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THE UTILIZATION OF FRESHWATER MUSSEL BIOASSAYS TO CHARACTERIZE SEDIMENT TOXICITY

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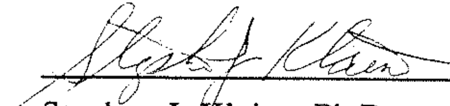
of
the Requirements for the Degree
Master of Science

by
Laurie A. Williams
May 1992

To the Graduate Council:

I am submitting herewith a thesis written by Laurie A. Williams entitled, "The Utilization of Freshwater Mussel Bioassays to Characterize Sediment Toxicity." I recommend that it be accepted for six hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biology.



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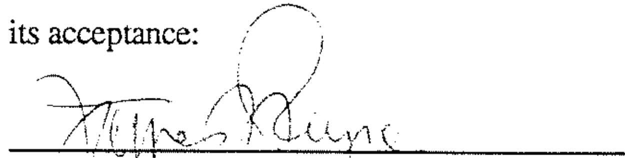

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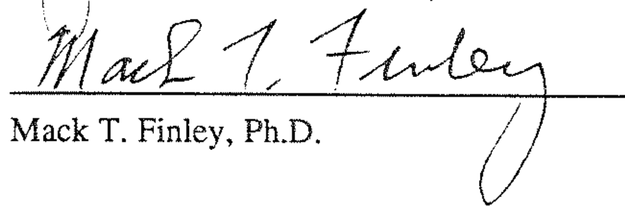

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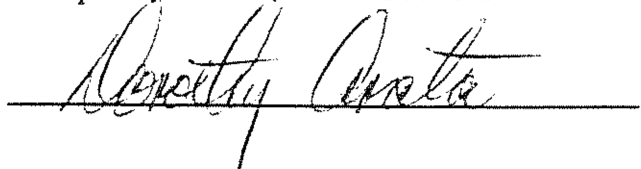

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ABSTRACT

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A 1989-90 study in the Kentucky Lake/Tennessee River system, showed that high Manganese (Mn) concentrations exist in mussel tissues at sites with the fewest species. The chemodynamics of dissolved oxygen (DO) and Mn mobility appears to exert a strong selection pressure on the diversity of mussel species. However, no overt toxicity in adult mussels caged at two sites in the Big Sandy region was apparent. Therefore, one hypothesis is that existing conditions may limit reproductive success. Laboratory and *in situ* bioassays with laboratory cultured juvenile mussels were developed. Results obtained from *in situ* assays conducted in the fall of 1990 with 6-week-old mussels indicated significant mortality at the two sites in Big Sandy: 96.9% mortality at a site nonsupportive of mussels (Site K) and 51.0% in a known mussel bed (Site C). Dissolved oxygen was ≥ 4.9 mg/l at the sediment-water interface at initiation and termination of the assay. Toxicity to juvenile mussels *in situ* correlates with the solubility of Mn at the sediment-water interface. Laboratory sediment toxicity assays, conducted with pore water extracted from sediment collected at the test sites, produced low toxicity to 7-day-old juveniles, with the highest mortality only 10% for Site K. Methods used to collect and transport sediment alter physical and chemical properties and influence toxicity. Results of this study illustrate the need for *in situ* assays to estimate sediment toxicity and suggest that laboratory sediment toxicity tests may misrepresent *in situ* toxicity to benthic organisms.

INTRODUCTION

Freshwater mussels (Class Pelecypoda, Family Unionidae) are harvested for their shells in the Kentucky Lake region of the Tennessee River. Over $8 million worth of shells were exported to Japan for use in the cultured pearl industry in 1988, resulting in a $22 million boost to the local economy (Tennessee Wildlife Resources Agency, 1990). Due to the occurrence of mussel die-offs and reduction of mussel species in recent years, studies were initiated to evaluate the health of the aquatic environment in this region (Scholla et al., 1987).

An interagency project investigated sediment chemistry (including manganese (Mn) diagenesis) in the Kentucky Lake/Tennessee River system during 1989-90. The study focused on the influences on unionid mussel populations from areas in the river channel to embayment sites in the Big Sandy region (Matthews et al., 1990; Pascuzzo et al., 1991). The chemodynamics of low dissolved oxygen (DO), and high Mn mobility appeared to exert a strong selection pressure on mussel species richness in this area (Table 1).

Mussels are bivalve, filter-feeding organisms that reside at the sediment-water interface. They are closely associated with and utilize sediment, overlying water, and pore water, and are therefore exposed to geochemical changes occurring at the benthic boundary. Hence, they may readily accumulate toxicants present in a variety of forms (Brooks and Rumsby, 1965; Luoma, 1989; Tessier and Campbell, 1987). Additionally, the sedentary nature of mussels limits their ability to avoid toxic substances.

The unique life cycle of most unionid freshwater mussels is dependent upon an intermediate fish host. A gravid adult releases mature larvae (glochidia) into the water through its excurrent siphon. The glochidia of *Anodonta imbecilis* possess a long thread

Table 1. Manganese concentrations and species richness at Kentucky Lake study sites (Pascuzzo et al., 1991). Figures are means of data collected during a two-year study.

SITE CHARACTERISTICS

Site	Mn concentration		Species richness
	Pore water (mg/L)	Mussel tissue (ug/g wet wt)	
A	1.22	365	11
C	2.20	1370	5
K	4.25	----	----

and hooked structures at the ventral tips of the shell which allow them attachment to the gills or fins of a fish. After encystment on the host (approx. eight days), most of the organs have formed and are functional, indicating transformation to the juvenile stage.

A. imbecilis is a hermaphroditic, "mud-loving" species, inhabiting areas of fine silt. This species has a large geographical distribution (Davis and Fuller, 1981) and a relatively long period of gravidity. In addition, this species is easily cultured and transformed from glochidium to juvenile in the laboratory (Wade et al., 1989). These characteristics make *A. imbecilis* a valuable bioassay organism.

Ecotoxicology studies have been conducted with adults of both marine (Goldberg et al., 1977; van Eck et al., 1989; Lakshmanan and Nambisan, 1989; Everaarts, 1990) and freshwater bivalves (Jones and Walker, 1979; Graney et al., 1984; Tessier et al., 1984; Czarnezki, 1987; Tevesz et al., 1989; Doherty, 1990). However, certain life stages of a given species may vary in their tolerances to adverse environmental conditions or to certain contaminants, such as trace metals.

Although embryonic, larval, and juvenile stages of marine species are widely used for toxicity testing (Calabrese, 1973; American Society of Testing Materials, 1980; Chapman and Morgan, 1983; Salazar, 1991; Morgan et al., 1986; Cherr et al., 1990), few studies have addressed early life stage responses of freshwater mussels to environmental toxicants (Farris, 1991; Wade, 1989; Wade et al., 1989; Schweinforth and Wade, 1990; Tennessee Wildlife Resources Agency, 1990; Keller and Zam, 1991). Parameters such as DO, pH, and redox potential (Eh) within the sediment and pore water are frequently altered during collection and transport to the laboratory. As a result, bioavailability of trace metals in the field may not be accurately predicted in laboratory tests, posing a need for more *in situ* studies (Livingston and Meeter, 1985; Sasson-Brickson and Burton, 1991). *In situ* toxicity tests using adult freshwater mussel species (Adams et al., 1981; Dickson et al., 1989; Hinch and Green, 1989; Metcalfe and Hayton, 1989) are used more frequently than

those using juveniles. *In situ* and laboratory tests utilizing juvenile mussels could be valuable tools to compliment sediment contaminant studies.

The objective of this study was to compare the results of three mussel bioassays as indicators of sediment-associated stress. In order to do this, the following tasks were accomplished: (1) a caged adult mussel bioassay was conducted at three sites, (2) an *in situ* juvenile mussel bioassay was developed, (3) juvenile mussel bioassays were conducted at the three sites, and (4) sediment samples at the three sites were collected, pore waters were extracted, and laboratory bioassays were conducted with juvenile mussels.

MATERIALS AND METHODS

Site Description

The Kentucky Lake/Tennessee River system flows north through western Middle Tennessee and includes approximately 184 miles of the Tennessee River (TNR) from Pickwick Dam (TNR mile 206.8) to Kentucky Lake Dam (TNR mile 22.5). Lotic conditions with steady flows and gravel substrate exist roughly 100 miles downstream from Pickwick Dam (Pascuzzo et al., 1991). Beginning 80 miles upstream of Kentucky Lake Dam, lentic conditions predominate, with slower currents and large embayments. Land use in this area is predominantly agriculture and forestry. Sediments are naturally high in Mn; geological records show widespread distribution of Mn minerals and sediments coated with iron and Mn oxides (Jewell, 1931; Russell and Parks, 1975).

The sites selected for this study were Sites A, C, and K (Figure 1), previously named by Pascuzzo et al. (1991) in their study of the influence of sediment and water column chemistry on mussel populations within the Kentucky Lake impoundment area. Site A, the most downstream site, is located along a channel ridge between TNR mile 60.5 and TNR mile 63.0. The area is lentic, near the main river channel, and has a diverse mussel community. Site A served as our reference site. Sites C (BSR mile 9/WS 0) and K (WS mile 3.5) are embayment sites in the Big Sandy region receiving runoff from over 250 square miles of agricultural land. Site C is located at the confluence of West Sandy Bay with Big Sandy Bay in the shallows near the southwest shoreline. Although it supports fewer numbers of species than Site A, a mussel bed is also found at Site C. Along with farming practices, wildlife management and dewatering areas are also present. Dewatering areas have had a profound impact on the hydrology in the Big Sandy and West Sandy

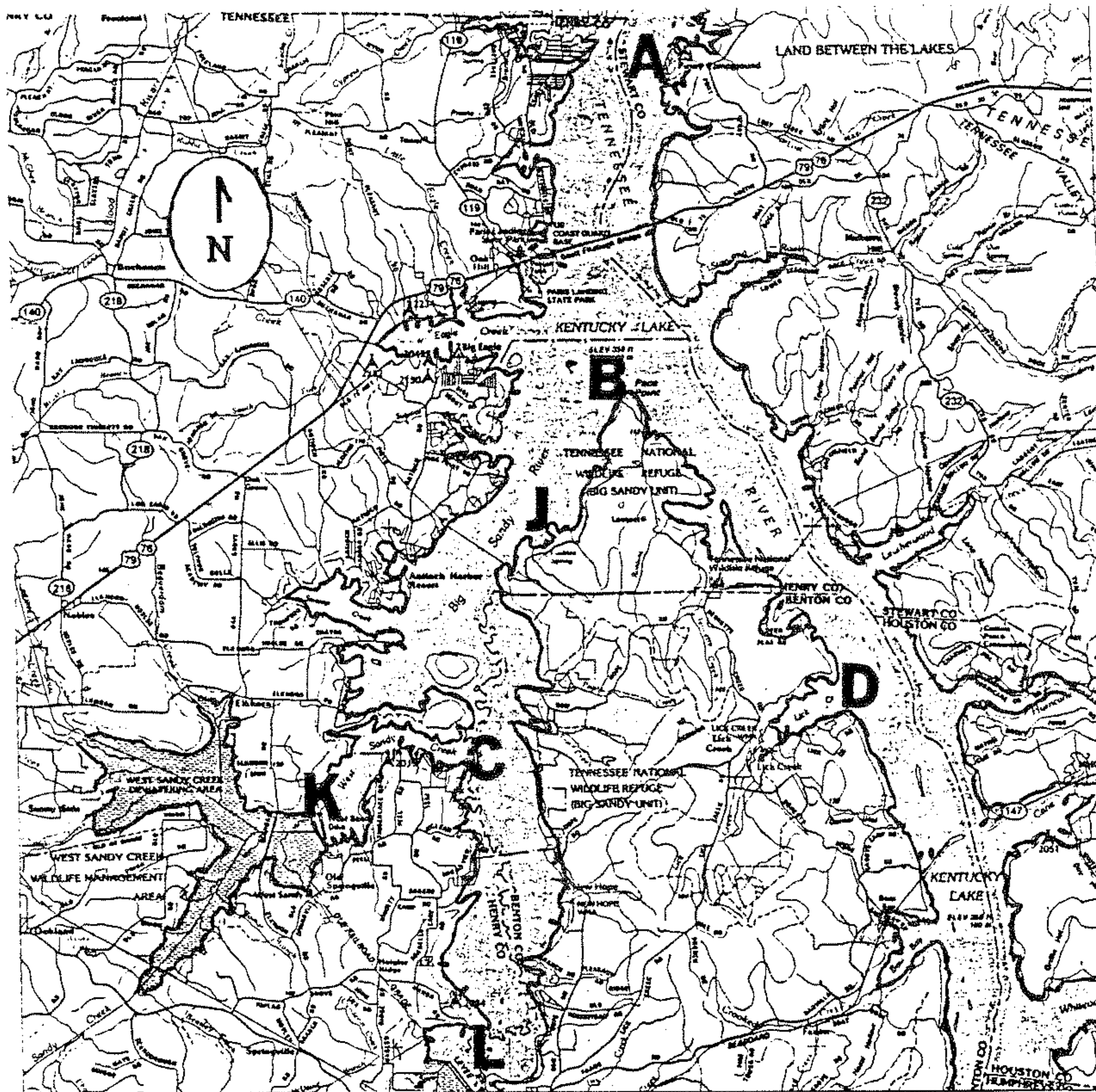

Figure 1. Study sites for mussel bioassays in the Kentucky Lake/Tennessee River system: **Site A**, Piney Campground, TNR mi. 63, Stewart Co., TN, (lat. 36°28'57", long. 87°34'49"); **Site C**, Mouth of West Sandy, BSR mi. 9/WS 0, Henry Co., TN, (lat. 36°19'15", long. 88°06'07"); **Site K,** West Sandy Bay, WS mi. 3.5, Henry Co., TN, (lat. 36°18'09", long. 88°08'34").

embayment areas (Barrass, 1989; 1990). Site K is located mid-bay in West Sandy, downstream of the West Sandy Dewatering area and Tennessee Valley Authority pumping station. A predominant characteristic of this site is a loose, very fine-textured organic suspension of sediment with a high percentage of organic carbon. Site K is considered non-supportive of mussels, although a small population of *Quadrula quadrula* (the Maple Leaf) has been reported.

The benthic environment in the Big Sandy region is not as conducive to mussel growth and colonization as are other lotic or lentic areas in the Kentucky Lake/Tennessee River system (A.J. Roman-Mas, United States Geological Survey, personal communication). Therefore, Sites C and K were selected from this embayment region for conducting toxicity tests with juvenile mussels. Characteristics of each study site are listed in Tables 1 and 2.

Caged Adult Mussel Bioassay

In July 1990, an *in situ* caged adult mussel bioassay was conducted in conjunction with Austin Peay State University in Clarksville, TN. Two species indigenous to the river system, *Quadrula quadrula* and *Fusconaia ebena* were collected by divers near the mouth of Piney Creek Bay on the eastern shoreline of the Tennessee River. Individuals three to five years of age (4 to 7 cm in length) were transplanted to sites A, C, and K. Cages were constructed from 1/4" vinyl aqua mesh netting reinforced with PVC tubing filled with sand. Two weighted cages (15 x 130 x 90 cm) marked with flotation devices were placed at each site. A total of 20 mussels of each species were placed in cages at Site A; 30 mussels of each species were placed in cages at Sites C and K. The assay began July 26 and was terminated November 7. Mussels were checked for mortality biweekly during the 14-week exposure period.

Table 2. Sediment parameters of study sites in Kentucky Lake. (Pascuzzo et al., 1991).

Site	Soil classification	% organic carbon	% water
A	Clay loam	0.524	21.5
C	Loam	0.399	18.6
K	Silt loam	8.530	50.8

Culture of Adult *Anodonta imbecilis*

Gravid *Anodonta imbecilis* were collected from a commercial catfish pond in Lawrence County, Alabama and transported to the Biology Department at Memphis State University. Mussels were held in 15 gallon aquaria containing "artificial lake water" (Millipore® water, 34 mg/l $NaHCO_3$, 18 mg/l NaCl, 59 mg/l $CaCl_2 \cdot 2\,H_2O$, 4 mg/l KCl) and a sand (mesh 24, 0.0227 cm opening) substrate. A concentrated diverse algal solution and, occasionally, fine silt were added to the water as a food source. The algal solution included *Selenastrum, Scenedesmus, Ankistrodesmus*, several blue green species, diatoms, and other unidentified algal species and microinvertebrates. At two week intervals, water and food were replaced. The water temperature in all aquaria was maintained at 22±1°C.

Juvenile Culture Procedure

A modification of a sterile tissue culture technique developed by Isom and Hudson (1982) was used to culture juvenile *A. imbecilis* in the laboratory. This procedure requires a complex medium that simulates the composition of fish gills. This technique is valuable in that it mediates the transformation of glochidia to juveniles without the need for the natural fish host. The medium consists of three major portions: minimal essential medium (MEM), antibiotics, and serum/serum replacements.

The MEM was prepared using a mixture of physiological salts (Unionid Ringers solution), amino acids, dextrose, vitamins, and phenol red, a pH indicator (Isom and Hudson, 1982). To maintain sterile conditions, the solution was filtered using a 0.45 µm sterile Nalgene® filter apparatus. Bicarbonate was autoclaved separately and added after sterile filtering to prevent loss during vacuum filtration. Three antibiotics (carbenicillin, gentimycin, rifampin), and amphotericin B, an antimycotic, were obtained from SIGMA. Each antibiotic was sterile filtered (0.45 µm) and added to MEM to reduce contamination during the culture period. The concentrations of these four components as used in the final complete medium are listed in Table 3.

Table 3. Composition of juvenile mussel culture medium (complete MEM).

Minimal Essential Medium (MEM)	
Dextrose	1.25 ml
Unionid ringers solution (below)	240.0 ml
Essential amino acids (SIGMA)	5.0 ml
Non-essential amino acids (SIGMA)	2.5 ml
100 X vitamins (SIGMA)	2.5 ml
Phenol red (SIGMA)	0.3 ml
Sodium bicarbonate ($NaHCO_3$)	0.55 g

Antibiotics (SIGMA)	
Carbenicillin	100 µg/ml
Gentamycin	5 µg/ml
Rifampin	10 µg/ml

Antimycotic (SIGMA)	
Amphotericin B	125 µg/ml

Unionid Ringers (UR) Solution

Solution A: 1.5 g $CaCl_2$, 1.0 g $MgCl_2 \cdot 6 H_2O$ in 100 ml milli-pore® water

Solution B: 0.765 g NaCl, 0.05 g KCl in 495 ml milli-pore® water

Sterilize both solutions. Add 5 ml Solution A to 495 ml Solution B to make UR solution.

The serum portion of the complete medium is added to MEM (with antibiotics) in a ratio of 1:2, serum:MEM. This portion consists of two serum replacements, TCH and TCM (Celox Corp.), and rabbit serum. A 12% concentration of TCH and TCM (0.6 ml TCH or TCM in 4.4 ml MEM) is added to rabbit serum (SIGMA) in a 1:1:1 serum:TCH:TCM ratio. This formulation eliminates the need to bleed fish and prepare fish plasma, and provides comparable transformation success (Hudson, 1990).

Juvenile cultures were established using sterilized instruments in a laminar flow hood. The outer marsupia (outer gills) of a gravid adult *A. imbecilis* were excised, placed into Unionid Ringers solution or "artificial lake water", and the glochidia were removed by breaking the gills apart with forceps and a probe. The larvae were rinsed three to four times by swirling in a beaker, allowing healthy glochidia to settle, and decanting water to remove gill tissue, dead larvae, bacteria, and protozoa (Isom and Hudson, 1982). Approximately 200 glochidia (250 µm) were introduced into a 15 x 60 mm culture dish containing 3 ml of *complete* media (2 ml MEM with antibiotics; 1 ml serum portion). Glochidia were cultured for eight days in a CO_2 incubator (4-5% CO_2) to maintain media pH in the range of 7.0 to 7.5. (Optimum physiological pH for transformation is 7.2 to 7.3). Although there is little or no increase in size during the transformation period, glochidia lose their attachment thread, and the foot, gills, and some organs develop. The transformed glochidia (juvenile mussels) were transferred along with media to "artificial lake water" and allowed to acclimate for 1 to 2 hours. Juveniles were then filtered through a 100 µm teflon mesh and added to approximately 2 L of "artificial lake water" containing a diverse algal mixture and 3 ml of 100 µm filtered silt from the site of adult mussel collection. Juveniles were kept in constant darkness with daily renewal of water and food until needed for toxicity tests.

***In situ* Test Design**

Six-week-old *A. imbecilis* juveniles were utilized for the *in situ* assays. Mussels of this age facilitate greater ease in observation and counting upon retrieval. Organisms

used in the *in situ* assay reported here were cultured using catfish plasma by Tennessee Valley Authority, Aquatic Research Laboratory near Decatur, Alabama.

In the fall of 1990, juveniles were transported to the test sites in Tennessee River water and were confined in small glass cylinders (3.8 cm x 2.0 cm diam) with teflon (fluorocarbon) mesh (100 μm) secured onto each end with 5.5 in. Bar-lok® nylon cable ties (Figure 2). The test containers were then secured with cable ties onto four sides of a weighted storage crate (13.5" x 15"), allowing exposure to sediment, pore water, and overlying water. Juveniles within the test vials were acclimated to the water at each test site for approximately 15 to 20 minutes. The test vials were then secured to the crate and the complete test apparatus was slowly lowered to the substrate and marked with a buoy. Twelve vials were placed at each site; six vials on the bottom (exposed to sediment and associated pore water) and six vials at mid-crate (exposed only to overlying water, 13 cm above the sediment-water interface). At each level, three vials held five mussels each and three held ten mussels each for a total of 45 mussels at each level. Thirty additional mussels were placed at Site K; one vial with ten and one vial with five mussels at both bottom and mid-crate levels.

Water temperature, pH, DO, and conductivity were measured at one meter increments from surface to the lake bottom (4 cm above the sediment-water interface) with a Hydrolab Surveyor II when juveniles were placed at the site and once again upon retrieval. Exposures were conducted at each site for seven days (9 October to 16 October), after which time the juveniles were harvested, placed in Ziploc bags containing water from the site, and transported to shore in a cooler. On shore, the juveniles were counted and observed for mortality with a dissecting scope within four hours of retrieval.

Laboratory Pore Water Bioassays

Sediment samples were collected with a petite ponar dredge on the afternoon of 24 September, 1991. Successive grabs (containing approximately 5 cm of bottom sediment)

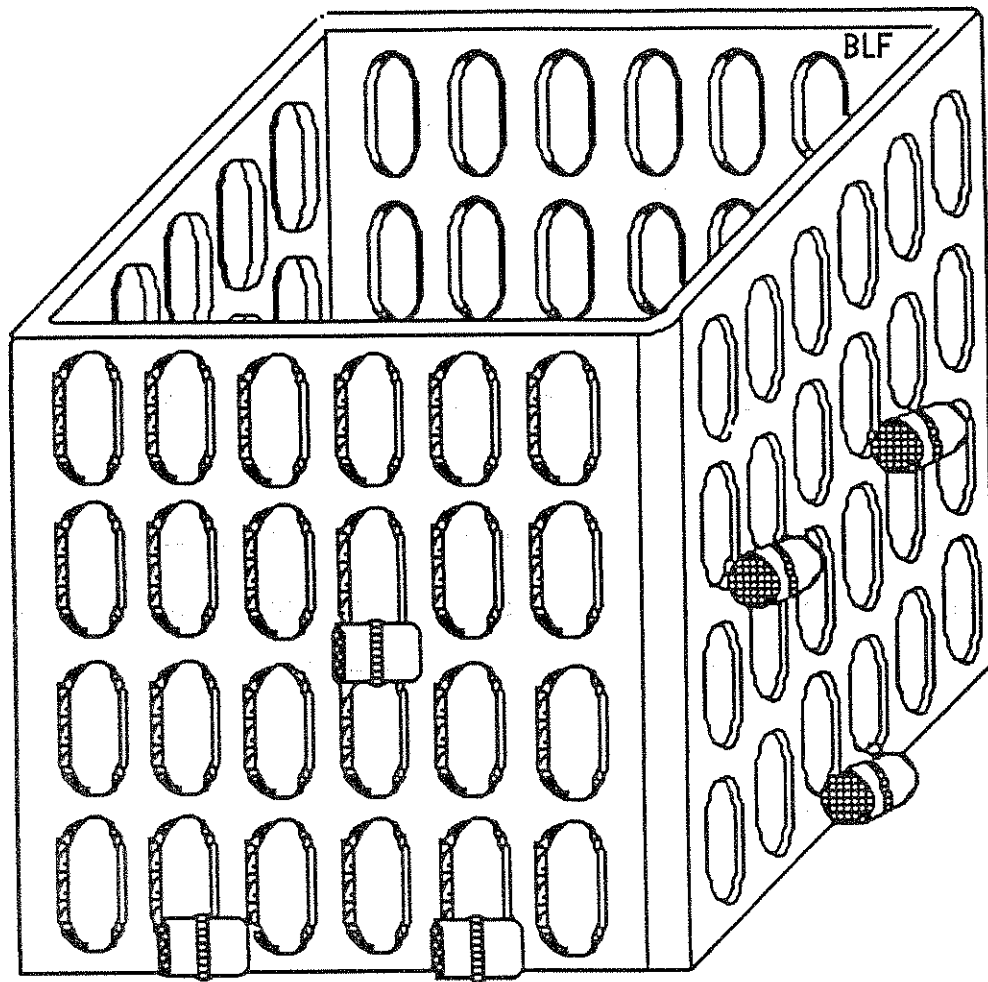

Figure 2. Diagram of *in situ* juvenile mussel bioassay test apparatus.

were pooled at each site. To ensure that an adequate amount of pore water was obtained for the laboratory test, the volume of sediment collected was determined on the basis of the percentage of water making up the bottom sediment at each site (Table 2). Site K sediment (approx. 7 L) was placed into two 5-gallon plastic buckets. Sediment collected from Sites A and C (approx. 20 L and 17 L, respectively) was contained in plastic Rubbermaid® tubs. After decanting overlying water, the sediment was transported to the laboratory and stored at 4°C until processed. Pore water was obtained from the sediment by centrifugation at 10,000 rpm for ten minutes in 250 ml polypropylene bottles. All pore water was extracted within four days of sediment collection.

A modification of the juvenile mussel 7-day static renewal test developed by Wade et al., (1989) was used. Juveniles were transformed and raised in the laboratory for seven days prior to the test. Each pore water concentration and the control were tested in five replicates with ten mussels per replicate. The serum portion of the culture media for this assay consisted of rabbit serum only. The culture yielded approximately 1,200 juveniles. Mussels were placed into small glass cylinders (3.8 cm in length, 2.0 cm diam), and mesh (100 µm) attached to one end with aquarium sealant to serve as the container bottom. The containers were placed in 30 ml plastic beakers with 20 ml of test solution. This test design allows rapid, daily transfer of vials to new beakers with fresh test solution without unnecessary handling of the juveniles.

Extracted pore water (100%) and two dilutions of 70/30 (v/v) and 90/10 (v/v) were prepared with "artificial lake water" for all three sites. "Artificial lake water" controls were tested in addition to the 100%, 30%, and 10% pore water concentrations. Diverse concentrated algae (0.6 ml) and 100 µm filtered silt slurry (0.2 ml) were added per 100 ml solution. The assay was conducted in total darkness at 25±1°C . All juveniles were observed each day for mortality and checked for lack of movement and absence of ciliary action in the digestive tract. Mussels were rinsed with and placed in "artificial lake water" for counting before being returned to new test solution.

Water quality parameters monitored throughout the test were pH, DO, conductivity, alkalinity, and hardness. A Fisher Accumet® pH meter Model 805 MP with Orion combination probe (or Corning 140 pH meter), YSI Model 57 oxygen meter, and a Hach® portable conductivity kit were used to determine their respective measurements. Alkalinity and hardness were determined by titration (American Public Health Association, 1985) or with a Hach® kit. All water quality parameters were recorded at the time of test solution renewal.

Data Analysis

Mortality data were analyzed for statistical significance using the Kruskal-Wallis test (combination of RANK and ANOVA procedures) for non-parametric analyses.

RESULTS AND DISCUSSION

Caged Adult Mussel Bioassay

Pascuzzo et al. (1991) observed that as pore water concentration of Mn ([Mn]) increased, greater Mn was measured in analyzed mussel tissue, and ascribed this to greater availability and bioaccumulation of the soluble form of the metal (Table 1). Bivalve molluscs bioaccumulate large amounts of Mn not readily displaced or exchanged, much of that being deposited in the gill tissue (Seah and Hobden, 1969). *Quadrula quadrula* , a tolerant species that is widespread throughout the Kentucky Lake/Tennessee River system, and individuals of the genus *Anodonta*, accumulate considerably high amounts of Mn with no apparent detrimental effects. In contrast, *Fusconaia ebena* appeared less tolerant to Mn, with lower amounts concentrated in soft tissues (Pascuzzo et al., in press). Both *Q. quadrula* and *F. ebena* are indigenous to Site A. *Q. quadrula* comprises 85.3% of the mussel fauna at Site C whereas *F. ebena* is absent. To further examine environmental conditions that may promote uptake and toxicity of Mn to mussel fauna in the Big Sandy region of the system, these two species were transplanted from Site A to Sites C and K .

All mussels survived at the reference site, and dissolved oxygen concentrations measured were above 6 mg/l throughout the assay. As expected, *F. ebena* exhibited high mortality at the non-supportive site (Site K); all individuals were dead by week eight (Figure 3). *Q. quadrula*, however, survived here over the same time period with only 16.7% mortality. A death rate of only 6.7% was observed in the number of *F. ebena* transplanted to Site C. Dissolved oxygen dropped below 5 mg/l during weeks 6 and 8 at Site C and during weeks 1 and 6 at Site K.

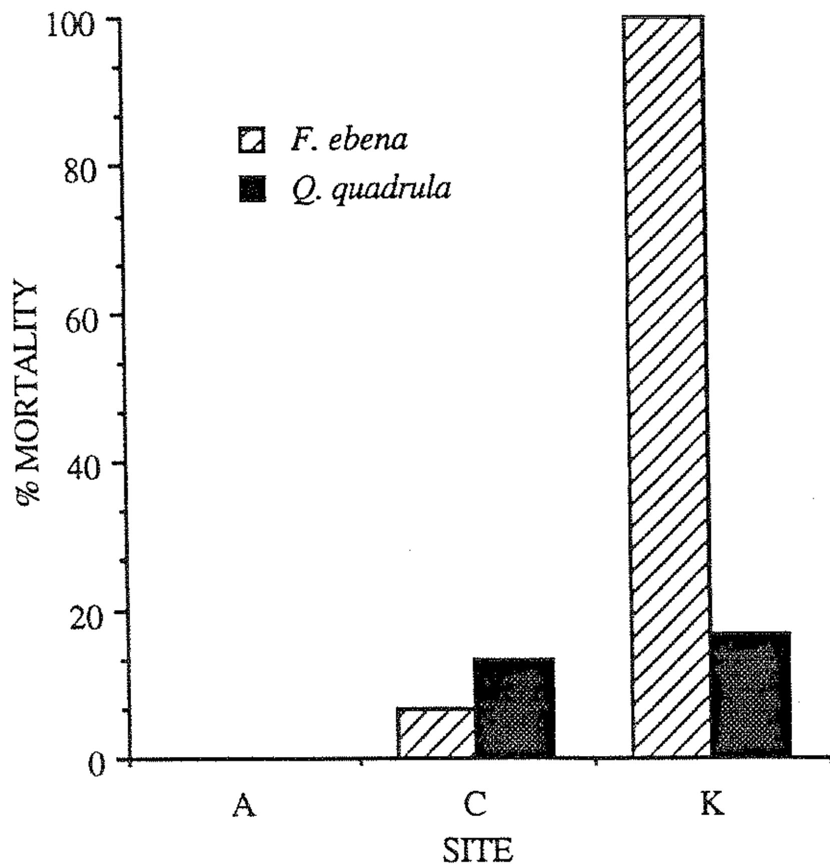

Figure 3. Percent mortality of *Q. quadrula* and *F. ebena* observed in adult caged mussel bioassay over 14 weeks.

Mortality results at Site K suggest a differential species sensitivity to conditions present at this site. Mortality results at Site C, however, indicate that there is little difference in survival of adults between the two test species at this site. Reduced species distribution at Site C may be a result of reduced recruitment for sensitive species. That is, the mobility of Mn or conditions which favor this process, may have substantial effects on the develop-ment and survival of early life stages of these and other freshwater mussel species. In turn, this could result in the differences in species composition observed within the Big Sandy Region of the river system.

Ford (1989) explains the use of indicator species in assessing environmental conditions as being based on the idea that the distribution of at least some species or groups of species are constrained to a narrow range of environmental conditions. Consequently, changes in chemical conditions will affect the occurrence of those species, eliminating some and encouraging colonization by others. Based on this approach, freshwater mussels, having restricted movement and confined to a limited area, are particularly suitable indicators of aquatic sediment toxicity. Utilization of the early life stage strengthens their use as an indicator species due to the potential for increased sensitivity to aquatic toxicants. To date, the majority of molluscs used as indicators of toxicity are marine organisms. However, Wade et al. (1989) have initiated the use of juvenile *A. imbecilis* as a freshwater indicator species.

***In situ* Juvenile Bioassay**

Exposure *in situ* revealed significant mortality of test organisms at Sites C and K, with conditions at Site K inducing the highest juvenile mortality (Figure 4). Mortality of those organisms at Site K exposed to both sediment and pore water (bottom of crate) was 96.86% (F=107.21, DF=1,8 P=0.0001), much higher than mortality at the reference site (21.2%). Low survival was expected since high mortality of the sensitive species was measured in the caged adult assay, and there was a lack of natural mussel populations at

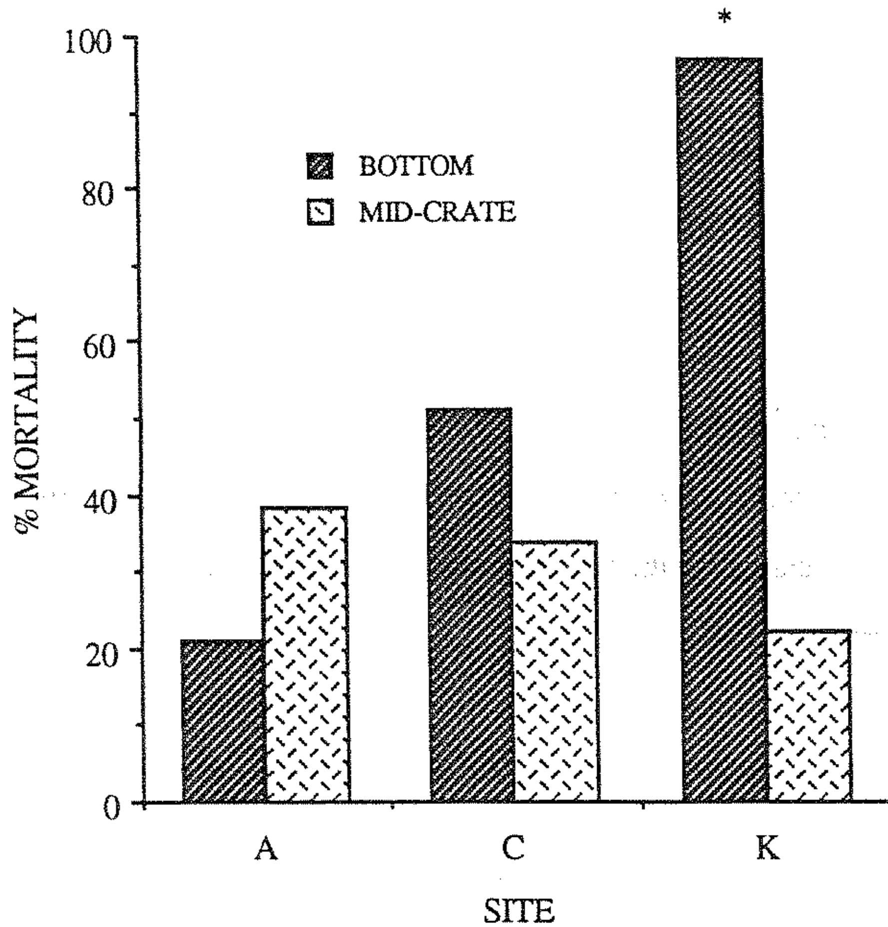

Figure 4. Percent mortality observed in *in situ* juvenile mussel bioassay of juveniles exposed to water column (mid-crate) and those exposed to sediment and pore water (bottom of crate).

this site. The relatively high mortality in sediment (51.0%) at Site C, although not statistically significant (F=3.67, DF=1,8 P=0.0917), indicates unfavorable conditions approaching West Sandy Bay. Decreased species diversity of adult mussels, and sensitivity of juveniles placed *in situ* at Site C suggest that the population may be limited by unsuccessful recruitment of certain species.

Juvenile mussels suspended in the water column showed greater survival than those exposed to sediment and associated pore water (Figure 4). Survival of those placed at mid-crate at Site K was significantly higher (F=95.17, DF= 1,8, P=0.0001) than survival of juveniles placed in the sediment at this site. This indicates toxicity at Site K is a function of exposure to sediment and associated pore water. No differences between crate levels at Sites A and C were observed. Additionally, there were no statistically significant differences in mortality between sites C and K at mid-crate. Dissolved oxygen was ≥ 4.9 mg/l at initiation and termination of the assay, although diel fluctuations over the duration of the test were not documented (Table 4).

Due to the escape of some juveniles as a result of inadequate security of mesh onto some test containers, an alternative test vial was developed. The bottom areas of glass scintillation vials (5.5 cm long, 2.5 cm diam) were cut off and the opening was covered with 100 μm mesh using aquarium sealant. An opening cut into the screw top (1.2 mm) covered with mesh allowed the chamber to be sealed. This design prevents the loss of test organisms and allows adequate water and sediment exchange across the mesh.

As a survival mechanism, juvenile mussels are metabolically active, and unlike adults, appear unable to shut down (by valve closure) to resist short-term environmental perturbations. As a result, juvenile mussels appear to be more sensitive to toxicants and serve as an ideal test organism for sediment toxicity studies (Wade et al., 1989). In addition, smaller mussels have relatively greater filtration rates and gill areas in proportion to mass and therefore have an increased potential to accumulate water-borne contaminants (Muncaster, 1990). Consequently, contaminants associated with pore water, suspended

Table 4. Water quality at *in situ* juvenile mussel bioassay sites.

Site	Depth	WATER PARAMETERS (Start / End[a]) pH	DO	Conductivity	ORP[b]
A	3.6	7.3 / 7.6	7.0 / 9.8	0.201 / 0.196	0.261 / 0.206
C	4.2	6.8 / 7.0	5.4 / 9.9	0.135 / 0.114	0.252 / 0.236
K	4.8	6.7 / 7.1	4.9 / 7.2	0.134 / 0.114	0.270 / 0.262

[a] test start date - 10/9/90; ending date - 10/16/90
[b] oxidation-reduction potential

solids or organic matter may contribute to mortality. Numerous studies exist in the literature concerning Mn diagenesis (Hem, 1964; Berner, 1980; Stumm and Morgan, 1981; Burdige and Gieskes, 1983; Chiswell and Mokhtar, 1986; Stone, 1987; Brookins, 1988). In the oxidized form, Mn is usually present in the solid phase as MnO_2, commonly occurring as a coating on minerals and finely dispersed particles in the sediment. However, under reduced conditions, Mn tends to be mobilized and partitioned into pore water as the soluble form (Mn^{+2}). Patterns of Mn diagenesis similar to those reported in the Kentucky Lake/Tennessee River system by Pascuzzo et al. (1991) have been reported to occur in the Great Lakes (Richardson and Nealson, 1989).

Site K exhibits high concentrations of soluble Mn in pore water and at the sediment-water interface, as well as high concentrations in the solid phase, probably a result of microbial oxidation (Gordon, 1989). Microorganisms can act as redox catalysts, reducing and dissolving Mn oxides with metabolites such as pyruvate and oxaloacetate (Stone, 1987). Additionally, dissolved Mn commonly builds up in interstitial waters of organic-rich, freshwater sediments due to the reduction of MnO_2 accompanying bacterial decomposition of organic matter (Berner, 1980). Site K differs from other sites in the Kentucky Lake/Tennessee River system in that it has the solid-phase [Mn] profile of an oxygenated site (e.g., Site A) and the soluble [Mn] profile of a more reduced site (e.g., Site C) (Pascuzzo et al., 1991).

Elevated water column metal concentrations have been correlated with organic matter decomposition, low flow conditions, and initial storm water flushing (Forstner, 1990). However, Mn levels in water column samples were, in all cases, less than 0.01 mg/l (Pascuzzo et al., 1991), indicating that Mn availability is associated with sediment and pore water. *In situ* mussel mortality at Site K occurred in a benthic environment that also exhibited elevated Mn in the pore water. Significantly lower mortality was observed in juveniles exposed only to overlying water. Toxicity of sediment at Sites C and K are consistent with conditions favoring the mobilization of Mn; low Eh at the sediment-water

interface, high organic carbon content, high sediment oxygen demand (SOD), and low DO create the reducing conditions that enhance Mn mobility (Pascuzzo et al., 1991). However, at Site C, these conditions are not as pronounced and may result in a reduction in species richness instead of mussel extinction.

Laboratory Pore Water Bioassay

Because pore water is widely considered to be the primary route of contaminant uptake by benthic organisms (especially filter feeding mussels), this phase was used to estimate sediment toxicity in the laboratory assay. Many studies have shown higher toxicities in pore water compared with solid phase or elutriate laboratory assays (Hoke and Prater, 1980; Cairns et al., 1984; Schuytema et al., 1984; Giesy et al., 1988; Sasson-Brickson and Burton, 1990). Also, greater concentrations of pollutants and other toxic components such as aluminum are more often found associated with pore water (Ankley et al., 1990).

Obvious differences in turbidity due to dissolved and suspended solids and organic carbon were observed in extracted pore water. Noticeably higher amounts of these components were present in water from Site C, and the pore water from Site K was a deep brown color, indicating high amounts of dissolved organic carbon. This correlates well with the findings of Pascuzzo et al. (1991) (Table 2). Dissolved oxygen concentrations were sufficient throughout the test (Table 5).

Low juvenile mussel mortality was measured in the pore water assay; the highest death rate occurred in the 100% concentration of pore water at Site K, with loss of only 10% of test organisms. Mussels are exposed to pore water from the top 5 cm of sediment, of which the upper 2 cm represent the biologically active portion. Sediments collected were well within this depth range; however, oxidation of sediment and pore water during collection and transport from field to laboratory alters the natural physical and chemical nature of the sediment (Burton, 1991). Soluble Mn present in pore water exposed to oxygen during manipulation of sediment for the laboratory assay was likely

Table 5. Water quality for juvenile mussel laboratory bioassay.

Concentration	DO	pH	Cond	Hardness[a]	Alkalinity[b]
Initial reading (100% pore water only)					
Artificial lake water	7.80	7.45	350	68.4	40
Site A	7.70	7.87	300	68.4	52
Site C	7.50	7.93	300	68.4	42
Site K	7.10	7.81	1300	68.4	78

Assay readings[c]

Concentration	DO	pH	Cond	Hardness[d]	Alkalinity
Control	7.40	7.52	309	142.72	---
Site A					
100%	7.05	7.58	180	124.88	---
30%	7.22	7.54	249	---	---
10%	7.36	7.51	263	---	---
Site C					
100%	6.96	7.42	176	115.96	---
30%	7.21	7.35	249	---	---
10%	7.21	7.43	261	---	---
Site K					
100%	5.94	7.22	166	115.96	---
30%	7.02	7.35	250	---	---
10%	6.94	7.44	264	---	---

[a]Readings determined with Hach kit
[b]Readings determined by titration (APHA, 1985)
[c]Average of 5 to 7 readings for DO, pH, and conductivity
[d]Readings determined by titration (APHA, 1985) with 100% concentrations only

precipitated to MnO_2 and bound to the sediment, thereby making it less available for uptake. This may explain the lack of toxicity observed in the laboratory assay when compared to the field study with juvenile *A. imbecilis*. Fungal hyphae introduced during transfer from media to holding water caused contamination of some test vials, but showed no detrimental effects to the juvenile mussels.

Although primary exposure is thought to occur via pore water, freshwater mussels may also ingest and bioaccumulate metals sorbed onto suspended solids taken in while feeding. The extremely high amount of organic matter and suspended solids in pore water extracted from sediment collected at Site K is likely the cause of mortality at the 100% concentration due to contaminants that were adsorbed to fine particles and possibly metabolized to a more toxic or bioavailable form (Luoma, 1989).

Previous studies have illustrated the utility of juvenile mussels as a laboratory test organism. Laboratory assays conducted by Wade et al. (1989) illustrate the toxic effects of Mn as well as a paper mill effluent, two herbicides and a larvicide on *A. imbecilis*. Responses to Mn were tested under both oxygenated and low DO conditions. They calculated an LC_{50} of 36.2 mg/l Mn in the presence of sufficient oxygen. However, an increase in toxicity was observed under pulsed low DO conditions (LC_{50} of 19.6 mg/l). Sublethal effects associated with exposure to Mn, such as retarded shell growth, have also been reported (Schweinforth and Wade, 1990). Keller and Zam (1991) found juvenile *A. imbecilis* to be as sensitive to some metals and metal mixtures as zooplankton, and more sensitive than commonly tested fish and aquatic insects in acute laboratory tests.

An acute static-renewal assay with *Ceriodaphnia dubia* indicated that the presence of humic acid lowers the toxicity of Mn. This suggests there may be other factors, particularly at Site K, causing mortality of mussels observed in the laboratory assay conducted as part of this study. An important consideration is the dependence of partitioning and thus, availability of contaminants on many chemical and physical processes. Results of laboratory pore water assays may be best used to assess the potential toxicity of

sediment under aerobic conditions (Geisy et al., 1990). Factors such as low DO which affect the mobilization of Mn are not a factor in standard laboratory bioassays. *In situ* assays, on the other hand, expose mussels to temporarily integrated changing conditions. Periods of anoxia during not only mid-day, but also in the early morning hours when photosynthetic activity is eliminated, may be directly or indirectly (by triggering metal mobilization) stressful to mussels.

Although no conclusive evidence exists that Mn is the cause of juvenile mortality, bioaccumulation of Mn (as evidenced in adult mussels of this area), juvenile survival data at study sites in the Big Sandy region of the Kentucky Lake/Tennessee River system, and documented geochemical changes in the benthic environment at these sites suggest Mn chemodynamics play an important role in mussel species distribution. It is possible that solubility and mobilization of other metals as well as the generation of hydrogen sulfide also play a significant role; the observed Mn associated processes may simply be an indicator of poor water quality in general.

Recently, an increasing number of *in situ* assays have been conducted (Burton, 1991), but few have focused directly on toxic effects of sediment (La Point et al., 1989). Although few in number, toxicity tests conducted with glochidial larvae (Farris, 1991) and laboratory cultured freshwater juvenile mussels (Wade et al., 1989; Keller and Zam, 1991) show much promise for the use of this sensitive life stage as a standard test organism for the assessment of sediment toxicity. The use of juvenile freshwater mussels as *in situ* test organisms may aid in the determination of sediment quality criteria, particularly in areas where mussels are of great economic importance.

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