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SCREENING TOXICITY EVALUATION OF WHEELER RESERVOIR SEDIMENTS USING JUVENILE FRESHWATER MUSSELS (ANODONTA IMBECILLIS SAY) EXPOSED TO SEDIMENT INTERSTITIAL WATER

by

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EXECUTIVE SUMMARY

Reservoir sediments (porewater) near several wastewater outfalls at Decatur, Alabama, were screened for acute (9-day) toxicity to 8-day old freshwater mussels. Sampling locations corresponded to four of five sites previously surveyed by the Alabama Wildlife Federation and Alabama mussel divers. A site located on the opposite (north) overbank where mussels are abundant was chosen as the study control. Reference sediments from an outdoor channel at TVA's Aquatic Research Laboratory (ARL) with flow-through Wheeler Reservoir water and from a downstream (Kentucky) reservoir were included in the study for comparative purposes.

Toxicity was observed at two of the Decatur sites (stations Alpha and Delta) and in the ARL channel. Sediments from the other two Decatur sites (stations Charlie and Echo), the north overbank (control), and Kentucky Reservoir were not toxic to the test animals.

Toxicity at station Alpha and from the ARL channel was correlated with un-ionized ammonia present in porewater during the test. Toxicity at station Delta was above the level explained by the regression model examined for ammonia. The site at Decatur having the greatest toxicity and ammonia concentration (Alpha) was located in the Dry Branch Embayment. Spatial extent of toxicity within the embayment or into the mainstream reservoir was not evaluated in this study. Elevated ammonia in sediment collected from ARL was attributed to natural events associated with high densities of aquatic macrophytes and forage/predator fish within the channel.

Additional investigations are recommended to focus on retesting sediments from stations Alpha and Delta with ammonia removed and added back in parallel sets of treatments and conducting porewater chemical analyses to evaluate the role of ammonia, or other chemicals, as causative agents. Screening of sediment toxicity out from these two sites should also be accomplished to delineate the extent of toxicity into Wheeler Reservoir.

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INTRODUCTION

As part of a continuing series of periodic water resources issues analyses (WRIAs) of local drainage basins that comprise the larger Tennessee River drainage, the Tennessee Valley Authority (TVA) recently published an initial assessment of the Wheeler Reservoir Watershed Region (Cox et al., 1990). Its intended use was to provide information for TVA, other Federal and State agencies, industries, lake user associations, citizen interest organizations and the general public to increase awareness about significant water resources problems within Wheeler Reservoir. Two issues identified in the WRIA assessment led TVA to act on a request by the Alabama Wildlife Federation (AWF) to evaluate aquatic habitat and the impact of pollution (toxicity) in Wheeler Reservoir near Decatur, Alabama. The two related WRIA issues addressed (1) the assimilative capacity of Wheeler Reservoir and effects of existing wastewater discharges and (2) questions about the reservoir mussel fishery with regard to existing populations and habitat quality. (The AWF with State mussel divers had previously surveyed benthic habitat adjacent to several industrial outfalls for mussels.)

A second reason for conducting the study was the current EPA draft policy guidance for controlling release of toxics which is being promulgated to establish sediment quality criteria for regulating wastewater discharges and evaluating their impacts (U.S. EPA, 1990). TVA

determined that screening of sediments for toxicity would provide important information about aquatic habitat in the continuing evaluation of Wheeler Reservoir water resources.

Since 1988 TVA has successfully developed and used a toxicity test utilizing juvenile (6-8 days old) mussels to evaluate environmental significance of specific chemicals (manganese), pesticides (2,4-D, Aquathol K, and BTI), complex effluents (ashpond and paper mill wastes), and sediments. Results of these investigations have been reported in several internal TVA reports and presented at the tenth annual meeting of the Society of Environmental Toxicology and Chemistry (Wade et al., 1989). Because of the mussel habitat and fishery issues identified in Wheeler Reservoir, the juvenile mussel test was the obvious choice to screen for toxicity near Decatur.

This assessment demonstrated effects of exposing 8-day old juvenile mussels (Anodonta imbecillis) to 100 percent interstitial water (porewater) extracted directly form Wheeler Reservoir sediments. Purpose of the study was to screen benthic habitats for acute (short-term) toxicity. Study design did not attempt to identify chronic (long-term) responses, or evaluate magnitude or extent of any toxicity observed in Wheeler Reservoir, or identify specific toxic agents. In a word, this study was designed to single out toxic sediments for future investigative activities. Identification of "problem toxicants" would not be an expected outcome of this study.

METHODS

Sample Collection

Sampling sites corresponded to four of five areas previously surveyed by the AWF and mussel divers on the south side of the Tennessee River adjacent to several wastewater outfalls (Figure 1). An additional site was chosen for a study control on the north side of the river from an area known to support reproducing populations of mussels. A reference sample was included in the study from an outdoor channel (114 m x 4.3 m x 2.1 m deep) with natural sediment and flowing lake water at TVA's Aquatic Research Laboratory (ARL). A second reference sample from another (Kentucky) reservoir, provided by the Tennessee Wildlife Resource Agency (TWRA), was also included in the test. Station designations and locations were as follows:

Station Designation	Location
Reference (ARL)	ARL Outdoor Channel
Control (WH)	TRM 302.0R (North Side of River)
Alpha	TRM 303 4L (Dry Branch Embayment)
Charlie	TRM 301.8L (Overbank)
Delta	TRM 301.1L (South End of Canal)
Echo	TRM 298.6L (Mouth of Small Embayment)
KY Reservoir	Big Sandy River Mile 11.0

Samples from Wheeler Reservoir were collected by area mussel divers (Darryl Shell and Jerry Brown) who were instructed to fill toxicity cleaned 19-L (5 gal) plastic buckets with fine sediment (silt/clay) from the top 3-8 cm (2-3 inches) of the bottom substrate. Sample containers were immediately returned to the boat, capped with plastic lids, placed in coolers, and surrounded with ice. Samples were collected from 0950-1212 CDT on August 27, 1990 and transported directly to ARL, where they were held at 4°C until processing.

The sample from Kentucky Reservoir was collected on August 26 by TWRA divers and transported on ice to ARL on August 27. The reference sample from the ARL channel was collected using a Ponar dredge and processed the week preceding collection of Wheeler Reservoir samples.

Following the toxicity test and during preliminary data analyses, a transect on the north overbank at TRM 297.9 (downstream of the control station) was sampled to provide additional physical/chemical information about Wheeler Reservoir sediments. Sediment was collected with a petite Ponar dredge at 500 m, 250 m, 100 m, and 30 m from the north shore on September 25, 1990, and returned to ARL for porewater extraction and chemical analyses.

Sample Processing

Sediment was placed into 250 mL cleaned plastic centrifuge tubes and centrifuged at 4°C and 10,000 rpm for 10 minutes to separate solid particles from the water component (porewater) of the sediments. Porewater was then stored at 4°C in cleaned glass 4-L (1-gal) jars and used as required during the toxicity test.

Processing of sediment from the Charlie station yielded only enough porewater for the initial two days of testing. Low porewater yield from this station was due to large amounts of sand in the sample rather than the silt/clay material desired. To provide enough sample for the entire 9-day test, an elutriate (4 parts lake water to 1 part sediment) was prepared on September 5 for use on days 3-9 of the test. Elutriate preparation included mixing water and sediment for a period of 30 minutes before separation of the water phase by centrifugation. All

samples (porewater and elutriate) was screened through a 100 µm Nitex® screen before use in toxicity tests.

Samples were processed the week before testing due to the time required for extracting porewater from approximately 132 L (35 gal) of sediment. Sample processing dates were:

Control (WH)	- August 31
Alpha	- August 29
Charlie	- August 29-30; September 5 (elutriate)
Delta	- August 30
Echo	- August 30
KY Reservoir	- August 28

Selection and Preparation of Test Organisms

The life history of freshwater mussels includes an obligate parasitic larval stage (glochidium) which becomes attached to gill and fin tissues of a host fish. The attached glochidium is soon covered by a layer of tissue and remains on the fish host throughout a transformation process which results in a free-living juvenile mussel. The transformed juvenile stage was chosen for testing because it is the first stage fully exposed and completely dependent upon the aquatic environment. The juvenile mussels are metabolically very active in order to meet immediate growth requirements for survival. Unlike adult mussels, juveniles appear unable to "clam up" to resist short-term environmental perturbations, making the juveniles sensitive and ideal for toxicity studies.

In order to provide sufficient juvenile mussels of known age for conducting the toxicity test, glochidia were taken from a gravid female and transformed *in vitro* in artificial culture medium as described by Isom and Hudson (1982), inventors of the technique which was patented by TVA (Isom and Hudson, 1984). *Anodonta imbecillis* was chosen as the test

species because its glochidia are available throughout a large portion of the year (Hudson et al., 1986) and because of past success in transforming, growing, and testing this species under laboratory conditions. This species is also widespread in the region and present in Wheeler Reservoir, preferring impounded waters and streams having mud or sand bottoms. Glochidia from a single female produced several thousand viable juveniles, more than enough for conducting the test. Juveniles used in the study were in culture from August 21-27, 1990 (7 days).

Toxicity Test

The static, daily renewal toxicity test, initiated September 4, 1990, utilized 318 juvenile (8-day old) mussels and was conducted over a 9-day exposure period. Fifteen juvenile mussels were added to each of 3 replicates per treatment. Test vessels were 250 mL crystallizing dishes each containing 200 mL of porewater, prescreened (100 µm) silt (approximately 800 mg dry silt/L), and 6 mL algal concentrate (from aquaria-bloomed indigenous flora inoculated out of the ARL channels) to provide food for the mussels. Both plankton and sediment are essential for juvenile mussel health and survival (Hudson and Isom, 1984). Within each dish, mussels were enclosed by a 50 mm-diameter glass cylinder fitted on the bottom with 100 µm Nitex® screen to allow exchange of water and also rinsing silt from the mussels for microscopic examination. The conditions for testing are described by the test protocol shown in Table 1.

Replicate samples were placed in random order into an incubator set at 24°C and exposed in the dark for 24-h long periods. After each

exposure period, enclosures with mussels were rinsed free of silt, placed into a petri dish with uncontaminated plankton water, and examined microscopically. Juvenile mussels were enumerated as either live/active, live/stressed, or dead. Following evaluation, enclosures and mussels were returned to the dishes and fresh porewater, silt and algae (warmed to test temperatures) were added.

Toxicity Endpoints

Criteria used to evaluate stress and differentiate between living and dead juveniles were:

1. Alive/Active - foot protrudes from shell within a minute of settling to the bottom of the dish; foot locates dish bottom and "flips" shell into an upright position; mussel glides along dish bottom on extended foot.
2. Alive/Stressed - shell remains closed or gapes open with foot immobile and protruding; close observation reveals only slight foot motion; ciliary action obvious.
3. Dead - Shell gaped open with detrital material adhered to soft tissues just within the shell (indicating absence of ciliary movement which sweeps this area clean in live, active and stressed, mussels); and/or decomposition of soft parts (indistinguishable foot, organs, etc., usually accompanied by protozoans within the shell); or empty shells.

Physical/Chemical Determinations

Initial measurements of dissolved oxygen, temperature, pH, alkalinity, hardness, and conductivity were made in each treatment before daily renewals. Final dissolved oxygen, temperature, and pH measurements were made in each replicate after 24-h exposures. Initial and final total ammonia concentrations were determined in each treatment several times during the test and used with corresponding temperature and pH values to calculate respective un-ionized ammonia concentrations. A sample of ARL reference porewater was monitored at 30-minute intervals to determine pH changes over a 24-h period during the test (for evaluating concentrations of un-ionized ammonia during exposure). Sufficient "dummy" replicates (identical to test medium, but without mussels) were included as necessary for each treatment to provide adequate volumes of water for final water chemistry analyses. North overbank sediments collected on September 25 were analyzed for total ammonia, hardness, conductivity, and pH.

Data Analysis

Statistical methods for determining toxicity endpoints such as LC50 or NOEC concentrations were not required because of the screening nature of the test (dilutions of porewater were not tested). Daily mortality was determined for each treatment (site) based on observations from replicate samples (Appendix A). These data were plotted against exposure (time) to identify the degree of toxicity with regard to numbers of mussels killed and time till death. Proportions of mussels surviving in replicate samples were transformed using an arc sine square root

transformation (U.S. EPA, 1989). Transformed means were compared using a One-way Analysis of Variance and Duncan's Multiple Range test (Steel and Torrie, 1960) to differentiate between toxic and non-toxic sediments.

Initial and final water chemistry data were placed into tables reporting both mean and range values. Total and un-ionized ammonia data were also placed into tables. Sample pH was plotted over time for a reference sample to indicate change over a 24-h exposure under test conditions.

RESULTS

Toxicity Evaluation

Nine-day exposure of juvenile mussels to sediment porewater resulted in 100 percent mortality at two of the seven locations tested (Table 2, Figure 2). Stations Alpha and Delta both exhibited a high rate of kill, requiring only three and five days, respectively, to eliminate all living mussels from the test. The reference sediment from ARL channels was also toxic, resulting in 40 percent mortality over the nine days tested. However, both percent killed and rate of death in the ARL sample (occurring on days 5-9 of the test) was much reduced compared to stations Alpha and Delta. Survival in porewater from stations Echo (87 percent) and Kentucky Reservoir (96 percent) and porewater/elutriate from station Charlie (100 percent) was not statistically different ($\alpha = 0.05$, Table 3) from the control (96 percent) collected from the north overbank of Wheeler Reservoir where mussels are abundant.

Because toxicity has been frequently correlated with ammonia in sediment porewater evaluations (Ankley et al., 1990), total and un-ionized ammonia concentrations were determined at intervals during the test to evaluate ammonia as a possible contributor to the effect observed. Because pH drifted upward during a 24-h exposure (Figure 3), it was not possible to select a representative pH value for calculating the un-ionized (toxic) form. Ankley et al. (1990) observed the same problem in evaluating Fox River/Green Bay (Wisconsin) sediment porewater toxicity. To circumvent this problem TVA used total ammonia, temperature, and pH measurements taken at sample renewal (initial) and after 24-h exposure (final) to calculate un-ionized ammonia and averaged these data for use in comparisons with toxicity. Average total and un-ionized ammonia ranged from 0.49-44.97 mg/L and 0.01-1.96 mg/L, respectively (Table 4), and appeared to correspond with much of the observed toxicity.

A plot of un-ionized ammonia against toxicity was made to evaluate the implied relationship (Figure 4). In order to express toxicity as a dimension of both time and magnitude, the 9-day (end-of-test) mortality data (expressed as a proportion) were divided by the minimum number of days required to reach that level and used in the ammonia/toxicity plot. The regression of un-ionized ammonia and toxicity showed a significant relationship ($r = 0.969$) for the complete data set, although more toxicity was present at station Delta than explained by the linear regression model. Removal of Delta from the data set improved the implied relationship ($r = 0.996$) which is also shown on Figure 4 as the better equation representing ammonia toxicity.

Test Porewater Physical/Chemical Characteristics

Although conducted in a temperature controlled environment, frequent adjustments of the incubator thermostat were required in an effort to compensate for a malfunctioning building air conditioner which caused large fluctuations in laboratory temperature over a 24-h period. Overall result was that exposure temperature (21.0-24.0°C; Table 5) was lower than that specified for the test (24 ± 1°C). Observations of juvenile mussels during the 9-day exposure revealed that movement of the mussels was reduced compared to previous testing under slightly warmer temperatures. While all mussels evaluated as alive/active achieved an upright mobile position within the allotted one minute, normally the total allotted time is not required. It is not felt that cool temperature during testing adversely affected toxicity results.

Dissolved oxygen concentrations ranging from 5.4-8.7 mg/L were adequate for testing mussels as was pH which ranged from 7.1-8.4 standard units. Average specific conductance (conductivity) ranged from a low of 166 µmhos at the control station to highs of 796 µmhos and 658 µmhos at stations Delta and Alpha, respectively, indicating alterations of sediment porewater quality (dissolved ionic matter) by pollution. Hardness was also lowest at the control station (69 mg/L) and highest at stations Alpha (330 mg/L) and Delta (276 mg/L). (An increase in water hardness is known to decrease toxicity of metals to aquatic life which was not the effect observed in this test.) Corresponding alkalinities at stations Alpha and Delta were 92 and 74 mg $CaCO_3$/L, respectively.

DISCUSSION

Control sediment from the north side of Wheeler Reservoir contained the lowest amount of ammonia and was not toxic to juvenile mussels. Station Alpha, with the greatest toxicity and levels of ammonia, was collected from the Dry Branch Embayment. Ninety-six hour LC50 values for un-ionized ammonia have been reported between 0.4 and 3.1 mg/L for fish (Colt and Armstrong, 1979), and a 96-h LC50 value of 2.9 mg/L has been reported for the fathead minnow (Pimephales promelas) and Ceriodaphnia (Ankley et al., 1990). Un-ionized ammonia concentrations of 0.35-0.6 mg/L have been shown to be toxic to fingernail clams (Anderson et al., 1978). Un-ionized ammonia concentrations of 0.1 to 0.3 mg/L have caused a 50 percent reduction in marine mollusc filtration rates (Colt and Armstrong, 1979). Un-ionized ammonia levels in sediment porewater collected from the Dry Branch Embayment (0.91-4.05 mg/L) was sufficiently high to have caused the toxicity observed, although other sediment contaminants which were not analyzed for in this screening test may have been present. Defining the role of ammonia and other sediment contaminants should be addressed in future work.

Cause(s) of toxicity at station Delta, located at the upper (south) end of a barge canal at TRM 301.1L, was not clearly defined. Ammonia was high at this site, but failed to account for the total effect observed. Exact contribution of ammonia to toxicity at both Alpha and Delta can only be determined through additional toxicity testing following removal of ammonia from the samples and from porewater chemical analyses. However, its contribution appeared to be substantial based on

the regression model examined and toxicity values reported in the literature (see above).

Intermediate ammonia concentrations and significant toxicity from the ARL outdoor channel may have resulted from sediment deposition of nitrogenous material from decomposition of large aquatic macrophyte infestations in the channel and from the confinement of grass carp and other fish species through excretion of urea and other nitrogen containing wastes. This hypothesis is supported by the limited chemical analyses of sediment porewater from the north overbank (TRM 297.9) collected on September 25 (Table 6). Porewater 500 m from shore showed similar characteristics as the control used in the present study. However, total ammonia, hardness, and conductivity increased slightly along a transect toward shore and dramatically at a point 30 m from shore. The point of dramatic increase corresponded to a contour with the greatest aquatic macrophyte infestation in the area. Ammonia toxicity in porewater has been shown to be a common problem in a variety of sediments (Baudo et al., 1990).

CONCLUSION AND RECOMMENDATIONS

Two of five stations in Wheeler Reservoir were toxic to juvenile freshwater mussels. Although this study was not designed to identify specific toxic agents within reservoir sediments, un-ionized ammonia concentrations in porewater appeared to have been sufficiently high to contribute to the toxic effects observed. However, this observation cannot be confirmed until further studies are conducted to evaluate role of ammonia and other sediment contaminants.

Additional investigations are recommended to focus on retesting sediments from stations Alpha and Delta with ammonia removed and added back to parallel sets of treatments and conducting porewater chemical analyses to evaluate the role of ammonia, and/or other chemicals, as causative agents. Screening of sediment toxicity out from these two sites should also be accomplished to delineate the extent of toxicity into Wheeler Reservoir.

REFERENCES

Anderson, K. B., R. E. Sparks, and A. A. Paparo. 1978. Rapid assessment of water quality, using the fingernail clam, Musculium transversum. WCR Research Report No. 133, Water Resources Center, University of Illinois, Urbana, IL, 115 pp.

Ankley, G. T., A. Katko, and J. Arthur. 1990. Identification of ammonia as an important sediment-associated toxicant in the lower Fox River and Green Bay, Wisconsin. Environ. Toxicol. Chem. 9:312-322.

Baudo, R., J. P. Giesy, and H. Muntau (ed.). 1990. Sediments: Chemistry and Toxicity of In-Place Pollutants. Lewis Publishers, Inc., 405 pp.

Colt, J. E., and D. A. Armstrong. 1979. Nitrogen toxicity to fish, crustaceans, and molluscs. Dept. of Civil Eng., Univ. of Calif., Davis. 30 pp.

Cox, J. P., M. P. Taylor, and D. C. Wade. 1990. Surface Water Resources and Issues Analysis: Wheeler Reservoir Watershed Region. Tennessee Valley Authority, TVA/WR/WQ-90/6, 121 pp.

Hudson, R. G. and B. G. Isom. 1984. Rearing juveniles of the freshwater mussels (Unionidae) in a laboratory setting. The Nautilus. 84(4):129-135.

Hudson, R. G., L. M. Koch, and B. G. Isom. 1986. Obligate parasitism and summer breeding in Anodonta imbecillis (Say 1829). Submitted for publication in Malacologia.

Isom, B. G. and R. G. Hudson. 1982. In vitro culture of parasitic freshwater mussel glochidia. The Nautilus. 96(4):147-151.

Isom, B. G. and R. G. Hudson. 1984. Culture of freshwater mussel glochidia in an artificial habitat utilizing complex liquid growth media. U.S. Patent 4,449,480. 18 pp.

Steel, G. D. and J .H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill. 481 pp.

U.S. EPA. 1989. Short-term methods for evaluating the chronic toxicity of effluents and receiving waters to freshwater organisms. 2nd Edition. EPA/600/4-89/001. 249 pp.

U.S. EPA. 1990. Technical support document for water quality-based toxics control. Draft. Office of Water, Washington, D.C., April 1990. 244 pp with Appendices.

Wade, D. C., R. G. Hudson, and A. D. McKinney. 1989. The use of juvenile freshwater mussels as a laboratory test species for evaluating environmental toxicity. Poster presented at Tenth Annual Meeting, Society of Environmental Toxicology and Chemistry, Toronto, Ontario, October 28-November 2, 1989.

Table 1. Protocol for Conducting Toxicity Tests Using Juvenile Freshwater Mussels.

TEST SPECIES	: Anodonta imbecillis (freshwater mussel, Unionidae)
TEST TYPE	: Solid phase, static, daily renewal
TEMPERATURE	: 24 ± 1°C
PHOTOPERIOD	: Dark
TEST CHAMBER VOLUME	: 250 mL
RENEWAL OF TEST SOLUTIONS	: Daily
AGE OF TEST ORGANISMS	: 6-10 days old*
NUMBER MUSSELS PER REPLICATE	: 15
REPLICATES PER CONCENTRATION	: 3
FEEDING REGIME	: Daily--Concentrated indigenous algae bloomed in dilution water. Also clean silt (filtered through 100 µm Nitex® to facilitate visual observations)
AERATION	: None
DILUTION WATER	: 5 µm bag filtered receiving water - No dilution required in screening study.
TEST DURATION	: 9 days
EFFECT MEASURED	: Stress - impaired movement Mortality - absence of ciliary action or empty shells

*Younger mussels can be used; however, by 6 days the activity of young mussels has increased to a level which insures inclusion of viable individuals into the test.

Table 2. Daily Mortality of Juvenile Freshwater Mussels (Anodonta imbecillis) Exposed to Sediment Porewater from Wheeler Reservoir Near Decatur, Alabama, September 4-13, 1990.

Source	Exposure (Days)								
	1	2	3	4	5	6	7	8	9
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
ARL REFERENCE	0.0	0.0	0.0	0.0	6.7	11.1	15.6	31.1	40.0
CONTROL (WH)	2.2	2.2	2.2	2.2	2.2	2.3	4.4	4.4	4.4
ALPHA	2.2	53.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0
CHARLIE*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DELTA	2.2	6.5	30.4	84.8	100.0	100.0	100.0	100.0	100.0
ECHO	0.0	0.0	0.0	0.0	0.0	2.1	4.3	10.4	12.8
KY RESERVOIR	0.0	0.0	0.0	0.0	0.0	0.0	2.2	4.4	4.4

*Exposed to sediment elutriate (1:4 sediment to water) on test days 3-9 because of inadequate amount of porewater extracted from sandy substrate.

Table 3. Ranking of Station Means Based on Proportion of Juvenile Mussels Surviving Nine Days Exposure to Sediment Porewater Collected from Wheeler Reservoir Near Decatur, Alabama, September 4-13, 1990.

Treatment	Proportion Surviving				Transformed Data*			
	R-1	R-2	R-3	Mean	R-1	R-2	R-3	Mean
ARL REFERENCE	.73	.40	.67	.60	1.024	0.685	0.959	0.889
CONTROL (WH)	.87	1.0	1.0	.96	1.202	1.441	1.441	1.361
ALPHA	.00	.00	.00	.00	0.129	0.129	0.129	0.129
CHARLIE	1.0	1.0	1.0	1.0	1.441	1.441	1.441	1.441
DELTA	.00	.00	.00	.00	0.129	0.129	0.129	0.129
ECHO	.80	.94	.88	.87	1.107	1.323	1.217	1.216
KY RESERVOIR	.87	1.0	1.0	.96	1.202	1.441	1.441	1.361

*$\sin^{-1}\sqrt{p}$; where p = the proportion surviving the test.

ANALYSIS OF VARIANCE

Source of Variation	df	Sum of Squares	Mean Square	F-Value
Among Locations	6	5.999	1.000	85.230
Within Locations	14	0.164	0.012	
Total	20	6.163		

RANKED STATION MEANS*

ALPHA	DELTA	ARL REF.	ECHO	KY RES.	CONTROL	CHARLIE
0.129	0.129	0.889	1.216	1.361	1.361	1.441

*Duncan's Multiple Range Test. Means underscored by the same line are statistically equal (α = .05).

Table 4. Total and Un-ionized Ammonia in Sediment Porewater During a Nine-Day Test to Evaluate Acute Toxicity of Wheeler Reservoir Sediments Near Decatur, Alabama, to Juvenile Freshwater Mussels (Anodonta imbecillis), September 4-13, 1990.

Source	Total Ammonia			Un-ionized Ammonia		
	Initial (mg/L)	Final (mg/L)	Combined* (mg/L)	Initial (mg/L)	Final (mg/L)	Combined* (mg/L)
ARL REFERENCE	11.12	6.02	9.08	0.24	0.31	0.26
CONTROL (WH)	0.54	0.42	0.49	0.01	0.02	0.01
ALPHA	46.90	41.20	44.97	0.91	4.05	1.96
CHARLIE**	2.46	2.62	2.52	0.09	0.17	0.12
DELTA	18.10	11.40	15.87	0.54	1.14	0.74
ECHO	6.20	3.72	5.20	0.14	0.38	0.24
KY RES	2.75	1.50	2.25	0.08	0.10	0.09

*Average of all measurements.

**Exposed to sediment elutriate (1:4 sediment to water) on test days 3-9 because of inadequate amount of porewater extracted from sandy substrate.

Table 5. Water Chemistry Mean Values and Ranges in Sediment Porewater During a 9-Day Test to Evaluate Acute Toxicity of Wheeler Reservoir Sediments Near Decatur, Alabama, to Juvenile Freshwater Mussels (Anodonta imbecillis), September 4-13, 1990.

Source	Temperature		Dissolved Oxygen		pH		Conductivity	Alk	Hard.
	Initial	Final	Initial	Final	Initial	Final	Initial	Initial	Initial
	(°C)	(°C)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(µmhos)	*	*
ARL REFERENCE	23.4 (21.9-22.7)	22.7 (21.0-24.0)	8.2 (7.2-8.5)	7.1 (5.9-8.0)	7.6 (7.2-7.9)	8.1 (7.7-8.2)	316 (306-340)	145 (135-160)	107 (98-115)
CONTROL (WH)	23.4 (22.0-24.0)	22.6 (21.1-23.8)	7.9 (7.5-8.3)	7.1 (6.7-8.6)	7.6 (7.3-7.9)	7.9 (7.8-8.0)	166 (150-178)	69 (67- 74)	69 (64- 77)
ALPHA	23.4 (22.2-24.0)	22.6 (20.7-22.4)	7.2 (5.4-7.8)	7.6 (7.4-8.0)	7.5 (7.1-7.7)	8.3 (8.3-8.4)	658 (640-681)	92 (91- 92)	330 (314-345)
CHARLIE**	23.5 (22.8-24.0)	21.6 (2170-23.6)	8.2 (7.2-8.5)	7.4 (7.0-8.0)	7.7 (7.4-7.9)	7.8 (7.6-8.2)	159 (139-264)	59 (43-102)	79 (43-120)
DELTA	23.3 (21.9-24.0)	22.1 (20.7-22.6)	8.2 (7.6-8.5)	7.3 (6.8-7.9)	7.9 (7.6-8.0)	8.4 (8.2-8.4)	796 (785-809)	74 (74- 74)	276 (276-276)
ECHO	23.2 (22.0-24.0)	22.6 (20.9-23.9)	8.0 (7.4-8.4)	7.2 (6.8-8.0)	7.7 (7.5-8.0)	8.4 (8.2-8.4)	450 (425-483)	162 (154-178)	223 (206-240)
KY RESERVOIR	23.4 (22.2-24.0)	22.3 (20.2-23.9)	8.1 (7.3-8.7)	7.3 (7.0-8.2)	7.7 (7.4-8.0)	8.2 (8.1-8.2)	231 (223-239)	95 (94- 96)	117 (114-121)

*mg/L as $CaCO_3$

**Exposed to sediment elutriate (1:4 sediment to water) on test days 3-9 because of inadequate amount of porewater extracted from sandy substrate. The change from porewater to elutriate on day 3 of the test is reflected in the mean and range values.

Table 6. Chemical Characteristics of Wheeler Reservoir Sediment Porewater Along a Transect on the North Overbank (TRM 297.9) from 500 Meters to Near Shore, September 25, 1990.

Parameter	Distance from Shore (meters)			
	30	100	250	500
TOTAL AMMONIA (mg/L)	4.7	0.87	0.73	0.46
HARDNESS (mg/L)	128	70	67	62
CONDUCTIVITY (µmhos)	327	201	181	176
pH (s.u.)	7.3	7.2	7.4	7.6

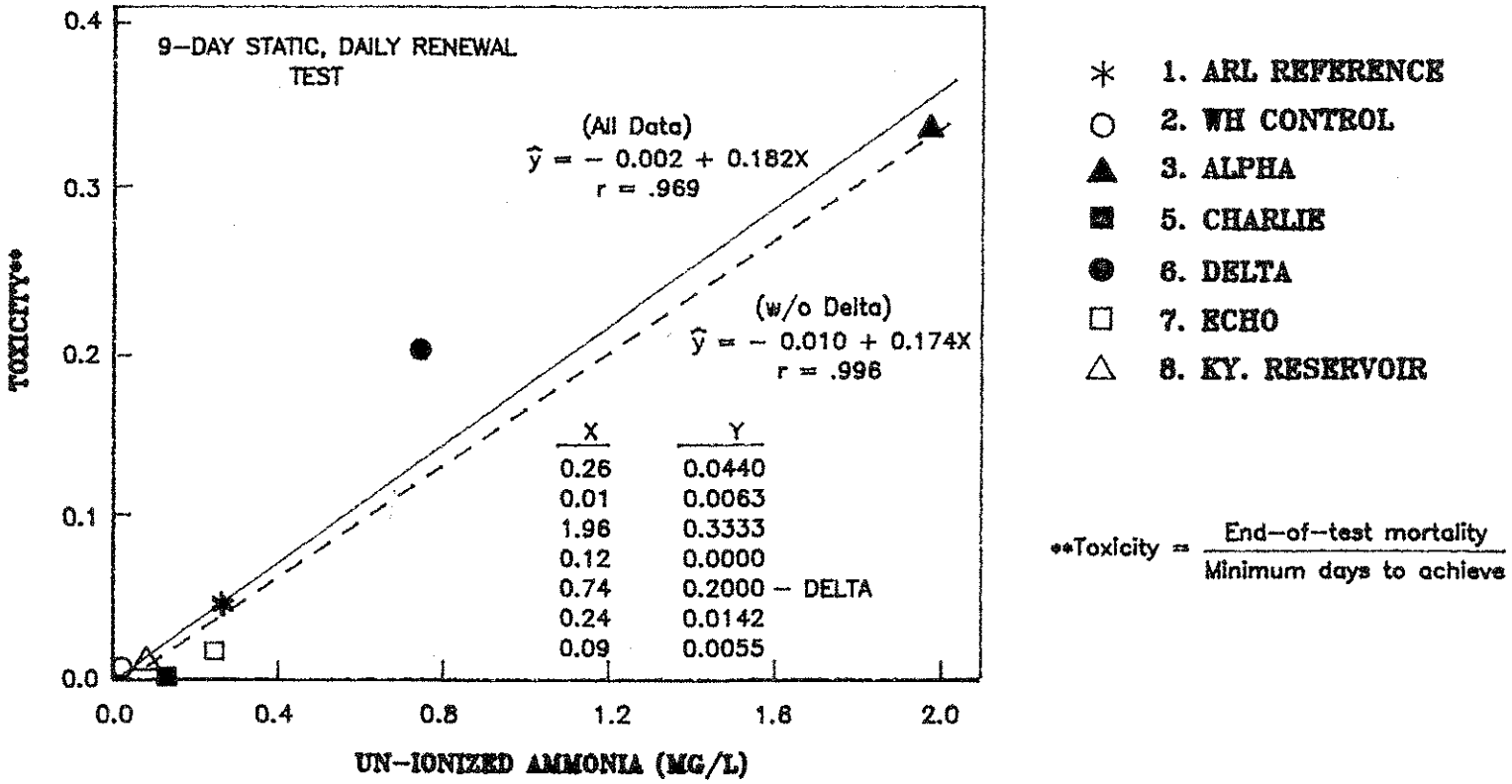

Figure 4. Relationship Between Unionized Ammonia in Wheeler Reservoir/Reference Sediment Interstitial Water and Toxicity to 8-Day Old Juvenile Freshwater Mussels (Anodonta imbecillis), September 4-13, 1990. (See Figure 1 for designated station locations.)

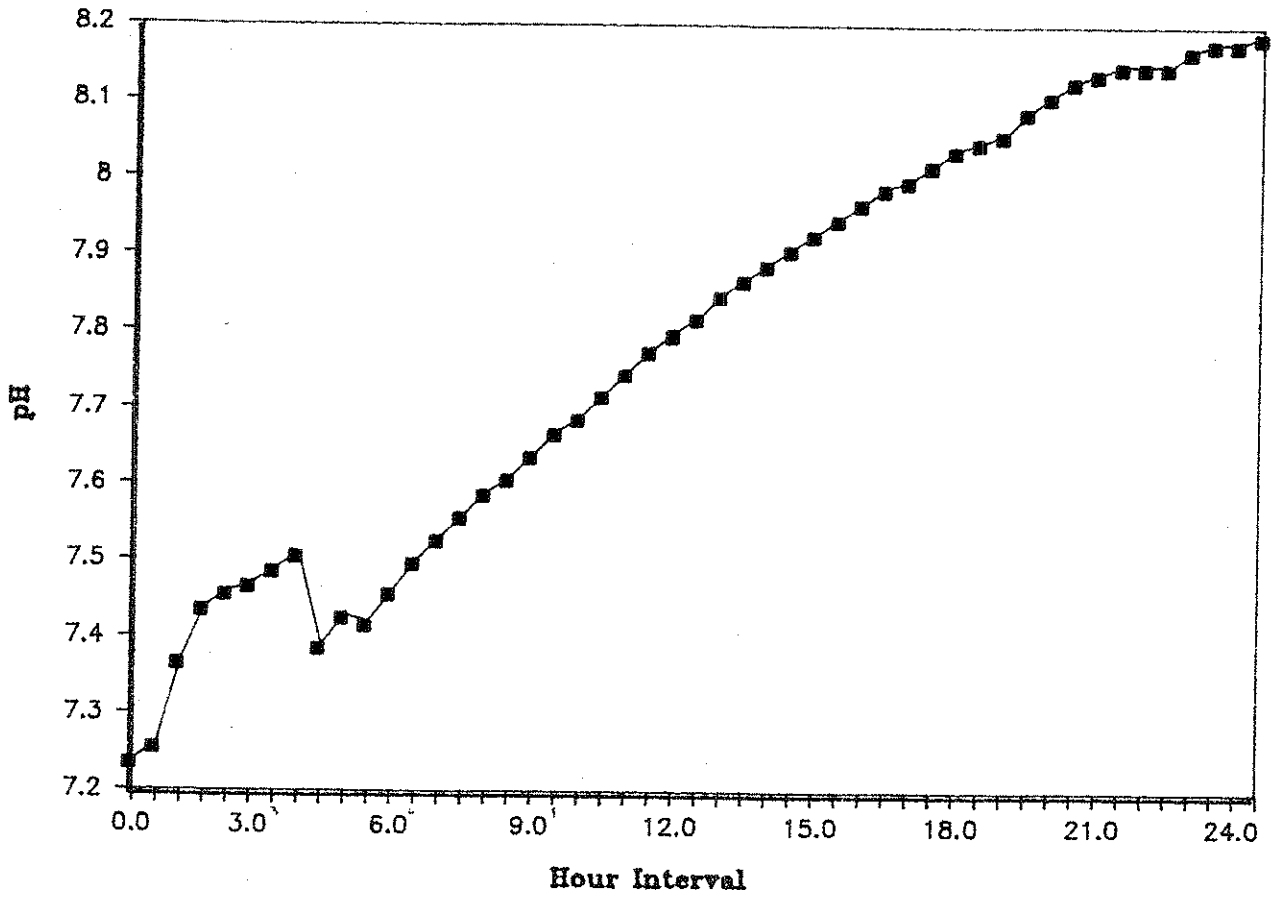

Figure 3. pH Change over 24 Hours in ARL Reference Sediment Interstitial Water, September 6-7, 1990.

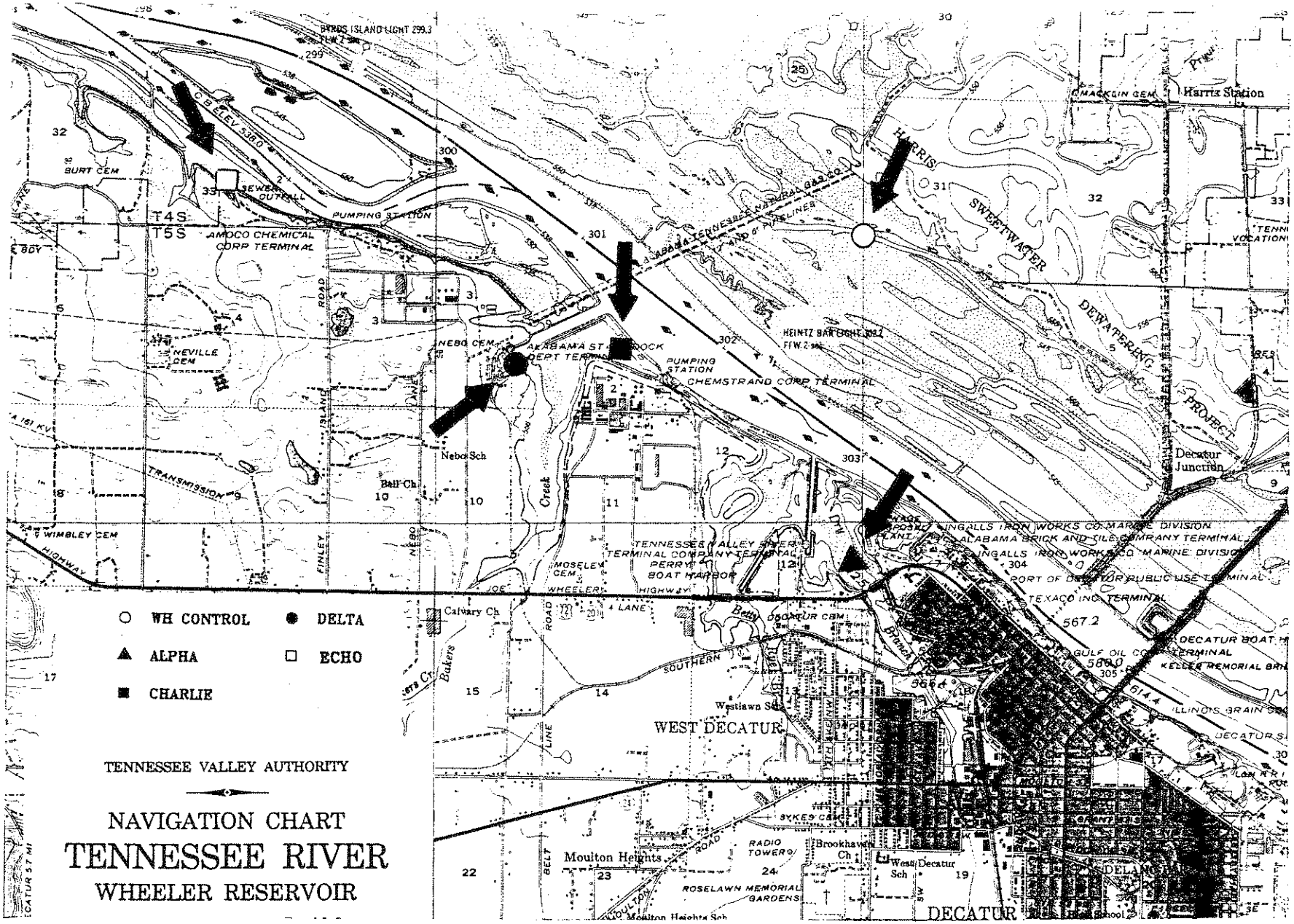

Figure 1. Wheeler Reservoir Locations Sampled for Sediment (Interstitial Water) Toxicity, August 27, 1990.

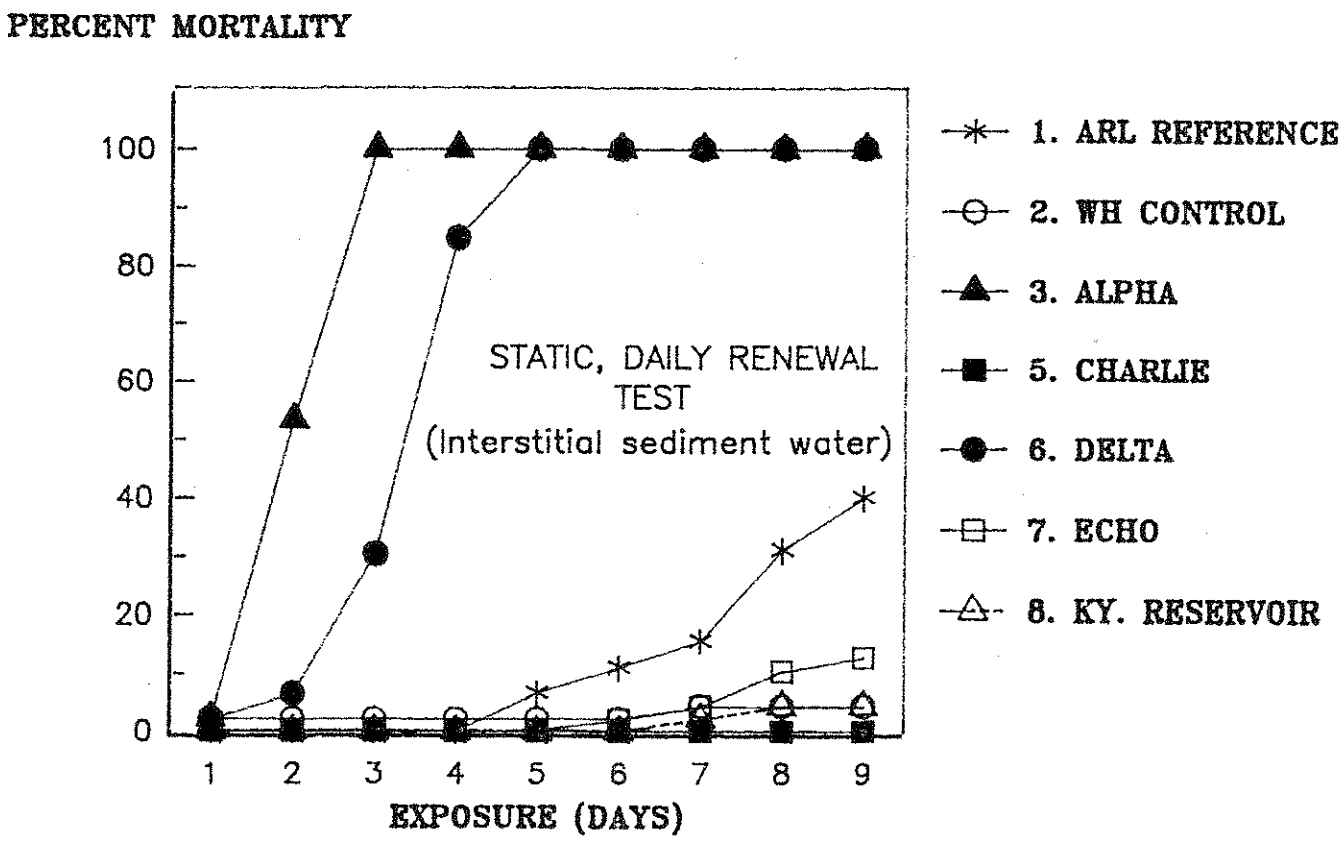

Figure 2. Toxicity of Wheeler Reservoir and Reference Sediment Interstitial Water to 8-Day Old Juvenile Freshwater Mussels (Anodonta imbecillis), September 4-13, 1990. (See Figure 1 for designated station locations.)

Appendix A. Percent Survival in Replicate Samples of Juvenile Freshwater Mussels (Anodonta imbecilis) During 9 Days Exposure to Sediment Porewater from Wheeler Reservoir Near Decatur, Alabama, September 4-13, 1990.

Treatment	Rep	Test Exposure (Days) 1	2	3	4	5	6	7	8	9
REF. (ARL)	1	100	100	100	100	87	87	87	80	73
	2	100	100	100	100	93	80	67	53	40
	3	100	100	100	100	100	100	100	73	67
	1-3	100	100	100	100	93	89	84	69	60
% Mortality:	1-3	0	0	0	0	7	11	16	31	40
CONTROL (WH)	1	93	93	93	93	93	93	87	87	87
	2	100	100	100	100	100	100	100	100	100
	3	100	100	100	100	100	100	100	100	100
	1-3	98	98	98	98	98	98	96	96	96
% Mortality:	1-3	2	2	2	2	2	2	4	4	4
ALPHA	1	100	47	0	0	0	0	0	0	0
	2	93	20	0	0	0	0	0	0	0
	3	100	73	0	0	0	0	0	0	0
	1-3	98	47	0	0	0	0	0	0	0
% Mortality:	1-3	2	53	100	100	100	100	100	100	100
CHARLIE	1	100	100	100	100	100	100	100	100	100
	2	100	100	100	100	100	100	100	100	100
	3	100	100	100	100	100	100	100	100	100
	1-3	100	100	100	100	100	100	100	100	100
% Mortality:	1-3	0	0	0	0	0	0	0	0	0
DELTA	1	100	93	67	20	0	0	0	0	0
	2	100	100	75	13	0	0	0	0	0
	3	93	87	67	13	0	0	0	0	0
	1-3	98	93	70	15	0	0	0	0	0
% Mortality:	1-3	2	7	30	85	100	100	100	100	100

Appendix A (Continued)

Treatment	Rep	Test Exposure (Days) 1	2	3	4	5	6	7	8	9
ECHO	1	100	100	100	100	100	94	94	81	80
	2	100	100	100	100	100	100	100	94	94
	3	100	100	100	100	100	100	94	94	88
	1-3	100	100	100	100	100	98	96	90	87
% Mortality:	1-3	0	0	0	0	0	2	4	10	13
KY RESERVOIR	1	100	100	100	100	100	100	93	87	87
	2	100	100	100	100	100	100	100	100	100
	3	100	100	100	100	100	100	100	100	100
	1-3	100	100	100	100	100	100	98	96	96
% Mortality:	1-3	0	0	0	0	9	0	2	4	4