niederlehner, N. Bowers, and J. Cairns, Jr. 1987a. Prediction of permissible concentrations of copper from microcosm toxicity tests. Tox. Assess. *2*:417–436.

Pratt, J., B. Niederlehner, N. Bowers, and J. Cairns, Jr. 1987b. Effects of zinc on freshwater microbial communities, P. 324–326. *In* S.E. Lindberg and T.C. Hutchinson (eds.), International Conference on Heavy Metals in the Environment, New Orleans. CEP Consultants Ltd., Edinburgh.

Pratt, J., N. Bowers, B. Niederlehner, and J. Cairns, Jr. 1988a. Effects of atrazine on freshwater microbial communities. Arch. Environ. Contam. Toxicol. *17*:449–457.

Pratt, J., B. Niederlehner, N. Bowers, and J. Cairns, Jr. 1988b. Effect of chlorine on microbial communities in naturally derived microcosms. Environ. Toxicol. Chem. *7*:679–687.

Rausch, P. 1981. The estimation of microalgae protein content and its meaning to the

evaluation of algal biomass, V: Comparison of methods for extracting protein. Hydrobiologia *78*:237–251.

Sayler, G., M. Puziss, and M. Silver. 1979. Alkaline phosphatase assay for freshwater sediments: application to perturbed sediment systems. Appl. Environ. Microbiol. *38*:922–927.

Sokal, R. and F. Rohlf. 1983. Biometry. W.H. Freeman, New York. 859 p.

Publication Agreement

Under U.S. law, the transfer of copyright from the author should be explicitly stated in writing to enable the publisher to disseminate the author's work to the fullest extent. Please complete a copy of the following Publication Agreement and *include it with all submissions to this journal.* Please note that if the manuscript was a work made for hire, this agreement must be signed by the employer, as author.

Publication Agreement

The undersigned author has submitted a manuscript entitled_____

_____

for publication in_____, published by John Wiley & Sons, Inc.

A. The author transfers to John Wiley & Sons, Inc. the exclusive rights comprised in the copyright of the article, if the article is accepted for publication, except that the author retains the following:

1. All proprietary rights, other than copyright, such as patent rights.
2. The right to make copies of all or part of the work for the author's use in classroom teaching.
3. The right to use, after publication, all or part of this material in a book by the author.
4. The right to make copies of the work for internal distribution within the institution which employs the author.
5. The right to use figures and tables from the work, and up to 250 words of text, for any purpose.
6. The right to make oral presentation of the material in any forum.
7. The right to veto or approve permission granted by the publisher to a third party to republish all or a substantial part of the article.

In the case of work prepared under U.S. Government contract, the U.S. Government may reproduce, royalty-free, all or portions of the article and may authorize others to do so, for official U.S. Government purposes only, if the contract so requires.

The author agrees that all copies made under any of the above conditions will include notice of the copyright of the publisher.

B. The author warrants that the manuscript is the author's original work. If the work was prepared jointly the author agrees to inform co-authors of the terms of this agreement. It is submitted only to this journal, and has not been published before. (If excerpts from copyrighted works are included, the author will obtain written permission from the copyright owners and show credit to the sources in the manuscript.) The author also warrants that the article contains no libelous or unlawful statements, and does not infringe on the rights of others.

_____
Author's signature and date

Check one:
_____Employer; work made for hire
_____Work of the U.S. Government
_____Author's own work

_____
Typed or printed name

_____
Institution or company

Note to Government Employees

A work prepared by a government employee as part of his/her official duties is called a "work of the U.S. Government," and is not copyrightable. If it is not part of the employee's official duties it may be copyrighted. If this is a work of the U.S. Government, the author may cross out Section A of this agreement and attach a copy of applicable policy of author's agency.

If the work was prepared jointly, and any co-author is not a U.S. Government employee, that

Hydrobiologia **188/189**: 5–20, 1989.
M. Munawar, G. Dixon, C. I. Mayfield, T. Reynoldson and M. H. Sadar (eds)
Environmental Bioassay Techniques and their Application.
© 1989 *Kluwer Academic Publishers. Printed in Belgium.*

The scientific basis of bioassays

John Cairns, Jr.[1] & James R. Pratt[2]
[1]*University Center for Environmental and Hazardous Materials Studies and Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, U.S.A.;* [2]*School of Forest Resources, The Pennsylvania State University, University Park, Pennsylvania 16802, U.S.A.*

Key words: bioassay, ecotoxicology, stress, microcosm, mesocosm

Abstract

The ultimate goal of ecotoxicological testing is to predict ecological effects of chemicals and other stressors. Since damage should be avoided rather than corrected after it occurs, the predictive value of such tests is crucial. A modest base of evidence shows that, in some cases, extrapolations from bioassays on one species to another species are reasonably accurate and, in other cases, misleading. Extrapolations from laboratory bioassays to response in natural systems at the population level are effective if the environmental realism of the bioassay is sufficiently high. When laboratory systems are poor simulations of natural systems, gross extrapolation errors may result. The problem of extrapolating among levels of biological organization has not been given the serious attention it deserves, and currently used methodologies have been chosen for reasons other than scientific validity. As the level of biological organization increases, new properties are added (e.g., nutrient cycling, energy transfer) that are not readily apparent at the lower levels. The measured responses (or end points) will not be the same at all levels of biological organization, making the validation of predictions difficult. Evidence indicates that responses of ecologically complex laboratory systems correspond to predicted and documented patterns in stressed ecosystems. The difficulties of improving the ecological evidence used to predict adverse effects are not insurmountable since the essence of predictive capability is the determination of effects thresholds at all levels of organization. The dilemma between basing predictive schemes on either traditional or holistic methods can only be solved by facing scientific and ethical questions regarding the adequacy of evidence used to make decisions of environmental protection.

'When very little is known about an important subject, the questions people raise are almost invariably ethical. Then as knowledge grows, they become more concerned with information and amoral, in other words more narrowly intellectual. Finally, as understanding becomes sufficiently complete, the questions turn ethical again. Environmentalism is now passing from the first to the second phase, and there is reason to hope that it will proceed directly on to the third.'

E. O. Wilson,
'The Conservative Ethic', 1984
Reprinted by permission
of Harvard University
Press, *Biophilia*, p. 119.

Introduction

The agricultural and industrial revolutions emerged from the need to manage the human environment; the environmental revolution now seeks to manage ecosystems for their necessary services. Ecosystem services include the 'free' provision of biomass (food and fiber) and the assimilation of wastes. Earlier in this century, the wholesale disposal of wastes and alteration of ecosystems made ecosystems undependable in providing expected services: too few fish, undesirable species, poor drinking water quality. We acted to prevent further degradation, and gross pollution is now much less common in the industrialized world. Now we are interested in improving our risk assessment techniques to minimize hazards. We have, indeed, passed into a new information age. Important questions remain about how we obtain information to estimate and manage environmental hazards.

Both biological and chemical evidence are needed to assess risk to ecosystems effectively. The ability to detect a compound does not ensure that biological effects can be predicted, and the failure to detect a released chemical does not preclude its effects. Where several chemicals interact, the integration of effects by biological material is the *only* reliable evidence for predicting or detecting adverse impacts.

A bioassay is a procedure that uses living material to estimate chemical effects. In ecotoxicology, we use bioassays to predict the levels of chemicals that produce no observable adverse effects on populations, communities, and ecosystems and to identify biological resources at risk. This discussion gives a brief overview of the use of standard bioassays, examines possible ecosystem effects of stress and means for detecting these, and summarizes some of the ethical questions motivating needed current and future research.

Bioassays: an overview

Newcomers to the field of ecotoxicology are startled to learn that non-biological methods have been considered superior to biological methods for estimating the hazard to the environment of chemicals and other stressors (e.g., heated wastewater, suspended solids). When one of us (Cairns) left graduate school in 1948 to work with Ruth Patrick, one of her primary tasks was to convince regulatory personnel that biological evidence should be used in addition to chemical and physical evidence in protecting the environment. The chemical and physical determinations that were then the primary basis for regulatory decisions were quantitative, used generally understood methodology often endorsed as standard methods, and were familiar to the sanitary engineers and chemists who then dominated the water pollution control field.

Only a few biologists made any effort to provide methodology suitable for pollution assessment. The single species fish bioassay was introduced by Hart *et al.* (1945). Following its endorsement and publication (Doudoroff *et al.*, 1951), biological evidence became more common and eventually was broadened to include not only fish but invertebrates and algae. Stream surveys above and below industrial or municipal discharge were also popular, but these were not predictive. Although they recorded biological degradation after it occurred (the damage could frequently be reversed), they did not provide as good a management tool as the more predictive bioassays.

Aquatic toxicology is an outgrowth of mammalian toxicology (Sloof, 1983) which had as its primary focus the protection of a single species, *Homo sapiens*. Because of this, there was unfortunate but general acceptance of single species bioassays as appropriate for environmental toxicology. The difficulties of extrapolating from one species to another are well recognized in mammalian toxicology and have even been adequately documented in aquatic toxicology (e.g., Kenaga, 1978; Doherty, 1983; Mayer & Ellersieck, 1986). The scientific basis for bioassays currently used is limited simply because most of the basic scientific questions about extrapolation from one level of biological organization (e.g., species to ecosystem) remain unanswered.

It was quite understandable for early toxicologists to determine the responses of individual species to toxicants under conditions that would permit replication by others. This inevitably meant low environmental realism (i.e., relatively simple test systems), focusing on a few well-understood test species, and developing tests that could be used by modestly trained people. Practically all of the funding for research in the United States came from the regulatory agencies and, as far as we can determine, there were similar circumstances elsewhere.

Regulatory agencies, understandably, established funding priorities to address regulatory, not scientific problems. Macek (1982) describes the attitude of the regulatory agencies beautifully. 'I think the reason that the regulators tend routinely to ask for the same kinds of answers (data) and can't assimilate other types of data into the regulatory process is that to do otherwise would require some kind of scientific judgment on the part of the people implementing the process. Making scientific judgments about a science in which few fundamental principles and underlying concepts exist can be a very risky business. Thus, those in regulatory positions shy away from making such judgments and accepting the responsibility for them. Why should they risk it? It's much safer for job security to mechanize the process, make it objective, and avoid having to make scientific judgments and taking the attendant risks.'

We examine problems further in this discussion. The regulatory stance in the United States has been to develop a few tests and test species for aquatic ecosystems and provide instruction for performing these tests. Those wishing a relatively recent view of the U.S. Environmental Protection Agency position would do well to read Wall & Hanmer (1987). Unfortunately, the scientific basis and/or fundamental questions underlying the problem of prediction of hazard are not examined in any depth in that article. This is distressing in a 'feature article' in one of the world's leading pollution journals.

In the 1970s in the United States, a technological solution to the pollution problem was attempted that generally occurred under the umbrella of BAT (best applicable technology) or BPT (best practical technology). The assumption was that, if the best technology presently available or the best practical technology was installed for waste treatment, nothing more could be done to protect natural systems. Since the best available technology was being used, environmental measurements were not necessary.

This is an over-simplification of the situation that is discussed in somewhat greater detail in Cairns (1983). However, some of the fallacies of this assumption are quite evident: (1) the size and assimilative capacity of the ecosystem into which the waste is discharged is not factored into the regulatory decision, (2) each discharge is treated as if it were the only one in existence whereas in many areas there are multiple discharges located quite close together, (3) there could easily be 'over-treatment' (i.e., treatment that provided no additional biological benefits) that would go unnoticed without biomonitoring, (4) since industry would be required to install the latest technology, there would be no incentive for new technologies to be developed because personnel would have to be constantly retrained in the use of this technology. This would be very expensive and would not necessarily increase environmental protection. The technology-based standards were eventually modified, and the use of bioassays returned.

The purpose of bioassays

Bioassays are usually carried out as a means of determining the no-adverse-biological effects concentration of a chemical in the environment. In relatively few cases, bioassays may be carried out to determine if a particular waste treatment process has reduced the toxicity of an effluent or to determine why chemicals in mixtures are acting differently than one would expect from their individual behavior.

Although these exercises are ultimately related to the environment, they do not require direct extrapolation of effects to natural systems. However, the majority of the bioassays are carried out

with the assumption that the test organisms are surrogates for the larger body of organisms comprising natural ecosystems. Basically, bioassays are intended to predict harm or no harm after exposure of living organisms to certain concentrations of a chemical (or mixture of chemicals) for certain periods of time. They are not reactive in the sense of documenting harm after it is done, which is much better accomplished by *in situ* surveys (e.g., Cairns, 1982). Therefore, the scientific basis for using bioassays must ultimately depend upon the degree to which the accuracy of the predictions mad. with bioassays can be validated or confirmed in natural systems. To be scientifically justifiable, indirect evidence is not enough – that is, failure to observe adverse effects in ecosystems at concentrations predicted to have no harm must consist of direct evidence, not absence of evidence due to inattention, lack of sufficiently detailed study, or failure to study the right end points or parameters.

There is, unquestionably, scientific justification for using living material to detect toxicity since no instrument devised by man will do so. Therefore, bioassays are superior to predictions made on the basis of chemical/physical measurements alone or assumptions of no harm based on the quality of the technology of the waste treatment system. However, the confidence we can place in predictions based on bioassays would be vastly improved if more attention were given to validating these predictions in natural systems or surrogates thereof.

The standard single species bioassay (both acute and chronic) has often been used for purposes not directly associated with ecosystem protection or impact prediction. For example, Tebo (1985) summarized the objectives of bioassay tests used by the U.S. Environmental Protection Agency for establishing chemical limits and we quote them as follows from pages 20 and 21* of *Multispecies Toxicity Testing* (Cairns, 1985):

"Screening

Tests should be rapid and inexpensive and should have wide applicability. Response should have high sensitivity to stress so that there will be low possibility of false-negatives.

Establishing Limitations

Tests should be of known precision with exposures that simulate environmental exposures and should be applicable to a wide range of site-specific situations.

The response should be directly related to environmental hazard and should be easy to interpret and meaningful to the public and courts.

Outputs should be directly translatable into specific decision criteria.

To avoid possibilities of varying interpretation, it is preferable that the end point be a discrete variable. If the end point is not a discrete variable (such as death), justifiable decision criteria should be provided.

Monitoring

Tests should be rapid, inexpensive, and of known precision.

Response should be sensitive and preferably related to the type of limitation imposed.

The desirable attributes of tests used for regulatory purposes are a function of these objectives."

These requirements, however, fail to note the limitations of bioassays. The most well-developed test methods (in terms of standardization) are acute bioassays. Inspection of the known precision of such tests shows that coefficients of variation are commonly 50–100% of the LC50/EC50 estimators (c.f., Mayer & Ellersieck, 1986). The measured response (death) is only environmentally meaningful in the grossest sense (dead animals are a problem), hence their public meaningfulness and utility in courts of law. Biologically, acute toxicity is uninteresting in the sense that rapid death and destruction, even when relatively widespread, results in short-term disruption of ecosystem services during the recovery period (Cairns *et al.*, 1971) unless the acute concentration approaches the expected environmen-

* Reprint with permission, Pergamon Press.

tal concentration over a long period. Long-term effects are more serious in their disruption of ecosystem processes and typically occur at concentrations near chronic toxicity test end points which are often near expected environmental concentrations of trace contaminants.

Experimental results have been translated into decision criteria using the aging application factor approach. Methods using bioassay data to estimate impacts on the larger biota require comparatively large data sets upon which interpretation of dose-response is made and only a few taxa (est. 5%) are allowed to be affected.

The result of these limitations has been a truncation of normal, healthy scientific debate over the ecological meaning of the measured response variables (typically mortality, reproductive output, and growth). In this paper we provide evidence useful for developing decision criteria based on direct measurement of ecosystem structures and functions. Interestingly, USEPA has supplemented discrete variable measures in recent short-term chronic fish growth tests (Horning & Weber, 1985). The relationship between the statistical analysis of these tests and any environmental impact is only implicit in the expression of results. Typically, chronic tests are used to estimate a no-effect level which is used in establishing water quality criteria.

In arguing against the use of new or more robust methods, scientists often argue either that the methods are not well developed or are too complicated. Agencies responsible for managing the environment control many of the research and development funds that might be directed toward developing and testing ecologically appropriate methods. When funding is inadequate to fully develop methods, reliance must again be placed on older methods. Sources funding basic ecological research often see research with practical application as 'too applied' for funding under their aegis even though useful ecological information results from 'applied' experiments. So poor is the link between 'basic' and 'applied' ecosystem scientists that the National Academy of Sciences (which includes few applied scientists) commonly goes outside its membership to assemble the appropriate blue-ribbon panel for important deliberations on the environment (e.g., NRC, 1981, 1986).

Tebo (1985, pp. 21–22) suggests the following as essentials for the predictive utility of bioassays.

> 'To be useful for regulation, the results of toxicity tests must be evaluated based on:
> - *Interpretation.* What does the laboratory response mean in terms of environmental hazard?
> - *Extrapolation.* Do responses in the laboratory simulate responses in the receiving system?
> - *Sensitivity.* Is the response sufficiently sensitive to avoid excessive false-negatives? The sensitivity desired is a function of the objective of this study.
> - *Variability.* Is the precision known (or can it be determined) and is it sufficiently high that impacts can be detected?
> - *Replicability.* For regulatory purposes, toxicity tests must be sufficiently simple and standardized that they can be carried out by governmental, academic, and private laboratories of widely varying capabilities. There must be sufficient quality control to allow consistent interlaboratory and intralaboratory levels of precision.'

Attention to each of these points is essential for scientists developing new or more robust bioassay methods. While each of the questions posed seems reasonable and appropriate, procedures currently used to estimate risk fail on one or more criteria. Many test methods, including those using nontraditional methodologies, could meet these criteria if properly developed and validated. Each of the criteria mentioned by Tebo is discussed below.

Interpretation. Death, growth, and reproductive success are usually easily understood measures used in single species toxicity tests. Effects of toxicants on organisms under laboratory conditions might reasonably be expected to correspond to observed effects in real ecosystems, assuming that the realized environmental concentration of

a toxicant in the compartment of interest was comparable to that tested in the laboratory and that the route of exposure was the same (typically in the water column). Where processes such as bioconcentration of toxicant in food, adsorption by sediments, or microbial degradation, are important, then single species laboratory tests may be poor predictors of ecosystem effects because they lack necessary environmental realism and biotic interactions.

Extrapolation. Correspondence between test results (and criteria based on test results) and environmental hazard is poorly understood. In general, chronic discharge criteria for particular chemicals are based on protection of sensitive or economically important species, usually fish. In combination with assumptions about minimal stream flows (see Biswas & Bell, 1984), discharges may infrequently reach or exceed criterion levels for a particular toxicant. Whole effluent bioassays are probably more representative of the total toxic effect of the complex effluent mixture, but often fail to simulate in-stream conditions including the upstream contaminant background or modification on the toxicant or mixture in the actual receiving system. Additionally, since ecosystems probably differ widely in their resistance to toxicants, and factors other than water hardness probably add to uncertainty about the action of a particular toxicant in a particular ecosystem, extrapolation of test results on individual compounds is not only problematic but unrealistic.

Sensitivity. Response sensitivity is a resolution problem. That is, the level of biological organization tested, the duration of the test, and the inherent variability of the measured response affect the outcome of the test procedures. Biological systems sensitive to toxicant action occur in all ecosystems, but these may be the standard test species (Cairns, 1986). Rather, they may be enzyme systems or microbial consortia or fish reproductive systems. Sound scientific judgement is required to determine which responses are the most valid and practical measures of adverse responses. However, the sensitivity of a response may have little meaning unless it is highly correlated with observable adverse ecological effects.

Variability. Determination of acceptable variability is a methodological and statistical problem (Giesy & Allred, 1985). Depending on the inherent variability of the measured response, the number of experimental units required to detect a given change or difference can be estimated (Sokal & Rohlf, 1981; Green, 1979). The size of the experimental array may conflict with the availability of resources, but this is a practical problem separate from the underlying science.

Replicability. Replicability of tests may be a statistical problem. Here, Tebo means repeatability: repeating the test gives the same results time after time. In addition to standard methods for single species tests, procedures for conducting standardized toxicological assessments of many types are reviewed and published by several organizations and professional journals (e.g., ASTM, *Toxicity Assessment*). The level of expertise required to carry out standard methods varies widely. However, we should not tolerate substandard performance from laboratories or establish test methodologies based on some least common denominator of performance. Not every laboratory will be capable of carrying out every applicable assessment method, as has been shown by efforts to assess interlaboratory variability of results from traditional test methods (Buikema, 1983).

Tebo (1985, p. 23) also summarizes his perceptions of decision criteria in setting limits based on bioassay results:

'Decision criteria

- *Social Relevance.* Is the response meaningful to the public and the courts?
- *Technical Relevance.* Does the response provide a realistic measure of population-, community-, or ecosystem-level impact? Is it possible to provide margins of safety based on objective criteria?
- *Legal Relevance.* Is the response (end point) usable for establishing limitations on the discharge of a substance? If the response is a continuous variable, is there an objective means of establishing a limiting exposure

concentration to avoid hazard? The 'no-effect' level in terms of mortality, as determined in single species tests, is an example of perhaps the only discrete variable resulting from toxicity tests.

- *Cost and Timing.* Is the cost reasonable in terms of the objectives of the test? Cost is largely a function of the time necessary to conduct the tests, the space required, and the level of expertise necessary both to conduct the test and to evaluate results. Decisions as to cost are largely a function of the degree of certainty required.'

Each of Tebo's points are discussed below.

Social relevance. Results of biological testing need to be communicated to the public and the courts. Obviously, a well-informed public (and its decision makers) will only understand bioassay results and the predicted risks of environmental stressors if they have a basic understanding of the underlying environmental science. The public and courts will understand the importance of environmental protection and the measures needed to assure it if they are provided with accurate and understandable information by responsible environmental scientists. It is incorrect to assume that the public, the courts, and non-scientist decision makers can only understand the grossest effects (death) on the most familiar organisms (fish and other vertebrates).

Technical relevance. To date, testing procedures and criteria have focused on the population level of biological organization. Methods to examine community- and ecosystem-level effects are being developed and need to be incorporated into risk assessment schemes.

Legal relevance. 'No-effect' levels reported for standard tests are statistically based and have been used to establish water quality criteria. Appropriate experimental designs and statistical tests are the only objective means of determining at which concentrations a chemical might be hazardous. The nature of the measured response (growth, reproduction, productivity, respiration, enzyme activity) is comparatively unimportant in the outcomes of statistical procedures except where the method of data collection or the nature of the collected data causes violation of the assumptions underlying statistical models. Discrete data (such as mortality) presents special problems in statistical analysis of experimental results. Other discrete variables measured in toxicity tests include numbers of offspring and numbers of species (in the case of microcosm experiments), but these can often be assumed to be continuous for purposes of statistical analysis.

Cost and timing. Costs of traditional and non-traditional tests are surprisingly similar for many types of procedures. Most tests last from 1 to 6 weeks, although life cycle tests may require several months to a year or more to complete. The costs of risk assessments of differing kinds have been summarized by Perez & Morrison (1985), and several authors have provided cost estimates and comparisons for single and multispecies tests (see discussions in Cairns, 1986). Most investigators have developed test methods that require common laboratory space and equipment. Additionally, the costs (time, space) of culturing standard test species are not always considered in comparisons of novel methods and traditional test methods.

With an estimated 63000 chemicals in daily use (Maugh, 1978) and literally millions on the American Chemical Society Computer Registry of Chemicals (personal communication), it is clear that society cannot wait for the perfection of a totally scientifically justifiable bioassay method. We know that chemical information alone can be misleading for the following reasons: (1) environmental quality mediates chemical toxicity (e.g., water hardness, pH, dissolved organics); (2) chemicals may act differently individually and in mixture; (3) chemicals may produce toxicological effects at concentrations below analytical capability; (4) various transformations may occur making the chemical more or less toxic; (5) chemicals undergo partitioning in the environment, and making the measurements in the wrong compartments or in only one compartment may produce misleading information; (6) concentrations of a chemical may vary, but living organisms integrate the toxicological effects of this continuous but

varied exposure much better than can be done with nonliving models. Despite the weaknesses of chemical information alone, the correct interpretation of bioassay results would be impossible without accompanying chemical information. Neither biological nor chemical information should be examined alone when determining toxicological response, and impacts at different levels of biological organization need to be estimated.

Predicting and detecting ecosystem changes

Many concepts but few general principles for system level responses to human-induced stress exist in basic ecological theory or in environmental science as a separate discipline. The burgeoning of concepts is illustrated by a survey of the British Ecological Society (Cherrett, 1988) in which 236 concepts were identified by fewer than 1000 ecologists. Recent accounts of the application of basic theory to environmental problems (National Research Council, 1986) are helpful case studies of stress and summarize applicable theoretical paradigms that may apply to particular situations. However, predictable patterns of stress effects are elusive.

Ecosystems are hierarchically structured (Webster, 1979). New properties emerge at increasing levels of biological complexity that are not simply the sum of structures and activities at lower levels. Properties of communities and ecosystems results from the simultaneous presence and functioning of many species. Many of these properties may only have meaning in the community or ecosystem context and are not predictable from properties of lower levels of organization. For example, predator-prey interactions only emerge from the concurrent activity of two species. The properties of such an interaction are not predictable from knowledge of the individual populations. Similar statements might be made for successional events and other complex interactions. Additionally, collective properties such as diversity, biomass allocation, or production summarize net effects in the whole community or ecosystem. However, these properties have meaning at more than one level of organization. Using population data from single species experiments (typically survival, growth, and reproduction) to imply effects on collective and emergent properties of systems is a conceptual leap that many ecologists are unwilling to make.

Odum (1985) has summarized predictions of system-level responses to stress that are drawn primarily from earlier work on successional trends in ecosystems (Odum, 1969). After many years of work on stress effects in freshwater lakes, Schindler (1987) has recently summarized the most useful indicators of anthropogenic stress. We summarize below (Tables 1–3) Odum's predictions for ecosystem change, Schindler's analysis of the detectability of these changes in the Experimental Lakes Area (ELA) of northern On-

Table 1. Predicted effects of stress on ecosystem energetics (Odum, 1969, 1985). Arrows indicate increases (↑) or decreases (↓) in system states or rates. Detectability refers to whether predicted changes are detectable (+) or not detectable (−) in microcosm experiments and ecosystem evaluations (Schindler, 1987).

Variable	Young → Mature Succession (Odum)	Stressed (Odum)	Detectability	
			Microcosm	Ecosystem (Schindler)
Community respiration	↑	↑	+	−
P R	approaches 1	unbalanced	+	−
P B or R B	↓	↓	+ (not as predicted)	−

Table 2. Predicted effects of stress on ecosystem nutrient cycling. See Table 1 legend.

Variable	Young → Mature Succession (Odum)	Stressed (Odum)	Detectability Microcosm	Ecosystem (Schindler)
Nutrient turnover	↓	↑	+	–
Nutrient loss	↓	↑	+	–

Table 3. Predicted effects of stress on community structure. See Table 1 legend.

Variable	Young → Mature Succession (Odum)	Stressed (Odum)	Detectability Microcosm	Ecosystem (Schindler)
Proportion of r-stategists	↓	↑	?	probably
Organism size (life-span)	↑	↓	+	+ (but opposite to predictions)
Food chains	lengthen	shorten	difficult	probably
Diversity	↑	↓ (usually)	+ (not always as predicted)	+
Symbiosis	mutualistic	parasitic	?	+

tario (Canada), and examples from experiments on laboratory ecosystems.

Several caveats accompany this summary, but it provides an indication of our ability to measure holistic, ecosystem-level changes. First, Odum's work is primary on terrestrial ecosystems, although much microcosm work has been carried out under his direction. Second, the ELA manipulations for nutrient addition and acidification mimic actual mechanisms in nature such as pulse dosing during discrete pollution events. Little ELA work has been carried out on toxics such as those found in industrial discharges to surface waters, expecially streams. However, Schindler has also reviewed the work of scientists conducting large experimental ecosystem manipulations.

Energetics. Patterns of energy flow in ecosystems often define the peculiar nature of the organisms and processes of that system. For example, many ecosystems (but not all) are photosynthetically dominated: most of the energy available in the ecosystem is fixed in reduced carbon compounds using light. Primary production is quickly passed to several groups of consumers. In such systems, primary producers dominate. However, small streams are often driven by dead organic matter on a much slower photosynthetic cycle: dead plant parts (chiefly leaves) fall into streams. The differential decay rates of these leaves power the stream for much of the year. Aging (succession) in the former ecosystems is accompanied by increases in dead material (in the soil or on lake bottoms, for example). In these systems respiration increases through time. Stress serves also to initiate increases in community respiration through shifts to maintenance (repair), although sometimes this shift can be caused by differential sensitivity of the primary producers.

Measuring such energetic shifts can be accomplished by one of several methods available for monitoring primary production or community re-

spiration. Primary production is usually monitored by some variant of the light-dark bottle method using either oxygen evolution or uptake of inorganic radioactive carbon by primary producers. Whole system can be monitored for diurnal cycles of primary production and respiration (e.g., Beyers, 1963; Giddings *et al.*, 1984; Stay *et al.*, 1985). Respiration is estimated by uptake of heterotrophically convertible substances, usually labeled, such as glucose or by the diurnal method noted above.

Similar methods are used for monitoring microcosms and larger ecosystems, but the timing of sampling to coincide with the presence of a stressor is important. In aquatic systems, primary production is dominated by rapidly reproducing (and rapidly recovering) populations of microorganisms.

Like many other researchers, we have observed shifts in energetic balance in microcosms using several stressors. For example, in experiments with atrazine, we noted elevation of primary production relative to biomass followed by nearly total collapse of photosynthesis (Pratt *et al.*, 1988). After 7 d, P/B ratios decreased dramatically as microecosystems shifted toward heterotrophy (Fig. 1). After 28 d, microcosms developed resistant floras that re-established primary production. Similar effects were seen by deNoyelles & Kettle (1985) in pond experiments and by Stay *et al.* (1985) in flask microcosms.

Schindler writes that energetic measures are poor indices of stress in large ecosystems, but we have observed altered production in large outdoor streams (USEPA Monticello Ecological Research Station) stressed with selenium. Low concentrations of selenium are known to stimulate algal growth. However, other researchers working in these streams with other stressors have not detected such functional shifts (Eaton *et al.*, 1985). While Odum predicted that P/B or R/B ratios would increase under stress (possibly due to loss of biomass), we have observed that P/B decreases with stress, probably indicating either direct effects on primary production or elevation in community respiration with stress.

Nutrient cycling. The behavior of nutrients in ecosystems is often difficult to monitor. Major nutrients are often in very low concentration; analytical methods are often inadequate to detect very low levels of biologically important nutrients, especially forms of nitrogen and phosphorus. Because nutrients are tightly cycled, even disruptions in the natural order of nutrient processing may be difficult to detect unless stress is severe. In flowing systems, disruption of nutrient cycles allows nutrients to be lost from the local system. Dramatic demonstration of losses of macronutrients is well known for small watersheds (Bormann & Likens, 1970). In lakes, nutrient cycle disruptions are more difficult to detect because of basin flushing times.

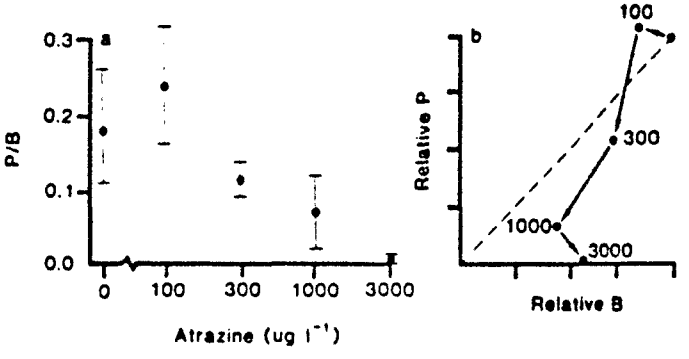


Fig. 1. A. Effect of atrazine on the ratio of primary production (P) to total biomass (B) in laboratory microcosms.
B. Phase plane plot of relative primary production (P) and total biomass (B) in atrazine treated microcosms. Control values for P and B were set to 1 (or 100%) and values for other treatments adjusted to this scale. Plotted points are values of nominal atrazine treatments ($\mu g\ l^{-1}$).

We observed altered nutrient cycling as changes in the recovery rate of macronutrients such as major cations and organic phosphorus by the alkaline phosphatase enzyme systems of microbes (Sayler *et al.*, 1979). Similarly, we have quantified changes in community nutrient pools in response to stress. In response to added zinc, microcosm communities lost cations (Table 4). Similarly, phosphorus was lost and alkaline phosphatase activity increased (Fig. 2), clear demonstration of stress effects on nutrient cycles. Similar effects have been reported for nitrate assimilation in synthesized microcosms (Taub *et al.*, 1986); in these experiments nitrate assimilation was inhibited by copper toxicity.

Community structure. Changes in the biotic composition of sampled communities has been considered to be direct evidence of adverse impacts on ecosystems. The most important changes reflect simplification of communities measured as the loss of species or as reduction in species diversity indices of several sorts. See Green (1979) for a discussion of the relative merits of species numbers versus mathematical diversity indices.

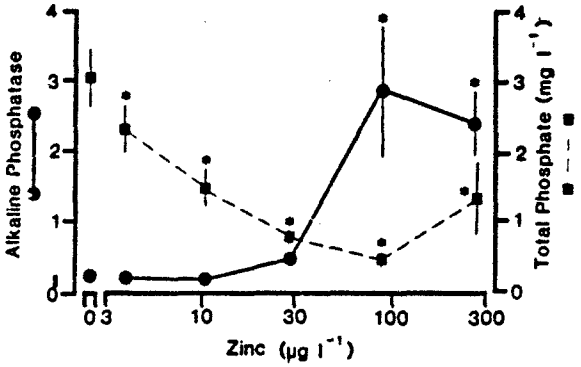


Fig. 2. Effect of zinc on phosphate retention and cycling in laboratory microcosms. Alkaline phosphatase activity is nmole p-NP mg protein^{-1} h^{-1}. Asterisks (*) identify values significantly different from controls ($p < 0.05$). Based on Pratt *et al.* (1987).

Many investigators have considered the changes that occur in stressed communities to represent a reversal of normal successional processes: reversion to smaller organisms with shorter life spans, higher reproductive rates

Table 4. Responses of microbial communities to zinc treatment in laboratory microcosms. Data are means (SD). Asterisks (*) denote values significantly different from controls ($p < 0.05$, Dunnet's test). Units for alkaline phosphatase activity are nmole p-NP mg protein^{-1} h^{-1}. Data from Pratt *et al.* (1987).

Average zinc dose µg l^{-1}	Species Number	Dry weight mg l^{-1}	In vivo fluor. FU	Total phosphate mg l^{-1}	Alkaline Phosphatase Activity
Control (<2.0)	33.7 (4.2)	179.7 (39.5)	507 (38)	3.06 (0.42)	180.0 (20.9)
4.2	30.3 (4.0)	139.9* (12.3)	472 (49)	2.27* (0.30)	181.1 (37.1)
10.7	28.7 (2.5)	89.7* (10.7)	290* (46)	1.46* (0.26)	176.3 (27.3)
29.8	26.7 (6.4)	36.7* (2.3)	88* (12)	0.75* (0.12)	461.3 (69.3)
89.2	22.7* (3.2)	25.3* (2.3)	18* (4)	0.54* (0.08)	2860* (936)
279.8	14.3* (0.6)	39.0* (8.2)	12* (2)	1.36* (0.58)	2389* (530)

among extant taxa, shorter food chains. However, nutrient enrichment in oligotrophic waters may be accompanied by increases in species diversity. Similarly, toxic stress that eliminates important controls in communities such as keystone predators or dominant taxa may result in increases in species richness (e.g., Fig. 3). In microcosms, atrazine addition resulted in reduction in producer species and a net increase in protozoa, most of which were heterotrophic. Perhaps the most general statement that can be made about stress effects on diversity is that diversity shifts from the nominal state. Usually, but not always, this shift is toward lower species richness (and diversity). It is unclear if species are actually eliminated from communities or if they are simply reduced to population sizes too small to detect. In the former case, the resilience of the ecosystem would be more seriously impaired.

Schindler reports that changes in the species richness of small, rapidly reproducing organisms such as algae were reliable indicators of ecosystem stress. He suggests that these are useful warning signals of stress effects. Unfortunately, such populations are rarely monitored in the field concomitant with fish and macroinvertebrates. In microcosm experiments, we routinely monitor protozoa since this group includes both photosynthetic (phytoflagellate algae) and heterotrophic forms. Stress typically reduces species number, and severe stress causes a return to heterotrophically dominated communities of small flagellates, similar to early successional communities (Henebry & Cairns, 1984).

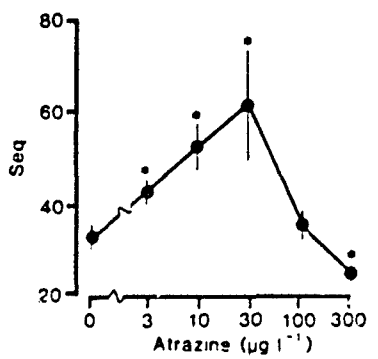


Fig. 3. Effect of atrazine on estimated equilibrium species number (Seq) for protozoa in laboratory microcosms. Asterisks (*) identify values significantly different from controls ($p < 0.05$). Based on Pratt *et al.* (1987).

Stress affects obvious symbiotic relationships, often assisting in the development of opportunistic diseases. Schindler reports that predictions of shifts in symbiosis toward parasitism are borne out by greater incidence of fish disease. Similarly, reservoir fish populations in Kentucky Lake (Tennessee River) during severe oxygen stress in 1986, there were many reported incidents of unexplained fish disease including both internal and external lesions (John Condor, Tennessee Wildlife Resources Agency, personal communication). Thermal stress is suspected of selecting for pathogenic strains of free-living amoebae that produce human meningitis.

Systematists tell us that there are between 3 and 30 million species, and every ecosystem is composed of at least several thousand of these. In the United States, decisions about pesticide licensing and chemical discharge limits are often based on the responses of three to six species. Typically, these species represent fish and invertebrates (often called fish food). Rarely are any primary producers tested (except in pesticide studies), and even more rarely are benthic, detritivorous species examined. This seems curious since it is well known that nearly 90% of all the carbon in most aquatic ecosystems is nonliving and much of this is in the detrital pool (Fenchel & Blackburn, 1979). While testing fish and planktonic invertebrates may ease the problem of communicating to the public and decision makers, it cannot be said to be scientifically justified, despite the claims of defenders of standard testing regimes.

The U.S. Federal Water Pollution Control Act prohibits the introduction of toxic substances in toxic amounts into U.S. waters. The introduction of a toxic chemical in any amount probably induces some local change in biological structure resulting in altered function. The question becomes one of determining if changes can be detected, if important processes are repeatedly or perpetually disrupted, and if species vital to the maintenance of system function are compromised. The protection of recreationally or

economically important species is a secondary biological question, but is very important economically and socially. Maintenance of water quality that is protective of human uses (e.g., drinking water) is similarly important.

Developing a scientific basis for bioassay methodology

The core of science is an explicit statement of a hypothesis and the design of an experiment that will validate or falsify it. To meet the criteria for scientific acceptance, direct rigorously controlled experimental evidence is highly desirable and is mandatory in most cases. Scientists are required to analyze how compatible their evidence and hypotheses are with evidence generated by others. Possibly because aquatic toxicology is an outgrowth of mammalian toxicology, the basic hypotheses are sometimes only implicit. This has held back development of the scientific basis for bioassays immeasurably because mammalian toxicology and aquatic toxicology or environmental toxicology do not share key hypotheses.

In mammalian toxicology, a hypothesis might state that one can extrapolate toxicological responses from a surrogate organism (e.g., rats, mice, *Rhesus* monkeys, etc.) to humans. While this hypothesis has not been validated as well as practitioners would like, the limitations of extrapolations based on certain species of experimental organisms to humans are now beginning to be fairly well understood so that corrections can be made or, more importantly, appropriate test species for a particular chemical can be selected on the basis of experimental evidence already generated. If there is an explicit statement of the hypothesis underlying ecotoxicological bioassays, it is not widely known to practitioners and is certainly difficult to find in the literature.

The annual review of the literature in the 'Journal of the Water Pollution Control Federation' (e.g., June 1987, vol. 59) has a variety of groupings based primarily on the kinds of organisms used, but an examination of the many years of annual reviews does not emphasize anywhere in the discussions that fundamental hypotheses are being tested. However, the key hypothesis is implicit when one examines the practices of the major U.S. regulatory agency (Wall & Hanmer, 1987). Clearly, it is the responsibility of these agencies to protect the environment, which most people would agree includes natural ecosystems. The practices presently endorsed are three short-term single species toxicity tests involving an alga, an invertebrate, and a fish. The implicit hypothesis is that, on the basis of bioassays carried out individually with three freshwater aquatic organisms (an alga, an invertebrate, and a fish), extrapolations to the response of an entire aquatic ecosystem can be effectively made so that standards for environmental concentrations of potentially toxic chemicals can be stated at or below which no significant adverse biological effects, in either structural or functional attributes, will occur at any level of biological organization (i.e., ecosystem to community to population and below). This hypothesis is difficult to find explicitly stated in the scientific literature; there is little evidence to support it.

Unfortunately, publications on single species toxicity tests (or even multispecies toxicity tests) rarely address a fundamental question: How will this information be used? If it is used primarily to compare relative toxicity, any species will do. If it is intended to be used to protect entire ecosystems, then some evidence must be provided about the reliability with which extrapolations can be made from the test organism to larger, more complex biological systems. The problem is that the scientific basis for applying information generated from bioassays has not expanded markedly in recent years and is unlikely to do so in the situation where funding is governed by regulatory agencies unlikely to ask fundamental questions and industries which must meet regulatory requirements. As Macek (1982) notes, 'There has been an incredible increase in data but virtually no increase in knowledge.'

The above hypothesis is so simply stated and seemingly so easily validated or falsified that it would be reasonable to wonder why it has not been done. To a certain degree, some checks have been made (e.g., Mount *et al.*, 1984, Crossland &

Wolff, 1985, Geckler *et al.*, 1976). Cairns & Cherry (1983) examined the correspondence of field and laboratory evidence at the same level of biological organization (single species) and found it to be relatively good. But as Cairns & Smith (1989) have noted, determining correspondence is not the same thing as validating a prediction. Furthermore, to ensure environmental protection, the validation should include higher levels of biological organization, such as community and ecosystem and both structural and functional attributes at each of the higher levels. But this would require the use of parameters or end points at the ecosystem or community level; ecologists as a profession have not formally endorsed such end points useful in determining ecosystem condition.

With regard to the scientific basis for the development of a predictive capability, ecologists are not on particularly solid ground either. As Harper (1982) notes ecology has tended to be highly descriptive in nature and has made little progress toward reaching maturity as a vigorous experimental and predictive science. Both ecologists and those developing bioassays may be so involved with the minutia of their methodology, such as solving highly site-specific problems, that they ignore the larger questions such as the scientific basis for their actions. Alternatively, since both fields are relatively young and still trying to acquire scientific respectability, investigators may fear to ask the larger questions because the answers may throw the spotlight on glaring deficiencies in the scientific justification for their present actions.

Ethical considerations

In the beginning of this discussion, we introduced the idea that ethical considerations motivated the human desire to manage, conserve, and protect environmental resources. Over the past 20 yr, ethical considerations were superceded by information needs (Wilson's amoral phase) leading to our present data rich/principle poor state (Macek, 1982). Recent syntheses of ethical-philosophical studies suggest that a significant debate on environmental ethics is now in progress (Lemons, 1985), Wilson's third phase. For ecotoxicologists, the timing and structure of this philosophical debate are particulary important. Important questions are being asked by ethicists about the way we assess environmental stresses and about the relation of these assessments to the important factors that influence ecological systems.

Two concepts drive much of ecological science (Golley, 1986). First, ecosystems and the interactions the organism has with its environment are hierarchically organized. Second, ecosystems are dynamic systems with flows and stores of energy, matter, and information. Individuals and whole systems are characterized by interactions among components and dynamic flows. Traditional ecotoxicological science has failed to account for ecosystem dynamics in its methods for predicting effects of stressors such as toxic chemicals on system health.

Debates about the adequacy and appropriateness of methods for estimating chemical effects in ecosystems are necessarily ethical. Where toxicological methods are incongruent with our world view and our concept of ecological functioning, decisions will necessarily be hotly debated. The time for these debates is now, the third phase of our developing awareness of ecological ethics.

Recent evidence indicates that our collective environmental ethic has shifted from theistic (God-centered) to humanistic (man-centered) – toward a reverence for life. Whether continuing development will bring us toward a deep ecological ethic (*sensu* Naess, Lemons, 1985; Golley, 1986) is uncertain. Clearly, protection of rare species, landscapes, and environmental aesthetics (e.g., scenic vistas) transcends ecological science. A greater discussion of environmental ethics among environmental scientists is needed to clarify competing world views as propounded by environmental laws and the missions of regulatory agencies. The information that we choose to use to predict environmental consequences of human activities will be determined from such discussions.

Acknowledgements

This work was supported in part by the U.S. Army Medical Research and Development Command under Contract No. DAMD17-88-C-8068. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army.

References

Beyers, R. J., 1963. The metabolism of twelve aquatic laboratory microecosystems. Ecol. Monogr. 33: 281–306.

Biswas, H. & B. A. Bell, 1984. A method for establishing site-specific stream design flows for wasteload allocation. J. Wat. Pollut. Cont. Fed. 56: 1123–1130.

Bormann, F. H. & G. E. Likens, 1970. Nutrient cycles of an ecosystem. Sci. Am. 220: 92–101.

Buikema, A. L., Jr., 1983. Inter- and intralaboratory variation in conducting static acute toxicity tests with *Daphnia magna* exposed to effluents and reference toxicants. American Petroleum Institute Publication 4362, Washington, D.C. 32 pp.

Cairns, J., Jr., 1982. Predictive and reactive systems for ecosystem quality control. In M. B. Fiering (ed.), Scientific Basis of Water-Resource Management. Geophysics Research Board, National Academy Press, Washington, D.C.: 72–84.

Cairns, J., Jr. (ed.), 1985. Multispecies Toxicity Testing. Pergamon Press, N.Y., 261 pp.

Cairns, J., Jr. (ed.), 1986. Community Toxicity Testing, STP 920. American Society for Testing and Materials, Philadelphia, Pa., 350 pp.

Cairns, J., Jr. (ed.), 1989. Functional Testing of Aquatic Biota for Estimating Hazards of Chemicals, STP 988. American Society for Testing and Materials, Philadelphia, Pa. 242 pp.

Cairns, J., Jr. & D. S. Cherry, 1983. A site-specific field and laboratory evaluation of fish and Asiatic clam population responses to coal fired power plant discharges. Water Sci. Tech. 15: 10–37.

Cairns, J., Jr. & E. P. Smith, 1989. Developing a statistic support system for environmental hazard assessment. Hydrobiologia. In press.

Cairns, J., Jr., J. S. Crossman, K. L. Dickson & E. E. Herricks, 1971. The recovery of damaged streams. Assoc. Southeast. Biol. Bull. 18: 79–106.

Cherrett, J. M., 1988. Ecological concepts – a survey of the views of the members of the British Ecological Society. Biologist 35: 64–66.

Crossland, N. O. & C. J. M. Wolff, 1985. Fate and biological effects of pentachlorophenol in outdoor ponds. Envir. Toxicol. Chem. 4: 73–86.

deNoyelles, F., Jr. & W. D. Kettle, 1985. Experimental ponds for evaluating bioassay predictions. In T. P. Boyle (ed.), Validation and Predictability of Laboratory Methods for Assessing the Fate and Effects of Contaminants in the Environment, STP 865. American Society for Testing and Materials, Philadelphia, Pa.: 91–103.

Doherty, F. G., 1983. Interspecies correlations of acute aquatic median lethal concentrations for four standard testing species. Envir. Sci. Technol. 17: 661–665.

Doudoroff, P., B. G. Anderson, G. E. Burdick, F. S. Galtsoff, R. Patrick, E. R. Strong, E. W. Surber & W. M. van Horn, 1951. Bio-assay for the evaluation of acute toxicity of industrial wastes to fish. Sewage Ind. Wastes 23: 1380–1397.

Eaton, J., J. Arthur, R. Hermanutz, R. Kiefer, L. Mueller, R. Anderson, R. Erikson, B. Nording, J. Rogers & H. Pritchard, 1985. Biological effects of continuous and intermittent dosing of outdoor experimental streams with chlorpyrifos. In R. C. Bahner & D. J. Hansen (eds.), Aquatic Toxicology and Hazard Assessment: Eighth Symposium, STP 891. American Society for Testing and Materials, Philadelphia, Pa.: 85–118.

Fenchel, T. & T. H. Blackburn, 1979. Bacteria and Mineral Cycling. Academic Press, N.Y., 225 pp.

Geckler, J. R., W. B. Horning, T. M. Neiheisel, Q. H. Pickering, E. L. Robinson & C. E. Stephan, 1976. Validity of laboratory tests for predicting copper toxicity in streams, EPA 600/3–76–116. National Technical Information Service, Springfield, Va.

Giddings, J. M., P. J. Franco, R. M. Cushman, L. A. Hook, G. R. Southworth & A. J. Stewart, 1984. Effects of chronic exposure to coal-derived oil on freshwater ecosystems: II. experimental ponds. Environ. Toxicol. Chem. 3: 465–488.

Giesy, J. P. & P. M. Allred, 1985. Replicability of aquatic multispecies test systems. In J. Cairns, Jr. (ed.), Multispecies Toxicity Testing, Pergamon Press, N.Y.: 187–247.

Golley, F. B., 1986. Deep ecology from the perspective of environmental science. Envir. Ethics 9: 45–55.

Green, R. H., 1979. Sampling Design and Statistical Methods for Environmental Biologists. J. Wiley & Sons, N.Y., 257 pp.

Harper, J. L., 1982. Beyond description. In E. J. Newman (ed.), The Plant Community as a Working Mechanism. Blackwell Scientific Publishers, London: 11–25.

Hart, W. B., P. Doudoroff & J. Greenbank, 1945. The Evaluation of the Toxicity of Industrial Wastes, Chemicals and Other Substances to Freshwater Fishes. Waste Control Laboratory, Atlantic Refining Co., Philadelphia, Pa. 376 pp.

Henebry, M. S. & J. Cairns, Jr., 1984. Protozoan colonization rates and trophic status of some freshwater wetland lakes. J. Protozool. 31: 456–467.

Horning, W. B. & C. I. Weber, 1985. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4–85–014. United States Environmental Protection Agency, Office of Research and Development, Cincinnati, Oh., 162 pp.

Kenaga, E. E., 1978. Test organisms and methods useful for

early assessment if acute toxicity of chemicals. Envir. Sci. Technol. 12: 1322–1329.

Lemons, J., 1985. Ecological stress phenomena and holistic environmental ethics – a viewpoint. Int. J. envir. Stud. 27: 9–30.

Macek, K. J., 1982. Aquatic toxicology: anarchy or democracy? In J. G. Pearson, R. B. Foster & W. E. Bishop (eds.), Aquatic Toxicology and Hazard Assessment. 5th Conference. American Society for Testing and Materials, Philadelphia, Pa.: 3–8.

Maugh, T. H., 1978. Chemicals: how many are there? Science 199: 162.

Mayer, F. L. & M. R. Ellersieck, 1986. Manual for Acute Toxicity: Interpretation and Data Base for 410 Chemicals and 66 Species of Freshwater Animals. U.S. Department of the Interior, Fish, and Wildlife Service, Resource Publication 160, Washington, D.C. 505 pp.

Mount, D. I., N. A. Thomas, T. J. Norberg, M. T. Barbour, T. H. Rousch & W. F. Brandes. 1984. Effluent and ambient toxicity testing and instream community response on the Ottowa River, Lima, Ohio, EPA 600/3–84–080. National Technical Information Service, Springfield, Va. 85 pp.

National Research Council. 1981. Testing for the Effects of Chemicals on Ecosystems. National Academy Press, Washington, D.C. 103 pp.

National Research Council, 1986. Ecological Knowledge and Environmental Problem-Solving: Concepts and Case Studies. National Academy Press, Washington, D.C., 338 pp.

Odum, E. P., 1969. The strategy of ecosystem development. Science 164: 262–270.

Odum, E. P., 1985. Trends expected in stressed ecosystems. BioScience 35: 419–422.

Perez, K. T. & G. E. Morrison, 1985. Environmental assessment from simple test systems and a microcosm: comparisons of monetary costs. In J. Cairns, Jr. (ed.), Multispecies Toxicity Testing. Pergamon Press, N.Y.: 89–95.

Pratt, J. R., B. R. Niederlehner, N. J. Bowers & J. Cairns, Jr., 1987. Effects of zinc on freshwater microbial communities. In S. E. Lindberg & T. C. Hutchinson (eds), International Conference on Heavy Metals in the Environment, Vol. 2. CEP Consultants Ltd., Edinburgh: 324–326.

Pratt, J. R., N. J. Bowers, B. R. Niederlehner & J. Cairns, Jr., 1988. Effects of atrazine on freshwater microbial communities. Arch. Envir. Contam. Toxicol. 17: 449–457.

Sayler, G. S., M. Puziss & M. Silver, 1979. Alkaline phosphatase assay for freshwater sediments: application to perturbed sediment systems. Appl. Envir. Microbiol. 38: 922–927.

Schindler, D. W., 1987. Detecting ecosystem responses to anthropogenic stress. Can. J. Fish. Aquat. Sci. 44 (Suppl. 1): 6–25.

Slooff, W., 1983. Biological Effects of Chemical Pollutants in the Aquatic Environment and their Indicative Value. Dissertation, University of Utrecht, The Netherlands, 191 pp.

Sokal, R. & F. Rohlf, 1981. Biometry. W. H. Freeman & Co., N.Y., 859 pp.

Stay, F. S., D. P. Larsen, A. Kato & C. M. Rohm, 1985. Effects of atrazine on community level responses in Taub microcosms. In T. P. Boyle (ed.), Validation and Prediction of Laboratory Methods for Assessing the Fate and Effect of Contaminants in Aquatic Ecosystems, STP 865. American Society for Testing and Materials, Philadelphia, Pa.: 75–90.

Taub, F. B., A. C. Kindig & L. L. Conquest, 1986. Preliminary results of interlaboratory testing of a standardized aquatic microcosm. In J. Cairns, Jr. (ed.), Community Toxicity Testing, STP 920. American Society for Testing and Materials, Philadelphia, Pa.: 93–120.

Tebo, L. B., Jr., 1985. Technical considerations related to the regulatory use of multispecies toxicity tests. In J. Cairns, Jr. (ed.), Multispecies Toxicity Testing. Pergamon Press, N.Y.: 19–26.

Wall, T. M. & R. W. Hanmer, 1987. Biological testing to control toxic water pollutants. J. Wat. Pollut. Cont. Fed. 59: 7–12.

Webster, J. R., 1979. Hierarchical organization of ecosystems. In A. Halfon (ed.), Theoretical Systems Ecology. Academic Press, N.Y.: 119–121.

Authorized Reprint from Standard Technical Publication 1096 - 1990
Copyright American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103

James R. Pratt[1]

Aquatic Community Response to Stress: Prediction and Detection of Adverse Effects

REFERENCE: Pratt, J. R., "Aquatic Community Response to Stress: Prediction and Detection of Adverse Effects," *Aquatic Toxicology and Risk Assessment: Thirteenth Volume, ASTM STP 1096*, W. G. Landis and W. H. van der Schalie, Eds., American Society for Testing and Materials, Philadelphia, 1990, pp. 16–26.

ABSTRACT: General ecological principles have been used to hypothesize community stress responses. A review of responses of microcosms, mesocosms, and ecosystems to chemical stressors indicated that changes in taxonomic and standing crop measures of community structure were usually reliable and sensitive indicators of stress responses. Changes in process rates showed less frequent response to chemical stressors. Ecosystem experiments have not demonstrated some predicted changes even at extreme levels of stress. Problems in demonstrating community and ecosystem responses may be a result of present methodologies or may identify failures of ecologists to accurately predict stress effects. Many ecosystem processes are substrate limited and robust even under severe stress. Although additional information is needed on the natural variability of measures of both structure and process, stress measures based on the composition of biological communities seem to have the greatest promise for evaluating ecosystem conditions.

KEY WORDS: stress, community, ecological effects, microcosm, mesocosm, aquatic toxicology

Improved methods for predicting the ecological effects of chemical stress on communities and ecosystems are needed to measure adverse effects on structures and processes. Just as individual test species are used in standard chemical testing as surrogates for other populations, patterns of structure and function in aquatic ecosystems are sufficiently repeatable to allow the use of surrogate ecosystems (microcosms and mesocosms) for prediction of adverse effects of stressors on important ecosystem structures and processes and to allow description of the degree of impairment of stressed ecosystems. Measurement of community- and ecosystem-level effects is becoming increasingly important in the regulation of toxic chemicals, especially pesticides.

Different authors have predicted a variety of patterns of stress effects in ecosystems (Table 1) [*1–3*]. Because ecological studies are time consuming and costly, it is useful to measure those ecosystem attributes most likely to provide evidence of adverse effects.

The purpose of this paper is to identify a limited number of responses which show general applicability to detecting ecosystem response to stress and to suggest possible reasons for the differential sensitivity of ecological variables; the thesis is that some common ecological measures may be incapable of providing sensitive measures of stress effects. For purposes of this paper, *stress* is defined as a disorganizing influence (*sensu* Odum [*4*]). Odum restricts the definition to negative responses and supplants the term *subsidy* for positive responses. However, an input producing an increase in a response (a subsidy *sensu* Odum and colleagues

[1] Assistant Professor of Aquatic Ecology, School of Forest Resources, The Pennsylvania State University, University Park, PA 16802.

TABLE 1—*Summary of several authors' predictions of ecosystem responses to stress.*

Effects on:	Odum [2]	Schindler [1]	Schaeffer et al. [3]
Structures	*r*-strategists increase Size of organisms decreases Life span of organisms decreases Food chains shorten Species diversity decreases and dominance increase	The most vulnerable species are organisms with short life cycles Sensitive indicators include life-table parameters, short-lived species, and changing community structure	Falling numbers of native species Changing standing crop biomass Overall regressive succession Changes in mineral macro-nutrient stocks
Processes	Community respiration increases P/R <>1 P/B, R/B increase Exported primary production increases Nutrient turnover increases Nutrient loss increases	Decomposition is a function of primary production and is unaffected by pollutants Nutrient cycling is not disrupted by toxicants	Changing gross or net primary production Changing relative energy flow to grazing and decomposer food chains Changes in mechanisms of, and capacity for, dampening undesirable oscillations

[4]) is very likely also a disorganizing influence [5]. Therefore the term *stress* should be applied equally to all inputs that produce deviations from the nominal state of the system.

Recent reports predict ecosystem stress responses [1–3,6] or review examples of these effects [7–9]. Some of these [2,3] provide little evidence of the sensitivity of ecological variables to stress. The extensive review by Sheehan and colleagues [8] provides evidence of ecosystem stress effects but little guidance for the selection of sensitive measures. Such measures have been likened to the process of medical diagnosis at the ecosystem level [10], but continuing improvement in the development and use of standard methods of ecological analysis is needed. Significant resources are spent measuring the chemical contaminants in water, but these measures fail to identify the linkages between stressors and ecosystem damage.

The several studies from the author's laboratory cited in this paper are based on experiments utilizing a standard microcosm method for detecting adverse ecological effects of stressors [11]. The methods are not repeated here; results are shown in Tables 2 to 4 and Figs. 1 and 2. In some cases laboratory methods have been applied or adapted to field or simulated field (mesocosm) studies. These studies (and others) measure stresses on ecosystem processes

TABLE 2—*Toxicant effects on community nutrient retention in laboratory microcosms (data from* [21], [22], [23]*). Values are means of triplicate microcosms. Asterisks denote significant differences from controls* ($p < 0.05$).

Zinc [21]		Copper [22]			Atrazine [23]		
Zn, µg/L	Total P, mg/L	Cu, µg/L	K, mg/L	Ca, mg/L	Atrazine, µg/L	K, mg/L	Ca, mg/L
Con.	3.06	Con.	7.0	102	Con.	2.0	24.1
4.2	2.27*	9.9	8.4	110	3.2	1.7	23.0
10.7	1.46*	19.9	6.7	76	10.0	2.6	21.8
29.8	0.75*	40	5.9	79	32.0	2.2	20.2*
89.2	0.54*	90	3.7*	44*	110	1.7	22.7
279	0.36*	205	2.8*	38*	337	1.1*	20.0*

TABLE 3—*Sensitivity of measured responses to toxic effects of selected chemicals in laboratory microcosms (based on* [21], [22], [23], [28]*). Table values are lowest observed effect concentrations (LOECs), the lowest tested toxicant concentration producing a response significantly different from the control. EPA criterion levels are shown for a hardness of 100 ppm.*

Variable	Toxicant Concentration, μg/L			
	Copper [22]	Zinc [21]	Atrazine [23]	Chlorine [28]
Species richness	9.9	89	3.2	6.1
Biomass				
Protein	9.9	4.2	3.2	100
Chlorophyll	19.9	10.7	3.2	2.1
Net primary production	90	4.2	3.2	25
Alkaline phosphatase activity	NS	89	100	6.1
EPA criterion	12	110	—	11

TABLE 4—*Responses showing sensitivity to toxicant action in laboratory ecosystems. Responses are listed in order of relative sensitivity. The number of responses is the number of experiments showing* any *significant response to a toxic chemical. The median coefficient of variation (CV) for each measurement is also given.*

Variable	Number of Tests	Number of Responses	%	CV, %
Species richness	14	11	79	9.6
Chlorophyll biomass	12	10	83	16–27
Dissolved oxygen	13	6	46	4.53
Alkaline phosphatase activity	11	4	36	18.4
pH	11	3	27	2.22
Protein biomass	12	3	25	23.6

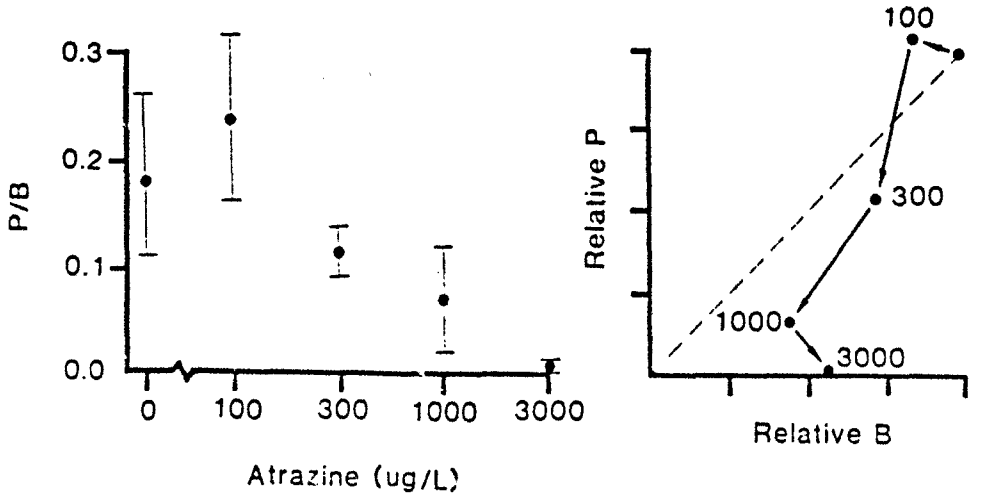


FIG. 1—*Effect of atrazine on biomass specific* (B) *primary production* (P) *of periphyton in laboratory microcosms. The left-hand figure shows the change in* P/B *ratio with atrazine dose. The right-hand figure shows the relative change in both* P *and* B *(full scale = control response). Adapted from* [23].

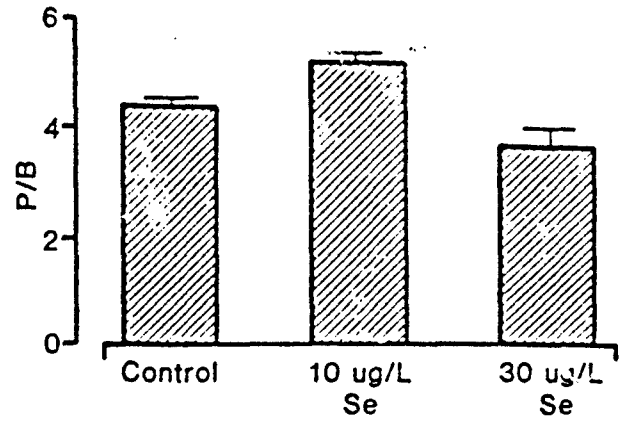


FIG. 2—*Effect of selenium on biomass specific* (B) *primary production* (P) *of periphyton in artificial streams at the U.S. EPA Monticello Ecological Research Station. The* P/B *ratio is calculated from daily primary production (as mg oxygen/m²/day) and algal biomass as mg chlorophyll-a/m². Based on* [17].

which may lead to altered metabolism. Effects on ecosystem structures are also examined and may include effects on populations, communities, and stores of materials. Predictions of stress effects by several authors are summarized in Table 1.

Ecosystem Processes

Net production is expected to change under stress. Odum [2] hypothesized that increased maintenance (respiration) would occur under stress and that ratios of production to biomass (P/B) and respiration to biomass (R/B) would increase because of imbalances in P/R. Energetic changes are measurable in laboratory-scale ecosystems at environmentally realistic toxicant levels, probably because confounding factors such as physical changes in the natural environment are less problematic. For example, diurnal cycles of pH or oxygen in microcosms have been shown to be related to stress [*12–14*]. Microcosm experiments on atrazine effects showed collapse of photosynthesis (Fig. 1). P/B fell as systems shifted toward heterotrophy. However, in lake phytoplankton stressed with copper, P/B increased with increasing stress [*15*]. In both cases, both production and standing crop decreased with increasing toxic dose, so changes in the individual measures may be more meaningful than the ratio of production to biomass.

In field experiments, de Noyelles and colleagues [*16*] observed shifts in production in replicated ponds. Periphyton P/B changed in replicated streams (U.S. EPA Monticello Ecological Research Station [*17*]) dosed with selenium (Fig. 2). Low concentrations of selenium stimulated algal growth. However, Schindler [*1*] reports no changes in planktonic production energetics in experimental lakes under either nutrient or acid stress. Similarly, experiments on chlorpyrifos effects in the Monticello streams yielded no evidence of energetic shifts [*18*].

The ability of ecosystems to retain and efficiently process nutrients is hypothesized to decrease with stress. Cycling patterns are affected if any of the key processors along a pathway are compromised by stress. Many nutrients in limited supply are typically tightly cycled; the availability of some nutrients limits production. Because of the low concentrations and rapid cycling of important nutrients, cycling processes are difficult to measure *in situ*, although the activities of many microbial enzyme complexes can be assayed [*19*].

Toxicants affect nutrient cycling and retention in experimental systems. For example, sediment phosphatase activity decreased in response to heavy metal toxicity [*20*]. In response

to added zinc [*21*], microbial communities lost phosphate and showed large increases in alkaline phosphatase activity (a measure of organic phosphorus recovery [Fig. 3]). Odum [*4*] calls such ecosystems "leaky," and evidence of the lack of macronutrient retention in relation to toxic action clearly demonstrates stress effects on nutrient cycles (Table 2). Related effects of a toxicant (copper) have been demonstrated by Taub and colleagues [*24*] for nitrate assimilation.

Field experiments do not regularly show effects of toxicants on nutrient cycles for limited perturbations [*8*]. Schindler [*1*] reports that neither added nutrients nor acidification affected nitrogen or phosphorus cycling. In a recent study of impacts of high ammonia and chlorine containing sewage wastewater on a stream, the author observed no effect on alkaline phosphatase activity despite readily observable alterations in community structure (Fig. 4). Because the wastewater contained total phosphate concentrations >1 mg/L, little effect was seen in phosphate recovery systems. In replicated artificial streams dosed with the insecticide

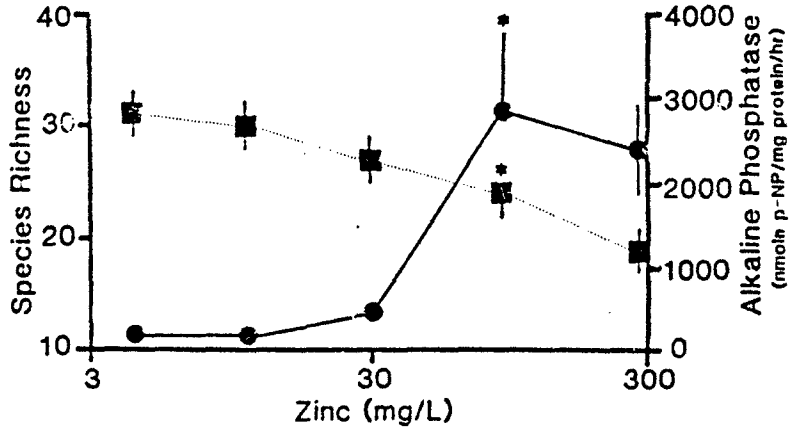


FIG. 3—*Effect of zinc on species numbers* (■) *and alkaline phosphatase activity* (●) *in laboratory microcosms* [21]. *Error bars are one standard deviation. Asterisks indicate treatments significantly different from controls* ($p < 0.05$). *Activity is expressed as nmol p-nitrophenol/mg protein/hour.*

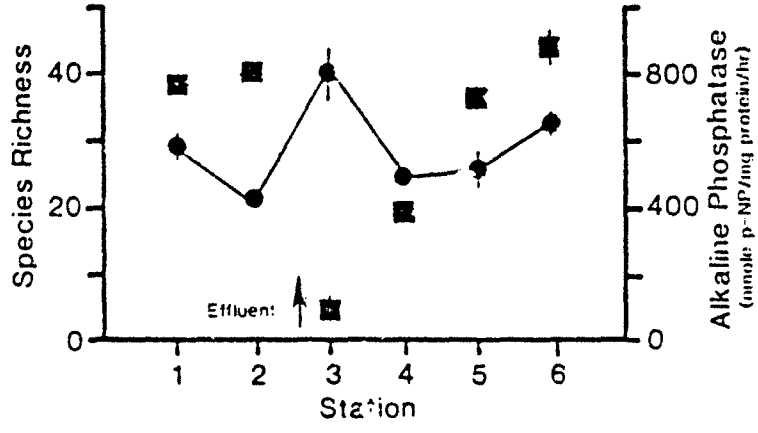


FIG. 4—*Effect of discharge of municipal wastewater containing ammonia and chlorine on species richness of protozoa* (■) *and on alkaline phosphatase activity* (●) *in Spring Creek, Centre County, Pennsylvania. Activity is expressed as nmol p-nitrophenol/mg protein/hour. Samples were from polyurethane foam artificial substrates colonized in the stream for seven days.*

chlorpyrifos. Eaton and colleagues [18] also observed no effects on microbial processes. Nutrient cycling changes often are obvious only under severe stress.

Ecosystem Structure

Changes in biotic composition have long been regarded as the primary evidence of environmental impact. In aquatic ecosystems, few species are long lived. Sensitive individuals and species are eliminated by stress and do not return until the stress is removed. Under toxic stress, the remaining species may exhibit broad tolerance, rapidly reproduction (opportunists or *r*-strategists), or resistance to the toxicant. In any of these cases, evidence of stress would be observed in species composition changes from the unstressed condition and, usually, the number of species declines. Such changes may lead to alterations in community function, to the shortening of food chains, and to a reversal of normal successional trends. Typical of the reversal of successional trends is the reversion to community dominance by smaller taxa with high reproductive rates, the *r*-strategists. Such changes have been observed in microcosm experiments [25].

Reduced species richness and altered standing crop are common results of stress. For example, in laboratory ecosystems the number and kinds of species change quickly in response to stress. Such changes are widely acknowledged as indicators of stress [1] and form the basis for some of the oldest and newest methods for evaluating the biological quality of waters [26,27]. Changes in biotic structure, including simple biomass estimators, are often among the most sensitive changes that occur in aquatic ecosystems (e.g., Tables 3, 4, and [1]).

Field evaluations of stress effects have often concentrated on observations of changing biological structure. We have regularly observed severe effects on species richness at lower levels of stress than nontaxonomic changes in structures and processes, suggesting that the measurement of subtle changes in ecosystems should include taxonomic investigations. For example, in the stream system impacted by wastewater (Fig. 4), only species number reflected inputs of high levels of ammonia and chlorine. Measurements of biomass (total and chlorophyll), microbial activity, and macronutrients showed no effects despite severe simplification of the stream community.

System-Level Trends

Effects on whole ecosystem characteristics are difficult to measure, although certain trends may be observed such as the apparent reversal of successional trends or the appearance of abnormal levels of parasitism. It seems likely that systems become less efficient and open in response to stress, but these trends are largely accounted for by alterations in species composition and processes. However, the appearance of parasitism is related to stress. For example, in Southern Indian Lake (Manitoba, Canada) impoundment affected several measures of water quality and was concurrent with the collapse of a whitefish fishery affected by nematode infections [29]. Schindler [1] also reported increased parasitic infections of fish in stress experimental lakes. In 1986, depletion of dissolved oxygen in Kentucky Lake (Tennessee River) coincided with recurring and widespread outbreaks of bacterial infections of catfish (J. Conder, Tennessee Wildlife Resources Agency, pers. comm.).

Measurement and Mechanisms of Stress

Sensitive measures of ecological effects of stress can be made in laboratory ecosystems developed to evaluate complex assemblages that mimic at least some of the properties of

natural ecosystems. However, care must be taken to ensure that such systems provide valid measures of effects, because laboratory systems may demonstrate effects that cannot be verified in the field. Small laboratory systems generally lack the cascading trophic effects caused by effects on key predators [*1*].

Laboratory systems for evaluating ecological effects of chemical stressors can produce erroneous predictions in opposing directions, "false negatives", when the test system is insensitive to stresses that affect sensitive species, and "false positives" when laboratory measures of effect cannot be verified in the field. The goal of ecological testing should be to minimize both errors.

In their present state of development, laboratory ecosystems appear to demonstrate effects of chemical stressors at environmentally plausible concentrations. Stress responses generally follow expected patterns, but field trials have not shown adverse effects at levels predicted to be deleterious by laboratory experiments. We might reasonably ask which response variables are broadly sensitive to stress and why laboratory responses may differ from field observations.

Sensitivity

Community structure consistently reflects stress effects (Tables 3 and 4). In the author's microcosm experiments, species richness is usually affected by toxicant action at toxicant concentrations expected to have limited effects in freshwater ecosystems. Other measures of stress effects, notably chlorophyll levels, have also been useful in evaluating stressors. Some other measures are considerably less useful in predicting stress effects. For example, alternative measures of biomass (including chitin), activities of several enzyme systems, and retention of macronutrients have not routinely been related to stress. Differences in the taxonomic composition of sampled species (as opposed to simple sums of species numbers) would be expected to provide even more sensitive measures of stress effects. The results of Blanck and colleagues [*30*] confirm these observations. They ranked changes in species composition of laboratory algal communities as more sensitive than nontaxonomic or production measures of arsenic stress. However, inferential statistical testing of changes in community structure has been difficult. Recent studies using permutation and simulation methods show promise for allowing direct significance testing of altered community structure [*31*].

Evidence that simple structural measures serve as useful indicators of toxic stress in fresh waters should be greeted with some caution. Firstly, taxonomic skill is required to evaluate the species richness of a sample. The greater the level of taxonomic precision, the greater the likelihood of detecting important changes caused by anthropogenic stress, but a significant time investment is required to measure community composition. Secondly, the author has frequently measured several different community responses including some that were at least as sensitive to stress as species richness but requiring considerably less effort. Depending on the type of stressor, logical choices of response variables can be made.

Laboratory-Field Comparisons

Ecologists have found detecting community and ecosystem change from the effects of toxic chemicals in the field a difficult task. The failure to detect certain community and system-level changes has three probable origins. Firstly, the natural range of variability of structural and process rate variables remains poorly known for most ecosystems. Secondly, measurement methods have limited sensitivity. Coefficients of variation for replicate measures of many structure and process variables commonly exceed 30%, making evaluation of differ-

ences problematic even when comparative information is available (and assumptions of statistical procedures can be met). Thirdly, often ecological sampling is not conducted with a knowledge of the power to detect effects given measurement and ecosystem variability [*32*].

Mechanisms of Stress

Processes—Changes in processing rates including primary productivity, decomposition, and nutrient cycling have been less sensitive to toxic stress. Ecological processes in surface waters are both substrate and capacity limited [*6*]; that is, many biological processes follow saturation kinetics. When the supply of needed substrates is high, conversion of substrate to product is limited by the biological machinery. When substrate supply is below the point at which the biological machinery is saturated (operating at maximum speed), the process is substrate limited.

In freshwater, photosynthesis serves as a good example of a process that can be capacity limited. The photosynthetic machinery is saturated at some fraction of incident light: increasing light produces no concomitant increase in photosynthesis because the process, at least with respect to light, is limited by the biological machinery—the abundance of algae. In fact, primary production, the elaboration of plant biomass, is usually limited by the supply of nutrients (commonly phosphorus in fresh water) which in turn limits algal abundance. Removing phosphorus limitation (as in eutrophication) leads to relaxation of production limitation and greater algal biomass.

Substrate limited processes should be less sensitive to toxicants than capacity limited processes (Table 5). Even though the identity of the biological machinery may change through the action of a toxicant, important substrate limited processes generally show little response, probably because substrate supply to a reduced set of taxa results in undetectable changes in processing rates [*1,6*]. Only under severe stress is the biological machinery significantly debilitated and capacity limitation revealed. However, relative process rates, mediated by resistant taxa, may actually increase on a unit biomass basis [*5,14*].

Effects on processes have been seen to conflict with structural measures of effects. Floessner [*33*] showed significant changes in macrobenthic community composition in a stream impacted by organic wastes even though production was unaffected. Untula [*34*] showed that invertebrate production in an acidified lake was greater than a reference lake, probably because the acidified lake had no fish. Kaushik and colleagues [discussion in *35*] report that

TABLE 5—*Predicted stress effects on ecological processes. Adapted from Levine* [6].

Adverse Effect	Expected Change	
	Capacity Limited Process	Substrate Limited Process
Functional Capacity		
Decreased capacity, some species	decrease	none
Increased capacity, some species	increase	none
Decreased abundance, some species	decrease	none
Increased abundance, some species	increase	none
Substrate Supply		
Decreased substrate available	none	decrease
Increased substrate available	none	increase
Chemical is a substrate	none	increase

primary production during recovery after atrazine application in limnocorrals recovered rapidly despite a shift in algal composition from micro- to nanoplankton.

Structure—Individual species show Gaussian responses [*36*] to environmental conditions, including toxic stress. This observation, coupled with the lognormal abundance pattern of species in most communities [*37,38*], leads to several expectations. The loss of sensitive taxa is especially important when that species is a keystone predator [*1*]. With increasing stress (e.g., toxicity), sensitive individuals are eliminated from local populations. Because there is no relationship between commonness and resistance to toxicants, some toxic stressors may result in the elimination of sensitive but common or important species; more often, toxicants lead to the elimination of relatively uncommon taxa. Significant changes in community structure following exposure to low levels of arsenic stress lead to increased community tolerance to subsequent, much greater stresses [*30*]. At low levels of stress, toxic effects should lead to replacement of sensitive species by more resistant taxa if the replacement taxa are capable of successfully competing at ambient nutrient levels.

In other words, the loss of "important" species and increased community resistance to stress have a common cause. A population ecologist viewing the toxic stress problem would observe lower population levels of some important or dominant taxa. A community ecologist would observe changes in dominance and the loss of some taxa. Replacement by more tolerant forms is the result of altered survival and growth either as a result of altered predation or competitive interactions. Changes in the composition of natural communities precede changes in processes.

Summary and Recommendations

Laboratory measures of ecological effects provide evidence of both the sensitivity of measurement variables and the nature of responses to anthropogenic stress. Stress can adversely affect community energetics by disrupting primary production and the balance between production and maintenance. Stressors may disrupt nutrient cycles by inhibiting the activities of important species, resulting in net loss of system nutrients. Stress affects the abundance and kinds of component species resulting in shifts in dominance and reversion of successional patterns. Adverse effects of stress may lead to increased parasitism and a reduction in linkages in ecosystems that allow efficient capture of matter and energy.

Aquatic communities and ecosystems typically display shifts in the number and kinds of component species prior to the appearance of effects on processes. As predicted, processes are robust (i.e., less sensitive to alterations in biological structure). Important ecological processes are often substrate limited, and the loss of taxonomic diversity alters patterns of use of substrates without affecting measured system function.

Improving the detection of stress effects at the community and ecosystem level assumes that additional effort and resources will be directed towards the study (not simply monitoring) of select ecosystems and the development of standard methods for predicting and measuring ecological effects using both process and structure variables. Regional classifications of ecosystems [*39*] may assist in limiting the number of ecosystems studied and may provide a framework for developing assessment tools for evaluating heavily used ecosystems. Regional biological assessment tools such as the Index of Biotic Integrity (IBI) [*27*] may provide valuable methods for systematically evaluating taxonomic data known to be sensitive to stress effects.

Biological assessments that are taxonomically based assume that investigators have appropriate taxonomic training and access to accurate, relevant, and up-to-date reference materials. At a time when greater importance will be placed on understanding the autecology of indicator organisms [*40*] our taxonomic works are, at best, out-dated and, at worst, unavail-

able. Treatises on the natural history, taxonomy, tolerances, and distribution of aquatic species ranging from fish to microbes appear with disquieting irregularity. Many regional floras and faunas have never been characterized, and those taxa expected to provide great indicator value are precisely the groups for which biological information is most needed: small, rapidly reproducing, poorly dispersing (algae, fungi, protozoa, worms, aquatic arthropods) [*1*].

Acknowledgments

The assistance of N. J. Bowers, R. W. Plotnikoff, and J. Balczon are gratefully acknowledged. Selenium studies were conducted with the assistance of the staff of the U.S. EPA Monticello Ecological Research Station (S. Hedtke, Director). Portions of this work were supported by the U.S. Army Medical Research and Development Command under Contract DAMD17-88-C-8068. Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

References

[*1*] Schindler, D. W., *Canadian Journal of Fisheries and Aquatic Sciences*, Vol. 44, Suppl. 1, 1987, pp. 6–25.
[*2*] Odum, E. P., *BioScience*, Vol. 35, 1985, pp. 419–422.
[*3*] Schaeffer, D., Herricks, E. E., and Kerster, H. W., *Environmental Management*, Vol. 12, 1988, pp. 445–455.
[*4*] Odum, E. P., Finn, J. T., and Franz, E. H., *BioScience*, Vol. 29, 1979, pp. 349–352.
[*5*] Pratt, J. R., Bowers, N. J., Niederlehner, B. R., and Cairns, J., Jr., *Archives for Environmental Contamination and Toxicology*, Vol. 17, 1988, pp. 449–457.
[*6*] Levine, S. N. in *Ecotoxicology: Problems and Approaches*, S. A. Levin, M. A. Harwell, J. R. Kelly, and K. D. Kimball, Eds., Springer-Verlag, New York, 1989, pp. 145–180.
[*7*] Barrett, G. W. and Rosenberg, R., Eds., *Stress Effects on Natural Ecosystems*, Wiley, Chichester, 1980, 305 pp.
[*8*] Sheehan, P. J. in *Effects of Pollutants at the Ecosystem Level*, P. J. Sheehan, D. R. Miller, G. C. Butler, and P. Bourdeau, Eds., Wiley, Chichester, 1984.
[*9*] Levin, S. A., Harwell, M. A., Kelly, J. R., and Kimball, K. D., Eds., *Ecotoxicology: Problems and Approaches*, Springer-Verlag, New York, 1989, 547 pp.
[*10*] Rappaport, D. J., Regier, H. A., and Thorpe, C. in *Stress Effects on Natural Ecosystems* G. W. Barrett and R. Rosenberg, Eds., Wiley, Chichester, 1981, pp. 269–280.
[*11*] Pratt, J. R. and Bowers, N. J., *Toxicity Assessment*, Vol. 5, 1990, pp. 189–205.
[*12*] Franco, P. J., Giddings, J. M., Herbes, S. E., Hook, L. A., Newbold, J. D., Roy, W. K., Southworth, G. R., and Stewart, A. J., *Environmental Toxicology and Chemistry*, Vol. 3, 1984, pp. 447–463.
[*13*] Stay, F. S., Larsen, D. P., Kato, A., and Rohm, C. M. in *Validation and Prediction of Laboratory Methods for Assessing the Fate and Effect of Contaminants in Aquatic Ecosystems*, *ASTM STP 865*, T. P. Boyle, Ed., American Society for Testing and Materials, Philadelphia, 1985, pp. 75–90.
[*14*] Shannon, L. J., Harrass, M. C., Yount, J. D., and Walbridge, C. T. in *Community Toxicity Testing*, *ASTM STP 920*, J. Cairns, Jr., Ed., American Society for Testing and Materials, Philadelphia, 1986, pp. 135–157.
[*15*] Wurtsbaugh, W. A. and Horne, A. J., *Canadian Journal of Fisheries and Aquatic Sciences*, Vol. 39, 1982, pp. 1636–1641.
[*16*] de Noyelles, F., Jr., Kettle, W. D., and Sinn, D. E., *Ecology*, Vol. 63, 1985, pp. 1285–1293.
[*17*] Pratt, J. R. and Bowers, N. J., "Effect of Selenium on Microbial Communities in Laboratory Microcosms and Outdoor Streams," *Toxicity Assessment*, in press.
[*18*] Eaton, J. A., Hermanutz, R., Kiefer, R., Mueller, L., Anderson, R., Erickson, R., Nordling, B., Rogers, J., and Pritchard, H. in *Aquatic Toxicology and Hazard Assessment: Eighth Symposium*, *ASTM STP 891*, R. C. Bahner and D. J. Hansen, Eds., American Society for Testing and Materials, Philadelphia, 1986, pp. 85–118.
[*19*] Lanza, G. R., Burton, G. A., Jr., and Dougherty, J. M. in *Functional Testing of Aquatic Biota for Estimating Hazards of Chemicals*, *ASTM STP 988*, J. Cairns, Jr., and J. R. Pratt, Eds., American Society for Testing and Materials, Philadelphia, 1988, pp. 41–54.

[20] Broberg, A. in *Proceedings*, 10th Nordic Symposium on Sediment, I. Bergstrom, J. Kettunen, and M. Stenmark, Eds., Helsinki University of Technology Report No. 26, Espoo, Finland, 1982, pp. 115–126.
[12] Pratt, J. R., Niederlehner, B. R., Bowers, N. J., and Cairns, J., Jr., in *International Conference on Heavy Metals in the Environment*, S. E. Lindberg and T. C. Hutchinson, Eds., CEP Consultants Ltd., Edinburgh, 1987, pp. 324–325.
[22] Pratt, J. R., Niederlehner, B. R., Bowers, N. J., and Cairns, J., Jr., *Toxicity Assessment*, Vol. 2, 1987, pp. 417–436.
[23] Pratt, J. R., Bowers, N. J., Niederlehner, B. R., and Cairns, J., Jr., *Archives of Environmental Contaminations and Toxicology*, Vol. 17, 1988, pp. 449–457.
[24] Taub, F. B., Kindig, A. C., and Conquest, L. L. in *Community Toxicity Testing, ASTM STP 920*, J. Cairns, Jr., Ed., American Society for Testing and Materials, Philadelphia, 1986, pp. 93–120.
[25] Cairns, J., Jr., Hart, K. M., and Henebry, M. S., *American Midland Naturalist*, Vol. 104, 1980, pp. 93–101.
[26] Kolkowitz, R. and Marsson, M., *International Revue des gesammten Hydrobiologie and Hydrographie*, Vol. 2, 1909, pp. 126–152.
[27] Karr, J. R., Fausch, K. D., Angermeier, P. L., Yant, P. R., and Schlosser, I. J., "Assessing Biological Integrity in Running Waters: A Method and its Rationale," Illinois Natural History Survey Special Publication No. 5, Champaign, Ill., 1986, 28 pp.
[28] Pratt, J. R., Niederlehner, B. R., Bowers, N. J., and Cairns, J., Jr., *Environmental Toxicology and Chemistry*, Vol. 7, 1988, pp. 679–687.
[29] Hecky, R. E., Newbury, R. W., Bodaly, R. A., Patalas, K., and Rosenberg, D. M., *Canadian Journal of Fisheries and Aquatic Sciences*, Vol. 41, 1984, pp. 730–732.
[30] Blanck, H., Wangberg, S.-A., and Molander, S. in *Functional Testing of Aquatic Biota for Estimating Hazards of Chemicals, ASTM STP 988*, J. Cairns, Jr., and J. R. Pratt, Eds., American Society for Testing and Materials, Philadelphia, 1988, pp. 219–230.
[31] Smith, E. P., Pontasch, K. W., and Cairns, Jr., Jr., "Community Similarity and the Analysis of Multispecies Environmental Data: A Unified Statistical Approach," *Hydrobiologia*, in press.
[32] Conquest, L. L., *International Journal of Environmental Studies*, Vol. 20, 1983, pp. 209–221.
[33] Floessner, D., *Limnologica*, Vol. 10, 1976, pp. 123–153.
[34] Untula, J. A. in *Effects of Acidic Precipitation on Benthos*, North American Benthological Society, Springfield, Ill., 1981, pp. 139–154.
[35] Kaushik, N. K., Solomon, K. R., Stephenson, G. L., and Day, K. E. in *Community Toxicity Testing, ASTM STP 920*, J. Cairns, Jr., Ed., American Society for Testing and Materials, Philadelphia, 1986, pp. 269–290.
[36] ter Braak, C. J. F. and Prentice, I. C., *Advances in Ecological Research*, Vol. [illegible], 1988, pp. 271–317.
[37] Preston, F. W., *Ecology*, Vol. 29, 1948, pp. 254–283.
[38] Patrick, R. and Strawbridge, D., *Journal of the Water Pollution Control Federation*, Vol. 25, 1963, pp. 151–161.
[39] Omernik, J. M., *Annals of the Association of American Geographers*, Vol. [illegible], 1987, pp. 118–125.
[40] Wilson, E. O., *Biodiversity*, National Academy Press, Washington, D.C., 1988, 521 pp.

APPENDIX D

Submitted Papers and Manuscripts

Significance of changes in community structure:

a new method for testing community differences.

J.R. Pratt[1] and E.P. Smith[2]

[1]School of Forest Resources and Graduate Program in Ecology
Penn State University
University Park, PA 16802

[2]Department of Statistics
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

ABSTRACT

Structural changes in biotic communities often precede detectable adverse effects on ecosystem process rates. Management concerns may focus on changes in abundance, the loss of important species, or changes in the composition or diversity of groups of taxa. Community structure data is high in information content, but there is a perception that it is difficult to analyze and interpret. Bioassessment methods have recommended use of multivariate procedures for examining differences in community similarity, but the number of species (variables) in community samples and the limited number of replications make application of multivariate methods problematic; the number of degrees of freedom will usually be fewer than the number of variables and the covariance matrix will be singular. Additionally, some species present at one site will be absent at other sites, especially impacted sites, invalidating assumptions of normality.

An analytical method is needed which can use community information for inferential analysis of environmental effects but which avoids the difficulties of violating assumptions of statistical models. To use community structure information, species data must first be reduced to measures of similarity (or distance) among replicates. Measures (indices) for assessing community similarity can be based on presence-absence, ranked or rated abundance, and relative or absolute abundance of taxa. A permutation procedure involving a large number of random switches of similarity measures is used to build a probability distribution of the ratio of mean between treatment or location similarity to mean within treatment similarity, analogous to analysis of variance procedures. Assuming that a null hypothesis of no difference among locations is rejected, follow-up analyses can indicate the locations or treatments that are different and can identify the influence of species whose presence, absence, or abundance greatly affects analyses.

We have used the permutation method and binary, ranked, and continuous data to examine community changes in multispecies laboratory experiments and field evaluations of periphyton and invertebrate communities. Unlike descriptive multivariate methods or rating/score methods, the permutation method provides an inferential test of hypothesized differences between reference sites to suspected impact sites and is an objective means of determining differences and identifying the taxa indicative of community differences.

INTRODUCTION

Evaluating biological changes in stressed ecosystems is based on our understanding that human influence alters the sustainability of ecosystem services. Controversy over the conceptual basis for ecosystem protection has followed the topical, favored paradigms of ecology: studies that once focused on describing the structure of ecosystems gave way to process studies examining sources and flows of energy and materials. Increasing evidence suggests, however, that aquatic ecosystem structure changes before process accomodations are detectable (Odum 1985, Schindler 1987, Schaeffer et al. 1988, Pratt 1990, Odum 1990). Process measures are robust and change primarily with the availability of substrates (nutrients, dead organic matter) rather than the biological machinery that processes these substrates. Where stresses do not significantly alter the supply of substrates, process measures show little impact (Levine 1989).

Evaluations of aquatic community structure have re-emerged as an important facet of environmental impact analysis and will soon be incorporated directly into the regulatory framework. The establishment of biological criteria for waters implies that methods are available to detect significant changes in community structure. Taxonomic lists incorporating the presence, absence, or abundance of species at particular locations of interest have high information content. The means for dealing with this information vary considerably depending on the study questions to be answered. In this paper, we present methods for examining community structure information using inferential procedures for testing hypotheses of community change.

Early in this century, the ubiquity of organic pollution and its effects on aquatic communities led to the classification of organisms by enrichment (or low oxygen) tolerance, the classic Saprobian system (Kolkwitz and Marsson 1908). Two decades ago, questions focused on changes in biotic diversity, and information theory (Shannon and Weaver 1949) was applied to the comparisons of community taxa abundance. However, information theory indices were often sensitive to the number of taxa (Green 1979) and aroused considerable debate about the relationship between biotic diversity and community stability (Hurlbert 1971).

Current impact analysis makes use of both our improved knowledge of stream ecology and our ability to deal with complex data sets. We now have a much clearer understanding of the distribution, tolerance, and habits of aquatic species (e.g., Lowe 1974, Hilsenhoff 1982, Cummins and Klug 1979, Karr et al. 1986). A variety of systematic procedures for evaluating taxonomic structure is available (Metcalfe 1989, Cairns and Pratt [in press]). Other descriptive procedures allow us to compare collections according to taxonomic composition and abundance using community similarity or multivariate distance measures (e.g., Gauch 1982, Pielou 1984, Digby and Kempton 1987). Such procedures have been used to examine community differences along

environmental gradients that include habitat alterations and pollutants (e.g., Pratt et al. 1985, Whittier et al. 1988). However, these descriptive procedures do not provide rigorous, inferential methods for testing differences among communities.

In 1986 the Environmental Protection Agency moved to standardize and improve biological methods for assessing and monitoring surface waters. An important product of this effort was a guidance document on rapid biological assessment focusing on community structure of fish, macroinvertebrates, and algae (Plafkin et al. 1989). Methods for assessing fish communities were based on the work of Karr and colleagues (1986) while those for macroinvertebrates were based both on historical methods and the development of additional methods analogous to those for fish. Much of this work was incorporated into methods now used by the state of Ohio in assessing biological water quality (Ohio EPA 1987).

Rapid bioassessment procedures are systematic means for collecting and evaluating community and habitat structure information. The procedures have various uses including determining attainability of uses of water and characterizing the degree of use impairment. The procedures recognize the potential for regional variation in ecosystem condition and performance (ecoregions, Omernik 1987). Site comparisons are made across limited environmental gradients to either an upstream, reference site community or to a regional reference community. Methods for assessing macroinvertebrate communities use similarity to the reference community as one of several community assessment metrics. While the rapid bioassessment procedures are rational means for evaluating community data, they are not inferential. The indices developed usually lack any estimate of variance and, therefore, cannot provide a statistical comparison among sites. Additionally, the use of multivariate procedures to compare communities violate assumptions of normality when taxa are present at some sites but not others.

Regardless of the mechanism by which biological criteria are developed, community comparisons and inferential procedures will be needed to detect changes in biological structure. The methods described in this paper can be used to compare communities to regional reference communities or to upstream reference sites and can provide an inferential test of hypotheses of no difference among communities. Studies of biological quality will need to demonstrate rigor in the quality of sampling, the identification of species, and the analysis of results.

METHODS

The analytical method presented below tests for differences in community structure among sites or treatments (see Smith et al. 1990). The analyses require the selection of a measure of community similarity-dissimilarity or distance. Following the contruction of a matrix comparing community measures by sites or treatments, a permutation procedure is used to repeatedly and randomly switch measures among the site or treatment categories. Switching similarity measures is equivalent to switching data vectors (i.e., switching all data for one treatment replicate). Statistical comparisons are based on the relationship of between treatment similarity to within treatment similarity, analogous to analysis of variance procedures. Follow-up analyses examine pairwise comparisons of treatment categories and identify the influence of particular species on the chosen community measure.

Community similarity - an overview

The array of methods for estimating community similarity is diverse and will not be reviewed in detail here. Legendre and Legendre (1983) list 27 community metrics. With reference to ecological studies, indices and methods for comparing communities are succinctly reviewed by Pielou (1984) and Digby and Kempton (1987). Certain indices are recommended by Plafkin et al. (1989), but most indices have uncertain or unknown statistical properties and few have been rigorously studied for their sensitivity in detecting community change.

Two aspects of the assessment of community similarity are worth noting. First, both similarity measures (comparisons of species overlap between physical samples) and distance measures (distance between samples in multidimensional species space, often the complement of similarity) are available to examine the relationship among replicates from different sites. Second, presence-absence (binary), relative abundance, and actual or absolute abundance are simply scales which weight the importance of a species. In binary data the weightings are 1 (present) and 0 (absent). Relative abdundance data may be presented as abundance rankings (usally scaled as integer values between 0 and 10) or as proportional abundance (scaled by the total number of individuals counted in each replicate). Actual or absolute abundance data weight individual species according to the actual number of individuals counted and weight different replicates by the total number of individuals. Other measures of abundance may be used and include such estimators as biomass, biovolume, cover, and importance value.

Binary data underestimate the importance of dominance changes, but are more closely related to expectations of falling species numbers in stressed communities. Binary data are comparatively easier to obtain because time is not consumed enumerating individuals, a laborious task for small taxa (e.g., algae, protozoa, micrometazoa). Where the scale of sampling is

large in comparison with the size of organisms, relatively complete sampling can be assumed. This is usually the case in sampling microorganisms. However, when communities of larger taxa such as macroinvertebrates or fishes are used, evidence of the adequacy of sampling (e.g., a species-area or species-effort curve) is needed to determine the appropriate scale and number of replicates. When the number of taxa is large, binary data are sufficient to detect changes in community structure.

The example analyses presented below use binary data or rated abundance, although the methods are applicable to indices derived from weightings with approximately continuous data such as individuals per species or continuous data such as biomass or biovolume per species. Obviously, enumeration of individuals ignores size differences among species; however, these estimates of population sizes withing replicate physical samples are appropriate for comparisons among sites or treatments. The analyses are appropriate for the analysis of multispecies laboratory experiments and for comparisons of replicate samples among field sites.

Data requirements

The starting point for analyses is the development of a taxonomic summary of species presence (or abundance) in replicate physical samples by treatment or site. Replicate physical samples of the within site or within treatment conditions are required. The number of replicates may vary, but at least three or four replicate samples per site is necessary to obtain adequate estimates of variability. Taxa may be identified to any practical taxonomic level, although the rigor of the analyses and the conclusions that can be drawn will depend on the level of taxonomic precision chosen. Family-level classification will likely increase similarity while species-level taxonomy will decrease similarity among replicates. Two example data sets are shown in Tables 1 and 2.

Table 1. Sample data set showing ranked abundance of taxa (scaled 1-5) by treatment and replicate. The ordered treatment values correspond to increasing concentrations of copper.

Taxon	Treatment 1	2	3	4	5	6
A sp1	0 0 1	0 0 0	0 1 0	0 1 0	3 3 3	0 4 4
A sp2	5 5 5	4 4 4	3 3 3	2 2 2	1 1 1	1 1 1
C sp1	1 1 1	1 1 1	0 1 1	1 1 0	1 0 1	0 1 0
C sp2	1 1 1	1 1 1	1 1 0	0 0 1	0 1 0	0 0 0
C sp3	0 0 0	1 0 0	0 0 0	1 1 1	1 0 0	1 1 1
C sp4	1 1 1	0 1 1	1 0 1	0 1 1	0 3 3	5 5 5
C sp5	1 1 1	1 1 1	1 1 0	1 1 0	0 0 0	1 0 0
D sp1	0 0 0	0 0 0	0 0 0	1 1 0	1 1 0	1 0 1
F sp1	1 1 1	1 1 1	0 1 1	0 1 1	1 1 1	1 0 1
F sp2	1 1 1	1 1 1	1 1 1	1 1 1	0 1 0	1 1 0
G sp1	0 0 1	0 0 0	0 0 1	0 2 2	3 3 3	0 4 4
G sp2	1 1 1	0 1 1	0 1 0	1 0 1	1 0 0	0 0 1
G sp3	1 1 1	0 1 1	1 0 0	1 1 0	0 0 1	0 1 0
G sp4	1 1 1	1 1 1	0 0 1	0 1 0	0 0 0	1 0 1
M sp1	1 1 1	1 1 1	1 0 1	1 0 0	0 0 1	1 0 0
M sp2	1 1 1	1 1 0	1 1 0	1 0 1	1 1 1	0 1 1
N sp1	5 5 5	4 4 4	3 3 3	2 2 2	1 1 1	1 1 1
N sp2	1 1 1	0 1 0	1 1 1	1 0 0	0 0 1	0 1 1
N sp3	1 1 1	0 1 1	0 0 1	0 1 1	1 0 0	0 0 0
N sp4	0 0 0	0 0 0	1 0 0	1 0 1	0 1 1	1 0 1
N sp5	1 0 0	1 1 1	1 0 1	0 0 0	0 0 0	0 0 0
N sp6	1 1 1	1 1 1	1 1 1	0 0 1	1 1 1	0 0 1
N sp7	1 1 1	1 1 1	0 0 1	1 0 1	0 0 0	1 1 1
P sp1	0 1 0	0 1 0	1 0 0	0 1 0	1 0 1	1 0 0
S sp1	1 0 0	1 0 0	1 1 0	0 0 0	0 0 0	1 1 0
S sp2	0 1 0	1 0 1	0 1 1	0 0 0	1 0 0	0 0 0
S sp3	0 1 0	1 1 0	0 0 0	1 1 0	0 0 1	0 0 0
S sp4	1 1 1	0 1 1	1 0 1	0 1 1	0 1 0	1 1 1
S sp5	1 1 1	1 1 0	1 1 0	1 1 0	1 1 0	1 1 0
S sp6	5 5 5	4 4 4	3 3 3	2 2 2	1 1 0	1 0 1
Total taxa	22 22 24	19 20 23	19 17 16	17 16 19	16 15 15	17 17 15

Table 2. Data from Table 1 rearranged by frequency of occurrence of taxa in replicates.

Taxon	Treatment 1	2	3	4	5	6	Frequency
A sp2	5 5 5	4 4 4	3 3 3	2 2 2	1 1 1	1 1 1	18
N sp1	5 5 5	4 4 4	3 3 3	2 2 2	1 1 1	1 1 1	18
S sp6	5 5 5	4 4 4	3 3 3	2 2 2	1 1 0	1 0 1	16
F sp2	1 1 1	1 1 1	1 1 1	1 1 1	0 1 0	1 1 0	15
F sp1	1 1 1	1 1 1	0 1 1	0 1 1	1 1 1	1 0 1	15
C sp4	1 1 1	0 1 1	1 0 1	0 1 1	0 3 3	5 5 5	14
M sp2	1 1 1	1 1 0	1 1 0	1 0 1	1 1 1	0 1 1	14
N sp6	1 1 1	1 1 1	1 1 1	0 0 1	1 1 1	0 0 1	14
C sp1	1 1 1	1 1 1	0 1 1	1 1 0	1 0 1	0 1 0	13
S sp4	1 1 1	0 1 1	1 0 1	0 1 1	0 1 0	1 1 1	13
S sp5	1 1 1	1 1 0	1 1 0	1 1 0	1 1 0	1 1 0	13
N sp7	1 1 1	1 1 1	0 0 1	1 0 1	0 0 0	1 1 1	12
C sp5	1 1 1	1 1 1	1 1 0	1 1 0	0 0 0	1 0 0	11
M sp1	1 1 1	1 1 1	1 0 1	1 0 0	0 0 1	1 0 0	11
N sp2	1 1 1	0 1 0	1 1 1	1 0 0	0 0 1	0 1 1	11
C sp2	1 1 1	1 1 1	1 1 0	0 0 1	0 1 0	0 0 0	10
G sp4	1 1 1	1 1 1	0 0 1	0 1 0	0 0 0	1 0 1	10
G sp2	1 1 1	0 1 1	0 1 0	1 0 1	1 0 0	0 0 1	10
G sp3	1 1 1	0 1 1	1 0 0	1 1 0	0 0 1	0 1 0	10
G sp1	0 0 1	0 0 0	0 0 1	0 2 2	3 3 3	0 4 4	9
N sp3	1 1 1	0 1 1	0 0 1	0 1 1	1 0 0	0 0 0	9
A sp1	0 0 1	0 0 0	0 1 0	0 1 0	3 3 3	0 4 4	8
C sp3	0 0 0	1 0 0	0 0 0	1 1 1	1 0 0	1 1 1	8
N sp4	0 0 0	0 0 0	1 0 0	1 0 1	0 1 1	1 0 1	7
P sp1	0 1 0	0 1 0	1 0 0	0 1 0	1 0 1	1 0 0	7
D sp1	0 0 0	0 0 0	0 0 0	1 1 0	1 1 0	1 0 1	6
N sp5	1 0 0	1 1 1	1 0 1	0 0 0	0 0 0	0 0 0	6
S sp1	1 0 0	1 0 0	1 1 0	0 0 0	0 0 0	1 1 0	6
S sp3	0 1 0	1 1 0	0 0 0	1 1 0	0 0 1	0 0 0	6
S sp2	0 1 0	1 0 1	0 1 1	0 0 0	1 0 0	0 0 0	6

Selection of community measure

As mentioned above, the selection of community association measure will, in part, influence the outcome of analyses. Similarity measures are based on comparisons of shared and unique taxa between pairs of samples (Fig. 1). Examples of commonly used measures of both similarity and distance are shown in Table 3. Negative matches (taxa failing to occur in both samples) are often ignored in similarity measures because the absence of a particular taxon may not be judged important (Roback 1979). However, when taxa have particular indicator value, the absence of a taxon in two samples may be judged to make the association between the samples stronger. This is especially useful when

important taxa are expected at study sites. For example, the absence of red oak trees at two sites might be considered important in associating those sites, and this importance should be reflected in the choice of community similarity measure. Binary, ranked, and continuous data can be used to form many of the indices.

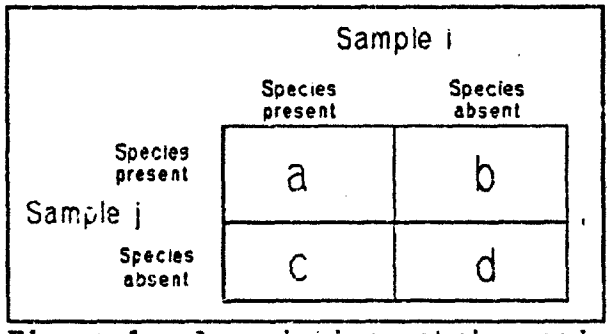


Figure 1 - Association matrix used to compute similarity measures

Table 3. Exemplary measures of community similarity. Formulas are based on terminolgy shown in Fig. 1. Measures whose complements satisfy the triangle inequality are termed metric. Those that do not are semimetric. Measures whose complements can take negative values are termed nonmetric.

Coefficient	Form	Class	Reference
Simple matching	$\frac{a + d}{a + b + c + d}$	metric	Digby & Kempton 1987
Jaccard	$\frac{a}{a + b + c}$	metric	Jaccard 1901
Czekanowski	$\frac{2a}{2a + b + c}$	semimetric	Czekanowski 1909 Sorensen 1948
Margalef	$\frac{a(a+b+c+d)}{(a+b)(a+c)}$	semimetric	Margalef 1958
Community Loss	$\frac{c}{a + b}$	nonmetric	Courtemanch & Davies 1987

Some measures or their complements are metric; that is, they satisfy the triangle inequality priciple. Other measures which fail to satifsy the triangle inequality are termed semimetric because they fail to satisfy the triangle inequality principle (Legendre and Legendre 1983). Semimetric measures may have less predictable statistical properties. A difference of a given magnitude between semimetric coefficients may not have the same meaning for all values of the coefficients. Still others are nonmetric because their complements can be negative, and the application of such coefficients may be problematic.

Many other measures of both community similarity or distance can be used (Legendre and Legendre 1983, Digby and Kempton 1987, Plafkin et al. 1990). Among the more familiar to ecologists and pollution biologists are Euclidian distance, Manhattan (city-block) distance, Bray-Curtis measure, Canberra measure, correlation coefficient complement, Morisita's index, and Pinkham and Pearson measure. A general form for community measures (and their complement distance measures) is given by Gower (1971).

Analysis method

A similarity matrix for all possible replicate pairs is contructed using a chosen metric. A test statistic is computed comparing the mean similarity of replicate objects within "treatments" to the between treatment similarity. If the test statistic for data is

$$L(\text{data}) = \overline{B}/\overline{W} \qquad (1)$$

where $\overline{B}$ is the mean between treatment similarity and $\overline{W}$ is the mean within treatment similarity, then this statistic can be compared to one derived from a permutation procedure in which coefficients in the similarity matrix are randomly switched a large number of times (>1000). A test statistic L(permute) is recalculated as above after each permutation of the matrix. The distribution of L(permute) can be used to determine if L(data) can be differentiated from L(permute) at a given alpha (α). For example, if 1000 random switches (permutations) are made then one would reject a null hypothesis of no difference in community similarity at α=0.05 if L(data) were more extreme than 950 (95%) of the L(permute) values. Because the total similarity (T) is a constant for any given matrix, the component B and W similarities may also be used as test statistics.

Similar arguments can be made for the use of distance measures rather than community similarity. However, in multidimensional space, the location of similar samples is associated with a small distance; dissimilar samples would be more widely separated. Therefore, the principles of using distance measures as measures of similarity would apply to testing community differences, but the expected direction of change in the randomly switched matrix would be opposite to expectations for similarity measures.

Follow-up analyses

Assuming that a hypothesis of community similarity among sites or treatments is rejected, several follow-up analyses are possible. One approach would be to conduct multiple comparisons using the permutation procedure on treatment pairs. This approach would test differences between individual treatments or sites. However, if the number of replicates is small (as it often is in field studies), detection power is limited by the

fact that only a small number of unique permutations of the similarity matrix are possible.

A second useful follow-up analysis is to determine the relative contribution of each taxon to community similarity. The effect of taxa on similarity can be determined by computing the effect on similarity of removing each taxon. In this analysis, removing common taxa will reduce total similarity. Taxa adding heterogeneity to the matrix (and so decreasing similarity) will increase total similarity when removed. Identification of these taxa permits an inspection of the data matrix to identify taxa that may appear or disappear in certain treatments or sites. When coupled with a data matrix sorted by the total frequency of occurrence of taxa, these identifications become easier (e.g., Table 2). Additional follow-up analyses are summarized by Smith et al. (1990).

Examples of community data analysis

To demonstrate a method for detecting changes in community composition between sites or treatments the hypothetical data set in Table 1 was constructed (based on actual data of effects of copper on periphyton algae). The presence of taxa was determined from 500 cell counts of preserved samples. The ordered treatments (1-6) ranged from controls (<10 ug Cu/L) to 80 ug Cu/L. The data matrix has been edited to include only the 30 most common taxa and to magnify abundance differences among taxa.

RESULTS

Example analysis - similarity

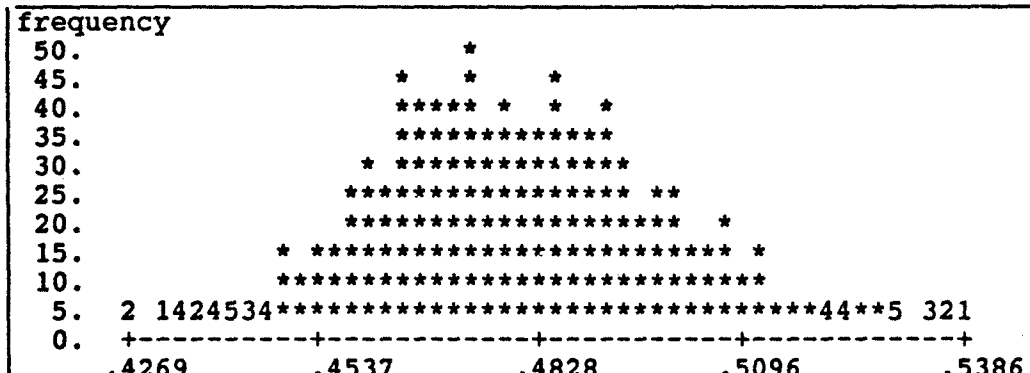
Based on presence-absence data shown in Table 1, a similarity matrix was constructed using the Jaccard measure. Communities have higher similarity if they share a greater proportion of their total species. A portion of this matrix is shown in Fig. 2. Rectangular blocks in the table mark similarity coefficients comparing samples between treatments. Triangular areas lying along the matrix diagonal are coefficients showing the within treatment similarity. Computation of within and between treatment similarity was followed by 1000 permutations randomly switching matrix coeffients to produce a hypothetical distribution of within treatment similarity (Fig. 3).

Treatment/Replicate													
1A	1B	1C	2A	2B	2C	3A	3B	3C	...	6A	6B	6C	
1	.80	.83	.64	.88	.78	.67	.58	.63	...	.50	.48	.44	1A
	1	.80	.62	.92	.75	.58	.56	.60	...	.48	.41	.43	1B
		1	.52	.80	.71	.54	.58	.63	...	.44	.54	.56	1C
			1	.62	.58	.48	.59	.50	...	.50	.36	.33	2A
				1	.75	.64	.50	.60	...	.48	.41	.43	2B
					1	.48	.46	.71	...	.44	.31	.38	2C
						1	.48	.40	...	.52	.43	.35	3A
							1	.38	...	.32	.41	.38	3B
								1	...	.42	.39	.48	3C

Figure 2 - Part of the matrix of Jaccard similarity coefficients from the examination of data in Tables 1 and 2. Triangular groups of values along the matrix diagonal are the within treatment coeffients. Rectangular groups away from the diagonal are between treatment similarities.

These analyses showed that the critical within treatment similarity based on $\alpha=0.05$ was 0.5155. This compared to the actual within treatment similarity of 0.5271. Further, the number of permutation similarity values that were more extreme that this observed similarity was 17 corresponding to a p-value of 0.018 ($p=(17+1)/1000$)). Therefore, the hypothesis of no difference in similarity among treatments is rejected.

A



Total similarity	73.7955	mean	0.4823
Between similarity	64.3072	mean	0.4763
Within similarity	9.4883	mean	0.5271

The critical value is the 950th value.
The critical mean within similarity is 0.51545.
Reject hypothesis of no effect if this value is smaller than the observed mean within similarity.
Number > observed within 17.

B

Lambda values for pairwise comparisons, ratios of mean between to mean within similarity. Values near 1 indicate little difference between groups.

	Treatment					
	1	2	3	4	5	6
1	1.0000	1.0051	.9690	.8659	.7022	.7608
2	1.0051	1.0000	1.0334	.8996	.6832	.7421
3	.9690	1.0334	1.0000	.9900	.9607	.9552
4	.8659	.8996	.9900	1.0000	1.0414	1.1360
5	.7022	.6832	.9607	1.0414	1.0000	.9639
6	.7608	.7421	.9552	1.1360	.9639	1.0000

Figure 3 - A. Distribution of permutations of within similarity (Jaccard coefficient) for the data from Fig. 2. B. Pairwise comparisons (Lambda values) showing progressive differences between treatments.

Follow-up analyses showed that similarity of treatments to controls (group 1) decreased with increasing copper levels (Fig. 4) and that common species strongly affected community similarity. Species with moderate negative or positive influence are typically those that are either eliminated in higher treatments (e.g., C. sp2) or that become more frequent in replicates at high copper levels (e.g., C. sp3, D. sp1). Based on multiple pairwise comparisons, only treatments 2 and 5 could be differentiated.

A

Species removed		Influence
A	sp2	-4.675
C	sp2	1.087
C	sp3	1.417
D	sp1	1.459
F	sp1	-1.956
F	sp2	-1.885
N	sp1	-4.675
N	sp3	1.332
N	sp4	1.331
N	sp5	1.774
N	sp6	-1.109
P	sp1	1.330
S	sp1	1.492
S	sp2	1.508
S	sp3	1.544
S	sp6	-2.735

B

The critical values below are for the multiple comparisons on the mean between similiarity. Reject if the mean between similarity is smaller than the critical value. Asterisk (*) denotes detected difference.

Trt.	Trt.	Critical value	Between similarity	
1	2	.38724	.73406	
1	3	.39258	.59541	
1	4	.39516	.52651	
1	5	.38613	.43858	
1	6	.39156	.47645	
2	3	.39058	.55150	
2	4	.39742	.47437	
2	5	.38703	.37148	*
2	6	.38590	.40479	
3	4	.38267	.40726	
3	5	.39143	.41105	
3	6	.39180	.41033	
4	5	.39739	.43892	
4	6	.38616	.48076	
5	6	.38786	.42378	

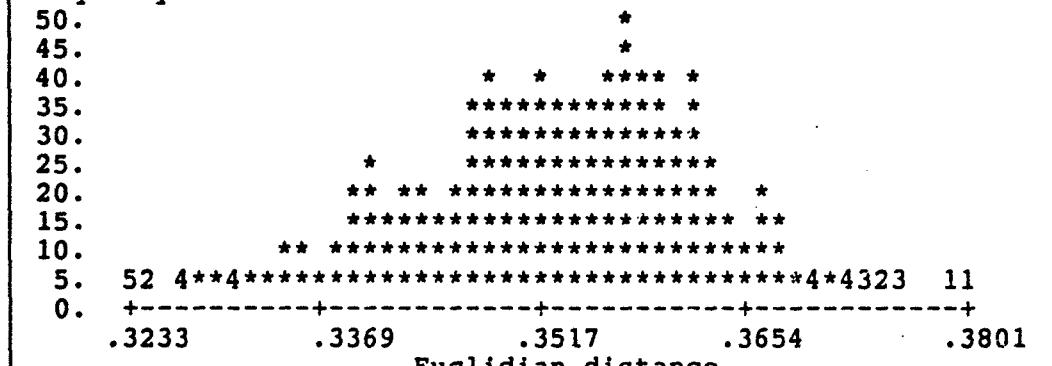
Figure 4 - Follow-up analyses. A. Species influence scores for select species from Tables 1 and 2. Scores indicate the magnitude and direction of effect on community similarity when the species is removed from the analysis. B. Pairwise tests of treatment differences based on between treatment similarity. Differences are detected at p=0.1.

Effect of measure

Comparable analyses to those presented above were done using only presence-absence data to determine Euclidian distance among treatments. Distance analyses provide estimates of community

dissimilary: dissimilar communities are more widely separated in multidimensional space. These analyses are essentially identical to those using presence-absence based similary (Fig. 5). The within treatment distance estimate is more extreme than 7 of 1000 permutation-based within treatment values (p=7+1/1000=0.007), so the hypothesis of no difference in distance among treatments is rejected.

A



Total distance	53.9703	mean	0.3527
Between distance	48.0703	mean	0.3561
Within distance	5.9000	mean	0.3278

The critical value is the 51st value.
The critical mean within distance is 0.33505.
Reject hypothesis of no effect if this value is smaller than the observed mean within distance.
Number < observed within 7.

B

Lambda values for pairwise comparisons, ratios of the mean between to the mean within distance. Values near 1 indicate little difference between groups.

	Treatment					
	1	2	3	4	5	6
1	1.0000	.9830	1.0712	1.1715	1.3650	1.2963
2	.9830	1.0000	.9841	1.0835	1.2646	1.2149
3	1.0712	.9841	1.0000	1.0057	1.0219	1.0236
4	1.1715	1.0835	1.0057	1.0000	.9789	.9296
5	1.3650	1.2646	1.0219	.9789	1.0000	1.0208
6	1.2963	1.2149	1.0236	.9296	1.0208	1.0000

Figure 5 - A. Distribution of permutations of within treatment distance for the example using binary data. B. Pairwise comparisons.

Follow-up analyses identified the same patterns found using analysis with Jaccard's coefficient (Fig. 6). Evaluation of these data using several other presence-absence based metrics

revealed essentially similar patterns, although other metrics weight common species more heavily and so produce coeffients of greater magnitude.

A

Species removed		Influence
A	sp2	.000
C	sp2	-2.157
C	sp3	-2.209
D	sp1	-1.896
F	sp1	-1.137
F	sp2	-1.155
N	sp1	.000
N	sp3	-2.142
N	sp4	-1.997
N	sp5	-2.048
N	sp6	-1.486
P	sp1	-1.954
S	sp1	-1.909
S	sp2	-1.928
S	sp3	-1.921
S	sp6	-.788

B

The critical values below are for the multiple comparisons of the mean between distance. Reject if the mean between distance is larger than the critical value. Asterisk (*) denotes detected difference.

Trt.	Trt.	Critical value	Between distance	
1	2	.38717	.24979	
1	3	.38667	.31570	
1	4	.38612	.35068	
1	5	.38813	.38352	
1	6	.38776	.37036	
2	3	.39018	.32776	
2	4	.38646	.36588	
2	5	.38768	.40385	*
2	6	.38789	.39370	*
3	4	.38773	.38046	
3	5	.38783	.36781	
3	6	.39041	.37327	
4	5	.38657	.35689	
4	6	.39088	.34328	
5	6	.38875	.35822	

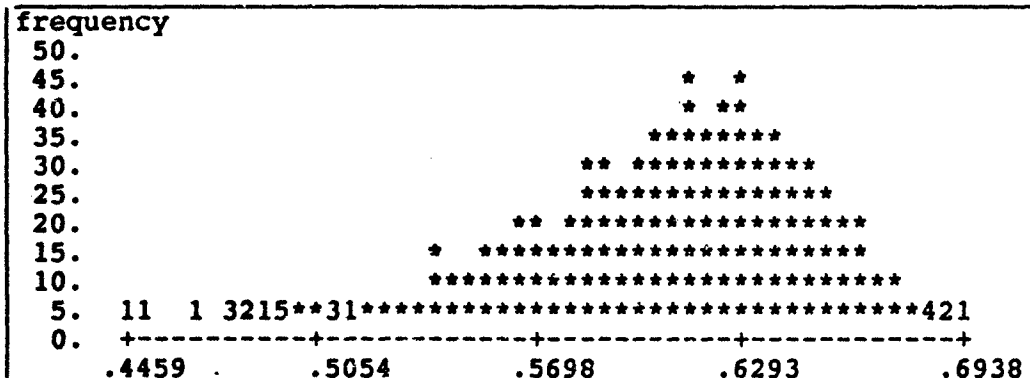
Figure 6 - Follow-up analyses. A. Species influence scores based on Euclidian distance. B. Pairwise tests of treatment differences based on between treatment similarity. Differences are detected at p=0.1.

Effect of data type

While presence-absence data were effective in detecting differences among treatments, data evaluated by rank abundance

resulted in greater detection power. For example, the rankings shown in Table 1 weight only six of 30 species; three become less abundant across treatments, and three become more abundant. Examination of the weighted data using Euclidian distance showed clear treatment differences (Fig. 7) and allowed detection of additional differences between treatment pairs (Fig. 8).

A



Total distance	92.8750	mean	0.6070
Between distance	86.1072	mean	0.6378
Within distance	6.7679	mean	0.3760

The critical value is the 51st value.
The critical mean within distance is 0.52928.
Reject hypothesis of no effect if this value is smaller than the observed mean within distance.
Number < observed within 0.

B

Lambda values for pairwise comparisons, ratios of the mean between to the mean within distance. Values near 1 indicate little difference between groups.

	Treatment					
	1	2	3	4	5	6
1	1.0000	1.2057	1.5910	2.0498	2.8655	2.5655
2	1.2057	1.0000	1.1144	1.4945	2.2802	2.1458
3	1.5910	1.1144	1.0000	1.1155	1.6803	1.7064
4	2.0498	1.4945	1.1155	1.0000	1.3238	1.4395
5	2.8655	2.2802	1.6803	1.3238	1.0000	1.1116
6	2.5655	2.1458	1.7064	1.4395	1.1116	1.0000

Figure 7 - A. Distribution of permutations of within treatment distance for the example using rank abundance data. B. Pairwise comparisons.

A

Species removed		Influence
A	sp2	-5.915
C	sp2	-.674
C	sp3	-.746
D	sp1	-.592
F	sp1	-.387
F	sp2	-.337
N	sp1	-5.915
N	sp3	-.699
N	sp4	-.640
N	sp5	-.696
N	sp6	-.489
P	sp1	-.669
S	sp1	-.659
S	sp2	-.667
S	sp3	-.686
S	sp6	-7.015

B

The critical values below are for the multiple comparisons of the mean between distance. Reject if the mean between distance is larger than the critical value. Asterisk (*) denotes detected difference.

Trt.	Trt.	Critical value	Between distance
1	2	.78949	.30638
1	3	.79348	.46888
1	4	.77944	.63923
1	5	.79077	.90158 *
1	6	.82022	.98559 *
2	3	.81480	.37119
2	4	.79738	.52340
2	5	.79219	.80488 *
2	6	.78752	.90666 *
3	4	.79658	.43593
3	5	.79791	.66137
3	6	.82326	.79031
4	5	.79434	.54372
4	6	.80127	.69137
5	6	.78662	.53696

Figure 8 - Follow-up analyses. A. Species influence scores based on Euclidian distance determined from rank abundance. B. Pairwise tests of treatment differences based on between treatment similarity. Differences are detected at p=0.1.

DISCUSSION

The structure of biological communities presents a host of potential adaptations and responses to the effects of environmental stressors ranging from pollutants to physical stresses to habitat modification. The analysis of populations within communities often presents equivocal data: some species increase in abundance while others decrease. In extreme cases, some species are locally extirpated, reducing community heterogeneity. In other cases, intolerant species are replaced by stress tolerant forms.

The use of community similarity and related analyses has provided a descriptive tool for comparing sites under differing conditions. The methods described above for critically examing replicate community at differing sites or in multispecies experiments provide a means of inferentially evaluating community change, detecting the sites or treatments at which significant change occurs, and identifying the species influencing the change in community structure.

Data type and effect of measure

Measures that are based on binary (presence-absence) data limit the ability to investigators to detect community differences. Those that place more weight on common species in community pairs (e.g., Czekanowski) increase the absolute value of the similarity index, but provide no greater detection power for comparing communities. Additionally, the complements of these measures are typically semimetric and are, therefore, less useful indicators of relative difference (distance) among communities. Measures such as the Coefficient of Community Loss are nonmetric and require additional investigation before they can be recommended, as they are now, for detecting community differences. Measures that place emphasis on missing species are only useful when the base species list includes important indicator species.

Measures based on binary data require species loss and gain to detect differences in community structure and are not sensitive to abundance changes. However, when the number of species examined is high (as for microbial communities) detection of community differences is greater than for less diverse assemblages such as fishes. Schindler (1987) has recently recommended small, rapid reproducing species with poor dispersal capabilities as efficient environmental monitors. Additional evidence suggests that structural changes in ecosystems consistently precede process changes (Pratt 1990)

Measures that incorporate ranked, relative, or absolute abundance as weightings for taxa in community samples have improved abilities to detect differences in structure. In these cases, measures are sensitive to changes in abundance not simply gain or loss of taxa. Distance measures are effective means of

detecting the separation of samples in multidimensional (multispecies) space; however, no rigorous analysis comparing the relative powers or sensitivities of these measures has been applied to detecting biotic community differences that result from stress.

Competing analytical methods

Several other methods for evaluating community structure data have been applied to the determination of ecosystem health. These methods include calculation of diversity indices or indices of biotic integrity and the comparison of communities using cluster analysis. A brief discussion of the advantages and disadvantages of each of these follows.

Diversity indices compare sites based on the information content of species abundance or some other measure of the distribution of individuals or biomass per species present. The most commonly used indices are based in information theory (e.g., Shannon and Weaver 1948). While these indices provide numerical values for diversity, including values for replicate samples at a site, the indices do not account for the identity of the component taxa. That is, two samples might have the same information theory diversity and share no species in common. Diversity indices have comparative value in comparing sites, and the computed index is often strongly correlated with the number of taxa (Green 1979) suggesting that species richness alone is a sufficient measure of diversity. The concept of species diversity is often ignored in investigations where problem taxa are pooled (e.g., chironomid midge larvae) so the resulting index is computed from a mixture of taxa, some species, some genera, some families.

Indices of biotic integrity provide a score for each of several biological criteria. Unlike diversity indices, where the identity of the taxa is ignored, the identity of collected taxa is of primary importance. Indices such as the fish-based index of biotic integrity (IBI, Karr et al. 1986) or the invertebrate community index (ICI, Ohio EPA 1987) are based on a combination of concepts. These indices are computed by assigning integer scores to community "metrics" which are conceptually categorized. For example, the IBI separates metrics according to species richness (all taxa and some indicators), trophic composition, and abundance and condition of specimens. Similar concepts are used in the ICI, although there is more reliance on indicator taxa. The scores assigned are derived from a subjective rating system based on determination of regional optima (i.e., expected values). Statistical comparisons are not possible, although community similarity to a reference site is one of the metrics used in the ICI. The term "metric" as applied to IBI and ICI is unfortunate because the assigned scores lack the characteristics of true metrics (sensu Legendre and Legendre 1983, Digby and Kempton 1987).

Cluster analyses share may common features with methods of community similarity and distance analysis (see Pielou 1984, Digby and Kempton 1987). Both hierarchical and nonheirarchical clustering methods are available, but clustering methods, like ordination procedures, are essentially graphical tools that allow visualization of community relationships. They provide no inferential analysis, but are simply ways of comparing several objects (samples) according to several descriptors (variables - species or other characters). As such, they may be useful companion tools to the analytical methods described in this paper. For most multivariate inferential procedures, the covariance matrix cannot be singular. However, when the number of samples is fewer than the number of variables(species) this will be the case (as it often will be in environmental analysis). Assumptions of normal distribution of variables are often violated when species abundances are zero at some sites.

Summary and conclusions

The choice of similarity measure affects the outcome of analyses. The numerical value of the similarity measure is influenced by functional form of the measure, transformations applied to the data prior to analysis and the type of data. Choice of data type limits what type of changes can be detected. For example, with presence absence data, one primarily is interested in changes in species composition. With abundance data, interest is more in decreases or increases in the species relative abundance. With some measures, dominant species may strongly influence the measure. Changes in the relative abundance of a dominant species may not result in the loss of a species. Thus measures based on presence absence may not reflect changes in the dominant species.In fact, if a species undergoes a large change but is not absent, the species may have a positive (stabilizing) effect on a presence absence measure. On the other hand, changes that result in loss of species may not be reflected in measures based on proportional abundance unless they are accompanied by strong changes in the relative abundance (the total relative abundance of the species absent must be moderate when they are present). Choice of data type and measure are important and should be based on the hypothesis of interest.

Biological criteria for evaluating individual and cummulative effets of stresses might be based on comparing communities to those of regional reference sites or to upstream reference communities, although often no acceptable upstream reference can be found. The usefulness of biological criteria for evaluating ecosystem health will be determined by the scientific adequacy of the analyses applied to detecting community change. At the present time, critical evaluations of analytical tools are needed to determine the appropriateness of using available measures for detecting community change. Critical detections cannot be made by indices that are only descriptive and lack measures of variability.

ACKNOWLEDGEMENTS

Portions of this work were supported by the US Army Biomedical Research and Development Laboratory, Fort Detrick, MD. The opinions and conclusions are those of the authors and do not necessarily represent those of the US Army.

LITERATURE CITED

Cairns, J. and J.R. Pratt. [in press]. A history of biomonitoring using inverebtrates. In V. Resh and D. Rosenberg, eds., Freshwater Biomonitoring and Benthic Macroinvertebrates, Chapman and Hall, New York.

Courtemanch, D.L., and S.P. Davies. 1987. A coefficient of community loss to assess detrimental change in aquatic communities. Wat. Res. 21:217-222.

Cummins, K. W., and M. J. Klug. 1979. Feeding Ecology of Stream Invertebrates. Ann. Rev. Ecol. Syst. 10:147-172.

Czekanowski, V. 1909. Zur differential diagnose der Neandertalgruppe. Korrespbl. dt. Ges. Antwop. 40:44-47.

Digby, P.G.N. and R.A. Kempton. 1987. Multivariate Analysis of Ecological Communities, Chapman and Hall, London.

Gauch, H. 1982. Multivariate Analysis in Community Ecology. Cambridge University Press, London.

Gower, R. 1971. A general coefficient of similarity and some of its properties. Biometrics 27:857-872.

Green, R.H. 1979. Sampling Design and Statistical Methods for Environmental Biologists. John Wiley and Sons, New York,

Hilsenhoff, W. 1982. Using a biotic index to evaluate water quality in streams. Tech. Bull. #132, Dept. Nat. Res., Madison, WI.

Hurlbert, S. H. 1971. The nonconcept of species diversity: a critique and alternative parameters. Ecology 52:577-586.

Jaccard, P. 1901. Distribution de la flore alpine dans le Bassin des Dranses et dans quelques régions voisines. Bull. Soc. vand. Sci. Nat. 37:241-272.

Karr, J., K.D. Fausch, P.L. Angermier, P.R. Yant, and I. Schlosser. 1986. Assessing biological integrity in running waters: a method and its rationale. Illinois Natural History Survey Special Publication 5, Champaign, IL.

Kolkwitz, R. and M. Marsson. 1908. Ökologie der pflanzlichen Saprobien. Berichte der Deutschen Botanischen Gesellschaft 26A:505-519.

Legendre, L. and P. Legendre. 1983. Numerical Ecology. Elsevier, Amsterdam.

Levine, S.N. 1989. Theoretical and methodological reasons for variability in the responses of aquatic ecosystem processes to

chemical stresses. pp. 145-180 in S.A. Levin, M.A. Harwell, J.R. Kelly, and K.D. Kimball, eds., Ecotoxicology: Problems and Approaches, Springer-Verlag, New York.

Lowe, R.L. 1974. Environmental Requirements and Pollution Tolerance of Freshwater Diatoms, US EPA 670/4-74-005.

Margalef, R. 1958. Information theory in ecology. General Systems 3:36-71.

Metcalfe, J.L. 1989. Biological water quality assessment of running waters based on macroinvertebrate communities: history and present status in Europe. Environ. Pollut. Ser. A 60:101-139.

Odum, E.P. 1990. Field experimental tests of ecosystem-level hypotheses. Trends in Ecology and Evolution 5:203-236.

Odum, E.P. 1985. Trends Expected in Stressed Ecosystems. BioScience 35(7):419-422.

Ohio Environmental Protection Agency. 1987. Biological Criteria for the Protection of Aquatic Life: Volume II. Users Manual for Biological Field Assessment of Ohio Surface Waters. Division of Water Quality Monitoring and Assessment, Columbus, OH.

Omernik, J.M. 1987. Ecoregions of the conterminous United States. Ann Assoc. Amer. Geogr. 77:118-125.

Pielou, E.C. 1984. The Interpretation of Ecological Data. Wiley, New York.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish. EPA/444/4-89-001, US EPA, Office of Water, Washington, DC.

Pratt, J.R. 1990. Aquatic community response to stress: prediction and detection of adverse effects, pp. 16-26 in W.G. Landis and W.H. van der Schalie, eds., Aquatic Toxicology and Risk Assessment, 13th Volume. American Society for Testing and Materials, Philadelphia, PA.

Pratt, J.R., J. Cairns, and P.M. Stewart. 1985. Development of microbial communities in mined lands. pp. 239-259 in R.P. Brook, D.E. Samuel, and J.B. Hill, eds., Wetlands and Water Management of Mined Lands, Pennsylvania State University, University Park, PA.

Roback, S.S. 1974. Insects (Arthropoda: Insecta), pp. 275-376 in C.W. Hart and S.L.H. Fuller, eds., Pollution Ecology of Freshwater Invertebrates. Academic Press, New York.

Schaeffer, D.J., E.E. Herricks, and H.W. Kerster. 1988. Ecosystem Health I. Measuring ecosystem health. Environ. Mgmt.

12:445-455.

Schindler, D.W. 1987. Detecting Ecosystem responses to anthropogenic stress. Can. J. Fish. Aquat. Sci. 44(Suppl.):6-25.

Shannon,C.E. and W. Weaver. 1949. The Mathematical Theory of Communication, University of Illinois Press, Urbana, IL.

Smith, E.P., K.W. Pontasch, and J. Cairns. 1990. Community similarity and the analysis of multispecies environmental data: a unified statistical approach. Water Research 24:507-514.

Sorensen, T. 1948. A method of establishing groups of equal amplitude in plant sociology based on similarity in species content. Biol. Skr. K. danske Vidensk. Selsk. 5:1-34.

Whittier, T.R., R.M. Hughes, and D.P. Larsen. 1988. Correspondence between ecoregions and spatial patterns is stream ecosystems in Oregon. Can. J. Fish. Aquat. Sci. 45:1264-1278.

Comparative Effects of Copper on Microcosms Developed From Different Ecosystems

J. R. Pratt, N. J. Bowers and J. M. Balczon
School of Forest Resources and Graduate Program in Ecology
Penn State University, University Park, PA 16802

W. H. Van der Schalie, T. Shedd and J. Madsen
U. S. Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701

Abstract

Ecological effects of copper on freshwater microbial communities on artificial substrates were compared using microcosms continuously dosed with copper at concentrations from 5-200 ug/L. Natural microbial communities were obtained from different streams and used to seed microcosms. Changes in species richness, biomass (protein, chlorophyll), phosphatase activity, production, and respiration were evaluated for 21 d. Effects of copper were similar in both experiments. Chlorophyll biomass was strongly affected by copper, and algal species richness fell with increasing copper dose. Similarity of algal and protozoan biotas changed with dose. Phosphatase activity was only slightly affected, but oxygen gain diminished with copper dose. Effect levels estimated both by hypothesis testing (NOEC, LOEC) and inverse prediction from regression (EC20) showed similarity of responses in the two experiments. LOEC's were 10.0 and 19.9 ug Cu/L for chloro-phyll biomass and 19.3 and 9.9 ug/L for species richness. LOEC's for other measures ranged from 10.0-90 ug/L. Over 90% of EC20's ranged from 3.0-39.2 ug/L. Significant differences in species composition were detected at nominal copper doses >20 ug/L. Coefficients of variation ranged from 1-250% for process measures and 3-172% for structure measures.

Introduction

Ecological evaluation of toxic chemicals has relied on testing individual sensitive species. The goal of such testing is to predict concentrations of toxicants that have only small effects on exposed species (including numerous untested species). Tests on individual species ignore the potential importance of ecological interactions and propagating effects that may result from adverse effects on key species or groups of sensitive taxa (NRC, 1981). While testing of sensitive species may predict concentrations of a contaminant that are broadly protective, standard bioassays cannot predict the types of ecological effects that may be realized in receiving ecosystems (Cairns, 1984; Kimball and Levin, 1985).

Artificial ecosystems share many properties with real ecosystems - succession, production, nutrient and mineral cycling - and range from small systems (microcosms) assembled from cultures of individual species (Taub, 1976) to large bounded portions of natural ecosystems (mesocosms) (Kaushik et al., 1986). The similarity and limitations of model ecosystems has been extensively examined (Giesy, 1980) and the utility of controlled laboratory and field systems in ecotoxicology predicted (Hammons, 1981).

In general, the number of microcosm and mesocosm designs for studying ecological and ecotoxicological problems has been large, reflecting the differing goals, backgrounds, and preferences of researchers so repeated experiments have been rare. Only recently have standard methods been proposed for artificial ecosystem test methods (Taub 1989, Leffler 1981, Giddings 1986, Pratt and Bowers 1990, Kaushik et al. 1986, Brazner et al. 1988, Touart 1986). Only a few of these methods have received significant testing for their ability to detect ecological effects and their ability to be conducted by several laboratories (Taub 1986; Shannon et al. 1986).

Both the SAM and MFC designs are static tests. The toxic material is introduced once although organisms are typically reintroduced throughout the test period. Both are planktonic communities, although the SAM design includes Gammarus and washed sand. Both systems are monitored over a long period (six weeks) ensuring that the systems become periphyton dominated. In fact, MFC experiments have remarked on the development of a detrital component as being

important in microcosm performance (Yount and Shannon 1989). Population and system-level responses are monitored in SAM while in the MFC process measurements are taken. Taxonomic diversity is low in both systems: the SAM protocol requires fewer than 20 species while MFC performance standards allow testing with as few as eight taxa. Replicate microcosms dosed with the test contaminant typically show initial dose-related displacement followed by recovery, even for conservative toxicants like copper. Both designs use the chelator EDTA in the test medium, which may alter the actual exposure.

An alternative to synthetic or cultured laboratory communities is the use of naturally derived portions of ecosystems. Giddings (1986) proposed using relatively intact pond littoral zone communities (sediment, microbes, plankton, periphyton, plants) in aquaria. These systems are replicable (by homogenizing and distributing collected materials) and reflect the types of communities often affected by point and nonpoint source pollutants. These systems have shown sensitivity to toxic organics (coal-derived phenolics) in comparison with standard bioassays (Giddings and Franco 1985) and in comparison with larger replicated ponds. Unfortunately, these microcosms have not been widely used in additional studies of sensitivity of ecosystem differences. Unlike the SAM and MFC methods, no culturing is required and the test medium is natural water. Like the SAM and MFC methods, tests are run statically, although experiments have used repeated dosing.

These most widely tested laboratory-scale ecosystems do not simulate the features of streams which are the most common recipient of toxic materials. Previous work using naturally derived periphyton communities in systems continuously dosed with toxic material have shown great sensitivity and replicability and can be adapted to use designs and equipment found in most ecotoxicological laboratories (Pratt and Bowers, 1990; Niederlehner et al. 1985; Pratt et al. 1987a).

In this paper, we report the effect of differing source ecosystems of the biological innoculum on predictions of adverse effects and examine the sensitivity and reproducibility of a standard method using continuously dosed, naturally derived laboratory ecosystems. Experiments

were carried out by two different laboratories with slightly differing analytical capabilities, so the standard design has been slightly modified by each lab.

Methods

Microcosm design

Experiments were carried out using the method of Pratt and Bowers (1990). Similar experiments using this method have been previously reported for tests of atrazine (Pratt et al. 1988a), copper (Pratt et al. 1987a), chlorine (Pratt et al. 1988b), phenol (Pratt et al. 1989), selenium (Pratt and Bowers in press), zinc (Pratt et al. 1987b), and chlorine-ammonia mixtures (Cairns et al. 1990). The basic method is described briefly below.

Microcosms were small glass tanks (< 6 L). A natural periphyton community (bacteria, algae, protozoa, rotifers, worms, sediment) was collected on replicate polyurethane foam artificial substrata colonized to species equilibrium (Cairns et al. 1979) at a reference site in a source ecosystem. These colonized substrata, used a species sources in the microcosms, were termed "epicenters". Each microcosm was randomly allocated one epicenter from the replicate collection of artificial substrata at the start of the experiment (Fig. 1). Three reference epicenters were sampled for selected parameters at the beginning of experiments. Three additional artificial substrata identical to the epicenters were placed in the microcosm container. The substrata were termed "islands" since they were initially barren and were colonized by species from the epicenter. Toxicant was supplied by a standard diluter (Benoit et al. 1982) which mixed a stock solution of copper with dechlorinated, charcoal filtered laboratory water. Toxicant-amended dilution water was supplied in sufficient volume to achieve approximately seven volume replacements per day.

The island substrata served as sampling devices. At the termination of experiments the epicenter substrata were also sampled. Microcosms were studied for several measures of ecological effects (Table 1) reflecting both the structure (biomass, nutrient pool, species composition) and function (production, respiration, nutrient uptake) of microcosm communities.

Production and respiration in the microcosms were estimated non-destructively using weekly three-point dissolved oxygen monitoring and by routine monitoring of microcosm pH.

Experiments were performed by two laboratories using different source communities. Experiments performed at Penn State University (PSU) were performed as described above with weekly sampling of island substrates. Experiments performed at the US Army Biomedical Research and Development Laboratory (BRDL) sampled microcosms only once after 21 d. Interlaboratory results are computed for samples taken after 21 d and for epicenter samples.

The PSU experiments used communities from Spring Creek (Centre County, PA) and were conducted in October 1988. BRDL experiments used communities from a spring-fed brook in Frederick County (MD) and were conducted in March 1989. A summary of the characteristics of tested communities and dilution water for each experiment is given in Table 2. The PSU experiment tested communities at nominal copper concentrations of 10, 20, 40, 80, and 160 ug Cu/L. BRDL experiments tested nominal copper concentrations of 5, 10, 20, 40, and 80 ug Cu/L. Samples from microcosms and the copper stock solution were analyzed weekly for total extractable copper by removing and fixing a 10 ml aliquot from each microcosm to pH < 2 using concentrated, redistilled (trace pure) nitric acid. Copper concentrations were determined by flame and flameless atomic absorption spectrophotometry (USEPA Methods 220.1, 220.2).

Because BRDL lacked expertise in organism identification, subsamples of reference epicenters were shipped in an insulated container by express carrier to the PSU lab and were examined for protozoa within 36 hr. of collection. The number of protozoan taxa was determined according to the method of Cairns et al. (1979). Preliminary experiments had shown no loss of taxonomic diversity from shipping. Subsamples from the termination of BRDL experiments were also shipped to the PSU lab for analysis. However, these subsamples did not arrive in sufficient time for valid examination of protozoa. To account for effects on biotic diversity, samples were prepared for examination of diatom algae using a modification of the method of Stevenson (1984). Fixed material from the PSU experiment was examined in an identical manner to provide comparable data on diatom algae. Prepared slides were examined to develop a qualitative list of

taxa. Then, the number of taxa encountered upon scanning 500 diatom valves was determined. Standard protistological references were used to identify the encountered organisms to the lowest practical taxon (usually genus, but often species).

Data were analyzed using single factor analysis of variance to detect treatment differences followed by paired comparisons (Dunnett's test, Dunnett 1955) to detect differences from controls. A no observable effect concentration (NOEC) was defined as the highest treatment level not significantly different from controls. A lowest observable effect concentration (LOEC) was defined as the lowest treatment level significantly different from controls. Because LOEC and NOEC determinations are limited by the chosen treatment levels, dose- response relationships were determined using ordinary least squares linear regression of response-treatment (exclusive of controls). Significant regressions were used to inversely predict effective concentrations (EC's) as proportional changes from the control response (Sokal and Rohlf 1983). For example, an EC20 was the copper concentration predicted to result in a 20% reduction in the control response.

Taxonomic data were also examined descriptively to examine the similarity of communities in different copper treatments. A matrix of similarity coefficients was developed based on presence-absence data. The matrix of similarity coefficients was used to develop dendrograms comparing the taxonomic similarity of samples using an average-linkage clustering method (SAS 1986). The taxonomic composition of samples was compared using the permutation method Smith et al. (1990). Sample similarity was determined using Jaccard's measure for presence and absence of taxa. The permutation method compared average within treatment similarity to average between treatment similarity and constructed a statistical distribution using 1000 random switches of similarity values among groups. Using this method, an inferential test of the relationship of between treatment and within treatment variability was obtained. Where between treatment similarity differed from within treatment similarity, follow up analyses identified the degree of divergence among treatment and the relative influence of individual species on sample similarities.

Results

Experimental communities

Protozoan species numbers were similar between tested communities (Table 2); BRDL communities had 72% bacterivorous species while PSU communities were 76% bacterivores. Biomass of communities in the BRDL was much greater. Protein biomass was nearly five times greater than communities used in PSU experiments and chlorophyll a biomass was more than an order of magnitude greater in BRDL experiments. This difference in biomass probably contributed to differences in dose-response relationships reported below. General water quality was similar in the two source ecosystems, and dilution water was quite similar for the two experiments. Mean measured toxicant concentrations were very near nominal doses in both experiments (Table 3).

Effects on structure

By 21 days after dosing, biomass on islands decreased strongly with copper dose in both experiments. Although BRDL epicenters had high initial biomass, protein biomass was higher and more strongly affected (in terms of dose-response slope) by copper in the PSU experiment (Fig. 2). However, chlorophyll biomass showed very similar dose responses in both experiments (Fig. 3). Both protein and chlorophyll standing crop were elevated over intermediate treatments in the BRDL experiments. While significant changes in taxonomic richness and composition occurred in relation to copper dose, comparatively diverse communities with elevated biomass at the highest copper dose in the BRDL experiment was not clearly related to the dominance of a particular algal taxon.

Taxonomic richness showed the strongest and least variable dose response among structural variables. Again, the dose-response was very strong for protozoan species richness in the PSU experiment, although comparable data were not available for the BRDL experiment (Fig. 4). In the PSU experiment, treatments $\geq$ 20 ug Cu/L (measured at 19.9 ug/L) had significantly fewer protozoan species than controls ($p<0.001$, ANOVA and $p<0.05$, Dunnett's Test). In the BRDL experiments, copper treatments $\geq$ 10 ug Cu/L (10.0 ug/L measured) were significandy

lower than controls ($p<0.001$, ANOVA; $p<0.05$, Dunnett's Test). Detection of effects for structural variables is summarized in Table 4.

Changes in composition of sampled communities was related to increasing copper stress. Decreasing protozoan species numbers showed a stronger response (in terms of the slope of the dose-response) than losses of algal taxa (Fig. 4). However, stressed communities were increasingly more similar to each other, presumably as copper-sensitive taxa were eliminated. Dendrograms from cluster analysis of pooled species occurrences in treatments show groupings of species assemblages that support other measures of adverse copper stress (Fig. 5). For protozoa in the PSU experiments, those communities receiving the lowest copper doses ($\leq$ 10 ug/L) did not cluster with communities showing consistent copper stress effects. Similarly, clustering of algal assemblages for both experiments showed clustering of treatments showing adverse responses to copper.

Effects on processes

Alkaline phosphatase activity did not differ significantly among treatments in the PSU experiment (Fig. 6). The BRDL experiment showed significant elevation of activity in treatments $\geq$ 40 ug Cu/L ($p<0.001$, ANOVA). Alkaline phosphatase activity is strongly influenced by available phosphate which was present in the PSU water supply at concentrations near 1 mg/L. BRDL dilution water had phosphate concentrations < 0.1 mg/L.

Levels of dissolved oxygen in late afternoon measurements are the result of oxygen input from primary production less diffusion and losses to respiration. Assuming the diffusion gradient was relatively constant for all microcosms, afternoon dissolved oxygen provides a sensitive measurement of relative net primary production. Strong, dose-related responses were seen in both experiments (Fig. 7). As noted above, high standing crops in the BRDL experiment may be related to the more shallow dose response.

Dose-response relationships

Each of the measured variables was not equally sensitive to the effects of copper on microcosms. In general, there was a strong negative relationship between the measured response and the logarithm of the measured copper dose. Significant linear dose-response relationships are reported in Table 4 as inverse predictions of effective concentrations, EC20's, which are presented for comparative purposes. The EC20 is reported because it represents a comparatively large effect on microbial communities. Additionally, most EC20 estimates were near estimators of the chronic value (the geometric mean of the NOEC and LOEC). EC20 estimates provide an indication of the strength of the dose response relative to the control response and also provide estimates of effect for variables where an LOEC (and, therefore, a chronic value) could not be determined.

In both experiments, structural variables (standing crop biomass and species number) were the most sensitive measures of copper toxicity. However, BRDL experiments showed that afternoon dissolved oxygen (a measure of net primary production) was very sensitive to the effects of copper. Standing crop measures for both island and epicenters were sensitive to copper stress, and in the PSU experiments epicenter biomass was more sensitive than that of islands. Both the enhanced sensitivity of epicenters and the failure to detect differences in dissolved oxygen in the PSU experiments was probably the result of lower biomass in the PSU microcosms.

Comparisons of the taxonomic compositions of samples showed that within treatment similarity differed significantly ($p < 0.001$) for both experiments. In the BRDL experiment within treatment similarity generally decreased with dose (Table 5), although the highest dose had relatively high within treatment similarity and diverged from controls by no more than the lowest treatment (5 ug Cu/l). In the PSU experiment, within treatment similarity increased slightly with dose, but divergence from controls increased with dose. Protozoan communities in the PSU experiment showed lower similarity than algal samples, primarily due to greater taxonomic richness. Divergence of treatments from controls increased strongly with dose. The removal of some species increased between treatment similarity (Table 5). These species were considered to

be those most strongly affecting analyses. In general, these were eliminated in higher doses resulting in decreased similarity to controls.

Discussion

Sensitivity

Ecological responses in these experiments occurred at concentrations from 4.7-60 ug Cu/L, corresponding to the reported range of single species chronic toxicity values (4-60 ug/L for soft water and 9-29 ug/L for hard water; Table 6) and to a hardness-based water quality criterion of approximately 20 ug Cu/L. Observed chronic values for several variables were near and below this level.

As expected, structural measures were sensitive indicators of toxicity. Algal biomass (as chlorophyll) was consistently very sensitive to copper effects and consistent between the two experiments (Table 4). Likewise, species richness and composition were consistently sensitive. Other measures showed sensitivity to copper effects, but were either not as sensitive as those measures reported above or shoed inconsistent sensitivity.

Process measures were less sensitive indicators of toxic effects even though measurement variability was low. Dissolved oxygen, indicative of the loss of chlorophyll biomass, had low variability and showed adverse effects at the lowest copper dose in BRDL experiments. Nutrient cycling as measured by alkaline phosphatase activity was high, but showed elevated activity in BRDL experiments where diluent phosphate was low.

Synthetic systems such as Taub's standard aquatic microcosm (SAM) have not shown sensitivity to toxic chemicals at environmentally realistic concentrations. For example, interlaboratory testing started at test concentrations of 500 ug Cu/L and often did not detect effects; the USEPA criterion for copper is about 20 ug/L in moderately hard water. Limited sensitivity of the SAM is to be expected since the component organisms are individually cultured and share no co-adaptation to the SAM environment (O'Neill and Waide 1981). The generic SAM design, like single species testing, does not reflect interactions in any natural ecosystem. Like the SAM design,

the Leffler mixed flask culture (MFC) involves culturing organisms in a defined medium. Unlike the SAM design, the stock MFC is obtained from a natural plankton community. Recent studies of the MFC (Stay et al. 1989b) suggest that the source ecosystem can play a role in producing different species sets for testing, but that sensitivity of similar ecosystems does not vary greatly. However, the taxonomic composition of the final MFC stock may bear little resemblance to that of the source ecosystem.

Similarity of response

Responses were not uniformly similar for the two tested communities although the response levels were quite similar for the spectrum of variables measured. Falling chlorophyll biomass and species richness, the most sensitive indicators of stress, showed surprisingly similar patterns in both experiments. Two different patterns of community composition change were observed. Within replicate similarity decreased for algae in the BRDL experiment and for protozoa in the PSU experiment, but algae in the PSU experiment showed increasingly similar community structure.

This finding can be interpreted in two ways. First communities might be expected to become more similar as stress eliminated sensitive species. Alternatively, stress might be seen as a disorganizing influence such that species similarity among replicates decreases due to stochastic events. Divergence of communities from controls was greatest for protozoan communities, although protozoan community similarity was uniformly low. Previous experiments (Pratt et al. 1987a) have showed progressive dissimilarity of communities under copper stress. Differences among algal communities may be a function of taxonomic resolution because only diatom algae were compared and soft algae ignored. This reduction in the taxonomic spectrum resulted in greater community similarity. Approximately double the number of algal taxa was enumerated in protozoan taxa, and communities were correspondingly less similar.

These experiments showed responses similar to those previously reported for microcosm responses to copper (Pratt et al. 1987a). In those experiments, conducted using communities from

soft water stream, copper affected standing crop, protozoan species richness, and community similarity at copper concentrations from 6.6 - 12.7 ug/L. When compared to single species tests, these community level microcosm responses lie in the sensitive range of observed effects of copper. However, SAM responses to a single dose of copper were not different from controls at copper concentrations of 500 ug/L (Taub et al. 1989).

Experiment variability

These experiments and a previous experiment using naturally derived microcosms show relatively low variability for measured variables (Table 7). Variability of species richness enumerations had the lowest variability among measures. In general, variability increased in copper-dosed microcosms. However, despite somewhat higher variability of standing crop measures or measures of nutrient retention standing crop changes were detectable adverse effects in microcosms where process measuresas compared to process measures were not. These observations correspond to other recent reports of stress effects at the community and ecosystem level (Schindler 1987, Levine 1989, Odum 1990). Nutrients supplying processes are unchanged by toxicants so processes change only after significant structural alteration. Dissolved oxygen production, a sensitive process measure, reflects the significant change in process capacity of phototrophic organisms.

The levels of copper producing adverse changes in microbial communities were surprisingly similar in each of the microcosm experiments even though experiments used different communities from different streams tested at different times of the year. The robust changes in species richness, a measure of biotic diversity, and standing crop occurred at copper concentrations below current water quality criteria, and the microcosm test provides an empirical estimate of effects not obtainable by testing individual species. The importance of these measures as indicators of ecosystem disease (Schaeffer et al. 1988) further emphasizes the importance of measurement of collective properties of surrogate ecosystems.

Similar to findings in this study, Stay et al. (1989a) reported the lowest CV's for MFC microcosms as those from oxygen measurements. CV's were typically <10%, but increased at high doses of toxicants. Organisms counts were more variable with CV's from 20-50% typical and ranging to over 100% in highly stressed microcosm communities. No data were given for P/R ratios or Eh measures.

Experiments such as those summarized by Stay et al. (1989a) display a continuing problem in microcosm assessments. Estimates of community biomass are not consistently given, so interpretation of functional changes such as oxygen production is problematic. Is less oxygen produced or consumed by the same biomass or is less biomass available for production and consumption? In the latter case, the functional changes may only reflect a change in the total amount of biological material, not the functional capacity of the standing stock. Organism counts, without species identification are less meaningful because population fluctuations are common (c.f., Perez et al. 1983), sizes of organisms in broad groups (zooplankton, algae, protozoa) are variable and often decrease with stress (Odum 1985). Population changes or changes in numbers within mixed populations are not reliable indicators of community biomass.

Microcosm experiments for hazard evaluation are approaching a new, sophisticated stage comparable to traditional toxicity testing. To adequately address ecological change, several ecologically meaningful endpoints need to be assessed (Odum 1985, Schindler 1987, Schaeffer et al. 1988), but data need to have sufficiently low variability that statistical tests can be powerful given the logistical limitations of microcosm tests (20-30 experimental units). Additionally, measured responses can probably not be relied upon to detect changes in mechanisms such as competition that have defied testing in multispecies arenas (Schoener 1982).

Naturally derived microcosms assembled from ecosystem samples are no more variable (and often less variable) than synthetic or cultured systems. The microcosm material is in limited supply so measurements need to be either non-destructive or focused on those responses of probable utility in detecting community change based on predicted response trends. Promising responses include:

- changing species richness
- changing standing crop
- altered nutrient pools and cycling rates
- altered production and respiration
- altered organism size.

Measures such as population fluctuation are unlikely to be helpful except for large taxa.

The consistency of the responses reported for this study suggests that minimal criteria might be needed for conducting valid microcosm toxicity assessments (see Pratt and Bowers 1990). That is, the several community responses measured could provide good probability for detecting adverse effects regardless of the time of year or source ecosystem, but considerably more experimentation is needed to critically evaluate the reproducability of community responses for a range of communities. However, only species richness was consistently sensitive in each experiment, although other measures of biotic structure and function showed the adverse effects of copper.

References

Benoit, D., V. Matson, and D. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Res. 16:457-464.

Brazner, J.C., S.J. Lozano, M.L. Knuth, S.L. Bertelsen, L.J. Heinis, D.A. Jensen, E.R. Kline S.L. O'Halloran, K.W. Sargent, D.K. Tanner, R.E. Siefert. 1988. The effects of chlorpyrifos on a natural aquatic system: A research design for littoral enclosure studies and final research report. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN, and University of Wisconsin-Superior, Center for Lake Superior Environmental Studies, Superior, WI. 194 pp.

Cairns, J. 1984. Are single species toxicity tests alone adequate for estimating environmental hazard? Hydrobiologia 100:47-57.

Cairns, J., Jr., D. Kuhn, and J. Plafkin. 1979. Protozoan colonization of artificial substrates, p. 34-57. In R.L. Weitzel (ed.), Methods and Measurements of Periphyton Communities: A Review, STP 690. American Society for Testing and Materials, Philadelphia, PA.

Cairns, J., Jr., B.R. Niederlehner and J.R. Pratt 1990. Evaluation of joint toxicity of chlorine and ammonia to aquatic communities. Aquatic Toxicology 16:87-100.

Dunnett, C. 1955. Multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. 50:1096-1121.

Giddings, J.M. 1986. A microcosm procedure for determining safe levels of chemical exposure in shallow-water communities, p. 121-134. In J. Cairns, Jr. (ed.), Community Toxicity Testing, ASM STP920. American Society for Testing and Materials, Philadelphia, PA.

Giddings, J.M. and P. Franco. 1985. Calibration of laboratory bioassays with results from microcosms and ponds. pp. 104-119. In T.P. Boyle (ed.) Validation and Predictability of Laboratory Models for Assessing the Fate and Effects of Contaminants in Aquatic Ecosystems, ASTM STP 865. American Society for Testing and Materials, Philadelphia, PA.

Giesy, J., Jr. 1980. Microcosms in Ecological Research. CONF-781101. National Technical Information Service, Springfield, VA.

Hammons, Anna S. 1981. Methods for Ecological Toxicology - A Critical Review of Laboratory Multispecies Tests. Ann Arbor Science Publishers, Inc. Ann Arbor, Michigan.

Kaushik, N.K., K.R. Solomon, G.L. Stephenson, and K.E. Day. 1986. Use of limnocorrals in evaluating the effects of pesticides on zooplankton communities, p. 269-290. In J. Cairns, Jr. (ed.) Community Toxicity Testing, ASTM STP 920. American Society for Testing and Materials, Philadelphia, PA.

Kimball, K.D., and S.A. Levin. Limitations of laboratory bioassays: The need for ecosystem-level testing. BioScience 35:165-177.

Leffler, J.W. 1981. Development of an ecosystem-level microcosm test for screening the effects of organic chemicals. Annual Meeting of the Society of Environmental Toxicology and Chemistry. Arlington, VA.

Levine, S.N. 1989. Theoretical and methodological reasons for variability in the responses of aquatic ecosystem processes to chemical stresses, pp. 145-180. In S.A. Levin, M.A. Harwell,

J.R. Kelly, and K.D. Kimball (eds.) Ecotoxicology: Problems and Approaches, Springer-Verlag, New York City, NY.

National Research Council (NRC). 1981. Testing for the Effects of Chemicals on Ecosystems. National Academy Press. Washington, D.C.

Niederlehner, B.R., J.R. Pratt, A.L. Buikema, Jr., and J. Cairns, Jr. 1985. Laboratory tests evaluating the effects of cadmium on freshwater protozoan communities. Environ. Tox. Chem. 4:155-165.

Odum, E.P. 1990. Field Experimental tests of ecosystem-level hypotheses. Trends in Ecology and Evolution 5:204-205.

Odum, E.P. 1985. Trends expected in stressed ecosystems. BioScience 35:419-422.

O'Neill R.O. and J.B. Waide. 1981. Ecosystem theory and the unexpected: implications for environmental toxicology. In B.W. Cornaby, ed., Management of Toxic Substances in our Environment. Ann Arbor Science, Ann Arbor, MI, pp. 43-73.

Perez, K.T., E.W. Davey, N.F. Lackie, G.E. Morrison, P.G. Murphy, A.E. Sopper, and D.L. Winslow. 1983. Environmental assessment of a phthalate ester, Di (2-Ethylhexyl) Phthalate (DEHP), derived from a marine microcosm, pp. 180-191. In Aquatic Toxicology and Hazard Assessment: Sixth Symposium, ASTM STP 802, W.E. Bishop, R.D. Cardwell, and B.B. Heidolph (eds.), American Society for Testing and Materials, Philadelphia, 1983.

Pratt, J.R., B. Niederlehner, N. Bowers, J. Cairns, Jr. 1987a. Prediction of permissible concentrations of copper from microcosm toxicity tests. Tox. Assess. 2:417-436.

Pratt, J.R., B. Niederlehner, N. Bowers, J. Cairns, Jr. 1987b. Effects of zinc on freshwater microbial communities, p. 324-326. In S.E. Lindberg and T.C. Hutchinson (eds.), International Conference on Heavy Metals in the Environment, New Orleans. CEP Consultants Ltd., Edinburgh.

Pratt, J.R., N. Bowers, B. Niederlehner, J. Cairns, Jr. 1988a. Effects of atrazine on freshwater microbial communities. Arch. Environ. Contam. Toxicol. 17:449-457.

Pratt. J.R., B. Niederlehner, N. Bowers, J. Cairns, Jr. 1988b. Effect of chlorine on microbial communities in naturally derived microcosms. Environ. Toxicol. Chem. 7:679-687.

Pratt, J.R., and N.J. Bowers. 1990. A microcosm procedure for estimating ecological effects of chemicals and mixtures. Tox. Assess. 5:189-205.

Pratt, J.R., N.J. Bowers, B.R. Niederlehner, and J. Cairns, Jr. 1989. Responses of laboratory ecosystems to environmental stress: effects of phenol. Tox. Assess. 4:161-174.

SAS Institute Inc. SAS User's Guide: Statistics, Version 5 Edition. Cary, NC: SAS Institute Inc., 1985. 956 pp.

Schaeffer, D., E.E. Herricks, and H.W. Kerster. 1988. Ecosystem health I. Measuring ecosystem health. Environ. Manage. 12:445-455.

Schindler, D.W. 1987. Detecting ecosystem response to anthropogenic stress. Can. J. Fish. Aquat. Sci. (Suppl. 1) 44:6-25.

Schoener, T.W. 1982. The controversy over interspecific competition. American Scientist 70:586-595.

Shannon, L.J., M.C. Harass, J.D. Yount, and C.T. Walbridge. 1986. A comparison of mixed flask culture and standardized laboratory model ecosystems for toxicity testing, pp. 135-157. In. J. Cairns, Jr. (ed.) Community Toxicity Testing, ASTM STP 920. American Society for Testing and Materials, Philadelphia, PA.

Smith, E.P., K.W. Pontasch, J. Cairns, Jr. 1990. Community similarity and the analysis of multispecies environmental data: A unified statistical approach. Water Res. 24:507-514.

Sokal, R., and F. Rohlf. 1983. Biometry. W.H. Freeman, New York. 859p.

Stay, F.S., T.E. Flum, L.J. Shannon, and J.D. Yount. 1989a. An assessment of the precision and accuracy of SAM and MFC microcosms exposed to toxicants, pp. 189-203. In Aquatic Toxicology and Hazard Assessment: 12th Volume, ASTM STP 1027, U.M. Cowgill and L.R. Williams, eds., American Society for Testing and Materials, Philadelphia, 1989.

Stay, F.S., A. Katko, C.M. Rohm, M.A. Fix, and D.P. Larsen. 1989b. The effects of atrazine on microcosms developed from four natural plankton communities. Arch. environ. Contam. Toxicol. 17:866-875.

Stevenson, R.J. 1984. Procedures for mounting algae in a syrup medium. Trans. Am. Microsc. Soc. 103-320-321.

Taub, F.B. 1989. Standardized aquatic microcosms. Environ. Sci. Tech. 23(9): 1064-1066.

Taub, F.B. 1986. Preliminary results of interlaboratory testing of a standardized aquatic microcosm, pp. 93-120. In J. Cairns, Jr. (ed.) Community Toxicity Testing ASTM STP 920. American Society for Testing and Materials, Philadelphia, PA.

Taub, F.B. 1976. Demonstration of pollution effects in aquatic microcosms. Int. J. Environ. Stud. 10:23-33.

Touart, L. 1986. Simulated aquatic ecosystem tests to support pesticide registrations. USEPA EBB/HED/DPP, draft document, 18pp.

United State Environmental Protection Agency. 1983. Methods for the chemical analysis of water and wastewater, EPA 600/4-79-020. National Technical Information Service, Springfield, VA.

Yount, J.D., and L.J. Shannon. 1989. State changes in laboratory microecosystems in response to chemicals from three structural groups. pp. 86-96. In J. Cairns, Jr. and J.R. Pratt (eds.) Functional Testing of Aquatic Biota for Estimating Hazards of Chemicals, ASTM STP 988. American Society for Testing and Materials, Philadelphia, PA.

Table 1: Variable measured in microcosms tests of copper toxicity.

Measured Effect	Ecological Estimate
Structure	
Protein	Total Biomass
Chlorophyll a	Algal Standing Crop
Calcium, Potassium, Magnesium	Nutrient Pool
Protozoan Taxa	Community Structure
Algal Taxa	Community Structure
Function	
Oxygen Production	Community Metabolism
Oxygen Respired	Community Metabolism
Alkaline Phosphatase Activity	Nutrient Cycling
pH	Community Metabolism

Table 2. Comparison of water chemistry and microbial communities for source ecosystem water and average water chemistry values for dilution water used in the PSU USABRDL microcosm tests. The microbial communities were collected on PF artificial substrates after 7 days of colonization and values shown are mean (SD) of three replicates.

	Location			
	BRDL		PSU	
Variable	Source Ecosystem	Dilution Water	Source Ecosystem	Dilution Water
Water quality				
pH	7.27	7.87	8.28	7.91
Dissolved oxygen (mg/L)	8.60	8.88	12.00	9.33
Temperature (°C)	13.4	19.7	9.5	16.2
Conductivity (umhos/cm)	596	570	325	262
Hardness (mg $CaCo_3$/L)	253	208	225	164
Alkalinity (mg $CACO_3$/L)	190	128	180	131
Source Community				
Protozoan Species	53 (5.66)		52 (3.60)	
% Bacterivores	72.0 (5.01)		75.6 (1.82)	
% Producers	14.0 (2.51)		7.7 (2.21)	
Protein (ug/mL)	595 (148)		129 (14.7)	
Chlorophyll (ug/L)	6989 (1915)		537 (93.4)	

Table 3. Copper concentrations in laboratory microcosms. Values are mean (SD).

Nominal Concentration ug Cu/L	Mean Measured Concentration BRDL	PSU
Control	2.2 (1.1)	< D.L.* -
5	4.7 (0.01)	-
10	10 (0.47)	9.9 (1.3)
20	19.3 (0.61)	19.9 (2.7)
40	36.8 (1.95)	40.0 (5.4)
80	77.7 (0.67)	90.0 (9.6)
160	-	205 (16.9)

* = Below detection limit

Table 4. Summary of effective concentrations for measured variables.
KEY: B: BRDL experiments, P: PSU experiments.

Var	Lab	NOEC[1]	LOEC[2]	ChV[3]	EC_{20}[4] (95% CI)
ISLANDS					
Protein	B	4.7	10.0	6.8	3.0 (0.0-10.9)
	P	40.0	90.0	60.0	43.7 (29.0-72.9)
Chl a	B	4.7	10.0	6.8	4.5 (0.0-4.9)
	P	9.9	19.9	14.0	11.6
Species	B	10.0	19.3	13.9	NL[5]
	P	-	9.9	(9.9)	10.8 (6.0-17.6)
APA[6]	B	19.3	36.8	26.7	11.4 (3.0-33.4)
	P	NS	NS	-	-
DO	B	NS	4.7	(4.7)	19.8 (5.0-77.0)
	P	40.0	90.0	60.0	232.0 (75.0-419.0)
C_2	B	NS	NS	-	NL
	P	40.0	90.0	60.0	25.1 (18.0-100.0)
K	B	36.8	77.7	53.5	NL
	P	40.0	90.0	60.0	39.2 (30.0-64.9)
EPICENTERS					
Protein	B	4.7	10.0	6.8	NL
	P	-	9.9	(9.9)	144.4 (57.0-861.0)
Chl a	B	4.7	10.0	6.8	5.2 (1.0-12.1)
	P	-	9.9	(9.9)	2.2 (0.0-3.2)

[1] No observable effect concentration (ug Cu/L)
[2] Lowest observable effect concentration (ug Cu/L)
[3] Chronic value (ug Cu/L) = geometric mean of NOEC and LOEC
Insert value sin parentheses are LOC if no NOEC was determined.
[4] EC_{20} = Effective concentration resulting in a 20% change in response relative to controls.
[5] NL = Non-linear response
[6] APA units are p-nitro phenol/mg protein/hr.

Table 5. Results of similarity analysis of treatments, within treatment similarity based on Jaccard's measure of similarity. Lambda (λ) values indicate the degree of difference between controls and treatments. Mean within and between similarity and p-values are based on randomized permutations of similarity coefficients. Species listed increased similarity among treatments when removed.

TRT	BRDL Algae Within Similarity	λ	TRT	PSU Algae Within Similarity	λ	PSU Protozoa Within Similarity	λ
Control	0.81	1.00	Control	0.59	1.00	0.43	1.00
5	0.66	1.02	10	0.48	0.93	0.37	0.81
10	0.44	0.92	20	0.63	0.81	0.48	0.70
20	0.42	0.72	40	0.67	0.76	0.34	0.76
40	0.48	0.76	80	0.70	0.77	0.33	0.44
80	0.62	0.92	160	0.66	0.83	0.27	0.35
Mean Within	0.57			0.62		0.37	
Mean Between	0.53			0.56		0.22	
p	< 0.001			< 0.001		< 0.001	

Species Influence:

BRDL Algae	PSU Algae	PSU Protozoa
Cymbella ventriculosa	Achnanthes sp.	Balladyna elongatum
Gomphonema parvulum	Cyclotella sp.	Chlorogonium elongatum
Neidium sp.	Neidium binode	Chlorogonium sp.
Nitzschia denticula	Nitzschia amphibia	Chroomonas caudata
Surirella sp.	Surirella sp.	Loxophyllum sp.

Table 6. Reported single-species chronic values for copper toxicity in hard water (USEPA 1985). All tests are life cycle tests.

Species	Hardness (mg/L $CaCO_3$)	ChV[1] (ug Cu/L)
Daphnia magna	104	29.330
D. magna	211	9.525
Pimephales notatus	194	8.798
P. promelas	198	21.870
P. promelas	200	27.710

[1] ChV = chronic value

Table 7. Coefficients of variation (%) for microcosm experiments conducted at PSU (P), BRDL (B), and Virginia Polytechnic Institute (V, see Pratt et al. 1987).

	Structure				Function	
	Species	Protein	Chl a	APA	Cation Nutrients	DO
Controls						
P	5.3[b] 7.0[a]	26.8	21.4	17.3	1.5-18.1	11.6
B	2.6[b]	37.5	31.5	14.5	6-28.4	7.1
V	8.6[a]	19.4[c]	4.6	ND[d]	8.6[e]	ND
Treatments						
P	2.6-7.5[b] 3.0-18[a]	7-40	16-172	7-77	2.5-43	3.7-8.5
B	3.7-10[a]	10.7-39	16.2-47.9	12-38	6.8-250	1.0-3.6
V	18-28[b]	7.6-46	9.9-38.6	ND	1.7-27.9	ND

[a] = algae

[b] = protozoa

[c] = ATP Biomass

[d] = ND = Not Determined

[e] = Potassium only

Figure Legends

Fig. 1. Location of epicenter and island artificial substrata in microcosms. Arrow marked "toxicant" indicates flow of diluent-toxicant mixture.

Fig. 2. Effect of copper on protein biomass in microcosms after 21 d of copper exposure. Symbols are means ± standard deviation.

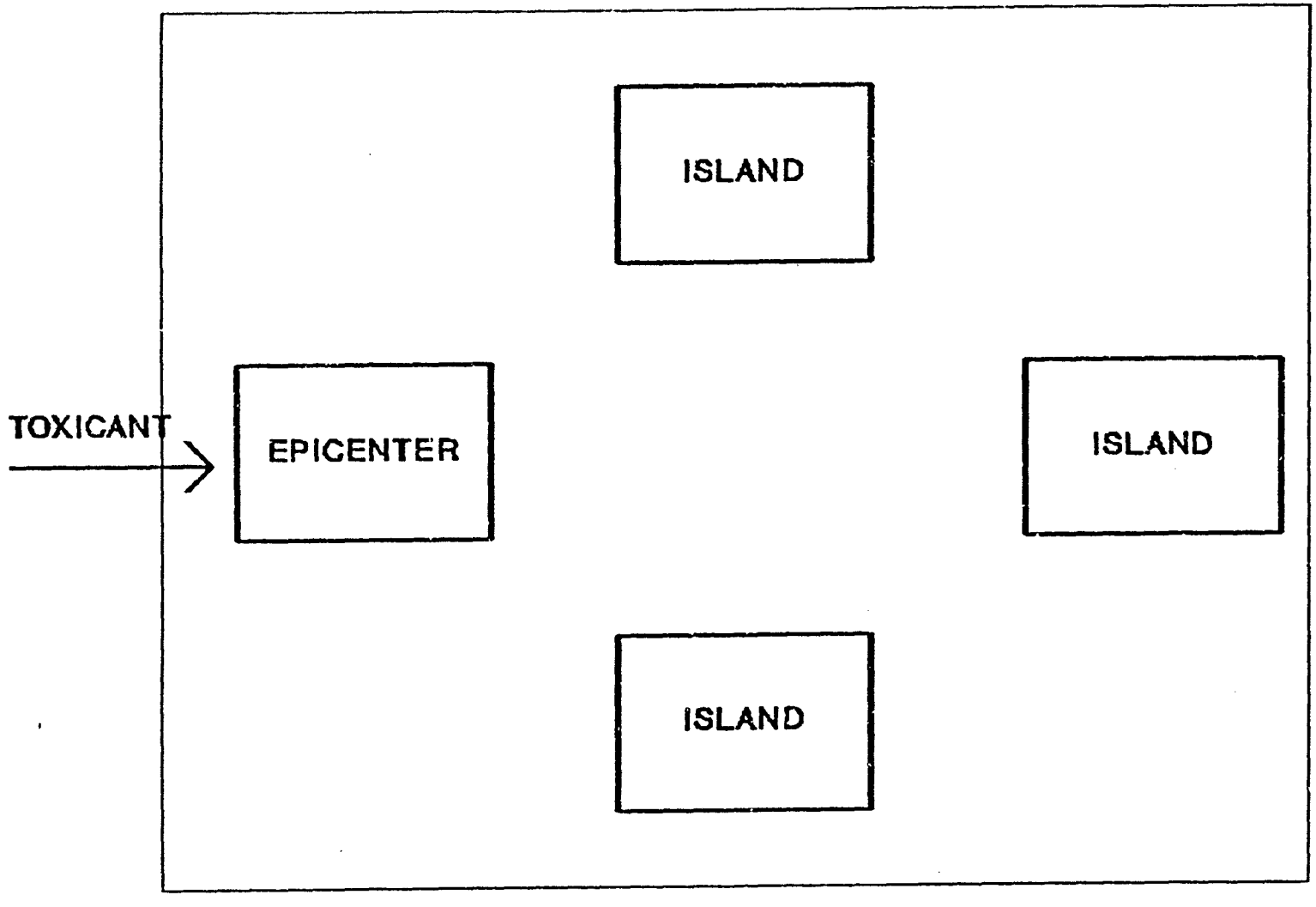
Fig. 3. Effect of copper on chlorophyll a biomass in microcosms after 21 d of copper exposure. Symbols are means ± standard deviation.

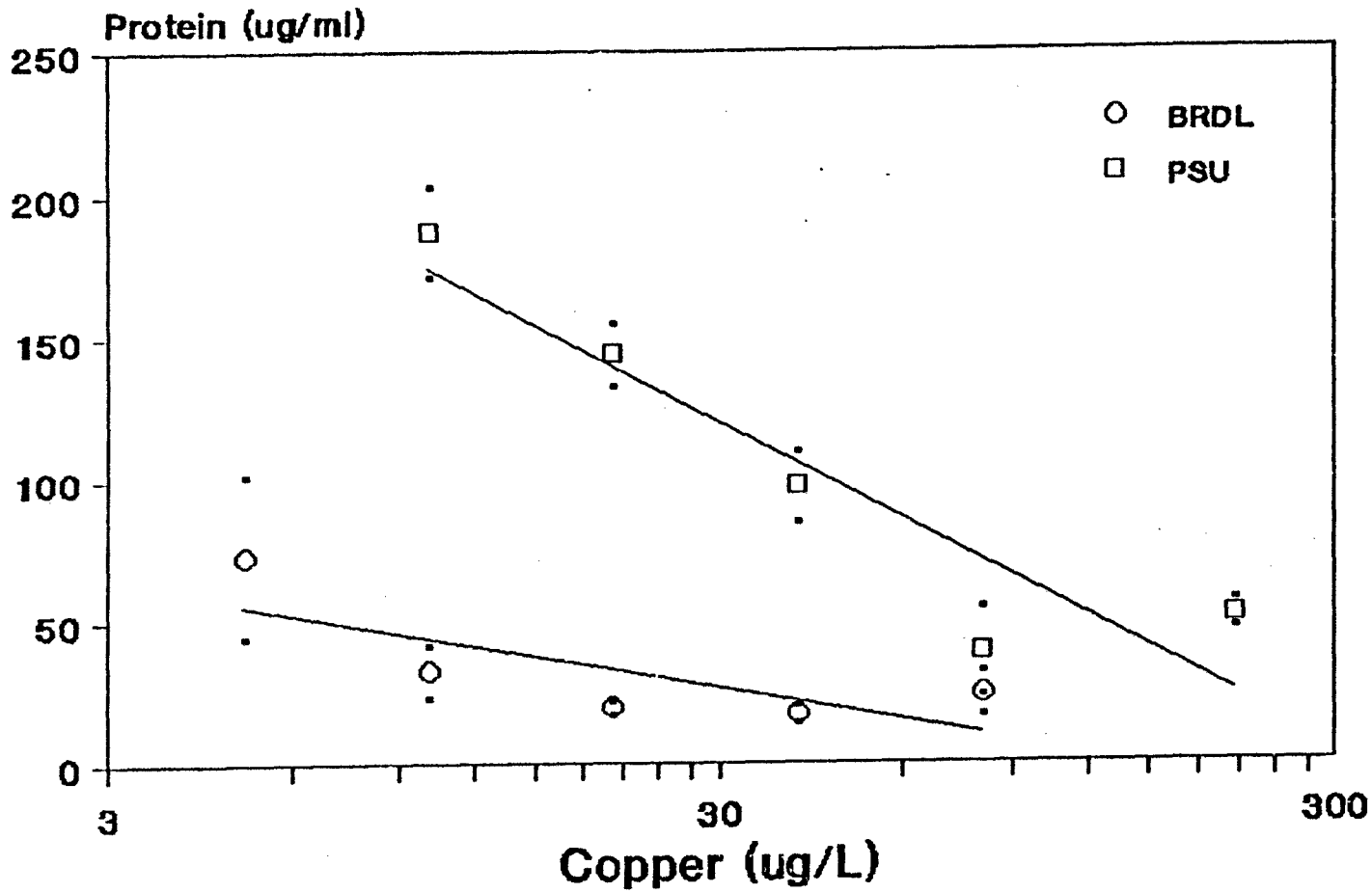
Fig. 4. Effect of copper on species richness of protozoa and diatom algae in microcosms after 21 d of copper exposure. Symbols are means ± standard deviation.

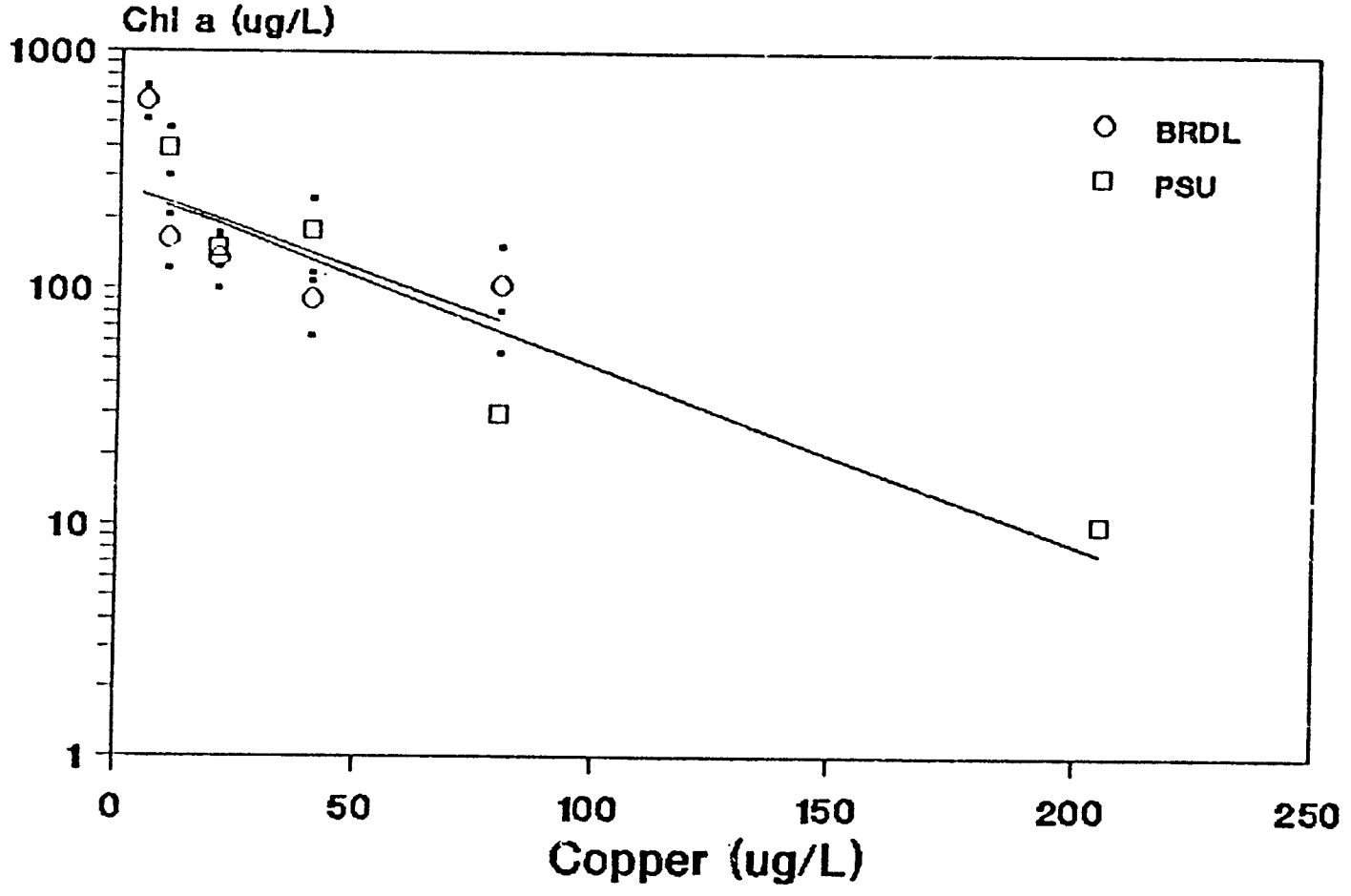
Fig. 5. Cluster diagram of relationship between algal and protozoan community structure and copper dose for microcosms after 21 d of copper exposure. Values in dendrograms are nomical copper doses (ug/L).

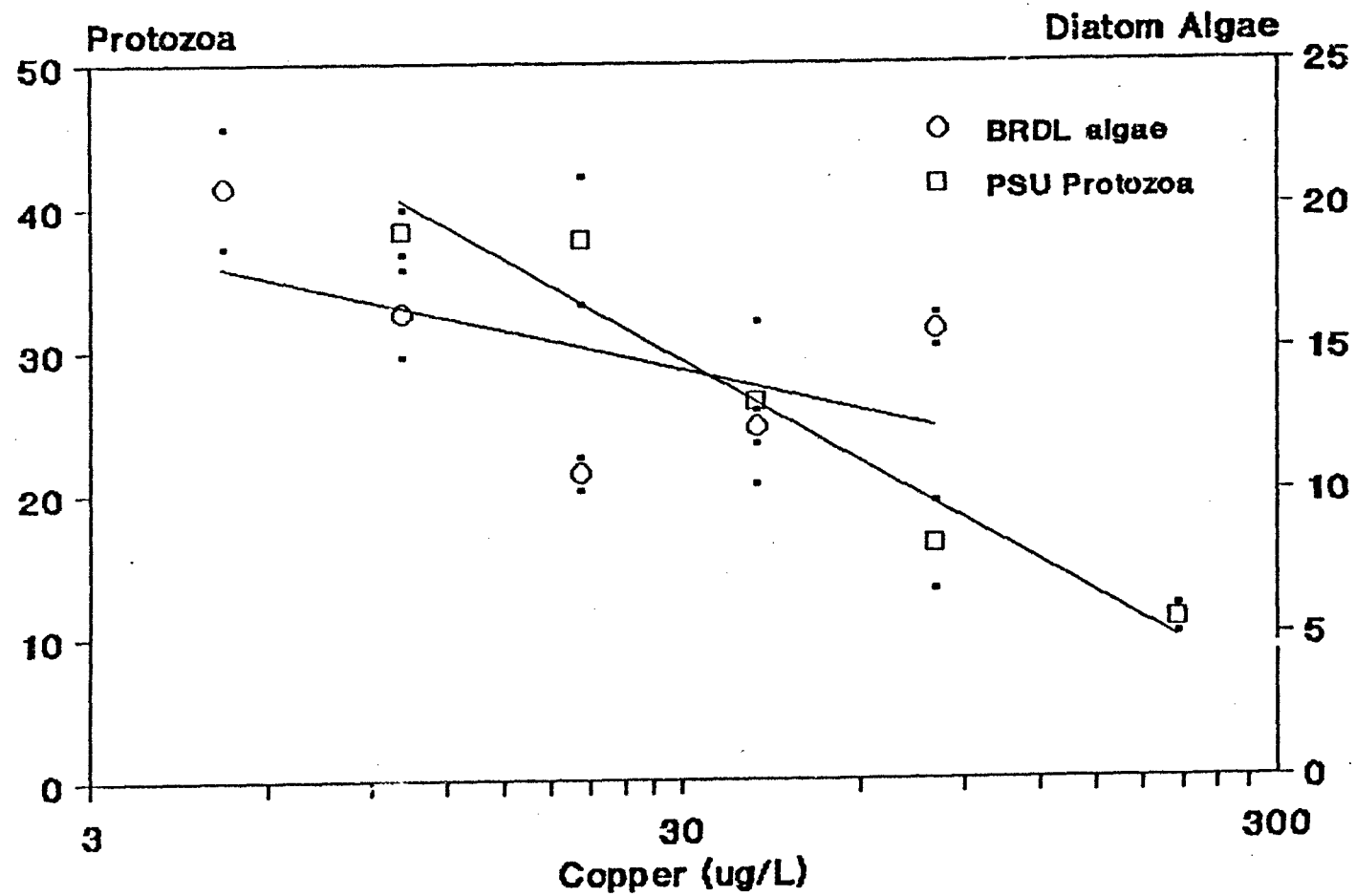
Fig. 6. Effect of copper on alkaline phosphatase activity (APA) in microcosms after 21 d of copper exposure. Symbols are means ± standard deviation. APA units are nmoles p-nitrophenol/mg protein/hr.

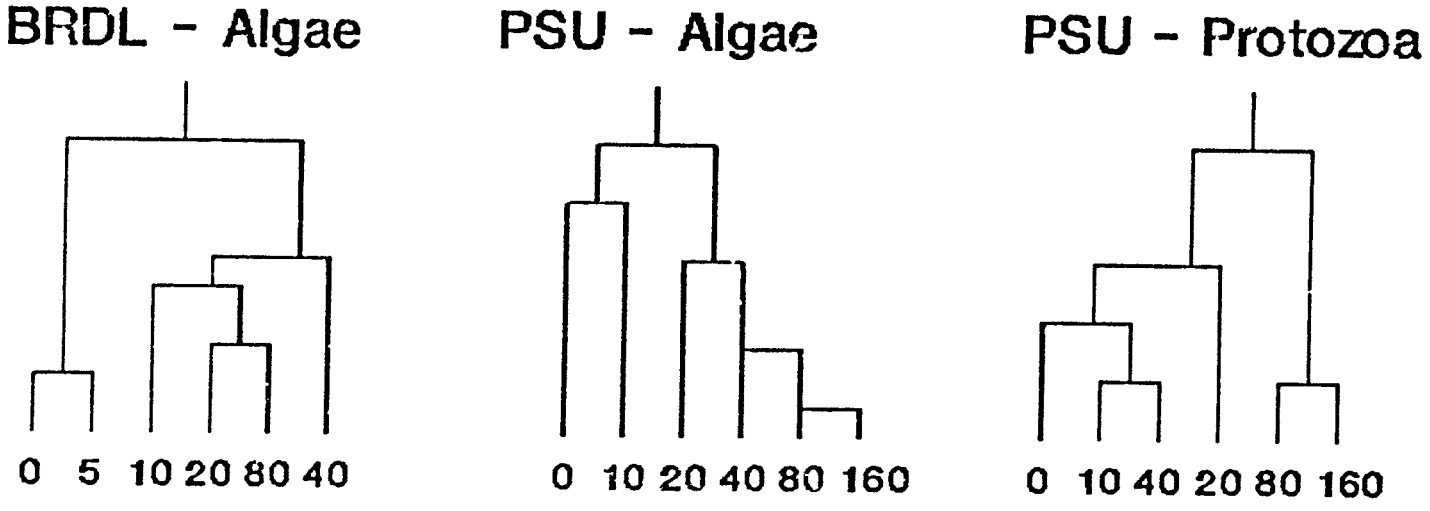
Fig. 7. Effect of copper on afternoon dissolved oxygen in microcosms after 21 d of copper exposure. Symbols are means ± standard deviation.

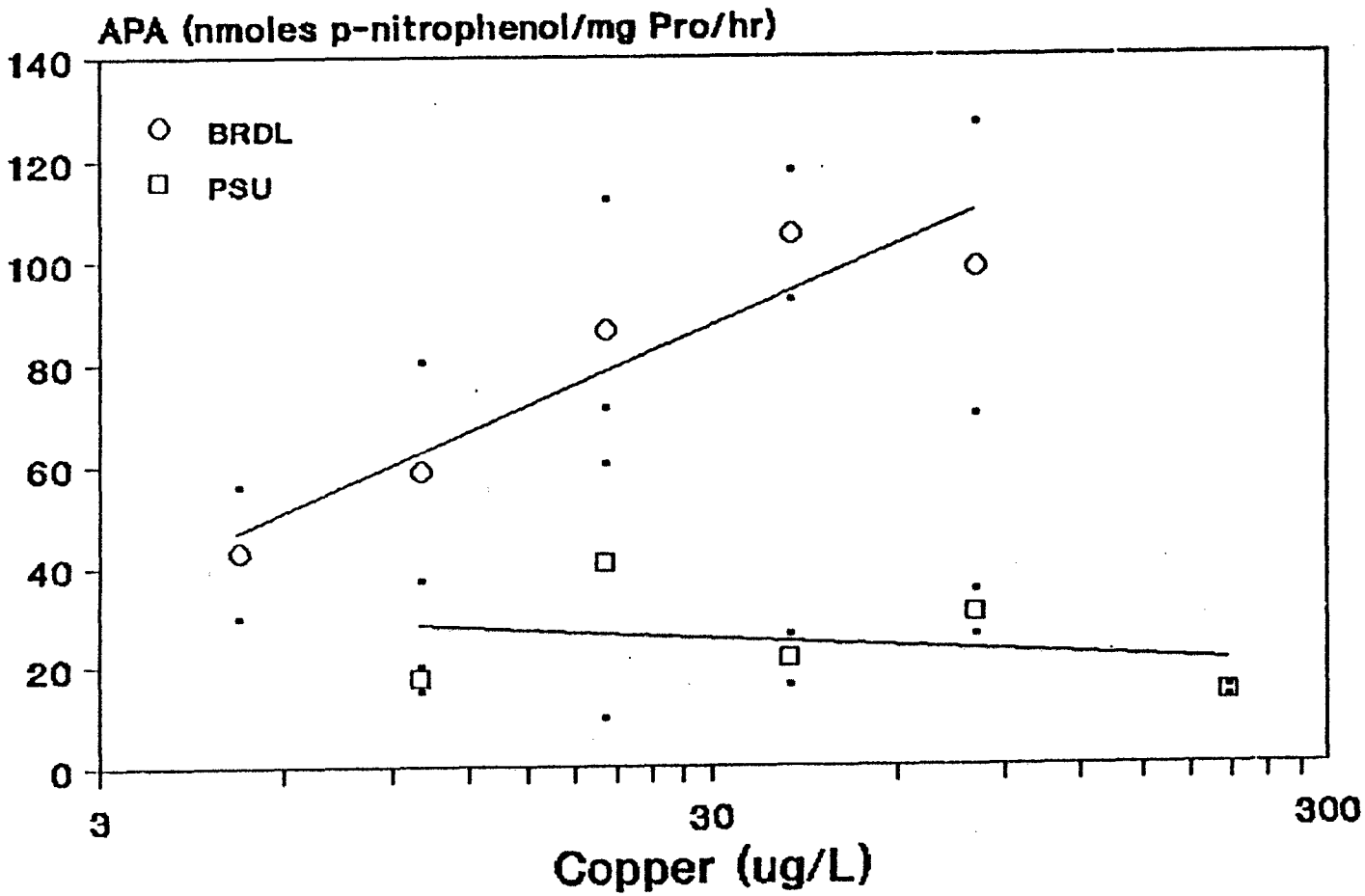

ISLAND
TOXICANT
EPICENTER
ISLAND
ISLAND

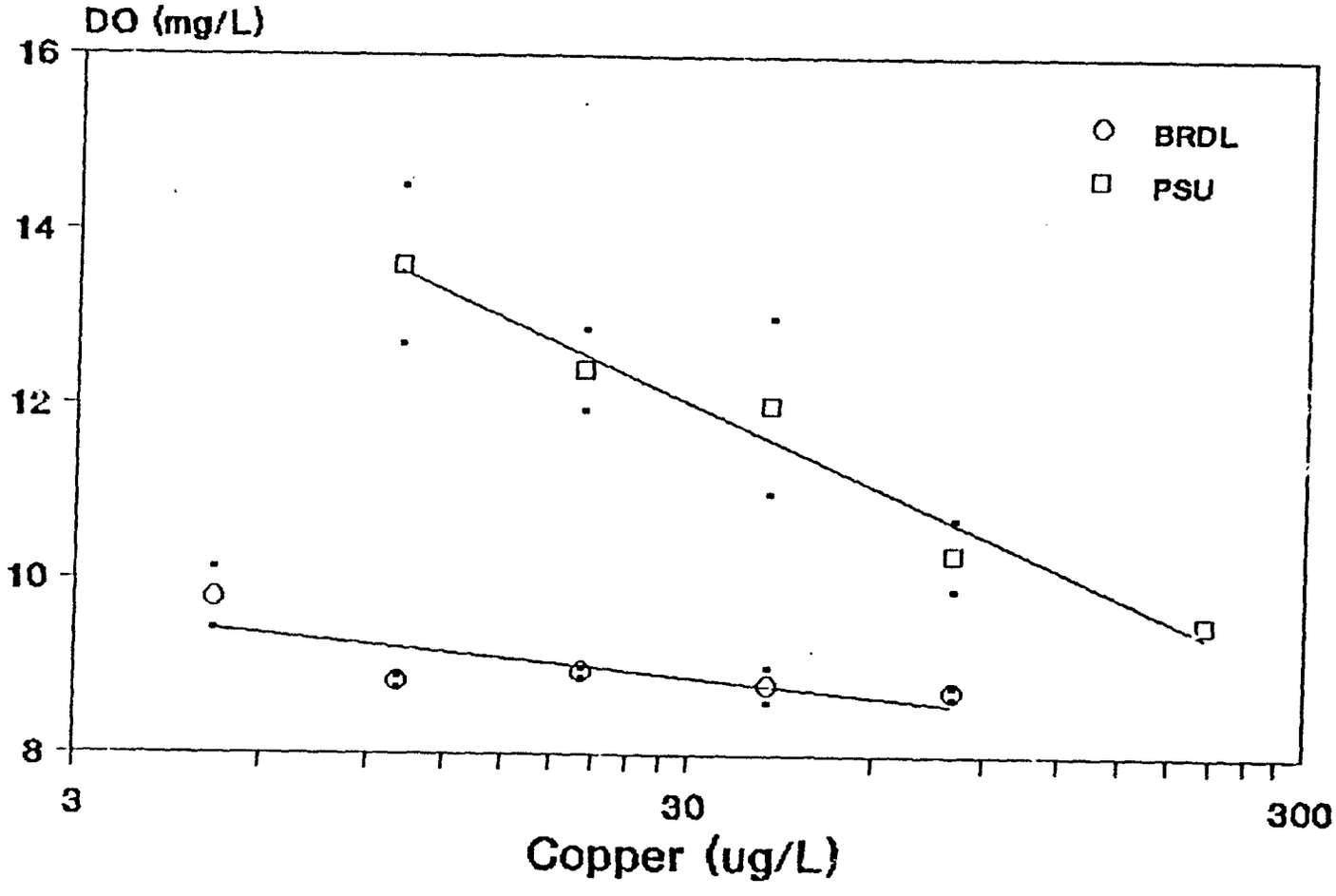

Protein (ug/ml)
250
200
150
100
50
0
BRDL
PSU
3
30
300
Copper (ug/L)


Chl a (ug/L)
1000
100
10
1
0
50
100
150
200
250
Copper (ug/L)
BRDL
PSU


Protozoa
Diatom Algae
50
40
30
20
10
0
25
20
15
10
5
0
BRDL algae
PSU Protozoa
3
30
300
Copper (ug/L)


BRDL - Algae
0 5 10 20 80 40
PSU - Algae
0 10 20 40 80 160
PSU - Protozoa
0 10 40 20 80 160


APA (nmoles p-nitrophenol/mg Pro/hr)
140
120
100
80
60
40
20
0
BRDL
PSU
3
30
300
Copper (ug/L)


DO (mg/L)
16
14
12
10
8
BRDL
PSU
3
30
300
Copper (ug/L)

Running Head: Variability of community metrics

Copyright and page proofs to: Dr. James R. Pratt
213 Ferguson Bldg.
The Pennsylvania State University
University Park, PA 16802
(814) 863-2095

Variability of community metrics: detecting changes in structure and function

J.R. Pratt and N.J. Bowers
School of Forest Resources, The Pennsylvania State University, University Park, PA 16802

(Received , Accepted)

Abstract - Increased environmental realism in toxicity testing has been advocated to better predict ecological effects of toxic chemicals, and several aquatic microcosm bioassays have been developed using both natural and synthetic organism assemblages. Additionally, greater emphasis is being placed on community changes as evidence of adverse effects on aquatic ecosystems. The ability to infer structural or functional changes in stressed versus unstressed communities is linked to statistical power which arises from the combination of experimental design and the underlying variability in chosen metrics. We evaluated several community response variables (metrics) in aquatic laboratory microcosm experiments and field studies to estimate metric variability. Variability of community metrics under laboratory conditions was similar to that observed in the field. Power curves were constructed to estimate the detectability of significant responses using different experimental designs. Using the median coefficient of variation for each measured response, we estimated the minimum detectable distance for power of 0.8 (β=0.2) and α=0.05 for each response. Community metrics with coefficients of variation < 30% allow detection of differences between means of 20 to 60% using as few as three replicates. Structural metrics such as species richness and standing crop are expected to prove more useful in detecting community and ecosystem changes. Process rates are affected by material supplies which may not change under stress.

Keywords - Microcosms Variability Community metrics

INTRODUCTION

Laboratory- and field-scale ecosystems have been recommended as logical and meaningful intermediates between traditional population-based laboratory experiments (single-species toxicity tests) and uncontrolled natural ecosystems for evaluating effects of anthropogenic stressors [1-3]. Recently, outdoor pond mesocosm studies have been required for testing ecological effects of pesticides for purposes of negating assumptions of unacceptable risk based on single species bioassays.

The choice of variables to be measured (metrics) is critical in any experiment directed at detecting adverse effects. Choosing metrics with high variability reduces the probability of detecting effects and can result in erroneous conclusions, namely a statistical type II error (incorrectly accepting a null hypothesis as true). Ecologically meaningful variables are often expected to have high variability, and variability is expected to increase with stress [4].

The stress responses of complex biological communities in artificial ecosystems may be estimated using indices of change in community structure, nutrient cycling, and energy flow [3]. Because of the natural variability, or noise, of even simple laboratory ecosystems, experiments attempting to examine stress effects under environmentally realistic conditions may lack appropriate statistical design, complicating the interpretation of stress effects [5-7]. Depending on the variability of the metrics chosen, the number of replicates required to detect differences among exposed, affected communities may be prohibitively large, suggesting that metrics with low variability and ecological meaning should be selected for measurement.

The purpose of this paper is to summarize information on the detectability of community-level responses measured in laboratory microcosms using single-factor analysis of variance designs which resemble traditional, single-species methods for evaluating chemical stress effects. Statistical procedures for testing hypothesis in analysis of variance designs assume equality of variances among treatments and this assumption is also necessary for the analysis that follows. We have summarized the coefficients of variation for several commonly measured responses and calculated minimal detectable differences (as a percentage of control values) possible based on

different experimental designs. A limited number of comparisons with other reports of response variability are provided. We have made recommendations for the selection of measurement variables and suggested additional studies needed to assess community metrics.

METHODS

Data from several experiments evaluating the effects of toxic chemicals on microbial community structure and function in laboratory microcosms have been summarized (Table 1). These experiments were conducted using naturally derived microbial communities collected on artificial substrata from several different ecosystems and examined effects on community structure (taxonomic richness, standing crop) and processes (production, respiration, nutrient cycling). The methods under which microbial communities were exposed to chemicals in the laboratory are described in Cairns et al. [8] and Pratt et al. [9]. The method is described briefly below, and a standardized procedure is reported in Pratt and Bowers [10]. In addition to laboratory results, similar responses of microbial communities collected under field conditions are also presented.

Experimental system

Microcosms were developed in small, shallow tanks with a maximum capacity of 8 l. Microbial communities were collected on polyurethane foam artificial substrata which had been exposed for 7 to 21 days in a natural ecosystem (e.g., [11]). Microbial communities collected in this manner included over 100 species of bacteria, algae, protozoa, fungi, rotifers, and larval insects. In the laboratory these communities displayed characteristics of ecosystems: high species diversity, production, nutrient cycling, competition, and succession.

Colonized substrata were collected and returned to the laboratory where they were randomly allocated to replicate microcosms filled with test medium (dechlorinated tap water or surface water amended with toxicant). In addition to the colonized substrata, initially barren substrata were placed in each microcosm to serve as colonizable habitat for continued community development and as sampling devices in experiments. Communities were exposed to 5 or 6

concentrations of toxicant under either static or continuous flow conditions. Experiments reported were about evenly divided between static and continuous flow. In each experiment, treatments were tested in triplicate and always included a control (undosed) treatment.

Measured variables

Microbial communities were sampled from each microcosm by removing a single, initially barren substratum and squeezing it to collect as much of its contents as possible. Samples were then subsampled for several analyses including structural measures such as taxonomic richness (the number of protozoan species), nontaxonomic measures of biomass and nutrients, and process measures such as primary production and enzyme activities. Daily evaluations of dissolved oxygen and pH were made as an index of net primary production, and in some experiments weekly evaluations of primary production and respiration were made using the whole microcosm as an experimental unit [12] to estimate the ratio of primary production to respiration (P/R). Because each physical sample was limited in volume and biomass and because the purposes of each experiment differed, the same suite of variables was not always measured.

Data Analysis

The purposes and dosing regimes differed among experiments, so only results from control communities were used in estimating the detectability of differences. Toxicant exposure can alter response variability, and we have observed response variability to increase, decrease, or remain the same depending on the response measured and the type of toxicant tested. Therefore, we have chosen to use control community data with the understanding that response variability under toxicant exposure may be altered. Variability of microbial community responses was estimated as the coefficient of variation (as a percent) for measures from triplicate control microcosms.

RESULTS

Coefficients of variation for metrics collected under laboratory and field conditions are shown in Table 1. Dissolved oxygen and pH were the least variable metrics, but the low CVs are due in part to the limited range of these parameters (pH 6-10 and dissolved oxygen 5-20 mg/L). Protozoan species richness also had low variability despite the availability often of over 100 species of protozoa in most of laboratory experiments and a greater species pool in the field. All of the metrics in Table 1 exhibited CVs of no more than 30% under both laboratory and field conditions.

Statistical power curves for several experimental designs at $\alpha = 0.05$ level were generated (see Figs. 1 and 2) using the relationship [13]:

$$(\delta/\sigma)^2 \geq 2/n\,(t_{\alpha,\nu} + t_{2(1-\rho),\,\nu})^2 \qquad (4)$$

where $t_{\alpha,\nu}$ = two-tailed value from a t-distribution with ν degrees of freedom corresponding to a level of significance α, $t_{2(1-\rho),\,\nu}$ = two-tailed value from a t-distribution that cuts off probability ρ to the left of the value where $\rho = 1-\beta$ is the power of the test, δ = an estimate of $|\mu_i - \mu_j|$ (minimum detectable difference), σ = an estimate of standard deviation, $\nu = k(n-1)$ (k=number of treatments), and n = the number of replicates.

With a knowledge of the variability (CV) of a given metric, the minimum detectable difference (MDD) between control and treatment means can be calculated by choosing a level of statistical power and estimate *delta/sigma* from the appropriate curve based on the experimental design using the relationship (from [13]):

$$MDD = \frac{CV}{\delta/\sigma} \qquad (5)$$

MDD's for several community responses measured in laboratory microcosms are shown in Tables 2 and 3 under several experimental designs using CV's based on control response. When the number of treatments is held constant (e.g., k = 6), increasing the number of replicates from two to

three decreases the MDD by approximately 9.1%, whereas increasing the number of replicates from three to four decreases the MDD by 16%. With an experimental design of six treatments and three replicates, the MDD for nine of the 14 metrics is less than 50%. The effect of increasing the number of treatments while maintaining the same number of replicates on the MDD is shown in Table 3. Increasing the number of treatments from two to three dramatically decreases the MDD by approximately 20%, although further increases in the number of treatments have much less effect (< 6%).

DISCUSSION

One of the major concerns of increased environmental realism in toxicity testing has been increased variability of measured variables and, therefore, difficulty in establishing effect levels [7]. There are three sources of metric variability: organismal, environmental, and methodological. The investigator has little or no control over the first two sources, but with prudent selection of metrics, careful analytical techniques, and replicate systems, variability can be managed so that community-level responses can be detected as statistically significant differences.

Based on our results from over 20 laboratory microcosm tests using naturally derived microbial communities, the variability of several ecosystem-level responses was sufficiently low to detect differences between control and exposed communities with simple experimental designs using minimal replication. Coefficients of variation for responses were similar whether collected in laboratory microcosms or under field conditions. An experimental design consisting of 5 to 6 treatments with 3 to 4 replicates provided enough statistical power to establish treatment effects using community metrics with CVs as high as 25%. Variability was similar for both structural and functional responses, although we did not evaluate species abundance data. Crow and Taub [14], however, reported that in their microcosms, measures of community "metabolism" (e.g., chlorophyll a, ash free dry weight [AFDW], dissolved oxygen) had less variability than organism counts.

Although the response variabilities reported refer to a specific microcosm method, other investigators using other laboratory microcosms have found similar response variability. Giddings and Eddlemon [15] monitored sediment/water microcosms inoculated with a natural source of algae and macrophytes for 5 weeks and found that CVs for most microcosm measures were 10 to 30% and that CVs decreased as microcosms matured. Brockway et al. [16] developed laboratory microcosms using pond sediment as a species source and measured nutrients, production/respiration (P/R), pH, and algal biomass. Coefficients of variation ranged from 0 to 99%, with the highest variability associated with orthophosphorus and nitrite measures, and static microcosms exhibited greater variability than flow-through microcosms. Coefficients of variation for chlorophyll a and AFDW measures of *Aufwuchs* collected on glass tubes in artificial streams were 13.6 and 6.96%, respectively [17].

Cautions

Observations of the detectability and importance of ecosystem changes correspond well to most of the measured variables reported, but the possibility of detecting differences because of low variability does not predict the probability of detecting ecological effects. Changing community structure and standing crop biomass are important and useful measures of adverse ecological effects [18]. Changes in nutrient cycling and primary production are more problematic and may not be detectable in stressed ecosystems [19, 20]. Additional environmental factors such as substrate supply affect processing rates more than the effects of toxicants. For example, we have observed comparatively low variability for alkaline phosphatase activity (APA), a measure of cycling rates of organic phosphorus. APA is affected by other variables such as the supply of inorganic phosphorous in dilution water. Ecological effects which might be interpretable as adverse effects of a toxicant on nutrient cycling often are observable only at extreme levels of stress (e.g., [21]). A knowledge of sampling and measurement precision is essential to understanding which variables will provide both the statistical power to detect ecological effects and heuristic power in interpreting results. Some variables have inherently low variability; for example, the

numbers of species vary over relatively narrow limits. Where variables are measured according to carefully controlled standard methods or standard operating procedures, variability will be reduced. Some response variables of inherent biological interest have low measurement precision because of methodological difficulties. Additionally, derived variables such as ratios often ignore the variability of the response variables used in their construction [22, 23]. The challenge in selecting response variables is to understand inherent variability. When the variability of response variables is estimated from pilot studies, the number of replicates can be controlled to achieve acceptable statistical power. When required replication is impractical, measurement error can often be controlled by improving sampling and analysis methods to achieve acceptable power.

Detection of ecosystem-level effects in multispecies laboratory microcosms has been shown to be possible, given prudent selection of metrics and experimental design. Microcosm experiments using natural communities optimize the probability of detecting effects [24]. Synthetic ecosystems relying on culture media and organisms that may not be co-adapted in any way should be expected to have higher variability. Further, results reported here utilize sampling from artificial substrata and standard methods to further reduce variability. Our experiments have been short-term measures of community response. Where longer experiment times and repeated measures have been used to characterize responses to pollutants, variability should be expected to be greater [26]. The repeatability of community-level experiments has yet to be demonstrated [26], although response variability is sufficiently low that standardization of laboratory testing and field measurements should allow sufficient power to detect adverse community responses to stress.

Experimental designs based on the analysis of variance assume that variances among treatments are equal [22]. Given the expectation of changing variance with stress [4], tests for variance homogeneity (e.g., Bartlett's test) should be conducted to assure that the assumptions of analysis of variance are validated. A pattern of increasing or decreasing variability can also be detected by plotting residuals from regression of response on dose or exposure (treatment). Such a pattern might be reflective of exposure variability or of important qualitative changes in the nature of the measured response. Although the number of replicates needed to detect effects of a given

magnitude can be determined a priori using reasonable estimates of variability, such estimates of numbers of replicates do not always match the availability of research resources (personnel, space, time). Therefore, sampling must be focused on those variables that convey ecotoxicological meaning and provide investigators with resolving power for finding differences. At the present time, these variables are primarily structural measures.

Acknowledgement

This work was supported in part by grants from the United States Environmental Protection Agency, Office of Research and Development (R-812813-01-0), the Air Force Office of Scientific Research (AFOSR-85-0324), and the United States Army Biomedical Research and Development Laboratory (DAMD17-88-C- 8068). Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the granting agencies.

REFERENCES

1. **Cairns, J., Jr.** 1984. Multispecies toxicity testing. *Environ.Toxicol. Chem.* 3:1-5.
2. **Kimball, K.D. and S.A. Levin.** 1985. Limitations of bioassays:the need for ecosystem-level testing. *BioScience* 35:165-171.
3. **Odum, E.P.** 1985. Trends expected in stressed ecosystems. *BioScience* 35:419-422.
4. **Odum, E.P., J.T. Finn, and E.H. Franz.** 1979. Perturbation theory and the subsidy-stress gradient. *BioScience* 29:349-352.
5. **Mount, D.** 1985. Scientific problems in using multispecies toxicity tests for regulatory purposes. In J.Cairns, ed., *Multispecies Toxicity Testing*. Pergamon Press, New York, NY, pp. 15-18.
6. **Tebo, L.B., Jr.** 1985. Technical considerations related to the regulatory use of multispecies toxicity tests. In J. Cairns, Jr., ed., *Multispecies Toxicity Testing*. Pergamon Press, New York, NY, pp. 19-26.
7. **Giesy, J.P.** 1985. Multispecies tests: research needs to assess the effects of chemicals on aquatic life. In R.C. Bahner and D.J. Hansen, eds., *Aquatic Toxicology and Hazard Assessment: Eighth Symposium*, STP 891. American Society for Testing and Materials, Philadelphia, PA, pp 66-77.
8. **Cairns, J., Jr., J.R. Pratt, and B.R. Niederlehner.** 1985. A provisional multispecies toxicity test using indigenous organisms. *Journal of Testing and Evaluation.* 13:316-319.
9. **Pratt, J.R., N.J. Bowers, B.R. Niederlehner, and J. Cairns, Jr.** 1988. Effects of atrazine on freshwater microbial communities. *Arch. Environ. Contam. Toxicol.* 17:449-457.
10. **Pratt, J.R. and N.J. Bowers.** 1990. A microcosm procedure for estimating ecological effects of chemicals and mixtures. *Toxicity Assessment*, 5:189-205.

11. **Cairns, J., Jr., D. Kuhn, and J. Plafkin.** 1979. Protozoan colonization of artificial substrates. In R. Weitzel, ed., *Methods and Measurement of Periphyton Communities: A Review*, STP 620. American Society for Testing and Materials, Philadelphia, PA, pp. 34-57.

12. **McConnell, W.** 1962. Productivity relations in carboy microcosms. *Limnol. Oceanogr.* **7**:335-343.

13. **Conquest, L.L.** 1983. Assessing the statistical effectiveness of ecological experiments: utility of the coefficient of variation. *Int. J. Environ. Stud.* **20**:209-221.

14. **Crow, M.E. and F.B. Taub.** 1979. Designing a microcosm bioassay to detect ecosystem level effects. *Int. J. Environ. Stud.* **13**:141-147.

15. **Giddings, J.M. and C.K. Eddelmon.** 1979. Some ecological and experimental properties of complex aquatic microcosms. *Jnt. J. Environ. Stud.* **13**:119-123.

16. **Brockway, D.L., J. Hill, J.R. Maudsley, and R.L. Lassiter.** 1979. Development, replicability and modelling of naturally derived microcosms. *Int. J. Environ. Stud.* **13**:149-158.

17. **Krieger, K.A., D.B. Baker, and J.W. Krammer.** 1988. Effects of herbicides on stream *Aufwuchs* productivity and nutrient uptake. *Arch. Environ. Contam. Toxicol.* **17**:299-306.

18. **Schaeffer, D.J., E.E. Herricks, and H.W. Kerster.** 1988. Ecosystem health: I. measuring ecosystem health. *Environ. Mgmt.* 12:445-455.

19. **Schindler, D.W.** 1987. Detecting ecosystem response to anthropogenic stress. *Can. J. Fish. Aquat. Sci.* **44**(Suppl.):6-25.

20. **Levine, S.N.** 1989. Theoretical and methodological reasons for variability in the responses of aquatic ecosystem processes to chemical stresses. In S.A. Levin, M.A. Harwell, J.R. Kelly, and K.D. Kimball, eds., *Ecotoxicology: Problems and Approaches.* Springer-Verlag, New York, NY, pp. 145-180.

21. **Pratt, J.R., B.R. Niederlehner, N.J. Bowers, and J. Cairns, Jr.** 1987. Effects of zinc on freshwater microbial communities. In S.E. Lindberg and T.C. Hutchinson, eds., *International Conference on Heavy Metals in the Environment*, Vol. 2, CEP Consultants Ltd., Edinburgh, pp. 324-327.

22. **Sokal, R.R. and F.J. Rohlf.** 1981. *Biometry*, second edition, W.H. Freeman and Co., New York, NY, 859 pp.

23. **Green, R.H.** 1989. Power analysis and practical strategies for environmental monitoring. *Environ. Res.* 50:195-205.

24. **O'Neill, R.O. and J.B. Waide.** 1981. Ecosystem theory and the unexpected: implications for environmental toxicology. In B.W. Cornaby, ed., *Management of Toxic Substances in our Environment*. Ann Arbor Science, Ann Arbor, MI, pp. 43-73.

25. **Giesy, J.P. and P.M. Allred.** 1985. Replicability of aquatic multispecies test systems. In J. Cairns, Jr., ed., *Multispecies Toxicity Testing*, Pergamon Press, New York, NY, pp. 187-523.

Table 1. Coefficients of variation (CV) for several microbial community metrics measured under laboratory and field conditions.[a]

	Laboratory Microcosms		Field Conditions	
Metric	n	CV	n	CV
Protozoan Species	23	7.29 (4.33)	11	6.57 (3.74)
Total Protein	24	17.6 (10.6)	11	19.5 (11.4)
Chlorophyll *a*	18	22.11 (12.0)	11	24.2 (11.1)
Alkaline Phosphatase Activity	23	18.0 (11.3)	11	13.2 (12.2)
Carbohydrate	6	26.0 (14.1)	9	20.2 (4.12)
Hexosamine	8	25.8 (11.7)	5	30.6 (11.8)
Ash Free Dry Weight	4	30.1 (18.1)	13	21.2 (16.8)
Electron Trans. System Activity		-	4	10.4 (6.51)
Magnesium	12	13.3 (10.8)		-
Calcium	12	11.4 (6.38)		-
Potassium	13	16.5 (7.43)		-
Phosphate	5	25.6 (20.1)		-
Dissolved Oxygen[b]	14	4.53 (3.33)		-
pH[b]	11	2.22 (1.44)		-
P/R[b]	8	13.9 (7.61)		-

[a] Values in parentheses are S.D. of CV
[b] Measured directly in microcosm

Table 2. Calculated minimum detectable differences (MDDs as percent of control) for several microbial community metrics based on coefficients of variation from laboratory microcosm measurements.[a] MDDs are shown for experimental designs of 6 treatments and n replicates using $\alpha = 0.05$ and $\beta = 0.2$.

Metric	n = 2	n = 3	n = 4	n = 6	n = 8	n = 12
Protozoan Species	20.0	18.1	15.2	12.1	10.4	8.46
Protein	48.2	43.8	36.8	29.4	25.2	20.4
Chlorophyll *a*	60.5	55.0	46.2	36.9	31.6	25.6
APA[b]	49.3	44.8	37.6	30.1	25.7	20.9
Carbohydrate	71.2	64.7	54.3	43.4	37.2	30.2
Hexosamine	70.6	64.2	53.9	43.1	36.9	29.9
AFDW[c]	82.5	74.9	62.9	50.3	43.0	34.9
Magnesium	36.4	33.1	27.8	22.2	19.0	15.4
Calcium	31.2	28.3	23.8	19.0	16.3	13.2
Potassium	45.2	41.1	34.5	27.5	23.5	19.1
Phosphate	70.1	63.7	53.5	42.7	36.6	26.7
Dissolved Oxygen	12.4	11.3	9.47	7.56	6.48	5.25
pH	6.08	5.53	4.64	3.71	3.17	2.57
P/R	38.1	34.6	29.0	23.2	19.9	16.1

[a] See [10] for references for analytical methods

[b] Alkaline phosphatase activity

[c] Ash free dry weight

Table 3. Calculated minimum detectable differences (MDDs as percent of control) for several microbial community metrics based on coefficients of variation from laboratory microcosm measurements.[a] MDDs are shown for experimental designs with 3 replicates and k treatments using $\alpha = 0.05$ and $\beta = 0.2$.

Metric	k = 2	k = 3	k = 4	k = 6	k = 8	k = 12
Protozoan Species	24.8	19.9	18.7	18.1	17.5	17.2
Protein	59.8	48.0	45.2	43.8	42.2	41.5
Chlorophyll *a*	75.1	60.3	56.8	55.0	53.0	52.1
APA[b]	61.2	49.1	46.3	44.8	43.2	42.5
Carbohydrate	88.3	71.0	66.8	64.7	62.4	61.4
Hexosamine	87.7	70.4	66.3	64.2	61.9	60.9
AFDW[c]	102.3	82.2	77.4	79.9	74.9	71.9
Magnesium	45.2	36.3	34.2	33.1	31.9	31.4
Calcium	38.7	31.1	29.3	28.4	27.4	26.9
Potassium	56.1	45.0	42.4	42.4	39.6	38.9
Phosphate	87.0	69.9	65.8	63.7	61.4	60.4
Dissolved Oxygen	15.4	12.4	11.6	11.3	10.9	10.7
pH	7.54	6.06	5.70	5.53	5.33	5.24
P/R	47.2	37.9	35.7	34.6	33.4	32.8

[a] See [10] for references for analytical methods

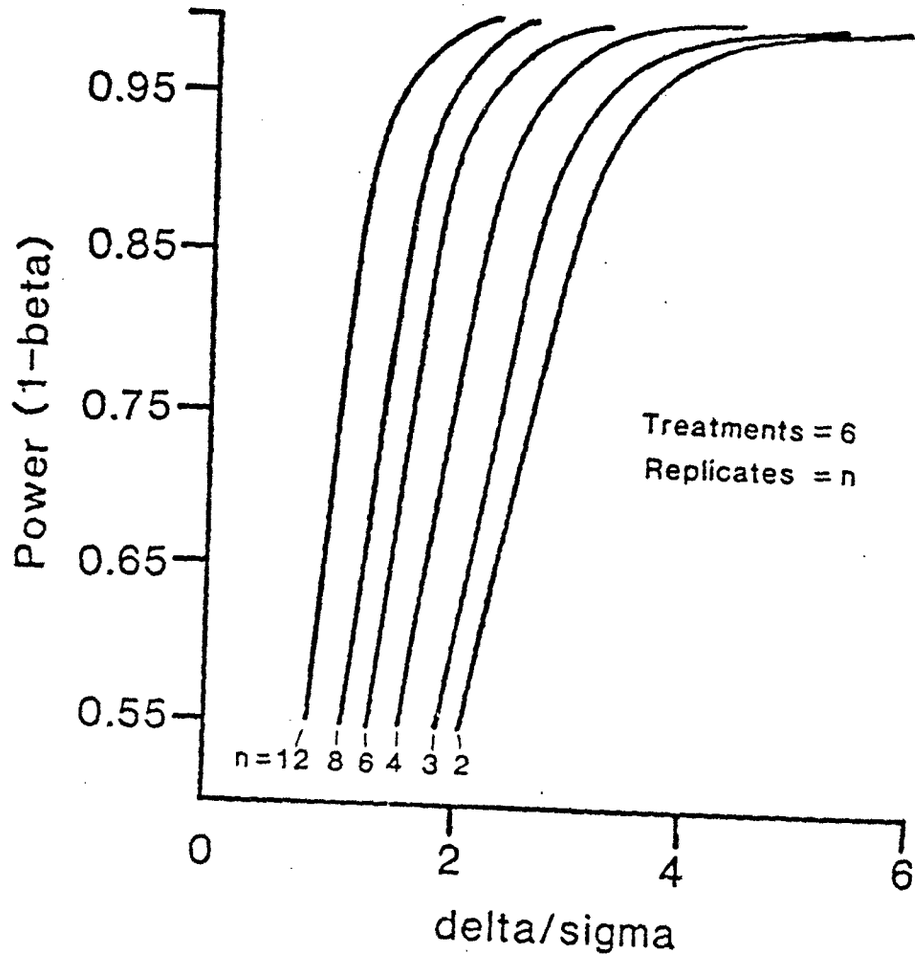
[b] Alkaline phosphatase activity

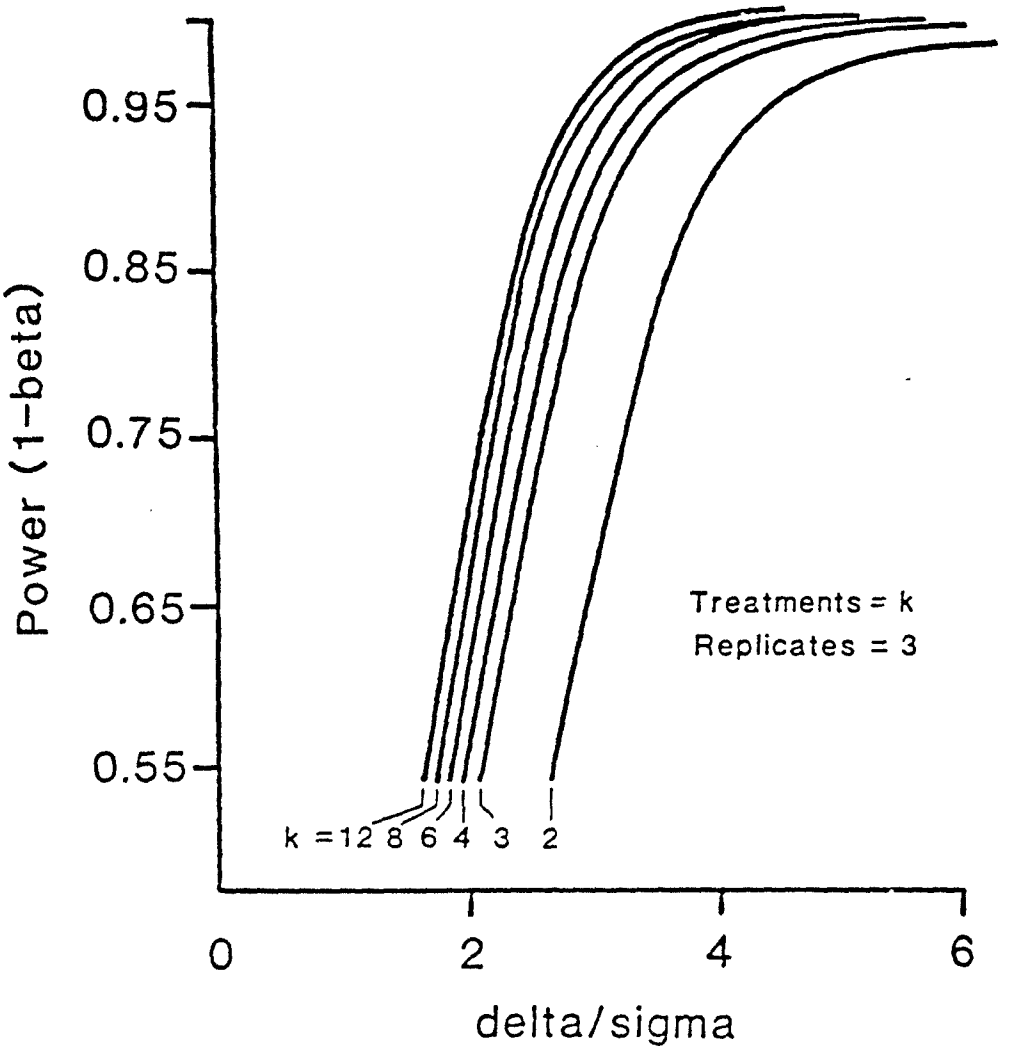
[c] Ash free dry weight

FIGURE LEGENDS

Fig. 1. Relationship among number of replicates (*n*), statistical power, and *delta/sigma* for experiments with six treatment levels (k=6).

Fig. 2. Relationship among number of treatments (k), statistical power, and *delta/sigma* (δ/σ) for experiments with three replicates per treatment (*n*=3).


0.95
0.85
0.75
0.65
0.55
Power (1-beta)
n=12 8 6 4 3 2
Treatments = 6
Replicates = n
0
2
4
6
delta/sigma


Power (1-beta)
0.95
0.85
0.75
0.65
0.55
k = 12 8 6 4 3 2
Treatments = k
Replicates = 3
0
2
4
6
delta/sigma

APPENDIX E

Personnel and Publications

APPENDIX E

PERSONNEL AND PUBLICATIONS
Contract DAMD17-88-C-8068

Personnel

Professional scientists

James R. Pratt, Ph.D.
James L. Roseberger, Ph.D.
Charles T. Morrow, Ph.D.
Stephen E. Tessler, Ph.D.

Graduate student assistant

Joseph M. Balczon (MS, supported by this contract)

Technical support personnel

Nancy J. Bowers
Karen L. Mortiz-Beard
Cynthia Kristine
Janice McClure
Thomas Ruscitti

Publications

Published papers:

Cairns, J. Jr. and J.R. Pratt. 1989. The scientific basis of bioassays. Hydrobiologia 188/189:5-20.

Pratt, J.R. and N.J. Bowers. 1990. A microcosm method for evaluating ecological effects of chemicals and mixtures. Toxicity Assessment 5:189-205.

Pratt, J.R. and N.J. Bowers. 1990. Effects of selenium on microbial communities in laboratory microcosms and outdoor streams. Toxicity Assessment 5:293-307.

Pratt, J.R. 1990. Aquatic ecosystem responses to stress. In Aquatic Toxicology and Risk Assessment, 13th Volume, W.G. Landis and W.H. van der Schalie, eds. American Society for Testing and Materials, Philadelphia, pp. 16-26.

Pratt, J.R. and E.P. Smith. 1991. Significance of change in community structure: a new method for testing differences. In Biological Criteria: Research and Regulation, Environmental Protection Agency, Washington, DC (in press).

Pratt, J.R. 1991. Making the transition from toxicology to ecotoxicology. Symposium on Organic Contaminants in Sediments, American Chemical Society, Lewis Publishers, Chelsea, MI (in press).

Graduate thesis:

Balczon, J.M. 1991. Response of two microcoecosystem to copper stress, MS Thesis, Graduate Program in Ecology, Pennsylvania State University.

Submitted manuscripts:

Pratt, J.R., N.J. Bowers, and J.M. Balczon. A microcosm using natural communities: comparative ecotoxicology. Symposium on Environmental Toxicology and Risk Assessment, American Society for Testing and Materials.

Abstracts and presentations:

Cairns, J. Jr. and J.R. Pratt. 1988. The scientific basis for bioassays. International Conference on Environmental Bioassay Techniques and Their Applications, Lancaster, England.

Pratt, J.R. and N.J. Bowers. 1989. Effects of selenium on microbial communities in laboratory microcosms and outdoor streams, International Symposium on Toxcity Testing Using Microorganisms, Las Vegas, NV. [This paper won the award for best presentation at the symposium.]

Pratt, J.R. 1990. Aquatic ecosystem responses to stress. Aquatic Toxicology and Risk Assessment, 13th Symposium, American Society for Testing and Materials, Atlanta, GA.

Pratt, J.R. and E.P. Smith. 1991. Significance of change in community structure: a new method for testing differences. Biological Criteria: Research and Regulation, Office of Water, Environmental Protection Agency, Alexandria, VA.

Pratt, J.R. 1991. Making the transition from toxicology to ecotoxicology. Symposium on Organic Contaminants in Sediments, American Chemical Society, Boston, MA.

Pratt, J.R. and W.H. van der Schalie. 1990. Interlaboratory comparison of copper effects in freshwater microcosms. Society of Environmental Toxicology and Chemistry Annual Meeting, Alexandria, VA.

Balczon, J.M., N.J. Bowers, and J.R. Pratt. 1990. Effects of complex effluent mixtures on microbial communities. Society of Environmental Toxicology and Chemistry Annual Meeting, Alexandria, VA.

Pratt, J.R. and N.J. Bowers. 1991. Ecological hazard assessment using microbes: repeatability of effects. North American Benthological Society Annual Meeting, Santa Fe, NM.

Smith, E.P. and J.R. Pratt. 1991. A new method for testing changes in community structure: effect of community measure. North American Benthological Society Annual Meeting, Santa Fe, NM.