ABIOTIC AND BIOTIC FACTORS INFLUENCING THE DECLINE OF NATIVE UNIONID MUSSELS IN THE CLINCH RIVER, VIRGINIA

by

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ABSTRACT

Declining unionid populations in the Clinch River are of concern due to the high endemism in the diverse fauna of the Cumberlandian region. Increase in agricultural and mining activities, as well as in industry and urbanization, are coupled with unionid declines throughout the watershed. In many reaches of the Clinch River, mussel populations exist which fail to show recruitment suggesting that this is the weak link in the complex life cycle. Two possible factors which could endanger the sensitive juvenile stage are the presence of sediment toxicants or adult *Corbicula fluminea* in the depositional areas, the preferred habitat of the juveniles.

Before investigating the impacts of these factors, it was necessary to characterize the relationship of the juveniles with the sediment they inhabit. Observations of feeding behavior using videotape, dye studies in a feeding chamber, and gut content analysis were used to determine mechanisms of feeding, the primary food source, and the origin of substances

taken up by juveniles. Exposure to sediment came not only through direct contact, but also through filtration of interstitial water and sediment-associated fine particulate organic matter. Juveniles used pedal locomotory and pedal sweep feeding behaviors to facilitate movement of particles into the pedal gape.

Intermittent sediment toxicity was found in laboratory bioassays using *Daphna magna* and *Chironomus riparius*. These data, along with fluctuating metals in the Clinch River sediments, indicated that acute insults existed from which recovery would depend on the frequency, intensity and duration of the events. Field studies revealed that the intermittent toxicity is reflected in the community structure of benthic macroinvertebrates and impairs growth of juvenile unionids in-situ studies. The intermittent toxicity which may be associated with rain events impairs stream biota and may prevent recruitment of juvenile unionids.

The presence of adult *C. fluminea* in sediments was found to decrease juvenile unionid growth and recovery from test sediments and to increase mortality and resuspension of juveniles into the water column. Both the presence of sediment-bound toxicants and *C. fluminea* may be contributing to unionid bivalve declines in the Clinch River, Virginia.

DEDICATION

This manuscript is dedicated to my Grandfather.
He shared with me his love of the rivers and he believed
I could make a difference in their future.

Francis Charles Creamer

1914 - 1992

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CHAPTER 1

INTRODUCTION

The native freshwater bivalve fauna of North America consists primarily of the families Sphaeriidae, Unionidae and Margaritiferidae. The sphaeriids, or fingernail clams, are comprised of 33 native and 4 introduced species which are found in very diverse habitats throughout the continental United States (McMahon, 1991). The freshwater mussels, Unionidae and Margaritiferidae, include approximately 300 species and subspecies found primarily in unpolluted shallow water habitats with rapid current and stable gravel to sand substrate. These filter-feeding bivalves remove suspended solids from the water column and stabilize the stream beds they colonize. They are also important as indicators of pollution due to their sensitivity to toxicants and siltation.

Throughout recent years, the decline of native mussels in the streams of North America has been well documented (Ortmann, 1918; Ellis, 1931; van der Schalie, 1938; Bates, 1962; Stansbery, 1964; Ahlstedt, 1984; Neves, 1987). What remains unclear are the roles and relationships of the factors instigating the decline of the unionids (Neves, 1987). Prior to the 1940's, the decline of mussels was attributed to

declining habitat quality from industrial and agricultural pollution, along with siltation and dam construction (van der Schalie, 1938). When the Asian clam, *Corbicula fluminea*, was introduced into the United States (Burch, 1944), it flourished and quickly broadened its range in the same aquatic habitats where native mussels were in decline. The introduction of this foreign competitor may further threaten the declining trends of mussels. Since its introduction the Asian clam has continued to spread into native mussel habitat, and the native mussels have continued to decline in spite of efforts to improve water quality and increase suitable habitat.

The declines in the native mussel populations have been of particular concern in the upper Mississippi River drainage area. Of the 300 species and subspecies of freshwater mussels in the United States, approximately 45 are found in the Cumberland Plateau region (Ortmann, 1924). This center of endemism and diversity includes the Clinch River, which is part of the upper Tennessee River drainage system. Of the 46 species found in the Clinch River, 11 are endangered, 21 are endemic, (Jacobson, 1990) and thirteen are thought to have already gone extinct (Ahlstedt, 1984). The declines in the "Cumberlandian fauna", particularly in the rich fauna of the Clinch River, have focused much attention on both this watershed, and on the ecology and life history of the unionid mussels.

In order to maintain existing mussel populations and to prevent further extirpations, the factors influencing mussel decline must be identified and controlled. For this to be accomplished, the life cycle and ecology of all life stages of the mussels need to be clearly understood. While the life cycle of unionids has been determined, the ecology of many parts of the unionid life cycle is not well defined. The habitat of adult unionids is gravel and sand substrata where they burrow with apertures extended into the water column to filter seston. However, even in what would be considered "good substratum", unionid distribution is patchy and clearly there are unknown factors which render some habitats more desirable for colonization. The juvenile life stage is even less well characterized than the adult. Juveniles are found most often in depositional areas behind large rocks and along stream banks (Neves and Widlak, 1987). While this habitat is likely to protect the juveniles from being resuspended in the water column, it also makes them more vulnerable to the effects of siltation. Juveniles were thought to exhibit similar feeding preferences and behaviors as their adult counterparts; however, this aspect of their ecology has not yet been investigated.

The glochidial and early juvenile stages of the unionid's life cycle are poorly understood, due in part to their small size and limited success in collection and maintenance of

these life stages in the laboratory. Neves and Widlak (1987) reported that the two greatest reasons for lack of recruitment in a mussel population are failure to contact a suitable fish host for encystment and detaching from the host into unsuitable habitat. It would seem then that factors affecting these two periods in the mussel life cycle would play key roles in decreasing mussel recruitment.

The incomplete knowledge of the most sensitive stages of the unionid life cycle impairs the elucidation of factors contributing to the declines in their abundance and diversity. While work done by Jacobson (1990), Varanka (1977), Goudreau (1988), Keller and Chrisman (1989), and others have shown the sensitivity of the larvae and juvenile mussels to toxicants, much about their ecology remains unknown. Information such as preferred habitat with respect to silt levels, organic content, and interstitial flow rate is unknown. Also undefined are feeding behaviors and diet of the juveniles. This information is necessary to understand the routes of uptake and modes of toxicity of many toxicants as well as in determining if toxicants are in fact causing impairment in populations or if physical alterations such as siltation could be the culprit.

As indicated, low recruitment may result from mussels falling into unsuitable habitat. However, the qualities which render the habitat unsuitable for mussel colonization are not

completely understood. Clearly poor water quality and unacceptable bottom type, such as bedrock, will prevent the establishment of a mussel population. There may be other factors which render an area unacceptable for mussel colonization. Sediment toxicity and competition with the Asian clam may also influence the ability of native mussels to exist in otherwise optimal habitats.

Several studies have indicated substrate preferences in various species of native unionid mussels (Lewis and Riebel, 1984; Kat, 1982; Harman, 1971) and in the Asian clam (Belanger et al., 1985). Although preference may exist, the substratum type does not appear to be the only factor affecting mussel distribution (Lewis and Riebel, 1984). Water velocity, temperature, food availability and water and sediment chemistry are also important in defining suitable mussel habitat.

While much is known about pollution contributions of industry, agriculture, and urbanization to the surface water, we have only begun to link these problems with sediment toxicology. Contaminants trapped in the sediments can damage the ecosystem long after the pollution source has been removed (Salomons, 1987). Mussels are especially vulnerable to toxins in sediments not only because they burrow but also because they are filter feeders which siphon at the sediment water interface. The juvenile mussels may be the most vulnerable to

toxic sediments because of their occurrence in depositional areas where fine particulates and sediment-bound toxicants accumulate. Toxic sediments can render otherwise suitable habitats inaccessible to unionid populations and decrease available habitat in an area.

Competition with the Asian clam may also be contributing to declining mussel habitats. Just as the Asian clam continues to increase its distribution in North America, the native mussels continue to decline (Gardner et al., 1976). Competition between native mussels and *C. fluminea* for habitat and food has been implicated (Gardner et al., 1976; Kraemer, 1979), due to the clams high rate of filtering efficiency, increased mobility, and less complex reproductive cycle (Britton and Morton, 1979). However, proof of adverse effects to the mussels due to the presence of *C. fluminea* is difficult to ascertain. The effects of the invading species on unionid populations may be more subtle than direct competition for limited resources. Recruitment of unionids may be hindered by impairment of juveniles by adult clams in depositional areas.

For the reasons previously stated and probably many others, native unionid mussels continue to decline throughout their respective ranges. Much work is needed, and quickly, to preserve the remaining populations and prevent further species extirpations. This research project is being undertaken in order to gain information which may be useful in slowing the

declining trends in unionid populations. The objectives of this work are threefold: (1) to determine the relationship of juvenile unionids with the sediment they inhabit; (2) to determine if sediment toxicity exists in the Clinch River and if this toxicity could impair recruitment in unionid populations; and (3) to examine the relationship between adult *C. fluminea* and juvenile unionids to determine whether recruitment of juveniles may be impaired by the presence of the Asian clam in depositional areas.

To specifically address the previously stated objectives, this study is divided into three sections. Chapter 2 describes procedures for culturing and maintaining juvenile *V. iris* in the laboratory. These methods were used throughout the work and are described here to streamline the methodology in later chapters. Chapter 3 addresses the first objective; i.e., to determine the relationship of juvenile unionids with the sediment they inhabit. In this chapter dye studies in a feeding chamber, gut content analysis, and video recordings of feeding were used to show the mechanisms of feeding, the primary food source, and the origin of substances taken up by juvenile *V. iris*. The second major objective, to determine if sediment toxicity exists in the Clinch River and if this toxicity could impair recruitment in unionid populations, is addressed in Chapters 4 and 5. Chapter 4 describes laboratory sediment tests using four species, including *V. iris*, to

determine if sediment toxicity is present at 11 sites on the Clinch River and one site in a tributary. Chapter 5 describes field validation of the laboratory findings which include mussel density surveys, invertebrate community analysis, and in-situ testing at four of the impaired sites. Chapter 6 examines the relationship between adult *C. fluminea* and juvenile unionids to determine whether recruitment of juveniles may be impaired by the presence of the Asian clam in depositional areas. In this study, ingestion of juvenile *V. iris* by adult *C. fluminea*, and the possibility of resuspension of juvenile *V. iris* by *C. fluminea*, were investigated. The final chapter summarizes all findings and describes their relevance to maintaining existing mussel populations and to preventing further extirpations, particularly in the Clinch River.

STUDY AREA

The Clinch River originates near Bluefield, Virginia and flows southwesterly through Virginia into Tennessee where it joins the Tennessee River at Watts Bar Reservoir (Tennessee River Mile 567.8) (Goudreau, 1988). A high diversity is also seen in the fish fauna of the Clinch River (Masnik, 1974).

In this watershed, the primary anthropogenic impacts are habitat degradation from both point (power plant effluent, coal mining drainage) and non-point (pesticide and fertilizer runoff from agriculture, municipal storm sewer input) sources.

Eleven sites on the Clinch River and one site on a tributary (Guest River) were chosen for this study (Table 1). Two of the twelve sites were considered reference sites because of abundant or diverse unionid mussel populations. The remaining ten sites were chosen for lack of unionid mussels in what appeared to be suitable habitat. Directions to the sites and specific locations of the sampling areas are given in Appendix I.

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Table 1. Description of study sites on the Clinch River which will be referred to in the text.

Clinch River Site	River Mile	Abbreviation
Pounding Mill	327.5	PM
Cedar Bluff	319.5	CB
Raven	310.0	RVN
Van Dykes Ford	305.5	VD
Hackneys Farm	269.0	HCK
Bulldozer Crossing	267.3	BDX
Carterton	264.1	CRTN
St. Paul	256.4	STP
Burtons Ford	249.7	BF
Dungannon	235.0	DGN
Clinchport	213.2	CPT
Guest River Site		
Coeburn	6.0	GST

CHAPTER 2

TECHNIQUES FOR PROPAGATION AND REARING OF JUVENILE UNIONIDS IN THE LABORATORY

INTRODUCTION

In response to declines in unionid bivalve populations, attempts have been made to culture unionids in the laboratory. The first of these attempts was in the early 1900's in response to declining populations in the Mississippi River resulting from overharvest by the pearl button industry (Oesch, 1984). Research on the reproductive biology of unionids conducted at the Fairport Biological Station in Fairport, Iowa between 1908 and 1917 provided juveniles for stocking. However, when the laboratory burned in 1917, research on unionid reproduction was suspended. Recent declines in unionid populations have once again promoted interest in methods for the successful culturing and maintenance of juveniles in the laboratory. Development of successful culturing techniques is critical for the survival of many unionid species. Juveniles metamorphosed and maintained in the laboratory can be used for the restocking of populations declining due to anthropogenic impacts and in toxicity tests which are designed to establish safe levels of pollutants for these sensitive species.

Most recent efforts in laboratory culture have focused on the development of methods to transform glochidia in vitro (Isom and Hudson, 1982). For maintaining these juveniles in the laboratory, the addition of silt to the cultures has been shown to improve growth and survival (Hudson and Isom, 1984). However, attempts at culturing juveniles beyond two or three months post-metamorphosis have resulted in extremely high mortality levels.

While the information herein does not solve all the problems of juvenile unionid culturing, it does contribute to the body of knowledge on unionid propagation and is a good starting point for individuals interested in joining the quest for suitable propagation methodology. The purpose of this chapter is to share techniques successful in the propagation and rearing of juvenile *Villosa iris*. The following is a qualitative description of the encystment and culture methods found to produce high quantities of healthy juveniles over the course of a three-year study.

ENCYSTMENT METHODS

Water Preparation

The water used throughout this study was dechlorinated tap water unless otherwise stated. The water was dechlorinated by the addition of small quantities of sodium thiosulfate and monitoring of the total residual chlorine after each addition to use the smallest amount necessary. The

alkalinity and hardness of the water were adjusted to approximately 180 mg/l $CaCO_3$ by the addition of sodium bicarbonate and calcium chloride. This water was used because of the large quantities needed; however, river water with a naturally high alkalinity and hardness would probably be more suitable due to the addition of zinc, fluoride and chlorine to the tap water. When natural river water was used, such as for feeding gravid females and juveniles, the water was plankton-net filtered Clinch River water which was collected at the same site as the gravid females.

Host Collection

Largemouth bass, ranging in size from 12.7 - 17.78 cm were obtained from Zett's Tri-State Fish Hatchery in Inwood, WV and maintained with ambient light at 18-20°C. Since fish will exhibit an immune response to encystment by glochidia, only fish which had not been previously exposed to glochidia were used. If fungal infection was evident, bass were treated with uniodized table salt at the rate of one tablespoon per 3.8 liters of water until such time as it was determined that they were in stable condition and able to survive encystment. Temperature of the water was critical since bass become territorial as they approach their spawning temperature (21.1°C)(Breder, 1936). If the water temperature remained above 21.0°C, the fish had to be kept isolated so that

fighting did not result in injury and death. Bass were maintained in both flow-through aquaria and 500-liter fiberglass boxes hereafter referred to as living streams. While some researchers have been successful holding fish in aquaria, the bass appeared less stressed when kept in living streams. There was no incidence of injury to their mouths from hitting the glass aquaria walls when frightened. When the bass were kept in aquaria, the tanks were covered with cardboard on the sides to prevent them from being startled by researchers working in the laboratory. This proved to be futile because, when left uncovered, the fish adapted to the movement around them and had less of a negative reaction to the siphoning of their tanks to remove debris.

The bass were fed one or two fathead minnows every two or three days. However, when feeder fish were not available, they were fed nightcrawlers purchased from the local baitshop.

Mussel Collection

Gravid *Villosa iris* were collected in the Clinch River and returned to the laboratory in water filled-coolers. Gravidity was determined in the field by the presence of swollen gills which were engorged with glochidia. The mussels were initially maintained in river water at 16°C in a recirculating waterbath. It was determined, however, that if cooled slowly with bi-weekly water change-overs, the gravid females could be

successfully held for at least six weeks in a refrigerator. The mussels were fed unfiltered river water and sediment interstitial water as well as a tri-algal mixture (Foe and Knight, 1986) until they were needed.

Procurement of Glochidia

Two different procedures were used to remove the glochidia from gravid mussels, with the latter being preferred. Initially, the marsupia were excised and placed in a petri dish containing dechlorinated tap water where they were teased apart using forceps and a dissecting needle, and the glochidia were rinsed out of the tissue. The valves were cleaned, labeled and saved for reference. It was subsequently determined that glochidia could be removed with minimal harm to the adult by gently prying the valves apart approximately 2-3 mm, inserting a water-filled syringe into the gorged gill chamber, and flushing the chamber with water. Several chambers of each marsupium could be emptied in this manner, and each female used would retain many glochidia and could be returned, unharmed, to the stream.

Determination of Viability

Prior to the bass exposure, six subsamples of glochidia were transferred to a deep-well dish, and the number of open and closed glochidia was recorded using a Zeiss dissecting microscope. A saturated NaCl solution was added to each well, and the number of open and closed glochidia was again

recorded. All glochidia which were open before the addition of the NaCl and closed afterward were recorded as viable (Zale and Neves, 1982). The glochidia which were closed prior to the addition of the NaCl or remained open afterward were recorded as dead. Glochidia would not have been used if less than 90% were viable, but this did not occurred during the course of this study.

Encystment

During early encystments of bass, glochidial density was monitored and attempts were made to maintain approximately 200 glochidia per liter in the aquaria. This was found to be unnecessary, as the density was irrelevant because the degree of encystment could be adjusted by altering the time of exposure. The easiest way to determine the appropriate density of encysted glochidia was to visually examine the gills at intervals during the encystment. While chemicals are available to anesthetize fish for examination, these were not necessary if fish were handled gently and grasped by the lower jaw so as not to dislodge any scales which could allow for infection. Bass were usually left in the encystment chamber until they had approximately 50-100 glochidia on each gill.

The size and shape of the encystment chamber were appropriate for the number of fish exposed simultaneously and the type of aeration used. A 38-L aquarium, with approximately 20 L of water was used to hold 5 - 7 bass during

encystment. The water was aerated using two air-bars to keep glochidia suspended in the water column. Bass were placed in the encysting chamber for up to 4 h, depending on the glochidial density. Fish were left in the encystment chamber until each gill arch had approximately one hundred glochidia attached. While over-encystment did not occur in this study, it has been reported by Lefevre and Curtis (1912) to occur at approximately 2,500 glochidia on a 10-12 cm largemouth bass. Even when these levels are not reached, encysted bass are vulnerable to fungal infections, from damage of the gill tissue, and to respiratory distress, due to the decreased surface area for gas exchange. In order to reduce the risk of host mortality while maintaining high levels of encystment, the water in the fish holding tanks must be kept clean and well aerated. After exposure, the bass were returned to their living streams.

Fish disease

On several occasions shipments of fish received were found to exhibit high incidence of fungal infections. As stated previously, these fish were treated with table salt until all evidence of the fungus was removed. Once the fungus was present, there was a high incidence of recurrence after infestation with glochidia from the injury inflicted on the gills by the encapsulating glochidia. If the bass became infected during the course of encystment, the spread of the

fungus was often controlled by isolating the affected individual and moving the remaining fish to clean water while the living stream was cleaned with salt prior to their return. If death was imminent, individuals were sometimes treated with copper at the rate of 100 ug/L for a 24-h period. The encysted glochidia have been shown to be resistant to this level of copper exposure (Jacobson, 1990). This technique had only a 30% success rate but was used only as a last resort, as the effect on the viability of juveniles retrieved from weakened fish as compared with those from healthy fish was unknown.

Juvenile collection

Depending on the water temperature, juveniles began dropping from the fish in 14 - 16 days and continued to fall off until about 21 days. In order to reduce the debris and ensure the health of juveniles, bass were fed for the last time about 12 days into the encystment. The tanks were thoroughly cleaned prior to juveniles dropping off the hosts. Juveniles were collected by siphoning the bottoms of the living streams and filtering the water through a 100-micron nitex mesh. The contents of the sieve were then rinsed into a petri dish, and juveniles were removed from the debris.

JUVENILE CULTURING

Juvenile rearing

Juveniles were held successfully for up to a month with

little mortality using several methods. The two most effective methods were placement in silt and algae, and placement in tubes which were placed in sediment. Juveniles were successfully held for up to 4 wk prior to testing in a 400 ml beaker with river sediment which was sieved to less than 53 microns and a tri-algal suspension containing Chlamydomonas, Ankistrodesmus and Chlorella (Foe and Knight 1986). Juveniles were rinsed and water in the beaker was changed every 3-4 days. Using water from the Clinch River instead of dechlorinated tap water resulted in greater survival of the juveniles. Whether this was due to the algal and detrital content of the river water or to the chemical constituents of the tap water is unknown.

Another successful method of holding juveniles was to place them in nitex tubes which were then placed in beakers containing water and sediment. The tubes were constructed using aquarium tubing which was cut away and fitted with 100 micron nitex mesh (Figure 1). The previously described tri-algal suspension and a detrital suspension consisting of the silt, clay and organic fractions of river sediment were added into the tube.

Juvenile mortality

Juveniles were held in the laboratory for up to 4 wk prior to their use in tests with little mortality. Some juveniles were held longer but the mortality increased despite

efforts to maintain them until all were dead, usually within two months. On several occasions a few individuals have lived for over 2 months. However, this was the exception; holding juveniles past 1 mo prior to use often caused the tests to be invalid due to control mortality. It is important to note that on many occasions juveniles were lost for no apparent reason. Sometimes they were siphoned dead from the living streams with no apparent cause of mortality. On several occasions, juveniles died in tests or holding beakers, and the cause of the mortality was unknown. Whether this mortality was due to dirty glassware, changes in the quality of the water, or some pathogen or disease which had been introduced to the rearing system, is unknown. Due to the sensitivity of the juveniles, any number of explanations are feasible.

Planaria

While the causes for juvenile mortality were sometimes obscure, more often than not they were obvious. Throughout the three-year study, the greatest number of juveniles were lost to predation by flatworms. While predation of platyhelminths on glochidia has been reported (Howard and Anson, 1922), the degree to which flatworms could damage a culture was not anticipated. On more than one occasion planaria, probably originating from the bass, were found in the living streams where they were siphoned and collected with the juveniles. Once in the cultures, they were devastating.

Within 3-4 days all juveniles collected could be consumed. Examination of holding chambers which were contaminated with flatworms would reveal numerous worms with mussels in the gut, often two mussels at a time, and on one occasion, three juveniles were seen in the gut of one planarian. After being consumed the mussels, not yet dead inside the flatworm, were visible inside the gut of the planarian (Figure 2), after approximately 20 min the mussel began to gape and finally opened completely inside the gut (Figure 3). When planaria were present in the living streams, they had to be siphoned at least twice daily, and then every juvenile mussel was carefully removed from the contaminated water and placed in filtered water. This had to be done quickly as the planarians had a voracious appetite and while the juveniles were being picked from a dish, the worms were devouring many and leaving empty shells in their place.

DISCUSSION

The ability to transform and hold juvenile unionids in laboratory cultures is critical to propagation of species in many areas of North America. Their sensitivity and vulnerability makes them difficult to maintain in the laboratory and critical for use in testing if anthropogenic impacts contributing to their demise are to be identified.

While more attention has focused on juvenile testing recently, a standard method for propagation of the juveniles

has not been published. Each researcher is forced to 're-invent the wheel', and this costs valuable time. Hopefully the ideas presented here can be modified and improved upon to aid other researchers. Of particular interest is the treatment of encysted bass with copper to prevent fungal infections while allowing maximum yield of juveniles per bass. In determining the sensitivity of encysted juveniles to copper, Jacobson (1990) found that *Saprolegenia spp.* was inhibited at concentrations of 100 ug Cu/L, thus resulting in less mortality in this treatment than in controls. The low success rate of the copper treatments in this study undoubtedly resulted from the seriousness of the infection prior to the treatment. Had copper been used sooner, or even routinely, the success rate surely would have been higher.

Attention now needs to be focused on getting the highest number of juveniles per fish without compromising the health of the juveniles. This number will be dependent on the health of host fish prior to encystment and the treatment of the fish during encystment. Perfection of these methods is ongoing, and this manuscript should generate some discussion on this issue.

Also of critical importance is that there is no comparison of the viability of juveniles metamorphosed *in vitro* as opposed to those encysted on host fish. Similarly, there is no information on whether or not the number of

glochidia on a fish will influence the viability of the juveniles when they excyst. These and many other areas of propagation and maintenance need further investigation.

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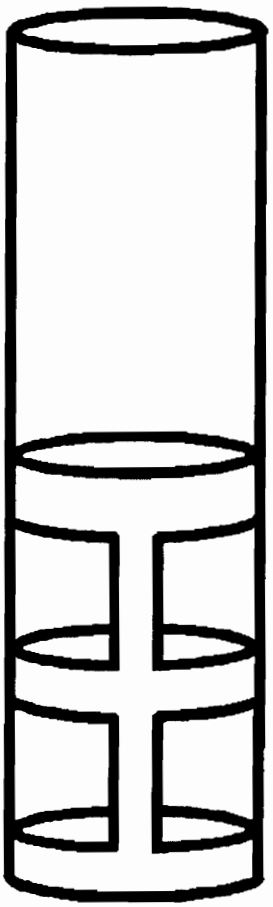

Fig. 1. Tubes constructed from PVC uplift tubing and nitex mesh for holding juvenile *V. iris* in sediments.

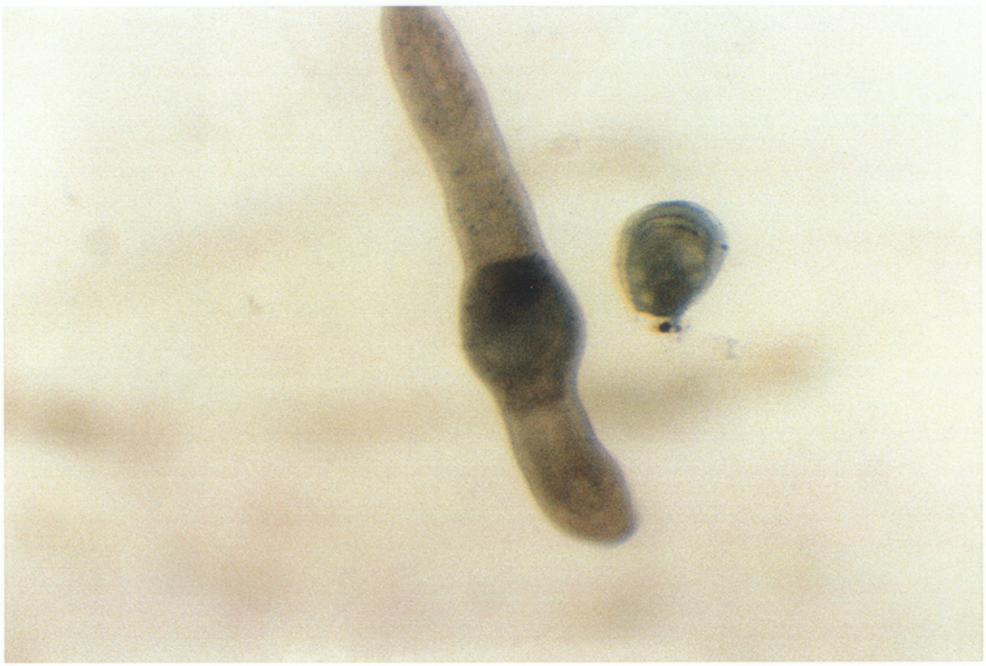

Fig. 2. Still-living juvenile *V. iris* in the digestive tract of a flatworm.

Fig. 3. Gaped juvenile *V. iris* in the digestive track of a flatworm.

CHAPTER 3

Feeding and Burrowing Behaviors of Juvenile Rainbow Mussels, *Villosa iris* (Bivalvia: Unionidae)

ABSTRACT

This study characterized the relationship between juvenile rainbow mussels (*Villosa iris*) and the sediment they inhabit. Dye studies in a feeding chamber, video recordings of feeding, and gut content analysis were used to determine mechanisms of feeding, the primary food source, and the origin of substances taken up by 1 - 14 d-old juveniles. Within 20 min, 98.5% of the juveniles were able to burrow in the sediment. Although juveniles burrowed < 1 cm into the sediment, they were not exposed to the overlying water. Exposure to sediment comes not only through direct contact, but also through filtration of interstitial water and sediment-associated fine particulate organic matter. Juveniles fed on bacteria and bacterial-sized particles as well as algae. Their predominant food source appears to vary with age. Pedal-locomotory and pedal-sweep feeding behaviors are used to facilitate movement of particles into the pedal gape. Pedal-locomotory behaviors were used more frequently in the presence of detrital particles while pedal-sweep feeding was most frequently observed in a tri-algal suspension.

INTRODUCTION

Although declines in freshwater mussel populations (Unionidae) in rivers are well documented, the reasons given for the plummeting numbers are largely speculative. Anthropogenic factors such as point and non-point discharges and siltation are likely contributors. However, studies on effects in specific stream reaches have been hindered by insufficient knowledge of the sensitive juvenile life stage which is seldom encountered in field collections (Kat 1982, Neves and Widlak 1987) and is difficult to culture in the laboratory (Hudson and Isom 1984).

Adult mussels are found typically in river reaches with sand and gravel substrate, while juveniles often inhabit depositional areas behind large rocks and along stream banks (Neves and Widlak, 1987). This latter habitat exposes the juvenile stage to an environment different from that of the adult. Decreased recruitment in areas that still support reproducing mussel populations suggests that the juvenile stage may be more susceptible to environmental degradation. Greater sensitivity of thin-shelled juveniles to contaminants (Jacobson et al. 1993a), and the fact that some toxicants remain in sediment while absent from the water column (Salomons et al. 1987) could account for decreased recruitment, particularly in areas with low but chronic inputs of toxicants. Sediment-bound contaminants are more persistent

and occur at higher concentrations than contaminants in the overlying water (Larson 1989). The mode of exposure to sediment-bound toxicants, whether by gill surface and body wall or by ingestion of sediment particles, can affect the bioavailability and ultimately the toxicity of these toxicants (Power and Chapman 1992).

Adult unionid mussels burrow in the substrate with siphons exposed for filter feeding, whereas the feeding behaviors of juvenile mussels are not well understood. Although it is known that juveniles burrow (Negus 1966), the depth at which they burrow and the origin of the water and particulate matter they take up is unknown.

The purpose of this study was to characterize the relationship of juvenile rainbow mussels (*Villosa iris*) with the sediment they inhabit. Specifically, this study determined the depth to which juveniles burrow and the origin of the water and particulate matter ingested.

METHODS

Juvenile procurement

Gravid *V. iris* were collected from the Clinch River at Cliffield, Virginia, and taken to the laboratory in water-filled coolers. The mussels were killed, the marsupia were removed and teased apart to release glochidia, which were checked for viability using a saturated salt solution (Zale and Neves 1982). Encystment onto 12-18 cm largemouth bass

(*Micropterus salmoides*) was accomplished by placing the fish in a well-aerated 18.9-L aquarium with the glochidia from both marsupia of a female. Exposure periods ranged from 20 - 45 min depending on glochidial density. The bass were transferred to 500-L fiberglass tanks which were siphoned daily once the juveniles began dropping from the hosts.

Feeding chamber

A feeding chamber filled with sediment from a depositional area in the Clinch River was used to simulate the habitat of juvenile unionids. The chamber was used to determine the depth to which juvenile unionids burrow into the sediments. A dye in the overlying water or in the sediment was used to determine the origin of water and particles taken up by the juveniles. A flow-through chamber was constructed from two plexiglass plates (28 cm x 26 cm). The plates were separated by 0.05 cm spacers which sealed the chamber on three sides. The chamber was filled with 15 cm of sediment which allowed 11 cm for water to flow across the sediment. Particle size composition of the sediment was determined gravimetrically. The sediment was 69.5% sand, 30.5% silt, and 0.04% clay. The water content of the sediment was 27.5% and the total organic carbon content was 0.38% ± 0.04%.

Four trials were run with juvenile mussels in the chamber. Trials were run under ambient light conditions (50 -

100 fc) and at room temperature (20 - 22°C). In each trial, newly metamorphosed juveniles (120 - 180 μm) were placed in the chamber and allowed to burrow for 20 min. Flow was then initiated by siphoning Clinch River water from a headbox through the chamber and then through a plankton net. Flow rate for each trial was controlled by clamps placed above and below the chamber (Table 1). Flow rate was measured by collecting water running through the chamber for 30-sec intervals in a 250-Ml graduated cylinder. The duration of the trials, number of juveniles used, and duration and route of exposure to neutral red dye varied in the 4 trials (Table 1). Neutral red dye is a vital stain which is specific for living tissue but does not harm the organisms (Jacobson et al. 1993b).

In trial 4, dye was placed in the sediment, but not in the overlying water. The dye was incorporated into the sediment by placing the dye in a beaker with sediment and water and allowing the overlying water to evaporate until only interstitial water remained. Initiation of flow in the chamber resulted in a temporary mixing zone at the sediment water interface; after flow was established, there was no evidence of dye leaching into the overlying water.

After each trial the entire chamber was placed in a freezer for 8 - 12 hours, to facilitate sectioning of the

sediments. A preliminary experiment was run without freezing to ensure that this procedure had no impact on the location of juveniles within the sediment. To locate juveniles, the chamber was opened and 1-cm sections were sliced longitudinally and swept with a brush into separate beakers. Juveniles were sorted from the sediment and counted; presence or absence of color in body tissues was noted.

Videotaping juveniles

Juveniles were placed in water on microscope slides with (1) a tri-algal suspension containing *Chlamydomonas*, *Ankistrodesmus* and *Chlorella* (Foe and Knight 1986) at a concentration of 3.2×10^7 cells/Ml, (2) a detrital suspension consisting of silt, clay and organic fractions of river sediment obtained by eluting these fractions from 50 Ml of the river sediment or (3) a mixture of (1) and (2). The movement and feeding of 11 juveniles were viewed under high and low magnification with an Olympus compound microscope, which was connected to an RCA video recorder and television. Juvenile feeding behaviors were recorded for 86 min. The frequencies of sweeping and pedal locomotory feeding behaviors were determined in each of the three food suspensions by recording the number of extensions and retractions of the foot by individual mussels.

Gut content analysis

Juvenile mussels were kept in 400-Ml beakers with sediment from the Clinch River site and the previously described tri-algal suspension. Juveniles were removed from the sediment by eluting the fine particles from the holding beaker and rinsing the suspension through a 100-μm nitex mesh sieve. The contents of the sieve were then rinsed into a petri dish and viewed with a dissecting microscope. Juveniles were transferred to sterilized water with pipettes, rinsed, and placed on microscope slides. They were compressed to fragment the shell and expose the gut contents, which were stained with acridine orange and viewed using epifluorescent microscopy. We used 18 juveniles for gut content analysis; of these, 8 were 3 - 5 d-old and 10 were 10 - 14 d-old. Bacteria and colloidal particles were measured with an ocular micrometer.

RESULTS

Feeding chamber

The percent of juveniles recovered from frozen sediment ranged from 82 to 96% in the four feeding trials (Table 2). All juveniles were recovered within the top 1 cm of sediment. In trials 1 - 3, juveniles failed to take up dye from the water column. A 1 - 2 mm zone of mixing between overlying water and sediment was revealed by the presence of dye within the interstitial spaces of this surface zone. Juveniles could

have been exposed to dye from the overlying water at this interface; however, the juveniles failed to take up the dye which indicates they were not in contact with this top layer. Juveniles did take up dye from interstitial water and sediment particles, as indicated by a pink/brown color in the gut of the animals in trial 4, with neutral red in the sediment. Juveniles burrowed rapidly to depths that minimized their resuspension in the water column. On average, < 1.5% of the juveniles failed to burrow in the 20 min before flow commenced in the chamber (Table 2).

Gut content analysis

The gut contents of all 8 of the 3 to 5 d-old juveniles contained primarily flagellated bacteria (2 to 5 μm) and detrital particles in the same size range. Diatoms (*Fragilaria sp.*) and *Chlorella* also were found in the gut contents but in lesser amounts. The gut contents of older juveniles (10-14 d) consisted principally of inorganic colloidal particles and tri-algal mix; bacteria were present only in small quantities.

Videotaping juveniles

Videotapes of the feeding behavior of 1 - 14 d-old juvenile mussels revealed that food intake is through the pedal gape, not the posterior apertures used in adult feeding. Currents created by cilia on the foot facilitate movement of organic and inorganic particles into the young mussels where

material is sorted for ingestion or egestion. Juvenile *V. iris* used the ciliary action of the foot to create an inhalant current through the pedal gape; i.e., they carried out suspension feeding on the interstitial water. The juveniles also used the foot in a sweeping motion to draw particles toward the pedal gape and inhalant current and to collect particles adhering to the foot and move them toward the pedal gape (Fig. 1). Foot sweeping occurred both posteriorly to anteriorly and anteriorly to posteriorly. The juveniles also fed by turning the shell upright with the umbo dorsal, extending the foot anteriorly, and pulling themselves along while picking up particles on the foot and transporting them to the pedal gape (Fig. 2). These two distinct types of deposit feeding observed for juvenile *V. iris* have been described as pedal sweep feeding and pedal locomotory feeding by Reid et al. (1992).

The 11 juveniles used either sweeping or pedal-locomotory behavior 47 times during 26 min of observation in algae, and 50 times during 30 min of observation in detritus. Pedal-locomotory behavior was used more frequently than foot sweeping in detritus ($\chi^2 = 40.1$, $\underline{n} = 50$, $\underline{p} < 0.01$), and foot sweeping was used more frequently than pedal-locomotory behavior in a tri-algal suspension ($\chi^2 = 28.7$, $\underline{n} = 47$, $\underline{p} < 0.01$). In the presence of both algae and detritus, juveniles

used pedal-locomotory feeding more frequently than pedal sweep feeding ($\chi^2 = 6.53$, $n = 30$, $p < 0.05$).

DISCUSSION

Results of the feeding chamber study showed that juveniles burrow quickly into the sediment and feed interstitially rather than from the water column. Mussels less than 0.5 mm are unlikely to withstand current in an exposed area such as a riffle. Depositional areas behind boulders and along stream banks afford juveniles protection from strong currents. Juvenile mussels which drop from fish hosts are probably dispersed and deposited in slow-flowing areas along with other suspended solids. Movement of the juveniles to the sediment-water interface increases their chance of being dislodged and perhaps relocated to unsuitable habitats. Although all the juveniles in this study were recovered from the top 1 cm of sediment, Neves and Widlak (1987) reported recovering juveniles (ages 0-3 yr) from the top 8 cm of sediment. Differences among species and sizes of juveniles, and current velocity may explain discrepancies in reported depths of capture.

Adult unionid mussels feed by filtering suspended particles from the water column through a posterior inhalant aperture (Kraemer 1979). The adult mussel diet is thought to consist primarily of phytoplankton and detritus; bacteria-

sized particles are not filtered efficiently (McMahon 1991). Gut content analysis showed that juvenile *V. iris* were ingesting bacteria, detritus, algae and colloidal particles which are found in the interstitial water. Ingestion of the inorganic colloidal particles and interstitial water could increase exposure to sediment-bound toxicants.

Based on the feeding chamber studies and the videotape analysis, we believe that juvenile *V. iris* are both deposit and suspension feeders. Lopez and Holopainen (1987) describe deposit feeders as those that use the ciliated foot to collect particles; suspension feeders siphon overlying or interstitial water. Juvenile mussels act as deposit feeders by using pedal-sweep feeding and pedal-locomotory feeding behaviors. Whether these juveniles use interstitial pedal feeding and pedal probe-feeding (Reid et al. 1992) is undetermined. Although the term "interstitial pedal feeding" has been used synonymously with "interstitial suspension feeding" (Lopez and Holopainen 1987, Reid et al. 1992), the latter term is best applied to our results. When the juveniles were filtering, without using foot sweeping or pedal locomotory behavior, the inhalant current was created by cilia on the foot but the foot was not extended as described in interstitial pedal feeding.

Many bivalve species exhibit these distinct modes of feeding (Caddy 1969, Lopez and Holopainen 1987, Reid et al.

1992). Some bivalves such as *Corbicula fluminea* (Reid et al. 1992), and *Pisidium spp.* (Lopez and Holopainen 1987) use these feeding behaviors throughout their lives, whereas other bivalves exhibit pedal feeding only as juveniles (Caddy 1969). Similar pedal feeding behaviors were described for juveniles of the marine lamellibranch *Macoma balthica* by Caddy (1969), who stated that the transition between juvenile and adult modes of feeding was gradual.

The increased frequency of foot sweeping in the presence of tri-algal suspension, and locomotory behavior in the presence of detritus, may result from differences in patterns of particle distributions in these suspensions. In the algal suspension the cells were uniformly distributed while in the detrital suspension the particles tended to aggregate into large masses. The small particles in the algal suspension are readily moved by the current created by sweeping and quickly diffuse back into close proximity of the juvenile following a sweep. Larger detrital particles are not easily moved by sweeping, so juveniles may move through the suspension to contact potential food particles. Using pedal locomotory behavior in a suspension of both tri-algal mix and detritus allows the juvenile to contact and take up both types of food. In a more natural environment, mode of feeding may depend on the particle size range of available food.

Juvenile mussels are associated with surface sediments

and, therefore, toxicants within the sediments. The possible routes of exposure of the juvenile mussel to sediment-sorbed contaminants are by ingestion of sediment particles and interstitial water as well as across body surfaces. Because of their feeding behaviors and location in depositional areas, juveniles may be exposed to toxicants to which adult mussels, filter feeding suspended solids from a gravel/cobble substrate, have only a limited exposure.

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Table 1. Experimental conditions of time, flow rate, sample size and dye use in feeding trials with juvenile mussels.

Trial number	Time of trial (hr)	Average flow (ml/s)	No. juveniles used	Location of dye	Time of dye exposure (hr)
1	2	4.2	200	water	1
2	4	3.75	100	water	2
3	2	3.5	82	water	1
4	2	3.16	75	sediment	2

Table 2. Recovery of juvenile mussels from the water column (juveniles which failed to burrow), and from the sediment following dye exposure in feeding trails.

Trial number	Percent recovered	No. juveniles which failed to burrow	Juveniles ingesting dyed sediment
1	82	4	no
2	95	1	no
3	94	0	no
4	96	4	yes

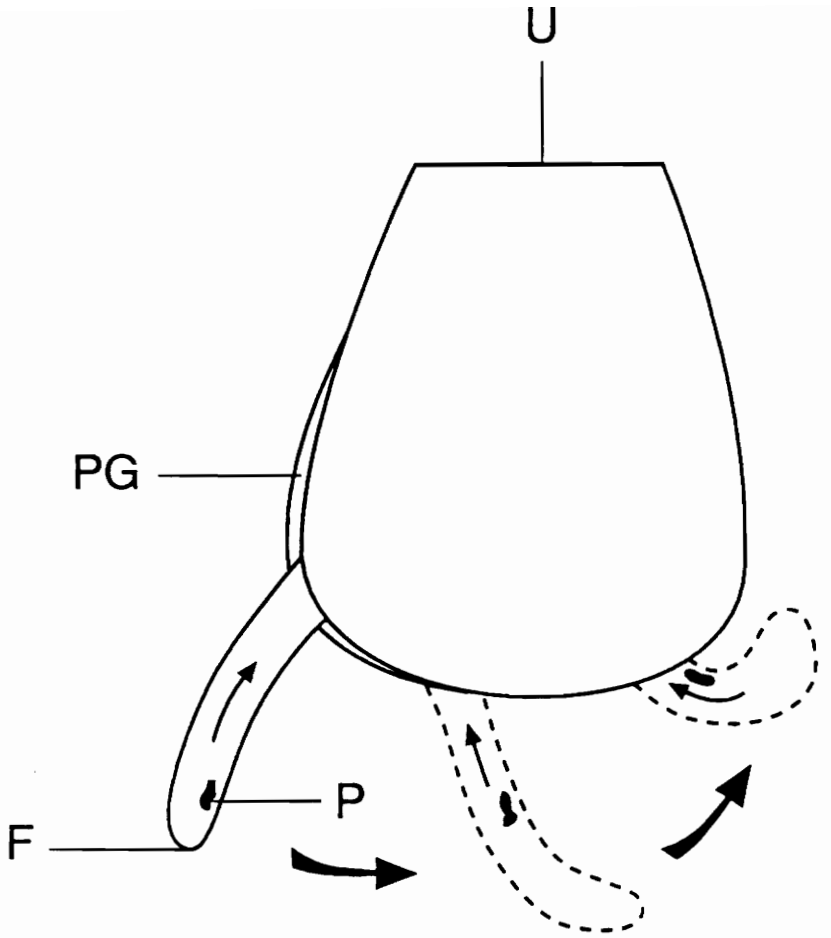

Fig. 1. Pedal-sweep feeding behavior of juvenile *Villosa iris*. Large arrows show the direction of a posterior to anterior sweep. Small arrows show the direction of particle movement. F = foot; P = particle taken up; PG = pedal gape; U = umbo.

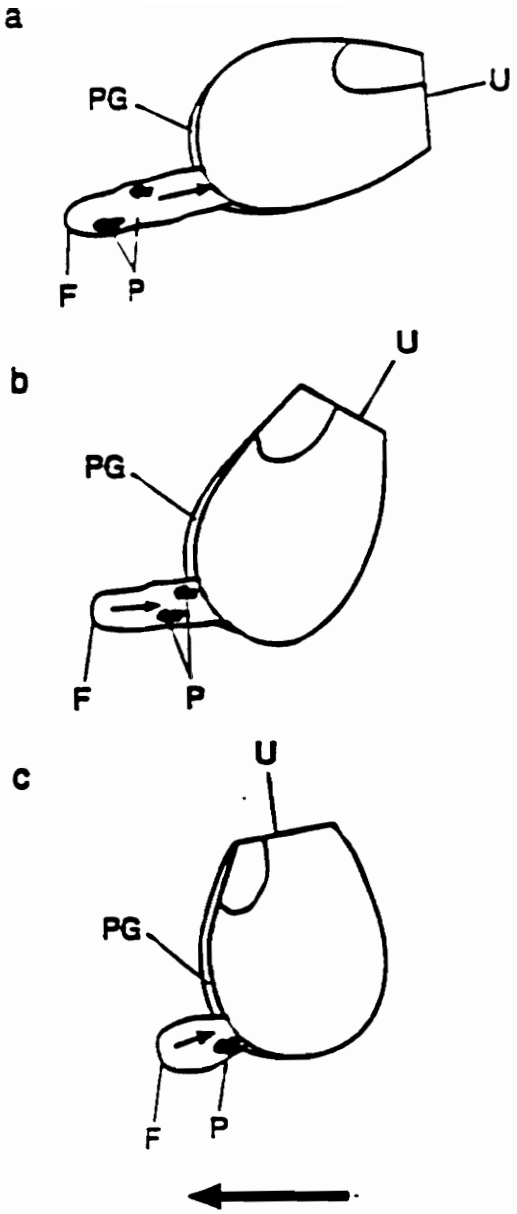

Fig. 2. Pedal-locomotory feeding behavior of juvenile *Villosa iris*. a. As the foot is extended, the valves move to a horizontal position. Detrital particles adhere to the foot and move toward the pedal gape. b. The valves move vertically as the foot begins to retract. c. The valves are pulled forward to enclose the foot resulting in forward motion. When the foot is retracted the valves are upright. Large arrow shows the direction of locomotion. Small arrows show the direction of particle movement. F = foot; P = particle taken up; PG = pedal gape; U = umbo.

CHAPTER 4

An Assessment of Sediment Contamination in the Clinch River, Virginia.

ABSTRACT

Due to the rapid declines in unionid bivalve populations in the Clinch River, Virginia, factors affecting their recruitment are being investigated. Sediment depositional areas in lotic systems have been identified as the habitat of juvenile unionids where they ingest interstitial water and detrital particles. Decreased recruitment in unionid populations may reflect the intimate association of the juvenile stage with sediments and therefore any sediment bound toxicants which may be present. Eleven sites on the Clinch River (CRM 213.2 - 319.5) and one site on a tributary, the Guest River, were examined for the presence of sediment contamination. In 10-day sediment tests using *Daphnia magna* and *Chironomus riparius* and 28-day sediment tests with *Hyallela azteca,* both enrichment and impairment were found. Intermittent toxicity identified at ten of the sites did not affect the daphnids and midges similarly. Several of the observed effects could be related to point source discharges from industrial and municipal effluents. Non-point sources such as runoff from agricultural and mining activities and

urbanization, which enter the system during rain events, may be responsible for much of the toxicity. Ten day sediment tests with juvenile *Villosa iris* and *Corbicula fluminea* did not confirm the toxicity seen previously on many of the testing dates. In situ testing which encompasses a rain event is recommended along with community structure analysis to determine if intermittent toxicity is causing alteration of instream biota.

INTRODUCTION

While much is known about pollution contributions of industry, agriculture, and urbanization to the surface water, we have only begun to link these problems with sediment toxicology. Contaminants trapped in the sediments can damage the ecosystem long after the pollution source has been removed (Salomons et al. 1987; Tessier and Campbell, 1987). Sediment toxicity testing has proven useful in identifying areas where sediment-bound toxicants have rendered benthic habitats intolerable to aquatic organisms (Nichols et al., 1979; Ginn and Pastorok, 1992; Huggett, et al., 1992; Sparks and Ross, 1992). Most of this work has focused on sediments with high contaminant levels which are acutely and consistently toxic to test organisms. Pinpointing low level toxicity and intermittent sediment toxicity is a much more challenging endeavor. Whether the toxicity is continuous or intermittent, it results in habitats which are unsuitable for colonization

by benthic organisms. Both point and non-point sources which intermittently impact a river are acute insults from which the system may not recover due to the frequency of the events.

One organism which would be affected by such intermittent sediment toxicity is the unionid mussel. The sensitive juvenile life stage of the unionid inhabits depositional areas in rivers where sediment accumulates (Neves and Widlak, 1987). A documented decline in the diverse Cumberlandian mussel fauna of the Clinch River has prompted research into factors instigating this decline. While much work in this river system has focused on water quality, the potential for impairment of mussel recruitment by sediment-bound toxicants has not been investigated. This study was undertaken to determine if sediment-bound toxicants were present in the Clinch River, and if these toxicants could impair recruitment in mussel populations. Attention was directed to the depositional areas in riffle reaches of the river because this is the known habitat of the sensitive juvenile life stage.

A triad approach was used to determine the presence of sediment toxicants which would render benthic habitat unsuitable for recruitment of mussel populations. A sediment quality triad involves chemical analysis, laboratory sediment toxicity testing, and field validation using in-situ studies, such as macrobenthic community structure analysis (Chapman et al., 1992). This study describes the chemical analysis,

physical characterization and laboratory toxicity testing of sediment from the Clinch River. The information gained from these two aspects of the triad approach will be combined with the third component, field validation, to determine if sediment toxicants are implicated in decreasing mussel recruitment in the Clinch River.

METHODS

Sediment tests were conducted from June, 1992 to October, 1993 to determine if sediment toxicity existed in the Clinch River, Virginia. Two of the twelve sites included in testing were considered reference sites (Fig. 1, Table 1). Pounding Mill and Hackneys Farm were chosen as reference sites because of abundant or diverse unionid mussel populations. Nine sites in the Clinch River and one site in the Guest River, a tributary of the Clinch, were selected for testing due to known or suspected impacts. Sediments for testing were sieved on site, to remove organisms and any gravel greater than 2 mm, and transported to the laboratory on ice. Five-day old *Daphnia magna* and second-instar *Chironomus riparius* were tested together for 10 days in 2-L beakers. A 1:4 sediment water ratio was maintained in all tests. *Daphnia magna* were fed a tri-algal suspension consisting of *Chlorella sp. Ankistrodesmus sp. and Chlamydomonas sp.* (Foe and Knight, 1986) at the rate of 3 ml/day for the first 5 days and 4 ml/day thereafter. Prior to September 1993, tests were

aerated and the chironomids were fed a tetramin suspension. After September 1993, midges were fed a trout chow suspension and tests were not aerated. The feeding rate for both tetramin and trout chow was 0.2 ml every other day. *Hyallela azteca* were tested in 350-ml bioassay dishes with 50 ml of sediment and 200 ml of Clinch River water for 28-day exposures. Amphipods were fed tetramin flakes prior to September 1993, and maple leaf disks thereafter.

Juvenile *Villosa iris* and *Corbicula fluminea* were tested in sediment from the Clinch River sites to determine whether sediments caused mortality or growth impairment in the bivalves, and to determine whether clams and mussels responded similarly so that juvenile *C. fluminea* could be used as a surrogate for unionids. Because juveniles were difficult to recover from sediments, they were tested in plastic tubes which had been fitted with 100um nitex over a large area to create a flow-through holding chamber. Ten 24-48 hour old clams and 6 3-5 day old mussels were used in each of three replicates from each of the 12 Clinch River sites. Clams and mussels were placed separately in the previously described tubes which were placed in 2-L beakers containing 200 ml of sediment and 800 ml of Clinch River water. The beakers were aerated gently during the 10-day test. The previously described tri-algal suspension was placed in the tubes at the rate of 1 ml per day. The organisms were fed during the test

primarily because organic content of the sediment was variable and if food had become limiting this could have affected growth of the organisms. After 10 days the tubes were removed and the organisms were rinsed into a petri dish and checked for mortality. Both mussels and clams were then preserved in 70% alcohol for measurement. Organisms were measured using an ocular micrometer on a Zeiss dissecting microscope. Growth was determined by subtracting original height (mussel) or width (clams) of the valves from the final measurement. The mussels quickly formed growth rings, so the original length of the mussel was determined by measuring the juvenile valve prior to growth rings. Since growth in juvenile clams was not readily seen, the original size was determined by averaging the widths of 50 juveniles preserved at the beginning of the test to give an estimate of initial length.

Mortality for all organisms, growth of the chironomids, *H. azteca*, *V.iris* and *C. fluminea,* and reproduction of the daphnids, were analyzed using a Kruskal-Wallis Test, and multiple comparisons were made between groups using a least significant differences procedure on the ranked data. A significance level of $p < 0.05$ was used for all statistical comparisons. Prior to September 1993, the reference site for statistical analysis was considered to be Pounding Mill or a nearby site, Cliffield. However, due to variation in the response of the organisms, a water reference was added in

later tests. These water references mimicked culture conditions for the three species and allowed comparisons to be drawn between test dates where the organisms exhibited similar degrees of fitness.

Particle size distribution was determined gravimetrically (Gee and Bauder, 1986). Percent water content (Black, 1986) and percent volatile solids (Plumb, 1981) were also determined for sediments from each of the Clinch River sites.

RESULTS/DISCUSSION

Physical characterization of the sediment from the twelve Clinch River sites revealed few differences. There was little variation in the percent water content of sediments which ranged from 19.98% - 24.54% water (Table 2). Similarly, there were no substantial differences in the silt and clay contents at the 12 sites. However, Clinchport, Raven and Cedar Bluff had significantly lower sand content than Pounding Mill. Volatile organic compounds were significantly higher than the upstream reference site at St. Paul (3.92% volatile compounds), which may account for the high chironomid weights at that site in the 9-3-93 test (Fig. 2) and the apparent enrichment in the screening test on 10-12-92 (Table 3). The Bulldozer Crossing and Carterton sites also had high volatile organics (2.57 and 2.33, respectively) which were not reflected in the daphnid and midge responses on test dates 6-12-92, 9-3-93, and 10-8-93 (Fig. 3), but may have been

reflected in the 7-26-92 screening test.

Only in rare instances was mortality of the organisms significantly high when testing Clinch River sediments. For this reason mortality was not useful as an endpoint. Significance in the growth and reproduction endpoints also was seen infrequently due to the tendency of the references to fall in the middle of enriched and impaired sites.

Screening tests run between July and November, 1992 revealed trends in sediment toxicity at the Clinch River sites. The Guest River site impaired both reproduction of *D. magna* and growth of *C. riparius* (Table 3). The Raven site consistently impaired the daphnids, having at least 50% mortality in both screening tests, but did not impair the midges. Cedar Bluff sediments impaired *D. magna* reproduction by greater than 50% in the 6-12-92 screening test, but impairment to midge growth was slight. However, in the 8-19-92 screening test, midge growth was impaired but *D.magna* reproduction was not. Sediment from the Carterton site also was intermittently toxic to both the daphnids and the midges but not simultaneously. In the 6-12-92 screening test, *D. magna* reproduction was almost 50% lower than the controls, while the midge weights were not lower than the reference organisms. In the 7-26-92 test, midge weights appeared impaired, while *D. magna* reproduction was greatest in sediments from the Carterton site. The high volatile organic

content (Table 2) of the St. Paul sediments may have been reflected in the high reproduction of the daphnids but was not reflected in the midge weight in the screening test on 10-22-92. Both organisms in the Burtons Ford sediments had higher mortality that the reference organisms. *D. magna* reproduction and *C. riparius* growth were impaired in sediment from this site as well.

The results of these early screening tests indicated two major trends: (1) the sites exhibited variability in the responses between the organisms and between test dates, and (2) the variability in the responses of the reference organisms was unacceptably high. It was clear from these results that sediments from the Clinch River impaired laboratory animals albeit inconsistently. It was also evident that clarification of the causes and results of sediment toxicity in the Clinch River would be complicated by the inconsistent toxicity. While intermittent toxicity does not render an area consistently uninhabitable, it would result in a cycle of disturbance and recovery events where the degree of recovery would be dependent on the frequency, duration and intensity of the insults.

Sediment tests were run to determine the degree of toxicity at some of the sites which showed intermittent impairment in the screening tests. Sites chosen for the more extensive testing were those that had the most consistent

impairment in screening tests. These sites were Cedar Bluff, Raven, Bulldozer Crossing and Guest River. It was thought that a serial dilution, where sediment from the impaired site was cut with clean sediment, would result in a dose dependent response. As seen in the screening tests, some tests impaired *D. magna* reproduction at the highest concentration in the dilution series but failed to cause a response in the *C. riparius* (Table 4). As expected from the intermittent impairment in screening tests, some tests had no impairment at the highest concentrations of potentially toxic sediments. Even though Guest River sediments had been toxic on the 6-12-92 testing date, with *D. magna* reproduction only 11% of the reference site reproduction (Table 3), three separate serial dilution tests failed to demonstrate any impairment to either organism in sediments from this site (Table 5). One of the more surprising results in these serial dilution tests was the tendency for increased toxicity in the intermediate range of the dilution series. This is seen in the daphnid reproduction at the 12.5% Bulldozer crossing sediments on the 7-12-92 test date (Table 4) and in the 25% Raven sediment on the 4-10-93 test date (Table 6). This increased toxicity in the intermediate concentrations was often consistent for *D. magna* reproduction, and *C. riparius* and *H. azteca* growth (Table 7). This toxicity may have been due to the mixing of the sediments which would have allowed any bound toxicants to release and

rebind in the mixed sediment until a new equilibrium was reached. While this process was underway, the toxicants may have been available to the organisms. Sediment characteristics such as particle size and total organic carbon would also have been altered and could have affected the toxicity. However, the physical parameters of the sediments were similar enough (Table 2) that this mode of exposure to toxicants warrants further investigation. If mixing with clean sediments increases toxicity in this manner, then impairment would be expected to be greater downstream of a point source impact where mixing occurs and similarly, toxicity may be expected to increase as toxicants leach from the sediments, creating open binding sites after an insult.

The intermittent toxicity observed in the Clinch River was thought to be related to rain events because of field notes regarding the weather and flow conditions. To further ascertain if rain events were involved, two additional screening tests incorporating all twelve sites were run. In the screening tests on 9-3-93 (Fig. 2) and 10-8-93 (Fig. 3) the daphnids in the water controls had an average of 298 and 331 neonates, respectively. The daphnids in test 9-3-93 all had between 90 and 135% of the reference brood size. The sediment for this test was collected under low flow conditions when the area had been without a significant rain event for several weeks. The sediment for the 10-8-93 screening test

was collected under higher flow conditions following a rain event. Daphnid reproduction throughout the test fell to between 40 and 80% of the water reference (Fig. 4). While midge growth dropped from upwards of 130% to more than 90% of the reference in the 10-8-93 test, the daphnid response was substantially greater. As was noted earlier, midges often failed to respond negatively when daphnid reproduction is impaired. The response of *H. azteca* was variable and did not appear to correlate with either the growth of the midge or the reproduction of the daphnids. *D. magna* has been found to be the most sensitive animal used for sediment tests, especially to metals (Nebeker et al., 1984). Both the daphnids and midges are more sensitive than *H. azteca* (Giesy, 1990).

Due to the intermittent nature of toxicity in the Clinch River sediments, it becomes necessary to test the sediment on several dates and compare test organism responses through time. A water reference allows for these comparisons by ensuring the consistency of the test organism fitness. When the Guest River site is examined through time (Fig. 5a) it is evident that both the midges and daphnids sometimes exhibited no differences from the reference organisms and at other times indicated impairment or enrichment. At the Carterton site, the daphnid reproduction was impaired on 7-8-92 and 10-8-93 but reproduction was elevated on 7-26-92 (Fig 5b). Similar variability of responses were seen at the Raven site were D.

magna reproduction was impaired on two test dates while the midge growth appeared to be enhanced here on two test dates (Fig. 5c). At the Bulldozer crossing site, both midge growth and daphnid reproduction were greater than the reference on three test dates but daphnid reproduction was impaired on 10-8-93, following a rain event (Fig 5d).

Not only do responses differ on different test dates but also within a test. At the Guest River site on 7-6-92 and 10-8-93, daphnid reproduction was impaired but the midge failed to respond negatively to the toxicity (Fig 5a). Similar results were seen at the Carterton site (Fig 5b). The differences in the midge and daphnid sensitivity may be due to the toxicants present which are often metals in the Clinch River. Metals analysis performed on sediments collected from each of the twelve sites on three different dates revealed fluctuating levels of metals such as copper, lead, and zinc (Fig. 6). These temporary changes in metal concentrations in the sediments may account for variation in the organism responses on different test dates. While some results such as enrichment at St. Paul and impairment at Carterton can be related to point source discharges from municipal and industrial effluents, non-point sources such as road and agricultural runoff and mining inputs, which enter the system during rain events, may account for a large part of the intermittent toxicity in the Clinch River.

The mussel and clam sediment testing did not result in clarification of the degree of sediment toxicity in the Clinch River. While growth of the mussels was impaired at the Raven site, it was not impaired at the Bulldozer crossing or Carterton sites, as would be expected from the toxicity in the previous tests (Fig. 7). The high growth at these two sites may reflect the high organic content of the sediments there. However, the growth impairment at the St. Paul site was unexpected due to the high organic content of the sediments there. The highest growth was seen at the Guest River site which had consistently shown impairment in previous testing. Due to the intermittent nature of the toxicity, more testing needs to be done with sediments from these sites do determine if sediments are toxic to unionids.

The differences between the responses of *V. iris* and *C. fluminea* were varied. While the asian clam juveniles also showed low growth at the St. Paul site, they were not impaired at the Raven site but exhibited low growth at the Cedar Bluff site (Fig. 8). Failure of the two bivalves to respond similarly in sediments from the Clinch River sites indicates that juvenile *C. fluminea* are not good surrogate test organisms for the native unionids. Little information on the sediment toxicity was gained in the mussel and clam bioassays, probably due to the intermittent toxicity. For this reason, in-situ testing using juvenile unionids is recommended during

a rain event to determine if the intermittent toxicity would impair recruitment in mussel populations. Benthic community structure analysis should also be examined to determine if intermittent toxicity is causing alteration of instream biota.

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Table 1. Sampling sites and locations, with abbreviations used on the map and graphs.

Clinch River Site	River Mile	Abbreviation
Pounding Mill	327.5	PM
Cedar Bluff	319.5	CB
Raven	310.0	RVN
Van Dykes	305.5	VD
Hackneys Farm	269.0	HCK
Bulldozer Crossing	267.3	BDX
Carterton	264.1	CRTN
St. Paul	256.4	STP
Burtons Ford	249.7	BF
Dungannon	235.0	DGN
Clinchport	213.2	CPT
Guest River Site		
Coeburn	6.0	GST

Table 2. Physical characteristics of the sediment from the Clinch River sites.

Site	% water content	% volatile compounds	% particle size distribution sand/clay/silt
Pounding Mill	21.81	1.627	86.2 / 0.1 / 14.6
Cedar Bluff	21.27	1.511	78.0* / 0 / 20.8
Raven	22.95	1.544	79.5* / 0.9 / 21.2
Van Dykes	22.92	1.656	83.7 / 0 / 16.3
Hackneys Farm	22.57	1.181	88.0 / 0 / 16.7
BDX	22.86	2.567*	84.1 / 0 / 17.7
Carterton	23.26	2.334*	84.2 / 0 / 13.1
St.Paul	24.54	3.920*	84.7 / 0.1 / 15.8
Burtons Ford	19.98	1.438	82.0 / 0 / 18.0
Guest	22.38	1.279	87.0 / 0 / 14.7
Dungannon	20.97	1.603	86.8 / 0 / 14.1
Clinchport	20.32	1.488	78.6* / 0 / 21.8

* indicates significant difference at the $p<0.05$ level

Table 3. Screening tests of the Clinch River sites on selected dates. Pounding Mill is the reference site.

Site	*D.magna* mortality (% mortality)	*D.magna* reproduction (avg. # neonates/rep)	*C.riparius* mortality (% mortality)	*C.riparius* growth (avg. wt. in grams)
		6-12-92		
Pounding Mill	10	324	15	1.141
Cedar Bluff	20	150	5	0.955
BDXL	0	331	5	1.122
Carterton	15	167	5	1.071
Guest	5	37	20	0.937
		7-26-92		
Pounding Mill	35	62	13	0.594
BDX	15	96	1	0.658
Carterton	5	106	4	0.549
Raven	60	47	14	0.582

Table 3. continued

		8-19-92		
Pounding Mill	0	72	3	0.742
Cedar Bluff	30	60	19	0.584
Raven	50	33	11	0.723
		10-22-92		
Pounding Mill	13	209#	6##	1.124
St. Paul	10	291	13	1.104
Burtons Ford	43	177	16##	.974

Two replicates had *Corbicula* in the sediment which apparently ingested some *D. magna* juveniles.

Indicates replicates disregarded due to the presence of dipterans.

Table 4. Serial dilution test using sediments collected from Bulldozer Crossing cut with sediments from Pounding Mill. Test date is 7-12-92.

Site	*D.magna* mortality (% mortality)	*D.magna* reproduction (total # neonates)	*C.riparius* mortality (% mortality)	*C.riparius* growth (avg. wt. in grams)
PM	5	727	30	0.981
6.25% BDX	30	507	11	0.874
12.5% BDX	40	404	5	0.969
25% BDX	10	670	22	1.002
50% BDX	25	597	16	0.997
100% BDX	30	337	2	1.020

Table 5. Serial dilution tests using sediments collected from the Guest River cut with sediments from Pounding Mill.

Site	*D. magna* mortality (% mortality)	*D. magna* reproduction (total # neonates)	*C.riparius* mortality (% mortality)	*C.riparius* growth (avg. wt. in grams)
		8-21-92		
Pounding Mill	5	482	6	0.7221
6.25% Guest	5	580	8	0.9391
12.5% Guest	10	398[#]	14	0.8711
25% Guest	5	547	8	0.6537
50% Guest	5	614	7	0.9637
100% Guest	5	548	10	0.7363
		10-25-92		
Pounding Mill	23	643	16	1.094
12.5% Guest	27	603	25	1.023
25% Guest	3	896	9[##]	1.009
50% Guest	37	593	13	0.959
100% Guest	33	633	8	1.007

[#] some individuals were lost when plankton net overflowed.
[##] This concentration had one replicate which had a dipteran in it and the midges may have been eaten. This replicate had 32% mortality and was not included in the analysis.

Table 6. Serial dilution test using sediments collected from the Raven site cut with sediments from Cliffield, VA. Test date is 4-10-93.

Site	*D.magna* mortality (% mortality)	*D.magna* reproduction (total # neonates)	*C.riparius* mortality (% mortality)	*C.riparius* growth (avg. wt. in grams)	*H.azteca* mortality (% mortality)	*H.azteca* growth (avg. wt. in grams)
Cliffield	15	164	26.7	0.961	24.0	0.938
12.5% RVN	5	310	24.0	1.131	27.0	0.968
25% RVN	40	58	24.0	1.021	3.3	0.902
50% RVN	15	169	10.7	1.032	7.0	1.034
100% RVN	5	256	0	1.133	0.0	1.093

Table 7. Serial dilution test using sediments collected from the Cedar Bluff site cut with sediments from Cliffield, VA. Test date is 4-1-93.

Site	*D.magna* mortality (% mortality)	*D.magna* reproduction (total # neonates)	*C.riparius* mortality (% mortality)	*C.riparius* growth (avg. wt. in grams)	*H.azteca* mortality (% mortality)	*H.azteca* growth (avg. wt. in grams)
Cliffield	23.3	548	3.3	1.606	10	0.789
12.5% CB	26.7	490	16.7	1.648	16.7	0.858
25% CB	36.7	372	4.4	1.266	33.3	0.750
50% CB	36.7	375	3.3	1.468	3.3	0.855
100% CB	46.7	437	6.7	1.289	26.7	0.943

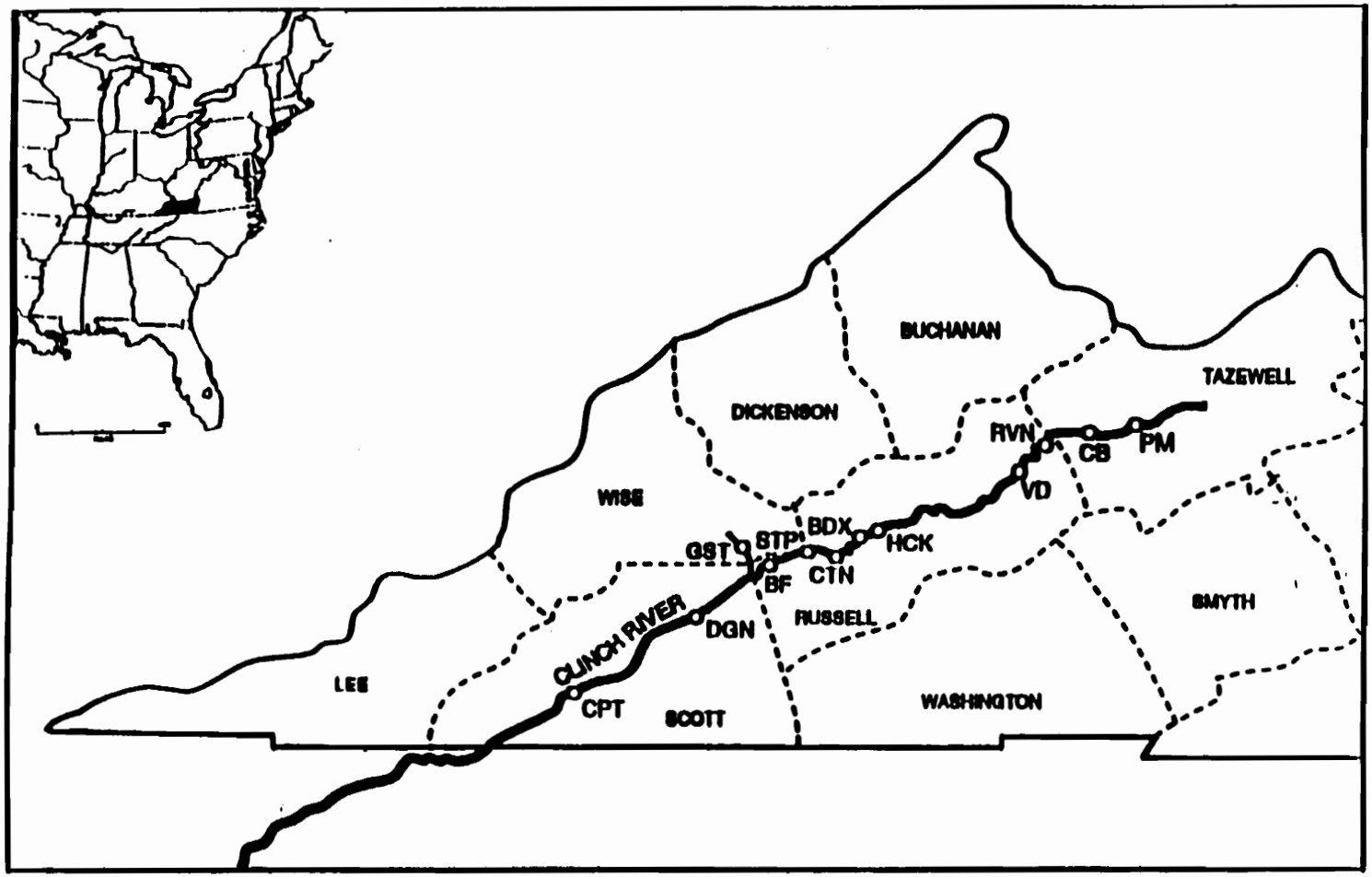

Fig. 1. Map depicting the location of the sampling area and the Clinch River sampling sites.

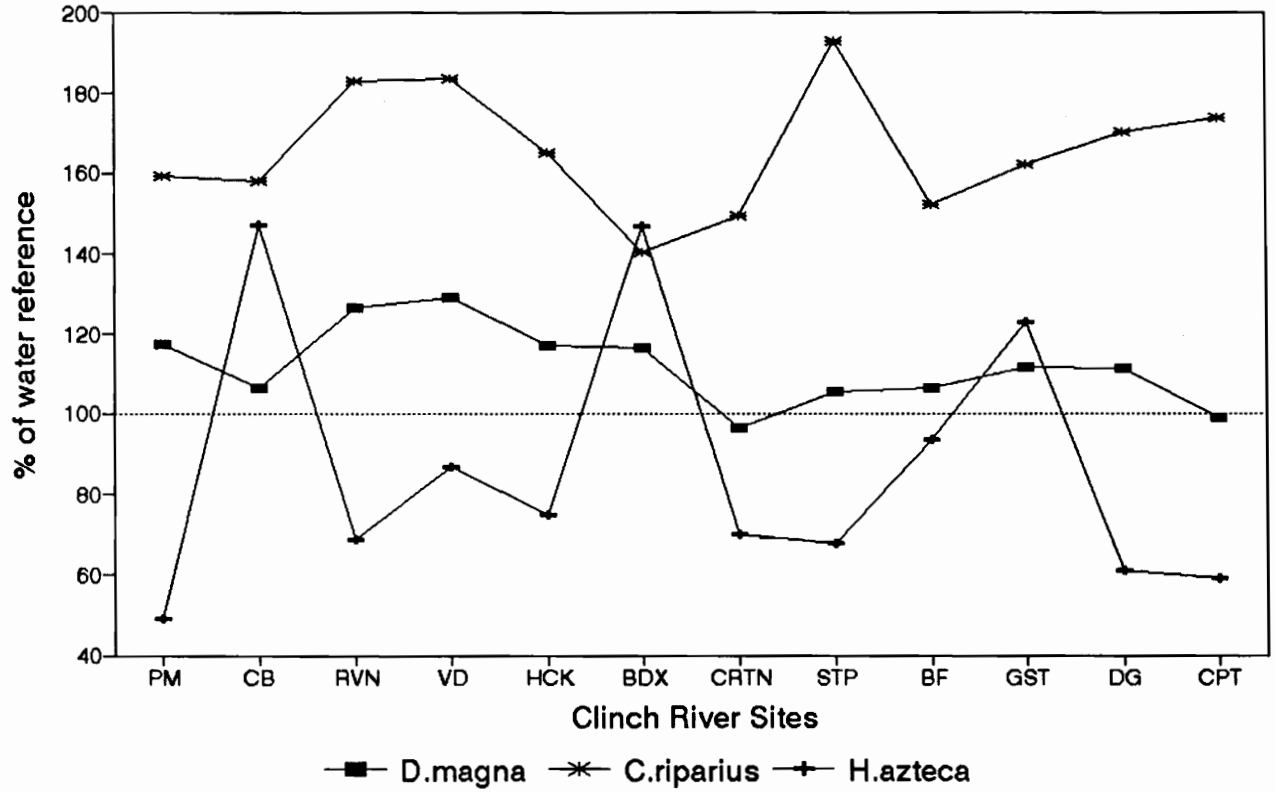

Fig. 2. Screening test using Clinch River sediments collected from each of the 12 sites on September 9, 1993. Sediments were collected under low flow conditions when there had been no significant rain event for 10 d. *C. riparius* and *H. azteca* growth and *D. magna* reproduction are expressed as a percentage of the water reference. Performance of the reference organisms is depicted with a line at 100%.

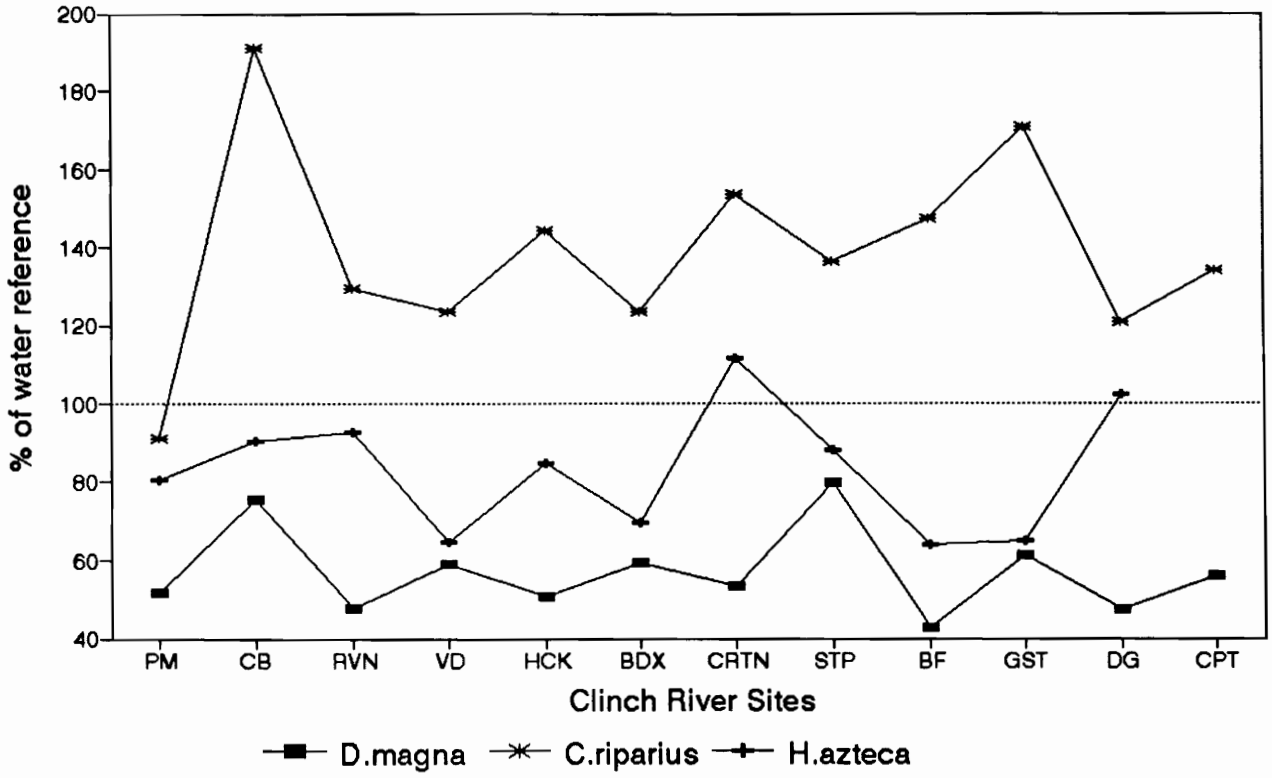

Fig. 3. Screening test using Clinch River sediments collected from each of the 12 sites on October 8, 1993. Sediments were collected under high flow conditions following a rain event. *C. riparius* and *H. azteca* growth and *D. magna* reproduction are expressed as a percentage of the water reference. Performance of the reference organisms is depicted with a line at 100%.

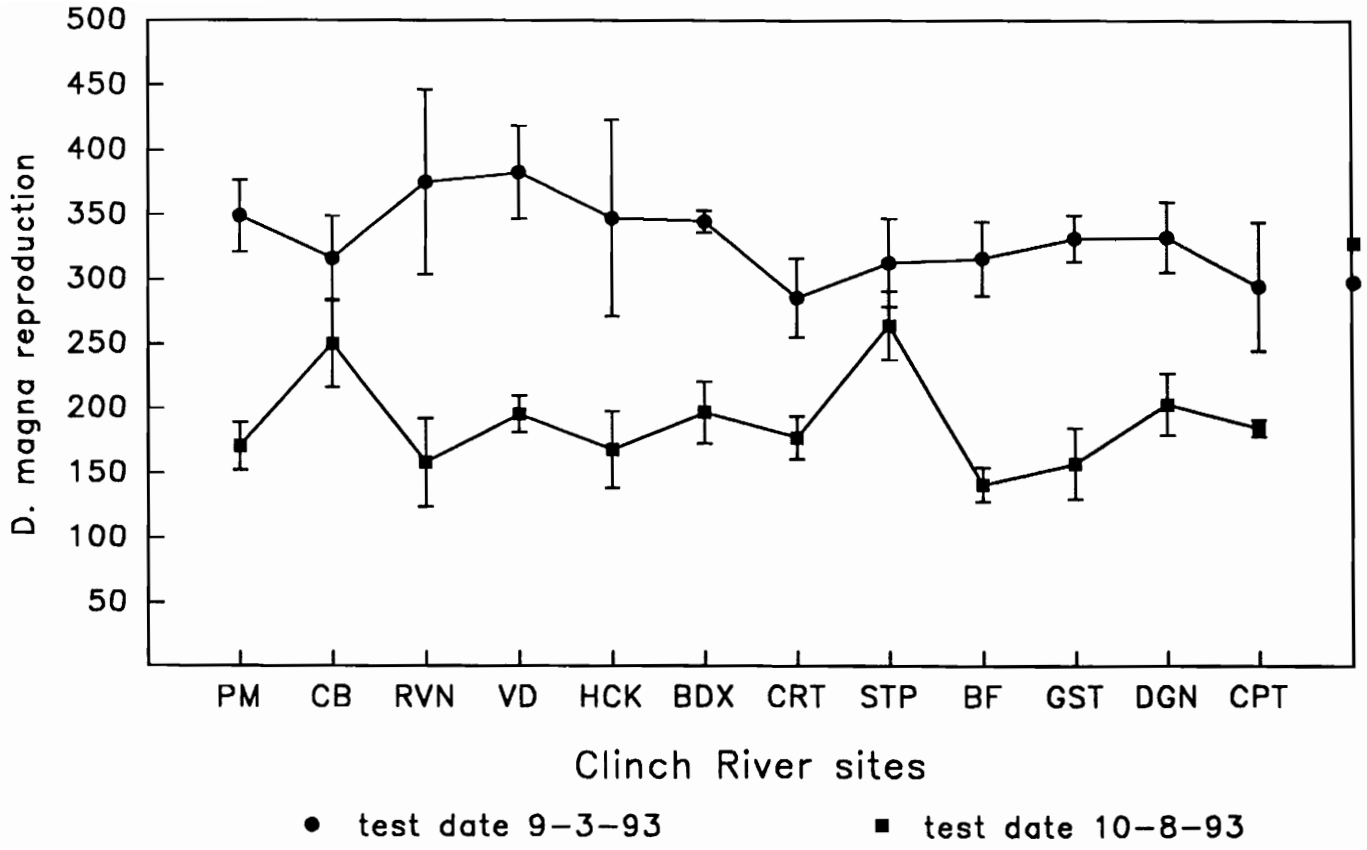

Fig. 4. Comparison of *D. magna* response to sediments from the 12 sites on two sampling dates. Sediments collected on September 9, 1993 were collected under low flow conditions. Sediments collected on October 8, 1993 were collected under high flow conditions following a rain event. Symbols on the right Y axis depict the response of the water references.

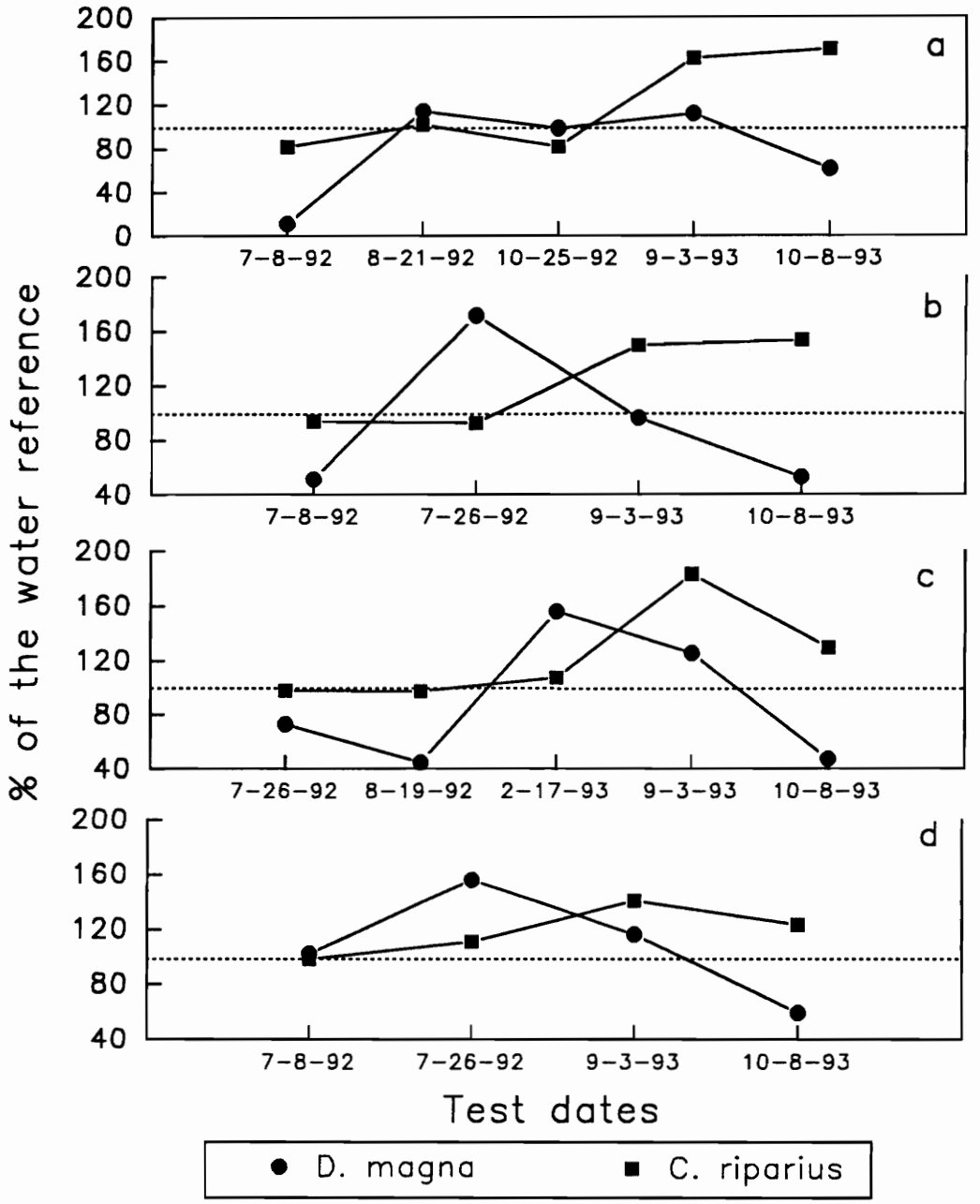

Fig. 5. Comparison of test organism response from Guest River (a), Carterton (b), Raven (c) and Bulldozer Crossing (d) on multiple test dates. *D. magna* reproduction and *C. riparius* growth are presented as percent of a water control. Performance of the reference organisms is depicted with a line at 100%.

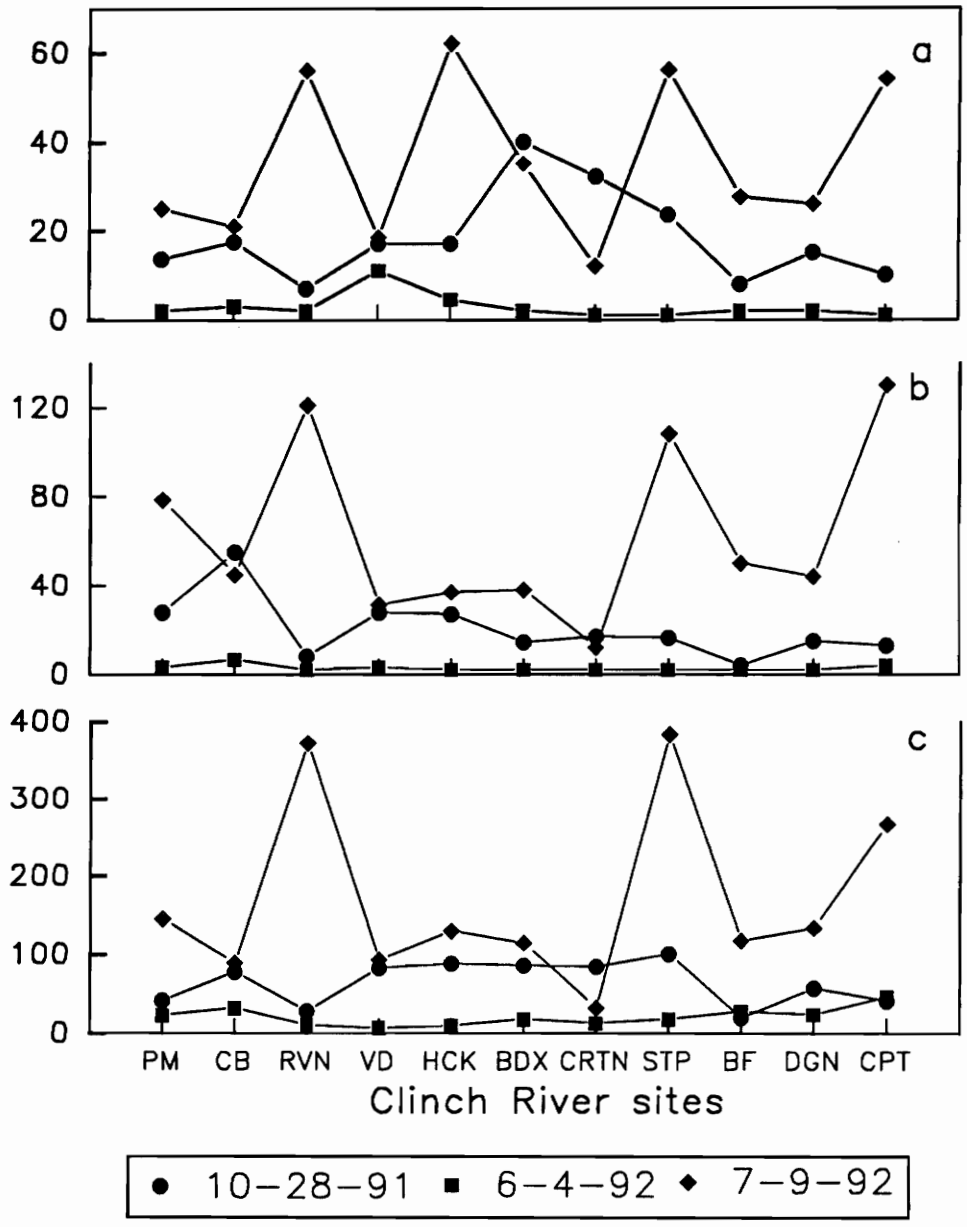

Fig. 6. Total recoverable metal concentrations of copper (a), lead (b) and zinc (c) from the unfiltered interstitial water collected at the Clinch River sites on three sampling dates.

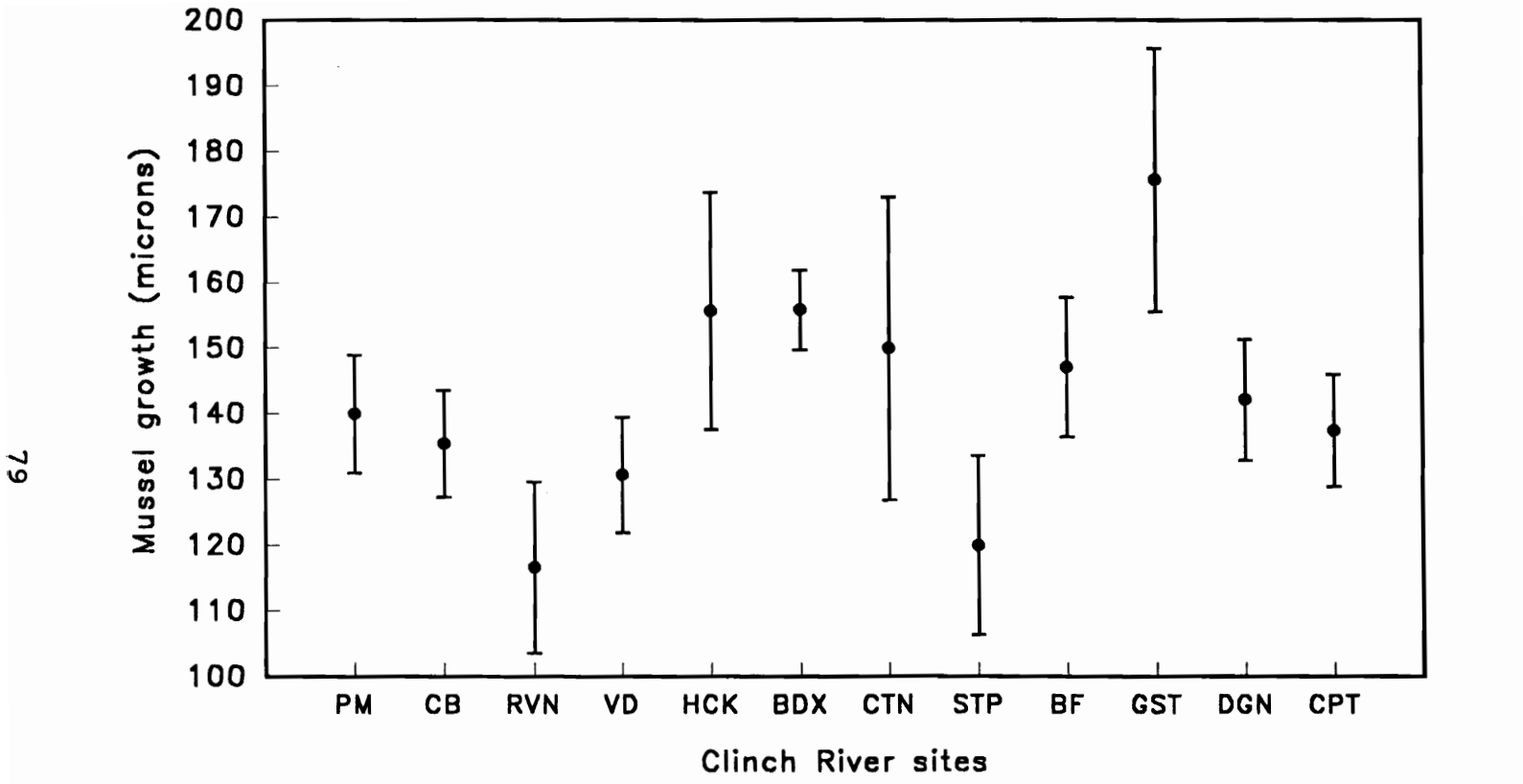

Fig. 7. Average growth and standard error of juvenile *V. iris* in a 10 day screening test using sediments from 11 sites on the Clinch River and 1 site on the Guest River.

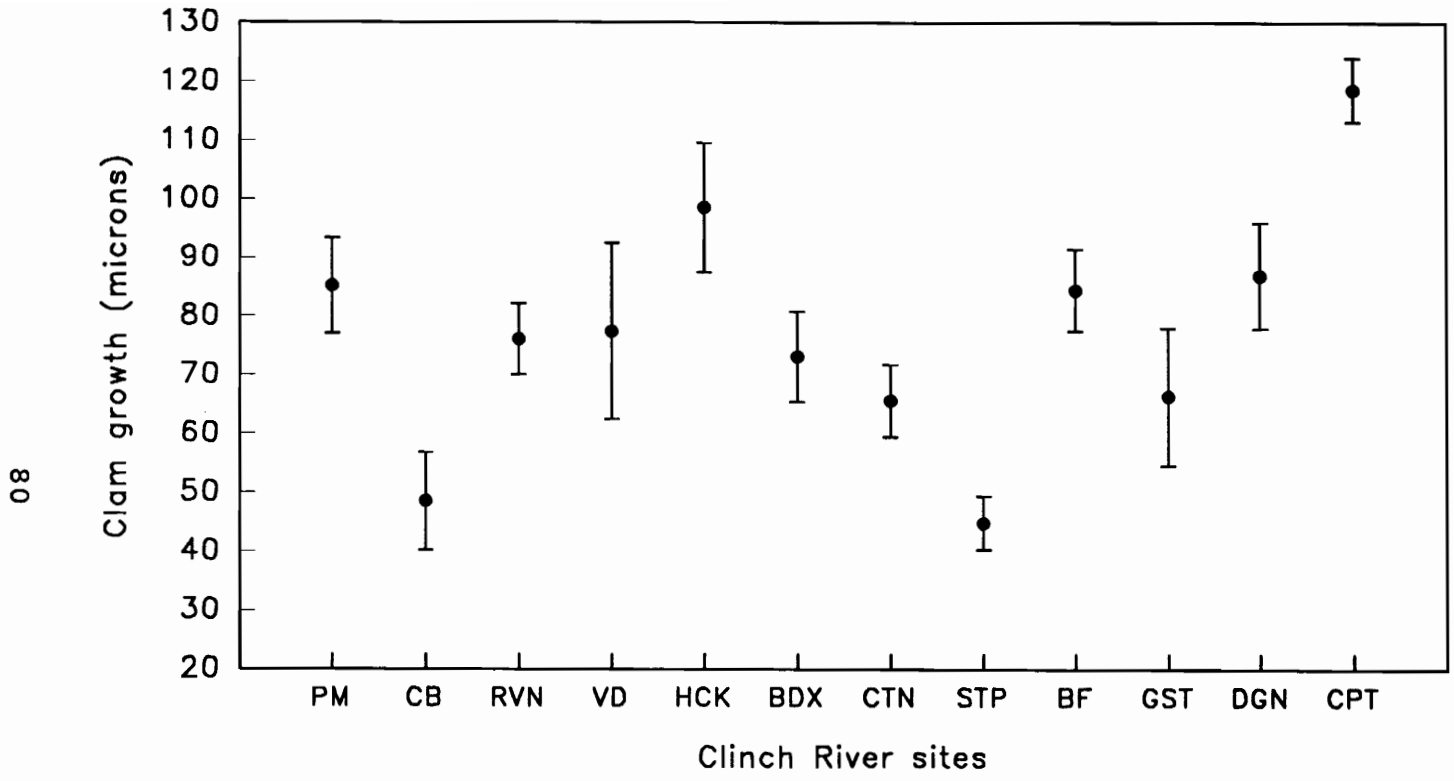

Fig. 8. Average growth and standard error of juvenile *C. fluminea* in a 10 day screening test using sediments from 11 sites on the Clinch River and 1 site on the Guest River.

CHAPTER 5

FIELD VALIDATION OF THE EFFECTS OF INTERMITTENT SEDIMENT TOXICITY ON BENTHIC COMMUNITY STRUCTURE AND GROWTH AND SURVIVAL OF *VILLOSA IRIS* (BIVALVIA: UNIONIDAE), IN THE CLINCH RIVER, VIRGINIA

ABSTRACT

In previous laboratory testing involving sediments from 11 sites in the Clinch River and 1 site in a tributary, the Guest River, intermittent sediment toxicity was found. Alteration of stream biota will be dependent on the frequency, duration and intensity of the acute insults which may be caused by runoff from rain events. This study validated laboratory findings by determining that intermittent toxicity was reflected in benthic invertebrate community structure and mussel density and richness. Significant reductions in invertebrate abundance were seen at 8 of the 12 study sites and 6 of these sites had reduced taxa richness as well. Mussel density and richness were also reduced at 10 of the 12 sites . In-situ testing using juvenile *V. iris* revealed significant mortality of test organisms at three sites and growth significantly lower than laboratory references at 10 of the 12 sites. Two of the sites, impacted by a coal-fired power generating facility, may be recovering following the

installation of a waste treatment facility which removes metals from the effluent. Quality of the upstream reference site appeared to decline throughout the two-year study period.

INTRODUCTION

Due to declines in the diverse Cumberlandian mussel fauna of the Clinch River, factors contributing these declines are being investigated. Because of the close association of juvenile mussels with depositional areas in river reaches with gravel and cobble substratum (Neves and Widlak, 1987), sediment-bound toxicants are a possible contributor to unionid declines. The propensity of sediments to bind toxicants could amplify low level chronic inputs or extend the period of exposure of juveniles to toxicants following acute insults (Salmons et al., 1987; Tessier and Campbell, 1987).

The type of toxicant present and the physical characteristics of the sediments can affect the bioavailability of toxicants in sediments; therefore, prediction of the impact on aquatic biota is difficult. For this reason, a triad approach is used (Chapman et al., 1992) to determine if sediment-bound toxicants are present and if the toxicants are affecting an ecosystem. This approach involves chemical and physical analysis of sediments, laboratory bioassays, and in-stream validation. The triad approach is particularly useful in areas of intermittent toxicity where the actual impacts on organisms and community

structure may be more severe than are indicated by laboratory bioassays and chemical analyses.

In the Clinch River, Virginia, intermittent sediment toxicity was found in laboratory sediment bioassays. Fluctuating levels of copper, lead and zinc indicate acute inputs of these metals which may persist in the sediments (Chapter 4). Industry, agriculture, mining and urbanization along the Clinch River may be contributing to point and non-point toxicant inputs. Collection of sediments in the river for laboratory testing, however, does not fully depict the impacts of intermittently toxic sediments on organisms and communities exposed to the intermittent toxicity.

The purpose of this research was to complete the triad approach in determining sediment toxicity by documenting if the intermittent toxicity seen in laboratory testing was reflected in the stream benthic community structure. In-situ juvenile mussel testing and community structure analysis of aquatic invertebrates were used to validate the laboratory findings. Mussel density estimates and benthic macroinvertebrate community structure were used to examine sites in the Clinch River where intermittent toxicity may be rendering habitat unsuitable for mussel survival. In-situ testing of juvenile mussels is the final determination of mussel recruitment inhibition by sediment-bound toxicants.

METHODS

Twelve sites, spanning 114.3 river miles, were examined based on the availability of background information indicating intermittent sediment toxicity at these sites (Chapter 4). Eleven sites on the Clinch River and one site in a tributary, the Guest River, were included in the field analysis (see Chapter 4: Fig. 1, Table 1). Two of the sites included in this study, Pounding Mill and Hackney's Farm, were considered reference sites.

Mussel Density and Richness

To determine mussel densities at the twelve sites, a 4 X 4 m grid was constructed from rope and divided into 4 quadrants each measuring 1 x 4 m. This grid was placed on the river bottom in a sand and gravel substratum and held in place using tent stakes. Mussel density was quantitatively determined by snorkeling through the quadrate and removing substratum to a depth of 15 cm. Mussels collected were measured, recorded and then replaced in the quadrant.

Benthic Macroinvertebrate Community Structure

Invertebrate samples were collected on August 10, 1993, using a Hess sampler to collect 1/10 m^2 samples at the twelve sites. Similar habitat was chosen in riffle reaches and the bottom of the river was raked using a hand-held gardening tool to dislodge the invertebrates. Four replicates were collected

at each site and immediately preserved in 10% formalin.

In the laboratory, samples were rinsed and the invertebrates were removed from the debris and placed in alcohol. All individuals were identified to the lowest feasible taxonomic level, usually genus. Site comparisons were for the made using analysis of variance procedures followed by the least significant differences method of multiple comparisons. The following parameters were compared for each site: abundance, taxa richness, Shannon-Wiener Index of species diversity (Shannon and Weaver, 1949), and Ephemerella/Plecoptera/Trichoptera (EPT) abundance and taxa richness. These EPT parameters are used to compare the groups of insects which are considered to be most sensitive to pollution (Plafkin et al., 1989).

Juvenile Mussel Procurement

Gravid *V. iris* were collected from the Clinch River at Cliffield, Virginia, and returned to the laboratory in water-filled coolers. The glochidia were removed with minimal harm to the adult by gently prying the valves apart approximately 2-3 mm, inserting a water-filled syringe into the gorged gill chamber, and flushing the chamber with water. The glochidia were checked for viability using a saturated salt solution (Zale and Neves 1982). Encystment onto 12-18 cm largemouth bass (*Micropterus salmoides*) was accomplished by placing the fish in a well-aerated 18.9-L aquarium for at least 30 min

with the glochidia from both marsupia of a female. When several fish were exposed together, they were left in the encystment aquarium for up to 120 min depending on glochidial density. The bass were kept in 500-L fiberglass tanks which were siphoned daily once the juveniles began dropping from the hosts.

In-situ Mussel Testing

Test chambers were constructed using aquarium uplift tubing which was cut away and fitted with 105 um nitex screening to create a flow-through holding chamber. Ten 1-2 week old *V. iris* were placed in each tube which was then fitted with a cotton plug and wired in place. Two sets of 4 tubes containing juveniles were maintained in a 2-L beaker of Clinch River water in the laboratory as a laboratory reference. These mussels were fed a detrital suspension containing silt, clay and organic fractions of sediment from an upstream reference site on the Clinch River, and a tri-algal suspension containing *Chlamydomonas*, *Ankistrodesmus* and *Chlorella* (Foe and Knight, 1986).

Sets of two tubes were placed in 1-L bottles of Clinch River water and transported to the river. Test tube racks, which were designed to hold nine tubes, were cut in half and wired onto bricks to secure the test chambers. On July 15 and 16, 1994, two bricks with two tubes, each containing 10 juveniles, were placed at the 11 sites in the Clinch River and

the Guest River site. Depositional areas behind large rocks were excavated and the bricks were placed in the area of low flow were the sediments would build up around the test chambers. Where necessary, depositional areas were created or augmented with boulders to create protected areas for the test chambers. The tubes were transferred underwater, to prevent the juveniles from being suspended and trapped in the cotton, into the test tube racks and wired into place. At the time of placement and recovery, water collected for water chemistry was transported on ice to the laboratory, and depth of the bricks and water temperature were recorded in the field. Due to high flow from rain events, retrieval of all of the test chambers was not possible after two weeks so all mussels were not in the river for the same amount of time (Table 1). However, an extra set of laboratory controls gave references for comparison of juveniles retrieved on July 29 and 31, 1994 (LR1) and August 6, 1994 (LR2). Replicates placed at the upstream reference site (Pounding Mill) were not useful for comparisons to other sites due to the impaired growth of juveniles at this site. Data were analyzed using a Kruskal Wallis procedure followed by least significant differences multiple comparisons of the ranked data.

RESULTS

Mussel Density and Richness

Mussels were found at seven of the twelve field sites

with the highest density of 8.5 mussels/m^2 at Pounding Mill. The second highest density, 1.75 mussels/m^2, was found at the intermediately located reference site, Hackneys Farm. These were followed by Dungannon, Clinchport, St. Paul, Van Dykes and Bulldozer Crossing each with less than 1 mussel/m^2. No unionids were found at Cedar Bluff, Raven, Carterton, Burtons Ford or the Guest River site (Fig 1).

Hackney's Farm had the highest species richness of all sites with 9 species collected there. Pounding Mill, St. Paul, Dungannon and Clinchport each had 4 species present while Van Dykes and Bulldozer Crossing had only one (Fig 1).

Mussels less than 20 mm were collected only at the reference sites (Table 2). Mussels less than 30 mm were collected at Pounding Mill, Van Dykes, Hackney's Farm, Bulldozer Crossing, St. Paul and Dungannon.

Benthic Macroinvertebrate Community Structure

Benthic macroinvertebrate abundance ranged from 460 to 3265 organisms per m^2 at the twelve sites. Pounding Mill had the highest abundance, with an average of 3265 organisms/m^2 (Fig. 2). Carterton, Burtons Ford and Hackneys Farm followed Pounding Mill with 2735, 2470, and 2298 organisms/m^2, respectively. All other sites had abundance significantly lower ($P<0.01$) than Pounding Mill, with the lowest abundances being found at Bulldozer Crossing, Van Dykes and the Guest

River site with 460, 553, and 650 organisms/m^2, respectively. Average abundance of species collected at each of the twelve sites is given in Appendix II.

Benthic macroinvertebrate taxa richness was also highest at Pounding Mill, which had 21 taxa collected (Fig. 3). Raven, Van Dykes, Bulldozer Crossing, St. Paul, Guest River and Dungannon were again significantly lower than the reference site ($p< 0.01$) with taxon richness ranging from an average of 11.5 taxa (Guest River) to an average of 15.5 taxa (St. Paul). Hackney's Farm, Cedar Bluff, Carterton, Burtons Ford and Clinchport were not significantly different from the upstream reference site.

Species diversity ranged from 1.957 (Van Dykes and Clinchport) to 1.105 (Guest River), with values of 1.893 and 1.695 at Pounding Mill and Hackney's Farm, respectively (Fig. 4). Only the Guest River site was significantly lower than the Pounding Mill site ($p<0.05$).

Stoneflies were collected only at Pounding Mill and Cedar Bluff. Mayflies were most numerous at the Burtons Ford site, while caddisflies were most abundant at the Guest River site. EPT taxa abundance was highest at Burtons Ford with 1770 organisms/m^2, followed by Hackneys Farm and Carterton having 1405 and 1335 organisms/m^2, respectively (Fig. 5). Pounding Mill had an average of 622.5 organisms/m^2 which was

significantly higher than Raven, Van Dykes, Bulldozer Crossing and the Guest River sites which had 342.5, 280.0, 237.5, and 202.5 organisms/m^2, respectively ($p<0.01$).

EPT taxa richness was highest (9 taxa) at the Burtons Ford site, followed by Pounding Mill with 8.75 EPT taxa present (Fig. 6). As seen in the total invertebrate taxa richness; Raven, Van Dykes, Bulldozer Crossing, St. Paul, Dungannon, and the Guest River site had the lowest EPT taxa richness values.

In-situ Mussel Testing

Due to the high flow conditions, not all tubes were retrieved on the same dates. The water depth was on average 32 cm higher when the juveniles were retrieved than when they were set out (Table 3). River temperature was cooler in the higher flow conditions. Except for higher conductivity at the Guest River site, there were no substantial trends in the water chemistry analysis of samples collected at the twelve sites during placement and recovery of mussels (Table 4). All of the tubes of mussels were retrieved except for the Raven and Van Dykes sites. At these sites two of the tubes were retrieved. Mussels retrieved on 7-29-94 and 7-31-94 were grouped for statistical analysis. Mortality was highest at the Clinchport site (58.1%) followed by Dungannon and Pounding Mill with 29.0% and 25%, respectively (Fig. 7). The mussels

retrieved after three weeks of exposure to Clinch River sediments had the highest mortality at the St. Paul site, which had a mortality of 6.25%. The laboratory references and the Burtons Ford site had no mortality.

Significant differences in growth occurred between the laboratory references and all sites retrieved after two weeks (Fig. 8). There were no significant differences after three weeks between the 3-week laboratory reference and the mussels retrieved from Burtons Ford and St. Paul. Growth was highest in the 3-week old laboratory reference which grew an average of 458 um. St. Paul and Burtons Ford #2 had the second highest growth rates which averaged 435 um and 425 um, respectively.

The 2-week laboratory reference had the highest growth of any mussels retrieved after two weeks (403 um) and was significantly higher than all sites in the river which were retrieved in this time period ($p < 0.05$) except for Burtons Ford #1 (Fig. 8). Mussels retrieved from Pounding Mill had the lowest average growth, 241 um, and subsequently this site was not used as reference for comparison to the others.

DISCUSSION

In previous laboratory testing, all sites except Cedar Bluff and St. Paul had intermittent impairment to *D. magna* in laboratory testing following a rain event. Cedar Bluff had demonstrated toxicity on separate testing dates. The toxicity

of sediment at these sites was intermittent resulting from a series of acute insults from which recovery would depend on the frequency, duration and intensity of the insults. The toxicity demonstrated may result from agricultural, mining and urban runoff which occurs during rain events. Research on the toxicity of urban runoff has shown that the primary exposure of runoff toxicity in rivers is through the river bed rather than the overlying water (Medeiros et al., 1983). Similarly, while the greatest amount of pesticide leaves the site in dissolved form, the greatest observed concentrations are often bound to suspended sediments (Douglas et al., 1993).

Field studies were conducted to determine whether the toxicity demonstrated in laboratory testing was reflected in the benthic community. Invertebrate community structure is widely used to evaluate the condition of lotic ecosystems and to characterize the areas of impact (Davis and Lathrop, 1992). In recent years it has been used as a sediment quality indicator (Burton, 1991). Invertebrate abundance was reduced at 8 of the 12 study sites and 6 of these also had impaired taxa richness. These data would indicate in-stream benthic impairment due to the previously described intermittent toxicity. Species diversity did not reflect the impairment seen in the previous indices with the exception of the Guest River site. The diversity index may not be lowered if the taxa richness and abundance are both decreased uniformly.

Richness and abundance of the sensitive EPT taxa are similar to the overall abundance and richness measurements. Raven, Van Dykes, Bulldozer crossing and Guest River consistently fall below the levels of the reference sites. Cedar Bluff, St. Paul, Dungannon and Clinchport also appear to be affected but to a lesser extent. Benthic community structure was not impaired at Burtons Ford and Carterton. The EPT taxa abundance is lower at Pounding Mill than at the intermediate reference site. While this is not significant alone, it may signify some type of impairment at this site allowing for the establishment of tolerant communities.

Mussel density and richness support the implications of the invertebrate data. Pounding Mill has the highest density and Hackney's Farm has the highest richness. The number of species should increase as the stream becomes larger, suggesting that the downstream stations should have higher richness than the upstream reference (Ahlstedt, 1984). This is not the case. St. Paul, Dungannon, and Clinchport all have 4 species like the upstream reference and do not reflect the trend towards higher richness seen at Hackneys Farm.

Van Dykes, St. Paul, Dungannon and Clinchport had only large mussels collected indicating no recruitment in the population. This would be expected in areas with intermittent sediment toxicity in the depositional areas. Both Pounding Mill and Hackneys Farm had juvenile mussels collected during

the snorkeling effort. A 2 - 3 year old *Lampsilis fasciola* was collected at the Bulldozer Crossing site. In the late 1960's and early 1970's two industrial spills from a coal-fired power generating facility located on the Clinch River decimated the mussel populations at the Bulldozer Crossing downstream to the Carterton Site. Due to these acute insults and chronic impacts from elevated copper concentrations in the power plant effluent, mussels have failed to recolonize here. In fact, it is probably the impacts from this power plant that have caused the impairment seen in the invertebrate community structure and the intermittent sediment toxicity. A multi-million dollar waste water treatment facility to remove metals went on line at the power plant in June of 1993 and has resulted in metal concentrations below background levels. The mussel found in this study was the first collected below the power plant since 1968 and may be evidence of recovery in that stream reach.

In-situ assays are an effective way to study the effects of sediment contamination and provide validation of laboratory results (Burton, 1991). In-situ bioassays are particularly useful when the toxicity is intermittent because they provide organism exposure to changing field conditions. Due to the intermittent nature of the previously observed sediment toxicity, it was hoped that the in-situ mussel testing would encompass a flow event. In fact, it rained in the area almost

continuously during the two week test. The river was rising as the test chambers were set into position and water levels did not recede until after the test was completed. While this made retrieval of the juveniles more difficult, it also provided maximum potential for point and non-point toxicant inputs due to run-off from agriculture, industry, mining and urbanization. Mortality was seen at Clinchport, Dungannon and Pounding Mill. While Pounding Mill had appeared to be a good reference in the previous summer's invertebrate sampling, it did not prove to be an adequate reference for this testing event. Runoff from a concrete facility and a nearby limestone quarry may be resulting in some impacts at this site. All sites, except St. Paul and Burtons Ford, were significantly lower than the laboratory reference which indicates that juvenile mussels are not reaching maximum size for that time period. Juveniles placed at the Bulldozer Crossing and Carterton had as much growth as Hackneys Farm, the intermediate reference site. This may be further evidence for recovery in that stream reach.

Due to the high flow conditions, some test chambers had to remain in the river for three weeks and did so without high mortality. In future studies, extending the length of the test may give more information on the chronic toxicity in a river system.

Field evidence indicates that the Cedar Bluff, Raven, Van

Dykes, Guest River, Dungannon, and to a lesser extent, St. Paul and Clinchport sites, appear to have some anthropogenic impacts which impair benthic macroinvertebrate community and mussel growth. The Bulldozer Crossing and Carterton have exhibited impairment but now show evidence of recovery. Burtons Ford, while not having signs of toxicity, does not have a healthy mussel population. Pounding Mill appears to be declining in habitat quality despite an apparently healthy mussel population. Overall, the field validation supports the findings of the laboratory sediment testing. Due to the intermittent nature of the toxicity, however, the in-stream assessment more accurately depicts the impacts of the toxicity.

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Table 1. Placement and retrieval dates, number retrieved and reference for comparison of the juvenile mussel in-situ test in the Clinch River.

Sites	Date placed in river	Date retrieved	# tubes retrieved	Reference for comparison
Pounding Mill #1	7-16-94	7-29-94	4	Lab reference #1
Pounding Mill #2	7-16-94	7-31-94	4	Lab reference #1
Cedar Bluff	7-16-94	7-29-94	4	Lab reference #1
Raven	7-16-94	7-29-94	2	Lab reference #1
Van Dykes	7-16-04	7-29-94	2	Lab reference #1
Hackneys	7-15-94	7-31-94	4	Lab reference #1
Bulldozer crossing	7-15-94	7-31-94	4	Lab reference #1
Carterton	7-15-94	7-31-94	4	Lab reference #1
St. Paul	7-15-94	8-6-94	4	Lab reference #2
Burtons Ford #1	7-15-94	7-31-94	2	Lab reference #1
Burtons Ford #2	7-15-94	8-6-94	2	Lab reference #2
Guest	7-15-94	7-29-94	4	Lab reference #1
Dungannon	7-15-94	7-31-94	4	Lab reference #1
Clinchport	7-15-94	7-31-94	4	Lab reference #1

Table 2. Mussels collected in the Clinch River, Virginia by snorkeling a 16 m^2 area.

	Number collected	Average length (mm)	Range (mm)
Pounding Mill			
Pleurobema oviforme	13	54.5	22 - 74
Medionidus conradicus	28	38.2	14 - 54
Villosa iris	93	37.8	25 - 52
Lampsilis fasciola	2	62	60 - 64
Van Dykes			
Villosa iris	2	29.5	29 - 30
Hackney's Farm			
Elliptio dilatata	8	84.3	66 - 93
Fusconaia barnesiana	3	69.3	57 - 76
Fusconaia cor	1	76	-----
Actinonaias pectorosa	4	99	85 - 107
Lampsilis ovata	1	112	-----
Medionidus conradicus	4	44.3	39 - 50
Villosa iris	2	22	16 - 28
Ptychobranchus subtentum	1	86	-----
Lampsilis fasciola	3	49.3	30 - 83
Bulldozer Crossing			
Lampsilis fasciola	1	24	-----
St. Paul			
Ptychobranchus fasciolaris	1	72	-----
Medionidus conradicus	1	25	-----
Amblema plicata	1	74	-----
Lampsilis fasciola	1	80	-----

Table 2. continued

Dungannon			
Fusconaia sp.	4	79.25	75 - 82
Amblema plicata	3	123	107 - 133
Ptychobranchus subtentum	3	88.7	70 - 111
Ptychobranchus fasciolaris	1	25	-----
Clinchport			
Actinonaias ligamentina	2	108.5	104 - 113
Cyclonaias tuberculata	1	101	-----
Actinonaias pectorosa	1	119	-----
Lampsilis fasciola	3	43.3	35 - 60

Table 3. Water depth and temperature at the 12 sites at the time of placement (P) and retrieval (R) of the in-situ test chambers.

Sites	Depth (cm)		Temp. (°C)	
	P	R	P	R
Pounding Mill	30.5	52.1	24	24
Cedar Bluff	16.5	38.1	24	22
Raven	19.1	55.9	24	24
Van Dykes	27.9	76.2	24	21
Hackneys Farm	19.1	38.1	28	24
Bulldozer Crossing	15.2	38.1	27	24
Carterton	30.5	76.2	27	24
St. Paul	39.4	58.4	27	24
Burtons Ford	33.0	67.3	27	24
Guest River	17.8	47.0	25	21
Dungannon	17.8	50.8	28	23
Clinchport	30.5	80.0	27	22

Table 4. Water chemistry from the 12 sites at the time of placement (P) and retrieval (R) of the in-situ test chambers.

Sites	Dissolved oxygen (mg/l)		pH		Conductivity (umhos)		Alkalinity (mg/l)		Hardness (mg/l)	
	P	R	P	R	P	R	P	R	P	R
PM	8.2	8.8	8.4	8.2	312	322	142	142	148	152
CB	8.5	8.8	8.5	8.2	294	281	134	88	140	116
RVN	7.85	8.5	8.0	8.0	320	292	105	120	140	130
VD	7.9	8.6	8.0	8.0	361	287	119	122	150	132
HCK	9.4	8.6	8.4	7.8	308	334	138	142	146	160
BDX	8.7	8.7	8.5	7.8	373	356	137	144	162	166
CTN	8.6	8.7	8.4	7.9	358	364	145	130	146	162
STP	9.5	8.6	8.3	8.0	365	314	144	128	152	146
BF	9.6	8.9	8.4	7.7	367	341	139	124	138	150
GST	9.5	8.7	8.5	7.7	543	302	110	60	232	120
DGN	8.9	8.8	8.3	7.7	493	352	138	132	208	156
CPT	9.2	8.6	8.3	7.7	384	322	135	122	166	144

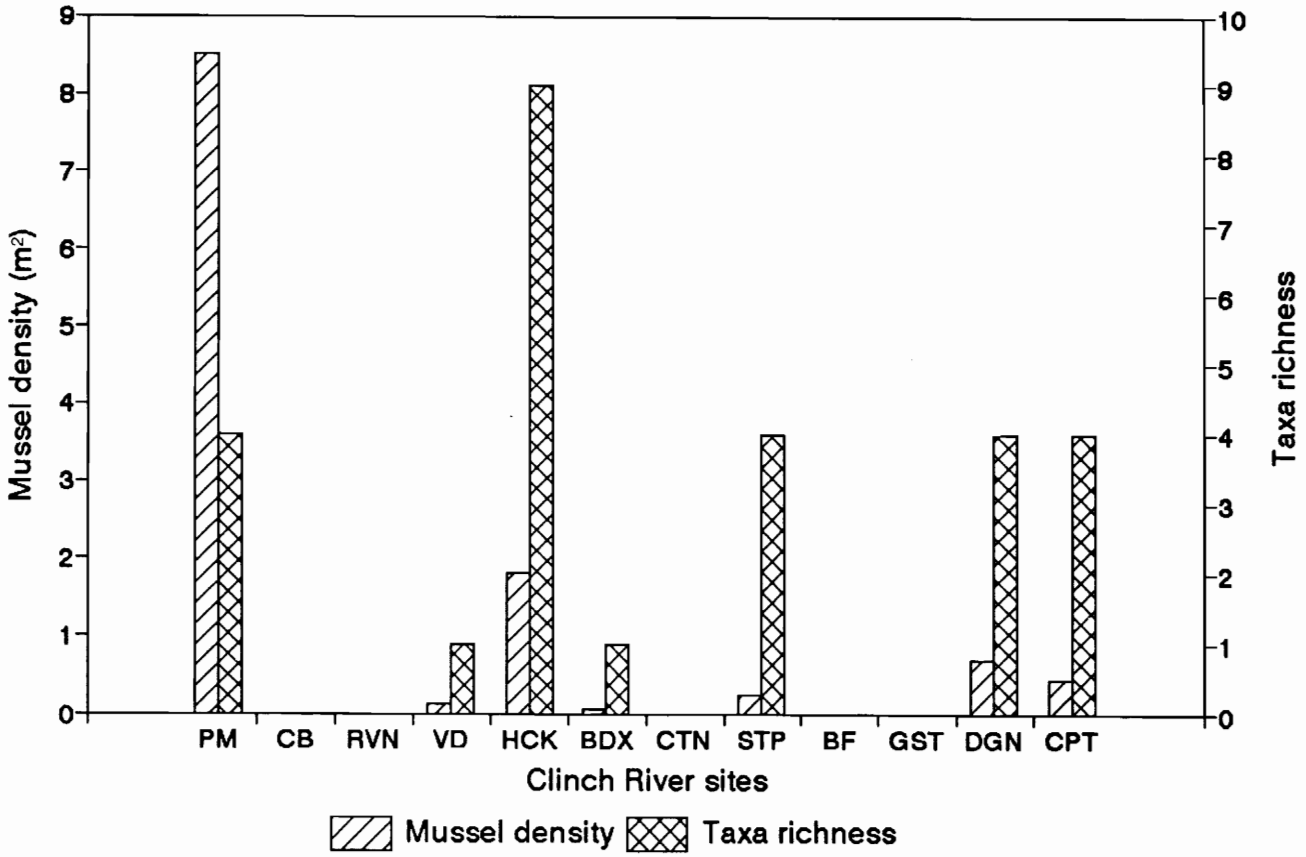

Fig. 1. Mussel density and richness at 11 sites on the Clinch River and 1 site on a tributary, the Guest River.

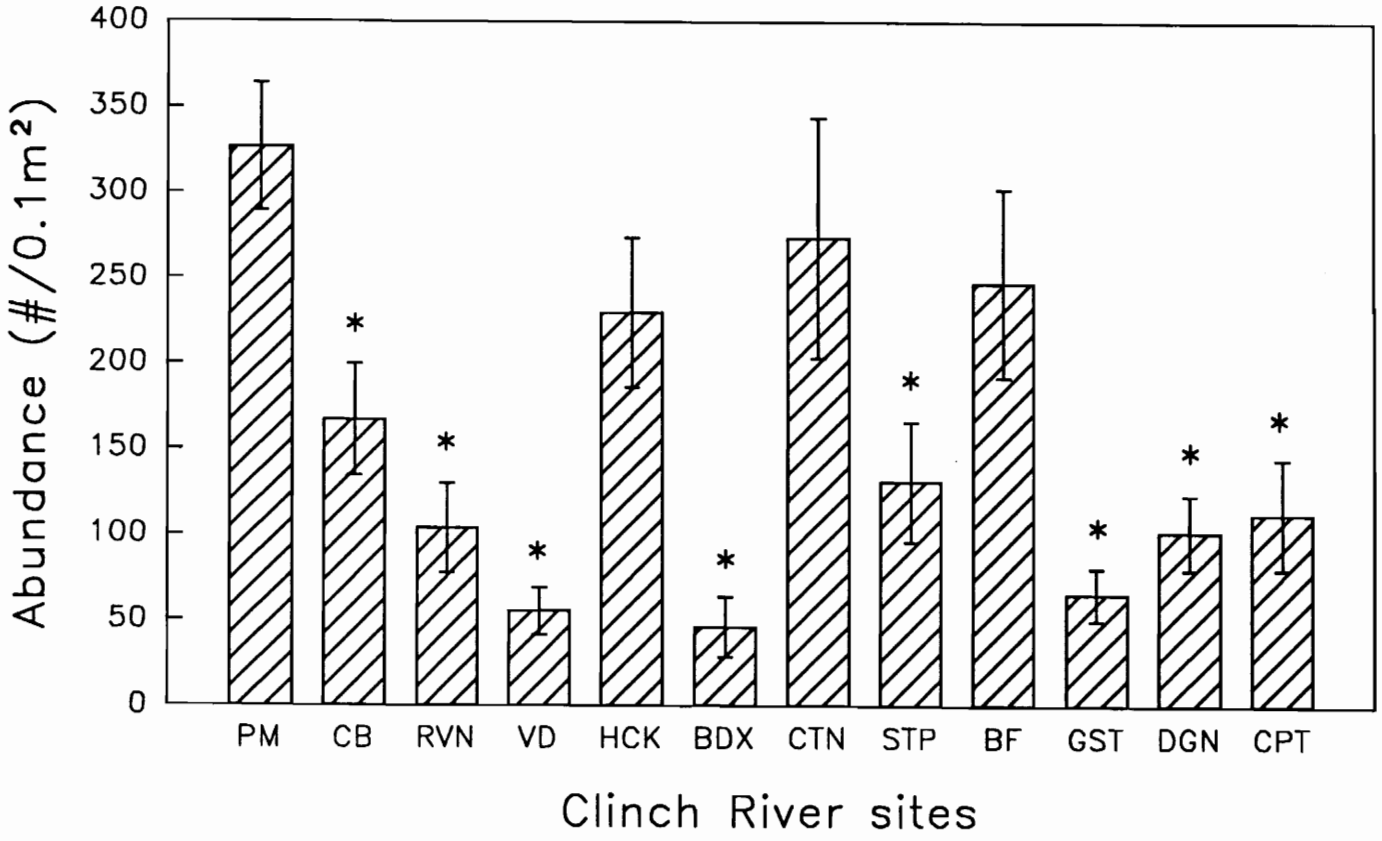

Fig. 2. Benthic macroinvertebrate abundance at the 12 sampling locations. Asterisk indicates significant difference from the Pounding Mill reference site at the p=0.05 level.

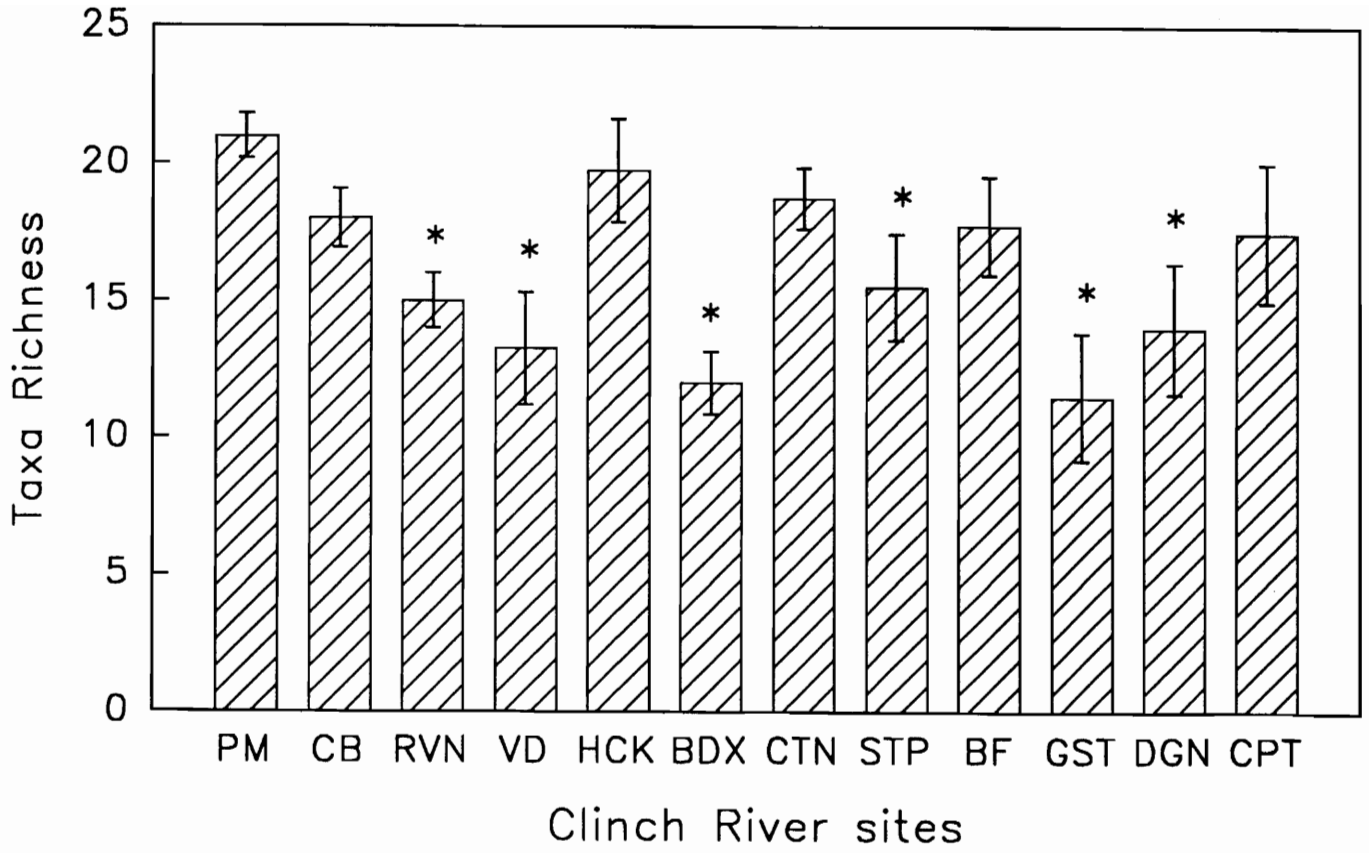

Fig. 3. Invertebrate taxa richness at the 12 sampling locations. Asterisk indicates significant difference from the Pounding Mill reference site at the p=0.05 level.

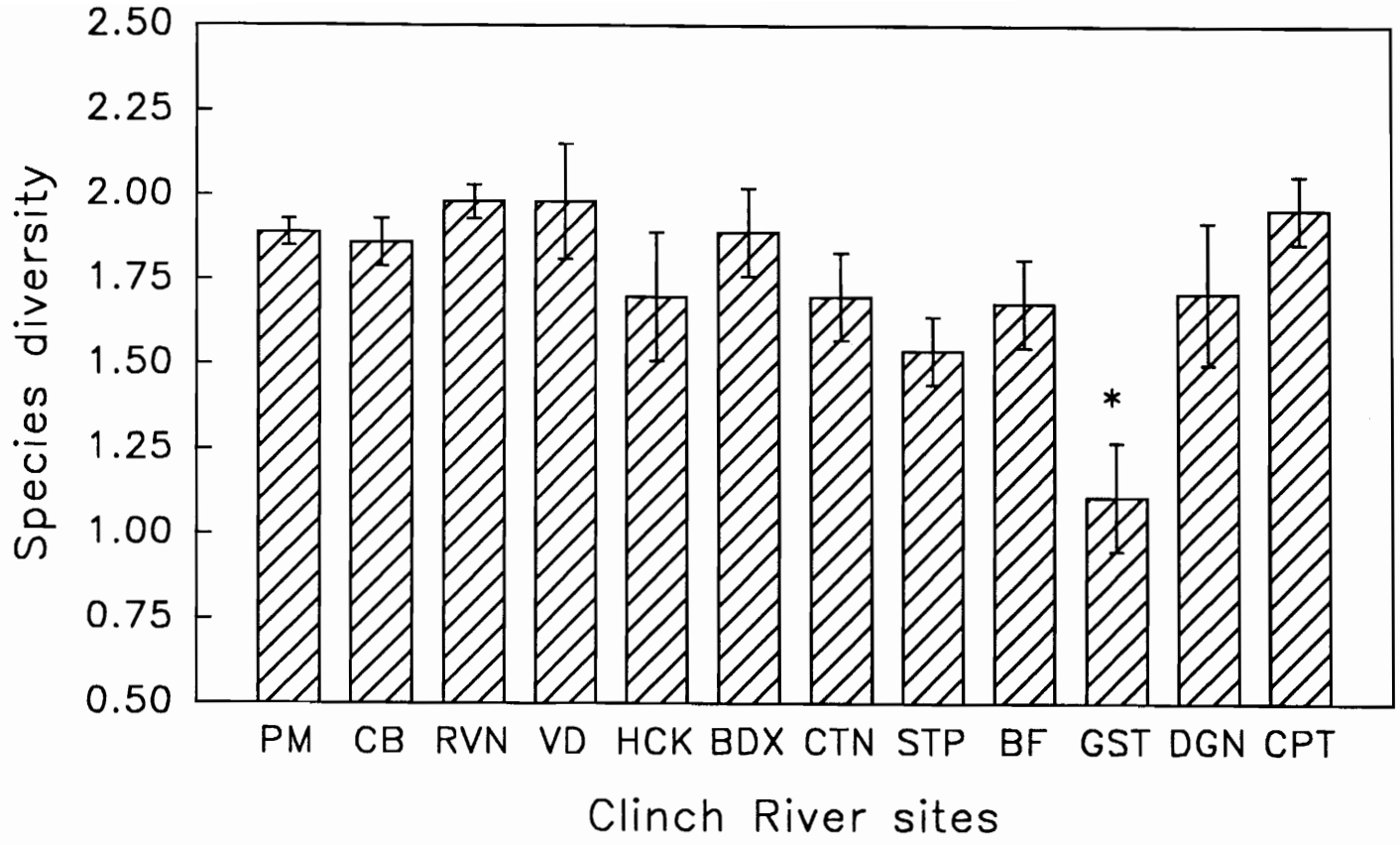

Fig. 4. Invertebrate species diversity at the 12 sampling locations. Asterisk indicates significant difference from the Pounding Mill reference site at the p=0.05 level.

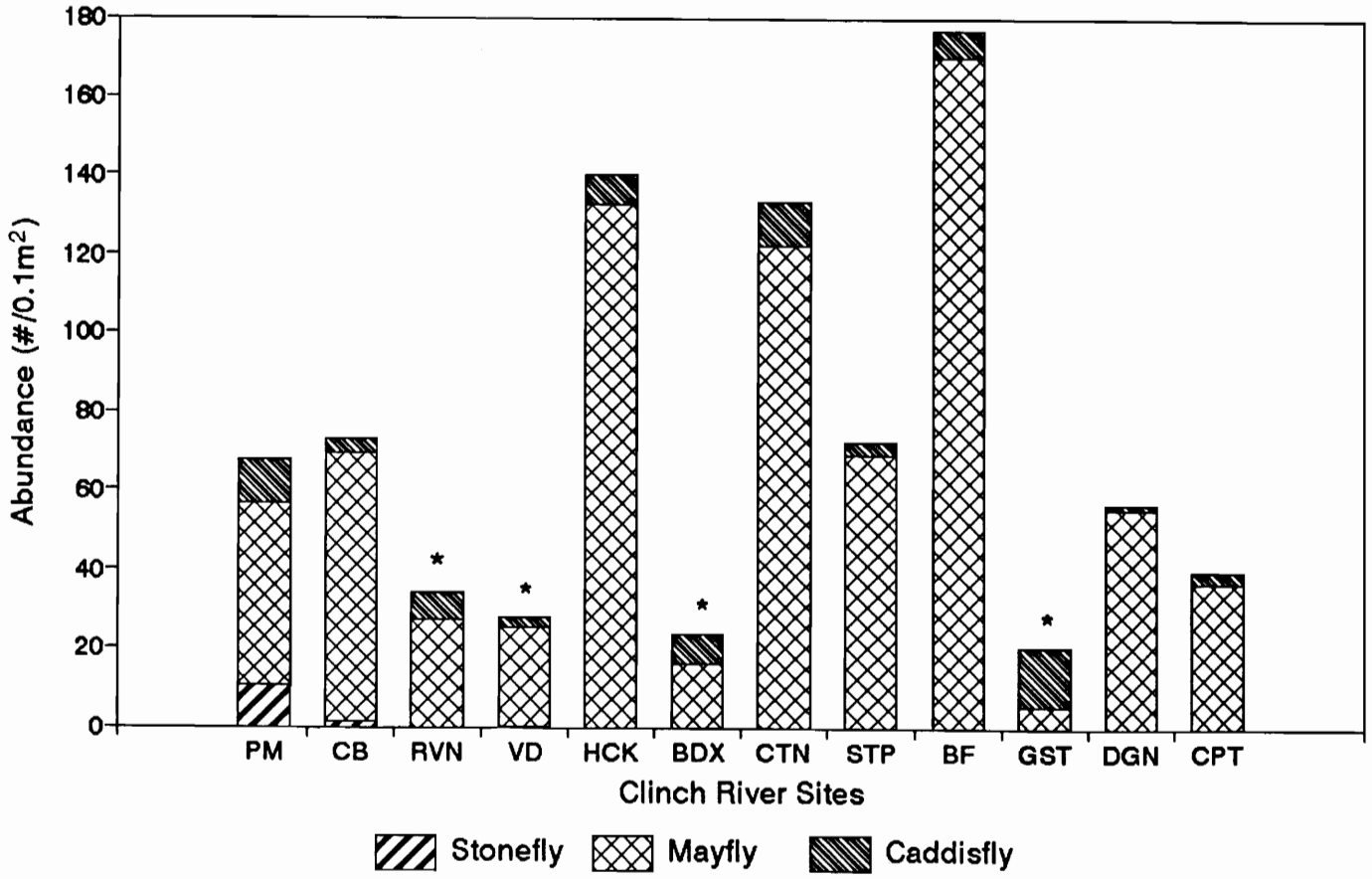

Fig. 5. Abundance of the EPT taxa at the 12 sampling locations. Asterisk indicates significant difference from the Pounding Mill reference site at the p=0.05 level.

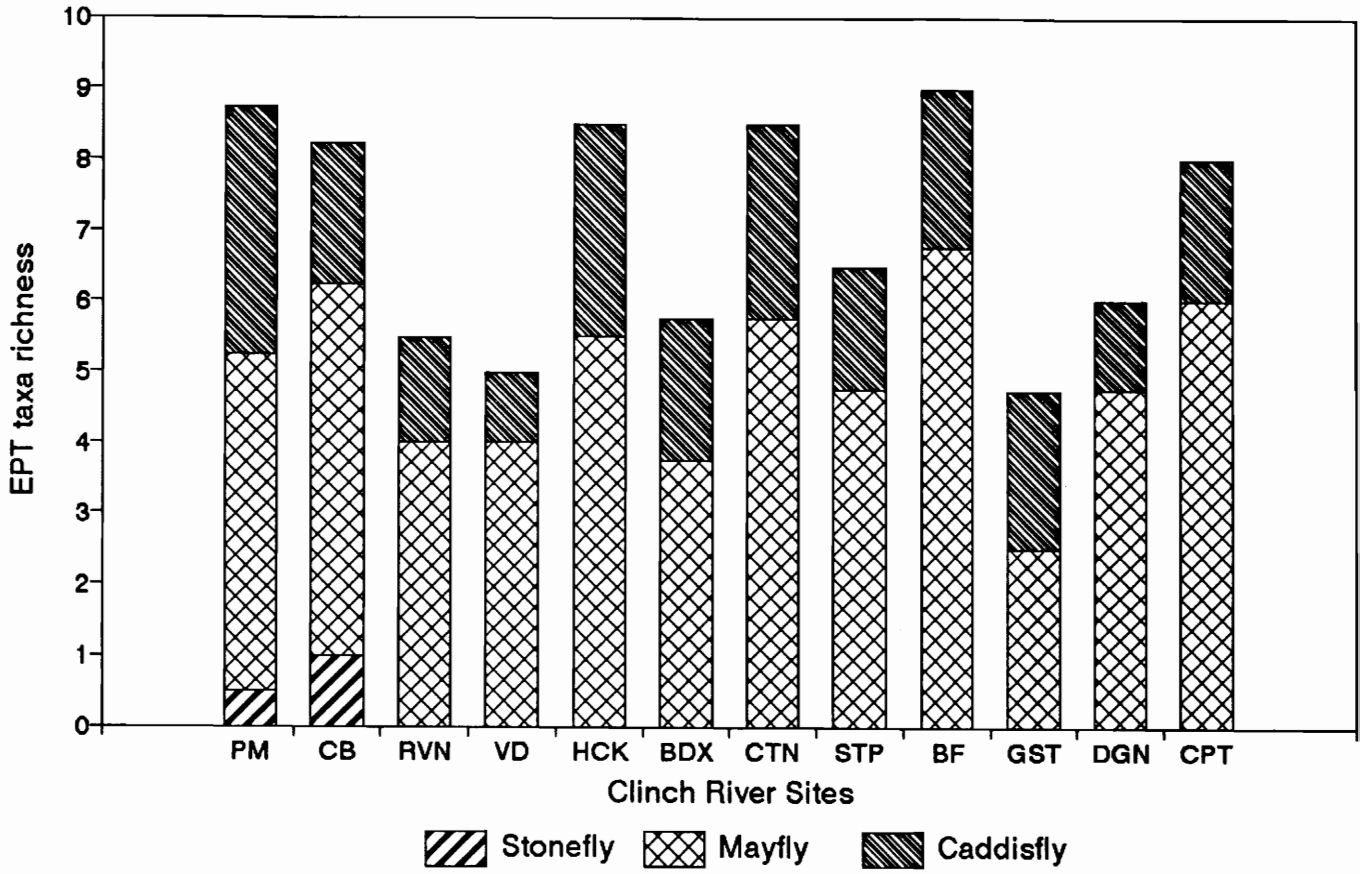

Fig. 6. Taxa richness of the EPT taxa at the 12 sampling locations.

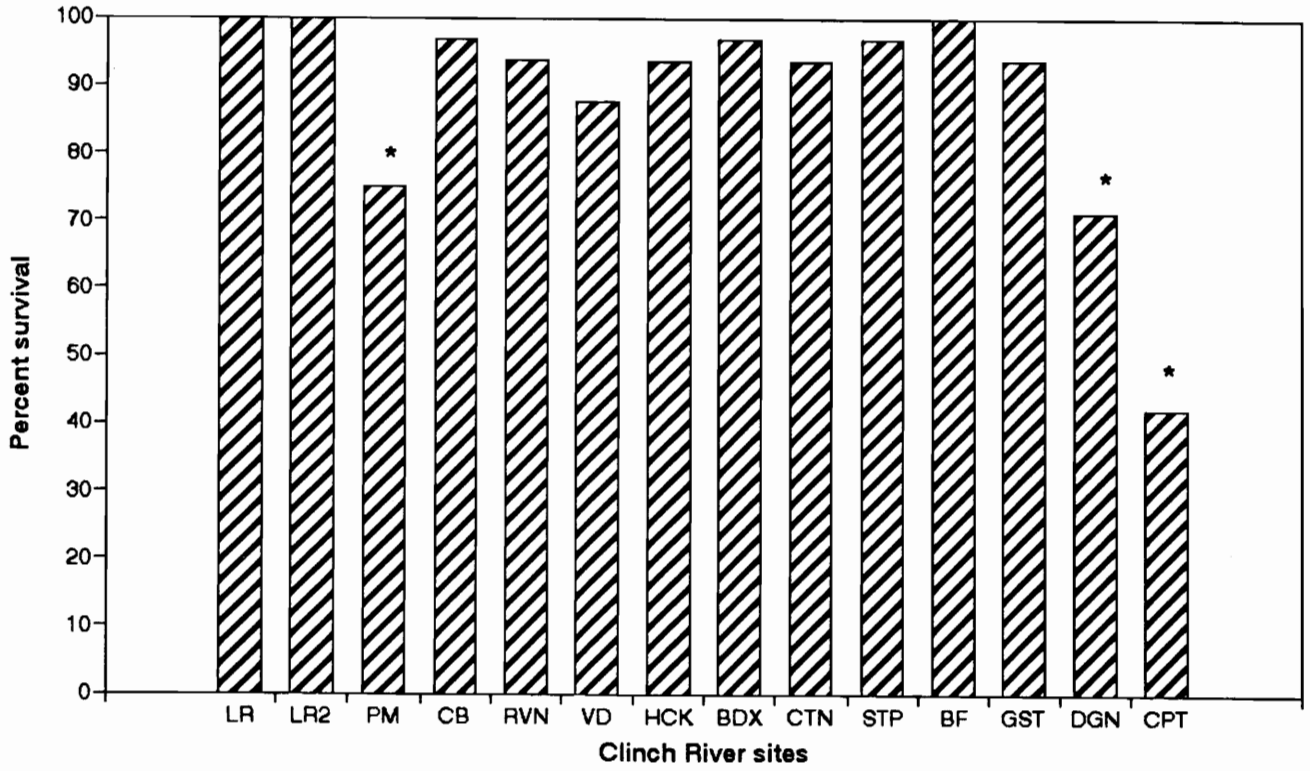

Fig. 7. Survival of juvenile *V. iris* from an in-situ test in the 11 Clinch River sites and 1 site in the Guest River. Asterisk indicates significant difference from the laboratory reference at the p=0.05 level.

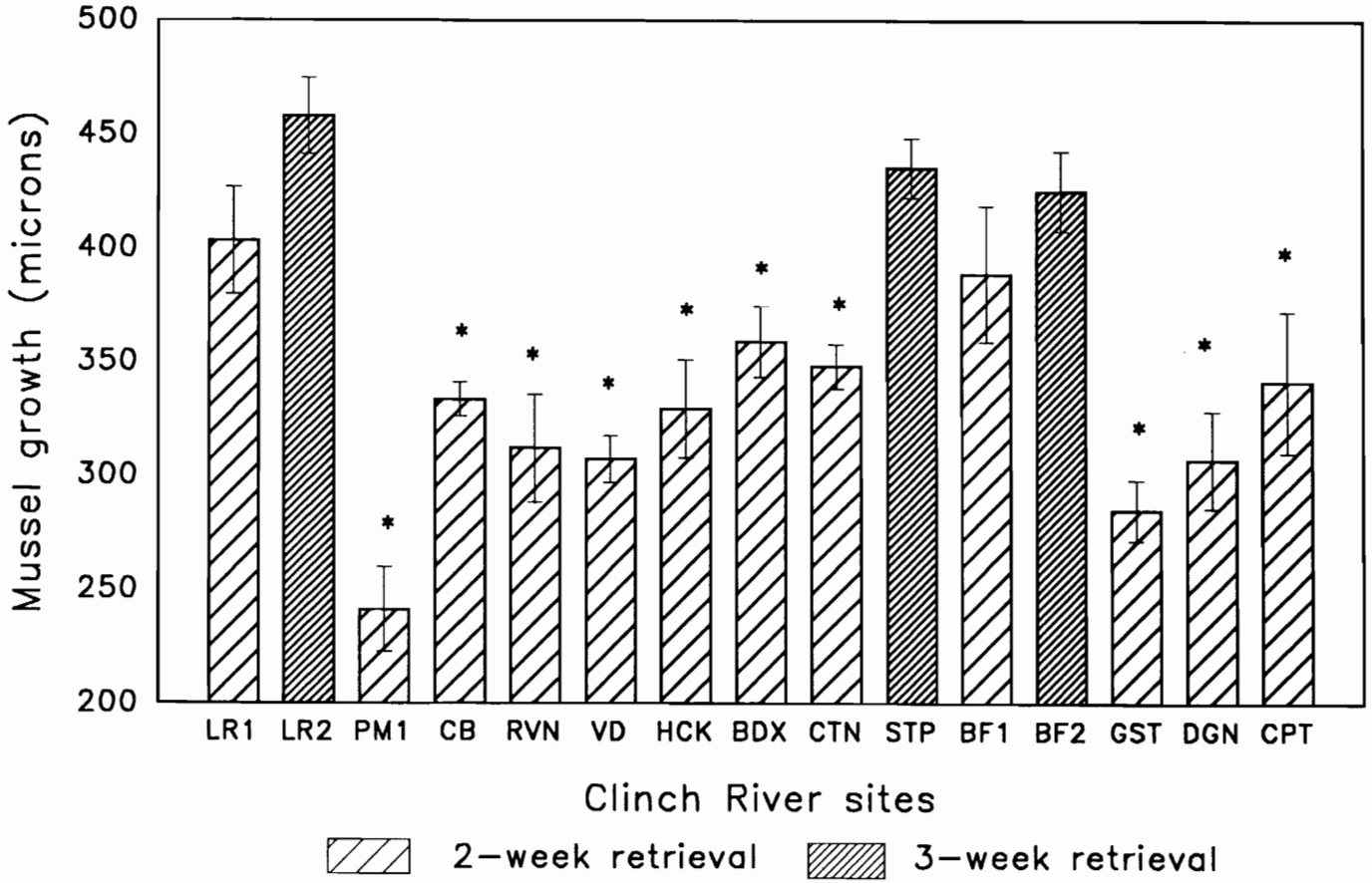

Fig. 8. Growth of juvenile *V. iris* during an in-situ test in the 11 Clinch River sites and 1 site in the Guest River. Asterisk indicates significant difference from the appropriate laboratory reference at the p=0.05 level.

CHAPTER 6

Impaired Growth and Ingestion of Juvenile *Villosa iris*, (Bivalvia: Unionidae), by Adult *Corbicula fluminea*

ABSTRACT

The interaction of adult *Corbicula fluminea* with juvenile unionids in depositional areas has been overlooked as a mechanism for impairment of unionid recruitment by the invading species. This study uses videotaping of interactions, flow-through testing, and resuspension experiments to determine whether the presence of adult *C. fluminea* can impair juvenile *V. iris*. Adult *C. fluminea* were found to ingest both glochidia and juvenile *V. iris* through siphoning and to collect them in mucous along the pedal gape when using pedal feeding. The presence of adult clams in flow-through sediment tests was found to decrease juvenile mussel growth and increase juvenile mussel mortality and the incidence of shell damage, resulting in lower recovery from the test chambers. In resuspension experiments, the presence of adult *C. fluminea* caused resuspension of 2 - 4 day old juveniles into the water column but did not have the same effect on 14 - 21 day old juveniles. Impairment of mussel recruitment by asian clams is plausible, especially in riffle

areas of stream reaches where depositional areas may be scarce.

INTRODUCTION

The decline of native mussels in the streams of North America has been well documented (Neves, 1987). Prior to the 1940's the decline of mussels was seen as a result of declining habitat quality due to increasing pollution from industrialization and agriculture, along with siltation and dam construction (van der Schalie, 1938). When the Asian clam, *Corbicula fluminea*, was introduced into the United States (Burch, 1944), it flourished and quickly broadened its range in the same aquatic habitats where native mussels were in decline. Since their introduction, the Asian clam has continued to spread into native mussel habitat. Native mussels have continued to decline in spite of efforts to improve water quality and increase suitable habitat for mussel recruitment.

C. fluminea's high tolerance to siltation and other anthropogenic impacts, along with the differences in life histories between the two bivalves, may explain the continued spread of the invading species into habitats unsuitable for unionids. However, in some areas which have supported unionid populations, the introduction of asian clams has been coupled with declines in unionid populations (Gardner et al. 1976). Competition between native mussels and *C. fluminea* for habitat

and food have been implicated (Gardner et al., 1976; Kraemer, 1979), due to the clams high rate of filtering efficiency, increased mobility, and less complex reproductive cycle (Britton and Morton, 1979). However, competition between the two bivalves has been difficult to demonstrate.

In many areas where *C. fluminea* and unionids cohabitate, adult unionids are present but recruitment of juveniles into the population is impaired. This may be due to the interaction between juvenile unionids and adult clams in depositional areas which are preferred habitats for unionid juveniles. This research was conducted to ascertain whether adult *C. fluminea* negatively impact juvenile unionids and could impair recruitment in unionid populations.

METHODS

C. fluminea Collection

Adult *C. fluminea*, ranging from 15 - 25 mm in length, were collected in the Clinch River, Virginia. Clams were held in river sediment and dechlorinated tap water in a flow-through fiberglass living stream and fed a tri-algal suspension containing *Chlamydomonas*, *Ankistrodesmus* and *Chlorella* (Foe and Knight 1986) until they were used in testing.

Juvenile Procurement

Gravid *V. iris* were collected from the Clinch River at Cliffield, Virginia, and returned to the laboratory in water-

filled coolers. The glochidia were removed with minimal harm to the adult by gently prying the valves apart approximately 2-3 mm, inserting a water-filled syringe into the gorged gill chamber, and flushing the chamber with water. The glochidia were checked for viability using a saturated salt solution (Zale and Neves 1982). Encystment onto 12-18 cm largemouth bass (*Micropterus salmoides*) was accomplished by placing the fish in a well-aerated 19-L aquarium for at least 30 min, with the glochidia from both marsupia of a female. When several fish were exposed together, they were left in the encystment aquarium for up to 120 min depending on glochidial density. The bass were kept in 500-L fiberglass tanks that were siphoned daily once the juveniles began dropping from the hosts.

Videotaping Juveniles

Both glochidia and juvenile *V. iris* were videotaped in the presence of adult *C. fluminea*. The glochidia were dyed with methylene blue so that they could be more easily seen. Adult clams, placed in 250-ml beakers with Clinch River water and in 400-ml bioassay dishes with Clinch River sediments and water, were filmed with the juveniles and the dyed glochidia. The previously described tri-algal suspension was added to induce siphoning by the clams. The interactions between bivalves were viewed using a Zeiss dissecting microscope connected to an RCA videorecorder and television for recording

activity. A total of 240 min of videotape was used to characterize the interaction.

Sediment Preparation

Sediments collected in the Clinch River, Virginia were sieved on site through a 2-mm stainless steel sieve to remove gravel and organisms. In the laboratory, sediments were sieved through a 1-mm sieve, and the fraction greater than 1 mm but less than 2 mm was retained for use in testing. The fraction of sediment less than 1 mm was sieved through a 53-um sieve to separate the silt fraction, less than 53 um. The silt fraction was added to the >1 mm fraction and became the test sediment. This separation allowed the juveniles to be more easily retrieved from the test chambers because they fell in the size range where the particles had been removed.

Flow-through Testing

Ten-day flow-through tests were conducted with 1 - 3 day old juvenile *V. iris* and adult *C. fluminea*. The test chambers were 600 ml plexiglass containers fitted with an inlet on the side of the container, 2.54 cm from the bottom. This inlet was above the sediment level (100 ml). Water flowed out of the chamber through a nitex mesh fitted over a 1-cm by 4-cm rectangular hole which was positioned on the front of the container so that the container held 550 ml. This allowed for an approximate sediment to water ratio of 1:4. A series of 5 treatments, containing 32, 16, 8, 4, and 2 clams, along with

a control containing no clams, were included with two replicates per treatment. Flow was created by siphoning dechlorinated tap water from a 500-L fiberglass headbox positioned on a shelf above the water bath where a 25°C±2°C temperature was maintained for the test beakers. Ambient laboratory lighting was used (50-100 fc) on a 16:8 light to dark schedule. Addition of tri-algal suspension to the headbox provided a uniform food supply to all test chambers. Flow into the test chambers averaged 63.4 ml/min (range 54.1 - 71.0 ml/min). Data were analyze using an ANOVA procedure followed by a Dunnetts test.

Resuspension Chambers

Experiments were conducted to determine if the presence of adult *C. fluminea* resulted in the resuspension of juvenile unionids into the water column. Flow-through troughs were created using 40.5-cm plastic gutters which were fitted on one end with an inlet hose. A 10-cm head space was created by cutting 1.8 cm off the top of a plastic gutter end and inserting it into the trough, which allowed water to flow evenly into the trough. The trough was filled with 1050 g of sediment, and water flowed out of the chamber over another plastic gutter end cut 1.8 cm below the trough height. The water flowing out of a chamber was collected in a funnel and passed through a plankton cup. Plankton cups were rinsed

daily, and the number of juvenile mussels washing out of the chambers was recorded. The experiment was run for seven days on two separate occasions. In the first experiment, 20 juvenile *V. iris* (14 - 21 days old) were placed in the troughs with 0, 10 or 20 adult clams. During the second experiment, each trough contained 75 juvenile mussels (2 - 4 days old) and either 0, 6 or 12 adult clams.

RESULTS

Videotaping Juveniles

Videotaping adult asian clams in the presence of dyed glochidia revealed that the mussels could be impacted in two ways. It was apparent that glochidia could be ingested by the clams through the siphons. Glochidia, some retaining the color of methylene blue and some clear, were present in the feces of clams observed siphoning the glochidia. The second way glochidia were impacted by the adult clams was by being collected in the mucous of pseudofeces which formed along the pedal gape of the clams. Similar results were seen when the adult clams were videotaped with juvenile *V. iris*. Again it was apparent that the juveniles were within the size range of food particles that were siphoned by the clams. While shell fragments were found in feces of the adult clams, many juveniles were rejected by being expelled from the siphon. Juvenile mussels were transported up the foot of the clam during pedal feeding and became trapped in the mucous at the

pedal gape. These mussels were not seen being ingested, and it is not known whether entrapment led to their ingestion.

Flow-through Test

Mortality in the 10-day test exhibited a clam density-dependent response, with mortality increasing as the number of clams in the test chambers increased (Fig. 1). The treatments containing 8, 16, and 32 clams all had 100% mortality of juvenile mussels, while the treatments with 2 and 4 clams each had 92.5% mortality. Mortality in one of the control replicates was 30%. However, one of the control replicates had 80% mortality, due to the interruption of flow from a clogged hose which resulted in lower dissolved oxygen. Similar interruption of flow occurred in one of the 32-clam treatment replicates. Both the replicates were included in the analysis. While these replicates could have been deleted, resulting in greater apparent impairment of the mussels by the clams, they were retained to give a more conservative estimate of the impairment. Even with the control survival at only 45%, the impact of the adult clams on juvenile *V. iris* mortality was apparent, as was the case in the additional test endpoints. However, the high variability in the controls and the low number of replicates prevent the differences in mortality from being statistically significant.

Recovery of shells decreased with increasing clam number (Fig. 1), except for the 2 clam treatment. In the controls,

90% of the mussel shells were recovered. The 2, 4, 8, 16 and 32 clam treatments had 62.5%, 85.0%, 62.5%, 42.5% and 37.5% recovery, respectively. All treatments except the 4 clam treatment had significantly lower recovery than the control ($p=0.05$).

As previously described, higher clam densities were correlated with mussel shell destruction. For this reason, the number of retrieved half shells was calculated. This number increased with increasing clam number in the 0, 2, 4 and 8 clam treatments (Fig.1). However, the number of half shells then began to decline. The proportion of shells recovered as half shells was only 13.8% in the controls and 28.0%, 30.9%, 44.2%, 32.3%, and 26.5% in the 2, 4, 8, 16, and 32 clam treatments, respectively.

Growth of the juvenile mussels prior to death could indicate the length of time the mussels coexisted with the clams before they died. Growth of the mussels, measured as shell height, was greatest in the controls which averaged 71.3 um over the 10-day period (Fig. 1). The second highest growth (40.0 um) was seen in the 2-clam treatment, which was not significantly different from the control. Growth in the 4, 8, 16, and 32 clam treatments was significantly lower than the control with averages of 15.9, 6.7, 8.8 and 5.0 um, respectively ($p=0.05$).

Resuspension Chambers

In the first resuspension experiment, using 14 - 21 day old *V. iris* juveniles, no trend was evident in resuspension of the juveniles. During the 7 d period, one mussel washed out in the 10-clam treatment and 1 half shell was recovered from the 20-clam treatment. No mussels were washed out of the control.

In the second experiment, using 2 - 4 day old juveniles, mussels were recovered from all three treatments, with more juveniles resuspended with greater numbers of adult clams (Fig. 2). At the end of 7 days, 9 (12%) juvenile mussels had been resuspended in the 0 clam treatment. During the same time period, 28% or 21 mussels and 40% or 30 mussels had been resuspended in the 6 and 12-clam treatments, respectively.

DISCUSSION

One of the most vulnerable periods in the life cycle of unionids is when the juvenile drops from the fish host and then must contact suitable habitat to survive (Neves and Widlak, 1987). While the exact constituents of a suitable habitat are not well defined, it is known that they inhabit depositional areas (Neves and Widlak, 1987) with low flow where they pedally feed and siphon interstitial water (Yeager et al., 1994). While Asian clams may be present in the gravel and cobble substrata which are the habitat of adult unionids, they preferentially inhabit sand and detrital areas (Gardner,

et al., 1976) more characteristic of the depositional areas in lotic systems. The presence of adult *C. fluminea*, which also deposit feed (Reid et al., 1992), may impair the recruitment of unionids by disturbance and resuspension of the juvenile unionids, and through ingestion of juvenile mussels by adult clams.

While juvenile *V. iris* and glochidia are slightly larger than the reported size range of particles ingested by *C. fluminea* (McMahon, 1991; Reid et al., 1992), it was shown that they could be ingested and passed through the gut of the adult clam. Clams foraging in depositional areas could ingest juvenile mussels which have settled there.

In the flow-through test, the presence of *C. fluminea* in the sediments was shown to impair survival and growth of unionids. Also, the presence of shell fragments from dead mussels indicates the outcome of interactions with the larger clams. Due to the persistence of the shells in sediments, it was possible to recover shells of dead organisms to ensure organisms considered dead were not lost. This also allowed determination of approximately how long mussels had coexisted with the clams by measuring how much growth had occurred prior to mortality.

Some shells had been destroyed passing through the gut of the clams during videotape analysis. I believed that greater numbers of clams in the treatment would result in lower

recovery of shells. This was the case with decreasing recovery of shells in the higher clam concentrations. This is possibly due to the destruction of shells either from passing through the gut of the clams, possibly on more than one occasion, or from physical abrasion by the clams foraging as they were pedal feeding in the sediments. This appears to be supported by the increasing number of half shells retrieved from higher clam concentrations, up to the 16 clam treatment. At the highest clam densities, the decline in half shell numbers most likely resulted from the overall decline in mussel shell recovery.

The first resuspension test with 14 to 21 day old juveniles failed to produce any resuspension of juveniles, regardless of the presence of clams. This is probably due to the size of 2 to 3 week old juveniles which were 400 - 600 um as opposed to the 220 - 250 um juveniles used in the second experiment. The lack of response may indicate that mussels of this size may be more resistant to the disturbance by the clams than the smaller juveniles.

Results of the second resuspension chamber experiments, using 2 to 4 day old juveniles, indicate that adult *C. fluminea* can resuspend juvenile *V. iris* into the water column from which they may not be redeposited into inhabitable areas. While the data look extremely convincing, conclusions are still tentative due to the lack of replication resulting from

the seasonal availability of juvenile unionids. The adult-larval interaction hypothesis (Woodin, 1976) predicts that discrete assemblages of deposit feeders can be maintained through adult-juvenile interactions. According to Hines et al. (1989), deposit feeders can ingest larvae in surface sediments and disrupt juveniles while searching for food. In riffle areas where depositional habitat is scarce, this may be an effective mechanism for impairing recruitment.

The effects of adult clams on juvenile unionids have previously been overlooked in discussions of competition between the two taxa. However, studies attempting to show competition between the two bivalves have produced some noteworthy conclusions. For example Kraemer (1979) states that asian clams have been present in the Buffalo River for 10 years and that they inhabit only some sandy patches of river bottom where indigenous mussels are rare. It is unknown whether unionid recruitment is occurring in this population. Similarly, Leff et al. (1990) investigated competitive interactions between *Elliptio complanata* and *C. fluminea* by examining seston removal rates and spatial distribution. While no competitive effect was shown, they report a slightly higher shell length in stream reaches with asian clams as opposed to an upstream reach without clams. These data may indicate no impairment of *E. complanata* growth by *C. fluminea*, or it may indicate an age class shift toward larger

individuals resulting from decreased recruitment in the stream reach with clams present. Without the age class structures it is impossible to draw conclusions. In light of current findings, however, many areas should be reevaluated to determine if impairment of juvenile recruitment could be the negative impact of *C. fluminea* eluded to by previous researchers. Certainly, ingestion of juveniles and resuspension of juveniles into the water column would result in decreased recruitment in unionid populations.

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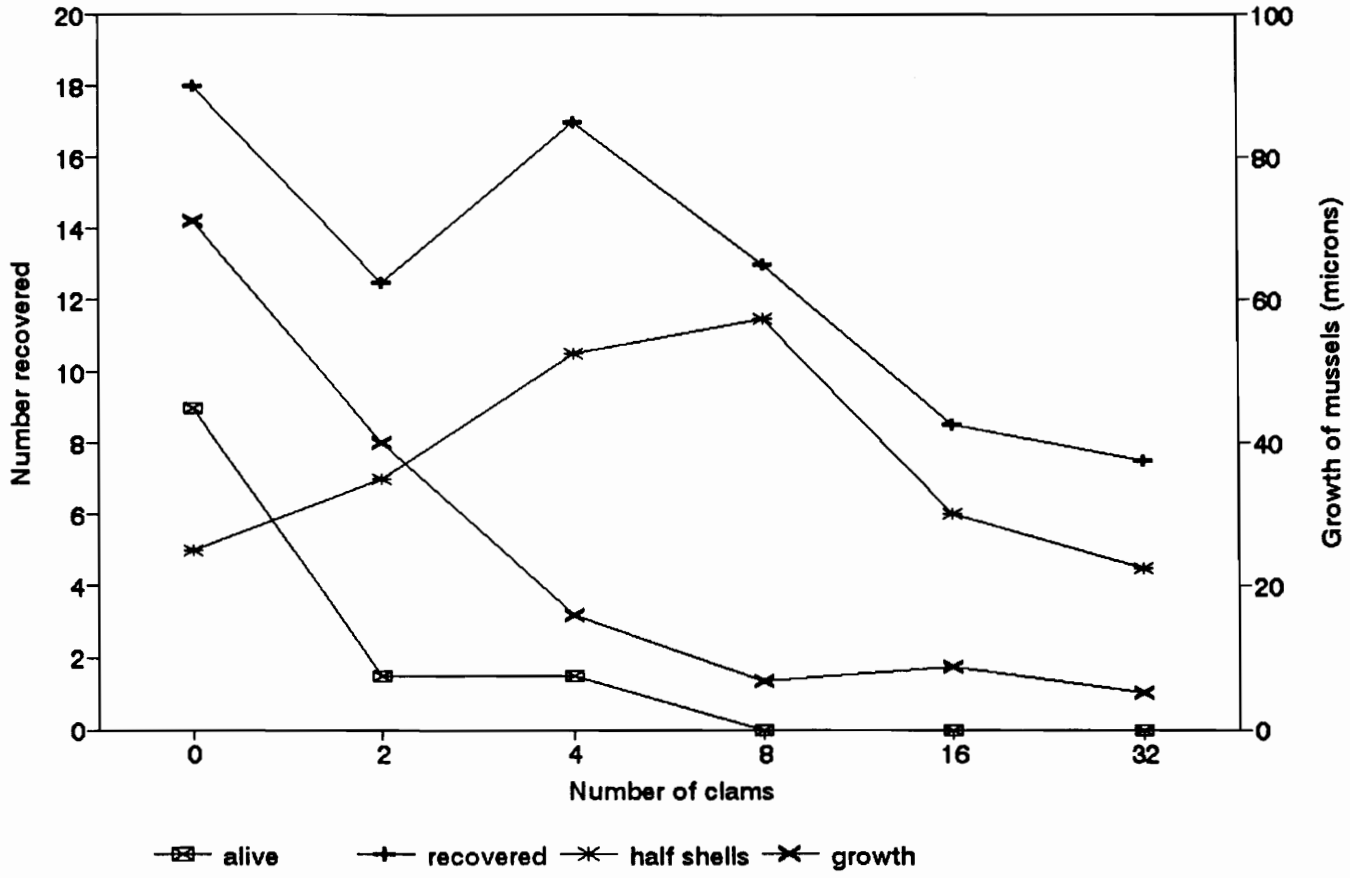

Fig. 1. Flow-through test showing the effect of adult *C. fluminea* on survival and growth of juvenile rainbow mussels, and the incidence of shell damage expressed as recovery of intact shells.

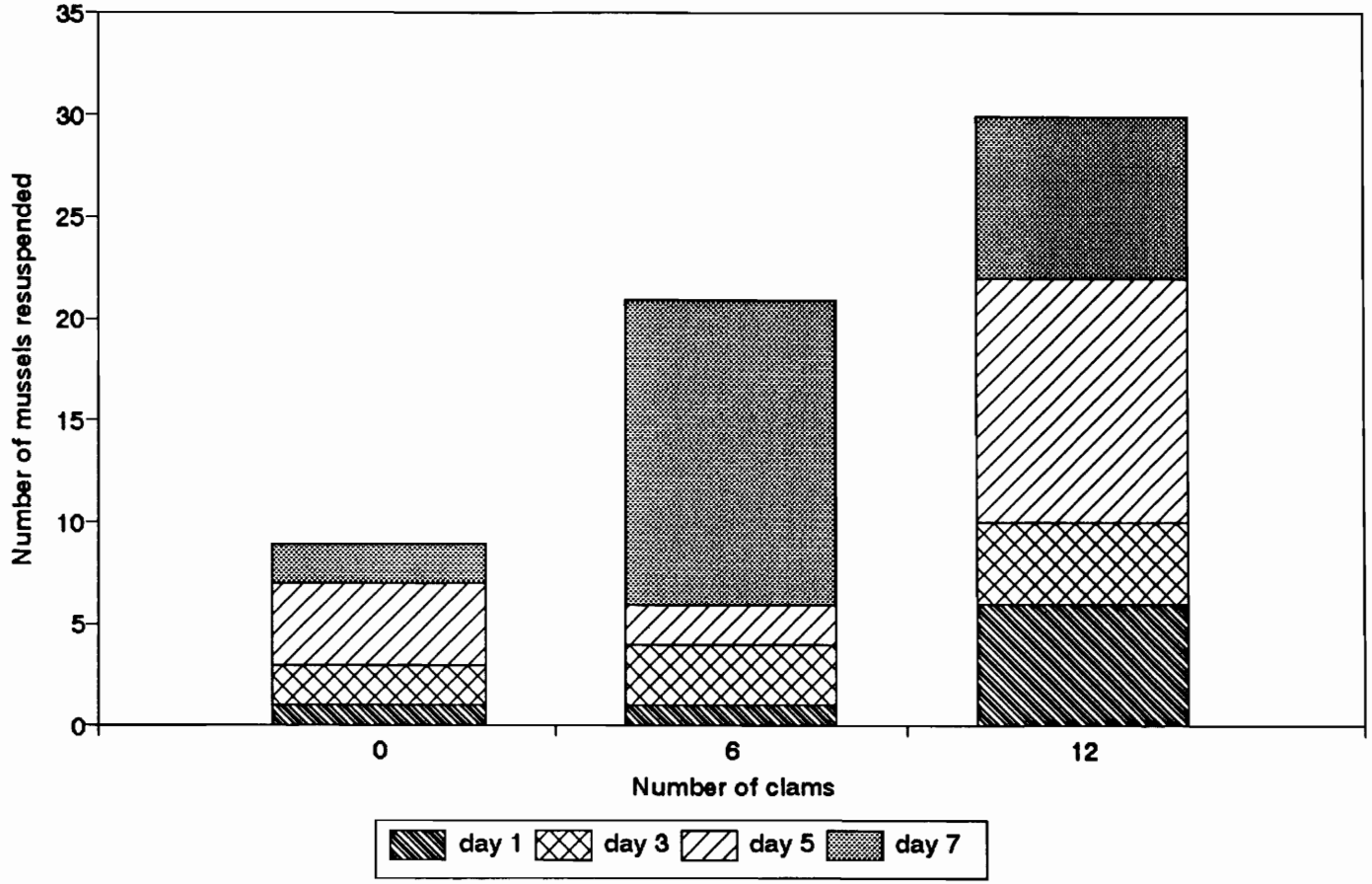

Fig. 2. Resuspension of juvenile *V. iris* by increasing densities of adult *C. fluminea* in flow-through troughs over a 7 day period.

CHAPTER 7

SUMMARY

While the causes of declining unionid populations may be largely site specific, there are many similarities in the changes of watersheds throughout the country. Increases in urbanization and industrialization, changing agricultural technology and increased demand for fossil fuels continue to contribute point and non-point discharges to rivers and streams. Furthermore, the introduction of exotic species continues to be a problem in many areas of the country and the ultimate effects of the invading species are as yet unknown. This study focused on possible factors contributing to the declines in unionid populations in the Clinch River, Virginia. However, the conditions that exist in this system may be applicable to other systems as well.

Many areas of the ecology and life history of unionid bivalves are not well characterized. Before unionids can be successfully used in testing to establish regulatory guidelines, a prerequisite to their protection, more information is necessary on propagation and culturing techniques. Chapter 2 outlines the methodology found to be useful in obtaining juveniles for use in the later chapters.

This is intended to be a starting point for future researchers who may continue to improve these techniques.

Chapter 3 is also an attempt to contribute information on the ecology and life history of unionids to the growing body of knowledge. This study characterized the relationship between juvenile rainbow mussels (*Villosa iris*) and the sediment they inhabit. Dye studies in a feeding chamber, observations of feeding using video tape, and gut content analysis were used to determine mechanisms of feeding, the primary food source, and the origin of substances taken up by the juveniles. Juveniles burrowed less than 1 cm into the sediment but they were not exposed to the overlying water. Exposure to sediment comes not only through direct contact, but also through filtration of interstitial water and sediment-associated fine particulate organic matter. Juveniles fed on bacteria and bacterial-sized particles as well as algae. Their predominant food source appears to vary with age. Pedal locomotory and pedal sweep feeding behaviors are used to facilitate movement of particles into the pedal gape.

Chapter 4 examined the depositional areas in the Clinch River to determine if sediment toxicity could be contributing to decreased recruitment in unionid populations. Sediment depositional areas in lotic systems have been identified as the habitat of juvenile unionids where they ingest

interstitial water and detrital particles. Decreased recruitment in unionid populations may reflect the intimate association of the juvenile stage with sediments and therefore any sediment bound toxicants which may be present. Eleven sites on the Clinch River (CRM 213.2 - 319.5) and one site on a tributary (Guest River) were examined to determine if sediment contamination exists in this river system. In 10-day sediment tests utilizing *Daphnia magna* and *Chironomus riparius* and 28-day sediment tests with *Hyallela azteca* both enrichment and impairment were found. Intermittent toxicity identified at ten of the sites did not affect the daphnids and midges similarly. Several of the observed effects could be related to point source discharges from industrial and municipal effluents. Non-point sources such as runoff from agricultural and mining activities and urbanization, which enter the system during rain events, may be responsible for much of the toxicity. Ten day sediment tests with juvenile *Villosa iris* and *Corbicula fluminea* did not reflect the toxicity seen previously on many of the testing dates. It was subsequently important to determine if the intermittent toxicity was causing alterations in the stream biota.

Chapter 5 addressed the alteration of stream biota resulting from the intermittent sediment toxicity found in laboratory testing. The ecosystem impacts are dependent on the frequency, duration and intensity of the acute insults

which may be caused by runoff from rain events. This study validated laboratory findings by determining if the intermittent toxicity was reflected in benthic invertebrate community structure and mussel density and richness. In-situ testing using juvenile *V. iris* was the final test of the effects of intermittent toxicity on unionid recruitment. The effects of intermittent toxicity were evident in the invertebrate community structure at 8 of the 12 study sites and in the mussel density and richness at 10 of the 12 sites. Two of the sites, impacted by a coal fired power generating facility, may be recovering following the installation of a waste treatment facility which removes metals from the effluent. Quality of the upstream reference site appeared to decline throughout the two year study period however the mussel population there does not yet reflect this decline.

While point and non-point discharges are certainly the greatest threat to unionids, the impacts of invading species becomes more controversial with each passing year. Competition between the Asian clam and native unionid bivalves has been implicated as a contributing factor in declining unionid populations. However, studies of interactions between the adult bivalves have failed to generate evidence of competition for food or space. The interaction of adult *C. fluminea* with juvenile unionids in depositional areas has been overlooked as a mechanism for impairment of unionid

recruitment by the invading species. In Chapter 6 videotaping of interactions, flow-through testing and resuspension experiments were used to determine if the presence of adult clams can impair juvenile *V. iris*. Adult *C. fluminea* were found to ingest both glochidia and juvenile *V. iris* via siphons and to collect them in mucous along the pedal gape when using pedal feeding. The presence of adult clams in flow-through sediment tests was found to decrease juvenile mussel growth and increase juvenile mussel mortality and the incidence of shell damage resulting in lower recovery from the test chambers. In resuspension experiments, the presence of adult clams caused resuspension of 2 - 4 day old juveniles into the water column but did not have the same effect on 14 - 21 day old juveniles. Impairment of mussel recruitment by *C. fluminea* is plausible especially in riffle areas of stream reaches where depositional areas may be scarce.

Declining unionid populations in the Clinch River are of concern due to the high endemism in the diverse fauna of the Cumberlandian region. Both the presence of sediment-bound toxicants and *C. fluminea* in the depositional areas, the preferred habitat of the sensitive juvenile life stage, are found to be potential contributors to unionid bivalve declines in the Clinch River, Virginia. Research into the contribution of run-off following rain events is needed to further define the impacts of non-point pollution in this river system.

APPENDIX I

Directions to the twelve sites beginning at the upstream site and specific locations of sampling areas at each site.

Pounding Mill - Approximately 11 miles west of Tazwell on U.S. Route 460 turn right on State Route 637. Pull off the road on the left before crossing the bridge over the Clinch River. A cement mixing facility is on the right side of the road. Walk down the bank and enter the river downstream of the bridge. The site is approximately 20 yards downstream of the bridge on the right bank and extends into the middle of the river. The left bank in this area is heavily silted.

Cedar Bluff - Exit U.S. Route 460 on to highway 609 at Cedar Bluff. As you exit the interstate turn left towards the town of Cedar Bluff and then take the first right which leads to a bridge crossing the river to a private residence. Pull off the road before crossing the bridge. Enter the river on the right bank downstream of the bridge. Walk downstream approximately 50 yards to the end of the island. The site is located in the main stream channel on the right side of the island. The area utilized in this study encompasses the area adjacent to the last 10 yards of the island and extends about 10 yards into the main channel.

Raven - After passing through Doran on U.S. Route 460 there is a stop light and a sign for Richlands Municipal Airport, veer left at this light. Pass the first left, which goes to the airport, and take the next left which crosses a bridge and

has a stop light. At the stop sign, turn right into a residential area and drive towards the school at the end of the road. Turn left on State Route 635 which is the second to last left before the school. Almost immediately the road forks, veer to the right. Travel for about half a mile and then on the right will be a small road going right into the river. Just downstream of the ford and on the right bank is a riffle area which was used in this research.

Van Dykes - From U.S. Route 460 west of Raven, turn left on State Route 67, then left on State Route 635. Route 635 forks and veering right puts you on State Route 633 heading south. On the left you will see and old two-story wooden house sitting in a curve and at the bottom of a hill. Beside this house is access to privately owned property which can be recognized by the railroad ties which serve as posts for the gates. Upon entering this property, drive straight on the road making no turns. You will cross the railroad tracks twice and finally find yourself at a ford across the Clinch River. Enter the river at the ford and turn left. Walking upstream for about 15 yards you will find a riffle on the right bank which was used in this research.

Hackney's Farm - When traveling south on State Route 661 from the town of Cleveland, the road changes to State Route 664.

Turning left from this road onto the Hackney's property gives river access to this site. Enter the river on the right bank and cross the river below the fall on the right bank. Walk upstream and on the left bank about 20 yards above the falls is a riffle with a large mussel bed. This is were samples were collected in this study.

Bulldozer Crossing - Take State Route 664 through Carbo and after the junction of 664 and 616 you are on State Route 665. This road runs around the power plant and along the river. On the left side of the road approximately .75 miles from the plant entrance a road goes down to the river. Pull down this road and park. Cross the river to the left bank and this riffle area was the study site.

Carterton - From the Bulldozer Crossing site continue on State Route 665. Just after the junction with State Route 614 turn left and cross the bridge. Turn right across a wooden bridge and then take another right across the railroad tracks to the public access boat landing. Enter the water and walk upstream to just below the entrance of the tributary on the left bank. The river is deep on the right bank, the riffle area on the left bank is the study site.

St. Paul - When traveling on U.S. Route 58, exit onto State Route 640 before arriving in the town of St. Paul. Turn right at the Frosty Bossie and continue to the top of the hill where you veer left. The road begins to run parallel to the river. Pass a church on the right and begin to look for a parking area on the left. Pull off the road and walk back about 50 yards. Enter the river there and cross the river to the right bank. The area between the island and the bank at the downstream end of the island is the riffle reach used in this study.

Burtons Ford - About one mile past St. Paul on U.S. Route 58 make a left onto State Route 611. Stay on this road until it ends at a guard rail and park there. Cross the guard rail and walk down the road to the river. Cross the river above the island. The site is located between the left bank and the island near the top of the island.

Guest River - Take U.S. Route 58 to Coeburn, then turn left on State Route 72 heading south. Pass through Maytown and cross bridge over the Guest River. On the south side of the bridge take an immediate left and pull into the grass. Walk past the gas well to the river. Approximately 20 yards downstream of the bridge in the center of the river is the collection site.

Dungannon - Take State Route 72 to Dungannon and continue south until you see a left turn on State Route 66 north. There should be a sign there for St. Paul. Go over the bridge crossing the Clinch River and then take the second right. Stay on this gravel road for about 2 miles and then pull off to the right in an unmarked parking area. At this point the bank is very steep and the river is about 30 feet below the road. You can see an island in the river. Climb down the bank and cross the river. Between the right bank and the island, at the top of the island, is the area used in this study.

Clinchport - From Dungannon take State Route 66 south towards Gate City and Clinchport. This road becomes State Route 65 and runs all the way into Clinchport. After crossing Stock Creek and a church on the right, turn left on a small road. This road should run right into the river after about 20 yards, turn left. When the paved road veers left, pull over and park there. Walk up the dirt road about 30 yard until the first big sycamore tree and enter the river there. There should be a swinging bridge downstream about 200 yards. Cross the river to the left bank and there is a riffle about 10 yards downstream from the sycamore tree entrance point. This is the area where collections were made for this study.

APPENDIX II

Species list with average abundance and standard error for 4 replicates of benthic macroinvertebrates samples collected at each of the twelve sites. Samples were collected on August 10, 1993 using a Hess sampler.

Site - Pounding Mill

Species	Mean	S.E.
Physidae	2.0	1.40
Pleuroceridae	28.5	7.70
Corbicula	1.5	0.87
OLIGOCHAETA	3.0	1.47
Stenonema	29.8	2.75
Isonychia	1.25	0.63
Caenis	7.5	2.78
Baetis	5.75	3.57
Hexagenia	2.0	0.91
Baetisca	0.25	0.25
Acroneuria	5.5	4.01
Perlodidae	5.0	3.00
Corydalus	1.0	0.57
Sialus	2.75	2.14
Hydropsyche	0.50	0.29
Cheumatopsyche	0.25	0.25
Helicopsyche	3.5	3.18
Brachycentrus	3.5	1.32
Limnephilidae	1.75	1.44
Glossosomatidae	1.75	1.11
Stenelmis	5.0	2.97
Optioservus	57.5	16.73
Ordobrevia	73.5	69.1
Dubiraphia	0.5	0.5
Promesia	0.25	0.25
Psephenus	23.25	6.01

Pounding Mill Continued		
Microcylloepus	0.5	0.29
Chironomidae	54.75	10.06
Antocha	0.25	0.25
Atherix	1.5	0.65
Tipula	3.0	0.82

Site - Cedar Bluff		
Species	Mean	S.E.
Physidae	3.5	2.53
Pleuroceridae	19.5	17.56
Ferrissia	1.75	0.75
Corbicula	0.5	0.29
OLIGOCHAETA	3.25	2.29
Stenacron	1.5	1.5
Stenonema	31.5	7.38
Isonychia	0.5	0.5
Caenis	21.0	6.79
Tricorythodes	11.0	3.11
Ephoron	0.25	0.25
Baetis	1.75	1.03
Siphlonurus	0.25	0.25
Hexagenia	0.25	0.25
Litobranchia	0.25	0.25
Acroneuria	0.25	0.25
Isoperla	0.25	0.25
Perlodidae	0.75	0.48
Pteronarcys	0.25	0.25
Corydalus	0.50	0.50
Sialus	1.50	0.65
Hydropsyche	0.25	0.25
Cheumatopsyche	1.75	0.75
Helicopsyche	0.25	0.25
Brachycentrus	0.75	0.75
Dolophilodes	0.25	0.25
Rhyacophila	0.25	0.25
Stenelmis	3.25	1.38

Cedar Bluff
continued

Optioservus	4.25	2.27
Ordobrevia	15.25	3.33
Dubiraphia	0.75	0.25
Psephenus	4.75	0.85
Chironomidae	33.75	9.99
Atherix	1.25	0.95
Tipula	0.50	0.50

Site - Raven		
Species	Mean	S.E.
Physidae	5.5	3.23
Pleuroceridae	3.5	2.84
Ferrissia	3.0	1.73
Corbicula	6.0	1.96
OLIGOCHAETA	1.0	0.71
Stenonema	19.3	5.65
Isonychia	1.75	.75
Caenis	3.5	1.85
Tricorythodes	0.5	0.29
Baetis	2.3	0.75
Hydropsyche	0.8	0.75
Ceratopsyche	0.8	0.75
Cheumatopsyche	5.5	1.85
Stenelmis	3.3	2.93
Optioservus	14.5	8.27
Ordobrevia	7.0	2.80
Dubiraphia	0.3	0.25
Ancyronyx	2.3	2.25
Psephenus	1.3	0.48
Macronychus	0.5	0.50
Microcylloepus	0.5	0.50
Chironomidae	18.5	7.89
Atherix	0.5	0.29

Site - Van Dykes		
Species	Mean	S.E.
Physidae	9.0	4.64
Pleuroceridae	4.3	3.59
Corbicula	1.3	0.48
OLIGOCHAETA	0.8	0.48
Cambarus	0.3	0.25
Stenacron	0.3	0.25
Stenonema	5.0	1.35
Caenis	7.3	5.62
Tricorythodes	9.5	5.39
Ephoron	1.3	1.25
Baetis	1.8	0.63
Drunella	0.3	0.25
Dromogomphus	0.3	0.25
Ophiogomphus	0.3	0.25
Nigronia	0.5	0.50
Hydropsyche	1.8	1.44
Cheumatopsyche	0.5	0.50
Orthotrichia	0.3	0.25
Optioservus	3.5	1.76
Ordobrevia	2.0	1.08
Psephenus	4.3	0.48
Chironimidae	0.8	0.48
Tipula	0.3	0.25

Site - Hackney's Farm		
Species	Mean	S.E.
Physidae	2.5	1.66
Pleuroceridae	8.8	4.15
Ferrissia	0.5	0.29
Corbicula	0.5	0.29
OLIGOCHAETA	1.0	0.71
Stenonema	9.8	3.07
Isonychia	0.5	0.29
Caenis	46.0	15.69
Tricorythodes	67.8	14.54
Baetis	5.0	4.34
Potamanthus	3.5	1.04
Ephemera	0.3	0.25
Dromogomphus	0.5	0.29
Protoneura	0.3	0.25
Corydalus	0.5	0.29
Nigronia	0.3	0.25
Sialus	0.5	0.50
Hydropsyche	2.0	1.00
Cheumatopsyche	2.3	2.25
Helicopsyche	2.5	1.85
Brachycentrus	0.3	0.25
Rhyacophila	0.3	0.25
Limnephilidae	0.3	0.25
Glossosomatidae	0.3	0.25
Stenelmis	2.8	1.70
Optioservus	6.3	1.93
Ordobrevia	8.8	4.50

Hackney's Farm
continued

Dubiraphia	1.0	0.71
Psephenus	15.3	11.40
Microcylloepus	0.3	0.25
Chironimidae	37.8	11.52
Antocha	0.3	0.25
Atherix	1.3	0.48

Site - Bulldozer Crossing		
Species	Mean	S.E.
Physidae	4.3	3.61
Pleuroceridae	3.8	2.50
Ferrissia	0.3	0.25
Corbicula	0.8	0.25
OLIGOCHAETA	0.8	0.48
Stenonema	3.0	1.47
Isonychia	4.75	4.11
Caenis	2.0	0.91
Tricorythodes	3.3	1.38
Baetis	2.25	1.65
Litobranchia	0.3	0.25
Baetisca	0.5	0.50
Dromogomphus	0.5	0.50
Nigronia	0.3	0.25
Hydropsyche	4.0	3.37
Ceratopsyche	1.5	1.50
Cheumatopsyche	1.5	0.87
Helicopsyche	0.3	0.25
Parapsyche	0.3	0.25
Brachycentrus	0.3	0.25
Stenelmis	0.3	0.25
Optioservus	0.3	0.25
Ordobrevia	0.8	0.25
Psephenus	3.0	3.00
Chironomidae	6.0	2.97
Atherix	0.5	0.29

Site - Carterton

Species	Mean	S.E.
Physidae	1.3	1.25
Pleuroceridae	7.5	2.63
Ferrissia	0.5	0.29
Corbicula	0.8	0.75
OLIGOCHAETA	4.8	1.89
Stenonema	17.0	5.12
Isonychia	1.8	1.03
Caenis	25.8	11.32
Tricorythodes	54.0	16.79
Baetis	12.5	10.51
Potamanthus	10.8	6.16
Ephemerella	0.5	0.50
Dromogomphus	0.5	0.29
Argia	0.5	0.50
Corydalus	1.0	0.41
Nigronia	0.3	0.25
Hydropsyche	2.8	1.70
Cheumatopsyche	7.3	4.66
Helicopsyche	0.3	0.25
Parapsyche	0.3	0.25
Orthotrichia	0.3	0.25
Rhyacophila	0.3	0.25
Philopotamidae	0.3	0.25
Stenelmis	9.5	4.84
Optioservus	6.5	2.87
Ordobrevia	10.5	4.63
Dubiraphia	0.8	0.48

Carterton
continued

Psephenus	52.3	33.94
Macronychus	1.5	1.50
Ectopria	0.3	0.25
Chironomidae	41.8	8.78

Site - St. Paul		
Species	Mean	S.E.
Physidae	1.3	0.48
Pleuroceridae	0.5	0.50
Ferrissia	0.5	0.50
Corbicula	3.8	2.78
OLIGOCHAETA	0.3	0.25
Stenacron	1.8	1.03
Stenonema	7.8	5.20
Caenis	8.0	4.02
Tricorythodes	27.3	8.50
Baetis	0.5	0.29
Potamanthus	21.8	12.40
Ephemera	0.3	0.25
Hexagenia	1.8	1.75
Dromogomphus	0.3	0.25
Ophiogomphus	0.3	0.25
Corydalus	0.5	0.29
Nigronia	0.3	0.25
Hydropsyche	0.5	0.29
Cheumatopsyche	2.0	1.23
Helicopsyche	0.3	0.25
Brachycentrus	0.3	0.25
Pycnospyche	0.8	0.75
Stenelmis	2.5	2.50
Optioservus	0.5	0.29
Ordobrevia	3.3	2.02
Dubiraphia	2.8	1.11
Promesia	0.3	0.25

St. Paul
continued

Psephenus	1.5	0.87
Macronychus	0.5	0.50
Microcylloepus	0.8	0.75
Ectopria	0.3	0.25
Chironomidae	36.8	20.75
Tipula	0.3	0.25

Site - Burtons Ford

Species	Mean	S.E.
Physidae	1.5	1.19
Pleuroceridae	0.3	0.25
Ferrissia	8.3	6.26
Corbicula	6.8	2.75
OLIGOCHAETA	6.5	3.75
Stenacron	4.8	3.47
Stenonema	24.8	10.13
Isonychia	0.3	0.25
Caenis	53.5	17.56
Tricorythodes	70.3	15.73
Baetis	1.0	0.41
Potamanthus	6.5	1.94
Ephemera	0.3	0.25
Hexagenia	0.5	0.29
Leptohyphes	7.8	7.75
Heptagenia	0.5	0.50
Dromogomphus	0.8	0.48
Argia	0.3	0.25
Nigronia	0.3	0.25
Sialus	1.8	1.03
Hydropsyche	1.5	0.96
Cheumatopsyche	4.3	2.18
Orthotrichia	0.8	0.25
Dolophilodes	0.5	0.50
Ordobrevia	7.3	5.36
Dubiraphia	0.8	0.48
Psephenus	2.8	1.11

Burtons Ford continued		
Microcylloepus	0.3	0.25
Ampumixis	0.5	0.29
Chironomidae	32.5	8.70

Site - Guest River		
Species	Mean	S.E.
Ferrissia	0.8	0.47
Corbicula	3.8	2.14
OLIGOCHAETA	5.5	4.27
Cambarus	0.5	0.29
Stenonema	0.3	0.25
Isonychia	0.5	0.50
Caenis	1.8	1.44
Tricorythodes	0.8	0.75
Baetis	1.3	0.95
Potamanthus	0.3	0.25
Centroptilum	0.3	0.25
Dromogomphus	0.3	0.25
Nigronia	0.3	0.25
Sialus	0.3	0.25
Hydropsyche	3.8	3.09
Ceratopsyche	0.3	0.25
Cheumatopsyche	9.8	8.11
Potamyia	1.3	1.25
Optioservus	0.3	0.25
Ordobrevia	2.0	1.08
Dubiraphia	0.3	0.25
Macronychus	0.3	0.25
Microcylloepus	0.5	0.50
Ampumixis	0.3	0.25
Ancyronyx	0.3	0.25
Chironomidae	29.5	3.10

Site - Dungannon

Species	Mean	S.E.
Pleuroceridae	1.3	0.75
Ferrissia	1.0	0.71
Corbicula	4.3	2.50
OLIGOCHAETA	6.3	3.68
Stenonema	14.3	4.27
Caenis	18.5	5.55
Tricorythodes	6.5	2.53
Baetis	1.3	1.25
Potamanthus	14.0	11.09
Ephemera	0.3	0.25
Hexagenia	0.5	0.29
Ophiogomphus	1.0	0.41
Protoneura	0.5	0.50
Corydalus	0.3	0.25
Hydropsyche	0.3	0.25
Ceratopsyche	0.3	0.25
Cheumatopsyche	0.3	0.25
Helicopsyche	0.3	0.25
Orthotrichia	0.3	0.25
Stenelmis	7.0	3.76
Optioservus	1.3	0.48
Ordobrevia	8.8	5.88
Psephenus	0.5	0.29
Microcylloepus	0.3	0.25
Chironomidae	12.5	1.94

Site - Clinchport

Species	Mean	S.E.
Physidae	12.8	11.76
Pleuroceridae	3.8	2.18
Ferrissia	0.3	0.25
Corbicula	31.0	21.72
Sphaeridae	0.3	0.25
OLIGOCHAETA	1.5	0.65
Stenacron	1.0	0.41
Stenonema	16.5	4.17
Isonychia	0.5	0.50
Caenis	5.0	2.55
Tricorythodes	4.5	0.96
Baetis	1.3	0.75
Potamanthus	3.3	1.03
Centroptilum	0.3	0.25
Leptohyphes	4.3	3.33
Dromogomphus	0.8	0.48
Argia	0.3	0.25
Sialus	0.3	0.25
Hydropsyche	0.3	0.25
Ceratopsyche	0.5	0.50
Cheumatopsyche	2.0	1.08
Orthotrichia	0.3	0.25
Dolophilodes	0.3	0.25
Pycnospyche	0.3	0.25
Stenelmis	4.3	1.70
Optioservus	0.8	0.48
Ordobrevia	9.8	4.96
Psephenus	0.5	0.29

Clinchport continued		
Macronychus	0.3	0.25
Ampumixis	0.5	0.50
Chironomidae	4.5	1.66
Antocha	0.3	0.25
Atherix	0.5	0.50
Tipula	0.5	0.29

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BIRTHDATE AND PLACE

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EDUCATION

Ph.D Program in Aquatic Ecology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 24061. June, 1991 to present.

M.S. in Aquatic Ecology, Marshall University, Huntington, West Virginia, July, 1991 Thesis title: An Analysis of Variation in a Disjunct Population of the Central Mudminnow, Umbra limi (Kirtland) in the Greenbottom Wildlife Management Area, Cabell County, West Virginia.

University of North Carolina-Charlotte, Charlotte, North Carolina 21 hours graduate course work, Fall 1988 - Spring 1989.

B.S. Biology, University of Charleston, Charleston, West Virginia. May 1987 (Cum Laude).

EMPLOYMENT

1988 - 1989
Graduate Teaching Assistant, Dept. of Biology, University of North Carolina-Charlotte, Charlotte, North Carolina

1989 - 1991
Graduate Teaching Assistant, Dept. of Biology, Marshall University, Huntington, West Virginia

1991 - 1992
Graduate Teaching Assistant, Dept of Biology, Virginia Polytechnic Institute and State University.

1992 - present
Graduate Research Assistant, Dept. of Biology, Virginia Polytechnic Institute and State University.

SPECIAL RECOGNITION

Travel award, Fourteenth annual meeting, Society of Environmental Toxicology and Chemistry; Matching funds award, Department of Biology, Total award, $600.00, November, 1993

Tuition Scholarship, VPI&SU, Academic Year 1992-1993, 1993-1994

Partial Tuition Scholarship, VPI&SU, Academic Year 1991-1992

Chemistry Award, Univ. of Charleston, Academic Year 1986-1987

PROFESSIONAL ORGANIZATIONS

Society of Environmental Toxicology and Chemistry
North American Benthological Society
Sigma Xi
Association of Southeastern Biologists
West Virginia Academy of Science

PUBLISHED ABSTRACTS AND PRESENTATIONS

Yeager, M.M., D. Tarter, T. Jones, and D. Cincotta. 1990. Discovery of the Central Mudminnow Umbra limi (Kirtland), in the Greenbottom Wildlife Management Area, Cabell County, West Virginia. West Virginia Academy of Science, Association of Southeastern Biologists, 1990. (Paper Presented).

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

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MANUSCRIPTS IN PREPARATION/REVIEW/PRESS

Yeager, M.M., D. Tarter, and T. Jones. (IN PRESS). The Reproductive Biology of a Disjunct Population of the Central Mudminnow, Umbra limi (Kirtland), in the Greenbottom Wildlife Management Area, Cabell County, West Virginia. Bulletin of the West Virginia Academy of Sciences.

Cherry, D.S., Van Hassel, J.H., M.M. Yeager, and J. Cairns, Jr. (IN PRESS). Avoidance of Twenty-Four Fish Species to Thermally Influenced, Chlorinated Water. Submitted to J. Aquatic Ecosystem Health.

Yeager, M.M., D. Tarter, M. Little, and M. Seidel. (IN PREP). An Analysis of Variation in a Disjunct Population of the

Central Mudminnow, Umbra limi (Kirtland), in the Greenbottom Wildlife Management Area, Cabell County, West Virginia. To be submitted to Transactions of the American Fisheries Society.

Cherry, D.S., Whitaker, J.B., Van Hassel, J.H., Stetler, D.A., Cairns, J. and M.M. Yeager. (IN PREP). Laboratory Avoidance, Acute Toxicity, and Gill Tissue Ultrastructure Responses of Fish to Acidic and Alkaline pH Exposures.

FUNDED GRANTS

Co-Principal Investigator: Sources of Pollutants Influencing Sediment Toxicity and the Mussel Fauna in the Clinch River Drainage System - An On-site Investigation. American Electric Power Service Corporation, Columbus, Ohio. $75,000 to November 30, 1994.

TECHNICAL REPORTS

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Cherry, D.S., Yeager, M.M., Dobbs, M.G., Bidwell, J.R., Farris, J.L., and Smith, E.P.. 1992. The Influence of Effluent Temperature on the Distribution of Fishes and Other Biota in the New River Near Hoechst Celanese Corporation, Virginia, Report and Proposal. Submitted to Hoechst Celanese Corporation on 6 November, 1992.

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Cherry, D.S., M.G. Dobbs, M.M. Yeager, S.R. Lynde, and J.F. Scott. 1994. Benthic Macroinvertebrate Assesssment of the North Impact Area in the La Cross River, Fort McCoy, Wisconsin.

Cherry, D.S., M.G. Dobbs, S.R. Lynde, M.M. Yeager. 1994. Benthic Macroinvertebrate Analysis of East Branch, Brandywine Creek for the Sonoco Products Company, Downington, Chester County, Pennsylvania.

PROFESSIONAL ACTIVITIES

1991

Duqesne Light Company, Beaver Valley, PA, Sediment testing as part of a larger project to assess fate and effects of a nuclear power plant outfall following application of a molluscicide.

1991 - 1993

Hoechst Celanese Corporation, Celco Plant, Narrows, VA, Fish sampling, fish identification and statistical analysis to evaluate the impact of heated effluent on the distribution of fishes in the New River, VA.

1992 - 1993

Hoechst Celanese Corporation, Celco Plant, Narrows, VA, Monitoring of chlorination for the control of *Corbicula fluminea* biofouling.

1992 - 1993

Hoechst Celanese Corporation, Celriver Plant, Rock Hill, South Carolina, Monitoring of chlorination for the control of *Corbicula fluminea* biofouling.

1993 - 1994

Hoechst Celanese Corporation, Celco Plant, Narrows, VA, Fish sampling, gonadal somatic indices, juvenile fry sampling and identification to determine the impact of heated effluent on fish reproductive success in a macrophyte habitat.

1994

Hoechst Celanese Corporation, Celco Plant, Narrows, VA, Monitoring of chlorination for the control of *Corbicula fluminea* biofouling.

Hoechst Celanese Corporation, Celriver Plant, Rock Hill, South Carolina, Monitoring of chlorination for the control of *Corbicula fluminea* biofouling.

Army Corps of Engineers, Waterways Experiment Station, Vicksburg, Mississippi. Rapid bioassessment of benthic macroinvertebrate community structure in the La Crosse River, Fort McCoy, Wisconsin.

Army Corps of Engineers, Waterways Experiment Station, Vicksburg, Mississippi. Rapid bioassessment of benthic macroinvertebrate community structure in two streams in the impact area at Fort Bragg, Fayeyyeville, North Carolina.

Sonoco Products Company, Hartsville, South Carolina. Determination of the source of acute toxicity in settling pond effluent.

RESEARCH INTERESTS

The overall objectives of my research are to determine factors contributing to the rapid declines of unionid mussel populations in the Clinch River, VA. This includes determination of the presence of toxic sediments in the Clinch River, and correlation of these sediments with declining unionid populations. A second objective is to determine if interactions of juvenile unionids with adult *Corbicula* could impair recruitment of the native mussels.

RESEARCH EXPERIENCE

Laboratory skills

Polyacrylamide gel electrophoresis
Starch gel electrophoresis
Enzyme bioassays
Invertebrate identification
Fish identification

Freshwater acute and chronic toxicity testing

Ceriodaphnia dubia
Pimephales promelas
Daphnia magna
Daphnia pulex

Sediment toxicity testing

Chironomus riparius
Chironomus tentans
Hyallela azteca
Pimephales promelas
Daphnia magna
Corbicula fluminea
Villosa iris

Field monitoring

Benthic invertebrate sampling
Fish sampling
Bivalve density sampling

Mary Melinda Yeager