Evolutionary Patterns in Trace Metal (Cd and Zn) Efflux Capacity in Aquatic Organisms

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Ⓢ *Supporting Information*

ABSTRACT: The ability to eliminate (efflux) metals is a physiological trait that acts as a major driver of bioaccumulation differences among species. This species-specific trait plays a large role in determining the metal loads that species will need to detoxify to persist in chronically contaminated environments and, therefore, contributes significantly to differences in environmental sensitivity among species. To develop a better understanding of how efflux varies within and among taxonomic groupings, we compared Cd and Zn efflux rate constants (k_e values) among members of two species-rich aquatic insect families, Ephemerellidae and Hydropsychidae, and discovered that k_e values strongly covaried across species. This relationship allowed us to successfully predict Zn efflux from Cd data gathered from aquatic species belonging to other insect orders and families. We then performed a broader, comparative analysis of Cd and Zn k_e values from existing data for arthropods, mollusks, annelids, and chordates (77 species total) and found significant phylogenetic patterns. Taxonomic groups exhibited marked variability in k_e magnitudes and ranges, suggesting that some groups are more constrained than others in their abilities to eliminate metals. Understanding broader patterns of variability can lead to more rational extrapolations across species and improved protectiveness in water-quality criteria and ecological assessment.
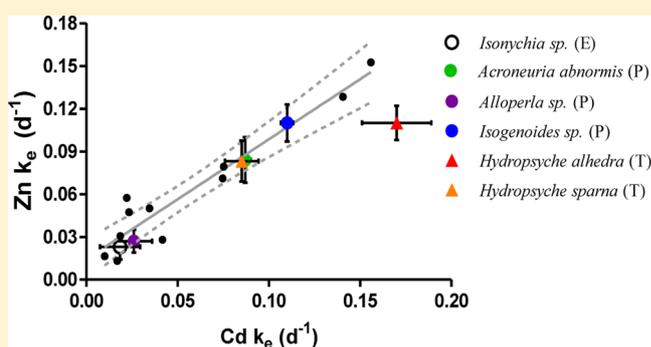
■ INTRODUCTION

The related fields of applied ecotoxicology and ecological bioassessment are challenged by the tremendous biodiversity that exists in nature. The root of this challenge is a fundamental lack of understanding of how patterns of sensitivity to environmental stressors are distributed within and among taxonomic groupings and phylogenetic lineages. In the case of applied ecotoxicology, the widespread use of surrogate species to represent entire taxonomic groups[1] largely stems from the practical reality that a relatively small set of available and convenient test species with standardized test methods have been developed for laboratory use. Regulatory entities are largely forced to assume that each test species represents its particular group, even when there is evidence to the contrary.[2] The regulatory community generally accepts that, when data from these species are combined, a distribution of sensitivities can be used to inform decisions about how best to protect real communities,[3−6] but the need for improvements has been noted.[7]

In the ecological bioassessment of real communities, the lumping of species, whether based on taxonomy (phylogenetic relationships) or function, is borne out of practical challenges, including both taxonomic uncertainties and a conceptual vagueness regarding how the presence of an organism in a sample represents larger groups. Our lack of clarity on the general issue of representativeness introduces major uncertainty in the final products of both applied ecotoxicology (pollution limits and standards) and ecological bioassessment as mandated by the Clean Water Act (U.S.) and related statutes.

One way to explore or refine our concept of representativeness is by explicitly examining interspecific variability in a trait of interest (e.g., a complex trait, such as sensitivity to a contaminant, or subordinate traits that contribute to sensitivity) in defined species groupings.[8] By doing so, we can begin to ask

questions relevant to environmental protection and/or assessment: How much does a trait of interest vary across members of a taxonomic grouping? How variable are the magnitudes and ranges of values for a particular trait across different groups? What is the likelihood that a given species represents the central tendencies or "outliers" of a given taxonomic grouping? Does variance parse phylogenetically? Can we identify emergent patterns that might eventually lead to rational extrapolation and prediction?

Here, we take a modest first step in attempting to answer these questions by examining variability within and among taxonomic groups in two physiological traits relevant to trace metal pollution. Specifically, we examine the ability of aquatic species to eliminate Cd and Zn from tissues as measured by the efflux rate constant (k_e).[9] Although not a proxy for metal sensitivity per se, this loss trait (k_e) has emerged from the biodynamic modeling literature as a major driver of metal bioaccumulation differences among co-occurring species.[10] These differences can be profound,[10,11] and as such, k_e is a primary determinant of the metal loads that a given species will need to detoxify or store to persist in a given exposure scenario.

In this study, we explore patterns in Zn and Cd loss (efflux) across aquatic organisms. Zn and Cd were used for this study because of their co-occurrence in nature, covariance in bioaccumulation parameters,[12−15] and abundance of values in the literature. We examined patterns of measured k_e values for Cd and Zn among several members of two common and species-rich aquatic insect families (Ephemerellidae and Hydropsychidae). We compared the ranges of Cd and Zn k_e values within and between families and asked if Cd k_e values could be used to predict Zn k_e values in other aquatic insect species. We also searched for patterns in Zn and Cd k_e values across and within other taxonomic groupings with an exhaustive compilation of values from the literature.

METHODS

Insect Collection and Handling. Insect larvae were field-collected from streams in Great Smoky Mountains National Park (GRSM) and Basin Creek (BC) (all from North Carolina) from July 2010 to August 2012 using a D-frame kicknet. All larvae were collected from cool, cobble-bottomed, riffle-pool type streams. Larvae were transported to the laboratory and acclimated in a manner described previously.[12] Only larvae that appeared healthy were used for experimentation. All species identifications were verified by taxonomic experts.

Collecting efforts focused upon the species-rich families Hydropsychidae (order: Trichoptera) and Ephemerellidae (order: Ephemeroptera) to enable comparisons among close relatives. Hydropsychid species are net-spinning collectors and had average sizes ranging from 10.5 to 67.3 mg. Ephemerellid species are collector-gatherers and ranged in average size from 3.4 to 7.4 mg.

Determination of Aquatic Insect Efflux Rate Constants. Efflux rate constants (k_e values) for all aquatic insects were determined using γ-emitting radioisotopes ^{65}Zn and ^{109}Cd as described elsewhere.[8,16,17] In vivo γ counting is nondestructive, enabling the repeated measurements of the same individual organisms. All experimental procedures used American Society for Testing Materials artificial very soft water (ASTM VSW) (12 mg L^{-1} NaHCO$_3$, 7.5 mg L^{-1} CaSO$_4$·2H$_2$O, 7.5 mg L^{-1} MgSO$_4$, and 0.5 mg L^{-1} KCl) because the native streams where insects were collected have very low Ca content. All experiments were performed at 12.7 ±

0.5 °C. Most experiments used dual-metal exposures because previous work with *Hydropsyche sparna* showed no difference in single- or dual-metal exposures with the concentrations used (see below). Larvae (5−10 individuals per species) were exposed to 102 kBq L^{-1} Zn and 29.5 kBq L^{-1} Cd, with the remainder of the metal being stable Zn in the form of ZnCl$_2$ and stable Cd in the form of CdCl$_2$. Total metal concentrations (stable metal plus tracer) were 3 μg L^{-1} Zn and 0.3 μg L^{-1} Cd. Environmentally, background concentrations rarely exceed 40 μg L^{-1} for Zn[11,18,19] and 0.5 μg L^{-1} for Cd;[20,21] however, these concentrations can be exceeded at anthropogenically affected sites. Therefore, our exposure concentrations were environmentally relevant. Exposures lasted for 4−5 days to ensure that an adequate radiolabel would be retained in tissues following 10 days of efflux and that accumulated tissue concentrations would not cause overt toxicity. The pH of each bulk solution was adjusted to 7.20 ± 0.02 using 0.1 N NaOH. Exposures occurred in aerated solutions in high-density polyethylene (HDPE) cups containing Teflon mesh as a substrate. Parafilm covers minimized evaporative loss.

After exposure to the dissolved solutions, we ensured that larvae had acquired an adequate signal from each isotope by rinsing them with VSW and assaying in vivo for radioactivity in scintillation vials containing 15 mL of VSW. Larvae were then placed in individual aerated 1 L HDPE containers containing 500 mL of VSW, Teflon mesh as a substrate, and Parafilm to reduce evaporative loss. Each replicate consisted of a single larva, with 5−10 replicates per species. Larvae were assayed for radioactivity daily for 10 days and returned to their individual VSW containers. Spot checking of the solutions for radioactivity ensured that re-uptake of released metals did not confound the interpretation of results. After insects were assayed on the 10th day, larvae were blotted dry and wet weights were determined. k_e was determined as the slope of the natural log of the proportion of metal retained in the body tissue and the time of depuration[22] after excluding days 0 and 1 as follows:

$$C_t = C_i e^{-k_e t}$$

where C_t is the tissue concentration at time (day) t, C_i is the initial tissue concentration, and t is the time (days).

Predictions of k_e Values in Aquatic Insects. To test whether the k_e of one metal was predictable based on the k_e of the other, we constructed a regression and 95% confidence interval of Zn k_e versus Cd k_e from the first 11 insect species tested (all from GRSM). We then obtained Cd k_e values for six additional species. Cd k_e values for three of these species [*Isonychia tuscalanensis* (Ephemeroptera: Isonychidae), *Acroneuria abnormis* (Plecoptera: Perlidae), and *Isogenoides hansoni* (Plecoptera: Perlodidae)] were previously published,[8] and the other three [*Alloperla* sp. (Plecoptera: Peltoperlidae), *Hydropsyche sparna* (Trichoptera: Hydropsychidae), and *Hydropsyche alhedra* (Trichoptera: Hydropsychidae)] were generated for this study. For experimentation, *H. alhedra* and *H. sparna* were collected from GRSM, while the remainder were collected from BC. On the basis of these Cd k_e values, we predicted Zn k_e values based on the regression line of the first 11 species for which we obtained Zn and Cd k_e values. These predictions were then tested by measuring Zn k_e values using a ^{65}Zn radiotracer in the same manner described above (but independent of Cd).

Data Compilation for Other Taxa. Zn and Cd k_e values were compiled from the literature for several aquatic species
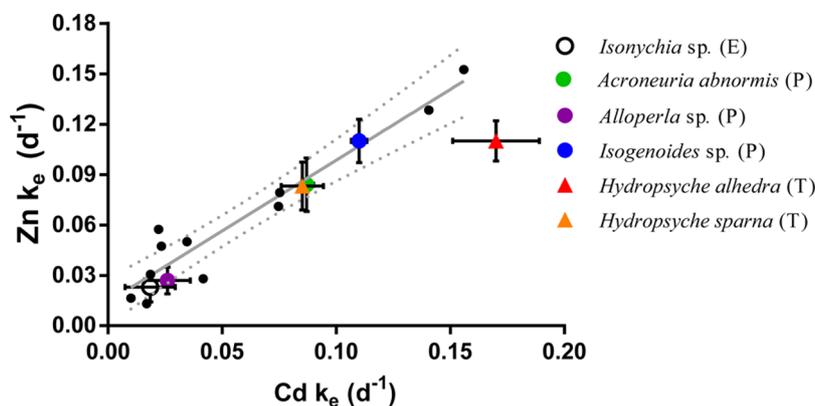
Figure 1. Predicted and measured values of Zn k_e values from six EPT species. Closed black circles represent Zn and Cd k_e values of 11 species within aquatic insect families Ephemerellidae and Hydropsychidae used to construct the regression line and associated 95% confidence interval. Five of the six species tested fell within the 95% confidence interval. Symbols represent mean ± standard error of measured Zn and Cd k_e values. E, Ephemeroptera; P, Plecoptera; and T, Trichoptera.
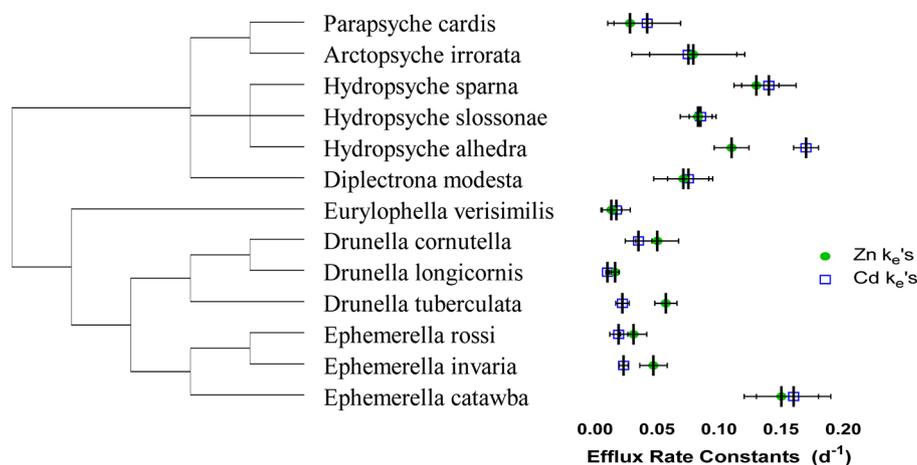
Figure 2. Zn and Cd k_e values for 13 aquatic insect species representing the families Ephemerellidae and Hydropsychidae. Pagel arbitrary branch lengths are depicted. The phylogenetic tree was constructed using Dendroscope.[46]

representing chordates, mollusks, arthropods, and annelids. Because much more data were available for k_e values using dissolved exposures, we preferred those values over dietary values when both were available. However, we did include dietary data when no dissolved data were available, provided that the dietary exposures did not result from single-pulse feedings (to analyze only true physiological loss). When multiple k_e values were reported for a given species, we used a mean value.

Data Analysis. All conventional statistical analyses were performed using GraphPad Prism (version 5.04). Results were considered statistically significant with a p value of <0.05. Regression analyses of k_e values from insects were performed using untransformed data, while data were log-transformed for comparisons between taxonomic groups.

Phylogenetic methods were employed to analyze log-transformed k_e values across the 13 aquatic insect species gathered for this study as well as across all aquatic organisms. Whereas conventional statistics treat species as independent units and assume complete unrelatedness, phylogenetic methods allow for the comparison of species while taking into account their evolutionary relatedness.[23] The K statistic by Blomberg et al.[24] was used to quantify the phylogenetic signal (the tendency for related species to resemble each other), and their randomization test based on the mean-squared error was

used to test for the significance of the phylogenetic signal. Phylogenetically independent contrasts were used to detect covariation of Zn and Cd k_e values.[25,26] Because body weight had no significant effect on the analyses, log-transformed traits were not corrected allometrically. See the Supporting Information for more information on the used phylogenetic methods.

■ RESULTS

Patterns of Zn and Cd k_e Values Across Aquatic Insects. Zn and Cd k_e values across 13 aquatic insect species from families Ephemerellidae and Hydropsychidae ranged over an order of magnitude for both Zn (0.013−0.15 day^{-1}) and Cd (0.010−0.17 day^{-1}) (Figure 1). Across all species, Zn and Cd k_e values were strongly correlated using conventional statistics (r = 0.93; $p < 0.0001$). Body weight was not a significant driver in k_e differences among species. Whether data were log-transformed or analyzed raw, correlations of Cd or Zn and body weight were not statistically significant and ranged from −0.36 to 0.15 in ephemerellids, from −0.05 to 0.47 in hydropsychids, and from 0.24 to 0.40 in all taxa. This finding suggests that species-specific physiological differences dwarfed any possible influence of body weight.

Across 13 species of aquatic insects, Cd k_e values exhibited a significant phylogenetic signal (the tendency for related species to resemble each other) ($K = 0.86$; $p = 0.013$) (Figure 2 and Table S3 of the Supporting Information). Although the phylogenetic signal did not reach statistical significance for Zn k_e values ($K = 0.67$; $p = 0.078$), this lack of significance is most likely an artifact of the small sample size.[24] Phylogenetically, Zn and Cd k_e values were strongly correlated ($r_{IC} = 0.90$; $p < 0.0001$).

Among seven ephemerellids, the k_e values of most species fell between 0.013 and 0.057 day^{-1} for Zn and between 0.010 and 0.035 day^{-1} for Cd. *Ephemerella catawba* was a clear outlier, with Zn and Cd k_e values of 0.15 and 0.16 day^{-1}, respectively. Excluding *E. catawba*, the mean ($\pm$standard deviation) k_e values for Zn and Cd were 0.036 ± 0.019 and 0.021 ± 0.008 day^{-1} respectively. Among six hydropsychids, Zn k_e values ranged from 0.028 to 0.13 day^{-1}, while Cd k_e values ranged from 0.042 to 0.14 day^{-1}. The mean ($\pm$standard deviation) k_e values across hydropsychids for Zn and Cd were 0.083 ± 0.034 and 0.098 ± 0.048 day^{-1}, respectively. Excluding *E. catawba* from the analysis, it is clear that, on average, ephemerellids eliminate Zn ($p = 0.014$) and Cd ($p = 0.003$) from tissues much more slowly than hydropsychids, despite the huge variances around the means for these groups.

Predictions of Zn k_e Values in Aquatic Insects. A regression of Zn k_e on Cd k_e based on the first 11 species that we tested (Figure 1) resulted in a line with the form

$$Zn(k_e) = 0.84[Cd(k_e)] + 0.014$$

$$(r^2 = 0.92;\ p < 0.0001)$$

The strength of this regression led us to test whether Zn k_e values could be predicted on the basis of Cd k_e values from a suite of other aquatic insect species. Predictions of Zn k_e values using the linear regression of Zn k_e versus Cd k_e fell within the 95% confidence interval of the linear regression in five of the six EPT (orders: Ephemeroptera, Plecoptera, and Trichoptera) species tested (Figure 1). Of the five species that fell within the 95% confidence interval, the experimental measurements deviated between 2.9 and 25% from the predicted Zn k_e values (*I. hansoni*, 2.9%; *H. sparna*, 3.2%; *A. abnormis*, 4.1%; *I. tuscalanensis*, 23%; and *Alloperla* sp., 25%). Although experimental values for *I. tuscalanensis* and *Alloperla* sp. differed the most from their predicted Zn k_e values, the difference between their predicted and experimental Zn k_e values was less than 1% per day in both cases (only 0.007 and 0.009 day^{-1}, respectively). *H. alhedra* did not fall within the 95% confidence interval, but it was also outside of the range of Cd k_e values used to generate the regression. *H. sparna* k_e values for Zn and Cd were identical for dual-labeled (Cd + Zn) and individually labeled metal experiments ($p > 0.05$).

On the basis of the success of this prediction exercise, we predicted Zn k_e values for an additional 22 species of EPT taxa based on published k_e values for Cd (see Table S1 of the Supporting Information). Data for other aquatic insect orders (Diptera and Megaloptera) were available; however, we only carried out predictions for EPT taxa because of our success in predicting Zn k_e values for EPT taxa previously.

Taxonomic Group Comparisons. To explore broad patterns in k_e values for other taxonomic groupings, we compiled Zn and/or Cd k_e values for all aquatic taxa available in the literature. Including the data generated as part of this study for insects, we compiled data from 77 species representing four

phyla (Zn k_e values for 45 species, Cd k_e values for 75 species, and k_e values for both metals for 43 species; see Tables S1 and S2 of the Supporting Information).

Aquatic insects had the most data available, allowing us to analyze the variance among species within taxonomic rankings ranging from genus to order. Mayflies (order: Ephemeroptera) had the most variance of the three orders analyzed, and the variance remained high across all levels of taxonomic rank. Within two genera, *Ephemerella* (family: Ephemerellidae) and *Drunella* (family: Ephemerellidae) each showed high variances of Cd k_e values, with coefficients of variation (CVs) of 100% ($n = 5$) and 86% ($n = 5$), respectively. Within the family Ephemerellidae, CVs for Cd and Zn k_e values were 96% ($n = 12$) and 89% ($n = 7$), respectively. Across Ephemeroptera, Cd and Zn k_e values had CVs of 99% ($n = 19$) and 100% ($n = 9$), respectively.

Caddisflies (order: Trichoptera) showed less variance in efflux than mayflies. Within genus *Hydropsyche* (family: Hydropsychidae), Cd k_e values had a CV of 31% ($n = 5$). Within Hydropsychidae, Cd and Zn k_e values had CVs of 53% ($n = 9$) and 42% ($n = 6$), respectively. Finally, Cd and Zn k_e values for Trichoptera had CVs of 53% ($n = 11$) and 42% ($n = 6$), respectively.

Stoneflies (order: Plecoptera) showed the least variance overall across taxonomic ranks that we were able to compare. In the family Perlidae, Cd k_e values exhibited a CV of only 3% ($n = 6$). Overall, Plecoptera had Cd k_e values that exhibited a CV of 28% ($n = 11$), again, much lower than other order-level variances among aquatic insects.

At the phylum level of classification, 53 Arthropoda species (10 orders and 18 families) exhibited the most variability of Zn and Cd k_e values, with CVs of 82 and 80%, respectively. Zn k_e values for arthropods ranged 100-fold (Figure 3A), and Cd k_e values for arthropods ranged 297-fold (Figure 3B). Arthropods also exhibited the largest mean k_e values. Arthropoda had a
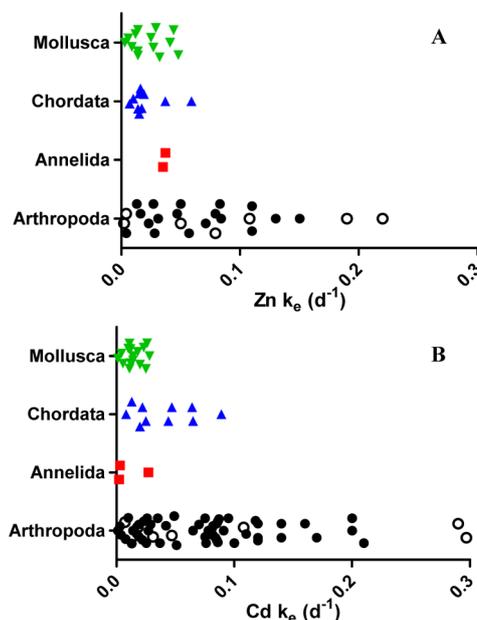
Figure 3. All compiled (A) Zn and (B) Cd k_e values from the literature. Values include both dietary and dissolved k_e values separately. In Arthropoda, closed circles, aquatic insects; open circles, other species.

mean Zn k_e value of 0.072 day^{-1} and a mean Cd k_e of 0.080 day^{-1}.

Across 13 species (5 orders and 10 families), Mollusca had the lowest CVs for both Zn and Cd k_e values at 65 and 55%, respectively. Zn k_e values ranged 16-fold (Figure 3A), and Cd k_e values ranged 25.4-fold (Figure 3B). Mollusca had the lowest average Zn and Cd k_e values of 0.022 and 0.015 day^{-1}, respectively.

Chordata, across 8 species (4 orders and 8 families), had CVs for Zn and Cd k_e values of 75 and 70%, respectively. Zn k_e values ranged 8.4-fold (Figure 3A), and Cd k_e values ranged 29.6-fold (Figure 3B). Chordates had a mean Zn k_e of 0.024 day^{-1} and a mean Cd k_e of 0.038 day^{-1}.

Across all four phyla, Arthropoda had significantly higher Zn k_e values than both Mollusca and Chordata ($p = 0.003$) and significantly higher Cd k_e values than Mollusca ($p = 0.0002$). Annelida did not have adequate representation to analyze trends in Zn and Cd k_e values, with values of only two and three species available, respectively (panels A and B of Figure 3). The phylogenetic signal was detected for Zn k_e values across 45 species ($K = 0.47$; $p = 0.014$) and Cd k_e values across 75 species ($K = 0.59$; $p < 0.0001$), signifying that both Zn and Cd k_e values agree with phylogeny (see Table S3 of the Supporting Information).

Correlations between Cd and Zn k_e Values. Efflux rate constants (log-transformed) for Zn and Cd in 17 species of aquatic insects (all available insect values) exhibited a strong covariance ($r = 0.92$; $p < 0.0001$). The inclusion of 4 non-insect arthropods (subphylum: Crustacea) in the analysis still showed significant correlation ($r = 0.81$; $p < 0.0001$) as did the inclusion of 12 molluscs, 8 chordates, and 2 annelids (43 species total) ($r = 0.73$; $p < 0.0001$). A phylogenetic correlation of Zn and Cd k_e values across all 43 taxa was similar ($r_{IC} = 0.61$; $p < 0.0001$). Efflux rate constants within 12 species of Mollusca and within 8 species of Chordata failed to correlate significantly, but an analysis of all 26 non-insect species showed a weaker correlation ($r = 0.48$; $p = 0.01$).

■ DISCUSSION

Here, we used metal efflux as a starting point to examine patterns of interspecific variability in a physiological trait that pertains to metal accumulation. Proximal sensitivity to metals is a complex trait, encompassing bioaccumulation (uptake and efflux), detoxification capacity,[27,28] and target site sensitivity (which remains poorly studied).[28] Other demographic traits related to recovery and dispersal also contribute to population persistence.[29,30] However, the ability to eliminate (efflux) acts as a major driver of bioaccumulation differences among species and plays a large role in determining the metal loads that species will need to detoxify in contaminated environments.

One goal of this study was to generate a data set of closely related species within two species-rich insect families to determine whether the phylogenetic signal previously observed at more coarse taxonomic levels[8] is retained at finer taxonomic levels. Despite finding a surprisingly wide range of k_e values within Ephemerellidae and Hydropsychidae, we found evidence of a statistically significant phylogenetic signal within the k_e values. Blomberg et al. found that the significance of the phylogenetic signal was strongly related to the number of species used in the study.[24] For instance, 92% of studies with >20 species showed a significant phylogenetic signal, while only 41% of studies with <20 studies showed a significant phylogenetic signal.[24] The analyses performed here only had

values for 13 species, thus decreasing the power of the statistical tests. However, these preliminary tests show that the phylogenetic signal does exist at the genus/species levels of taxonomic rank in metal bioaccumulation parameters.

Using conventional statistics, we found that these values covaried strongly enough to allow us to accurately predict Zn k_e values from existing Cd k_e values for five of six additional aquatic insect species, including large (average 142 mg) predatory and small (average 5 mg) shredder stoneflies as well as other mayfly and caddisfly species. This strong pattern of covariation among insect Zn and Cd k_e values suggests similar trafficking and binding within subcellular pools of the animals. This is the first time to our knowledge that a correlation between the k_e values of two metals has been used for the prediction of an unknown metal bioaccumulation value.

A second goal of this study was to explore patterns of previously published Cd and Zn k_e values across broader taxonomic groups. We limited our analysis to efflux values in this study for several reasons. k_e values generally are more robust to exposure route differences,[9,31,32] exposure concentration,[33,34] and water chemistry[10,35] than other metal bioaccumulation parameters. Robust comparisons of other bioaccumulation parameters (e.g., k_u) would require a more standardized exposure regime than was present in the available literature (but see ref 8). Several patterns emerged from our analysis. The ability of organisms to efflux metal differed within taxonomic groupings, and the ranges of efflux values within and across taxonomic groups also varied. Despite this, we still observed a significant phylogenetic signal in both Cd ($K = 0.59$) and Zn ($K = 0.47$) k_e values. We also found a strong covariation using both conventional ($r = 0.73$) and phylogenetic ($r = 0.61$) statistics. The phylogenetic signal present in Zn and Cd k_e values indicates that phylogenetic statistics should be used to analyze the data further;[23,26] therefore, further analyses or extrapolations should rely on phylogenetic statistics.

We note that interlaboratory differences in experimental procedures can influence physiological parameters and could have introduced variance into our compiled data set. In particular, differences in laboratory temperatures likely influenced metabolic rate, which, in turn, has been shown to affect physiological traits dictating metal accumulation,[36,37] including efflux.[38] For k_e values obtained in this study for aquatic insects, all insects were acclimated to the same experimental temperatures (12.7 °C). However, among all values obtained in the literature, the temperature was variable, ranging from 10 to 25 °C. This range of experimental temperatures likely contributed to wider physiological variation within the compiled data set.

Overall, it is clear that taxonomic groups have different ranges (capacities) with respect to their ability to eliminate Cd and Zn from tissues. For example, the fastest k_e values in mollusks would be considerably below the average rate for aquatic insects. Species only tolerate metal exposures through a combination of effluxing and detoxifying or storing the metal.[28] Previous studies in insects revealed an inverse relationship between the ability to eliminate and detoxify Cd[8] (e.g., species that stored metal effectively did not efflux quickly). It remains unclear whether slow metal efflux is the result of metal being tightly bound (detoxified) in physiologically inert structures or if stronger detoxification capacity arises when organisms are poor effluxers. At a subcellular level, caddisfly species store significantly less metal (Cd) in metallothionein-like proteins

(MTLPs) than mayfly species[17] while maintaining higher k_e values (as seen in this study). Mollusks are known to accumulate extremely high concentrations of metals, likely a result of their overall low rates of efflux and abilities to sequester Cd and Zn in metal-rich granules.[22,39,40]

We also observed that variances do not scale similarly within a given taxonomic rank. For instance, there is more variation among k_e values in some individual insect genera than we observed within the whole mollusk phylum. Numerous potential sources of variability occur among taxonomic groupings (e.g., phyla) that remain poorly explored. It may be the case that physiological variance increases with the species richness (evolutionary radiation). Arthropods are by far the most species-rich phylum, with a species richness estimated at between 5 and 10 million species (including both aquatic and terrestrial species).[41] Mollusca has a total estimated species richness of 50 000−200 000.[42] However, within insects, we observe that mayflies vary more widely than stoneflies, despite both groups being comparable with respect to lineage age and biodiversity. It appears as though age of a given evolutionary lineage (clade) is not a good predictor of physiological variability. Mollusks (545 million years ago[43]), mayflies (290 million years ago[44]), and stoneflies (360 million years ago[45]) are each ancient lineages, but only mayflies showed widely varying k_e values, corresponding to a wider array of morphological bauplans (body plans). We have no explanation for the presence of outliers (e.g., *E. catawba*) based on ecology, morphology, or life history.

If variation in other more complex traits (e.g., sensitivity to a particular contaminant) is similarly distributed, then it calls to question whether taxonomic rank is a reasonable criterion on which to base practical decisions (e.g., use of single representatives of eight families[1] for the development of water-quality criteria and the use of family-level taxonomic work in bioassessments). The ability of a single species to adequately represent a larger taxonomic group is inversely related to the amount of variance that occurs in that taxonomic group, unless it is known *a priori* that the particular representative is at the sensitive (protective) end of the range. It is more likely, for example, that a randomly selected mollusk is reasonably close to the mollusk mean than a randomly selected insect would be to the insect mean for these k_e measures. It is clear that species-rich groups (such as insects) cannot be adequately represented by single test species.

By exploring variation within and across taxonomic groups, we can begin to develop a better understanding of the issue of representativeness, which is so deeply embedded in practical environmental management and assessment. Examining the variance that occurs in this one subordinate trait begins to give us an idea of just how much variation exists in the physiological traits that influence sensitivity of aquatic organisms. Although it is impossible to test toxicological characteristics of every species for every contaminant, there are likely larger patterns (and hypotheses) that can be gleaned from existing data. Ultimately, incorporation of phylogenetically based methodologies to explore broader patterns among species has the potential to improve our ability to make rational extrapolations across species and taxonomic groups and lead to more robust environmental standards and ecological assessments.

■ ASSOCIATED CONTENT

⑤ Supporting Information

Phylogenetic methods, Cd and Zn efflux rate constants (k_e values) for 47 species of aquatic insects (Table S1), Cd and Zn efflux rate constants (k_e values) for 30 aquatic species (Table S2), and analyses of the phylogenetic signal for Cd and Zn k_e values, including comparisons of the natural log likelihoods for each set of arbitrary branch lengths compared to that of a star phylogeny (indicating no hierarchal structure to the data) (Table S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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