Powell River Project FY2019 – Annual Report
Chronic Toxicity of Trace-Metal Mixtures to Juvenile Freshwater Mussels

Investigators
Anthony J. Timpano, Fish and Wildlife Conservation, Virginia Tech (Co-Principal Investigator)
and
Jess W. Jones, U.S. Fish and Wildlife Service (Co-Principal Investigator)

Preface

Funding from Powell River Project in FY2019 provided partial support for completion of data analysis and communication of findings from a prior ecotoxicological study funded by The Nature Conservancy (TNC). Our communications included oral presentations at two scientific meetings, three webinar presentations to federal agency staff, preparation of a final technical report, and preparation of a manuscript for publication in a peer-reviewed journal. Completed analyses of study results and sharing of findings should aid efforts to conserve and restore mussel populations in the Clinch and Powell rivers, thus advancing the environmental science mission of the Powell River Project to enhance management and restoration of environmental resources affected by mining in the Appalachian coalfield.

This Annual Report contains a summary of research findings from the study supported by TNC, followed by a summary of activities described above that were supported directly by Powell River Project.

Summary of Research Findings

The Clinch and Powell River systems of southwestern Virginia and northeastern Tennessee are among the most biodiverse freshwater ecosystems in North America. Rich freshwater mussel assemblages are major contributors to the highly valued biological status and high conservation priority of these two rivers. Currently, 46 extant freshwater species occur in these systems, with 20 species classified as federally threatened or endangered under the Endangered Species Act.

Freshwater mussel assemblages in some river segments influenced by coal mining land use have exhibited significant declines over decades. Scientific investigations have been conducted for the purpose of identifying specific stressors, toxicants, or combinations of such that are responsible for those observed declines, anticipating that results would aid current conservation and restoration initiatives. Despite those efforts, the specific stressors or toxicants that have been or are causing ongoing severe impairments of freshwater mussel communities in the Clinch and Powell Rivers have not been identified.

Toxicity from trace metals of coal-mining origin (e.g., alkaline drainage from valley fills) is a candidate cause of mussel decline, as several metals are elevated above background concentrations in the Clinch and Powell Rivers, but those metals have not been observed at levels exceeding their respective U.S. Environmental Protection Agency-recommended ambient chronic water quality criteria (WQC) for aquatic life protection. Given that many trace metals co-occur in the Clinch and Powell Rivers, it is hypothesized that multiple-metal mixtures may be exerting a combined effect on mussels that is more toxic than would be predicted based solely on the concentration of any single metal within the mixture.

The objective of this study was to test the combined-effects hypothesis in an environmentally-relevant context by exposing juveniles of the common species rainbow mussel (*Villosa iris*) to trace metals in the presence of elevated major ions at levels comparable to those found in the Clinch River as a result of alkaline coal-mine drainage (**Table 1**). We exposed mussels for 42 days to water-column copper (Cu), nickel (Ni), and zinc (Zn) individually and as three-metal mixtures at concentrations approximately equal to or less than chronic WQC for individual metals (**Table 2**).

Table 1. Water chemistry of test exposure water approximating annual maxima in Clinch River.

Water Quality Parameter	Units	Clinch Target	Actual†
Specific Conductance	µS/cm	476	521
Sum of Major Ions	mg/L	359	337
Ca	mg/L	36.8	34.6
Mg	mg/L	17.3	16.7
K	mg/L	2.64	4.10
Na	mg/L	22.7	37.2
Cl	mg/L	16.3	17.2
HCO_3	mg/L	203	165
SO_4	mg/L	60.6	60.5
Hardness (as $CaCO_3$)	mg/L	163	155
Ca:Mg mass ratio		2.13	2.07
Cl:SO4 mass ratio		0.27	0.28
Dissolved organic carbon	mg/L	2.1	1.7*

†Mean values measured across 6 treatments on Days 1 and 7 during each week of the 42-day exposure (n = 12 measurements per treatment). With the exception of specific conductance, all values are total dissolved concentrations (0.45 µm filter).

*Concentration in diluted water from the pond at the Freshwater Mollusk Conservation Center at Virginia Tech, which was used to prepare dilution water for treatments; not adjusted.

We standardized metal exposure concentrations to toxic units (TU) to enable comparisons of individual and combined toxic effects. A TU is the concentration of a toxicant, or effect concentration, that causes a specific effect to the test organisms. Although the numeric effect concentration for a given toxicological effect may vary greatly among toxicants, each one should cause the same response at the respective concentration equivalent to 1 TU. This standardized approach allowed evaluation of whether metal mixtures had a greater effect than would be predicted by single-metal concentrations. Toxic units were selected based on a review of effect concentrations in the best available literature on freshwater mussel water-column toxicity for each metal. We established each TU such that 1/3 TU would be a concentration below the USEPA National Recommended Water Quality Criteria for Aquatic Life freshwater criteria continuous concentration (CCC). The 1/3 TU treatments were intended to constrain metal concentrations to levels lower than CCCs, and the 1/9 TU treatment contained metals at concentrations approximately 30% of their respective CCCs (**Table 2**).

Metal exposure did not reduce mussel survival after 42 days (≥ 93%), but growth was suppressed in all metal treatments for the duration of the experiment (Figure 1). At 42 days, growth was significantly inhibited relative to controls, as measured by changes in length (Figure 2) and dry weight (Figure 3). Combined effects were evident, as the three-metal mixture inhibited growth more than single-metal exposures of similarly-toxic concentrations: 95% inhibition for the 1/3 EA three-metal mixture versus 61 – 74% for the individual metals (Figure 4). A mixture of three metals with individual concentrations approximately 30% of chronic WQC inhibited growth by more than 50% relative to the control treatment (1/9 EA treatment in Figure 4).

Table 2. Metal concentrations and water hardness for each treatment, with water quality criteria.

Treatment Toxic Units (TU) fraction, with target concentrations (μg/L); and Water Quality Criteria	**Measured concentration mean and range (μg/L, dissolved)†**			
	Cu	Ni	Zn	**Hardness (mg/L as $CaCO_3$)**
Experimental Treatments				
Control‡	0.6 0.1 - 4.4	0.5 0.3 - 0.9	6.7 0.1 - 29.3	155 144 - 162
1/3 TU Cu (8 Cu)	8.0 6.4 - 10.3	0.7 0.4 - 1.6	5.6 0.1 - 13.9	156 149 - 165
1/3 TU Ni (60 Ni)	0.5 0.2 - 1.5	63.5 52.4 - 69.6	5.5 0.1 - 11.1	153 146 - 162
1/3 TU Zn (150 Zn)	0.5 0.1 - 1.7	0.6 0.4 - 1	193.5 138.8 - 245.6	157 147 - 170
1/3 TU each metal (8 Cu, 60 Ni, 150 Zn)	7.2 6.1 - 10.8	65.3 50.8 - 71.3	183.1 116.4 - 223	156 149 - 162
1/9 TU each metal (2.7 Cu, 20 Ni, 50 Zn)	3.4 2.3 - 6.3	21.8 17.6 - 24.1	62.1 47.8 - 78.2	155 147 - 162
Water Quality Criteria (at mean exposure water hardness of 155 mg/L as $CaCO_3$)				
USEPA CCC[1] (Chronic)	13.0	75.4	170.0	
USEPA CMC[2] (Acute)	20.3	678.0	170.0	

† Treatment concentration is mean of total dissolved (0.45 μm filter) values measured in a 5-replicate composite sample on Days 1 and 7 during each week of the 42-day exposure (n = 12 measurements per treatment).

‡ Metal present in diluted water from the pond at the Freshwater Mollusk Conservation Center at Virginia Tech, which was used to prepare dilution water for treatments. [1]Criteria Continuous Concentration; [2]Criteria Maximum Concentration

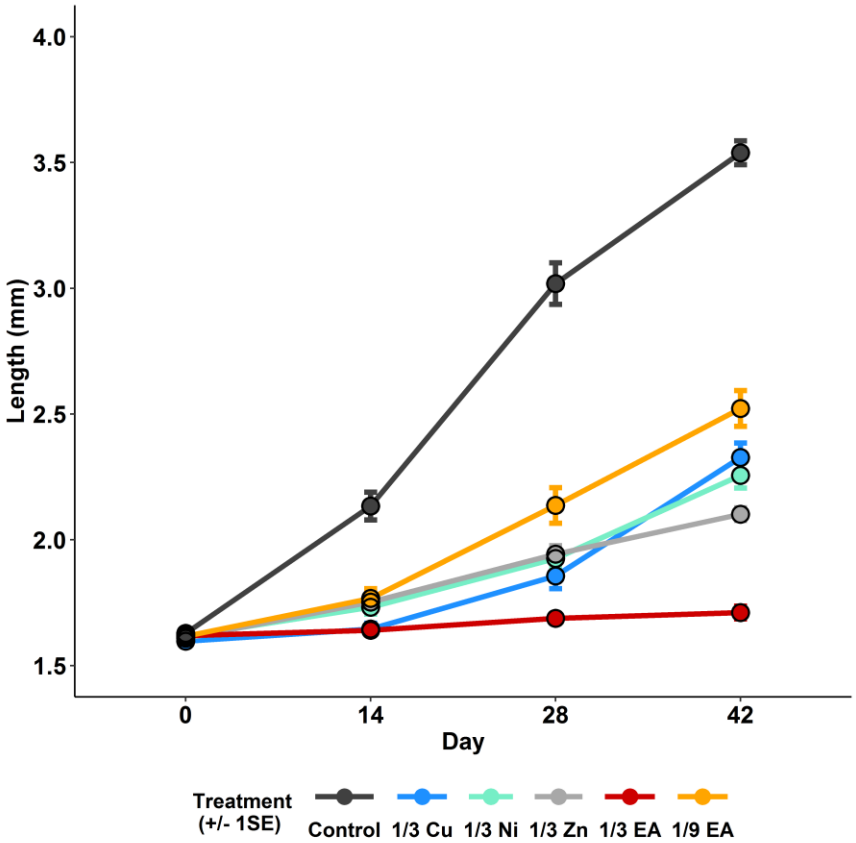

Figure 1. Length of mussels (mean ± 1 SE) over 42-day test duration depicting suppressed growth in metal treatments relative to control. Note greater suppression in the 3-metal mixture (1/3 EA) relative to individual metal treatments (1/3 Cu, 1/3 Ni, 1/3 Zn). Metal exposures are based on Toxic Units as defined in the text and Table 2.

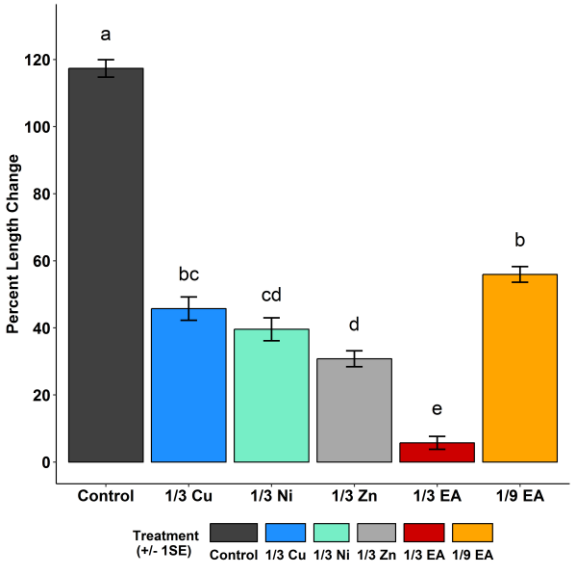

Figure 2. Percent length change at Day 42 (mean ± 1 SE). Treatments with different letters are significantly different from one another (Tukey's HSD, $p < 0.05$). Metal exposures are based on Toxic Units as defined in the text and Table 2.

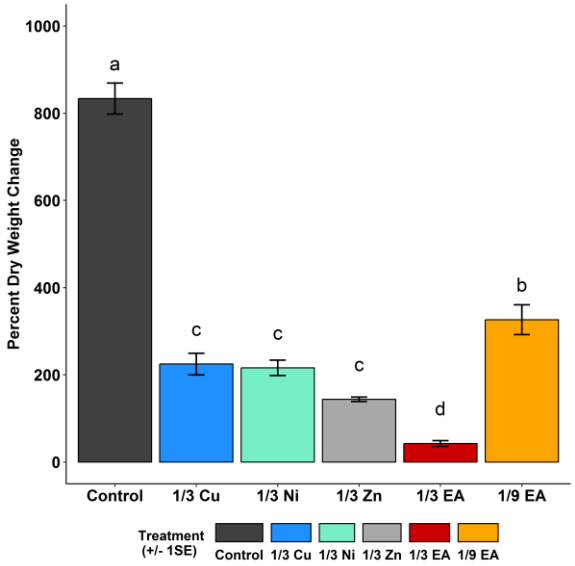

Figure 3. Percent dry weight change at Day 42 (mean ± 1 SE). Treatments with different letters are significantly different from one another (Tukey's HSD, $p < 0.05$). Metal exposures are based on Toxic Units as defined in the text and Table 2.

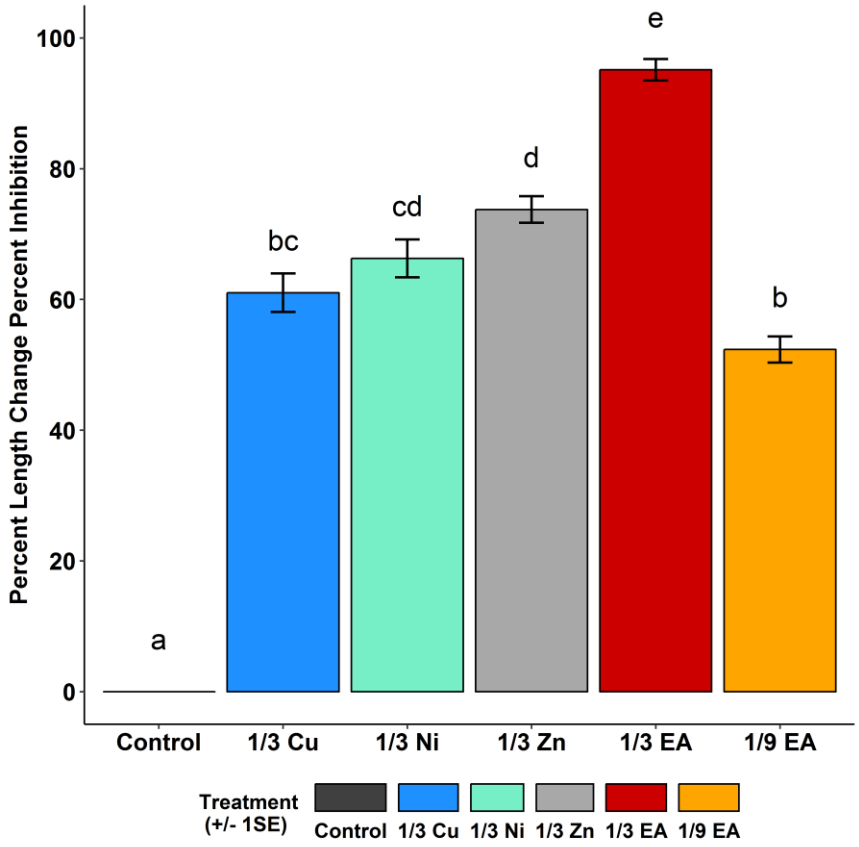

Figure 4. Percent inhibition of length change at Day 42 (mean ± 1 SE). Treatments with different letters are significantly different from one another (Tukey's HSD, $p < 0.05$). Note greater inhibition of growth in the 3-metal mixture (1/3 EA) relative to individual metal treatments (1/3 Cu, 1/3 Ni, 1/3 Zn). Metal exposures are based on Toxic Units as defined in the text and Table 2.

These results suggest two general conclusions. First, combined effects of metal mixtures is a plausible mechanism of metal toxicity causing chronic suppression freshwater mussel growth in the Clinch and other rivers receiving alkaline coal-mine drainage such as the Powell. Second, substantial mussel growth inhibition at metal concentrations only a fraction of their CCCs in an environmentally-relevant context (e.g., elevated major ions as occur in coalfield rivers) suggests that CCCs for certain metals may be under-protective of freshwater mussels in coal-mining influenced rivers. Our findings justify considering concentrations of all trace elements together when evaluating toxicity potential to mussels within the Clinch and Powell Rivers. They also highlight the need for further study of effects of multiple stressors on freshwater mussels.

Our results can be used to inform further research to identify causes of mussel decline in Clinch and Powell Rivers. Findings can also aid development of water quality criteria that account for combined effects of metal mixtures. These outcomes advance the environmental science mission of Powell River Project by generating knowledge that will aid management and restoration of Powell River water quality and mussel populations, both of which are important environmental resources affected by mining in the Appalachian coalfield.

Summary of Activities Supported Directly by Powell River Project

Presentations at Scientific Meetings

Dr. Timpano attended two scientific meetings to make oral presentations of findings. Attendees included researchers, state and federal agency personnel, and aquatic resource conservation practitioners working in the Appalachian region and nationally. Presentation details are below:

Timpano, A.J., J.W. Jones, B. Beaty, M. Hull, D.J. Soucek, C.E. Zipper. Combined Effects of Trace-metal Mixtures on Juvenile Rainbow Mussels (*Villosa iris*). Oral presentation at the Freshwater Mollusk Conservation Society 2019 Symposium. San Antonio, Texas. 14-18 April 2019.

Timpano, A.J., J.W. Jones, B. Beaty, M. Hull, D.J. Soucek, C.E. Zipper. Chronic Toxicity of Trace-Metal Mixtures to Rainbow Mussels (*Villosa iris*). Oral presentation at Association of Mid-Atlantic Aquatic Biologists 2019 Workshop. Berkeley Springs, West Virginia. 26-28 March 2019.

Presentations to Stakeholders

We have shared our findings with US EPA personnel working to address water quality and freshwater mussel conservation challenges nationally, as well as in mining-influenced streams of Appalachia. Dr. Timpano has made three oral webinar-style presentations to EPA staff from Regions 3 and 5, EPA Headquarters, and the Whole-Effluent Toxicity Coordinators working group. We have also briefed TNC and US Fish and Wildlife Service on our findings.

Scientific Reports and Manuscripts

A draft final technical report of findings will be submitted to the original study sponsor (TNC) for review and comment, with final report anticipated in Q4 2019. That report will be shared with Powell River Project as soon as it is available.

A scientific manuscript will be prepared based on the final technical report. It is anticipated that the manuscript will be submitted to an appropriate journal (e.g., Environmental Toxicology and Chemistry) shortly after completion of the final technical report.

Grant Proposals

In partnership with the Clinch-Powell Clean Rivers Initiative, we submitted in April 2019 a pre-proposal to the U.S. Office of Surface Mining Reclamation and Enforcement Applied Science program that would support further investigation of toxicity of environmentally-relevant trace-metal mixtures to juvenile freshwater mussels in the Clinch and Powell River basins.

Title: Chronic Toxicity of Environmentally-Relevant Trace-Metal Mixtures to Juvenile Freshwater Mussels Native to Clinch and Powell Rivers

Investigators: Anthony J. Timpano, Virginia Tech; Jess W. Jones, US Fish and Wildlife Service, Cooperative Unit at Virginia Tech; Braven Beaty, The Nature Conservancy; Matthew Hull, Virginia Tech; David J. Soucek, University of Illinois; Carl E. Zipper, Virginia Tech

Status: Pending indefinitely, contingent on agency program funding.