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Original Article

Environmental Toxicology

Calibration of a laboratory-based chronic toxicity model to nickel effects on stream invertebrates in the field

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Abstract

Predicting effects of metals on stream invertebrate communities can be hindered by spatial and temporal variation in toxicity-modifying factors, a paucity of laboratory toxicity data for stream taxa (mainly insects), and variation in benthic invertebrate community structure related to habitat and factors other than the stressor of interest. We addressed these challenges by combining laboratory-based chronic toxicity data with field-based biological monitoring data to build a lab-to-field stressor-response relationship. A laboratory-based toxicity model for *Ceriodaphnia dubia* was used to translate field nickel (Ni) concentrations into predicted toxicity, and a quantile regression model of field data from a mine-affected watershed was then used to describe the limiting effect of Ni toxicity on the benthic invertebrate community. Many taxa showed no evidence of Ni effects up to the highest studied exposure (30 µg/L dissolved Ni, or 92% effect to *C. dubia* reproduction). The most sensitive metric was percent Ephemeroptera, reflecting declines in abundance of some sensitive mayflies (e.g., *Baetis*, *Epeorus*) and concurrent increases in abundance of some tolerant non-mayfly taxa (e.g., *Rheocricotopus*, *Eukiefferiella*) across the gradient of Ni toxicity in the field. The field 10% effect concentration for percent Ephemeroptera occurred at a 22% effect of Ni to *C. dubia* reproduction. This finding supports previous estimates of 20% as a critical effect size in laboratory test organisms that could be predictive of discernible effects on sensitive invertebrates in the field.

Keywords: nickel; macroinvertebrates; Ephemeroptera; mining

Introduction

Biological assays have been the gold standard for measuring chemical toxicity for more than a century (Van Noordwijk, 1989), and for the vast majority of environmental contaminants, the only information on toxicity comes from a small number of standardized laboratory tests (Persoone & Janssen, 1994). The ability of this information to accurately predict ecological effects in nature is often assumed but has rarely been tested (Cairns & Pratt, 1989; Persoone & Janssen, 1994; Pontasch et al., 1989). As noted by Cairns and Pratt (1989), "[t]he problem of extrapolating among levels of biological organization has not been given the serious attention it deserves, and currently used methodologies have been chosen for reasons other than scientific validity." Subsequent reviews have agreed that laboratory to field extrapolation remains a major challenge in ecotoxicology (Mebane, 2010; Vignati et al., 2007).

There are several excellent discussions of why it is challenging to validate laboratory tests as meaningful in the field (e.g., Cairns & Pratt, 1989; La Point & Waller, 2000; Mebane, 2010; Persoone & Janssen, 1994). Notably, real communities contain many taxa, the most abundant of which are often not well represented in toxicity datasets (Brix et al., 2005), and it is unclear how well model species represent the sensitivity of native species. Real communities also live in much more complex and dynamic physical, chemical, and biological environments than can (or should) be simulated by standardized laboratory conditions. This challenge is particularly important for contaminants with strong toxicity-modifying factors (TMFs), especially if TMF effects differ among species. In addition, real communities are subject to many sources of variability and other stressors that are intentionally controlled in standardized testing. Natural variability can obscure patterns of response to a stressor of interest in the field and other stressors can hinder the attribution of responses, especially if stressors are collinear (Peters et al., 2014; Takeshita et al., 2019).

We attempted to overcome the challenges summarized above and test how well a model laboratory test species could predict the effects of nickel (Ni) on stream invertebrate communities in the field. We applied approaches developed by others to evaluate the relative sensitivity of laboratory test species and native species (Besser et al., 2021; Mebane et al., 2020; Soucek et al., 2000),

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to model TMF effects using laboratory data (Croteau et al., 2021; Van Geest et al., 2025), to identify and remove correlated stressor effects in field data (Van Geest et al., 2025), and to detect a pattern of response to Ni in the context of natural variability and uncontrolled confounding factors (Cade et al., 1999; Cade & Noon, 2003). The outcome of our analysis is a stressor-response relationship that links a laboratory toxicity model to effects on stream benthic invertebrates in the field.

Methods

Study area

The Elk River watershed in southeast British Columbia, Canada is an area of historical and current metallurgical coal mining. The watershed contains unaffected reference areas and areas with a range of exposure to mine-related effects on water quality and physical habitat. Important contaminants in this watershed are nitrate from blasting residue and weathering products from waste rock, including major ions (predominantly calcium, magnesium, sulfate, and bicarbonate), selenium, and Ni. Major ion concentrations in some areas are sufficiently high to cause localized calcification of the streambed. Contaminant concentrations vary widely among watercourses according to the type and degree of mine influence, and generally decline with distance from mining within each watercourse. Monitoring has been conducted at dozens of sites throughout the watershed over more than a decade to evaluate effects to water quality, physical habitat, and benthic invertebrate communities (BIC).

Toxicity model

We predicted Ni toxicity from water quality monitoring data using a *Ceriodaphnia dubia* multiple linear regression (MLR) model described in a companion paper (Van Geest et al., 2025). In brief, we compiled reproductive median effect concentrations (EC50s) from previous studies ($n = 46$) and conducted supplemental testing with Ni-spiked site waters and formulated waters ($n = 55$) to expand the range of tested TMF conditions for hardness (15–1,020 mg/L as $CaCO_3$), dissolved organic carbon (DOC: 0.4–40 mg/L), pH (6.6–8.7), and bicarbonate (8.0–366 mg/L HCO_3^-). We used MLR to derive models for Ni toxicity to *C. dubia* reproduction. As in some previous studies (Puttaswamy & Liber, 2012; Santore et al., 2021), we found evidence that high bicarbonate concentrations increase Ni toxicity to *C. dubia*. Van Geest et al. (2025) concluded that including bicarbonate concentration in the MLR improved model performance, especially in the very hard and alkaline waters in our study area. We therefore adopted a model form with coefficients for hardness, DOC, and bicarbonate to predict Ni toxicity to invertebrates at our field study sites. Use of the *C. dubia* MLR to predict toxicity of Ni to sensitive benthic invertebrates is supported by the broad overlap in Ni sensitivities between Cladocera and Ephemeroptera (Besser et al., 2021; Mebane et al., 2020) and similar patterns of TMF effects observed for *C. dubia* and the mayfly *Neocloeon triangulifer* (Besser et al., 2021; Van Geest et al., 2025).

Field dataset

We described patterns of response to Ni in the field by combining data from field studies conducted between 2012 and 2020 at sites across the Elk Valley and conducting supplemental sampling in 2021 in a sub-watershed with relatively high Ni concentrations. The combined BIC dataset included 576 sampling events from 146 study sites, each representing an average of 1–5 replicate samples collected in September per Canadian Biomonitoring Network kick-and-sweep methods (Environment Canada, 2012). All BIC samples had paired habitat information, including measures of streambed calcification. Reported BIC metrics were percent Ephemeroptera (%E), percent Ephemeroptera, Plecoptera, and Trichoptera (%EPT), taxonomic richness, and total invertebrate abundance, as well as abundances of all taxa identified to the lowest practical level of taxonomy.

The limiting factor approach we applied to our field dataset (see *Limiting Factor Analysis*) is designed to detect a stressor-response relationship in the context of other sources of variability, including other stressors. However, attribution of responses to the stressor of interest can be confounded by collinear stressors (Peters et al., 2014). To mitigate this issue in the present dataset, we used concentration-response relationships for *C. dubia* reproductive effects from nitrate and sulphate exposure (Van Geest et al., 2025; see also online supplementary material) and the multi-ion toxicity model of Mount et al. (2019) to identify and remove sampling events with predicted effects of other mine-related stressors. Because *C. dubia* is similarly sensitive to sulfate (Elphick et al., 2011) and more sensitive to nitrate (Baker et al., 2017) than the mayfly *N. triangulifer* (Soucek & Dickinson, 2015), this screening was expected to reduce potential effects on sensitive BIC metrics from stressors collinear with Ni. We also removed all sampling events with a streambed calcification index exceeding the range observed at reference sites to reduce the potential for effects on BIC metrics from alteration of physical habitat. An additional 13 sampling events were removed due to missing data for Ni and/or collinear stressors. After screening, the dataset contained 434 sampling events from 77 mine-affected and 43 reference study sites. Dissolved Ni concentrations in the screened field dataset ranged from <0.5 to 30 μg/L, hardness 88 to 648 mg/L as $CaCO_3$, DOC <0.5 to 2.4 mg/L, and bicarbonate 89 to 397 mg/L HCO_3^-. The full unscreened dataset, details of screening, and exposure and response data used in the analysis are available in the online supplementary material.

Limiting factor analysis

We characterized the response of BIC metrics to predicted Ni toxicity by applying the ecological concept of limiting factors, following a theoretical approach described by Cade et al. (1999) and Cade and Noon (2003). The central concept in this approach is that many factors contribute to variability in ecological condition, and this complexity can hinder attempts to test the effect of one factor using conventional approaches like regression analysis of the mean or median response. A more powerful analytical approach for such data is to test for changes near the upper bound of the response variable, rather than at the center of the response distribution. In the presence of confounding factors, a large range of responses may be observed at low levels of the stressor of interest, reflecting variability in the response variable related to natural factors or other stressors. As levels of the stressor of interest increase, the maximum achievable response will be progressively limited by that stressor.

Following previous authors (e.g., Crane et al., 2007; Peters et al., 2014; Schmidt et al., 2012) and recommendations of the U. S. Evironmental Protection Agency (USEPA; 2023), we used quantile regression to describe a limiting effect. For the stressor gradient we used predicted effect of Ni to *C. dubia* reproduction (see *Toxicity Model*) for each sampling event, thereby accounting for spatial and temporal variation in Ni and its TMFs. For the response variable, we tested all reported BIC metrics (see *Field Dataset*) and all reported taxa that were sufficiently widespread and abundant to allow quantile regression analysis. A conceptual overview of the analysis steps is provided in online supplementary material Figure S1.

To support the selection of taxa for inclusion, we first calculated the maximum proportion of each taxon observed across all samples (see online supplementary material Table S2) and retained taxa that made up at least 1% of total abundance in at least one sample. We then compared each taxon's occurrence (percent of samples that contained the taxon) at reference sites, mine-affected sites unlikely to have Ni effects (defined as less than a 10% effect concentration [EC10] for *C. dubia* reproduction), and mine-affected sites with potential Ni effects (defined as ≥EC10 for *C. dubia* reproduction). This comparison (see online supplementary material Figure S2) indicated that taxa tended to occur at a similar frequency in all three categories of sites (i.e., widespread taxa tended to be widespread across all sites). Therefore, we included taxa in the limiting factor analysis if they occurred in at least 20% of mine-affected sites with potential Ni effects, which ensured that there were at least 14 detected values in that category to support the quantile regression fit. Of the 266 taxa detected at least once in our field dataset, 64 met these criteria (see online supplementary material Table S2).

We used the nlrq function of the quantreg package in R (R Core Team, 2022) to fit a logistic curve (R function sslogis) to each quantile from 0.80 to 0.95 in increments of 0.01, expressed as:

$$y_i = \frac{Asym}{1 + e^{((Mid - x_i)/Scal)}} \quad \text{(Eq. 1)}$$

where y_i is the BIC metric selected as a response variable, x_i is predicted effect of Ni to *C. dubia* reproduction, *Asym* is the upper asymptotic value of *y*, *Mid* is the value of *x* at the inflection point of the curve, and *Scal* is a scale parameter that inversely defines the slope of the curve. Total invertebrate abundance and taxon abundances exhibited strongly skewed distributions and therefore were $\log_{10}(x+1)$ transformed to stabilize residual variance and reduce the leverage of individual observations.

In addition to the default starting values automatically calculated by sslogis, each analysis was also run with manually selected starting values. Model runs with manually selected starting values were more often able to converge. *Asym* was started at the percentile of the data matching the quantile being fitted. Starting values for *Mid* and *Scal* were selected for each taxon by inspecting data plots. If a pattern was apparent in the upper bound of the data, starting values were selected to approximate this pattern. Otherwise, *Mid* was started at the approximate midpoint of the exposure gradient (50% effect) and *Scal* was started at a value that produced a shallow but discernible negative (if the upper bound of the data appeared to decline) or positive (if the upper bound of the data appeared to increase) slope in $\log_{10}$-transformed abundances. Model fits were considered credible if *Asym* varied monotonically across quantiles (as it should, if it is correctly defining the asymptotic value of the desired quantile), and if *Mid* and *Scal* varied monotonically or were consistent across quantiles (defined as having a coefficient of variation less than 20%). Relatively small and/or monotonic variation in model parameters would indicate that different quantile fits are describing similar patterns of response, increasing confidence that the analysis is correctly estimating the true underlying exposure-response relationship. *Asym* and *Mid* were required to be positive numbers, but *Scal* was permitted to be negative (consistent with a direct effect of Ni) or positive (consistent with an indirect effect of Ni [Fleeger et al., 2003] or some non-Ni factor). Anomalous parameter sets that did not meet these criteria were not considered credible and were not retained.

$\log_{10}$-transformed abundance metrics were back-transformed prior to calculating EC_X values. We calculated the EC10, EC20, and EC50 for each credible quantile curve, representing the level of Ni toxicity to *C. dubia* associated with a 10%, 20%, or 50% change in the tested quantile of the untransformed BIC response variable. Results for each EC_X were summarized as the mean (± standard deviation) *C. dubia* reproductive effect across tested quantiles with credible models. Results were also converted from *C. dubia* effects to dissolved Ni concentrations normalized to a standard set of median TMFs for our study area (hardness 231 mg/L as $CaCO_3$; DOC 1 mg/L; bicarbonate 191 mg/L as HCO_3^-). For comparison to other studies, results were also converted to dissolved Ni concentrations normalized to typical hardness (48.3 mg/L as $CaCO_3$) and DOC concentrations (2.5 mg/L) for Lake Monate, Italy (per Mebane et al., 2020 and others). A bicarbonate concentration for Lake Monate (50.6 mg/L as HCO_3^-) was calculated from alkalinity (0.83 mEq/L) reported in the European water quality database described in Schmidt-Kloiber et al. (2013).

Results and discussion

Effect of Ni on stream invertebrates

Application of the *C. dubia* toxicity model (Van Geest et al., 2025) to our field water quality data described a range of predicted Ni effects from zero to 92%, with 76 sampling events having ≥10% predicted effect to *C. dubia* reproduction and 23 sampling events having ≥50% predicted effects (Table 1). The maximum predicted Ni effect in our dataset would be associated with a dissolved Ni concentration of 46 μg/L under Lake Monate TMFs, which is slightly higher than the 50th percentile of the Mebane et al. (2020) species sensitivity distribution (SSD). Thus, approximately half of tested species would be expected to exceed an EC10 for Ni within our range of study exposures.

Distributions of BIC metrics were most variable at <10% predicted Ni toxicity: total invertebrate abundance at reference and low-Ni sites spanned 2 orders of magnitude, richness extended from 20 to 60 detected taxa, and proportional abundance metrics extended from <20% to >80% (Table 1), reflecting natural variability and effects of stressors not collinear with Ni (Cormier et al., 2018; Peters et al., 2014; Sylvestre et al., 2005). According to Cade et al. (1999) and Cade and Noon (2003), limiting effects of Ni on a BIC metric would cause a compression of the distribution of values in Table 1, resulting in a linear or nonlinear negative trend on the upper bound of the data plotted in Figures 1–5.

Despite the expectation that metal contamination should lead to a decrease in richness and abundance caused by the loss of sensitive species (Clements et al., 2000), no such trend was apparent for taxonomic richness or total abundance of invertebrates (Figure 1) and a model could not be fit to these metrics by quantile regression. Takeshita et al. (2019) similarly did not detect an effect of Ni on richness or total abundance metrics, but did report significant q90 regressions for total wet biomass of invertebrates and total abundance of filter feeders. In contrast, Peters et al. (2014) found an effect of bioavailable Ni on richness and total abundance metrics in a dataset of stream BIC from England and Wales, and Mebane et al. (2020) reported effects of Ni on taxonomic richness of invertebrates in Ni-dosed experimental streams.

Mebane et al. (2020) reported that Ni reduced richness in their experimental streams by 20% at 53 μg/L under their study TMF conditions. Applying the Van Geest et al. (2025) toxicity model to these Ni concentrations and TMFs predicts that the EC20 for richness occurred at 91% effect to *C. dubia* reproduction. Thus, if organisms at our field sites were as sensitive as those in the experimental streams, we would expect to see a near 20% decline in richness at the high end of our modeled range of Ni effects.

Table 1. Summary statistics for exposure and benthic invertebrate community metrics for three categories of Ni exposure.

Parameter	Dissolved [Ni] (μg/L)	Modeled effect on *C. dubia* (%)	Total abundance	Taxonomic richness	%E	%EPT	E abundance	EPT abundance
Reference sites (*n* = 131)								
Minimum	<0.5	0	260	20	11	26	173	248
P5	<0.5	0	540	25	28	63	250	487
P25	<0.5	0	3,268	30	42	77	1,267	2,530
P50	<0.5	<1	6,066	35	51	84	3,033	4,840
P75	<0.5	<1	10,085	39	60	91	5,400	8,274
P95	1.25	8.6	22,732	44	76	97	11,873	17,720
Maximum	1.25	12.1	36,940	48	83	98	21,280	36,100
Mine-exposed sites, modeled Ni effect <10% (*n* = 230)								
Minimum	<0.5	0	340	23	2.1	14	29	317
P5	<0.5	0	2,031	28	13	43	666	1,320
P25	<0.5	<1	4,686	33	30	63	1,733	3,340
P50	0.5	<1	7,740	36	42	74	2,800	5,661
P75	1.0	1.5	12,267	40	55	84	4,940	8,053
P95	2.2	6.1	25,380	46	73	93	11,393	16,640
Maximum	3.7	9.9	57,360	60	83	95	28,500	49,420
Mine-exposed sites, modeled Ni effect ≥10% (*n* = 73)								
Minimum	1.5	10	2,692	25	2.1	7.1	260	853
P5	2.6	11	4,402	28	4.0	14	575	2,158
P25	3.6	17	8,811	33	18	45	1,755	3,730
P50	4.5	36	11,687	37	34	65	3,084	5,784
P75	7.9	57	16,637	39	44	75	5,638	10,122
P95	20	88	26,882	49	60	87	10,813	20,063
Maximum	30	90	40,420	53	79	92	17,900	26,380

Note. E = Ephemeroptera; EPT = Ephemeroptera, Plecoptera, and Trichoptera.

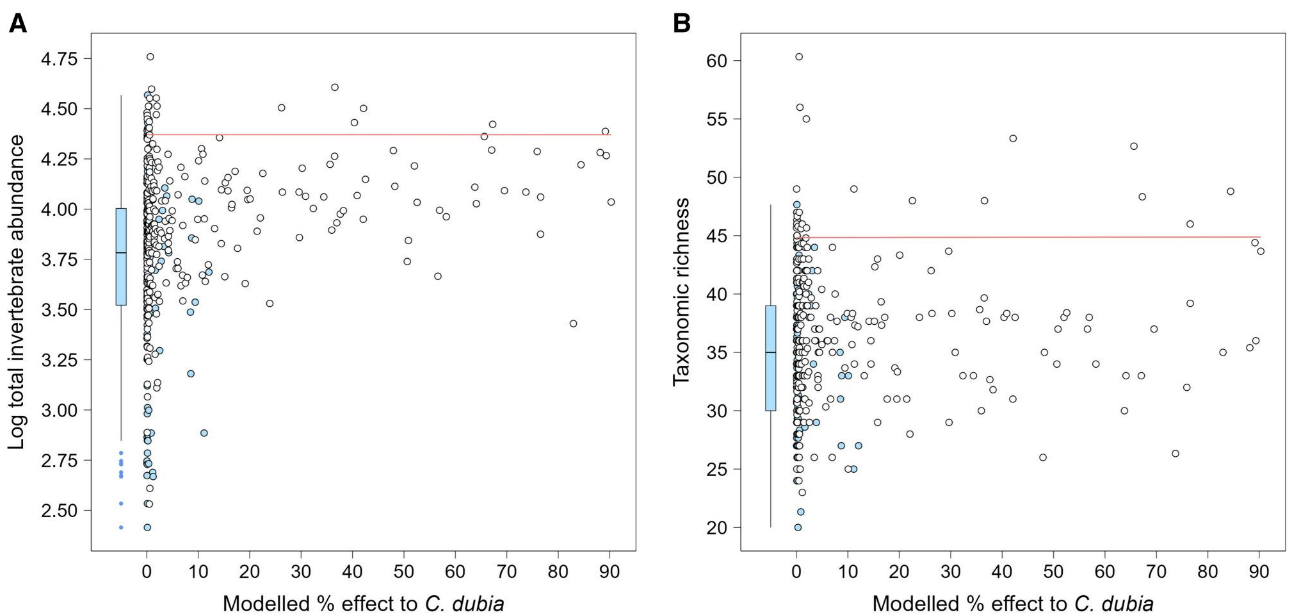

Figure 1. Absence of a limiting effect of Ni on (A) total abundance and (B) taxonomic richness of stream invertebrates at reference sites (blue symbols and boxplot) and mine-exposed sites (open symbols). Colored line is example upper quantile.

Quantile regression may not be sensitive enough to detect an effect of this magnitude in the context of the variability apparent in Figure 1B. Alternatively, recolonization or some other factor may have made richness less sensitive to Ni in our field invertebrate communities compared to the experimental stream study.

In contrast to total abundance and richness, a negative trend was apparent in upper quantiles of %E and %EPT (Figure 2). Credible logistic models could be fit to all tested quantiles for %E (Table 2) and for quantiles up to q85 for %EPT (higher quantiles did not converge; see online supplementary material Table S3). The more sensitive metric was %E, with a mean EC10 of 22% effect to *C. dubia* (7.6 μg/L under Lake Monate TMFs; 4.3 μg/L under median TMFs in our study area). A trend was also apparent in upper quantiles of total mayfly abundance (Table 2; Figure 3A) and EPT abundance (Figure 3B) and credible logistic models could be fit to most tested quantiles for these abundance metrics (see online supplementary material Table S3). Percent Ephemeroptera was a more sensitive metric than total mayfly abundance because %E reflects both direct effects of Ni on total mayfly abundance and correlated increases in abundance of some non-mayfly taxa (discussed below).

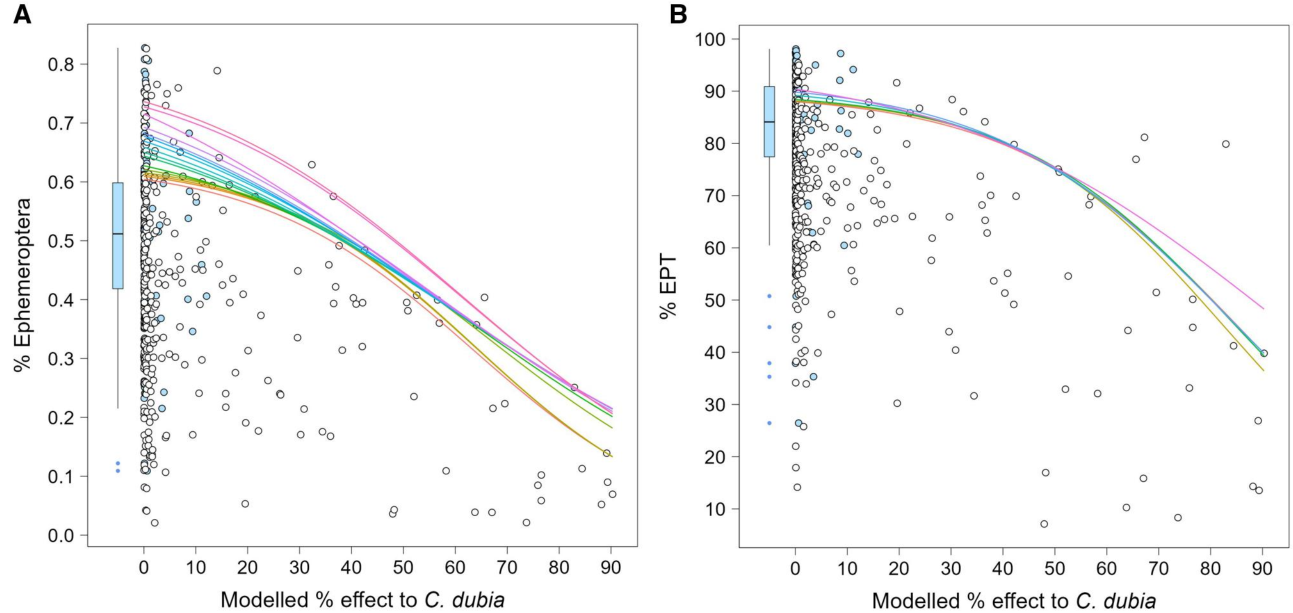

Figure 2. Limiting effects of Ni on (A) proportional abundance of Ephemeroptera and (B) proportional abundance of Ephemeroptera, Plecoptera, and Trichoptera (EPT) at reference sites (blue symbols and boxplot) and mine-exposed sites (open symbols). Colored lines are credible upper quantile models.

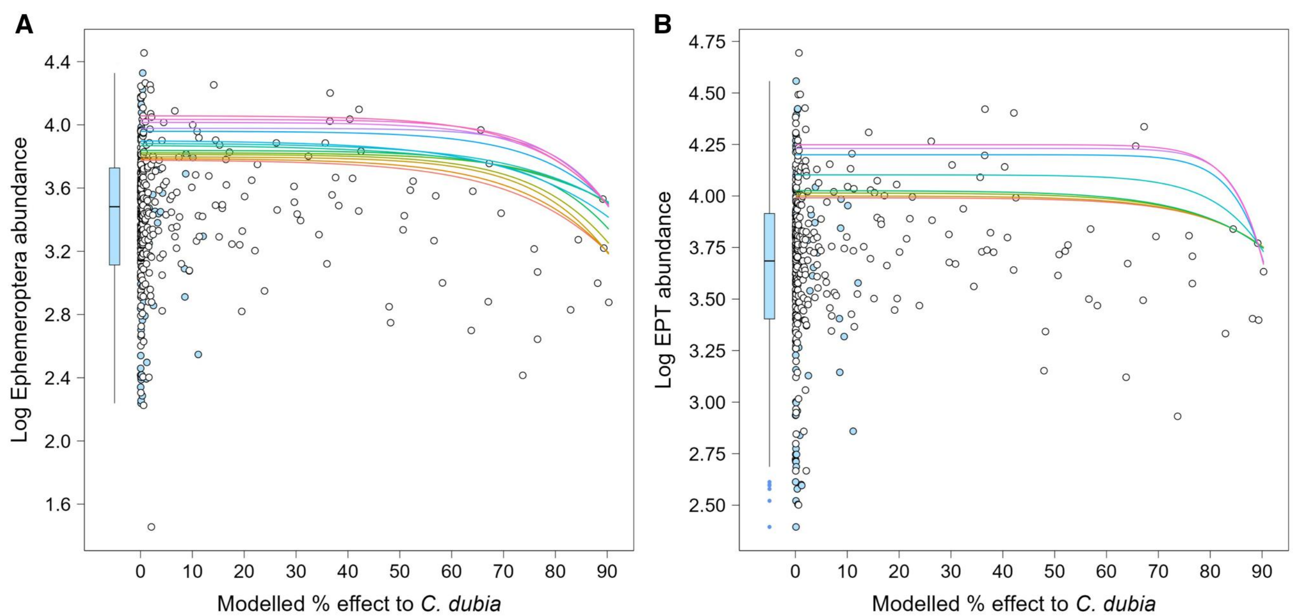

Figure 3. Limiting effects of Ni on (A) abundance of Ephemeroptera and (B) abundance of Ephemeroptera, Plecoptera, and Trichoptera (EPT) at reference sites (blue symbols and boxplot) and mine-exposed sites (open symbols). Colored lines are credible upper quantile models.

Sensitivity of mayfly metrics to Ni toxicity in the field is not surprising, given the preponderance of mayflies at the low end of the SSD (Mebane et al., 2020). Ephemeroptera are often reported to be sensitive to metals in field studies, with mayfly richness or EPT richness (e.g., Carlisle & Clements, 1999; Clements et al., 2000; Iwasaki et al., 2018; Takeshita et al., 2019) and abundances of certain mayfly taxa (e.g., Heptageniidae and Ephemerellidae [Schmidt et al., 2012; Iwasaki et al., 2018]) reported as sensitive metrics (but cf. Peters et al., 2014; Takeshita et al., 2020). Mebane et al. (2020) reported declines in total mayfly abundance of 10% at 15 μg/L Ni under their study TMF conditions (which corresponds to 43% predicted effect to *C. dubia* per Van Geest et al., 2025) and 20% at 26 μg/L Ni (which corresponds to 70% predicted effect to *C. dubia*). These values compare favorably to the present study, in which the mean EC10 for upper quantiles of total mayfly abundance was 56% predicted effect to *C. dubia* (16 μg/L Ni under Lake Monate TMFs) and the mean EC20 was 67% predicted effect to *C. dubia* (20 μg/L Ni under Lake Monate TMFs).

Aside from Mebane et al. (2020), few studies have attempted to describe effects of Ni on mayfly abundance in the field. Crane et al. (2007) reported effects of Ni on q99 Ephemeroptera abundance at <1 μg/L and q99 EPT abundance at 2 μg/L, although these estimates have been questioned because they did not consider TMF effects and were not expressed relative to reference data (Peters et al., 2014). Crane et al. (2007) also did not account for collinear stressors, which may have confounded their attribution

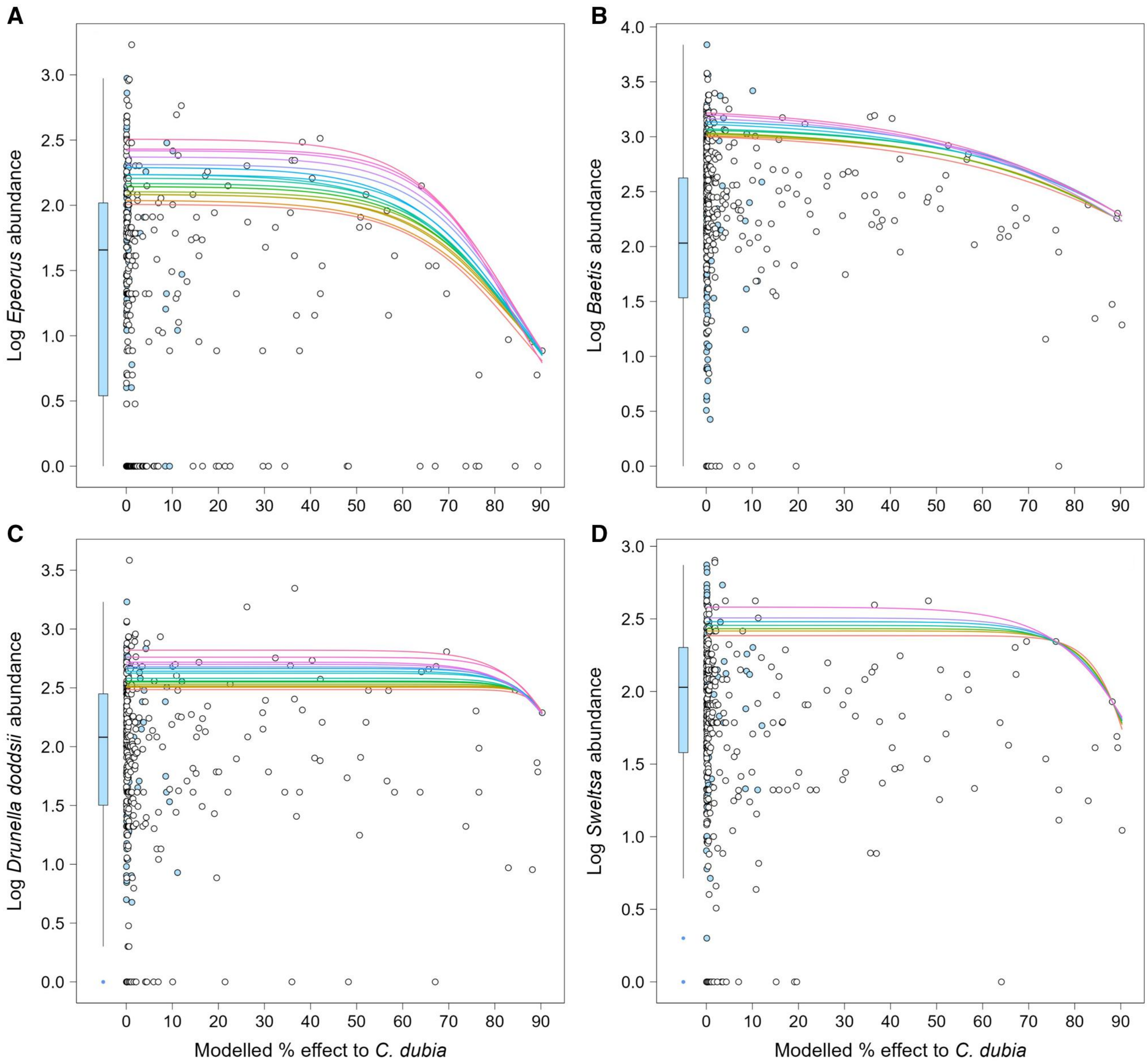

Figure 4. Limiting effects of Ni on abundances of (A, B, C) Ephemeroptera and (D) Plecoptera species at reference sites (blue symbols and boxplot) and mine-exposed sites (open symbols). Colored lines are credible upper quantile models.

of effects to Ni (Peters et al., 2014; Takeshita et al., 2019). Peters et al. (2014) acknowledged this limitation in their own field dataset and reported that Ni concentrations were correlated with copper, zinc, arsenic, chromium, ammonia, and phosphate. The inconsistency of findings from these field studies highlights the effect that collinear stressors can have on quantile regression.

Of the 64 taxa with sufficient data to attempt quantile regression, credible models indicating a limiting effect of Ni (Table 3) were obtained for four species (*Epeorus* sp., *Baetis* sp., *Drunella doddsii*, *Sweltsa* sp.; Figure 4) and three families (Heptageniidae, Ameletidae, Taeniopterygidae; Figure 5) of Ephemeroptera and Plecoptera. Four chironomid species (*Orthocladius complex*, *Eukiefferiella* sp., *Pagastia* sp., *Rheocricotopus* sp.) and one trombiformid mite (*Sperchon* sp.) exhibited increasing abundance across the gradient of Ni toxicity (see online supplementary material Figure S3). For the remaining taxa, no pattern of abundance across the gradient of Ni toxicity could be detected.

The most sensitive mayfly in our study was *Baetis*, with a mean EC50 for upper quantile abundance at 60% effect to *C. dubia* (17 μg/L normalized to Lake Monate TMFs; Table 3). This analysis indicated greater sensitivity than the EC50 of 44 μg/L under experimental stream TMFs (37 μg/L under Lake Monate TMFs) reported by Mebane et al. (2020) for *B. tricaudatus*. The most sensitive EC50s reported by Mebane et al. (2020) were for the baetid mayfly *Diphetor hageni* (22 μg/L under experimental stream TMFs) and the ephemerellid mayflies *Ephemerella infrequens* (27 μg/L under experimental stream TMFs) and *Drunella doddsi* (30 μg/L under experimental stream TMFs). The mean EC50 for upper quantile abundance of *Drunella doddsi* in our study area was 89% effect to *C. dubia* (39 μg/L normalized to Lake Monate TMFs), which compares favorably to the EC50 reported by Mebane et al. (2020). The sensitivity of *Diphetor hageni* could not be evaluated in the present study because this genus was absent at reference sites in our study area and present in very low abundance (≤0.3% of total abundance) in only 6 of 434 samples overall. *Ephemerella* also could not be evaluated in our study because it was detected in only 15% of samples with ≥10% predicted Ni toxicity, including only three samples associated with ≥50% predicted effects to *C.*

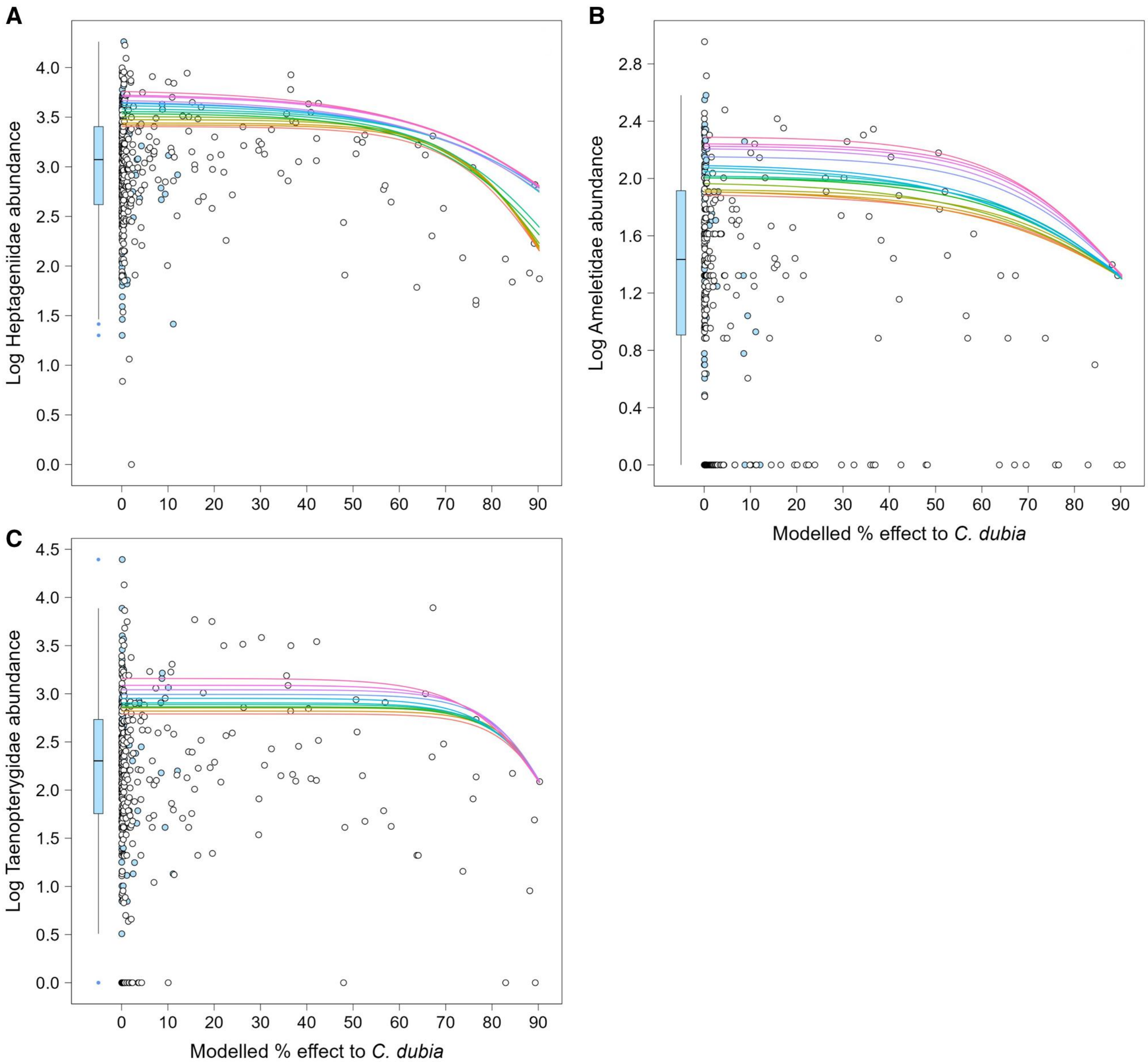

Figure 5. Limiting effects of Ni on abundances of (A, B) Ephemeroptera and (C) Plecoptera families at reference sites (blue symbols and boxplot) and mine-exposed sites (open symbols). Colored lines are credible upper quantile models.

dubia. However, *Ephemerella* was detected in 30% of samples from reference and low-Ni sites. Assuming that *Ephemerella* in our study area are as sensitive to Ni as *E. infrequens* in the Mebane et al. (2020) study, direct effects of Ni could have contributed to the reduced occurrence of this taxon in high-Ni versus low-Ni sites.

There were three other taxa besides *Ephemerella* that occurred in ≥20% of samples from reference and low-Ni sites but <20% of samples from high-Ni sites: *Arctopsyche* sp. (Trichoptera: Hydropsychidae), *Orthocladius* sp. (Diptera: Chironomidae), and *Chelifera/Metachela* sp. (Diptera: Empididae). *Orthocladius complex* increased in abundance across the gradient of Ni toxicity (Figure S3) and Mebane et al. (2020) reported an unbounded EC50 of >253 μg/L for *Arctopsyche grandis*, suggesting that these taxa had low occurrence in our study area for reasons unrelated to Ni. It is unknown whether Ni may have contributed to reduced occurrence of *Chelifera/Metachela* at high-Ni sites in our study area.

Increases in abundance of some non-mayfly taxa across the gradient of Ni toxicity could result from an indirect ecological effect via Ni-induced changes in behavior, competition, or predation by other taxa (Fleeger et al., 2003). However, for at least some of the taxa that exhibited positive upper quantile slopes (e.g., *Rheocricotopus*), increases in abundance appeared to occur at lower levels of Ni (<10% effect to *C. dubia*) than were associated with detectable changes in abundance of even the most sensitive mayfly taxa we could evaluate. Other possible explanations could include a stimulatory effect of a water quality factor (e.g., micronutrient-related shifts in biofilm composition) or physical factor collinear with Ni in our study area. Regardless of the cause, the simultaneous declines in abundance of some mayfly taxa and increases in abundance of some non-mayfly taxa caused %E to be a more sensitive metric (albeit one with a less clear interpretation) than individual or total mayfly abundances.

The remaining 52 taxa exhibited no pattern of abundance relative to Ni toxicity up to the highest concentration in our study. Little can be said about the sensitivity to Ni of taxa with low overall abundances and/or uneven distribution of occurrence across the gradient of Ni toxicity. However, many taxa were widespread

Table 2. Model parameters and effects concentrations from quantile regression of mayfly metrics as a function of modeled Ni toxicity to *C. dubia*.

Quantile	% Ephemeroptera						Ephemeroptera abundance					
	EC10	EC20	EC50	*Asym*	*Mid*	*Scal*	EC10	EC20	EC50	*Asym*	*Mid*	*Scal*
q80	26.0	39.1	65.1	0.63	63.3	−20.5	47.9	59.7	78.4	3.78	117	−15.9
q81	27.3	40.4	65.7	0.63	64.2	−19.7	51.2	62.1	79.1	3.79	114	−14.4
q82	27.0	40.1	65.4	0.64	63.9	−19.8	56.8	66.0	80.5	3.80	110	−12.2
q83	26.8	39.9	65.3	0.64	63.7	−19.9	57.3	66.8	81.5	3.81	112	−12.5
q84	25.7	40.1	69.7	0.66	67.0	−24.0	61.7	72.4	89.1	3.82	124	−14.1
q85	22.3	37.3	71.3	0.70	65.4	−29.6	60.1	69.2	83.5	3.84	113	−12.0
q86	20.3	35.0	70.1	0.74	62.0	−31.9	50.3	64.3	86.7	3.87	134	−19.3
q87	18.5	32.9	68.8	0.77	58.1	−34.3	52.4	65.4	85.9	3.88	129	−17.6
q88	19.2	33.6	68.6	0.77	59.5	−32.6	47.5	60.4	81.0	3.90	125	−17.7
q89	18.8	33.0	67.9	0.78	58.5	−32.7	–	–	–	–	–	–
q90	18.0	32.1	67.1	0.80	56.6	−33.5	–	–	–	–	–	–
q91	17.9	31.9	66.8	0.81	56.2	−33.4	59.5	69.1	84.2	3.96	116	−12.7
q92	17.5	31.2	66.0	0.83	55.0	−33.6	67.1	74.2	85.1	3.98	108	−9.2
q93	21.7	35.9	67.5	0.78	62.6	−27.1	60.1	69.0	82.9	4.02	112	−11.7
q94	21.4	35.4	66.7	0.80	61.8	−27.0	61.3	69.7	82.8	4.03	111	−11.1
q95	20.3	34.2	65.7	0.83	60.0	−27.6	55.9	65.7	81.1	4.06	114	−13.0

Note. Dashes indicate that no credible model could be fit.
EC10 = 10% effects concentration; EC20 = 20% effects concentration; EC50 = 50% effects concentration.

Table 3. Effects concentrations from quantile regression of stream invertebrate metrics.

Metric	Modeled Ni effect to *C. dubia* reproduction (%)			[Ni] (μg/L) Lake Monate TMFs			[Ni] (μg/L) Median study area TMFs		
	EC10	EC20	EC50	EC10	EC20	EC50	EC10	EC20	EC50
%E	22 ± 3.6	36 ± 3.3	67 ± 1.9	7.5 ± 0.8	11 ± 0.7	20 ± 0.9	4.3 ± 0.4	6.1 ± 0.4	12 ± 0.5
%EPT	40 ± 2.1	56 ± 0.9	87 ± 3.8	12 ± 0.5	16 ± 0.3	37 ± 9.1	6.7 ± 0.3	9.2 ± 0.2	21 ± 5.2
E abundance	56 ± 5.8	67 ± 4.2	83 ± 3.0	16 ± 1.9	20 ± 1.9	31 ± 3.5	9.3 ± 1.1	12 ± 1.1	18 ± 2.0
EPT abundance	71 ± 4.8	78 ± 2.0	90 ± 2.9	22 ± 2.6	26 ± 1.5	42 ± 6.6	13 ± 1.5	15 ± 0.9	24 ± 3.8
Epeorus	41 ± 1.9	50 ± 1.5	64 ± 1.2	12 ± 0.5	14 ± 0.4	19 ± 0.5	6.9 ± 0.3	8.1 ± 0.2	11 ± 0.3
Baetis	22 ± 3.3	35 ± 3.9	60 ± 3.6	7.6 ± 0.7	10 ± 0.9	17 ± 1.3	4.4 ± 0.4	6.0 ± 0.5	10 ± 0.7
Drunella doddsii	79 ± 5.2	83 ± 4.0	90 ± 2.0	28 ± 4.4	31 ± 4.3	40 ± 3.9	16 ± 2.6	18 ± 2.5	23 ± 2.2
Heptageniidae	37 ± 9.0	48 ± 7.0	67 ± 3.0	11 ± 2.1	14 ± 1.9	20 ± 1.3	6.3 ± 1.2	7.8 ± 1.1	11 ± 0.7
Ameletidae	36 ± 3.9	48 ± 3.3	67 ± 3.0	11 ± 0.9	14 ± 0.9	21 ± 1.4	6.1 ± 0.5	7.9 ± 0.5	12 ± 0.8
Taeniopterygidae	65 ± 4.6	71 ± 3.8	80 ± 2.7	19 ± 1.8	22 ± 1.9	28 ± 2.2	11 ± 1.0	13 ± 1.1	16 ± 1.2
Sweltsa	69 ± 5.9	74 ± 4.6	83 ± 2.4	21 ± 2.7	24 ± 2.7	31 ± 2.4	12 ± 1.6	14 ± 1.6	18 ± 1.4

Note. Values are mean ± standard deviation across quantiles with credible models.
E = Ephemeroptera; EC10 = 10% effects concentration; EC20 = 20% effects concentration; EC50 = 50% effects concentration; EPT = Ephemeroptera, Plecoptera, and Trichoptera; TMF = toxicity-modifying factor.

and abundant at high-Ni sites (see online supplementary material Table S2), indicating an EC10 higher than the Ni concentrations that occurred in our study area and a position on the SSD above the 50th percentile. Mebane et al. (2020) also concluded that many taxa in their study, including most or all non-mayfly taxa, were relatively tolerant of Ni.

Ceriodaphnia dubia as a surrogate for sensitive stream invertebrates

The bottom quartile of the SSD contains 12 aquatic taxa with species mean chronic values (SMCVs, all normalized to Lake Monate TMFs) of 4.3 to 19.4 μg/L, four of which are Cladocera and four of which are Ephemeroptera (Mebane et al., 2020). These two taxa also occupy the next quartile in similarly high proportions. The second and third most sensitive species on the SSD are *C. dubia* (SMCV = 4.8 μg/L) at the 4th percentile and *Ephemerella infrequens* (SMCV = 7.7 μg/L) at the 6th percentile. The most sensitive taxon in our analysis (*Baetis*, EC10 = 7.6 μg/L) would fall between these two percentiles, and the next-most sensitive taxa (Heptageniidae and Amelitidae, EC10 = 11 μg/L for both) would fall near the 9th percentile. This broad overlap in sensitivity between Cladocera and Ephemeroptera indicates that *C. dubia* should be a useful surrogate for the mayfly species that frequently dominate stream communities. A comparison of TMF effects between *C. dubia* and *N. triangulifer* (Van Geest et al., 2025) supports this interpretation, as does the conclusion of Besser et al. (2021) that effects of Ni on the mayfly *N. triangulifer* were well described by a model developed using *C. dubia* toxicity data.

Additional support for *C. dubia* as a useful surrogate for stream invertebrates comes from studies finding a positive association between ambient toxicity to cladoceran test species and effects to macroinvertebrate communities in the field (Iwasaki et al., 2018; Pontasch et al., 1989; Soucek et al., 2000). Pontasch et al. (1989) found significant effects of a complex effluent on chronic *C. dubia* reproduction at 3% effluent, which they concluded to correspond well with reduced abundance of some mayfly taxa observed at 1%–10% effluent both in microcosms and field sites. Soucek et al. (2000) similarly found that sites with the greatest acute toxicity to *C. dubia* from continuous exposure to acid mine drainage had significantly lower EPT richness, %E, and total richness compared to reference sites. Soucek et al. (2000) were able to derive MLR models to predict taxonomic richness, EPT richness, and %EPT from toxicity test results. Iwasaki et al. (2018) found a significant association of ambient toxicity to *C.*

dubia (chronic) and *D. magna* (acute) with effects to abundances of heptageniid and ephemerellid mayflies and mayfly richness in the field. These findings support the conclusion that toxicity testing with appropriately sensitive taxa can provide a useful effects-based method for evaluating effects on biological communities from metals, and a key advantage of such testing is its ability to consider TMF effects (Brix et al., 2023).

Critical effect sizes for lab-to-field extrapolation

The EC_X estimates for %E and total mayfly abundance varied across quantiles but showed little systematic difference across the tested quantile range (Table 2). The EC10 for %E decreased slightly across quantiles, from approximately 25% *C. dubia* reproductive effect for q80–q85 to approximately 20% *C. dubia* reproductive effect at quantiles >q85. Conversely, the EC10 for total mayfly abundance increased slightly across quantiles, from 50%–60% *C. dubia* reproductive effect for q80–q88 to ≥60% *C. dubia* reproductive effect for q91–q95 (Table 2). These patterns (and those for individual taxa in online supplementary material Table S3) indicate that any arbitrarily selected quantile is likely to provide reasonable EC_X estimates, but that averages across multiple quantiles (e.g., the mean EC10 for %E of 22% effect to *C. dubia*) may be more robust estimates. The convergence of effect levels for %E, the most sensitive taxon in our field data (*Baetis* sp.), and the laboratory and mesocosm data summarized in the lowest decile of the SSD, supports our field-based approach as similar to other methods in power to detect effects.

The EC_X estimates in Tables 2 and 3 are levels of effect in a laboratory test that could be associated with a discernible effect on biota in the field, which can be viewed as a type of critical effect size (CES), sensu Dekkers et al. (2001). Our analysis indicates that a shift in stream invertebrate community structure and declines in abundance of sensitive taxa could be discernible when chronic, sublethal effects on a sensitive laboratory species and endpoint (herein, *C. dubia* reproduction) exceed about 20%. Few other studies have attempted to estimate a CES for lab-to-field extrapolation. The primacy of EC10s and EC20s in deriving regulatory criteria, either directly or via their incorporation in an SSD, implies a de facto CES in the range of 10% to 20%. The USEPA (1999) attempted to validate an EC20-based criterion for ammonia using a field study that measured macroinvertebrate abundances and survival and growth of multiple fish species in experimental streams dosed with ammonia (Hermanutz et al., 1987; Zischke & Arthur, 1987). The USEPA (1999) focused on fish as the more sensitive taxon and concluded that effects were generally absent below the criterion and often present above the criterion, which supported 20% as a CES. More recently, Mebane (2010) conducted an extensive meta-analysis of population models, mesocosm and whole-lake experiments, and field surveys to identify laboratory effect sizes that could be "biologically acceptable" for biological communities exposed to cadmium. Mebane (2010) proposed a CES of 20% for stream invertebrates, supported by generally good agreement between laboratory-based predictions and effects observed in field surveys and ecosystem experiments. The convergence of these various approaches on a CES near 20% suggests that EC20s may be an appropriate basis on which to set regulatory criteria.

Conclusion

Our analysis was able to detect and quantify effects of Ni on sensitive invertebrates in the field using toxicity information from a model laboratory organism. This successful lab-to-field extrapolation was possible because previous investigators have developed powerful approaches to modeling TMF effects, removing correlated stressor effects, and detecting stressor-response patterns in noisy field datasets. Our analysis had the further advantage of a rich laboratory dataset for *C. dubia*, which appeared to be a good surrogate for native mayfly taxa in terms of both sensitivity to Ni and its TMF effects. It seems likely that a similar analysis could be conducted for other stressors and other receptors, provided these same advantages are in place.

One useful outcome of our study was a set of novel EC_X estimates for Ni effects to several previously unstudied taxa. Mebane et al. (2020) added EC_X estimates from their experimental stream study to SSDs for several metals, increasing the taxonomic richness and greatly improving the representation of aquatic insects in those SSDs. The Ni SSD would be further enhanced by adding the field-based EC_X estimates derived herein. More generally, our approach could be a useful way to bolster SSDs for contaminants with lower richness of tested species. Our results indicate that careful analysis of field data can provide toxicity information similar in quality, although with different strengths and weaknesses, to the information from laboratory and mesocosm studies commonly used in SSDs.

A second useful outcome of our lab-to-field extrapolation was an estimated effect size in laboratory test organisms that would be predictive of discernible effects on invertebrates in the field. Our results indicated a CES near 20% for the most sensitive stream invertebrates in our study area, aligning with previous analyses (Mebane, 2010; USEPA, 1999) and supporting the use of EC20s and EC20-based SSDs in setting regulatory criteria to protect stream invertebrate communities. In our study area, a Ni criterion based on a *C. dubia* EC20 would be approximately equal to an estimated effects threshold for percent Ephemeroptera and the most sensitive mayfly taxon (*Baetis*), lower than thresholds for other metrics and taxa for which Ni effects could be described (*Epeorus*, *Drunella doddsii*, *Sweltsa*, Heptageniidae, Ameletidae, and Taeniopterygidae), and presumably much lower than thresholds for total abundance, taxonomic richness, and the 52 taxa for which no effects were apparent up to the highest Ni concentrations in our study. We would suggest that similar logic may be useful in setting and characterizing the protectiveness of regulatory criteria in other areas.

Supplementary material

Supplementary material is available online at *Environmental Toxicology and Chemistry*.

Data availability

Data, associated metadata, and calculation tools are available in the online supplementary material and from the corresponding author (adrian.debruyn@outlook.com).

Author contributions

Adrian M.H. de Bruyn (Conceptualization, Formal analysis, Investigation, Methodology, Writing—original draft, Writing—review & editing), Jordana L. Van Geest (Investigation, Methodology), Jennifer C. Arens (Formal analysis, Investigation), Jennifer Ings (Investigation), Nick Manklow (Project administration), Kevin V. Brix (Conceptualization, Writing—review & editing), and Mariah Arnold (Funding acquisition, Project administration, Writing—review & editing)

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Conflicts of interest

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Disclaimer

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