Environmental Toxicology and Chemistry, Vol. 7, pp. 701–713, 1988
Printed in the USA. Pergamon Press plc

0730-7268/88 $3.00 + .00

Farris et al 1988

APPLICATION OF CELLULOLYTIC ACTIVITY OF ASIATIC CLAMS (*CORBICULA* SP.) TO IN-STREAM MONITORING OF POWER PLANT EFFLUENTS

JERRY L. FARRIS,* JOHN H. VAN HASSEL, SCOTT E. BELANGER, DONALD S. CHERRY and JOHN CAIRNS, JR.
Department of Biology and University Center for Environmental Studies, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

(*Received* 3 *April* 1987; *Accepted* 15 *December* 1987)

Abstract—Rigorous testing schemes in field-located artificial streams and in-stream monitoring provided evidence for use of *Corbicula* cellulolytic activity as a highly sensitive and efficient approach to effluent assessment. Cellulolytic (exo- and endocellulase) activity of the Asiatic clam, *Corbicula* sp., determined in 30-d, field-located artificial stream exposures at the New River, Virginia to single components of power plant effluents (copper [Cu] and zinc [Zn] separately) was compared with cellulolytic responses in caged clams from within an impacted area of the Clinch River, Virginia below power plant effluents. Cellulolytic responses were then compared to conventional biomonitoring responses (Hester-Dendy macroinvertebrate community structure), water quality monitoring in the Clinch River, and laboratory artificial stream bioassays. Clam enzyme activity was significantly reduced in 10 to 20 d ($\alpha = 0.05$) at 16 and 87 μg Cu and Zn/L, respectively, in field-located artificial streams. Cellulolytic activity of clams caged at stations within power plant outfalls (metal concentrations of 47–78 μg Zn/L and 80–345 μg Cu/L) was significantly reduced to levels as low as 9 to 52% of upstream activity levels. Reduction in cellulolytic activity in *Corbicula* was more sensitive after 14 d of in-stream monitoring than reduction in diversity of macroinvertebrate assemblages after 28 d. Bioassay exposures as long as 30 d were needed to provide toxicity data comparable to enzyme impairment seen as early as 10 d.

Keywords—Cellulase Biomonitoring Heavy metals *Corbicula* Effluent

INTRODUCTION

Assessment of the extent and severity of impacts from industrial effluents usually involves either documenting qualitative and quantitative shifts in resident populations (structural measurements of fish and/or invertebrate present) or predicting effects based on the presence of priority pollutants in the effluents or on the results of effluent toxicity testing. Major constitutents within a variety of complex power plant effluents that could potentially affect aquatic ecosystems include thermal and pH excursions from ambient conditions, suspended particles associated with fly ash, settling pond discharges, heavy metals and other metalloids such as arsenic and selenium from ash ponds or cooling tower blowdowns, and chlorine [1–3]. These components may cause ash siltation of benthic habitat, heavy metal bioaccumulation in invertebrates and fish, and alter osmoregulatory capacities of fish and macroinvertebrates [4]. The effects of such perturbations include simplification of food chains, reduction in number and diversity of heterotrophic bacteria populations, reductions in diversity and density of benthic macroinvertebrate communities, and reductions in fish populations [5]. However, measurements of shifts in resident populations fail to provide information on sensitive stress responses among individuals that precede population-level effects or recovery potential of surviving individuals. Similarly, determination of body burdens of accumulated metals may help assess the level of exposure or persistence but may not necessarily relate to effects or to the relative health of surviving organisms [6]. The current use of laboratory bioassays to predict effects on aquatic ecosystems is being challenged as not providing an accurate representation of the responses of resident aquatic biological communities [7]. Attempts to overcome limitations have in-

*To whom correspondence may be addressed.
J.H. Van Hassel's current address is Environmental Engineering Division, American Electric Power Service Corporation, Columbus, Ohio 43216.

Presented at the Symposium on Population and Community Level Responses to Environmental Perturbations, Seventh Annual Meeting of the Society of Environmental Toxicology and Chemistry, Alexandria, Virginia, November 2–5, 1986.

cluded direct in-stream measurement of biological response [8], integrated field-laboratory approaches to toxicity testing [9], and simultaneous toxicity testing at several levels of biological organization [10].

A number of biochemical tests for diagnosis of acute or chronic pollutant stress has been successfully developed [11-13]. Although these tests are sensitive at sublethal exposures, their usefulness in monitoring complex effluents is complicated by the need to correlate sensitive internal changes in individuals with adverse effects occurring in entire populations in receiving systems. Studies emphasizing enzyme inhibition within aquatic organisms from heavy metal cations have presented possible applications to monitoring [14]. Yet no reliable combination of measurements has proven to validate dose-effect responses to all pathways of uptake. Brown [14] found that some heavy metals may activate cellular enzymes or enhance their activity at low concentrations while being inhibiting at higher concentrations. Indeed, this may suggest that the most sensitive indicator of effect may not always be the most interpretable in terms of discerning real-world impact upon aquatic systems.

In addition, an inherent difficulty exists in relating biological responses to critical components of complex effluents. Fetterolf et al. [15] noted that consideration of changes in effluents through time must include variability in composition and discharge rates. It is clearly more difficult to assess complex effluents in receiving systems than single chemicals in the laboratory. Therefore, careful consideration must not only be given to the distribution and concentration of the effluent as it interacts with the receiving system, but also to changes in response by receiving system biota relative to the distribution of the effluent [16].

In this study, we incorporated a sensitive functional measurement (response of cellulolytic activity in *Corbicula* sp. using field-located artificial stream tests) with data from laboratory and field testing to examine effects from selected components of power plant effluents (copper [Cu] and zinc [Zn]). Cellulolytic indices from individual component studies were compared to cellulolytic responses in clams transplanted within receiving zones of power plant effluents. These responses were then compared to conventional in-stream biological responses (macroinvertebrate community structure) and water quality monitoring to determine the impact of a power plant effluent containing elevated concentrations of Cu and Zn.

MATERIALS AND METHODS

Bioassays

Short (96-h) and long-term (30-d) bioassays were conducted in the Ecosystem Simulation Laboratory (ESL) at Virginia Tech and at the Glen Lyn field (outdoor) site, Glen Lyn, Virginia, under conditions ranging from static to artificial stream flow-through tests from 17 July 1984 to 28 June 1986. Adult *Corbicula* (14.5-20.0 mm shell length) were collected from the New River, Virginia (river mile 100). Clams were either transported 13 km to the Glen Lyn Power Plant where they were acclimated in 20-L oval artificial stream systems receiving New River water [17], or they were transferred to the ESL for acclimation to dechlorinated municipal tap water.

Two static, 96-h exposures to Zn (as $ZnSO_4 \cdot 7H_2O$) at [illegible] replicated concentrations ranging from 500 to 50,000 μg/L were performed by placing 10 clams per replicate on a raised Plexiglas platform in a 15-L polycarbonate container as described by Belanger et al. [18].

Two 30-d flow-through artificial stream toxicity tests using clams at the Glen Lyn field site were conducted using the aforementioned artificial streams continually renewed with New River water at 1.2 L/min. Peristaltic pumps were used to continuously dose $ZnSO_4$ (100 to 5000 μg Zn/L) or $CuSO_4$ (6-26 μg Cu/L) at a rate of 0.5 ml/min. Stock solutions were held in 25-L carboys and were changed every other day during testing. Thirty clams were randomly distributed in artificial streams filled to a depth of 2 cm with coarse sand sediment. Additional static tests for Cu were discontinued since *Corbicula* did not have observed mortality in any of the short-term metal tests at levels <10,000 μg/L.

Probit analysis [19,20] was used to determine LC50 estimates (± 95% feducial limits) when appropriate.

30-d artificial stream tests

Copper and zinc. In fall 1984, Zn was metered into artificial streams (three replicates per concentration) at Glen Lyn from $ZnSO_4 \cdot 7H_2O$ stocks at target concentrations of control (no addition), 50, 500, and 1000 μg/L with subsequent realized mean concentrations of 27, 56, 633, and 1186 μg/L (Table 1). For the summer 1986 test, Cu was added to streams from $CuSO_4 \cdot 7H_2O$ stocks at target concentrations of control, 12, 25, and 50 μg/L with subsequent realized concentrations of 5 (control), 16, 21, and 26 μg/L.

Table 1. Water chemistry of 30-d Zn and Cu tests at Glen Lyn, Virginia and Clinch River Hester-Dendy artificial substrate stations used with *Corbicula* monitoring (SE in parentheses)

Study	Targeted exposure or station	Measured Zn concentration (μg/L)	Measured Cu concentration (μg/L)	Temperature (°C)	pH	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Conductivity (μmhos)
Glen Lyn Fall 1984 Zn	Control	27 (1)	–	20.6 (1.9)	8.31 (0.12)	88.8 (2.3)	56.2 (0.7)	164.3 (4.0)
	50 μg/L	56 (8)	–	–	8.27 (0.09)	88.3 (2.6)	55.5 (0.8)	160.6 (3.7)
	500 μg/L	633 (66)	–	–	8.20 (0.05)	87.1 (2.6)	59.7 (1.1)	163.0 (3.3)
	1000 μg/L	1186 (30)	–	–	8.14 (0.02)	88.3 (2.3)	56.1 (0.7)	167.3 (4.1)
Glen Lyn Summer 1986 Cu	Control	–	5 (1)	24.7 (0.4)	8.44 (0.03)	71.2 (1.4)	46.5 (0.7)	136.0 (1.3)
	12 μg/L	–	16 (1)	–	8.31 (0.03)	70.6 (1.1)	45.3 (0.7)	134.7 (1.4)
	25 μg/L	–	21 (2)	–	8.40 (0.04)	71.7 (0.7)	46.4 (0.7)	134.2 (1.5)
	50 μg/L	–	26 (2)	–	8.36 (0.03)	70.3 (0.5)	46.9 (0.7)	131.6 (1.5)
Clinch River Fall 1985	1	17[a]	<15	24.0 (0.5)	8.42 (0.03)	149.2 (4.1)	133.8 (2.9)	305.3 (1.8)
	8	78 (67)	345[b]	24.3 (1.2)	8.34 (0.06)	256.4 (53.5)	121.6 (14.2)	468.5 (53.6)
	11	47 (21)	80 (40)	24.5 (1.3)	8.46 (0.09)	189.2 (11.4)	124.2 (4.5)	394.8 (17.0)
	15	28[c]	20[d]	24.2 (0.6)	8.45 (0.07)	159.2 (5.6)	134.4 (2.4)	328.2 (3.5)
Clinch River Fall 1986	2	17 (3)	<15	22.6 (0.8)	8.45 (0.05)	155.8 (5.4)	156.0 (9.1)	299.9 (7.0)
	8	81 (20)	104 (18)	21.8 (1.2)	8.36 (0.03)	186.6 (13.4)	117.0 (5.9)	366.2 (19.5)
	11	42 (14)	47 (11)	22.3 (1.2)	8.17 (0.21)	185.0 (8.8)	142.6 (13.3)	360.6 (16.3)
	14A	15 (0)	22 (0)	22.7 (0.8)	8.41 (0.14)	160.2 (5.6)	164.8 (12.2)	320.5 (7.9)
	15A	15 (2)	<15	22.6 (0.7)	8.32 (0.16)	165.8 (4.3)	159.6 (10.6)	323.7 (7.1)

BDL = below detection limit. Detection for Cu ≧ 15 μg/L; Zn ≧ 10 μg/L.
[a]Four Zn measurements of 5 were BDL for Station 1.
[b]One Cu measurement of 2 was BDL for Station 8.
[c]One Zn measurement of 2 was BDL for Station 15.
[d]One Cu measurement of 2 was BDL for Station 15.

Twelve measurements of total Zn or acid-soluble Cu were made at each target concentration (four measurements from each of the three replicates). Analyses (water chemistry and enzyme activity) were performed on days 0, 10, 20 and 30.

Acid-soluble Zn and Cu concentrations were determined by flame and graphite furnace atomic absorption spectrophotometry using a Perkin Elmer Model 310 AAS [21,22]. Total hardness and alkalinity (as mg/L $CaCO_3$) were measured titrimetrically, conductivity by a YSI Model 33 conductivity meter, and pH by an Altex Model 3560 pH meter by procedures given by the U.S. Environmental Protection Agency (EPA) [21]. Stream temperatures were monitored at least twice weekly.

Enzyme activity. Six clams from each treatment during the 30-day tests were chosen randomly on each sample day and transferred to the laboratory for dissection and weighing. Enzyme extracts from individual clams were prepared from whole

body homogenates. Samples were homogenized in 0.15 M phosphate buffer at pH 6.0 at a wet mass to buffer ratio of 0.2 g/ml. Homogenates were centrifuged for 15 min at 15,000 *g*. Supernatants (extracts) were decanted and the final extract volume recorded. Pellets were recovered for dry mass measurements.

Cellulase activity was expressed as a relativized control to treated product index

$$\text{Relativized product index} = 1 + \frac{\begin{pmatrix}\text{treatment}\\ \text{product}\end{pmatrix} - \begin{pmatrix}\text{control}\\ \text{product}\end{pmatrix}}{\text{control product}} \times 100$$

that incorporated both endo- and exocellulase estimates (and their synergistic interaction) to compare units of activity of clams exposed to metal in relation to control activities. One unit of the enzyme is defined in this context as the amount of enzyme required to liberate 1 mg of reducing sugar equivalent to that of glucose per hour using CMC (carboxymethylcellulose) as a substrate. As reviewed by Farris [17], the degradation of crystalline cellulose requires the participation of several enzymes referred to as cellulases. Therefore, both a viscometric assay using 1% (w/v) CMC (Hercules type 7H3SF) solution [23] and a colorimetric reducing sugar determination using dinitrosalicylic acid (DNS) as a reagent [24] were used. Details of these assays are reported by Sinsabaugh [25] and Sinsabaugh et al. [26].

One-way analysis of variance (ANOVA) was used to evaluate the effects of Cu and Zn on *Corbicula* cellulolytic activity in artificial stream tests [20]. Significance was inferred at $\alpha = 0.05$ and Duncan's multiple range test was used to determine means that were significantly different from control activities.

Clinch River testing

Corbicula cellulolytic activity. Thirty adult *Corbicula* were placed in nylon mesh cages (2-mm^2 mesh size) containing precolonized cobble from the Clinch River, Virginia. One cage was tied to an iron stake at each of four predetermined stations coinciding with macroinvertebrate sampling stations 1, 8, 11, and 15 on September 12, 1985 (Fig. 1). These stations were selected to represent one upstream uninfluenced site, two stations under the influence of plant discharges, and one downstream station representative of recovery. Six clams on day 0 were transferred to Virginia Tech for enzyme analysis as previously described. Following 14 d in the field, 6 clams were retrieved from each station for enzyme analysis.

During fall 1986 macroinvertebrate sampling period (August 20 to September 18), caged clams were similarly placed at Hester-Dendy stations 2, 8, 11, 14A, and 15A on August 20. Following 10 and 30 d in the river, clams were retrieved from all stations and analyzed as in 1985.

Statistical analysis of cellulolytic activity in caged *Corbicula* was identical to that for artificial stream tests except that the independent variable was station location and not metal concentration.

Hester-Dendy invertebrate sampling. Benthic invertebrate monitoring using Hester-Dendy multiplate samplers [27] was carried out at 15 sampling stations above, within, and below Appalachian Power Company's Clinch River Plant (Carbo, Virginia) effluent discharges to the Clinch River (Fig. 1) from August 15 to September 12, 1985. Stations 1, 2, 5, 7, 10, 14, and 15 were located on the right side (facing downstream) of the river; stations 3, 6, 8, and 11 on the left side; and stations 9, 12, and 13 in the middle. Stations 1 to 3 were upstream reference sites. To determine potential plant impacts, stations 6, 8, and 11 (left side of the river) were within effluent discharges from plant outfalls designated 003, 004, and 005 (Fig. 1), respectively. Stations 9, 10 and 12 to 15 were selected to measure the downstream extent of effluent effects. Potential confounding effects of a coal-washing facility on Dumps Creek, a small Clinch River tributary opposite the Clinch River Plant, were monitored using stations 4 (Dumps Creek proper), 5 (Clinch River just below Dumps Creek), and 7 (Clinch River below station 5 on the same side).

A second survey was performed from August 20 to September 17, 1986. Six stations used in 1985 were deleted, and four stations were added during the 1986 sampling period in an attempt to eliminate siltation, vandalism, and data redundancy encountered in the first survey. Sampling was performed at stations 1, 2, 3, 4A, 5A, 6, 7, 8, 10, 11, 13, 14A, and 15A in 1986.

For both surveys, three replicate samplers were placed at each location by securing each sampler to iron stakes driven into the river sediment. The samplers were suspended in the water column ~5 cm above the substrate for a colonization period of 4 weeks. Samplers were retrieved, washed, and sieved in the field with a 500-μm sieve and preserved with 10% formalin. In the laboratory, sam-

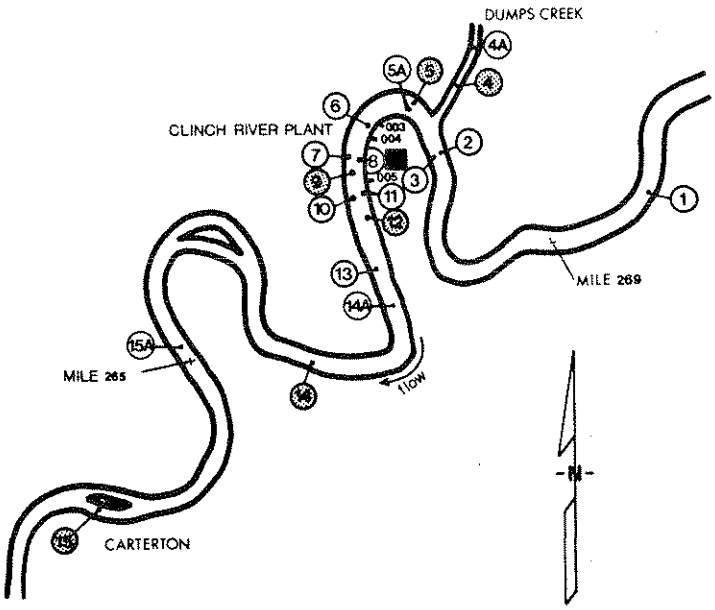

Fig. 1. Schematic representation of the relationships between Clinch River Plant, plant discharges (003, 004, and 005) and stations where *Corbicula* and Hester-Dendy monitoring were accomplished in 1985 and 1986.

ples were randomly selected, sorted to taxonomic orders, and placed in 70% ethanol. Chironomids were identified to tribe by characteristics given in Merritt and Cummins [28]. This key allowed identification of remaining aquatic insects to genus. Mollusc identification was based upon family characteristics given in Burch [29]. Summaries of trophic relationships were compiled from Merritt and Cummins [28].

Invertebrate data were statistically analyzed by ANOVA using the Statistical Analysis System [20]. The effects of sample location on total abundance, taxon diversity, taxon richness, and functional group classifications were tested with significance inferred at $\alpha = 0.05$. Duncan's multiple range test was applied to group means to determine significant differences.

Table 1 lists types of water chemistry analyses performed on samples from mid-depth at each sampling station. All measurements were made in the field except total recoverable Cu and Zn. For these, 200-ml river samples were preserved with 1 ml HNO_3, digested, and analyzed using flame atomic absorption spectroscopy with graphite furnace detection [22]. These two metals were chosen due to their preponderance in the Clinch River Plant effluents. Temperature and conductivity were measured using a YEW Model SC51 Conductivity Meter. A Beckman 21 pH meter was used to measure pH. Hardness and alkalinity were measured using a Hach Kit.

RESULTS

Bioassays

Corbicula was resistant to all exposures of Zn with significant mortality occurring only in 30-d exposures (50% mortality at 1,101 μg Zn/L in flow-through artificial streams). No significant mortality was observed in any short-term bioassay conducted at either Glen Lyn or the ESL, with only 10% mortality occurring at the highest Zn concentration tested (40,000 μg Zn/L). Thirty-day Cu toxicity tests in flow-through artificial stream exposures at Glen Lyn resulted in an LC50 of 19.2 $\pm$ 0.7 μg Cu/L.

Enzyme activity response to Zn and Cu

Total cellulolytic activity, as represented by the relativized exo- and endocellulase product indices, declined with high metal exposures over time for both 30-d exposures in the artificial stream tests (Figs. 2A and B). A significant decline in cellulolytic activity from exposure to 633 and 1186 μg Zn/L was evident as early as day 10 (12 and 14% of control activity, respectively). Cellulolytic activity in clams exposed to 56 μg Zn/L was reduced significantly [166.7 $\pm$ 66 (units/g dry wt)^2] from control clam activities [2307.29 $\pm$ 569 (units/g dry wt)^2] by day 20, and remained so thereafter.

Cellulolytic activity was sensitive to all levels of Cu tested and was significantly reduced within the first 10 d at the lowest exposure of 16 μg Cu/L

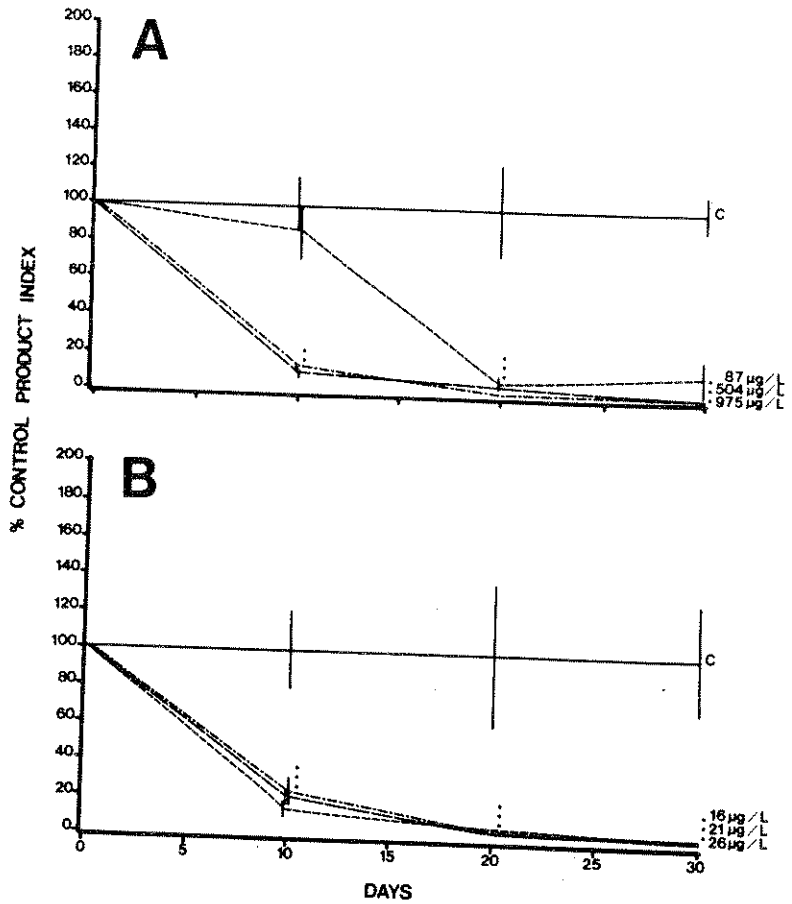

Fig. 2. Response of *Corbicula* cellulolytic complex to Zn (A) and Cu (B) in 30-d artificial stream exposures at Glen Lyn, Virginia. Means ± 1 SE are given on each day. Means that are significantly different from the control are indicated by an asterisk(*).

[487.1 ± 162 (units/g dry wt)^2 or 14% of control cellulolytic activity; Fig. 2B]. By day 30, cellulolytic activity measured from clams in all Cu exposures was ~3% of control clam activity.

Clinch River tests

Cellulolytic activity. Clams held upstream of plant outfalls at station 1, where measured metal concentrations averaged <15 µg Cu/L and <17 µg Zn/L (Table 1) during the 1985 monitoring, had the highest cellulolytic activity levels observed [2141 ± 375 (units/g dry wt)^2; Table 2]. Activity levels measured in clams from effluent influenced stations 8 (345 µg Cu/L and 78 µg Zn/L) and 11 (80 µg Cu/L and 47 µg Zn/L) were significantly reduced to 52% and 9%, respectively, of levels measured in clams from station 1. Cellulolytic activity in clams from station 15 (28 µg Cu/L and 20 µg Zn/L) had a 34% reduction in activity [1409 ± 309 (units/g dry wt)^2] that was not significantly different relative to station 1.

Cellulolytic activity of clams held at stations 8 (105 µg Cu/L and 81 µg Zn/L) and 15A (<15 µg Cu/L and 16 µg Zn/L) during the 1986 monitoring was not significantly reduced below that of clams from upstream station 2 (<15 µg Cu/L and 17 µg Zn/L) by day 10 (Table 2). Clams from effluent-influenced station 11 (47 µg Cu/L and 42 µg Zn/L) had significantly higher activity levels [2822 ± 674 (units/g dry wt)^2] than upstream clams by day 10. This doubling in cellulolytic activity, however, was then reduced to 2% of upstream activity by day 30. Clams from station 14A (23 µg Cu/L and 15 µg Zn/L) had significantly reduced cellulolytic activity by day 30 (35% of station 2 activity). Cellulolytic activity of clams taken

Table 2. Analysis of variance by Kruskal-Wallis for *Corbicula* enzyme activity as 10^5 units/g dry weight with SE (in parentheses, percent activity relative to station 1 with SE) and macroinvertebrate species richness (in parentheses, percent richness relative to station 1) from 30-d in-stream monitoring of the Clinch River. Means that are significantly different from upstream stations 1 and 2 are indicated by an asterisk (*)

		Exo- × endo-cellulase product (% station 1 activity)	Invertebrate species richness (% richness to station 1)
Summer 1985 study—after 14 d			30 d
1	2141 ± 375 (100 ± 18)		17.7 ± 1.8 (100 ± 10)
8	1119 ± 199 (52 ± 9)*		7.0 ± 1.0 (40 ± 6)*
11	183 ± 38 (9 ± 2)*		9.7 ± 1.3 (50 ± 7)*
15	1409 ± 309 (66 ± 14)		10.3 ± 1.2 (59 ± 7)
Summer 1986 study—after 10 d		30 d	30 d
2	1320 ± 292 (100 ± 22)	1852 ± 551 (100 ± 30)	16.7 ± 0.7 (100 ± 4)
8	1117 ± 256 (85 ± 19)	1089 ± 134 (59 ± 7)	7.9 ± 2.5 (42 ± 15)*
11	2822 ± 674 (213 ± 51)*	2 ± 1 (0.1 ± 0.1)*	9.3 ± 2.4 (56 ± 14)*
14A	898 ± 639 (68 ± 48)	649 ± 284 (35 ± 15)*	13.3 ± 0.7 (80 ± 4)
15A	892 ± 135 (68 ± 10)	1771 ± 389 (96 ± 21)	16.0 ± 1.0 (96 ± 6)

from stations 8 and 15A did not differ significantly from activity levels measured in clams from station 2.

Hester-Dendy sampling. In 1985, the number of organisms collected varied substantially among the three replicates at some sampling stations but very little at others. Station 8 had the fewest number of organisms (mean = 29.7 per sampler), while midriver station 13 (which averaged 17.5 μg Zn/L and 22.5 μg Cu/L and was 0.1 km downstream of outfall 005) produced the most (mean = 573 per sampler, Fig. 3A). No station differed significantly in abundance from one or more of the upstream reference stations except station 13, which produced significantly higher abundance than all other stations. Diversity of aquatic invertebrates was lowest at station 15 (1.98) followed by stations 8, 5, 7, 4, and 11 (2.25–2.54). Richness was lowest at station 8 followed by 4, 11, 15, and 5 (7–10.7 taxa per sampler). All of these stations had significantly lower taxonomic richness than the reference sites.

Dipterans were the most abundant organisms at all sampling stations representing 43 to 86% of all organisms collected (Fig. 4A). Other major insect orders collected, in decreasing order of abundance, were Ephemeroptera (0–47%), Trichoptera (1–33%), Coleoptera (0–9%), and Odonata, Plecoptera, and Megaloptera (0–7% each). The proportion of mayflies in the collections ranged from none collected at station 8 to 47.2% of all organisms collected at upstream station 2. Mayfly proportions at stations 4, 8, 11, 13, 14, and 15 were significantly less than at the three reference stations. Non-insect taxa (nematodes, oligochaetes, bivalve molluscs, and snails) comprised a low percentage of collections at all sampling stations (0–6%). Functional feeding group proportions at downstream stations did not differ from stations 1 to 3 except for the presence of higher proportions of predator organisms at stations 4, 5, 8, 11, and 14.

In 1986, the number of organisms collected varied substantially among replicates at stations 1, 4A, 7, 10, and 15A, while stations 2, 3, and 14A had low replicate variability. The fewest number of organisms was found at station 8 (mean = 13.0 per sampler; Fig. 3B) where measured metal concentrations were greatest. Midriver station 5A, where metal concentrations were <12 μg Zn/L and <19 μg Cu/L, had the greatest number of individuals (mean = 240 per sampler). No station differed significantly in abundance from one or more of the upstream stations. Diversity of aquatic invertebrates was lowest at station 5A (1.79) followed by stations 4A, 8, 6, 11, and 13 (2.19–2.61; Fig. 3B). Only stations 4A and 5A differed significantly from upstream diversity values. Taxonomic richness was similar among replicates at most sampling stations, but means ranged from 7.0 taxa per sampler at station 8, to 19.3 taxa at station 1 where metal concentrations averaged 35.0 μg Zn/L and <15 μg Cu/L. Only station 8 had significantly different taxonomic richness than the reference stations.

As in 1985, dipterans (primarily chironomids) were the most abundant organisms in the 1986 collections, representing 20 to 83% of all organisms collected at individual sampling stations (Fig. 4B).

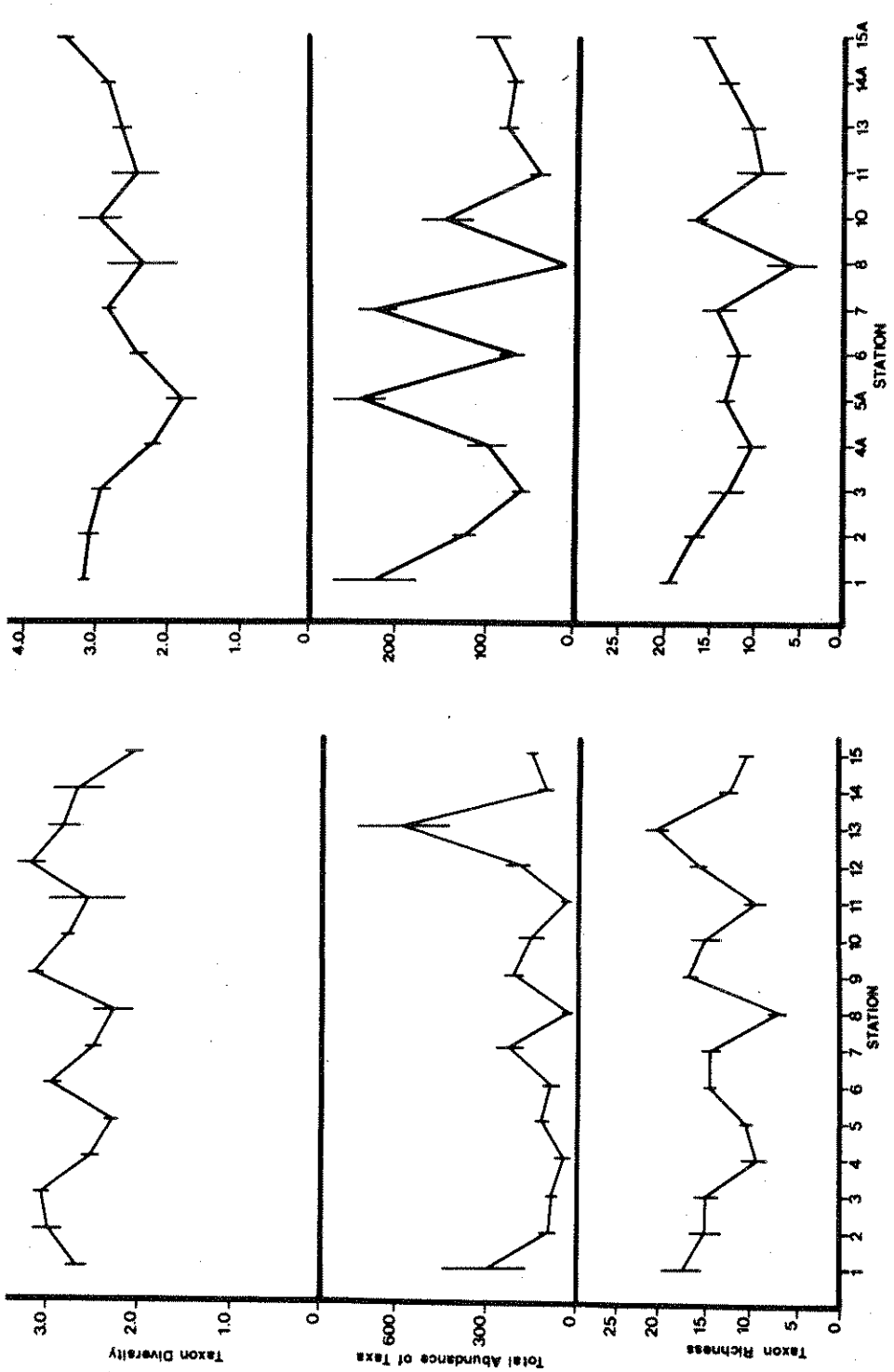

Fig. 3. Taxon diversity, total taxon abundance, and taxon richness at Hester-Dendy collection stations in the Clinch River and Dumps Creek for 1985 (A) and 1986 (B). The mean ± 1 SE is indicated for each parameter.

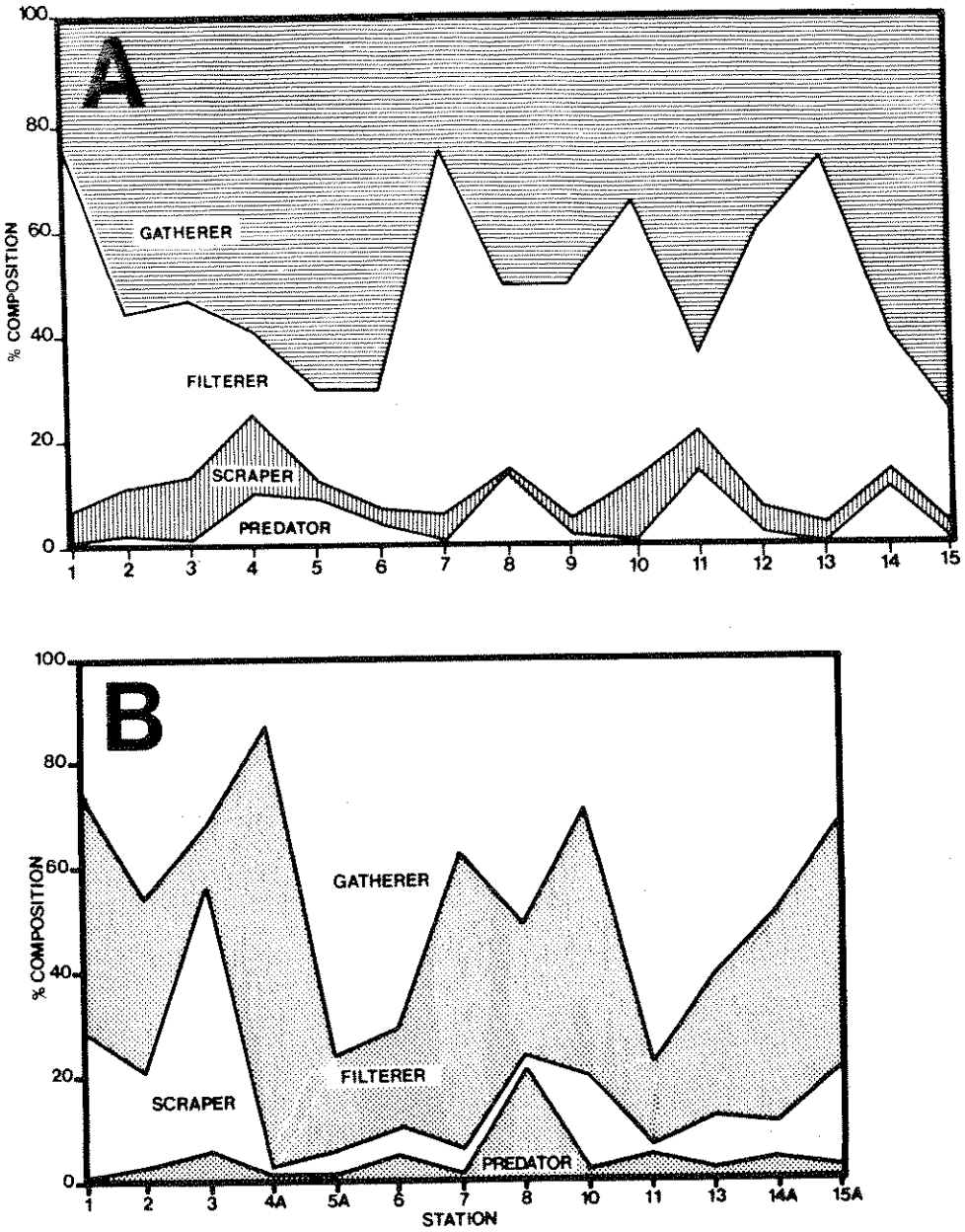

Fig. 4. Percent composition of individuals in major invertebrate orders comprising each Hester-Dendy sample at stations in the Clinch River for 1985 (A) and 1986 (B).

The other major insect orders collected, in decreasing order of abundance, were Ephemeroptera (3–73%), Trichoptera (2–34%), Coleoptera (0–6%), and Odonata, Plecoptera, and Megaloptera (0–8% each). Mayfly contribution to the collections ranged from <5% of collections at stations 4A and 13 to 74.1% of all organisms collected at station 5A. Non-insect taxa again comprised a low percentage of collections at all sampling stations (1–11%). Within-site variability among replicates was great enough that no significant differences in composition were found between downstream and refer-

ence stations. Functional feeding group proportions varied between stations, but no meaningful upstream-downstream differences were found.

DISCUSSION

Bioassays

Although laboratory bioassays incorporating a single component of a complex discharge are pertinent to assessment of certain effluents [30,31], laboratory testing of single metals removes many of the confounding physico-chemical and ecological factors known to affect complex effluent effects on aquatic ecosystems (e.g., changes in TSS, temperature, pH, hardness–toxicant interactions, and food availability). Most often, metals may be only a few of the many components of an effluent that affect bioavailability and/or toxicity [32].

Our toxicity tests involved both Cu and Zn since both were measured at elevated concentrations in Clinch River Plant discharges. Resistance of *Corbicula* in short-term (96-h) exposures to both metals in our tests suggests caution using bivalves in acute toxicity tests for effluent assessments or criteria development. Contradictory findings regarding acute metal toxicity to *Corbicula* have been reported. Harrison et al. [33] found no mortality at 12,000 μg Cu/L in 10 d, while a 96-h LC50 490 μg Cu/L was obtained by Rodgers et al. [34]. This may in part be attributed to the ability of *Corbicula* to isolate tissue from exposure for extended periods. This behavioral avoidance mechanism can be further affected by a number of natural parameters including presence of particulates and food [35]; all pertinent to the bioavailability of metals.

Bivalves have been shown to react to toxicants by incorporating changes in valve adduction, with adjustments in filtration rates, as well as depressing normal burrowing behavior [36]. These adjustments are pertinent to affecting acute metal toxicity by a number of different routes. In studies reported by Belanger and coworkers [18,37], growth of *Corbicula* could be affected by metal exposure at as early as 10 d; however, extending tests to 20 to 30 d increased reliability by use of repeated measurements. This consistency in growth response over 20 to 30 d of exposure suggests that longer term tests with *Corbicula* are most useful for ecotoxicological investigations.

Enzyme responses to Cu and Zn

The value of the cellulolytic product index as a useful, sensitive stress indicator was demonstrated by significantly reduced enzyme activity found in *Corbicula* in 30-d field exposures to Cu and Zn. Both New River artificial stream and Clinch River in-stream data show that cellulolytic activity is significantly affected at or below EPA chronic water quality criteria levels. Significantly reduced activity was measured as early as day 10 at 16 μg Cu/L the lowest concentration tested, and by day 20 for Zn exposures as low as 56 μg/L. From 30-d cellulolytic responses of *Corbicula* in field-located artificial streams, the calculated no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) were 5 and 16 μg Cu/L, respectively. The maximum acceptable toxicant concentration (MATC) of 9 μg/L as defined by McKim [38] calculated from our data compares to the EPA chronic criterion of 11 μg/L at a hardness of 71 mg/L [22]. These calculated concentrations are comparable to life cycle chronic effect data for brook trout *Salvelinus fontinalis* (12.9 μg/L) [39]; bluntnose minnow *Pimephales notatus* (8.8 μg/L) [40]; and snails *Campeloma decisum* and *Physa integra* (10.9 μg/L) [41]. In the Clinch River, with 160 mg/L upstream water hardness, the LOEC and NOEC for Cu determined from cellulolytic activity monitored in 1986 were 47 and 15 μg/L, respectively. The 39 μg Zn/L MATC calculated from exposures in artificial streams was well below the site-specific chronic criterion of 79 μg/L calculated for the New River. Copper elicited the most drastic reductions in activity during 30-d exposures when compared with low-level (i.e., <50 μg/L) Zn tests conducted in field-located artificial streams. In tests by Belanger et al. [37, 42], this strong reaction to Cu in long-term testing of *Corbicula* was demonstrated by immediate cessation of growth at 8.4 to 26.7 μg Cu/L compared to slight but significantly inhibited growth at 25 to 38 μg Zn/L.

Results of long-term exposures of metals to *Corbicula* conducted to date in laboratory artificial streams receiving dechlorinated tap water with introduced algal supplements have been inconclusive due to variability caused by laboratory holding stress attributed to insufficient diet [17]. Dietary requirements were met in field-located exposures where suspended periphyton were transported to artificial streams via river water. This assumption was supported by the consistent trends in clam cellulolytic activity presented in this study and by simultaneous growth analysis performed upon these *Corbicula* as reported by Belanger et al. [42]. The latter study showed that algal growth in artificial streams, together with transported suspended phytoplankton, supported growth in *Corbicula*

held during 30-d field tests. Dauble et al. [43] have reported that algal densities of 1,000 cells/ml are required to support tissue and shell growth of *Corbicula* under flow-through laboratory conditions.

Although it is reported that most filter-feeders, such as the bivalve molluscs, will take up metals rapidly from solution or from food, the latter route is noted as most important for metals not having preference for particulate association [44]. This may explain in part the striking contrast in responses observed in laboratory metal-exposure systems, in which dechlorinated water does not offer a continual renewed particulate or food resource, versus field-located systems where particulate phases most likely dominate uptake routes. The cellulolytic index was sufficiently sensitive to detect this difference in *Corbicula*, and toxicity testing results presented here support the selection of this species and an enzymatic test specific to feeding responses as a sensitive tool for environmental impact assessment.

Application to site-specific criteria development

Cellulolytic activity of *Corbicula* used in monitoring the Clinch River was affected differently with respect to sample location when compared to the macroinvertebrate parameters measured. While identical trends were apparent with respect to gross effects (e.g., mortality, barren artificial substrates, etc.) directly below effluents followed by recovery downstream, stations at or directly below effluents did not conform to this pattern during both 1985 and 1986. Gradation of effects seen downstream of station 8 was apparent from the Hester-Dendy sampling and was always consistent with measured metal concentrations in the water column for each station. However, clams not subject to drift and more highly dependent upon suspended food particulates that accumulate metal while floating downstream than upon water-borne metals, demonstrated their most significantly decreased activity after 30 d at station 11, ~275 m downstream of station 8 (where water-column metal concentrations were greatest). Effects on cellulolytic activity were also greater at downstream station 14A than at station 8. The importance in contrasting the results from the colonization study with that of the cellulolytic response is found in the route of metal uptake characteristic of different organisms. This indicates that clam cellulolytic activity represents a reliable field monitoring tool for effects acting upon compartments other than those reflected by total recoverable metal concentrations in the water column. Similarly, Belanger et al. [37] reported maximal accumulations of Cu in clams at station 11 during the 1986 monitoring of the Clinch River. High levels of Cu were measured in clams as far downstream as station 15A and were attributed to uptake from algal food sources since downstream Cu concentrations were below detection.

In order to better assess the derivation and effectiveness of site-specific criteria as provided by the EPA [45], it is intended that test species directly integrate differences in the bioavailability and toxicity of effluents and provide direct measures of site-water capacity to affect toxicity values relative to values obtained in reference water. One such site-specific study by Carlson et al. [46] combined with other receiving and effluent water studies [47] has shown that the national and site-specific water quality criteria derived for Cu in a Connecticut river is protective of the river's aquatic community. However, the appropriateness of station-specific criteria could not be determined in their studies due to an absence of criteria exceedences at stations with healthy communities.

Van Hassel and Gaulke [48] related data from in-stream monitoring to criteria or impact estimates in the Clinch River and demonstrated advantages of an integrated field-laboratory assessment of power plant effluents over laboratory toxicological approaches. Their study combined effluent and ambient chemical data with benthic surveys. The Cu criterion of 34.2 μg/L calculated in their study compared well with the mean upstream Clinch River Cu concentration of 30.1 μg/L. A site-specific chronic Cu criterion of 17.5 μg/L (proposed national chronic Cu criterion of 17.7 μg Cu/L for water hardness of 172 mg/L) was recalculated by elimination of nonresident species from the EPA Cu acute toxicity data base.

Certain refinements upon the field-derived, site-specific criterion can be made by examination of cellulolytic activity in *Corbicula* as applied to field monitoring. They include reducing the variability in biological response parameters, providing enzymatic responses through time that are sensitive to bioavailability, and providing a sensitive index that can be applied to experimental manipulations involving bioassays.

Acknowledgement—We thank D. Hamerdorfer for help with chemistry analyses. This research was supported by the American Electric Power Service Corporation, Columbus, Ohio.

REFERENCES

1. **Abbe, G.R.** and **J.G. Sanders.** 1986. Condenser replacement in a coastal power plant: Copper uptake

and incorporation in the American oyster, *Crassostrea virginica*. *Mar. Environ. Res.* **19**:93–113.

2. **Cherry, D.S., R.K. Gutherie, S.R. Larrick** and **F.F. Sherberger.** 1979. The influence of coal ash and thermal discharges upon the distribution and bioaccumulation of aquatic invertebrates. *Hydrobiologia* **62**:257–267.
3. **Cherry, D.S., S.R. Larrick, K.L. Dickson, R.C. Hoehn** and **J. Cairns, Jr.** 1977. Significance of hypochlorous acid in free residual chlorine to the avoidance response of spotted bass (*Micropterus punctulatus*) and rosyface shiner (*Notropis rubellus*). *Can. J. Fish. Aquat. Sci.* **34**:1365–1372.
4. **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.
5. **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.
6. **Mehrle, P.M.** and **R.L. Mayer.** 1980. Clinical tests in toxicology: State of the art. *Environ. Health Perspect.* **34**:139–143.
7. **Kimball, K.D.** and **S.A. Levin.** 1985. Limitations of laboratory bioassays: The need for ecosystem-level testing. *BioScience* **35**:165–171.
8. **Weber, C.I.** 1981. Evaluation of the effects of effluents on aquatic life in receiving waters—An overview. In J.M. Bates and C.I. Weber, eds., *Ecological Assessments of Effluent Impacts on Communities of Indigenous Aquatic Organisms*. American Society for Testing and Materials, Philadelphia, PA, pp. 3–13.
9. **Cherry, D.S., J. Cairns, Jr., M.L. Failla** and **A.E. Linkins.** 1986. A site-specific variance demonstration for the Glen Lyn plant effluents (bottom ash, fly ash, heavy metals and acidic-alkaline pH) by validation of field and laboratory studies–Final report. Prepared for the American Electric Power Company, Canton, OH.
10. **Cairns, J., Jr.** 1983. The case for simultaneous toxicity testing at different levels of biological organization. In W.E. Bishop, R.D. Cardwell, and B.B. Heidolph, eds., *Aquatic Toxicology and Hazard Assessment*. American Society for Testing and Materials, Philadelphia, PA, pp. 111–127.
11. **Bitton, G.** 1982. Bacterial and biochemical tests for assessing chemical toxicity in the aquatic environment: A review. *CRC Critical Reviews in Environmental Control* **13**:51–67.
12. **Payne, J.F.** 1984. Mixed-function oxygenases in biological monitoring programs: Review of potential usage in different phyla of aquatic animals. In G. Personne, E. Jaspers, and C. Claus, eds., *Ecotoxicological Testing for the Marine Environment*. State University of Ghent and Institute for Marine Scientific Research, Bredene, Belgium, pp. 625–655.
13. **Neff, J.M.** 1985. Use of biochemical measurements to detect pollutant-mediated damage to fish. In R.D. Cardwell, R. Purdy, and R.C. Bahner, Eds., *Aquatic Toxicology and Hazard Assessment*. American Society for Testing and Materials, Philadelphia, PA, pp. 155–183.
14. **Brown, G.W., Jr.** 1976. Effects of polluting substances on enzymes of aquatic organisms. *J. Fish. Res. Board Can.* **33**:2018–2022.
15. **Fetterolf, C.M., Jr., R.M. Atlas, D.M. DiForo, S.E. Herbes, J.A. Leenheer, J.F. Paul, A. Spacie** and **T. Stuart.** 1986. Discussion synopsis: Exposure assessment for effluents. In H.L. Bergman, R.A. Kimerle, and A.W. Maki, eds., *Environmental Hazard Assessment of Effluents*. Pergamon Press, Elmsford, NY, pp. 191–205.
16. **Dickson, K.L.** and **J.H. Rodgers, Jr.** 1986. Assessing the hazards of effluents in the aquatic environment. In H.L. Bergman, R.A. Kimerle, and A.W. Maki, eds., *Environmental Hazard Assessment of Effluents*. Pergamon Press, Elmsford, NY, pp. 209–215.
17. **Farris, J.L.** 1986. Cellulolytic responses to heavy metal accumulation in *Corbicula fluminea* and *Mudalia dilatata*. Ph.D. thesis. Virginia Polytechnic Institute and State Univeristy, Blacksburg, VA.
18. **Belanger, S.E., D.S. Cherry** and **J. Cairns, Jr.** 1986. Uptake of chrysotile asbestos fibers alters growth and reproduction of Asiatic clams. *Can. J. Fish. Aquat. Sci.* **43**:43–52.
19. **Daum, R.J.** 1970. A revision of two computer programs for probit analysis. *Bull. Entomol. Soc. Am.* **16**:10–15.
20. **SAS Institute, Inc.** 1985. *SAS® User's Guide: Statistics*. Version 5 Edition. Cary, NC.
21. **U.S. Environmental Protection Agency.** 1983. *Methods for Chemical Analysis of Water and Wastes*. EPA-600/4-79-020. Cincinnati, OH.
22. **U.S. Environmental Protection Agency.** 1985. *Ambient Water Quality Criteria for Copper–1984*. EPA-440/5-84-031. Washington, DC.
23. **Almin, K.E.** and **K.E. Eriksson.** 1967. Enzymatic degradation of polymers: I—Viscometric method for the determination of enzymatic activity. *Biochem. Biophys. Acta* **139**:238–247.
24. **Miller, G.L.** 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **31**:426–428.
25. **Sinsabaugh, R.L.** 1980. Distribution of microbial and macroinvertebrate cellulolytic activity in relation to leaf processing in a headwater stream. M.S. thesis. Virginia Polytechnic Institute and State University, Blacksburg, VA.
26. **Sinsabaugh, R.L., A.E. Linkins** and **E.F. Benfield.** 1985. Cellulase digestion and assimilation by three leaf-shredding aquatic insects. *Ecology* **66**:1464–1471.
27. **Hester, E.E.** and **J.S. Dendy.** 1962. A multiple-plate sampler for aquatic macroinvertebrates. *Trans. Am. Fish. Soc.* **91**:420–421.
28. **Merritt, R.W.** and **K.W. Cummins.** 1984. *An Introduction to the Aquatic Insects of North America*. Kendall/Hunt Publishing Co., Dubuque, IA.
29. **Burch, J.B.** 1982. *Freshwater Snails (Mollusca:Gastropoda) of North America*. EPA-600/3-82-026. Cincinnati, OH, p. 294.
30. **Adams, W.J., R.A. Kimerle** and **R.G. Mosher.** 1985. Aquatic safety assessment of chemicals sorbed to sediments. In R.D. Cardwell, R. Purdy, and R.C. Bahner, eds., *Aquatic Toxicology and Hazard Assessment*. American Society for Testing and Materials, Philadelphia, PA, pp. 429–453.
31. **Mount, D.I.** and **T.J. Norberg.** 1984. A seven-day

life-cycle cladoceran toxicity test. *Environ. Toxicol. Chem.* 3:425–434.

32. **Carlson, A.R.** and **T.H. Roush.** 1985. Site-specific water quality studies of the Straight River, Minnesota: Complex effluent toxicity, Zn toxicity, and biological survey relationships. PB85-160703. U.S. Environmental Research Laboratory, Duluth, MN, p. 56.
33. **Harrison F.L., J.P. Knezovich** and **D.W. Rice, Jr.** 1984. The toxicity of copper to the adult and early life stages of the freshwater clam, *Corbicula manilensis*. *Arch. Environ. Contam. Toxicol.* **13**:85–92.
34. **Rodgers, J.H., Jr., D.S. Cherry, R. L. Graney, K.L. Dickson** and **J. Cairns, Jr.** 1980. Comparison of heavy metal interactions in acute and artificial stream bioassay techniques for the Asiatic clam (*Corbicula fluminea*). In J.G. Eaton, P.R. Parrish, and A.C. Hendricks, eds., *Aquatic Toxicology*. American Society for Testing and Materials, Philadelpia, PA, pp. 266–280.
35. **Bayne, B.L.** 1976. Aspects of physiological condition in *Mytilus edulis* with special reference to the effects of oxygen tension and salinity. In H. Barnes, ed., *Proceedings, 9th European Marine Biology Symposium*. Aberdeen University Press, Aberdeen, Scotland, pp. 213–238.
36. **Phillips, D.J.H.** 1980. *Quantitative Aquatic Biological Indicators: Their Use to Monitor Trace Metal and Organochlorine Pollution*. Applied Science Publishers Ltd., London.
37. **Cherry, D.S., J.L. Farris** and **S.E. Belanger.** 1988. Use of ecological, biochemical, and toxicological procedures for identifying and quantifying the extent of stressful constituents in power plant effluents. Final Report Prepared for the American Electric Power Co., Columbus, OH.
38. **McKim, J.M.** 1985. Early life stage toxicity tests. In G.M. Rand and S.R. Petrocelli, eds., *Fundamentals of Aquatic Toxicology: Methods and Applications*. Hemisphere Publishing, Washington, DC, pp. 58–95.
39. **McKim, J.M.** and **D.A. Benoit.** 1971. Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (*Salvelinus fontinalis*). *J. Fish. Res. Board Can.* **28**:655–662.
40. **Horning, W.B.** and **T.W. Neiheisel.** 1979. Chronic effect of copper on the bluntnose minnow, *Pimephales notatus* (Rafinesque). *Arch. Environ. Contam. Toxicol.* **8**:545–554.
41. **Arthur, J.W.** and **E.N. Leonard.** 1970. Effects of copper on *Gammarus pseudolimnaeus, Physa integra*, and *Campeloma decisum* in soft water. *J. Fish. Res. Board Can.* **27**:1277–1286.
42. **Belanger, S.E., J.L. Farris, D.S. Cherry** and **J. Cairns, Jr.** 1986. Growth of Asiatic clams (*Corbicula* sp.) during and after long-term Zn exposure in field-located and laboratory artificial streams. *Arch. Environ. Contam. Toxicol.* **15**:427–434.
43. **Dauble, D.D., D.S. Daly** and **C.S. Abernathy.** 1985. Factors affecting growth and survival of the Asiatic clam, *Corbicula* sp., in controlled laboratory conditions. In R.D. Cardwell, R. Purdy, and R.C. Bahner, eds., *Aquatic Toxicology and Hazard Assessment*. American Society for Testing and Materials, Philadelphia, PA, pp. 134–144.
44. **Phillips, D.J.H.** 1976. The common mussel *Mytilus edulis* is an indicator of pollution by Zn, cadmium, lead and copper. II. Relationship of metals in the mussel to those discharged by industry. *Mar. Biol.* **38**:71–80.
45. **U.S. Environmental Protection Agency.** 1983. Guidelines for deriving site-specific water quality criteria. In *Water Quality Standards Handbook*. Office of Water Regulations and Standards, Washington, DC.
46. **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.
47. **Mount, D.I., T.J. Norberg-King** and **A.E. Steen,** eds. 1986. Validity of effluent and ambient toxicity tests for predicting biological impact, Naugatuck River, Waterbury, Connecticut. EPA/600/8-86/005. U.S. Environmental Research Laboratory, Duluth, MN.
48. **Van Hassel, J.H.** and **A.E. Gaulke.** 1986. Water quality-based criteria from in-stream monitoring data. *Environ. Toxicol. Chem.* **5**:417–426.