

TECHNIQUES FOR EVALUATING POWER PLANT DISCHARGES
USING IN-SITU LEAF BREAKDOWN AND FLOW-THROUGH LABORATORY
SEDIMENT BIOASSAYS

by

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(ABSTRACT)

This research is divided into two main parts. Chapters 1 and 2 address the effect of thermal addition on the breakdown of Boxelder leaves (*Acer negundo*) in in-situ and artificial stream studies. In both studies the rates for the decline in penetrance values decreased significantly in the thermally influenced treatments. Reduction in mass-loss rate coefficients was significant in the in-situ study, but not in the artificial streams. This result was due to differences in flow and macroinvertebrate colonization.

Chapters 3 and 4 address the development of a methodology for testing 24-48 hr old juvenile Asiatic clams (*Corbicula fluminea*) to sediments in 10-day bioassays. These organisms had acceptable survivorship in the controls (>70%), excellent

Abstract

recovery of all exposed organisms (97.5%) and were sensitive to copper-spiked sediments. Comparative sensitivity of this age class demonstrated them to be equally or more sensitive than other commonly used sediment test organisms including *Chironomus tentans*, *Daphnia magna* and *Hyaella azteca* for survival and growth endpoints in 10-day exposures. Survival and growth effects for *Corbicula* juveniles were seen at 25 ppb copper in the sediment.

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along with culturist extraordinaire Lisa Gotow, exhibited classic work avoidance behavior with daily 15-min "hack-breaks".

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FORWARD

Anthropogenic influences on lotic and lentic ecosystems have been documented for many years. It is the focus of this work to address some effects commonly associated with industries using natural waters for system processes. Superficially, the following chapters seem disjointed, breaking down into two rather discrete sections, the first addressing thermal addition from power production and its influence on the breakdown of leaves and the second examining the toxicity of copper-spiked sediments from cooling tower blowdown effluent. However, both of these topics are tied together in that both are problems associated with cooling-water discharges. The conditioning of leaves is governed partially by the colonization of bacteria and fungus on the leaf surface and has been well documented. The rates of conditioning and breakdown of leaves is examined in the first two chapters. It was also hypothesized that the rates of colonization on leaf disks might be a useful structural indicator for examining sediments. Sediment toxicity of various organisms to copper is examined in Chapters 3 and 4. Microbial colonization showed little promise in preliminary studies; it was dropped, leaving the latter half of this thesis without the microbial component. The sediment toxicity objectives provided more substantial input and promise in the testing of power production discharge upon benthic dwelling organisms.

CHAPTER 1

The Effect of Power Plant Thermal Addition and Benthic Macroinvertebrates on Leaf Breakdown in the East River

INTRODUCTION

The breakdown of terrestrial litter plays an important role in many lotic systems by contributing large amounts of allochthonous energy. Leaves are the primary component of terrestrial litter and enter the water in large quantities during the fall (Hobbie and Likens 1973), providing a substrate for the colonization fungal decomposers, bacteria, algae, and invertebrates and a primary energy source for stream foodwebs. The breakdown also releases nutrients into the stream that other organisms can assimilate. A large quantity of literature has been accumulated on the breakdown as subsequent fragmentation of leaves (Peterson and Cummins 1974, Iversen 1975, Webster and Benfield 1986, D'Angelo and Webster 1992), and the physical and biological methods by which they are degraded (Cummins and Klug 1979, Boulton and Boon 1991). However, most effort has been spent studying headwater streams and low order streams, rather than middle and high order tributaries (Paul et al. 1983). Allochthonous input is perceived to be greatest in small streams (Peterson and Cummins 1974); although, its importance in the energy budget for all streams and rivers should not be overlooked (Kaushik and Hynes 1971).

It is generally recognized that leaf breakdown occurs in three stages: leaching of soluble components leading to large mass loss, colonization of leaves by microorganisms (conditioning), and degradation by invertebrate feeders and physical abrasion (Webster and Benfield 1986, Boulton and Boon 1991). Factors affecting the rate at which these processes occur include environmental parameters (stream flow, substrate type, and temperature) and exposure techniques (Webster and Benfield

1986).

Studies of breakdown and the factors that cause it can be accomplished by the use of artificial leaf packs. The mesh bag method of leaf packs uses a known quantity of leaves that are placed in an open mesh bag (Paul et al. 1983). This method has been used extensively in the literature (Peterson and Cummins 1974, Iversen 1975, Paul et al. 1978, 1983, Benfield et al. 1979, Benfield and Webster 1985, Boulton and Boon 1991, Bird and Kaushik 1992) and assumes that: 1) conditions within the artificial leaf packs are identical to conditions outside the packs, 2) benthic organisms grow on, in, or around the leaf packs is negligible and can be easily removed, and 3) large amounts of leaf material will not escape (Boulton and Boon 1991). Leaf breakdown has been quantified as the amount of mass loss experienced by the pack over a period of time. This method can be subject to high initial handling losses. Penetrance is a measure of leaf toughness and was first described for the conditioning of terrestrial litter (Feeny 1970), but has been used successfully in aquatic habitats to measure conditioning in natural and artificial leafpacks. A semi-qualitative sample of macroinvertebrates can be collected from colonized leaf packs and used to characterize the benthic community.

The primary objectives of this study were to determine the effect of a power plant thermal discharge on leaf breakdown as measured by mass loss and decline in penetrance; to determine if those effects were due solely to differences in temperature regime, or if other biotic or abiotic factors contributed significantly. Secondary objectives included determining the differences, if any, in the rates of colonization of macroinvertebrates on leaf packs at the different sites. Finally I was interested in

determining differences in macroinvertebrate colonization of packs with leaves in them.

METHODS

Study Site

The study site was located in the East River (mile 95) in Glen Lyn, Virginia. The East River is one receiving system for Appalachian Power Company's Glen Lyn power station, a coal fired power plant located near the confluence of the East and New Rivers. The Glen Lyn Plant has two cooling water systems, one for each power unit, both of which draw water from the pumphouse located on the New River. One unit discharges back into the New River while the other discharges into the East River (Figure 1-1). Three stations were set up in the East River to assess the thermal effects of the discharge on leaf pack colonization and degradation. An upstream reference site (REF) was located 0.5 miles upstream from the discharge canal where there was no influence of the discharge; a middle station (MIX), at the margin of the heated plume in an area of partial influence; and a station (PIPE), near the mouth of the canal, in an area of total thermal influence (Figure 1-2). The stations were located in such a way as to try and keep the parameters of flow and water chemistry as close to the reference site as possible.

Study Design

Site Characterization

Each station was characterized by measuring abiotic factors such as stream velocity and accumulation of degree-days, as well as other standard water chemistry including: conductivity, pH, dissolved oxygen, alkalinity and hardness (American Public Health Association et al. 1992). These data are summarized in Table 1-1. Substantial

differences in some water chemistry parameters between reference and the other two sites are attributable to the origin of the cooling system discharge water. Three replicate measurements of stream velocity were made at each station using a General Oceanics velocity meter. A max/min thermometer was placed at each station to monitor the temperature. Degree days were determined by taking the average temperature measured and multiplying by the number of days since the previous measurement.

Leaf Pack Sampling

Artificial leaf packs were constructed by placing ten grams of freshly abscised boxelder (*Acer negundo*) leaves into three millimeter mesh pecan bags. Boxelder was chosen for its rapid rate of decay (Paul et al. 1983) that would limit the effects of macroinvertebrate colonization. Bags were attached to "strings" of 14 gauge insulated copper wire and spaced so that no bag would interfere with another. Empty packs (ie bags without leaves) were also randomly intermixed with full packs and attached to the strings to help determine how much colonization was due to the presence of a new substrate versus that from a food source. Twenty full packs and twelve empty packs were randomly placed on each of four replicate "strings". These strings were attached to concrete blocks which were placed in the stream bed and held in place by 0.4 meter steel spikes. One full pack was randomly sampled from each replicate string on days 0, 7, and 21 with the remaining eight packs sampled on day 35. The additional replicates on day 35 were to help explain the increasing variation associated with these types of time studies (Boulton and Boon 1991). Four empty packs were sampled as above on days 0 and 7, with the remaining packs sampled on day 35.

A pack was sampled by easing a kicknet around the pack and then removing it. This technique prevented the loss of insects and leaf material to the disturbance associated with sampling. Each pack and its associated material was placed in a 1-L Ziplock® storage bag, and stored on ice and transported to the laboratory. At the laboratory packs were maintained in the refrigerator at four degrees Celsius until they could be analyzed.

Empty leaf packs were washed into a 250-micron sieve to remove any associated macroinvertebrates. These organisms were preserved in 70% ethanol and later keyed to functional feeding groups (Merritt and Cummins 1984, Cummins and Wilzbach 1985).

Full leaf packs were cleaned by rinsing the bag into a 250-micron sieve, then removing the contents of the bag and carefully rinsing each piece of remaining leaf material of silt and macroinvertebrates. The macroinvertebrates were preserved and keyed as above. Penetrance, a measure of leaf toughness, was measured twice on each of five leaves from a pack by the method reported by Feeny et al. (1970) for a total of 10 measurements on each pack. The leaf material was then air-dried at room temperature and weighed to determine the percent mass loss. The weights were corrected for siltation by homogenizing the sample in a Wiley mill, and then ashing three replicate subsamples (0.24-0.26 g) at 510 °C (Paul et al. 1983).

Benthic Macroinvertebrate Sampling

Benthic macroinvertebrates were sampled quantitatively at each station in order to obtain a general idea of habitat quality throughout the study period. Three replicate benthic samples were taken from each station per sampling day during the study.

Macroinvertebrates were sampled using a 0.5-m² Surber sampler. Two people collected each sample by disturbing the sample area for a period of two minutes, at which time the sample was preserved in formaldehyde and returned to the laboratory for analysis. The organisms in each sample were picked by hand and keyed to functional feeding groups (Merritt and Cummins 1984, Cummins and Wilzbach 1985).

Statistical Analysis

All mass and penetrance data were transformed to fit the exponential decay model generally accepted for leaf breakdown data (Jenny et al. 1949, Olson 1963, Peterson and Cummins 1974, Webster and Simmons 1978, Webster and Benfield 1986). The transformed data for each station were fit with a simple linear regression to obtain breakdown coefficients. Regressions for breakdown data were compared by analysis of covariance with station as the covariate. Colonization rates for leaf pack macroinvertebrates were obtained by fitting the data to a simple linear regression. Regressions for colonization rates were compared by analysis of covariance with station as the covariate. Benthic macroinvertebrate data were log transformed and compared using Tukey's technique for multiple comparisons. All comparisons were carried out at an alpha level of 0.05.

RESULTS AND DISCUSSION

Abiotic Factors

Water chemistry parameters for the three stations varied substantially, but these differences were attributed to the origin of the cooling system discharge water (Table 1-1). The New River had marked differences in conductivity, alkalinity and hardness when compared to the same parameters in the East River. New River water had similar pH values but had lower conductivity, alkalinity and hardness values. Parameters for this site were in line with other published water quality data on the New River (Paul et al. 1983). Parameters measured at the Pipe station reflect values typically found in the New River, whereas measurements taken at the Reference station reflect natural conditions in the East River. Measurements of water quality taken at the Mix were median to the other two stations. Stream velocity measurements were higher at the Mix station than at either of the other two stations which did not appear to influence the experiment.

Degree-days accumulated much slower at the Reference station than at either of the other stations (Figure 1-3). Although most water chemistry parameters indicated that the Mix station was only partially influenced by the discharge, it accumulated degree-days at almost the same rate as the Pipe station. This was most likely due to the transfer of heat energy from the New River water of the discharge to the water of the East River. Measuring differences in average temperatures at the three stations was best done by the accumulation of degree-days. Although just plotting the average temperature would suffice, the effect of thermal addition on leaf breakdown appeared

to be an additive effect and by using a time-temperature measurement resulted in a cumulative parameter. This measure has been used by others (Webster 1983, Hanson et al. 1984, Short et al. 1984, D'Angelo and Webster 1992) and seems to be a logical solution to getting a more accurate measure of time-temperature based relationships.

Benthic Macroinvertebrates

The abundance of certain groups of macroinvertebrates has been used as an indicator of the relative "health" of a system by many (Hilsenhoff 1981, 1982, 1987, 1988, Specht et al. 1984, Plafkin et al. 1989). Abundance data are typically restricted to the more sensitive groups of aquatic insects, such as the mayflies, stoneflies and caddisflies as well as the total number of organisms present. The total number of macroinvertebrates collected in 0.5 m² Surber samples were significantly ($\alpha=0.05$) lower at the Pipe station than at any other station (Table 1-2). Mayfly and stonefly abundances were significantly reduced at both the mix and the pipe stations. Caddisflies were also reduced at both stations, but not significantly at the mix station.

Examining the contribution of each of the groups to the total number of organisms in the samples, I found that the percent mayflies at the Mix station was not significant from the Reference station (Table 1-3) when it was found to be significant based on abundance (Table 1-2). The number of stoneflies in the samples and their contribution to the sample's size were too small (0.17- 3.42 per 0.5 m²) and showed no significance at any station.

Caddisfly percentages showed the same pattern of significance as their abundance data (Tables 1-2, 1-3). The Pipe station was significantly different from the Reference station, but the Mix station was not significant different than the other two.

Midges made up 24.43% of the samples collected at the Mix station. This is significantly higher than either of the other two stations. This large standing population had a significant effect on the colonization of the leafpacks which is discussed later (Table 1-3).

When these data were grouped by functional feeding groups to look at the structure of that assemblage, I found similar results (Table 1-4). While there were no differences between stations with respect to collector abundance; scrappers, shredders and predators were all significantly reduced at both lower stations, except that shredder abundance was not significantly different from the Reference or Pipe stations.

In general, the Pipe station showed an overall reduction in the numbers of macroinvertebrates indicating an impact on the receiving system. The Mix station, however, showed no reduction in the overall number on macroinvertebrates but did see some intermediate reduction in the abundance of mayflies (Table 1-2).

Macroinvertebrate Colonization

Colonization rates of macroinvertebrates on empty leafpacks were not significantly different for any station during the study, nor were rates for these packs significantly different than full packs located at the Reference station (Table 1-5). There were no significant differences in the total number of colonizing macroinvertebrates nor in relative abundances of mayflies and caddisflies (Table 1-6). Stonefly abundance was significantly higher at the Reference station relative to the Pipe station near the discharge. This difference is attributed to leaves accumulating around the packs at the Reference station.

Colonization rates of full leaf packs was significantly higher at the Mix and Pipe

stations when compared to the Reference station (Table 1-5). Colonization at both of these stations was dominated by collectors, primarily midges, using the packs as a substrate and presumably a food source (Table 1-7). Full packs at the Reference station contained significantly higher numbers of shredders, 29.96%, than did either of the other stations.

Leaf Breakdown

The major variable influencing leaf breakdown rates in this study was thermal addition. This influences microbial, fungal, and macroinvertebrate activity, as well as direct physical and chemical breakdown by the water (Peterson and Cummins 1974). The decay coefficients for mass and penetrance data represent the slope of the regression for the transformed data. The slope of the line represents the rate at which the process was occurring, in this case the breakdown of leaves (Table 1-8). As the slope (decay coefficient) increases the rate at which the leaves breakdown increases. Differences in the rates are reflected as differences in the slopes of the regressions.

Analysis of covariance on the regressors for mass loss showed significantly greater breakdown at both the Mix and Pipe station, when days was used as one of the regressors (Table 1-8). Substituting degree-days for days in the model revealed no significant differences between any of the regressions. The degree-day substitution, while not explaining much more of the variation within each regression, did explain all significant differences between the regressions.

Analysis of Covariance on the regressors for penetrance showed a significantly greater rate of decline in penetrance measurements at both stations relative to the Reference site, $\alpha=0.05$, (Table 1-8) when days was used as one of the independent

variables. When degree-days was used as one of the regressors; (correcting for temperature) no significant differences occurred in the rate functions. As with mass loss, the correction for temperature explained all of the significant differences between the stations.

CONCLUSIONS

The impact on the East River from the thermal addition associated with the cooling water discharge was summarized by evaluating the differences in the benthic macroinvertebrate community as well as the effect on increased temperature on the breakdown of allochthonous materials. An overall reduction in the "standing" population of benthic macroinvertebrates, including significantly reduced numbers of mayflies, stoneflies and caddisflies was found. The breakdown of allochthonous materials was significantly increased in the influenced areas and fully attributable to the thermal addition. This was concluded by the removal of significant slope differences when the substituting the degree-day term for the time term in the regression models, in effect correcting for temperature effects. Although leaf packs were colonized by significantly more collectors (mainly midges) in the influenced areas, the overall number of colonizers was not significant between stations. The Reference station which had the slowest breakdown rates for leaf packs maintained a significantly higher percent of its colonizing population as shredders. This is another indication of the significant effect that increased water temperatures played in breaking down the leaves in the packs.

LITURATURE CITED

- American Public Health Association, American Water Works Association and Water Environment Federation (eds.). 1992. Standard methods for the examination of water and wastewater, 18th ed. - American Public Health Association, Washington.
- Benfield, E. F. and Webster, J. R. 1985. Shredder abundance and leaf breakdown in an Appalachain Mountain stream. - *Freshwater Biol.* 15: 113-120.
- Benfield, E. F., Paul, R. W., Jr. and Webster, J. R. 1979. Influence of exposure technique on leaf breakdown rates in streams. - *Oikos* 33: 386-391.
- Bird, G. A. and Kaushik, N. K. 1992. Invertebrate colonization and processing of maple leaf litter in a forested and agricultural reach of a stream. - *Hydrobiologia* 234: 65-77.
- Boulton, A. J. and Boon, P. I. 1991. A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over a new leaf? - *Aust. J. Mar. Freshwater Res.* 42: 1-43.
- Cummins, K. W. and Klug, M. J. 1979. Feeding ecology of stream invertebrates. - *Annual Rev. Ecol. Systemat.* 10: 147-172.
- Cummins, K. W. and Wilzbach, M. A. 1985. Field procedures for analysis of functional feeding groups of stream macroinvertebrates. - University of Maryland, College Park, MD.
- D'Angelo, D. J. and Webster, J. R. 1992. Natural and constraint-induced factors influencing the breakdown of dogwood and oak leaves. - *Hydrobiologia* 237: 39-46.
- Feeny, P. 1970. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth catapillars. - *Ecology* 51: 565-581.
- Hanson, B. J., Cummins, K. W., Barnes, J. B. and Carter, M. W. 1984. Leaf litter processing in aquatic systems: a two variable model. - *Hydrobiologia* 111: 21-29.
- Hilsenhoff, W. L. 1981. Aquatic insects of Wisconsin. - Natural History Council, University of Wisconsin-Madison, Madison, WI.
- Hilsenhoff, W. L. 1982. Using a biotic index to evaluate water quality in streams. - Wisconsin Department of Natural Resources, Madison.
- Hilsenhoff, W. L. 1987. An improved biotic index of organic stream pollution. - *Great Lakes Entomologist* 20: 31-39.

Hilsenhoff, W. L. 1988. Rapid field assessment of organic pollution with a family-level biotic index. - J. N. Am. Benthol. Soc. 7: 65-68.

Hobbie, J. E. and Likens, G. E. 1973. Output of phosphorous, dissolved organic carbon, and fine particulate carbon from Hubbard Brook watersheds. - Limnol. Oceanogr. 18: 734-742.

Iversen, T. M. 1975. Disappearance of autumn shed beech leaves placed in bags in small streams. - Verh. Internat. Verein. Limnol. 19: 1687-1692.

Jenny, H., Gessel, S. P. and Bingham, F. T. 1949. Comparative study of decomposition rates of organic matter in temperate and tropical regions. - Soil Sci. 68: 419-432.

Kaushik, N. K. and Hynes, H. B. N. 1971. The fate of dead leaves that fall into streams. - Arch. Hydrobiol. Beih. 68: 465-515.

Merritt, R. W. and Cummins, K. W. (eds.). 1984. An introduction to the aquatic insects of North America, 2nd ed. - Kendall/Hunt Publishing Company, Debuque.

Olson, J. S. 1963. Energy storage and the balance of producers and decomposers in ecological systems. - Ecology 44: 322-332.

Paul, R. W., Jr., Benfield, E. F. and Cairns, J., Jr. 1978. Effects of thermal discharge on leaf decomposition in a river ecosystem. - Verh. Internat. Verein. Limnol. 20: 1759-1766.

Paul, R. W., Jr., Benfield, E. F. and Cairns, J., Jr. 1983. Dynamics of leaf processing in a medium-sized river. - In: Fontaine, T. D., III and Bartell, S. M. (eds.), Dynamics of Lotic Ecosystems. Ann Arbor Science, Ann Arbor, pp. 403-423.

Peterson, R. C. and Cummins, K. W. 1974. Leaf processing in a woodland stream. - Freshwater Biol. 4: 343-368.

Plafkin, J. L., Barbour, M. T., Porter, K. D., Gross, S. K. and Hughes, R. M. 1989. Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish. - United States Environmental Protection Agency, Office of Water, Washington.

Short, R. A., Smith, S. L., Guthrie, D. W. and Stanford, J. A. 1984. Leaf litter processing rates in four Texas, USA streams. - Journal of Freshwater Ecology 2: 469-474.

Specht, W. L., Cherry, D. S., Lechleitner, R. A. and Cains, J., Jr. 1984. Structural, functional, and recovery responses of stream invertebrates to fly ash effluent. - Can. J.

Fish. Aquat. Sci. 41: 884-896.

Webster, J. R. 1983. The role of benthic macroinvertebrates in detritus dynamics of streams: A computer simulation. - Ecol. Monogr. 53: 383-404.

Webster, J. R. and Benfield, E. F. 1986. Vascular plant breakdown in freshwater ecosystems. - Annual Rev. Ecol. Systemat. 17: 567-594.

Webster, J. R. and Simmons, G. M., Jr. 1978. Leaf breakdown and invertebrate colonization on a reservoir bottom. - Verh. Internat. Verein. Limnol. 20: 1587-1596.

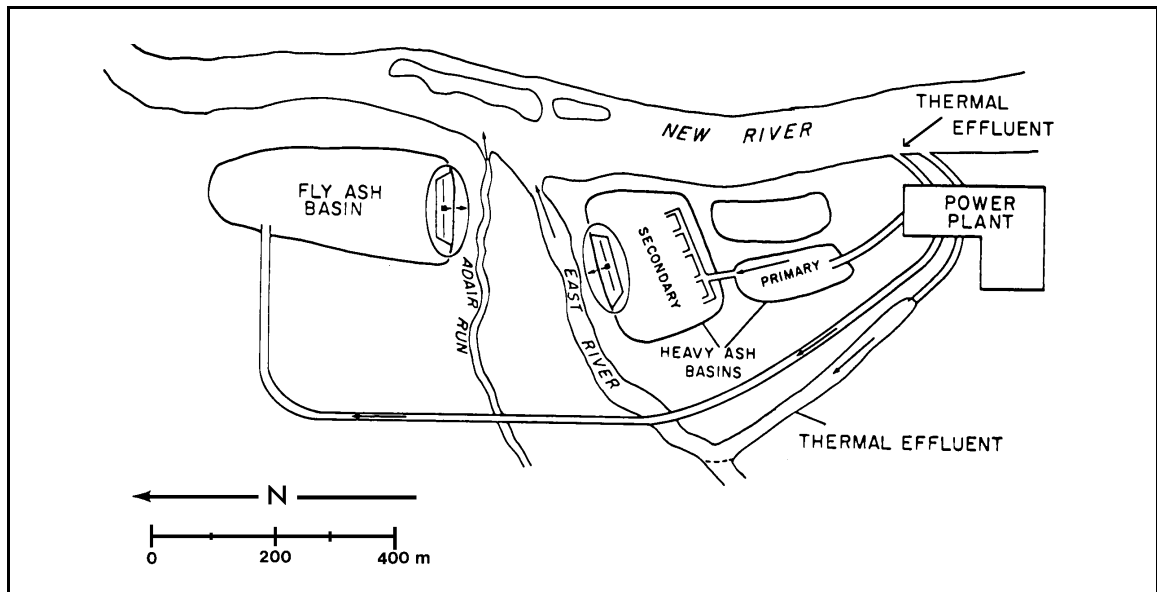


Figure 1-1. Schematic of the Glen Lyn power station located at the confluence on the New and East Rivers in Glen Lyn, Virginia. Modified from Specht et al (1984).

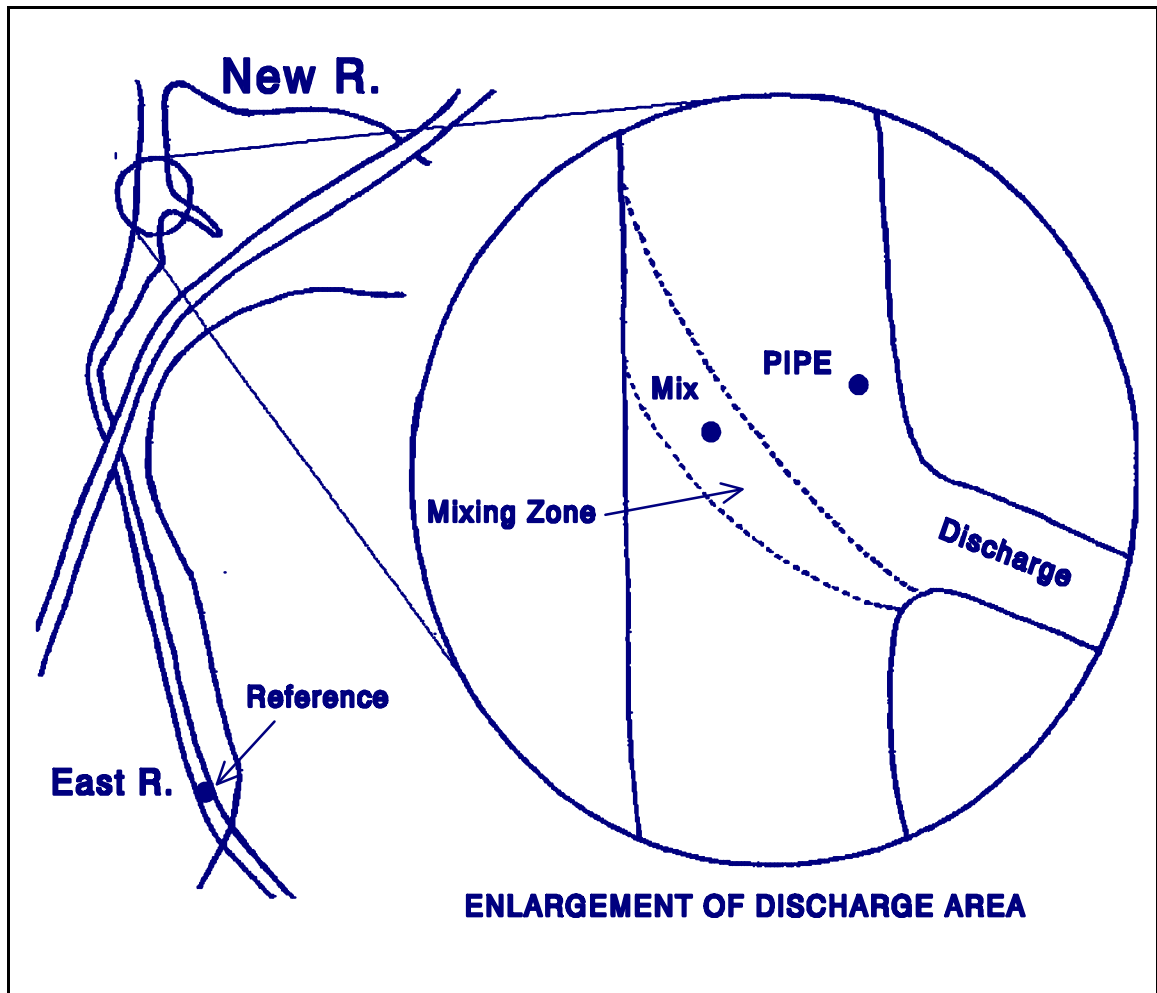


Figure 1-2. Schematic of the East River stations. The discharge canal enters into the right.

Degree Day Accumulation East River Stations

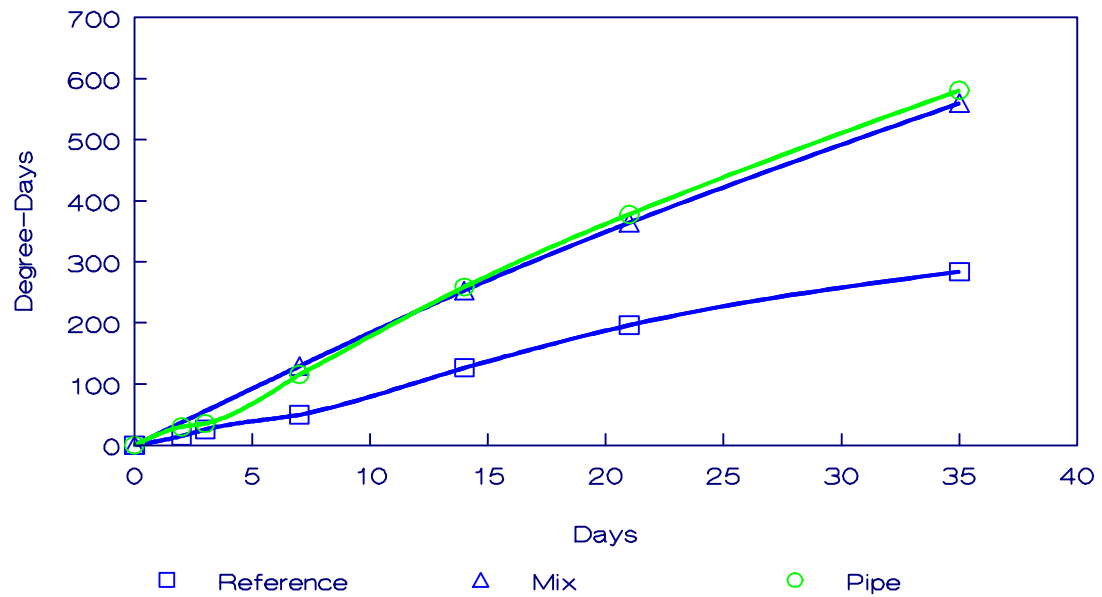


Figure 1-3. Degree-day accumulation for the three East River Stations during the fall of 1992. Degree-days accumulated significantly slower at the Reference station than at either of the other two stations.

Table 1-1. Water chemistry parameters for the East River Stations during the study period and typical ranges for those parameters for the New River. Numbers in parentheses represent one standard error of the mean (n=3).

Station	pH (s.u.)	Cond (μ mhos)	Alkalinity (mg/L as CaCO ₃)	Hardness (mg/L as CaCO ₃)	Flow cm/s
Reference	7.1 (0.6)	250 (10)	149 (16)	191 (2)	40.1 (3.5)
Mix	7.5 (0.5)	200 (60)	126 (34)	142 (37)	90.5 (12.3)
Pipe	7.9 (0.4)	97 (7)	66 (7)	68 (3)	64.0 (7.5)
New River	7.5-8.5	100-150	40-70	40-60	-

Table 1-2. Mean abundances and Duncan's Multiple Range comparisons ($\alpha=0.05$) of macroinvertebrates in Surber samples taken from the East River, Virginia. Numbers in parentheses represent ± 1 standard error (n=12).

	Mayflies		Stoneflies		Caddisflies		Total	
	mean	DMR ¹	mean	DMR	mean	DMR	mean	DMR
Ref	85.08 (16.36)	A A	3.42 (1.26)	A A	35.67 (12.90)	A A	235.00 (37.03)	A A
Mix ²	38.91 (5.71)	B B	1.00 (0.43)	B B	28.82 (7.74)	A B A B	154.25 (43.02)	A A
Pipe	18.67 (7.40)	B B	0.17 (0.17)	B B	3.83 (0.55)	B B	80.33 (15.07)	B B

¹means with the same letter are not significantly different.

²n=11

Table 1-3. Percent composition of macroinvertebrates in Surber samples taken from the East River, Virginia and Duncan's Multiple Range comparisons ($\alpha=0.05$). Numbers in parentheses represent ± 1 standard error (n=12).

	% Mayflies		% Stoneflies		% Caddisflies		% Midge	
	mean	DMR ¹	mean	DMR	mean	DMR	mean	DMR
Ref	35.90 (3.82)	A A	1.38 (0.73)	A A	12.88 (2.80)	A A	8.32 (2.36)	B B
Mix ²	27.85 (6.17)	A B A B	0.82 (0.48)	A A	18.37 (5.27)	A B A B	24.43 (8.15)	A A
Pipe	19.77 (7.69)	B B	0.41 (0.41)	A A	6.05 (0.68)	B B	4.68 (1.18)	B B

¹means with the same letter are not significantly different.

²n=11

Table 1-4. Macroinvertebrate functional feeding group abundances and Duncan's Multiple Range comparisons ($\alpha=0.05$) in Surber samples taken from the East River, Virginia. Numbers in parentheses represent ± 1 standard error.

	Collectors		Scrapers		Shredders		Predators	
	mean	DMR ¹	mean	DMR	mean	DMR	mean	DMR
Ref	129.75 (19.41)	A A	87.75 (11.50)	A A	1.42 (0.73)	A A	16.42 (4.55)	A A
Mix ²	120.09 (27.79)	A A	43.73 (6.18)	B B	0.18 (0.12)	A B A B	4.27 (0.94)	B B
Pipe	58.58 (9.80)	A A	21.33 (5.56)	B B	0.00 (0.00)	B B	0.50 (0.23)	B B

¹means with the same letter are not significantly different.

²n=11

Table 1-5. Colonization rates for macroinvertebrates for empty and full leafpack bags. Values having matching letters are not significantly different from each other. All comparisons were carried out at the $\alpha=0.05$ level.

	Empty Packs (no./pack/day)	Duncan's	Full Packs	Duncan's
Reference	3.5703	A	3.0097	A
Mix	2.3342	A	8.5446	B
Pipe	3.1276	A	4.5890	B

Table 1-6. Mean abundances and Duncan's Multiple Range comparisons ($\alpha=0.05$) of macroinvertebrates on empty leafpack bags placed in the East River. Numbers in parentheses represent ± 1 standard error.

	Mayflies		Stoneflies		Caddisflies		Total	
	mean	DMR ¹	mean	DMR	mean	DMR	mean	DMR
Ref	3.50 (1.26)	A A	17.25 (4.70)	A A	7.63 (1.91)	A A	64.25 (17.25)	A A
Mix ²	3.20 (1.36)	A A	1.60 (0.67)	B B	6.30 (2.13)	A A	83.50 (30.33)	A A
Pipe	6.29 (2.08)	A A	0.28 (0.19)	B B	5.93 (1.91)	A A	80.29 (22.02)	A A

¹means with the same letter are not significantly different.

²n=11

Table 1-7. Full leafpack functional feeding group abundances and Duncan's Multiple Range comparisons ($\alpha=0.05$) in samples taken from the East River, Virginia. Numbers in parentheses represent ± 1 standard error.

	Collectors		Scrapers		Shredders		Predators	
	mean	DMR ¹	mean	DMR	mean	DMR	mean	DMR
Ref	34.70 (6.55)	A A	0.70 (0.02)	A A	19.50 (0.04)	A A	0.80 (0.37)	A A
Mix ²	147.61 (35.80)	B B	1.28 (0.00)	A A	4.78 (0.02)	B B	0.33 (0.14)	A A
Pipe	101.70 (22.83)	A B A B	2.30 (0.01)	A A	0.70 (0.00)	B B	0.35 (0.15)	A A

¹means with the same letter are not significantly different.

²n=11

Table 1-8. Rate Coefficients of the regressions for mass loss and decline of penetrance in leaf packs. Values with the same letter associated with them are not significantly different from each other at the $\alpha=0.05$ level.

	Days (g/day)	Duncan's	Degree-Days (g/degree-day)	Duncan's
Mass Loss in Leaf Packs				
Reference	0.0228	A	0.0026	A
Mix	0.0666	B	0.0052	A
Pipe	0.0673	B	0.0040	A
Decline in Penetrance				
Reference	0.0587	A	0.0070	A
Mix	0.1100	B	0.0087	A
Pipe	0.1317	B	0.0079	A

Table 1-9. Full leafpack functional feeding group percentages and Duncan's Multiple Range comparisons ($\alpha=0.05$) in samples taken from the East River, Virginia. Numbers in parentheses represent ± 1 standard error.

	Collectors		Scrapers		Shredders		Predators	
	mean	DMR ¹	mean	DMR	mean	DMR	mean	DMR
Ref	64.82 (3.71)	A A	2.36 (1.52)	A A	29.96 (3.94)	A A	1.81 (0.88)	A A
Mix ²	95.51 (1.47)	B B	0.65 (0.24)	A A	3.36 (1.50)	A B A B	0.40 (0.27)	A A
Pipe	97.04 (0.80)	B B	1.57 (0.57)	A A	0.68 (0.32)	B B	0.60 (0.37)	A A

¹means with the same letter are not significantly different.

²n=11

Table 1-10. Macroinvertebrate abundances in Surber samples taken from the East River stations during the fall of 1992. Values in parentheses represent one standard error. All statistical comparisons were carried out at the $\alpha=0.05$ level.

	Day	Reference	Mix	Pipe
% Mayflies	0	39.56 (4.72)	41.11 (2.03)	14.66 (2.66)
	7	38.62 (10.04)	32.38 (13.70)	14.67 (4.22)
	21	40.88 (7.51)	26.13 (2.98)	7.49 (3.57)
	35	24.52 (5.98)	11.78 (2.92)	42.26 (14.81)
% Stoneflies	0	1.31 (0.45)	0 (0)	0 (0)
	7	3.47 (1.45)	1.85 (0.85)	0 (0)
	21	0.22 (0.22)	1.42 (0.50)	1.63 (1.63)
	35	0.51 (0.51)	0 (0)	0 (0)
% Caddisflies	0	6.40 (2.85)	8.66 (1.19)	8.37 (1.29)
	7	19.80 (7.77)	29.44 (2.23)	6.26 (1.56)
	21	11.17 (5.69)	25.28 (9.62)	4.49 (2.84)
	35	14.14 (7.89)	10.11 (4.07)	5.06 (0.98)
Total	0	209.67 (43.44)	107.33 (32.77)	48.00 (1.15)
	7	209.67 (81.66)	68.67 (35.03)	67.00 (29.67)
	21	177.00 (63.06)	176.33 (29.92)	88.00 (40.67)
	35	343.67 (88.19)	264.67 (106.84)	118.33 (16.56)

CHAPTER 2

The Use of Artificial Streams in leaf Breakdown Experiments

INTRODUCTION

Artificial stream systems have been used both to test contaminants to a variety of organisms as well as used as a tool to model lotic ecosystems. There is extensive literature on the exposure of organisms in artificial streams ranging from heavy metals exposure (Rodgers et al. 1980, Graney et al. 1983, Clements et al. 1988, Farris et al. 1989, Belanger et al. 1990) to organics (Belanger et al. 1993, Fairchild et al. 1993). They have also been used to test other environmental stressors such as the effect of pH on macroinvertebrates (Peters et al. 1985).

These stream systems offer a variety of advantages over other types of test systems. The greatest advantage of artificial stream systems lies in the ability to manipulate stream parameters and model a range of lotic systems. Unlike static systems artificial streams systems can be used to test the same parameter over multiple flow regimes, ranging from velocities comparable to lentic systems to velocities of high gradient mountain streams. Another advantage is in the ability to test with different levels of environmental realism. These conditions can range from systems with high environmental realism but little control to systems with high control and little realism

The breakdown of terrestrial litter plays an important role in many lotic systems by contributing large amounts of allochthonous energy. Leaves are the primary component of terrestrial litter and enter the water in large quantities during the fall (Hobbie and Likens 1973), providing a substrate for the colonization fungal decomposers, bacteria, algae, and invertebrates and a primary energy source for

stream foodwebs. The breakdown also releases nutrients into the stream that other organisms can assimilate. A large quantity of literature has been accumulated on the breakdown as subsequent fragmentation of leaves (Peterson and Cummins 1974, Iversen 1975, Webster and Benfield 1986, D'Angelo and Webster 1992), and the physical and biological methods by which they are degraded (Cummins and Klug 1979, Boulton and Boon 1991). However, most effort has been spent studying headwater streams and low order streams, rather than middle and high order tributaries (Paul et al. 1983). Allochthonous input is perceived to be greatest in small streams (Peterson and Cummins 1974); although its importance in the energy budget for all streams and rivers should not be overlooked (Kaushik and Hynes 1971).

When looking at the possible effects of an effluent on a receiving system it is favorable to generate some data on the effects of that effluent to specific organisms. This is generally done by exposing sensitive organisms in acute and chronic bioassays, and is used by the USEPA as a method to regulate the levels of permitted effluents allowed to be discharged into any given receiving system (Webber et al. 1989, Webber 1993).

The objective of this study was to assess the artificial stream's ability to model and predict the effect of thermal addition on the breakdown of leaves in in-situ studies. This was accomplished by a comparative study of breakdown rates for leaves in artificial stream systems and those in leafpacks placed in the thermal discharge of a coal fired power plant.

METHODS

Study Site

The in-situ study site was located in the East River (mile 95) in Glen Lyn, Virginia. The East River is one receiving system for Appalachian Power Company's Glen Lyn power station, a coal fired power plant located near the confluence of the East and New Rivers. The Glen Lyn Plant has two cooling water systems, one for each power unit, both of which draw water from the pumphouse located on the New River. One unit discharges back into the New River while the other discharges into the East River (Figure 2-1). Located next to the New River by the pumphouse (not shown) is an artificial stream laboratory which houses a series of fiberglass artificial streams.

Study Design

Artificial Stream System

Each artificial stream consists of a doughnut shaped tub capable of holding approximately 60 liters of water. Current is provided by a motor driven paddle wheel. Stream velocity was not calculated for these streams and was significantly less than those velocities seen in the in-situ. Each stream was fed by a water pumped directly from the New River by a submersible well pump. A flow rate of 60 L/day renewed each stream approximately every 48 hours. A series of three stream units was dedicated to this experiment. Each stream was heated with submersible aquarium heaters to try and match the temp at the three in-situ stations, and labeled accordingly as either (Unheated, Medium or High Heat). A max/min thermometer was placed in each stream to monitor the temperature. Degree days were determined by taking the

average temperature measured and multiplying by the number of days since the previous measurement.

In-Situ Stations and Site Characterization

Three stations were set up in the East River to assess the thermal effects of the discharge on leaf pack colonization and degradation. An upstream reference site (REF) was located 0.5 miles upstream from the discharge canal where there was no influence of the discharge; a middle station (MIX), at the margin of the heated plume in an area of partial influence; and a station (PIPE), near the mouth of the canal, in an area of total thermal influence (Figure 2-2). The stations were located in such a way as to try and keep the parameters of flow and water chemistry as close to the reference site as possible.

Each station was characterized by measuring abiotic factors such as stream velocity and accumulation of degree-days, as well as other standard water chemistry including: conductivity, pH, dissolved oxygen, alkalinity and hardness (American Public Health Association et al. 1992). These data are summarized in Table 2-1. Substantial differences in some water chemistry parameters between reference and the other two sites are attributable to the origin of the cooling system discharge water. Three replicate measurements of stream velocity were made at each station using a General Oceanics velocity meter. A max/min thermometer was placed at each station to monitor the temperature. Degree days were determined by taking the average temperature measured and multiplying by the number of days since the previous measurement.

Leaf Pack Construction and Sampling Design

Artificial leaf packs were constructed by placing ten grams of freshly abscised boxelder (*Acer negundo*) leaves into three millimeter mesh pecan bags. Boxelder was chosen for its rapid rate of decay (Paul et al. 1983) that would limit the effects of macroinvertebrate colonization. 16 leaf packs were placed in each artificial stream and sampled on days 0,7,21 and 35 of the experiment. On day 0, 4 packs from each stream were sampled; 3 packs were sampled on days 7 and 21; and the remaining 6 packs were sampled from the artificial streams on day-35. In the in-situ study bags were attached to "strings" of 14 gauge insulated copper wire and spaced so that no bag would interfere with another. These strings were attached to concrete blocks which were placed in the stream bed and held in place by 0.4 meter steel spikes. One pack was randomly sampled from each replicate string on days 0,7, and 21 with the remaining eight packs sampled on day 35. The additional replicates on day 35 were to help explain the increasing variation associated with these types of time studies (Boulton and Boon 1991).

In the East River a pack was sampled by easing a kicknet around the pack and then removing it. This technique prevented the loss of insects and leaf material to the disturbance associated with sampling. In the Artificial stream study the packs were randomly sampled by hand. Each pack and its associated material was placed in a 1-L Ziplock® storage bag, and stored on ice and transported to the laboratory. At the laboratory packs were maintained in the refrigerator at four degrees Celsius until they could be analyzed.

Leaf packs were cleaned by rinsing the bag into a 250-micron sieve, then removing the contents of the bag and carefully rinsing each piece of remaining leaf

material of silt and macroinvertebrates. The macroinvertebrates were preserved and keyed as above. Penetrance, a measure of leaf toughness, was measured twice on each of five leaves from a pack by the method reported by Feeny et al. (1970) for a total of 10 measurements on each pack. The leaf material was then air-dried at room temperature and weighed to determine the percent mass loss. The weights were corrected for siltation by homogenizing the sample in a Wiley mill, and then ashing three replicate subsamples (0.24-0.26 g) at 510 °C (Paul et al. 1983).

Statistical Analysis

All mass and penetrance data were transformed to fit the exponential decay model generally accepted for leaf breakdown data (Jenny et al. 1949, Olson 1963, Peterson and Cummins 1974, Webster and Simmons 1978, Webster and Benfield 1986). The transformed data for each station were fit with a simple linear regression to obtain breakdown coefficients. Regressions for breakdown data were compared by analysis of covariance with station as the covariate. Colonization rates for leaf pack macroinvertebrates were obtained by fitting the data to a simple linear regression. Regressions for colonization rates were compared by analysis of covariance with station as the covariate. Benthic macroinvertebrate data were log transformed and compared using Duncan's Multiple Range Test for multiple comparisons. All comparisons were carried out at an alpha level of 0.05.

RESULTS AND DISCUSSION

Abiotic Factors

New River water had similar pH values but had lower conductivity, alkalinity and hardness values, than did stations East River stations in the comparative study (Table 2-1). Parameters for the streams were in line with other published water quality data in the New River (Paul et al. 1983). Parameters measured at the East River Pipe station reflected values typically found in the New River. Measurements of water quality taken at the Mix were median to the other two stations, and reflected the same values seen in the artificial streams.

Degree-days accumulated much slower in the unheated stream than in either of the other streams (Figure 2-3). Measuring differences in average temperatures at the three stations was best done by the accumulation of degree-days. Although just plotting the average temperature would suffice, the effect of thermal addition on leaf breakdown appeared to be an additive effect and by using a time-temperature measurement resulted in a cumulative parameter. This measure has been used by others (Webster 1983, Hanson et al. 1984, Short et al. 1984, D'Angelo and Webster 1992) and seems to be a logical solution to getting a more accurate measure of time-temperature based relationships.

Macroinvertebrate Colonization

Artificial Steam Study

Colonization of macroinvertebrates on leafpacks was not significant for any stream during the study. There were only a few colonizing macroinvertebrates (less

than 1 per pack per station) and those were chironomid larvae which managed to enter through the system. Since numbers of these insects were so small, no follow-up statistical analysis was conducted and for all practical purposes these numbers are assumed to be zero. Table 2-2 shows the mean number of macroinvertebrates colonizing these packs as zero, representing the slope of the regression line for these data.

In-Situ Study

Colonization of East River leaf packs was significantly higher at the Mix and Pipe stations when compared to the Reference station (Table 2-2). Colonization at both of these stations was dominated by collectors, primarily midges, using the packs as a substrate and presumably a food source. Full packs at the Reference station contained significantly higher numbers of shredders, 41% by day 35, than did either of the other stations.

Leaf Breakdown in Artificial Streams

The major variable influencing leaf breakdown rates in this study was thermal addition. This influences microbial, fungal, and macroinvertebrate activity, as well as direct physical and chemical breakdown by the water (Peterson and Cummins 1974). The decay coefficients for mass and penetrance data represent the slope of the regression for the transformed data (Table 2-3). The slope of the line represents the rate at which the process is occurring, in this case the breakdown of leaves. As the slope (decay coefficient) increases the rate at which the leaves breakdown increases. Differences in the rates are reflected as differences in the slopes of the regressions.

Mass Loss

The artificial streams did not model well what happened in the East River with regards to the loss of mass from the leafpacks. The artificial stream studies did not show any significant reduction in mass loss when either days or degree-days was used as the regressor. Increased temperature did not cause an increase in the loss of mass in the streams. However in the in-situ East River stations the opposite happened. There was a significant increase in the amount of mass lost in the influenced stations. This can be seen in Table 2-3 when days was used as the regressor. I know that these significant differences are due to temperature differences because the same regression using Degree-days as the regressor (correcting for temperature) revealed no significant differences, however the rates for the Mix and the Pipe station were still higher.

Two other possible explanations for these differences involve factors which may be indirectly associated with the increased temperature at the two influenced in-situ stations. Stream velocities were higher at the Mix and Pipe stations because of the input of the heated discharge into the streams. It has been shown that stream flow and velocity can play a significant role in the physical breakdown of allochthonous input (Webster and Benfield 1986). Another alternative lies in the increased numbers of collectors colonizing the leafpacks in the thermally influenced stations. These were primarily midges which while not directly breaking the leaves down were using some of the material for building cases, helping with the physical breakdown of the leaves.

Decline in Penetrance

The artificial stream system did model well the decline in penetrance values that were shown in the East River Stations. In the artificial streams both heated streams

were significantly different from the Unheated stream. Likewise in the in-situ study, both thermally influenced stations were significantly different from the reference station when days is used as the regressor (no temperature correction), and in both systems when correcting for temperature all significance was removed.

CONCLUSIONS

The use of artificial stream systems in examining the fate of allochthonous input in receiving systems which are impacted by thermal additions is warranted. These systems can accurately model the receiving system with respect to the decline in penetrance values. This is important because the level of conditioning of leaves is considered of primary importance in the food value of the leaf. Highly conditioned (softer) leaves are of more food value than lesser conditioned leaves.

While the system did not accurately model the receiving system with respect to mass loss in leaves, it provides a good tool for controlling for certain stream characteristics. In these experiments the effect of macroinvertebrates was controlled for in the artificial streams. While it was shown that the loss of mass in the in-situ study was explained by the higher temperatures, there was no effect of increased temperatures on mass in the artificial stream studies. This indicates that some other factor covarying with temperature was responsible. It is suggested by the data that either stream velocity or collector abundance may partially explain the differences.

LITERATURE CITED

- American Public Health Association, American Water Works Association and Water Environment Federation (eds.). 1992. Standard methods for the examination of water and wastewater, 18th ed. - American Public Health Association, Washington.
- Belanger, S. E., Farris, J. L., Cherry, D. S. and Cairns, J., Jr. 1990. Validation of *Corbicula fluminea* growth reductions induced by copper in artificial streams and river systems. - Can. J. Fish. Aquat. Sci. 47: 904-914.
- Belanger, S. E., Davidson, D. H., Farris, J. L., Reed, D. and Cherry, D. S. 1993. Effects of cationic surfactant exposure to a bivalve mollusc in stream mesocosm. - Environ. Tox. Chem. 12: 1789-1802.
- Boulton, A. J. and Boon, P. I. 1991. A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over a new leaf? - Aust. J. Mar. Freshwater Res. 42: 1-43.
- Clements, W. H., Cherry, D. S. and Cairns, J., Jr. 1988. Impact of heavy metals on insect communities in streams: a comparison of observational and experimental results. - Can. J. Fish. Aquat. Sci. 45: 2017-2025.
- Cummins, K. W. and Klug, M. J. 1979. Feeding ecology of stream invertebrates. - Annual Rev. Ecol. Systemat. 10: 147-172.
- D'Angelo, D. J. and Webster, J. R. 1992. Natural and constraint-induced factors influencing the breakdown of dogwood and oak leaves. - Hydrobiologia 237: 39-46.
- Fairchild, J. F., Dwyer, J. F., La Point, T. W., Burch, S. A. and Ingersoll, C. G. 1993. Evaluation of a laboratory-generated NOEC for linear alkylbenzene sulfonate in outdoor experimental streams. - Environ. Tox. Chem. 12: 1736-1775.
- Farris, J. L., Belanger, S. E., Cherry, D. S. and Cairns, J., Jr. 1989. Cellulytic activity as a novel approach to asses long-term zinc stress to *Corbicula*. - Wat. Res. Bull. 23: 1275-1283.
- Feeny, P. 1970. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth catapillars. - Ecology 51: 565-581.
- Graney, R. L., Jr., Cherry, D. S. and Cairns, J., Jr. 1983. Heavy metal indicator potential of the Asiatic clam *Corbicula fluminea* in artificial stream systems. - Hydrobiologia 102: 81-88.
- Hanson, B. J., Cummins, K. W., Barnes, J. B. and Carter, M. W. 1984. Leaf litter processing in aquatic systems: a two variable model. - Hydrobiologia 111: 21-29.

Hobbie, J. E. and Likens, G. E. 1973. Output of phosphorous, dissolved organic carbon, and fine particulate carbon from Hubbard Brook watersheds. - *Limnol. Oceanogr.* 18: 734-742.

Iversen, T. M. 1975. Disappearance of autumn shed beech leaves placed in bags in small streams. - *Verh. Internat. Verein. Limnol.* 19: 1687-1692.

Jenny, H., Gessel, S. P. and Bingham, F. T. 1949. Comparative study of decomposition rates of organic matter in temperate and tropical regions. - *Soil Sci.* 68: 419-432.

Kaushik, N. K. and Hynes, H. B. N. 1971. The fate of dead leaves that fall into streams. - *Arch. Hydrobiol. Beih.* 68: 465-515.

Olson, J. S. 1963. Energy storage and the balance of producers and decomposers in ecological systems. - *Ecology* 44: 322-332.

Paul, R. W., Jr., Benfield, E. F. and Cairns, J., Jr. 1983. Dynamics of leaf processing in a medium-sized river. - In: Fontaine, T. D., III and Bartell, S. M. (eds.), *Dynamics of Lotic Ecosystems*. Ann Arbor Science, Ann Arbor, pp. 403-423.

Peters, G. T., Cherry, D. S. and Cairns, J., 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.

Peterson, R. C. and Cummins, K. W. 1974. Leaf processing in a woodland stream. - *Freshwater Biol.* 4: 343-368.

Rodgers, J. H., Jr., Cherry, D. S., Graney, R. L., Dickson, K. L. and Cairns, J., Jr. 1980. Comparison of heavy metal interactions in acute and artificial stream bioassay techniques for the Asiatic clam *Corbicula fluminea*. - In: Eaton, J. G., Parrish, P. R. and Hendricks, A. C. (eds.), *Aquatic Toxicology*. American Society for Testing and Materials, Philadelphia, pp. 266-280.

Short, R. A., Smith, S. L., Guthrie, D. W. and Stanford, J. A. 1984. Leaf litter processing rates in four Texas, USA streams. - *Journal of Freshwater Ecology* 2: 469-474.

Webber, C. I. (ed.). 1993. *Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms*, 4th ed. - United States Environmental Protection Agency, Cincinnati.

Webber, C. I., Peltier, W. H., Norberg-King, T. J., Horning, W. B., II, Kessler, F. A. and Menkedick, J. R. (eds.). 1989. *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms*, 2nd ed. - United States

Environmental Protection Agency, Cincinnati.

Webster, J. R. 1983. The role of benthic macroinvertebrates in detritus dynamics of streams: A computer simulation. - *Ecol. Monogr.* 53: 383-404.

Webster, J. R. and Benfield, E. F. 1986. Vascular plant breakdown in freshwater ecosystems. - *Annual Rev. Ecol. Systemat.* 17: 567-594.

Webster, J. R. and Simmons, G. M., Jr. 1978. Leaf breakdown and invertebrate colonization on a reservoir bottom. - *Verh. Internat. Verein. Limnol.* 20: 1587-1596.

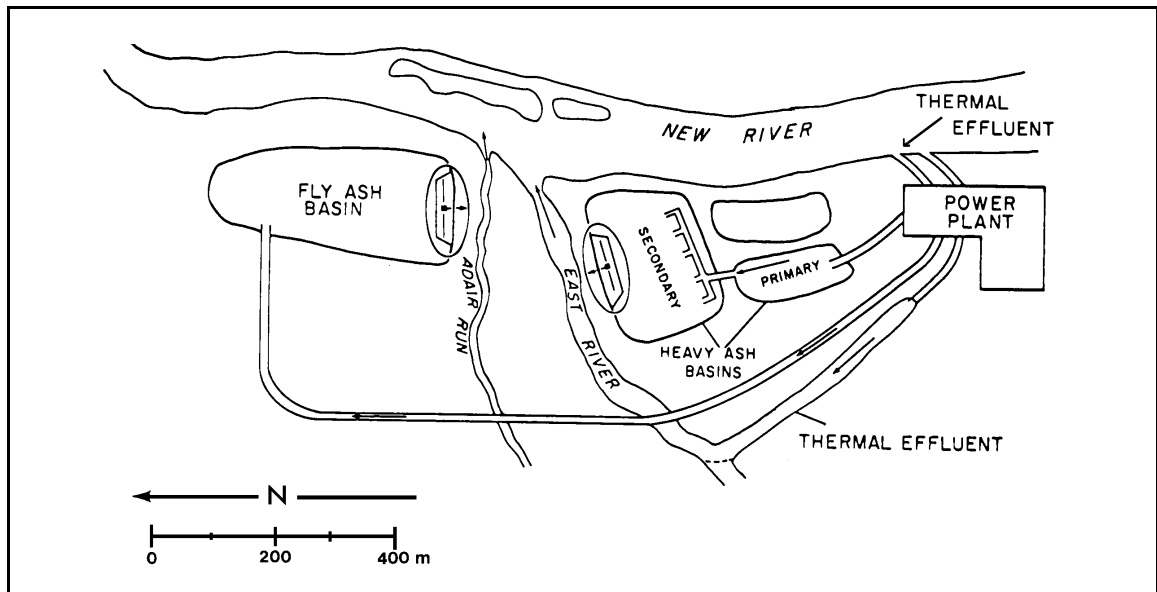


Figure 2-1. Schematic of the Glen Lyn power station located at the confluence on the New and East Rivers in Glen Lyn, Virginia. Modified from Specht et al (1985).

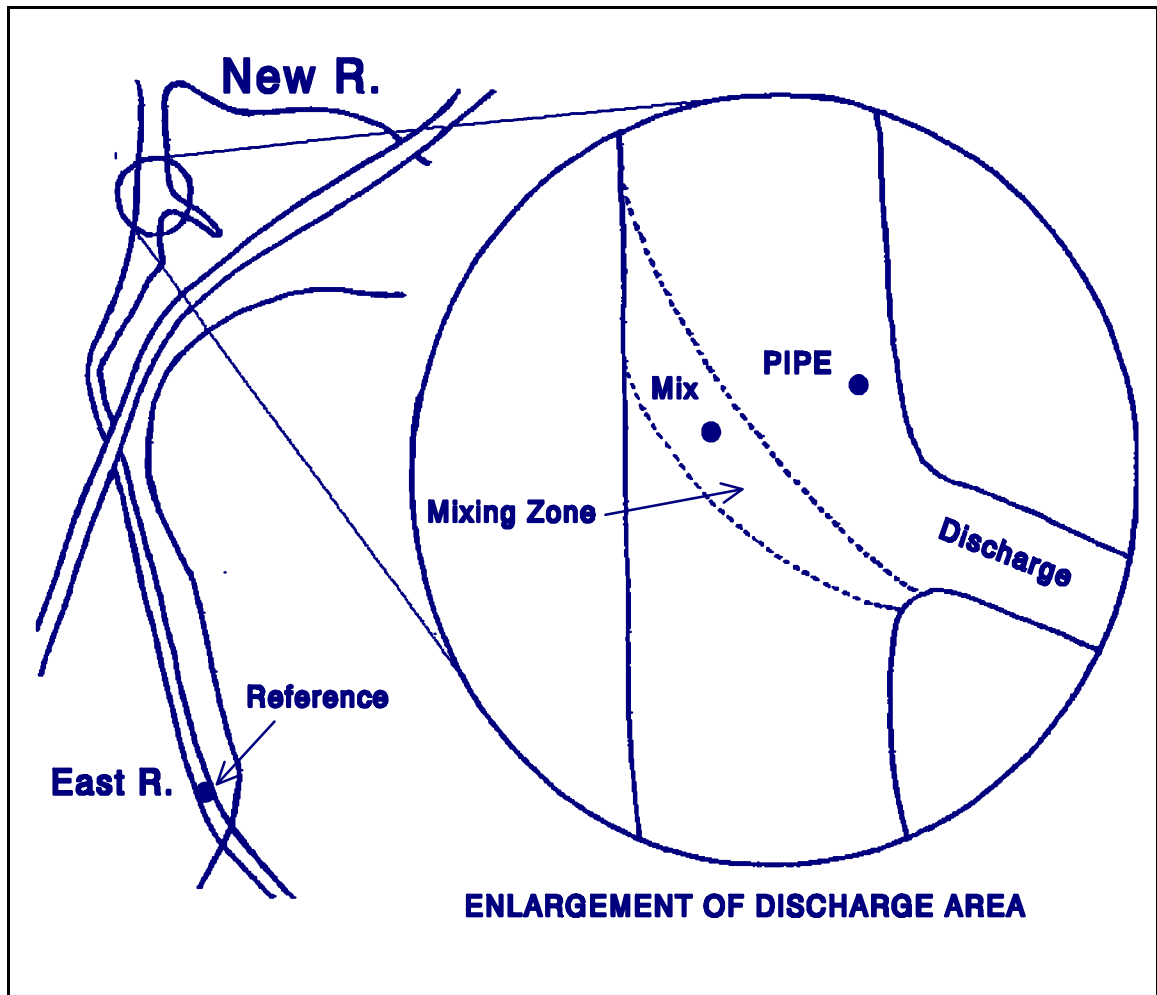


Figure 2-2. Schematic of the East River stations. The discharge canal enters into the right.

Degree Day Accumulation Artificial Steam Study

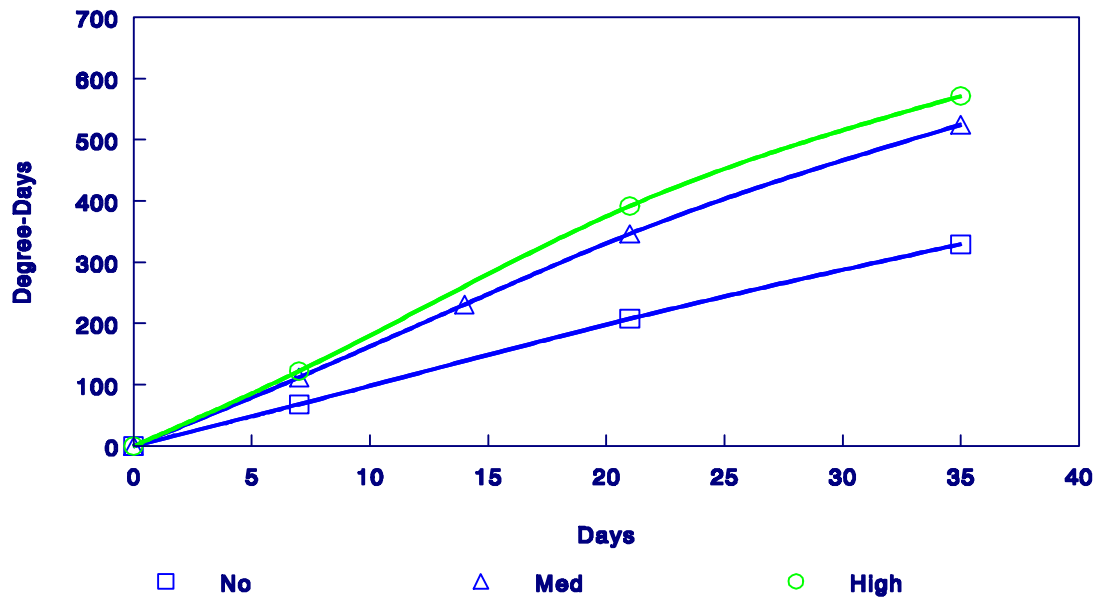


Figure 2-3. Degree-day accumulation in the artificial streams during the fall of 1992. Degree-days accumulated significantly slower in the unheated stream (No) than at either of the other two stations.

Table 2-1. Water chemistry parameters for the East River Stations during the study period and typical ranges for those parameters for the New River. Numbers in parentheses represent one standard error of the mean. n=3.

Station	pH (s.u.)	Cond (μ mhos)	Alkalinity (mg/L as CaCO ₃)	Hardness (mg/L as CaCO ₃)	Flow cm/s
Reference	7.1 (0.6)	250 (10)	149 (16)	191 (2)	40.1 (3.5)
Mix	7.5 (0.5)	200 (60)	126 (34)	142 (37)	90.5 (12.3)
Pipe	7.9 (0.4)	97 (7)	66 (7)	68 (3)	64.0 (7.5)
New R.	7.5-8.5	100-150	40-70	40-60	-

Table 2-2. Mean macroinvertebrates colonization rates for leafpacks. Values having matching letters are not significantly different from each other. All comparisons were carried out at the $\alpha=0.05$ level.

	Artificial Stream Study		East River Study	
	Colonization (inverts/day)	Duncan's	Leafpacks (inverts/day)	Duncan's
Reference	0.0000	-	3.0097	A
Mix	0.0000	-	8.5446	B
Pipe	0.0000	-	4.5890	B

Table 2-3. Rate Coefficients of the regressions for mass loss and decline of penetrance in leaf packs. Values with the same letter associated with them are not significantly different from each other at the $\alpha=0.05$ level.

	Mass Loss in Leaf Packs				Decline in Penetrance			
	Days (g/day)	D M R	Degree- Days (g/degree- e-day)	D M R	Days (g/day)	D M R	Degree- Days (g/degree- day)	D M R
ARTIFICIAL STREAMS								
Unheated	0.0170	A	0.0021	A	0.0717	A	0.0088	A
Medium	0.0219	A	0.0017	A	0.1250	B	0.0084	A
High	0.0224	A	0.0015	A	0.1627	B	0.0117	A
EAST RIVER STATIONS								
Ref.	0.0228	A	0.0026	A	0.0587	A	0.0070	A
Mix	0.0666	B	0.0052	A	0.1100	B	0.0087	A
Pipe	0.0673	B	0.0040	A	0.1317	B	0.0079	A

CHAPTER 3

Development of a Flow-through Technique for Exposing the Juvenile
Lifestage of the Introduced Asiatic Clam (*Corbicula fluminea*) in
Sediment Toxicity Tests.

INTRODUCTION

"Sediments are the ultimate sink for pollutants. However before these sediments become part of the sedimentary record (e.g. deeply buried) they are able to influence the composition of surface waters." (Salomons et al. 1987)

The above statement typifies the concerns with scientists, environmentalists, and regulatory agencies in their efforts to protect water quality and mitigate contaminants produced by industrial and municipal processes. Regulatory agencies including the US EPA are currently developing methods for testing sediments in order to begin including them in discharge permits as they have done with water-only bioassays, but to date have released little guidance on these issues.

History of Sediment Toxicity

Regulatory and scientific concerns with the disposal and toxicity of sediment and sediment-associated contaminants began in earnest with elevated contaminant levels recognized in dredged materials. The Federal Water Quality Administration (precursor to the current EPA) developed limited criteria for disposal in the early 1970's known as the Jenson Criteria. The EPA and Army Corps of Engineers developed methods to evaluate dredge material as a result of the Marine Protection, Research and Sanctuaries Act (PL-92-532) (Burton 1991). This resulted in the release of multiple reports and guidance documents for these materials and stimulated research and the development of a new toxicological field.

As this field was developing, there was the realization that the interaction between sediments, overlaying water and contaminants was much more complex than

first thought. Equilibrium partitioning theory helps to explain availability of non-polar organic compounds to the water column, and hence the organism, but assumes the toxicity occurs only by exposure to the aqueous fraction. (Kadeg and Pavlou 1987, Word et al. 1987) There has also been a correlation made between the ratio of the acid volatile sulfide (AVS) concentration and the acid extractable metals concentration in explaining metals toxicity in sediments (Allen et al. 1991, Di Toro et al. 1992, Gonzalez 1992), and these methods represent an improvement in the understanding of possible mechanisms of toxicity. For a full review see Burton (1991, 1992), Salmons (1987) and Huang (1993).

Sediment Test Methods

As in aqueous testing there are several modes which have been considered appropriate depending on the toxicant and study objectives. Static testing is suitable for non-degrading, non-volatile acute studies. They have the advantage of being easy to set-up, and with minimal maintenance. Their usefulness in long term testing (> 10-days), however, is limited because of changes in water quality (Ingersoll and Nelson 1990, Zumwalt et al. 1993), they and have the least environmental realism of any of the laboratory studies. A static renewal can help maintain water quality and but it still has very little environmental realism, and causes a shift in the partitioning of the toxicant between the sediment-water interface. It does not, however, address the potential for leaching of the toxicant in lotic systems. Flow-through technology allows for increased environmental realism in lotic systems, maintains water quality, and addresses the issues concerning contaminants leaching from the substrate.

Objectives

The objective of this study was to develop a flow-through technique for examining survival and growth of juvenile Asiatic clams (*Corbicula fluminea*) in 10-day exposures. Juvenile *Corbicula* were chosen for several reasons. They exhibit many of the characteristics considered favorable in organisms used to assess sediments. They are easy to collect from gravid adults taken from the field and easy to manipulate in the laboratory. This introduced exotic species has a nearly cosmopolitan North American distribution and is considered to be a nuisance species usually, requiring no permits to collect them. Their biennial spawning periodicity can provide unlimited juveniles nearly all summer. As benthic organisms juveniles mollusks are closely associated with the sediments, actively burrowing and feeding in the substrate (Yeager et al. 1994). Pedal feeding in adult *Corbicula* has also been documented (Reid et al. 1992). They exhibit dramatic growth during a 10-day exposure period and appear to be very sensitive. Asiatic clams have also been used for many years as surrogates for rare and endangered native unionid mussels, especially in the Clinch River (upper Tennessee River drainage basin) where native mussel populations are in rapid decline (Rodgers, J. H., Jr et al. 1980, Graney et al. 1983, Belanger et al. 1990).

METHODS

Construction of the Flow-through Test System

A system of peristaltic pumps was designed to deliver a constant flow of overlaying water from a reservoir to test chambers that contained dosed sediments (Figure 3-1). Most test organisms can be exposed directly to the sediment in these chambers; however, juvenile *Corbicula* (24-48 hours old) are translucent and are difficult to recover from the sediment. In order to enhance recovery of juveniles a second exposure tube was designed to hold the clams but still allowed for exposure to the sediment.

Three Cole-Palmer peristaltic pumps were used to deliver the dilution water to the test chambers. One pump was fitted with 4 single heads and the other two with 10-channel multi-heads. This provided the 24 supply lines needed to run the tests. The intake for each supply line was attached to a glass tube which reached to the bottom of the reservoir.

Sediments were tested in chambers constructed from 1 qt ($\approx$ 980 ml) clear polystyrene wide mouthed jars which were modified with an inlet and overflow for flow-through testing (Figure 3-2). The inlet was made by drilling a 1 cm hole in the jars approximately 3 cm above the base of the chamber. The hole was threaded and fitted with a nylon nipple to which the supply line could be attached.

Collection of Clean Sediments

A source of contaminant-free sediment was chosen from the Clinch River at Clifffield, Virginia (Tazwell County) and was no more than 14 days prior to the initiation

of a test (Figure 3-3). Sediments were collected by removing the upper few centimeters of sediment with a nalgene scoop and placing it into a stainless steel 2-mm stainless steel sieve. The sediment was then sieved into a precleaned acid-rinsed bucket. When a sufficient quantity of sediment was collected, the bucket was sealed, and returned to the laboratory and stored at 4°C until used in a test.

Clinch River Sediment was an ideal sediment for this work because this river, and in particular the Clifffield Site maintains a healthy population of both adult and juvenile *Corbicula*. I have measure densities ranging from several hundred to several thousand per meter square at this site, and the sediment characteristics of the sieved sediment (Table 3-1) match closely the preferred sediment of this organism (Cherry et al. 1980, Belanger et al. 1985).

Collection of Dilution Water

Dilution water for these tests came from Sinking Creek in Newport, Virginia at the town park. Sinking Creek water is a known source of clean water which has been used in this laboratory and others for testing and culturing. Water was pumped from the river into a precleaned 570-liter water tank by a gasoline powered water pump. This water was transferred to a 1900-liter storage container at the Ecosystem Simulation Laboratory, where it was aerated and stored until used in the tests. Dilution water was collected and no more than 14 days prior to the initiation of a test.

Collection of Juvenile *Corbicula*

Juvenile *Corbicula* were collected using the same type of modified downweller system that was described by Doherty et al (1986) in his doctoral research. This

system consists of recirculating water which flows through an adult holding area, entrains naturally released juveniles and carries them into a collection chamber (Figure 3-4).

Dosing Sediments with Copper Chloride

Sediments were dosed with copper chloride in the following manner. One hundred ml of homogenized sediment was measured in a tared 100-ml beaker. This volume was then weighed to the nearest gram and that mass of homogenized sediment was weighed into the test chambers. Since the test chambers held approximately 500 ml of water, the 100 ml volume maintained the 1:4 sediment:water ratio suggested in the literature (Nebeker et al. 1984, USEPA 1992, USEPA Sediment Oversight Technical Committee 1992). Measuring these volumes on a weight basis allowed for a more accurate measurement of test sediment. A stock solution of copper chloride 12.5 times the highest test concentration was prepared using acidified distilled water. Sediment was dosed by adding 8, 4, 2, 1, or 0.5 ml of the stock solution to the appropriate test chambers, starting at the highest concentration and working down to the controls which was not dosed with the stock solution at all. When each concentration was dosed, the replicates in that concentration were each mixed for 30 sec and allowed to stand for at least 10 min.

Test Chambers

The test chambers were constructed from 1-quart (946-ml) polystyrene wide mouth jars. These jars were modified by cutting an overflow in the side of the jar so that it would contain approximate 500 ml of liquid (Figure 3-2). The overflow was

covered on the inside with a piece of 105 micron nitex screening so that animals could not be flushed from the test chamber. Each chamber was fitted with an inlet (0.3 mm nylon nipple) 3.81 cm from the base of the jar on the opposite side from the overflow. The inlet was placed such that incoming water would pass just over the surface of the sediment near in the bottom of the chamber. These test chambers hold 100 ml of sediment and 400 ml of overlaying water, providing a test chamber suitable for testing any of the more commonly used sediment test organisms, which maintains the 1:4 sediment water ratio set up for static tests.

Juvenile Clam Exposure Tubes

Because of their size and translucency, juvenile clams cannot be readily retrieved from sediment after placement into the chambers for exposure. For this reason exposure tubes were created to aid in the retrieval of the clams placed in the sediment (Figure 3-2). Tubes were constructed of 2.54 cm pvc uplift tubing and nitex screening. A 15 cm piece of tubing was used for the body of the tube. It was screened on the bottom and sides with 105-micron nitex screening, secured with pvc cement. Tubes were allowed to cure for a minimum of 48 hours before conditioning. Tubes were then acid washed and allowed to condition by soaking them in dilution water or an additional 24-48 hours.

Flow Rates

The general idea of these experiments was to maintain the water column metal concentrations at or near the background concentration for the dilution water. This required that the flow rates for each stream to be relatively high. For this reason I

chose a rate which would replace 95% of the water column every 6 hrs, or 240 ml/hr (Webber 1993). Flow rates were calculated by combining the input from all lines coming from a pump, and measuring the output for 5 minutes and dividing by the number of lines present.

Sediment Analysis for Copper

At the end of a test the sediment remaining in the test chamber was dried at 105°C and a 2.5-g portion was subsampled and analyzed for copper concentrations on the Atomic Absorption Spectrophotometer. The extraction used was a 16-hr, 0.5 N HCl extraction used to remove the total non-residual (TNR) fraction of the bound sediment (Chester et al. 1985). The non residual fraction is that which is loosely bound to the sediment particle and therefore available to biological organisms.

Water Column Analysis of Copper

On days -1, 0, 1, 3, 5, 7, and 10; 10 ml was removed from each replicate and preserved with 0.01 ml of metals grade nitric acid to be analyzed for water column copper concentrations.

Test Initiation

On day -1 of the test, after dosed sediments were placed in the test chambers, they were connected to the peristaltic pumps and the pumps started. The system was allowed to run for 24 hours with no organisms to allow for flushing of the initial pulse of copper from the system.

The initial exposure of the organisms to the dosed sediments was on day 0. Juvenile clams were collected from the juvenile collection chamber and 20 clams were

placed in each of the exposure tubes which were partially submerged in dilution water. This was done by pipetting them in 50 µl of water under a dissecting microscope. This volume is sufficient to move anywhere from 1 to 10 clams.

After the juvenile clams were placed in the exposure tubes, each exposure tube was randomly placed in a test chamber such that the bottom of the tube was in contact with the sediment surface and was held in place by a short piece of plastic coated wire.

Test Termination

At the end of the ten-day exposure period, the tubes were removed from the test chambers and the clams were rinsed into a petri dish using distilled water. The clams were checked under a dissecting microscope and the number of live, dead and total number of clams recovered. Live clams were transferred to small glass vials and preserved in 70% ethanol. These were measured later using an optical micrometer. A summary of test conditions is presented in Table 3-2.

Standard water chemistry parameters on the overlaying water were measured and recorded. Water samples for metals analysis were also taken. Sediments from the test were air dried and stored in plastic ziplock bags for later metals analysis.

Statistical Analysis

Survival data were transformed using the square-root transformation and tested for normality using a Shapiro Wilks test. These data were also tested for homogeneity of variance using Bartley's test (Gulley et al. 1991). Duncan's Multiple Range test was used under the general linear models procedure was used to compare differences

between treatments and the control (SAS Institute Inc. 1989).

The length of the surviving clams was measured in microns under a dissecting stereomicroscope and was used as a measure of growth for the organisms. While in stricter terms growth would be considered to be the initial length minus the final length, it was not for these experiments, In this case, I wanted to do the statistical analysis the same as they are done for other sediment organisms (Chironomus, Hyalella, and D. magna), where growth is considered to be analogous to the final mass of the organism. That is, for these organisms the assumption that the initial mass the organism is has very little variation is made. This assumption holds true for juvenile asiatic clams with respect to length. I measured 30, 24-hour old clams using an optical micrometer and the average length was 180 microns with a standard error of 1.7. These data were also tested for homogeneity of variance using Bartley's test (Gulley et al. 1991). Duncan's Multiple Range test was used under the general linear models procedure to compare differences between treatments and the control (SAS Institute Inc. 1989).

$$n = \frac{2*(Z_{1-\alpha} + Z_{1-\beta})^2 * \sigma^2}{(\mu_1 - \mu_2)^2}$$

Where:

(3-1)

σ^2 = *estimation of population variation*

α = *Type I rate*

β = *Type II rate*

$\mu_1 - \mu_2$ = *difference of the means*

A power analysis was used to determine if there was an adequate number of replicates to obtain the desire sensitivity with a type I error rate of 0.05 and a type II error rate of 0.80 (Eckblad 1991). The number of replicates needed to achieve this

level of statistical power was computed using the Power Equation (3-1) which requires some estimation of the natural variation in the population (σ^2). Population variation was determined by taking the sample variation associated with the control replicates ($n=51$). The Type I and Type II error rates used (α and β respectively) were chosen because they are considered to be typical for biological data (α level=0.05 and $\beta=0.80$). The difference in mean final length of 5% was considered to be significant ($\mu_1-\mu_2$) and was determined by taking the mean control length minus 0.95 times the mean length.

RESULTS AND DISCUSSION

Sediment Dosing

The total non-residual fraction (TNR) of copper measured at the end of the 10-day test period was very close to the nominal concentrations dosed at the beginning of the test (Figure 3-5). A linear regression on the data indicates a very tight fit with an r-value of 0.995. This indicates that the method of dosing the sediments was sufficient for providing the gradient of concentrations needed to test the method.

Recovery Data

When testing sediments with most of the commonly used sediment test organisms, including *Chironomus* sp, *Hyalella* sp, and *Hexagenia* sp, survival data are normally considered to be the ratio of recovered live organisms to exposed organisms (Equation 3-2). This is because organisms that die during the test are often very small and hard to recover, as in the case of some chironomids and amphipods, or they are soft bodied, die beneath the surface of the sediment and decay too rapidly before the end of the test. Regardless of the reasons, many organisms which die may not be recovered at the end of the test. That leads us to make the assumption, "If you can't find it, it's dead".

$$\text{Survival} = \frac{\text{Number of Recovered Organisms}}{\text{Number of Exposed Organisms}} \quad (3-2)$$

This assumption may not be entirely accurate. There are other reasons that test organisms may not be recovered at the end of the exposure period. The most common of which is that the correct number of organisms are miscounted in the test initially. This is easily detected in water only tests where there is no substrate to

interfere and the test organisms are visible throughout the testing period. Sediments create another problem in that once the organisms are placed in the test chambers, they may not be seen until the termination of the test. Therefore a greater importance is placed on making sure the correct number of organisms is exposed.

Another less common problem is that when recovering the organisms at the termination of the test, adequate time is not placed in searching the sediments to find the organism. It is not uncommon to spend several hours searching for the test organisms, only recover 80% of those exposed, and then return to it several minutes later and find the other 20%. This may be most important when checking the last few containers after breaking down large tests over many hours.

Making this assumption then may lead to erroneously high mortality data (low survival) in that organisms some organisms may be alive and unrecovered or may never have been exposed at all. Either way, mortality data for these test concentrations may be high leading to oversensitivity of the test. This, however, is a necessary assumption when dealing with soft-bodied organisms.

$$Survival = \frac{\text{number of surviving organisms}}{\text{number of recovered organisms}} \quad (3-3)$$

Testing sediments with organisms which leave a shell when they die, such as mussels and clams, provides a recoverable "residue" which allows survival to be calculated as in equation 3-3. *Corbicula* adults (10-12 mm) have been used in 30-day+ exposures in artificial streams to measure survival, growth and bioaccumulation of copper and other metals (Rodgers, J. H. et al. 1977, Graney et al. 1983, Belanger et al. 1990). This lifestage grows too slow for use in a short-term (10-day) study. Also

the younger lifestage has been shown to be as sensitive a lifestage to copper (Jacobson 1990).

Recovery of this lifestage from sediment is not feasible without the use of the Juvenile Clam Exposure Tubes due to their small size (averaging 180 μm) and translucent coloration. These data, however, show that using the Juvenile Clam Exposure Tubes will aid in recovery of all organisms to range from 93 to 100% after the 10-day exposure, with an overall mean recovery of $97.5 \pm 0.95\%$ (Table 3-3). These organisms were recovered spending only 15 to 45 min per test chamber. Recovery averaging almost 98%, calculating survival based solely on recovery (Equation 3-3), is warranted. This eliminates the unrecovered/dead assumption and reduces the variability introduced by that assumption.

Survival Data

Survival of the control juveniles was very good except for one replicate in which there were two surviving (Table 3-4). There was no indication as to why this replicate did not survive, however even with the high mortality in this one replicate there was still 70% survival in the controls (Table 3-4). If this replicate is dropped because of the anomalous mortality, the control survival jumps to $91.7 \pm 1.77\%$. Survival was significantly impaired at all toxicant concentrations when compared to the control using Duncan's Multiple Range test to compare the means. The organisms showed a good dose response to the copper dosed sediments (Table 3-4) with an LOEC at the 25 ppm level. The NOEC for these data could not be generated because all concentrations were significantly different from the controls, but was between the 6.8

ppm background concentration and the 26.2 ppm (25 nominal).

In 48-hour acute exposures of the native mussel juvenile, *Actinonaias pectorosa*, to copper, Jacobson et al. (1990) found acute toxicity to occur between 51-53 ppb copper. While these data are for another organism the relative sensitivities of these lifestages are considered similar. Data generated from the flow-through sediment bioassays indicate toxicity well below the acute values for water-only exposures, except for the two highest concentrations (Table 3-4). These concentrations (200 and 400 ppm) which showed acutely toxic water column levels for the first 48-96 hours (Figure 3-6).

Growth Data

Surviving juveniles exposed to all levels of copper-spiked sediments showed significant reductions in growth when compared to the controls (Table 3-5). Growth was significantly impaired at all toxicant concentrations when compared to the control using Duncan's Multiple Range test to compare the means. The organisms showed a good dose response to the copper dosed sediments (Table 3-5) with an LOEC at the 25-ppm level. The NOEC for these data could not be generated since all concentrations were impaired, but was between the 6.8 ppm background concentration and the 26.2 ppm (25 nominal). Control organisms grew from an average of 180 to 287.9 microns in 10 days. This is a 59.9% increase in length over the 10-day period, and suggests that at this lifestage, juveniles grow rapidly enough to be useful test organisms. The minimum detectable difference in shell length was 20 microns because of the magnification of the microscope used. This translates in the ability to

detect differences of 11% in the smaller 24-hr juveniles and 7% in the 10-day old control animals. These detection limits were suitable for these experiments where the average control animal grew nearly 60%, making it possible to detect over 8 distinct levels of growth in individual juveniles.

Power Analysis

Result of the power analysis indicated that a 5% difference in growth could be detected using 4 replicates. However, when 3 replicates are used the ability to detect differences only rises to 5.72%. Since the change in detection differences are so small and the time involved in setting up and breaking down the additional replicate is significant, the additional replicate is not warranted.

CONCLUSIONS

Juvenile Corbicula have many of the characteristics which make it an ideal sediment testing organism. They are easy to collect from gravid adults taken from the field and easy to manipulate in the lab. This introduced exotic species has a nearly cosmopolitan North American distribution and is considered to be a nuisance, requiring no permits to collect or remove. Their biennial spawning periodicity can provide unlimited juveniles nearly all summer. As benthic organisms juveniles are closely associated with the sediments, actively burrowing and feeding in the substrate. They exhibit dramatic growth during a 10-day exposure period and have shown to be very sensitive.

It has become apparent that their most difficult aspect in testing juveniles is recovery from the test sediment. Their translucent coloration and small size prohibits recovery from sediments in a manner which is economically feasible, until now. It is standard practice in sediment toxicology that unrecovered organisms be presumed dead. If recovery is low then mortality data can be falsely inflated. The use of the Juvenile Clam Exposure Tubes described in this chapter allows for recovery of 97% of the organisms tested. Recovery values this close to 100% means that we can eliminate that assumption and calculate mortality (or survival) directly from recovered organisms, eliminating one source of variability in the testing process. When testing sediments, we like to challenge several types organisms to the same sediment in order to get a generalized response. By using these tubes, we do not need to change our

test system in order to challenge other organisms. All organisms can still be challenged using the same test chamber, flow rates etc. This allows us to remove other variables in the testing scheme such as chamber artifacts.

With respect to survival, juvenile corbicula were shown to be very sensitive with and LOEC of 25 ppm. Corbicula have also shown to be equally sensitive with respect to estimating chronic endpoints with an LOEC of 25 ppm.

Flow-through technology increases the environmental realism of testing sediments in lotic systems. It also allows us to separate out effects of the watercolumn by continually replacing it. In aquatic systems, especially lotic systems, contaminants in the sediment leach out into the overlaying water. Flow-through technology gives us a method of examining how this leaching process effects both the overlaying water and the sediment. Even though total recoverable copper in the water column was below 20 ppb throughout the testing period, effects on survival and growth were detected.

LITERATURE CITED

- Allen, H. E., Fu, G., Boothman, W., DiToro, D. M. and Mahony, J. D. 1991. Draft analytical method for determination of acid volatile sulfide in sediment. - United States Environmental Protection Agency, Washington, DC.
- Belanger, S. E., Farris, J. L., Cherry, D. S. and Cairns, J., Jr. 1985. Sediment preference of the freshwater Asiatic clam, *Corbicula fluminea*. - *The Nautilus* 99: 66-73.
- Belanger, S. E., Farris, J. L., Cherry, D. S. and Cairns, J., Jr. 1990. Validation of *Corbicula fluminea* growth reductions induced by copper in artificial streams and river systems. - *Can. J. Fish. Aquat. Sci.* 47: 904-914.
- Burton, G. A., Jr. 1991. Assessing the toxicity of freshwater sediments. - *Environ. Tox. Chem.* 10: 1585-1627.
- Burton, G. A., Jr. 1992. Assessing contaminated aquatic sediments. - *Environ. Sci. Technol.* 26: 1862-1863.
- Cherry, D. S., Rodgers, J. H., Jr., Graney, R. L. and Cairns, J., Jr. (eds.). 1980. Dynamics and control of the Asiatic clam in the New River, Virginia. - Virginia Water Resources Research Center, Blacksburg.
- Chester, R., Kudoja, W. M., Thomas, A. and Towner, J. 1985. Pollution reconnaissance in stream sediments using non-residual trace metals. - *Environ. Pollut. Ser. B.* 10: 213-238.
- Di Toro, D. M., Mahony, J. D., Hansen, D. J., Scott, K. J., Carlson, A. R. and Ankley, G. T. 1992. Acid volatile sulfide predicts the acute toxicity of cadmium and nickel in sediments. - *Environ. Sci. Technol.* 26: 96-101.
- Doherty, F. G. 1986. A multidisciplinary study of the Asiatic clam, *Corbicula fluminea*, from the New River, Virginia. - Ph.D. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg.
- Eckblad, J. W. 1991. How many samples should be taken? - *Bioscience* 41: 346-348.
- Gonzalez, A. M. 1992. Heavy metal sorption in anoxic sediments: an experimental study of sediment organic carbon as a metal-binding phase under anoxic conditions. - MS Thesis, Manhattan College, Riverdale, NY.
- Graney, R. L., Jr., Cherry, D. S. and Cairns, J., Jr. 1983. Heavy metal indicator potential of the Asiatic clam *Corbicula fluminea* in artificial stream systems. - *Hydrobiologia* 102: 81-88.

Gulley, D. D., Boelter, A. M. and Bergman, H. L. 1991. Toxstat - version 3.3. - Fish Physiology and Toxicology Laboratory, Department of Zoology and Physiology. University of Wyoming. Laramie, Wyoming.

Huang, P. M. 1993. An overview of dynamics and biotoxicity of metals in freshwater environment. - Water Poll. Res. J. Canada 28: 1-5.

Ingersoll, C. G. and Nelson, M. K. 1990. Testing sediment toxicity with *Hyalella azteca* (Amphipoda) and *Chironomus riparius* (Diptera). - In: Landis, W. G. and Van Der Schalie, W. H. (eds.), Aquatic Toxicology and Risk Assessment, Vol. 13. American Society for Testing and Materials, Philadelphia, pp. 93-109.

Jacobson, P. J. 1990. Sensitivity of early life stages of freshwater mussels (Bivalvia: Unionidae) to copper. - ms Thesis, Virginia Polytechnic Institute and State University.

Kadeg, R. D. and Pavlou, S. P. 1987. Reconnaissance field study for verification of equilibrium partitioning: nonpolar hydrophobic organic chemicals. - Office of Water Regulations and Standards, Criteria and Standards Division, United States Environmental Protection Agency, Washington.

Nebeker, A. V., Cairns, M. A., Gakstatter, J. H., Malueg, K. W., Schuytema, G. S. and Krawczyk, D. F. 1984. Biological methods for determining toxicity of contaminated freshwater sediments to invertebrates. - Environ. Tox. Chem. 3: 617-630.

Reid, R. G., McMahon, D. O., Foighil, D. O. and Finnigan, R. 1992. Anterior inhalent currents and pedal feeding in bivalves. - The Veliger 35: 93-104.

Rodgers, J. H., Cherry, D. S., Dickson, K. L. and Cairns, J., Jr. 1977. Invasion, population dynamics and elemental accumulation of *Corbicula fluminea* in the New River, Virginia. - In: (), Proceedings of the First International Corbicula Symposium. Texas Christian University, Fort Worth, pp. 99-110.

Rodgers, J. H., Jr., Cherry, D. S., Graney, R. L., Dickson, K. L. and Cairns, J., Jr. 1980. Comparison of heavy metal interactions in acute and artificial stream bioassay techniques for the Asiatic clam *Corbicula fluminea*. - In: Eaton, J. G., Parrish, P. R. and Hendricks, A. C. (eds.), Aquatic Toxicology. American Society for Testing and Materials, Philadelphia, pp. 266-280.

Salomons, W., de Rooij, N. M., Kerdijk, H. and Bril, J. 1987. Sediments as a source for contaminants? - Hydrobiologia 149: 13-30.

SAS Institute Inc. 1989. SAS/STAT users guide, version 6, 4th ed., Vol. 2. - SAS Institute Inc., Cary, NC.

USEPA. 1992. Proceedings, Tiered testing issues for freshwater and marine sediments. - United States Environmental Protection Agency, Office of Water, Office of Science and Technology, and Office of Research and Development, Washington.

USEPA Sediment Oversight Technical Committee (ed.). 1992. Sediment Classification Methods Compendium. - United States Environmental Protection Agency, Office of Science and Technology, Washington.

Webber, C. I. (ed.). 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 4th ed. - United States Environmental Protection Agency, Cincinnati.

Word, Q. J., Ward, J. A., Franklin, L. M., Cullin, V. I. and Kiesser, S. L. 1987. Evaluation of the equilibrium partitioning theory for estimating the toxicity of the nonpolar organic compound DDT to the sediment dwelling organism *Rhepoxynius abronius*. - Office of Water Regulations and Standards, Criteria and Standards Division, United States Environmental Protection Agency, Washington.

Yeager, M. M., Cherry, D. S. and Neves, R. J. 1994. Feeding and burrowing behaviors of juvenile rainbow mussels, *Villosa iris* (Bivalvia: Unionidae). - J. N. Am. Benthol. Soc. 13: 217-222.

Zumwalt, D. C., Dwyer, F. J., Greer, I. E. and Ingersoll, C. G. 1993. A water-renewal system that accurately delivers small volumes of water to exposure chambers. - Environ. Tox. Chem. 13: 1311-1314.

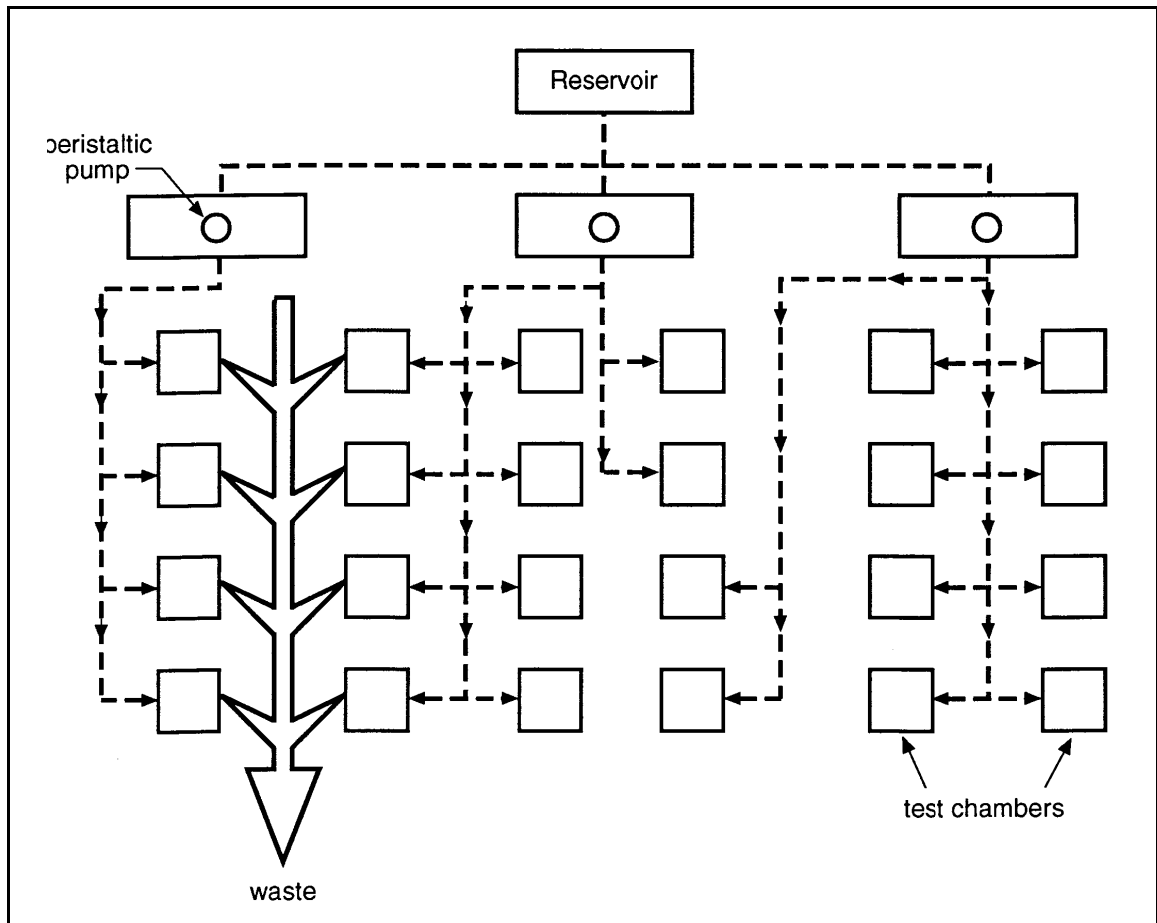


Figure 3-1. Schematic of the flow-through system. Peristaltic pumps deliver dilution water from the reservoir to each test chamber. Waste flows over the edge of the test chamber and is drained away.

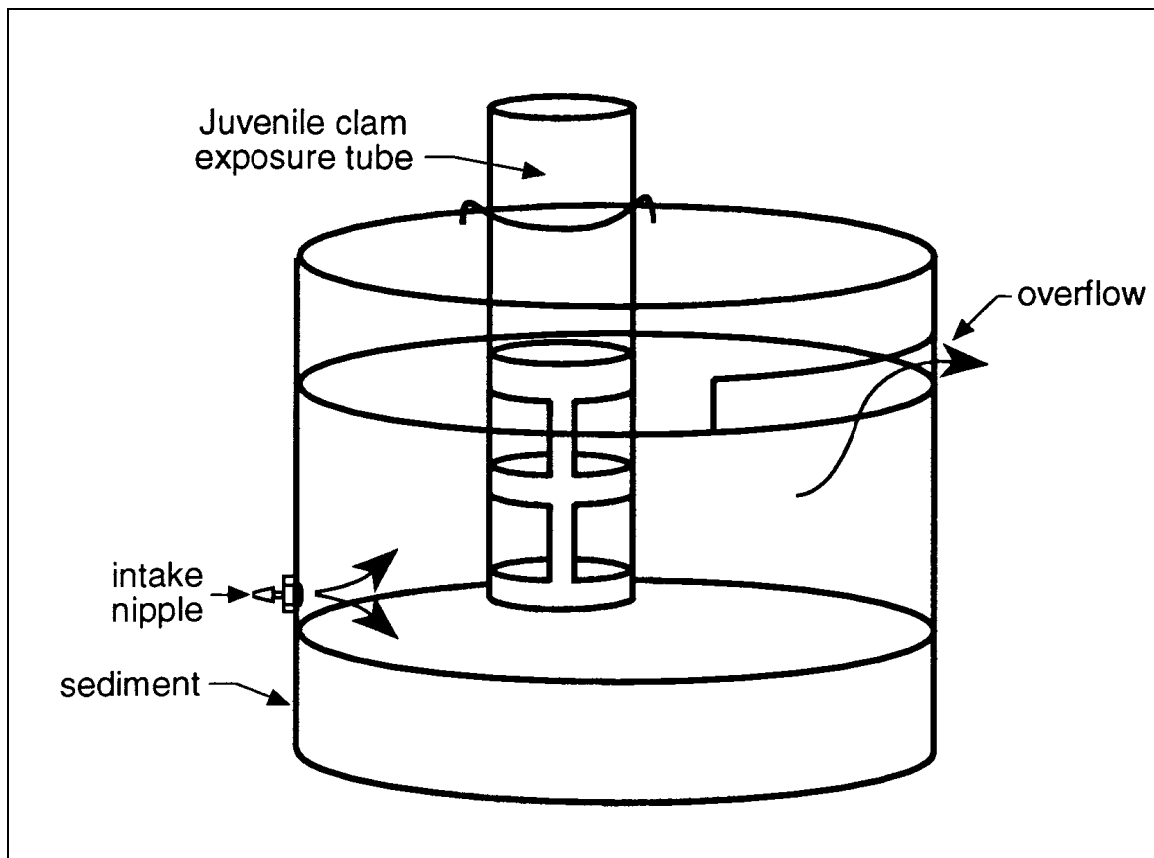


Figure 3-2. Flow-through sediment test chamber with a Juvenile Clam Exposure Tube. Dilution water enters through the intake nipple and exits through the screened overflow. Clams are placed in the screened exposure tube.

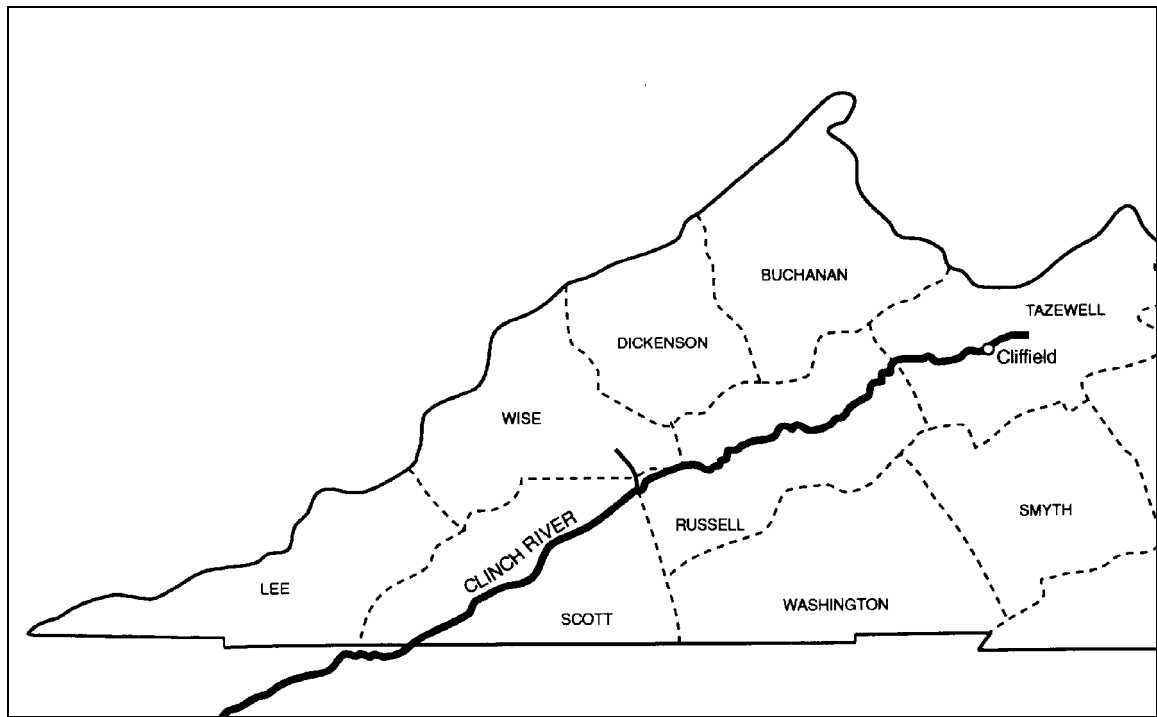


Figure 3-3. Map of southwestern Virginia showing the Clinch River flowing southwest from Tazewell County and the location of the Clifffield site. Sediments collected from this site were dosed with copper chloride to test juvenile clams.

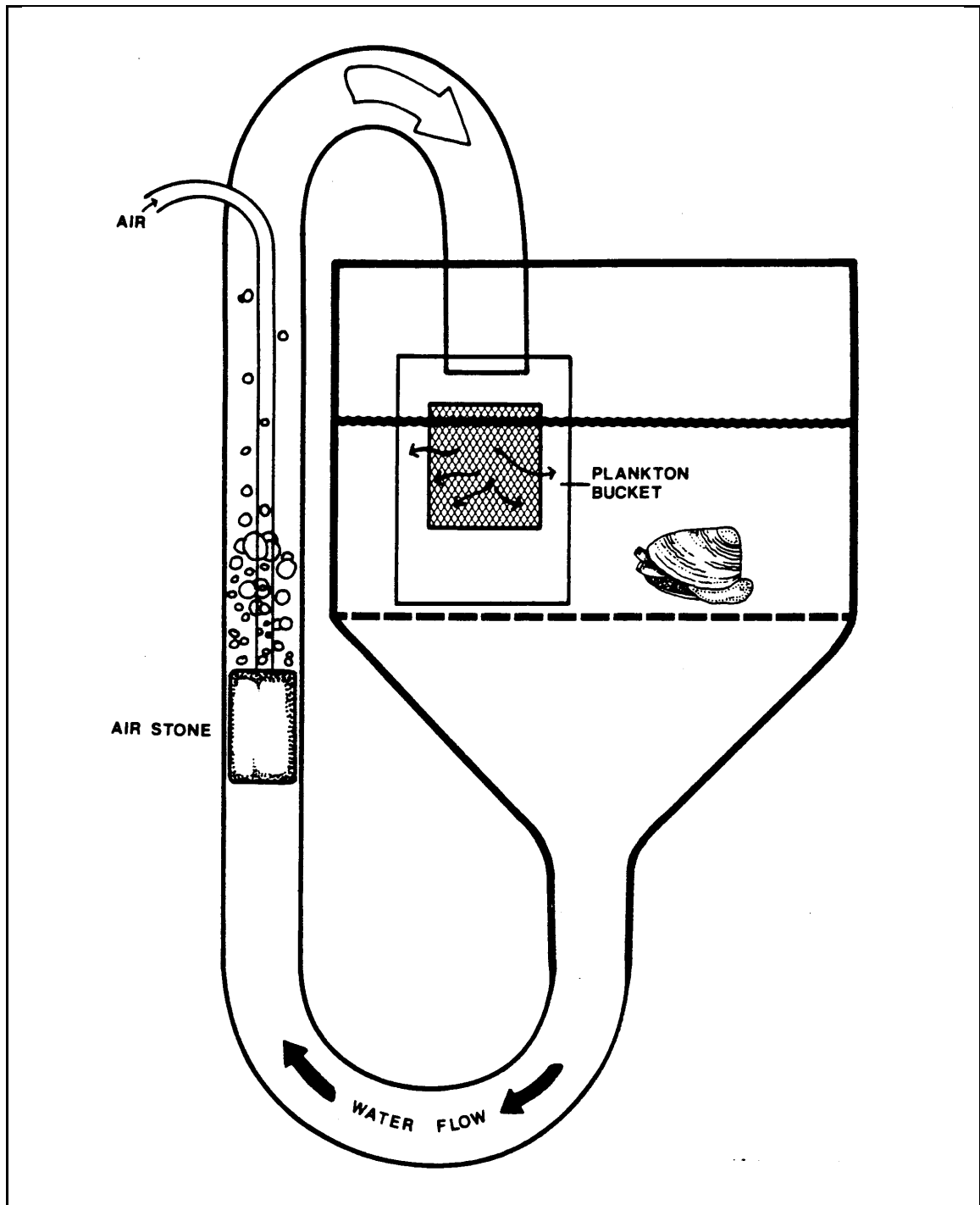


Figure 3-4. Juvenile *Corbicula* collection chamber - a modified downweller designed to entrain juvenile clams naturally released from gravid adults. Figure from Doherty et al. (1986).

Flow-through technique for
exposing the juvenile clams

Sediment Copper Concentration

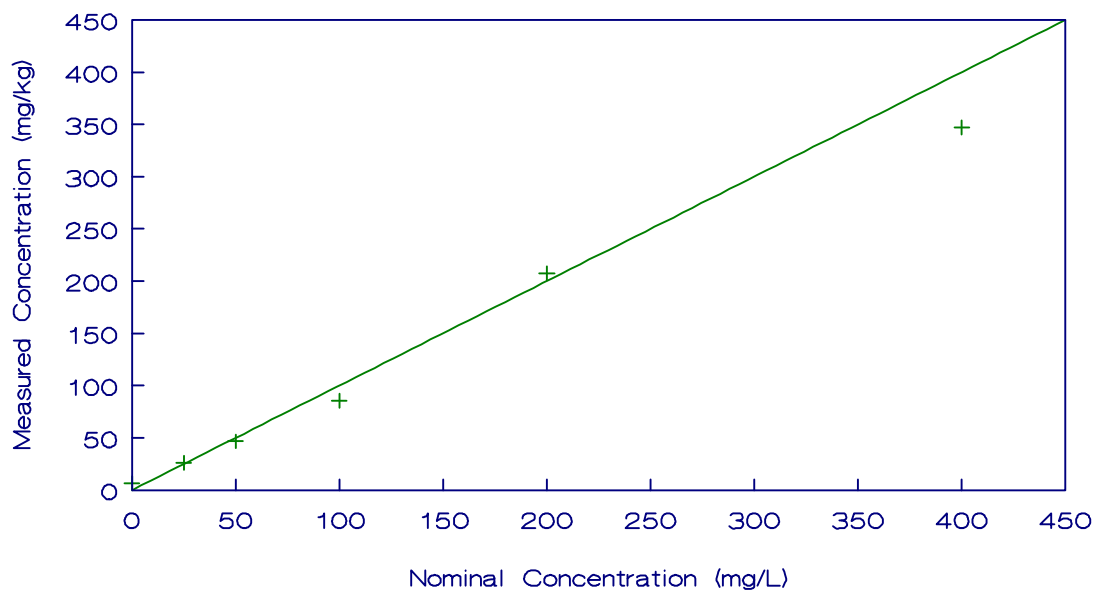


Figure 3-5. Measured sediment copper concentrations (mg cu/kg dry sediment) are shown against nominal concentrations (mg cu/L wet sediment) used in dosing Clinch River sediment. Error bars are not visible in this graph.

Total Recoverable Copper

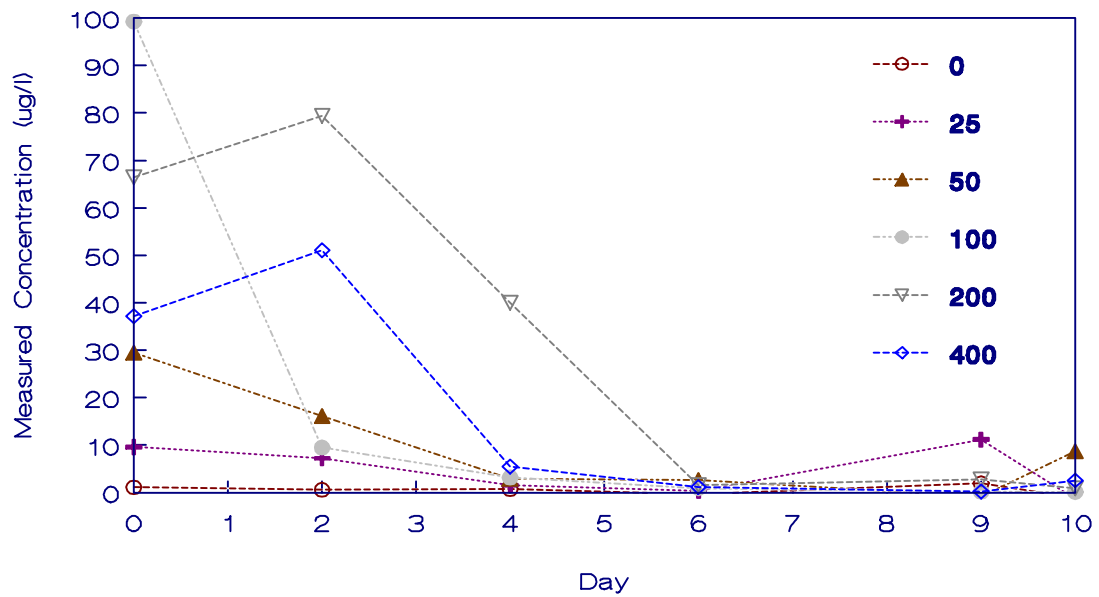


Figure 3-6. Total recoverable copper concentrations ($\mu\text{g/L}$) are shown for each nominal concentration over test duration.

Table 3-1. Sediment characterization of sediments dosed for flow-through sediment bioassays using juvenile Asiatic clams in 10-day exposures.

Parameter	Mean Value	Standard Error	n
Sediment pH (su)	7.69	na	1
Moisture (%)	22.42	0.71	3
Loss on Ignition (%)	1.41	0.13	3
Total Organic Carbon (%)	0.432	0.023	3
Cationic Exchange Capacity (meq/100 g)	4.327	0.408	3
Particle Size Distribution			
Sand (%)	69.452	1.720	3
Silt (%)	31.410	1.994	3
Clay (%)	-0.498	0.308	3

Table 3-2. Summary table of test conditions for conducting flow-through sediment bioassays with naturally released juvenile Asiatic clams (*Corbicula fluminea*).

Test Type	Flow-through
Test Temperature	20°C ±2
Light Quality	Ambient Laboratory Light
Light Intensity	50-100 ft c
Aeration	As needed
Test chamber size	≈1 liter
Juvenile exposure Chamber	1 inch screened pvc tube
Sediment Water Ratio	1:4
Replicates per Treatment	4
Organisms per Replicate	20
Test Endpoints	Survival and Growth
Acceptable Control Survival	70%
Mean 10-day juvenile length	205 microns

Table 3-3. Mean Total Recoverable Copper concentrations in the water column of a flow through sediment test using the juvenile Asiatic clam (*Corbicula fluminea*).

Nominal Sediment Concentration (mg/kg)	Total Recoverable Copper (ppb)	Standard Error	Range
0	<1.0	0.42	<1.0 - 1.95
25	4.97	2.14	<1.0 - 9.60
50	9.87	4.61	<1.0 - 29.46
100	18.87	16.14	<1.0 - 99.26
200	31.86	14.42	0.84 - 79.43
400	16.29	9.01	<1.0 - 51.05

Table 3-4. Survival of juvenile Asiatic clams to Clinch River sediments spiked with copper chloride in a flow through bioassay.

Nominal Sediment Concentration (mgCu/kgSed.)	Measured Sediment Concentration (mgCu/kgSed.)	Survival (%) ^{§¥}	Recovery (%)
0	6.8	70.0 (20.1)	97.5 (2.5)
25	26.2	33.6 (17.8)*	96.3 (3.8)
50	46.7	28.8 (15.2)*	100.0 (0)
100	85.7	8.0 (6.3)*	93.8 (2.4)
200	207.2	2.3 (1.3)*	97.5 (2.5)
400	346.9	1.7 (1.7)*	100 (0)
Overall Mean Recovery:			97.5 (0.95)

[§] Numbers in parentheses represent 1 standard Error.

[¥] Numbers followed by an asterisk represent values significantly different from the control using Duncan's Multiple Range test at $\alpha=0.05$ level.

Table 3-5. Growth of juvenile Asiatic clams to Clinch River sediments spiked with copper chloride in a flow through bioassay.

Nominal Sediment Concentration (mgCu/kgSed.)	Measured Sediment Concentration (mgCu/kgSed.)	Mean Length (µm) ^{§*}	Range (µm)
0	6.8	287.9 (5.0)	220 - 340
25	26.2	263.4 (12.1)*	220 - 300
50	46.7	249.9 (6.4)*	240 - 280
100	85.7	254.0 (6.0)*	240 - 260
200	207.2	250 (-)*	240 - 260
400	346.9	240 (-)*	na

[§] Numbers in parentheses represent 1 standard Error.

^{*} Numbers followed by an asterisk represent values significantly different from the control using Duncan's Multiple Range test at $\alpha=0.05$ level.

CHAPTER 4

Relative Sensitivity of Juvenile *Corbicula fluminea* and Other Sediment
Test Organisms When Exposed to Copper Chloride.

INTRODUCTION

"Sediments are the ultimate sink for pollutants. However before these sediments become part of the sedimentary record (e.g. deeply buried) they are able to influence the composition of surface waters." (Salomons et al. 1987)

The above statement typifies the concerns with scientists, environmentalists, and regulatory agencies in their efforts to protect water quality and mitigate contaminants produced by industrial and municipal processes. Regulatory agencies including the US EPA are currently developing methods for testing sediments in order to begin including them in discharge permits as they have done with water-only bioassays, but to date have released little guidance on these issues.

History of Sediment Toxicity

Regulatory and scientific concerns with the disposal and toxicity of sediment and sediment-associated contaminants began in earnest with elevated contaminant levels recognized in dredged materials. The Federal Water Quality Administration (precursor to the current EPA) developed limited criteria for disposal in the early 1970's known as the Jenson Criteria. The EPA and Army Corps of Engineers developed methods to evaluate dredge material as a result of the Marine Protection, Research and Sanctuaries Act (PL-92-532) (Burton 1991). This resulted in the release of multiple reports and guidance documents for these materials and stimulated research and the development of a new toxicological field.

As this field was developing, there was the realization that the interaction between sediments, overlaying water and contaminants was much more complex than

first thought. Equilibrium partitioning theory helps to explain availability of non-polar organic compounds to the water column, and hence the organism, but assumes the toxicity occurs only by exposure to the aqueous fraction. (Kadeg and Pavlou 1987, Word et al. 1987) There has also been a correlation made between the ratio of the acid volatile sulfide (AVS) concentration and the acid extractable metals concentration in explaining metals toxicity in sediments (Allen et al. 1991, Di Toro et al. 1992, Gonzalez 1992), and these methods represent an improvement in the understanding of possible mechanisms of toxicity. For a full review see Burton (1991, 1992), Salomons et al. (1987) and Huang (1993).

Sediment Test Methods

As in aqueous testing there are several modes which have been considered appropriate depending on the toxicant and study objectives. Static testing is suitable for non-degrading, non-volatile acute studies. They have the advantage of being easy to set-up, and with minimal maintenance. Their usefulness in long term testing (> 10-days), however, is limited because of changes in water quality (Ingersoll and Nelson 1990, Zumwalt et al. 1993), and they have the least environmental realism of any of the laboratory studies. A static renewal can help maintain water quality and but it still has very little environmental realism, and causes a shift in the partitioning of the toxicant between the sediment-water interface. It does not, however, address the potential for leaching of the toxicant in lotic systems. Flow-through technology allows for increased environmental realism in lotic systems, maintains water quality, and addresses the issues concerning contaminants leaching from the substrate.

Objectives

Juvenile *Corbicula* were chosen for several reasons. They exhibit many of the characteristics considered favorable in organisms used to assess sediments. They are easy to collect from gravid adults taken from the field and easy to manipulate in the laboratory. This introduced exotic species has a nearly cosmopolitan North American distribution and is considered to be a nuisance species, usually, requiring no permits to collect them. Their biennial spawning periodicity can provide unlimited juveniles nearly all summer. As benthic organisms juveniles mollusks, they are closely associated with the sediments, actively burrowing and feeding in the substrate (Yeager et al. 1994). Pedal feeding in adult *Corbicula* has also been documented (Reid et al. 1992). They exhibit dramatic growth during a 10-day exposure period and appear to be very sensitive. Asiatic clams have also been used for many years as surrogates for rare and endangered native unionid mussels, especially in the Clinch River (upper Tennessee River drainage basin) where native mussel populations are in rapid decline (Rodgers et al. 1980, Graney et al. 1983, Belanger et al. 1990). The objective of this research was to evaluate the sensitivity of juvenile Asiatic clams by comparing them to three other commonly used sediment test organisms in 10-day flow-through bioassays. *Chironomus riparius*, *Daphnia magna* and *Hyalella azteca* were chosen because they are commonly used in sediment bioassays and are readily available (Cairns et al. 1984, Schuytema et al. 1984, Nebeker et al. 1986, 1988, Nebeker and Miller

1988, USEPA Sediment Oversight Technical Committee 1992).

METHODS

Construction of the Flow-through Test System

A system of peristaltic pumps was designed to deliver a constant flow of overlaying water from a reservoir to test chambers that contained dosed sediments (Figure 1). Most test organisms can be exposed directly to the sediment in these chambers; however, juvenile *Corbicula* (24-48 hours old) are translucent and difficult to recover from the sediment. In order to enhance recovery of juveniles a second exposure tube was designed to hold the clams but still allowed for exposure to the sediment. A summary of the test conditions is located in Table 4-1.

Three Cole-Palmer peristaltic pumps were used to deliver the dilution water to the test chambers. One pump was fitted with 4 single heads and the other two were with a 10 channel multi-head system. This provided the 24 supply lines needed to run the tests. The intake for each supply line was attached to a glass tube which reached to the bottom of the reservoir.

Sediments were tested in chambers constructed from 1 qt ($\approx$ 980 ml) clear polystyrene wide mouthed jars which were modified with an inlet and overflow for flow-through testing (Figure 4-82). The inlet was made by drilling a 1 cm hole the jars approximately 3 cm above the base of the chamber. The hole was threaded and fitted with a nylon nipple to which the supply line could be attached.

Collection of Clean Sediments

A source of contaminant-free sediment was chosen from the Clinch River at Clifffield, Virginia (Tazwell County) and was no more than 14 days prior to the initiation

of a test (Figure 3). Sediments were collected by removing the upper few centimeters of sediment with a nalgene scoop and placing it into a 2 mm stainless steel sieve. The sediment was then sieved into a precleaned acid-rinsed bucket. When a sufficient quantity of sediment was collected The bucket was sealed and returned to the laboratory and stored at 4°C until used in a test.

Clinch River Sediment was an ideal sediment for this work because this river, and in particular the Clifffield Site maintains a healthy population of both adult and juvenile *Corbicula*. I have measured densities ranging from several hundred to several thousand/m² at this site, and the sediment characteristics of the sieved sediment (Table 4-2) match closely the preferred sediment of this organism (Cherry et al. 1980, Belanger et al. 1985).

Collection of Dilution Water

Dilution water for these tests was collected from Sinking Creek near the town park in Newport, Virginia (Montgomery County). Sinking Creek water is a known source of clean water which has been used in this laboratory and others for testing and culturing. Water was pumped from the river into a precleaned 570-liter water tank by a gasoline powered water pump. This water was transferred to a 1900-liter storage container at the Ecosystem Simulation Laboratory, where it was aerated and stored until used in the tests. Dilution water was collected no more than 14 days prior to the initiation of a test.

Test Organisms

Chironomus riparius

Midges, *Chironomus riparius*, were cultured in mass cultures. Each culture consisted of a substrate of boiled paper towels and covered with Clinch River water. Midges were fed a diet of trout chow and ground paper towels. Egg masses were removed from the cultures daily and hatched in 50-ml beakers of dilution water. The newly hatched larvae were placed in small individual cultures similar to the mass cultures. These were maintained until the midges reached 2nd instar.

Juvenile Corbicula

Juvenile *Corbicula* were collected using a modified downweller system from field collected gravid adults. This method was described by Doherty et al. (1986) in his doctoral research. This system consisted of recirculating water which flowed through an adult holding area, entrained naturally released juveniles and carried them into a collection chamber (Figure 4-4).

Daphnia magna

Daphnia magna were cultured in Clinch River water for a minimum of two weeks prior to the initiation of a test. Prior to test initiation on day -5, approximately 60-80 adult daphnids were removed from the mass cultures and placed in a new culture. Every 24 hours these adults were transferred to a new culture and the neonates released during that 24-hour period were maintained until they reached a suitable age for testing. These organisms were fed 7 ml of a mixture of three green algae (*Chlamydomonas*, *Ankistrodesmus*, and *Chlorella*) at a concentration of 3.2×10^7 cells per ml daily.

Hyalalella azteca

Mass cultures of *Hyalella* were maintained in Clinch River water with a substrate of maple and sycamore leaves. Their diet was supplemented periodically with Tetra-min fish food and heat killed daphnids. Prior to the initiation of a test organisms were removed from these cultures by sieving them through a 1.0 and 0.6 mm stainless steel sieve. This provided organisms that were 2-3 weeks of age for testing.

Dosing Sediments with Copper Chloride

Sediments were dosed with copper chloride in the following manner. One hundred-ml of homogenized sediment was measured in a tared 100-ml beaker. This volume was then weighed to the nearest gram and that mass of homogenized sediment was weighed into the test chambers. Since the test chambers held approximately 500 ml of water, the 100-ml volume maintained the 1:4 sediment:water ratio recommended in the literature (Nebeker et al. 1984a, USEPA 1992, USEPA Sediment Oversight Technical Committee 1992). Measuring these volumes on a weight basis allowed for a more accurate measurement of test sediment. A stock solution of copper chloride 12.5 times the highest test concentration was prepared using acidified distilled water. Sediment was dosed by adding 8, 4, 2, 1, or 0.5 ml of the stock solution to the appropriate test chambers, starting at the highest concentration and working down to the controls which were not dosed with the stock solution at all. When each concentration was dosed, the replicates in that concentration were each mixed for 30 sec and allowed to stand for at least 10 min.

Test Chambers

Relative sensitivities of various
organisms to copper chloride

The test chambers were constructed from 1-quart (946-ml) polystyrene wide mouth jars. These jars were modified by cutting an overflow in the side of the jar so that the jar would contain approximate 500 ml of liquid (Figure 4-2). The overflow was covered on the inside with a piece of 105-micron nitex screening so that animals could not be flushed from the test chamber. Each chamber was fitted with an inlet (3 mm nylon nipple) 3.81 cm from the base of the jar on the opposite side from the overflow. The inlet was placed such that incoming water would pass just over the surface of the sediment near the bottom of the chamber. These test chambers hold 100 ml of sediment and 400 ml of overlaying water, providing a test chamber suitable for testing any of the more commonly used sediment test organisms, which maintains the 1:4 sediment water ratio set up for static tests.

Juvenile Clam Exposure Tubes

Because of their size and translucency juvenile clams cannot be readily retrieved from sediment after placement into the chambers for exposure. For this reason exposure tubes were created to aid in the retrieval of the clams placed in the sediment (Figure 4-2). Tubes were constructed of 2.54 cm pvc uplift tubing and nitex screening. A 15 cm piece of tubing was used for the body of the tube. It was screened on the bottom and sides with 105 micron nitex screening, secured with pvc cement. Tubes were allowed to cure for a minimum of 48 hours before conditioning. Tubes were then acid washed and allowed to condition by soaking them in dilution water or an additional 24-48 hours.

Flow Rates

Relative sensitivities of various
organisms to copper chloride

The study was designed to maintain the water column metal concentrations at or near the background concentration for the dilution water. This required the flow rates for each stream to be relatively high. I chose a rate which would replace 95% of the water column every 6 hrs, or 240 ml/hr (Webber 1993). Flow Rates were calculated by combining the input from all lines coming from a pump, and measuring the output for 5 min and dividing by the number of lines present.

Sediment Analysis for Copper

At the end of a test the sediment remaining in the test chamber was dried at 105°C and a 2.5-g portion was subsampled and analyzed for copper concentrations Atomic Absorption Spectrophotometry. The extraction used was a 16-hr, 0.5 N HCl extraction used to remove the total non-residual (TNR) fraction of the bound sediment (Chester et al. 1985). The non-residual fraction is that fraction which is loosely bound to the sediment particle and therefore available to biological organisms.

Water Column Analysis of Copper

On days 0, 1, 3, 5, 7, and 10; 10 ml of overlaying water were removed from each replicate and preserved with 0.01 ml of trace metals grade nitric acid to be analyzed for total extractable copper concentrations.

Test Initiation

On day -1 of the test, after dosed sediments were placed in the test chambers, the test chambers were connected to the peristaltic pumps and the pumps were started. The system was allowed to run for 24 hours with no organisms to allow for flushing of any initial pulse of copper from the system.

Placing Organisms in test

The initial exposure of the organisms to the dosed sediments was on day 0. Juvenile *Corbicula* were collected from the juvenile collection chamber and 20 clams were placed in each of the exposure tubes by pipetting them in 50 µl of water under a dissecting microscope. This volume is sufficient to move anywhere from 1 to 10 clams. Tubes were maintained partially submerged in dilution water.

Chironomus riparius, *Hyaella azteca*, and *Daphnia magna* tests were initiated by pipetting 10 organisms carefully into 30-ml polystyrene cups with 20 ml of dilution water. One organism was added to each cup until each cup contained the appropriate number of organisms.

After the test organisms were placed in their appropriate cups or tubes, each exposure tube was randomly placed in a test chamber such that the bottom of the tube was in contact with the sediment surface and was held in place by a short piece of plastic coated wire. Polystyrene cups were carefully emptied into test chambers as to stress them as little as possible.

Test Termination

Corbicula fluminea

At the end of the ten-day exposure period, the tubes were removed from the test chambers and the clams were rinsed into a petri dish using distilled water. The clams were checked under a dissecting microscope and the number of live, dead and total number of clams recovered was tabulated. Mortality was recorded as the total

number of organisms exposed minus number recovered (Equation 4-1). Live clams were transferred to small glass vials and preserved in 70% ethanol. These were measured later using an optical micrometer.

$$\text{Survival} = \frac{\text{number of surviving organisms}}{\text{number of recovered organisms}} \quad (4-1)$$

Hyalella azteca

Hyalella were removed from the test chambers by sieving the sediments through a 0.6-micron stainless steel sieve. Mortality was recorded as the total number of organisms exposed minus number exposed (Equation 4-2). Recovered organisms were placed in plastic weigh boats, dried for 24 hours at 60 °C, and weighed to the nearest mg.

$$\text{Survival} = \frac{\text{Number of Recovered Organisms}}{\text{Number of Exposed Organisms}} \quad (4-2)$$

Chironomus riparius

Midges were removed from the test chambers by sieving the sediments through a 0.6-micron stainless steel sieve. Mortality was recorded as the total number of organisms exposed minus number exposed (Equation 4-1). Recovered organisms were placed in plastic weigh boats, dried for 24 hours at 60 °C, and weighed to the nearest mg.

Daphnia magna

Daphnids were removed from the test chambers by pipetting the adults with wide-bore pipette. Mortality was recorded as the total number of organisms exposed minus number exposed (Equation 4-1). Neonates were recovered by siphoning off the

overlay water and daphnids and filtering through a plankton cup. Neonates were rinsed into a small beaker with dilution water and counted.

Water Chemistry

Standard water chemistry parameters on the overlaying water were measured and recorded. Water samples for metals analysis were also taken. Sediments from the test were oven dried and stored in plastic ziplock bags for later metals analysis.

Statistical Analysis

Survival data were transformed using the square-root transformation and tested for normality using a Shapiro Wilks test. These data were also tested for homogeneity of variance using Bartley's test (Gulley et al. 1991), and Duncan's Multiple Range test under the general linear models procedure was used to compare differences between treatments and the control (SAS Institute Inc. 1989).

Growth or reproduction data generated from Chironomus, Hyalella, and Daphnia data were checked for normality using a Shapiro-Wilks test. The data were also tested for homogeneity of variance using Bartley's test (Gulley et al. 1991), and Duncan's Multiple Range test under the general linear models procedure was used to compare differences between treatments and the control (SAS Institute Inc. 1989).

The length of the surviving clams was measured in microns under a dissecting stereomicroscope and was used as a measure of growth for the organisms. While in stricter terms growth would be considered to be the initial length minus the final length, it was not for these experiments. I wanted to do the statistical analysis the same way it is done for other sediment organisms (Chironomus, Hyalella, and D. magna) where

growth is considered to be analogous to the final mass of the organism. That is, the initial mass the of organisms is assumed to have little variation. This assumption holds true for juvenile Asiatic clams with respect to length. I measured 30, 24-hour old clams using an optical micrometer and the average length was 180 microns with a standard error of 1.7. These data were also tested for homogeneity of variance using Bartley's test (Gulley et al. 1991), and Duncan's Multiple Range test under the general linear models procedure was used to compare differences between treatments and the control (SAS Institute Inc. 1989).

RESULTS AND DISCUSSION

Sediment Dosing

The total non-residual fraction (TNR) of copper measured at the end of the 10-day test periods was very close to the nominal concentrations dosed at the beginning of the tests (Figures 4-5 and 4-6). The data fit the linear regression well with an r-value of 0.995 for the test with *Hyaella* and *Corbicula* (Figure 4-5). The data for *Chironomus* and *D. magna* fit the linear regression as well with an r-value of 0.998, however the measured concentration tended to fall below the nominal concentration at the higher concentrations (Figure 4-6). These data indicate that the method of dosing the sediments was sufficient for providing the gradient of concentrations needed to test the method.

Water Column Copper Concentrations

Water column copper concentrations were measured as total recoverable copper. In both tests there was an initial pulse of copper into the water column which declined soon after initiation of the test and remained low thereafter (Figures 4-7 and 4-8). In the *Corbicula* and *Hyaella* test the initial pulse was sustained longest in the 200-ppm concentration, followed by the 400-ppm concentration (Figure 4-7). It is believed that the water column copper in 200-ppm level was due to bioturbation caused by the movement of *Hyaella* in the test chambers (Table 4-3). Mean total copper concentration for this concentration was 31.8 ppb. In the *Chironomus* and *Daphnia* test the initial pulse had subsided prior to the initiation of the test (Figure 4-8) and except for the 1000 ppm concentration, mean copper levels for the test did not

exceed 16.67 ppb (Table 4-4).

Survival Data

Corbicula fluminea

Survival of the control juveniles was high except for one replicate in which there were two surviving (Table 4-5). There was no indication as to the reason this replicate did not survive. When taking into account the high mortality in this one replicate, control survival was still 70% (Table 4-5). If this replicated is dropped because of the anomalous mortality the control survival increased to $91.7 \pm 1.77\%$. Survival was significantly impaired at all toxicant concentrations when compared to the control using Duncan's Multiple Range test to compare the means. The organisms showed a dose response to the copper dosed sediments (Table 4-5) with an LOEC at the 25 ppm level. The NOEC for these data could not be generated because all concentrations were significantly different from the controls, but would be between the 6.8 ppm background concentration and the 26.2 ppm (25 nominal).

In 48-hr acute exposures of the native mussel juvenile *Actinonaias pectorosa* to copper, Jacobson et al. (1990) found acute toxicity to occur between 51-53 ppb copper. While these data represent another organism, the relative sensitivities of these lifestages are considered similar. Data generated from the flow-through sediment bioassays indicate toxicity well below the acute values for water only exposures, except in the two highest concentrations. These concentrations (200 and 400 ppm) showed acutely toxic water column levels for the first 48-96 hrs (Figure 4-6).

Hyalella azteca

Control survival in the Hyalella test was only 65% in the ten-day exposure to copper dosed sediment (Table 4-6). The NOEC for survival was 100 ppm and the LOEC for survival was 200 ppm. There was much variability in these data with survival at the lowest concentration ranging from 50-90%. Under the assumptions used in these tests, survival=recovered live organisms, which may not be the case here. Recovery of these organisms at this life stage was difficult due to their small size. Normally these tests run 28 days (Cairns et al. 1984, Nebeker and Miller 1988); however, I wanted to compare all of these organisms in 10-day studies. In order to use this organism I recommend continuing using them in 28-day bioassays.

Chironomus riparius

Chironomus riparius survival data showed a relatively steep dose response (Table 4-7). Control survival was 87.5% after the 10-day exposure. The NOEC for these data was 125 ppm and the LOEC was at 250 ppm. All of the organisms died at the 500 and 1000 ppm concentrations. These values are well below the 10-day LC50 of 857 ppm reported by Cairns et al. (1984) for 18-20 day old Chironomus sp. (presumed to be Chironomus tentans) in static tests. These differences may be species dependant but are more likely due to differences in sediment characteristics. The Soap Creek Pond sediment used in this paper contained 3% total organic carbon as compared to the 0.43% in Clinch River sediment. Organic carbon plays a role in mitigating the toxicity of metals in sediment and soils (Gonzalez 1992).

Daphnia magna

Daphnia survival to these sediments was more sensitive than any of the other

organisms tested except for *Corbicula* (Tables 4-5 and 4-8). There was 100% control survival and a steep dose response (Table 4-8). The NOEC for survival occurred at 62.5 ppm and the 125 ppm was the LOEC. There was a 100% kill at the 500 and 1000 ppm concentrations. These values are well below the 10-day LC50 of 681 ppm reported by Cairns et al. (1984) for 5-day old daphnids exposed in static bioassays. These differences are more likely due to differences in sediment characteristics. The Soap Creek Pond sediment used in this paper contained 3% total organic carbon as compared to the 0.43% in Clinch River sediment. Organic carbon plays a role in mitigating the toxicity of metals in sediment and soils (Landrum and Robbins 1990, Gonzalez 1992).

Growth Data

Corbicula fluminea

Surviving juveniles exposed to all levels of copper-spiked sediments showed significant reductions in growth when compared to the controls (Table 4-9). Growth was significantly impaired at all toxicant concentrations when compared to the controls using Duncan's Multiple Range test to compare the means. The organisms showed a dose response to the copper dosed sediments (Table 4-9) with an LOEC at the 25 ppm level. The NOEC for these data could not be generated for these data because all concentrations were impaired, but was between the 6.8 ppm background concentration and the 26.2 ppm (25 nominal). Control organisms grew from an average of 180 microns to 287.9 microns in 10 days. This is a 59.9% increase in length over the 10-day period, and when considered, at this lifestage, juveniles grow rapidly

enough to be useful test organisms. The minimum measurable difference in shell length was 20 microns because of the magnification of the microscope used. This translates in the ability to measure differences of 11% in the smaller 24-hour juveniles and 7% in the 10-day old control animals. These limits were suitable for these experiments where the average control animal grew close to 60%. We should have been able to detect over 8 distinct levels of growth in individual juveniles.

Hyalella azteca

Growth in *Hyalella* was impaired at the lower concentrations (25 and 50 ppm); however, they were not impaired at the higher concentrations (Table 4-10). At those concentrations where survival was significant (200-400 ppm) the mean mass of the organisms was almost equal to or greater than that of the controls. It is possible that this is due to an increased availability of resources with increased mortality. This may also account for the lack of significance at the 100 ppm level.

Chironomus riparius

The LOEC for growth in the midge was at the 125 ppm concentration (Table 4-11). The NOEC for growth in these organisms occurred at 62.5 ppm. At the 125 ppm concentration the mean aqueous total recoverable copper concentration was 17.9 ppb with a range in values from 54.93 ppb to 2.71 ppb over the 10-day exposure period. Williams et al. (1986) reported a 96-hour LC50 of 13 ppm for *riparius* to cadmium. A 12-day LC50 of >529 ppb was reported by Nebeker et al. (1984b) for *chironomus tentans*. These additional data show that *riparius* was more sensitive in these flow-through bioassays than in other reported literature.

Daphnia magna

Daphnia reproduction was as sensitive as midge growth in these studies (Tables 4-11 and 4-12). The NOEC for reproduction was 62.5 ppm and the LOEC was at 125 ppm copper. At the 125 ppm concentration the mean aqueous total recoverable copper concentration was 17.9 ppb with a range in values from 54.93 ppb to 2.71 ppb over the 10-day exposure period. These values are near the chronic value of 17.5 ppb for *Daphnia magna* in a 21-day life cycle test (USEPA 1985), but in a much shorter period.

CONCLUSIONS

Juvenile Corbicula have many of the characteristics which make it an ideal sediment testing organism. They are easy to collect from gravid adults taken from the field and easy to manipulate in the laboratory. This introduced exotic species has a nearly cosmopolitan North American distribution and is considered to be a nuisance, requiring no permits to collect or remove. Their biennial spawning periodicity can provide unlimited juveniles nearly all summer. As benthic organisms, juveniles are closely associated with the sediments, actively burrowing and feeding in the substrate. They exhibit dramatic growth during a 10-day exposure period and have shown to be very sensitive to copper in acute and chronic exposures.

With respect to survival, juvenile corbicula were shown to be more sensitive to copper than any other species tested with and LOEC of 25 ppm as compared to 125, 200, and 250 for *Daphnia magna*, *Hyalella azteca* and *Chironomus riparius* respectively.

Corbicula has shown to be equally of more sensitive than these organisms with respect to estimating chronic endpoints of copper with an LOEC of 25 ppm as compared to the 25 ppm for *Hyalella* and 125 ppm for both *Daphnia* and *Chironomus*. However, *Hyalella* did exhibit the highest control mortality and had some scattered mortality.

Flow-through technology increases the environmental realism of testing sediments in lotic systems. It allows us to segregate effects associated with the water-

column by continually replacing it. In aquatic systems, especially lotic systems, contaminants in the sediment leach out into the overlaying water. Flow-through technology gives us a method of examining how this leaching process effects both the overlaying water and the sediment. Even though total recoverable copper in the water column was below 20 ppb throughout the testing period, effects on survival and growth were detected to less than 25 ppm in the total non-residual fraction of the sediment.

LITERATURE CITED

- Allen, H. E., Fu, G., Boothman, W., DiToro, D. M. and Mahony, J. D. 1991. Draft analytical method for determination of acid volatile sulfide in sediment. - United States Environmental Protection Agency, Washington, DC.
- Belanger, S. E., Farris, J. L., Cherry, D. S. and Cairns, J., Jr. 1985. Sediment preference of the freshwater Asiatic clam, *Corbicula fluminea*. - *The Nautilus* 99: 66-73.
- Belanger, S. E., Farris, J. L., Cherry, D. S. and Cairns, J., Jr. 1990. Validation of *Corbicula fluminea* growth reductions induced by copper in artificial streams and river systems. - *Can. J. Fish. Aquat. Sci.* 47: 904-914.
- Burton, G. A., Jr. 1991. Assessing the toxicity of freshwater sediments. - *Environ. Tox. Chem.* 10: 1585-1627.
- Burton, G. A., Jr. 1992. Assessing contaminated aquatic sediments. - *Environ. Sci. Technol.* 26: 1862-1863.
- Cairns, M. A., Nebeker, A. V., Gakstatter, J. H. and Griffis, W. L. 1984. Toxicity of copper-spiked sediments to freshwater invertebrates. - *Environ. Tox. Chem.* 3: 435-445.
- Cherry, D. S., Rodgers, J. H., Jr., Graney, R. L. and Cairns, J., Jr. (eds.). 1980. Dynamics and control of the Asiatic clam in the New River, Virginia. - Virginia Water Resources Research Center, Blacksburg.
- Chester, R., Kudoja, W. M., Thomas, A. and Towner, J. 1985. Pollution reconnaissance in stream sediments using non-residual trace metals. - *Environ. Pollut. Ser. B.* 10: 213-238.
- Di Toro, D. M., Mahony, J. D., Hansen, D. J., Scott, K. J., Carlson, A. R. and Ankley, G. T. 1992. Acid volatile sulfide predicts the acute toxicity of cadmium and nickel in sediments. - *Environ. Sci. Technol.* 26: 96-101.
- Doherty, F. G. 1986. A multidisciplinary study of the Asiatic clam, *Corbicula fluminea*, from the New River, Virginia. - Ph.D. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg.
- Gonzalez, A. M. 1992. Heavy metal sorption in anoxic sediments: an experimental study of sediment organic carbon as a metal-binding phase under anoxic conditions. - MS Thesis, Manhattan College, Riverdale, NY.
- Graney, R. L., Jr., Cherry, D. S. and Cairns, J., Jr. 1983. Heavy metal indicator potential

of the Asiatic clam *Corbicula fluminea* in artificial stream systems. - *Hydrobiologia* 102: 81-88.

Gulley, D. D., Boelter, A. M. and Bergman, H. L. 1991. Toxstat - version 3.3. - Fish Physiology and Toxicology Laboratory, Department of Zoology and Physiology. University of Wyoming. Laramie, Wyoming.

Huang, P. M. 1993. An overview of dynamics and biotoxicity of metals in freshwater environment. - *Water Poll. Res. J. Canada* 28: 1-5.

Ingersoll, C. G. and Nelson, M. K. 1990. Testing sediment toxicity with *Hyaella azteca* (Amphipoda) and *Chironomus riparius* (Diptera). - In: Landis, W. G. and Van Der Schalie, W. H. (eds.), *Aquatic Toxicology and Risk Assessment*, Vol. 13. American Society for Testing and Materials, Philadelphia, pp. 93-109.

Jacobson, P. J. 1990. Sensitivity of early life stages of freshwater mussels (Bivalvia: Unionidae) to copper. - ms Thesis, Virginia Polytechnic Institute and State University.

Kadeg, R. D. and Pavlou, S. P. 1987. Reconnaissance field study for verification of equilibrium partitioning: nonpolar hydrophobic organic chemicals. - Office of Water Regulations and Standards, Criteria and Standards Division, United States Environmental Protection Agency, Washington.

Landrum, P. F. and Robbins, J. A. 1990. Bioavailability of sediment-associated contaminants to benthic invertebrates. - In: Baudo, R., Geisy, J. P. and Muntau, H. (eds.), *Sediments: chemistry and toxicity of in-place pollutants*. Lewis Publishers, inc, Ann Arbor, pp. 237-263.

Nebeker, A. V. and Miller, C. E. 1988. Use of the amphipod crustacean *Hyaella azteca* in freshwater and estuarine sediment toxicity tests. - *Environ. Tox. Chem.* 7: 1027-1033.

Nebeker, A. V., Cairns, M. A., Gakstatter, J. H., Malueg, K. W., Schuytema, G. S. and Krawczyk, D. F. 1984a. Biological methods for determining toxicity of contaminated freshwater sediments to invertebrates. - *Environ. Tox. Chem.* 3: 617-630.

Nebeker, A. V., Cairns, M. A. and Wise, C. M. 1984b. Relative sensitivity of *Chironomus tentans* life stages to copper. - *Environ. Tox. Chem.* 3: 151-158.

Nebeker, A. V., Onjukka, S. T., Cairns, M. A. and Krawczyk, D. F. 1986. Survival of *Daphnia magna* and *Hyaella azteca* in cadmium-spiked water and sediment. - *Environ. Tox. Chem.* 5: 933-938.

Nebeker, A. V., Onjukka, S. T. and Cairns, M. A. 1988. Chronic effects of contaminated

sediment on *Daphnia magna* and *Chironomus tentans*. - Bull. Environ. Contam. Toxicol. 41: 574-581.

Reid, R. G., McMahon, D. O., Foighil, D. O. and Finnigan, R. 1992. Anterior inhalent currents and pedal feeding in bivalves. - The Veliger 35: 93-104.

Rodgers, J. H., Jr., Cherry, D. S., Graney, R. L., Dickson, K. L. and Cairns, J., Jr. 1980. Comparison of heavy metal interactions in acute and artificial stream bioassay techniques for the Asiatic clam *Corbicula fluminea*. - In: Eaton, J. G., Parrish, P. R. and Hendricks, A. C. (eds.), Aquatic Toxicology. American Society for Testing and Materials, Philadelphia, pp. 266-280.

Salomons, W., de Rooij, N. M., Kerdijk, H. and Bril, J. 1987. Sediments as a source for contaminants? - Hydrobiologia 149: 13-30.

SAS Institute Inc. 1989. SAS/STAT users guide, version 6, 4th ed., Vol. 2. - SAS Institute Inc., Cary, NC.

Schuytema, G. S., Nelson, P. O., Malueg, K. W., Nebeker, A. V., Krawczyk, D. F., Ratcliff, A. K. and Gakstatter, J. H. 1984. Toxicity of cadmium in water and sediment slurries to *Daphnia magna*. - Environ. Tox. Chem. 3: 293-308.

USEPA. 1985. Ambient water quality criteria for copper - 1984. - United States Environmental Protection Agency, Washington.

USEPA. 1992. Proceedings, Tiered testing issues for freshwater and marine sediments. - United States Environmental Protection Agency, Office of Water, Office of Science and Technology, and Office of Research and Development, Washington.

USEPA Sediment Oversight Technical Committee (ed.). 1992. Sediment Classification Methods Compendium. - United States Environmental Protection Agency, Office of Science and Technology, Washington.

Webber, C. I. (ed.). 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 4th ed. - United States Environmental Protection Agency, Cincinnati.

Williams, K. A., Green, D. W. J., Pascoe, D. and Gower, D. E. 1986. The acute toxicity of cadmium to different larval stages of *Chironomus riparius* (Diptera: Chironomidae) and its ecological significance for population regulation. - Oecologia 70: 362-366.

Word, Q. J., Ward, J. A., Franklin, L. M., Cullin, V. I. and Kiesser, S. L. 1987. Evaluation of the equilibrium partitioning theory for estimating the toxicity of the nonpolar organic compound DDT to the sediment dwelling organism *Rhepoxynius*

abronius. - Office of Water Regulations and Standards, Criteria and Standards Division, United States Environmental Protection Agency, Washington.

Yeager, M. M., Cherry, D. S. and Neves, R. J. 1994. Feeding and burrowing behaviors of juvenile rainbow mussels, *Villosa iris* (Bivalvia: Unionidae). - J. N. Am. Benthol. Soc. 13: 217-222.

Zumwalt, D. C., Dwyer, F. J., Greer, I. E. and Ingersoll, C. G. 1993. A water-renewal system that accurately delivers small volumes of water to exposure chambers. - Environ. Tox. Chem. 13: 1311-1314.

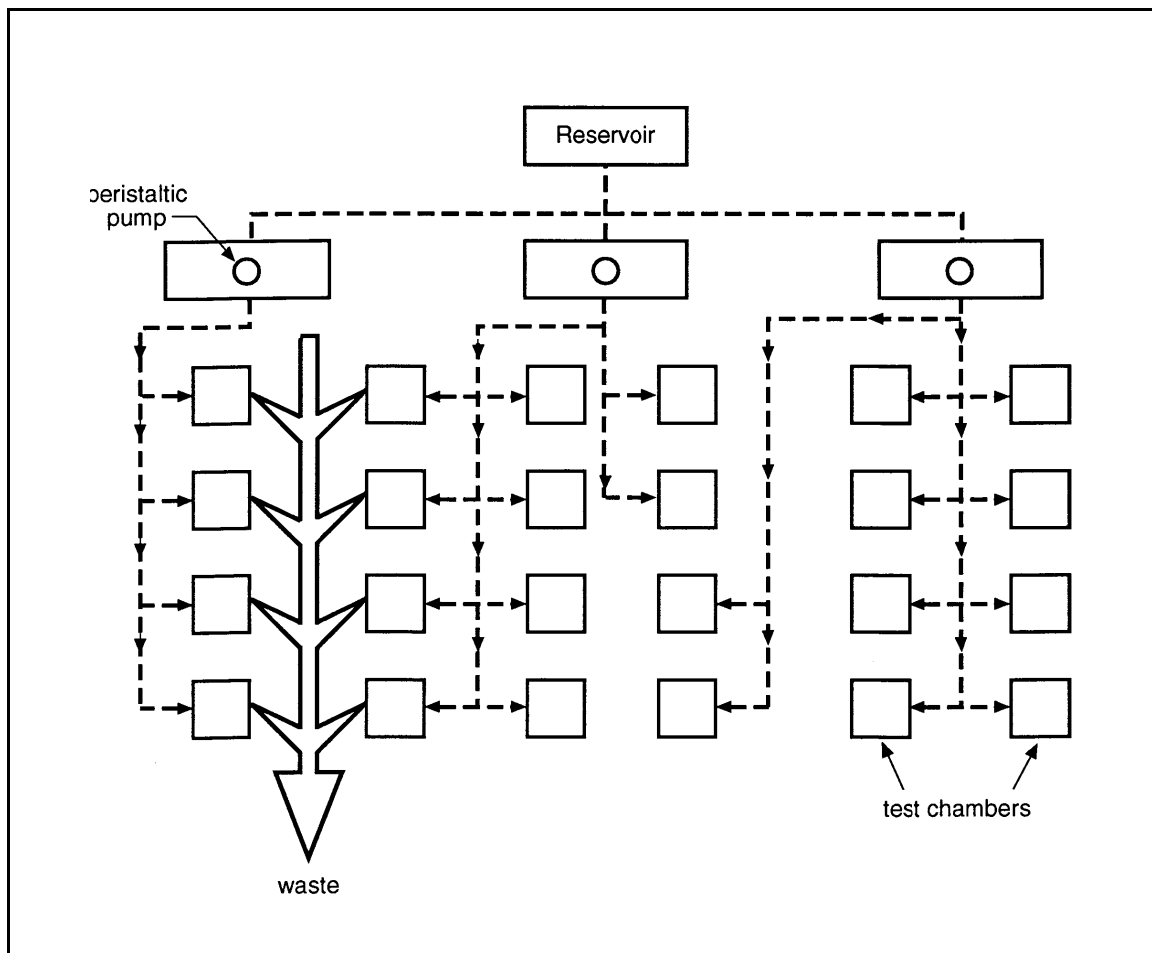


Figure 4-1 Schematic of the flow-through system. Peristaltic pumps deliver dilution water from the reservoir to each test chamber. Waste overflows the edge of the test chamber and is drained away.

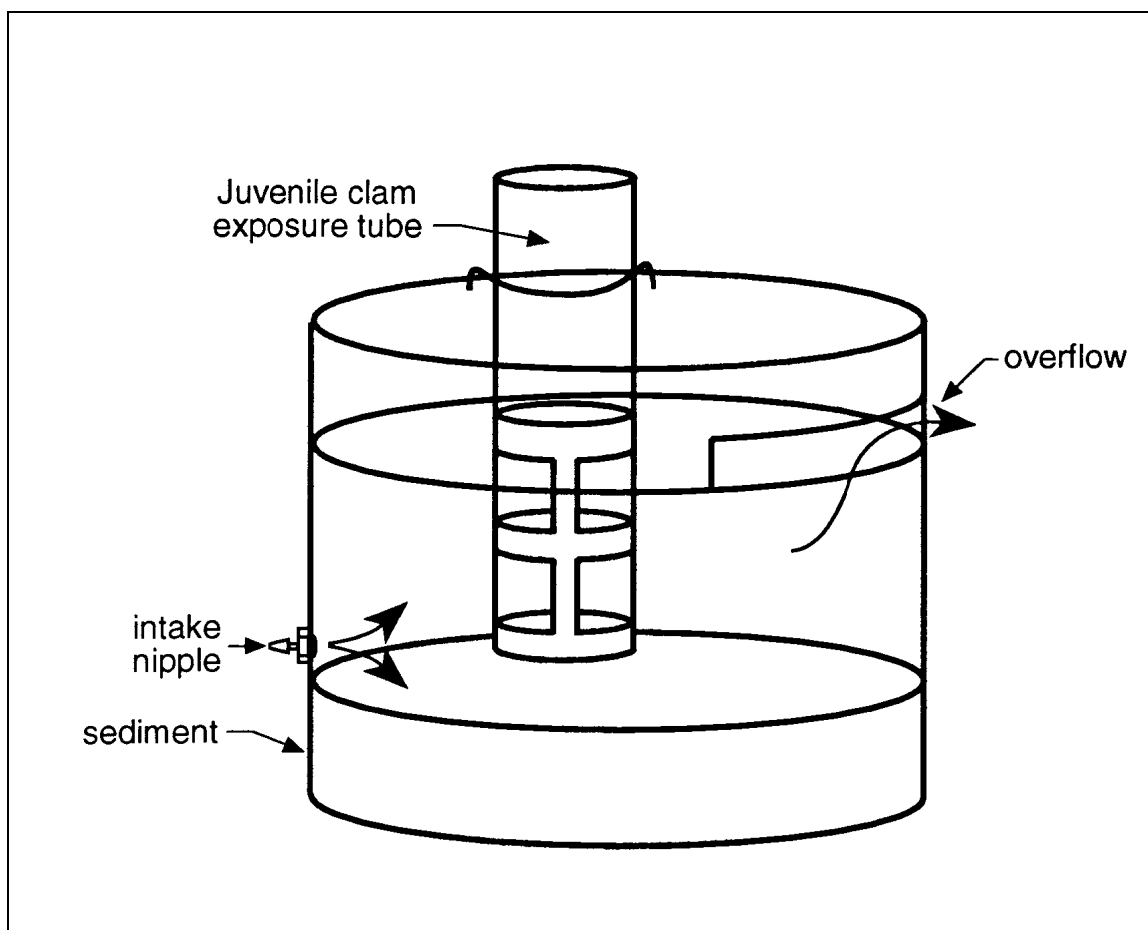


Figure 4-2 Flow-through sediment test chamber with a Juvenile Clam Exposure Tube. Dilution water enters through the intake nipple and exits through the screened overflow. Clams are placed in the screened exposure tube.

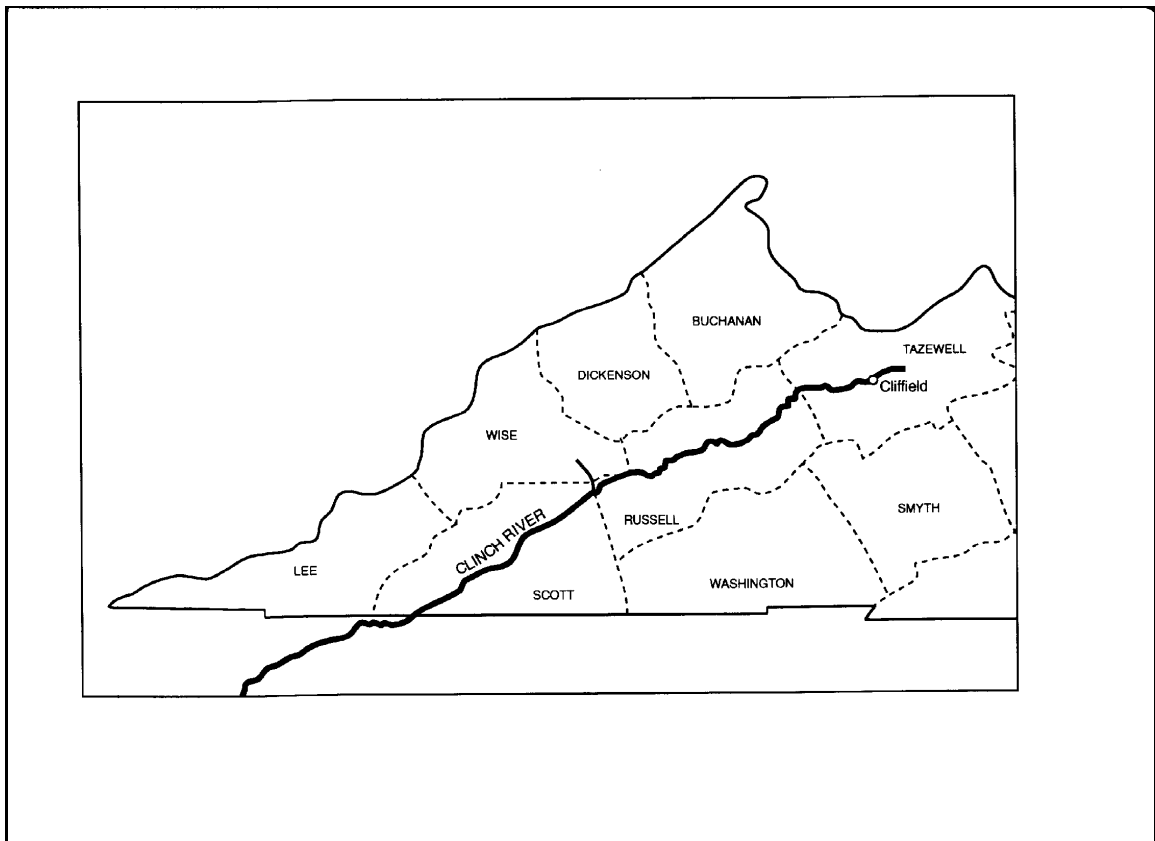


Figure 4-3 Map of southwestern Virginia showing the Clinch River flowing southwest from Tazewell County and the location of the Clifffield site. Sediments collected from this site were dosed with copper chloride to test juvenile clams.

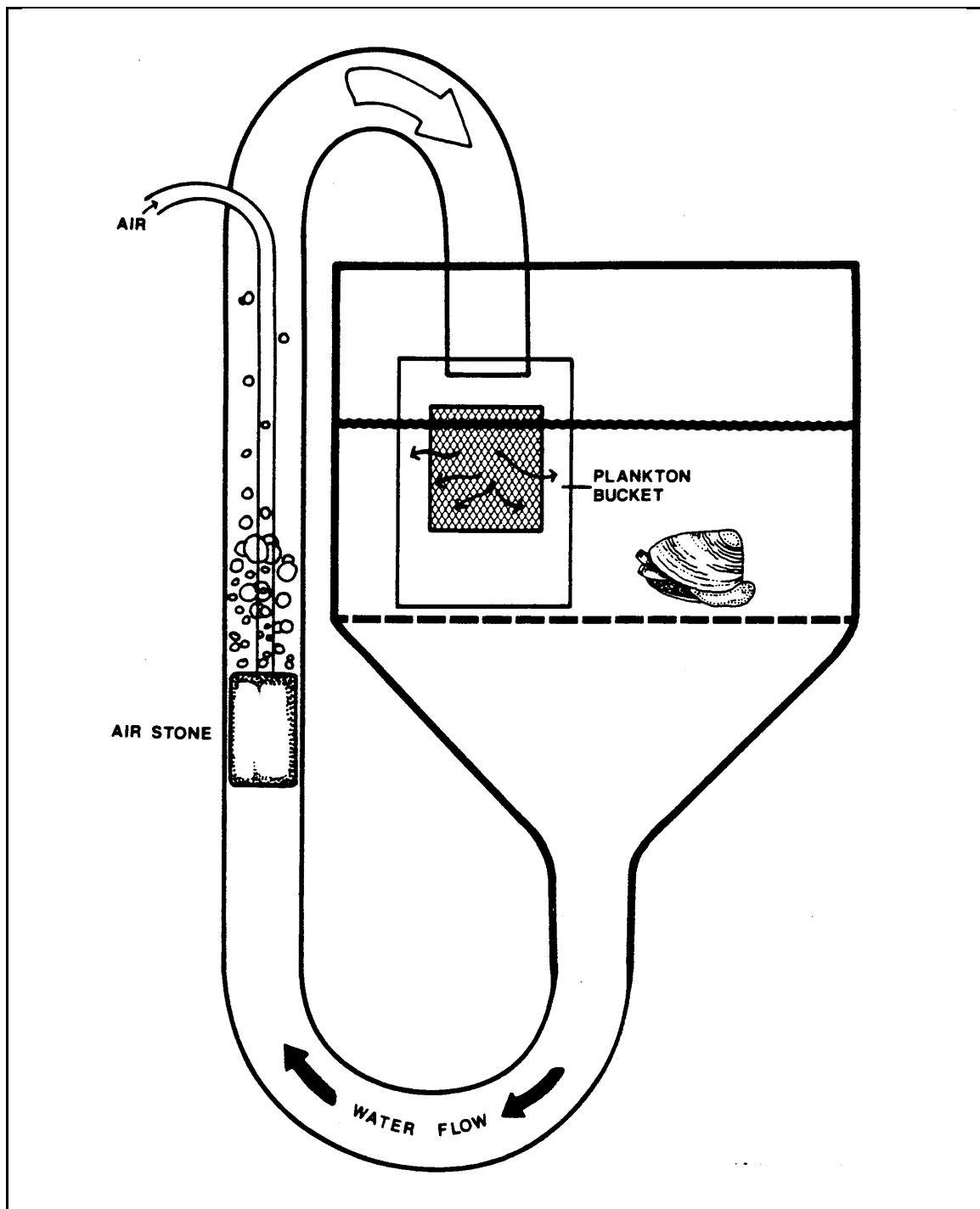


Figure 4-4 Juvenile *Corbicula* collection chamber - a modified downweller designed to entrain juvenile clams naturally released from gravid adults. Figure from Doherty et al. (1986).

Sediment Copper Concentration

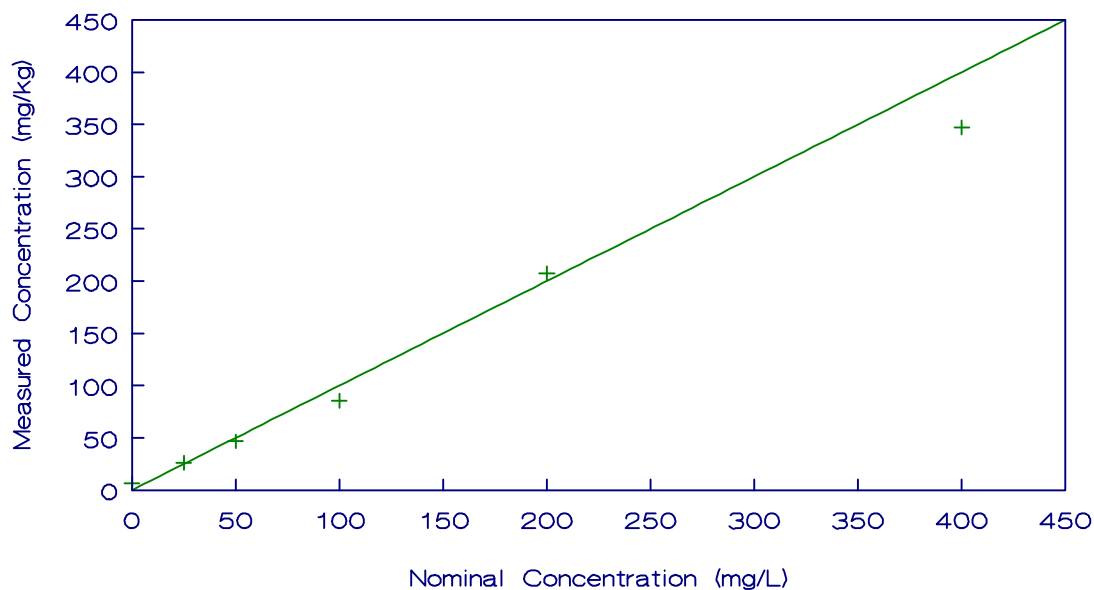


Figure 4-5. Measured sediment copper concentrations (mg cu/kg dry sediment) are shown against nominal concentrations (mg cu/L wet sediment) used in dosing Clinch River sediment. Error bars are not visible in this graph.

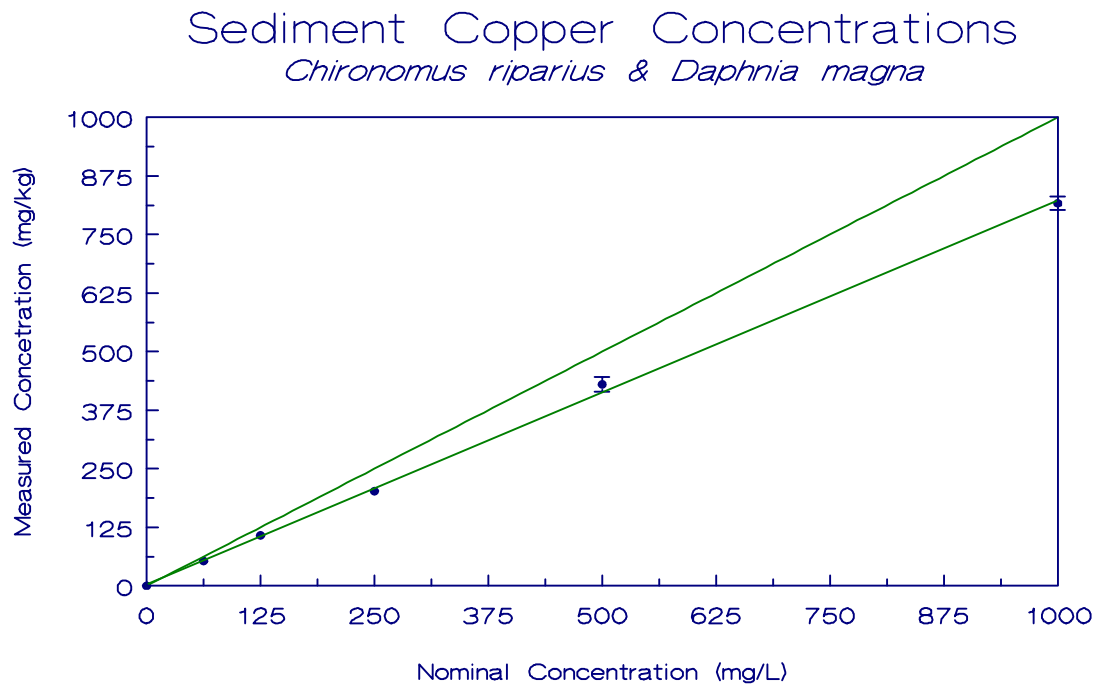


Figure 4-6. Measured sediment copper concentrations (mg cu/kg dry sediment) are shown against nominal concentrations (mg cu/L wet sediment) used in dosing Clinch River sediment. Error bars are not visible in this graph.

Total Recoverable Copper

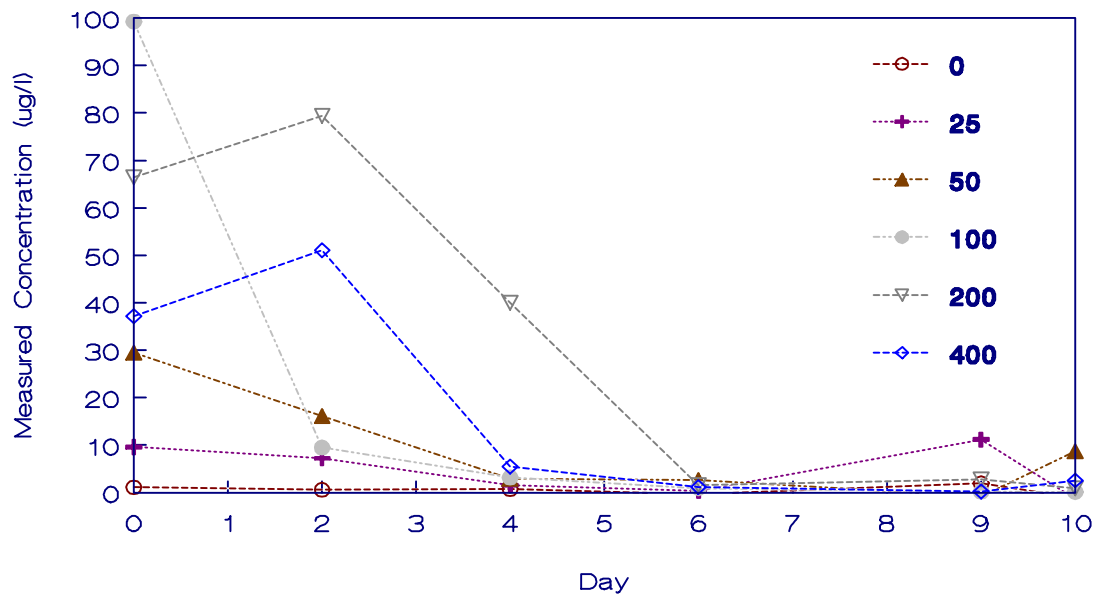


Figure 4-7. Total recoverable copper concentrations ($\mu\text{g/L}$) for the Corbicula and Hyalella test are shown for each nominal concentration over test duration.

Sediment Copper Concentrations

Chironomus riparius & *Daphnia magna*

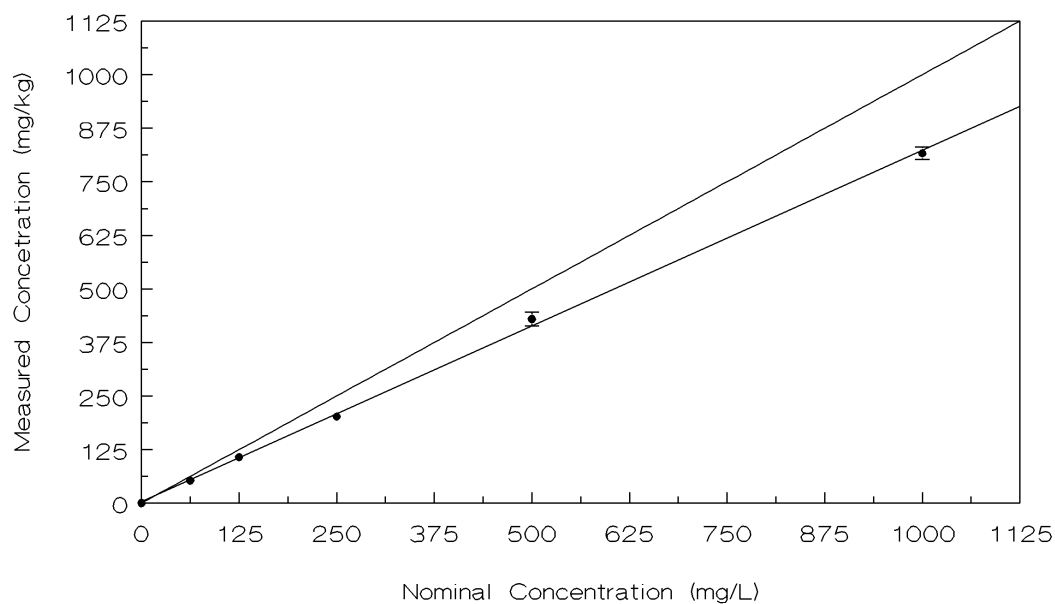


Figure 4-8. Total recoverable copper concentrations ($\mu\text{g/L}$) for the *Chironomus* and *Daphnia* test are shown for each nominal concentration over the test duration.

Table 4-1. Summary table of test conditions for conducting flow-through sediment bioassays with naturally released juvenile Asiatic clams (*Corbicula fluminea*).

Test Type	Flow-through
Test Temperature	20°C ±2
Light Quality	Ambient Laboratory Light
Light Intensity	50-100 ft c
Aeration	None
Test chamber size	≈ 1 liter
Juvenile Clam Exposure Chamber	2.54 cm screened pvc tube
Sediment Water Ratio	1:4
Replicates per Treatment	4
Organisms per Replicate	10 (20 for <i>Corbicula</i>)
Test Endpoints:	
<i>Chironomus riparius</i>	Survival and Growth
<i>Corbicula fluminea</i>	Survival and Growth
<i>Daphnia magna</i>	Survival and Reproduction
<i>Hyalella azteca</i>	Survival and Growth
Acceptable Control Survival	
<i>Chironomus riparius</i>	75%
<i>Corbicula fluminea</i>	70%
<i>Daphnia magna</i>	80%
<i>Hyalella azteca</i>	75%

Table 4-2. Sediment characterization of sediments dosed for flow-through sediment bioassays using juvenile Asiatic clams in 10-day exposures.

Parameter	Mean Value	Standard Error	n
Sediment pH (su)	7.69	na	1
Moisture (%)	22.42	0.71	3
Loss on Ignition (%)	1.41	0.13	3
Total Organic Carbon (%)	0.432	0.023	3
Cationic Exchange Capacity (meq/100 g)	4.327	0.408	3
Particle Size Distribution			
Sand (%)	69.452	1.720	3
Silt (%)	31.410	1.994	3
Clay (%)	-0.498	0.308	3

Table 4-3. Mean Total Recoverable Copper concentrations in the water column of a flow through sediment test using *Corbicula fluminea* and the amphipod *Hyalella azteca*.

Nominal Sediment Concentration (mg/kg)	Total Recoverable Copper (ppb)	Standard Error	Range
0	<1.0	0.42	<1.0 - 1.95
25	4.97	2.14	<1.0 - 9.60
50	9.87	4.61	<1.0 - 29.46
100	18.87	16.14	<1.0 - 99.26
200	31.86	14.42	0.84 - 79.43
400	16.29	9.01	<1.0 - 51.05

Table 4-4. Mean Total Recoverable Copper concentrations in the water column of a flow-through sediment test using *Chironomus riparius* and *Daphnia magna* .

Nominal Sediment Concentration (mg/kg)	Total Recoverable Copper (ppb)	Standard Error	Range
0	4.16	0.94	2.11-6.78
62.5	16.48	5.57	2.42-40.81
125	17.90	7.82	2.71-54.93
250	13.37	4.54	3.87-34.01
500	16.67	5.33	7.01-41.60
1000	38.05	16.02	7.93-113.98

Table 4-5. Survival of juvenile Asiatic clams to Clinch River sediments spiked with copper chloride in a flow through bioassay.

Nominal Sediment Concentration (mgCu/kgSed.)	Measured Sediment Concentration (mgCu/kgSed.)	Survival (%) ^{§¥}	Recovery (%)
0	6.8	70.0 (20.1)	97.5 (2.5)
25	26.2	33.6 (17.8)*	96.3 (3.8)
50	46.7	28.8 (15.2)*	100.0 (0)
100	85.7	8.0 (6.3)*	93.8 (2.4)
200	207.2	2.3 (1.3)*	97.5 (2.5)
400	346.9	1.7 (1.7)*	100 (0)
Overall Mean Recovery:			97.5 (0.95)

[§] Numbers in parentheses represent 1 standard Error.

[¥] Numbers followed by an asterisk represent values significantly different from the control using Duncan's Multiple Range test at $\alpha=0.05$ level.

Table 4-6. Survival of *Hyaella azteca* exposed to Clinch River sediments spiked with copper chloride in a flow through bioassay.

Nominal Sediment Concentration (mgCu/kgSed.)	Measured Sediment Concentration (mgCu/kgSed.)	Survival (%) ^{§¥}	Range (%)
0	6.8	65.0 (2.9)	60-70
25	26.2	67.5 (8.5)	50-90
50	46.7	47.5 (7.5)	40-70
100	85.7	50 (14.1)	10-70
200	207.2	37.5 (4.8)*	30-50
400	346.9	10 (4.1)*	0-20

§

Numbers in parentheses represent 1 standard Error.

¥

Numbers followed by an asterisk represent values significantly different from the control using Duncan's Multiple Range test at $\alpha=0.05$ level.

Table 4-7. Survival of *Chironomus riparius* to Clinch River sediments spiked with copper chloride in a flow-through bioassay.

Nominal Sediment Concentration (mgCu/kgSed.)	Measured Sediment Concentration (mgCu/kgSed.)	Survival (%) ^{§¥}	Range (%)
0	0.43	87.5 (9.5)	60-100
62.5	52.90	90.0 (4.1)	80-100
125	107.74	82.5 (4.8)	70-90
250	202.25	45.0 (9.6)*	20-60
500	429.86	0 (0)*	---
1000	816.36	0 (0)*	---

§

Numbers in parentheses represent 1 standard Error.

¥

Numbers followed by an asterisk represent values significantly different from the control using Duncan's Multiple Range test at $\alpha=0.05$ level.

Table 4-8. Survival of *Daphnia magna* exposed to Clinch River sediments spiked with copper chloride in a flow-through bioassay.

Nominal Sediment Concentration (mgCu/kgSed.)	Measured Sediment Concentration (mgCu/kgSed.)	Survival (%) ^{§¥}	Range (%)
0	0.43	100.0 (0)	100-100
62.5	52.90	92.5 (4.8)	80-100
125	107.74	42.5 (11.1)*	10-60
250	202.25	2.5 (2.5)*	0-10
500	429.86	0 (0)	---
1000	816.36	0 (0)	---

§

Numbers in parentheses represent 1 standard Error.

¥

Numbers followed by an asterisk represent values significantly different from the control using Duncan's Multiple Range test at $\alpha=0.05$ level.

Table 4-9. Growth of juvenile Asiatic clams to Clinch River sediments spiked with copper chloride in a flow through bioassay.

Nominal Sediment Concentration (mgCu/kgSed.)	Measured Sediment Concentration (mgCu/kgSed.)	Mean Length (µm) ^{§¶}	Range (µm)
0	6.8	287.9 (5.0)	220 - 340
25	26.2	263.4 (12.1)*	220 - 300
50	46.7	249.9 (6.4)*	240 - 280
100	85.7	254.0 (6.0)*	240 - 260
200	207.2	250 (-)*	240 - 260
400	346.9	240 (-)*	na

§

Numbers in parentheses represent 1 standard Error.

¶

Numbers followed by an asterisk represent values significantly different from the control using Duncan's Multiple Range test at $\alpha=0.05$ level.

Table 4-10. Growth of *Hyaella azteca* exposed to Clinch River sediments spiked with copper chloride in a flow-through bioassay.

Nominal Sediment Concentration (mgCu/kgSed.)	Measured Sediment Concentration (mgCu/kgSed.)	Mean Mass (mg) ^{§¥}	Range (mg)
0	6.8	0.133 (0.016)	0.028-0.373
25	26.2	0.071 (0.007)*	0.031-0.147
50	46.7	0.058 (0.007)*	0.022-0.132
100	85.7	0.086 (0.013)	0.038-0.246
200	207.2	0.132 (0.033)	0.010-0.178
400	346.9	0.244 (0.244)	---

§ Numbers in parentheses represent 1 standard Error.
¥ Numbers followed by an asterisk represent values significantly different from the control using Duncan's Multiple Range test at $\alpha=0.05$ level.

Table 4-11. Growth of *Chironomus riparius* exposed to Clinch River sediments spiked with copper chloride in a flow-through bioassay.

Nominal Sediment Concentration (mgCu/kgSed.)	Measured Sediment Concentration (mgCu/kgSed.)	Mean Length (µg) ^{§¥}	Range (µg)
0	0.43	0.879 (0.033)	0.543-1.317
62.5	52.90	0.773 (0.065)	0.201-1.420
125	107.74	0.448 (0.031)*	0.176-0.881
250	202.25	0.349 (0.067)*	0.077-1.002
500	429.86	--- (---)	---
1000	816.36	--- (---)	---

§

Numbers in parentheses represent 1 standard Error.

¥

Numbers followed by an asterisk represent values significantly different from the control using Duncan's Multiple Range test at $\alpha=0.05$ level.

Table 4-12. Reproduction of *Daphnia magna* exposed to Clinch River sediments spiked with copper chloride in a flow-through bioassay.

Nominal Sediment Concentration (mgCu/kgSed.)	Measured Sediment Concentration (mgCu/kgSed.)	Mean Reproduction (number/day) ^{§¥}	Range (number/day)
0	0.43	34.25 (3.97)	26.3-43.4
62.5	52.90	25.05 (3.23)	16.4-31.0
125	107.74	3.675 (0.87)*	2.0-5.5
250	202.25	0 (0)	---
500	429.86	0 (0)	---
1000	816.36	0 (0)	---

§

Numbers in parentheses represent 1 standard Error.

¥

Numbers followed by an asterisk represent values significantly different from the control using Duncan's Multiple Range test at $\alpha=0.05$ level.

CURRICULUM VITA

Stuart R. Lynde

August, 1994

Birthdate and Place: October 8, 1964, Charlottesville, Virginia.

Marital Status: Married to Katherine L. Snell, April 2, 1988.

Children: William Joseph Harris, born December 21, 1981.
 Elizabeth Harrington Lynde, born February 7, 1992.

Home Address: 2503 Capistrano St.
 Blacksburg, Virginia 24060
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Education:

MS program in Zoology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 1991-present.

BS in Biological Science, Lynchburg College, Lynchburg VA, 1991.

Undergraduate Curriculum:

Cell Biology	Organic Chemistry
General Biology	Physics
General Chemistry	Physical Geology
General Ecology	Plant Systematics
Genetics	Survey of the Plant Kingdom
Invertebrate Zoology	Tropical Botany
Vertebrate Zoology	

Graduate Curriculum:

Biometry	Freshwater Ecology
Hazard Evaluation	Soil Chemistry
Aquatic Ecotoxicology	Aquatic Entomology
Aquatic Vascular Plants	

Awards:

Betty Chamberlain / Smith Mountain Lake Association, 1990. (\$500)
Honor Roll (QPA > 3.0), 1989-91. Two semesters.
Tuition Waiver, VPI & SU, Spring 1992. (\$700)
Tuition Waiver, VPI & SU, 1992. (\$1500)
Tuition Waiver, VPI & SU, 1993. (\$1500)

Professional Activities:

Researching Aquatic Ecotoxicologist, 1990, North Carolina Department of Transportation. Monitoring of water quality standards of the Nanthahala River.

Efficacy Evaluator, 1991, Celanese Fibers Corporation, Narrows, Virginia. Monitoring of chlorination and impact upon the Asiatic Clam (*Corbicula*) control.

Aquatic Biologist, 1991, Procter & Gamble Corporation, Cincinnati OH. Collection and transport of the Asiatic Clam (*Corbicula*).

Efficacy Evaluator, 1992, Celanese Fibers Corporation, Narrows, Virginia. Monitoring of chlorination and impact upon the Asiatic Clam (*Corbicula*) control.

Efficacy Evaluator, 1993, Celanese Fibers Corporation, Narrows, Virginia. Monitoring of chlorination and impact upon the Asiatic Clam (*Corbicula*) control.

Consulting Aquatic Ecotoxicologist, 1994, Sonoco Products Company, Downingtown, Pennsylvania. Benthic Macro invertebrate Analysis of East Branch, Brandywine Creek, Downingtown, Chester County Pennsylvania.

Data Collector as an Aquatic Ecotoxicologist, 1994, Army Corps of Engineers, Waterways Experimental Station, Vicksburg, Mississippi. Benthic Macroinvertebrate Assessment of the North Impact Area in the La Crosse River, Fort McCoy, Wisconsin.

Data Collector as an Aquatic Ecotoxicologist, 1994, Army Corps of Engineers, Waterways Experimental Station, Vicksburg, Mississippi. Benthic Macroinvertebrate Assessment of the Impact Area, Fort Bragg, Fayetteville, North Carolina.

Professional Societies:

Society of Environmental Toxicology and Chemistry
Society of Environmental Toxicology and Chemistry, Chesapeake and Potomac Regional Chapter
North American Benthological Society
Association of Southern Biologists
Virginia Academy of Science
American Chemical Society - Vice-president, 1989-1991, Lynchburg College Chapter of student affiliates.

Positions Held:

Graduate Research Assistant. Virginia Polytechnic Institute & State University, Blacksburg, Virginia. Summer 1992 - present. Lab Quality Assurance/Quality Control Officer. Responsibilities included developing and implementing all QA/QC guidelines associated with the laboratory and grant related work. This includes SOPs and QAPs as well as certification requirements for the state of Florida's Department of Environmental Regulation and the State of Virginia. These certifications allowed us to acquire over \$65,000 in permit funding to date.

Graduate Teaching Assistant. Virginia Polytechnic Institute & State University, Blacksburg, Virginia. 1991-92. Instructional include teaching laboratory sections in General Biology, preparing, administering, and grading laboratory exams, quizzes and exercises.

Graduate Research Assistant. Virginia Polytechnic Institute & State University, Blacksburg, Virginia. Summer 1991.

Laboratory Technician. Lynchburg College, Lynchburg Virginia. 1990. Duties included general lab maintenance and set-up of General Biology Lab.

Tutor. 1990-91. Instruction in General Biology and Chemistry to undergraduate majors and non-majors at two colleges.

Cash Vault Teller. Central Fidelity Banks Inc., Lynchburg, Virginia. 1986-1988. Duties included shipping and receiving of large currency transactions between corporate customers, branches and the Federal Reserve. Bonded to 2.5 million dollars.

Promotional Director / Announcer. WLVA Radio, Lynchburg, Virginia. 1986. Managed promotional "Passport to Riches" campaign, hosted evening radio program.

Department Manager. Taylor Brothers Inc., Lynchburg, Virginia. 1985-86. Duties included departmental purchasing of stock and inside sales.

Grants:

Co-Investigator: Cherry, D.S., S.R. Lynde, J.R. Bidwell, M.G. Dobbs, G.P. Schoenig, and A.S. Fenster. December 1993. Development of a study plan to test toxicity and environmental effects of Didecyldimethylammoniumchloride (DDAC) to non-target organisms for biofouling control. (\$108,000)

Published Abstracts and Presentations:

Lynde, S. R. and O. O. Stenroos. 1991. A Three Month Study of Fecal Coliform Levels in Lake Water from Three Areas of Smith Mountain Lake, Virginia. Virginia Journal of Science. Vol 42:2 Summer 1991. (Presentation)

Cherry, D.S., J.L. Farris, J.R. Bidwell, A. Mikailoff, M.M. Yeager, S.R. Lynde, R.L. Shema, J.W. McIntire. 1992. Environmental Effects of Molluscicide Application for Corbicula Control in a Nuclear Power Plant: A Two-Year Study. Abstracts of the 13th Annual Meeting of the Society of

Environmental Toxicology and Chemistry. Cincinnati, Ohio. (Presentation).

Lynde, S.R., K.J. Reubush, J.M. Roper, W.J. VanWart, J.R. Webster, and D.S. Cherry. 1993. The Use of Artificial Streams in Evaluating Leaf Degradation: a Comparison of Breakdown Rates Based on Thermal Addition. 54th Annual Meeting of the Association of Southeastern Biologists. Virginia Beach, Virginia. The ASB Bulletin. Vol 40:2 (Presentation).

Lynde, S.R., K.J. Reubush, J.M. Roper, W.J. VanWart, J.R. Webster, and D.S. Cherry. 1993. The Effects of Temperature and Macroinvertebrates on the breakdown of Boxelder (*Acer negundo*) Leaves in a Tributary of the New River, Virginia. Abstracts of 41st Annual Meeting of the North American Benthological Society. Calgary, Alberta. (Presentation).

Lynde, S.R., D.S. Cherry, A.L. Buikema, J.R. Lauth. 1993. Comparison of Invertebrate Impairment and Microbial Colonization of Leaves in River Sediment Exposed to Copper. Abstracts of the 14th Annual Meeting of the Society of Environmental Toxicology and Chemistry. Houston, Texas. (Poster Presentation).

Lynde, S.R. and D.S. Cherry. 1994. Testing Sediments with Juvenile Bivalves: a Methodology for Static and Flow-Through Bioassays. 42nd Annual Meeting of the North American Benthological Society. Orlando, Florida. (Invited Presentation).

Cherry, D.S., M.M. Yeager, S.R. Lynde, and N.D. Shepard. 1994. The Influence of Freshwater Mussels by Ammonia Toxicity from Population Crashes of Asiatic Clams. 42nd Annual Meeting of the North American Benthological Society. Orlando, Florida. (Invited Presentation).

Lynde, S.R. and D.S. Cherry. 1994. Development of a Mussel Larvae Testing Chamber to Evaluate Sediment Toxicity to Heavy Metals. Abstract of the 15th Annual Meeting of the Society of Environmental Toxicology and Chemistry. Denver, Colorado. (Poster Session).

J.L. Scheller, D.S. Cherry, M.M. Yeager, S.R. Lynde and N.D. Shepard. 1994. Water and Sediment Toxicity of Freshwater Mussels from Population Crashes of Asiatic Clams. Abstract of the 15th Annual Meeting of the Society of Environmental Toxicology and Chemistry. Denver, Colorado. (Poster Session).

D.S. Cherry, J.R. Bidwell, M.M. Yeager, M.G. Dobbs and S.R. Lynde. 1994. Comparative Efficacies of Oxidizing and Nonoxidizing Molluscicides at Different Temperatures. Abstract of the 15th Annual Meeting of the Society of Environmental Toxicology and Chemistry. Denver, Colorado. (Invited Presentation).

J.R. Bidwell, D.S. Cherry, S.R. Lynde, M.G. Dobbs and M.A. Yeager. 1994. Monitoring Non-target Impact of a Surfactant-Based Biocide. Abstract of the 15th Annual Meeting of the Society of Environmental Toxicology and Chemistry. Denver, Colorado. (Invited Presentation).

Publications:

Lynde, S.R., K.J. Reubush, J.M. Roper, W.J. VanWart, J.R. Webster, and D.S. Cherry. (In-Prep). The Use of Artificial Streams in Evaluating Leaf Degradation: a Comparison of Breakdown Rates Based on Thermal Addition. 54th Annual Meeting of the Association of Southeastern Biologists. (To be Submitted to Environmental Toxicology and Chemistry)

Lynde, S.R., K.J. Reubush, J.M. Roper, W.J. VanWart, J.R. Webster, and D.S. Cherry. (In-Prep). The Effects of Temperature and Macroinvertebrates on the breakdown of Boxelder (*Acer negundo*) Leaves in a Tributary of the New River, Virginia. (To Be Submitted to Journal of the North American Benthological Society)

Technical Reports (Limited Distribution):

Cherry, Donald S., Joseph R. Bidwell, Annick Mikailoff, Mindy M. Yeager, and Stuart R. Lynde. 1992. 1991 Corbicula Control Program Environmental Fate and Effects Studies - Summer and Fall Dosing Studies. Submitted to Duquesne Light Company on February 14, 1992.

Cherry, Donald S., Michael G. Dobbs, Annick Mikailoff, Stuart R. Lynde, Joseph R. Bidwell, Mindy M. Yeager and Jennifer C. Fisher. 1992. Acute Toxicity and Chronic Impairment Testing of *Daphnia pulex*, *Ceriodaphnia dubia* and Fathead Minnow (*Pimephales promelas*) to Robins Air Force Ramp - Phase II Effluent - September 1992 Tests.

Cherry, Donald S., Michael G. Dobbs, Mindy M. Yeager, Stuart R. Lynde, and Jennifer F. Scott. 1994. Benthic Macroinvertebrate Assessment of the North Impact Area in the La Cross River, Fort McCoy, Wisconsin.

Cherry, Donald S., Michael G. Dobbs, Stuart R. Lynde, Mindy M. Yeager. 1994. Benthic Macroinvertebrate Analysis of East Branch, Brandywine Creek for the Sonoco Products Company, Downingtown, Chester County, Pennsylvania.