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# ASSESSING ACCUMULATION AND SUBLETHAL EFFECTS OF LEAD IN A UNIONID MUSSEL

**Shad Mosher[1], W. Gregory Cope[1], Frank X. Weber[2], Thomas J. Kwak[3, 4], Damian Shea[1, 3]**

[1]Department of Environmental and Molecular Toxicology, North Carolina State University, Box 7633, Raleigh, North Carolina 27695 USA
phone: (919) 541-1548; email: mosher.shad@epa.gov

[2]RTI International, Research Triangle Park, Raleigh, North Carolina 27709 U.S.A.

[3]Department of Biology, North Carolina State University, Box 7617, Raleigh, North Carolina 27695 U.S.A.

[4]U.S. Geological Survey, North Carolina Cooperative Fish and Wildlife Research Unit, North Carolina State University, Box 7617, Raleigh, North Carolina 27695 U.S.A.

**ABSTRACT**

Lead (Pb) contamination of the environment remains a global problem. Previous studies have demonstrated that Pb deposited onto roadside sediments from the past use of leaded gasoline in vehicles may be mobilized into rivers and streams, thereby resulting in exposure to aquatic biota. The aims of this study were to conduct a 28-day laboratory toxicity test with Pb and adult Eastern Elliptio (*Elliptio complanata*; family Unionidae) mussels to determine uptake kinetics and to assess several potential non-lethal biomarkers of Pb exposure. Mussels were collected from a relatively uncontaminated reference site and exposed to a control and eight concentrations of Pb (as lead nitrate) ranging from 1 to 251 µg/L, as a static renewal test. There were five replicates per treatment with one mussel per replicate. The hemolymph of mussels from four of the replicates was repeatedly sampled (days 7, 14, 21, and 28) for analysis of Pb and ion (Na+, K+, Cl-, Ca2+) concentrations. The mussels in the fifth replicate per treatment were only sampled on day 28 and served as a comparison to the repeatedly sampled mussels. The accumulation of Pb in mussel tissue was also evaluated during the study. No mussels died during the test. We found that measured concentrations of Pb in mussel hemolymph suggested regulation of the heavy metal up to 66 µg/L by day 14, whereas concentrations in tissue proved to be strongly correlated ($R2 = 0.98$; $p < 0.0001$) throughout the 28-day exposure, displaying concentration dependent uptake. The concentration of Pb in mussel hemolymph, which can be sampled and measured non-lethally, is a suitable marker of recent Pb exposure in mussels. In contrast, none of the ion concentrations measured in the hemolymph from the repeatedly sampled mussels was significantly changed with increasing concentrations of Pb, whereas the mussels from the fifth replicate sampled only on day 28 showed altered calcium concentrations. The activity of δ-aminolevulinic acid dehydratase (ALAD), a demonstrated Pb-specific biomarker in vertebrates and some invertebrates, which was also evaluated as a potential endpoint in an initial evaluation for this study, proved to be an unsuitable biomarker in *Elliptio complanata*, with no detectable activity observed. This finding was in contrast to a second freshwater, but non-unionid bivalve tested, the Asian Clam *Corbicula fluminea*, which had detectable ALAD activity.

**KEY WORDS** ALAD, Bioavailability, Biomarkers, *Elliptio complanata*, Lead, Unionidae

## INTRODUCTION

Lead (Pb) contamination is a global environmental problem. Many studies have demonstrated excess levels of Pb in roadside sediments (Latimer et al., 1990; Mielke, 1999; Sutherland & Tolosa, 2000; Sutherland, 2003; Weiss et al., 2006) and other ecosystem compartments associated with the past use of leaded gasoline. Most of the Pb in sediment is found in the small grain fraction (< 63 µm), which is more likely to be re-suspended or eroded into rivers and streams adjacent to roads (Angelo et al., 2007; Sutherland & Tolosa, 2000; Weiss et al., 2006). Native freshwater mussels belonging to the family Unionidae are suspension and deposit-feeding, long-lived (10-100 yr) organisms that reside burrowed in sediments of streams and rivers (McMahon & Bogan, 2001) and, therefore, may be among the groups of aquatic organisms adversely affected by persistent, low-level exposure to Pb in our surface waters.

Unionids are one of the most imperiled faunal groups in the world, especially in North America, where almost 70% of the nearly 300 native species are considered vulnerable to extinction or are already extinct

(Bogan, 1993; Williams et al., 1993; Graf & Cummings, 2007). Unionids are also recognized as one of the most sensitive groups of organisms that have been tested to date with certain contaminants like ammonia and copper, compared to other commonly tested aquatic organisms like fish and crustaceans (Augspurger et al., 2003; March et al., 2007). Mussels, being suspension and deposit feeders, are exposed to a wide variety of contaminants, including Pb, throughout their life (Cope et al., 2008), and are considered to be good sentinels for assessing environmental conditions (Metcalfe-Smith et al., 1996; Gundacker, 2000; Dobrowolski & Skowrońska, 2002; Yap et al., 2004). A recent study found that while the Na+,K+-ATPase enzyme was present and inhibited by Pb in the unionid mussel, Eastern Elliptio, *Elliptio complanata* (Lightfoot, 1786), results were variable at environmentally relevant concentrations (Mosher et al., 2010) and, therefore, recommended that more specific, non-lethal biomarkers should be assessed. As there is mechanistic understanding for Ca-Pb interactions (Grosell et al., 2006), it is possible that the presence of Pb would interfere with the transport and uptake of this and other ions. Changes in Ca2+ and other ion concentrations (Dietz, 1985) could affect pH and result in reduced shell formation.

Because unionids are such an imperiled fauna, it is critical to develop non-lethal sampling techniques and associated biomarkers of toxicant exposure and effect (Newton & Cope, 2006), when available. Fortunately, the extraction of hemolymph (the circulatory fluid) has been shown to be a suitable non-lethal sampling approach for assessing health and condition of mussels (Gustafson et al., 2005a). Therefore, this study utilized the repeated, non-lethal sampling of mussel hemolymph to evaluate the adverse effects of Pb. The specific aims of this study were to conduct a 28-day laboratory toxicity test with Pb and adult *Elliptio complanata* to determine uptake kinetics and to assess potential non-lethal biomarkers of Pb exposure and effect in mussel hemolymph, focusing on Pb and ion (Na+, K+, Cl-, Ca2+) concentrations. The accumulation of Pb in mussel tissue, in relation to the non-lethal measurements, was also evaluated during the study. One of the classic biomarkers of Pb exposure in mammals, fish, and some invertebrates is δ-aminolevulinic acid dehydratase (ALAD) activity (Schmitt et al., 2002; Ahamed et al., 2005; Schmitt et al., 2005; Aisemberg et al., 2005), but it has not been demonstrated in unionid mussels. Because of the positive results with ALAD and the non-unionid freshwater bivalve the Asian Clam, *Corbicula fluminea* (Pallas, 1769), reported by Company et al. (2008), an additional aim of this study was to determine if ALAD activity is present in the hemolymph of *Elliptio complanata* and assess its use as a potential Pb biomarker.

## MATERIALS AND METHODS

### *Collection, Transport, and Holding of Mussels*

A total of 53 adult *Elliptio complanata* were collected from a rural, forested, and relatively uncontaminated section of the Eno River near Hillsborough, North Carolina, USA (NCDENR 2009). Test mussels averaged 77 mm in total length, ranging from 68 to 88 mm, and had a mean wet weight of 69 g, ranging from 40 to 98 g. Upon collection, mussels were promptly placed in ice chests to maintain their temperature near the 21°C river water, covered with damp mesh bags to prevent desiccation and temperature change, and transported directly to the laboratory (30 min transport time). Upon arrival at the laboratory, each mussel was scrubbed with a soft-bristle brush and rinsed with deionized water. Five mussels were randomly chosen for baseline measurements of the test endpoints (Pb and ion (Na+, K+, Cl-, Ca2+) concentrations in hemolymph, Pb concentrations in body tissue) for comparative purposes. These five mussels were each weighed, measured for total length, gently pried open, had ~ 1 mL of hemolymph extracted from the anterior adductor muscle, and were then bagged and stored frozen (-20°C) for Pb analysis. The hemolymph was divided into two cryotubes, with one frozen at -80°C for analysis of ion concentrations, and the other at -20°C for Pb analysis. The remaining mussels were placed into individual 3-L glass aquaria. The aquaria each contained 2 L of ASTM soft water (ASTM 1993) that was gently aerated (5-15 bubbles/s) by a central aeration unit (Sweet Water Air Pump SL24 Aquatic Eco-Systems, Inc., Apopka, FL, USA). For the toxicity test, there were nine target Pb treatment concentrations (0, 1.95, 3.9, 7.8, 15.6, 31.25, 62.5, 125 and 500 µg/L), and five replicates per treatment with one mussel per replicate. The 45 test mussels were then acclimated to test conditions for 72 h prior to initiation. Immediately prior to the start of the test on day 0, the mussel in each aquarium was fed 20 mL of a suspension containing 2 mL of Instant Algae® Shellfish Diet and 1 mL *Nannochloropsis* concentrate (Reed Mariculture, Campbell, CA, USA) in 1 L of deionized water. The mussel in each jar was allowed to siphon and feed for 2 h, after which a complete water renewal and toxicant spiking commenced. Mussel feeding and water and toxicant renewals were conducted three times per week during the 28-day test in this same manner.

### *Experimental Procedures*

#### *Pb Exposure Study*

All laboratory methods followed ASTM guidelines for conducting laboratory toxicity tests with freshwater mussels (ASTM 2006), with modifications for testing adult mussels. Water samples (5 mL) were taken from three of the five replicate test aquaria per treat-

ment concentration every 0, 48, and 72 h post-renewal throughout the 28-day exposure for verification of Pb concentration. These samples were stored preserved (75 µL of concentrated trace metal grade nitric acid) until analysis. Alkalinity, hardness, pH, temperature, and dissolved oxygen were all measured in each aquarium before test initiation and then three times per week thereafter. Water temperature and dissolved oxygen were measured with a calibrated multi-probe (YSI Model 556 MPS, Yellow Springs Instruments, Yellow Springs, OH, USA). Water pH was measured with a calibrated Beckman Model Φ 240 meter (Beckman Instruments, Fullerton, CA, USA). Alkalinity and hardness were measured by standard titrametric methods (APHA et al. 1995). Physiochemical characteristics of test water averaged 21.0°C (range 20.9 – 21.29) for temperature, dissolved oxygen 8.3 mg/L (range 7.9 – 8.7), pH 8.0 (range 7.8 – 8.1), alkalinity 30 mg/L as CaCO3 (range 28 – 32), and hardness 42 mg/L (range 40 – 44).

On day 0 of the test, each mussel was removed from its aquarium, gently pried open, a 25 gauge syringe was used to withdraw 0.25 mL of hemolymph from the anterior adductor muscle, and was then immediately returned to the aquarium. Hemolymph was composited from the first four mussels (replicates) from each test concentration, including the control, to achieve 1 mL total volume. Hemolymph was then divided into aliquots of 0.5 mL for ion (Na+, K+, Cl-, Ca2+) analysis stored at -80°C, and 0.5 mL for Pb analysis stored at -20°C. The sampling of hemolymph from these same first four mussel replicates of each treatment was repeated weekly on days 7, 14, 21 and 28 in the same manner. The fifth and final mussel (replicate) for each test concentration was not sampled until the end of the experiment (d 28) as a control for the repeated, weekly hemolymph sampling. Water and toxicant renewals were conducted three times per week. Before each renewal, the mussel in each aquarium was fed and allowed to siphon for 2 h, as previously described. Each aquarium was then siphoned and renewed (~ 90%) with fresh ASTM soft water. Aquaria were then spiked with Pb from a concentrated stock solution (1,000 mg/L) prepared from lead nitrate to generate the final target Pb concentrations.

*Analytical Procedures*

All samples of mussel hemolymph, body tissue, and test water were analyzed for Pb concentrations with standard methods at RTI International (Research Triangle Park, NC, USA) according to good laboratory practices and strict quality assurance protocols. Briefly, mussel tissues were lyophilized and homogenized, with a nominal weight of 250 mg aliquoted and heated with a mixture of concentrated nitric and hydrochloric acids. Hydrogen peroxide was added to aid in the decomposition of organic material. The hemolymph samples had a 0.2 mL aliquot transferred to an acid washed 15 mL plastic centrifuge tube, and 4.8 mL of a 2.5% HCl-2.5% HNO3 acid extraction solution was added to each sample and vortex mixed. Samples were placed in a water bath at 60°C for 30 min, then vortex mixed, allowed to cool to room temperature, and centrifuged for 30 min at 2,800 RPM. A 3 mL aliquot of the supernatant liquid was removed for analysis. Samples were then analyzed by magnetic sector inductively coupled mass spectrometry (Thermo Element 2 Magnetic Sector ICP-MS). The average percent recovery of Pb from spiked mussel tissue samples was 101%, and ranged from 99-103%. Recovery of Pb in samples of hemolymph averaged 97% and ranged from 87-102%.

All hemolymph samples were analyzed for ion concentrations at the Analytical Service Laboratory in the Department of Soil Sciences at North Carolina State University (Raleigh, NC, USA) according to standard methods, good laboratory practices and strict quality assurance procedures with two Dionex Ion Chromatographs (DX-500 and 4000, Dionex Corporation, Sunnyvale, CA, USA). Using an autosampler (Dionex AS-50) fitted with two injection valves, the samples were simultaneously analyzed for anions and cations. Chloride analysis was performed using a separation column AS 22 and conductivity detection for the cations analysis was done on a Dionex CS 12A column. The concentration of each analyte was determined by comparing the peak area in the chromatograms (using Dionex Software Peak Net 5.21) to those generated with standard solutions.

Because ALAD has recently been found in the freshwater *Corbicula fluminea* (Company et al. 2008), an initial assessment with *Corbicula fluminea* was conducted alongside *Elliptio complanata* for the presence and relative detectability of ALAD among the bivalve species. To ensure data quality and validation for ALAD analysis in the previously untested mussel tissues, the fathead minnow, *Pimephales promelas*, was selected as the test organism to serve as a positive control in the ALAD analysis because this fish has been shown to provide consistent ALAD responses (Spokas et al. 2006). In the initial assessment of ALAD detectability, three *Elliptio complanata*, two *Corbicula fluminea* (collected from the same stream) and one *Pimephales promelas* were analyzed. The bivalves were each weighed, measured, and hemolymph, gill, mantle, foot and viscera samples were taken for analysis and stored at 80°C. All laboratory materials (e.g., glass pipettes, cryotubes) used for hemolymph and fish blood collection were heparinized before use to minimize clotting. The ALAD assay in this assessment utilized methods

to increase sensitivity in order to detect the presence of ALAD in each species. It was modified from that of Schmitt et al. (2005) for use with mussel hemolymph and tissue using a microplate assay. Mussel tissue was sonicated to minimize clotting. In detail, mussel tissues and fish blood samples were removed from the -80°C freezer and placed in a 4°C refrigerator to thaw, along with one cryotube of a porphobilinogen (PBG) stock solution (221 µM PBG). Six centrifuge tubes were labeled for each sample: blank A, B and C, and aminolevulinic acid (ALA) A, B and C. To each blank tube, 50 µL of assay buffer (0.2% Triton X-100 in 0.1M phosphate buffer (pH 6.2)) was added. To each ALA tube, 50 µL of ALA buffer (670 µg ALA·HCl/mL) was added. 75 µL of assay buffer were added to six of the seven PBG standard curve tubes, along with the controls. Blood samples were pipetted and weighed, and an equal volume (10 µg = 10 µL) of deionized water was added. Blood dilutions and hemolymph samples were then both sonicated for 10 min. PBG serial dilution was prepared from the thawed stock solution, 150 µL was pipetted into the empty tube, and a 1:1 serial dilution of the remaining six tubes was prepared transferring 75 µL at a time and vortexing. Then 200 µL of each sonicated sample was added per tube for that sample, vortexed for five seconds, and incubated for 4 h in a 37°C water bath. Modified Ehrlich's reagent was prepared by weighing out and mixing the appropriate amount of p-dimethylamino benzaldehyde to Ehrlich's reagent (e.g. 0.545 g to 30 mL Ehrlich's reagent) for the number of samples being run per batch. After removing the samples from the water bath, the reaction was terminated by the addition of 200 µL stop solution (TCA/n-ethylmaleimide solution) to each tube. Samples were vortexed and centrifuged at 1,000 x g for 10 min. A 100 µL aliquot of supernatant from each sample was pipetted into a 96-well plate, and 100 µL of modified Ehrlich's reagent was added to each well. The plate was placed on a plate shaker for 15 min of color development, and the absorbance was read on a FusionTM Universal Microplate Analyzer (A153600 Meriden, CT, USA) at 540 nm. To normalize the ALAD results among the different tissues and organisms, the Bradford Protein assay (IBI-Shelton Scientific, Peosta, IL, USA), a kit containing 0.5 mg/mL bovine serum albumin (BSA), 0.15 M NaCl and a Bradford Reagent consisting of Coomassie blue, a dye that binds protein, was used to determine the protein concentration in samples by generating a BSA linear standard curve plotting absorbance at 595 nm (Spectronic® Genesys™, Milton Roy Company, Rochester, NY, USA) versus protein concentration. ALAD activity was then reported as uM PBG/min/mg protein.

*Statistical Analysis*

Data were analyzed with StatCrunch software (www.statcrunch.com, Department of Statistics, University of South Carolina, Columbia, SC, USA). One way Analysis of Variance and Simple Linear Regressions were performed with statistical significance determined at $\alpha = 0.05$ for all tests.

## RESULTS

During the 28-d test with Pb, the average measured Pb concentration in samples of test water (n = 3) at time 0 and immediately following each renewal was 73% of the target concentration. Concentrations decreased with time in each replicate to an average of 10.5% of the target by 48 h and 7.4% by 72 h before the next renewal. The average daily exposure concentration was calculated as the weekly average of 3 time 0 h (T0), 3 time 48 h (T48) and 1 time 72 h (T72) measurement(s), to be 0.9, 1.3, 3.2, 6.4, 10.5, 25.9, 66.3 and 250.8 µg Pb/L, and were the values used hereafter to denote the actual measured Pb treatment groups.

No mussels died during the test. The average Pb concentration in mussel tissue at the end of the 28-d study was strongly correlated to exposure concentrations (Fig. 1) with an R2 = 0.98 and $p < 0.0001$. Because the tissue samples from the replicates for a treatment were composited for analysis, variation is not reported.

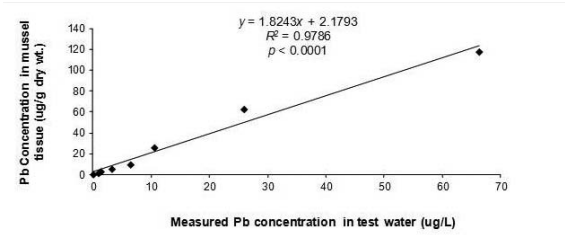


**FIGURE 1**

Relation between waterborne Pb exposure concentrations from control to 251 µg/L and concentrations of Pb in mussel tissue at the end of the 28-d laboratory test.

Concentrations of Pb in hemolymph of the repeatedly sampled mussels (replicates 1 – 4), which were composited per treatment group to obtain sufficient volume, had several different trends over the 28-d study depending on their Pb exposure. These results are summarized for measured exposures of 0 – 66 µg/L (Fig. 2A) to show better endpoint resolution, and for all concentrations from 0 to 251 µg/L (Fig. 2B). For Pb exposures of ≤ 6 µg/L, mussels slowly increased concentrations of Pb in their hemolymph over time, never exceeding three times their exposure concentration. For exposures of 11 – 66 µg/L, concentrations plateau around d 14 with the 11 µg/L treatment group at 3.6 times its exposure concentration and the 26 and 66 µg/L exposures at 1.4 and 1.2 times exposure concentrations, respectively. However, for the greatest exposure concentration of 251 µg/L, hemolymph concentrations never plateau, but appeared to bioconcentrate with rapid, linear accumulation, as shown by the best fit line with an R2 = 0.98, and p = 0.0009, to five times the exposure concentration. The replicate 5 mussels (i.e., those not sampled until d 28) had Pb hemolymph concentrations similar to their corresponding treatment group replicates 1 through 4 mussels on day 28, except for the greatest exposure which had a hemolymph concentration greater than 1,700 µg/L. This was over 500 µg Pb/L above the concentration of the repeatedly sampled mussels in that treatment group.

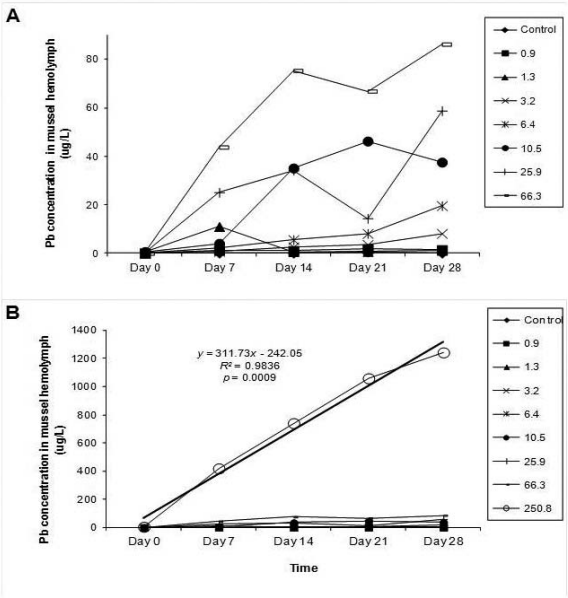


**FIGURE 2**

Concentrations of Pb in hemolymph of the repeatedly sampled mussels (replicates 1 – 4) over the (A) low range (0 – 66 µg Pb/L; provided for endpoint resolution) and (B) full range (0 – 251 µg Pb/L) of exposures at each time point sampled through the 28-d laboratory test. Because the tissue samples from the replicates for a treatment were composited for analysis, variation is not reported.

None of the ion (Na+, K+, Cl-, Ca2+) concentrations measured in the hemolymph from the repeatedly sampled mussels was significantly changed with increasing concentrations of Pb, whereas the mussels from the fifth replicate sampled only on day 28 showed altered calcium concentrations. The Ca2+ concentrations in hemolymph from the non-repeatedly sampled replicate 5 mussels were found to be below the lower 95% confidence interval (CI) of 12.85 mg/dL, which was derived from our five baseline mussels, for low Pb exposures of 1 – 3 µg/L, and above the CI (16.23 mg/dL) for high Pb exposures of 11, 66 and 251 µg/L (Table 1). Because the control treatment remained within the CI, this suggests that Pb exposure altered Ca2+ within the hemolymph.

**TABLE 1**

Hemolymph calcium (Ca2+) concentrations (in mg/dL) of each treatment group of the non-repeatedly sampled replicate 5 mussels on d 28 of the test compared to the 95% confidence interval (CI) of the baseline measurements taken before test initiation.

| Measured Pb in Test Water (µg/L) | Hemolymph Calcium (mg/dL) | Baseline 95% CI (12.85 – 16.23) |
|---|---|---|
| **Control** | 15.5 | = |
| **0.9** | 12.4 | < |
| **1.3** | 12 | < |
| **3.2** | 12.1 | < |
| **6.4** | 13.3 | = |
| **10.5** | 19.2 | > |
| **25.9** | 15.8 | = |
| **66.3** | 18.4 | > |
| **250.8** | 16.9 | > |

Results from the ALAD comparison assay showed significant activity (180 uM PBG/min/mg protein) in the positive control fish, Pimephales promelas ($p < 0.0001$) compared to the negative controls. However, no significant ALAD activity was detected at any time in Elliptio complanata, neither in hemolymph nor in tissue (gill, mantle, foot or viscera). In contrast, when comparing species for activity, the non-unionid Corbicula fluminea had significant ALAD activity ($p = 0.0002 – 0.0098$), producing levels of 0.72 – 0.93 uM PBG/min/mg protein, or an average of 46.7 ng PBG/min/mg protein at 37°C compared to the results of Company et al., (2008) of 1.5 ng PBG/min/mg protein at room temperature. Therefore, ALAD activity in Elliptio complanata was not considered a viable biomarker for the unionid tested and was not measured in mussels from the 28 d Pb exposure study.

## DISCUSSION

We found that *Elliptio complanata*, a freshwater mussel of the family Unionidae, accumulated waterborne Pb extremely rapidly by ventilation across the gills in our laboratory study, a finding that was supported by rapidly declining concentrations of Pb measured in test water following each renewal, and concomitant increasing Pb concentrations in mussel hemolymph and body tissue throughout the test. To place the accumulation of Pb by mussels in this study into an environmentally relevant perspective, we found that the lowest exposure concentration of 0.9 µg/L for 28 d resulted in an average tissue concentration of 1.5 µg/g dry weight, which is similar to the average tissue concentration (1.6 µg/g dry wt.) measured in *Elliptio complanata* (n =240) sampled from natural populations at 40 stream sites across North Carolina (Mosher, 2008). The fact that mid-range Pb exposures resulted in a plateau of Pb concentration in the mussel hemolymph by d 14, whereas the greatest exposure concentrations resulted in rapid accumulation throughout the exposure duration, suggests that metabolic regulation of Pb was occurring in the mussels. Because the concentrations of Pb in test water were being depleted just as rapidly by the end of the experiment as they were in the beginning, it is unlikely that the mussels reduced uptake appreciably over time. This suggests that either the mussels started transporting the lead from hemolymph into tissue and/or shell, or they started eliminating it more efficiently in lysosomes through urine and pseudo-feces (Amiard et al., 1995; Marigómez et al., 2002), where it would then be bound and settle on the floor of the aquaria.

Calcium concentrations in hemolymph of the non-repeatedly sampled mussels (replicate 5 from each treatment) appeared to be altered by Pb exposure. When comparing the 95% CI reference values for Ca2+ levels in *Elliptio complanata* (13.1 – 23.7 mg/dL) generated by Gustafson et al. (2005b), the lowest three Pb

exposures in this test also resulted in a decrease below this lower limit. Because this observation was based only on a single mussel per concentration, this relation is uncertain and requires additional research. However in another 28-day Pb exposure study by Mosher et al. (2010) in which mussels were sampled terminally (rather than repeatedly) at the same 0, 7, 14, 21, and 28 day time intervals, they found similar results with significant increases in Ca2+ at the greatest Pb exposure of 245 µg/L. While no trends were determined between Pb exposure and ion levels in hemolymph of the mussels repeatedly sampled in this study, this may have been due to the damaging effects of repeated puncturing of the anterior adductor muscle during sampling rather than to the exposure to Pb. Even though repeated hemolymph sampling of three times over seven months has been determined to be non-lethal and pose no apparent physiological harm (Gustafson et al., 2005a), the repeated sampling of five times over one month may have been causing additional stress, as well as possibly allowing direct transport of ions into and out of the adductor muscle via the tracts left by the 25 gauge needle. By the end of the experiment, some of the adductor muscles had visible holes in the side from tearing, as a result of weakening from multiple punctures with little time for recovery. We believe that five repeated sampling periods of test mussels for hemolymph within 28 d was too aggressive, causing tissue damage in some cases, and, therefore, potentially masking any trends in ion levels with Pb exposure concentrations. A future study with an experimental design that would provide for greater numbers of non-repeatedly sampled mussels to be analyzed would be recommended to determine if Ca2+ concentrations are unequivocally adversely affected by Pb exposure.

For the potential development and application of an assay to rapidly and sub-lethally assess Pb exposure in native freshwater mussels, we found that simple measurements of Pb concentration in mussel hemolymph were highly indicative of real-time and recent exposures. This type of application could be easily applied to field monitoring situations in which mussels could be sampled and replaced in their habitat without damaging populations. In addition, we found that Ca2+ concentrations in hemolymph may be useful in determining the overall health impact of a mussel population to Pb exposure, given enough individuals were sampled to reduce variability. Moreover, additional research is needed in assessing Ca2+ concentrations in response to other environmental stressors before such a monitoring assessment and link could be made.

Finally, our results indicated that while we confirmed the presence of ALAD in *Corbicula fluminea* (e.g., Company et al., 2008), it does not appear to be a suitable biomarker in the unionid mussel *Elliptio complanata* due to the lack of detectable activity at baseline metabolic conditions. Mollusks can contain either hemocyanin or hemoglobin for oxygen transport within the hemolymph, or have no respiratory proteins at all (Mangum et al., 1987; Alyakrinskaya, 2003), depending on the genera. While the absence of iron hemoglobin in hemolymph does not necessarily negate its presence in other tissues of bivalves (Alyakrinskaya, 2003), we found no evidence of ALAD activity in *Elliptio complanata* hemolymph, gill, mantle, foot or visceral tissue.

*Conclusion*

Overall, we found that measurements of Pb concentration in mussel hemolymph were highly indicative of real-time and recent exposures, and may provide a suitable marker to rapidly and sub-lethally assess Pb exposure and toxicity in native freshwater mussels in both laboratory and stream settings. Ca2+ concentrations in hemolymph could potentially be adversely affected by Pb exposure in non-repeatedly sampled mussels, although further assessment is needed to confirm this relationship. Concentrations of Pb measured in body tissue were strongly correlated with the full range (0-251µg/L) of Pb exposure concentrations. Thus, freshwater mussels appear to accumulate Pb in a concentration dependent manner and begin to actively regulate Pb uptake by d 14 of exposure, based on measured hemolymph concentrations.

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# WALKERANA The Journal of the Freshwater Mollusk Conservation Society



## OUR PURPOSE

The Freshwater Mollusk Conservation Society (FMCS) is dedicated to the conservation of and advocacy of freshwater mollusks, North America's most imperiled animals. Membership in the society is open to anyone interested in freshwater mollusks who supports the stated purposes of the Society which are as follows:

1) Advocate conservation of freshwater molluscan resources;

2) Serve as a conduit for information about freshwater mollusks;

3) Promote science-based management of freshwater mollusks;

4) Promote and facilitate education and awareness about freshwater mollusks and their function in freshwater ecosystems;

5) Assist with the facilitation of the National Strategy for the Conservation of Native Freshwater Mussels (Journal of Shellfish Research, 1999, Volume 17, Number 5), and a similar strategy under development for freshwater gastropods.

## OUR HISTORY

The FMCS traces it's origins to 1992 when a symposium sponsored by the Upper Mississippi River Conservation Committee, USFWS, Mussel Mitigation Trust, and Tennessee Shell Company brought concerned people to St. Louis, Missouri to discuss the status, conservation, and management of freshwater mussels. This meeting resulted in the formation of a working group to develop the National Strategy for the Conservation of Native Freshwater Mussels and set the ground work for another freshwater mussel symposium. In 1995, the next symposium was also held in St. Louis, and both the 1992 and 1995 symposia had published proceedings. Then in March 1996, the Mississippi Interstate Cooperative Research Association (MICRA) formed a mussel committee. It was this committee (National Native Mussel Conservation Committee) whose function it was to implement the National Strategy for the Conservation of Native Freshwater Mussels by organizing a group of state, federal, and academic biologists, along with individuals from the commercial mussel industry. In March 1998, the NNMCC and attendees of the Conservation, Captive Care and Propagation of Freshwater Mussels Symposium held in Columbus, OH, voted to form the Freshwater Mollusk Conservation Society. In November 1998, the executive board drafted a society constitution and voted to incorporate the FMCS as a not-for-profit society. In March 1999, the FMCS held it's first symposium "Musseling in on Biodiversity" in Chattanooga, Tennessee. The symposium attracted 280 attendees; proceedings from that meeting are available for purchase. The second symposium was held in March 2001 in Pittsburgh, Pennsylvania, the third in March 2003 in Raleigh, North Carolina, the fourth in St. Paul, Minnesota in May 2005, the fifth in Little Rock, Arkansas in March 2007, and the sixth in Baltimore, Maryland in April 2009. The society also holds workshops on alternating years, and produces a newsletter three times a year.

## FMCS SOCIETY COMMITTEES

Participation in any of the standing committees is open to any FMCS member. Committees include:

Awards

Environmental Quality and Affairs

Gastropod Distribution and Status

Genetics

Guidelines and Techniques

Information Exchange - Walkerana and Ellipsaria

Mussel Distribution and Status

Outreach

Propagation and Restoration

## TO JOIN FMCS OR SUBMIT A PAPER

Please visit our website for more information at http://www.molluskconservation.org

Or contact any of our board members or editors of WALKERANA to talk to someone of your needs. You'll find contact information on the back cover of this publication.

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## FMCS 2011-2013 Officers

*President* **Caryn Vaughn**
Oklahoma Biological Survey
University of Oklahoma
111 E Chesapeake
St. Norman, OK 73019
**cvaughn@ou.edu**

*President Elect* **Patricia Morrison**
Ohio River Islands NWR
3982 Waverly Road
Williamstown, WV 26187
**patricia_morrison@fws.gov**

*Secretary* **Greg Zimmerman**
EnviroScience, Inc.
6751 A-1 Taylor Rd.
Blacklick, Ohio 43004
**gzimmerman@enviroscienceinc.com**

*Treasurer* **Heidi L. Dunn**
Ecological Specialists, Inc.
1417 Hoff Industrial Park
O'Fallon, MO 63366
636-281-1982 Fax: 0973
**Hdunn@ ecologicalspecialists.com**

*Past President* **W. Gregory Cope**
North Carolina State University
Department of Environ. & Molecular Toxicology
Box 7633
Raleigh, NC 27695-7633
**greg_cope@ncsu.edu**