Mussels Toxicity Testing Workshop
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Session I: Introduction

Session II: Background

"Overview of North American freshwater mussels," Chris Barnhart, Southwest Missouri State University

Authors: Chris Barnhart and Dick Neves.

Freshwater mussels (Order Unionoida) are among the most widespread, diverse, and historically abundant animals in freshwaters. About 300 species are found in North America, with <10 species per state in the West to over 100 species per state in the Southeast. Unfortunately, much of this diversity is threatened by a variety of human impacts. At least 35 North American species are extinct, and more extinctions are impending. Nonetheless, mussels are a dominant component of the benthic community in rivers, with peak abundance reaching 100 individuals and 10 kg per m^2. Mussels are important ecologically as suspension feeders, as food and habitat for other animals, and as structural components of the substratum. Bead production from U.S. mussel shell sustains pearl culture in oysters, and development of pearl culture in freshwater mussels has recently revolutionized the multi-billion dollar pearl industry.

Adult mussels are suspension feeders that live partially or completely buried in sediments. Mussels filter prodigious volumes of water, capturing particles down to 5 microns in size and ingesting algae, zooplankton, bacteria and detritus. They are therefore intimately exposed to both dissolved and suspended contaminants. Young juvenile mussels are very small (<400 microns) and occupy interstitial spaces in sediments where they ingest both deposited and suspended particles. At this stage, juveniles are likely exposed primarily to contaminants in pore water and associated with sediment particles. As they grow, most species occupy the water-sediment interface and draw water mainly from the water column. However, adults may also filter pore water when they burrow below the surface.

Most mussels reproduce annually. Males release spherical aggregates of sperm called "spermatozeugmata", which swim by beating of the sperm flagella and remain intact for hours. Sensitivity of this life stage to pollutants has not been examined. Spermatozeugmata are captured from the water by the female and fertilization takes place internally. The developing eggs are brooded within the female's gills. Depending on species, brooding may continue for weeks to nearly

a year before the larvae are released. Little is known of coupling between solute concentrations in the gills, ambient water and female body fluids during brooding.

Mussels produce tens of thousands to millions of glochidia larvae, which vary in size among species from about 60 to 330 microns. In some species glochidia are released from the egg immediately as they leave the female. In others the glochidia remain within the eggs, which are clustered in masses called conglutinates that act as bait for the host. Because of variety in these adaptations, eggs or glochidia may be exposed to the water for seconds to days before encountering a host. Therefore, the ecological relevance of direct exposure of glochidia to water contaminants depends on the species.

Glochidia are briefly but obligately parasitic on the gills or skin of particular species of fish. Within hours after attachment the glochidium is encysted by migration of host tissue. The solute environment of encysted glochidia is mainly dependent on host physiology. Although this isolation protects encysted glochidia from exposure to many water-borne toxicants, limited evidence suggests that host body burdens of certain toxicants may affect encysted glochidia. Metamorphosis to the juvenile life stage requires days to weeks depending on species and temperature. After completing metamorphosis, juveniles excyst and fall into the substrate. Growth to sexual maturity requires approximately 2-6 years.

Unionids are among the longest lived animals, with most species living for decades and some to over a century. Therefore, the duration of 4 to 28-d toxicity tests outlined in the draft ASTM standard for conducting toxicity tests with mussels may not be overly protective. The combination of complex life cycle, exposure to the vertical range of aquatic and benthic environments, species diversity and range, ecological importance, and widespread imperilment of native mussels make this taxon uniquely attractive for study of the effects of water and sediment pollution.

Session III: Propagation and Culturing

"Methods for culturing freshwater mussels," Richard Neves, Virginia Polytechnic Institute and State University

Authors: Richard Neves and Chris Barnhart.

The propagation and culture of freshwater mussels was initiated in the mid-1990s in response to recovery needs of federally endangered species. Production of juvenile mussels have been on going at Virginia Tech for about 10 years, with the first release of endangered juveniles in 1997. The Freshwater Mollusk Conservation Center began production of juveniles of multiple species in 2000, with an average of 100,000 endangered juvenile mussels produced each year for

culture and release to augment populations and re-introduce species to historic ranges. Closed system recirculating aquaculture tanks are supplied by well water and treated municipal water, and a suite of algal species are reared to provide diets suitable for all stages in the life cycle. All juveniles are produced by induced infestations of glochidia on previously identified fish hosts, and resultant juveniles are moved to culture tanks with sediment, and fed an algal diet of circa 30,000 cells/ml of water. Depending on availability of space, juveniles typically are cultured from 2 weeks to 2 months before release to the wild. We have propagated 11 federally endangered species and 10 common species over the last 5 years. In addition to our facility, there are now many other culture facilities scattered throughout the Mississippi drainage and eastern U. S., conducting culture and grow-out for regional faunas in need of conservation. These facilities include flow-through hatcheries with spring or river water, piped pond water to culture tanks, riverside house trailers adapted for culture, and various other facility designs. Southwest Missouri State University has recently developed a simple bucket culture system for indoor culture of juveniles. The feasibility and success of juvenile culture methods is sufficiently tested that production on demand, particularly for common species, is now possible to meet the need for juvenile mussels in toxicology work.

"Propagation and Culture of Freshwater Mussels: *In Vitro*," Author: Cristi Bishop, EA Engineering; Presented by Richard Neves, Virginia Polytechnic Institute and State University

Authors: Cristi Bishop, Robert Hudson, and Jerry Farris.

Some of the earliest known reports to propagate juvenile freshwater mussels began in the early 1900s, which employed techniques similar to those still used today. The successful development of an artificial culture medium (*in vitro*) in the early 1980s not only allowed scientists to visually monitor development (organ development, shell growth, etc), but also provided an understanding for specific requirements that support development and growth at this early life stage. Juvenile transformation, *in vitro*, has produced viable juveniles of numerous species that met project objectives including toxicity testing and reintroduction efforts into recovering streams. The science of juvenile propagation and culture has advanced within the last decade; researchers now have a better understanding of the sublethal (shell growth, foot movement, heartbeat, etc) and lethal responses that occur when juveniles are exposed to certain contaminants

Session IV: Methods for Conducting Laboratory Toxicity Tests

"ASTM International standard guide for conducting laboratory toxicity tests with freshwater mussels," Chris Ingersoll, Columbia Environmental Research Center, U.S. Geological Survey

Authors: Chris Ingersoll, Tom Augspurger, Chris Barnhart, Joe Bidwell, Cristi Bishop, Marsha Black, Greg Cope, Robert Bringolf, Jim Dwyer, Eugene Greer, Anne Keller, Greg Linder, Dick Neves, Teresa Newton, Rob Pepin, Andy

Roberts, Cindy Roberts, Mike Salazar, Alan Samel, Chuck Stephan, John Van Hassel, Ning Wang, and Tom Watters.

Committee E47 on Biological Effects and Environmental Fate recently approved a new standard entitled a S*tandard guide for conducting laboratory toxicity tests with freshwater mussels* (E2455-05) through the ASTM International. The standard describes methods for conducting laboratory toxicity tests with early life stages of freshwater mussels including glochidia and juvenile mussels in water-only exposures. Future revisions to the standard may describe methods for conducting toxicity tests with (1) adult freshwater mussels and (2) contaminated sediments using various life stages of freshwater mussels. Recommended test conditions for conducting these toxicity tests and test acceptability criteria outlined in the standard are based on various methods published in the literature and on the conditions used to conduct an inter-laboratory toxicity test with glochidia and juvenile mussels. The standard states that toxicity tests with glochidia and juvenile mussels should be conducted at 20°C with a 16L:8D photoperiod at an illuminance of about 100 to 1000 lux. Toxicity tests with glochidia are typically started within 2 h of isolation from the gills of the female mussels; however, some toxicity tests have been conducted with glochidia isolated from female mussels about 24 h before the start of a test. The endpoint measured in toxicity tests with glochidia is survival (viability) as determined by the response of organisms to the addition of a solution of NaCl. Glochidia that close their valves with the addition of a salt solution are classified as alive (viable) in a toxicity test. For most species, the duration of a toxicity test conducted with glochidia should be up to 24 h with survival measured at 6 and 24 h. Control survival is typically >90% at the end of 24-h toxicity tests conducted with glochidia. Longer duration toxicity tests with glochidia (e.g., 48 h) can be conducted as long as control survival >90% is achieved. However, toxicity tests conducted with glochidia for more than 24 h with glochidia may not be as ecologically relevant. Effect concentrations are typically calculated based on the percentage of viable glochidia in the control at a particular sampling time. Survival can be determined throughout the toxicity test by subsampling each replicate. Toxicity tests with juvenile mussels are typically started with organisms <5 d after release from the host; however, some toxicity tests have been started with 2- to 4-month-old juvenile mussels. Acute toxicity tests with juvenile mussels are typically conducted for 96 h with survival measured at 48 and 96 h. Chronic toxicity tests started with 2- to 4-month-old juvenile mussels have been conducted for 21 to 28 d with measures of survival (based on movement of the foot) and growth (based on shell length). Control survival is typically >90% at the end of 96-h toxicity tests conducted with juvenile mussels and is typically >80% at the end of 10- to 28-d toxicity tests conducted with juvenile mussels. Juvenile mussels are not typically fed during toxicity tests conducted for up to 10 d. Algae have been used as a food source in toxicity tests conducted for 10 to 28 d.

“Control survival of mussels in toxicity tests,” Chris Ingersoll, Columbia Environmental Research Center, U.S. Geological Survey

Authors: Chris Ingersoll, Ning Wang, Chris Barnhart, and Dick Neves.

Control survival is typically >90% at the end of 24-h toxicity tests conducted with glochidia. Longer duration toxicity tests with glochidia (e.g., 48 h) can be conducted as long as control survival >90% is achieved (ASTM International *Standard guide for conducting laboratory toxicity tests with freshwater mussels* (E2455-05)). However, toxicity tests conducted for >24 h with glochidia may not be as ecologically relevant. Control survival is typically >90% at the end of 96-h toxicity tests conducted with juvenile mussels and is typically >80% at the end of toxicity tests conducted for 10 to 28 d with juvenile mussels (ASTM International S*tandard guide for conducting laboratory toxicity tests with freshwater mussels* (E2455-05)). As a general rule, acute toxicity tests should be conducted for at least 24 h for glochidia and 96 h for juvenile mussels. However, shorter time periods for glochidia toxicity tests might be needed for a particular species depending on the survival time of the glochidia. The relatively short duration of toxicity tests with glochidia is based on the relatively short duration between release of glochidia into the water column and encystment on the host and is based on the relatively short survival time of glochidia after isolation from the female mussel. If the life history of the glochidia for a particular species is not known (e.g., the host required for encystment or how long glochidia released from a female mussel can remain in the water column before encysting on a host), it might be appropriate to conduct toxicity tests with glochidia for longer than 24 h. Regardless of exposure time, the 90% control survival acceptability criteria must be achieved. Longevity of glochidia after release and before attachment to a host may exceed one week and may be dependent on temperature. Glochidia of some species released in conglutinates remain viable for days or weeks after release into the environment. Glochidia of several species, including *Anodonta* spp., remain viable while free in the environment for 7 to 14 d. The ASTM International *Standard guide for conducting laboratory toxicity tests with freshwater mussels* (E2455-05) provides a summary of laboratory studies that have evaluated survival times of glochidia after removal from the marsupium of the female or survival time based on results reported in toxicity tests conducted with glochidia. For example, the viability of glochidia of *V. iris* was >75% for 8 d at 10^{o}C and 2 d at 25^{o}C and viability of glochidia of *A. pectorosa* was >75% for 13 d at 10^{o}C and 5 d at 25^{o}C. Similarly, glochidia of *Utterbackia imbecillis* may survive up to 19 d, but exhibit 50% mortality within 13.5 d. Survival of isolated glochidia from many species is typically >90% after 2 to 3 d; however, the viability of glochidia for a particular species should be determined before the start of an exposure. For example, glochidia of *Lampsilis teres* and *Epioblasma capsaeformis* were viable for only 4 to 6 h, glochidia of *Megalonaias nervosa* and *Quadrula quadrula* were viable for 1 d after removal from the marsupium of the female. Therefore, 24 h is a reasonable time period to conduct toxicity tests with glochidia of many species at 20^{o}C, although shorter or longer tests might be needed for a particular species depending on glochidia

survival time and the life history characteristics of the species (i.e., survival of glochidia must be >90% at the toxicity test as outlined in the standard). Longer exposure periods may be required for older life stages of mussels that are capable of avoiding exposure for short periods of time (older juvenile mussels and adult mussels; ASTM International Guide E729). Juvenile mussels have been successfully raised in the laboratory and met survival acceptability criteria. Barnhart (2005) described a 15-L recirculating system for rearing newly-released juvenile freshwater mussels. Newly-released juvenile mussels of 8 species were held in these systems for several months and fed continuously by drip with a monoculture of algae (*Neochloris oleoabundans*). River water filtered to remove particles >30 μm was used to culture juvenile mussels to provide a natural community of microorganisms which may aid in digestion. Survival rates were higher than most previous reports for captive juvenile mussels. Survival of newly-released *Lampsilis siliquoidea* and *L. reeveiana* exceeded 95% over 2 months. Changes in shell length in these two species were about linear ranging from 4.2 to 12.5 μm/day at 22°C. These growth rates are similar to or higher than previous reports of growth of juvenile mussels in recirculating systems.

"Precision of acute copper and ammonia toxicity tests conducted with glochidia or juvenile mussel," Ning Wang, Columbia Environmental Research Center, U.S. Geological Survey

Authors: Ning Wang, Chris Barnhart, Joe Bidwell, Greg Cope, Steve Geis, Chris Ingersoll, and Teresa Newton.

The ASTM International *Standard guide for conducting laboratory toxicity tests with freshwater mussels* (E2455-05) describes results of intra- or inter-laboratory acute toxicity tests with copper on glochidia and juvenile mussels. The US Geological Survey in Columbia, Missouri, also evaluated variability in intra-laboratory acute toxicity tests with ammonia and chlorine with glochidia and juvenile mussels. Test conditions for conducting all of the toxicity tests were in accordance with the recommended test conditions outlined in the standard and all of the toxicity tests met the test acceptability requirements outlined in the standard (e.g., >90% control survival). Copper intra-laboratory toxicity tests were conducted with glochidia of *Actinonaias ligamentina* and *Lampsilis siliquoidea*. Intra-laboratory variability of EC50s for toxicity tests conducted with copper, ammonia, or chlorine over exposure periods of 24 and 48 h, expressed as the coefficient of variation (CV), ranged between 13 and 36% for toxicity tests conducted with glochidia of *A. ligamentina* and between 14 and 34% for toxicity tests conducted with glochidia of *L. siliquoidea*. Intra-laboratory variability of EC50s for toxicity tests conducted with juvenile *L. siliquoidea* and copper over exposure periods of 48 and 96 h expressed as the CV ranged from 13 to 26%. Copper inter-laboratory toxicity tests were conducted with glochidia and juvenile *L. siliquoidea*. The testing laboratories included 2 federal facilities and 3 university facilities. Inter-laboratory variability of EC50s for glochidia, expressed as the CV, was 13% for the 24-h EC50s and was 24% for the 48-h EC50s. Inter-laboratory variability of EC50s for juvenile mussels, expressed as the CV, was

22% for the 48-h EC50s and was 42% for the 96-h EC50s. These measures of intra- and inter-laboratory precision in glochidia or juvenile mussel toxicity tests were at or below the variation reported for previous inter-laboratory studies with commonly-tested organisms in water-only exposures or in sediment exposures (e.g., CVs ranging from 14 to >100%). Results of these studies indicated that toxicity tests with glochidia and juveniles can be repeated within or between laboratories.

Session V: Relative Sensitivity of Laboratory Toxicity Tests

"Acute toxicity of ammonia to freshwater mussels: How new data and emerging data quality guidelines affect data synthesis," Tom Augspurger, Ecological Services Field Office, U.S. Fish and Wildlife Service

Authors: Tom Augspurger, Jim Dwyer, Chris Ingersoll, and Ning Wang.

In 2003, acute ammonia toxicity data for freshwater mussels were compiled, screened against data quality objectives, and summarized to derive estimates of concentrations that would be safe for mussels. Since then, additional tests have been performed and a standard method for conducting mussel toxicity tests has been balloted. We compared the number of toxicity tests, the number of species tested, and the magnitude of the results among (1) the 2003 dataset, (2) that same dataset as modified by applying test acceptability recommendations from the draft *ASTM International standard guide for conducting laboratory toxicity tests with freshwater mussels*, and (3) a 2005 dataset which includes new data that also meet the test acceptability recommendations in the draft ASTM guide. The 2003 dataset included 30 acute tests from 7 laboratories, covering 10 mussel species in 8 genera. Genus mean acute values (GMAVs; geometric mean EC50s by genus) for the 8 mussel genera were all lower than (more sensitive than) the GMAVs of other invertebrates and fishes in the acute database of the *1999 Update of ambient water quality criteria for ammonia*. Applying test acceptability recommendations outlined in the draft ASTM guide to the 2003 dataset results in culling some tests of longer duration and slightly lower control survival (the draft ASTM guide recommends 24-hour glochidia tests and control survival of >90%). Data available in 2003 that meet the draft ASTM recommendations include 18 acute tests from 6 laboratories, covering 7 mussel species in 6 genera. The mussel GMAVs rank as 6 of the 7 most sensitive when compared to the acute database of the *1999 Update of ambient water quality criteria for ammonia*. When results from tests meeting test acceptability recommendations outlined in the draft ASTM guide conducted since 2003 are added, there are 47 acute tests from 7 laboratories, covering 12 mussel species in 8 genera. The mussel GMAVs rank as 8 of the 9 most sensitive when compared to the acute database of the *1999 Update of ambient water quality criteria for ammonia*. Federal agencies, States, Tribes and others may employ different data quality objectives in reviewing freshwater mussel ammonia toxicity tests for inclusion in databases for such purposes as deriving water quality

criteria, water quality standards, permit limits, clean-up values, toxicity reference values, or other ammonia toxicity guidelines. Our comparison of datasets demonstrates that mussel toxicity data for ammonia are reliable and that mussels routinely rank among the more sensitive organisms to ammonia.

"Acute and chronic effects of ammonia and copper on 2-month old juvenile mussels," Ning Wang, Columbia Environmental Research Center, U.S. Geological Survey

Authors: Ning Wang, Chris Ingersoll, Chris Barnhart, Dick Neves, Tom Augspurger, Jim Dwyer, and Cindy Kane.

Chronic toxicity tests were conducted for 28 d starting with 2-month-old juvenile *Villosa iris* (rainbow mussel) and copper or ammonia and with *Lampsilis siliquoidea* (fatmucket) and copper. A chronic toxicity test with ammonia is ongoing with *L. siliquoidea*. Endpoints measured were 28-d survival (based on foot movement) and growth (based on shell length). Mussels were fed an instant algae mixture twice daily. The instant algae mixture was prepared from commercial Instant Algae® brand non-viable microalgae concentrates (Reed Mariculture, Campbell, CA; *Nannochloropsis* concentrate and a Shellfish Diet concentrate [mix of four marine microalgae: *Isochrysis, Pavlova, Tetraselmis, Thalassiosira weissflogii*]). Control survival at the end of the 28-d exposures with *V. iris* was 88% in the copper test and was 100% in the ammonia test. Control survival at the end of the 28-d exposure with *L. siliquoidea* was 98% in the copper test. The 28-d chronic values (ChV; geometric mean of the no-observed-effect concentration and the lowest-observed-effect concentration) for *V. iris* based on survival were 8.8 ug Cu/L and 1.4 mg total ammonia-N/L. Mean length of *V. iris* in the controls increased 46% in the ammonia test and 69% in the copper test during the 28-d exposures. The ChVs for *V. iris* based survival and growth were 4.4 ug Cu/L and <0.5 mg total ammonia-N/L. The acute to chronic ratio (ACR) for copper and *V. iris* was 5.4 based on survival and growth (compared to 2.7 for survival only) and ACR for ammonia and *V. iris* was >20 based on survival and growth (compared to 7.6 for survival only). The 28-d ChV for *L. siliquoidea* based on survival and growth was 8.8 ug Cu/L. Mean length of *L. siliquoidea* in the controls increased 83% during the 28-d exposure. The ACR for copper and *L. siliquoidea* was 6.8 based on survival and growth. Results of the ongoing chronic ammonia toxicity test with *L. siliquoidea* will also be discussed.

"Comparison of the acute toxicity of ammonia and copper of freshwater mussels and surrogate species ," Ning Wang, Columbia Environmental Research Center, U.S. Geological Survey

Authors: Ning Wang and Chris Ingersoll.

The acute toxicity of ammonia and copper was evaluated with freshwater mussels and several commonly-tested surrogate species. Glochidia (2-d exposures) and juvenile mussels (4-d exposures) of up to 15 species of mussels

were evaluated. Surrogate species evaluated included *Daphnia magna* and *Ceriodaphnia dubia* (cladocerans, 2-d exposures), *Hyalella azteca* (amphipod, 2-d exposures), *Pimephales promelas* (fathead minnows, 4-d exposures), and *Oncorhynchus mykiss* (rainbow trout, 4-d exposures). Tests were conducted in reconstituted water (hardness 170 mg/L as $CaCO_3$, pH 8.3) at a temperature of 12^oC for the trout and 20^oC for the other species. Conditions for conducting all of the toxicity tests were in accordance with the recommended test conditions outlined in the ASTM International standards for testing these surrogate species and in the ASTM International *Standard guide for conducting laboratory toxicity tests with freshwater mussels* (E2455-05) and all of the toxicity tests met the test acceptability requirements (e.g., >90% control survival). The EC50s for ammonia ranged from 13 to >16 mg total ammonia-N/L for the surrogate species and ranged from 3.0 to 13 mg total ammonia-N/L for glochidia and from 2.3 to 11 mg total ammonia-N/L for juvenile mussels (EPA acute water quality criterion is 5.6 mg total ammonia-N/L at pH 8 [including salmonids]). The EC50s for copper ranged from 15 to >100 ug Cu/L for the surrogate species and from 7 to 86 ug Cu/L for glochidia and from 6.8 to 60 ug/L for juvenile mussels (EPA acute water quality criterion is 23 ug Cu/L at a hardness of 170 mg/L). Results of this study indicate that ammonia and copper were frequently toxic to mussels at concentrations that were not toxic to the surrogate species.

"Comparison of the sensitivity of glochidia and juvenile mussels to ammonia, metals, and chlorine," Chris Ingersoll, Columbia Environmental Research Center, U.S. Geological Survey

Authors: Chris Ingersoll, Ning Wang, Tom Augspurger, Jim Dwyer, and Cindy Kane.

Effects of ammonia, chlorine, copper, zinc, lead, and cadmium on glochidia and juvenile mussels were evaluated for several species in acute toxicity tests. The toxicity endpoint measured in 6-h, 1-d, or 2-d glochidia toxicity tests was survival based on valve closure in response to the addition of NaCl. The toxicity endpoint measured in 2-, 4-, or 10-d juvenile toxicity tests was survival based foot movement. For copper, ammonia, and chlorine, the strongest correspondence in EC50s was observed between the 2-d glochidia test and the 4-d juvenile test for several species. For zinc, lead, and cadmium, glochidia in 2-d tests were much less sensitive than juveniles in 4-d tests with *Lampsilis siliquoidea* (fatmucket). Short-term tests with glochidia may be useful for screening the sensitivity of mussels to some chemicals (i.e., copper, ammonia, and chlorine), but response of juvenile mussels may be more ecologically relevant. Use of glochidia to screen the relative sensitivity of a particular mussel species to select chemicals would be particularly useful when evaluating species where only a limited number of adult mussels are available for methods development or for generating juvenile mussels for toxicity testing. Moreover, the host fish for some species of mussels or techniques for transforming juvenile mussels in the laboratory may be unknown for some species. Additional studies are needed comparing effects of select chemicals across multiple life stages of mussels. Specifically, these

comparisons should include acute or chronic toxicity tests with (1) brooding females, (2) glochidia, (3) newly-released juveniles, (4) older juveniles, and (5) adult mussels. Endpoints measured in these exposures should include bioaccumulation and (1) the ability of glochidia to survive and successfully transform on a host fish; (2) survival, growth and behavior of juvenile mussels; and (3) survival, growth, behavior and reproduction of adult mussels.

"Comparison of the sensitivity of early life stages of native freshwater mussels to current use pesticides," Robert Bringolf, Department of Environmental and Molecular Toxicology, North Carolina State University

Authors: Robert B. Bringolf, W. Gregory Cope, Peter R. Lazaro, Chris Eads, Chris Barnhart, and Damian Shea

Native freshwater mussels (family Unionidae) are among the most imperiled faunal groups in North America. Approximately 67% of the nearly 300 freshwater mussel species are considered vulnerable to extinction or already extinct. Although numerous stressors have been implicated in the decline of freshwater mussels, the effects of pesticides on native mussels are largely unknown. Timing of pesticide application combined with the unique life history and reproductive strategy of mussels makes all life stages of mussels susceptible to pesticide exposure. The objective of this study was to determine the hazards of pesticides to early life stages of freshwater mussels. In the first phase, we performed acute toxicity tests with glochidia (7 species) and juveniles (6 species) exposed to a suite of technical grade current use pesticides (atrazine, fipronil, pendimethalin, and permethrin) and a reference toxicant (NaCl). Our results indicate that technical grades of these pesticides, at concentrations approaching water solubility, were not acutely toxic to the species of glochidia and juveniles tested. However, in a 21-d chronic toxicity test performed with juvenile *Lampsilis siliquoidea* exposed to technical grade atrazine, the 14-d LC50 was 15.8 mg/L (95% confidence interval 12.0 – 19.5) and the 21-d LC50 was 4.3 mg/L (95% confidence interval 2.8 – 5.8). Additionally, exposure to atrazine significantly reduced the growth of juvenile mussels during the chronic test. In the second phase of the project, we compared the acute and chronic toxicity of pesticide formulations to technical grade pesticides with glochidia and juvenile mussels. Roundup (glyphosate formulation) was substantially more acutely toxic to both glochidia and juveniles than was technical glyphosate. However, LC50s for formulations of atrazine, chlorpyrifos, and permethrin were similar to those of the technical grades of the same pesticides. In all, our results indicate that some pesticides and their formulations are acutely toxic to glochidia and juveniles of freshwater mussels. Additionally, a pesticide formulation may be more toxic than the technical grade, as in the case of Roundup and glyphosate. Results of juvenile chronic tests indicate that atrazine may be impacting mussel populations and warrants further investigation, as does the assessment of chronic toxicity of other pesticides, their formulations, and pesticide mixtures.

“Effects of hypoxia on juvenile mussels,” Brianna Kaiser, Southwest Missouri State University

Authors: Brianna Kaiser and Chris Barnhart.

Similar to other aerobic organisms, unionids are dependent on adequate concentrations of dissolved oxygen (DO). Determination of protective DO criteria is complicated by the complex unionid life cycle and the variety of habitats occupied by different life stages. Adult mussels occupy the sediment-water interface and are generally able to draw water from the water column. Adults have limited ability to regulate aerobic metabolism in hypoxia, exhibiting a hyperbolic relationship between oxygen consumption and DO. Oxygen consumption of most of 9 species studied by Chen et al. (2001) declined steeply below about 2-3 mg/L but also showed significant slope at higher DO. Studies of the effects of hypoxia on survivorship of adults are apparently lacking. Other life stages may be more sensitive. Female unionids brood their eggs and developing embryos in their marsupial gills. In many species these structures are massive, containing hundreds of thousands to millions of eggs. It appears likely that developing embryos may experience lower DO than that in the water column, but data are lacking. By analogy with other aquatic organisms, developing embryos may be particularly sensitive to hypoxia. It is generally recognized that females of some unionids abort the developing embryos when placed in stagnant water.

Juvenile mussels are very small and occupy interstitial spaces that are potentially depleted of oxygen. We tested the effect of DO on mean days to death (DTD) of young juveniles of 4 species of lampsiline unionids (*Lampsilis abrupta, L. reeveiana, L. rafinesqueana, and Ligumia recta*). Juveniles were held without substrate in flow-through containers at 20 C and were not fed during the trials, which lasted up to 45 d. Seven levels of DO were tested, and survival was assessed at 2-d intervals. The relationship between DTD and DO was fitted by regression. Critical DO was defined as the DO at which predicted DTD fell to 90% of that at 7 mg/L. At the lowest DO tested (<0.2 mg/L), mean DTD of newly excysted (0-mo) juveniles of the 4 species was 4.2 d (range 2.2 -7.6 d). Critical DO increased with juvenile age. Mean critical DO of the 4 species was 0.65 mg/L (range 0.43-0.91 mg/L) at 0-mo and increased to 1.08 (range 0.79-1.55) mg/L at 3-mo. Although juveniles tolerate low DO, it must be remembered that interstitial DO is lower than that in the water column. DO criteria for salmonid eggs assume that interstitial DO is 3 mg/L lower than the water column (U.S.E.P.A. 1986). This differential is based on measurements in salmon redd gravels, which are typically coarse and relatively open to flow. Young juvenile mussels probably occupy finer sediments where the DO differential could be larger.

“The relative sensitivity of mussels to sulfates,” David Soucek, Illinois Natural History Survey

Authors: David Soucek, Chris Ivey, Chris Ingersoll, Ning Wang

We compared the response of juvenile freshwater mussels (*Lampsilis siliquoidea*) to elevated sulfate concentrations with those of the cladoceran *Ceriodaphnia dubia,*the amphipod *Hyalella azteca ,*and the freshwater clam *Sphaerium simile*. When diluent chloride concentrations were 25 to 33 mg/L and hardness was approximately 100 mg/L as CaCO3, juvenile mussels were the least sensitive of the four organisms tested, with an LC50 of nearly 3,500 mg SO42-/L compared to values ranging from about 1,900 to 2,600 mg/L for the other three organisms. Variation in water hardness and chloride concentrations had substantial effects on sulfate toxicity to *Ceriodaphnia,* and *Hyalella,* and to a lesser extent, *Sphaerium*. Neither water hardness nor chloride had a substantial influence on sulfate toxicity to juvenile fatmuckets; however, sulfate was more toxic to juvenile fatmuckets in water with a lower Ca:Mg ratio at the same water hardness.

Session VI: Lab-to-Field, Water Chemistry-to-Sediments

"Sensitivity of juvenile unionids to ammonia in sediment and water-only toxicity tests," Teresa Newton, Upper Midwest Environmental Sciences Center (UMESC) West, U.S. Geological Survey

Authors: Teresa J. Newton and Michelle R. Bartsch.

Recent data suggests that unionids are sensitive to ammonia relative to other organisms. Ammonia preferentially accumulates in sediments and pore water, suggesting that this may be an important exposure route for benthic, pedal feeding juvenile unionids. Given that juveniles naturally reside and probably feed in sediments, we wanted to compare their sensitivity between sediment and water-only exposures. We conducted two 10-d sediment tests, six 96-h sediment tests, and four 96-h water-only tests with juvenile *Lampsilis cardium* (8 tests) and *L. higginsii* (4 tests). Ammonium chloride was delivered to each experimental unit by peristaltic pump. Twenty juveniles were placed in chambers that were buried 2.5 cm into reference sediments to approximate pore water exposure (sediment tests) or elevated 2.5 cm above the bottom of the experimental unit (water-only tests). Survival and growth were measured on the last day of exposure. All data are reported as total ammonia nitrogen (TAN) normalized to pH 8. Survival exceeded 95% in the controls across all tests. Differences in toxicity between species were minimal. In the 10-d sediment tests, LC50s were 3.4 and 3.8 mg/L and growth EC50s were 2.8 and 3.4 mg/L based on concentrations in pore water. In the 96-h sediment tests, LC50s ranged from 4.9 to 6.7 mg/L when based on concentrations in pore water (pH -7.2) and ranged from 20.8 to 29.5 mg/L when based on concentrations in the water overlying sediments (pH -8.1). From profiles, we know that sediments modify pH such that pH is -8.0 in the overlying water, -7.8 at the sediment water interface, and -7.2 at a sediment depth of 2.5 cm; the degree to which sediments modify toxicity is unknown. Clearly, understanding where juveniles reside in these sediment exposures is critical. In the 96-h water-only tests (pH -7.1), LC50s ranged from 5.7 to 11.1 mg/L—values similar to those in the sediment tests when

based on pore water concentrations and within the ranges reported by previous investigators in water-only tests (0.6 to 19.7 mg/L). In the control treatments, growth was consistently greater in the sediment tests than in the water-only tests. We also conducted a series of 4, 10, and 28-d in situ exposures of juvenile *L. cardium* at 12 sites in the St. Croix River (Minnesota and Wisconsin). In general, survival was negatively correlated and growth was positively correlated with TAN concentrations in pore water. Differences between the laboratory and the field were not unexpected given the multitude of factors that may influence survival and growth in the field. Clearly, more research on exposure routes and effects of ammonia on early life stages of unionids is needed to determine if pervasive, low-level contamination is contributing to widespread declines in this imperiled faunal group.

"Cultural importance of freshwater mussels to Native American tribes: a field study of caged mussels," Meredith Garvin, Tribal Environmental Management Services

Author: Meredith Garvin.

Freshwater mussels have important cultural significance to Native American tribes. Historically mussels have been used by tribes as food, utensils, and ornaments. Large "shell middens" found by archaeologist suggest that villages were located along streams and rivers of the southeastern portion of the U.S. not by the presence of big game buy by the abundance of freshwater mussels living in the water. Mussel shells can be seen today adorning tribal clothing and jewelry worn at powwows, ceremonies, and gatherings. In April 2992, eight tribes of northeastern Oklahoma (Quapaw, Peoria, Eastern Shawnee, Wyandotte, Seneca/Cayuga, Miami, Ottawa, and Modoc) joined together to conduct a field study of caged freshwater mussels in Spring River which drains portions of Missouri, Kansas, and Oklahoma. The purpose of this study was to determine the toxic effects of heavy metals coming from lead and zinc mining in the Tri-State Superfund areas. A control population was established in Lake Skiatook. Samples were drawn at three month intervals for evaluation of heavy metals.

"Potential sources of variability in unionid mussel metal burdens in the great rivers confluence area," David Soucek, Illinois Natural History Survey

Authors: D. Soucek, J. Esarey, J. Levengood, and S. Gallo.

We examined spatial and interspecific variability in metal concentrations and stable nitrogen and carbon isotope ratios in unionid mussels from the Mississippi and Illinois Rivers confluence area. Threeridge mussels (*Amblema plicata*) collected from the MS River below the IL River confluence had significantly higher whole body concentrations of copper, manganese, iron, zinc, and selenium but significantly lower concentrations of lead, compared to those collected from MS and IL locations above the confluence. No significant differences were observed among sites in *Amblema plicata* length, height, tissue condition index (TCI), dry soft tissue

weight, shell weight, or age. At the MS downstream site, inter-specific differences were observed in some metal concentrations. For example tissue concentrations of zinc, strontium and manganese were significantly higher in threeridge mussels than in mapleleafs (*Quadrula quadrula*), while washboard mussels (*Megalonaias nervosa*) had intermediate values for each. There are two potential explanations for these trends. First, metal concentrations were positively correlated with organism age, so either older individuals had a longer time to accumulate metals, or younger species existed under lower contamination levels than those previously experienced by older individuals. An alternative explanation is that there are differences among species in filtering rates, food selection (because of gill morphology), or assimilation/depuration rates. This hypothesis is supported by the fact that individuals that were more enriched with 15N, an indicator of trophic level, or potentially, feeding rate, had higher concentrations of zinc and strontium in their tissues.

"Residual effects of lead and zinc mining on freshwater mussels in the Tri-State Mining District," Robert Angelo, Kansas Department of Health and Environment

Authors: Bob Angelo, Steve Cringan, Diana Chamberlain, Steve Haslouer, and Tony Stahl

The Tri-State Mining District of southeastern Kansas and adjoining areas of Missouri and Oklahoma historically ranked among the nation's most productive lead and zinc mining regions. Although ore extraction and processing operations largely ceased in this district a half-century ago, persistent environmental contamination problems have prompted a series of recent investigations and remedial actions designed to identify and mitigate mining related damages to the region's terrestrial and aquatic resources.

Our study examined the lingering effects of this industry on freshwater mussels in the Spring River Basin, a 6,600 km^2 watershed encompassing most of the historical mining district. Qualitative mussel surveys were performed in 23 stream reaches distributed throughout the basin and above and below former mining sites. Quantitative surveys were conducted in the Spring River at one upstream reference location and one downstream location to estimate local mussel densities and identify locally dominant unionid species. Concentrations of selected metal contaminants in the soft tissues of mussels and Asian clams (Mollusca: Corbiculidae: *Corbicula fluminea*) were evaluated at most survey sites. Additional metal analyses were performed on fluvial sediment samples and on surface water samples obtained during base flow and peak flow synoptic surveys.

Sites on the Spring River near the Kansas-Missouri border were found to support at least 20-23 species of mussels. These included the federal candidate species, *Lampsilis rafinesqueana*, and six additional taxa currently listed as threatened or endangered by one or more states in the region. Sites near the lower terminus of

the river in Oklahoma yielded evidence of only six extant mussel taxa and no state or federally listed unionid species. Mussel density and taxa richness declined abruptly in the river below the mouths of Center and Turkey creeks. The downstream reaches of these streams and three other surveyed tributaries seemingly were devoid of live mussels and other aquatic mollusks. Elsewhere in the basin, concentrations of cadmium, lead, and zinc in mussel tissues closely paralleled the local environmental (sediment and peak flow) concentrations of these metals. Metal contaminant burdens in *C. fluminea* were strongly correlated with levels documented in mussels, suggesting that this exotic (but widely distributed) bivalve could serve as an effective surrogate for mussels in future tissue contamination studies.

We conclude that pollution attributable to former mining practices continues to degrade environmental quality and impede the recovery of mussel communities in a large portion of the Tri-State Mining District. Additional environmental studies focusing on the mussel fauna of this region, and involving various other governmental agencies and academic institutions, are now underway or planned for the near future.

Session VII: Discussion of Salient Issues and Closing Remarks