COMMUNITY RESPONSES OF AQUATIC MACROINVERTEBRATES TO HEAVY METALS IN LABORATORY AND OUTDOOR EXPERIMENTAL STREAMS

by

William H. Clements

Dissertation submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Biology

APPROVED:

Donald S. Cherry,
Co-chairman

John Cairns, Jr.,
Co-chairman

Ernest F. Benfield

Eric P. Smith

Joseph R. Voshell

May, 1988

Blacksburg, Virginia

COMMUNITY RESPONSES OF AQUATIC MACROINVERTEBRATES TO HEAVY METALS IN LABORATORY AND OUTDOOR EXPERIMENTAL STREAMS

by

William H. Clements

Committee Co-Chairmen: Donald S. Cherry and John Cairns, Jr.

Zoology

(ABSTRACT)

This research describes aquatic macroinvertebrate community responses to heavy metals (copper, zinc) in experimental streams and at metal-impacted sites in the field. Experiments employed substrate-filled trays which were colonized in the field and then transferred to laboratory or outdoor streams.

Laboratory experiments conducted over three seasons showed that acute (96 h) exposure to copper (Cu) at 15-32 ug Cu/L significantly reduced macroinvertebrate abundance and number of taxa during each season. Owing to differences in sensitivity among taxa, the percent composition of dominant groups varied between control and dosed streams. Mayflies were quite sensitive to Cu, particularly during the summer when water temperatures were higher.

Community responses to Cu and Zn in outdoor experimental streams were similar to those observed at metal-impacted sites in the field. Control streams and field reference

stations were dominated by mayflies and Tanytarsini chironomids. In contrast, treated streams and impacted field sites were dominated by net-spinning caddisflies (Hydropsychidae) and Orthocladiini chironomids. The similarity of these experimental results to those observed in the field suggest that macroinvertebrate community responses to heavy metals are highly predictable.

Responses of these communities to Cu were greatly influenced by water quality. Effects were more severe in New River streams, where water hardness and alkalinity were low, compared to Clinch River streams, where hardness and alkalinity were higher. In soft water streams, abundance was reduced by 84% after 10 d exposure to Cu (measured concentration = 13 ug/L). In contrast, abundance was reduced by only 45% in hard water streams after 10 d at similar Cu levels. These results demonstrate the importance of accounting for water quality characteristics of receiving systems when establishing site-specific criteria for metals.

Chronic exposure (14 d) to sublethal levels of Cu (< 6 ug/L) increased vulnerability of caddisflies (*Hydropsyche morosa* and *Chimarra* sp.) to predation by the stonefly, *Paragnetina fumosa*. Caddisflies were also the major component of stonefly diets and were consumed significantly more frequently in dosed streams than controls. These results demonstrate that single species bioassays were inadequate for predicting effects of toxicants on community level processes.

ACKNOWLEDGMENTS

I am deeply indebted to my dissertation committee co-chairs, Drs. Donald S. Cherry and John Cairns Jr., who provided considerable insight, encouragement, and necessary financial support during various stages of this research. I am especially grateful to Don for his friendship and support, which went beyond the realm of traditional advisor responsibilities. I thank my committee members, Drs. Fred Benfield, Eric Smith, and Reese Voshel for stimulating discussions during numerous committee meetings, comments on manuscripts, and for their overall criticisms of this project.

This research could not have been conducted without considerable field support. I am particularly grateful to and for assistance transporting cinderblocks across raging riffles and pounding re-bar into impenetrable bedrock. designed and constructed the experimental stream system employed in this research and also provided field assistance. Water samples were analyzed by and . I thank , , and the staff of the Clinch River Power Plant for logistical support during the biomonitoring studies.

Thoughtful discussions with fellow graduate students in aquatic ecology (, , , , , and) during various stages of this research greatly improved interpretation of results as well as the quality of my stay in Blacksburg. I would also like to thank my parents, , for their support during this project. Finally, a special thanks to whose companionship over the last two years has been a continued source of inspiration. This work could not have been completed without her help.

Funding for this research was provided in part by a grant-in-aid of research from Sigma Xi; the Virginia Academy of Science; the Department of Biology at VPI & SU, who provided matching funds for these grants as well as various tuition waivers, teaching assistantships, and a Mark S. Maly Scholarship; a Cunningham Dissertation Research Fellowship from VPI & SU; Water Resources Research Funds; the E. I. Du Pont de Nemours Educational Foundation; and American Electric Power Service Corporation, Columbus OH, 43216.

TABLE OF CONTENTS

List of Tables

List of Figures

General Introduction

Investigations into the effects of toxicants on aquatic organisms have become increasingly sophisticated in recent years. Perhaps one of the more significant and controversial developments in the field of aquatic toxicology was the recognition that the single species bioassay, which has been the "workhorse" of environmental regulatory agencies (Cairns 1981), may not be appropriate for predicting effects of toxicants on higher levels of biological organization (Cairns 1983; 1986; Odum 1984; Kimball and Levin 1985). These simple tests cannot, for example, predict effects of chemicals on species interactions, nutrient cycling, or energy flow in aquatic systems.

Arguments against development of more sophisticated procedures, such as multispecies tests and field experimentation, include the greater costs and loss of replicability associated with these techniques. Boyle (1983) notes that there is generally an inverse relationship between simplicity or repeatability of these approaches and the degree of environmental realism (Fig. 1). As a compromise between the lack of realism of single species bioassays and the cost of field experimentation, Odum (1984) advocates the use of mesocosms, or "bounded and partially enclosed outdoor experimental setups", for testing effects of toxicants.

Production of heavy metals and their discharge into aquatic receiving systems have increased greatly in recent

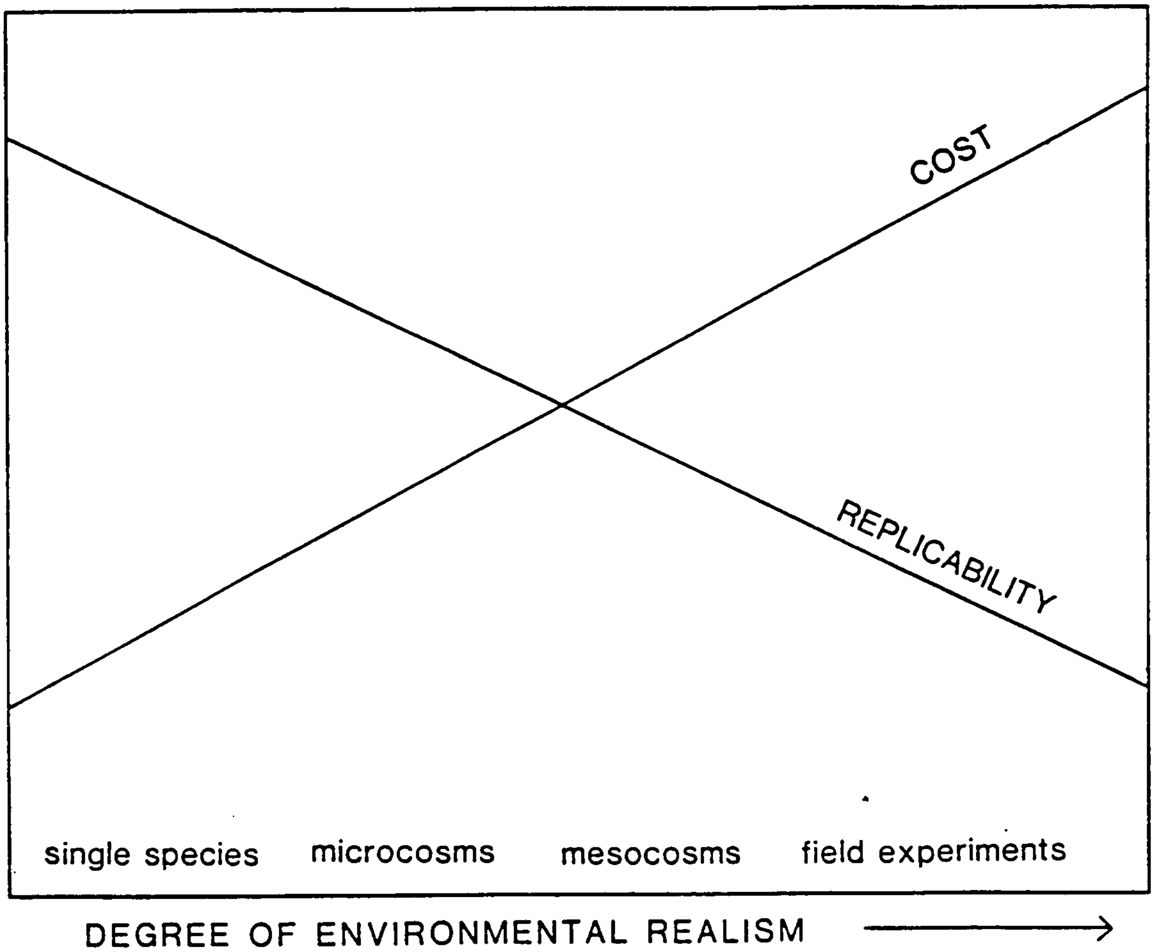

Figure 1. Hypothetical relationship between cost, replicability, and the degree of environmental realism of testing procedures (after Boyle, 1983).

years (Moore and Ramaoorthy 1984). Effects of these effluents on aquatic organisms have been well documented in the laboratory (Warnick and Bell 1969; Brown 1977; Spehar et al. 1978; Kosalwat and Knight 1987). Although results of these experiments show considerable variability between species, in general most aquatic invertebrates are quite sensitive to metals (Hodson et al. 1979).

Since publication of Hynes´ (1960) classic text, The Biology of Polluted Waters, aquatic insects have had a major role as indicators of the condition of freshwater systems. Owing to their importance in freshwater food webs, short generation time, and diversity of responses to toxicants, aquatic insects are frequently included in pollution surveys (Hart and Fuller 1974). Quantitative sampling of these organisms has, however, posed serious problems for benthic ecologists (Downing 1979; Green 1979; Morin 1985). Descriptive surveys of benthic communities in streams are often complicated by variability in natural substrate composition, thus making it difficult to isolate the role of any single environmental parameter (e.g. pollution). Introduced substrates are frequently employed in stream surveys to reduce this variability and improve statistical reliability of benthic samples (Cairns 1982). Recently these devices have been employed in microcosms to test effects of toxicants on colonization rate of protozoans (Cairns et al. 1980) and in experimental streams to determine the impact of sewage on aquatic insects communities (Burks and Wilhm 1977).

The research reported here describes macroinvertebrate community responses to heavy metals (Cu, Zn) in outdoor and laboratory experimental streams. In chapter one, I describe the colonization dynamics and sampling variability of the introduced substrates employed in this research. The usefulness of these substrates for biomonitoring benthic communities is also discussed. In chapter two, I document the responses of macroinvertebrate communities to copper in laboratory streams over three seasons. Chapter three compares community responses to metals in outdoor experimental streams to those observed at impacted sites in the field. In chapter four, the influence of water quality on these responses is examined by comparing results of experiments conducted in streams receiving diluent water from different sources. Finally, in chapter five, the effects of chronic Cu exposure on predator-prey interactions among stream insects are examined.

CHAPTER ONE

Colonization, variability, and the use of substratum-filled trays for sampling benthic communities

Abstract

Sampling variability and colonization rate of introduced substrates (plastic trays filled with pebble and cobble) in two southwestern Virginia streams are described. Substrates were rapidly colonized by aquatic macroinvertebrates, but colonization rates differed between years, possibly due to annual variability in macroinvertebrate abundance. To examine the applicability of using these substrates for biomonitoring benthic communities, trays were placed at several locations in a river receiving power plant discharges. Only six samples were necessary to detect a 15% reduction in macroinvertebrate density and a 12% reduction in number of taxa at effluent sites. Benthic communities established on rock-filled trays and multiplate samplers collected from the same stations during the same period were compared. Although multiplate samplers were more variable than rock trays and were selective for different taxa, both substrate types showed significant differences in community parameters among locations. Rock trays at all sites were dominated by <u>Cheumatopsyche</u> sp., whereas chironomids were more abundant on multiplate samplers. The relative abundance of mayflies was reduced at the effluent site on both substrate types.

Introduction

Descriptive surveys of benthic communities in streams are often complicated by variability in substrate composition, which contributes to difficulties in isolating effects of any single environmental parameter (e.g., pollution). In addition, since the distribution of benthic organisms is usually aggregated and highly variable (Resh, 1979; Downing, 1979), a large number of samples may be necessary to obtain reasonably precise estimates of macroinvertebrate abundance. Allan (1984) states that "sampling precision is one of the most fundamental problems limiting quantitative studies of benthos." Artificial substrates are frequently employed in stream surveys to reduce this variability and to improve statistical reliability of benthic samples (Cairns, 1982). Traditionally, these devices have received the most attention from environmental biologists comparing distribution and abundance of aquatic organisms at control and impacted sites. Critics note that artificial substrates are often selective for certain taxa, and, therefore, their usefulness in benthic research is limited (Minshall & Minshall, 1977; Roby et al., 1978; Khalaf & Tachet, 1980; Chadwick & Canton, 1983). Selectivity of artificial substrates is usually attributed to the specific materials from which these devices are constructed. Minshall & Minshall (1977) suggest that closer agreement between communities established on natural and introduced substrates could be obtained if introduced substrates were composed of streambed materials with similar composition and packing as the natural substrate. Rosenberg & Resh (1982) discuss several advantages and disadvantages of using artificial substrates for sampling macroinvertebrates and conclude that the most serious drawback of these devices is the lack of knowledge of colonization dynamics.

The purpose of this research was to describe the sampling variability and colonization dynamics of rock-filled trays from two southwestern Virginia streams. To determine the applicability of using these substrates for biomonitoring benthic communities, trays were sampled from several locations in a river receiving power plant effluents. Macroinvertebrate density and species composition of these trays were compared to multiplate samples collected from the same sites during the same period.

Materials and Methods

Description of introduced substrates

Introduced substrates employed in these studies consisted of 10 x 10 x 6-cm plastic (polypropylene) trays filled with pebble and small cobble (2-6 cm diameter). Substrate was collected from dry portions of the streambed several months prior to the start of each experiment, and a similar particle size distribution was placed into each tray. Three, 2-cm^2 holes were drilled into each side of the trays to increase water flow and to facilitate colonization by aquatic insects. The trays were held in place in the stream by wedging them between two wooden strips attached to a 1-m section of shelving board. Corner irons were attached to each end of the shelving board and the entire assembly was anchored in the stream with cinderblocks placed over each corner iron (Fig. 2). Five to six trays were placed into each rack, and large cobble was then packed around the racks.

Variability and colonization rate

To examine sampling variability and colonization rates, rock-filled trays were placed in Adair Run, a second-order tributary to the New River located in Giles County, Virginia. The stream is 2-3 m wide and water depth ranges from 0.1 to 0.4 m under normal flow conditions. The substrate is composed primarily of pebble and cobble with some small boulders. During March, 1985, 45 trays were placed at nine locations (five trays per location) with different current velocities along a 50-m section of Adair Run. Current was measured 10 cm above the substrate using a pygmy current meter. Three locations were assigned to each of the following categories: slow (21-24 cm sec^{-1}), moderate (36-41 cm sec^{-1}), and fast (51-58 cm sec^{-1}) current. After 30-d colonization, a 500-um mesh net was placed directly downstream of each tray to prevent the loss of organisms, and the trays were removed from the stream. High water during the second week of colonization resulted in the loss of 14 trays, primarily from locations

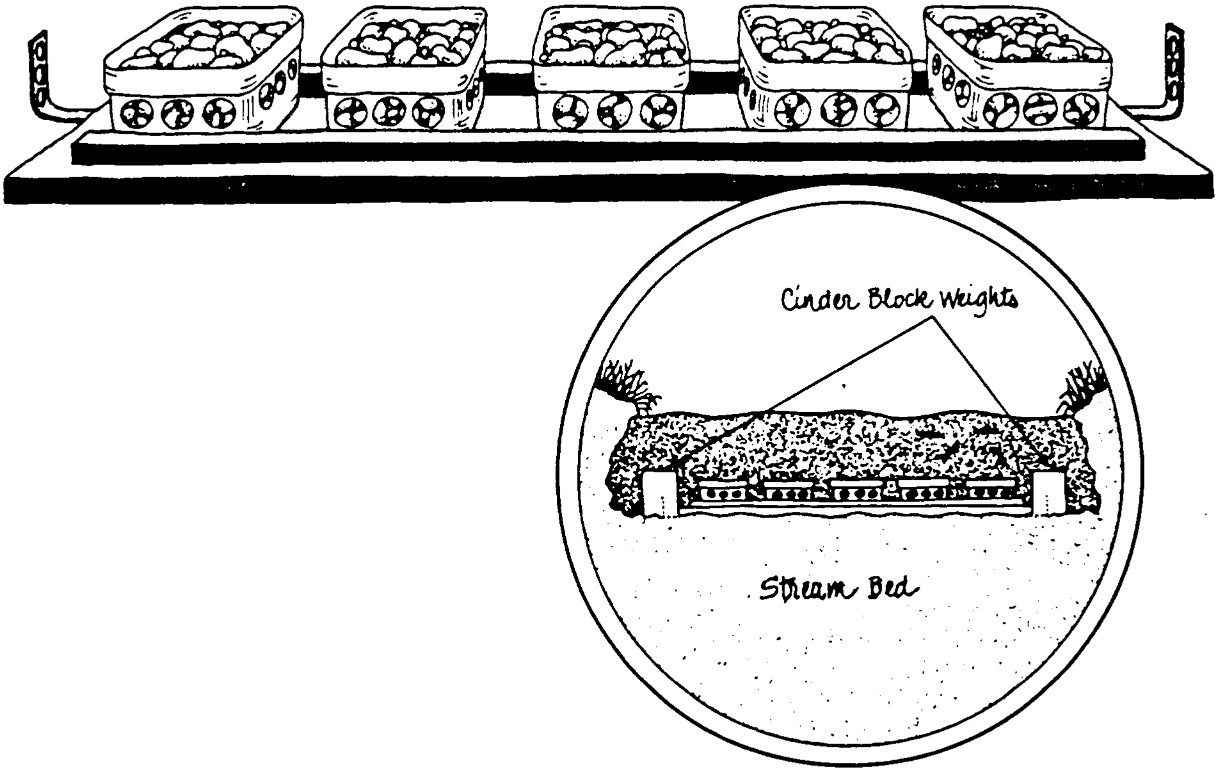

Figure 2. Schematic of the introduced substrates described in this study.

with fast currents. Contents of each of the remaining trays (n=31) were washed through a 500-um sieve and preserved in 10% formalin. In the laboratory, organisms were sorted in a white enamel pan and identified to genus (except for chironomids, which were identified to tribe).

Colonization experiments were conducted during spring of 1985 and 1986. On 22 April, 1985, 30 trays were placed at five different locations with moderate current velocity (32-36 cm sec^{-1}). One tray was randomly selected from each of the five locations after 4, 8, 12, 18, 23, and 29 d of colonization. On 1 April, 1986, 25 trays were placed at the same locations, and five trays were recovered after days 5, 10, 15, 20, and 28. All trays were removed from the stream and processed as described above.

Biomonitoring studies

In order to examine the applicability of using rock-filled trays for biomonitoring benthic communities, substrates were placed upstream and downstream of a coal-fired power plant located on the Clinch River (Fig. 3). This stream is a fourth-order tributary to the Tennessee River located in Russell County, Virginia. The stream is ~60 m wide, and water depth ranged from 0.5 to 0.8 m. The substrate is dominated by large pebble to intermediate cobble. During September 1985, six trays were placed at each of two upstream control sites and one downstream site that received heavy metal effluents.

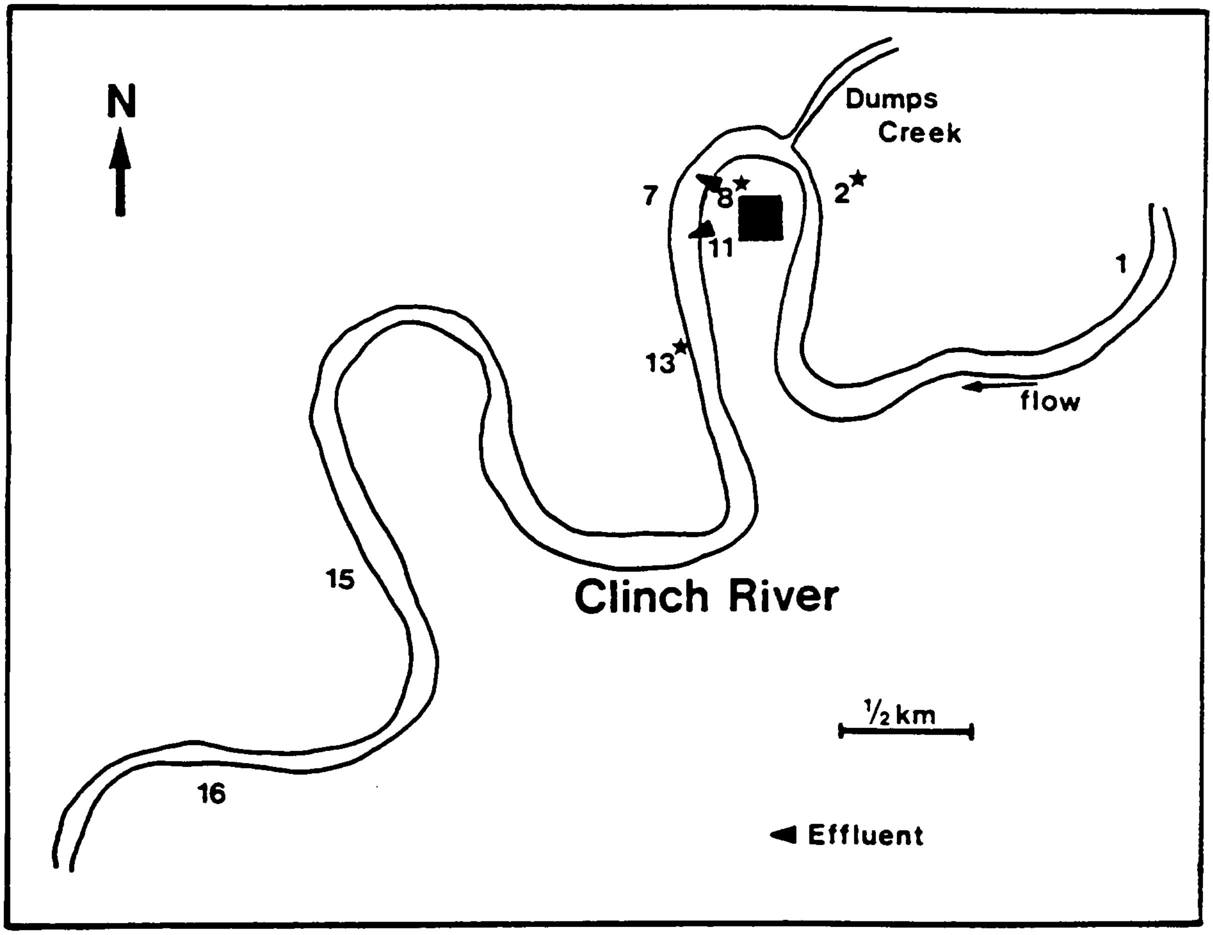

Figure 3. Map of sampling stations at the Clinch River. Arrows refer to locations of effluent discharges.

Stations 1 and 2 were located ~3.0 and 1.0 km upstream of the plant, respectively. Station 8 was located 50-m downstream of a cooling tower blowdown discharge. Current velocity ranged from 44-50 cm s^{-1} at each station. Three multiplate samplers (Hester & Dendy, 1962) were suspended in the water column 30 cm above the substrate adjacent to the rock-filled trays at each site. After 30-d colonization, all substrates were removed and processed as described above.

Statistical analyses

Estimates of sampling precision and required number of samples were determined as described by Green (1979). In this procedure, precision is defined as the percent difference between two sets of samples that can be detected with 95% confidence. The equation is

$$p=t_{(0.05)}\, s\, n^{-1/2}$$

where p=the desired level of precision (expressed as a percent of the mean); t=Student's t-statistic; s=standard deviation; and n=number of samples.

The cumulative percent of total taxa collected from rock trays in Adair Run was estimated by randomly selecting a single sample from the pool of all samples collected during March, 1985 (n=31) and recording the number of new taxa that occurred. This procedure was repeated 10 times for sample sizes 1 to 10.

Differences in macroinvertebrate abundance and number of taxa between locations in Adair Run and between control and effluent sites on the Clinch River were analyzed using one-way ANOVA. In order to compare sampling variability and precision of rock trays and multiplate samplers, three of the six trays from each site were randomly selected and included in the analysis. All data were transformed using a ln(n+1) transformation to stabilize variances. If differences between locations were detected ($p<0.05$), Duncan´s Multiple Range Test was employed to determine which sites were significantly different from others.

Results

Variability and colonization rate

Rock-filled trays collected from Adair Run during March, 1985 were dominated by chironomids and mayflies at each site (Table 1). Macroinvertebrate abundance ranged from 47 to 132 individuals per tray and varied between locations with different current velocity. Although the total number of individuals per tray was reduced at locations with fast current velocity, this difference was not significant ($p=0.189$) owing to the low number of trays ($n=4$) recovered from these sites. The number of taxa per tray ranged between 8 and 16 and was significantly lower at locations with slow currents ($p=0.016$).

Estimates of sampling precision, based on means and standard deviations of these data, indicated that both

Table 1. Mean macroinvertebrate abundance, number of taxa, and abundance of dominant taxa from locations with slow (n=12), moderate (n=15), and fast (n=4) current velocity in Adair Run during March 1985. Standard deviations are given in parentheses. Means with the same letter are not significantly different based on Duncan´s Multiple Range Test.

	Current Velocity (cm/s)			
	21-23	36-41	51-58	F-value
Mean Abundance	86.9 (23.3)	83.4 (26.9)	62.5 (15.3)	1.77
Number of Taxa	10.6 (2.5) a	12.9 (2.0) b	12.8 (1.3) b	4.80*
Baetis sp.	8.3 (6.4)	8.8 (7.1)	6.2 (5.5)	0.20
Eurylophella sp.	2.9 (2.6) a	1.8 (2.2) a	0.0 (0.0) b	4.12*
Paraleptophlebia sp.	2.8 (2.2)	1.4 (1.4)	2.0 (2.7)	1.23
Amphinemura sp.	0.7 (1.2) a	1.7 (2.4) ab	3.3 (0.5) b	4.80*
Isoperla sp.	0.6 (1.2)	1.1 (1.1)	1.8 (1.0)	3.11
Cheumatopsyche sp.	0.9 (1.5)	2.9 (3.6)	1.0 (1.1)	2.39
Orthocladiini	57.8 (19.4)	52.5 (21.5)	36.5 (13.4)	2.06
Chironomini	2.5 (1.6)	1.8 (1.7)	0.5 (0.6)	3.25

* $p<0.05$

macroinvertebrate abundance and number of taxa were estimated more precisely from locations with faster currents. Depending on location, a 30-40% difference in macroinvertebrate abundance and a 13-29% difference in number of taxa could be detected with five samples ($p<0.05$).

Variability in abundance of dominant taxa was considerably greater than variability of these community level parameters. Of the eight dominant taxa collected from Adair Run, two taxa showed significant differences between locations (Table 1). Eurylophella sp. was significantly more abundant at locations with slow-moderate currents ($p=0.027$), whereas Amphinemura sp. ($p=0.016$) was more abundant on trays in faster currents. Both Chironomini ($p=0.054$) and Isoperla sp. ($p=0.060$) showed moderate, but nonsignificant, differences between locations.

The cumulative percent of total taxa collected on rock trays from Adair Run in 1985 increased rapidly between one and six samples, after which the curve slowly became asymptotic (Fig. 4). With six samples, ~65% of the total taxa that colonized trays were represented. Between six and ten samples the cumulative percent of taxa collected increased by only 5%.

Colonization dynamics of rock trays in Adair Run differed between years (Fig. 5). In 1985 experiments, macroinvertebrate abundance increased rapidly during the first two weeks of colonization and then declined slightly for the

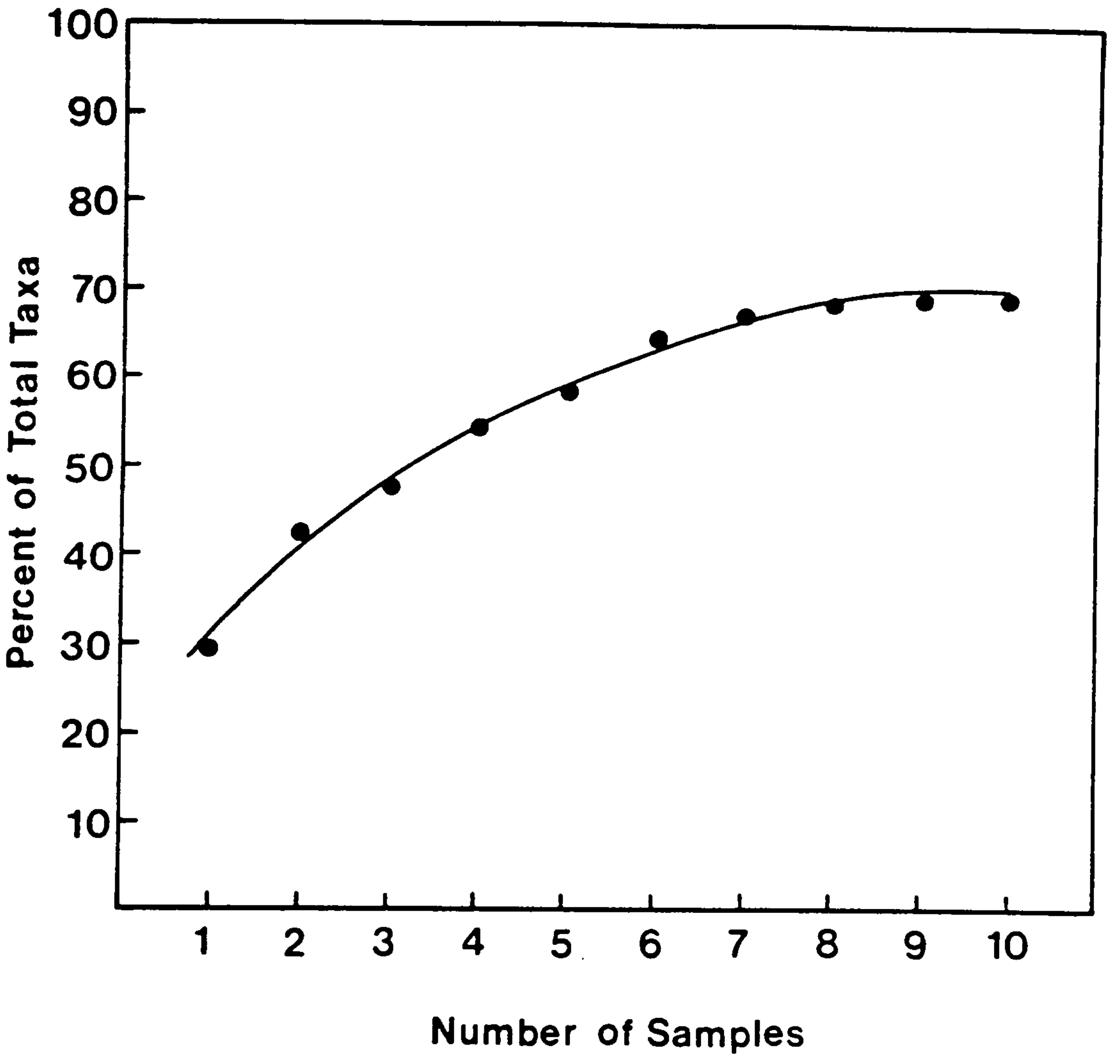

Figure 4. Cumulative percent of total taxa collected on rock tray substrates from Adair Run as a function of the number of samples.

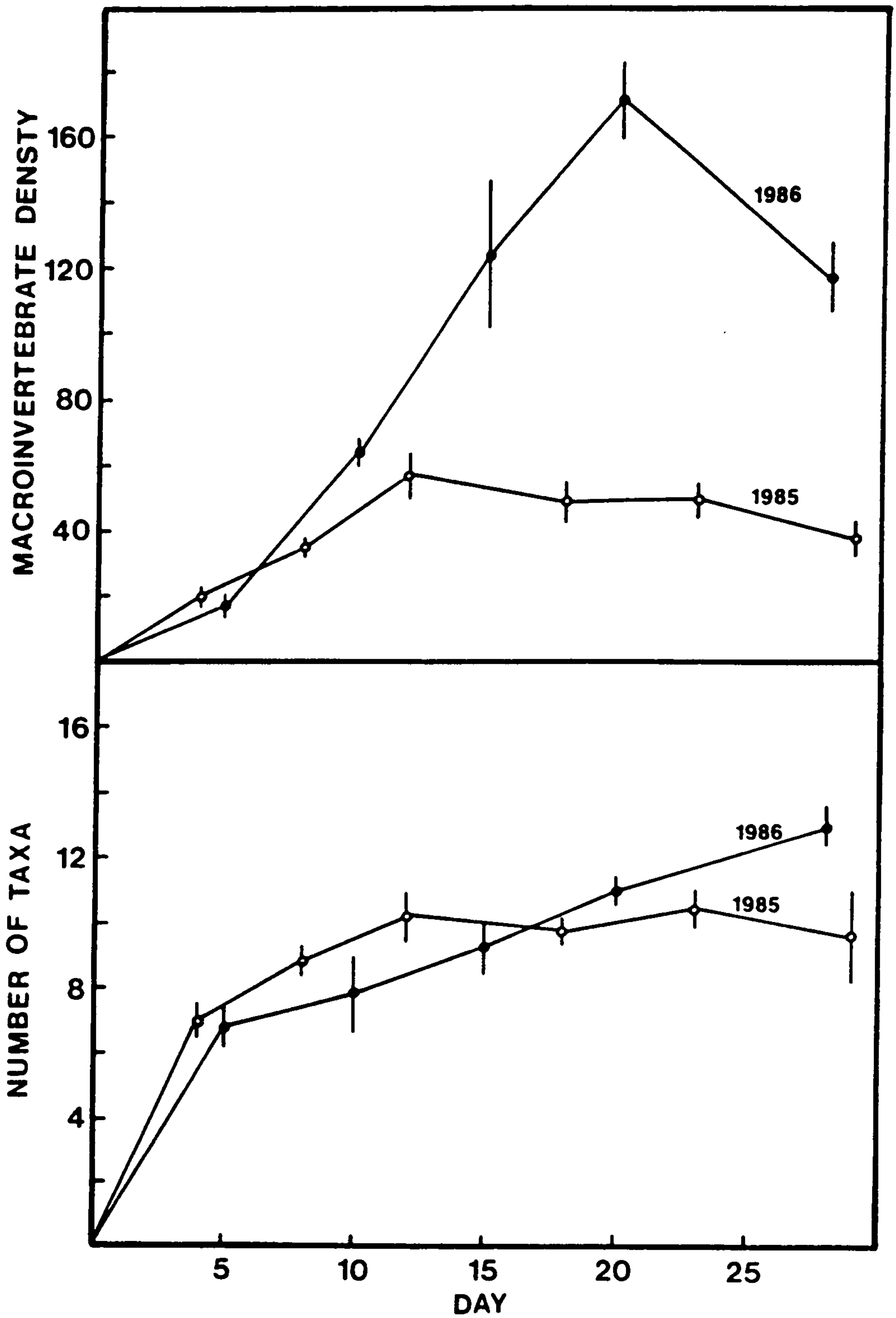

Figure 5. Colonization rate of rock tray substrates from Adair Run during spring of 1985 and 1986. Each point represents the mean ± standard error.

remaining period. In 1986, the number of individuals per tray increased until day 20 and then declined between days 20 and 28. By day 28, the total number of organisms that colonized trays was significantly greater in 1986 than in 1985 (Student´s t-test, p=0.001). The shape of colonization curves for number of taxa was similar during the first two weeks of colonization in both years, after which the number of taxa per tray remained constant in 1985 but continued to increase in 1986. By day 28, however, the number of taxa did not differ significantly between years.

Clinch River biomonitoring

Mean macroinvertebrate density, number of taxa, and Shannon diversity were generally less variable on rock trays than on multiplate samplers (Table 2). Results of ANOVA indicated that with both substrate types I detected significant differences in abundance and number of taxa between upstream and downstream stations. Diversity, however, was significantly reduced at the downstream station only on rock trays. Abundance of most dominant taxa varied between stations, but these differences were dependent on substrate type and could not always be attributed to plant discharges. Chironomini (p=0.002) and *Isonychia* sp. (p=0.001) were reduced on rock trays but not on multiplate samplers. Of the five taxa showing significant differences between stations on multiplate samplers, only three were reduced at the down-

Table 2. Mean macroinvertebrate abundance, number of taxa, and diversity of rock trays and multiplate samplers from Stations 1, 2 (upstream), and 8 (effluent) on the Clinch River. Standard deviations are given in parentheses. Means with the same letter are not significantly different based on Duncan's Multiple Range Test.

	Rock Trays				Multiplate Samplers			
	Station				Station			
	1	2	8	F-value	1	2	8	F-value
Abundance	139.0^{a} (14.2)	107.7^{a} (18.0)	32.0^{b} (5.3)	88.8**	273.0^{a} (228.2)	108.7^{a} (16.3)	27.0^{b} (1.8)	15.1*
Number of Taxa	18.0^{a} (1.0)	18.7^{a} (2.1)	7.7^{b} (0.6)	106.1**	17.7^{a} (3.1)	15.7^{a} (2.1)	7.0^{b} (1.7)	23.3**
Diversity	3.14^{a} (0.07)	3.42^{a} (0.18)	1.79^{b} (0.27)	51.2**	2.69 (0.17)	2.93 (0.19)	2.25 (0.43)	3.9

* $p<0.05$; ** $p<0.001$

stream site compared to both controls. On rock tray samples, seven taxa showed significant differences between stations, and six of these taxa were reduced at the downstream site relative to both upstream sites.

The relative abundance of dominant taxa also varied among stations and substrate types in the Clinch River (Table 3). Nine taxa accounted for 82-96% of the total organisms collected on both trays and multiplate samplers. At all sites, the relative abundance of chironomids, particularly tube-building groups such as Chironomini and Tanytarsini, was much greater on multiplate samplers than on rock trays. The net-spinning trichopteran, Cheumatopsyche sp., was more abundant on trays than on multiplate samplers at all stations. The relative abundance of mayflies was reduced at the downstream station compared to upstream reference stations on both substrate types. The decrease in relative abundance of mayflies on rock trays at the impacted site resulted in increased dominance by hydropsychid caddisflies, which accounted for 75% of the total number of individuals on these substrates.

Table 3. Relative abundance of dominant taxa on rock trays and multiplate samplers from stations 1, 2 (upstream), and 8 (effluent) in the Clinch River.

Taxa	Rock Trays Station			Multiplate samplers Station		
	1	2	8	1	2	8
Stenonema sp.	10.1	8.4	1.0	3.2	5.5	0.0
Tricorythodes sp.	3.4	11.8	0.0	5.1	36.5	7.4
Isonychia sp.	8.1	11.1	2.1	1.7	1.8	0.0
Caenis sp.	4.8	3.4	0.0	0.4	6.4	0.0
Cheumatopsyche sp.	40.8	26.3	67.7	10.5	2.8	14.8
Hydropsyche sp.	2.9	3.4	7.3	8.5	0.9	6.3
Tanytarsini	11.3	16.7	1.0	55.6	23.6	0.0
Chironomini	1.2	4.0	0.0	7.0	13.5	49.3
Orthocladiini	1.7	7.1	0.0	4.1	0.9	3.7

Discussion

Results of these studies showed that rock-filled trays were rapidly colonized by aquatic macroinvertebrates and that only 5-6 samples were necessary to account for a significant amount of variation in macroinvertebrate density and number of taxa. Morin (1985) noted that the number of samples required to estimate macroinvertebrate abundance increased with variance and aggregation, but decreased with mean density and sampler size. The choice of sampler size and number of samples collected in benthic studies are of considerable importance, particularly given the amount of time required to process these samples. Based on the relationship between mean density and variance obtained from a large number of benthic studies, Morin (1985) concluded that small samples are more cost-effective than large ones, especially at higher densities. Allan (1984) also noted that many small samples are often preferable to few larger ones, but cautions that this generalization does not apply when samples are so small that zero counts are common. The 100-cm^2 trays employed in our study sampled approximately 11% of the total area of a Surber sampler and usually had higher densities of organisms per m^2. Specht et al. (1984) reported peak density of macroinvertebrates of 1,700 m^{-2} in Surber samples from Adair Run during spring of 1980. Mean densities of macroinvertebrates from rock trays in Adair Run ranged between 6,250-11,800 m^{-2} in the spring of 1985 and 1986.

Introduced substrates are often less variable than natural substrate samplers. Shaw & Minshall (1980) compared variability in macroinvertebrate abundance from pebble-filled trays to Hess samples and concluded that coefficients of variation were generally lower on the introduced substrates. Morin (1985) showed that, while artificial substrates in general were as variable as conventional sampling devices, substratum-filled trays and baskets were significantly less variable than average. In Surber samples taken from Adair Run during March 1980 and April 1981, coefficients of variation ranged from 45-50% for macroinvertebrate density and from 19-32% for number of taxa (Specht 1985). These estimates were considerably higher than coefficients of variation for rock trays collected from Adair Run during spring of 1985 and 1986 (19-32% for density and 11-24% for number of taxa). In my studies using rock trays on the Clinch River, highly significant differences in macroinvertebrate density, number of taxa, and abundance of several dominant taxa were detected with only three samples per station. Based on estimates of mean and variance from Station 2 (the most variable upstream station), I could detect a 15% reduction in macroinvertebrate density and a 12% reduction in number of taxa with only six samples ($p=0.05$). Multiplate samplers also showed significant differences between stations in these community parameters, but, because of their greater variability, more multiplate samples than rock trays would be necessary to detect subtle differences between stations.

Colonization dynamics of rock trays in Adair Run differed between years, even though trays were placed in the same locations and during the same season each year. This finding complicates results of colonization studies conducted over a single season during a single year. Differences in colonization dynamics between years were most likely the result of annual variation in abundance of benthic macroinvertebrates. Specht et al. (1984) reported considerable annual variation in macroinvertebrate density and number of taxa from Adair Run between 1979 and 1981. Despite the observed differences between years, colonization by macroinvertebrates levelled off within 2-3 weeks each year, suggesting that these communities had reached equilibrium conditions. These results support the fingings of De Pauw et al. (1986) who recommended a 3-week exposure period for artificial substrates. I feel that this period of time was necessary to ensure adequate colonization and that longer periods would simply increase the risk of loss of substrates due to vandalism, floods, etc.

Although selectivity of artificial substrates may preclude their application for some types of benthic research (e.g., productivity, functional group analyses), these devices will be most useful for experimental purposes or for biomonitoring benthic communities (Minshall & Minshall 1977;

Shaw & Minshall 1980). Furthermore, if the primary objective of a study is to delineate zones of impact in a stream, selectivity of artificial substrates may not be a severe disadvantage. In my studies at the Clinch River, relative abundance of dominant taxa differed between substrate types. Rock tray samples at all sites, including the downstream station, were dominated by Cheumatophyche sp. Multiplate samples at Station 1 were dominated by Tanytarsini, whereas Tricorythodes sp. was the dominant organism at Station 2. Differences in relative abundances of dominant taxa between substrate types may be explained by the specific placement of these devices in the field (i.e., rock trays were placed on the bottom while multiplate samplers were held in the water column). Despite these differences, both substrate types detected highly significant differences in macroinvertebrate abundance and number of taxa between stations and were, therefore, adequate for describing the impact of plant effluents. In addition, both substrate types showed a decrease in relative abundance of Ephemeroptera at the downstream site. This finding supports the proposal of Van Hassel & Gaulke (1986) that the percent reduction in mayflies at effluent sites on the Clinch River could be employed to establish on-site water quality criteria. In the present study, the percent reduction in mayflies at the downstream station relative to upstream controls was less variable on rock trays (88-91%) than on multiplate samplers (29-85%), suggesting that rock trays may be more useful for this purpose.

Although it is unlikely that the rock tray substrates I have described can be employed in deeper rivers, I feel that these devices are quite appropriate for shallow, rocky-bottom streams. Substratum-filled trays were both less variable and more precise than multiplate samplers. In addition, coefficients of variation of our substrates were considerably less than those reported in the literature for Surber samplers. As a result, fewer samples were necessary to detect differences in community parameters (density, number of taxa, diversity) between locations. In addition, since samples were small and did not accumulate large amounts of organic detritus, the length of time required for complete laboratory processing (1-1.5 h) was often less than for conventional sampling devices (Clements, personal observation). Therefore, if it is necessary to detect subtle differences between stations, or if a researcher is interested in comparing abundances of less common taxa, a large number of samples can be processed in a relatively short period of time.

CHAPTER TWO

Structural Alterations in Aquatic Insect Communities Exposed to Copper in Laboratory Streams

Abstract

The effects of copper on aquatic insect communities were examined using rock-filled trays colonized in the field for 30 days, transferred to laboratory streams, and dosed with $CuSO_4$. Each stream was randomly assigned to one of three treatments: control (0 ug/L), low dose (15-32 ug/L), and high dose (135-178 ug/L). Experiments were replicated over three seasons. Exposure to copper for 96 h significantly reduced both the total number of individuals and number of taxa during each season, with greatest effects observed in summer. Owing to differences in sensitivity to copper, the percent composition of dominant orders of aquatic insects varied among treatments. The relative abundance of Ephemeroptera decreased in treated streams during each season. The response of other aquatic insects, including Diptera and Plecoptera, varied between seasons, but these groups were generally less sensitive to copper exposure. These results indicate that the artificial substrates employed in this study are amenable to experimental manipulation and will provide a unique opportunity to examine the community responses of aquatic insects to toxicants under environmentally realistic conditions.

Introduction

Aquatic toxicologists employ a variety of techniques, ranging from single species laboratory bioassays to experimental introduction of toxicants into natural systems, to examine the responses of freshwater organisms to pollutants. At present, there is considerable controversy over which approach is more useful (Cairns 1983; Kimball and Levins 1985). Boyle (1983) notes that there is generally an inverse relationship between the simplicity or repeatability of these approaches and the degree of environmental realism. Single species toxicity tests are routinely employed to establish water quality criteria and to predict the environmental impact of hazardous materials on aquatic communities. Owing to their inherent simplicity, these tests are relatively inexpensive and have a high degree of replicability and statistical precision. Recently, however, some researchers have questioned the adequacy of single species tests for predicting the effects of toxicants on natural communities in the field (Cairns 1983; 1986; Odum 1984; Kimball and Levins 1985). Although some studies have demonstrated good agreement between single species tests and field data (Hansen and Garton 1982; Adams et al. 1983) others have shown effects of toxicants in field experiments at considerably lower concentrations than indicated by single species bioassays (Dewey 1986). Kimball and Levins (1985) present several examples that illustrate the limitations of single species bioassays but note the historical preference for these simpler tests. For several reasons, toxicologists have been reluctant to incorporate more environmentally realistic testing procedures into their repertoire of experimental techniques. Arguments against the use of multispecies bioassays and field experimentation include the greater costs, loss of replicability, and potential for environmental damage associated with experimental introduction of toxicants into natural systems. However, since the objective of hazard assessment is to predict effects of pollutants beyond the level of single species (Cairns 1983), it follows that community or ecosystem level tests should be considered.

Since publication of the classic text by Hynes (1960) on the biology of polluted waters, aquatic insects have had a major role as indicators of the condition of freshwater systems. Owing to their ecological importance in freshwater food chains, short generation time, and diversity of responses to toxicants, aquatic macroinvertebrates are frequently included in pollution studies (Hart and Fuller 1974). Descriptive surveys of stream organisms are complicated by variability of natural substrates (Resh 1979; Downing 1979), thus making it difficult to isolate the effects of single factors (e.g. pollution). Artificial substrates are frequently employed in stream surveys to reduce sampling variability between locations. These devices have also been used colonization rate and community composition of protozoans (Cairns et al. 1980). Burks and Wilhm (1977) employed artificial substrates in experimental streams to document the effects of sewage effluents on aquatic macroinvertebrates.

The purpose of this research was to examine the effects of copper on abundance, number of taxa, and species composition of aquatic insect communities established on artificial substrates. Copper was chosen because it is a ubiquitous pollutant in freshwater systems and because of the availability of field and laboratory data in the literature on the responses of aquatic organisms to this toxicant. Experiments were replicated over three seasons (winter, spring, and summer) in order to examine seasonal variation in the responses of aquatic communities to copper.

Materials and Methods

Artificial substrates (described in Chapter one) were colonized in Adair Run, a second-order tributary to the New River located in Giles County, Virginia. Forty trays were recovered from Adair Run on three occasions: 22 January 1986 (winter), 28 April 1986 (spring), and 24 July 1986 (summer) after 30 d of colonization. Previous experiments conducted in Adair Run during spring of 1985 and 1986 showed that these trays were rapidly colonized by aquatic insects and that the number of individuals and number of taxa per tray became asymptotic within 2-3 weeks (Clements et al. in press a).

Trays were collected by placing a 500-um mesh net directly downstream to prevent loss of organisms and then gently removing each tray from the stream. Contents of 10 trays were were washed through a 500-um sieve, and the organisms retained were preserved in 10% formalin in the field. These samples (field controls) were used to estimate initial macroinvertebrate abundance and community composition. The remaining 30 trays were placed into 7-L coolers (two trays per cooler) filled with stream water. Each of the 15 coolers was supplied with an airstone attached to a 12-volt air pump. Trays were then transferred to the Virginia Tech Ecosystem Simulation Laboratory and the two trays from each cooler were placed into an artificial stream. Each oval, flow-through stream received dechlorinated tap water (drawn from the New River) at a rate of 0.3 L/min. Turnover time in the 13-L streams was approximately 43 min, and water depth in each stream was 10 cm. Current was provided by paddle wheels that maintained an average current velocity of 35.0 cm/sec. A 12L:12D photoperiod was maintained in laboratory experiments with fluorescent lights.

After a 48-h acclimation period, each stream was randomly assigned to one of three treatments: laboratory controls (0 ug/L copper), low dose (25 ug/L copper), and high dose (150 ug/L copper). Peristaltic pumps dripped stock solutions of $CuSO_4$ from separate 20-L carboys into each treated stream at a rate of 6.0 ml/min. After 96 h, trays were removed and the contents gently washed through a 500-um net. All living organisms retained were removed and preserved in 10% formalin.

Water temperature, pH, hardness, alkalinity, and conductivity were measured in both Adair Run and in laboratory streams (Table 4) at the start of each experiment according to U.S. EPA standards (U.S. EPA 1979). In addition to these water quality parameters, the concentration of total copper was measured in each laboratory stream using flameless atomic absorption spectrophotometry (Perkin-Elmer Model 370). In low dose streams, copper concentrations were 32, 17, and 15 ug/L during winter, spring, and summer experiments, respectively. In high dose streams, these concentrations were 178, 168, and 135 ug/L, respectively.

A model I one-way ANOVA with subsamples (Sokal and Rohlf 1981) was employed to test the hypothesis that exposure to copper reduced the number of taxa, number of individuals, Shannon's diversity, and abundance of major orders during each season. Data were transformed using a ln(n+1) transformation since most variables showed a relationship between the mean and variance. If significant differences were detected ($p<0.05$), a Duncan's Multiple Range Test was employed to test for differences between treatments.

Table 4. Water quality parameters from Adair Run (field) and laboratory streams. Laboratory values represent the means of 15 streams. Field data were collected from Adair Run at the start of each experiment.

	Winter	Spring	Summer
Temperature (°C)			
Laboratory	8.3	14.3	20.8
Field	6.5	15.0	21.0
pH			
Laboratory	7.85	8.38	8.44
Field	7.60	8.29	7.80
Hardness (mg/L)			
Laboratory	69.7	64.3	76.7
Field	70.0	60.0	90.0
Alkalinity (mg/L)			
Laboratory	40.8	46.7	65.5
Field	37.2	38.4	68.4
Conductivity (umhos)			
Laboratory	120	115	173
Field	160	131	200

Results

The total number of taxa and number of individuals per tray did not differ significantly between field and laboratory controls during any season (Fig. 6). After 6 d in laboratory control streams, the number of individuals was reduced by only 10 to 12%, and the number of taxa was 0 to 3% lower than in field controls. These results show that colonized substrates can be maintained in laboratory streams for short periods of time with no evidence of control mortality.

Exposure of aquatic insect communities to copper in laboratory streams significantly reduced both the number of taxa and number of individuals in all three seasons (Fig. 6). With one exception (the number of taxa in winter experiments), both parameters were significantly reduced in high dose streams compared to low dose streams, indicating a dose-dependent response to increasing copper concentration. The greatest percent reduction in the number of taxa and number of individuals was observed in summer experiments. Species diversity was also reduced in treated streams, but this parameter was less sensitive to copper exposure than the number of taxa and number of individuals (Table 5). Species diversity did not differ significantly between treatments during the winter and was significantly reduced in the spring only in high dose streams.

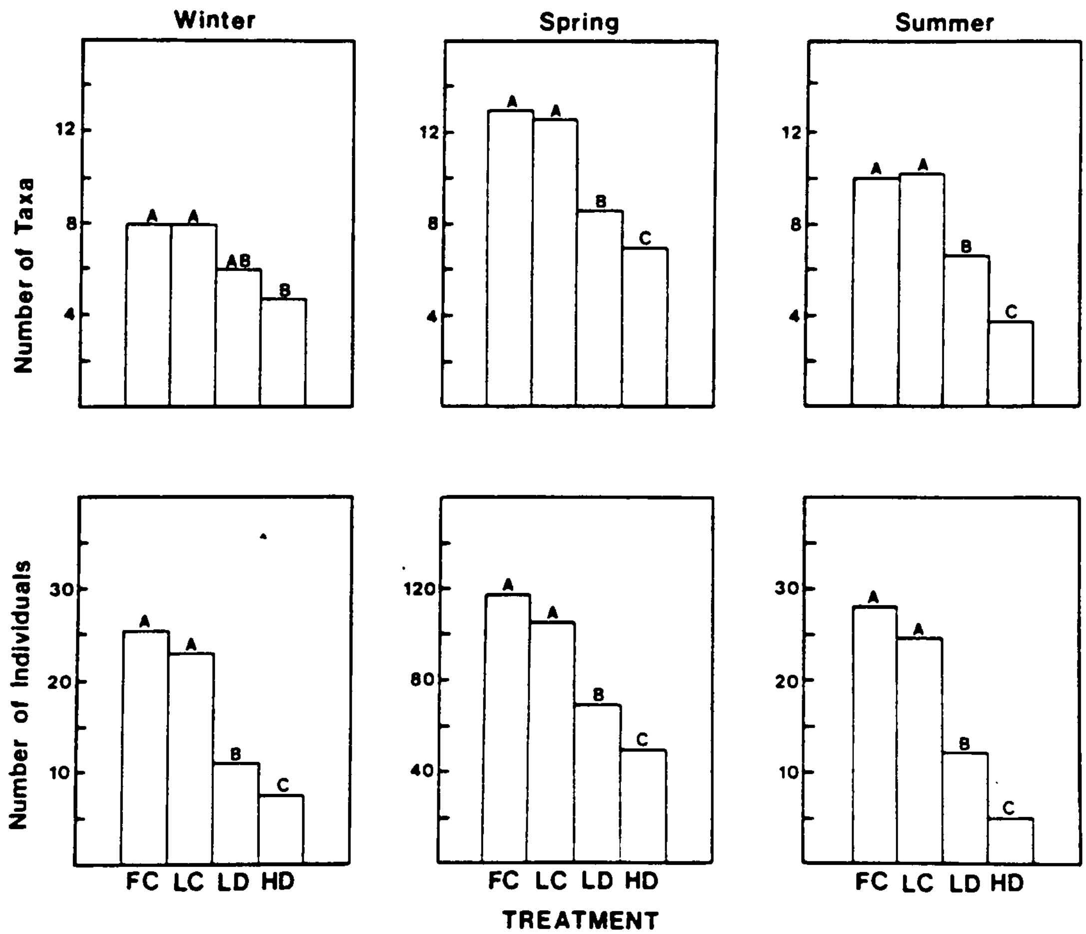

Figure 6. Mean number of taxa and number of individuals per tray from field controls (FC), laboratory controls (LC), low dose (LD), and high dose (HD) treatments. Treatments with the same letter were not significantly different based on Duncan's Multiple Range Test.

Table 5. Mean diversity values from field controls, laboratory controls, low dose, and high dose treatments during each season. Standard deviations are given in parentheses. Means with the same letter were not significantly different based on Duncan´s Multiple Range Test.

	Field controls	Laboratory controls	Low dose	High dose	F-value
Winter	2.47 (0.43)	2.47 (0.37)	2.29 (0.53)	1.98 (0.56)	2.87
Spring	1.85 a (0.49)	1.80 a (0.17)	1.54 a (0.53)	1.09 b (0.32)	6.52*
Summer	2.91 a (0.24)	2.99 a (0.18)	2.67 b (0.30)	1.85 c (0.21)	72.74**

* $p<0.01$
** $p<0.0001$

The effects of copper exposure on the three dominant orders of aquatic insects varied between seasons and groups (Fig. 7). Ephemeroptera were highly sensitive to copper exposure in laboratory streams. These organisms were significantly reduced in both low and high dose streams during each season, with the greatest effects observed in summer experiments. Dipterans were significantly reduced in treated streams during spring and winter experiments. Seasonal differences in the response of Diptera to copper are difficult to explain since chironomids, the dominant group, were identified only to the level of tribe. Plecoptera were significantly reduced in treated streams compared to laboratory controls only during the spring experiment. Owing to differences in sensitivity to copper, the percent composition of Ephemeroptera, Diptera, Plecoptera, and other aquatic insects varied among treatments. The relative abundance of mayflies decreased in dosed streams during each season, whereas dipterans increased in winter and spring experiments. Similarly, the percent composition of plecopterans increased in treated streams during winter and summer experiments.

The relative abundance of dominant taxa within these groups also varied between treatments and seasons (Table 6). Baetis sp., the dominant mayfly collected, was reduced by 85 to 98% in low dose streams during each season and was eliminated in high dose streams in spring and summer experiments. The response of other common mayflies was variable, but these

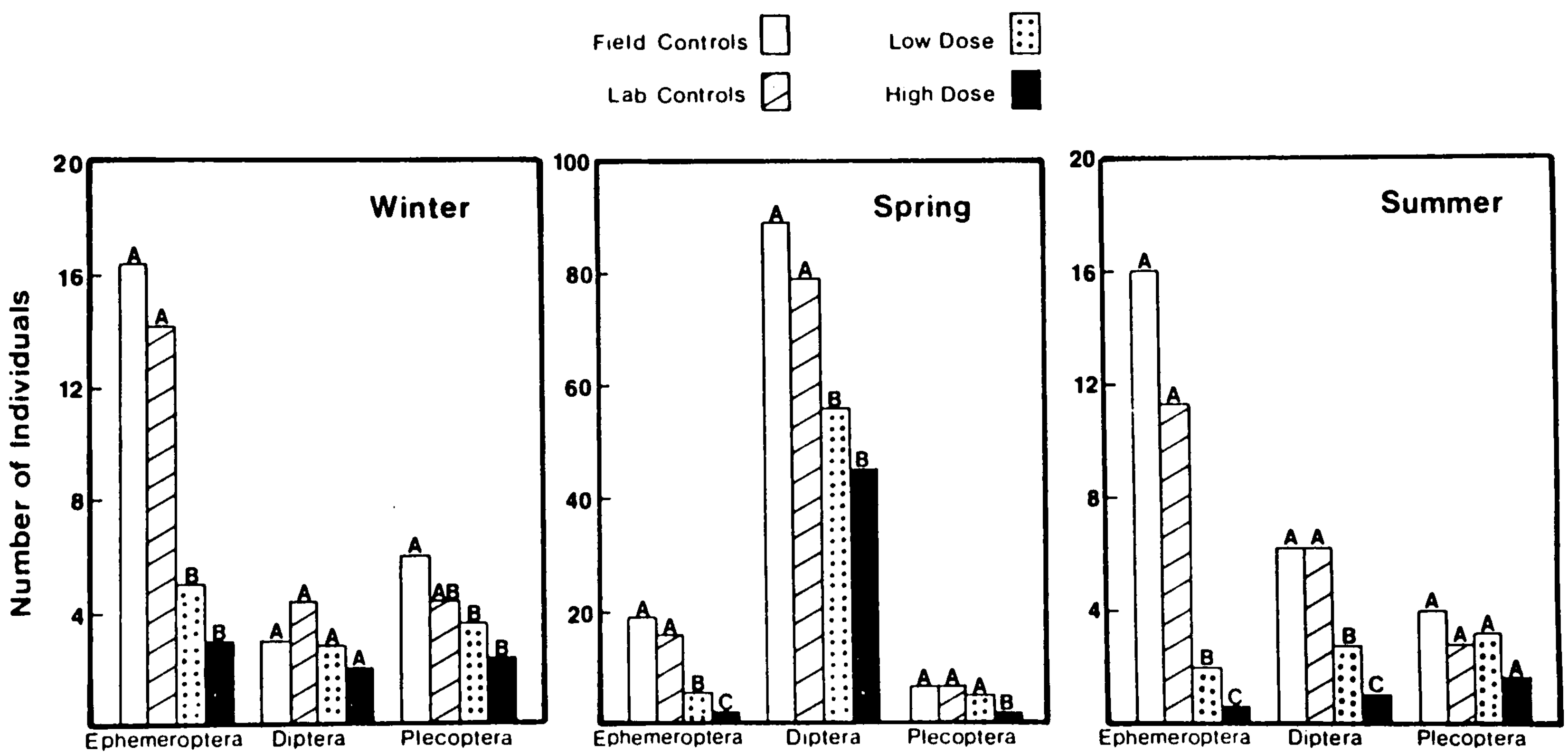

Figure 7. Mean number of Ephemeroptera, Diptera, and Plecoptera per tray from field controls, laboratory controls, low dose, and high dose treatments. Treatments with the same letter were not significantly different.

Table 6. Relative abundance of dominant taxa from field controls, laboratory controls, low dose, and high dose streams. Only those taxa that comprised >1.0% in field controls were included.

	Field controls	Laboratory controls	Low dose	High dose
Winter				
Ephemeroptera				
Baetis sp.	38.3	43.2	18.0	13.3
Pseudocloeon sp.	4.7	3.9	3.6	5.3
Ameletus sp.	6.6	2.6	3.6	2.7
Isonychia sp.	5.0	3.9	4.5	1.3
Plecoptera				
Allocapnia sp.	21.1	16.2	27.9	29.3
Diptera				
Orthocladiini	6.6	14.0	15.3	18.7
Prosimulium sp.	4.7	3.0	6.3	8.0
Spring				
Ephemeroptera				
Baetis sp.	7.1	5.5	0.1	0.0
Habrophlebiodes sp.	3.2	2.3	2.0	0.4
Heptagenia sp.	2.8	2.9	0.9	0.0
Eurylophella sp.	0.9	0.6	3.1	2.2
Plecoptera				
Perlesta sp.	3.3	3.6	2.8	0.0
Amphinemura sp.	1.5	2.2	3.2	1.0
Diptera				
Orthocladiini	69.3	70.7	75.7	81.9
Chironomini	3.1	1.5	3.4	3.4
Tanypodini	2.0	1.6	2.5	2.6
Summer				
Ephemeroptera				
Baetis sp.	26.7	16.3	2.6	0.0
Heptagenia sp.	13.2	7.7	5.7	4.1
Ephemera sp.	4.3	17.0	9.8	4.1
Stenonema sp.	3.6	2.8	4.9	2.0
Plecoptera				
Acroneuria sp.	4.6	4.5	4.9	20.4
Diptera				
Orthocladiini	5.0	9.3	5.7	10.2
Chironomini	8.2	11.0	11.5	12.2
Megaloptera				
Nigronia sp.	7.1	4.5	11.5	12.3

organisms were generally less sensitive to copper than Baetis sp. The relative abundance of Orthocladiini, the dominant chironomid collected, increased in treated streams relative to controls in winter and spring experiments. Seasonal differences in the response of plecopterans to copper may be explained by the taxonomic composition of this group, which varied among experiments. Winter and summer samples were dominated by Allocapnia sp. and Acroneuria sp., respectively, which were relatively insensitive to copper. In contrast, spring experiments were dominated by Perlesta sp., which was reduced by 50% in low dose streams and completely eliminated in high dose streams.

The percent composition of functional feeding groups also varied between seasons and treatments (Fig. 8). Collector-gatherers dominated field and laboratory control streams during each season. The percent composition of this group decreased in treated streams in winter and summer experiments but increased slightly in the spring. Collector-gatherers in spring experiments were dominated by chironomids, which were less sensitive to copper than the mayflies that dominated winter and summer samples.

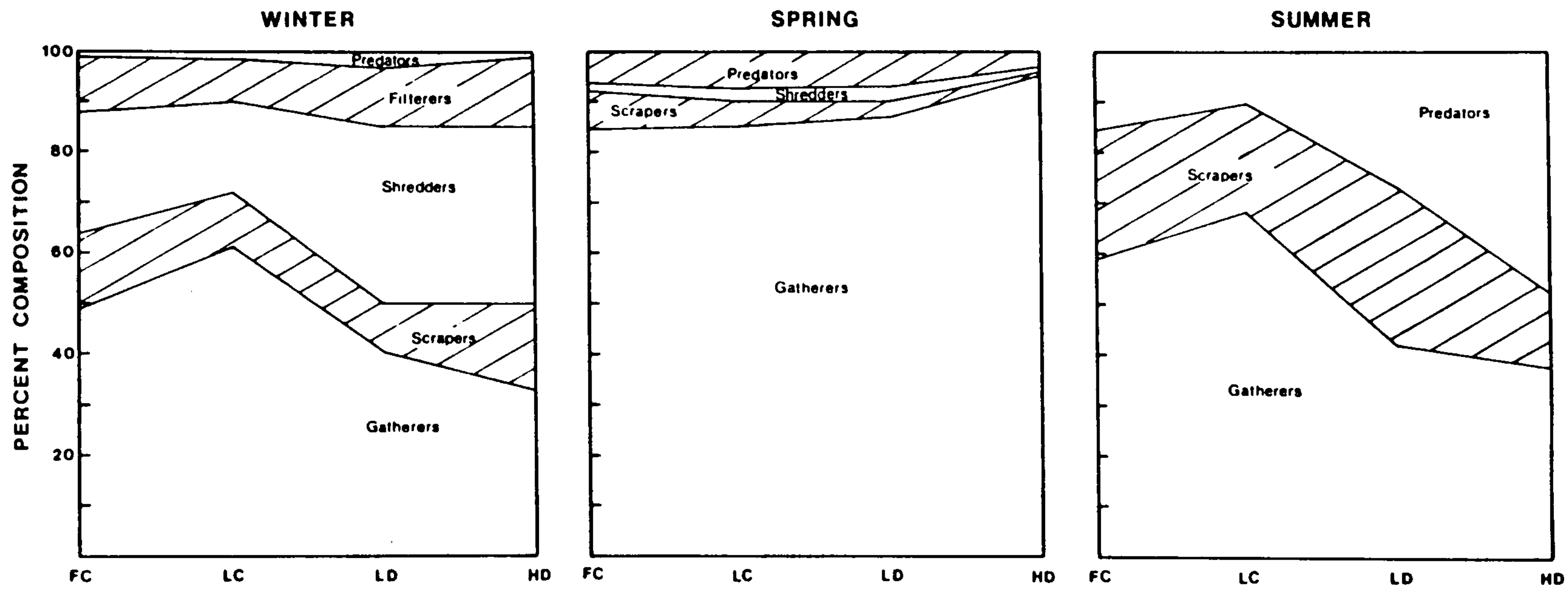

Figure 8. Percent composition of dominant functional feeding groups from field controls (FC), laboratory controls (LC), low dose (LD), and high dose (HD) treatments.

Discussion

Results of this study demonstrate that simple community level parameters, such as number of taxa and number of individuals, were highly sensitive to copper exposure. Within 96 h in low dose streams (15-32.0 ug/L), the number of taxa per tray was reduced by 24 to 36% and the number of individuals was reduced by 35 to 52% relative to laboratory controls. Winner (1975) compared the sensitivity of several community level parameters (number of individuals, number of taxa, Shannon's diversity, Margalef's diversity) and concluded that simpler indices, such as the number of taxa, were most useful for predicting the impact of copper in an experimentally polluted stream. In my experiments, species diversity was significantly reduced in low dose streams only during the summer. Derived indices, such as species diversity, are less useful for demonstrating the impact of heavy metals since they often show little variation between control and impacted sites (Lapoint et al. 1984). Unlike many forms of organic pollution, particularly nutrient enrichment, in which macroinvertebrate abundance may actually increase owing to dominance by a few tolerant species, heavy metals are usually toxic to most aquatic organisms. Consequently, these derived variables are less sensitive to the impact of metals.

The effects of copper on Ephemeroptera, the dominant aquatic insects in winter and summer experiments, showed some seasonal variation. These organisms, particularly _Baetis_ sp., were greatly reduced in treated streams during each season but were especially sensitive during summer when water temperatures were higher. Several other studies have demonstrated the sensitivity of mayflies to metals in both the field (Winner et al. 1975, 1980; Geckler et al. 1976; LaPoint et al. 1984; Van Hassel et al. 1986; Clements et al. in press b) and laboratory (Warnick and Bell 1969; Specht et al. 1984). Warnick and Bell (1969) reported a 96 h-LC_{50} of 320 ug/L copper for *Ephemerella subvaria* while Specht et al. (1984) calculated an LC_{50} of 180 ug/L copper for *Stenonema pudicum*. In experiments described here, exposure of 15 to 32 ug/L copper reduced the total number of mayflies by 64 to 82%, suggesting that these communities were considerably more sensitive to metals than indicated by single species tests. The effects of copper exposure on abundance of dipterans and plecopterans varied seasonally, but was generally less severe than for mayflies.

Differences in relative sensitivity of aquatic insects to heavy metal pollution may result in shifts in their percent composition at field impacted sites. For example, Geckler et al. (1976) reported reduced abundance of most macroinvertebrate groups but increased numbers of chironomids at sites impacted by copper. Changes in relative abundance of aquatic insects between control and impacted sites in the field have been proposed as an index of heavy metal pollution

(Winner et al. 1975, 1980; LaPoint et al. 1984; Van Hassel and Gaulke 1986). For example, Winner et al. (1980) showed that the percent chironomids in benthic samples was highly correlated with copper concentration in impacted streams and concluded that the macroinvertebrate community exhibited a "predictable, graded response to heavy metals." In my experiments, the percent chironomids increased in treated streams during winter and spring, but was relatively constant during summer. Van Hassel and Gaulke (1986) proposed that the percent reduction in mayflies at stations impacted by copper and zinc could be used as an index of metal pollution and to establish site-specific copper criteria. In my experiments, the percentage of mayflies was reduced in treated streams during each season; however, because of species specific differences in sensitivity to copper (e.g. Table 6), seasonal changes in the relative abundance of dominant taxa may complicate the usefulness of this index.

The use of changes in macroinvertebrate community structure and distribution in the field to assess the impact of heavy metals is an important supplement for single species bioassays. Owing to complex interactions between aquatic organisms and various habitat features, particularly current and substrate, reduced abundance and/or species richness observed downstream of a particular effluent does not actually demonstrate that the effluent is responsible. LaPoint et al. (1984) argue that experimental studies are necessary to show direct cause and effect relationships. The experimental approach employed by Geckler et al. (1976) at Shayler Run is certainly the most direct method of estimating community and ecoysytem level responses to toxicants. Although experimental introduction of toxicants into natural streams can provide the strongest evidence for cause and effect relationships between the presence of toxicants and macroinvertebrate community structure (LaPoint et al. 1984; Cuffney et al. 1985; Hall et al. 1985), there are a number of serious problems associated with this approach that will limit its usefulness (Cairns 1981). In addition, Hurlbert (1984) discusses the statistical problems of field experimentation that require replication of both control and treated streams. The approach that I have outlined here, using colonized artificial substrates for toxicity testing, may represent an alternative to field experimentation and an important supplement to single species tests. This approach provides a unique opportunity to examine community level responses to toxicants under both replicable and environmentally realistic conditions and may be particularly useful during initial screening of an effluent, prior to its release into the environment. For example, substrates could be colonized in the proposed receiving system, transferred to outdoor experimental streams, and dosed with the proposed effluent. Changes in macroinvertebrate community structure, number of taxa, and number of individuals observed in treated streams could then be employed to predict the impact of these effluents in the field.

CHAPTER THREE

The Impact of Heavy Metals on Macroinvertebrate Communities: A Comparison of Observational and Experimental Results

Abstract

Exposure of natural assemblages of aquatic macroinvertebrates to low levels of copper and zinc (mean concentrations = 12 ug/L and 15 ug/L, respectively) in outdoor experimental streams significantly reduced the number of taxa, number of individuals, and abundance of most dominant taxa within 4 d. After 10 d, macroinvertebrate responses to metals in these streams were similar to those observed at the Clinch River, a system receiving heavy metal effluents. The effects of these effluents varied among groups of aquatic insects in both the field and in experimental streams. Ephemeroptera and Tanytarsini chironomids were the most sensitive groups examined. *Isonychia bicolor*, one of the dominant mayflies collected, was reduced by 99% within 4 d in treated streams. In contrast, Orthocladiini chironomids were highly tolerant of heavy metals. Abundance of these organisms did not differ significantly between reference and low effluent sites in the field, and abundance was significantly greater in treated experimental streams than in controls after 10 d. Heavy metal tolerance of Hydropsychidae was intermediate between these groups. After 10 d in treated streams, abundance of these organisms was reduced by ~50%. Caddisflies were, however,

more sensitive to heavy metals in the field than in experimental streams. The similarity of my experimental results to those obtained from Clinch River field sites suggests that outdoor stream mesocosms may be employed to predict community responses of aquatic insects to heavy metals.

Introduction

Production of heavy metals and their discharge into aquatic receiving systems have increased greatly in recent years (Moore and Ramamoorthy 1984). The effects of these effluents on aquatic invertebrates have been well documented in the laboratory (Warnick and Bell 1969; Brown 1977; Spehar et al. 1978; Kosalwat and Knight 1987). Although results of these experiments show considerable variation between species, in general most aquatic invertebrates are quite sensitive to heavy metals (Hodson et al. 1979). Several investigators however, have noted the limitations of laboratory bioassays and have suggested that these tests be supplemented with more environmentally realistic procedures, including field sampling and experimentation (Cairns 1983, 1986; Odum 1984; Kimball and Levin 1985). Traditionally, most field research on the impact of heavy metals has been purely descriptive (Sprague et al. 1965; Armitage 1980; Ramusino et al. 1981; LaPoint et al. 1984; Chadwick et al. 1986; Clements et al. in press a), and, consequently, few attempts have been made to predict changes in macroinvertebrate communities exposed to these effluents. An important exception to the descriptive approach employed by most field researchers is the work conducted at Shayler Run by Winner et al. (1975; 1980). These investigators monitored changes in macroinvertebrate communities exposed to copper (Cu) in experimentally-polluted streams and concluded that these communities showed predictable changes in structure along a gradient of heavy metals stress.

While such experimental manipulations are the most direct method of determining cause and effect relationships between toxicants and macroinvertebrate community structure (LaPoint et al. 1984), logistical and economic considerations make this an unlikely approach on a large scale. This conclusion is particularly meaningful given the recent emphasis on development of site-specific water quality criteria (Carlson et al. 1986; Van Hassel and Gaulke 1986). An alternative approach, advocated by Odum (1984), involves the use of mesocosms or "bounded and partially enclosed outdoor experimental" systems.

I used laboratory experimental streams containing natural assemblages of aquatic insects (Clements et al. in press b) to show that community-level parameters (number of taxa, number of individuals) were highly sensitive to Cu exposure and suggested that this approach may be employed to predict effects of heavy metals in the field. Here I examined macroinvertebrate community responses to heavy metals, Cu and zinc (Zn), in outdoor experimental streams. To demonstrate the predictive ability of my approach, I compared structural alterations of these communities to those observed at the Clinch River, a system receiving heavy metals effluents.

Materials And Methods

Study site

Eight sampling stations were selected at the Clinch River, a fourth-order tributary to the Tennessee River located in Russell County, Virginia (see Fig. 3, chapter 1). The river is ~60 m wide and depth ranges from 0.5-0.8 m at each station. Substrate is dominated by large pebble and intermediate cobble. Reference stations 1 and 2 were located upstream, and reference station 7 was located directly across from a coal-fired electric generating plant. Station 8 was located 50 m downstream of a cooling tower blowdown effluent, and Station 11 was located 30 m below a second, low volume waste discharge. These two stations represented high metals sites, whereas station 13, located 1.0 km farther downstream, was noted as a low metals site. Recovery stations 15 and 16 were located 3.0 and 4.5 km downstream of the plant, respectively.

Colonization dynamics and Clinch River biomonitoring

To determine the impact of heavy metals on colonization rate, in August, 1986, 36 trays (10 x 10 x 6 cm) containing pebble and small cobble substrate were placed at each of stations 2 (reference), 13 (low metals), and 8 (high metals). Previous studies at the Clinch River (Clements et al. in press a) showed that only six of these trays were necessary to detect a 15% reduction in number of individuals and a 12% reduction in number of taxa at effluent stations. Six trays were sampled from each station after 6, 12, 18, 30, 36, and 42 d of colonization by placing a 500-um mesh net directly downstream and then removing the tray from the stream. Contents of each tray were washed through a 500-um sieve and preserved in 10% formalin. In the laboratory, organisms were sorted in white enamel pans and, except for chironomids, identified to the level of genus or species. Chironomids were identified to tribe.

Six trays were also collected from stations 1, 7, 11, 15, and 16 on day 30 of the colonization study to examine the effects of heavy metal effluents on the spatial distribution of benthic macroinvertebrates in the Clinch River. All samples were collected and processed as described above.

<u>Experimental streams and Clinch River study</u>

Benthic communities for the experimental stream studies were established on trays placed at reference station 2 in the Clinch River. After 30-d colonization, 48 trays were removed and placed into 12 separate 7-L coolers (4 trays per cooler) filled with stream water. Each cooler was supplied with an airstone and attached to a 12-V air pump. Trays were transferred to the Glen Lyn Research Site, located on the banks of the New River in Giles County, Virginia, and the trays in each cooler were placed into a separate experimental stream. Each oval, flow-through stream (76 x 46 x 14 cm) was supplied with unaltered water drawn from the New River at a rate of 1.6 L/min, resulting in a turnover time of 13 min. Current in the streams was provided by paddle-wheels, which maintained a constant velocity of 42 cm/s.

After 2-d of acclimation, streams were randomly assigned to one of three treatments: controls (ambient Cu and Zn levels), low metals (12 ug/L Cu and Zn), and high metals (50 ug/L Cu and Zn). These target values of Cu and Zn in high metals streams were lower than concentrations previously measured at station 8. Reduced target concentrations were used in experimental streams because of lower water hardness of the New River, which generally increases toxicity of metals (Winner and Gauss 1986). Peristaltic pumps dripped stock solutions of Cu and Zn sulfate from 20-L carboys into each treated stream at a rate of 6 mL/min. After 4 and 10 d of exposure to metals, two trays from each stream were removed and processed as described above. Note that trays collected on days 4 and 10 corresponded to days 36 and 42 of the colonization study, respectively. Thus, trays collected from control streams were compared to trays from reference station 2 at the Clinch River. Similarly, trays collected from treated streams were compared to those from effluent sites in the field.

Water quality

Water temperature, dissolved oxygen, current velocity, pH, conductivity, hardness, alkalinity, and metals concentrations were measured weekly at all field sites, except for station 16. Water samples were collected from this station only on day 30, and metals concentrations were not determined. Water quality data were collected from each experimental stream on days 4 and 10. Concentrations of total Cu and Zn were measured with a flameless atomic absorption spectrophotometer (Perkin-Elmer Model 370).

Statistical analyses

Design of the experimental streams studies included three treatments, four replicate streams, and two subsamples within each stream. Differences between treatments in these streams and between sampling stations in the field were determined using one-way ANOVA. All analyses were performed on ln(n+1) transformed data, as preliminary analyses revealed a relationship between means and variances for most variables. If differences in the overall F-test were detected ($p<0.05$), Duncan´s Multiple Range Test was used to determine which treatments (or stations) were significantly different from others.

I estimated equilibrium numbers of individuals and taxa for the colonization study using a nonlinear regression procedure. Colonization data were fitted to a negative exponential growth equation and estimated equilibrium values were compared to observed values.

Results

Water quality

Water temperature, dissolved oxygen, current velocity, and pH were similar at the Clinch River and in experimental streams (Table 7). Other parameters, however, varied between these locations. Conductivity and alkalinity were both greater in the Clinch River. More importantly, water hardness was approximately twice as high in the Clinch River as in experimental streams.

Mean Cu and Zn concentrations at effluent stations in the Clinch River were generally higher than in treated experimental streams (Table 7). The highest concentrations in the field were recorded from stations 8 and 11. Metals concentrations were greatly reduced at station 13, the low effluent site, and were comparable to upstream reference sites at station 15. Measured concentrations of Cu and Zn in low metals experimental streams approximated target values, whereas measured values were ~50% less than target concentrations in high metals streams.

Table 7. Water chemistry data from the Clinch River and from outdoor experimental streams. Field values represent the means of weekly samples (n=5) collected in August and September, 1986. Experimental streams data (n=8) were collected on days 4 and 10.

	Clinch River								Experimental streams		
	1	2	7	8	11	13	15	16[a]	Control	Low	High
Temperature (°C)	22.4	22.6	21.6	21.8	22.3	22.7	22.6	21.0	22.1	22.4	22.0
Dissolved Oxygen (mg/L)	8.1	8.0	7.9	7.9	7.9	7.9	8.1	8.2	9.3	8.9	8.4
Current (cm/s)	12.9	45.9	35.5	47.5	30.2	46.0	33.8	38.2	42.0	42.0	42.0
pH	8.40	8.45	8.43	8.36	8.17	8.41	8.32	8.46	8.91	8.93	8.90
Conductivity (umohms)	298	299	318	364	357	327	323	315	145	146	146
Hardness (mg/L)	159	156	170	186	185	164	166	168	90	85	85
Alkalinity (mg/L)	145	156	156	117	143	162	160	141	56	57	57
[Cu] (ug/L)	nd[b]	3	nd	105	47	9	nd	-[c]	nd	12	20
[Zn] (ug/L)	14	10	12	81	42	18	6	-	nd	15	27

[a] n=1; [b] nd=not detected; [c] -=no data collected.

Colonization dynamics

After 6 d, and on each subsequent sampling occasion, both the number of taxa and number of individuals per tray were significantly greater at reference station 2 than at either effluent stations 8 or 13 (Fig. 9). Colonization at the reference station was rapid during the first 18 d, after which the number of taxa and number of individuals became asymptotic and little additional increase occurred after day 30. Both the observed number of taxa and individuals per tray were within the 95% confidence intervals of estimated equilibrium values by day 30, as determined by nonlinear regression analyses (Table 8).

Trays collected from station 2 on days 6 and 12 were dominated by Orthocladiini and Chironomini, which accounted for 43-46% of the total organisms. These early colonists were gradually replaced by net-spinning caddisflies (*Hydropsyche morosa* and *Cheumatopsyche* sp.), which increased in relative abundance from 3% on day 6 to 38% on day 42. The relative abundance of mayflies (*Isonychia bicolor*, *Baetis brunneicolor*, *Stenonema modestum*, *Tricorythodes* sp., *Caenis* sp., *Pseudocloeon* sp., and *Potamanthus* sp.) also increased from 20% on day 6 to 32% on day 42.

Macroinvertebrate abundance was significantly greater at station 13 than at station 8 on each sampling date, except day 18 (Fig. 9). Abundance at this low effluent site was greatest on day 36, but was still reduced to only 32% that of

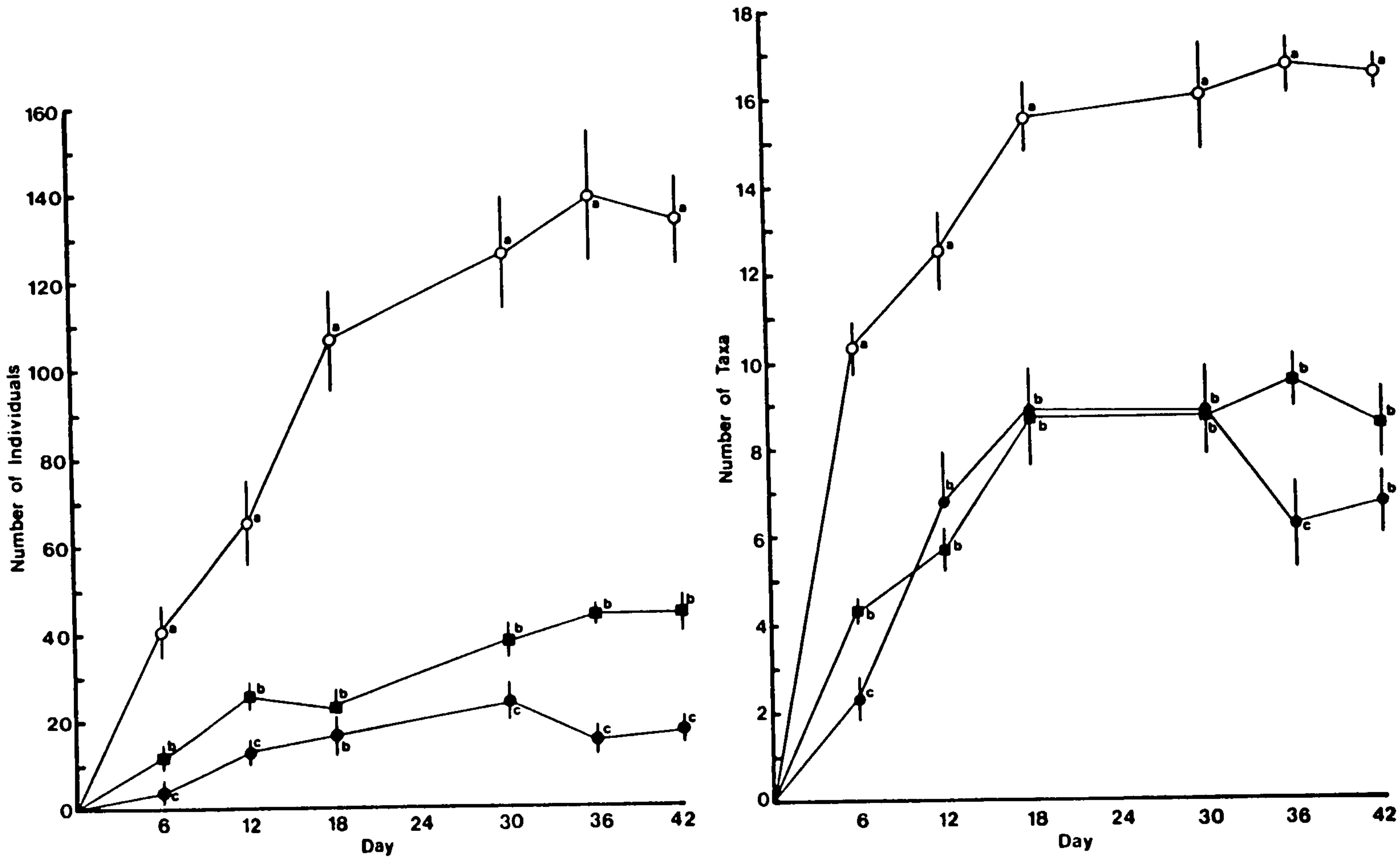

Figure 9. Colonization of introduced substrates at stations 2 (open circles), 8 (closed circles), and 13 (closed squares). Each point represents the mean number per tray (n=6) $\pm$SE. Means with the same letter were not significantly different on a given day.

Table 8. Results of nonlinear regression analysis for estimating the equilibrium number of individuals and number of taxa for the Clinch River colonization study.

	Number of individuals		Number of taxa	
	Observed	Estimated (95% C.I.)	Observed	Estimated (95% C.I.)
Station 2	126.3	157.4 (119.3-195.5)	16.0	16.4 (15.4-17.4)
Station 13	38.0	55.6 (38.2-73.1)	8.7	9.2 (8.1-10.4)
Station 8	23.8	20.0 (13.4-26.7)	8.8	7.7 (6.3-9.1)

the reference station. Significant differences in number of taxa between these effluent stations occurred only on days 6 and 36. Trays from station 13 were dominated by Orthocladiini, which accounted for 42-66% of all organisms collected. Relative abundance of hydropsychid caddisflies increased from 8% on day 6 to 29% on day 42, with a concomitant decrease in Chironomini. Mayflies were rare at this station (<2 individuals per tray), and therefore trends in their relative abundance were not apparent. Similarly, trends in relative abundance of dominant macroinvertebrates at the high effluent site were not obvious due to reduced total number of individuals.

Clinch River biomonitoring

Both the number of taxa and number of individuals per tray were significantly reduced at all effluent stations compared to upstream reference sites (Fig. 10). The greatest reduction in both variables was observed at station 11, the second effluent site. The number of taxa increased at downstream recovery sites, but was still slightly lower than at upstream reference stations. Duncan's Multiple Range Test indicated that the number of taxa at station 16 was significantly less than reference stations 1 and 7. Macroinvertebrate abundance also increased downstream and was slightly, although not significantly, greater at recovery stations than at reference sites. This increase was primarily the result of

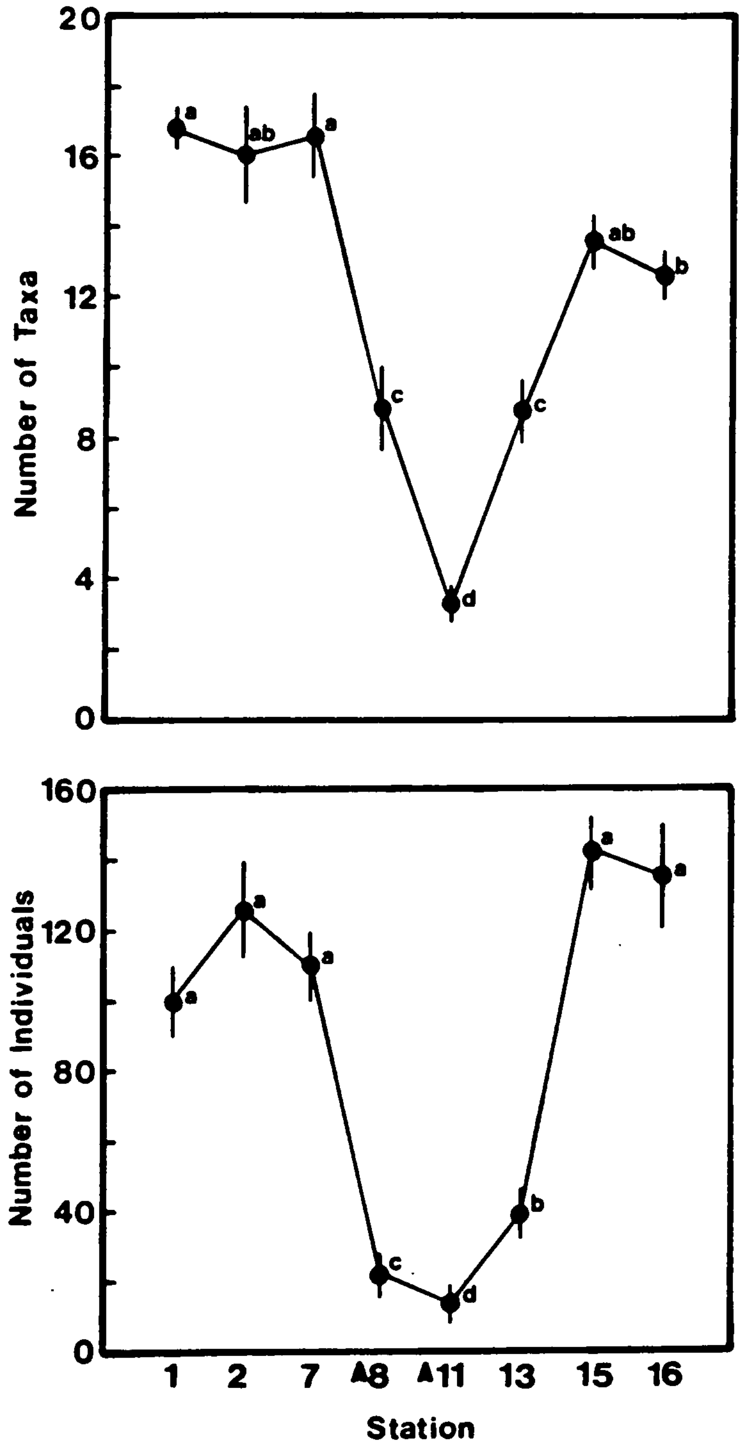

Figure 10. Number of taxa and number of individuals at sampling stations in the Clinch River. Each point represents the mean number per tray (n=6) ±SE. Means with the same letter were not significantly different.

greater abundance of Hydropsychidae and Orthocladiini at stations 15 and 16.

The spatial distribution of dominant groups at the Clinch River was clearly influenced by heavy metal effluents (Fig. 11). Except for Orthocladiini, abundance of each group was significantly reduced at metals sites compared to upstream reference stations. In contrast to other groups, Ephemeroptera showed no indication of recovery at station 13, the low effluent site. While mayfly abundance increased at station 15, complete recovery did not occur until station 16.

Tanytarsini chironomids were also significantly reduced at all metals stations; however, their distribution among both reference and recovery sites was highly variable. For example, abundance was significantly greater at reference station 2 than either stations 1 or 7. Complete recovery of Tanytarsini occurred at station 15, but abundance was again reduced at station 16.

The number of Hydropsychidae also varied among upstream reference sites and was significantly reduced at station 1 compared to stations 2 and 7. Caddisflies recovered at station 15, and abundance was significantly greater at both recovery sites than reference stations 1 and 2.

Orthocladiini chironomids were considerably more tolerant of heavy metal effluents that other groups. The only pairwise comparison showing significant differences among upstream reference and high effluent sites were stations 7

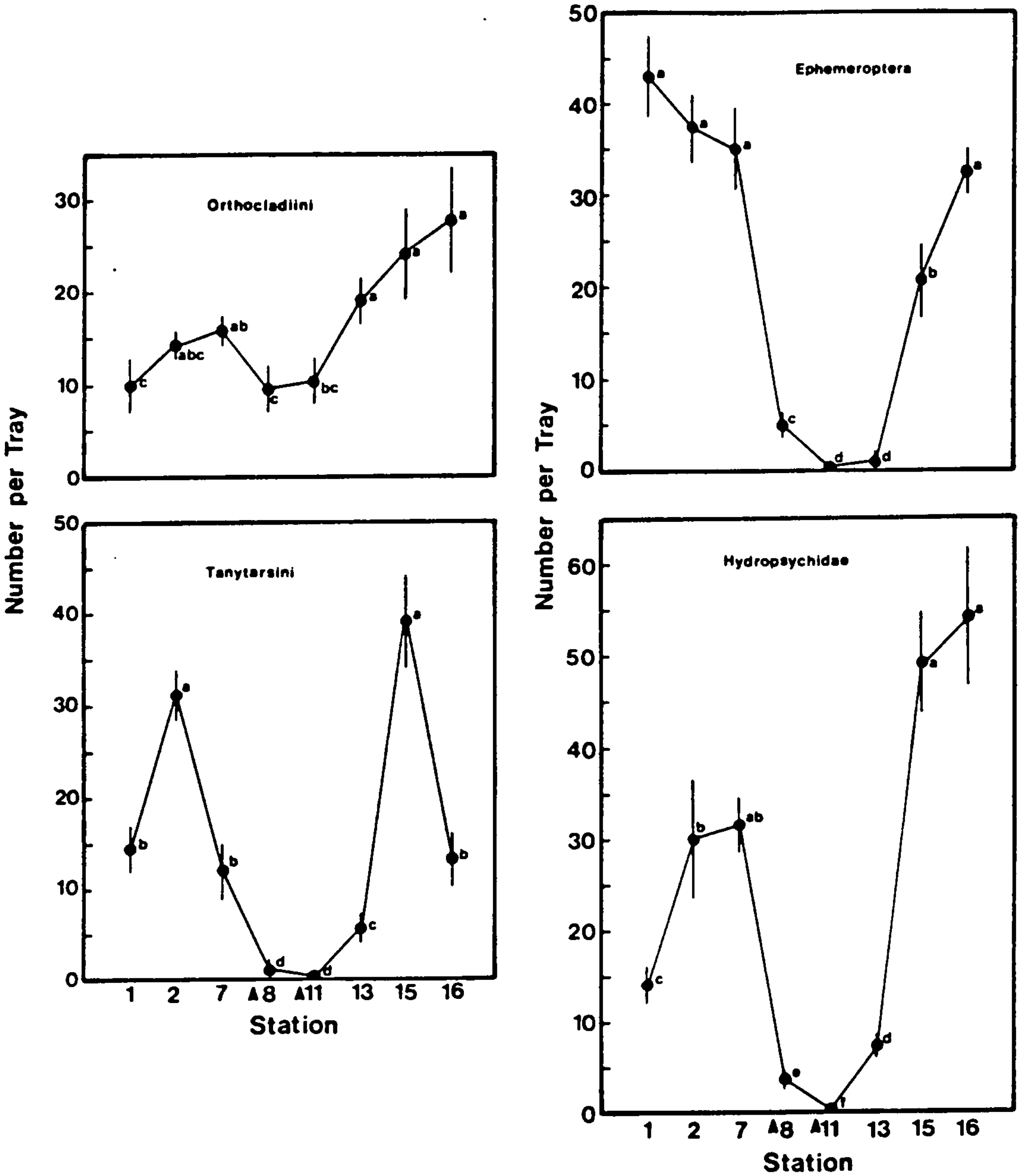

Figure 11. Numbers of Ephemeroptera, Hydropsychidae, Orthocladiini, and Tanytarsini at sampling stations in the Clinch River. Key same as figure 10.

and 8. Abundance of Orthocladiini was actually greater at station 13, the low effluent site, than at reference station 1, and abundance continued to increase downstream.

Comparison of Clinch River and experimental streams

Macroinvertebrate community responses to heavy metals in experimental streams were similar to those in the Clinch River (Fig. 12). Within four days, both the number of taxa and number of individuals were significantly reduced in treated streams compared to controls (Table 9). In addition, macroinvertebrate abundance was actually greater in control experimental streams than at the reference station on both sampling days. Despite greater abundances in these streams, the shapes of the curves for the experimental streams and Clinch River field sites were virtually identical by day 10.

The responses of dominant groups of aquatic insects (Ephemeroptera, Hydropsychidae, Orthocladiini, and Tanytarsini) to heavy metals in experimental streams were also similar to those observed at the Clinch River, particularly on day 10 (Fig. 13). Ephemeroptera and Tanytarsini were the most sensitive organisms examined. Both groups were significantly reduced in low metals streams and at the low effluent site on days 4 and 10. Although abundance of Tanytarsini decreased in control experimental streams on day 10, this response was also observed in the field. Mayfly abundance increased in control streams on day 10 and was greater in

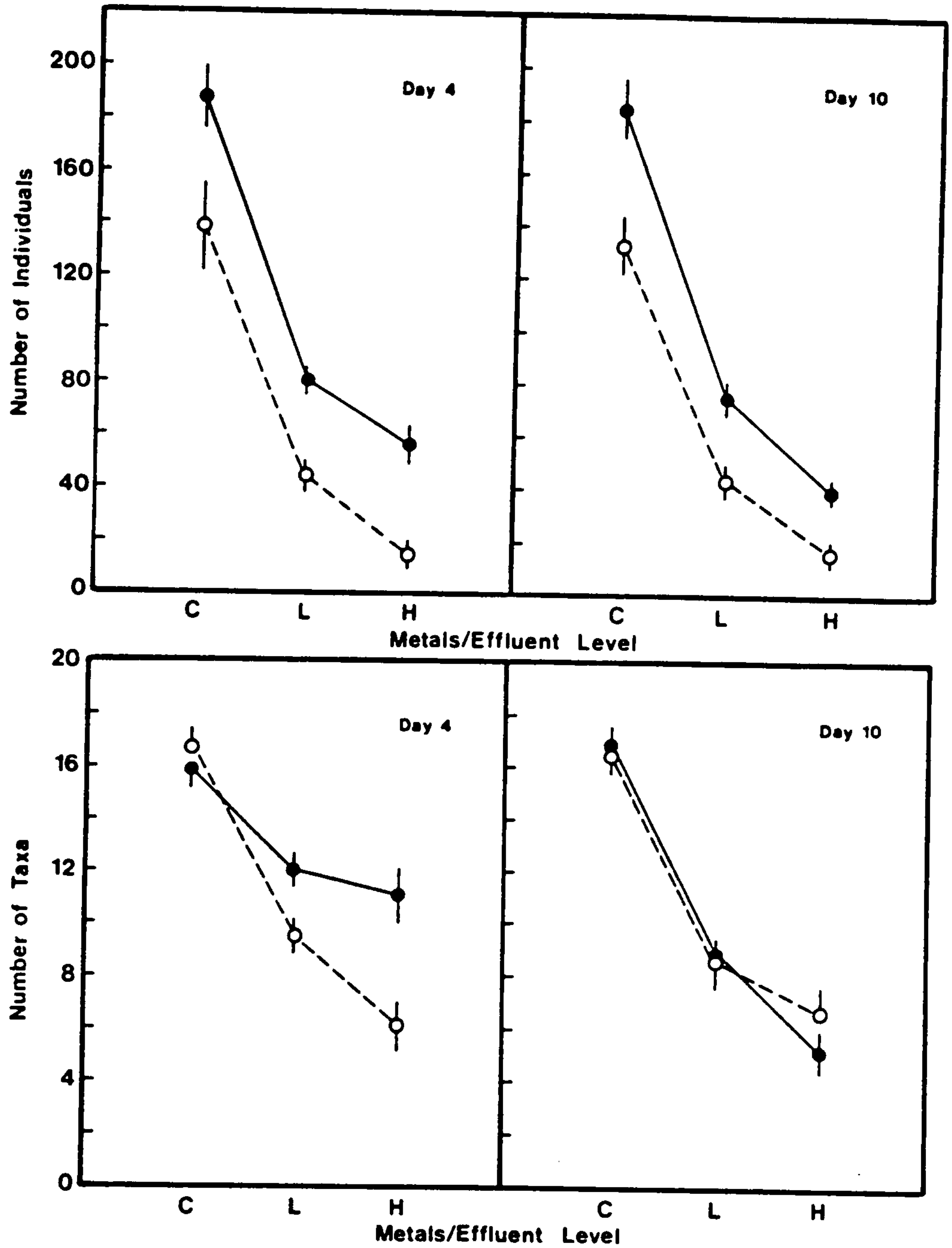

Figure 12. Effects of heavy metals in experimental streams (solid lines) and the Clinch River (dashed lines) on days 4 and 10. C, L, and H refer to control, low, and high metals/ effluents levels, respectively. Each point represents the mean (n=4 for experimental streams; n=6 for the Clinch River samples) ±SE.

Table 9. Results of one-way ANOVA and Duncan's Multiple Range Test for the Clinch River and experimental streams comparisons. Given are F-values and levels of significance for number of individuals, number of taxa, and dominant groups. C=control, L=low, and H=high metals/effluent levels. levels. Underlined treatments were not significantly different.

	Day 4				Day 10			
	Clinch River		Experimental streams		Clinch River		Experimental streams	
Individuals	84.2^{a}	CLH	97.6^{a}	CLH	65.3^{a}	CLH	135.8^{a}	CLH
Taxa	24.5^{a}	CLH	18.0^{b}	CLH	27.3^{a}	CLH	31.1^{a}	CLH
Ephemeroptera	36.7^{a}	CHL	79.9^{a}	CLH	114.9^{a}	CHL	157.1^{a}	CLH
Hydropsychidae	53.7^{a}	CLH	4.8^{c}	CLH	31.0^{a}	CLH	9.8^{c}	CLH
Tanytarsini	64.3^{a}	CLH	20.9^{a}	CLH	24.9^{a}	CLH	250.3^{a}	CLH
Orthocladiini	9.9^{c}	LCH	21.9^{a}	CLH	7.0^{c}	LCH	11.1^{c}	LCH

$^{a}p<0.0001$; $^{b}p<0.001$; $^{c}p<0.01$.

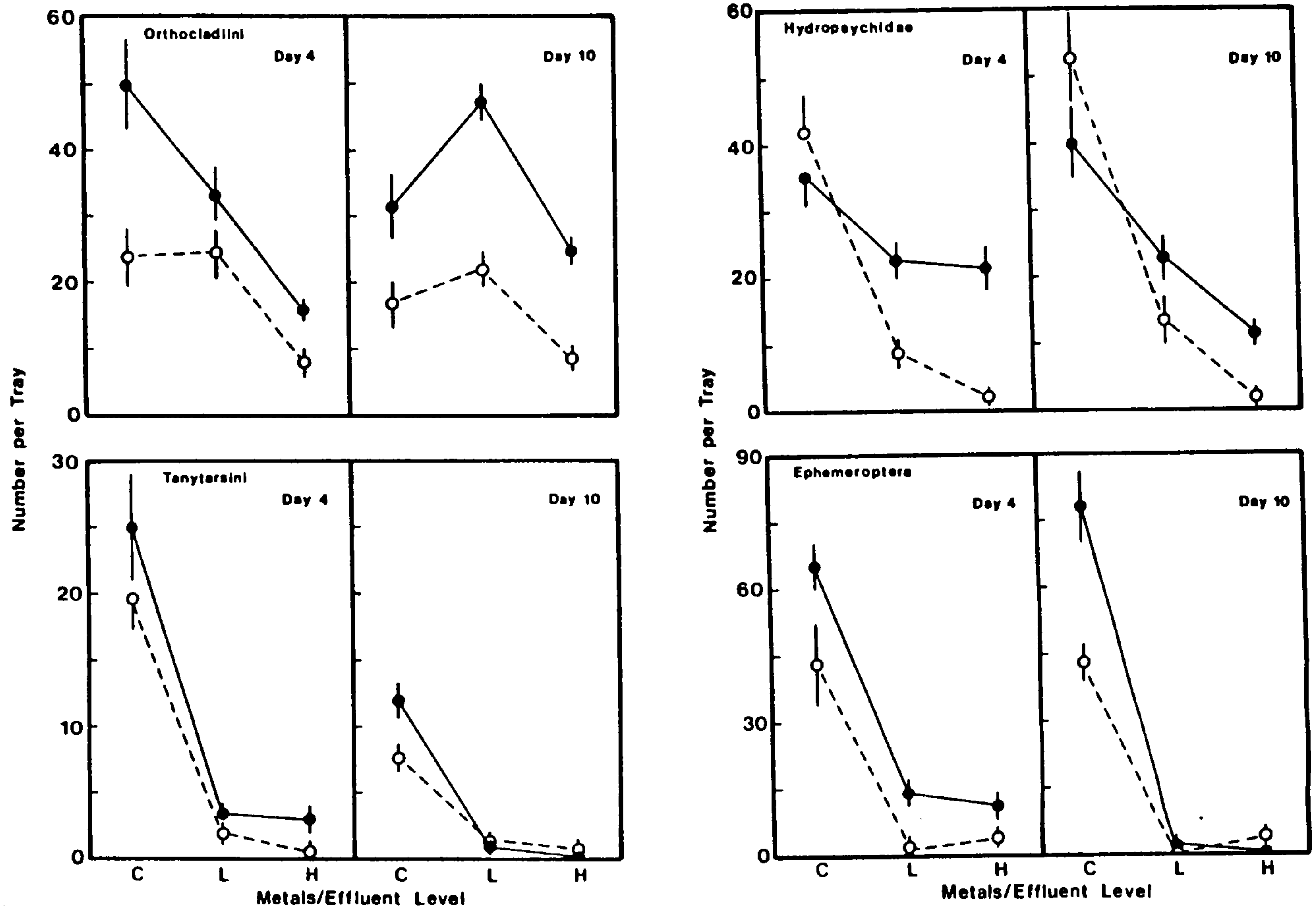

Figure 13. Effects of heavy metals on dominant groups of organisms in experimental streams and the Clinch River. Key same as figure 12.

these streams than at the reference site on both days.

There was only slight variation in responses to heavy metals between mayfly taxa. Within 4 d, each of the dominant species was significantly reduced in low metals streams. Isonychia bicolor was particularly sensitive, being reduced by 99% in low metals streams on day 4. Stenonema modestum and Baetis brunneicolor, the other dominant taxa collected, were somewhat less sensitive, each being reduced by 74% in these same streams.

Hydropsychidae (Hydropsyche morosa and Cheumatopsyche sp.) were more tolerant of heavy metals than either Ephemeroptera or Tanytarsini, particularly in experimental streams. Caddisflies were reduced only by 35-40% in treated streams on day 4, compared to 75-85% for these more sensitive groups. The impact of heavy metals on caddisflies at the Clinch River was similar between days, but varied between days in experimental streams. In particular, abundance in high metals streams was reduced on day 10 compared to day 4.

The response of Orthocladiini chironomids to heavy metals was quite different from that observed for other groups. In the field, abundance was not significantly different between reference and low effluent sites on either day. Although the number of Orthocladiini in treated streams was reduced on day 4, abundance was significantly greater in low metals streams than in either controls or high metals streams on day 10.

Due to differences in sensitivity to heavy metals, the percent composition of dominant macroinvertebrates in both experimental streams and at the Clinch River varied between metals/effluent levels (Fig. 14). Within levels, however, the percent composition was similar between experimental streams and the field. At the reference station and in control experimental streams, sensitive taxa such as Tanytarsini, *Isonychia bicolor*, *Stenonema modestum*, and *Baetis brunneicolor* accounted for 29-39% of the total organisms collected. In contrast, at the low metals site and in low metals experimental streams, these groups comprised only 3-17% of total abundance. In treated streams and at effluent sites in the field, these sensitive taxa were replaced by more tolerant groups, such as Orthocladiini and Hydropsychidae, which accounted for 60-90% of total individuals. The percent composition of these tolerant groups in high metals streams increased from 65% on day 4 to greater than 90% on day 10.

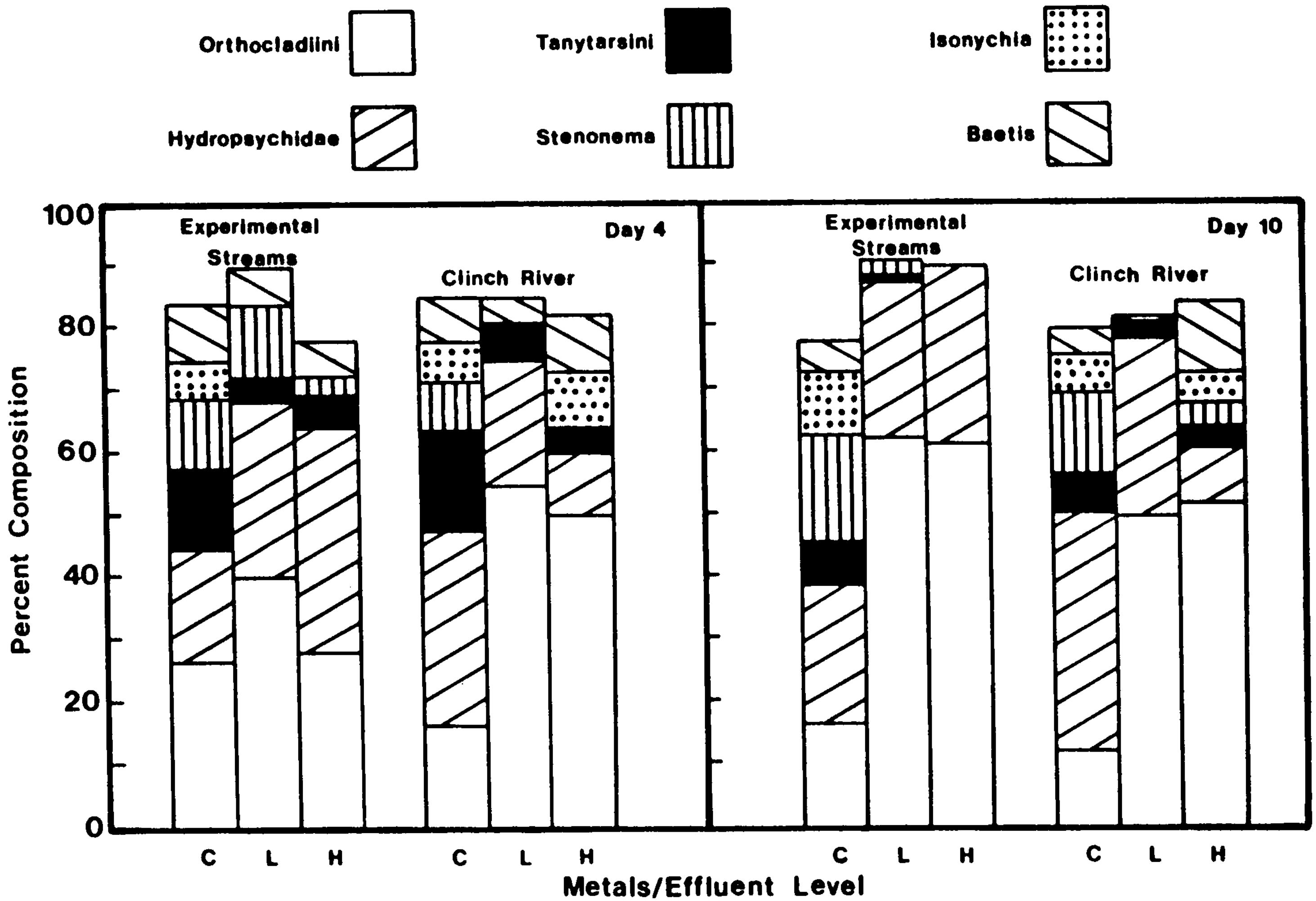

Figure 14. Relative abundance of dominant taxa in experimental streams and the Clinch River.

Discussion

Results of my experimental studies and field biomonitoring support the findings of other researchers who have shown major alterations of macroinvertebrate community structure in streams receiving heavy metals (Winner et al. 1980; LaPoint et al. 1984; Chadwick et al. 1986; Clements et al. in press a). More importantly, the similarity of these results to those obtained from the Clinch River indicate that macroinvertebrate responses to heavy metals are, as suggested by Winner et al. (1980), highly predictable. Within 10 d, number of organisms, number of taxa, and abundance of dominant groups in control and treated experimental streams reflected values observed at reference and effluent stations in the Clinch River, respectively. These responses of macroinvertebrate communities to heavy metals in experimental streams occurred rapidly. Within 4 d in low metals streams, macroinvertebrate abundance and number of taxa were reduced by 57% and 24%, respectively. These findings suggest that my approach may provide a cost-effective alternative to actual field experimentation for predicting the impact of toxicants on benthic communities.

Responses of dominant macroinvertebrates to heavy metals

Field and laboratory evidence suggests decreased heavy metals tolerance from chironomids, to caddisflies, to mayflies (Wiederholm 1984), and my results support these findings. In my experiments, mayflies were virtually eliminated after 10 d of exposure in low metals streams. Although there was little variation among taxa, *Isonychia bicolor* was somewhat more sensitive to heavy metals than other species. In both laboratory and field tests, *I. bicolor* was found to be more sensitive to Cu than either *Stenonema* sp. or *Ephemerella* sp. (W. H. Clements, unpublished data). Estimated 96-h LC50 values for this species ranged from 5-10 ug/L Cu, indicating that these are probably among the most sensitive aquatic insects examined.

The spatial distribution of mayflies at reference, effluent, and recovery stations in the Clinch River also reflected the impact of heavy metals on these organisms. Ephemeroptera was the only group significantly reduced at station 15, the first recovery station. Abundance of mayflies at station 8, where Cu and Zn concentrations were highest, was greater than stations 11 and 13. This difference was a result of greater numbers of highly mobile taxa, such as *I. bicolor* and *Baetis brunneicolor*, which probably arrived from upstream reference stations via drift and were not permanant residents on the substrates.

Several investigators have reported the tolerance of chironomids for heavy metals (Surber 1959; Sprague et al. 1965; Winner et al. 1975, 1980; Armitage 1980; Waterhouse and Farrell 1985; Chadwick et al. 1986). While my results support these findings, it is important to note that responses to heavy metals differed greatly among groups of chironomids. For example, Tanytarsini were highly sensitive to heavy metals in both the field and in experimental streams. Anderson et al. (1980) reported a 28-d LC50 of 3 ug/L Cu for this group. Armitage (1980) found that Tanytarsini dominated low Zn sites in a mine drainage area, whereas Orthocladiini were more abundant at high Zn sites. In my study, Orthocladiini were the dominant organisms collected at all effluent stations in the field and in treated experimental streams. The tolerance of Orthocladiini for heavy metals is well established. Surber (1959) reported increased abundance of _Cricotopus bicinctus_ at sites receiving Cu and chromium. Waterhouse and Farrell (1985) also found that two species of Orthocladiini, _C. bicinctus_ and _C. infuscatus_, dominated stations impacted by heavy metals. Thus, it appears that the response of Orthocladiini to heavy metals may be analogous to that of some oligocheates (e.g. _Tubifex tubifex_ and _Limnodrilus hoffmeisteri_) to organic pollution.

Although tolerance of this group for heavy metals may explain their dominance relative to other, more sensitive organisms, it does not explain why abundance was greater in treated streams and at effluent sites than controls. Increased abundance of Orthocladiini may have resulted from reduced numbers of potential competitors that were eliminated from treated streams and effluent sites. Alternatively, these organisms may have responded to increased abundance of a particular resource. Surber (1959) noted that Orthocladiini feed on resistant blue-green algae that often dominate metals polluted areas. At effluent sites in the Clinch River and in treated experimental streams, blue-green algae was abundant and may have provided a resource not available at reference sites and in control streams. The unique response of Orthocladiini to metals and their potential usefulness as an indicator of metals pollution requires further investigation.

Hydropsychidae was also tolerant of heavy metals in both the field and in experimental streams. In low metals streams, caddisfly abundance did not vary between days 4 and 10, indicating that these organisms could survive at relatively low concentrations of Cu and Zn. In the field, Hydropsychidae were significantly reduced at effluent sites, but not to the extent observed for Ephemeroptera and Tanytarsini. The number of caddisflies also increased significantly at stations 15 and 16 compared to upstream reference sites. As with Orthocladiini, increased abundance of Hydropsychidae may have resulted from either reduced abundance of other groups or increased availability of some resource.

Advantages of this approach

Presently, most multispecies toxicity research involves simply testing groups of aquatic organisms simultaneously, with little concern for species interactions (Mount 1985).

These assemblages are often highly synthetic and are frequently selected out of convenience and need for standardization rather than ecological relevance. While this approach is, by definition, a multispecies test, I feel that its only advantage over conventional single species tests is in the efficiency of testing several taxa simultaneously. Niederlehner et al. (1985) discuss the importance of using naturally-derived communities of organisms for toxicity testing. We feel that the main advantage of our procedure is the ability to predict responses of macroinvertebrate communities to toxicants. For example, this approach could be employed to validate site-specific water quality criteria prior to the actual release of an effluent. This method could also be useful in situations where logistical problems make it impossible to measure impact directly from field sampling. Each of these situations will require making a prediction that should be based on environmentally realistic procedures (Van Hassel and Gaulke 1986).

The approach described here is also more environmentally realistic than that employed in previous studies. Organisms were collected with their substrate and placed into outdoor experimental streams receiving unaltered river water. Since these trays accumulate both detritus and periphyton during colonization (W. H. Clements, personal observation), a natural food source is available. Experimental conditions in stream mesocosms were highly favorable for maintaining aquatic insect populations, as indicated by the greater abundance of most organisms in control experimental streams compared to the reference station in the field. There are several potential explanations for these apparent increases, including oviposition by adults, immigration via the diluent water, and growth of early instars beyond 500 um during the study. While the exact explanation is uncertain, the important point is that not only can these communities be maintained in experimental streams, but macroinvertebrate abundances were actually greater at the end of the experiment. In addition, the percent composition of dominant groups in control experimental streams and at the reference field site remained similar.

Finally, my approach will provide an opportunity to test organisms that are rarely included in standard laboratory bioassays. For example, many of the taxa that colonize these trays, especially early instars of some mayflies, cannot be tested using conventional procedures because of difficulties collecting and maintaining them in the laboratory. These early life stages of aquatic insects often comprise a significant portion of benthic communities and are usually quite sensitive to pollution. It therefore follows that these organisms should be included in toxicity testing and establishment of water quality criteria.

CHAPTER FOUR

Site-Specific Validation of Macroinvertebrate Community Responses to Copper: the Role of Water Quality

Abstract

Experiments were conducted in artificial streams to examine the influence of water quality on macroinvertebrate community responses to copper (Cu). Macroinvertebrate communities were established on substratum-filled trays placed in the Clinch River and transferred to two artificial stream systems receiving water from different sources. Alkalinity and hardness in Clinch River (CR) streams were approximately 2-3 x´s greater than in New River (NR) streams.

Effects of Cu were more severe in NR than in CR streams. After 4 d, total macroinvertebrate abundance was reduced by 32% in NR streams (measured concentration = 6 ug Cu/L) and by and 25% in CR streams (measured concentration = 15 ug Cu/L). After 10 d Tanytarsini chironomids, the dominant and most sensitive organisms collected, were eliminated from NR streams at 13 ug Cu/L but reduced by only 35% in CR streams at 12 ug Cu/L. Results of canonical discriminant analysis also revealed greater effects of Cu in NR streams. After 10 d, macroinvertebrate communities in 9 ug Cu/L NR streams were similar to those in 24 ug Cu/L CR streams.

Responses to Cu in both experimental streams and at impacted field sites were highly variabile among taxa.

Orthocladiini chironomids and net-spinning caddisflies were quite tolerant of Cu in experimental streams and were the only groups that recovered in the field. Our results demonstrate the importance of accounting for both water quality differences as well as composition of the resident fauna for establishing site-specific water quality criteria.

Introduction

Toxicity and availability of heavy metal effluents to aquatic organisms are greatly influenced by water quality characteristics of receiving systems. In particular, the inverse relationship between heavy metals toxicity and water hardness or alkalinity has received considerable attention (Cairns and Scheier 1957; U.S. EPA 1980). Various chemical and biological explanations for the observed detoxification of metals by hardness and alkalinity have been proposed, including reduced availability, reduced uptake, and increased excretion rates (Pascoe et al. 1986). Most research documenting this relationship has been conducted either with fish (Miller and Mackay 1980; Pascoe et al. 1986) or daphnids (Winner and Gauss 1986), whereas few studies have shown how water quality affects toxicity of metals to aquatic insects. Gauss et al. (1985) reported reduced copper toxicity for *Chironomus tentans* at greater water hardness, but concluded that our understanding of how water quality affects toxicity and bioavailability for different species and life stages is incomplete.

Because of these potential modifying effects of water quality on metals toxicity, a number of researchers have suggested that water quality criteria should be derived on a site-specific basis (Cairns 1957; Carlson et al. 1986; Van Hassel and Gaulke 1986). In 1983, the U.S. Environmental Protection Agency published guidelines for establishing such criteria for metals, based on bioassays using both laboratory and site water (U.S. EPA 1983). More recently, Van Hassel and Gaulke (1986) employed field biomonitoring data to calculate site specific water quality criteria for copper in the Clinch River, a system receiving heavy metals from a coal-fired electric generating plant.

While there has been considerable research documenting how hardness-alkalinity affects metal toxicity in the laboratory, I can find no experimental studies showing how water quality influences toxicity in the field. Strict reliance on single species, laboratory bioassays for establishment of water quality criteria has recently been criticized, and a number of researchers have suggested that these tests be supplemented with more environmentally realistic approaches (Cairns 1983; Odum 1984; Kimball and Levins 1985). Clements et al. (in press b) exposed natural assemblages of aquatic macroinvertebrates to copper in laboratory streams and demonstrated that communuity level parameters (number of individuals, number of taxa, abundance of dominant taxa) were highly sensitive indicators of metal stress. This approach has also been employed in outdoor experimental streams to predict effects of copper and zinc on macroinvertebrate communities in the field (Chapter four). Here I use a similar approach and compare macroinvertebrate community responses to copper in two outdoor experimental stream systems receiving water from different sources. My objectives were: 1) to show how water quality, particularly hardness and alkalinity, affected Cu toxicity in outdoor experimental streams; 2) to compare responses among several dominant species of aquatic insects; and 3) to compare responses in experimental streams to those observed at impacted field sites.

Materials and Methods

To document the effects of heavy metals on macroinvertebrate communities in the field, during August 1987, substratum-filled trays were colonized at six stations in the Clinch River (see Fig. 3, chapter 1). Station 2, the reference station, was located 1 km upstream from a coal-fired electric generating plant. Impacted stations 13, 13A, and 13B were located 1.0, 1.2, and 2.0 km downstream from the plant's cooling tower blowdown discharge, respectively. Copper concentrations of this effluent ranged from 300-400 ug/L. Recovery stations 15 and 16 were located 3.0 and 4.5 km downstream from the plant, respectively. After 30 d colonization, six trays were sampled from each station. To prevent the loss of organisms, a 500-um mesh net was placed directly downstream from each tray and the tray was removed from the stream. Contents were washed through a 500-um sieve and the organisms retained were preserved in 10% formalin. In the laboratory, organisms were sorted in white enamel pans and, except for chironomids, identified to genus or species. Chironomids were identified to tribe.

During this same period, macroinvertebrate communities for the experimental stream studies were established on substratum-filled trays placed at station 2. After 30 d colonization, 84 trays were removed and placed into separate 7-L coolers (4 trays per cooler) which were supplied with airstones and attached to a battery-operated air pump. Thirty-six of these trays were then transferred to nine outdoor experimental streams located at the Clinch River plant, and the four trays in each cooler were placed into a separate stream. Current in each oval, flow through stream (95 x 71 x 14 cm) was provided by paddlewheels, which maintained a constant current velocity of 38 cm/s. Each stream received water drawn directly from the Clinch River, upstream from the plant effluents, at a rate of 1.5 L/min, resulting in a turnover time of ~30 min.

The forty-eight remaining trays were transferred to 12 experimental streams (4 trays per stream) located at the Glen Lyn Research Site. These streams (76 x 46 x 14 cm) received water directly from the New River at a rate of 1.5 L/min, resulting in a turnover time of ~14 min.

After 2-d acclimation, the nine Clinch River (CR) streams were randomly assigned to one of three treatments: controls, 12, and 25 ug Cu/L. Similarly, the 12 New River (NR) streams were randomly assigned to one of four treatments: controls, 6, 12, and 25 ug Cu/L. Peristaltic pumps dripped stock solutions of copper sulfate from 20-L carboys into each treated stream. Two trays were removed from each stream after 4 and 10 d exposure to copper and processed as described above.

To estimate additional colonization of these trays from river water, two uncolonized trays were placed into each of three separate CR streams and sampled after 12 d. To estimate macroinvertebrate abundance in the field during these experiments, six colonized trays were collected from station 2 on days 4 and 10 (after 36 and 42 d colonization in the Clinch River). All samples were collected and processed as described above.

Water samples were collected from all experimental streams on days 4 and 10 and from each field station at the beginning and end of the biomonitoring study. Alkalinity, hardness, conductivity, and total Cu concentration were measured in the laboratory. Acid-soluble Cu was analyzed using inductively coupled plasma emission spectrometry. Temperature, pH, and dissolved oxygen were measured directly in the experimental streams and at each field site.

Differences between treatments in CR and NR experimental streams and between stations in the Clinch River were analyzed using one-way ANOVA. All analyses were performed on ln(n+1) transformed data, as plots of residuals indicated heterogeneity of variances among treatments. If differences in the overall F-test were significant, Duncan's Multiple Range Test was employed to determine which treatments (or stations) were different from others.

Macroinvertebrate community responses to copper in experimental streams were also analyzed using canonical discriminant analysis. This multivariate technique separates treatments, based on linear combinations of variables (in this case dominant taxa), and identifies which variables were responsible for this separation. The procedure also provides a graphical representation of separation and overlap among treatments.

Results

Water quality

While water temperature, pH, and dissolved oxygen were similar between stream systems, conductivity, alkalinity and hardness were 2-3 times greater in CR than in NR streams (Table 10). Measured Cu concentrations in CR streams approximated target values, whereas concentrations in NR streams

Table 10. Water quality in New River and Clinch River experimental streams on days 4 and 10. Each value is the mean of 3 streams. C (control), 6, 12, and 25 refer to target copper concentrations.

		New River				Clinch River		
		C	6	12	25	C	12	25
Alkalinity (mg/L)	Day 4	49	49	49	49	144	146	144
	Day 10	60	60	61	60	137	137	138
Hardness (mg/L)	Day 4	57	57	53	53	153	150	150
	Day 10	60	60	60	60	153	153	157
Conductivity (umohms)	Day 4	132	131	132	132	297	298	299
	Day 10	158	155	159	160	296	297	302
pH	Day 4	8.62	8.53	8.32	8.27	8.36	8.35	8.36
	Day 10	9.46	9.46	9.28	8.99	8.98	8.82	8.70
Temperature (oC)	Day 4	19.8	19.7	20.0	19.7	19.7	19.7	20.0
	Day 10	22.7	22.0	22.0	23.0	21.0	22.0	22.0
Dissolved oxygen (mg/L)	Day 4	10.2	10.6	9.6	9.7	9.9	10.1	9.7
	Day 10	11.2	11.1	10.0	10.0	11.5	10.4	9.7
[Copper] (ug/L)	Day 4	b.d.	3	6	19	b.d.	15	33
	Day 10	b.d.	2	9	13	b.d.	12	24

were less than target values. Below, all results will be presented using only measured copper concentrations.

Water quality at Clinch River reference and recovery stations was similar to that in CR control streams. Copper concentrations were greatest at station 13 (33 ug/L), the first impacted site, and decreased rapidly downstream. Copper concentrations were below the limit of detection (<2 ug/L) at station 13B.

Clinch River biomonitoring

Heavy metal effluents had a severe impact on benthic communities at the Clinch River (Table 11). Total macroinvertebrate abundance was reduced by 80-90% at the three impacted sites. While some evidence of recovery was apparent at station 16, the furthest downstream site, macroinvertebrate abundance was still only 36% that of the reference station. The total number of taxa was also significantly reduced at impacted stations, but recovered at station 16.

Mayflies and Tanytarsini chironomids, which comprised ~80% of the upstream community, were particularly sensitive to heavy metal effluents. These organisms were eliminated from impacted stations and showed little indication of recovery at the furthest downstream site. Impacted stations were dominated by Orthocaldiini chironomids, which accounted for 41-82% of the total individuals. Relative abundance of this group was reduced at recovery stations 15 and 16. The

Table 11. Mean macroinvertebrate abundance, number of taxa, and abundance of dominant taxa at biomonitoring stations in the Clinch River (n=6). Results of one-way ANOVA and Duncan´s Multiple Range Test are also included. Means with the same letter were not significantly different.

	Station						
	2	13	13A	13B	15	16	F-value
Orthocladiini	27.8^{a}	23.5^{a}	9.5^{c}	26.2^{a}	11.5^{bc}	23.5^{ab}	5.3*
Tanytarsini	218.3^{a}	0.0^{e}	0.0^{e}	2.8^{d}	12.7^{c}	24.0^{b}	463.4***
Cheumatopsyche sp.	18.3^{a}	0.2^{d}	2.3^{c}	9.2^{b}	17.5^{a}	16.0^{ab}	36.9***
Hydropsyche bifida	8.3^{b}	0.2^{d}	1.8^{c}	5.0^{b}	33.2^{a}	35.7^{a}	27.9***
Baetis brunneicolor	19.0^{a}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	1.7^{b}	136.1***
Stenonema modestum	11.3^{a}	0.0^{c}	0.0^{c}	0.0^{c}	0.3^{c}	0.8^{b}	89.0***
Isonychia bicolor	5.0^{a}	0.0^{b}	0.0^{b}	0.2^{b}	0.0^{b}	1.3^{ab}	4.3*
Number of individuals	342.3^{a}	28.5^{d}	23.0^{d}	56.8^{c}	99.3^{b}	124.8^{b}	102.7***
Number of taxa	17.2^{a}	3.7^{d}	7.0^{c}	8.2^{c}	11.7^{b}	14.5^{a}	61.3***

* $p<0.01$; ** $p<0.001$; *** $p<0.0001$;

downstream community at these stations was dominated by net-spinning caddisflies, Cheumatopsyche sp. and Hydropsyche morosa. This latter species was the only taxa showing a significant increase downstream compared to the upstream reference station.

Experimental streams

Macroinvertebrate communities in both CR and NR experimental streams responded quickly to Cu exposure (Fig. 15). Within 4 d, total abundance was reduced by 58-65% in streams receiving the highest copper concentrations. The number of taxa was reduced in treated NR streams on both sampling days and in CR streams after 10 d exposure. Results of Duncan's Multiple Range Test indicated that the lowest measured Cu concentration in NR streams (2-3 ug/L) had no effect on macroinvertebrate abundance on day 4, or number of taxa on either sampling day. Abundance was, however, significantly reduced in these streams on day 10.

Macroinvertebrate abundance in control experimental streams after 4 and 10 d was 50-100% greater than on trays removed from station 2 after 36 and 42 d colonization. The greater abundance in experimental streams was not a result of additional colonization of CR trays from river water. After 12 d, previously uncolonized trays contained only 18.7 organisms per tray (n=3; s.d.=5.3), and chironomids accounted for 93% of these organisms.

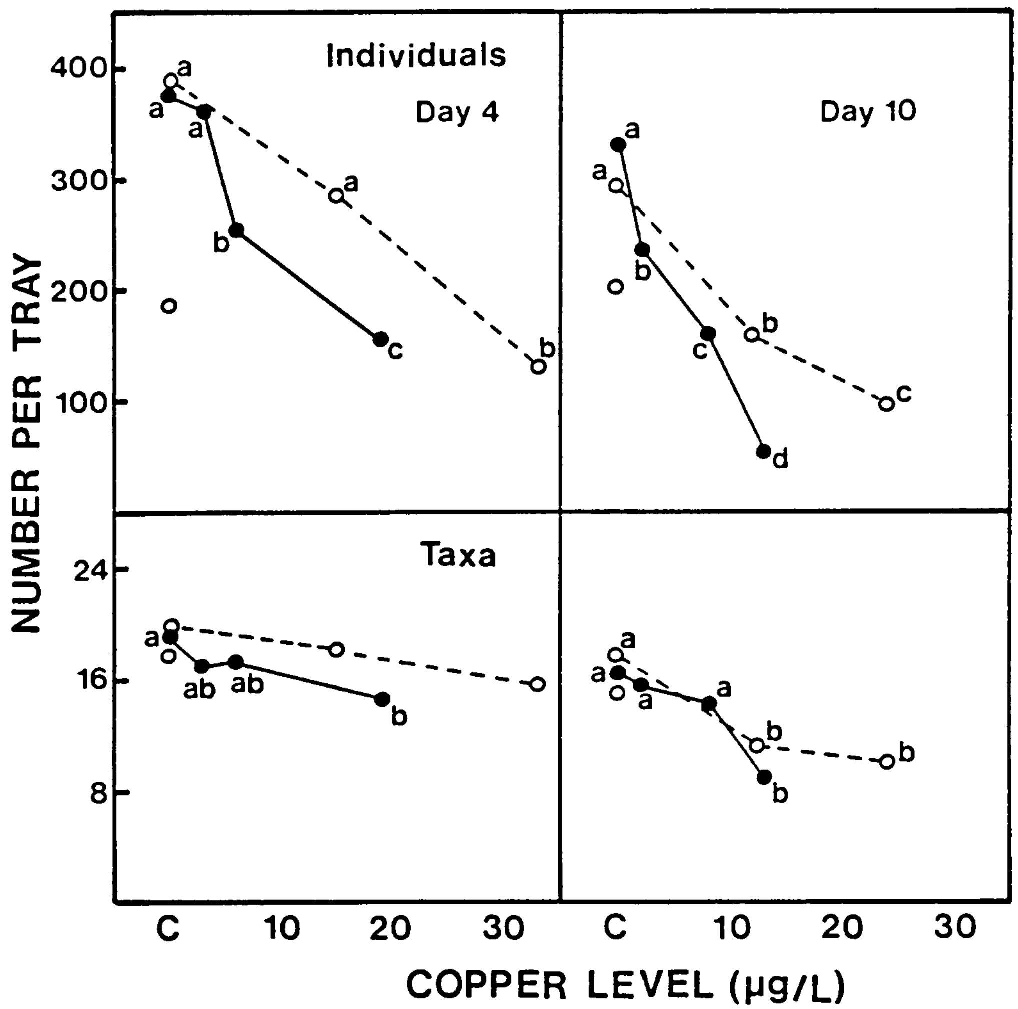

Figure 15. Effects of copper on number of individuals and number of taxa in NR (solid lines) and CR (broken lines) experimental streams. Data from days 4 and 10 in experimental streams and from reference station 2 at the Clinch River (open circles) are included. Each point represents the mean number per tray. Within stream systems, treatments with the same letter were not significantly different.

Effects of Cu were more severe in NR streams on both sampling days, as indicated by lower macroinvertebrate abundance in these streams compared to CR streams at similar Cu concentrations. Four day LC50 values for total individuals, estimated by inspection of Fig. 15, were ~25 and 14 ug Cu/L in CR and NR streams, respectively. After 10 d exposure, these values decreased to ~14 and 8 ug Cu/L in CR and NR streams.

The response of Tanytarsini chironomids, the dominant organisms collected, was also greater in NR than in CR experimental streams (Fig. 16). Within 4 d, abundance of Tanytarsini was reduced by 91% in NR streams at 19 ug Cu/L. In CR streams, there was no significant difference in abundance of Tanytarsini between controls and the 15 ug Cu/L streams. In CR streams at 33 ug Cu/L, these organisms were reduced by 79%. Estimated 4 d LC50 values for this group were 8 and 23 ug/L in NR and CR streams, respectively.

The dominant mayflies collected (*Baetis brunneicolor*, *Stenonema modestum*, and *Isonychia bicolor*) were highly sensitive to copper exposure (Fig. 16). Each species was significantly reduced in CR and NR treated streams on day 4, and two taxa, *B. brunneicolor* and *I. bicolor*, were eliminated after 10 d. *Stenonema modestum* were more tolerant of Cu exposure than other mayfly taxa, particularly in CR streams, and was the only species remaining after 10 d exposure to the highest Cu levels. There was also some indication that effects of Cu on *S. modestum* were greater in NR streams, but

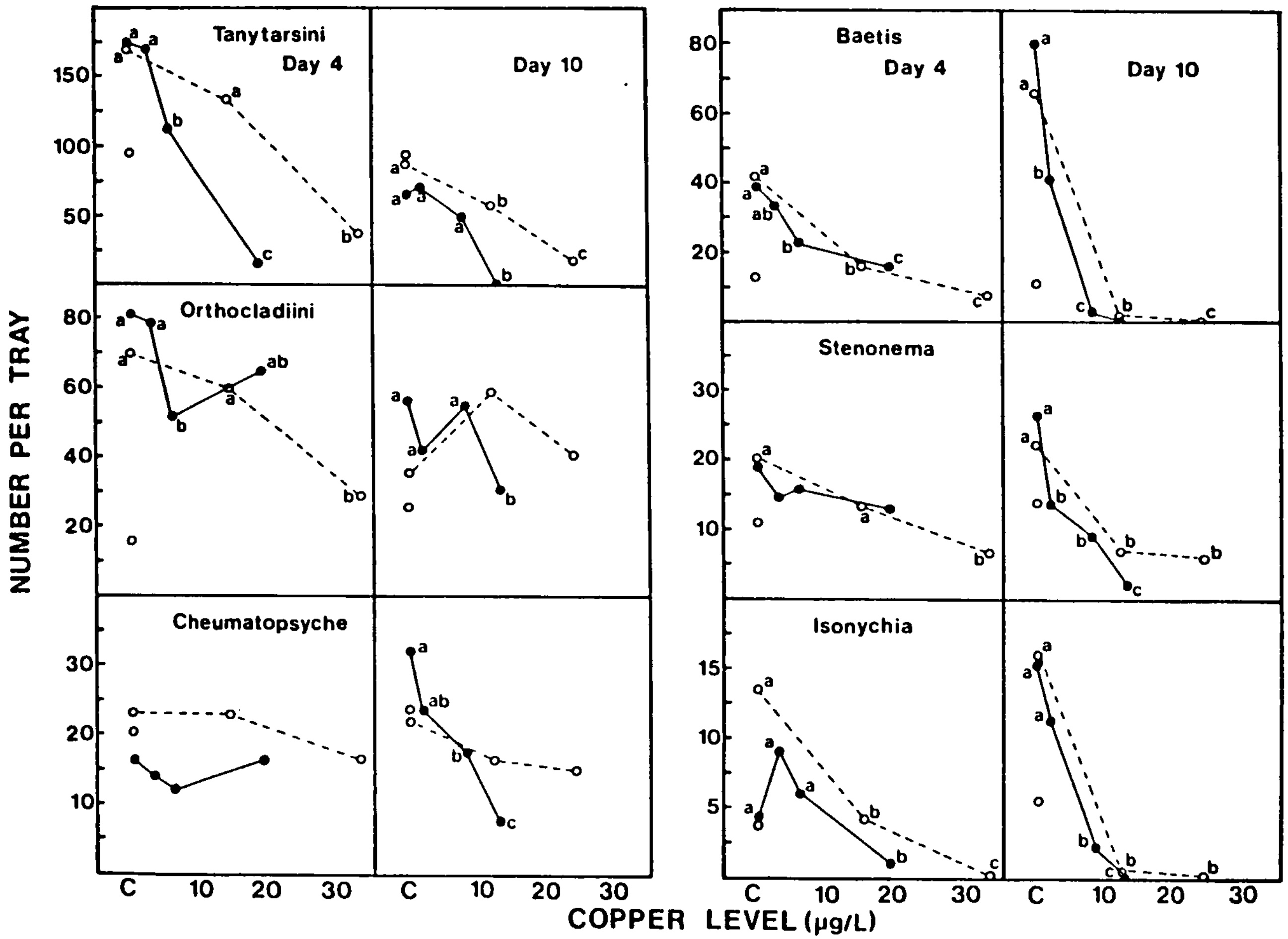

Figure 16. Effects of copper on dominant taxa in NR (solid lines) and CR (broken lines) experimental streams. (after figure 15).

in general mayfly responses were similar between systems.

Net-spinning caddisflies, Cheumatopsyche sp., and Orthocladiini chironomids were quite tolerant of Cu exposure. Significant treatment effects were observed for Cheumatopsyche sp. only in NR streams after 10 d exposure. These organisms were also more sensitive to copper in NR than in CR streams on day 10. The response of Orthocladiini to Cu was quite different from that observed for other groups and was highly variable between CR and NR streams. In general, these organisms did not show a typical dose-dependent response to increased Cu, as peak abundance often occured at intermediate to high Cu concentrations.

Multivariate analysis of these data was employed to compare treatment and water quality effects in CR and NR streams. This analysis, which was based on abundance of the six dominant taxa, showed the degree of separation and overlap among treatments in CR and NR streams, where greater overlap indicated greater similarity of macroinvertebrate communities. Results showed three distinct groups on both sampling days (Fig. 17). On day 4, Group I consisted of all control streams, 3 ug Cu/L NR streams, 6 ug Cu/L NR streams, and the 15 ug Cu/L CR streams. Groups II and III consisted of 33 ug Cu/L CR streams and 19 ug Cu/L NR streams, respectively. Thus on day 4, only the two highest Cu treatments were distinctly different from other treatments. Separation of these groups was based primarily on abundance of Tanytar-

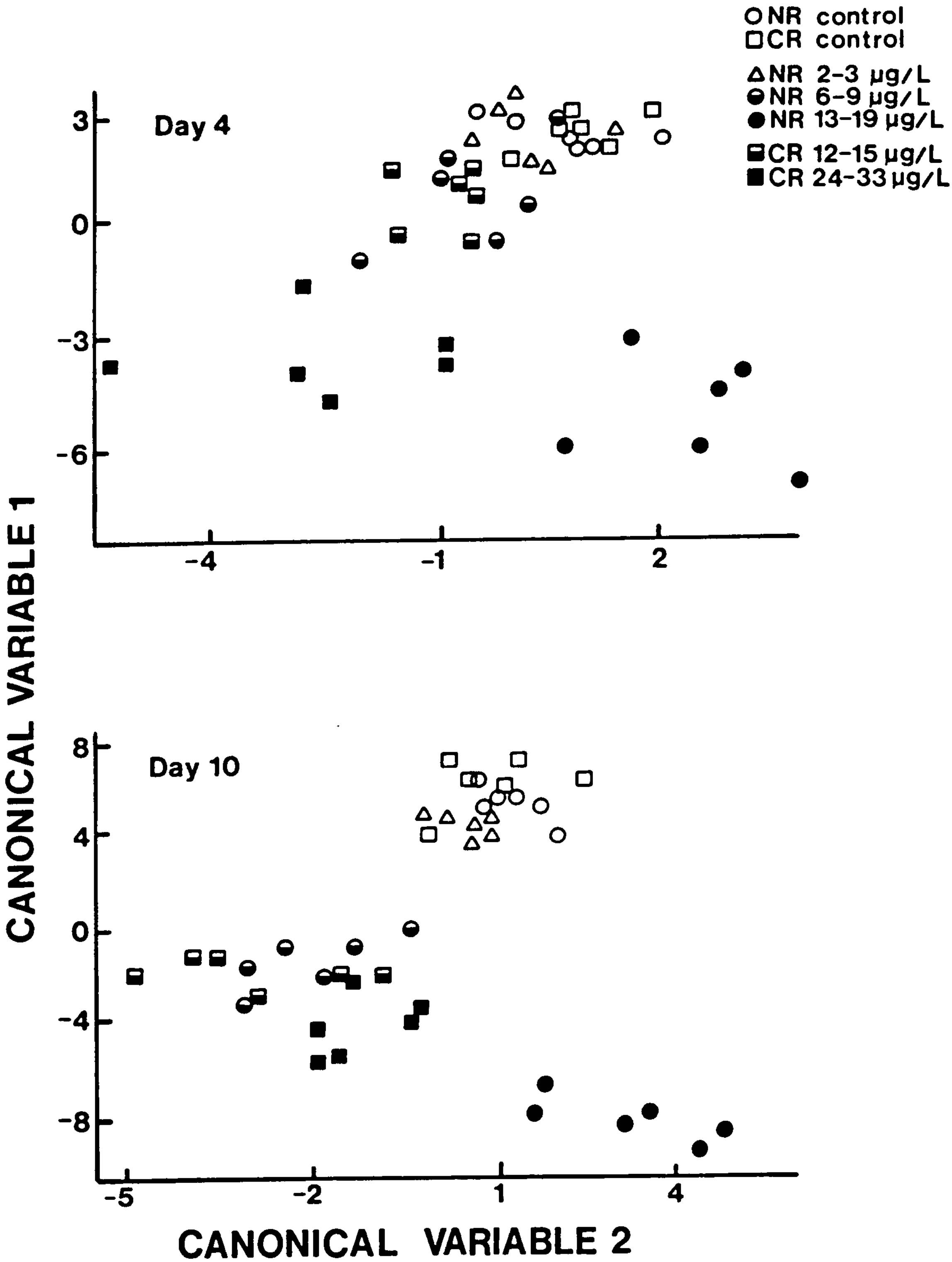

Figure 17. Canonical discriminant analysis of community responses to copper in NR and CR experimental streams.

sini, which was the dominant component of canonical variable 1, and Orthocladiini, which was the dominant component of canonical variable 2. The distribution of treatments among groups changed after 10 d exposure to Cu. On day 10, Group I consisted only of control streams and the 2 ug Cu/L NR streams. The remaining treatments, except for the 13 ug Cu/L NR streams, were included in Group II. Separation of groups on day 10 was based primarily on abundance of B. brunneicolor (canonical variable 1) and reduced abundance of Tanytarsini (canonical variable 2).

Discussion

These experimental results demonstrate the importance of accounting for water quality characteristics of the receiving system as well as composition of the resident fauna in establishing site-specific criteria for metals. Effects of Cu on benthic communities were greater in NR than in CR streams on both sampling days. Tanytarsini chironomids, which dominated control streams and the field reference site, were eliminated from 13-19 ug Cu/L NR streams but reduced by only 23-35% in CR streams at similar concentrations. Multivariate analyses of these data also revealed the greater impact of Cu in NR streams, as indicated by inclusion of the 8 ug Cu/L NR streams with the 12 and 24 ug Cu/L CR streams on day 10.

The greater alkalinity and hardness of Clinch River water most likely accounted for the reduced Cu toxicity observed in CR streams. Gauss et al. (1985) compared effects of Cu on chironomids in hard and soft water which was similar to water in my CR and NR streams. They reported 96 h LC50 values of 17 ug Cu/L in soft (43 mg/L CaCO3) water and 98 ug Cu/L in hard (172 mg/L CaCO3) water. Winner and Gauss (1986) also observed reduced metals toxicity in hard water for daphnids, but noted that the presence of humic acid complicates this relationship. Since dissolved organic carbon was not measured in my streams, the influence of these materials on observed Cu toxicity is unknown.

My experimental results demonstrated significant variability in Cu toxicity among groups of aquatic insects. Tanytarsini chironomids and the mayflies B. brunneicolor and I. bicolor were especially sensitive to Cu, whereas Orthocladiini and Cheumatopsyche sp. were quite tolerant. These findings were consistent with those reported elsewhere (Surber 1959; Sprague et al. 1965; Winner et al. 1975; Peterson and Peterson 1983; Chadwick et al. 1985), and show that macroinvertebrate community responses to heavy metals are, as suggested by Winner et al. (1980), highly predictable.

Observed differences in Cu sensitivity among taxa may be related to differences in trophic habits, as food ingestion is potentailly an important route of metals uptake. Smock (1983a) showed that differences in feeding habits of aquatic insects affected bioconcentration of metals. The trays employed in my experiments were colonized by a diverse assemblage of aquatic insects representing several functional feeding groups (sensu Merritt and Cummins 1978), thus providing an opportunity to examine the influence of feeding habits on Cu sensitivity. In general, sensitivity to Cu was unrelated to these trophic relationships. Tanytarsini and Orthocladiini, which were among the most and least sensitive groups, are both classified as collector-gatherers (Merritt and Cummins 1978). Similarly, Cheumatopsyche sp. and I. bicolor, which also differed greatly in their sensitivity to Cu, are collector-filterers.

Certain morphological characteristics of aquatic insects may influence their susceptibility to heavy metals. Hodson et al. (1979) note that variation in acute toxicity among invertebrate taxa is related to the nature of the body covering. For example, stoneflies and coleopterans, which possess heavily sclerotized plates, are highly tolerant of heavy metals (Warnick and Bell 1969; Nehring 1976; Spehar et al. 1978). Although stoneflies were uncommon in my samples, Stenelmis sp., the dominant coleopteran collected, was significantly more abundant at impacted station 13B than any other site.

Owing to their membranous structure and permeability, gills are an important target for heavy metals injury in aquatic organisms (Simpson 1980; Young et al. 1981). Peters et al. (1987) reported that alkaline pH disrupted chloride cell ultrastructure in gills of *I. bicolor*. I speculate that the external, plate-like gills of these organisms may have influenced their sensitivity to Cu in my experiments.

Organism size influences toxicity and bioaccumulation of heavy metals, and the high surface to volume ratio of aquatic insects makes them especially sensitive (Kosalwat and Knight 1987). Smock (1983b) reported an inverse relationship between body size and metals concentration in mayflies, and suggested that surface adsorption was primarily responsible. In my experiments there was little evidence that size affected sensitivity to Cu between taxa. Within taxa, however, there was some evidence that Cu toxicity was greater among small individuals, a finding which has been reported by several investigators (Clubb et al. 1975; Hodson et al. 1979; Gauss et al. 1985). On day 10, trays from control streams were dominated by very small, early instar mayflies. Since these organisms did not colonize streams during the experiments, they were obviously present on day 4 but represented by eggs or first-instars which passed through the 500-um sieve. Therefore the greater effects of observed Cu on day 10 were, in part, a result of greater numbers of these highly sensitive organisms, which dominated control streams but were eliminated from treated streams.

Differences in sensitivity to Cu exposure among aquatic insects were also evident from field biomonitoring at the Clinch River. Mayflies were completely eliminated from impacted stations and represented by < 5 individuals per tray at the furthest downstream site. Impacted stations were dominated by Orthocladiini chironomids, which were gradually replaced by net-spinning caddisflies further downstream. The significant increase in abundance of Hydropsyche morosa at stations 15 and 16 was also observed during the 1986 survey of the Clinch River (Chapter 4). These results were similar to those reported by Sprague et al. (1965), in which caddisflies were found to be 2.5 times more abundant at metal impacted sites than at upstream reference sites.

Macroinvertebrate communities failed to recover at the furthest downstream stations, even where Cu concentrations approximated upstream, reference station values. This suggests that reduced macroinvertebrate abundance at these stations was not due to direct toxic effects of metals per se, but rather to some indirect mechanism. Since colonization of substrates occurs primarily via drift (Townsend and Hildrew 1976), failure of these downstream communities to recover may have been a result of reduced abundance of potential colonists from upstream, impacted sites.

My experimental results suggest that heavy metal effluents, discharged at similar concentrations, would have greater impact on benthic communities in the New River than in the Clinch River. These findings support the recent trends toward development of site-specific criteria for metals. I recommend, however, that these criteria be developed using environmentally realistic procedures and based on responses of resident organisms. On day 10 of my experiments, both NR and CR control streams were dominated by highly sensitive, early instar mayflies. Since these organisms are rarely included in standard laboratory bioassays, criteria based on these simple laboratory tests may be underprotective. I feel that my experimental approach, which exposes natural assemblages of aquatic organisms to toxicants in systems receiving site water, will be useful for establishing these site-specific criteria.

CHAPTEE FIVE

The Effects of Copper Exposure on Predator-Prey Interactions in Macroinvertebrate Communities

Abstract

Experiments were conducted in artificial streams to examine the influence of chronic levels of copper exposure on predator-prey interactions between stoneflies, <u>Paragnetina fumosa</u>, and their invertebrate prey. Substrate-filled trays colonized by aquatic insects were transferred to 12 artificial streams, which were randomly assigned to one of four treatments: controls, no predators; controls, with predators; dosed, no predators; and dosed, with predators.

Effects of Cu (target concentration = 6 ug/L) on macroinvertebrate communities were relatively moderate, as abundance in dosed streams (no predators) was only 12% lower than controls. In contrast, stoneflies significantly reduced total abundance and abundance of most dominant taxa. Of the nine dominant taxa examined, <u>Serratella</u> sp., <u>Pseudocloeon</u> sp., and Chironomini were most affected by predation. Vulnerability to predation was significantly greater in dosed streams for two net-spinning caddisflies (<u>Chimarra</u> sp. and <u>Hydropsyche morosa</u>), but predation on the remaining groups was not affected by copper exposure. Results of stonefly stomach analyses showed that Hydropsychidae was the major component of the

diet and that the number of Hydropsychidae per stonefly gut was significantly greater in dosed streams than controls.

Introduction

The influence of toxicants on predator-prey interactions in fish has received considerable attention (Goodyear 1972; Hatfield and Anderson 1972; Kania and O´Hara 1974; Sullivan et al. 1978; Woltering et al. 1978). These interactions, which integrate important behavioral components of both predators and prey (Heath 1987), are often quite sensitive to stress. Sullivan et al. (1978) reported greater vulnerability of fathead minnows to predation by largemouth bass following chronic exposure of prey to cadmium. Effects were observed at concentrations well below the estimated maximum allowable toxicant concentration (MATC) for fathead minnows. Similar results have been reported for mercury (Kania and O´Hara 1974) and insecticides (Hatfield and Anderson 1972), and are usually attributed to alteration in predator avoidance behavior of exposed prey.

When predator and prey are simultaneously exposed to a toxicant, the situation most likely to occur in the field, different results may be obtained. Exposure of both predator and prey to toxicants may result in either similar, increased, or decreased predation intensity (Fig. 18). Variation in predation intensity is most likely to occur if the behavior of predator or prey is differentially affected by

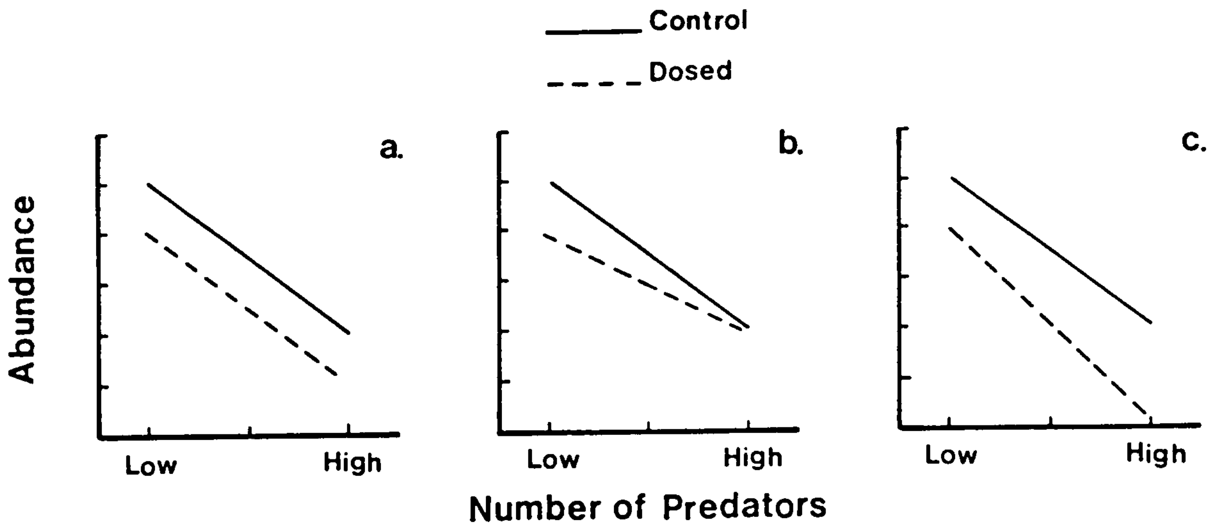

Figure 18. Hypothetical model of the combined effects of toxicants and predators on macroinvertebrate abundance. a) null model- no interaction between toxicants and predators; b) predation lower in dosed streams; c) predation greater in dosed streams.

toxicants. Woltering et al. (1978) noted decreased vulnerability of mosquitofish to bass predation when both were exposed to ammonia. These results were explained by the greater impact of ammonia on bass than on mosquitofish.

The lack of evidence showing that community or ecosystem level processes can be predicted from single-species tests is probably the most oft-cited justification for development of more sophisticated testing procedures. I can find no examples in the literature of how species interactions among stream invertebrates are affected by toxicants, despite the evidence that these interactions are important in structuring benthic communities (Allan 1982; McAuliffe 1984; Hart 1985; Peckarsky 1985). Predacious stoneflies for example are particularly effective at reducing prey populations in streams (Peckarsky 1985). Since stoneflies are highly tolerant of heavy metals, whereas their prey (mayflies, caddisflies, chironomids) are often more sensitive (Warnick and Bell 1969; Nehring 1976; Spehar et al. 1978; Clements et al. in press b), there is the potential for metals to influence predator-prey interactions among these organisms.

In this study, I examine the influence of copper (Cu) on predator-prey interactions between stoneflies (Paragnetina fumosa) and their invertebrate prey. Experiments were conducted in replicate artificial streams to test the hypothesis that predation intensity would be greater in streams dosed with Cu.

Materials and Methods

Macroinvertebrate communities were established on substratum-filled trays placed in the New River (Giles Co., Virginia) during October, 1987. Previous experiments have shown that these trays are rapidly colonized by a diverse assemblege of organisms (Clements et al. in press a). After 30 d colonization, each tray was recovered by placing a 500-um mesh net directly downstream to prevent the loss of organisms. Trays were removed from the stream, placed into 7-L coolers filled with stream water (4 trays per cooler), and transferred ~ 1 km to the Glen Lyn Research Laboratory. The four trays from each cooler were placed into one of 12 experimental streams. These flow-through, indoor streams (76 x 46 x 14 cm) received unaltered water directly from the New River at a rate of 1.5 L/min, resulting in a turnover time of 14 min. Current in each stream was provided by paddlewheels, which maintained a constant velocity of 38 cm/s. A 12L:12D photoperiod was maintained in the laboratory using fluorescent lights.

After 2 d acclimation, the streams were randomly assigned to one of four groups (Fig. 19): controls, no predators (C-NP); controls, with predators (C-P); dosed, no predators (D-NP); and dosed, with predators (D-P). Dosed streams received Cu at target concentrations of 6 ug/L. This concentration was chosen because previous experiments showed that 6 ug/L had only moderate impact on macroinvertebrate communi-

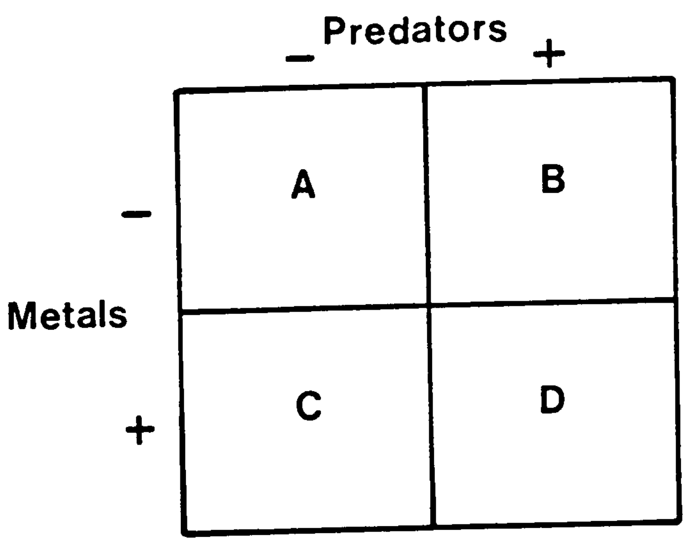

Figure 19. Experimental design employed to detect effects of metals, predators, and interaction of these factors on macroinvertebrate community structure.

ties (chapter 4). Peristaltic pumps delivered stock $CuSO_4$ solution from 20-L carboys to each dosed stream. Six stonefly predators (Paragnetina fumosa) were placed into each predation stream. Stoneflies (13-20 mm) were collected from nearby Sinking Creek, a third-order tributary to the New River.

After 14 d, all trays were removed and the contents washed through a 500-um sieve. Organisms retained were preserved in 10% formalin, sorted in white enamel pans, and, except for chironomids, identified to genus or species. Chironomids were identified to tribe. To estimate initial macroinvertebrate abundance and community composition, six trays were also sampled from the New River after 30 d colonization and preserved in the field. These samples were processed as described above.

Stomach contents of stoneflies (n = 34) were examined at the end of the experiment. The foregut of each individual was removed and prey were identified and counted using a dissecting scope.

Water samples were collected from experimental streams after 7 and 14 d exposure to Cu and analyzed for hardness, alkalinity, and Cu concentration. Total acid-soluble Cu was analyzed using inductively-coupled, plasma emission spectrometry. Temperature, pH, dissolved oxygen, and conductivity were measured in each stream on the same days.

Differences among predator and Cu treatments in experimental streams were analyzed using two-way ANOVA. Significant interaction between the two main factors in this model indicates that predation effects were influenced by Cu exposure.

Results

Except for Cu concentration, the various water quality parameters examined were similar among streams (Table 12). Measured Cu concentrations approximated target values in dosed streams and were below the limit of detection (<2 ug/L) in controls.

Exposure to Cu reduced macroinvertebrate abundance and Shannon's diversity, but had no effect on the number of taxa (Fig. 20). Although the effect of Cu on abundance was significant (Table 13), the total number of organisms in D-NP streams was only 12% less than in C-NP streams, indicating that these effects were relatively moderate. Most dominant groups were not affected by Cu, and only two taxa, *Serratella* sp. and *Chimarra* sp., were significantly reduced in dosed streams. Since abundance of *Chimarra* sp. in C-NP and D-NP streams was similar, the significant Cu effect observed for this group was a result of the significant metals x predation interaction. Abundances of *Isonychia bicolor*, *Hydropsyche morosa* and Chironomini were reduced in dosed streams, but these treatment effects were not significant ($p=0.068$, 0.053, and 0.067, respectively).

Table 12. Water quality in experimental streams. Values represent the means per treatment for both sampling days combined (n=6). b.d. = below detection.

	Controls		Dosed	
	No predators	Predators	No Predators	Predators
Temperature (°C)	14	14	14	14
pH	8.59	8.60	8.61	8.60
Dissolved oxygen (mg/L)	11.4	11.5	11.4	11.4
Alkalinity (mg/L)	64	64	63	66
Hardness (mg/L)	87	90	87	88
Conductivity (umohms)	161	161	161	160
[Cu] (ug/L)	b.d.	b.d.	5.6	5.3

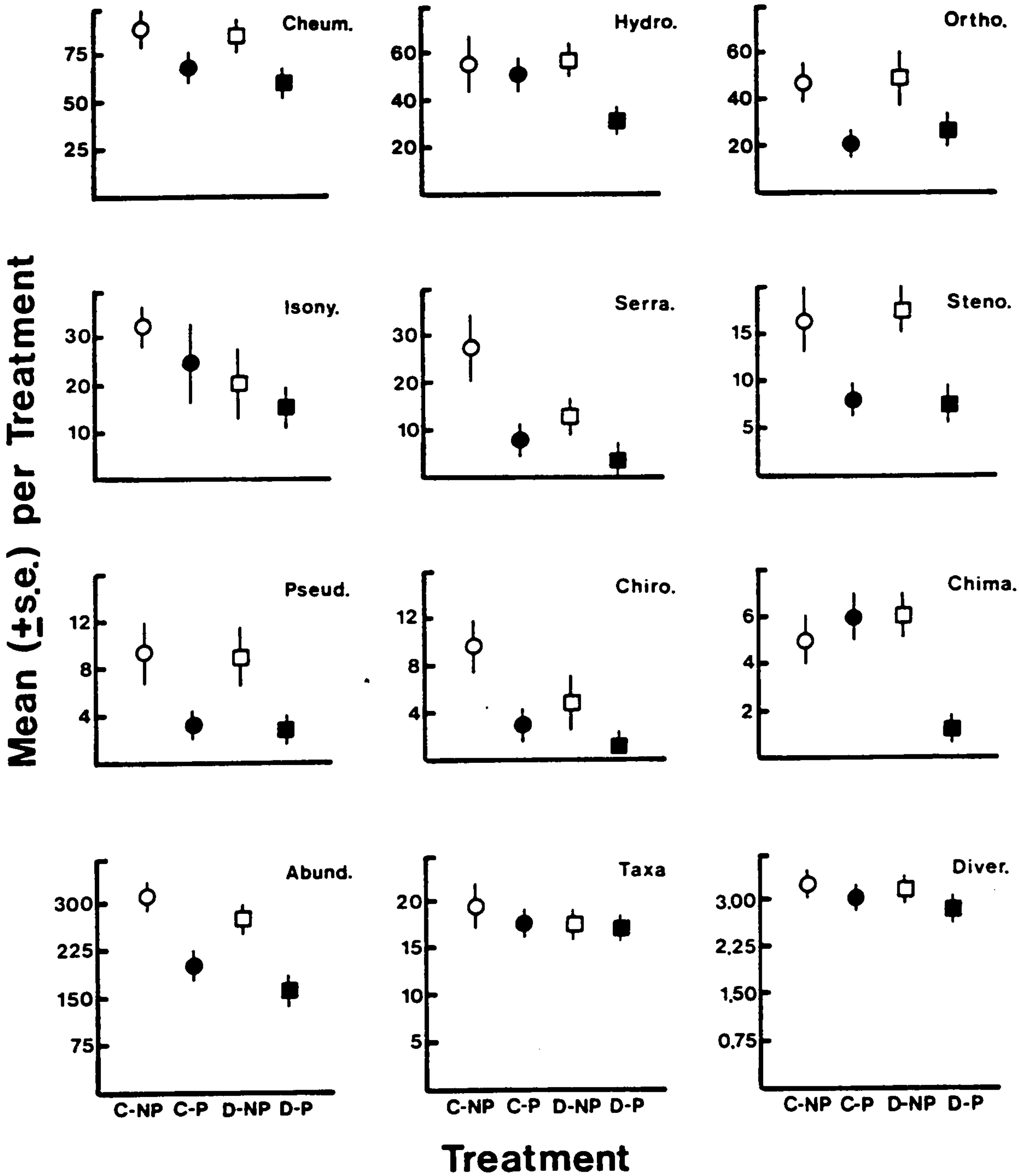

Figure 20. Effects of Cu and predation on macroinvertebrate abundance, number of taxa, Shannon's diversity, and abundance of the nine dominant taxa. Open circles = control, no predators (C-NP); closed circles = control, with predators (C-P); open squares = dosed, no predators (D-NP); and closed squares = dosed, with predators (D-P). Cheum.=<u>Cheumatopsyche</u> sp.; Hydro. = <u>H. morosa</u>; Ortho. = Orthocladiini; Isony.=<u>I. bicolor</u>; Serra.=<u>Serratella</u> sp.; Steno.=<u>S. modestum</u>; Pseud.= <u>Pseudocloeon</u> sp.; Chiro=Chironomini; and Chima.=<u>Chimarra</u> sp.

Table 13. Results of two-way ANOVA. F-values for the two main factors and interaction effects are included.

	Metals	Predation	Interaction
Cheumatopsyche	1.79	18.43**	0.36
Hydropsyche	5.14	9.94*	5.64*
Orthocladiini	0.64	18.70*	0.42
Isonychia	4.45	1.88	0.04
Serratella	5.91*	17.99**	0.08
Stenonema	0.00	10.11**	0.11
Pseudocloeon	0.26	26.14**	0.00
Chironomini	4.47	7.28*	0.01
Chimarra	8.23	7.90*	15.07**
Total abundance	27.63**	181.96***	2.64
Number of taxa	2.58	1.49	0.66
Diversity (H´)	6.71*	21.74**	0.00

$*p<0.05$; $**p<0.005$; $**p<0.0001$

In contrast to the moderate effects of Cu, stoneflies had a major impact on macroinvertebrate communities, significantly reducing abundance, diversity, and abundance of most dominant groups (Fig. 20; Table 13). In streams with predators (both C-P and D-P) the total number of organisms was reduced by 35-42% compared to streams without predators. Of the nine dominant taxa examined, only *I. bicolor* failed to show a significant response to stonefly predation. The mayflies, *Serratella* sp. and *Pseudocloeon* sp., and Chironomini were most affected by predation. These organisms were reduced by 66-72% in C-P and D-P streams compared to those without predators.

Significant metals x predator interaction was observed for two taxa. Predation on the caddisflies *Hydropsyche morosa* and *Chimarra* sp. was significantly greater in dosed streams. For both taxa, predation had little affect in control streams but greatly reduced abundance in dosed streams. For the remaining taxa, as well as the community level parameters examined, predation intensity was similar among control and dosed streams.

The greater vulnerability of Hydropsychidae to predation in dosed streams was also evident from analysis of stonefly stomach contents (Table 14) The mean number of Hydropsychidae per stonefly gut was significantly greater in dosed streams than in controls (Student´s $t=2.13$, $p=0.05$). The total number

Table 14. Results of stomach contents analysis of *Paragnetina fumosa* from control (n=16) and dosed (n=18) streams. Values given are the mean per stonefly ± standard deviation.

	Controls	Dosed
Number of prey	1.6 (±) 0.6	1.9 (±) 0.4
Number of Hydropsychidae	1.1 (±) 0.3	1.5 (±) 0.1
Percent Hydropsychidae	70 (±) 9	81 (±) 18

of prey per stonefly was also greater in dosed streams, but this difference was not significant. Stoneflies showed strong preference for caddisflies, particularly Hydropsychidae. On day 14, these organisms comprised 70% and 81% of the stomach contents of stoneflies from C-P and D-P streams, respectively.

Discussion

These results demonstrated that chronic exposure of benthic communities to Cu (< 6 ug/L) significantly increased predation on two of the nine dominant taxa examined. Results of gut analyses also showed that predation on Hydropsychidae was significantly greater in dosed streams. For the remaining groups, as well as the various community level parameters examined, predation intensity was similar in control and dosed streams.

Both taxa showing significant metals x predation interaction were net-spinning caddisflies. While the number of *Chimarra* sp. in experimental streams was relatively low, *Hydropsyche morosa* was the second most abundant species collected and accounted for ~18% of total individuals in control streams. Hydropsychid caddisflies were also the major component of stonefly diets. The preference of a related species, *Paragnetina media*, for net-spinning caddisflies has been reported elsewhere (Fuller and Hynes 1987).

Abundance of Hydropsychidae was similar in C-NP and D-

NP streams, indicating the tolerance of these organisms for heavy metals (Fig. 20). Several other investigators have noted that caddisflies are resistant to heavy metals (Winner et al. 1975; Chadwick and Canton 1986; Clements et al. in press a), thus the significant metals x predation interaction for this group was surprising. Greater vulnerability of Hydropsyche to predation in dosed streams may have resulted from a change in behavior of these organisms; however, since my experiments did not include behavioral observations, I can only speculate about the mechanism responsible for this interaction. There is evidence that heavy metals disrupt silk-spinning in Hydropsychidae and result in anomalies in capture net structure (Petersen and Petersen 1983). If these alterations caused Hydropsyche to spend more time outside of their retreats, maintaining or repairing capture nets, then predation pressure on these organisms would be increased. Hershey and Dodson (1985), for example, reported that vulnerability of chironomids to predation was a function of the amount of time these organisms spent outside their tubes. This explanation is complicated by the fact that predation on Cheumatopsyche sp., the dominant caddisfly in my streams, was not affected by Cu.

While gut analyses revealed that caddisflies were the major component of stonefly diets, abundance of most taxa was also reduced by predation, suggesting that these organisms consumed a wide variety of prey. In C-P and D-P streams, total macroinvertebrate abundance was reduced by 107-116 organisms compared to streams without predators. Peckarsky (1985) reported that reduced prey abundance in cages containing stoneflies was a result of either predator avoidance and/or actual prey consumption. Since prey could not emmigrate out of my experimental streams, differences between C-NP and C-P streams were a direct result of prey consumption. Per capita predation rates, estimated from the above values, were 1.27 and 1.38 prey/stonefly/day in control and dosed streams, respectively. These values were comparable to those obtained from stonefly gut analyses in the present study (Table 14) as well as those reported in the literature for related species (Peckarsky 1985).

The importance of biotic interactions in structuring benthic communities is often influenced by levels of disturbance (Menge 1976; Dayton 1971). Peckarsky (1983) proposed a conceptual model of stream community structure and hypothesized that biotic interactions among stream invertebrates would become less important as physical and chemical conditions become harsh. My experimental results were in contrast to this prediction, as species interactions among stoneflies and caddisflies were actually greater in treated streams. It must be noted, however, that levels of disturbance in these experiments were moderate, as indicated by the lack of Cu effects on most taxa. Previous experiments have shown that higher levels of Cu exposure (12-25 ug/L) have severe effects on macroinvertebrate communities (Clements et al. in press b). I hypothesize that at these higher levels of stress, the importance of predation will be reduced and physical/chemical conditions will determine the structure of benthic communities.

Summary of Results

Chapter one

1. Substrate-filled trays were rapidly colonized by a diverse assemblege of aquatic macroinvertebrates and approached equilibrium conditions within 2-3 weeks.
2. Observed differences in colonization rates during 1985 and 1986 were most likely the result of differences in macroinvertebrate density between years.
3. Substrate-filled trays were less variable than either multiplate or Surber samplers; only six samples were necessary to detect a 15% reduction in abundance and a 12% reduction in number of taxa at metal-impacted field sites.
4. Since these samples were relatively small and did not accumulate large amounts of organic detritus, the time required to process samples was greatly reduced.

Chapter two

1. After 6 d in control experimental streams, macroinvertebrate communities on substrate-filed trays were similar to those in the field, indicating that these communities can be maintained in the laboratory with little evidence of control mortality.
2. Acute exposure of these communities to Cu significantly reduced abundance and number of taxa and altered community composition during each season.
3. Ephemeroptera were quite sensitive to Cu exposure,

particularly during summer experiments when water temperatures were higher; other seasonal differences in community responses to Cu were attributed to differences in species composition of introduced substrates.

4. The introduced substrates employed in these studies are amenable to experimental manipulation and will provide a unique opportunity to examine community responses to toxicants.

Chapter three

1. Colonization of introduced substrates was faster at reference stations than at metal-impacted sites in the Clinch River.
2. Chironomids were the earliest colonists on all trays; at the reference station, these organisms were gradually replaced by caddisflies and mayflies, whereas at impacted stations Orthocladiini chironomids dominated trays during the entire 42-d study.
3. Abundance of Hydropsychidae increased significantly at recovery stations 15 and 16 compared to upstream reference sites.
4. Tanytarsini chironomids and mayflies were eliminated from impacted field sites in the Clinch River.
5. Macroinvertebrate community responses to Cu and Zn in experimental streams were similar to those observed in the field, suggesting that these responses were highly

predictable.

6. Increased abundance of Orthocladiini in dosed experimental streams and at impacted field sites may be analagous to the response of _Tubifex tubifex_ to organic pollution.
7. The use of natural assembleges of aquatic insects for toxicity testing provides an opportunity to examine the responses of organisms rarely included in standard laboratory bioassays.

Chapter four

1. The reference site at the Clinch River was dominated by mayflies and Tanytarsini chironomids, which were eliminated at impacted sites.
2. In contrast to results of the 1986 benthic survey, these sensitive organisms showed little indication of recovery at stations 15 and 16.
3. Impacted and recovery sites were dominated by Orthocladiini and the net-spinning caddisflies, _Hydropsyche morosa_ and _Cheumatopsyche_ sp.
4. The greater impact of Cu in New River streams was most likely a result of the lower hardness and alkalinity of New River water compared to Clinch River water.
5. Multivariate analyses of these data also revealed the greater impact of Cu in New River streams. After 10 d of exposure to Cu, macroinvertebrate communities in New River streams at 9 ug Cu/L were similar to those in Clinch River

streams at 24 ug Cu/L.

6. Considerable variation in response to Cu exposure was observed among taxa in experimental streams. The mayflies, *Isonychia bicolor* and *Baetis brunneicolor*, were highly sensitive to Cu whereas Orthocladiini chironomids and hydropsychid caddisflies were quite tolerant.
7. These results demonstrate the importance of accounting for both water quality and composition of the resident fauna when establishing criteria for metals.

Chapter five

1. Predation by *Paragnetina fumosa* in experimental streams had a major impact on macroinvertebrate communities. Total abundance was reduced by 35-42% in streams with predators.
2. Stonefly predation on two taxa, *Hydropsyche morosa* and *Chimarra* sp., was significantly greater in dosed streams than controls.
3. Analysis of stonefly stomach contents also revealed greater vulnerability of Hydropsychidae to predation in dosed streams.

References

Adams, W. J., R. A. Kimerle, B. B. Heidolph, and P. R. Michael. 1983. Field comparison of laboratory derived acute and chronic toxicity data. In W. E. Bishop, R. D. Cardwell, and B. B. Heidolph, [eds]. Aquatic toxicology and hazard assessment: Sixth Symposium, STP 802. Amer. Soc. Test. Mat., Philadelphia, PA. pp. 367-385.

Allan, J. D. 1982. Feeding habits and prey consumption of three setipalpian stoneflies (Plecoptera) in a mountain stream. Ecology 63:26-34.

Allan, J. D. 1984. Hypothesis testing in ecological studies of aquatic insects. In V. H. Resh and D. M. Rosenberg [eds.] The ecology of aquatic insects. Praeger Scientific, New York, NY. pp. 484-507.

Anderson, R. L., C. T. Walbridge, and T. J. Fiandt. 1980. Survival and growth of Tanytarsus dissimilis (Chironomidae) exposed to copper, cadmium, zinc, and lead. Arch. Environ. Contam. Toxicol. 9:329-335.

Armitage, P. D. 1980. The effects of mine drainage and organic enrichment on benthos in the River Nent System, Northern Pennines. Hydrobiologia 74:119-128.

Boyle, T. P. 1983. Role and application of semicontrolled ecosystem research in the assessment of environmental chemicals. In W. E. Bishop, R. D. Cardwell, and B. B. Heidolph, [eds]. Aquatic toxicology and hazard assessment: Sixth Symposium, STP 802. Amer. Soc. Test. Mat., Philadelphia, PA. pp. 406-413.

Brown, B. E. 1977. Uptake of copper and lead by a metal-tolerant isopod Asellus meridianus Rac. Freshw. Biol. 7: 235-244.

/Brown, B. E. 1977. Effects of mine drainage on the River Hayle, Cornwall A) Factors affecting concentrations of copper, zinc and iron in water, sediments and dominant invertebrate fauna. Hydrobiologia 52:221-233.

Burks, S. L. and J. L. Wilhm. 1977. Bioassays with a natural assemblage of benthic macroinvertebrates. In F. L. Mayer and J. L. Hamelink [eds], Aquatic toxicology and hazard evaluation, ASTM STP 634. Amer. Soc. Test. Mat., Philadelphia, PA. pp. 127-136.

Cairns, J., Jr. 1957. Environment and time in fish toxicity. Industr. Wastes 2:1-15.

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

Cairns, J. Jr. 1982. Artificial substrates. Ann Arbor Science Publ., Ann Arbor, MI. 279 pp.

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

Cairns, J., Jr. 1986. The myth of the most sensitive species. BioScience 36:670-672.

Cairns, J., Jr. and A. Scheier. 1957. The effects of temperature and hardness of water upon the toxicity of zinc to the common bluegill (Lepomis macrochirus Raf.). Nat. Acad. Nat. Sci. Phila. 299:1-12.

Cairns, J. Jr., K. M. Hart, and M. S. Henebry. 1980. The effects of a sublethal dose of copper sulfate on the colonization rate of freshwater protozoan communities. Amer. Midl. Nat. 104:93-101.

Carlson, A. R., H. Nelson, and D. Hammermeister. 1986. Development and validation of site-specific water quality criteria for copper. Environ. Toxicol. Chem. 5: 997-1012.

Chadwick, J. W., and S. P. Canton. 1983. Comparison of multiplate and surber samplers in a Colorado Mountain stream. J. Freshwat. Ecol. 2:287-292.

Chadwick, J. W., S. P. Canton, and R. L. Dent. 1986. Recovery of benthic invertebrate communities in Silver Bow Creek, Montana, following improved metal mine wastewater treatment. Water Air Soil Pollut. 28:427-438.

Clements, W. H., J. H. Van Hassel, D. S. Cherry, and J. Cairns, Jr. In press a. Colonization, variability, and application of substratum-filled trays for biomonitoring benthic communities. Hydrobiologia.

Clements, W. H., D. S. Cherry, and J. Cairns, Jr. In press b. Structural alterations in aquatic insect communities exposed to copper in laboratory streams. Environ. Toxicol. Chem.

Clubb, R. W., A. R. Gaufin, and J. L. Lords. 1975. Acute cadmium toxicity studies upon nine species of aquatic insects. Environ. Res. 9:332-341.

Cuffney, T. F., J. B. Wallace, and J. R. Webster. 1985. Pesticide manipulation of a headwater stream: invertebrate responses and their significance for ecosystem processes. Freshw. Biol. 3:153-171.

Dayton, P. K. 1971. Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. Ecol. Monogr. 41:351-389.

De Pauw, N., D. Roels, and A. P. Fontoura. 1986. Use of artificial substrates for standardized sampling of macroinvertebrates in the assessment of water quality by the Belgian Biotic Index. Hydrobiologia 133:237-258.

Dewey, S. L. 1986. Effects of the herbicide atrazine on aquatic insect community structure and emergence. Ecology 67:148-162.

Downing, J. A. 1979. Aggregation, transformation, and the design of benthos sampling programs. J. Fish. Res. Bd. Can. 36:1454-1463.

Fuller, R. L. and H. B. N. Hynes. 1987. Feeding ecology of three predacious aquatic insects and two fish in a riffle of the Speed River, Ontario. Hydrobiologia 150:243-255.

Gauss, J. D., P. E. Woods, R. W. Winner and J. H. Skillings. 1985. Acute toxicity of copper to three life stages of Chironomus tentans as affected by water hardness-alkalinity. Environ. Pollut. 37:149-157.

Geckler, J. R., W. B. Horning, T. M. Neiheisel, Q. H. Pickering, E. L. Robinson, and C. E. Stephan. 1976. Validity of laboratory tests for predicting copper toxicity in streams. Ecological Research Series, EPA-600/3-76-116, U. S. Environmental Protection Agency, Duluth, MN.

Goodyear, C. P. 1972. A simple technique for detecting effects of toxicants or other stresses on a predator-prey interaction. Trans. Amer. Fish. Soc. 101:367-370.

Green, R. H. 1979. Sampling design and statistical methods for environmental biologists. Wiley and Sons, N.Y., NY. 257 pp.

Hall, R. J., C. T. Driscoll, G. E. Likens, and J. M. Pratt. 1985. Physical, chemical, and biological consequences of episodic aluminum additions to a stream. Limnol. Oceanogr. 30:212-220.

Hansen, S. R. and R. R. Garton. 1982. The effects of diflubenzuron on a complex laboratory stream community. Arch. Environ. Contam. Toxicol. 11:1-10.

Hart, C. W. Jr. and S. L. H. Fuller. 1974. Pollution ecology of freshwater invertebrates. Academic Press, New York, NY.

Hart, D. D. 1985. Causes and consequences of territoriality in a grazing stream insect. Ecology 66:404-414.

Hatfield, C. T. and J. M. Anderson. 1972. Effects of two insecticides on the vulnerability of atlantic salmon (Salmo salar) parr to brook trout (Salvelinus fontinalis) predation. J. Fish. Res. Bd. Can. 29:27-29.

Heath, 1987. Water pollution and fish physiology. CRC Press, Inc. Boca Raton, FL.

Hershey, A. E. 1987. Tubes and foraging behavior in larval Chironomidae: implications for predator avoidance. Oecologia (Berlin) 73:236-241.

Hester, F. E., and J. S. Dendy. 1962. A multiplate sampler for aquatic macroinvertebrates. Trans. Amer. Fish. Soc. 91:420-421.

Hodson, P. V., U. Borgmann, and H. Shear. 1979. Toxicity of copper to aquatic biota, p. 308-372. In J. O. Nriagu [ed.] Copper in the environment, II: Health effects. John Wiley & Sons, New York, NY.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54:187-211.

Hynes, H. B. N. 1960. The biology of polluted waters. Liverpool University Press, Liverpool, England.

Kania, H. J. and J. O´Hara. 1974. Behavioral alterations in a simple predator-prey system due to a sublethal exposure to mercury. Trans. Amer. Fish. Soc. 103:134-136.

Khalaf, G., and H. Tachet. 1980. Colonization of artificial substrata by macroinvertebrates in a stream and variations according to stream size. Freshw. Biol. 10:475-482.

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

Kosalwat, P., and A. W. Knight. 1987. Acute toxicity of aqueous and substrate-bound copper to the midge, Chironomus decorus. Arch. Environ. Contam. Toxicol. 16:275-282.

LaPoint, T. W., S. M. Melancan, and M. K. Morris. 1984. Relationships among observed metal concentrations, criteria, and benthic structural community responses in 15 streams. J. Water Pollut. Contol Fed. 56:1030-1038.

McAuliffe, J. R. 1984. Competition for space, disturbance, and the structure of a benthic stream community. Ecology 65:894-908.

Menge, B. A. 1976. Organization of the New England rocky intertidal community: role of predation, competition, and environmental heterogeneity. Ecol. Monogr. 46:355-393.

Merritt, R. W., and K. W. Cummins (eds.). 1978. An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Co., Dubuque, IA.

Miller, T. G. and W. C. Mackay. 1980. The effects of hardness, alkalinity, and pH of test water on the toxicity of copper to rainbow trout (Salmo gairdneri). Water Res. 14:129-133.

Minshall, G. W., and J. N. Minshall. 1977. Microdistribution of benthic invertebrates in a Rocky Mountain (U.S.A.) stream. Hydrobiologia. 55:231-249.

Moore, J. W., and S. Ramamoorthy. 1984. Heavy metals in natural waters: Applied monitoring and impact assessment. Springer-Verlag, New York, NY.

Morin, A. 1985. Variability of design estimates and the optimization of sampling programs for stream benthos. Can. J. Fish. Aquat. Sci. 42:1530-1534.

Mount, D. I. 1985. Scientific problems in using multispecies toxicity tests for regulating purposes, p. 13-18. In J. Cairns, Jr. [ed.] Multispecies toxicity testing. Pergamon Press, New York, NY.

Nehring, R. B. 1976. Aquatic insects as biological monitors of heavy metal pollution. Bull. Environ. Contam. Toxicol. 15:147-154.

Niederlehner, B. R., J. R. Pratt, A. L. Buikema, Jr., and J. Cairns, Jr. 1985. Laboratory tests evaluating the effects of cadmium on freshwater protozoan communities. Environ. Toxicol. Chem. 4:155-165.

Odum, E. P. 1984. The mesocosm. BioScience 34:558-562.

Pascoe, D., S. A. Evans, and J. Woodworth. 1986. Heavy metal toxicity to fish and the influence of water hardness. Arch. Environ. Contam. Toxicol. 15:481-487.

Peckarsky, B. L. 1983. Biotic interactions or abiotic limitations? A model of lotic community structure. In Dynamics of lotic ecosystems. S. M. Bartell and T. D. Fontaine III [eds]. Ann Arbor Science Publishers, Inc., Ann Arbor, MI. pp. 131-160.

Peckarsky, B. L. 1985. Do predaceous stoneflies and siltation affect the structure of stream insect communities colonizing enclosures? Can. J. Zool. 63:1519-1530.

Peters, G. T., D. S. Cherry, and J. Cairns, Jr. 1985. Responses of Isonychia bicolor to alkaline pH: an evaluation of survival, oxygen consumption, and chloride cell ultrastructure. Can. J. Fish. Aquat. Sci. 42:1088-1095.

Petersen, L. B.-M., and R. C. Petersen. 1983. Anomalies in hydropchchid capture nets from polluted streams. Freshw. Biol. 13:185-191.

Ramusino, M. C., G. Pacchetti, and A. Lucchese. 1981. Influence of chromium (IV) upon Ephemeroptera in the Pre-Alps. Bull. Environ. Contam. Toxicol. 26:228-232.

Resh, V. H. 1979. Sampling variability and life history features: basic considerations in the design of aquatic insect studies. J. Fish. Res. Bd. Can. 36:290-311.

Roby, K. B., J. D. Newbold, and D. C. Erman. 1978. Effectiveness of an artificial substrate for sampling macroinvertebrates in small streams. Freshw. Biol. 8:1-8.

Rosenberg, D. M., and V. H. Resh. 1982. The use of artificial substrates in the study of freshwater benthic macroinvertebrates. In J. Cairns Jr. [ed], Artificial substrates. Ann Arbor Science Publishers, Ann Arbor, MI. pp. 175-235.

Shaw, D. W., and G. W. Minshall. 1980. Colonization of an introduced substrate by stream macroinvertebrates. Oikos 34:259-271.

Simpson, K. W. 1980. Abnormalities in the tracheal gills of aquatic insects collected from streams receiving chlorinated or crude oil wastes. Freshw. Biol. 10:581-583.

Smock, L. A. 1983a. The influence of feeding habits on whole-body metal concentrations in aquatic insects. Freshw. Biol. 13:301-311.

Smock, L. A. 1983b. Relationships between metal concentrations and organism size in aquatic insects. Freshw. Biol. 13:313-321.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry, 2nd edition. W. H. Freeman and Company, San Francisco, CA.

Specht, W. L. 1985. Alteration and recovery of a stream macroinvertebrate community exposed to fly ash effluent and an analysis of the causative factors. Ph.D. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, VA. 208 pp.

Specht, W. L., D. S. Cherry, R. A. Lechleitner, and J. Cairns, Jr. 1984. Structural, functional, and recovery responses of stream invertebrates to fly ash effluent. Can. J. Fish. Aquat. Sci. 41:884-896.

Spehar, R. L., R. L. Anderson, and J. T. Fiandt. 1978. Toxicity and bioaccumulation of cadmium and lead in aquatic invertebrates. Environ. Pollut. 15: 195-208.

Sprague, J. B., P. F. Elson, and R. L. Saunders. 1965. Sub-lethal copper-zinc pollution in a salmon river - a field laboratory study. Air Water Pollut. 9:531-543.

Sullivan, J. F., G. J. Atchinson, D. J. Kolar, and A. W. McIntosh. 1978. Changes in predator-prey behavior of fathead minnows (Pimephales promelas) and largemouth bass (Micropterus salmoides) caused by cadmium. J. Fish. Res. Bd. Can. 35:446-451.

Surber, E. W. 1959. Cricotopus bicinctus, a midgefly resistant to electroplating wastes. Trans. Am. Fish. Soc. 89: 111-116.

Townsend, C. R. and A. G. Hildrew. 1976. Field experiments on the drifting, colonization, and continuous redistribution of stream benthos. J. Anim. Ecol. 45:759-772.

U. S. Environmental Protection Agency. 1979. Methods for chemical analysis of water and wastes. Environmental monitoring and support laboratory, Cincinnati, OH. pp. 120.1-1-310.1-3.

U.S. Environmental Protection Agency. 1980. Ambient water quality criteria for copper. EPA-440/5-80-036. Washington, DC, U.S. Government Printing Office.

U.S. Environmental Protection Agency. 1983. Water quality standards handbook. Office of Water Regulations and Standards, Washington, DC. pp. 4-1-2-20.

Van Hassel, J. H., and A. E. Gaulke. 1986. Site-specific water quality criteria from in-stream monitoring data. Environ. Toxicol. Chem. 5:417-426.

Warnick, S. L., and H. L. Bell. 1969. The acute toxicity of some heavy metals to different species of aquatic insects. J. Water Pollut. Control Fed. 41: 280-284.

Waterhouse, J. C., and M. P. Farrell. 1985. Identifying pollution related changes in chironomid communities as a function of taxonomic rank. Can. J. Fish. Aquat. Sci. 42: 406-413.

Wiederholm, T. 1984. Responses of aquatic insects to environmental pollution. p. 508-557. In V. H. Resh and D. M. Rosenberg [eds.] The ecology of aquatic insects. Praeger, New York, NY.

Winner, R. W., and J. D. Gauss. 1986. Relationship between chronic toxicity and bioaccumulation of copper, cadmium, and zinc as affected by water hardness and humic acid. Aquat. Toxicol. 8:149-161.

Winner, R. W., J. Scott Van Dyke, N. Caris, and M. P. Farrell. 1975. Response of macroinvertebrate fauna to a copper gradient in an experimentally-polluted stream. Int. Verein Theoret. Angewan Limnol. Verhan. 19: 2121-2127.

Winner, R. W., M. W. Boesel, and M. P. Farrell. 1980. Insect community structure as an index of heavy-metal pollution in lotic ecosystems. Can. J. Fish. Aquat. Sci. 37: 647-655.

Woltering, D. M., J. L. Hedtke, and L. J. Weber. 1978. Predator-prey interactions of fishes under the influence of ammonia. Trans. Amer. Fish. Soc. 107:500-504.

Young, J. S., R. R. Adee, I. Piscopo, and R. L. Buschbom. 1981. Effects of copper on the sabellid polychaete, Eudistylia vancouveri, II. Copper accumulation and tissue injury in the branchial crown. Arch. Environ. Contam. Toxicol. 10:87-104.

The ___5___ page vita has been removed from the scanned document

The vita has been removed from the scanned document

The vita has been removed from the scanned document

The vita has been removed from the scanned document

The vita has been removed from the scanned document