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Responses of Aquatic Insects to Cu and Zn in Stream Microcosms: Understanding Differences Between Single Species Tests and Field Responses

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Supporting Information

ABSTRACT: Field surveys of metal-contaminated streams suggest that some aquatic insects, particularly mayflies (Ephemeroptera) and stoneflies (Plecoptera), are highly sensitive to metals. However, results of single species toxicity tests indicate these organisms are quite tolerant, with LC50 values often several orders of magnitude greater than those obtained using standard test organisms (e.g., cladocerans and fathead minnows). Reconciling these differences is a critical research need, particularly since water quality criteria for metals are based primarily on results of single species toxicity tests. In this research we provide evidence based on community-level microcosm experiments to support the hypothesis that some aquatic insects are highly sensitive to metals. We present results of three experiments that quantified effects of Cu and Zn, alone and in combination, on stream insect communities. EC50 values, defined as the metal concentration that reduced abundance of aquatic insects by 50%, were several orders of magnitude lower than previously published values obtained from single species tests. We hypothesize that the short duration of laboratory toxicity tests and the failure to evaluate effects of metals on sensitive early life stages are the primary factors responsible for unrealistically high LC50 values in the literature. We also observed that Cu alone was significantly more toxic to aquatic insects than the combination of Cu and Zn, despite the fact that exposure concentrations represented theoretically similar toxicity levels. Our results suggest that water quality criteria for Zn were protective of most aquatic insects, whereas Cu was highly toxic to some species at concentrations near water quality criteria. Because of the functional significance of aquatic insects in stream ecosystems and their well-established importance as indicators of water quality, reconciling differences between field and laboratory responses and understanding the mechanisms responsible for variation in sensitivity among metals and metal mixtures is of critical importance.

INTRODUCTION

Water quality criteria for trace metals and other contaminants in aquatic ecosystems are based primarily on results of single species toxicity tests conducted in the laboratory. For decades the usefulness of these experiments for assessing effects of contaminants in natural systems have been the subject of considerable debate.[1] Although water quality criteria are assumed to be protective of most aquatic organisms, some groups are poorly represented in the database used to establish these values.[2,3] Most notably, laboratory data for aquatic insects, which are often the dominant organisms in many freshwater ecosystems, are very limited. The data that are available suggest that most aquatic insects are highly tolerant to metals, with LC50 values several orders of magnitude greater than those for standard test organisms (e.g., cladocerans and fathead minnows).[4,5] In contrast, field surveys of metal-contaminated streams indicate that some aquatic insects, especially mayflies (Ephemeroptera) and stoneflies (Plecoptera), are at least as sensitive to metals as fish[6,7] and may be affected at concentrations below existing water quality criteria.[8]

Reconciling differences in sensitivity of aquatic insects between field and laboratory results and understanding mechanisms responsible for these differences has been a significant focus of ecotoxicological research for several years. Buchwalter et al.[9] attributed the high tolerance of aquatic insects to metals in the laboratory to the short duration of exposure, which limited metal accumulation. Failure to assess dietary exposure to metals in laboratory experiments also underestimates real ecological effects.[10,11] Microcosm and field experiments conducted with aquatic insect communities showed that metals may alter susceptibility to predation.[12−14] In addition to direct toxic effects, we also know that metals alter

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critical ecosystem functions such as detritus processing and primary production.[15,16] These alterations in ecosystem function, which are rarely included in laboratory experiments, have important consequences for the flow of material and energy through aquatic food webs. Finally, with few exceptions, laboratory experiments conducted with aquatic insects routinely employ mature life stages or larger instars, which are considerably more tolerant to metals than early instars.[17,18]

Because aquatic insects are poorly represented in the database used to establish water quality criteria for metals, we know relatively little about the level of protection provided for these organisms. Furthermore, although differences in toxicity of trace metals are well established in the literature for other groups, there is little information on the relative toxicity of metals, individually or in combination, to aquatic insects. Because sensitivity to metals varies greatly among taxa,[6,8,18−20] it is possible that criterion values for one metal may be underprotective for some organisms whereas those derived for another metal may be overprotective. Using a stream microcosm technique that exposes natural communities of aquatic insects to metals and metal mixtures under controlled conditions, we established concentration−response relationships for several dominant EPT taxa (Ephemeroptera, Plecoptera, Trichoptera) and several macroinvertebrate metrics. The specific goals of this research were to (1) identify factors that may be responsible for differences between laboratory toxicity tests and field responses to metals; and (2) quantify the relative toxicity of Cu alone and in combination with Zn to aquatic insect communities.

■ MATERIALS AND METHODS

Three stream microcosm experiments were conducted using natural aquatic insect communities collected from the South Fork of the Michigan River (SFMR), a third order stream located in Routt National Forest, Colorado. Headwaters of the SFMR are located in a wilderness area and the stream has no history of metal contamination. All experiments were conducted in late summer or early fall. Communities were collected using 10 × 10 × 6 cm colonization trays filled with small cobble and pebble. Trays were deployed in the field for 30−40 days and colonized by a diverse community of aquatic insects. We have previously shown that the composition of communities that colonize these trays is similar to and representative of natural communities in Colorado streams.[13] Colonized trays were collected and placed into small insulated containers (four trays per container) and transported to the Stream Research Laboratory (SRL) located at Colorado State University. The SRL consists of 18 stream microcosms housed in a greenhouse that receives natural water directly from a deep, mesotrophic reservoir. Water quality in the microcosms (Supporting Information (SI) Table 1) is typical of montane Rocky Mountain streams and was characterized by low hardness (30−38 mg/L $CaCO_3$), alkalinity (25−29 mg/L $CaCO_3$) and dissolved organic carbon (2.5−3.0 mg/L), cool temperature (12−16.6 °C), circumneutral pH (6.7−7.8) and moderate conductivity (57−89 μmhos). Current in the 20 L microcosms is provided by paddlewheels that maintain a constant current velocity of 0.45 m/s. Each flow-through stream receives water from a headbox at 1.0 L/min, resulting in a turnover time of approximately 20 min. The four colonized trays in each container were placed in a single stream microcosm and randomly assigned to a treatment. To prevent emigration of aquatic insects from the microcosms, all standpipes were covered with a fine mesh. We have previously used the SRL to quantify responses of aquatic insect communities to natural and anthropogenic stressors, including trace metals,[20] low pH,[21] UV-B radiation[22] and stonefly predation.[13]

The first two microcosm experiments were conducted in fall 2007 and employed a regression design to establish concentration−response relationships to a range of Cu or Cu+Zn concentrations. Target concentrations in the Cu only experiment represented 0, 1, 2, 3, 6, 12, 25, 50, and 100× the U.S. EPA hardness-adjusted criterion value (~ 5 μg/L) at a water hardness of 35 mg/L $CaCO_3$.[23] We used hardness-based criterion values for these analyses instead of the more recent biotic ligand model (BLM, [24]) because (1) a BLM-based criterion value for Zn has not been approved by the U.S. EPA; and (2) a BLM-based approach to quantify effects of metal mixtures has not been developed. However, we note that using water quality characteristics in our stream microcosms, the criterion value for Cu based on the BLM (6.2 μg/L) was very similar to the hardness-based criterion value. Target concentrations in the Cu+Zn experiment represented 0, 2, 4, 6, 10, 20, 40, 75, 150× the criterion values for Cu and Zn (48 μg/L). Streams were treated with metals in duplicate (n = 2) using peristaltic pumps that dripped stock solutions from 20 L carboys (10 mL/min). A third microcosm experiment conducted in fall 2010 also examined the relative toxicity of Cu and Zn to benthic communities. However, in this experiment we employed a factorial design in which microcosms were assigned to one of four treatments (n = 4): control, Cu only, Zn only, and Cu + Zn. Target concentrations in metal-treated streams were 10× the hardness-adjusted criterion values in the Cu only (50 μg/L) and Zn only (480 μg/L) treatments and 5× these value in the Cu+Zn treatment. Benthic communities were exposed to metals for 10 days in all three experiments, after which trays were removed and surviving organisms were counted and identified.

In addition to these community-level experiments, single species toxicity tests were conducted concurrently with the mayfly *Drunella grandis* (Ephemeroptera: Ephemerellidae). The purpose of these experiments was to compare community-level responses in stream microcosms to those observed using standard laboratory procedures with single species. To verify that differences between single species experiments and whole community responses were not a result of differences in water quality characteristics, single species tests were conducted simultaneously at the Colorado Parks and Wildlife (CPW) Aquatic Toxicology Laboratory and the SRL. Mayflies were collected in fall 2007 from the SFMR at the same time that colonization trays were collected. We followed typical protocols for testing with aquatic insects and collected mature, late instars for each experiment. Organisms were transferred to the CPW laboratory or to the SRL in aerated and chilled plastic containers. Mayflies in the SRL experiments were placed in cylindrical nylon mesh (600 μm) chambers (10 cm × 13 cm) and randomly assigned to each microcosm (n = 2). Chambers contained 5−6 mayflies each and were suspended in the same microcosms with the community-level experiments. Therefore, these organisms were exposed to the same water quality characteristics as in the community-level experiments described above. Exposure chambers in the CPW laboratory experiments consisted of 1.25 L, cylindrical, polypropylene containers equipped with an air-lift system that provided continuous, circular flow in the exposure chamber. Nitex mesh (1000 μm)

Table 1. Nominal and Mean Measured Concentrations (μg/L) of Cu and Zn in the Two Regression Experiments (Cu only and Cu+Zn) and the ANOVA Experiment[a]

Cu Only (regression)			Cu + Zn (regression)					Cu + Zn (ANOVA)			
nominal	measured		nominal	measured	nominal	measured		measured		measured	
Cu	Cu	CCU	Cu	Cu	Zn	Zn	CCU	treatment	Cu	Zn	CCU
5	7.1	1.4	5	10.3	50	41.4	2.9	Cu only	36.3		7.2
10	12.9	2.6	10	13.5	100	93.0	4.6	Zn only		520.0	10.8
15	22.7	4.5	15	18.8	150	176.9	7.4	Cu+Zn	21.8	285.0	10.3
30	38.0	7.6	25	22.8	300	306.1	10.9				
60	58.1	11.6	30	31.2	625	658	19.9				
125	121.5	24.3	60	55.8	1250	1382.7	40.0				
250	224.4	44.9	125	125.7	2500	2574.2	78.8				
500	494.5	98.9	250	264.3	5000	5531.6	168.1				

[a]CCU = the sum of the measured concentration for each metal divided by the U.S. EPA hardness-adjusted criterion values for Cu (5.0 μg/L) and/or Zn (48 μg/L).

was placed in the exposure chambers as substrate. Source water at the CPW consisted of a mixture of well water and reverse osmosis water. A conductivity controller maintained water hardness near 36 mg/L to approximate water quality conditions in the microcosms. Organisms were exposed using a continuous-flow serial diluter that delivered five concentrations of metals with a 50% dilution ratio and a control. Target concentrations in the Cu only experiment (0, 31, 62, 125, 250, 500 μg Cu/L) and Cu+Zn experiment (0, 16, 31, 62, 125, 250 μg Cu/L + 0, 312, 625, 1250, 2500, 5000 μg Zn/L) bracketed those in the microcosm experiments. Five *D. grandis* nymphs were randomly assigned to each exposure chamber (n = 4 in the Cu only experiment; n = 2 in the Cu + Zn experiment). Organisms were not fed during the 7 day exposure, and mortality was recorded at the end of the experiment.

To determine concentrations of Cu and Zn, water samples were collected on days 2 and 7 from stream microcosms and collected daily from CPW laboratory exposure chambers. Water samples were filtered through a 0.45 μm filter, acidified to a pH of <2.0 and analyzed using flame or furnace (depending on expected concentration) atomic adsorption spectrophotometry. Water samples for metals analyses included sample splits and spikes collected during each sampling event to verify reproducibility and to quantify analytical recovery.

Data Analyses. All statistical analyses were conducted using SAS (SAS Institute Inc., Cary, NC). Because the experiments involved exposure to individual or mixtures of metals, an additive measure of toxicity was used to express concentrations relative to the U.S. EPA hardness-adjusted criterion values. The cumulative criterion unit (CCU), defined as the ratio of the measured dissolved metal concentration to the hardness-adjusted criterion value and summed for each metal, is given as

$$\mathrm{CCU} = \sum m_{\mathrm{i}}/\mathrm{c}_{\mathrm{i}}$$

where m_i = the measured concentration of the ith metal and c_i = the hardness-adjusted criterion value for the *i*th metal. This approach has been used in previous studies to quantify effects of metal mixtures.[6,25]

Probit analysis (PROC PROBIT) was used to estimate LC50 values for the single species toxicity tests conducted with *Drunella*. We developed general linear models (PROC GLM) in SAS (SAS Institute, Cary, NC) to estimate the relative toxicity of Cu and Cu+Zn and to establish concentration−response relationships between CCU and benthic community responses in the two regression experiments. We examined the effects of metal concentration (as CCU), treatment (Cu versus Cu+Zn) and the CCU x treatment interaction term. In this analysis a significant interaction term would indicate differences in the slope of the concentration−response relationships between the Cu alone and Cu+Zn treatments. For those metrics that showed a significant interaction, we also ran a separate analysis using Cu concentration alone as a predictor variable. In this analysis, a significant interaction between Cu and treatment would suggest synergistic or antagonistic effects of Zn. All data were log transformed (ln+1) to improve the fit of these models. Using parameters from the regression models, we estimated EC50 values, defined as the concentration that resulted in a 50% reduction in abundance or richness, for all macroinvertebrate metrics. One-way ANOVA was used to characterize toxicity of Cu, Zn, and Cu+Zn in microcosm experiment 3 (the factorial design experiment).

RESULTS

Concentrations of Cu and Zn were consistently within 10% of target levels in laboratory single species experiments conducted with *D. grandis*. Concentrations of metals were more variable in stream microcosms, but approximated target concentrations in the two regression experiments (Table 1). Measured concentrations of Cu and Zn also approximated target levels in the Zn only and Zn+Cu treatments of the ANOVA experiment. However, measured Cu was 27% less than the target concentration in the Cu only treatment.

Results of single species toxicity tests conducted with the mayfly *D. grandis* showed that responses to metals in stream microcosms and the laboratory were very similar (Figure 1). Control survival ranged from 80 to 100% in all experiments and decreased with increasing Cu or Cu+Zn concentration. Results of both laboratory and microcosm experiments showed that Cu alone was significantly more toxic to *Drunella* than the combination of Cu and Zn, despite the fact that exposure concentrations represented theoretically similar toxicity levels. Estimated LC50 values (and the associated 95% fiducial limits) were similar between laboratory and microcosm experiments, but were much greater for the Cu+Zn experiment compared to the Cu only experiment.

Macroinvertebrate communities in control microcosms were diverse, averaging 26.7 (±4.9) taxa and 932.6 (±204) individuals per stream across the three experiments. Exposure of these communities to metals resulted in highly significant reductions in abundance and showed strong concentration−

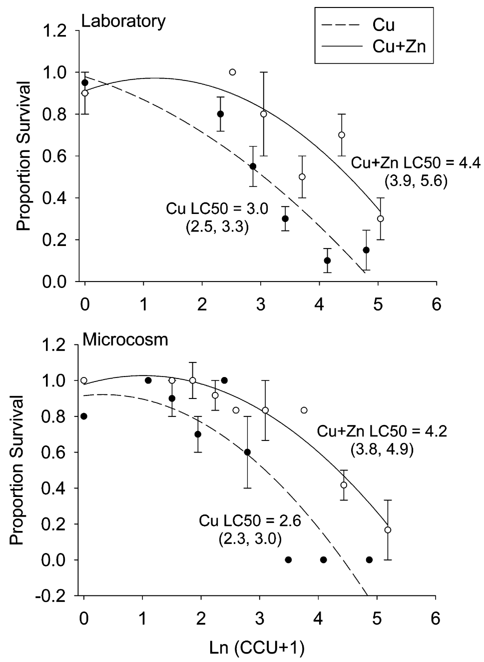

Figure 1. Results of single species toxicity tests conducted with the mayfly *Drunella grandis* (Ephemeroptera: Ephemerellidae) in the laboratory and in stream microcosms. The figure shows the mean proportion survival (±s.e.) after 7 days exposure to Cu or Cu+Zn. LC50 values with the associated 95% fiducial limits are also shown. CCU = the ratio of the measured dissolved metal concentration to the hardness-adjusted criterion value and summed for each metal (see Materials and Methods for details).

response relationships for most taxa (Figure 2; SI Tables 2 and 3). Metal concentration was a significant predictor for all dominant EPT taxa and a simple model that included concentration, treatment (Cu only versus Cu+Zn) and the treatment x concentration interaction term explained 52−87% of the variation. Compared to results of our single species tests, the four dominant mayflies (*Baetis*, *Serratella*, *Rhithrogena*, and *Cinygmula*) were highly sensitive to metals in stream microcosms. In the Cu only experiment, most mayflies were eliminated when Cu exceeded 40 μg/L (approximately 7.5× the hardness-adjusted CCU). *Zapada* and *Sweltsa*, the dominant stoneflies in our experiment, also showed significant responses to metals; however, the magnitude of these effects differed between the two experiments. Although concentration−response relationships for the dominant caddisflies (*Brachycentrus* and *Micrasema*) were significant, these taxa were much less sensitive to metals. EC50 values, defined as the metal concentration that reduced abundance by 50% compared to controls, were generally lower for mayflies and stoneflies than for caddisflies (Figure 2; SI Table 3).

Each of the macroinvertebrate abundance and richness metrics that we examined also showed a highly significant concentration response relationship; however, effects of metals differed among metrics (Figure 3; SI Table 3). Total abundance of Ephemeroptera was highly sensitive to metals, and was reduced by >50% at the U.S. EPA hardness-adjusted criterion value of 5 μg/L Cu. Plecoptera showed an intermediate response to metals and most Trichoptera survived concentrations that exceeded 100 times the criterion value. Comparisons of EC50 values for these metrics showed that total macroinvertebrate abundance was considerably more sensitive to metals than species richness.

One of the most consistent results of these experiments was that Cu alone was significantly more toxic to aquatic insects than a mixture of Cu and Zn. Across most dominant taxa and metrics that we examined, EC50 values in the Cu+Zn experiment were greater than in the Cu only experiment (SI Table 3; Figures 2, 3). The metal x concentration interaction term was significant for five variables (*Serratella*, *Rhithrogena*, *Sweltsa*, total abundance of mayflies and species richness of mayflies), indicating greater effects in the Cu only experiment. We observed similar results for these metrics when we conducted the analyses using Cu concentration instead of CCU. Cu was less toxic to *Serratella* ($p = 0.0010$), *Sweltsa* ($p <$

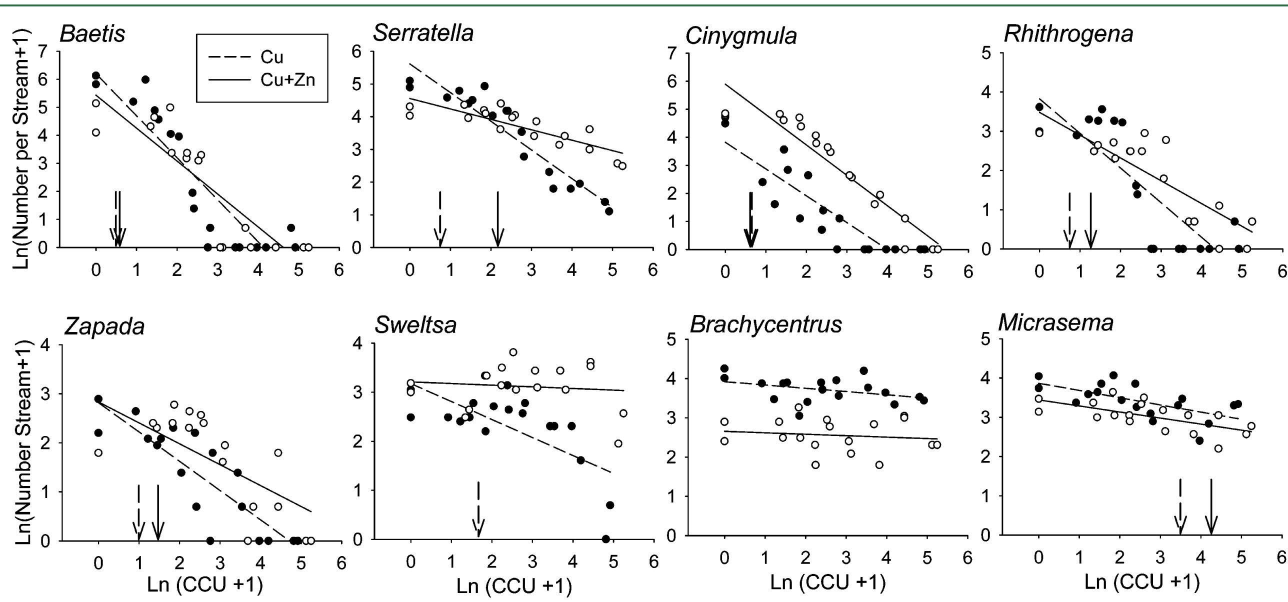

Figure 2. Effects of Cu alone (solid circles) or Cu+Zn (open circles) on abundance of dominant mayflies, stoneflies and caddisflies in stream microcosms. Arrows indicate the estimated EC50 values, defined as the metal concentration that reduced abundance by 50%, for the Cu (dashed line) and Cu+Zn experiments. CCU as defined in Figure 1.

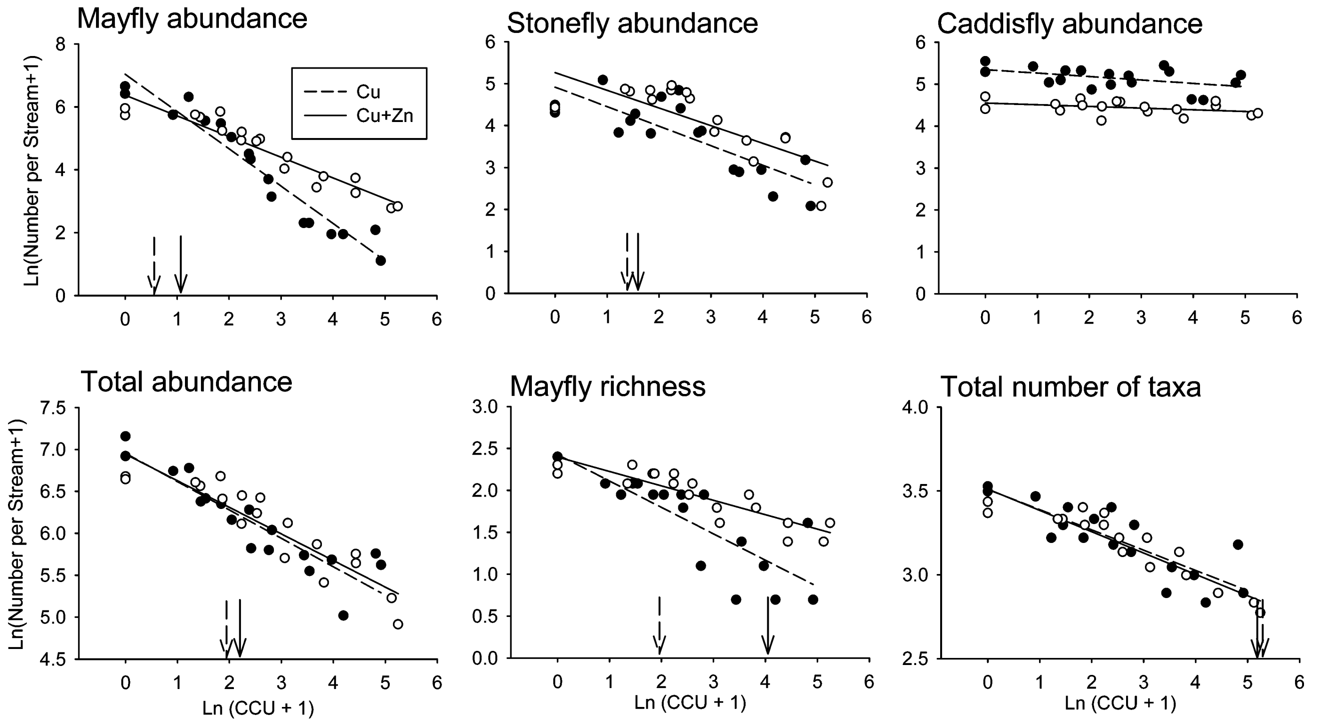

Figure 3. Effects of Cu alone (solid circles) or Cu+Zn (open circles) on abundance and species richness in stream microcosms. Arrows indicate the estimated EC50 values, defined as the metal concentration that reduced abundance or richness by 50%, for the Cu (dashed line) and Cu+Zn experiments. CCU as defined in Figure 1.

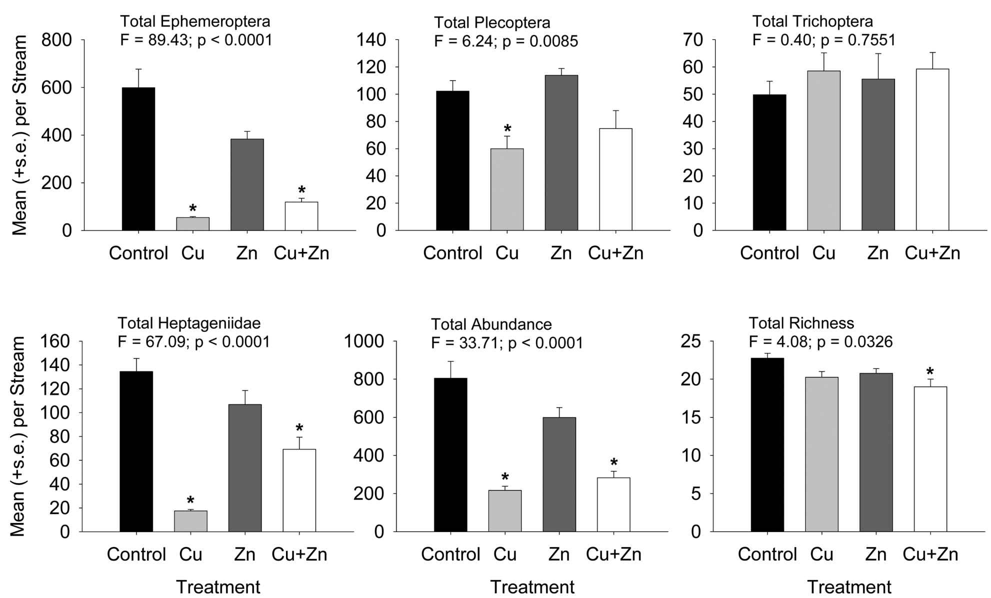

Figure 4. Effects of Cu, Zn or Cu+Zn on macroinvertebrate communities in stream microcosms. The figure shows mean (±s.e.) abundance or richness and the results of 1-way ANOVA. Target concentrations in all metal treatments were 10× the hardness-adjusted criterion value in the Cu only and Zn only treatments and 5× each metal in the Cu+Zn treatment. * = significantly different from control streams.

0.0466), total abundance of mayflies ($p < 0.0123$) and species richness of mayflies ($p < 0.0858$) when Zn was present.

In microcosm experiment 3, we employed an ANOVA design to compare the relative toxicity of Cu and Zn, individually and in combination. Assuming a simple additive model, target concentrations in treated streams represented equally toxic levels of these metals. Therefore, we hypothesized that effects would be similar across metal treatments. Results of this experiment were consistent with the 2 regression experiments, especially for mayflies (Figure 4). Exposure of macroinvertebrate communities to Cu alone (CCU = 7.2) and Cu+Zn (CCU = 10.3) resulted in 90% and 80% reductions in total mayfly abundance, respectively. Using parameters obtained from the Cu and Cu+Zn regression experiments (SI Table 3), we estimated that these same concentrations reduced total mayfly abundance by 93% and 80%, respectively. With the exception of caddisfly abundance, all metrics showed a significant reduction in the Cu only and/or Cu+Zn treatments compared to controls. Effects were generally greater in the Cu only treatments, despite a lower than expected measured Cu concentration (Table 1). Although abundance of some groups was lower in the Zn only treatments, these differences were not significant for any metric.

■ DISCUSSION

The most significant finding of this study was that relatively short-term (10 day) exposure of macroinvertebrate communities to Cu at concentrations near the U.S. EPA hardness-adjusted criterion value significantly reduced abundance and altered community composition in stream microcosms. Consistent with previous microcosm experiments[18,20] and field studies,[7,8] the greatest effects were observed on mayflies, which were reduced by approximately 50% at 5 μg Cu/L in the Cu only experiment and essentially eliminated at 36 μg Cu/L in experiment 3 (the ANOVA experiment). Although some aquatic insect groups (e.g., caddisflies) were considerably more tolerant to Cu, other nonmayfly taxa were reduced at low Cu concentrations. For example, exposure to 15 μg Cu/L reduced total abundance of stoneflies by approximately 50%. The sensitivity of mayflies and some stoneflies to Cu is important because these organisms are dominant in many western streams and are an important food resource for trout and other species.

Results of our microcosm experiments are in agreement with several spatially extensive field studies that reported alterations in macroinvertebrate communities at low metal concentrations. In a survey of 153 streams in Colorado, Schmidt et al.[8] observed negative effects of metals on benthic communities at concentrations near the water quality criteria. Clements et al.[6] reported significant effects on macroinvertebrate communities when metal levels exceeded 2× the hardness-adjusted criterion value and some evidence of effects on metal-sensitive groups when concentrations were between 1 and 2× this level. Using data from macroinvertebrate surveys of over 400 sites on three continents, Iwasaki and Ormerod[26] estimated safe metal concentrations for Cu and Zn were 6.6 μg/L and 34 μg/L, respectively. Results of our microcosm experiments and these field studies were strikingly different from laboratory experiments that measured toxicity of metals to aquatic insects. In a review of laboratory studies conducted with numerous taxa, Brix et al.[3] reported that 96 h LC50 values were several orders of magnitude greater for aquatic insects than for other groups examined. In laboratory experiments conducted with the mayfly *Rhithrogena hageni*, a common species in Colorado, Brinkman and Johnston[4] reported 96 h LC50 values of 137 and 50 500 μg/L for Cu and Zn, respectively. These concentrations are approximately 25 and 850 times greater than the hardness-based criteria values for these metals. Similarly high LC50 values have been reported for mayflies and other aquatic insect taxa exposed to metals in the laboratory.[5,27−29]

How Do We Reconcile Differences in Sensitivity among Field, Microcosm and Laboratory Studies? The absence of many aquatic insect groups from moderately contaminated streams and the large discrepancies among laboratory, microcosm and field studies suggest that single species toxicity tests may underestimate effects of metals on some aquatic insects. Results of our single species tests conducted with the mayfly *Drunella grandis* in stream microcosms showed that LC50 values were approximately 17 and 36 times greater than EC50s estimated for total mayfly abundance in the Cu only and Cu+Zn microcosm experiments, respectively. Two primary hypotheses have been proposed to explain the greater tolerance of aquatic insects observed in single species tests. First, laboratory experiments generally do not account for dietary uptake of metals, which is the predominant route of exposure for some organisms.[10,11,30] Although macroinvertebrate communities in our microcosms were exposed to metals primarily through water, some uptake may have occurred through consumption of metal-contaminated periphyton and detritus. The second hypothesis to explain discrepancies between field and laboratory results emerged from research on biodynamic models.[31,32] Because of the short duration (96 h) of most laboratory toxicity tests, it is unlikely that metal concentrations in aquatic insects reach steady state conditions, which may require several weeks or even months for some taxa.[9] Other investigators have reported considerably lower LC50 values for aquatic insects when experiments were conducted for longer duration.[33,34] Although the longer duration of our microcosm experiments (10 days) may partially explain the greater effects observed on these natural communities, our experiments were still relatively short-term compared to field exposures. Thus, we hypothesize that other factors related to life history characteristics of aquatic insects are also responsible.

Because most aquatic insects cannot be cultured in the laboratory (but see ref 35 as an important exception), single species toxicity tests are typically conducted with field-collected organisms. For practical reasons, these experiments usually employ larger instars, which are easier to collect in the field and considerably more tolerant to handling stress. However, laboratory[36,37] and microcosm[17,18] experiments have consistently shown that larger instars of aquatic insects are significantly more tolerant to metals. In contrast to single species toxicity tests, our microcosm experiments are typical of natural field conditions and expose numerous species across a wide range of size classes to metals. Although we did not quantify size distributions of dominant taxa in this study, we observed the greatest effects on small, early instars of mayflies (particularly *Baetis* and *Cinygmula*). In summary, we hypothesize that the short duration of laboratory toxicity tests and the failure to evaluate effects on highly sensitive, early life stages are the primary factors responsible for high LC50 values generated from single species tests with aquatic insects. This hypothesis is supported by early life stage experiments conducted with parthenogenetic mayflies[35] showing that small instars are at least as sensitive to metals and other contaminants as the cladoceran *Ceriodaphnia dubia*.[38]

Variation in sensitivity to contaminants among instars and life stages of aquatic insects has important implications for bioassessment and the establishment of water quality criteria. Because growth and maturation of aquatic insects are closely associated with phenology, sensitivity to metals and other contaminants may also vary among seasons.[18,39] For example, we have previously shown that communities collected in late summer, which in Rocky Mountain streams are dominated by small, early instars, were significantly more sensitive to metals than communities collected in spring which were dominated by larger individuals.[18] Ultimately, a better understanding of the complex relationship between insect phenology and seasonal changes in abiotic factors that influence metal bioavailability (e.g., DOC, pH, water hardness) will be necessary to predict effects of metals in the field.

Differences between Cu and Zn Toxicity. It is well established that Cu is significantly more toxic to aquatic organisms than Zn, and water quality criteria for metals reflect these differences.[23] By expressing metal treatments in our experiments as a function of hardness-based criteria values, hypothetically we should be able to compare relative toxicity of individual metals and metal mixtures. In other words, assuming no metal interactions, we predicted that exposure to a similar CCU for Cu, Zn, and Cu+Zn should have approximately similar effects on aquatic insect communities. In contrast to these predictions, we found that Cu was much more toxic than Zn alone or a mixture of Cu and Zn. This conclusion was supported by the comparison of EC50 values from the two regression experiments, the significant metal x concentration interaction term for several metrics, and results of microcosm experiment 3 which showed highly significant effects of Cu but relatively modest effects of Zn. Our findings also suggest that the hardness-adjusted criterion value for Zn was protective of aquatic insects, at least in short-term (10 day) experiments. With the exception of mayflies, most aquatic insect groups showed relatively little response to Zn at approximately 10× the hardness-adjusted criterion value (520 μg/L). In contrast, the criterion value for Cu may not be sufficiently protective, especially for longer-term exposure of early instars. Although exposure to mixtures of metals is common in nature, surprisingly few studies have examined effects of metal interactions on natural aquatic insect communities. Clements[20] reported that macroinvertebrate community responses to a mixture of Cd+Cu+Zn were greater than Zn alone or Cd+Zn. The specific mechanisms responsible for these differences were not identified, but our current findings suggest that the presence of Cu in this mixture was likely responsible for greater toxicity. Regardless, these results suggest that caution is required when interpreting responses to metal mixtures. Our inability to predict differences in relative toxicity based on hardness-adjusted criterion values for metals should not be surprising given that aquatic insects are so poorly represented in the database used to establish these criteria.[3] Because of the functional significance of aquatic insects in stream ecosystems and their well-established importance as indicators of water quality, additional research to understand the mechanisms responsible for variation in sensitivity to metals and metal mixtures is of critical importance.

In summary, previous experiments conducted in our stream microcosm facility have allowed us to quantify direct and indirect effects of metals on natural communities,[12,13] study interactions of metals with other stressors[21,22] and establish causal relationship between metals and community-level responses.[20] Our current study contributes to our understanding of the effects of metals and metal mixtures on natural communities and provides support for the hypothesis that differences between field and laboratory responses are influenced by life history characteristics of aquatic insects. The vast difference between Cu and Zn toxicity, particularly when these metals are expressed as a function of toxic units, is additional evidence that ecologically realistic experiments with aquatic insects should be included in the development and validation of water quality criteria.

■ ASSOCIATED CONTENT

Supporting Information

The following information is available. Supporting Table 1 shows average water quality characteristics from the 3 microcosm experiments. Supporting Table 2 shows mean abundance of other dominant and non-EPT taxa in 3 stream microcosm experiments. Supporting Table 3 shows results of statistical analyses from the 2 regression experiments. 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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