TOXICANT-RELEASING SUBSTRATES: A NEW METHOD FOR DELIVERING COPPER TO MICROBIAL COMMUNITIES IN SITU

by

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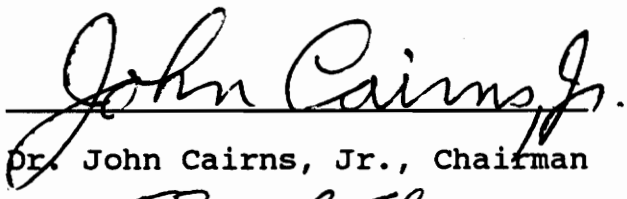
Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

BIOLOGY

APPROVED:



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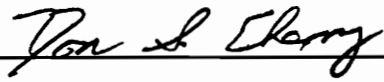

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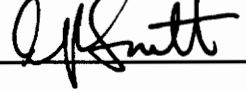

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May, 1993

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(ABSTRACT)

Currently, protocols for investigating the effects of chemical pollutants on periphyton communities under natural conditions are statistically flawed and/or potentially harmful to the ecosystems in which the studies are conducted. Toxicant-releasing substrates have been proposed to allow the delivery of different levels of chemical pollutants to replicate microbial communities in situ while minimizing the amount of toxicant released into the aquatic ecosystems under investigation. The purpose of this research was to compare the copper-induced responses of laboratory periphyton communities in artificial streams to those generated using standard, laboratory toxicity testing protocols during a summer and winter experiment. Chemical-releasing substrates were successfully used to deliver copper to periphyton communities in a predictable manner, over a broad range of doses, and at fairly constant rates during one week exposure

periods. Periphyton community structure (in the summer and the winter) and community function (in the summer only) were significantly affected by copper, in agreement with the scientific literature. The decrease in the ratio of chlorophyll a to biomass served as the most consistent and sensitive indicator of copper stress. In general, copper induced a shift in community structure in the direction of heterotrophically dominated communities. The season in which the community-level toxicity test was conducted influenced the sensitivity of total community biomass to copper and the pattern of recovery of community chlorophyll a following the termination of copper stress. Retention of copper by the communities two weeks after the exposure phase may have hindered the recovery of some periphyton structural attributes in communities previously exposed to high levels of stress. Most importantly, none of the measured responses to or recoveries from copper stress were statistically different for different methods of toxicant delivery. Thus, chemical-diffusing substrates generate toxicologically interpretable responses to copper in attached periphyton communities and may be of great use in future field studies of the effects of chemical stress on aquatic microbial communities.

Acknowledgements

I would like to thank my advisor, Dr. John Cairns, Jr., for initiating me into the field of ecology as an undergraduate and providing me with continual advice and support since then. I have also received extremely useful academic guidance from my other committee members, Drs. Donald S. Cherry and Eric P. Smith.

Dr. Paul McCormick, a supervisor and associate of mine, deserves a large amount of credit for this research; his creative thinking and experimental knowledge, in large part, led to the proposal of the method with which I worked in the present study. Other members of the biology faculty, including Dr. Bruce C. Parker, Dr. Albert C. Hendricks, and Dr. Arthur L. Buikema, have been graciously willing to lend their time and resources to aid me in my research.

Several graduate students also deserve thanks. Jay Comeaux provided me with a large amount of guidance and assistance in remodelling the artificial stream system used in my studies, and he gave me much help in the form of constructive criticism and encouragement. I wish to thank my colleagues within the department who have provided me with their academic curiosity, cross-disciplinary scientific advice, and friendship. In particular, I would like thank Matt Walker, Kevin Simon, Mark Miller, Barb Lubinski, and my office mates Rob Atkinson, Stephanie Hill, Karen Holl, David Jones, Bobbie Niederlehner, and Mara Sabre. Finally, I wish

to give special thanks to Eric Schiedt and Mike McMunigal, who assisted me directly with much of my data collection.

This research was supported by funds from the United States Air Force (AFSOR-91-0379), the Virginia Academy of Science, the Graduate Student Assembly at VPI, and matching funds from Dr. Joe R. Cowles and the Biology Department.

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Chapter 1

Introduction

This research involved the development and laboratory validation of a new, toxicant-releasing, clay substrate, which is colonized by microbial communities in aquatic ecosystems. This device allows the delivery of toxic concentrations of inorganic pollutants to these communities under ambient environmental conditions, while greatly minimizing the amount of toxicant released to the surrounding ecosystem. By allowing *in situ* manipulations with one class of anthropogenic stress (i.e., that resulting from inorganic toxicant discharge), chemical-releasing substrates have several potential applications to the investigation of problems facing aquatic resource managers.

Rivers and streams initially receive the majority of all point source waste discharges in the United States (Cairns and Dickson, 1975). Cost effective attempts by society to mitigate the degradation of aquatic natural resources rely on scientifically sound predictions of the impact of toxic chemicals, among other potential ecological stresses, on the biota in the receiving systems. Currently, water quality criteria for protecting aquatic ecosystems are largely derived from single-species, laboratory toxicity tests (Stephan et

al., 1985). This approach is indispensable for initially assessing the environmental hazards of the great number of toxic substances discharged into flowing waters today. However, these tests lack environmental realism and can fall short of generating reliable predictions of the impact of toxicants to interacting populations and communities of organisms in complex natural ecosystems (Cairns, 1983; Kimball and Levin, 1985; Moore and Winner, 1989; Suter et al., 1993).

Although current national water quality criteria for specific toxicants are dependent on a few physicochemical characteristics, such as temperature, pH, and water hardness (e.g., USEPA, 1985a and USEPA, 1985b), they rely on the assumption that ecosystems which are abiotically similar, but which are comprised of different species of organisms, will respond similarly to stress. Thus, there is a need for increased knowledge of the hazard of chemical pollutants under different environmental conditions. Furthermore, Koeman (1982) discusses the need to incorporate global and regional differences in the species compositions of specific ecosystem types, among other ecological variables, into the first stages of sequential or tiered hazard assessment protocols.

Improvements in the predictability of ecotoxicological methods and, thus, the effectiveness of resource management decisions, can be achieved in two ways. First, extensive validation studies must be conducted to determine if "safe"

concentrations of an individual chemical compound or a complex effluent, as predicted from single species, laboratory toxicity tests, result in "acceptable" protection of ecosystem structural and functional integrity (Cairns, 1986). Secondly, the extent to which community response to stress varies in different ecosystems must be more fully characterized.

Laboratory microcosm studies of the effects chemical toxicants on assemblages of organisms (e.g., protistan communities) provide preliminary data on the validity of predictions of environmental risk generated from single species toxicity tests (e.g., Cairns and McCormick, 1991). Furthermore, outdoor experimental manipulations in constructed, ecosystem models and replicate enclosures of real ecosystems, termed mesocosms, have been used to further test the responses of complex ecological systems to human-induced perturbations (Odum, 1984). Mesocosm studies combine a high degree of environmental realism with the experimental control of replicated units. In addition, they utilize enclosed or semi-enclosed systems, so impact to the modelled, natural environment is prevented or minimized. However, mesocosms are only ecosystem surrogates. Conclusions of ecological risk drawn from mesocosm studies possess significant uncertainty due to the artificial nature of the test systems, as well as uncertainty in the form of type I and, more importantly, type II experimental error rates (Kleinbaum et al., 1988).

Investigators studying the effects of industrial effluents on stream and river ecosystems commonly estimate the extent of damage by comparing downstream, impacted reaches to upstream, reference reaches (USEPA, 1989) and/or making comparisons before and after construction of an industry. However, this approach is statistically flawed, as there is no true control or replication (Hurlbert, 1984; Smith et al., in press.).

Some of the problems associated with the assessment of hazard for existing point source discharges can be overcome by experimentally perturbing real ecosystems. Schindler's (1987) classic investigations at the Experimental Lakes Area in northwestern Ontario employed eutrophication and acidification as manipulations of entire, lentic (non-flowing) ecosystems. These experiments have contributed information to ecotoxicological data bases and have been important in the formulation of theories of ecosystem stress indicators. However, with the global human population increasing by 92 million people annually and habitat destruction occurring in all biogeographic regions of the world (Brown et al., 1992), the adverse consequences of manipulating natural ecosystems must be carefully weighed against the scientific benefits of such studies.

Streams and rivers present an added complexity to investigators planning ecosystem-level experimental

manipulations with pollutants: toxic chemicals released into flowing water, rather than impoundments, readily move out of the study area due to hydrology, in addition to the biological vectors found in all ecosystems (e.g., biomagnification, emigration, etc.).

A five-year, manipulative study conducted by the USEPA was an early attempt at characterizing the robustness of hazard assessment predictions based on single species, copper toxicity tests (Geckler, 1976). Although the mortality of some species in Shayler Run, the copper-treated stream in this study, agreed with laboratory-generated predictions, the experiment effectively demonstrated the failure of the toxicity tests to predict a more complex response -- the avoidance behavior demonstrated by several fish species. However, this experimental design required 120 μg/L of copper, a persistent toxicant, to be maintained in Shayler Run for 33 months.

More recently, Leland and Carter (1984) demonstrated significant effects of copper on 1) periphyton microsuccession on introduced substrates and 2) species composition on natural substrates in sections of an oligotrophic, Sierra Nevada stream by maintaining copper at 2.5, 5, and 10 μg/L in stream reaches for one year. Clearly, studies such as these are very important in predicting and validating the environmental hazard of toxic chemicals. However, future efforts in

developing manipulative toxicological experiments in natural ecosystems must be focussed, in part, on ameliorating the potential impact of these studies.

Microbial Communities in Toxicological Studies

Studies which aim to validate the results of laboratory, aquatic toxicity tests require ecotoxicologists to choose one or more populations or assemblages of organisms as subjects for experimental manipulations. Benthic, microbial communities are structurally diverse assemblages which are generally composed of bacteria, fungi, heterotrophic protozoans, metazoans, and algae. Stevenson and Lowe (1986) and, more recently, Cairns and McCormick (1991) list several advantages of using microbial communities for toxicity testing (Table 1). These include: 1) the important ecological role (e.g., mineral cycling and autochthonous primary production) that this group of organisms plays in aquatic ecosystems; 2) the great trophic diversity contained in microbial communities, all facets of which are exposed to stress during toxicological investigations; and 3) the rapid generation times of microorganisms, which results in many life stages and even generations being exposed to manipulations lasting days rather than the months or years required for life cycle toxicity tests using vertebrates.

As indicated by Stevenson and Lowe (1986), diatoms (Bacillariophyceae), often the most abundant algae in freshwater periphyton communities (a name given to benthic microbial assemblages), are easily preserved and identified and are generally sensitive to slight changes in water quality. These characteristics, along with the small spatial and temporal scales at which periphyton structure and function can be measured, serve to keep research costs low for this assemblage of organisms. Finally, experimental data exists which demonstrates that the functional and structural attributes of this community type exhibit sensitivities to stress that are similar to those of other ecosystem compartments (Niederlehner et al., 1985; Pratt et al., 1988a; Pratt et al., 1988b).

Historical Use of Chemical-Releasing Substrates

The development of a variety of artificial substrates has greatly facilitated the elucidation of structural (e.g., the lognormal distribution of diatom species relative abundance) and functional (e.g., protozoan colonization dynamics) characteristics of aquatic microbial communities (Patrick et al., 1954; Cairns and Henebry, 1982). By simultaneously introducing identical artificial substrates to the same microhabitat in an aquatic ecosystem, similar microbial communities may be obtained for study in an otherwise

heterogeneous environment. In a comparative study, Tuchman and Stevenson (1980) showed that diatom communities growing on clay tiles (the colonized surface of the chemical-releasing substrates employed in this study) introduced into a stream in southeastern Michigan were structurally more reproducible than those growing on introduced, sterilized rock substrates or natural rocks. Indeed, relatively powerful statistical analyses of field data rely on a low level of variability among such replicate communities.

More recently, several investigators have used nutrient-releasing, artificial substrates to experimentally manipulate the chemical microenvironment of derived periphyton communities in natural ecosystems (Pringle and Bowers, 1984; Fairchild et al., 1985; Lowe et al., 1986; Gibeau and Miller, 1989). Table 2 summarizes the specific chemical-releasing substrates which have been used effectively for characterizing nutrient limitations in aquatic ecosystems. The extremely small quantities of nutrient salts released by the substrates, relative to the size (i.e., dilution factor) of the ecosystems studied, has prevented eutrophication of the systems used in these field manipulations. Although the modified use of nutrient-releasing substrates for experimental acidification has been proposed (E. D. Keithan, unpublished), no methods employing substrate-released (non-nutrient) toxicants in field manipulations have been published.

The ability to deliver chemical stresses, other than inorganic nutrients, to natural, periphyton communities would allow the effects of toxicants on these assemblages to be validated under natural conditions (Stevenson and Lowe, 1986). In addition, with such a method, replicate communities could be stressed with different toxicant treatments at a given field site instead of treating an entire, manipulated ecosystem as a single experimental unit. Furthermore, the extension of this approach to several, different ecosystems would allow one to characterize the variation in the sensitivities of microbial communities in different lotic environments to particular toxic chemicals (Paul McCormick, pers. comm.). There is much need for future research in this area.

Ecosystem Responses to Stress

Two measures of ecosystem susceptibility to stress which have gained acceptance among ecologists are resistance and resilience (e.g., Margalef, 1968; Cairns and Dickson, 1977; Connell and Slatyer, 1977; Webster et al., 1983). Resistance is an ecosystem's ability to remain structurally and functionally unaltered by stress, and resilience refers to the rate at which an ecosystem returns to its pre-disturbance state upon termination of the stress. Clearly, these properties influence the manner in which succession occurs

following disturbance of an ecosystem (Connell and Slayter, 1977). Moreover, Stauffer et al. (1978) and Webster et al. (1983) have used resistance and resilience as concepts for comparing the sensitivities of different ecosystems to human-induced stress.

The different structural and functional attributes of a biological community or an ecosystem do not respond in concert to a given perturbation -- some static and dynamic characteristics are more resistant and resilient, when stressed, than others. Well designed community- and ecosystem-level, toxicological studies employ several measures of structure and function, based on their ecological importance, sensitivity to stress, and feasibility of measurement. A large number of microbial responses to toxicants have been effectively used in laboratory studies to quantify the effects of toxic chemicals on periphyton communities (e.g., Niederlehner et al., 1985; McCormick et al., 1986; Pratt et al., 1988a; Pratt et al., 1988b), and they can be used with varying degrees of success as indicators of stress in natural systems.

Several prominent ecologists have hypothesized general, ecosystem responses to stress (Woodwell, 1970; Rapport et al., 1985; Odum, 1985; Schindler, 1987). Although many of their postulated, ecosystem-level effects are the same, some controversies exist (Table 3). Most of these investigators

agree that ecosystem functional properties, although more robust than structural characteristics, change in response to stress. However, Schindler (1987) has found that phytoplankton production, microbial decomposition, and nutrient cycling were not disrupted by a variety of perturbing agents in several lakes located in northwestern Ontario. While he does not conclude from this work that these rate processes generally show no change in response to stress in various ecosystem types, he does call for an increased focus on structural responses as general, early warning signs of ecosystem-level stress, a position not held by the other investigators. The lack of change that Schindler observed for bacterial and protistan functional responses in lakes, however, may not have occurred in other ecosystems or for differently-scaled measurements (e.g, deviation in the productivity of communities of longer lived organisms).

Although a unified theory of predicted responses of different natural systems to stress has not been and probably cannot be developed, the abundance of evidence that supports some general ecosystem responses to stress, both structural (e.g., a reduction in species diversity) and functional (e.g., a shift in the ratio of productivity to respiration), serves as a solid foundation for ecotoxicological experimental design. A comprehensive approach to community- and ecosystem-level toxicological studies employs a variety of uncorrelated

functional and structural measurements, which are analyzed in light of the concepts of resistance (i.e., stress-induced change in a biological attribute) and resilience (i.e., recovery following the termination of stress). In this manner, 1) ecologically meaningful and/or predictively robust deviations of community attributes from their pre-disturbance states and 2) the community dynamics following the termination of the disturbance and its relevance to ecological succession and restoration may be elucidated.

Objectives and Hypotheses

The primary objectives of the following thesis were, first, to design a new system for delivering toxicants *in situ* to periphyton communities in a manner which is benign to the ecosystem as a whole, and, second, to validate that the proposed method for doing so would generate both predictable and meaningful toxicological responses to a well-studied chemical pollutant, copper. More specifically, the study had three stages.

The purpose of the first stage was to develop a chemical-releasing artificial substrate which would deliver chemical stresses to periphyton communities in streams and allow an array of functional and structural responses to be measured. This necessitated characterization of the diffusion, or lack thereof, for several potential toxicants through the diffusing

surface of the novel substrates and the development of clear and opaque recirculating chambers, required for the measurement of periphyton productivity, respiration rate, and nutrient uptake rate.

The second objective involved dosing periphyton communities in artificial streams with a metal toxicant (copper) using both the proposed method and a standard method of toxicant delivery (via aqueous solutions of copper added to the water column). Copper is bioconcentrated by the organisms which constitute periphyton communities (Klotz, 1981; International Copper Association, Ltd., 1992); thus, the magnitude of bioconcentration was used as the explanatory variable for community responses to both methods of dosing. Two comparative studies were conducted -- one in the summer and one in the winter. The responses of the periphyton communities in the laboratory mesocosms dosed by releasing copper from the novel substrates were compared with those dosed by dripping copper solutions directly into the water column. This was done to characterize any methodological artifacts associated with the proposed method of copper delivery and to illuminate unanticipated difficulties associated with using chemical-releasing substrates in field manipulations -- the ultimate purpose of developing these substrates. The results obtained in the summer were compared to the winter results.

Finally, the measured structural and functional responses of periphyton communities to, and their recoveries from, copper stress in artificial stream mesocosms were analyzed. This was done in order to complement existing knowledge of the toxicity of copper to aquatic microorganisms (e.g., algae) for which there is a paucity of controlled, community-level studies.

The predictions to be tested were:

1) Copper can be delivered to periphyton communities in a predictable manner for at least one week using the toxicant-releasing substrates.

2) Copper which diffuses into periphyton communities from the chemical-releasing substrates generates dose-dependent responses, and these responses are not statistically different from those generated by additions of soluble copper to the water column surrounding the communities.

3) Bioconcentration of copper by periphyton communities is a season-dependent phenomenon, predictable within a given season.

4) Periphyton communities exposed to copper in laboratory artificial streams are altered both structurally and functionally, in agreement with previous studies, and seasonal environmental differences in the artificial streams affect the responses of these communities.

5) After the termination of copper stress, microbial

communities exhibit different patterns of short term recovery, depending on the season and the community attribute of interest.

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Table 1 - Advantages of utilizing periphyton communities in ecotoxicological investigations. See text for further discussion.

Attribute	Citations
1) Ecological Importance	Stevenson and Lowe, 1986 Cairns and McCormick, 1991
2) High Structural and Functional Diversity	Cairns and McCormick, 1991
3) Small Spatial, Temporal, and Financial Scale of Investigations	Stevenson and Lowe, 1986 Cairns and McCormick, 1991
4) Ubiquitous Occurrence	Cairns and McCormick, 1991
5) Rapid Reproduction Rates	Stevenson and Lowe, 1986 Cairns and McCormick, 1991
6) Sensitivity Similar to that Exhibited by Other Groups of Organisms	Niederlehner et al., 1985 Pratt et al., 1988a Pratt et al., 1988b Cairns and McCormick, 1991
7) Ease of Sampling and Preserving the Diatoms (Bacillariophyceae) and High Sensitivity of this Group of Algae	Stevenson and Lowe, 1986

Table 2 - Chemical-releasing substrates used in published, ecological field experiments. See text for further discussion.

Publication	Substrate Composition	Focus of Study
Pringle and Bowers, 1984	Petri dishes containing agar, dissolved nutrient salts, and sand	Nutrient limitation for epipsammic diatom communities in a northern Michigan stream
Fairchild et al., 1985	Clay flower pots filled with nutrient-agar solutions	Nutrient limitations for dominant algal species in periphyton communities in Douglas Lake, Michigan
Lowe et al., 1986	Clay flower pots filled with nutrient-agar solutions	Effects of nutrients on the structure of algal periphyton communities in clearcut and forested streams in the Coweeta Hydrologic Laboratory, North Carolina
Gibeau and Miller, 1989	Porous porcelain and silica substrates filled with nutrient-agar solutions	Nutrient limitations for epilithic periphyton periphyton communities in a tundra stream in the Brooks Range, Alaska

Table 3 - Some predicted responses of ecosystems to stress.

Response	Citations
Structural Responses	
1) Species diversity decreases; resistant organisms dominate	Woodwell, 1970; Rapport et al., 1985; Odum, 1985; Schindler, 1987
2) Food chains simplify due to reduced energy flow at higher trophic levels and/or greater sensitivity of predators	Woodwell, 1970; Odum et al., 1985
3) Nutrient standing stocks decrease	Woodwell, 1970; Rapport et al., 1985; Odum, 1985
4) Reduction in the average size of some organisms	Woodwell, 1970; Rapport et al., 1985; Odum, 1985
5) Proportion of r strategists (and rapidly reproducing organisms) increases	(Woodwell, 1970); Odum, 1985
6) Increase in the abundance of parasites	Rapport et al., 1985; Odum, 1985; Schindler, 1987
Functional Responses	
1) Increase in community respiration	Woodwell, 1970; Odum, 1985
2) Shifts (increases or decreases) in the ratio of productivity to respiration	Woodwell, 1970; Rapport et al., 1985; Odum, 1985
3) Nutrient turnover increases	Odum, 1985

(continued on next page)

Table 3, continued

Response	Citations
System-Level Trends	
1) Retrogression (the reversal of autogenic succession) occurs	Rapport et al., 1985; Odum, 1985
2) Functional properties deviate less (i.e., are more redundant) than structural properties	Rapport et al., 1985; Odum, 1985; Schindler, 1987
3) Fluctuations in population sizes increase	Rapport et al., 1985; Schindler, 1987

Chapter 2

Laboratory Characterization of the Performance of Toxicant-Releasing Substrates: Summer and Winter Copper Toxicity Tests

Introduction

Predictions of the adverse effects of toxic chemicals on stream communities are largely based on the results of laboratory toxicity tests. However, these tests cannot incorporate all of the complexities of natural ecosystems and often fall short of generating reliable assessments of risk (Cairns, 1983; Kimball and Levin, 1985; Moore and Winner, 1989). The uncertainty associated with laboratory-generated predictions of toxicant-induced effects can be reduced by validating these predictions in outdoor surrogate ecosystems and, ultimately, in natural ecosystems (Cairns et al., 1978; Odum 1984; Suter et al., 1993). Although the information generated by field studies is indispensable to regulators of toxic chemicals, both impact assessments for existing toxic discharges into stream ecosystems and unreplicated stream manipulations are statistically limited (Hurlbert, 1984; Smith et al., in press.).

Recently, several investigators have utilized nutrient-releasing, artificial substrates in controlled, field investigations of nutrient limitation in periphyton communities (Pringle and Bowers, 1984; Fairchild et al., 1985; Lowe et al., 1986; Gibeau and Miller, 1989). These substrates are permeable to many nutrient salts, which can be delivered in effective concentrations to periphyton communities colonizing the substrates. The slow rate of diffusion from these substrates and the dilution capacity of aquatic ecosystems prevents one nutrient treatment from significantly affecting adjacent periphyton communities receiving different or less concentrated nutrient treatments. Thus, replication at one location in a stream or lake can be achieved with these substrates.

Paul McCormick (pers. comm.) recently proposed a modified substrate which would allow periphyton communities to be experimentally stressed with chemical toxicants under natural conditions and then recover from the stressing agent. Prototype substrates were shown to release several inorganic chemicals (e.g., cupric chloride, sodium hypochlorite, sulfuric acid, and zinc sulfate) in a replicable manner in static, laboratory diffusion studies (data not shown).

This research used cupric chloride as a model toxicant, in order to evaluate the performance of the proposed substrates in delivering toxic chemicals to established

periphyton communities. The mechanisms of copper toxicity to bacteria and algae (Table 4) and the effects of copper on periphyton communities (Table 5) have been well characterized relative to other toxic chemicals. The standard method by which periphyton communities are dosed with copper in both laboratory (e.g., Hedtke, 1984) and field (e.g., Leland and Carter, 1984) toxicity tests is by adding aqueous copper to the water column of the test system. Therefore, the measured responses of periphyton communities dosed by releasing copper from the novel substrates were compared to the responses of communities in flow-through streams to which aqueous solutions of copper were added dropwise at known rates.

The primary purpose of this investigation was to characterize any effects of the proposed method of copper delivery on periphyton response to this toxicant and to expose any other artifacts associated with using chemical-releasing substrates. Copper is an essential trace element for both prokaryotes (Cabral, 1989) and eukaryotes (International Copper Association, Inc., 1992), and is therefore accumulated from the environment by living organisms. Comparisons across dosing methods were possible in this study because bioconcentration of copper by periphyton communities served as a single measure of dose, valid for both methods of copper delivery.

It is well known that periphyton community structure

differs in different seasons (Neel, 1968; Hoagland et al., 1982), and that environmental factors such as pH, dissolved $CaCO_3$, chelating agents, and temperature modify the toxicity of copper to aquatic microorganisms (Cairns et al., 1978; Klotz, 1981; Campbell and Stokes, 1985; Peterson and Healy, 1985; USEPA, 1985). However, controlled, community-level studies of the adverse effects of copper on periphyton communities inhabiting flowing water are lacking in the scientific literature.

Comparisons of the responses of periphyton communities to substrate-released copper with those of communities dosed in the standard manner were made in artificial streams during both the summer and winter, in order to strengthen the conclusions made about the performance of the novel substrates. In addition, statistical assessments were made of the effects of season on periphyton response to copper. Finally, the short term recovery of community attributes following removal of the stress were investigated in order to add much needed information to the ecotoxicological data base.

Materials and Methods

Artificial Substrate and Recirculating Chamber Design

Chemical-releasing, artificial substrates were constructed from unglazed clay tiles and 750 ml Falcon,

polystyrene tissue culture flasks (Fig. 1). 4.2 mm thick clay tiles, composed of red, terra-cotta clay fired at 1062 °C, were sealed with silicone sealant (Dow Corning) over 10 cm x 10 cm square holes cut in the flasks. Completed substrates had an exposed tile area of 105.7 cm^2 and contained a volume of 790 ml. In previous, laboratory diffusion experiments, copper diffused out of substrates constructed in this manner in proportion to the internal concentration of aqueous copper (II) chloride.

Recirculating chambers were modified from those used by previous investigators (Bott et al., 1978; Rodgers et al., 1978; Crossey and La Point, 1988) to seal over the chemical-releasing substrates during the measurement of community functional parameters (Fig. 1). The chambers were constructed of clear acrylic with external dimensions of 12 cm (length) x 12 cm (width) x 4 cm (height). A tight seal was achieved between the chambers and the substrates through the use of flat silicone gaskets on both the bottom of the chambers and around the clay tiles. Flat gaskets were made by allowing the silicone beads around the clay tiles and silicone beads placed on the bottom of the recirculating chambers to cure while pressed against glass plates covered with commercial dishwashing detergent.

Four, heavy-duty rubber bands were used to seal each chamber to a substrate, during the measurement of functional

parameters, with the resulting silicone gaskets adjacent to one another. In this manner, the periphyton communities growing on the tiles and known volumes of water could be isolated from the artificial stream microcosms, enabling oxygen production and consumption and ammonium uptake rates to be measured with flow across the clay substrates.

Faller brand, miniature submersible pumps (Edmund Scientific), which require 0.26 amperes of a 12-16 volt alternating current, were used to recirculate the water enclosed by the chambers. A portable power supply (Fig. 1) was constructed using a 70DT-72 Dura Power II marine battery (Delco Remy), a Tripp Lite Model PV-550B DC to AC inverter (Trippe Manufacturing Co.), and a Staco type 221-B variable transformer (Staco Energy Products Co.).

The flow across the tile surfaces was made fairly uniform by means of triangular baffles sealed over the inflow and outflow ports of the recirculating chambers. A 60 ml Becton Dickinson syringe (Fisher Scientific), modified with internal flow interrupters to insure mixing within the syringe, was placed in-line with each submersible pumps and aided in removing air bubbles from the chambers prior to incubation. In addition, they allowed an initial water sample to be removed for dissolved oxygen analysis without introducing additional air bubbles. With the syringes depressed to the 10 ml level, the chambers enclosed a total volume of 790 ml.

Summer and Winter Artificial Stream Experiments

Flow-through toxicity tests with copper were conducted on microbial communities growing on chemical-releasing substrates in nine laboratory artificial streams, beginning in the late summer and early winter of 1992. The streams consisted of 95 cm (long axis) x 70 cm (short axis) x 19 cm (depth) elliptical, fiber glass channels, in which water velocities of 10 cm/s were established by means of rotating paddle wheels (Fig. 2). Carbon dechlorinated, municipal water, which is derived from the New River, Montgomery Co., VA, flowed from a headbox and into each stream at a rate of 1 l/min. The water left each stream through a 12.5 cm high standpipe, which served to retain 58 liters of water in each channel.

Periphyton covered rocks collected from two sites (Pembroke, Giles Co., VA and McCoy, Montgomery Co., VA) in the New River, were placed in the headbox and replaced weekly during the two experiments. These natural communities served as sources of microbial colonizers for the artificial streams. Macroinvertebrate grazers were not excluded from the headbox due to their role in dislodging living cells and promoting downstream periphyton colonization (McCormick et al., 1992), but they were kept from entering the artificial stream channels by means of a 0.23 mm mesh screen covering the outflow pipe of the headbox. The screen also prevented large clumps of periphyton from leaving the headbox and clogging the

spigots entering the individual streams.

Current was established in the headbox using a 100 watt, Little Giant, submersible pump (Edmund Scientific). The artificial stream channels and headbox were each illuminated with two Vita-Lite bulbs (CRI > 90, Durotest Corp.). Incident light intensity at the surface of the water was 47.5 $\mu E/m^2/sec$, and the photoperiod closely approximated the natural photoperiod for the New River at the beginning of each experiment.

The first toxicity test was initiated on September 4, 1992 by placing six, randomly chosen, chemical-releasing substrates, filled with filtered (0.2 μm pore size) stream water, into each of the nine stream channels. Substrates were secured in place using velcro strips, which were attached to both the bottom of the artificial streams and the chemical-releasing substrates with silicone sealant. The photoperiod for the summer test was 13L:12D. The winter toxicity test was similarly started with six initially barren substrates per stream on December 26, 1992, the photoperiod being 10L:12D.

Both experiments were conducted at ambient, in-flowing water temperatures; mean temperatures were 20.8 °C (n=31; S.E.=0.08) and 8.3 °C (n=16; S.E.=0.2) for the summer and winter experiments, respectively. In addition to temperature, several physicochemical characteristics of the water in the headbox were periodically monitored during the two tests.

The pH was monitored potentiometrically with a Fisher Acumet 1003 pH meter and Fisher 13-620-111, gel-filled, pH electrode with automatic temperature compensation. Water hardness and alkalinity, reported as mg/L of $CaCO_3$, were assessed using the EDTA titrimetric method and using potentiometric titration with 0.01N sulfuric acid to an endpoint pH of 4.5, respectively (APHA et al., 1989). Conductivity was monitored with a YSI Model 33 S-C-T meter.

Soluble phosphate, nitrate, sulfate, chloride, and fluoride were measured as anions in filtered samples (0.45μm) using a Dionex Series 2000i/SP ion chromatograph and a Dionex 4270 Integrator. Soluble nitrite and ammonium in filtered samples (0.45μm) were determined in the summer using the Colorimetric Method and Phenate Method, respectively (APHA et al., 1989). Neither form of nitrogen was detected (detection limit = 10 μg/L), so these measurements were not repeated in the winter. Silica, a macronutrient for diatoms, was assessed using a LaMotte Chemical, Single-Parameter Colorimeter Kit, which employs the Molybdosilicate Method (APHA et al., 1989).

Finally, free and combined residual chlorine were determined using amperometric titrations with 0.00564N phenylarsine oxide and a Wallace & Tiernan Titrator in order to monitor the performance of the carbon dechlorinator used in the artificial stream system (APHA, 1989). Table 6 summarized the results of theses analyses and indicates those parameters

for which two-sample t-tests, corrected for unequal variances when appropriate, demonstrate statistically significant differences.

During the summer and winter toxicity tests, colonization and community growth was allowed to proceed in the absence of copper stress for 365 light hours (approximately 4 weeks for the summer test and 5 weeks for the winter test). Rather than allowing periphyton communities to reach peak biomass before copper dosing, this relatively short colonization time was chosen so that the communities would be actively growing and, therefore, very adherent to the artificial substrates during all experimental manipulations.

At the end of the colonization phase, one randomly-selected substrate was removed from each stream for analysis of nontaxonomic, baseline community structure. The microbial community attached to the clay tile portions of the chemical-releasing substrates was removed using a stiff bristle tooth brush and filtered stream water, and it was homogenized with a Biomixer (Fisher Scientific). Known aliquots of the homogenate were used for structural analyses.

Immediately after a substrate was taken out of a stream and the microbial community removed and homogenized, a subsample was filtered onto a 47 mm diameter, Supor-200, 0.2 μm membrane filter (Gelman Sciences), from which ATP was extracted in boiling Tris (0.02 M, pH 7.5), frozen at -20 °C,

and later analyzed using the luciferin-luciferase method (APHA et al., 1989).

Next, a sample for analysis of chlorophylls a, b, and c was filtered onto a 24 mm diameter, Fisherbrand, G6 glass fiber filters (mesh size 1.5 μm), macerated using a Wheaton, Potter-Elvehjem tissue grinder (Fisher Scientific,) and extracted in 90% buffered acetone overnight at 4 °C in the dark. The following day chlorophyll a was determined in the presence of pheophytin a using a Perkin Elmer Lambda 6 UV/VIS spectrophotometer; chlorophylls b and c were determined concurrently using the Trichromatic Method (APHA et al., 1989).

Finally, a known subsample of the periphyton homogenate was filtered onto a 55 mm diameter, Fisherbrand, G6 glass fiber filter for the determination of the total organic content of the microbial communities. This was measured as the ash-free dry weight or the difference between the weights of dried samples (24 hr. at 105 °C) before and after combustion at 500 °C for 1 hour (APHA et al., 1989). Combusted samples were wetted with distilled, deionized water prior to drying to ensure the proper chemical hydration of the ash. The periphyton homogenate remaining after all subsamples were processed was preserved with 4 ml of modified M3 preservative (3.8% glacial acetic acid; 19.2% formalin, 0% KI) per 100 ml homogenate (APHA et al., 1989).

After completion of the baseline structural sampling, the seven-day, copper exposure phase was initiated. All five remaining chemical-releasing substrates were removed from each of four randomly chosen streams and filled with a predetermined solution of copper (II) chloride made up in filtered (0.2 μm) headbox water. The concentrated solutions of copper (II) chloride were prevented from running over the periphyton communities by tipping the chemical-releasing substrates back while being filled. Any toxicant solution that ran over the polystyrene portions of the artificial substrates was rinsed off several times with distilled water before returning the substrates to the artificial streams.

Each of the four treatment levels (i.e., chemical-releasing substrate internal concentration) was allocated to a different artificial stream. Copper solutions were added to the water column of four additional, randomly chosen streams using a MasterFlex Model 7520-10 peristaltic pump, fitted with four pump heads. The five substrates remaining in these streams were removed, drained, filled with filtered (0.2 μm) headbox water, and returned to their original positions in the streams to control for the effect of removing colonized, toxicant-releasing substrates from the streams.

One of the nine streams served as a reference stream and received no copper. Again, the filtered water in the five substrates in the reference stream was replenished to control

for periphyton sloughing. No visible loss of biomass, including fine chlorophycean filaments, occurred during either the summer or winter tests due to the experimental manipulation of the substrates.

Dissolved copper in the water column of each stream was monitored during the week of copper exposure. An aliquot of stream water was removed daily from each stream, acidified to a final concentration of 0.15% nitric acid (trace metals grade), and analyzed electrothermally with a Perkin Elmer 110 Atomic Adsorption Spectrophotometer and a Perkin Elmer HGA-300 furnace programmer (APHA et al., 1989).

After exposing the periphyton communities growing on artificial substrates to four different levels of copper stress for each of two methods of copper delivery (e.g., released from the substrate or through the water column), all five flasks in each of the nine streams were removed, one stream at a time, and the enclosed, filtered stream water or copper solution four of the five flasks was replaced with filtered headbox water. Concurrently, copper delivery to the water column of the four streams, selected for this method of dosing, was terminated. The four flasks filled with filtered water were returned to their original positions in the appropriate streams, and the periphyton community on the fifth, randomly chosen substrate was initially sampled using the same protocol described for the baseline assessments. In

addition to the measurements previously described, copper bioconcentration, bacterial biomass, and three community functional parameters were measured at the end of the one-week exposure phase.

Copper content, reported as milligrams of copper per gram of total organic content, was determined in the aliquots removed for the measurement of ash-free dry weights. After being filtered onto the glass fiber filters, ash-free dry weight subsamples were washed several times with filtered stream water to remove soluble copper. After combustion at 500°C, copper was extracted from the ash for one week in concentrated, trace metals grade nitric acid (APHA et al., 1989). Following the extraction, the samples were diluted to a final concentration of 1.0 % nitric acid and analyzed via atomic absorption spectrometry as before.

Preserved samples were used for the analysis of bacterial biomass. Known volumes of the preserved periphyton were: 1) stained with sterile Acridine Orange (0.01% final concentration); 2) filtered onto 25 mm diameter, 0.2 μm pore size, black, polycarbonate membrane filters (Poretics Corporation); and 3) destained with sterile, distilled water (Hobbie et al., 1977). The filters were then placed on a drop of fluorescence-critical immersion oil (Fisher Scientific) and covered with another drop of oil, followed by a coverslip.

Bacteria in forty, randomly selected 0.00264 mm^2 fields

were counted at 1000X magnification using epifluorescence microscopy (Hobbie et al., 1977). The relevant dimensions of 10 randomly selected bacteria in each morphological class per sample (i.e., cocci, rods) were used in the calculation of average bacterial biovolume, which was converted to an estimate of bacterial biomass using 0.22×10^{-14} gC/μm^3 as a conversion factor (Bratbak, 1985; see also Edwards et al., 1990).

Upon completion of the structural measurements, three substrates were randomly chosen from the four remaining substrates in each of the streams for measurement of community functional parameters. Respiration rate and primary productivity were measured as changes in dissolved oxygen concentration in the water enclosed by opaque and clear recirculating chambers, respectively, during in situ incubations. Water in the artificial streams contained no visible tychoplankton (i.e., dislodged periphyton), so no effort was made to filter the water used in these incubations. Artificial stream water, which was at or near oxygen saturation, was deoxygenated by bubbling nitrogen gas through it prior to incubations in clear chambers (2-2.5 hr.). This was not done to the water used in dark chamber incubations (approx. 1.5 hr.). The equations of Wetzel (1983, p. 152) for the "light- and dark-bottle techniques for estimating primary productivity" were used to calculate respiration rate, net

primary productivity, and gross primary productivity.

Ammonium uptake rate was measured as the reduction in the concentration of ammonium in clear, recirculating chambers during incubations for known periods of time (approx. 0.5 hour). Nutrient uptake is an important functional attribute of communities dominated by autotrophs. Ammonium is reduced relative to other ionic nitrogen species and is, thus, the inorganic form of nitrogen most readily sequestered by the majority of algal taxa (Dr. Bruce Parker, pers. comm). The chambers used for this assessment were spiked with aqueous ammonium chloride, resulting in an initial total concentration of 0.5 mg/L (as the cation). This is more than an order of magnitude below the acute (one-hour average) national criteria for total ammonia for the summer (19.2 mg/L) and winter (15.5 mg/L) toxicity tests (USEPA, 1985a). The ash-free dry weight of communities chosen for the functional assays was measured, and all measures of function were normalized for total community biomass.

One substrate remained in each stream after two weeks of recovery. The periphyton community growing on these substrates were removed at this time and processed in the same manner as those substrates sampled before beginning the seven-day copper exposure phase. Copper bioconcentration in the periphyton communities was measured again to assess retention of copper by the periphyton communities after two weeks of

recovery.

Data Analysis

Differences in the physicochemical quality of the diluent water in the summer and winter were assessed by comparing means using two sample t-tests. These tests were corrected, when appropriate, for unequal variances found by Bartlett's F-test. No extreme, temporal trends in the measured parameters were observed during the individual experiments, so no time graphs were plotted. Two sample t-tests, corrected for unequal variances if necessary, were used as well to test for differences in the baseline, nontaxonomic structure of the periphyton communities.

Seven-day plots of water column copper concentration served as qualitative indicators of diffusion characteristics. Copper bioconcentration in periphyton communities dosed through the water column was linearly regressed against average water column concentration to assess the relationship between dose and the binding and uptake of copper by periphyton communities. The slopes of the regression lines, corresponding to the summer and winter toxicity tests, were compared via an analysis of covariance. In the model for this analysis, season was a class variable, water column copper concentration was the covariate, and an interaction between season and copper bioconcentration was used to test for a

difference between the summer and winter slopes of this relationship (SAS Institute Inc., 1982; Kleinbaum et al., 1988). Similar analyses were performed to assess seasonal differences in the relationship between copper bioconcentration or in-stream copper concentration and the concentration of copper used in the chemical-releasing substrates.

The community-level, toxicological effects of copper were analyzed individually for the summer and winter experiments using the combined set of data for both methods of toxicant delivery, including the reference stream datum. In all cases, the explanatory variable (copper bioconcentration) was $\log_{10}$-transformed to linearize the response and reduce variance heterogeneity. Of the structural measurements, only ATP and bacterial biomass required $\log_{10}$-transformations. All of the functional measurements were $\log_{10}$-transformed. Small positive numbers, approximately an order of magnitude below the lowest datum for a given response, were added to the data to allow values of zero to be included in the analyses. Specifically, 0.01, 0.1, and 0.1 were added, respectively, to the respiration, gross primary productivity, and ammonium uptake data. All of the structural data were positive and non-zero. Significant toxicological responses were determined through simple, linear regressions of the transformed data (Kleinbaum et al., 1988).

An analysis of covariance approach was used to assess differential responses of aquatic microbial communities to method of copper delivery in different seasons (summer 1992 vs. winter 1993). The same $\log_{10}$ transformations used for the toxicological regression analyses were also used in this analysis. The model employed for all responses included the class variables "method" and "season" and the covariate, $\log_{10}$ copper bioconcentration (see Table 8 for the statistical model). In addition, method-by-bioconcentration, season-by-bioconcentration, and method-by-season-by-bioconcentration interactions constituted the univariate, covariance models.

Method-by-bioconcentration and method-by-season-by-bioconcentration interactions were used to test the null hypotheses: 1) that the responses of periphyton communities to copper was not affected by method of toxicant delivery and 2) that any significant dependence was itself consistent in different seasons. For ease of comparison and interpretation, all models employed the same terms. Although as many as nine dependent variables were analyzed for a given data set, Bonferroni corrections were not used for any of the hypothesis tests because: 1) the toxicological analyses were primarily done due to the scarcity of some of the measured responses to copper in the scientific literature and not to generate predictions of ecological hazard; and 2) Bonferroni corrections result in conservative hypothesis testing -- any

effects of the method of copper delivery on the toxicological endpoints that are approaching statistical significance are of interest in this study.

Significant recovery of community attributes and differential recoveries across method of copper delivery were assessed using a repeated measures model (SAS Institute Inc., 1989). Substrates were removed from the streams immediately after the end of a one week exposure to copper and after two weeks of recovery; thus, phase was a within-stream factor. The statistical model incorporated: 1) the $\log_{10}$ of copper bioconcentration, measured for communities sampled at the end of the seven-day exposure phase; 2) method copper delivery; and 3) the interaction between bioconcentration and method. A multivariate approach using type III sums of squares was taken to test within-stream effects and related interactions (SAS Institute Inc., 1989).

Results

Water Chemistry and Baseline Community Structure

Several physicochemical properties of the headbox water were different in the summer and winter experiments (Table 6). One of the primary differences was water temperature, the means for which were approximately 20°C in the summer and 8°C in the winter ($p<0.0001$). While pH was significantly lower

(p=0.0401) in the summer ($\bar{X}_S$+/-S.E. = 7.35+/-0.02) than in the winter ($\bar{X}_W$+/-S.E. = 7.48+/-0.05), the magnitude of the difference was small.

The artificial stream water was relatively soft throughout both experiments -- all individual measurements fell between 37 and 54 mg/L as $CaCO_3$. However, the mean water hardness for the summer toxicity test was approximately 10 mg/L higher than that found for the winter test ($p<0.0001$). Alkalinity differed by a similar amount during the summer ($\bar{X}_S$+/-S.E. = 38.6+/-0.8 mg/L as $CaCO_3$) and winter ($\bar{X}_W$+/-S.E. = 30.7+/-2.0 mg/L as $CaCO_3$) experiments (p=0.0006). The pH, water hardness, and alkalinity in the individual streams did not vary significantly with water column copper concentration (Jay Comeaux, pers. comm.).

Conductivity, a measure of total dissolved ions, was significantly greater ($p<0.0001$) in the summer ($\bar{X}_S$+/-S.E. = 114+/-0.9 μS) than in the winter ($\bar{X}_W$+/-S.E. = 78.6+/-1.1 μS). Mean summer and winter levels of soluble phosphate ($\bar{X}$+/-S.E. = 88.5+/-30 μg/L and 166+/-12 μg/L, respectively) and nitrate ($\bar{X}$+/-S.E. = 1.90+/-0.20 and 3.09+/-0.66 mg/L, respectively) were not significantly different. Nitrite and ammonium were not detected (detection limit = 10 μg/L) during the summer toxicity test, so assays for these ions were not conducted during the winter test. Significantly more sulfate (p=0.0208) was dissolved in the stream water in the winter ($\bar{X}_W$+/-S.E. =

22.0+/-4.8 mg/L) than in the summer ($\bar{X}_S$+/-S.E. = 6.20+/-1.5 mg/L).

Free hypochlorite was never detected (detection limit = 0.010 mg/L) during either the summer or the winter experiments. Combined residual chlorine, a much less toxic form of chlorine, was undetectable (detection limit = 0.010 mg/L) in the summer (n = 19) in all but two water samples, which contained 0.010 and 0.015 mg/L, but was detected in all 12 measurements in the winter ($\bar{X}_W$+/-S.E. = 0.020+/-0.001 mg/L). The higher concentration of combined residual chlorine in the winter may have been caused by increased chlorination of the municipal water, and thus, a greater chlorine load on the artificial stream system's dechlorinator, which was repacked with activated carbon before the initiation of each experiment.

Baseline characteristics of the periphyton were assessed just prior to the initiation of the week-long dosing phase. Baseline total biomass was significantly higher ($p<0.0001$) in the summer ($\bar{X}_S$+/-S.E. = 0.852+/-0.0548 mg/cm^2) than in the winter ($\bar{X}_W$+/-S.E. = 0.315+/-0.0312 mg/cm^2). The ratio of chlorophyll a to biomass was also significantly higher ($p=0.0144$) in the summer ($\bar{X}_S$+/-S.E. = 14.7+/-2.29 mgChla/gAFDW) than in the winter experiment ($\bar{X}_W$+/-S.E. = 7.54+/-1.25 mgChla/gAFDW). Thus, phototrophs appear to have made up a greater proportion of pre-disturbance community

biomass in the summer than in the winter. On the other hand, the amount of ATP measured per unit biomass in the baseline assemblages was relatively variable ($\bar{X}_s$+/-S.E. = 0.206+/-0.0409 mg/gAFDW; $\bar{X}_w$+/-S.E.= 0.0994+/-0.0200 mgATP/gAFDW). No significant difference between these means could be detected at the 5% level.

Total copper concentrations in the water columns of the individual artificial streams are shown in Figures 3 and 4, for the seven-day exposure phases of the summer and winter toxicity tests. The high, initial concentrations of copper in the streams dosed via the chemical-releasing substrates was caused by small amounts of extremely concentrated copper solutions (0.25 - 10 g/L of Cu) which had spilled onto the outside of the flasks while they were being filled. Qualitatively, these figures illustrate that by day three the rates of copper diffusion from the substrates were constant and remained so for the rest of the week. Water samples taken from the stream containing substrates filled with 0.5 g/L Cu in the winter contained more copper than expected but dropped continually during the week. This may have been due to a slow leak in one of the substrates in this stream and/or heavier than usual initial contamination with copper.

Periphyton Bioconcentration of Copper

In artificial streams to which toxicant was added dropwise, periphyton communities bioconcentrated copper in direct proportion to its ambient concentration (R^2=0.988 and $p<0.0001$ for both the summer and winter linear regression models; see Fig. 5). However, the slope of this linear relationship was significantly greater during the winter experiment ($p<0.0001$), indicating that the winter microbial communities accumulated a greater relative amount copper than those in the summer.

Quadratic models without linear terms best explained the copper bioconcentration of communities dosed at different levels of toxicant released from the artificial substrates (Fig. 6). The values of R^2 for the summer and winter quadratic models were 0.968 and 0.998, respectively, and the p-values were $p=0.0024$ and $p<0.0001$. However, for diffuser-dosed communities in the two different seasons, no significant difference ($p=0.8296$) was found between the parameter estimates for the quadratic terms. Because the replenishment of water was maintained at 1 liter per minute during both experiments and all streams contained the same volume, the equilibrium concentration of copper in the substrate-dosed streams served as an indirect measure of the rate of diffusion of copper from the colonized substrates. Again, quadratic models lacking linear terms best explained the relationship

between the copper concentration in the water column at the end of the exposure phase (i.e., equilibrium concentration) and the concentration of copper placed in the diffusing substrates at the beginning of the week (Fig. 7). In this case, the values of R^2 for the summer and winter quadratic models were 0.993 and 0.796, respectively, and the p-values were p=0.0002 and p=0.0419. Again, there was no statistically significant difference (p=0.3815) between the parameter estimates for the quadratic terms in these models.

Structural and Functional Responses to Copper

After one week of exposure, several structural and functional attributes of the microbial communities were significantly affected by copper. Table 7 summarizes the results of the simple linear regression models for the various untransformed or $\log_{10}$-transformed response variables and the explanatory variable, $\log_{10}$ copper bioconcentration. Copper-induced changes in community structure in the summer were reflected by significant decreases in: 1) total biomass, measured as the ash-free dry weight (p=0.0261; Fig. 8.1); 2) the ratio of chlorophyll a to biomass (p=0.0095; Fig. 10.1); and 3) the ratio of chlorophyll c to biomass (p=0.0412; Fig. 12.1).

The standard logarithm of ATP relative to total biomass decreased with increasing copper bioconcentration (Fig. 9),

but the decrease was marginally insignificant ($p=0.0568$). Two structural parameters, the measured ratio of chlorophyll b to total biomass (Fig. 11.1) and the standard logarithm of bacterial biomass relative to total biomass (Fig. 13.1), showed significant increases with copper bioconcentration during the summer experiment.

During the winter toxicity test, total biomass ($p=0.0413$; Fig. 8.2), the relative amount of ATP ($p=0.0067$; Fig. 9), and the relative amount of chlorophyll a ($p=0.0125$; Fig. 10.2) decreased significantly, while the measured quantity of chlorophyll b relative to community biomass increased ($p<0.0001$; Fig. 11.2). The $\log_{10}$-transformed ratio of bacterial biomass to total community biomass increased in response to bioconcentrated copper, although the increase was marginally insignificant ($p=0.0503$; Fig. 13.2). The amount of chlorophyll c in the microbial communities in the winter did not change in response to the toxicant ($p=0.6006$; Fig. 12.2).

Periphyton community function was significantly impaired in the summer but was not significantly altered by copper in the winter. Figures 14.1 and 14.2 show the relationships between untransformed community oxygen consumption and $\log_{10}$-transformed copper bioconcentration for the summer and winter tests, respectively. In the summer, copper induced a significant decrease ($p=0.0182$; $R^2=0.573$) in community respiration (measured as periphyton oxygen consumption).

Although community oxygen consumption in the winter appeared to increase with copper bioconcentration throughout the range of low and intermediate concentrations of copper (Fig. 14.2), the linear model for this transformed response (data combined across method of toxicant delivery) had a slightly negative, but nonsignificant slope ($p=0.3798$). When the spurious datum corresponding to the highest bioconcentration in the winter was ignored, the slope of the relationship between oxygen consumption and copper bioconcentration was positive yet still statistically insignificant for either the $\log_{10}$-transformed ($p=0.121$) or untransformed ($p=0.110$) response variable. Summer and winter rates of community respiration were similar at low levels of copper bioconcentration.

Gross primary productivity decreased with increasing copper bioconcentration (Fig. 15); the linear regression model was statistically significant in the summer ($p=0.0026$ and $R^2=0.748$) but insignificant in the winter ($p=0.1020$; $R^2=0.336$). Again, at low levels of copper bioconcentration, the rates of gross primary productivity in the summer and winter were similar (see Fig. 15).

Changes in the untransformed rate of ammonium uptake by the periphyton communities with increasing copper bioconcentration are shown in Figure 16 for the summer and winter experiments. Log_{10}-transformed ammonium uptake

decreased significantly with increasing copper bioconcentration in the summer ($p=0.0217$; $R^2=0.552$), but the decrease in the transformed ammonium uptake rate in the winter was not significant ($p=0.1840$; $R^2=0.237$). The uptake of ammonium by the microbial communities at low levels of copper bioconcentration (see Figs. 16) appears to have occurred at a much higher rate in the winter (≈4 mgNH^+/gAFDW/hr) than in the summer (≈0.35 mg NH_4^+/gAFDW/hr).

The inability to detect a significant relationship between ammonium uptake and copper bioconcentration in the winter may have been due in large part to the influence of a single outlier at the second highest level of copper delivered through the water column (Fig. 16.2). After the removal of this point, the linear regression model for the log-transformed ammonium uptake rate more closely approached statistically significance ($p=0.0618$; $R^2=0.467$).

Summer and Winter Differences in Periphyton Response to Copper

Analysis of covariance revealed significant differences in response during the summer and winter experiments (i.e., significant season effects) for community ash-free dry weight ($p<0.0001$; Fig. 8), chlorophyll a ($p<0.0001$; Fig. 10), chlorophyll c ($p=0.0004$; Fig. 12), and bacterial biomass ($p=0.0025$; Fig. 13). Ash-free dry weight and the relative amounts of chlorophyll a and chlorophyll c decreased with

increasing toxicant concentration during both experiments but were consistently higher in the summer than in the winter across the entire range of copper concentrations. Conversely, relative bacterial biomass increased with copper bioconcentration in both seasons and was consistently greater in the winter across all copper concentrations in the communities. The ratios of chlorophyll a and chlorophyll c to biomass were lower in the winter than in the summer across all copper concentrations. Of all these seasonally dependent variables, only ash-free dry weight responded differently (i.e., with different slopes) during the two experiments ($p=0.0489$). None of the other structural or functional responses demonstrated a significant seasonal effect or a season-by-copper bioconcentration interaction (Table 8).

Effects of Method of Copper Delivery on Toxicological Responses

Some of the responses of periphyton communities dosed in a bottom-up fashion via the chemical-releasing substrates appeared to be shifted along the abscissa, as if these assemblages were slightly more tolerant of copper. Specifically, this was observed during the summer experiment for ash-free dry weight (Fig. 8.1), chlorophyll a (Fig. 10.1), and chlorophyll c (Fig. 12.1). However, none of these apparent shifts was significant ($p>0.29$ for the method term in

all ANCOVA models). In addition, after accounting for the effects of method, alone, there was no significant method-by-copper bioconcentration interaction (Table 8). Finally, after the statistical models had accounted for the lower order interactions and individual effects, only community respiration demonstrated a method-by-season-by-bioconcentration interaction ($p=0.0217$; see Table 8). The same three-way interaction was marginally significant for gross primary productivity ($p=0.0514$).

Periphyton Recovery from Copper Stress

Structural measurements were made on periphyton communities immediately following a one week exposure to stress and on communities from the same artificial streams after two weeks of recovery. Analysis of these data was performed using a repeated measures model. The single measurement of copper bioconcentration made at the end of the seven-day exposure period was used for each pair of exposure phase and recovery phase responses. The analysis revealed significant effects of the class variable phase on several structural responses of the microbial communities (Table 9).

Total community biomass generally increased two weeks after the termination of stress in the summer ($p=0.0099$) and winter ($p=0.0021$). However, the greatest increase in biomass following the removal of stress occurred at low copper

bioconcentration (Fig. 8). This was especially true during the winter experiment, for which this analysis revealed a significant interaction between phase and copper bioconcentration ($p=0.0151$). Thus, after two weeks community biomass had not measurably recovered from stress across all concentration of copper. Measured quantities of ATP in the microbial communities were extremely variable two weeks after the copper stress (data not plotted). Thus, no significant phase effect or higher order interactions with phase were found for this response (Table 9).

The changes in the relative amount of chlorophyll a in the periphyton communities after two weeks of recovery were different during the two experiments (Fig. 10). The amount of chlorophyll a in the summer communities decreased after termination of the copper stress ($p=0.0047$) but increased in the winter ($p=0.0034$) relative to communities stressed with copper for one week. Again, apparent recovery occurred primarily at low and intermediate concentrations of copper and not at the highest concentrations. The slope of the summer relationship between chlorophyll a and $\log_{10}$-transformed bioconcentration at the end of the seven-day copper exposure phase was statistically indistinguishable from the slope of the same relationship after two weeks of recovery (i.e., the interaction between phase and bioconcentration was not significant). In the winter experiment, however, the slope of

the linear relationship between community chlorophyll a content and $\log_{10}$-transformed copper bioconcentration after two weeks of recovery was significantly greater ($p=0.0236$) than that at the end of the seven-day exposure phase. The reason for this change in slope appears to be a lack of increase in the ratio of chlorophyll a to biomass in communities dosed with high (as opposed to low and intermediate) levels of copper stress during the winter exposure phase.

Changes in the relative amounts of chlorophyll c following the two week recovery period were similar to those of chlorophyll a. The amount of chlorophyll c in the communities after two weeks of recovery decreased in the summer relative to communities assayed at the end of the one-week exposure period ($p=0.0139$) and increased in the winter, although not significantly ($p=0.0548$). There were no significant interactions between phase and log-transformed bioconcentration for chlorophyll c in the summer or winter.

Measured quantities of chlorophyll b decreased in many of the streams during the two-week recovery phase, but the decreases were not significant at the 5% level (Fig. 11). The relative proportion of total community biomass contributed by heterotrophic bacteria decreased significantly after two weeks of recovery at all concentrations of copper during the summer ($p=0.0050$; Fig. 13.1) and winter ($p=0.0242$; Fig. 13.2) experiments. However, the decrease occurred approximately

equally over almost all copper concentrations, and no interactions between phase and bioconcentration were found during either experiment (Table 9). Three-way, phase-by-bioconcentration-by-method interactions were not significant in any of the models for the structural measurements made during either experiment; thus, method of copper delivery had no significant effect on the direction and magnitude of the deviations of any measured community structural attributes two weeks after termination of copper stress.

Discussion

Water Chemistry and Baseline Community Structure

Periphyton communities which developed in the artificial streams after 365 light hours of colonization were structurally different in the summer and in the winter. Total biomass (living and dead) was greater in the summer than in the winter, though mean concentrations of soluble reactive phosphate and dissolved nitrate were not significantly different from one another during the two toxicity tests. The artificial stream system receives fairly high levels of soluble nitrogen and phosphorus. Thus, it is likely that the low water temperature limited community growth in the winter.

Baseline communities in the summer contained a greater amount of chlorophyll a relative to biomass than those in the

winter. Periphyton communities are known to undergo seasonal succession on natural substrates (Neel, 1968) and seasonally-influenced primary succession on introduced substrates (Hoagland et al., 1982), so it is not surprising that the amounts of chlorophyll a relative to community biomass in the summer and winter were different. In fact, because photosynthesis is generally less temperature-dependent than respiration at lower temperatures, Cairns et al. (1975) hypothesized that, in general, decomposition rates in colder aquatic microbial communities may be low relative to production rates, leading to the accumulation of organic matter. An increase in the relative amount of nonliving organic matter in the winter compared to the summer could have been responsible for the decrease in the ratio of chlorophyll a to total biomass observed for the winter baseline communities.

Mean adenosine triphosphate content was highest in the summer. However, because of high variability in baseline ATP measurements, the summer and winter mean ratios of ATP to biomass could not be distinguished from one another. Due to the lability of some of the measured structural parameters (e.g., chlorophyll a, ATP), periphyton removed from one substrate had to be processed completely before removing another substrate from the artificial streams. For this reason, baseline sampling lasted several hours. Therefore,

some of the variability in the ATP measurements may have been due to real diurnal fluctuations in the average community ATP content (Cole et al., 1967; Holm-Hansen, 1973). Although extremely variable in the baseline and recovery samples, community ATP did respond significantly to copper in the winter (Fig. 9). Indeed, because it is a good indicator of living biomass, the measurement of ATP is often worthwhile in toxicological studies, especially when interpreted in conjunction with other estimates of periphyton community standing crop (Stevenson and Lowe, 1986).

Periphyton Bioconcentration of Copper

Bioconcentration of copper by periphyton communities dosed through the water column was a linear function of copper concentration. According to the limited data gathered here, more copper was bioconcentrated by winter communities than by summer communities. However, this finding was strongly influenced by a single point in the winter.

Several possible causes exist for differences in periphyton bioconcentration of copper in different seasons, given that they are real. These include important differences in periphyton community structure or physicochemical differences in water quality during the two experiments. Based on chlorophyll a measurements, autotrophs were more abundant relative to total community biomass in the summer

than in the winter. In addition, water temperature was 12°C colder and pH was slightly, but significantly, higher in the winter. In a previous study by Klotz (1981), algae were cultured from epilithic periphyton samples taken from a third order Connecticut stream, placed in dialysis bags, and incubated in a copper contaminated reach. Algal bioconcentration of copper was found to be positively correlated with ambient water temperature. In the present study, more copper was bioconcentrated by the intact, laboratory communities in colder rather than warmer water. At the same in-stream copper concentration, Klotz (1981) measured less copper bioconcentration for individual algal cells in aqueous culture than measured during either season in the artificial streams. Periphyton communities typically contain large amounts of extracellular organic material (e.g., Hoagland et al., 1982). This extracellular material may bind relatively more copper than free algal cells, leading to greater measurements for copper bioconcentration in intact periphyton communities than algal isolates.

Furthermore, the affinity of organic ligands for metals decreases with decreasing pH (Campbell and Stokes, 1985). This supports the hypothesis that more copper was bound by the extracellular organic material in the periphyton communities in the winter than the summer, due to increased pH and, possibly, decreased water temperature. Furthermore, in a

recent review of copper bioconcentration studies on wide variety of organisms, bioconcentration values reported for heterotrophic bacteria are generally higher that those reported for eukaryotic algae (International Copper Association, Inc., 1992). In the winter periphyton communities heterotrophic bacteria were relatively more abundant. Thus, two postulated mechanisms for the observed seasonal difference in periphyton copper accumulation are: 1) the dependence of abiotic copper binding (e.g., by organics) on environmental factors and 2) the dependence of copper bioconcentration on community taxonomic composition.

Chemical-releasing substrates also delivered copper to attached, microbial communities in a predictable manner. The rates of diffusion of copper from the colonized substrates appeared to increase to constant levels two days after being filled with solutions of copper (II) chloride and remained at these levels for the rest of the week. The slow initial rate of diffusion from the substrates, if real, may have been a result of copper binding to the walls of the pores in the clay tiles and/or from an initial impediment to diffusion by the mucilaginous periphyton communities. For a given level of copper bioconcentration, the resulting concentration of copper in an artificial stream dosed by releasing toxicant from the novel substrates was much less than the required concentration of copper in a water column dosed stream. The artificial

stream system used in this study had a slow flow-through rate (1 L/min) relative to natural streams; thus, impact to streams used in ecotoxicological manipulations (e.g., Geckler et al., 1976; Leland and Carter, 1984) would be greatly minimized through the use of chemical-releasing substrates.

Bioconcentration of copper and the resulting concentration of copper in the water column, both indirect measures of diffusion from the chemical-releasing substrates, were proportional to the square of the concentration of aqueous copper inside the flasks. It is expected, however, that the diffusion of copper from the substrates would become asymptotic at a high enough internal copper concentration due to physical and chemical limitations of the clay tiles to diffusion. However, bioconcentration did not even begin to approach an asymptote at internal copper concentrations as high as 10 g/L (summer experiment). The bioconcentration by periphyton growing on a toxicant-releasing substrate filled with 10 g/L of aqueous copper was more than an order of magnitude greater than the bioconcentration of copper by periphyton growing in a stream in which copper was maintained at about 200 μg/L (summer experiment). In previous studies, as little as 30 μg/L of copper measurably altered the structure and function of periphyton communities (Hedtke, 1984; Leland and Carter, 1984). Therefore, copper can be delivered to periphyton communities over a wide range of

ecotoxicologically significant levels using the novel substrates.

The summer and winter relationships between bioconcentration and the copper concentrations inside the substrates were statistically indistinguishable. Because a reduction in water temperature from 21°C to 8°C has a very small effect on the density of water, the calculated Reynolds number for the flows across the tiles changed little during the two experiments. Therefore, the flows were dynamically similar during the summer and winter experiments (Streeter, 1948), and no significant differences in the diffusion of copper away from the substrate-stream interface would be expected.

Lower water temperature and higher pH during the winter experiment might have resulted in a stronger chemical interaction between cupric ions and binding sites in the terra-cotta clay. The result would have been a reduction in the diffusion of copper through the clay tiles and, hence, in copper bioconcentration, but this was not detected by the analysis of covariance, which was relatively weak due to poor interspersion of the data.

On the other hand, it is believed than an artifact of sampling was the primary reason that the relationships between copper bioconcentration and copper concentration inside the artificial substrates were indistinct during the summer and

winter toxicity tests. More specifically, the method of periphyton removal -- scrubbing with a stiff bristle toothbrush and rinsing with water -- resulted in small amounts of clay being included in the homogenized samples. Consequently, the aliquots used for measuring copper bioconcentration contained this clay. Because of the extremely high concentrations of toxicant used in the flasks, the clay tiles of the chemical-releasing substrates abiotically bound far more copper than the clay tiles in the water column dosed streams (Fig. 17). This additional copper probably masked differences in the actual bioconcentration of this metal by substrate-dosed communities. Incidently, this artifact of sampling might be related to the apparent but insignificant shifts to the right observed for the some of the summer attributes of diffuser-dosed communities (i.e., ash-free dry weight, chlorophyll a, and chlorophyll c).

Structural and Functional Responses to Copper

Many of the periphyton responses to copper stress in the summer and winter experiments were qualitatively similar to those reported by other investigators (Tables 4 and 5). Total community biomass decreased with increasing copper bioconcentration (Fig. 8). However, community biomass after seven days of exposure to the highest concentrations of copper employed in these experiments was approximately the same as

the pre-exposure level. Thus, it appears that community production (i.e., colonization and growth) during the seven-day exposure phase was inversely related to copper bioconcentration and that high concentrations of copper halted net community growth.

Copper induced significant decreases in the ratio of chlorophyll a to biomass during the summer and winter toxicity tests and in the ratio of chlorophyll c to biomass during the summer test. The reduction in the relative amount of these phytopigments may have been caused by adverse responses of attached algae to copper at the organismal level, such as copper degradation of chlorophyll a (Robinson and Choi, 1989) or a community-level response, such as algal senescence with a concomitant increase in the abundance of heterotrophs (Hedtke, 1984), or a combination of these two processes.

Measured ratios of chlorophyll b to biomass increased in response to copper in the summer and winter. However, Robinson and Choi (1989) found that chlorophyll b is more susceptible to degradation by copper than chlorophyll a. Chlorophyll b occurs in the Chlorophyta and the Euglenophyta, and the ratio of chlorophyll a to chlorophyll b in members of these algal divisions varies between 2:1 and 3:1 (Lee, 1989). Thus, a relative increase in chlorophyll b in communities for which chlorophyll a is decreasing is probably an invalid finding. Apparent increases in chlorophyll b may have

resulted from spectrophotometric interference of the trichromatic method by copper. Such an interference has not previously been reported (APHA, 1989) and merits further study.

During both experiments relative bacterial abundance increased in response to copper stress, and the adenosine triphosphate content of periphyton communities decreased. However, when the increase in relative bacterial biomass was significant (summer only), the decrease in community ATP was insignificant, and vice versa. Viable bacteria contribute to the total ATP pool of aquatic microbial communities. A significant increase in relative bacterial abundance in response to copper, for example, may have slightly offset the decrease in community ATP content, resulting in the lack of a significant relationship between community ATP content and copper bioconcentration in the summer. This relationship is highly speculative due to the fact that the two nonsignificant linear models (e.g., those involving ATP in the summer and bacterial abundance in the winter) approached statistical significance at the 5% level.

The positive relationship between the relative abundance of bacteria and bioconcentration in the summer was probably the result of the increased availability of reduced carbon in the form of dead or senescing algal cells and not a direct effect of copper. Although a few bacteria may be sensitive to

low levels of copper (Bringmann and Kühn, 1980) some bacteria typically found in aquatic ecosystems can tolerate far higher concentrations of copper (Flemming and Trevors, 1989) than those tolerated by most aquatic eukaryotes (USEPA, 1985b).

Community functional attributes were impaired by copper in the summer, but no significant relationships between the measured functional parameters and copper bioconcentration were found in the winter. Community gross primary productivity and ammonium uptake were significantly impaired by copper in the summer in agreement with previously characterized mechanisms of copper toxicity. It is known, for example, that copper inhibits photosystem I (Droppa and Horváth, 1990) and photosystem II (Renganathan and Bose, 1989) in a wide variety of photoautotrophs and that it reduces the rate of uptake of ammonium by Scenedesmus quadricauda (Peterson and Healy, 1985). No significant dose-response relationships occurred for community functional attributes in the winter.

Throughout this study, measured functional parameters were more variable than microbial community structure. Manipulation of the communities with the functional assay chambers (e.g., addition of ammonium in the light with circulation) was labor intensive and fairly susceptible to methodological errors. Thus, the greater variability of the functional measurements and the lack of significant functional

deviations in the winter may have been primarily methodological in origin rather than truly reflective of community functional redundancy. A decrease in the ratio of chlorophyll a to biomass was consistently the most sensitive response of periphyton communities to copper, followed by the decrease of total biomass (measured as ash-free dry weight).

The algae in the periphyton communities were structurally and functionally altered by copper in the summer and may have declined in relative abundance due to copper stress during both the summer and winter toxicity tests. In addition, because copper induced an increase in relative bacterial abundance, it appears that the microbial communities shifted in the direction of heterotrophically structured communities in response to copper.

Community oxygen consumption decreased with increasing copper concentration in the summer. In a previous study of the effects of copper on aerobic respiration in aquatic sediments, 2.5 mg/L of dissolved copper caused a 50% reduction in sediment oxygen consumption (Flemming and Trevors, 1989). In the present study, measurable oxygen consumption was completely inhibited at the highest bioconcentration of copper in the summer (Fig. 14.1), even though bacterial biomass had increased (Fig. 13.1). In addition to impairing algal oxygen consumption, copper may have impaired heterotrophic bacterial respiration. However, it is also possible that community

bacterial respiration was resistant to copper but made a negligible contribution to periphyton oxygen consumption.

Methodological problems, which were encountered during the winter oxygen consumption assay, may have been responsible in part for the apparent differences between the summer and winter responses of community respiration to copper. Although the winter data strongly suggest an increase in community oxygen consumption in response to increasing copper (especially for the chemical diffuser dosed streams), the winter relationship (data combined across method of copper delivery) between respiration and copper was insignificant. Thus, future experiments should be conducted to investigate whether or not copper stress may cause an increase in lotic (flowing water) periphyton oxygen consumption under certain environmental conditions.

Summer and Winter Differences in Periphyton Response to Copper

The season in which the toxicity tests were conducted was an important factor governing the relationships between several of the structural attributes of the periphyton communities and copper bioconcentration. The summer ratio of chlorophyll a to biomass (Fig. 10) at the end of the seven-day exposure phase was smaller than the winter ratio across all concentrations of copper. However, the slopes of the summer and winter relationships were statistically indistinguishable.

Although communities contained relatively less chlorophyll a in the winter, probably as a result of differential primary microsuccession (Hoagland et al., 1982), the sensitivity of chlorophyll a to copper did not differ in the two experiments. On the other hand, heterotrophic bacteria contributed more to total community biomass in the winter across all copper concentrations (Fig. 13). Again, the slopes of the dose-dependent responses of bacterial biomass are not statistically different during the two experiments, and it is concluded that the increase in bacteria was similarly induced by copper in both seasons.

Total community biomass was greater on an areal basis in the summer than in the winter across all concentrations of copper (Fig. 8). However, the slope of the relationship between total biomass and copper bioconcentration was significantly greater in magnitude in the winter (Table 7). Although community colonization and growth was allowed to proceed for 365 light hours during both the summer and winter toxicity tests, higher water temperatures in the summer were probably responsible for greater biomass accumulation. When stressed with copper, the summer periphyton communities may have lost relatively more biomass, due to sloughing of cells and dead organic matter, when compared to the more adherent winter communities. Thus, unlike chlorophyll a or bacterial biomass, total community biomass was impaired more in the

summer than the winter.

The ratio of chlorophyll c to biomass decreased significantly with increasing copper bioconcentration in the summer but remained unchanged in the winter (Fig. 12). Thus, there appears to be a seasonal difference in the response of community chlorophyll c to copper as well. However, the slopes of this response are not significantly different, and conclusions on the seasonal differences in the relationship between chlorophyll c and copper cannot be drawn from data generated in this study.

Effects of Method of Copper Delivery on Toxicological Responses

In contrast to the season in which the toxicity test was conducted, the method of copper delivery did not affect any of the structural and functional responses of periphyton communities. In the analysis of covariance models tested to assess differences in the responses of periphyton communities to method of copper delivery (Table 8), method, alone, was never significant. Although no significant method-by-bioconcentration interactions were found, a three-way interaction between method, season, and bioconcentration was significant for community oxygen consumption. However, methodological problems occurred during the winter functional assays, and no strong conclusions concerning winter community

functional responses to copper can be drawn. In general, the summer functional responses and summer and winter structural response of periphyton communities to substrate-released copper agree with previously published results and cannot be statistically distinguished from those of communities dosed through the water column. This important result indicates that no significant methodological artifacts hinder the application of chemical-releasing substrates in studies of the effects of copper (and perhaps other inorganic toxicants) on periphyton communities in natural streams.

Periphyton Recovery from Copper Stress

Two patterns of short-term recovery following stress are postulated for previously undisturbed microbial communities. First, for a community attribute which is at steady state prior to a perturbation, full recovery following removal of the stressing agent would be achieved when the attribute reached its pre-disturbance state. In this case, significant recovery from a stressed state, indicated by a negatively-sloped dose-response relationship for example, would be assessed as a reduction in the magnitude of the slope of the relationship with no change in the intercept (Fig. 18). An analogous process occurred in the first experimental confirmation of the MacArthur-Wilson equilibrium theory of island biogeography conducted by Simberloff and Wilson (1969);

250 days after mangrove islands were defaunated with a methyl bromide fumigant species richness attained pre-fumigation levels, even though the species composition had changed somewhat. In this example, methyl bromide can be thought of as a stressing agent, albeit a severe one, and arthropod species richness can be viewed as the steady state community attribute of interest.

A different pattern of short-term recovery would be expected if the microbial communities are actively growing, or otherwise not at steady state, when the stress is initiated. In this second case, initial recovery from a stressed state, again indicated by a negatively-sloped dose-response relationship, would be assessed statistically as an increase in the intercept of the relationship with no change in slope (Fig. 18). This would occur if the colonization and growth of periphyton communities were impaired by a toxicant in a dose-dependent fashion but proceeded at all previous stress levels following removal of the toxic agent. As the community attribute of interest approached steady state, the slope would approach zero, and final recovery could be assessed as a change in both the intercept and the slope of the relationship (Fig. 18). Assessing recovery by statistically testing for changes in slope and/or intercept would also be valid for community attributes which increase in magnitude due to stress.

Repeated measures analysis (Table 9) was used to effectively compare 1) the slopes and intercepts of the relationships between community structural parameters after two weeks of recovery and copper bioconcentration at the end of the seven day exposure period with 2) those calculated for copper-exposed communities before recovery. The periphyton communities were actively growing when they were exposed to copper, so significant shifts of the two week relationships along the ordinate without changes in the slopes of the relationships served as indicators of recovery. Such shifts were identified as significant phase effects, which were found for several structural parameters in both the summer and winter (Table 9).

Although copper impaired net community growth, similar biomass accumulation occurred across most of the range of copper bioconcentrations shortly after the termination of copper stress (Fig. 8). Conversely, bacterial biomass decreased during the recovery period in all but the most stressed communities (Fig. 13). Because copper induced an increase in the relative abundance of heterotrophic bacteria, the shifts in bacterial biomass following the exposure phase can be interpreted as significant recovery of this structural attribute in both the summer and winter communities.

In the summer, the ratios of chlorophyll a and c to total community biomass after two weeks of recovery were generally

less than they were immediately after a week of exposure to copper (Figs. 10.1 and 12.1). On the other hand, the relative amounts of community chlorophylls a and c in the winter were generally greater after the recovery phase than they were at the end of the copper exposure phase (Figs. 10.2 and 12.2). During 365 hours of pre-stress colonization, summer communities accumulated more biomass than winter communities, and the natural onset of algal senescence in the more mature summer communities may have brought on the decrease in chlorophyll during the recovery phase. The relative amounts of these phytopigments also decreased in the reference stream in the summer. Thus, the shift in this microbial community attribute may be interpreted as recovery at low levels of copper stress. However, the amounts of chlorophyll a and c in moderately to highly stressed communities in the summer was greatly reduced relative to the reference stream. Thus, recovery of community chlorophylls did not occur over the entire range of copper concentrations in the summer.

On the other hand, community chlorophyll a in the winter increased significantly across all doses of copper, shortly (i.e., two weeks) after the removal of stress. Clearly, the composition of periphyton communities, which is seasonally influenced, and/or seasonal differences in water chemistry affect phytopigment recovery following copper stress. The ratio of ATP to total biomass was extremely variable two weeks

after removal of copper stress, and significant recovery was not detected for this aspect of community structure.

Changes in the slope of the relationships between community structural attributes and copper bioconcentration after two weeks of recovery were only observed in the winter (Table 9). Instead of following the expected pattern for the recovery of steady state communities (Fig. 18), the slopes of the winter relationships for total community biomass (Fig. 8.2) and the ratio of chlorophyll a to biomass (Fig. 10.2) were steeper at the end of the two-week recovery phase than at the end of the exposure phase.

Table 10 lists the concentration of copper in periphyton communities at the end of the seven-day exposure period and in similarly exposed communities after two weeks of recovery. Although communities lost copper during the recovery phase, the communities treated with high concentrations of this toxicant retained significant copper (>1 milligram per gram of total biomass) after two weeks. This retention of copper may have been responsible for the delay in the recovery of total biomass and the chlorophyll a content of communities previously exposed to high concentrations of toxicant. Although both the substrate-dosed periphyton assemblages and the clay tiles of the toxicant-releasing substrates may have retained more copper than communities and tiles in the water column dosed streams (Table 10; Fig. 17), no significant

effects of copper delivery method on community recovery were observed (Table 9). The novel substrates appear to allow the recovery of aquatic microbial assemblages from copper stress to proceed in a manner indistinguishable from the recovery of communities exposed to copper in the water column.

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Table 4 - Relative toxicity of copper to aquatic microorganisms in the laboratory and the elucidated mechanisms of copper toxicity.

Result	Citation
Pseudomonas putida (a prokaryote) is more sensitive than Entosiphon sulcatum (a heterotrophic protozoan), which is more sensitive than Scenedesmus quadricauda (an alga). [Lab growth inhibition tests]	Bringmann and Kühn, 1980
Uptake of Ammonium, nitrate, and phosphate by S. quadricauda is inhibited by copper (e.g., 6.4 μg/L Cu caused a 50% inhibition of ammonium uptake at pH=7). Inhibition was reduced at lower pH (perhaps via competition between the free metal and H^+). [Lab chemostat studies]	Peterson and Healy, 1985
The mechanisms of ionic copper toxicity to algae include: 1) suppression of mitosis via rxn with glutathione; 2) inhibition of catalase activity; 3) inhibition of photosynthesis; and 4) rxn with -SH. Toxicity of copper to algae is ameliorated by trivalent metal cations. [Lab investigations using two marine diatoms and Chlorella pyrenoidosa]	Stauber and Florence, 1987
Cu^{++} induces leakage of K^+ from Pseudomonas syringae cells and some algae (discussed).	Cabral, 1989
35 - 45 mg/L Cu caused a 50% reduction in respiration of Aeromonas hydrophila. [Lab oxygen consumption experiments]	Flemming and Trevors, 1989
Nuisance, planktonic bluegreen algae were found to be much more sensitive to copper (EC_{100} = 16 - 95 μg/L Cu) than planktonic diatoms (EC_{100} = 250 μg/L Cu) and S. quadricauda (EC_{100} = 3200 μg/L Cu). [Lab growth inhibition experiments]	Lüdernitz and Nicklisch, 1989

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Table 4, continued

Result	Citation
Copper inhibits photosystem II. [Data presented from lab experiments with Pisum sativum and scientific literature is reviewed]	Renganathan and Bose, 1990
Copper degrades chlorophylls a and b; chlorophyll a is more resistant to photo-oxidation and/or other degradation processes than chlorophyll b. [Lab manipulation of naturally derived Lemna]	Robinson and Choi, 1989
Copper inhibits photosystem I. [Literature review]	Droppa and Horváth, 1990

Table 5 - Effects of copper on laboratory mesocosm assemblages and natural, aquatic microbial communities.

Result	Citation
Scenedesums acutiformis and Chlorella fusca isolated from Ontario lakes contaminated with copper and nickel were found to be tolerant of these metals. [Lab growth impairment experiments]	Stokes et al., 1973
A mesotrophic lake in New York responded to low levels of copper addition (max. 5 µg/L) with: 1) an increase in colonial, coccoid bluegreen algae; 2) a decrease in Anabaena flos-aquae (possibly due to impairment of nitrogen fixation); and 3) a decrease in heterotrophic bacteria (with a rapid recovery).	Effler et al., 1980
Resistant Chlamydomonas, Chlorella, and Scenedesmus spp. were isolated from a third order stream. These algae were more resistant to copper in the summer than in the winter; copper bioconcentration was positively correlated with temperature. [Field and laboratory manipulations with field-isolated algae]	Klotz, 1981
In lentic microcosms in the laboratory, treatment with 9.3 µg/L Cu resulted in decreases in primary production, DOC production, and macroalgal growth; microcosms containing 30 µg/L Cu shifted from predominantly autotrophic to heterotrophic systems.	Hedtke, 1984
An oligotrophic, Sierra Nevada stream was continuously dosed with copper for one year; densities of Lyngbya spp. were reduced at 2.5 µg/L Cu; Spirogyra and Cladophora spp. were reduced at 5 µg/L Cu; Achnanthes minutissima and Calothrix spp. were more abundant at 10 µg/L Cu than in the control reach.	Leland and Carter, 1984
2.5 mg/L Cu caused a 50% reduction in respiration of naturally-derived, freshwater sediment. [Lab oxygen consumption experiments]	Flemming and Trevors, 1989

Table 6 - Physicochemical attributes of the diluent water (carbon dechlorinated tap water) used in the summer and winter artificial stream experiments. Differences between summer and winter means were assessed using two sample t-tests (see text), and significant differences at the 0.05, 0.001, and the 0.0001 alpha probability levels are indicated with *, **, and ***, respectively.

Attribute	Summer Mean (StdErr)	Winter Mean (StdErr)
Temperature (°C)	20.8 (0.0810) *** n=31	8.34 (0.214) *** n=16
pH	7.35 (0.0153) * n=16	7.48 (0.0523) * n=10
Hardness (mg/L) as $CaCO_3$	49.1 (0.776) *** n=11	38.0 (0.842) *** n=4
Alkalinity (mg/L) as $CaCO_3$	38.6 (0.814) ** n=11	30.7 (1.98) ** n=4
Conductivity (μS)	114 (0.865) *** n=19	78.6 (1.12) *** n=8
PO_4^{3-} (μg/L)	88.5 (29.8) n=4	166 (11.6) n=4
NO_3^- (mg/L)	1.90 (0.202) n=4	3.09 (0.655) n=4
NO_2^- (μg/L)	b.d. (< 10) n=4	___
NH_4^+ (μg/L)	b.d. (< 10) n=4	___
SO_4^{2-} (mg/L)	6.20 (1.54) * n=4	22.0 (4.85) * n=4
SiO_2 (mg/L)	6.02 (0.325) n=2	7.05 (0.55) n=2
Cl^- (mg/L)	1.61 (1.29) n=2	4.13 (1.19) n=4
Fl^- (mg/L)	1.37 (0.416) n=2	0.651 (0.202) n=4

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Table 6, continued

Attribute	Summer Mean (StdErr)	Winter Mean (StdErr)
Free Residual OCl^- (mg/L)	b.d. (< 0.01) n=19	b.d. (< 0.01) n=12
Comb. Residual OCl^- (mg/L)	< 0.010 *** n=19	0.020 (0.001) *** n=12

Table 7 - Statistical results of simple linear regressions of the listed response variables on the explanatory variable (log_{10} Cu bioconcentration) using pooled data within each season (n=9). Significant regression models at the 0.05, 0.01, and 0.0001 alpha probability levels are indicated with *, **, and ***, respectively.

SUMMER EXPERIMENT

Response Variable	Parameter Estimates Slope	Intercept	R^2	p-value
*AFDW(mg/cm^2)	-0.22	+1.27	0.530	0.0261 *
log_{10}ATP(mg/gAFDW)	-0.50	-0.63	0.426	0.0568
**Chla(mg/gAFDW)	-6.8	+24	0.642	0.0095 **
*Chlb(mg/gAFDW)	+1.3	+0.34	0.572	0.0184 *
*Chlc(mg/gAFDW)	-0.98	+4.2	0.471	0.0412 *
*log_{10}Bacterial Biomass(μg/gAFDW)	+0.14	+1.8	0.500	0.0333 *
*log_{10}Respiration (mgO_2/gAFDW/hr)	-0.62	+0.42	0.573	0.0182 *
**log_{10}Gross 1° Productivity (mgO_2/gAFDW/hr)	-0.52	+0.75	0.748	0.0026 **
*log_{10}Ammonium Uptake ($mgNH_4^+$/gAFDW/hr)	-0.18	-0.30	0.552	0.0217 *

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Table 7, continued

WINTER EXPERIMENT

Response Variable	Parameter Estimates Slope	Intercept	R^2	p-value	
*AFDW(mg/cm^2)	-0.12	+0.49	0.471	0.0413	*
**log_{10}ATP(mg/gAFDW)	-0.68	-0.97	0.674	0.0067	**
*Chla(mg/gAFDW)	-2.4	+7.0	0.614	0.0125	*
***Chlb(mg/gAFDW)	+0.88	+0.55	0.904	<0.0001	***
Chlc(mg/gAFDW)	+0.072	+0.73	0.0412	0.6006	
log_{10}Bacterial Biomass(μg/gAFDW)	+0.24	+2.3	0.4431	0.0503	
log_{10}Respiration (mgO_2/gAFDW/hr)	-0.20	+0.54	0.112	0.3798	
log_{10}Gross 1° Productivity (mgO_2/gAFDW/hr)	-0.35	+0.75	0.336	0.1020	
log_{10}Ammonium Uptake ($mgNH_4^+$/gAFDW/hr)	-0.30	+0.24	0.237	0.1840	

Table 8 - Results of the analyses of covariance using models to assess differences in the responses of periphyton communities to copper stress across method of copper delivery and in the different seasons. METHOD and SEASON are nominal variables which describe two states each. LOG_{10} Cu BIOCONCENTRATION is an interval variable equivalent to the standard logarithm of the copper bioconcentration measured in mg Cu/g AFDW. Significance at the 0.05, 0.01, and 0.0001 alpha probability levels are indicated with *, **, and ***, respectively. See text for further explanation.

MODEL: Y = METHOD SEASON LOG_{10}BIOC METHOD*LOG_{10}BIOC SEASON*LOG_{10}BIOC METHOD*SEASON*LOG_{10}BIOC

	Overall Model	p-values for Model Terms		
Response	p-value	METH*logBIOC	SEAS*logBIOC	METH*SEAS*logBIOC
AFDW (mg/cm^2)	<0.0001***	0.5833	0.0489*	0.1753
log_{10} ATP (mg/gAFDW)	0.1064	0.2897	0.9597	0.8955
Chl a (mg/gAFDW)	0.0005**	0.0812	0.0776	0.9633
Chl b (mg/gAFDW)	0.0412*	0.7386	0.2792	0.2176
Chl c (mg/gAFDW)	0.0096**	0.3425	0.2190	0.7327
Bacteria (µg/gAFDW)	0.0312*	0.9123	0.8088	0.9844
log_{10} Respiration (mgO_2/gAFDW/hr)	0.0302*	0.9249	0.0617	0.0217*
log_{10}Gross 1° Productivity (mgO_2/gAFDW/hr)	0.0236*	0.5306	0.1473	0.0514
log_{10}Ammonium Uptake ($mgNH_4^+$/gAFDW/hr)	0.2795	0.3617	0.5133	0.6488

Table 9 - Results of a repeated measures analysis, the purpose of which was to determine those variables which significantly recovered two weeks after termination of copper stress and to discover any interactions between recovery and method of copper delivery. Phase (either "exposure" or "recovery") was a within-stream factor. The $\log_{10}$ of copper bioconcentration, method type (either "water column" or "chemical diffuser"), and the interaction between bioconcentration and method were between-stream factors. Significant effects at the 0.05, 0.01, and 0.005 alpha probability levels are indicated with *, **, and ***, respectively.

STATISTICAL MODEL FOR USE WITH SAS SOFTWARE (SAS Inst. Inc., 1989):

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Y1 Y2 = METHOD  LOG10BIOCONC  METHOD*LOG10BIOCONC;
  REPEATED PHASE;
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SUMMER EXPERIMENT

	p-values for Model Terms		
RESPONSE	PHASE	PHASE*$\log_{10}$BIOC	PHASE*$\log_{10}$BIOC*METHOD
AFDW (mg/cm^2)	0.0099 **	0.1403	0.6543
$\log_{10}$ATP (mg/gAFDW)	0.3184	0.3642	0.2444
Chl a (mg/gAFDW)	0.0047 ***	0.0940	0.6361
Chl b (mg/gAFDW)	0.7693	0.3637	0.2079
Chl c (mg/gAFDW)	0.0139 *	0.3184	0.2815
$\log_{10}$Bacteria (μg/gAFDW)	0.0050 ***	0.0647	0.3926

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Table 9, continued

WINTER EXPERIMENT

	p-values for Model Terms		
RESPONSE	PHASE	PHASE*LOG_{10}BIOC	PHASE*LOG_{10}BIOC*METHOD
AFDW (mg/cm^2)	0.0021 ***	0.0151 *	0.7562
log_{10}ATP (mg/gAFDW)	0.7917	0.2992	0.4517
Chl a (mg/gAFDW)	0.0034 ***	0.0236 *	0.4682
Chl b (mg/gAFDW)	0.2244	0.8071	0.3290
Chl c (mg/gAFDW)	0.0548	0.1279	0.7453
log_{10}Bacteria (μg/gAFDW)	0.0242 *	0.1898	0.5357

Table 10 - Copper bioconcentration by laboratory periphyton communities during summer and winter toxicity tests. Data are reported for the bioconcentration after seven days of exposure to copper and the bioconcentration in similarly exposed communities after two weeks of recovery. "WC" indicates the average copper concentration delivered to the communities through the water column, and "CD" indicates the initial copper concentration placed in chemical-diffusing substrates.

SUMMER

	Cu Bioconcentration (mg Cu/g AFDW)	
Treatment	Just After Exposure	After Two Weeks of Recovery
Reference stream	0.150	0.0802
WC 7.2 µgCu/L	0.694	0.170
WC 20.3 µgCu/L	0.994	0.152
WC 69.1 µgCu/L	4.89	0.480
WC 211.2 µgCu/L	14.2	1.05
CD 0.50 gCu/L	0.907	0.825
CD 1.0 gCu/L	5.51	1.35
CD 5.0 gCu/L	20.0	15.8
CD 10 gCu/L	251	160

WINTER

	Cu Bioconcentration (mg Cu/g AFDW)	
Treatment	Just After Exposure	After Two Weeks of Recovery
Reference stream	0.241	0.267
WC 3.6 µgCu/L	0.481	0.296
WC 14.6 µgCu/L	1.64	0.451
WC 66.1 µgCu/L	9.44	0.833
WC 641.8 µgCu/L	227	7.56
CD 0.25 gCu/L	0.309	0.464
CD 0.5 gCu/L	2.36	1.23
CD 2.0 gCu/L	9.71	5.80
CD 4.0 gCu/L	36.5	10.8

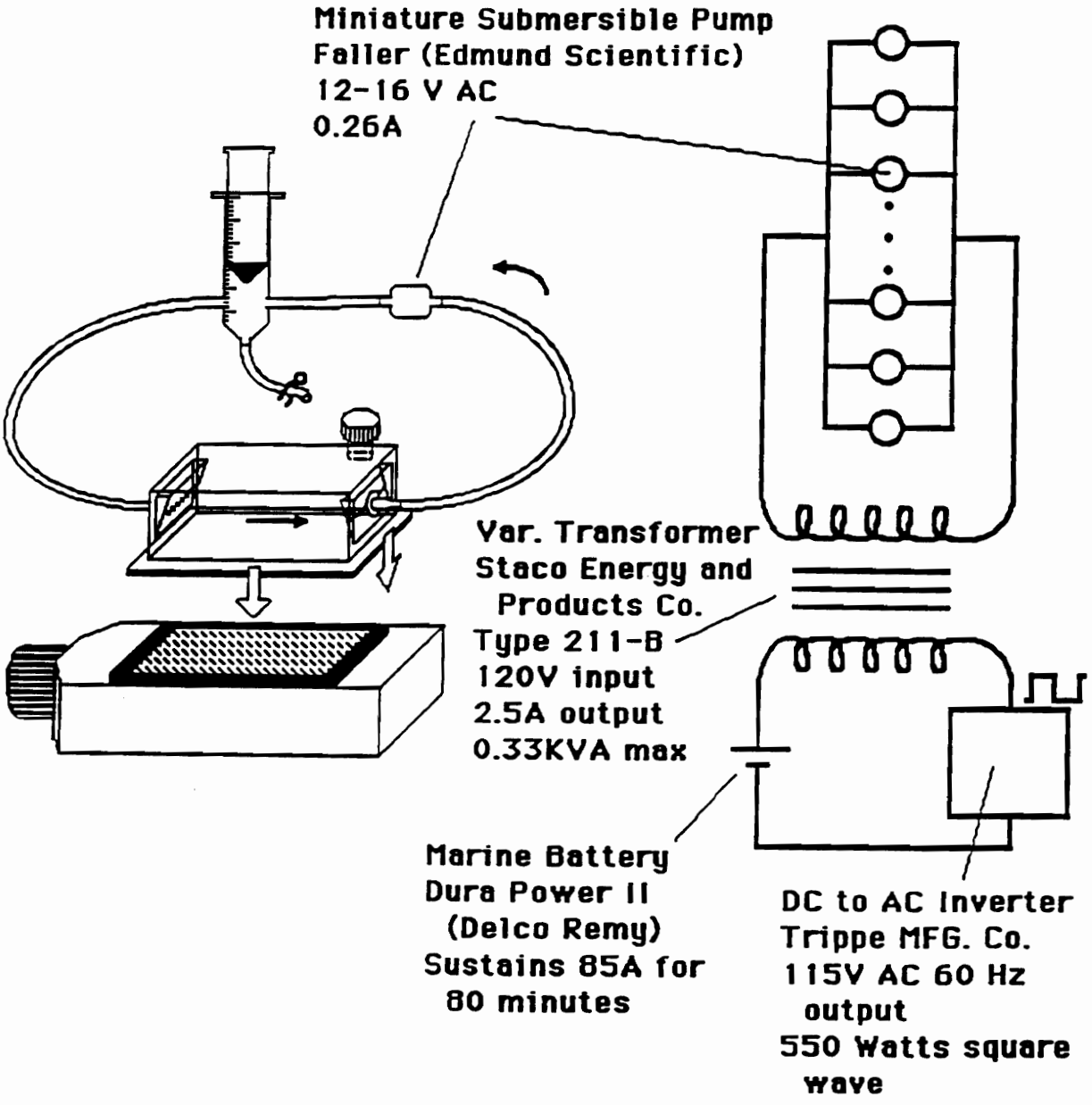


Figure 1. Illustrations of a chemical-releasing substrate (left, below), a recirculating chamber required for the measurement of community functional attributes (left, above), and the circuit diagram for the portable power supply required by the recirculating chambers (right).

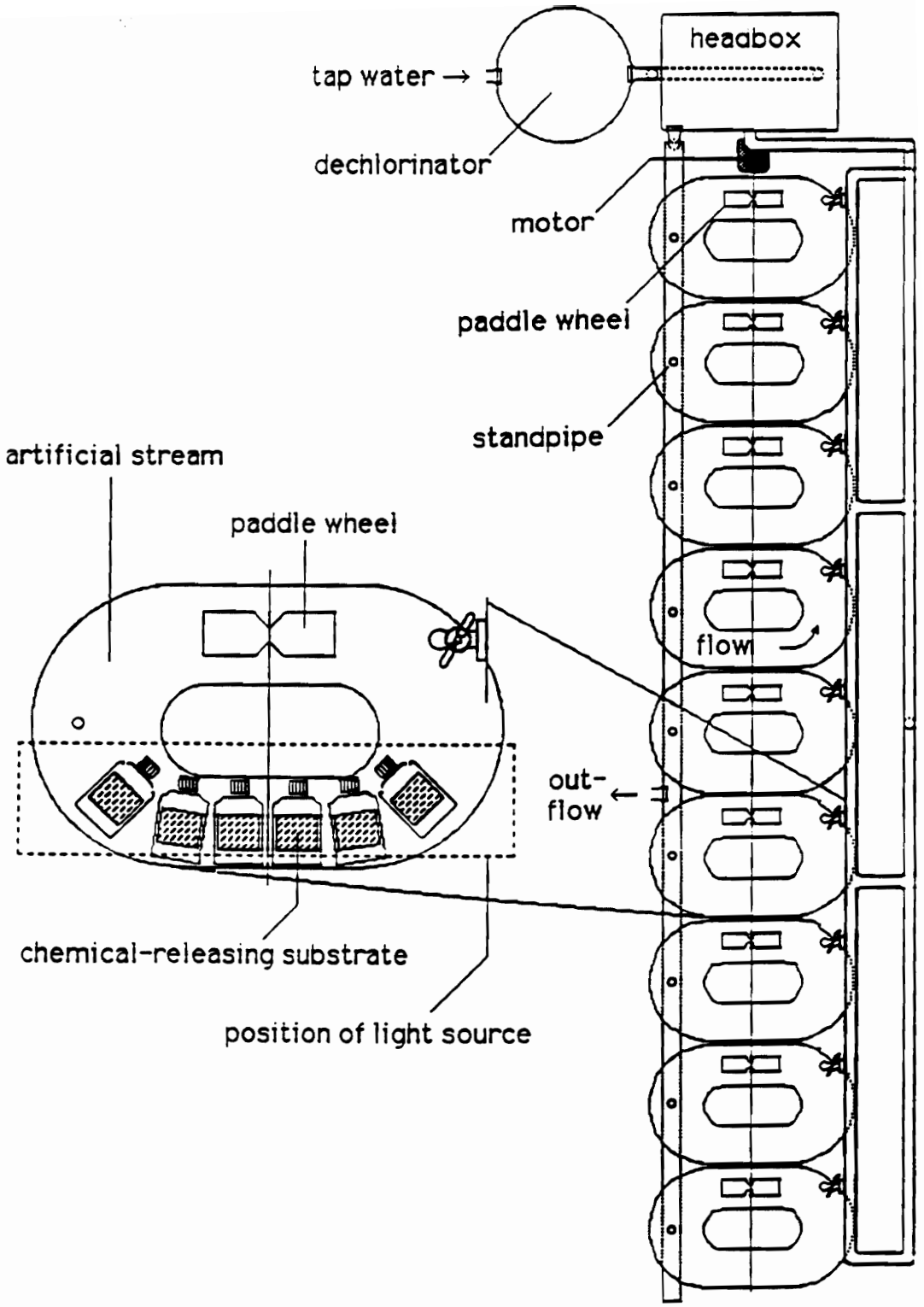
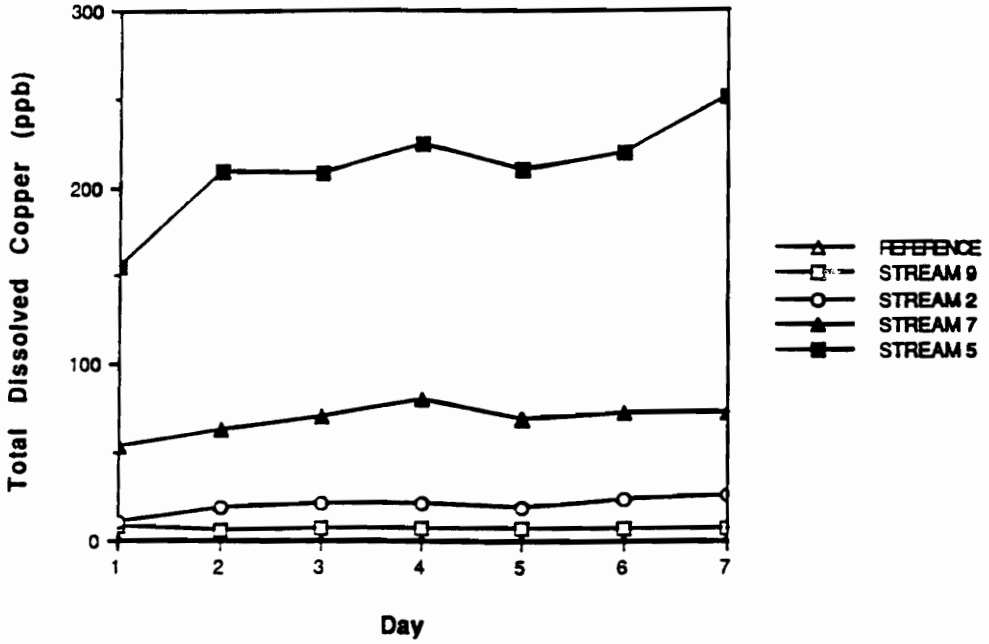


Figure 2. Diagram of the nine channel, flow-through, artificial stream system (top view).

EXPOSURE PHASE WATER COLUMN COPPER CONCENTRATIONS

3.1 WATER COLUMN DOSED STREAMS - SUMMER



3.2 CHEMICAL DIFFUSER DOSED STREAMS - SUMMER

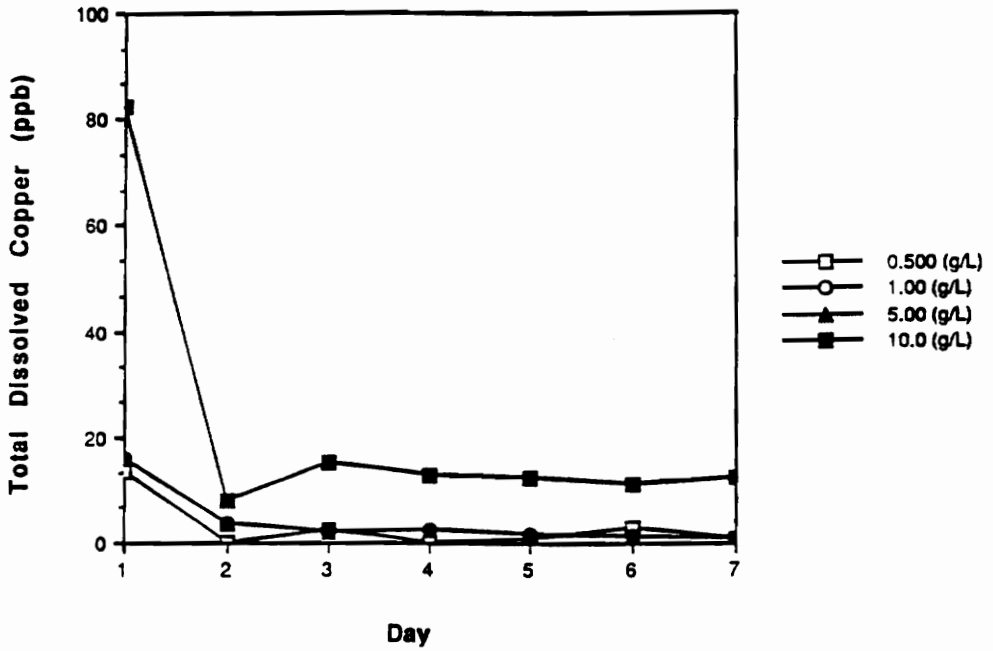
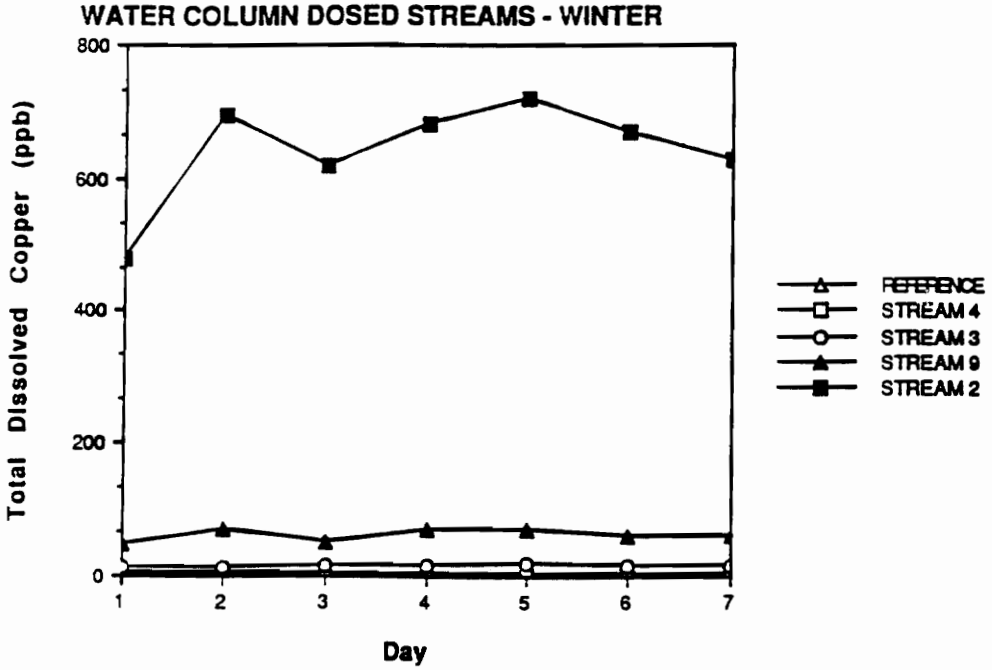


Figure 3. In stream, water column concentrations of copper during the seven-day exposure phase of the summer experiment for streams dosed through the water column (3.1) and via the chemical-releasing substrates (3.2). Each point represents the copper concentration measured for a single water sample.

EXPOSURE PHASE WATER COLUMN COPPER CONCENTRATIONS

4.1



4.2

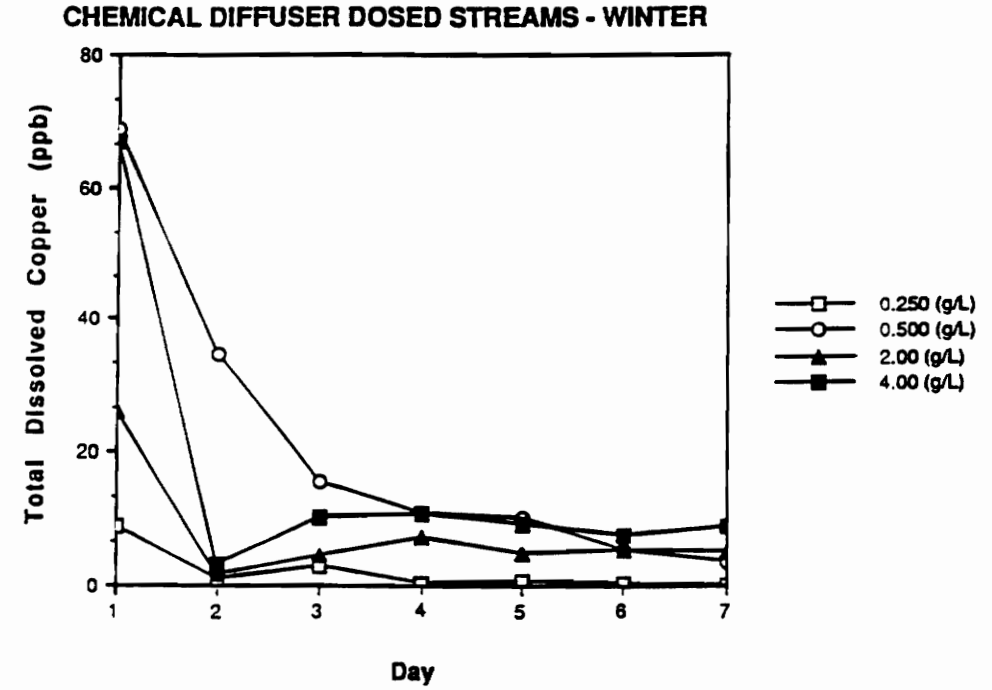


Figure 4. In stream, water column concentrations of copper during the seven-day exposure phase of the winter experiment for streams dosed through the water column (4.1) and via the chemical-releasing substrates (4.2). Each point represents the copper concentration measured for a single water sample.

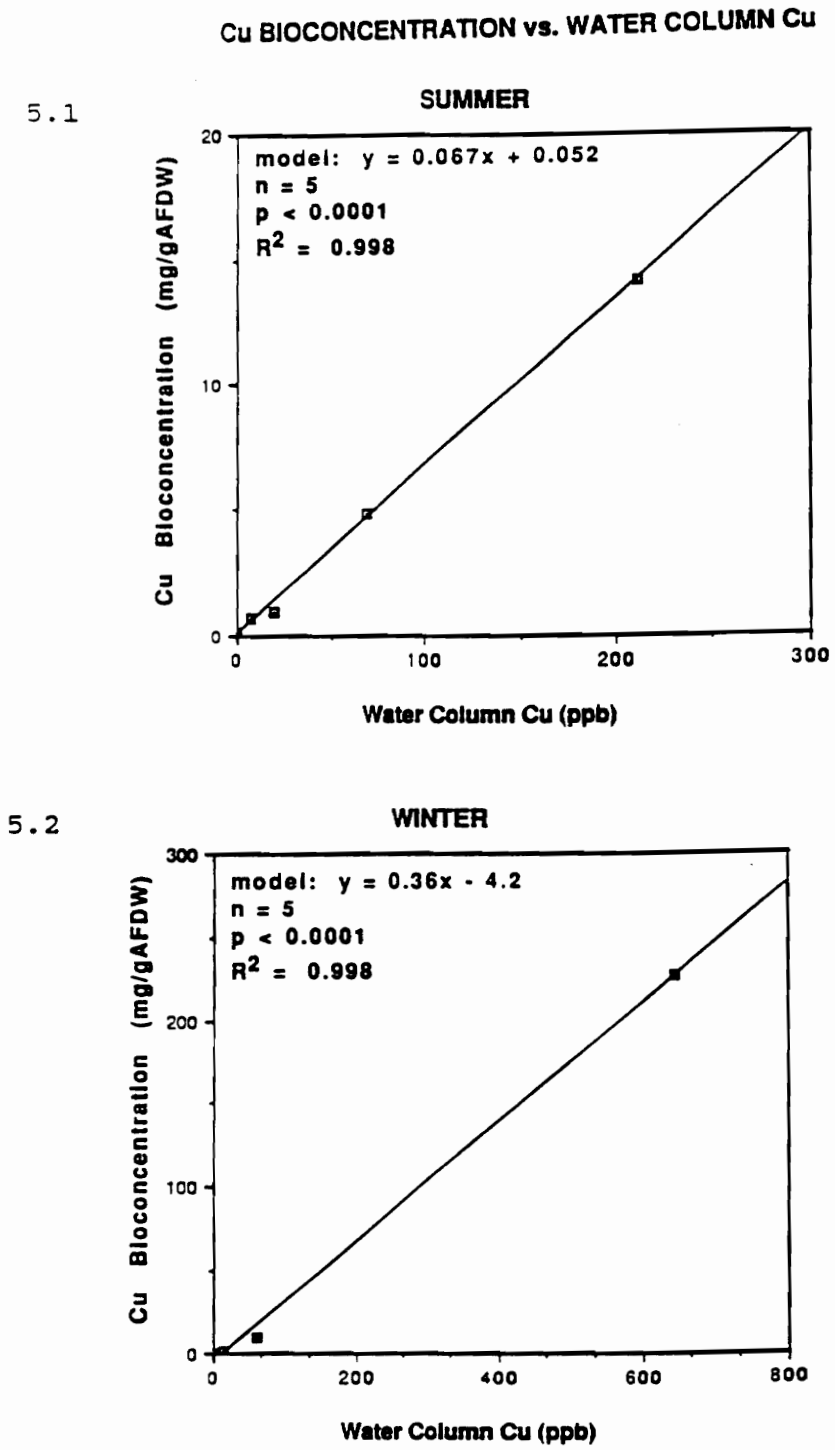


Figure 5. Summer (5.1) and winter (5.2) periphyton bioconcentration of copper in streams dosed through the water column. The scales are different in order to best display the positions of the individual data points. Note that the slope of the winter model is greater than the slope of the summer model. This difference is significant ($p<0.0001$), but the single datum at the highest water column copper concentration has a strong influence on the comparison of slopes. See text for further discussion.

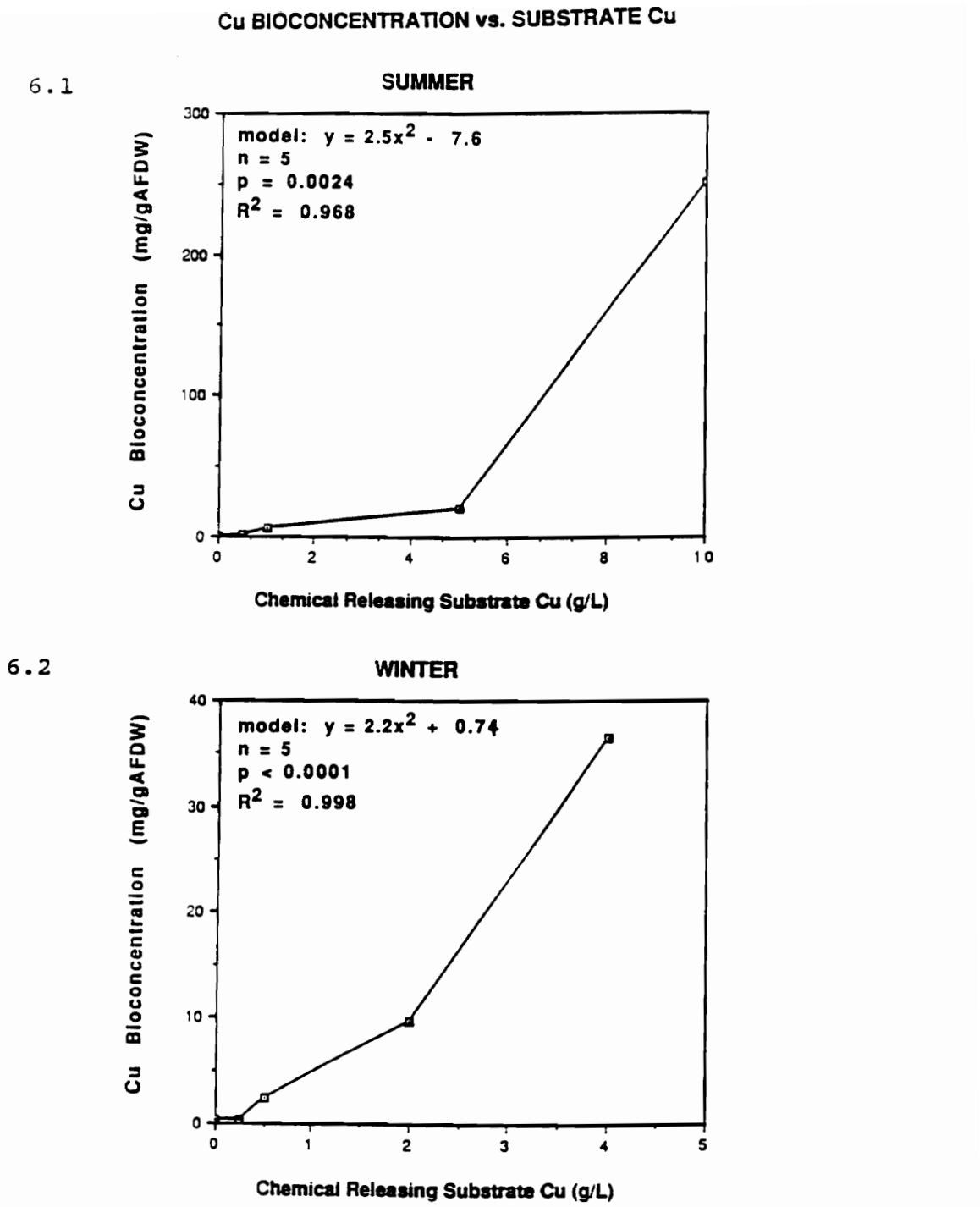


Figure 6. Summer (6.1) and winter (6.2) periphyton bioconcentration of copper in streams dosed using toxicant-releasing substrates. The scales are different in order to best display the positions of the individual data points. The quadratic terms for the two models are not significantly different from one another (p=0.830). See text for further discussion.

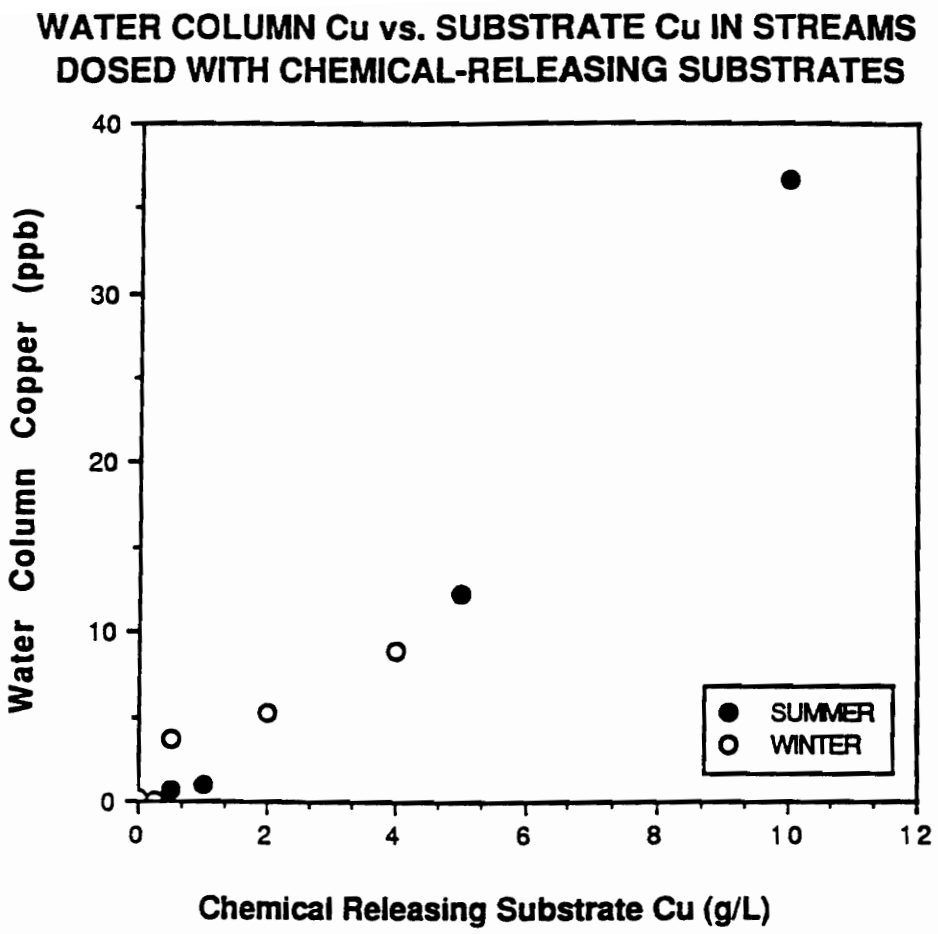


Figure 7. Resulting in-stream copper concentration as a function of the concentration of copper placed in the chemical-releasing substrates during the summer (n=5) and winter experiments (n=5).

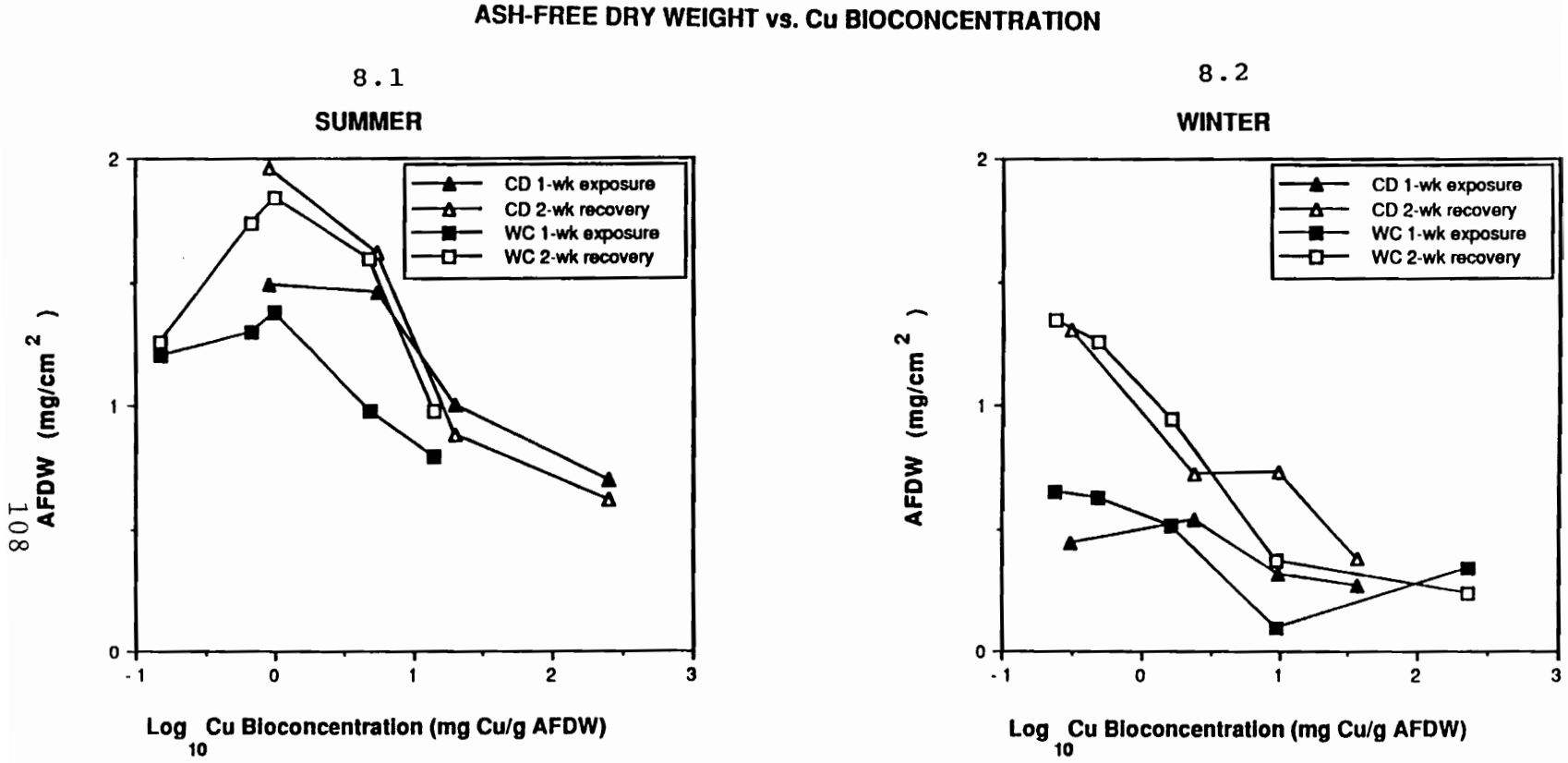


Figure 8. Summer (8.1) and winter (8.2) areal ash-free dry weight as a function of the $\log_{10}$ of copper bioconcentration. In the legend, CD refers to "chemical diffuser" dosed streams (triangles), and WC refers to "water column" dosed streams (squares) during the seven day exposure phase (dark points) and after two weeks of recovery (open points). Each point represents a single, independent measurement and not the mean of several measurement values.

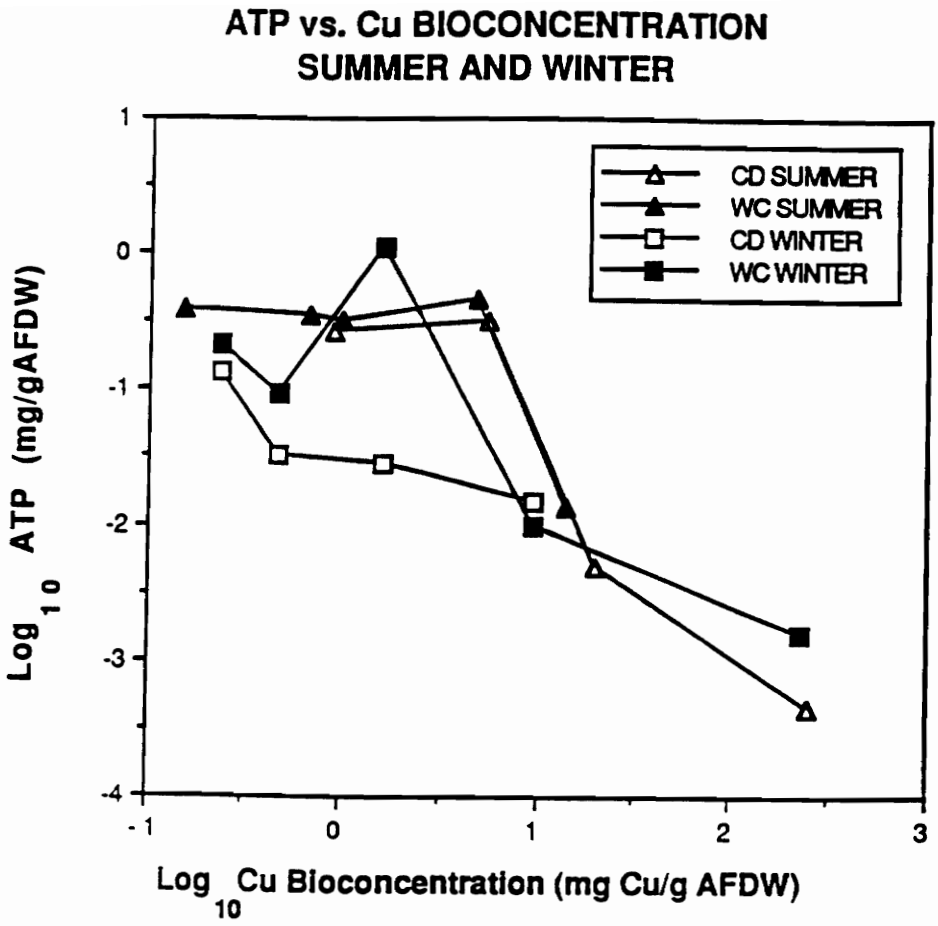


Figure 9. Log_{10}-transformed ATP as a function of the log_{10} of copper bioconcentration. In the legend, CD refers to "chemical diffuser" dosed streams (open points), and WC refers to "water column" dosed streams (dark points), during the summer (triangles) and winter (squares) experiments. Each point represents a single, independent measurement and not the mean of several measurement values.

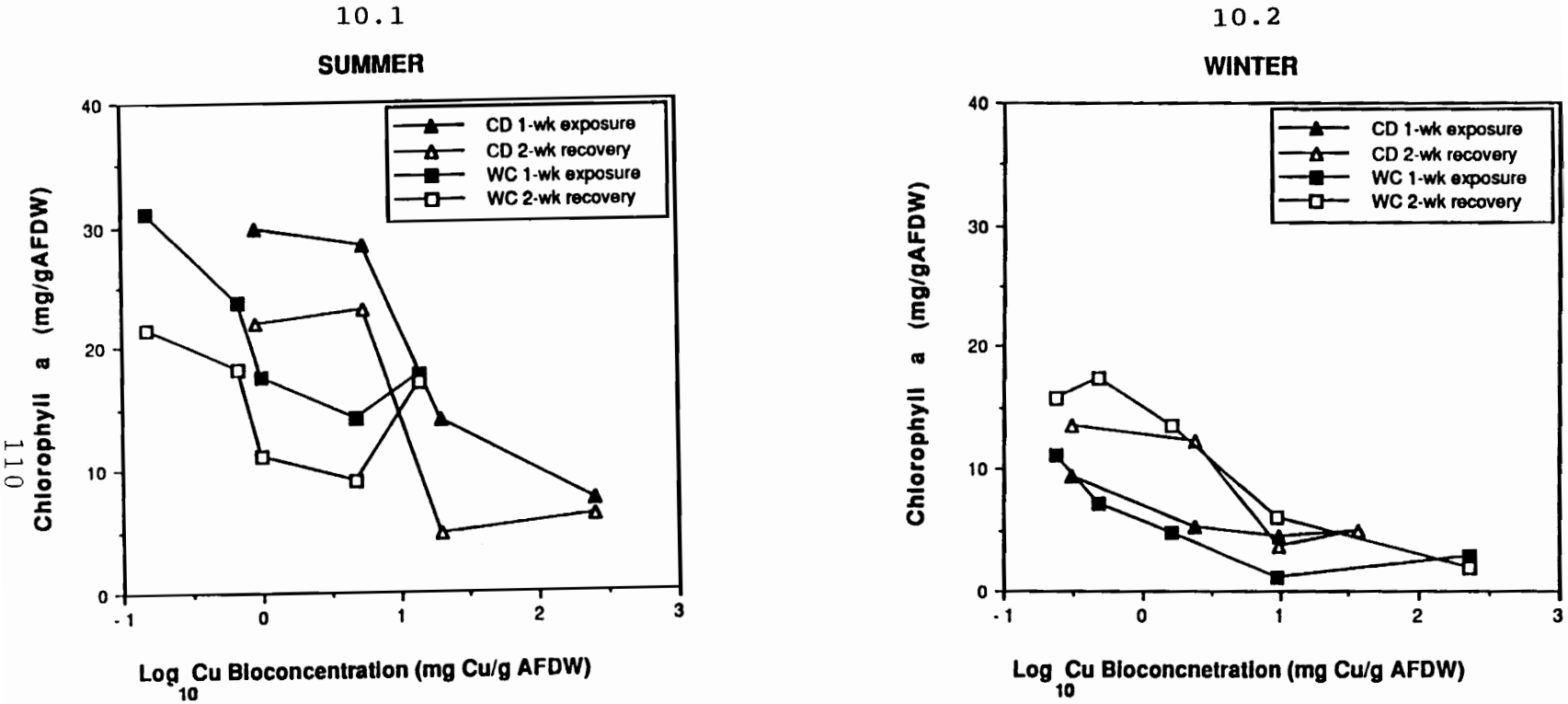


Figure 10. Summer (10.1) and winter (10.2) community chlorophyll a content as a function of the $\log_{10}$ of copper bioconcentration. In the legend, CD refers to "chemical diffuser" dosed streams (triangles), and WC refers to "water column" dosed streams (squares), during the seven day exposure phase (dark points) and after two weeks of recovery (open points). Each point represents a single, independent measurement and not the mean of several measurement values.

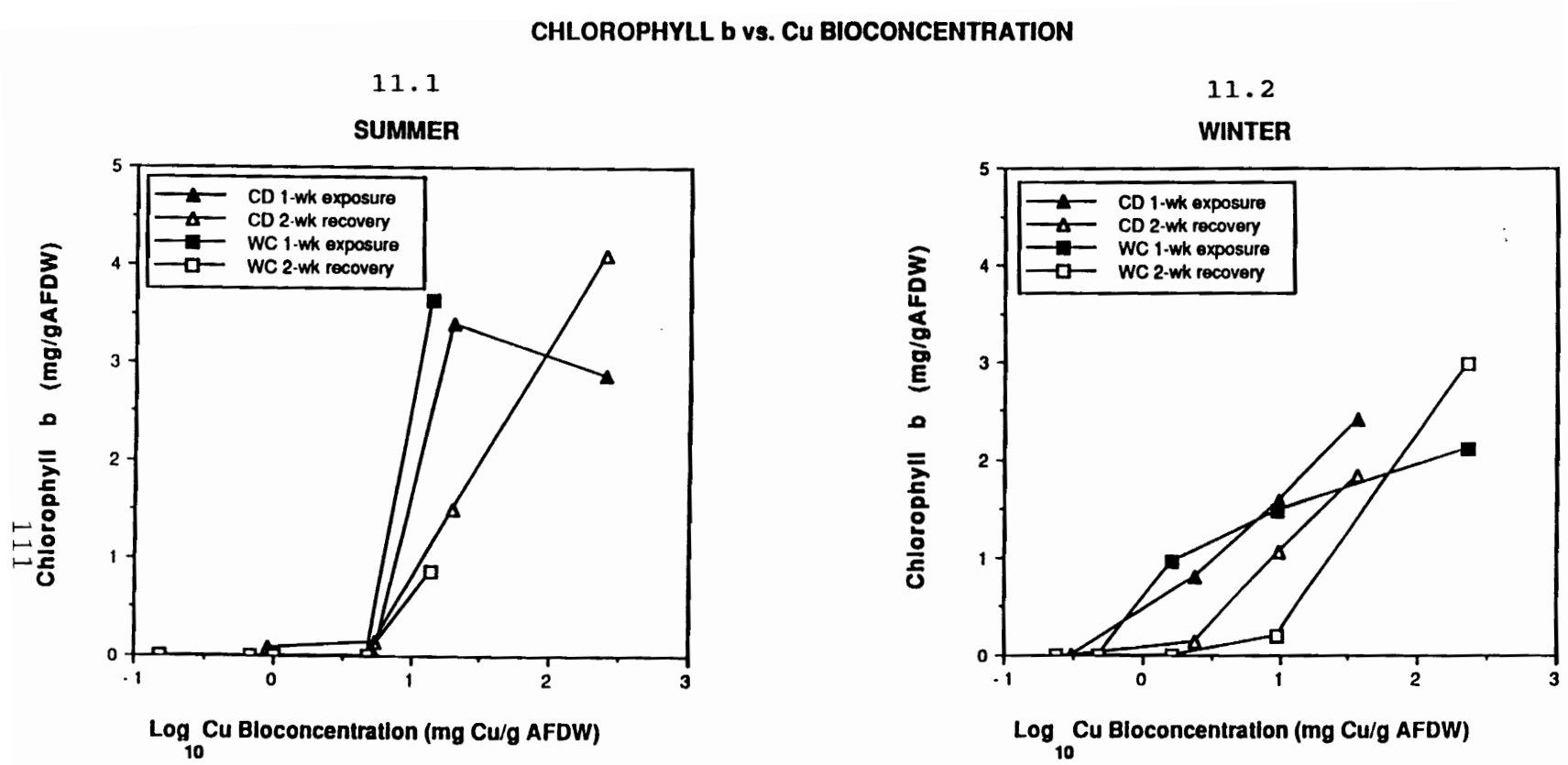


Figure 11. Summer (11.1) and winter (11.2) community chlorophyll b content as a function of the $\log_{10}$ of copper bioconcentration. In the legend, CD refers to "chemical diffuser" dosed streams (triangles), and WC refers to "water column" dosed streams (squares), during the seven day exposure phase (dark points) and after two weeks of recovery (open points). Each point represents a single, independent measurement and not the mean of several measurement values.

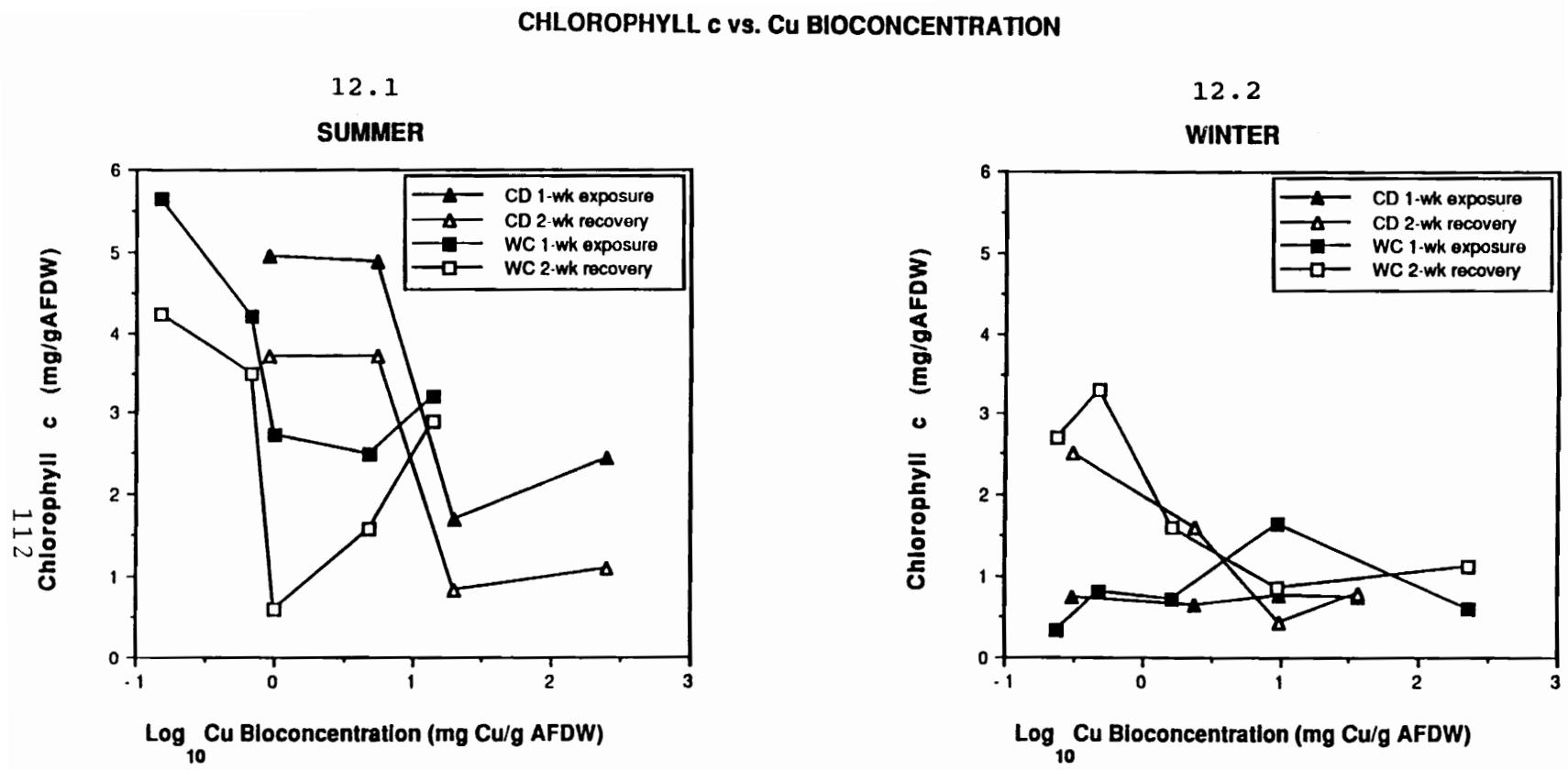


Figure 12. Summer (12.1) and winter (12.2) community chlorophyll c content as a function of the $\log_{10}$ of copper bioconcentration. In the legend, CD refers to "chemical diffuser" dosed streams (triangles), and WC refers to "water column" dosed streams (squares), during the seven day exposure phase (dark points) and after two weeks of recovery (open points). Each point represents a single, independent measurement and not the mean of several measurement values.

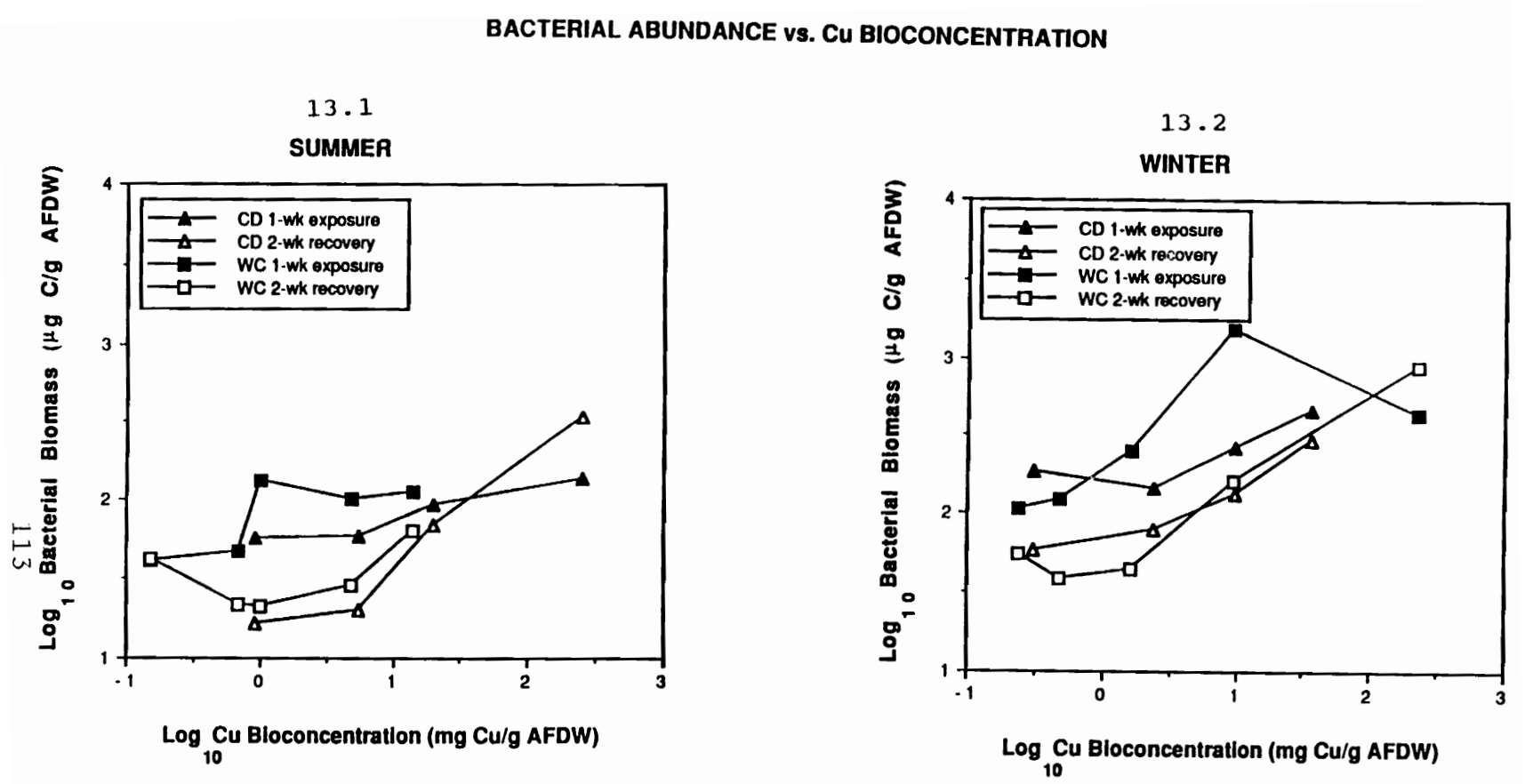


Figure 13. Summer (13.1) and winter (13.2) $\log_{10}$-transformed bacterial biomass as a function of the $\log_{10}$ of copper bioconcentration. In the legend, CD refers to "chemical diffuser" dosed streams (triangles), and WC refers to "water column" dosed streams (squares), during the seven day exposure phase (dark points) and after two weeks of recovery (open points). Each point represents a single, independent measurement and not the mean of several measurement values.

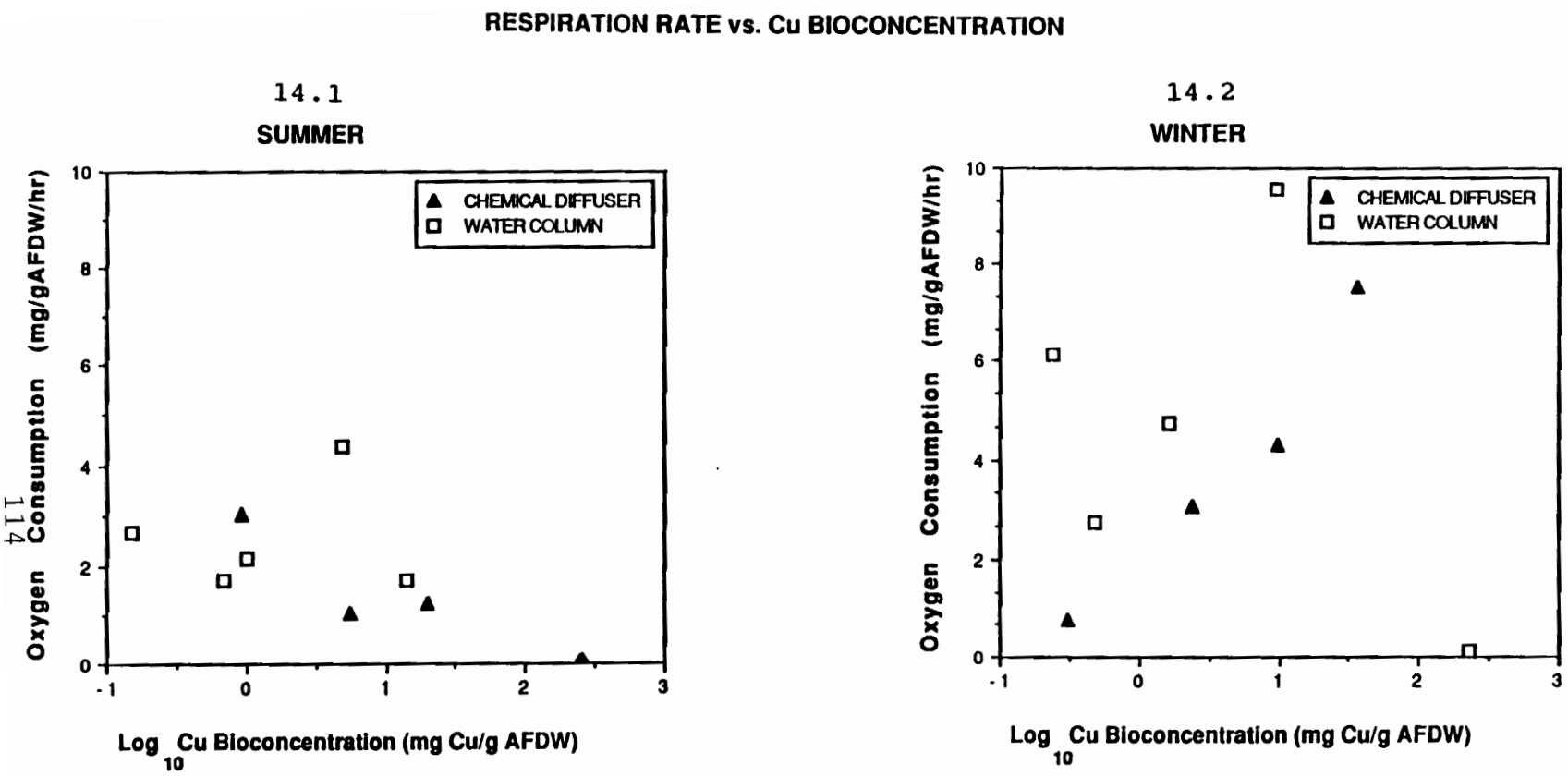


Figure 14. Summer (14.1) and winter (14.2) community oxygen consumption as a function of the $\log_{10}$ of copper bioconcentration for communities dosed using "chemical diffusers" (dark triangles) or by dripping solutions of copper into the "water columns" of the artificial streams (open squares). Each point represents a single, independent measurement and not the mean of several measurement values.

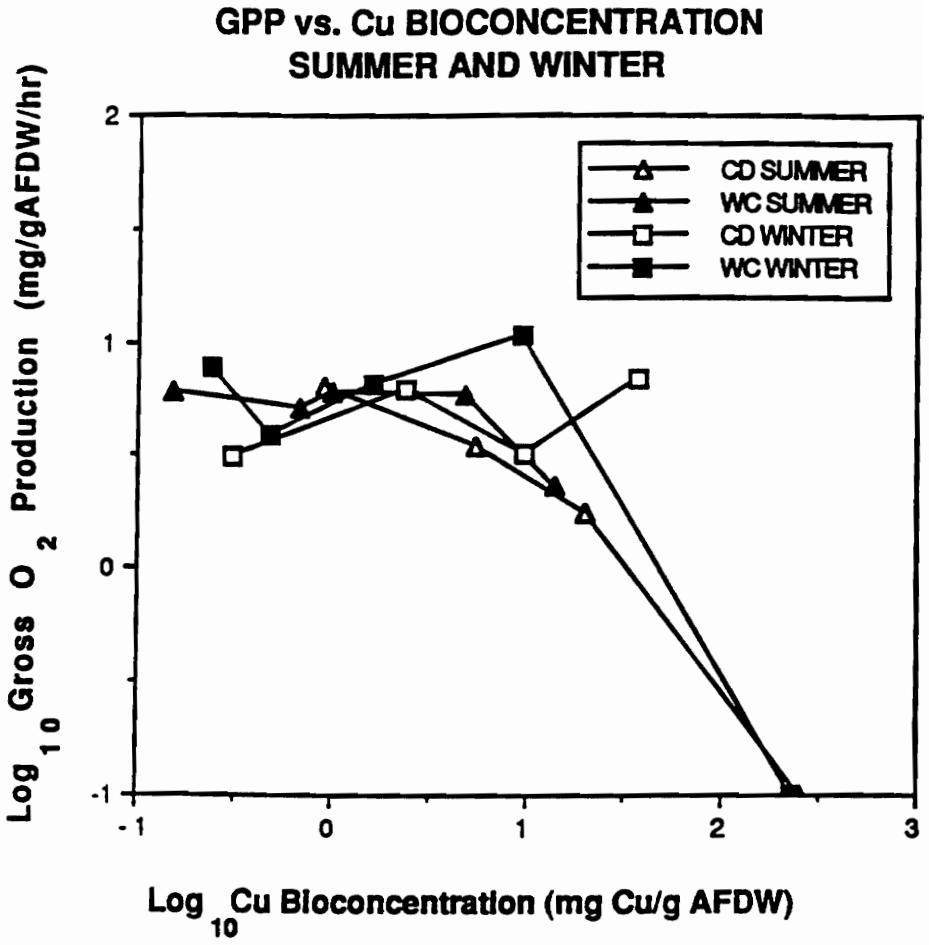


Figure 15. Log_{10}-transformed gross oxygen production as a function of the log_{10} of copper bioconcentration. In the legend, CD refers to "chemical diffuser" dosed streams (open points), and WC refers to "water column" dosed streams (dark points), during the summer (triangles) and winter (squares) experiments. Each point represents a single, independent measurement and not the mean of several measurement values.

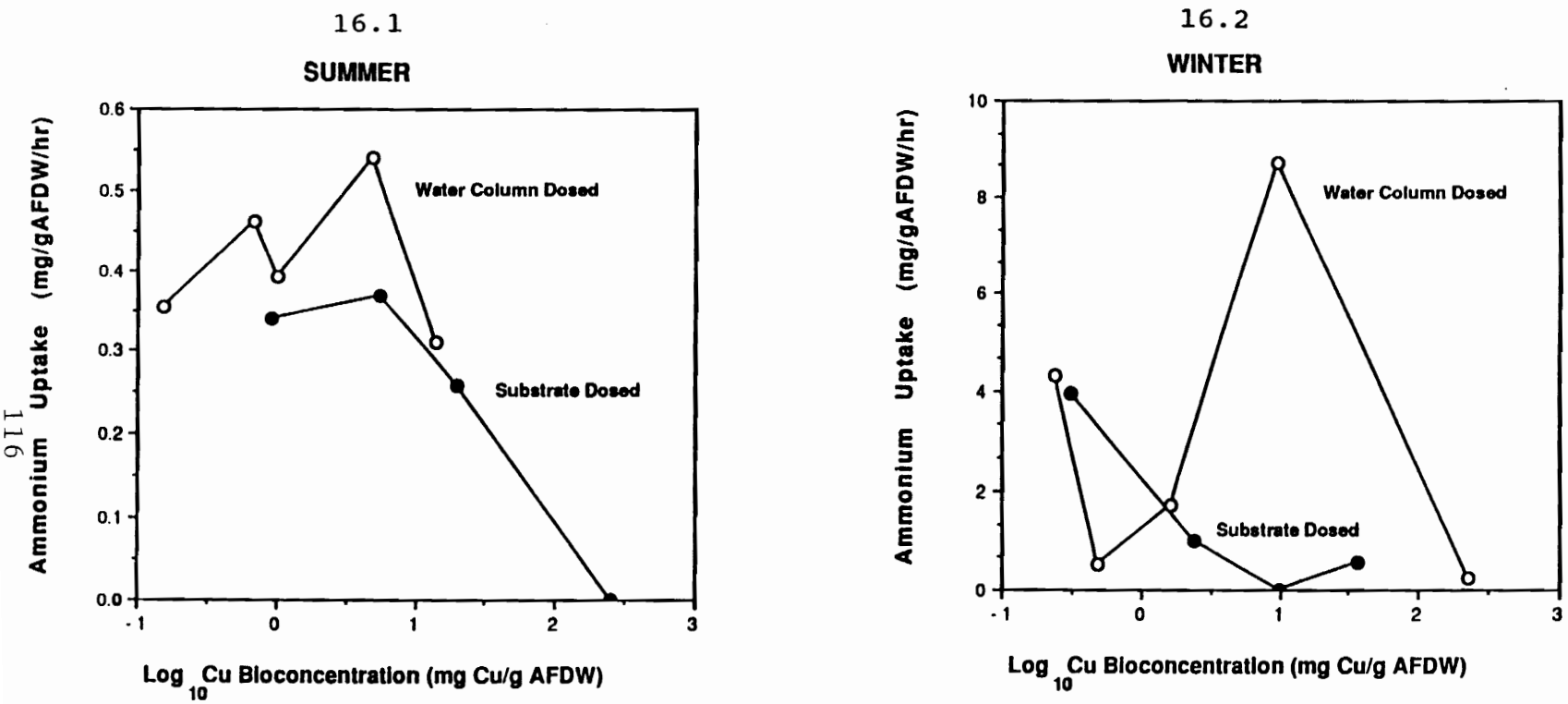


Figure 16. Summer (16.1) and winter (16.2) ammonium uptake by periphyton communities as a function of the $\log_{10}$ of copper bioconcentration. Open circles indicate data collected for streams dosed through the water column, and closed circles indicate data collected for streams dosed via the toxicant-releasing substrates. Each point represents a single, independent measurement and not the mean of several measurement values.

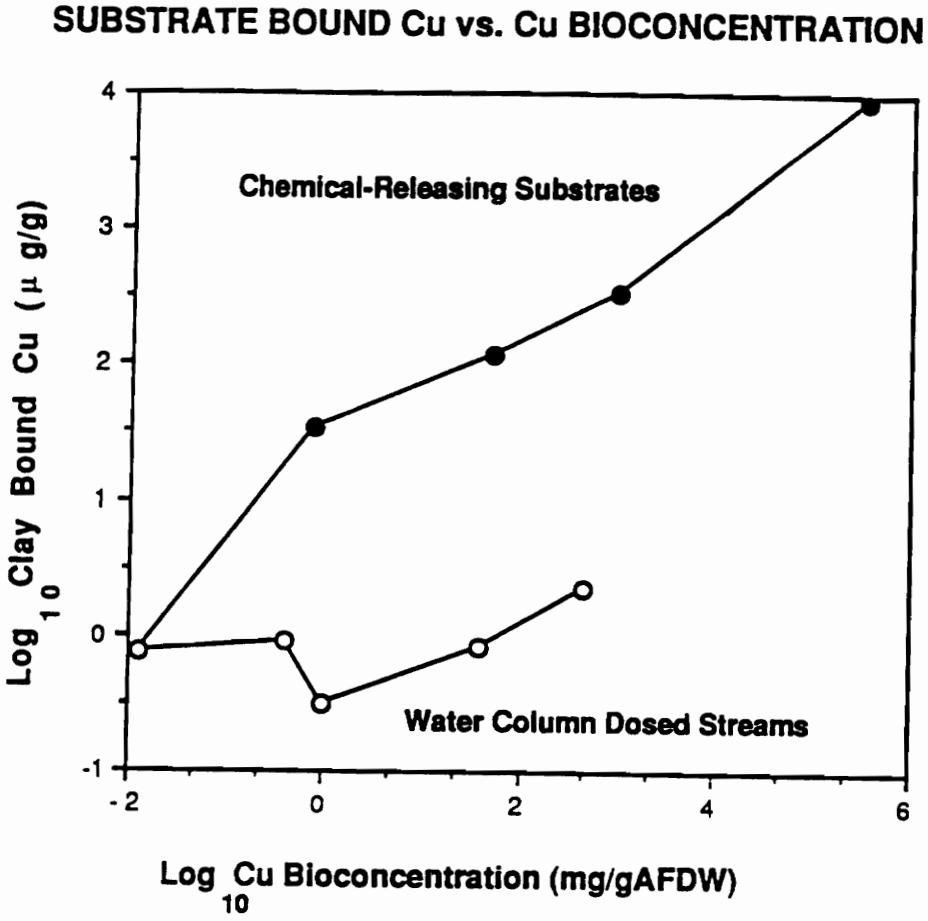
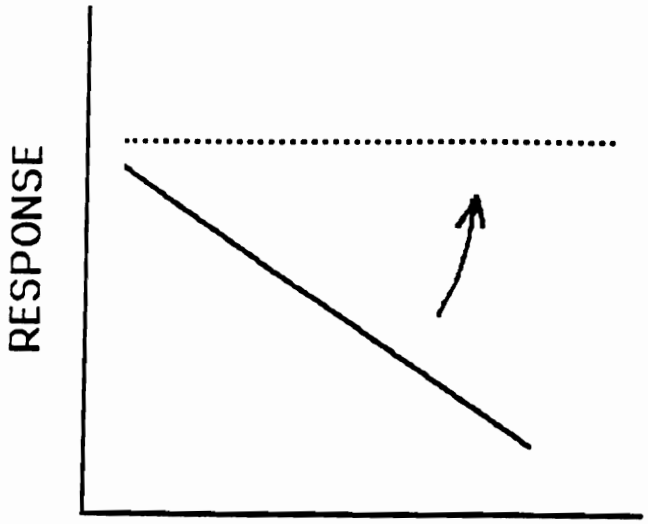


Figure 17. Abiotic binding of copper to the terra-cotta clay tiles as a function of periphyton stress intensity in streams dosed through the water column (open circles) and in streams dosed via the chemical-releasing substrates (dark circles) during the summer experiment.

18.1 Steady State Communities



18.2 Growing Communities

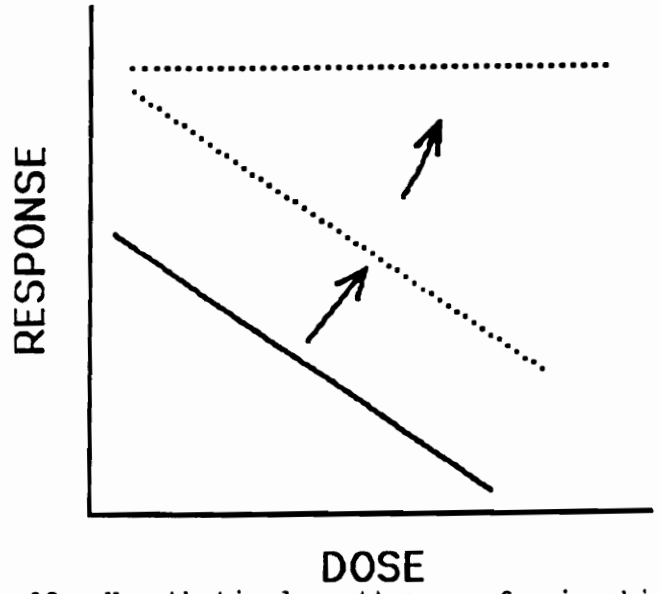


Figure 18. Hypothetical patterns of microbial community recovery following the removal of a stressing agent. Figure 8.1 (above) illustrates the recovery of communities which were at steady state just prior to the initiation of stress; significant recovery would be assessed as a change in slope without a change in the intercept. Figure 8.2 (below) illustrates the recovery of communities which were actively growing just prior to the initiation of stress. In this case, significant recovery would initially be assessed as a change in the intercept without a change in slope, and final recovery to a steady state condition would be assessed as a change in both the slope and the intercept.

Chapter 3

Conclusions

Investigators studying the effects of toxicants on the species and communities that make up aquatic ecosystems often utilize laboratory studies so that natural variability can be reduced, experimental treatments replicated, and hypotheses tested. Although the conclusions drawn from indoor studies are valid for the test systems from which the data are gathered, they become less certain when used to assess hazard in real ecosystems. Mesocosms are more environmentally complex and can be replicated (Odum, 1984), but the responses of these ecosystem surrogates may also fail to reveal unexpected ecosystem behavior following stress. Ultimately, obtaining the highest degree of confidence for predictions of ecological risk requires ecosystem-level validation of the results obtained from simplified, artificial test systems (Kimball and Levin, 1985; Cairns and McCormick, 1991).

There are several drawbacks to studying the effects of chemical toxicants in stream ecosystems. Assessing the ecological effects of an existing chemical pollutant relies on comparing impacted reaches with upstream reference sites. However, in doing so, the statistical assumption of

independence among samples is violated (Hurlbert, 1984; Smith et al., in press.). Similarly, manipulative studies in which toxicants are added to entire stream ecosystems (e.g., Geckler et al., 1976; Leland and Carter, 1984) may also be statistically flawed. In addition, these studies require the release of large quantities of toxic substances which may persist in the environment.

Toxicant-releasing substrates have been proposed by Dr. Paul McCormick (pers. comm.) as a means of delivering chemical toxicants to microbial communities in a controlled and predictable manner. The main goal of this investigation was to characterize the delivery of copper to periphyton communities growing on the novel substrates and the resulting response and recovery dynamics of these assemblages. The standards by which the performance of the toxicant-releasing substrates was assessed and to which the behavior of the colonized substrates was compared were the results of concomitant toxicity tests performed on identically-derived periphyton communities. In these parallel tests, copper was added to the water column surrounding the communities instead of being released from the substrates.

During both summer and winter artificial stream experiments, the responses of microbial communities to copper delivered from the novel substrates did not differ from community responses to copper delivered initially to the water

column of the test systems. Specifically, in characterizing the performance of the toxicant-releasing substrates it was found that:

1) After an initial two day phase when the rate of copper diffusion is (probably) low, the novel substrates release copper at constant rates for at least four days.

2) Measured bioconcentration of copper by periphyton communities growing on the novel substrates is a quadratic function of internal copper concentration in the substrate.

3) The chemical-releasing substrates can be used over a broad range of toxicologically significant copper doses without extinction of diffusion.

4) The sampled surfaces of toxicant-releasing substrates bind large amounts of copper relative to terra-cotta tiles used in streams to which copper is added dropwise.

5) Most importantly, however, neither the binding of copper to the clay tiles nor any other methodological feature of the toxicant-releasing substrates significantly affect the outcomes of toxicity tests conducted using the novel substrates. Although periphyton communities responded differently to (measured) copper bioconcentration in the summer and winter, no differences in response were due to the method of copper delivery. Binding of copper to the chemical-diffusing substrates might also be expected to delay recovery, but this phenomenon, too, was independent of toxicant delivery

method.

Sampling from the copper-releasing substrates inevitably removes some clay-bound copper. Because this could conceivably bias the results of future measurements of bioconcentration in substrate-dosed communities, minimization of this artifact should be considered. If necessary, efforts such as decanting homogenized algae away from denser clay particles or washing samples with a dilute EDTA solution (Campbell and Stokes, 1985) might effectively remove the contribution of clay-bound copper to measured community bioconcentration.

The toxicological responses of periphyton communities treated with copper using either of the two experimental dosing methods were in good agreement with the scientific literature (Tables 4 and 5). However, data does not exist for many of the community-level responses of lotic (flowing water) periphyton to copper. This study expands knowledge of the effects of copper on microbial communities by providing evidence that:

5) Non-taxonomic periphyton structure in unstressed communities differs in different seasons, and temperature is probably a significant factor controlling this difference.

6) Bioconcentration in copper-stressed periphyton communities may be a seasonally-dependent phenomenon (although this conclusion is based on two small data sets generated in

this study and, thus, is tenuous). The seasonal difference in copper bioconcentration may be due to the dependence of bioconcentration on community structure or due to differential abiotic binding of copper by extracellular organic matter under different physicochemical conditions.

7) Young periphyton communities in streams respond to seven days of copper stress by structural shifts in the direction of more heterotrophically-dominated communities.

8) In the test system employed in this study, reduction in the ratio of chlorophyll a to community biomass is consistently the most sensitive response of periphyton assemblages to copper, followed by a reduction in ash-free dry weight relative to unstressed communities.

9) Measurements of periphyton community function as changes in oxygen or nutrient content in recirculating chambers in situ are often more variable, labor intensive, and prone to methodological errors, when made on small periphyton community sample sizes, than are structural measurements.

10) Season (e.g., seasonally-dependent community structure and water chemistry) influences the response of community structure (e.g., total biomass) to copper stress.

11) Significant community recovery from copper begins to occur two weeks after removal of low levels of copper stress, but retention of copper by communities exposed to high concentrations of copper may delay recovery for at least two

weeks.

Chemical-releasing substrates allow the resistance and resilience of periphyton communities to copper stress, and perhaps to several other inorganic toxicants, to be assessed *in situ*. In addition, the structural and functional responses of these communities to copper delivered using the novel substrates are not method-dependent and may be compared to the results of standard toxicity tests in a straightforward manner.

Toxicant-releasing substrates have several potential uses including: 1) community-level validation of the results of laboratory toxicity tests or confirmation of upstream-downstream studies under natural conditions; 2) investigation of the effects of synergistic or antagonistic combinations of toxicants on microbial communities; and 3) comparison of the sensitivities of periphyton communities in different ecosystems (Paul McCormick, pers. comm.). For example, experiments employing identical, experimentally introduced stresses in different streams would generate data useful for industrial site planners or relevant to the study of ecological adaptation to stress in natural ecosystems (Cairns, 1987). The results of this work indicate that these substrates will effectively aid microbial ecologists in elucidating the important factors governing periphyton community behavior following stress.

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Smith, E. P., D. R. Orvos, and J. Cairns, Jr., "Impact Assessment Using the Before-After-Control-Impact (BACI) Model: Concerns and Comments," Canadian Journal of Fisheries and Aquatic Sciences, in press.

Curriculum Vitae

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May, 1993

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Place of Birth: Portland, Oregon

Education

M.S. Biology, in progress, VPI&SU, Blacksburg, Virginia
Thesis topic: Predicting the hazard of toxicants to microbial communities in streams
Major Professor: Dr. John Cairns, Jr.

Department of Chemical Engineering, University of Karlsruhe, Karlsruhe, Federal Republic of Germany
Fulbright Fellowship

B.S. Biology, magna cum laude, 1989, VPI&SU, Blacksburg, Virginia

Summer Study, 1987, Rocky Mountain Biological Laboratory, Gothic, Colorado

Professional Employment

present Graduate Research Assistant, University Center for Environmental and Hazardous Materials Studies, VPI&SU, Blacksburg, Virginia
Duties include: 1) construction of chemical-diffusing substrates and productivity/respiration chambers; 2) the corresponding work in the laboratory (e.g., nutrient analyses and algal counts) and field (e.g., placement and

retrieval of the substrates and collection of water samples) necessary for experimentation with these substrates; and 3) data analysis and presentation.

1991 Graduate Teaching Assistant, Department of Biology, VPI&SU, Blacksburg, Virginia
Instructed General Biology Laboratory.

1988-89 Physical Science Aid, Chemistry Division, Naval Research Laboratory, Washington, D.C.
Synthesized several precursors of conductive organic polymers.

1988-89 National Science Foundation Research Experience for Undergraduates Grant, Laboratory for the Molecular Biology of Plant Stress, VPI&SU, Blacksburg, Virginia
Acquired skills in several molecular biological techniques used in the genetic manipulations of Erwinia carotovora.

Honors and Awards

1989 Fulbright Fellowship

1988 National Science Foundation Research Experience for Undergraduates Grant

Honorary Society Memberships

Phi Beta Kappa, National Honor Society

Professional Organizations

American Institute of Biological Sciences

North American Benthological Society

Virginia Academy of Science

Grant Proposals

1992 Virginia Academy of Science Small Project Research Funds Grant (funded $523.00; $500.00 matched by the Biology Department)

1991 Graduate Research Development Project, Support for Degree Contingent Research (funded $150.00; $150.00 matched by the Biology Department)

1991 Sigma Xi Grants-in-Aid of Research Award (not funded)

1991 Cunningham Master's Summer Thesis Fellowship (not funded)

1990 Air and Waste Management Association Graduate Study Scholarship (not funded)

Publications

Arnegard, M.E. and J. Cairns, Jr. 1992. A new method for assessing the effects of inorganic pollutants on algal communities in streams. Proceedings of the Third Annual Environment Virginia Symposium. pp. 42-46.

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McCormick, P.V., M.E. Arnegard, and J. Cairns, Jr. 1993. An in situ experimental method for assessing aquatic ecosystem responses to chemical stressors. Abstracts of the Third International Conference of Aquatic Ecosystem Health & the Ecological Significance of Bioassay Techniques.

Presentations

1993	Third International Conference of Aquatic Ecosystem Health & the Ecological Significance of Bioassay Techniques.
1993	71st Annual Meeting of the Virginia Academy of Science, Old Dominion University, Norfolk, VA
1992	Annual meeting of the North American Benthological Society, Louisville, Kentucky.
1992	Third Annual Environment Virginia Symposium, VMI, Lexington, Virginia
1991	69th Annual Meeting of the Virginia Academy of Science, VPI&SU, Blacksburg, Virginia
1990	Invited talk at the Sustainable Economic and Environmental Development (SEED) Conference, Hollins College, Hollins, Virginia

Extracurricular Activities

1993	Ecological consultant for the College of Architecture and Urban Studies' entry for the international Sustainable Communities and Ecological Restoration Competition (in progress)
1992-93	Laboratory Health and Safety Officer for the University Center for Environmental and Hazardous Materials Studies
1992-93	Member of the 1992-93 Botany Seminar Planning Committee

1991-	Active member of the Biology Organization of Graduate Students
1991-92	Elected Graduate Student Assembly Representative, Commission on Faculty Affairs
1990	Member of the Leadership Panel, Sustainable Economic and Environmental Development (SEED) Conference, Hollins College, Virginia Represented the youth perspective toward sustainable development.