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Critical Tissue Residue Approach Linking Accumulated Metals in Aquatic Insects to Population and Community-Level Effects

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ABSTRACT: Whole body Zn concentrations in individuals (n = 825) from three aquatic insect taxa (mayflies *Rhithrogena* spp. and *Drunella* spp. and the caddisfly *Arctopsyche grandis*) were used to predict effects on populations and communities (n = 149 samples). Both mayflies accumulated significantly more Zn than the caddisfly. The presence/absence of *Drunella* spp. most reliably distinguished sites with low and high Zn concentrations; however, population densities of mayflies were more sensitive to increases in accumulated Zn. Critical tissue residues (634 μg/g Zn for *Drunella* spp. and 267 μg/g Zn for *Rhithrogena* spp.) caused a 20% reduction in maximum (90th quantile) mayfly densities. These critical tissue residues were associated with exposure to 7.0 and 3.9 μg/L dissolved Zn for *Drunella* spp. and *Rhithrogena* spp., respectively. A threshold in a measure of taxonomic completeness (observed/expected) was observed at 5.4 μg/L dissolved Zn. Dissolved Zn concentrations associated with critical tissue residues in mayflies were also associated with adverse effects in the aquatic community as a whole. These effects on populations and communities occurred at Zn concentrations below the U.S. EPA hardness-adjusted continuous chronic criterion.

INTRODUCTION

Weathering of mineralized rocks and the associated release of toxic metals and acids into aquatic ecosystems is a global phenomenon.[1,2] Aquatic insects are sensitive to metals and their predictable responses are often used to determine injury to ecosystems.[3,4] Increasingly the bioaccumulation (accumulation of a substance in an organism) of metals is used to describe the biological fraction of metal in the environment that causes effects in aquatic insect populations (occurrence and density).[5−7]

The accumulation of metals by aquatic organisms can occur by association with sediment, directly from the water column, or through dietary exposure (Figure 1).[8] Small-scale laboratory studies have accurately described the biological cycling of metals accumulated from the environment (Figure 1).[9−12] These studies hypothesize that toxicity occurs when the accumulation of metabolically available metal, defined as the fraction of metal that is internalized but not eliminated or detoxified, exceeds a threshold.[13] This metabolically available or toxic fraction of internalized metal is correlated with whole body metal concentrations observed in field-collected and laboratory-exposed organisms.[6,14,15] Presumably this accumulated metal causes decreased individual fitness, with subsequent declines in metal-sensitive populations.[10,16]

Field assessments of the effect of metal contamination on aquatic populations often make implicit links among exposure, accumulation, and effects on populations (Figure 1). This is usually accomplished through independent correlations among concentrations of metal in the environment and accumulated metals in tissues, food resources, bioassays, and population densities, while invoking a weight of evidence to support assumptions.[6,17−19] Quantitative statistical models that explicitly link concentrations of metal in aquatic insects to changes in aquatic ecosystem health are rarely used in biological assessment.

Our objective was to use a tissue residue effects approach to develop critical tissue thresholds of zinc (Zn) in aquatic insects as an indicator of effects on populations and communities.[7,20,21] We evaluated the accumulation of Zn by the mayflies *Rhithrogena* spp. (Heptageniidae) and *Drunella* spp. (Ephemerellidae) and the caddisfly *Arctopsyche grandis* (Hydropsychidae), and determined the critical tissue residue of Zn that causes an adverse change in population densities. Our aim was to determine if a critical tissue residue of Zn can be linked to community-level taxa loss. All three taxa are ubiquitous in Rocky Mountain streams and are often considered sentinels of metal contamination in this region.[4,6,22]

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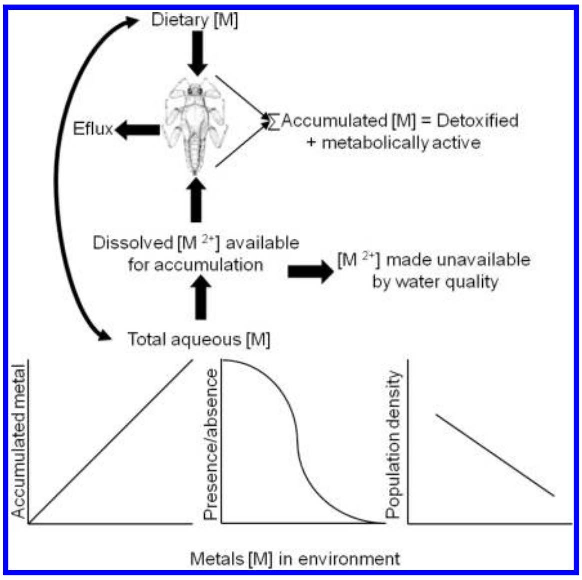

Figure 1. Our empirical understanding of how metals are accumulated by aquatic insects, how those metals are dynamically controlled once internalized, and how population responses are often used to develop implicit links among exposure, accumulation, and effects in populations. Modified from Prusha and Clements.[51]

EXPERIMENTAL SECTION

Study Area. The study area is central Colorado from Wyoming to the New Mexico border, an area of approximately 55,000 km^2 that includes most of the Rocky Mountains in Colorado representing about 20% of the land area in that state. This area is inclusive of a geologic feature called the Colorado Mineral Belt that has been mined for the past 150 years. Sample locations ranged from 2330 to 3550 m above sea level. The climate is temperate continental, and generally receives 50 cm of precipitation annually, especially at higher altitudes. Much of this precipitation occurs as snow in winter or as rain between June and August. Vegetation ranges from deciduous cover at lower altitudes and in riparian zones, to conifer forests, and open tundra at the highest altitudes.[23] Soils within the study area are thin (rarely greater than 10 cm) to nonexistent, occurring in areas dominated by bedrock outcrops. Thicker (up to 1 m or more) immature soils, as well as unconsolidated overburden, occur intermixed at lower elevations and along streams.[24]

Small catchments (1st−3rd order) predominantly underlain by a single rock type were sampled and categorized on the basis of predominant mineral deposits. The purpose of this sampling strategy was to target a large variety of water quality conditions resulting from interaction with the underlying rocks of the watershed and to develop geochemical and biological baselines based on rock type.[25,26] Targeted catchments were otherwise unaffected by other anthropogenic stressors (i.e., least impacted) except for the influences of mining. Using this approach, we collected geochemical and benthic macroinvertebrate samples from 125 discrete locations during summer base flow conditions (July to September) from 2003 through 2007. To capture interannual variability, 12 of these locations were targeted for annual sampling, resulting in an additional 24 or a total of 149 samples from 125 unique locations. A subset of these data representing catchments that drain watersheds containing no detectable mineralization, ore deposits, or mining activity, was used to define background conditions representative of water quality at undisturbed sites.[26]

Chemical Analysis. Water samples were collected and preserved in the field at all sites using methods described by Wilde et al.[27] One aliquot of water was filtered through a 0.45-μm filter and acidified with ultrapure HNO_3 to a pH of ∼1 for cation analysis. A filtered, unacidified aliquot was also collected for anion analysis. Field blanks were prepared by analyzing doubly deionized water that was taken from the laboratory to the field, and treated as a sample at a field site. Sample analysis was conducted at the laboratories of the USGS Laboratory in Denver, Colorado. Concentrations of Zn were analyzed by inductively coupled plasma mass spectrometry (Perkin-Elmer Sciex Elan 6000 ICP-MS). Analytical methods for 2003 differed from the above in that Zn was analyzed by furnace atomic absorption (Perkin-Elmer model 372) at the Colorado State University Department of Fish, Wildlife, and Conservation Biology. The minimum detection limits were 2 μg/L Zn in 2003 vs 0.5 μg/L in 2004−2007; 1/2 detection was substituted for results below the detection limit.

To check the quality of the data all analytical results were run through the PHREEQC program[28] to calculate charge balance and saturation-index values for minerals. In most cases, charge balance was within ±10%. Field duplicates (two samples collected at the same time) or field blanks comprised 10% of the samples collected. Results of analyses of the field blanks were below detection. Field duplicate samples collected the same day showed good reproducibility (usually within ±10%), although errors increased if elements were present near detection limits.

Critical Tissue Residue Determination. *Arctopsyche grandis*, *Drunella* spp., and *Rhithrogena* spp., (n = 1−9 individuals per site) were collected for the determination of whole body Zn concentrations at most sites. This sampling effort occurred immediately following the collection of quantitative benthic samples (see below) in the same reach of stream. Large individuals were targeted to ensure enough tissue was collected to limit analytical error due to low sample weight. Cobbles and small boulders were removed from the stream by hand and individuals of each taxon were collected. Individual organisms were placed into a 50-mL test tube filled with site water and kept at 4 °C for 24−48 h to purge gut contents, which may contain metals that are not accumulated into the tissues of the organism.[3] Individuals were then placed into a 15-mL polypropylene test tube filled with 5 mL of 0.01 M EDTA (ethylenediaminetetraacetic acid) for 30 s to remove externally bound trace metal[3] and then removed and placed into a new tube and frozen until digestion. Samples were dried at 60 °C for 72 h and then placed in a desiccator until they returned to room temperature. Individuals were weighed to the nearest tenth of a milligram using a Sartorius BP 110S balance. Concentrated nitric acid (16 M) was added to each tube for 24 h and then heated to 60 °C for 4 h. Once test tubes were cooled, distilled hydrogen peroxide was added to each sample and heated again to 60 °C for 4 h. Samples were cooled again to room temperature, and double-distilled nitric acid was added to each test tube to a final volume of 2 mL. Chemical analysis was done using ICP-MS (Perkin-Elmer Sciex Elan 6000) from a 5% HNO_3 solution (20:1 dilution:dilution) of the entire sample. To quantify recovery of metals, NIST standard 1577b, bovine liver was digested with sample batches over the course of the study. The recoveries were near 100%. Mean ± standard error (SE) of whole body Zn concentrations were calculated when multiple individuals of the same taxon were collected from the same stream.

Characterization of Populations and Communities. At each stream location, five replicate benthic samples were collected using a 0.1-m^2 Hess sampler (350-μm mesh net) from shallow riffle areas (<0.5 m). Overlying substrate was scrubbed and disturbed to a depth of approximately 10 cm and the remaining material was washed through a 350-μm mesh sieve. All organisms retained were preserved in 80% ethanol in the field and enumerated in the laboratory. In the laboratory, samples were processed to remove debris and subsampled until 300 organisms (±10%) were removed from the sample following methods described by Moulton et al.[29] Invertebrates were identified to the lowest practical taxonomic level (genus or species for most taxa).[22,30] Means of the five replicate benthic samples were used to calculate the density (number of individuals/0.1 m^2) of taxa at each site.

We used a previously developed River Invertebrate Prediction and Classification System (RIVPACS) type predictive model[31] for Colorado[32] to determine the dissolved Zn concentration associated with community level effects. RIVPACS type predictive models measure taxonomic completeness as the ratio of observed (O) taxa collected at each site to the modeled taxa expected (E) to occur at each site.[33] Values of O/E near 1.0 imply that the observed taxa found at a site closely resemble the taxa that were predicted (E) to occur; whereas, O/E < 1.0 implies some degree of taxa loss. O/E values were calculated using a probability-of-capture threshold of 0.5.[33,34] Descriptions of predictive model construction are detailed elsewhere (e.g., refs 31 and 33). Details of the predictive model used herein are described in Hawkins.[32]

Data Analysis. All statistics were performed using R software (version 2.7.2).[35] Simple linear-regression models were developed to determine the relationship between dissolved Zn and whole body Zn concentrations in *A. grandis*, *Drunella* spp., and *Rhithrogena* spp. No differences were observed in body concentrations between *D. doddsii* or *D. coloradensis* or between *R. hageni* or *R. robusta* so these taxa were pooled at the genus level. These regressions were used to predict population-level end points (presence/absence and density) as a function of accumulated metal.

Logistic regression (generalized linear model with binomial link function) was employed to predict occurrences of *A. grandis*, *Drunella* spp., and *Rhithrogena* spp. as a function of whole body Zn concentration.[36] The results of this analysis were then plotted versus dissolved Zn to depict changes in the probability of detection of each taxa as a function of accumulated metal at streams with increasing dissolved Zn concentrations. Model performance was measured by area under the curve (AUC).[37] AUC is quantified by plotting model sensitivity (fraction of "presence" correctly classified) verses specificity (fraction of absences correctly classified). AUC ranges from 0 to 1, were values <0.5 indicate models that perform no better than chance (do not discriminate presence from absence) whereas a value of 1.0 indicates a model with perfect discrimination.

We determined the critical tissue residue of Zn associated with an adverse effect to population density. An adverse effect was defined as a 20% change in the 90th quantile of population density at sites with background levels of Zn.[38−40] Regression quantiles are a series of ascending planes above a proportion of sample observations increasing with quantiles.[41,42] This property of regression quantiles facilitates the estimation of a rate of change (slope) for any quantile or resource value (e.g., 90th quantile of density) as a function of a stressor.[39,40,43] The maximum observable density can be constrained by accumulated Zn at some locations while other limiting factors (e.g., habitat, life history, and other metals) may not permit this maximum value. Therefore, the 90th quantile is the maximum density that could be observed at a site in the absence of other limiting factors. The 90th quantile regression was estimated using the quantreg package in R software.[44] Percent change in density was calculated as follows:

$$\textit{Percent change in 90th quantile} = \frac{\textit{Density}_i - \textit{Density}_j}{\textit{Density}_i}$$

where Density_i is the 90th quantile of density at whole body Zn concentrations associated with background dissolved Zn concentrations (0.9 μg/L) (determined using data and methods described by Schmidt et al.[26]) and Density_j is the 90th quantile where a 20% loss in density was observed. Dissolved Zn concentrations associated with the adverse effect were calculated by solving the whole body Zn linear regression models for dissolved Zn.

We then compared the dissolved Zn concentrations associated with the critical tissue residues of Zn to threshold effects observed in a measure of taxonomic completeness (O/E). Piecewise linear regression[45] following methods described in Schmidt et al.[46] was used to determine the dissolved Zn concentration associated with an abrupt change in O/E.

RESULTS

Critical Tissue Residue. Whole body Zn concentrations were measured in *A. grandis* (344 individuals from 56 locations), *Drunella* spp. (393 individuals from 63 locations), and *Rhithrogena* spp. (88 individuals from 14 locations). Simple linear regression models of dissolved Zn versus whole body Zn concentrations for *A. grandis* (R^2 = 0.53), *Drunella* spp. (R^2 = 0.68), and *Rhithrogena* spp. (R^2 = 0.78) were highly significant ($p \leq 0.001$) (Figure 2). As metal concentrations increased, *Rhithrogena* spp. and *Drunella* spp. accumulated more metal (slopes 0.55 and 0.38, respectively) than *A. grandis* (slope = 0.11). These regressions were used to estimate whole body Zn concentration at sites where we were unable to collect organisms to measure Zn bioaccumulation.

Effects on Populations. Populations of *A. grandis, Drunella* spp., and *Rhithrogena* spp. were present at 55.6%, 82.5%, and 78.5% of the sites, respectively. The estimated probabilities of occurrence ranged 0.17−0.72 for *A. grandis*, 0.01− 0.96 for *Drunella* spp., and 0.05−0.87 for *Rhithrogena* spp. (Figure 3). Logistic regression results indicated that *Drunella* spp. (area under curve AUC = 0.89) occurrence was a good indicator of metals contamination, whereas AUC values for *A. grandis* (AUC = 0.67) and *Rhithrogena* spp. (AUC = 0.68) indicated discrimination somewhat above chance (AUC = 0.5).

Whole body Zn limited the 90th quantile of the mayfly populations but not *A. grandis*, where the regression slope was not significantly different from zero (Figure 4). The background concentration of Zn (0.9 μg/L) was associated with 333 and 119 μg/g Zn in *Drunella* spp. and *Rhithrogena* spp., respectively. A 20% reduction in density occurred at 726 and 267 μg/g Zn in *Drunella* spp. and *Rhithrogena* spp., respectively. These reductions in density were associated with exposure to 7.0 and

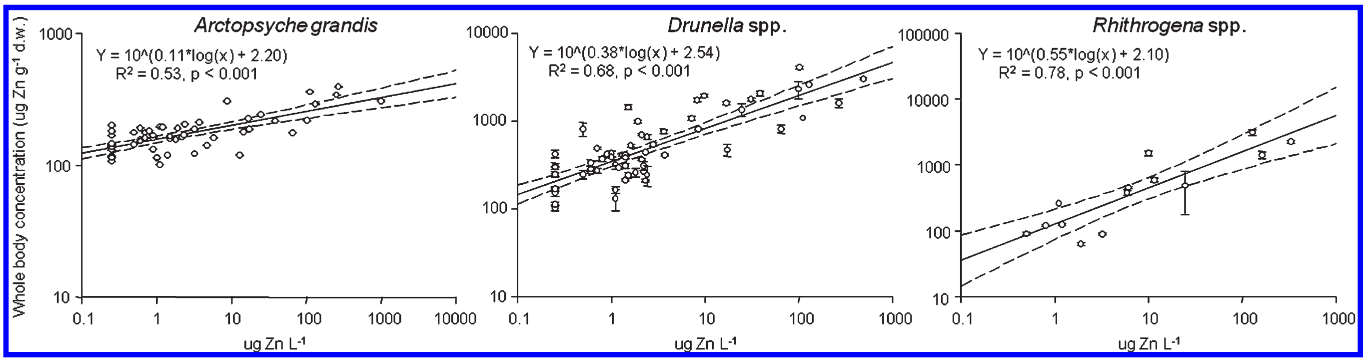

Figure 2. Simple linear regression lines and 95% confidence intervals describing the relationship between aquatic insect whole body Zn concentrations and dissolved Zn concentrations in water. Circles represent the whole body tissue Zn (mean ± SE) per site for each taxa.

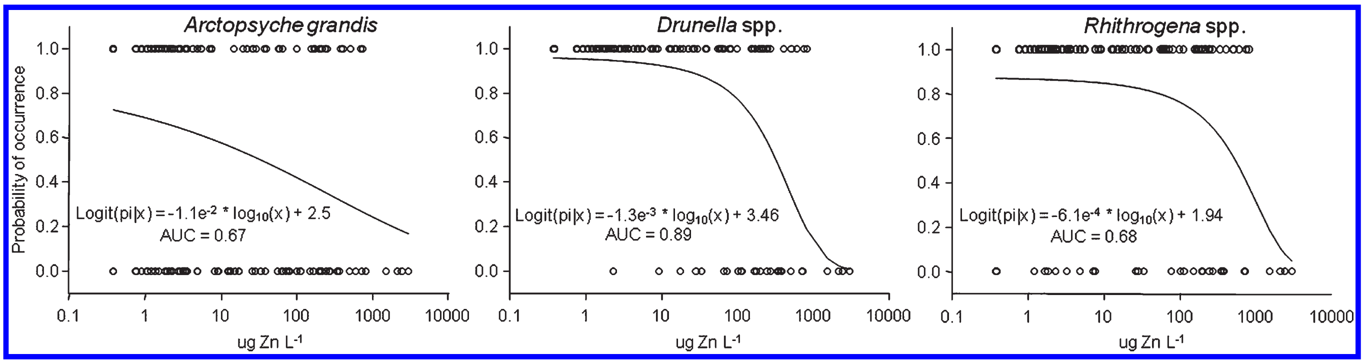

Figure 3. Logistic regressions depicting the probability of taxa occurrence (pi) as a function of dissolved Zn in water. The solid curve shows the probability of detection for each taxon. Circles show the observed presence (1.0) or absence (0.0) of taxa. X in the equations is the estimated whole body Zn concentration developed from regressions in Figure 2. AUC is the area under the curve.

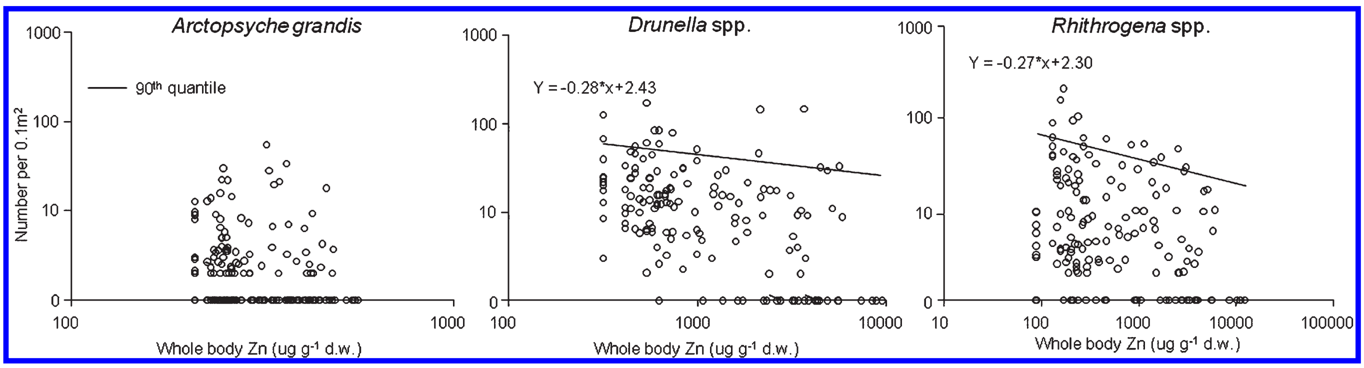

Figure 4. Ninetieth regression quantiles depicting change in maximum insect density as a function of whole body Zn. Circles are the mean number of organisms per location. No regression quantile was plotted for *Arctopsyche grandis* because the slope was not significantly different from zero. X in the equations is the estimated whole body Zn concentration developed from regressions in Figure 2.

3.9 μg/L dissolved Zn, concentrations that were well below the average (all sites) hardness-adjusted U.S EPA continuous chronic criterion value (55 μg/L[47]).

Effects on Communities. Taxonomic completeness (O/E) of the entire data set ranged from 0.06 to 1.34 (Figure 5). The median O/E value at background sites (1.0 ± 0.15 SD) was very similar to the reference site data used to develop the predictive model for the mountains bioregion.[32] A threshold describing an abrupt reduction in O/E was measured at 5.4 μg/L Zn (95% confidence intervals: 1.8−251.2 μg/L Zn). The O/E at 5.4 μg/L Zn was 0.98. The O/E at the critical tissue residues for *Drunella* spp. and *Rhithrogena* spp., was 0.96 and 0.99, respectively.

DISCUSSION

We used a critical tissue residue approach and quantitative statistical models to estimate adverse effects in aquatic insect populations and communities exposed to dissolved Zn. The approach identifies mechanistic links among exposure, accumulation, and adverse effects in aquatic ecosystems caused by metal

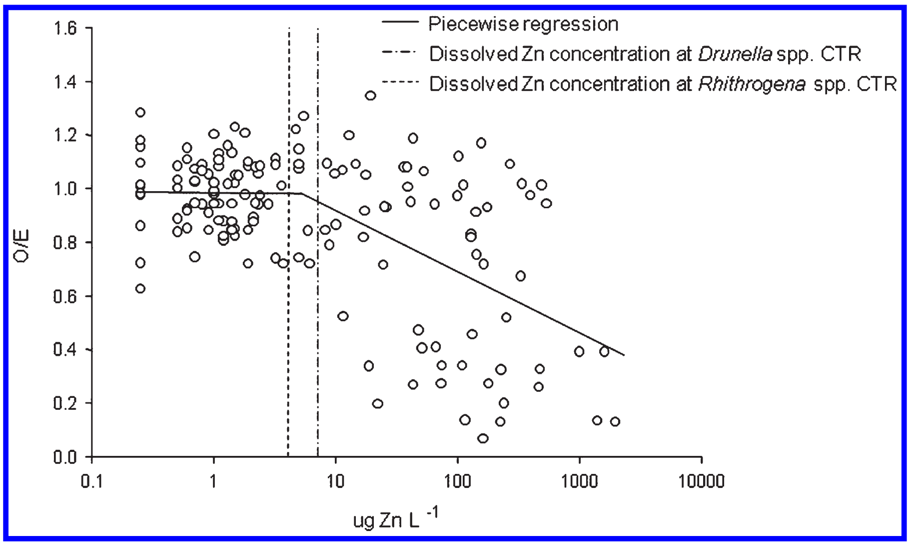

Figure 5. Piecewise regression depicting the threshold in taxonomic completeness (O/E) at 5.4 μg/L Zn (95% confidence intervals: 1.8−251.2) in comparison to the concentration of dissolved Zn associated with the critical tissue residue in *Drunella* spp. and *Rhithrogena* spp. (7.0 and 3.9 μg/L Zn, respectively). O/E is the ratio of observed (O) taxa collected at each site to the modeled taxa expected (E) to occur at each site. CTR is the critical tissue residue for Zn.

contamination. Each taxon was observed at a wide range of dissolved Zn concentrations making it likely that population densities and whole body Zn concentrations could be integrated in a regional-scale assessment of the effects of mining on streams. Further, we determined that critical tissue residues in mayflies provide good estimates for effects on aquatic invertebrate communities as a whole.

Although differences in feeding habits exist among the three taxa (*A. grandis* is a collector−filterer, *Rhithrogena* spp. is a scraper, and *Drunella* spp. is a predator/scraper[30]), it is unknown to what extent dietary metals contribute to whole body metal concentrations for these organisms. Previous studies suggest that the predominant route of metal accumulation varies among families within the same order of aquatic insects, with some families accumulating metals mostly from the water column while others accumulate nearly their entire metal load via diet.[11] Acknowledging our lack of understanding about the role of dietary sources of metals in these taxa, differences in uptake and elimination rates from the water column offer reasonable explanations for the observations made in this study. Previous investigators have shown that *Rhithrogena* spp. and *A. grandis* have divergent physiological strategies for coping with metal exposure.[12] While *Rhithrogena* spp. accumulates metals at slower rates than the other two taxa, it also eliminates metals at a much slower rate. In contrast, *A. grandis* eliminates metals at a faster rate than the mayflies. Consequently, whole body Zn concentrations in *A. grandis* were lower than in the mayflies when exposed to similar aqueous concentrations. We speculate that lower metal load in *A. grandis* reduces the potential to suffer toxic effects of Zn or the need to expend energy detoxifying or storing metals.

Population-level responses of mayflies were reliable indicators of metal contamination and were related to whole body and dissolved Zn concentrations. Both taxa accumulated significant levels of Zn across a wide range of dissolved concentrations, suggesting whole body metal concentrations in these organisms could be useful for indicating metal contamination in the study area. Because these mayflies do accumulate an appreciable amount of Zn, it is likely that Zn could reduce individual fitness and result in lower population densities. The occurrence of *Drunella* spp. accurately distinguished metal-contaminated sites from reference sites; however, the absence of a taxa from a stream could result from numerous other factors in addition to metal contamination (e.g., habitat sampled or life history attributes).

Critical tissue residues of Zn in the mayflies were conservative benchmarks for determining the effect of dissolved Zn on the aquatic invertebrate community as a whole. An abrupt reduction in taxonomic completeness (O/E) was observed at 5.4 μg/L dissolved Zn, similar to the concentration observed to cause adverse effects in mayfly populations (7.0 and 3.9 μg/L for *Drunella* spp. and *Rhithrogena* spp., respectively). Although there is some error associated with these thresholds, they are remarkably comparable. These findings are consistent with previous work comparing critical tissue residues of copper to changes in aquatic insect communities.[7] The good agreement between critical tissue residues and community responses to metal contamination supports the idea that analysis of metal bioaccumulation could be implemented in broader biomonitoring studies. The presence or absence of multiple taxa, such as *Drunella* spp. and *Rhithrogena* spp., combined with an analysis of whole body metal concentrations, would be a reliable and inexpensive indicator of the ecological effects of metal contamination and perhaps more accurate than water sampling alone.[17]

Our findings have important implications for monitoring programs using species richness metrics or other approaches that predict the probability of species occurrence (presence/absence or O/E type predictive models). Although probable occurrences of all three taxa declined as metal accumulation increased, complete elimination of these taxa was not observed until dissolved Zn greatly exceeded the U.S. EPA hardness-adjusted chronic criterion value. Although the presence of sensitive taxa such as *Drunella* spp. or *Rhithrogena* spp. may indicate that Zn does not likely contaminate a site, the absence of these taxa could be driven by other variables, such as habitat or time of sampling. Although measures of taxonomic completeness (O/E) such as RIVPACS type predictive models[31] account for differences among sites that influence the probability of detecting a taxon at any given location, this measure was less sensitive for indicating Zn contamination than population density. The dissolved Zn concentrations associated with the critical tissue residues that caused a 20% loss in mayfly density resulted in marginal changes in the probability of occurrence for mayflies and only a 1−4% reduction in O/E. Many other studies have also noted that the densities of metal-sensitive taxa such as mayflies decline at greater rates than observed for richness metrics.[4,46,48] We suggest that species richness, presence/absence, and measures of taxonomic completeness (O/E) are not as sensitive to metal contamination as changes in taxa-specific densities.

Recently researchers have been interested in developing field-based environmental quality standards for stressors.[39,40,43,49] However, the statistical tools necessary to deal with the limitations associated with field-based data have only recently been introduced and utilized in ecology.[40,42] Here, quantile regression was used to estimate the concentration of metals that causes an unacceptable change in an ecological resource (maximum density, 90th quantile).[40,43] We observed a 20% reduction in maximum densities of two mayfly taxa exposed to dissolved Zn at concentrations below the U.S. EPA hardness-adjusted continuous chronic criterion. These results suggest that if the chronic criterion for Zn were selected as a benchmark for successful mine restoration, one should expect lower mayfly densities and somewhat fewer taxa compared to streams at background Zn concentrations. This is not to say that water quality criteria are not protective of aquatic communities when applied appropriately. However, application of U.S. EPA continuous chronic criterion as remedial end points for mine land restoration projects may result in a less successful restoration than expected.

Use of quantile regression in ecotoxicological investigations should be encouraged. Not only does the technique isolate effects on the maximum response values protected by environmental standards, but it offers an unbiased measure of the effect of a stressor on populations in the presence of other potential limiting factors.[39,40,42,43] For example, metal mixtures often influence streams and it is possible cadmium (Cd) was a colimiter of insect density in our study.[50] Co-limitation of insect density by Cd and Zn would cause density to fall below expected values given Zn alone, biasing the average or other response levels below the maximum limiting function.[39] The 90th quantile is a projection of the maximum observable density in the absence of other limiting factors and is less biased by these confounding variables than mean-based estimators of effects (e.g., one-way analysis of variance or ordinary least-squares regression).[39,42]

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