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Development of a field bioassay with juvenile mussels

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Abstract. Two embayment sites in Kentucky Lake, an impoundment of the Tennessee River, have low species diversity of mussels. A field bioassay with 6-wk-old laboratory-cultured juvenile mussels (*Utterbackia imbecillis* Say) was developed and used to compare these sites with a reference site. Mussels exposed to sediment at the test sites exhibited higher mean mortality than those exposed only to overlying water. Results showed significant mortality at both low-diversity sites: 97% mortality at a site where mussels were absent, and 51% in a mussel bed with limited species diversity. Mortality of sediment-exposed juvenile mussels at a reference site with diverse species was only 21%. Based upon the results of this study, field assays with juvenile mussels can be used to assess stress in aquatic ecosystems.

Key words: juvenile mussels, field bioassays, *Utterbackia imbecillis*, Unionidae, sediment toxicity.

Most ecotoxicology studies of bivalves in freshwater environments have been conducted using adult organisms (Jones and Walker 1979, Graney et al. 1984, Tessier et al. 1984, Czarnezki 1987, Hemelraad et al. 1987, Hemelraad and Herwig 1988, Kramer et al. 1989, Tevesz et al. 1989, Doherty 1990). However, adults of many species are known to be less sensitive to contaminant burdens than early life stages (McKim 1984). Only a few studies have addressed early life stage responses of freshwater mussels to environmental toxicants and only in the laboratory (Keller and Zam 1991, Jacobson et al. 1993). In contrast, embryonic, larval, and juvenile stages of bivalve species are widely used for toxicity testing in marine systems (ASTM 1980, Chapman and Morgan 1983, Morgan et al. 1986, Cherr et al. 1990). Consequently, little is known about the responses of early life stages of freshwater bivalves under actual field conditions.

The sedentary nature of mussels limits their ability to avoid toxic substances, increasing the length of exposure to toxic sediment. Filter-feeding juvenile unionid mussels reside at the sediment-water interface and, because of their small size, are closely associated with sediment and overlying water. These organisms are affected by biogeochemical processes occurring at the benthic boundary and are exposed to a variety of toxicants that may be readily accumulated (Brooks and Rumsby 1965, Tessier and Campbell 1987, Luoma 1989). Pore water and sediment particles can be taken in through the incurrent siphon, passed through the gills, and directed by ciliary action to the digestive tract where food particles are processed. As a result, pore water is a likely route of exposure for juvenile mussels, and uptake of aqueous and particulate contaminants may contribute to increased body burden in the soft tissues.

Laboratory toxicity tests may not accurately predict the field bioavailability of contaminants such as trace metals. Variables such as dissolved oxygen (DO), pH, and redox potential (E_H) within the sediment and pore water are frequently altered during collection and transport of samples to the laboratory (Burton 1991). The

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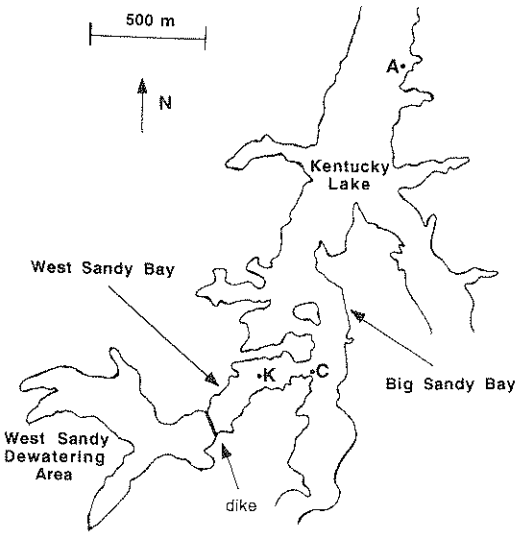

FIG. 1. Map of the Kentucky Lake study area showing the reference site (Site A) and the two test sites (sites C and K).

method and length of storage following collection may also influence the stability of sediment conditions (Hulbert and Brindle 1975, Wiederholm and Dave 1989). One alternative is the development and use of field bioassays (Livingston and Meeter 1985, Sasson-Brickson and Burton 1991). Although field assays have used adult freshwater mussel species (Adams et al. 1981, Hinch and Green 1989, Metcalfe and Hayton 1989), use of the juvenile stage has not been reported.

The objective of this study was to develop a field bioassay using laboratory-cultured juvenile freshwater mussels. The assay was conducted in an impoundment of the Tennessee River, Kentucky Lake, at three sites that displayed a range of adult species diversity. Historical evidence suggests that healthy, diverse mussel populations once existed throughout this system (Ortmann 1925, van der Schalie and van der Schalie 1950, Isom 1969). By the mid-1980s, however, more than half the species described by Isom (1969) were considered rare, endangered, or missing (Sickel et al. 1983, Bates and Dennis 1985). To test the field bioassay, we had to find sites that appeared to be chemically or physically impaired. Previously, researchers in the laboratory at Memphis State University observed a decrease in both mussel abundance and species diversity in Kentucky Lake as they sampled from the main channel into the Big Sandy and West Sandy bays (Table 1, unpublished data). The hypothesis of our research was that field bioassays using a sensitive life stage could detect adverse conditions that may have contributed to the decrease in the number of species present.

Methods

Study site

The Kentucky Lake reach of the Tennessee River system flows north through northwestern Tennessee and includes approximately 300 km of the Tennessee River (TNR) (Fig. 1). Site A, the reference site (36°28′57″N, 87°34′49″W), lies near Panther Bay along a channel ridge adjacent to Piney Campground in the Land Between the Lakes Recreational Area. Sites C and K are embayment sites in Henry Co. and receive runoff from ~650 km² of agricultural land. Wildlife management and wetland dewatering areas are present in these catchments. Site C (36°19′15″N, 88°06′07″W) is in the shallows near the southwest shoreline of the confluence of West Sandy Bay and Big Sandy Bay. Site K (36°18′09″N, 88°08′34″W) is mid-bay in West Sandy Bay, downstream of the West Sandy Dewatering area that is adjacent to a pumping station managed by the Tennessee Valley Authority. Characteristics of each study site are listed in Table 1.

Field juvenile mussel assay

Utterbackia imbecillis Say, formerly *Anodonta imbecillis* Say (Hoeh 1990), is a hermaphroditic, pond species inhabiting areas of fine silt. This species has a broad geographical distribution (Davis and Fuller 1981) and is found in the TNR

TABLE 1. Mussel species richness and sediment variables at Kentucky Lake study sites.

Site	No. of species	Sediment variables: Soil classification	% organic carbon	% water
A	11	Clay loam	0.524	21.5
C	5	Loam	0.399	18.6
K	0	Silt loam	8.53	50.8

system. *Utterbackia imbecillis* has been successfully cultured and transformed from glochidium to juvenile in the laboratory for use in toxicity testing (Wade 1989, Keller and Zam 1991).

Organisms used for our field assay were cultured at the Tennessee Valley Authority Aquatic Research Laboratory near Decatur, Alabama, using a tissue culture technique developed by Isom and Hudson (1982). Age of the juveniles was determined beginning with the time they were transferred from culture media to TNR water. Juvenile mussels were shipped overnight to Memphis State University and were maintained in TNR water under static conditions and constant darkness at 22 ± 1° C. Water was renewed 3–4 times/wk, at which time the juveniles were fed a diverse algal mixture and silt ad libitum. The algal mixture was cultured by seeding Bold's Basal Algal Medium with pond water in a 20-L glass jar under constant light and aeration. Because 6-wk-old juveniles are easier to observe and count upon retrieval, this age was used for the field assays.

Juveniles were taken to the study sites in small glass vials (cylinders 3.8 × 2.0 cm diam) with 105-μm teflon mesh attached to each end with plastic cable ties. Juveniles were exposed to fresh TNR water from each site for ~30 min before vials were secured to the crate. The vials were then attached horizontally to all four outer sides of a standard plastic storage crate (38.1 × 34.3 × 27.0 cm) using cable ties (Fig. 2). Six vials were attached to the bottom edge of the crate (exposed to sediment) 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 C: one vial with five and one vial with ten mussels at both bottom- and mid-crate levels. At each site, the complete apparatus was weighted with a brick, slowly lowered to the substrate in an upright position, and marked with a buoy.

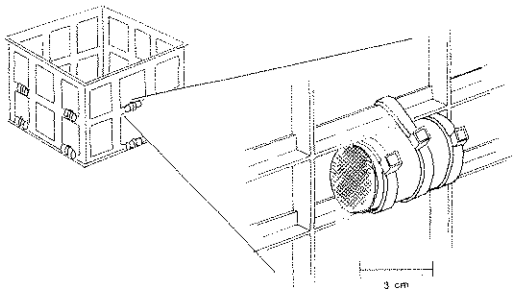

FIG. 2. Diagram of the complete apparatus used for the field juvenile mussel bioassay. Enlarged area shows method of attachment of vials to crate with cable ties.

Water temperature, pH, DO, conductivity, and E_H were measured at the lake bottom with a Hydrolab Surveyor II when juveniles were placed at the site and once again upon retrieval (Table 2). The mussels remained at the three sites for 7 d. Upon retrieval, vials containing juveniles were placed in resealable plastic bags containing water from the site and taken to shore in a cooler. Juveniles were counted and observed for mortality with a dissecting scope within 4 h of retrieval. Lack of movement, with absence of ciliary action in the digestive tract, and deterioration or absence of soft tissues indicated death.

Results

Data were typically non-normal and had heteroscedastic variances. Hence, juvenile mortality at each site was analyzed for statistical significance using the nonparametric Kruskal-Wallis test (SAS 1985). Some juveniles escaped from vials, and vials from which all juveniles escaped were excluded from analysis. Mortality of sediment-exposed mussels at both of the test

TABLE 2. Initial and final (I/F) water physicochemical variables for the field juvenile-mussel bioassay. Readings were taken at the sediment-water interface at the indicated depth.

Site	Depth (meters)	Water temp (°C)	pH	DO (mg/L)	Conductivity (μmhos)	E_H
A	3.6	23.3/19.3	7.3/7.6	7.0/9.8	201/196	0.261/0.206
C	4.2	22.0/18.4	6.8/7.0	5.4/9.9	135/114	0.252/0.236
K	4.8	21.8/18.6	6.7/7.1	4.9/7.2	134/114	0.270/0.262

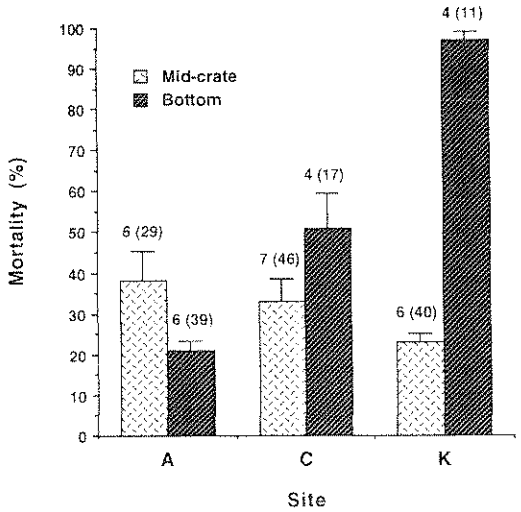

FIG. 3. Mean percentage mortality observed in the field bioassay. Mid-crate refers to juvenile mussels exposed to water column only; bottom refers to juveniles also exposed to the sediment. Error bars represent sample standard error. Numbers above each bar show the number of replicate vials, and numbers in parentheses show the total number of juveniles recovered. See text for further details.

sites (C and K) was significantly higher ($p < 0.05$) than that at the reference site ($F = 12.75$; df = 1,12; $p = 0.0038$). Although confounded by the escape of study organisms, mortality observed at Site K was still significantly higher than mortality at Site C ($F = 6.59$; df = 1,6; $p = 0.0425$), indicating more adverse conditions within West Sandy Bay. Mid-crate mortality within replicate vials ranged from 0 to 100% at sites A and C, and from 0 to 38% at Site K. Mortality ranges of sediment-exposed mussels were 0–33% at Site A, 0–80% at Site C, and 88–100% at Site K.

Although not significant at the $p = 0.05$ level, mortality of juvenile mussels at the two test sites tended to be higher among those exposed to sediment than among those placed in the water column (mid-crate). Site A had expected "background" mortality of 21–38%, encompassing the range seen in the mid-crate organisms at sites C and K (Fig. 3). Elevated mortality was evident with sediment exposures at sites C and K. The greatest mean mortality of juvenile mussels suspended in the water column (mid-crate) at the test sites was 33% at Site C, while mean mortality at bottom-crate level at this site was 51%. Mortality of those placed at mid-crate at Site K was much lower (23%) than mortality of juveniles placed in the sediment at this site (97%).

Discussion

Low survival of juveniles was expected at Site K because of the absence of mussel populations at this site. Low numbers of resident adult mussels at Site C, and mortality of juveniles placed there, suggest that mussel populations may be limited by unsuccessful recruitment and survival of some species. Higher mortality of juveniles at bottom-crate level as opposed to mid-crate level, particularly at Site K, suggests that toxicity in this embayment is associated with the sediment. Mortality observed at the reference site (21–38%) is probably a combination of handling stress (although handling was minimal) and natural mortality, which is known to be high during the first year of life (Howard 1922). Mean mid-crate mortality at sites C and K were within the range of "background" mortality observed for the reference site (A), indicating that elevated mortality was restricted to vials in contact with the sediment.

During the course of our field assay, some of the sediment-exposed mussels escaped because the mesh was inadequately attached to the vial. Therefore, an alternative test vial has been developed for future field tests using glass scintillation vials. The bottom surface of the vial (5.5 cm long, 2.5 cm diam) is removed and the opening is covered with 300-μm-mesh netting secured with silicone aquarium sealant. A screw top with a mesh-covered opening (1.2 cm) seals each vial. Vials are secured onto small storage crates (21.5 × 18.5 × 15.5 cm) in a vertical position to allow maximum exposure to the sediment. Subsequent studies using the improved caging method have shown it to be adequate for use in field toxicity testing with juvenile mussels.

Several other studies have illustrated the utility of juvenile *U. imbecillis* as a test organism in laboratory situations. Wade et al. (1989) examined toxicity of a larvicide to 6–10-d-old *U. imbecillis* in laboratory exposures. Keller and Zam (1991) reported juvenile *U. imbecillis* to be as sensitive as zooplankton to some metals and metal mixtures in acute laboratory tests, and more sensitive than commonly tested fish and aquatic insects. However, water quality vari-

ables such as low DO, as well as changes in speciation of trace metals, resulting in the flux of bioavailable contaminants across the sediment water interface, are not manifested in standard laboratory bioassays. Field assays, on the other hand, expose mussels to more natural conditions over time. Therefore, field assays should yield more realistic estimates of effects than laboratory assays.

Juvenile mussels are metabolically active and, unlike adults, appear less capable of "shutting down" (by valve closure) to resist short-term environmental perturbations. As a result, juvenile mussels appear to be more sensitive to toxicants and can serve as a better test organism for sediment toxicity studies than adults. In addition, smaller mussels have relatively greater filtration rates and gill areas in proportion to mass and therefore have a higher potential to accumulate water-borne contaminants than adults (Muncaster et al. 1990). Consequently, contaminants associated with pore water, suspended solids, or dissolved organic carbon may contribute to juvenile mortality observed in field assays.

More field assays have been conducted recently, but only a few have focused directly on toxic effects of sediment. Our study shows that juvenile *U. imbecillis* can be used successfully in field toxicity testing. Additional studies using juvenile mussels are needed to examine their potential use as test organisms for field assays that aid in the determination of water and sediment quality criteria, particularly in areas where mussels should make up a significant portion of a healthy benthic community.

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